

STUDIES ON HIGHER SUGARS

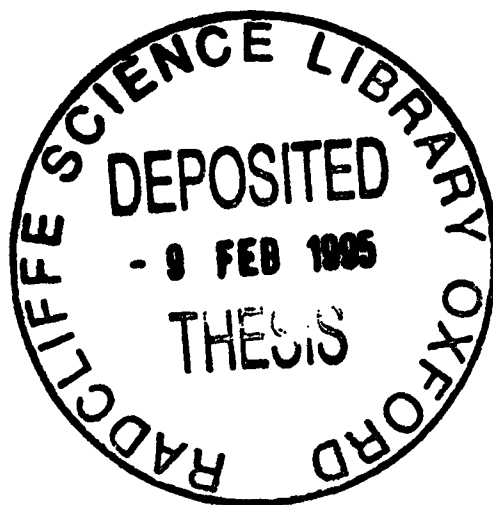
by

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A thesis submitted in partial fulfilment of
the requirements of the degree of D. Phil.



Studies on Higher Sugars.

Annabel R. Beacham, Wolfson College, Oxford

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Abstract.

This thesis describes the synthesis of three novel seven carbon bicyclic mimics of α -L-fucose, and of two new pyrrolidine amino sugars.

2,7-Anhydro-1-deoxy- β -L-*gulo*-heptulopyranose and 1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose were both synthesised from L-gulono-1,4-lactone. The addition of one equivalent of methyllithium to the diacetone of L-gulono-1,4-lactone gave a keto-sugar, 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose. The anomeric configuration of this compound was determined by equilibrium nOe measurements. Hydrolysis in aqueous trifluoroacetic acid caused simultaneous deprotection, isomerisation and dehydration to yield 2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose, a highly stable, rigid bicyclic system. The structure of the bicyclic system was confirmed by X-ray crystallographic studies on a crystalline derivative.

The introduction of nitrogen at C-6 of L-gulono-1,4-lactone was achieved via the azide displacement of the known bromide, 6-bromo-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone. Protection of the C-5 hydroxyl group as its silyl ether was followed by the addition of one equivalent of methyllithium to the carbonyl group to give a keto-sugar, 7-azido-6-*O*-*tert*-butyldimethylsilyl-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose. Removal of the protecting groups followed by reduction of the azide functionality gave the bicyclic hemiaminal, 1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose, a stable but hygroscopic solid.

A third bicyclic system, 2,7-anhydro-1,2,6-trideoxy-2,6-imino- β -L-*gulo*-heptulopyranose, was synthesised from diacetone-D-mannose via the known keto-sugar, 6-azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose. Removal of the protecting groups from this keto-sugar, followed by reduction of the azide functionality, gave the target system. Analysis of the NMR spectra showed that this existed as an equilibrium mixture of the closed, bicyclic hemiaminal form and the monocyclic imine form, with the bicyclic form predominating in all solvents investigated.

The sodium borohydride reduction of 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose gave a single product, the heptitol 7-deoxy-1,2;4,5-di-*O*-isopropylidene-L-*glycero*-D-*gluco*-heptitol. This was converted into two novel pyrrolidine amino sugars, 1,2,5-trideoxy-2,5-imino-L-*glycero*-L-*allo*-heptitol and 1,2,5-trideoxy-2,5-imino-L-allitol. The two free hydroxyl groups in the heptitol were converted into leaving groups and one was then displaced selectively with sodium azide. Reduction of the azide functionality gave an amine which cyclised onto the remaining leaving group to form the pyrrolidine framework. Complete deprotection of this product gave 1,2,5-trideoxy-2,5-imino-L-*glycero*-L-*allo*-heptitol, the structure of which was confirmed by X-ray crystallographic studies on a crystalline derivative. Removal of the primary acetone from the cyclisation product and subsequent periodate cleavage gave an aldehyde which was then reduced to an alcohol. Deprotection then gave the second pyrrolidine amino sugar 1,2,5-trideoxy-2,5-imino-L-allitol.

The effect of all five target compounds on eleven human liver glycosidase enzymes was investigated, and these results are also reported.

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Firstly, I must thank my supervisor at Oxford, Dr. George Fleet, for his help, advice and boundless enthusiasm, and for believing that "this must work". Many thanks are also due to my industrial supervisor, Dr. Keith Biggadike, for his constant support and practical suggestions.

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Abbreviations

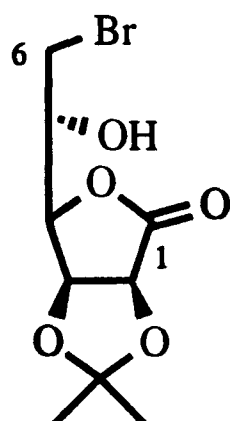
The following abbreviations are used in this thesis:

Ac	Acetyl
Aq.	Aqueous
Ar	Aryl
Bn	Benzyl
br	Broad
bz	Benzoate
c.	Concentrated
<i>ca.</i>	Circa
CSA	DL-10-Camphorsulphonic acid
d	Doublet
DBU	1,5-Diazabicyclo[5.4.0]undecane
dd	Double doublet
DCM	Dichloromethane
DFJ	Deoxyfuconojirimycin
DHAP	Dihydroxyacetone-3-phosphate
DIBAL	Diisobutylaluminium hydride
DMAP	4-(<i>N, N</i> -Dimethylamino)-pyridine
DMDP	2 <i>R</i> ,5 <i>R</i> -Dihydroxymethyl-3 <i>R</i> ,4 <i>R</i> -dihydroxy-pyrrolidine
DMF	<i>N, N</i> -Dimethylformamide
DMJ	Deoxymannonojirimycin
DMSO	Dimethylsulphoxide
dq	double quartet
dt	double triplet
Et	Ethyl
GDP	Guanidine diphosphate
h	Hour(s)

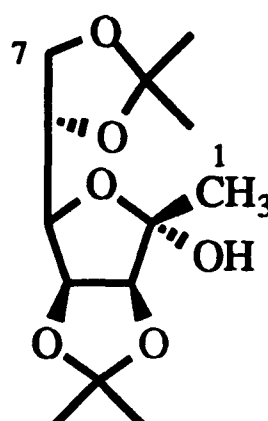
HFJ	Homofuconojirimycin
IR	Infra red
Lit.	Literature
m	Multiplet
Me	Methyl
min	Minutes
M.p.	Melting point
Ms, mesyl	Methanesulphonyl
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
PCC	Pyridinium chlorochromate
Ph	Phenyl
py	Pyridine
q	Quartet
qu	Quintet
quant.	Quantitative
s	Singlet
t	Triplet
TBAF	Tetra- <i>n</i> -butylammonium fluoride
TBDMS	<i>tert</i> -Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
t.l.c.	Thin layer chromatography
Ts, tosyl	<i>para</i> -Toluenesulphonyl
Tf, triflate	Trifluoromethanesulphonyl
Z	Benzyloxycarbonyl

Note concerning nomenclature

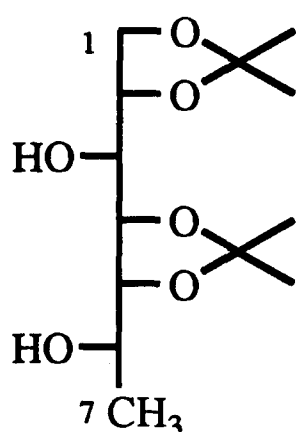
The compounds in this thesis are numbered according to the systems shown for the examples below.



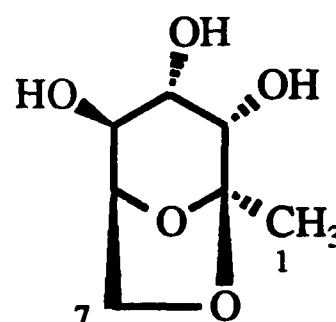
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2,3-*O*-isopropylidene-
L-gulono-1,4-lactone



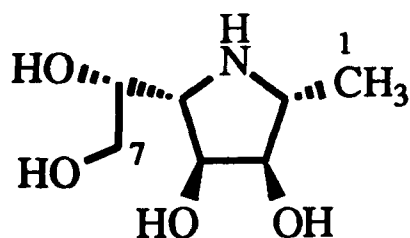
1-Deoxy-3,4;6,7-di-
O-isopropylidene-
 β -L-*gulo*-heptulfuranose



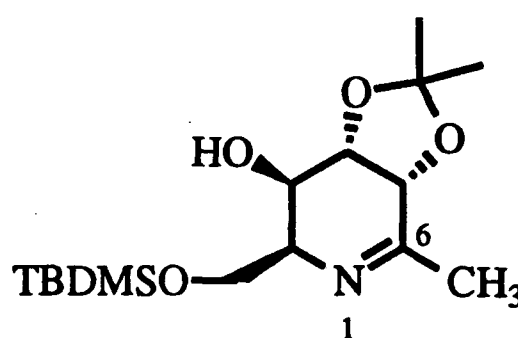
7-Deoxy-1,2;4,5-di-
O-isopropylidene-
L-*glycero*-D-*gluco*-heptitol



2,7-Anhydro-1-deoxy-
 β -L-*gulo*-heptulopyranose



1,2,5-Trideoxy-2,5-imino-L-
glycero-L-*allo*-heptitol



(2*S*,3*R*,4*S*,5*R*)-2-(*O*-*tert*-
Butyldimethylsilylhydroxymethyl)-
4,5-*O*-isopropylidene-6-methyl-
2,3,4,5-tetrahydropyridine-
3,4,5-triol

Chapter 1

Introduction.

1.1 General Introduction.

L-Fucose (1.1) is a naturally occurring sugar which is a constituent of many oligosaccharides of biological importance such as glycolipids, blood-group antigens and complex polysaccharides.¹ It is usually prepared by hydrolysis of the *Fucus* species of seaweed.² As this is an expensive process, several syntheses of L-fucose (1.1) from more common carbohydrates have been reported.³ L-Fucose is a 6-deoxy hexose, specifically the 6-deoxy derivative of L-galactose (1.2) (Figure 1).

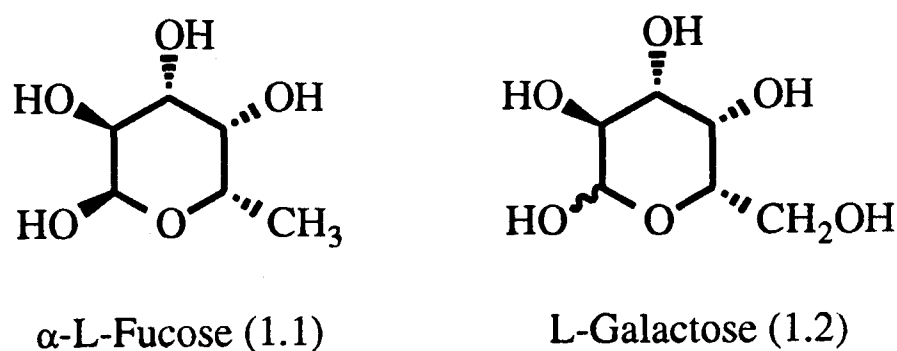


Figure 1

Analogues of fucose are of interest as potential inhibitors of fucose processing enzymes; for example the fucosyltransferases which are associated with the biosynthesis of fucose containing macromolecules, and the fucosidases which facilitate the hydrolysis of fucose from oligosaccharides. They may also be of interest as metabolically stable mimics of fucose for incorporation into synthetic oligosaccharides, thus enabling investigation of the role of fucose in carbohydrate-protein interactions.

Many naturally occurring and synthetic amino sugars have been investigated as glycosidase inhibitors.⁶ These are analogues of monosaccharides where the ring oxygen has been replaced by nitrogen, rendering the compounds metabolically stable, but not usually preventing their recognition by glycosidases and other carbohydrate-recognising proteins. The most potent fucosidase inhibitors so far reported are the amino sugars deoxyfuconojirimycin (DFJ)⁸ (1.5) and α -L-homofuconojirimycin (α -L-HFJ)⁹ (1.6). Both are highly specific, competitive inhibitors of the enzyme (Figure 3).

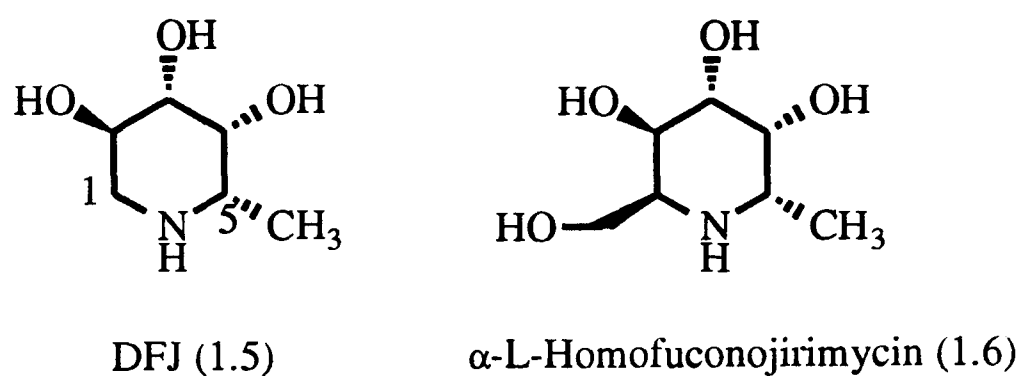
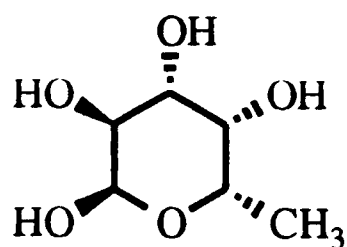


Figure 3

DFJ (1.5) has a K_i of 1×10^{-8} M against the human liver enzyme¹⁰ and a K_i of 4×10^{-11} M against a canine fucosidase.¹¹ α -HFJ (1.6) has a K_i of 1×10^{-8} M against human neutrophil fucosidase.⁹ A study into the structural requirements for fucosidase inhibition by analogues of DFJ (1.5) found that piperidine amino sugars with the same absolute configuration of the secondary hydroxyl groups as L-fucose (1.1) are powerful inhibitors of most fucosidases (Figure 4).¹⁰



α-L-Fucose (1.1)

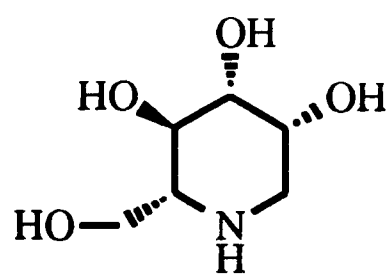
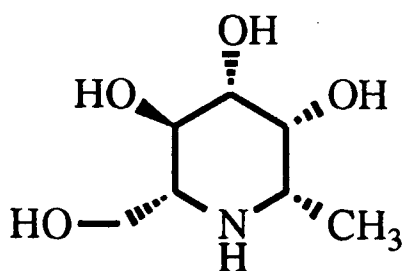
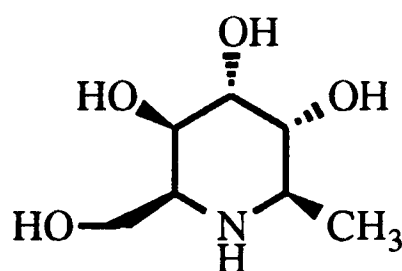
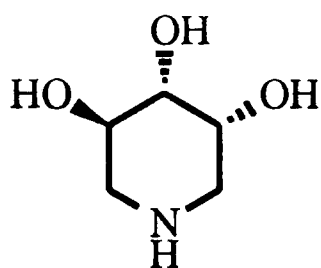
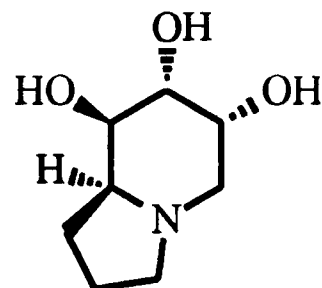
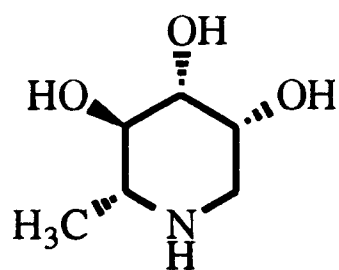
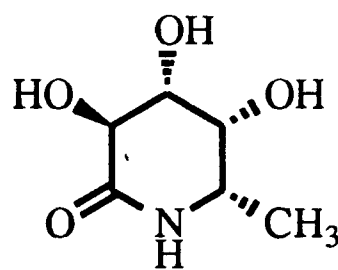
DMJ (1.7)
 K_i 5×10^{-6} Mβ-L-Homofuconojirimycin (1.8)
 K_i 1×10^{-8} M6-Epi-α-L-homofuconojirimycin (1.9)
 K_i 5×10^{-6} M2,3,4-Trihydroxy piperidine
 K_i 8×10^{-6} M1-Deoxy-6,8a-diepicastanospermine
 K_i 1.3×10^{-6} MD-Rhamnojirimycin
 K_i 7×10^{-5} ML-Fuconic-δ-lactam
 K_i 4×10^{-4} M

Figure 4

Different substituents in either configuration at C-1 or C-5 may alter the potency or specificity but do not destroy inhibition of α-L-fucosidase. For example, deoxymannonojirimycin (DMJ) (1.7), the D-mannose (1.10) analogue of DFJ

In general, pyrrolidine analogues of fucose (1.1) are less potent inhibitors of fucosidases than are the piperidine analogues.¹⁴ The most potent pyrrolidine inhibitor so far reported is compound (1.12), which has a K_i of 1.4×10^{-6} M against the bovine kidney enzyme.¹⁵ The stereochemical requirements for fucosidase inhibition by pyrrolidine amino sugars are less stringent than those for the piperidine fucose analogues, with a range of diastereomeric five membered amino sugars all showing similar inhibitory effects (Figure 6).^{15,16}

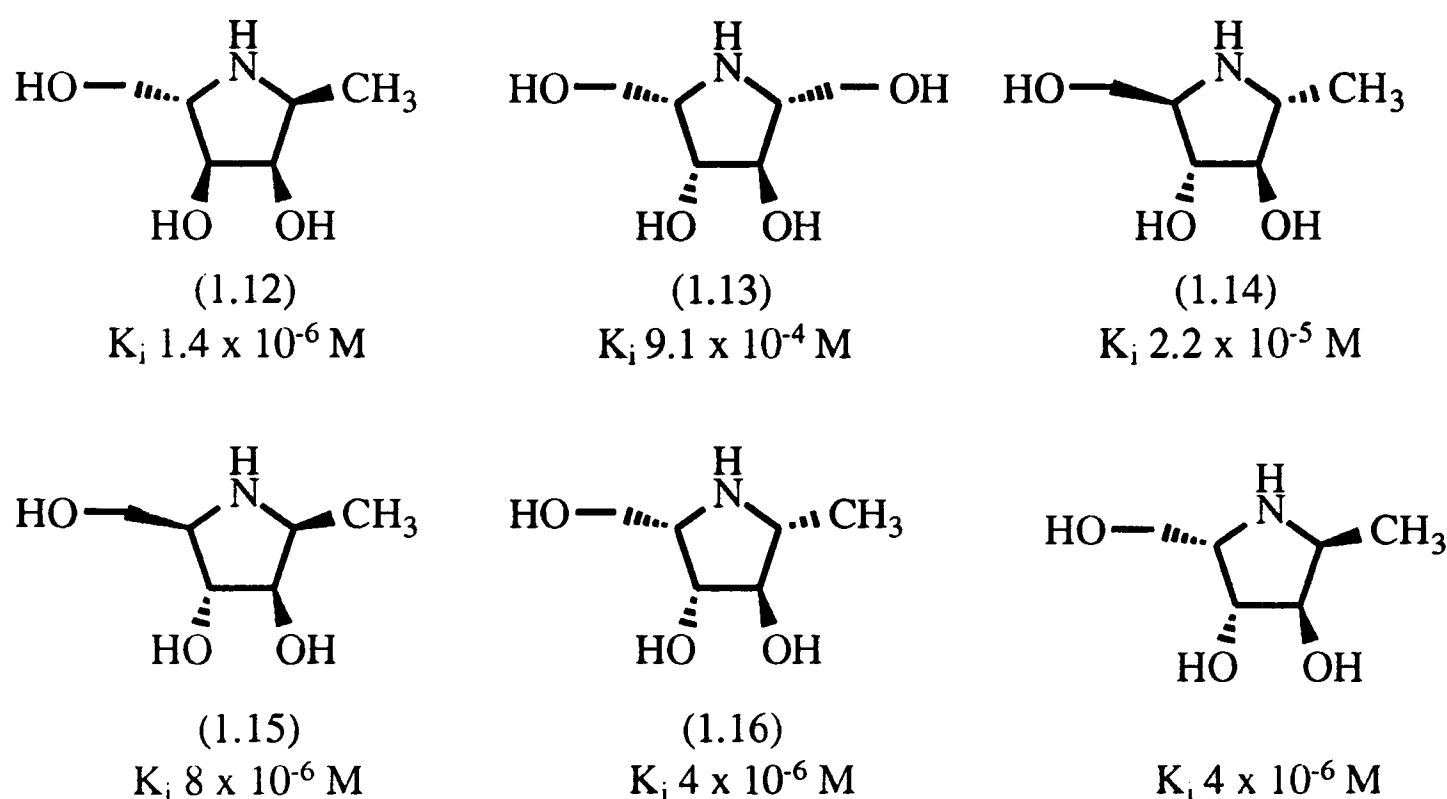


Figure 6

These compounds adopt an envelope conformation which is postulated to be a good mimic of the flattened half-chair conformation of the glycosyl cation.¹³ The stereochemistry of the hydroxyl groups has less effect on the potency and specificity of these inhibitors than it does for piperidine amino sugars. They therefore tend to exhibit inhibition of a range of glycosidases.¹⁴ For example, the compound (1.13) inhibits α -glucosidase ($K_i 3 \times 10^{-6}$ M), β -glucosidase ($K_i 8 \times 10^{-6}$ M), α -galactosidase ($K_i 5 \times 10^{-5}$ M) and α -mannosidase ($K_i 3 \times 10^{-3}$ M) in addition to α -fucosidase.¹⁷

A range of other fucose analogues, such as L-fucal (1.17) (K_i 5×10^{-5} M) and thio-fucose¹⁸ (1.18) (K_i 8×10^{-5} M) have been tested as fucosidase inhibitors but none are as potent as the amino sugars described above (Figure 7).¹⁶

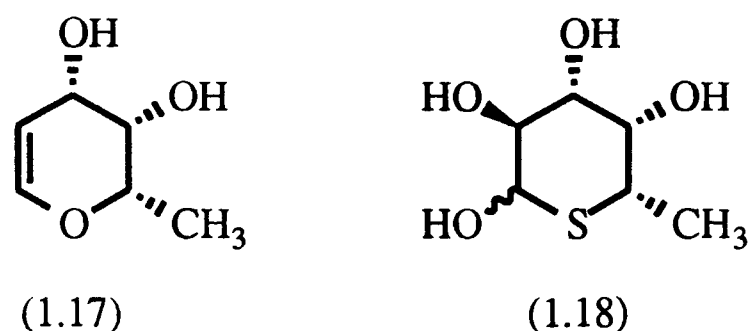
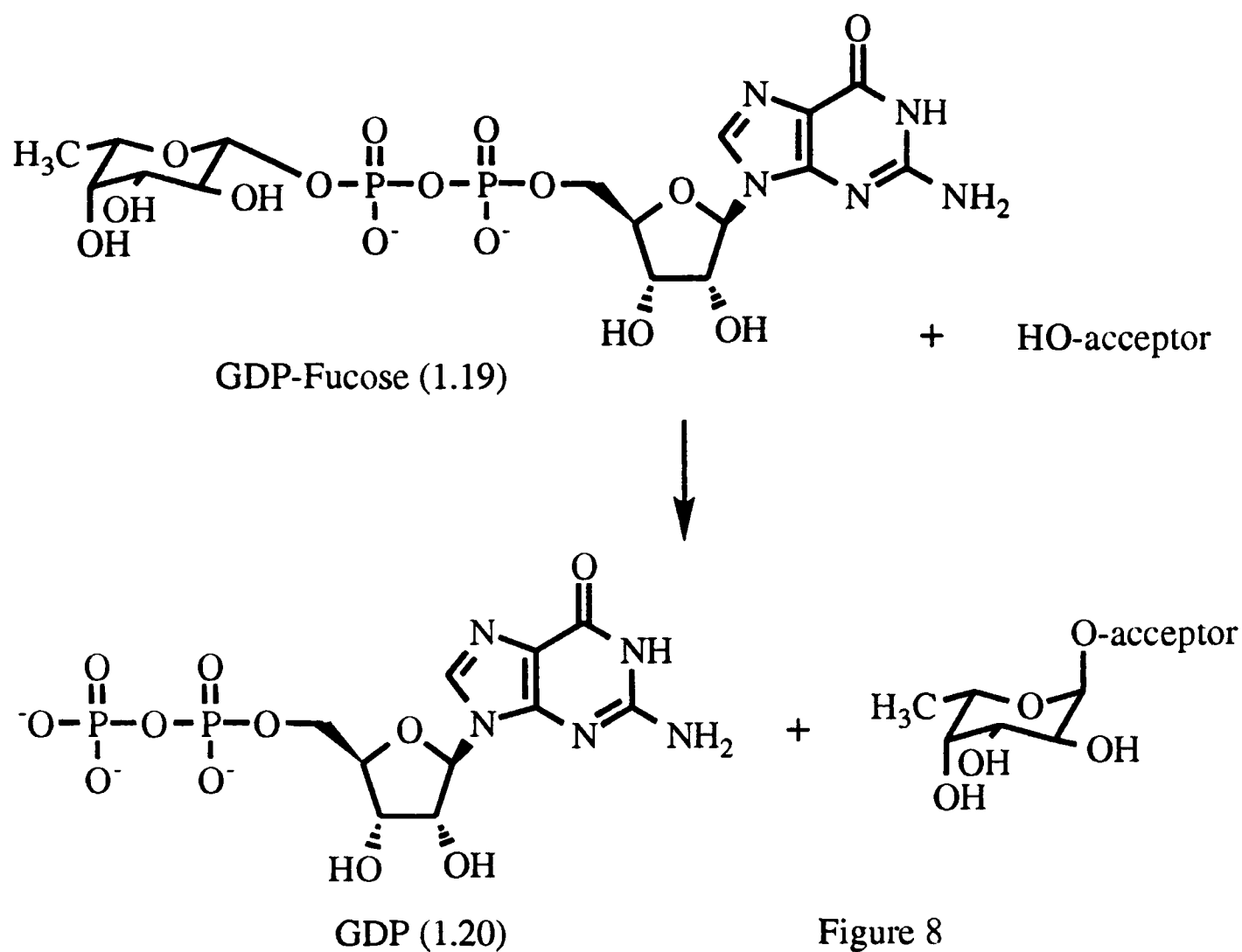


Figure 7

1.3 Fucosyltransferase Inhibition.

Inhibition of fucosyltransferases may allow control of the biosynthesis of fucose containing macromolecules. However, there is much less knowledge of the structural requirements for fucosyltransferase inhibition than there is for fucosidase inhibition. There are a number of fucosyltransferases which catalyse the addition of fucose to a range of acceptors in a variety of different linkages.¹⁹ Each fucosyltransferase is usually quite specific for one particular acceptor substrate and one type of linkage. However, β -linked GDP-fucose (1.19) is always the carbohydrate donor (Figure 8).



Guanidine diphosphate (GDP) (1.20), the by-product of the enzyme catalysed reaction, is itself an inhibitor of a recombinant human $\alpha 1 \rightarrow 3$ fucosyltransferase. It has an IC_{50} of 5×10^{-5} M, with lactose-*N*-acetate (1.21) as the acceptor.²⁰ Analogues (1.22) of GDP-fucose (1.19) have been synthesised as potential fucosyltransferase inhibitors, but as yet no results have been reported (Figure 9).²¹

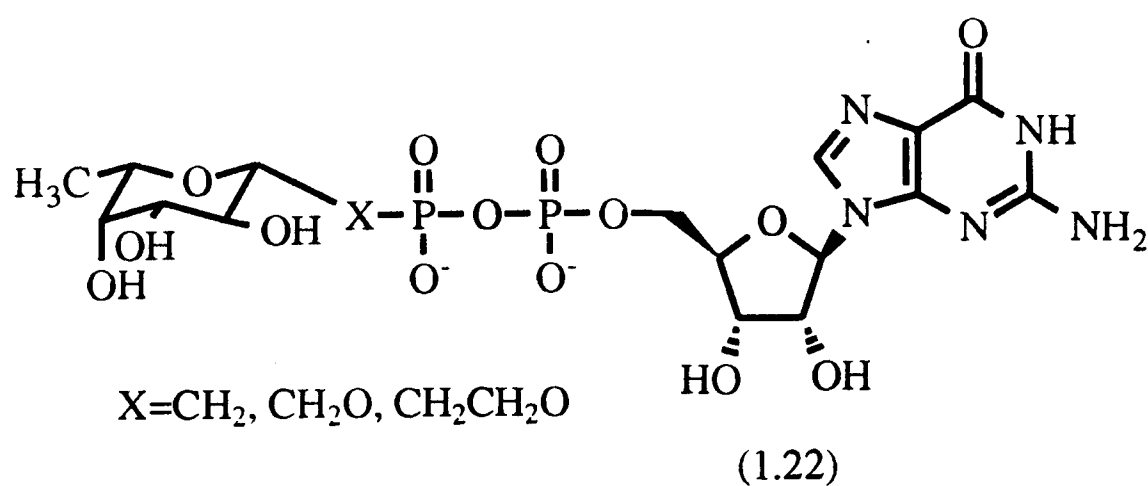


Figure 9

Some of the pyrrolidine fucose mimics which are inhibitors of fucosidase also exhibit moderate fucosyltransferase inhibition. Compound (1.12) has an IC_{50} of 8×10^{-2} M, and compound (1.16) an IC_{50} of 3.4×10^{-2} M, against a human $\alpha 1 \rightarrow 3$ fucosyltransferase, both with lactose-*N*-acetate (1.21) as the acceptor (Figure 10).²⁰

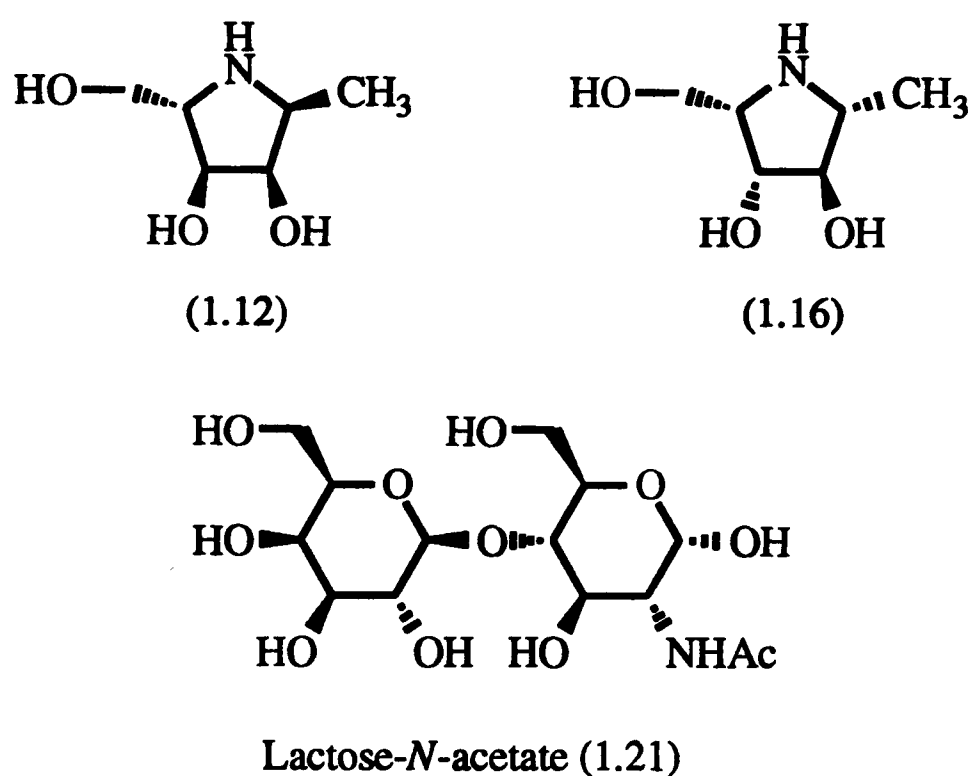


Figure 10

More interestingly, a profound synergistic inhibition is observed with either of these amino sugars in the presence of GDP (1.20). Approximately 90% of enzyme activity was inhibited by 5×10^{-5} M of GDP (1.20) and 3.4×10^{-2} M of (1.16) in the presence of 1×10^{-3} M each of ^{14}C -GDP-Fuc (1.19) and lactose-*N*-acetate (1.21) as the acceptor. It is postulated that the amino sugar (1.16) and GDP (1.20) form a complex in the enzyme active site which mimics the transition state of the fucosyl transfer reaction.²⁰

1.4 Recognition Processes.

Pyranosides of α -L-fucose play an important role in the interaction of carbohydrates with proteins. One such example is sialyl Lewis X (1.3), a fucose containing tetrasaccharide which occurs at the terminus of glycolipids on the surface of leucocytes (white blood cells) (Figure 11).^{4,22}

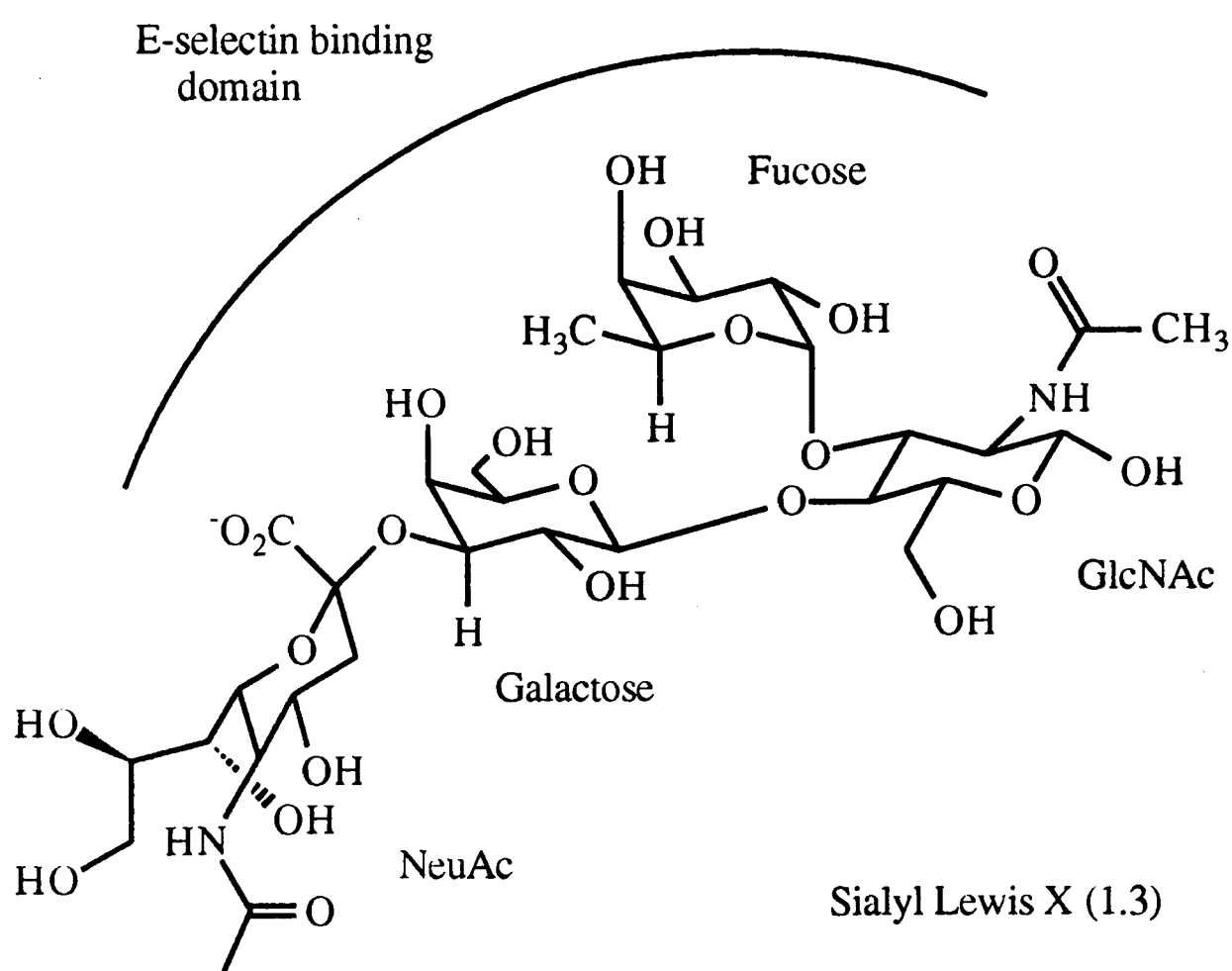


Figure 11

When tissue is injured or infected, the normal body repair mechanisms come into effect. In the inflammatory response, leucocytes are attracted to the site of injury where they destroy damaged tissue and pathogenic micro-organisms. The recruitment of leucocytes to injured tissue is partially mediated by sialyl Lewis X (1.3). In response to injury, cell adhesion molecules, E-selectins, are produced on the surface of the endothelial cells which line the blood vessel walls. These molecules recognise sialyl Lewis X (1.3) on the surface of the leucocytes, causing the leucocytes to adhere weakly to the blood vessel walls. Once at the

capillary wall, the leucocytes can squeeze between the endothelial cells, out of the blood vessel and into the site of injury.

However, sometimes leucocytes destroy normal tissue. This occurs in conditions such as septic shock, chronic inflammatory diseases such as rheumatoid arthritis and psoriasis, and in tissue reperfusion injury.²³ Cell adhesion and sialyl Lewis X (1.3) are also implicated in the metastasis of some forms of cancer.^{4,24} High levels of sialyl Lewis X (1.3) have been found on the surface of certain tumour and cancer cells, suggesting that cancer cells may exploit cell adhesion phenomena to spread round the body via the blood stream.

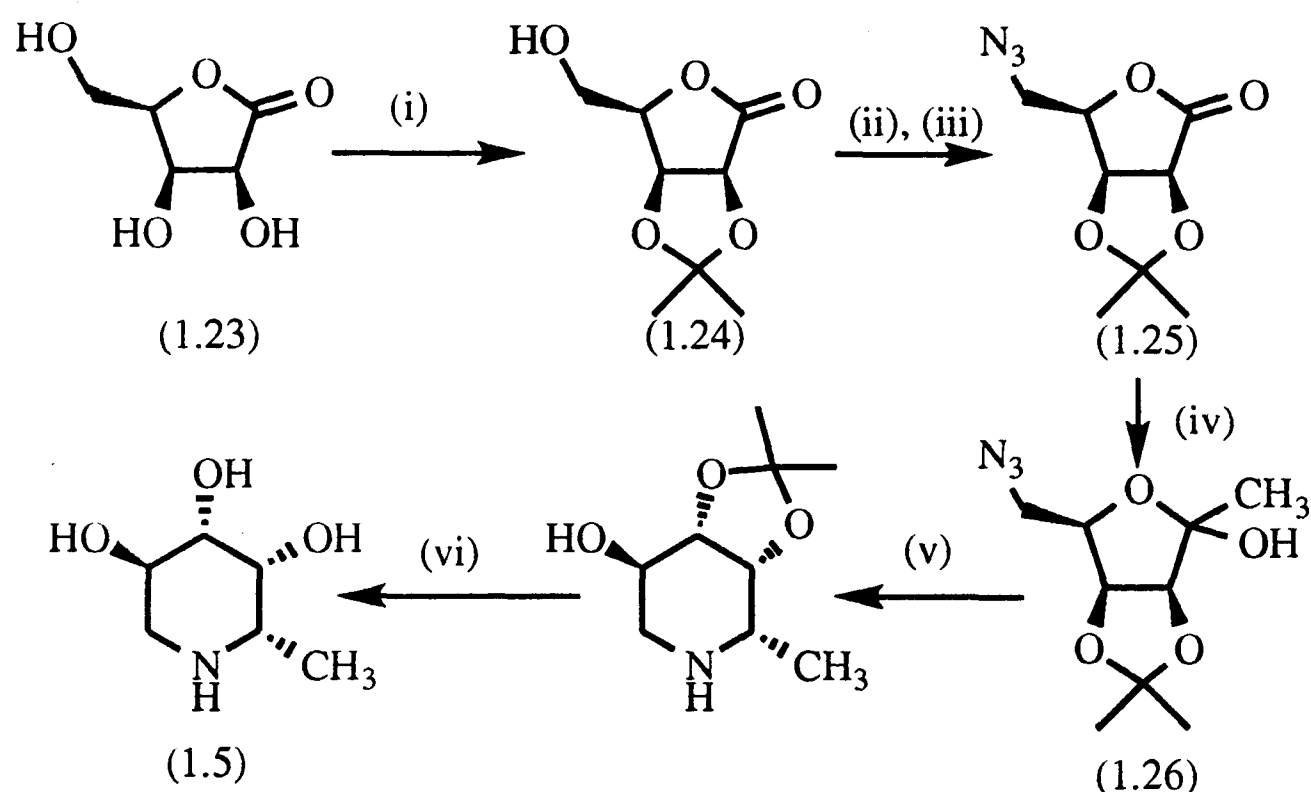
Much work has been focused on the synthesis^{25,26} and properties²⁶ of the sialyl Lewis X ligand (1.3) in an attempt to understand the inflammatory response. It may be possible to use analogues of L-fucose (1.1) in the synthesis of metabolically stable mimics of sialyl Lewis X (1.3).²⁷ The last stage in the biosynthesis of this ligand is the addition of L-fucose (1.1), catalysed by a fucosyltransferase. Inhibition of this enzyme might be a way to control the production of sialyl Lewis X (1.3), and hence the inflammatory response.

1.5 The synthesis of Amino Sugars as Fucosidase Inhibitors.

Carbohydrates are ideal starting materials for the synthesis of amino sugars as they are highly functionalised, possess a number of contiguous chiral centres and are readily available in enantiomerically pure forms. Care must be taken to ensure the stereochemical integrity of the products, as the presence of even one part in a million of a diastereomer may have profound effects on the results obtained in enzyme inhibition assays.

i. Piperidine amino sugars.

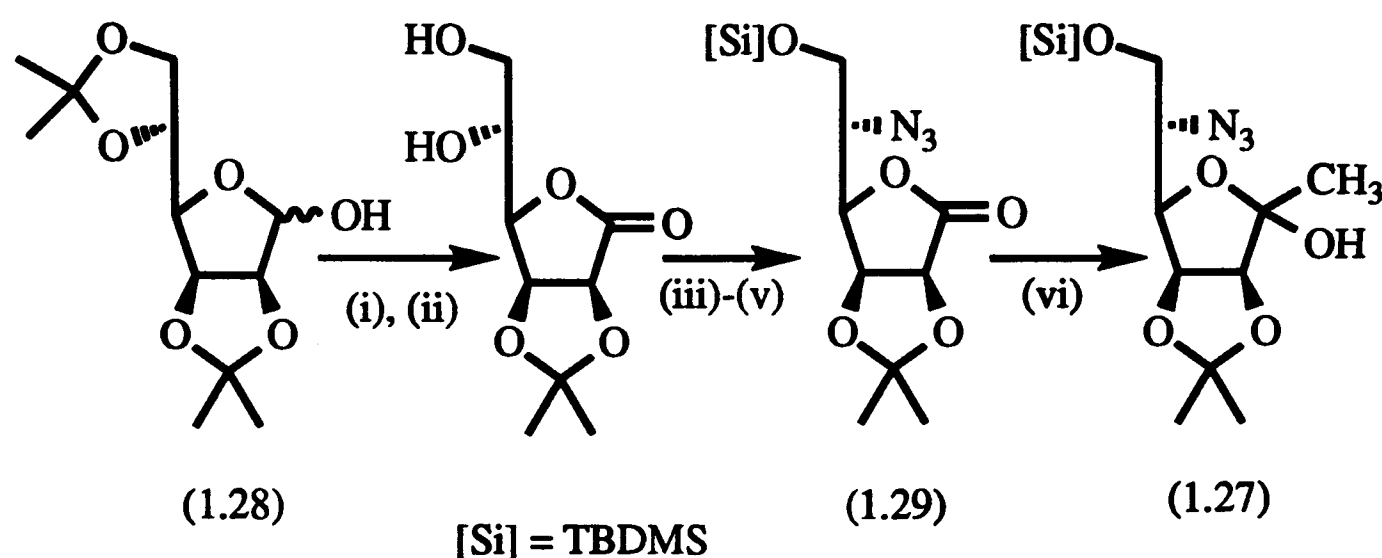
Several syntheses of DFJ (1.5) have been reported,^{8,11} with the most efficient route starting from D-lyxonolactone (1.23).²⁸ This was protected as its 2,3-acetonide (1.24) and nitrogen was introduced at the primary position via displacement of a triflate ester with sodium azide. Addition of one equivalent of methyllithium to the lactone carbonyl group of (1.25) gave the keto-sugar (1.26) as a single anomer, the anomeric configuration of which was not determined. Reduction of the azide functionality by catalytic hydrogenation, followed by removal of the acetonide protecting group, gave DFJ (1.5) in an overall yield of 41% (Scheme 2).



- (i) Acetone, anhydrous CuSO_4 , 60%, (ii) Tf_2O , py, DCM,
 (iii) NaN_3 , DMF, 0 °C, 89% over 2 steps,
 (iv) MeLi , THF, -78 °C, 97%, (v) H_2 , Pd-black, EtOH, 83%,
 (vi) aq. TFA, ion exchange chromatography, quant.

Scheme 2

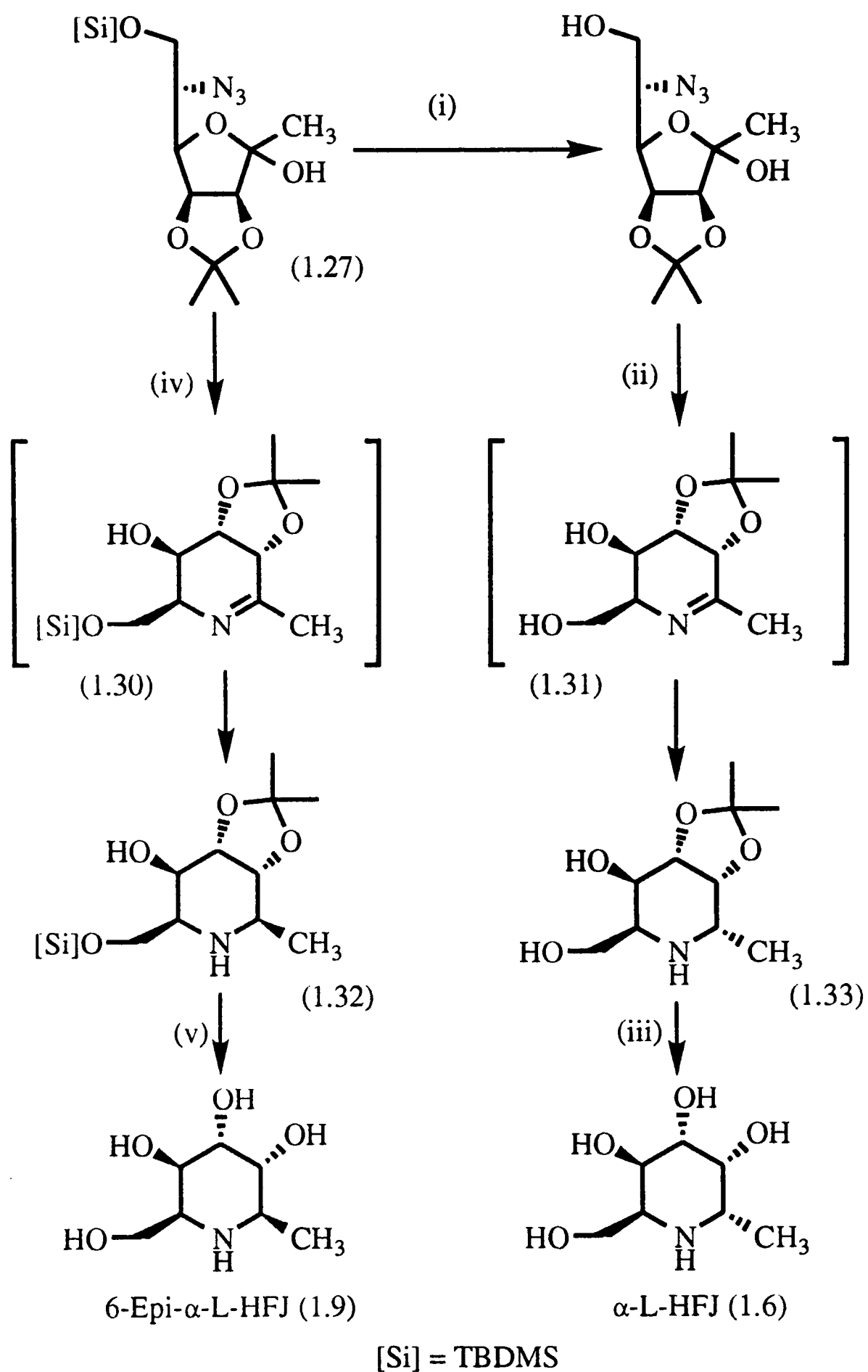
Both α -L-homofuconojirimycin⁹ (1.6) and 6-epi- α -L-homofuconojirimycin (6-epi- α -L-HFJ)²⁹ (1.9) have been synthesised from a common intermediate, the protected keto-sugar (1.27), which in turn was synthesised from diacetone-D-mannose (1.28) (Scheme 3).²⁹



(i) PCC, molecular sieves, DCM, (ii) Aq. H⁺, (iii) TBDMSCl, DMF, imidazole, (iv) Tf₂O, py, DCM, (v) NaN₃, DMF, 65% over 3 steps, (vi) MeLi, THF, 82%.

Scheme 3

As in the synthesis of DFJ (1.5) mentioned above, the methyl group was introduced via addition of methyllithium to a lactone (1.29). Reduction of the azide functionality to a primary amine was followed by *in situ* cyclisation onto the ketone to give an imine (1.30) or (1.31), which was then further reduced to a secondary amine, (1.32) or (1.33) (Scheme 4). The stereochemical outcome of this second reduction depended on the presence or absence of the bulky silyl protecting group on the primary hydroxyl group. If the silyl group was present, the major product was the protected 6-epi- α -L-HFJ derivative (1.32)²⁹ whilst in the absence of the silyl group, the major product was the protected α -L-HFJ derivative (1.33).⁹ Acidic hydrolysis, followed by purification by ion exchange chromatography, then yielded the target amines (1.9) and (1.6).



- (i) F^- , (ii) H_2 , 5% Pd-C, EtOH,
 (iii) aq. TFA, ion exchange chromatography, 66% over 2 steps,
 (iv) H_2 , PtO_2 , EtOAc, 47%, (v) aq. TFA, ion exchange chromatography, quant.

Scheme 4

ii. Pyrrolidine amino sugars.

One of the pyrrolidine amino sugars discussed on page 6, 6-deoxy-DMDP (6-deoxy-2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxy-pyrrolidine) (1.14), is naturally occurring (Figure 12). This thesis also reports the synthesis of two new pyrrolidine amino sugars, analogues of 6-deoxy-DMDP (1.14).

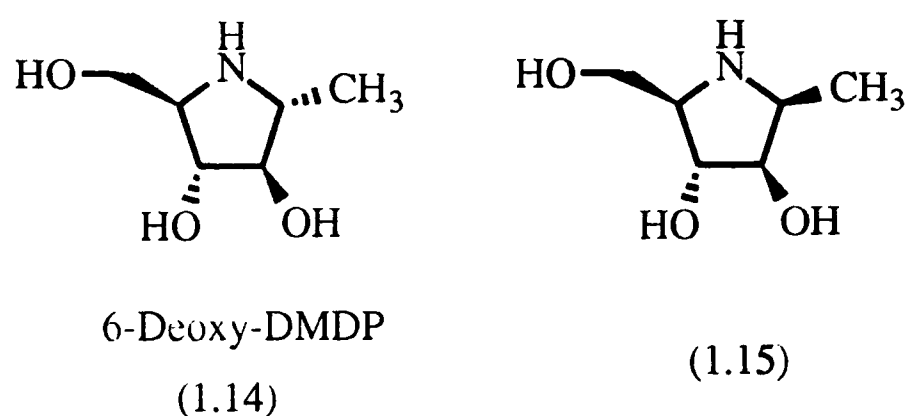
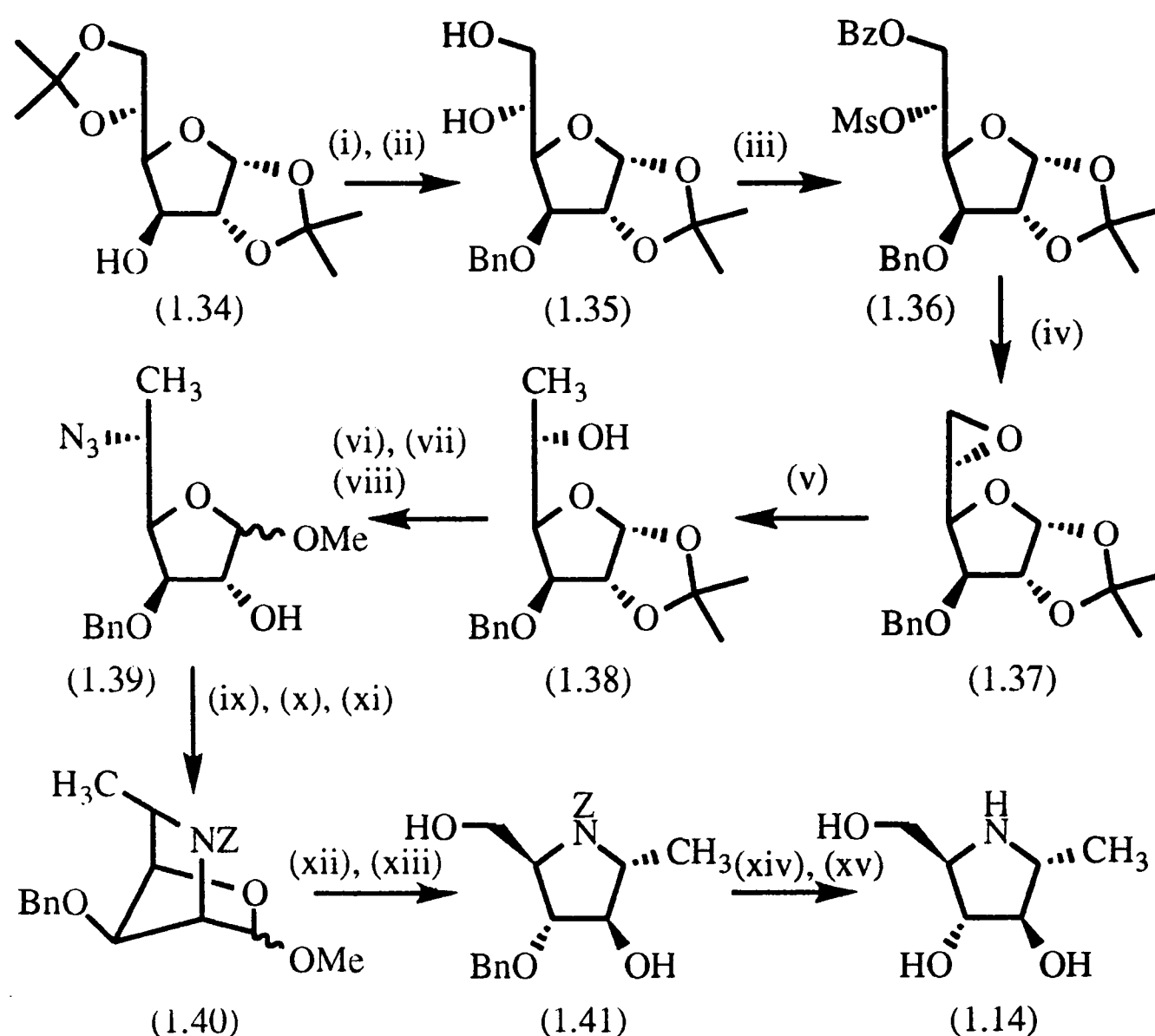


Figure 12

6-Deoxy-DMDP (1.14) was isolated from the seeds of the African legume *Angylocalyx pynaertii*, and its structure established by a stereochemically unambiguous synthesis.³⁰ Both chemical and chemo-enzymatic techniques have been employed to produce the other compounds.^{15,16,30}

Fleet and co-workers have published syntheses of 6-deoxy-DMDP (1.14) and its epimer (1.15), both starting from diacetone-D-glucose (1.34), and utilizing a common intermediate, the diol (1.35).³⁰ Benzylation of the free hydroxyl group in diacetone-D-glucose (1.34) was followed by selective hydrolysis of the terminal acetonide to give the diol (1.35) in 71% yield over two steps (Scheme 5).



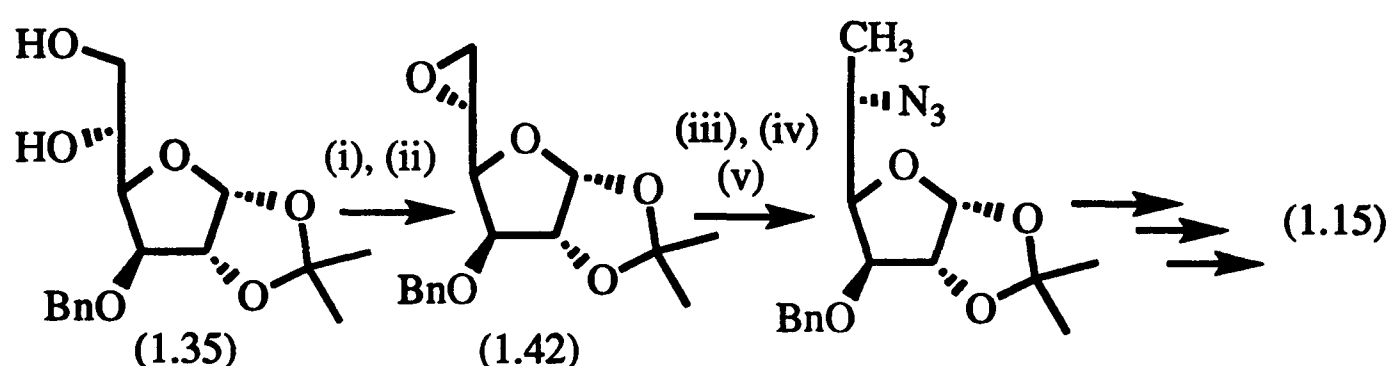
- (i) BnBr, 18-crown-6, THF, KOH, (ii) aq. AcOH, dioxan, 72% over 2 steps,
 (iii) BzCl, py, then MsCl, DMAP, 70%, (iv) NaOMe, DMF, 83%,
 (v) LiAlH₄, THF, 94%, (vi) Tf₂O, py, DCM, (vii) NaN₃, DMF,
 82% over 2 steps, (viii) MeOH, HCl, 91%, (ix) Tf₂O, py, DCM,
 (x) H₂, Pd-C, NaOAc, MeOH, (xi) ZCl, aq. NaHCO₃, Et₂O, 62-81% over 3 steps,
 (xii) aq. TFA, (xiii) NaBH₄, EtOH, 54-61% over 2 steps,
 (xiv) H₂, Pd-black, AcOH, (xv) ion exchange chromatography, quant.

Scheme 5

For the synthesis of the natural product (1.14), nitrogen was introduced at C-5 of D-glucose with retention of configuration (Scheme 5). The primary hydroxyl group in the diol (1.35) was protected as its benzoate ester and the secondary hydroxyl group was converted to a mesylate (1.36). Basic hydrolysis of the benzoate group liberated the primary oxyanion which displaced the mesylate to form the epoxide (1.37). The epoxide was opened with a hydride reducing agent to give (1.38) and nitrogen was introduced at C-5 via azide displacement of a

triflate ester. The remaining acetonide was hydrolysed in acidic methanol to form the methyl furanoside (1.39), as a mixture of anomers. The remaining free hydroxyl group was converted to its triflate ester and displaced with the amine formed by reduction of the azide group at C-5, to give a bicyclic system. The secondary amine thus produced was protected as its Z ($Z=CO_2Bn$) derivative (1.40) for ease of handling. Hydrolysis of the methyl furanoside liberated an aldehyde which was then reduced to the alcohol (1.41) using sodium borohydride. Deprotection then yielded the target compound (1.14).

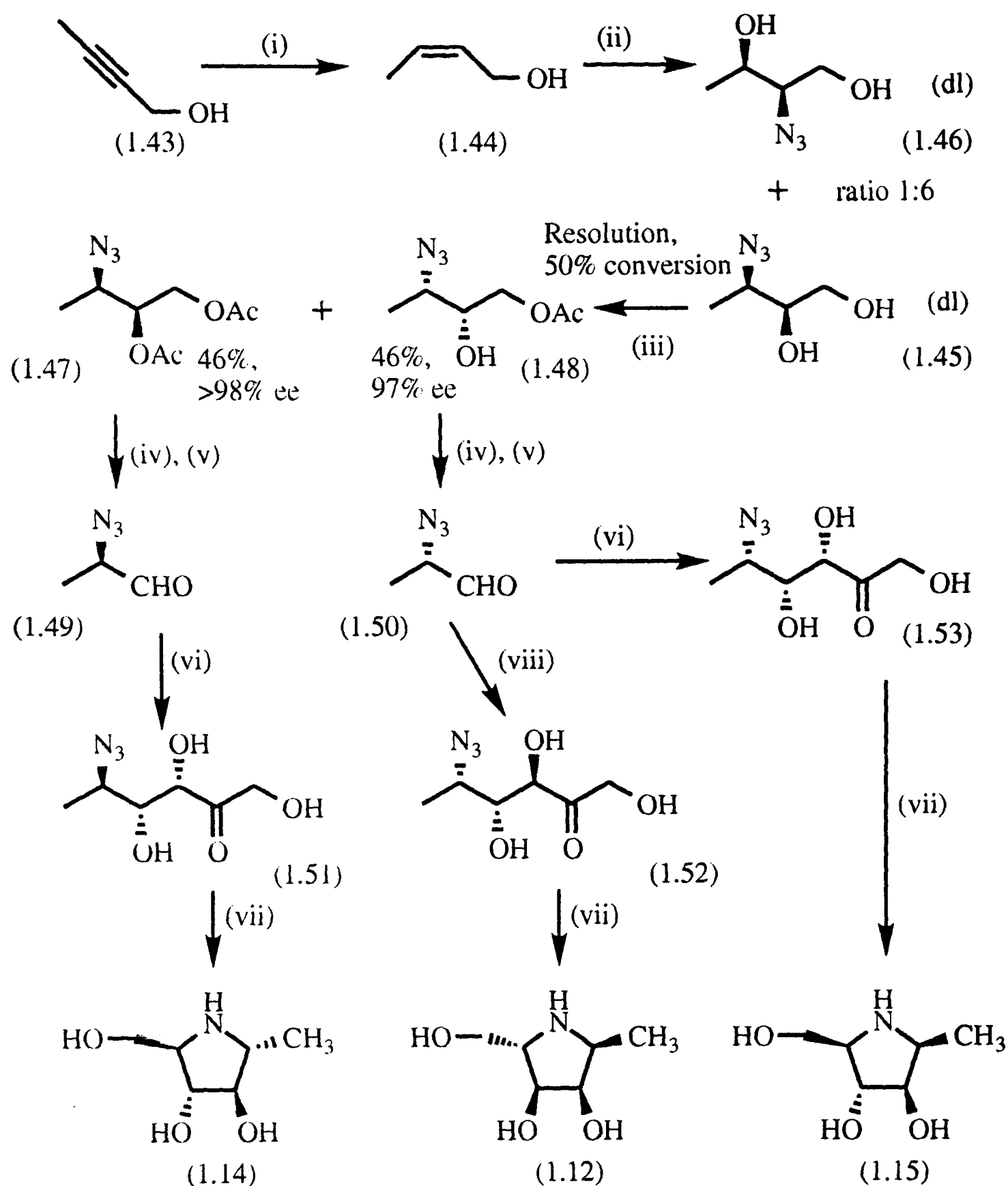
An analogous route was used to form the epimeric compound (1.15) (Scheme 6). In this case nitrogen was introduced with inversion of configuration via the epimeric epoxide (1.42). Both these routes suffer from the formation of a mixture of anomers in the middle stages, which complicates the experimental procedure.



(i) TsCl, py, 95%, (ii) NaH, DMF, 95%, (iii) LiAlH₄, THF, 77%,
 (iv) MsCl, DMAP, py, (v) NaN₃, DMF, 75%.

Scheme 6

Wong and co-workers have synthesised a number of amino sugars, including the two discussed above, (1.14) and (1.15), using a combination of chemical and enzymatic steps.^{14,15,16} The key step is an enzyme catalysed asymmetric aldol reaction (Scheme 7).¹⁵



(i) H_2 , Lindlar catalyst, > 95%, (ii) H_2O_2 , benzonitrile, then NaN_3 , pH 7.5, 78% ,
 (iii) Lipase PSL, vinyl acetate, (iv) hydrolysis, (v) NaIO_4 , (vi) DHAP,
 FDP aldolase, then acid phosphatase, 31%, (vii) H_2 , Pd-C, (viii) DHAP,
 Fuc-1-P aldolase, then acid phosphatase, 10%.

Scheme 7

The starting material was 2-butyn-1-ol (1.43) which was hydrogenated to the *cis* alkene (1.44) over Lindlar catalyst. Epoxidation followed by ring opening using sodium azide gave the desired azido diol (1.45) in racemic form plus the

unwanted regioisomer (1.46), in a ratio of 6:1. The desired azido diol (1.45) was kinetically resolved using the enzyme lipase PSL with vinyl acetate as an acetate donor. The 2*S*, 3*R* enantiomer (1.47) was diacylated under these conditions whilst the 2*R*, 3*S* enantiomer (1.48) only underwent monoacylation. This allowed separation of the enantiomers in 97-98% ee. Hydrolysis of the acetate groups followed by periodate cleavage of the diol unit led to the enantiomeric azido aldehydes (1.49) and (1.50).

Condensation of the azido aldehydes (1.49) and (1.50) with dihydroxyacetone-3-phosphate (DHAP) catalysed by either fucose-1-aldolase (Fuc-1-P aldolase) or fructose-1,6-diphosphate aldolase (FDP-aldolase), followed by removal of the phosphate group, gave the azido ketones (1.51), (1.52) and (1.53) in low to moderate yield. Reductive amination³¹ then yielded the target pyrrolidines (1.12), (1.14) and (1.15).

The yields of the enzyme catalysed aldol reaction are not high, and the final reductions may not be completely stereospecific, making this route unsuitable for the production of large quantities of these compounds.

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Chapter 2

The Synthesis of Two Novel Bicyclic Mimics of α -L-Fucose.

2.1 Introduction.

The principal aim of this project was the synthesis of a number of novel bicyclic mimics of α -L-fucose (1.1), of general structure (1.4). The initial target (2.1) was the system in which both X and Y in structure (1.4) were oxygen atoms (Figure 1).

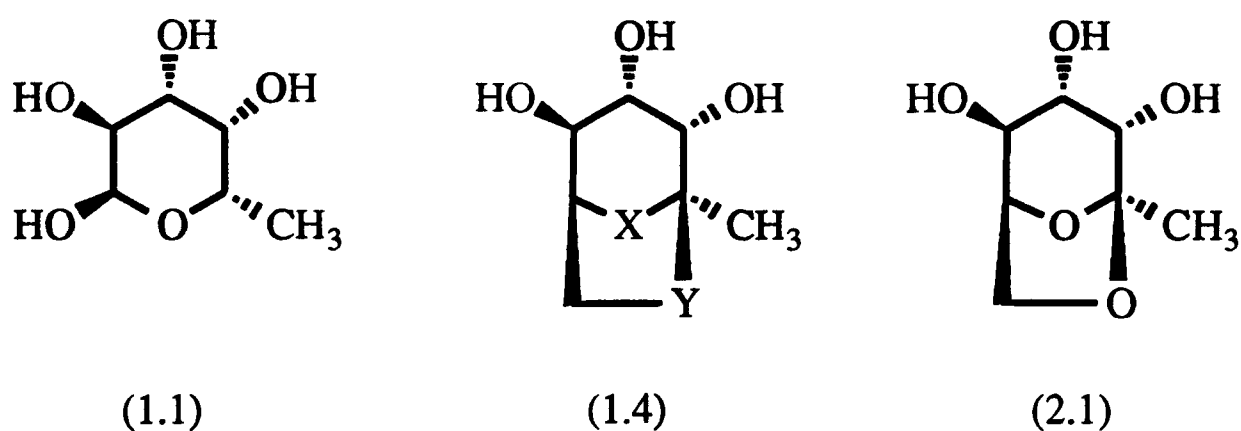
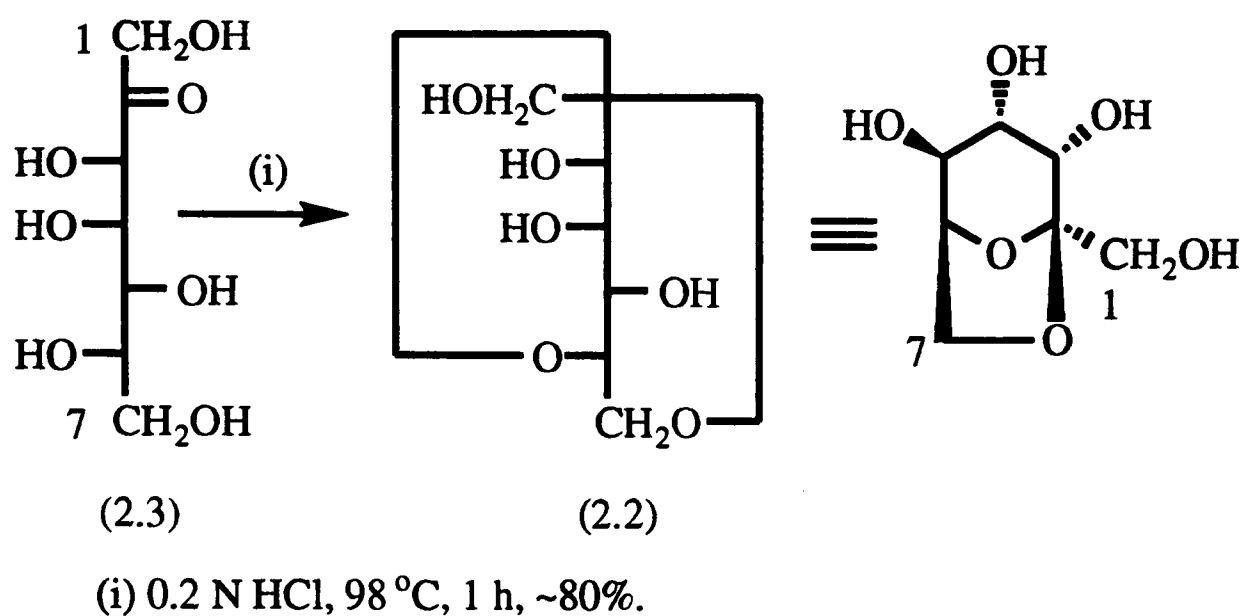


Figure 1

An analogous system (2.2), hydroxylated at C-1, has been reported in the literature.¹ When L-*gulo*-heptulose (2.3) was treated with dilute aqueous acid, the major component of the resultant equilibrium mixture was found to be the anhydro sugar (2.2) (Scheme 1). Such bicyclic systems therefore appear to be stable under acidic conditions. It was envisaged that the target system (2.1), which was deoxy at C-1, would be obtained from a suitable precursor under similar conditions.



Scheme 1

It was also hoped to synthesise the analogous system (2.4) with nitrogen in the bridging position in place of oxygen (Figure 2).

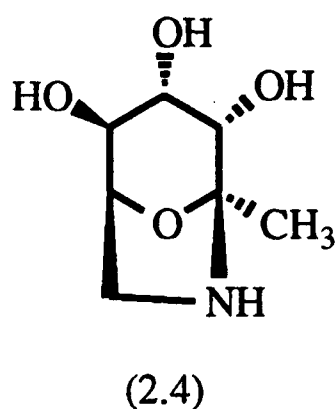
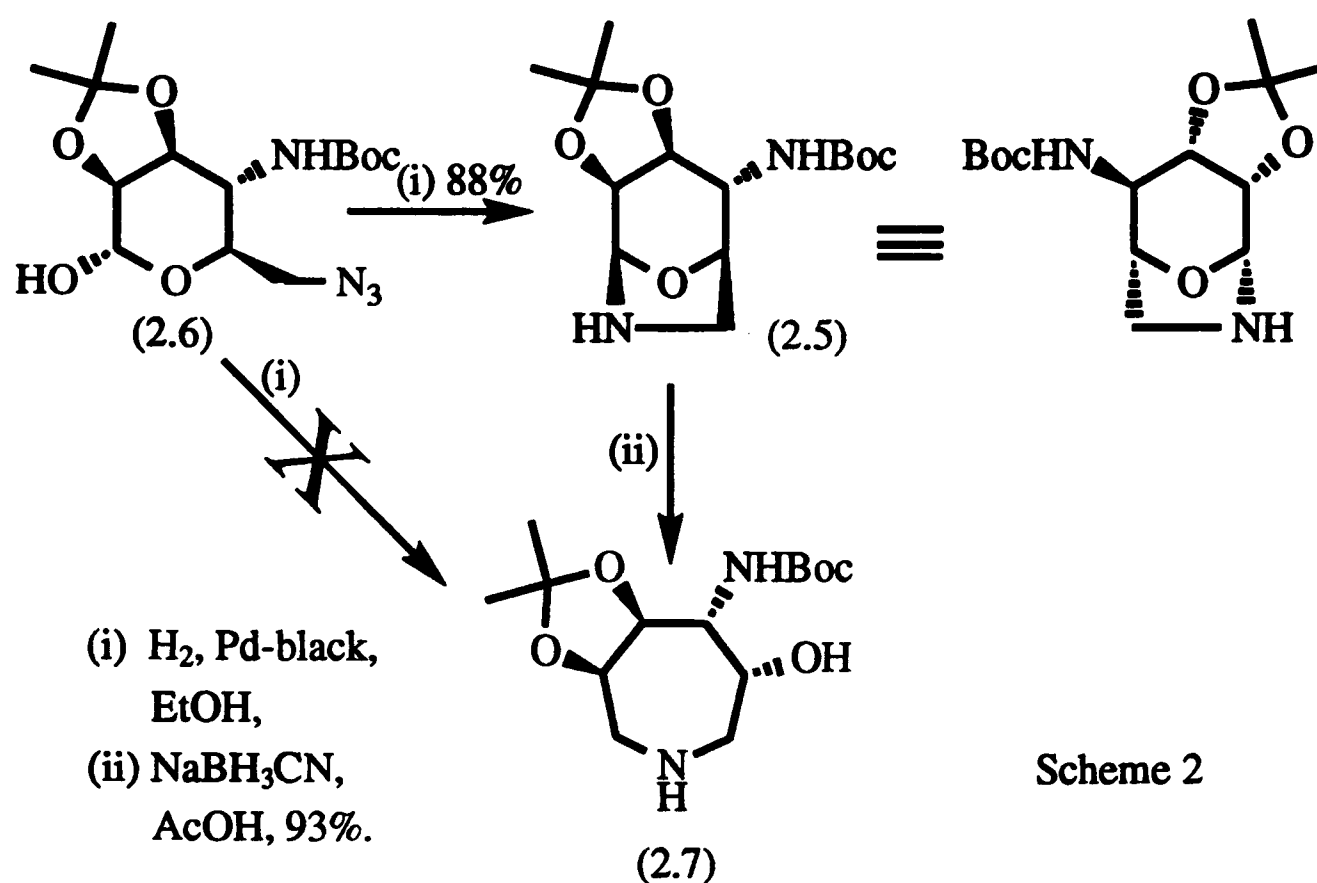
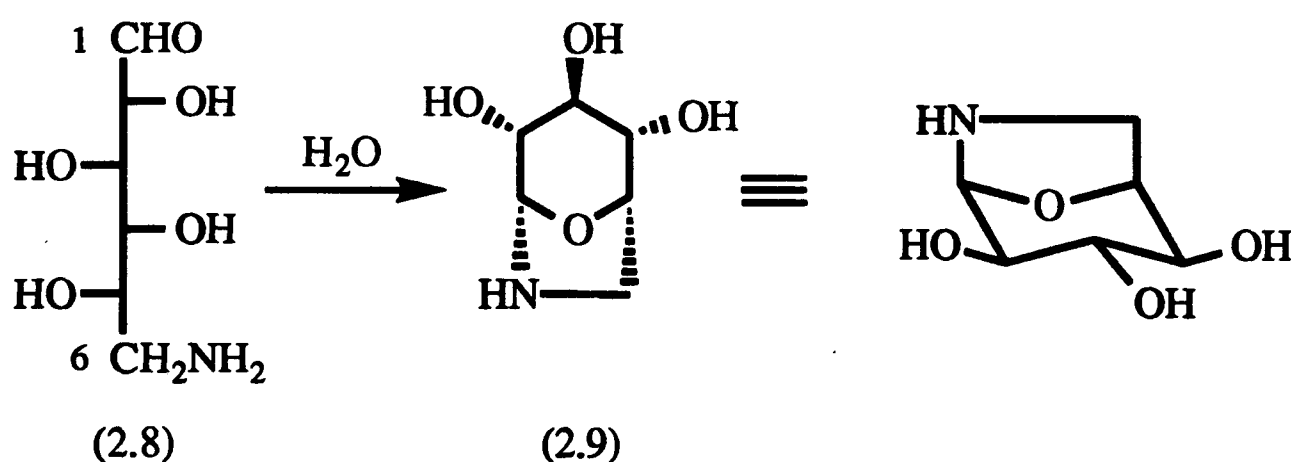


Figure 2

A similar bicyclic hemiaminal (2.5) with D-mannose (1.10) stereochemistry has been reported in the literature and was found to be stable.² Catalytic hydrogenation of the azido-lactol (2.6) gave the bicyclic system (2.5) as the major product, rather than the expected seven membered ring amino sugar (2.7). The bicyclic system underwent a reductive ring opening with sodium cyanoborohydride in acetic acid to give the desired amino sugar (2.7) (Scheme 2).



6-Amino-6-deoxy-L-idose (2.8) is reported to cyclise to a 1,6-anhydro derivative (2.9) on concentration of an aqueous solution of the free base.³ The ease of cyclisation is ascribed to the fact that all the hydroxyl groups can adopt an equatorial conformation in the anhydro compound (2.9) (Scheme 3).



Scheme 3

L-Gulono-1,4-lactone (2.10) is the ideal starting material for the construction of analogues of L-fucose (1.1). Fischer projections of L-gulono-1,4-lactone (2.10), α -L-fucose (1.1), and the first target system (2.1) are shown in Figure 3.

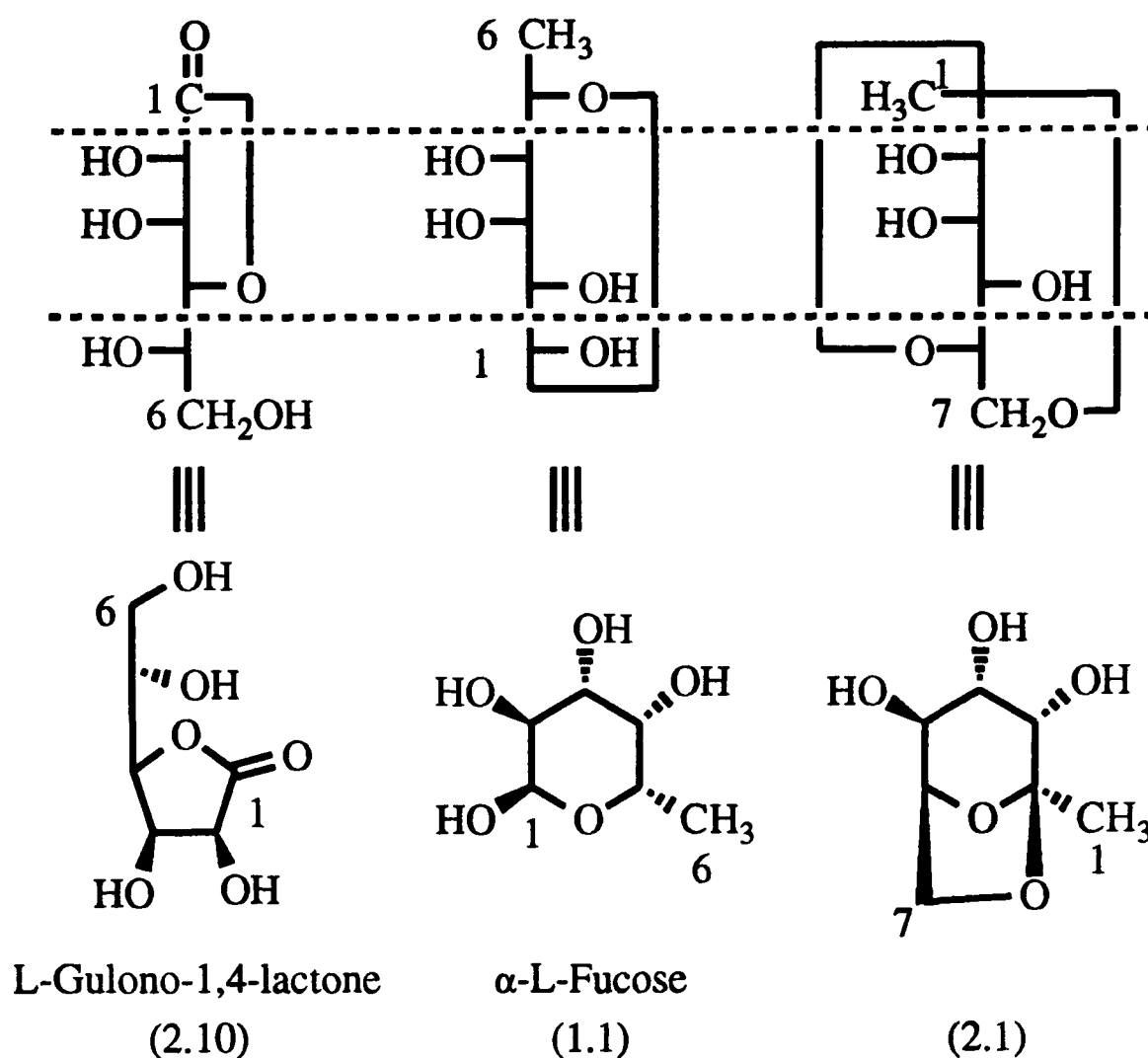


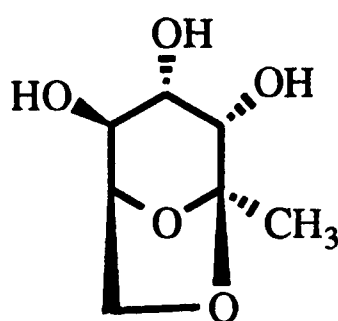
Figure 3

It can be seen that L-gulonolactone (2.10) possesses the correct stereochemistry of the C-2, C-3 and C-4 hydroxyl groups for L-fucose mimics. Addition of a methyl equivalent to C-1 of L-gulonolactone (2.10) should yield the required seven carbon skeleton of the target system (2.1). In the syntheses of DFJ (1.5)⁴ and α-L-homofuconojirimycin (1.6),⁵ described in the previous chapter, a methyl group was introduced via the addition of one equivalent of methyllithium to a lactone carbonyl group. Such additions are well documented in the literature for sugar lactones,^{6,7} and the same strategy was adopted for the synthesis of the target systems (2.1) and (2.4).

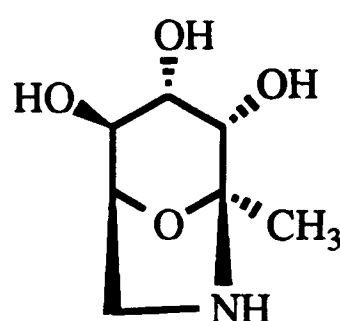
2.2 Results and Discussion.

The work will be discussed in the following three parts:

- i. The synthesis of 2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1).
- ii. The synthesis of 1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.4).
- iii. The evaluation of the above compounds, (2.1) and (2.4), as glycosidase inhibitors.



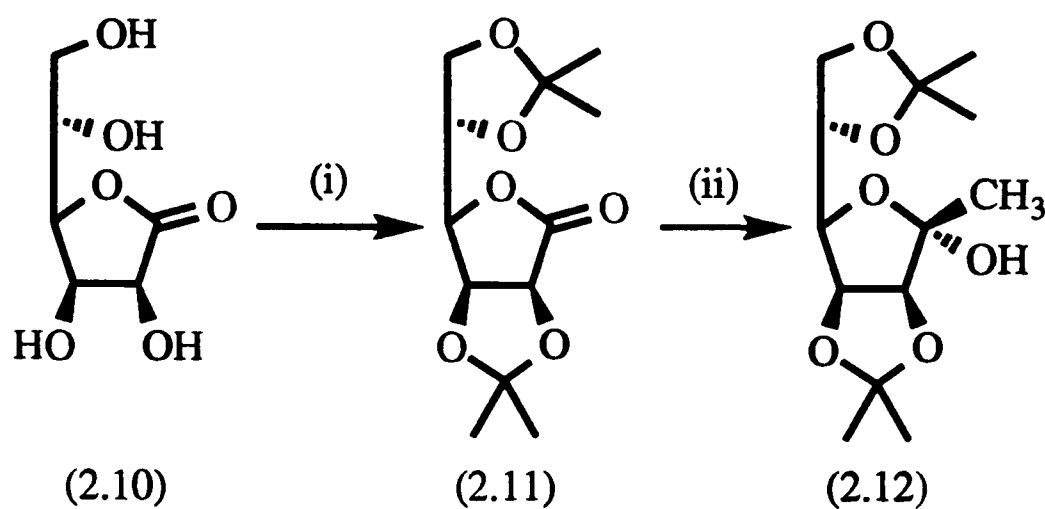
(2.1)



(2.4)

i. The synthesis of 2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1).

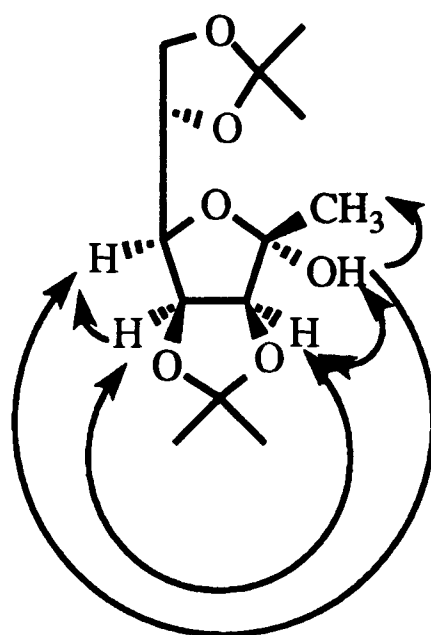
L-Gulono-1,4-lactone (2.10) is a cheap and readily available starting material, produced commercially via the hydrogenation of vitamin C⁸ or of D-glucurono-6,3-lactone.⁹ It was initially protected as its diacetonide (2.11),⁷ in 71% yield, by treatment with acidic acetone and 2,2-dimethoxypropane. This reaction was easily performed on a 30 g scale, and the product purified by recrystallisation. Addition of one equivalent of methyllithium to the diacetonide at -70 °C in tetrahydrofuran gave the keto-sugar (2.12) in excellent yield (Scheme 4). This product was sufficiently pure to use crude in the next reaction.



(i) Acetone, CSA, 2,2-dimethoxypropane, RT, 71%,
(ii) MeLi, THF, -70 °C, 89%.

Scheme 4

Only one product was isolated from the methyllithium addition reaction. The proton and carbon NMR and the IR spectra indicated that it was a cyclic derivative; no trace of any open chain compound was observed. This is in agreement with the results reported in the literature for the addition of lithium acetylides to the diacetonide of L-gulonolactone (2.11).⁷ The anomeric configuration of the product (2.12) was determined by equilibrium nOe measurements. In the proton NMR spectrum in d_6 -DMSO, large nOe enhancements from the hydroxyl proton to the ring protons were observed, indicating that they were on the same face of the furanose ring (Figure 4).

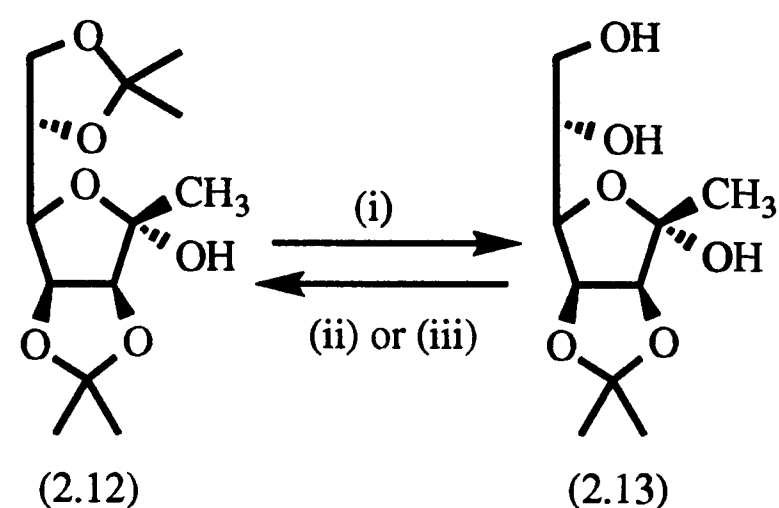


Observed nOe enhancements

Figure 4

The product isolated is therefore that of formal attack by methyllithium onto the more hindered face of the lactone carbonyl group. A similar result has previously been reported in the literature for the addition of lithiated dithiane to (2.11).¹⁰ In that case, the configuration of the anomeric centre was determined by X-ray crystallographic analysis.

The primary acetone was selectively removed by hydrolysis with aqueous acetic acid, to give a compound (2.13) which appeared to still be in the furanose form (Scheme 5). The acetone could be replaced under either kinetic or thermodynamic conditions to give the fully protected keto-sugar (2.12).

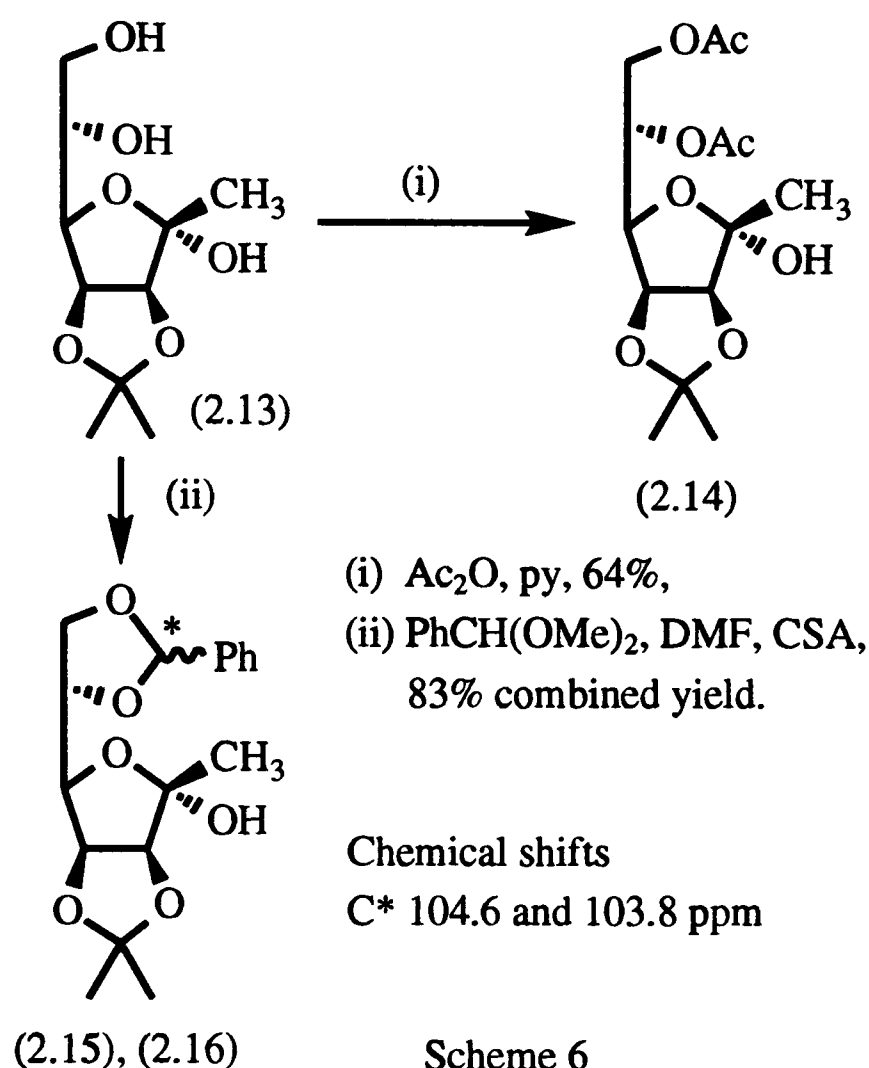


- (i) Aq. AcOH, 50 °C, 89%,
- (ii) acetone, CSA, 59%,
- (iii) 2,2-dimethoxypropane, DMF, CSA, 90%.

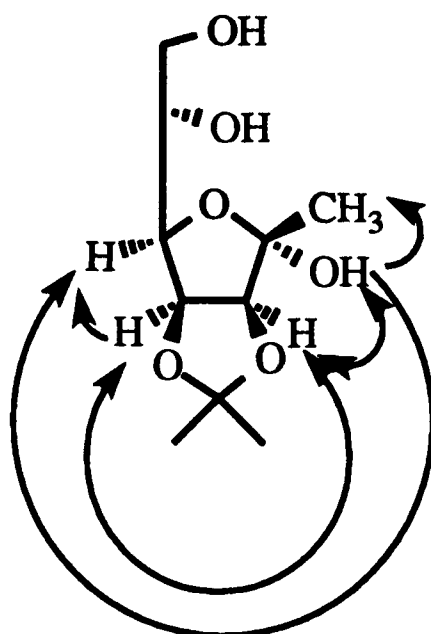
Scheme 5

Treatment of the monoacetone with acetic anhydride in pyridine gave a diacetate (2.14) in 64% yield (Scheme 6). From the downfield shifts in the proton NMR of the signals for H-6, H-7 and H-7' on acylation, it appeared that the acetate groups had gone onto the C-6 and C-7 hydroxyls, supporting the furanose ring form of the monoacetone (2.13). Reaction of the monoacetone (2.13) with benzaldehyde dimethyl acetal in *N,N*-dimethylformamide with a catalytic amount of camphorsulphonic acid gave a mixture of two products, (2.15) and (2.16). From the carbon NMR shifts of the acetal carbons, (δ 104.6 and

103.8 ppm), these were assigned as the two epimeric five ring benzyldienes, as drawn. Values of the chemical shifts for the acetal carbons of $\delta \sim 99$ ppm are typical for dioxane acetals, whilst shifts of $\delta \sim 105$ ppm are typical of dioxolane acetals.¹¹



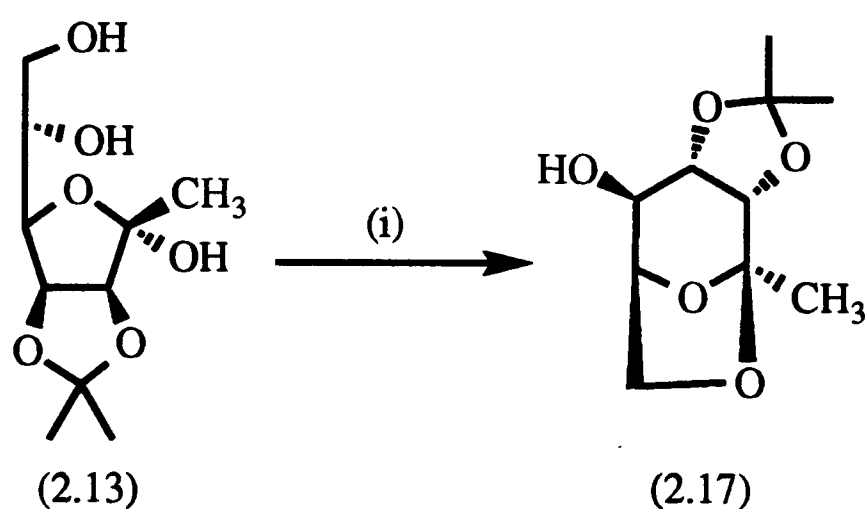
The proton NMR spectrum of the monoacetone (2.13) in d_6 -DMSO showed coupling between two hydroxyl protons and the protons assigned as H-6, H-7 and H-7', indicating that neither the C-6 or the C-7 oxygen were involved in the intramolecular hemiketal. No such coupling between an OH and H-5 was observed, supporting the conclusion that it was the C-5 oxygen that was involved in the hemiketal. All the above observations imply that no isomerisation of the ring size accompanied the hydrolysis of the primary acetone. Equilibrium nOe measurements in d_6 -DMSO indicated that the anomeric configuration was the same as in the fully protected compound (2.12) (Figure 5).



Observed nOe enhancements

Figure 5

Warming of the monoacetonide (2.13) in anhydrous *N,N*-dimethylformamide with a catalytic amount of camphorsulphonic acid led to simultaneous isomerisation and dehydration to give the bicyclic system (2.17) in 67% yield (Scheme 7).

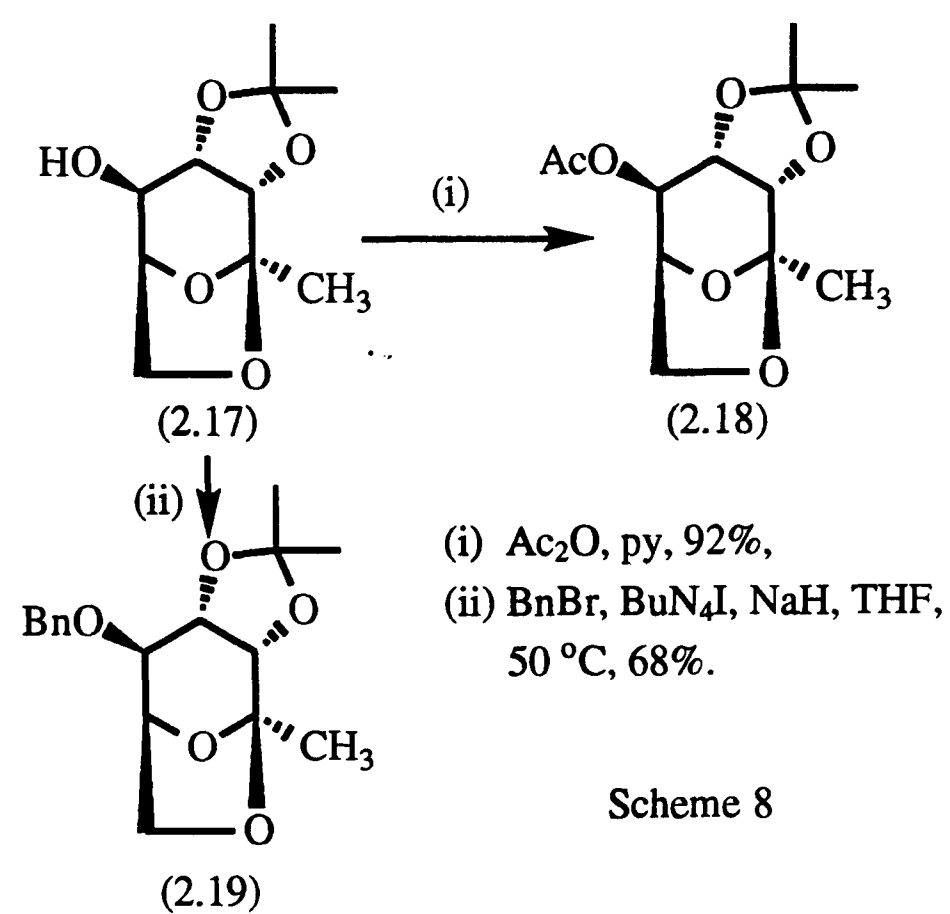


(i) CSA, DMF, 70 °C, 67%.

Scheme 7

This bicyclic system was further characterised as its acetate derivative (2.18), formed by treatment with acetic anhydride in pyridine (Scheme 8). From the downfield shift of the signal for H-5 in the proton NMR on acylation, it appeared that the C-5 hydroxyl group was acylated and therefore was not involved in the anhydro structure. This was confirmed by single crystal X-ray diffraction studies on the benzylated compound (2.19) (see Appendix 1).¹² The benzyl ether of the

free hydroxyl group in the monoacetone (2.17) was formed on reaction with sodium hydride, benzyl bromide and tetra-*n*-butylammonium iodide in tetrahydrofuran. The crystal structure of this compound (Figure 6) showed that the required bicyclic framework had indeed been formed.



Scheme 8

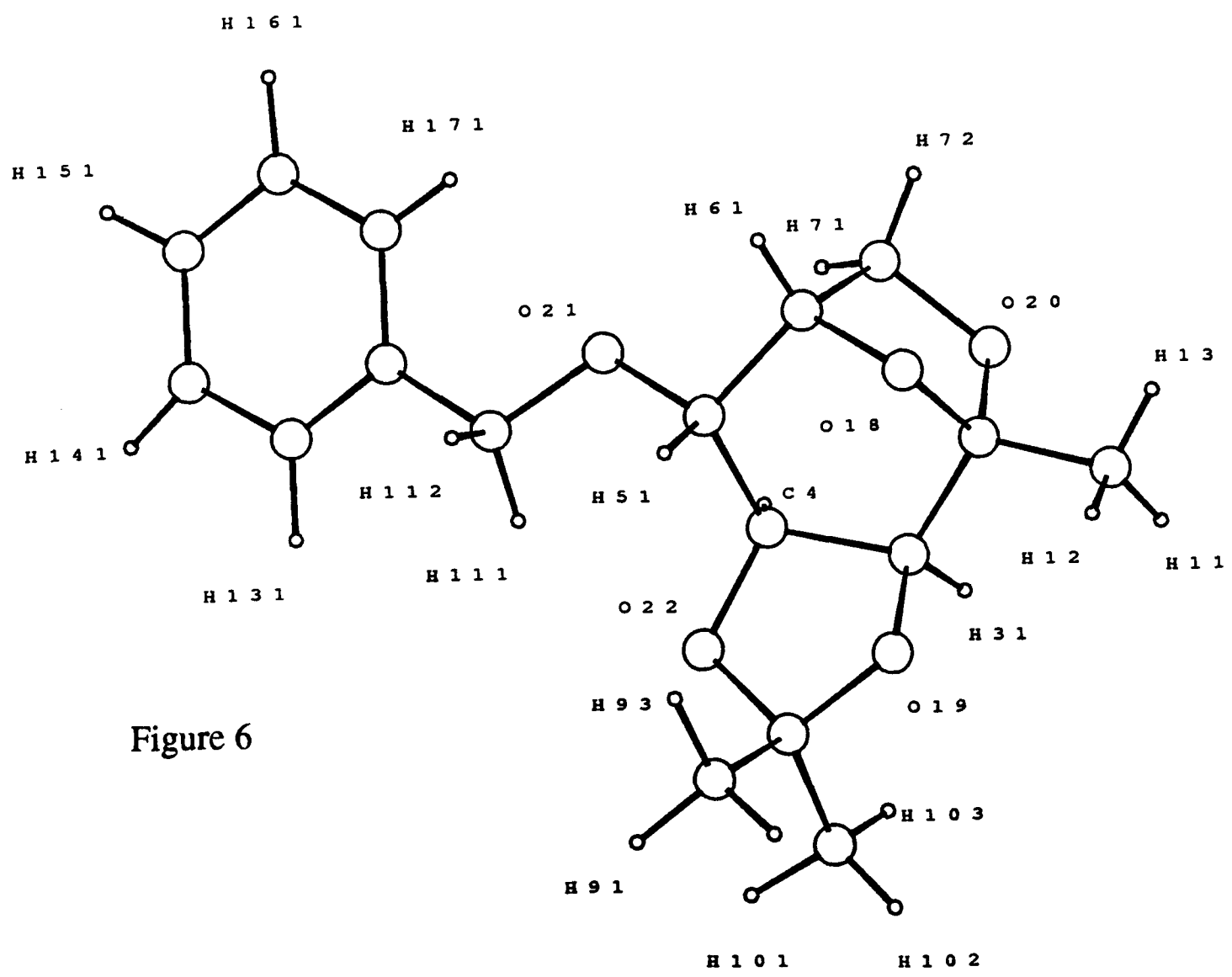
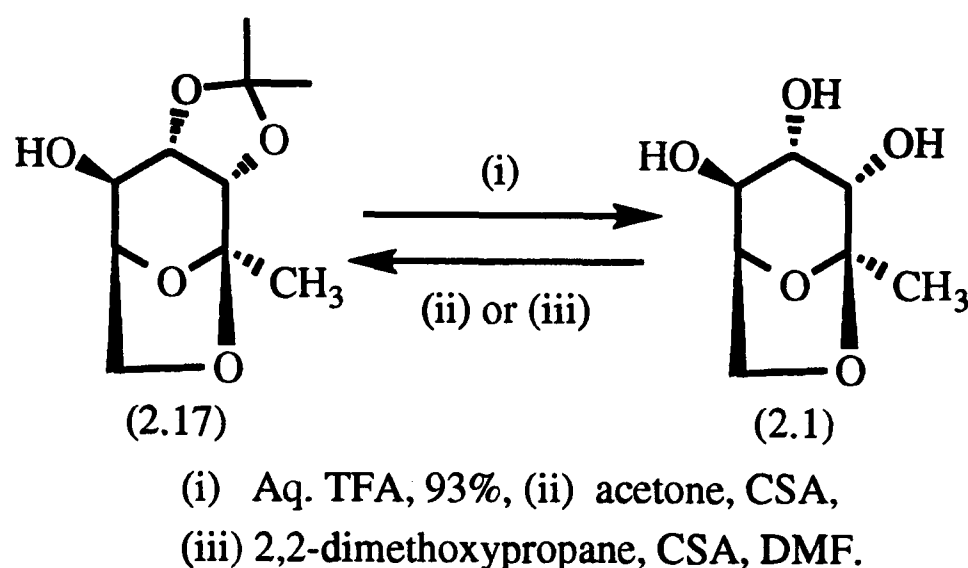


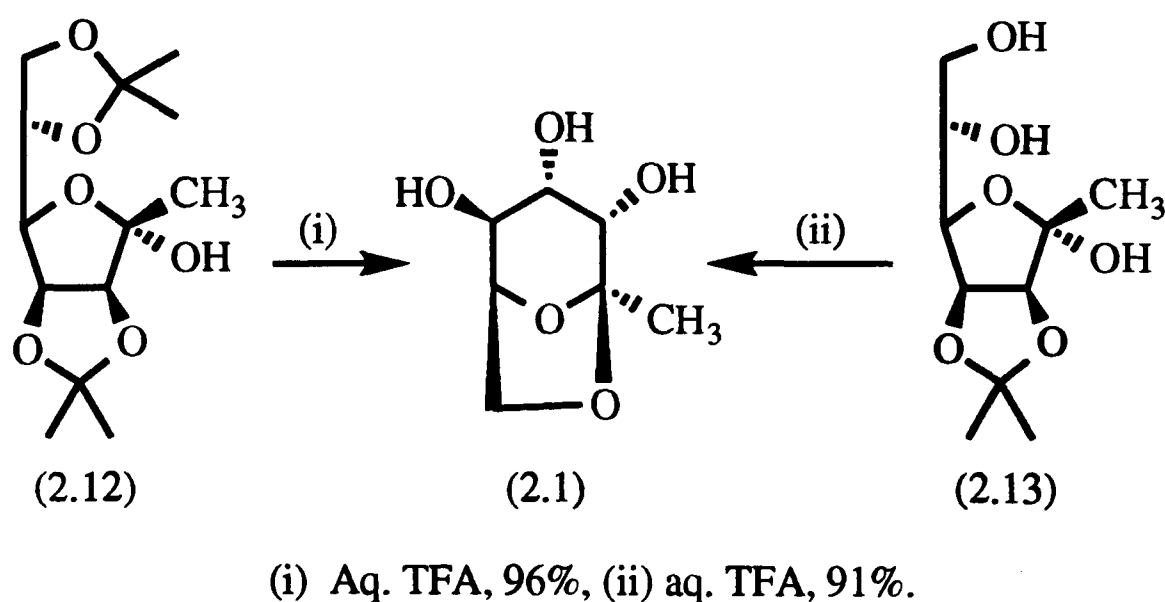
Figure 6

The bicyclic monoacetone (2.17) was completely deprotected on hydrolysis in aqueous trifluoroacetic acid to give the triol (2.1). The anhydro structure appeared to be stable under these conditions. To confirm that no isomerisation of the bicyclic framework had occurred, the monoacetone was reformed from the triol (2.1) under both kinetic and thermodynamic conditions. In both cases, the monoacetone formed was identical to compound (2.17) (Scheme 9).



Scheme 9

The bicyclic triol (2.1) was also the product if either the fully protected keto-sugar (2.12) or the monoacetone (2.13) were hydrolysed with aqueous trifluoroacetic acid (Scheme 10). The target compound (2.1) was therefore synthesised in three steps from L-gulono-1,4-lactone (2.10) in an overall yield of 61%.



Scheme 10

All the bicyclic systems mentioned above had highly characteristic proton NMR spectra due to the rigid nature of the carbon framework (Figure 7). The coupling constants between H-4 and H-5 were usually large (5.7-9.8 Hz), due to the dihedral angle between these two protons being close to 180° . Similarly, H-6 and one of the methylene protons showed very small coupling constants (0-1.1 Hz), due to a dihedral angle of close to 90° . Similar results have been observed in the proton NMR spectra of a number of 1,6-anhydrohexopyranoses.¹³

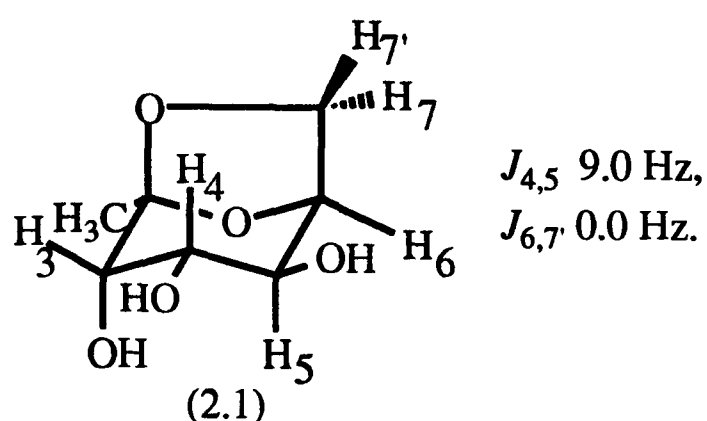


Figure 7

It was originally hoped that the anhydro bridge in the triol (2.1) could be opened to allow access to derivatives of α -L-homofucose (2.20) (Figure 8).

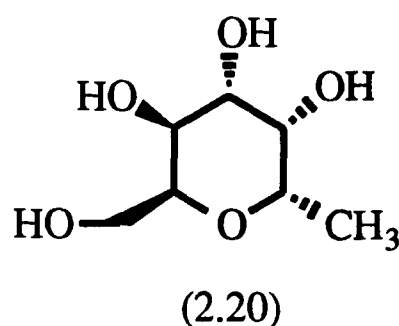
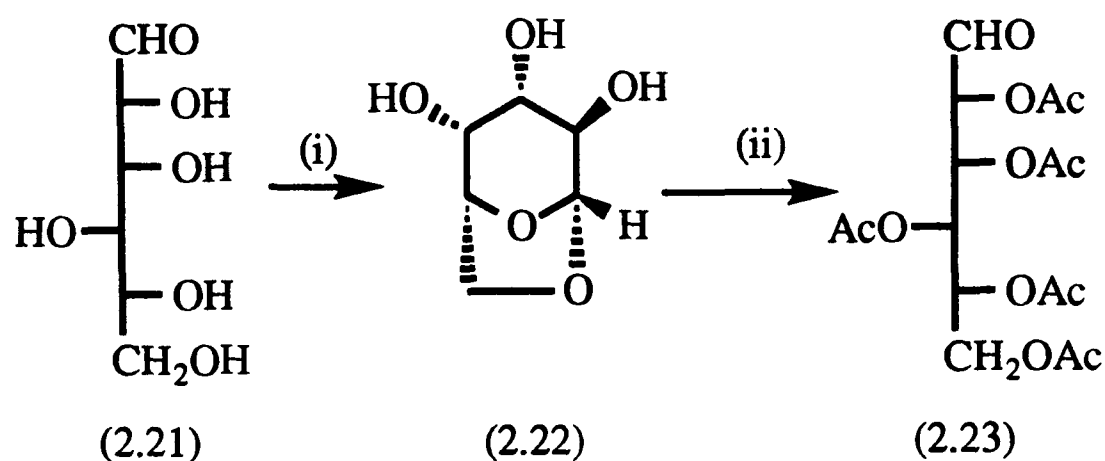


Figure 8

However, under a number of different conditions reported in the literature for the opening of such intramolecular ketals,¹³ the triol (2.1) could not be opened. Thus, treatment with strong acids,¹⁴ or with lithium aluminium hydride in the presence

of a Lewis acid,¹⁵ lead to either recovery of the starting material or complete decomposition.

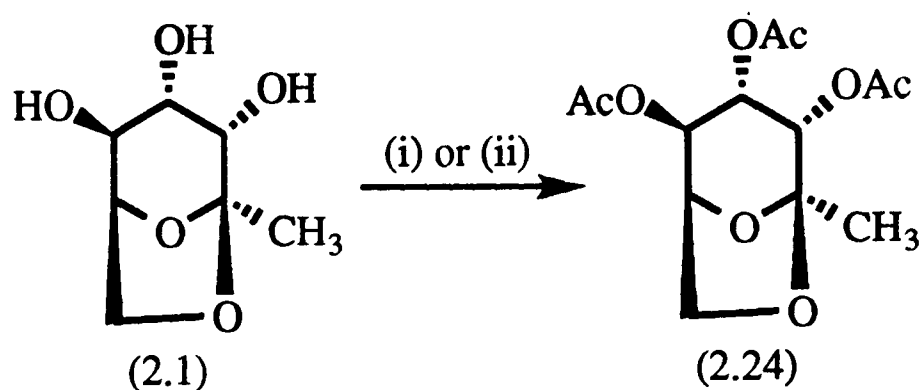
D-Gulose (2.21) forms an anhydride (2.22) on treatment with aqueous acid. This anhydride (2.22) was opened to D-gulose pentaacetate (2.23) by treatment with acetic anhydride and concentrated sulphuric acid (Scheme 11).¹⁶



(i) 0.5 N H_2SO_4 , 100 °C, 43%, (ii) Ac_2O , c. H_2SO_4 .

Scheme 11

It was therefore decided to attempt the opening of the triol (2.1) under similar conditions. However, treatment of the triol (2.1) with a mixture of acetic anhydride, acetic acid and concentrated sulphuric acid gave only the triacetate (2.24) in 94% yield. The same triacetate (2.24) was formed from the triol (2.1) in 92% yield by reaction with acetic anhydride in pyridine (Scheme 12).



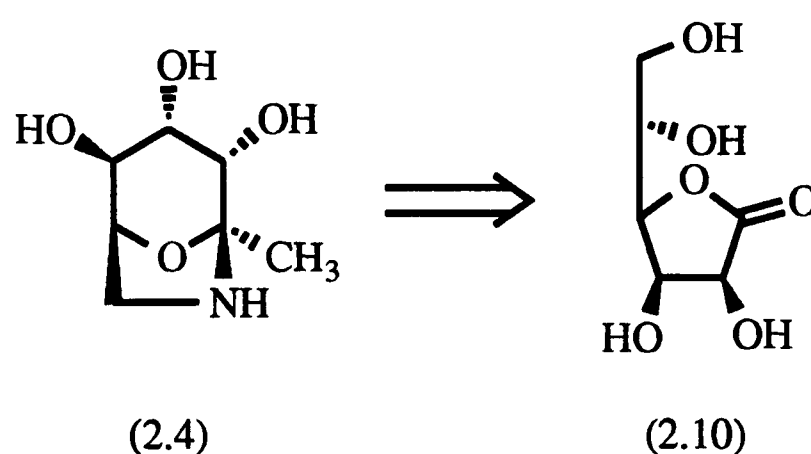
(i) Ac_2O , AcOH , c. H_2SO_4 , 94%,
(ii) Ac_2O , py, 92%.

Scheme 12

Treatment of the triacetate (2.24) with further acetic anhydride in the presence of either sulphuric acid and acetic acid or of boron trifluoride etherate¹⁷ led to decomposition of the starting material. Attempted opening of the triacetate (2.24) with trimethylsilyl iodide and DBU in toluene¹⁸ led only to recovery of the starting material.

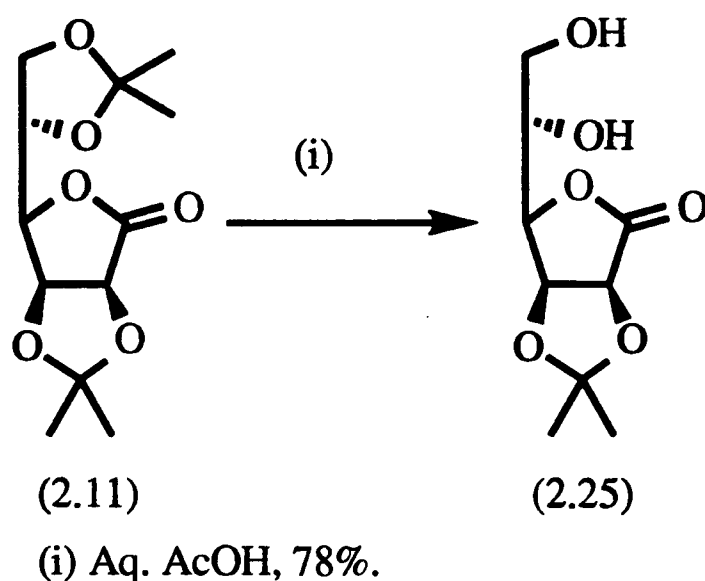
ii. Synthesis of 1,2,7-trideoxy-2,7-imino- β -L-gulo-heptulopyranose (2.4).

A similar strategy to that used for the synthesis of (2.1) was adopted for the synthesis of the nitrogen containing target (2.4). The starting material was again L-gulonolactone (2.10), and for this target nitrogen had to be introduced at the primary position (Scheme 13).



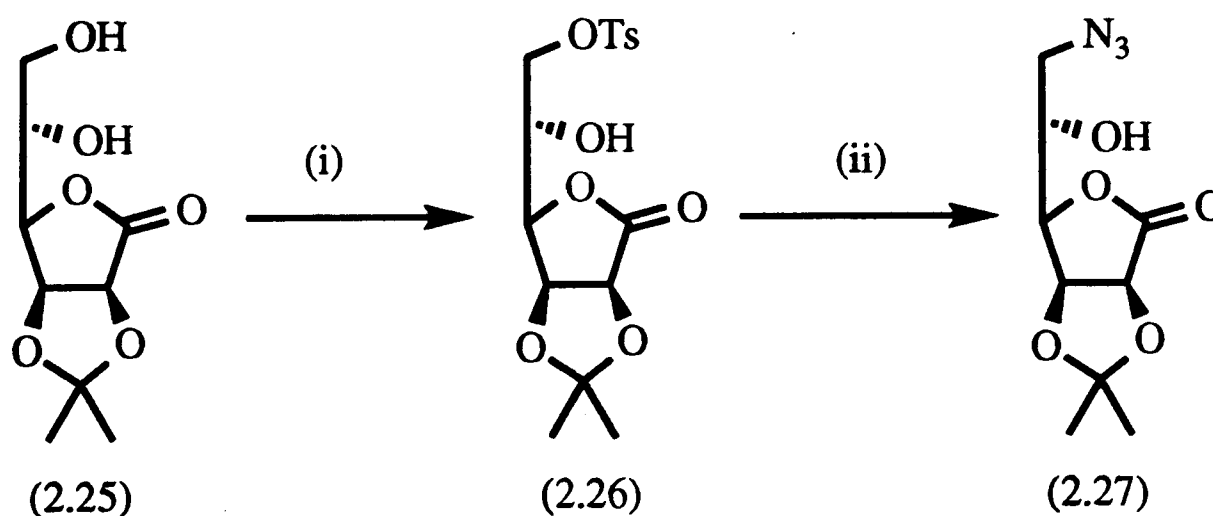
Scheme 13

As in the previous synthesis, the first step was to protect L-gulonolactone (2.10) as its diacetonide (2.11).⁷ The primary acetonide was then removed selectively by hydrolysis in aqueous acetic acid to give the diol (2.25),¹⁹ in 78% yield (Scheme 14).



Scheme 14

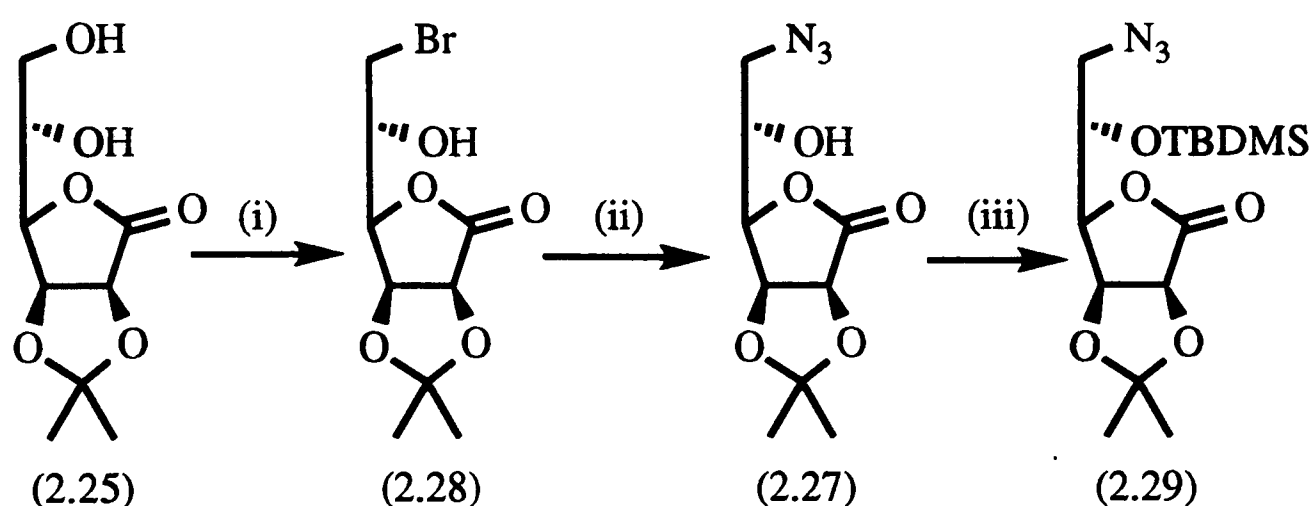
It was envisaged that selective conversion of the primary hydroxyl into a leaving group which could then be displaced using a nitrogen nucleophile, would lead to introduction of nitrogen at the required position. Initial attempts employed a tosylate as the leaving group. Thus, treatment of the diol (2.25) with tosyl chloride in pyridine in the presence of a catalytic amount of 4-(*N,N*-dimethylamino)-pyridine gave the primary tosylate (2.26) in 43% yield (Scheme 15). The tosylate group was then displaced by azide ion to give the primary azide (2.27) in 35% yield. Introduction of nitrogen at the primary position had been achieved but the yields of these two steps were poor. It was therefore decided to investigate an alternative leaving group.



(i) TsCl, DMAP, py, 43%, (ii) NaN₃, DMF, 35%.

Scheme 15

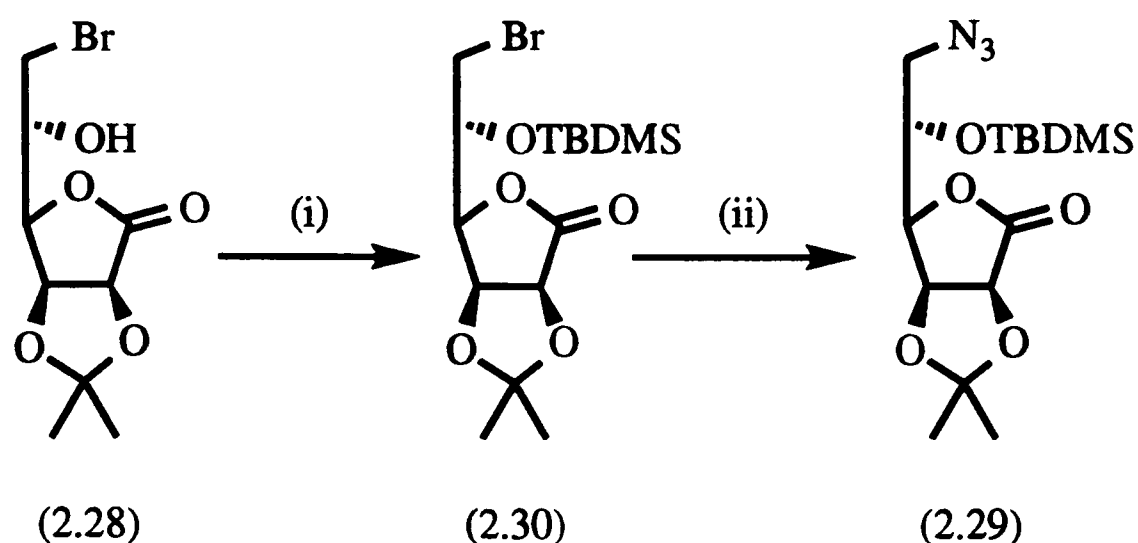
The primary bromide (2.28)²⁰ was synthesised following a literature procedure.²¹ Treatment of the diol (2.25) with carbon tetrabromide and triphenyl phosphine in tetrahydrofuran gave the bromide (2.28) in 66% yield (Scheme 16). The bromide was displaced by azide ion on heating at 60 °C in *N,N*-dimethylformamide with sodium azide. The azide (2.27) formed was identical to that formed via displacement of the tosylate (2.26), but the yield (95%) of the reaction was much higher. The remaining free hydroxyl group was then protected as its silyl ether (2.29).



(i) CBr₄, PPh₃, THF, 66%, (ii) NaN₃, DMF, 60 °C, 95%,
(iii) TBDMSCl, imidazole, DMF, 60 °C, 88%.

Scheme 16

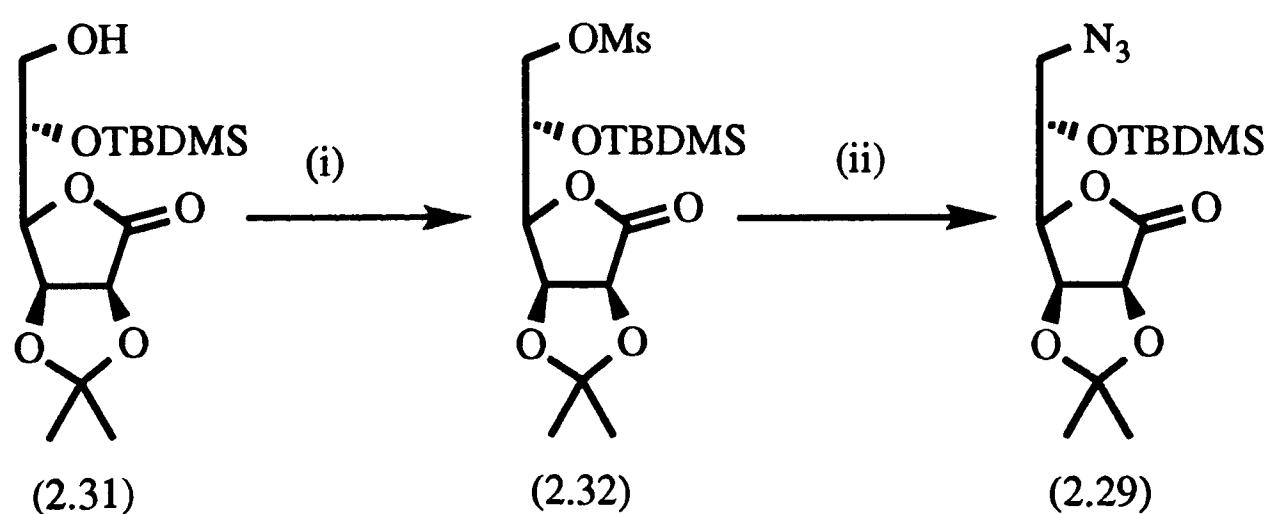
The primary bromide (2.28) and the primary azide (2.27) co-ran in all the t.l.c. systems investigated, making the azide displacement reaction difficult to monitor experimentally. Practically, it proved easier to first protect the free hydroxyl in the bromide (2.28) as its silyl ether (2.30) and then perform the azide displacement (Scheme 17). The two silylated compounds, (2.29) and (2.30), did not co-run on t.l.c., making this reaction easier to follow experimentally. The azide formed was identical to that produced in Scheme 16.



(i) TBDMSCl, imidazole, DMF, 50 °C, 80%, (ii) NaN_3 , DMF, 60 °C, 94%.

Scheme 17

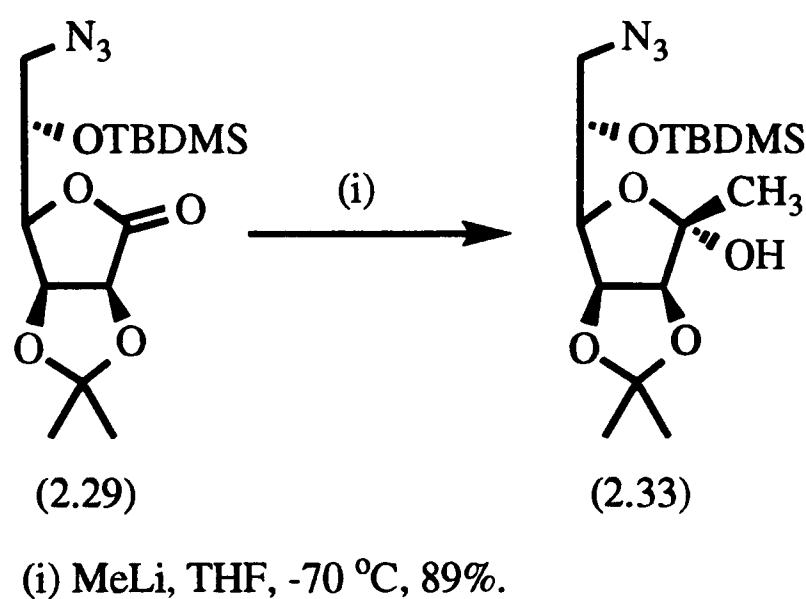
Some 5-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-L-gulono-1,4-lactone (2.31) was available at this time.²² Attempts at making the triflate ester of the primary hydroxyl group were unsuccessful. However, the mesylate (2.32) was formed in good yield on treatment with mesyl chloride and 4-(*N,N*-dimethylamino)-pyridine in pyridine. The mesylate underwent displacement with sodium azide to give the same silyl azide (2.29) as above (Scheme 18).



(i) MsCl , DMAP, py, 0 °C, 76%, (ii) NaN_3 , DMF, 60 °C, 85%.

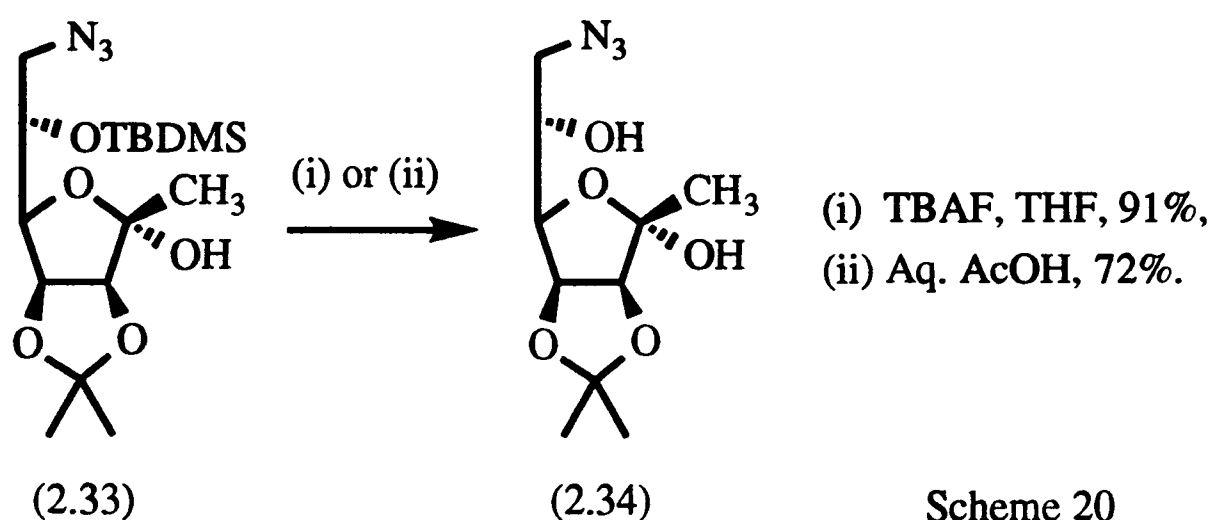
Scheme 18

As nitrogen had been introduced at the required position, the next step was introduction of the methyl group at C-1. This was achieved in good yield via the addition of one equivalent of methyllithium to the fully protected lactone (2.29) (Scheme 19). As in the previous synthesis, only one product (2.33) was isolated from this reaction. NMR and IR data indicated that it was still in a closed ring form, and the anomeric configuration was assigned by analogy to the previous case (2.12).



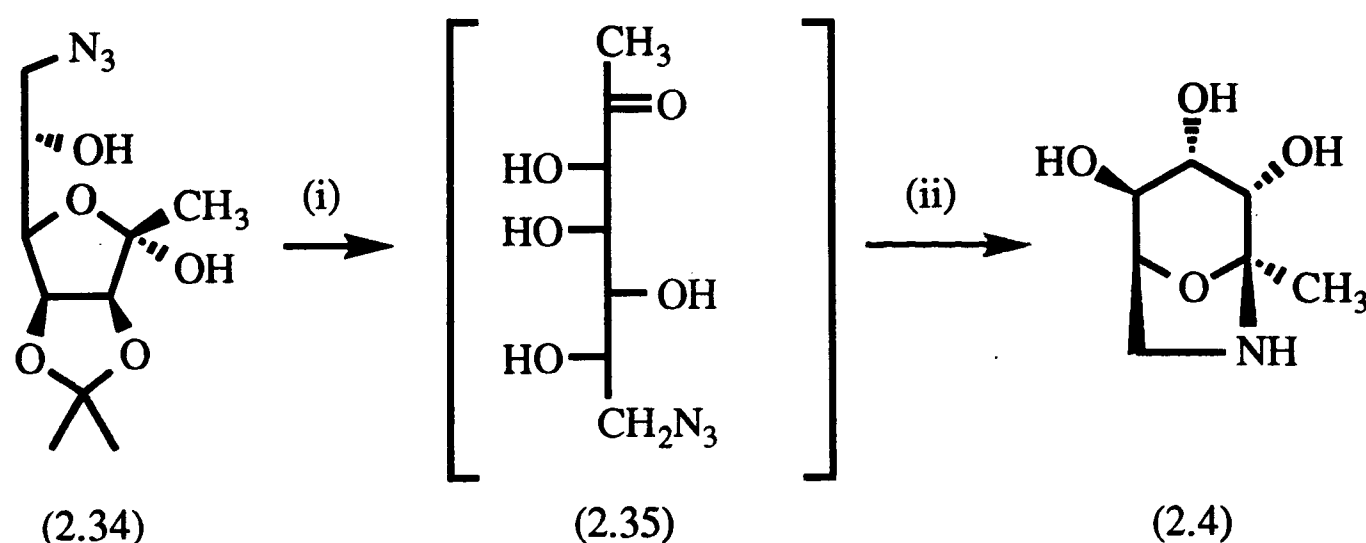
Scheme 19

The silyl protecting group was readily removed on treatment of the keto-sugar (2.33) with tetra-*n*-butylammonium fluoride in tetrahydrofuran to give the alcohol (2.34) as a single compound. NMR data again showed that this existed in a closed ring form. In the proton NMR in d_6 -DMSO, coupling between a hydroxyl proton and H-6 was observed, indicating that the compound was still in the furanose ring form, and that no isomerisation of the ring size had occurred during the deprotection. The same product (2.34) was also obtained if the silyl group was removed by acidic hydrolysis (Scheme 20).



Scheme 20

The acetonide was hydrolysed on stirring (2.34) with acidic ion exchange resin in water. From the proton and carbon NMR data, the crude product (2.35) appeared to exist in a mixture of forms, but it was unstable and was not readily purified. Therefore, the fully deprotected azide (2.35) was not isolated but was carried straight through to the next step. After the hydrolysis, the ion exchange resin was removed by filtration and the filtrate was subjected to a catalytic hydrogenation over palladium black. The product was purified by ion exchange chromatography to give the target hemiaminal (2.4) in 85% yield over the two steps (Scheme 21).

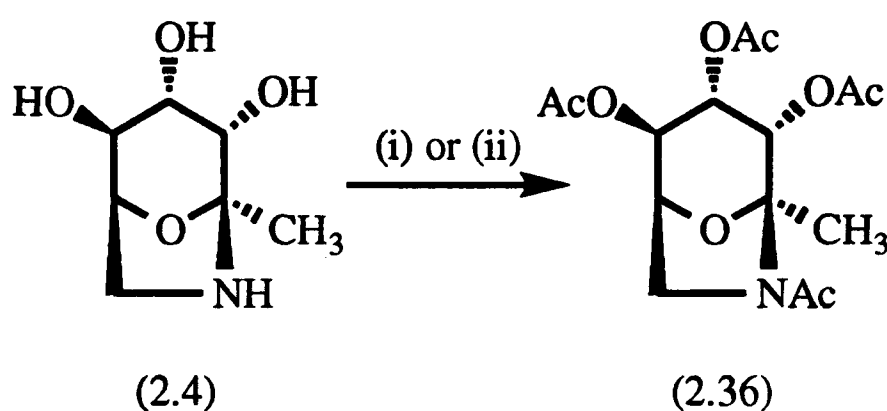


(i) H^+ ion exchange resin, H_2O , (ii) H_2 , Pd-black, H_2O , 7 h, 85% over 2 steps.

Scheme 21

This bicyclic system was remarkably stable, although it was highly hygroscopic. It was further characterised as its tetraacetate (2.36), formed by reaction with acetic anhydride in pyridine (Scheme 22). The same tetraacetate

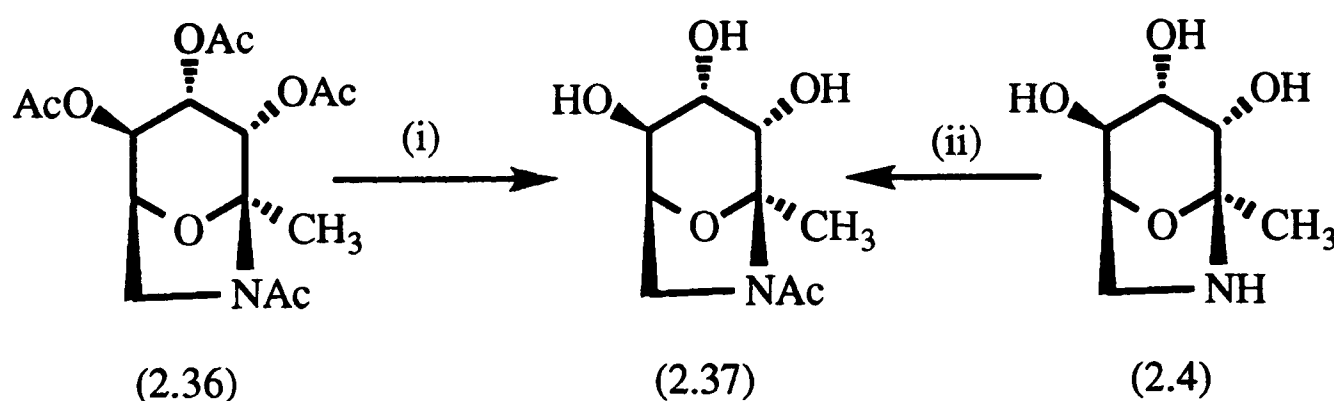
(2.36) was also the major product, although in much lower yield, if the bicyclic system (2.4) was treated with acetic anhydride, acetic acid and concentrated sulphuric acid.



(i) Ac_2O , py, 79%, (ii) Ac_2O , AcOH, c. H_2SO_4 , 46%.

Scheme 22

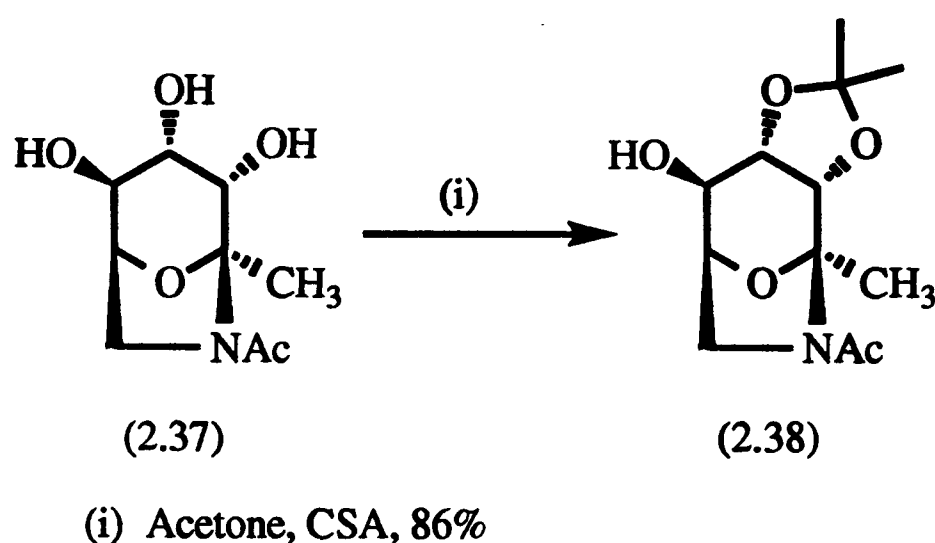
Selective removal of only the *O*-acetates was achieved by hydrolysis with triethylamine in methanol to give the amide (2.37). The same amide (2.37) was also formed when the target system (2.4) was treated with 1.5 equivalents of acetic anhydride in methanol. Such selective *N*-acylations are well documented for sugar derivatives.²³ The proton NMR of the amide (2.37) in d_6 -DMSO showed that the hydroxyl protons were coupled to the protons assigned as H-3, H-4 and H-5, supporting a pyranose anhydro structure as drawn (Scheme 23).



(i) Et_3N , MeOH, quant., (ii) Ac_2O , MeOH, 76%.

Scheme 23

As expected, the amide (2.37) formed a single mono-acetonide (2.38) on treatment with acetone and camphorsulphonic acid, confirming that there were two *cis* hydroxyl groups in the bicyclic structure (Scheme 24).



Scheme 24

All the nitrogen containing bicyclic systems mentioned above had highly characteristic proton NMR spectra, similar to those observed for the oxygen systems mentioned earlier. The coupling constants between H-4 and H-5 were large (9.3-9.8 Hz), due to the dihedral angle between these two protons being close to 180°. In addition, H-6 and one of the methylene protons showed no coupling, due to a dihedral angle of almost 90° (Figure 9). These results are also in agreement with a pyranose anhydro structure, as was demonstrated for the oxygen system by the X-ray analysis of compound (2.19).

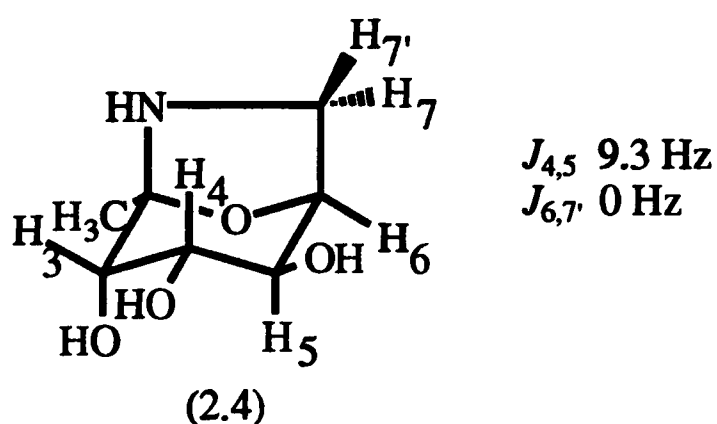


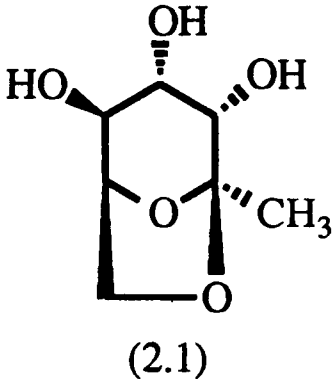
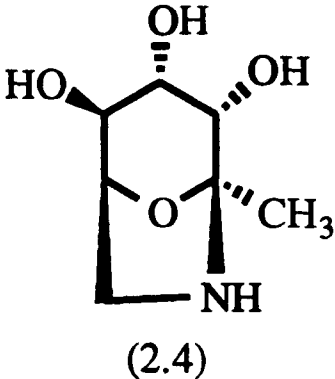
Figure 9

iii. The evaluation of compounds (2.1) and (2.4) as glycosidase inhibitors.

The effect of the target compounds (2.1) and (2.4) on a range of human liver glycosidases was investigated, and the results are shown in the table below.²⁴ The results show the percentage inhibition of the enzymes at a 1×10^{-3} M concentration of the test compounds. It can be seen that the oxygen containing system (2.1) has no effect on α -fucosidase, and no significant effect on any of the other glycosidases. However, the nitrogen containing system (2.4) is a potent and specific inhibitor of α -fucosidase, with a K_i value of 6×10^{-6} M. This is as potent as the best pyrrolidine fucosidase inhibitors and in addition the compound is highly specific for α -fucosidase. However, it is less potent than the best piperidine inhibitors such as DFJ (1.5) and α -L-homofuconojirimycin (1.6).

Inhibition (%) at 1 mM

(A negative value signifies inhibition)

Enzyme	 (2.1)	 (2.4)
α -mannosidase pH 4	-5	-1
α -mannosidase pH 6.5	-1	-1
β -mannosidase	+3	-10
α -glucosidase	+55	+12
β -glucosidase	-29	-3
α -galactosidase	+19	-3
β -galactosidase	+6	+12
α -fucosidase	+4	-94
β -hexosaminidase	-1	-14
β -glucuronidase	-13	+11
β -xylosidase	-16	-14
α -arabinosidase	-1	-6

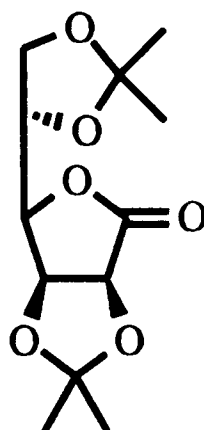
2.3 GENERAL EXPERIMENTAL.

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (at 200 MHz) or Brucker AC 200 (at 200 MHz) or Brucker AM 500 (at 500 MHz) spectrometers. ^{13}C Nuclear magnetic resonance spectra (δ_C) were recorded on Varian Gemini 200 (at 50 MHz) or Brucker AM 500 (at 125 MHz) spectrometers. Multiplicities were assigned using the DEPT sequence. All proton nuclear magnetic resonance spectra were recorded at 200 MHz, and carbon spectra at 50 MHz, unless otherwise stated. All chemical shifts are quoted on the δ -scale using residual solvent as an internal standard. ^{13}C Spectra run in D_2O had 1,4-dioxane (δ 67.8 ppm) added as an internal standard. Infra red spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrometer. Mass spectra were recorded on VG 20-250, ZAB 1F, or TRIO-1 GCMS (DB-5 flash) spectrometers using desorption chemical ionisation (NH_3 , DCI) or chemical ionisation (NH_3 , CI), as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis services of the Dyson Perrins laboratory. Thin layer chromatography was carried out on aluminium sheets coated with 60 F₂₅₄ silica or glass plates coated with silica blend GF₂₅₄. Plates were developed using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid or 0.5% ninhydrin in methanol (for amines). Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; dichloromethane was refluxed over and distilled from calcium hydride; pyridine was distilled from calcium hydride and stored over potassium hydroxide; tetrahydrofuran was distilled, under nitrogen, from a solution dried in the presence of benzophenone. *N,N*-Dimethylformamide was distilled under reduced pressure from calcium

hydride or purchased dry from the Aldrich Chemical Company in Sure-Seal™ bottles. Hexane was distilled at 68 °C before use to remove involatile fractions. All solvents were removed *in vacuo*.

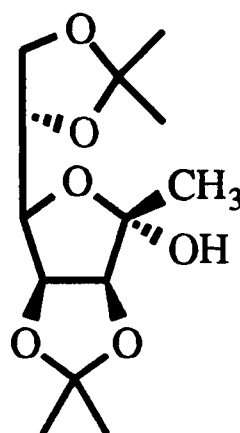
2.4 Experimental.

2,3;5,6-Di-*O*-isopropylidene-L-gulono-1,4-lactone (2.11).



L-Gulono-1,4-lactone (2.10) (28.62 g, 161 mmol) was stirred in acetone (500 ml), with 2,2-dimethoxypropane (73 ml, 595 mmol, 3.7 mol equivalents) and DL-10-camphorsulphonic acid (3.74 g, 16.1 mmol, 0.1 mol equivalents) for 28 h, when t.l.c. (ethyl acetate) revealed no starting material (baseline) and one major product (R_f 0.8). The reaction mixture was stirred with an excess of sodium hydrogen carbonate (1.43 g, 17 mmol), filtered through a pad of Celite and evaporated. The crude residue was dissolved in dichloromethane (200 ml), washed with water (3 x 100 ml), dried (magnesium sulphate) and filtered. The solvent was removed to give a solid which was recrystallised from ethyl acetate to give 2,3;5,6-di-*O*-isopropylidene-L-gulono-1,4-lactone (2.11) (29.56 g, 115 mmol, 71% yield) as a white crystalline solid. M.p. 153-154 °C (ethyl acetate) (lit.⁷ 153-154 °C); $[\alpha]_D^{20} +75.8$ (c , 1.1 in CHCl_3) (lit.⁷ $[\alpha]_D^{24} +91.5$ (c , 1.0 in CHCl_3)); δ_H (CDCl_3): 1.37, 1.39 (2 x 3H, 2 x s, 2 x CH_3), 1.46 (6H, s, 2 x CH_3), 3.78-3.86 (1H, m), 4.18-4.25 (1H, m), 4.41-4.46 (2H, m), 4.73-4.77 (1H, m), 4.84 (1H, d, J 5.6 Hz).

1-Deoxy-3,4:6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.12).



Method 1.

2,3;5,6-Di-*O*-isopropylidene-L-gulono-1,4-lactone (2.11) (11.13 g, 43 mmol) was dissolved in dry tetrahydrofuran (210 ml) and the solution cooled to -70 °C under nitrogen. Methyl lithium (1.4 M in diethyl ether, 34 ml, 1.1 mol equivalents) was added and the reaction mixture was stirred for 10 min, by which time t.l.c. (diethyl ether:hexane, 4:1) showed complete conversion of the starting material (R_f 0.2) to a single product (R_f 0.3). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution and the solvents were evaporated. The crude residue was partitioned between water (50 ml) and ethyl acetate (150 ml). The aqueous layer was washed with a further portion of ethyl acetate (150 ml). The organic extracts were combined, dried (magnesium sulphate), filtered and the solvent removed to give *1-deoxy-3,4;6,7-di-O-isopropylidene- β -L-gulo-heptulofuranose* (2.12) (10.19 g, 37 mmol, 89% yield) as a white solid which was sufficiently pure to use crude in the next reaction. A small portion was recrystallised for characterisation. M.p. 139-141 °C (diethyl ether/hexane); $[\alpha]_D^{20} +4.3$ (c, 1.0 in CHCl_3); ν_{max} (KBr): 3921 (br, OH) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.30, 1.40, 1.46, 1.47, 1.57 (5 x 3H, 5 x s, 5 x CH_3), 3.71 (1H, dd, H-7, $J_{7,7'}$ 8.3 Hz, $J_{7,6}$ 7.7 Hz), 4.09 (1H, dd, H-5, $J_{5,6}$ 8.4 Hz, $J_{5,4}$ 4.1 Hz), 4.22 (1H, dd, H-7', $J_{7,7}$ 8.3 Hz, $J_{7,6}$ 6.6 Hz), 4.35 (1H, ddd, H-6, $J_{6,5}$ 8.4 Hz, $J_{6,7}$ 7.7 Hz, $J_{6,7'}$ 6.6 Hz), 4.47 (1H, d, H-3, $J_{3,4}$ 5.9 Hz). 4.72 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 4.1 Hz); δ_{H} (d_6 -DMSO, 400 MHz): 1.18, 1.24, 1.29, 1.31, 1.33

(5 x 3H, 5 x s, 5 x CH₃), 3.68 (1H, t, H-7, *J* 8 Hz), 3.85 (1H, dd, H-5, *J*_{5,6} 8 Hz, *J*_{5,4} 4 Hz), 3.99 (1H, dd, H-7', *J*_{7',7} 8 Hz, *J*_{7',6} 7 Hz), 4.08 (1H, q, H-6, *J* 7.5 Hz), 4.24 (1H, d, H-3, *J*_{3,4} 6 Hz), 4.72 (1H, dd, H-4, *J*_{4,3} 6 Hz, *J*_{4,5} 4 Hz), 5.92 (1H, s, OH); δ_C (CDCl₃): 22.3 (q, C-1), 24.7, 25.3, 25.9, 26.6 (4 x q, 2 x (CH₃)₂C), 66.0 (t, C-7), 75.6, 80.7, 81.4, 85.5 (4 x d, C-3, C-4, C-5, C-6), 105.8 (s, C-2), 109.9, 113.1 (2 x s, 2 x (CH₃)₂C); *m/z* (NH₃, CI): 292 (M+NH₄⁺, 2%), 257 (M+H⁺-H₂O, 100%). (Found C, 56.67; H, 8.23. C₁₃H₂₂O₆ requires C, 56.92; H, 8.08%).

Method 2.

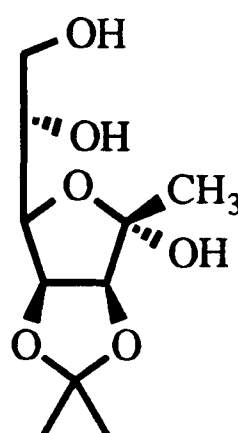
1-Deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13) (19 mg, 0.08 mmol) was stirred at room temperature in acetone (1 ml) with a catalytic amount of DL-10-camphorsulphonic acid. After 3.5 h t.l.c. (ethyl acetate) showed conversion of the starting material (*R*_f 0.1) to a single product (*R*_f 0.7). The reaction was quenched by the addition of saturated aqueous sodium hydrogen carbonate solution and the solvents were evaporated. The residue was dissolved in ethyl acetate (15 ml) and washed with water (5 ml) and brine (5 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:2 then 3:1) to yield 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.12) (13 mg, 0.05 mmol, 59% yield) as a white solid, identical to the material prepared above.

Method 3.

1-Deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13) (25 mg, 0.1 mmol) was stirred at room temperature in dry *N,N*-dimethylformamide (1 ml) with 2,2-dimethoxypropane (39 μ l, 3 mol equivalents) and a catalytic amount of

DL-10-camphorsulphonic acid. After 3 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.1) to a single product (R_f 0.7). The reaction was quenched by the addition of triethylamine and the solvents were evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:2 then 2:1) to yield 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.12) (26 mg, 0.09 mmol, 90% yield) as a white solid, identical to the material prepared above.

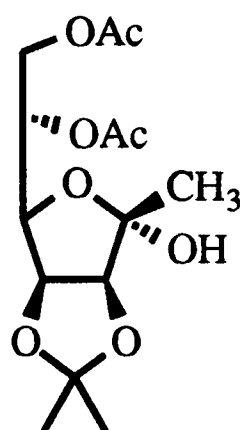
1-Deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13).



1-Deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.12) (1.793 g, 6.5 mmol) was dissolved in water (5.4 ml) and acetic acid (12.6 ml) and the reaction mixture heated at 50 °C. After 1 h t.l.c (diethyl ether:hexane, 4:1) showed complete conversion of the starting material (R_f 0.3) to a single product (baseline). The solvents were evaporated and the residue co-evaporated with toluene (3 x 5 ml). The resulting crude product was purified by flash chromatography (ethyl acetate) to give 1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13) (1.361 g, 5.8 mmol, 89% yield) as a colourless gum. $[\alpha]_D^{20} +28.7^\circ$ (c , 1.02 in CH_3CN); ν_{max} (neat): 3400 (br, OH) cm^{-1} ; δ_{H} (CD_3CN): 1.27 (3H, s, CH_3), 1.41 (6H, s, 2 x CH_3), 3.52 (1H, dd, H-7, $J_{7,7'}$ 11.4 Hz, $J_{7,6}$ 5.8 Hz), 3.64 (1H, dd, H-7', $J_{7',7}$ 11.4 Hz, $J_{7',6}$ 3.5 Hz), 3.85 (1H, ddd, H-6, $J_{6,5}$ 7.4 Hz, $J_{6,7}$ 5.8 Hz, $J_{6,7'}$ 3.5 Hz), 3.95 (1H, dd, H-5, $J_{5,6}$ 7.4 Hz, $J_{5,4}$ 3.6 Hz), 4.46

(1H, d, H-3, $J_{3,4}$ 6.0 Hz), 4.76 (1H, dd, H-4, $J_{4,3}$ 6.0 Hz, $J_{4,5}$ 3.6 Hz); δ_{H} (d_6 -DMSO, 500 MHz): 1.22, 1.32, 1.34 (3 x 3H, 3 x s, 3 x CH_3), 3.40 (1H, dt, H-7, $J_{7,7'}$ 11.2 Hz, $J_{7,6}$ 5.8 Hz, $J_{7,\text{OH}}$ 5.7 Hz), 3.50 (1H, ddd, H-7', $J_{7',7}$ 11.3 Hz, $J_{7',\text{OH}}$ 5.8 Hz, $J_{7',6}$ 2.9 Hz), 3.64 (1H, ddt, H-6, $J_{6,5}$ 8.5 Hz, $J_{6,7}$ 5.7 Hz, $J_{6,\text{OH}}$ 5.7 Hz, $J_{6,7'}$ 2.8 Hz), 3.86 (1H, dd, H-5, $J_{5,6}$ 8.4 Hz, $J_{5,4}$ 3.6 Hz), 4.22 (1H, d, H-3, $J_{3,4}$ 5.9 Hz), 4.43 (1H, t, OH-7, J 5.8 Hz, D_2O exchanges), 4.58 (1H, d, OH-6, J 5.0 Hz, D_2O exchanges), 4.66 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.6 Hz), 5.86 (1H, s, OH-1, D_2O exchanges); δ_{C} (CD_3CN): 21.4 (q, C-1), 23.8, 25.3 (2 x q, $(\text{CH}_3)_2\text{C}$), 63.1 (t, C-7), 71.1, 78.6, 80.9, 86.1 (4 x d, C-3, C-4, C-5, C-6), 104.3 (s, C-2), 112.2 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 252 ($\text{M}+\text{NH}_4^+$, 6%), 217 ($\text{M}+\text{H}^+-\text{H}_2\text{O}$, 100%). (Found C, 50.96; H, 7.82. $\text{C}_{10}\text{H}_{18}\text{O}_6$ requires C, 51.27; H, 7.75%).

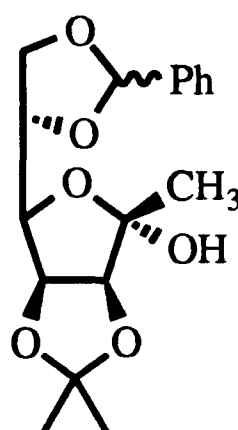
6,7-Di-*O*-acetyl-1-deoxy-3,4-*O*-isopropylidene- β -L-gulo-heptulofuranose (2.14).



1-Deoxy-3,4-*O*-isopropylidene- β -L-gulo-heptulofuranose (2.13) (117 mg, 0.5 mmol) was dissolved in dry pyridine (1.5 ml) at room temperature and acetic anhydride (0.6 ml) was added. After stirring for 2.5 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.2) to a single product (R_f 0.8). The solvents were evaporated and the residue dissolved in ethyl acetate (30 ml). The organic layer was washed with dilute aqueous hydrochloric acid (15 ml) and brine (15 ml), dried (magnesium sulphate), filtered and evaporated. The crude product

was purified by flash chromatography (diethyl ether/hexane, 1:1) to give *6,7-di-O-acetyl-1-deoxy-3,4-O-isopropylidene-β-L-gulo-heptulofuranose* (2.14) (102 mg, 0.32 mmol, 64% yield) as a gum. $[\alpha]_{\text{D}}^{20} +20.4$ (c, 0.93 in CHCl_3); ν_{max} (neat): 3460 (OH), 1746 (C=O) cm^{-1} ; δ_{H} (CD_3CN , 500 MHz): 1.30, 1.47, 1.51 (3 x 3H, 3 x s, 3 x CH_3), 2.07, 2.09 (2 x 3H, 2 x s, 2 x CH_3CO_2), 4.26-4.31 (2H, m, H-5, H-7), 4.46 (1H, d, H-3, $J_{3,4}$ 5.9 Hz), 4.50 (1H, dd, H-7', $J_{7',7}$ 12.5 Hz, $J_{7',6}$ 2.6 Hz), 4.77 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.7 Hz), 5.31 (1H, ddd, H-6, J 8.8 Hz, J 4.3 Hz, $J_{6,7'}$ 2.6 Hz); δ_{C} (CDCl_3): 20.6, 21.0, 22.2 (3 x q, C-1, 2 x CH_3CO_2), 24.7, 26.0 (2 x q, $(\text{CH}_3)_2\text{C}$), 62.9 (t, C-7), 71.2, 76.9, 80.3, 85.7 (4 x d, C-3, C-4, C-5, C-6), 105.4 (s, C-2), 113.1 (s, $(\text{CH}_3)_2\text{C}$), 170.7, 171.1 (2 x s, 2 x C=O); m/z (NH_3 , CI): 336 ($\text{M}+\text{NH}_4^+$, 9%), 301 ($\text{M}+\text{H}^+-\text{H}_2\text{O}$, 100%). (Found C, 53.01; H, 7.30. $\text{C}_{14}\text{H}_{22}\text{O}_8$ requires C, 52.82; H, 6.97%).

6,7-O-Benzylidene-1-deoxy-3,4-O-isopropylidene-β-L-gulo-heptulofuranose
(2.15, 2.16).



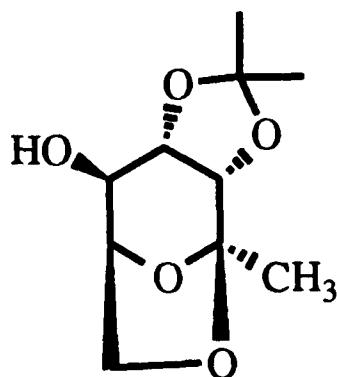
1-Deoxy-3,4-*O*-isopropylidene-β-*L-gulo*-heptulofuranose (2.13) (173 mg, 0.74 mmol) was dissolved in dry *N,N*-dimethylformamide (3 ml) and benzaldehyde dimethylacetal (0.33 ml, 3 mol equivalents) was added. DL-10-Camphorsulphonic acid was added to adjust the pH of the solution to 3. After stirring at room temperature for 1.5 h, t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.2) to a number of products (R_f 0.7-0.8). The reaction mixture was adjusted to pH 8 by dropwise addition of triethylamine

and the solvents were removed. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:2) to yield two products, (2.15, 2.16), (R_f 0.3 and 0.4, (diethyl ether:hexane, 4:1)), both white solids, in a combined yield of 83%.

Less polar (major) product, (R_f 0.4) (127 mg, 0.39 mmol, 52% yield). M.p. 109-111 °C (diethyl ether/hexane); $[\alpha]_D^{20} +9.3$ (c , 1.05 in CHCl_3); ν_{max} (KBr): 3230 (br, OH) cm^{-1} ; δ_{H} (CDCl_3): 1.32, 1.44, 1.57 (3 x 3H, 3 x s, 3 x CH_3), 3.88 (1H, dd, J 8.1 Hz, J 7.1 Hz), 4.20-4.31 (2H, m), 4.42-4.54 (2H, m), 4.77 (1H, dd, J 5.9 Hz, J 4.1 Hz), 5.85 (1H, s, PhCH), 7.35-7.53 (5H, m, ArCH); δ_{C} (CDCl_3): 22.2 (q, C-1), 24.7, 25.9 (2 x q, $(\text{CH}_3)_2\text{C}$), 66.9 (t, C-7), 76.8, 80.9, 85.5 (3 x d, C-3, C-4, C-5, C-6), 104.6 (d, PhCH), 105.9 (s, C-2), 113.1 (s, $(\text{CH}_3)_2\text{C}$), 127.0, 128.5, 129.6 (3 x d, ArCH), 137.2 (s, ArC); m/z (NH_3 , CI): 340 ($\text{M}+\text{NH}_4^+$, 6%), 323 ($\text{M}+\text{H}^+$, 17%), 305 ($\text{M}+\text{H}^+-\text{H}_2\text{O}$, 100%). (Found C, 63.64; H, 7.14. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires C, 63.34; H, 6.88%).

More polar (minor) product, (R_f 0.3) (74 mg, 0.23 mmol, 31% yield). M.p. 128-130 °C (diethyl ether/hexane); $[\alpha]_D^{20} -5.0$ (c , 1.13 in CHCl_3); ν_{max} (KBr): 3234 (br, OH) cm^{-1} ; δ_{H} (CDCl_3): 1.31, 1.47, 1.53 (3 x 3H, 3 x s, 3 x CH_3), 3.78 (1H, dd, J 8.1 Hz, J 7.0 Hz), 4.25 (1H, dd, J 8.1 Hz, J 4.0 Hz), 4.36-4.54 (3H, m), 4.78 (1H, dd, J 7.9 Hz, J 4.2 Hz), 5.97 (1H, s, PhCH), 7.35-7.53 (5H, m, ArCH); δ_{C} (CDCl_3): 22.3 (q, C-1), 24.7, 25.9 (2 x q, $(\text{CH}_3)_2\text{C}$), 67.3 (t, C-7), 76.2, 80.2, 80.6, 85.5 (4 x d, C-3, C-4, C-5, C-6), 103.8 (d, PhCH), 105.9 (s, C-2), 113.2 (s, $(\text{CH}_3)_2\text{C}$), 126.8, 128.4, 129.4 (3 x d, ArCH), 137.6 (s, ArC); m/z (NH_3 , CI): 340 ($\text{M}+\text{NH}_4^+$, 2%), 323 ($\text{M}+\text{H}^+$, 15%), 305 ($\text{M}+\text{H}^+-\text{H}_2\text{O}$, 100%). (Found C, 63.40; H, 7.14. $\text{C}_{17}\text{H}_{22}\text{O}_6$ requires C, 63.34; H, 6.88%).

2,7-Anhydro-1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.17).



Method 1.

1-Deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13) (911 mg, 3.9 mmol) was dissolved in dry *N,N*-dimethylformamide (10 ml) and a catalytic amount of DL-10-camphorsulphonic acid was added. The reaction mixture was heated at 70 °C for 45 min at which time t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.2) to one major product (R_f 0.7). The reaction was quenched by the addition of triethylamine (1 ml) and the solvents evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:1) to yield 2,7-anhydro-1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.17) (566 mg, 2.6 mmol, 67% yield) as a white solid. M.p. 95-97 °C (diethyl ether/hexane); $[\alpha]_D^{20}$ -46.3 (*c*, 0.94 in CHCl_3); ν_{max} (KBr): 3351 (br, OH) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz + COSY): 1.35, 1.52, 1.58 (3 x 3H, 3 x s, 3 x CH_3), 2.32 (1H, br d, OH, J 3.3 Hz, D_2O exchanges), 3.77 (1H, ddd, H-7, $J_{7,7'}$ 7.8 Hz, $J_{7,6}$ 5.7 Hz, $J_{7,5}$ 1.0 Hz), 3.95 (1H, dt, H-5, J 6.2 Hz, $J_{5,7}$ 1.0 Hz), 3.98 (1H, dd, H-7', $J_{7',7}$ 7.9 Hz, $J_{7',6}$ 1.0 Hz), 4.02 (1H, d, H-3, $J_{3,4}$ 6.6 Hz), 4.13 (1H, t, H-4, J 6.2 Hz), 4.43 (1H, dt, H-6, J 5.6 Hz, $J_{6,7}$ 1.0 Hz); δ_{C} (CDCl_3): 20.3 (q, C-1), 26.1, 28.0 (2 x q, $(\text{CH}_3)_2\text{C}$), 63.7 (t, C-7), 71.4, 75.7, 78.1, 78.9 (4 x d, C-3, C-4, C-5, C-6), 105.5 (s, C-2), 111.5 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 234 ($\text{M}+\text{NH}_4^+$, 8%), 217 (100%). (Found C, 55.86; H, 7.53. $\text{C}_{13}\text{H}_{22}\text{O}_6$ requires C, 55.55; H, 7.46%).

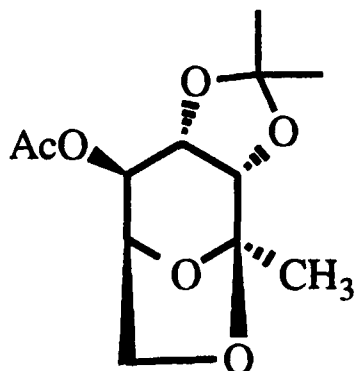
Method 2.

2,7-Anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (24 mg) was stirred at room temperature in acetone (1 ml) with a catalytic amount of DL-10-camphorsulphonic acid. After 3 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.1) to a single product (R_f 0.7). The reaction was quenched by the addition of saturated aqueous sodium hydrogen carbonate solution and the solvents were evaporated. The residue was dissolved in diethyl ether (15 ml) and washed with water (5 ml) and brine (5 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:1) to yield 2,7-anhydro-1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.17) as a white solid, identical to the material prepared above.

Method 3.

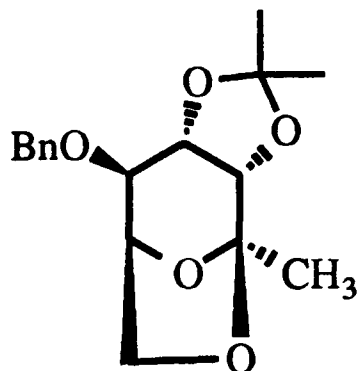
2,7-Anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (21 mg) was stirred at room temperature in dry *N,N*-dimethylformamide (1 ml) with 2,2-dimethoxypropane (80 μ l, 6 mol equivalents) and a catalytic amount of DL-10-camphorsulphonic acid. After 1 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.1) to a single product (R_f 0.7). The reaction was quenched by the addition of triethylamine and the solvents were evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:1) to yield 2,7-anhydro-1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.17) as a white solid, identical to the material prepared above.

5-O-Acetyl-2,7-anhydro-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.18).



2,7-Anhydro-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.17) (54 mg, 0.25 mmol) was dissolved in pyridine (1 ml) at room temperature and acetic anhydride (0.25 ml) was added. After stirring for 1 h t.l.c. (diethyl ether:hexane, 4:1) showed conversion of the starting material (R_f 0.3) to a single product (R_f 0.4). The solvents were evaporated and the residue dissolved in ethyl acetate (30 ml). The ethyl acetate was washed with dilute aqueous hydrochloric acid (15 ml) and brine (15 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:1) to give 5-O-acetyl-2,7-anhydro-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.18) (66 mg, 0.23 mmol, 92% yield) as a white solid. M.p. 141-142 °C; $[\alpha]_D^{20}$ -77.0 (c, 1.0 in CHCl_3); ν_{max} (KBr): 1741 (C=O) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz + COSY): 1.35, 1.54, 1.59 (3 x 3H, 3 x s, 3 x CH_3), 2.10 (3H, s, CH_3CO_2), 3.76 (1H, ddd, H-7, $J_{7,7'}$ 7.9 Hz, $J_{7,6}$ 5.5 Hz, $J_{7,5}$ 1.2 Hz), 3.90 (1H, dd, H-7', $J_{7',7}$ 7.9 Hz, $J_{7',6}$ 0.9 Hz), 4.06 (1H, d, H-3, $J_{3,4}$ 6.3 Hz), 4.28 (1H, t, H-4, J 6.3 Hz), 4.60 (1H, dt, H-6, J 5.2 Hz, $J_{6,7'}$ 0.8 Hz), 4.99 (1H, ddd, H-5, $J_{5,4}$ 6.3 Hz, $J_{5,6}$ 4.8 Hz, $J_{5,7}$ 1.2 Hz); δ_{C} (CDCl_3): 20.3, 20.9, 26.2, 27.8 (4 x q, C-1, $(\text{CH}_3)_2\text{C}$, CH_3CO_2), 64.1 (t, C-7), 73.0, 73.3, 75.2, 78.3 (4 x d, C-3, C-4, C-5, C-6), 105.8 (s, C-2), 111.8 (s, $(\text{CH}_3)_2\text{C}$), 170.4 (s, C=O); m/z (NH_3 , CI): 259 ($\text{M}+\text{H}^+$, 4%), 85 (100%). (Found C, 55.62; H, 7.08. $\text{C}_{12}\text{H}_{18}\text{O}_6$ requires C, 55.81; H, 7.02%).

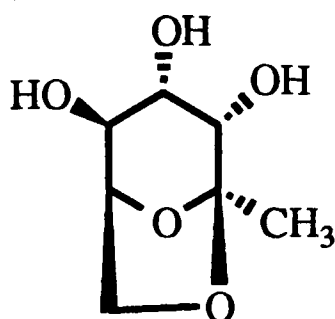
2,7-Anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19).



Sodium hydride (60% dispersion in oil, 32 mg, 1.4 mol equivalents) was washed with hexane (5 ml) and dry tetrahydrofuran (1 ml) was added. The resulting suspension was cooled to 0 °C and 2,7-anhydro-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.17) (122 mg, 0.56 mmol) in dry tetrahydrofuran (3 x 1 ml) was added dropwise. The reaction mixture was stirred at 0 °C for 0.5 h and then a catalytic amount of tetra-*n*-butylammonium iodide was added. The mixture was allowed to warm to room temperature and benzyl bromide (0.1 ml, 1.3 mol equivalents) was added. The reaction mixture was then warmed to 50 °C for 1.5 h by which time t.l.c. (diethyl ether:hexane, 4:1) showed complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.7). After cooling to room temperature, the reaction was quenched by careful dropwise addition of methanol until gas evolution ceased, and was then heated to 50 °C for 2 h. Diethyl ether (10 ml) was added and the reaction mixture was filtered through a Celite plug, eluting with further diethyl ether. The organic extracts were washed with water (15 ml), dried (magnesium sulphate), filtered and evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:10) to yield 2,7-anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19) (116 mg, 0.38 mmol, 68% yield), as a white crystalline solid. M.p. 75-77 °C (methanol/water); $[\alpha]_D^{20}$ -19.8 (*c*, 1.05 in CHCl₃); $\nu_{\max}$ (KBr): 2973, 2911 (CH stretch) cm⁻¹; δ_H (CDCl₃, 500 MHz): 1.36, 1.48, 1.57 (3 x 3H, 3 x s, 3 x CH₃), 3.70 (1H, ddd, H-6, $J_{6,7}$ 5.8 Hz, $J_{6,5}$ 4.8 Hz, $J_{6,7'}$ 1.0 Hz), 3.74 (1H, ddd, H-7, $J_{7,7'}$ 7.7 Hz, $J_{7,6}$ 5.7 Hz, $J_{7,5}$ 1.0

Hz), 3.99 (1H, dd, H-7', $J_{7',7}$ 7.7 Hz, $J_{7',6}$ 1.1 Hz), 4.04 (1H, d, H-3, $J_{3,4}$ 6.4 Hz), 4.28 (1H, t, H-4, J 6.1 Hz), 4.44 (1H, dt, H-5, J 5.7 Hz, $J_{5,7}$ 1.0 Hz); δ_C (CDCl₃): 20.4 (q, C-1), 26.2, 27.9 (2 x q, (CH₃)₂C), 64.3 (t, C-7), 71.6 (t, CH₂Ph), 74.2, 77.6, 77.9, 78.3 (4 x d, C-3, C-4, C-5, C-6), 105.6 (s, C-2), 111.2 (s, (CH₃)₂C), 128.1, 128.6 (2 x d, ArCH), 138.2 (s, ArC); m/z (NH₃, CI): 324 (M+NH₄⁺, 10%), 307 (M+H⁺, 100%), 91 (C₇H₇⁺, 50%). (Found C, 66.55; H, 7.39. C₁₃H₂₂O₆ requires C, 66.65; H, 7.24%).

2,7-Anhydro-1-deoxy- β -L-gulo-heptulopyranose (2.1).



Method 1.

2,7-Anhydro-3,4-*O*-isopropylidene-1-deoxy- β -L-gulo-heptulopyranose (2.17) (213 mg, 1.0 mmol) was stirred in trifluoroacetic acid (2 ml) and water (2 ml) at room temperature. After 0.5 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.7) to a single product (R_f 0.1). The solvents were removed and the residue co-evaporated with toluene (2 x 2 ml). The crude product was purified by flash chromatography (methanol:ethyl acetate, 1:20) to give 2,7-anhydro-1-deoxy- β -L-gulo-heptulopyranose (2.1) (164 mg, 0.93 mmol, 93% yield) as a white solid. M.p. 163.5-164.5 °C (methanol/ethyl acetate); $[\alpha]_D^{20}$ -46.1 (c , 1.0 in H₂O); $\nu_{\max}$ (KBr): 3306, 3427 (OH) cm⁻¹; δ_H (D₂O, 500 MHz): 1.39 (3H, s, CH₃), 3.60 (1H, d, H-3, $J_{3,4}$ 4.6 Hz), 3.62 (1H, dd, H-4, $J_{4,5}$ 9.0 Hz, $J_{4,3}$ 4.6 Hz), 3.65 (1H, dd, H-7, $J_{7,7'}$ 8.1 Hz, $J_{7,6}$ 4.9 Hz), 3.72 (1H, dd, H-5, $J_{5,4}$ 9.0 Hz, $J_{5,6}$ 4.1 Hz), 3.91 (1H, d, H-7', $J_{7',7}$ 8.1 Hz), 4.37 (1H, t, H-6,

J 4.5 Hz); δ_{C} (D_2O): 20.5 (q, C-1), 65.4 (t, C-7), 70.0, 70.8, 74.0, 76.8 (4 x d, C-3, C-4, C-5, C-6), 109.3 (s, C-2); m/z (NH_3 , CI): 194 ($\text{M}+\text{NH}_4^+$, 100%), 177 ($\text{M}+\text{H}^+$, 95%). (Found C, 47.69; H, 6.77. $\text{C}_7\text{H}_{12}\text{O}_5$ requires C, 47.73; H, 6.87%).

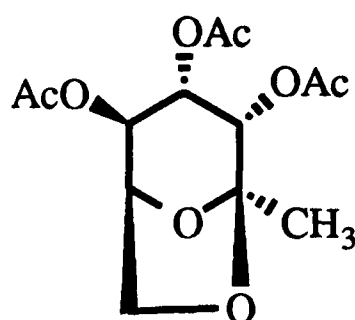
Method 2.

1-Deoxy-3,4;6,7-di-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.12) (1.570 g, 5.73 mmol) was stirred in trifluoroacetic acid (5 ml) and water (5 ml) at room temperature. After 1 h t.l.c. (methanol:ethyl acetate, 1:9) showed conversion of the starting material (R_f 0.8) to a single product (R_f 0.2). The solvents were removed and the residue co-evaporated with toluene (3 x 5 ml). The crude product was purified by flash chromatography (methanol:ethyl acetate, 1:20) to give 2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (965 mg, 5.48 mmol, 96% yield) as a white solid, identical to the material prepared above.

Method 3.

1-Deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.13) (98 mg, 0.42 mmol) was stirred in water (1 ml) and trifluoroacetic acid (1 ml) at room temperature. After 1 h t.l.c. (methanol:ethyl acetate, 1:9) showed conversion of the starting material (R_f 0.3) to a single product (R_f 0.2). The solvents were removed and the residue co-evaporated with toluene (3 x 1 ml). The crude product was purified by flash chromatography (methanol:ethyl acetate, 1:20) to give 2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (67 mg, 0.38 mmol, 91% yield) as a white solid, identical to the material prepared above.

3,4,5-Tri-*O*-acetyl-2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.24).

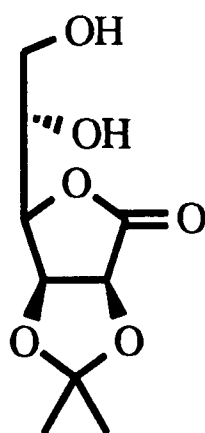


Method 1.

2,7-Anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (164 mg, 0.93 mmol) was dissolved in acetic acid (2 ml) at room temperature under nitrogen and acetic anhydride (2 ml) and concentrated sulphuric acid (1 drop) were added. After stirring for 1 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.2) to a single product (R_f 0.8). The solvents were evaporated and the residue was co-evaporated with toluene (3 x 2 ml). The crude product was purified by flash chromatography (diethyl ether:hexane, 1:1) to give 3,4,5-tri-*O*-acetyl-2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.24) (265 mg, 0.88 mmol, 94% yield) as a colourless gum. $[\alpha]_D^{20}$ -3.5 (c , 0.98 in CHCl_3); ν_{max} (KBr): 1752 ($\text{C}=\text{O}$) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.44 (3H, s, CH_3), 1.98, 2.06, 2.15 (3 x 3H, 3 x s, 3 x CH_3CO_2), 3.81 (1H, ddd, H-7, $J_{7,7'}$ 8.0 Hz, $J_{7,6}$ 4.8 Hz, $J_{7,5}$ 1.0 Hz), 4.08 (1H, d, H-7', $J_{7',7}$ 8.0 Hz), 4.58 (1H, t, H-6, J 4.4 Hz), 5.19 (1H, ddd, H-5, $J_{5,4}$ 9.8 Hz, $J_{5,6}$ 4.2 Hz, $J_{5,7}$ 1.0 Hz), 5.20 (1H, d, H-3, $J_{3,4}$ 4.7 Hz), 5.33 (1H, dd, H-4, $J_{4,5}$ 9.8 Hz, $J_{4,3}$ 4.7 Hz); δ_{C} (CDCl_3): 19.9 (q, C-1), 20.5, 20.6, 20.7 (3 x q, 3 x CH_3CO_2), 65.2 (t, C-7), 67.7, 68.8, 71.6, 73.3 (4 x d, C-3, C-4, C-5, C-6), 106.5 (s, C-2), 170.1, 170.3, 170.4 (3 x s, 3 x $\text{C}=\text{O}$); m/z (NH_3 , CI): 320 ($\text{M}+\text{NH}_4^+$, 100%), 303 ($\text{M}+\text{H}^+$, 5%). (Found C, 51.83; H, 6.29. $\text{C}_{13}\text{H}_{18}\text{O}_8$ requires C, 51.65; H, 6.00%).

Method 2.

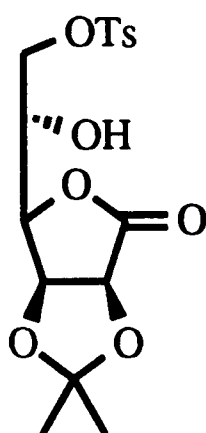
2,7-Anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.1) (1.688 g, 9.6 mmol) was dissolved in dry pyridine (20 ml) at room temperature under nitrogen and acetic anhydride (9 ml) was added. After stirring for 3 h t.l.c. (ethyl acetate) showed conversion of the starting material (R_f 0.2) to a single product (R_f 0.8). The solvents were evaporated and the residue dissolved in diethyl ether (80 ml). The diethyl ether solution was washed with dilute aqueous hydrochloric acid (40 ml) and brine (20 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:1) to give 3,4,5-tri-*O*-acetyl-2,7-anhydro-1-deoxy- β -L-*gulo*-heptulopyranose (2.24) (2.66 mg, 8.8 mmol, 92% yield) as a colourless gum, identical to the material prepared above.

2,3-*O*-Isopropylidene-L-gulono-1,4-lactone (2.25).

2,3;4,5-Di-*O*-isopropylidene-L-gulono-1,4-lactone (2.11) (13.74 g, 53 mmol) was stirred at room temperature in acetic acid (70 ml) and water (30 ml) for 24 h at which time t.l.c. (ethyl acetate:hexane, 2:1) showed no starting material (R_f 0.5) and one product (R_f 0.1). The solvents were evaporated and the residue co-evaporated with toluene (3 x 30 ml). The crude product was purified by flash chromatography (ethyl acetate:hexane, 3:1 then ethyl acetate) to give

2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.25) (9.04 g, 41.5 mmol, 78% yield) as a white crystalline solid. M.p. 141-143 °C (ethyl acetate) (lit.¹⁹ 139-141 °C); $[\alpha]_{\text{D}}^{25} +80.0$ (c, 1.01 in acetone) (lit.¹⁹ $[\alpha]_{\text{D}}^{20} +76.2$ (c, 1.0 in acetone)); δ_{H} (CD₃CN): 1.35, 1.39 (2 x 3H, 2 x s, (CH₃)₂C), 2.97 (1H, t, OH, *J* 5.7 Hz, D₂O exchanges), 3.44 (1H, d, OH, *J* 4.8 Hz, D₂O exchanges), 3.61-3.66 (2H, m), 3.84-3.93 (1H, m), 4.48 (1H, dd, *J* 7.8 Hz, *J* 2.9 Hz), 4.88-4.90 (2H, m).

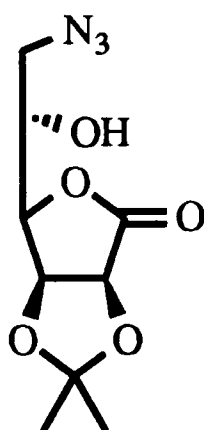
2,3-*O*-Isopropylidene-6-*O*-toluenesulphonyl-L-gulono-1,4-lactone (2.26).



2,3-*O*-Isopropylidene-L-gulono-1,4-lactone (2.25) (1.236 g, 5.7 mmol) was dissolved in dry dichloromethane (20 ml) and pyridine (1.15 ml, 14.2 mmol, 2.5 mol equivalents), 4-(*N,N*-dimethylamino)-pyridine (693 mg, 5.7 mmol, 1 mol equivalent) and *para*-toluenesulphonyl chloride (1.297 g, 6.8 mmol, 1.2 mol equivalents) were added. The reaction mixture was stirred for 5 days at room temperature, at which time t.l.c. (ethyl acetate:hexane, 2:1) showed partial conversion of the starting material (*R_f* 0.1) to one major product (*R_f* 0.5). The reaction mixture was diluted with dichloromethane (20 ml) and washed with dilute aqueous hydrochloric acid (20 ml) and brine (20 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (ethyl acetate:hexane, 1:5 then 1:4 then 1:3 then 1:1 then ethyl acetate) to give 2,3-*O*-isopropylidene-6-*O*-toluenesulphonyl-L-gulono-1,4-lactone (2.26) (905 mg, 2.4 mmol, 43% yield) as a colourless gum.

$[\alpha]_{\text{D}}^{25} +46.8$ (*c*, 1.0 in CHCl_3); ν_{max} (neat): 3500 (OH), 1790 (C=O), 1359 (SO_2) cm^{-1} ; δ_{H} (CDCl_3): 1.39, 1.47 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.48 (3H, s, ArCH_3), 2.90 (1H, d, OH, *J* 3.4 Hz), 4.16-4.38 (3H, m), 4.60 (1H, dd, *J* 5.9 Hz, *J* 3.0 Hz), 4.74-4.91 (2H, m), 7.39 (2H, d, ArCH , *J* 8.2 Hz), 7.83 (2H, d, ArCH , *J* 8.4 Hz); δ_{C} (CDCl_3): 21.6 (q, ArCH_3), 25.6, 26.6 (2 x q, $(\text{CH}_3)_2\text{C}$), 70.0 (t, C-6), 68.9, 75.9, 76.4, 78.6 (4 x d, C-2, C-3, C-4, C-5), 114.4 (s, $(\text{CH}_3)_2\text{C}$), 128.1, 130.0 (2 x d, ArCH), 132.0, 145.4 (2 x s, ArC), 173.6 (s, C=O); *m/z* (NH_3 , CI): 390 ($\text{M}+\text{NH}_4^+$, 66%), 373 ($\text{M}+\text{H}^+$, 6%), 218 ($\text{M}+\text{H}^+-\text{Ts}$, 34%), 178 (100%). (Found C, 51.46; H, 5.16. $\text{C}_{16}\text{H}_{20}\text{O}_8\text{S}$ requires C, 51.61; H, 5.41%).

6-Azido-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.27).



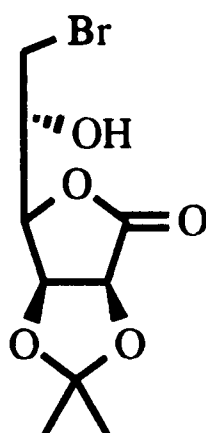
Method 1.

2,3-*O*-Isopropylidene-6-*O*-toluenesulphonyl-L-gulono-1,4-lactone (2.26) (486 mg, 1.31 mmol) was dissolved in *N,N*-dimethylformamide (5 ml) and sodium azide (255 mg, 3.92 mmol, 3 mol equivalents) was added. The reaction was stirred at room temperature for 3 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.2, uv active) and one product (R_f 0.25, not uv active). The solvents were evaporated and the residue partitioned between ethyl acetate (20 ml) and water (15 ml). The aqueous layer was washed with ethyl acetate (15 ml) and then the combined organic layers were washed with brine (15 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was

purified by flash chromatography (diethyl ether:hexane, 2:1, then 4:1, then diethyl ether) to give *6-azido-6-deoxy-2,3-O-isopropylidene-L-gulono-1,4-lactone* (2.27) (111 mg, 0.46 mmol, 35% yield) as a clear oil. $[\alpha]_{\text{D}}^{24} +33.9$ (*c*, 1.0 in CHCl_3); ν_{max} (neat): 3468 (OH), 2106 (N_3), 1790 ($\text{C}=\text{O}$) cm^{-1} ; δ_{H} (CDCl_3): 1.41, 1.49 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.96 (1H, d, OH, *J* 3.0 Hz), 3.53 (1H, dd, H-6, *J*_{6,6'} 13.0 Hz, *J*_{6,5} 4.9 Hz), 3.64 (1H, dd, H-6', *J*_{6',6} 13.0 Hz, *J*_{6',5} 4.1 Hz), 4.19-4.29 (1H, m, H-5), 4.57 (1H, dd, H-4, *J*_{4,5} 6.8 Hz, *J*_{4,3} 3.5 Hz), 4.85 (1H, dd, H-3, *J*_{3,2} 5.4 Hz, *J*_{3,4} 3.5 Hz), 4.91 (1H, d, H-2, *J*_{2,3} 5.4 Hz); δ_{C} (CDCl_3): 25.7, 26.6 (2 x q, $(\text{CH}_3)_2\text{C}$), 52.2 (t, C-6), 70.0, 76.0, 76.5, 79.6 (4 x d, C-2, C-3, C-4, C-5), 114.9 (s, $(\text{CH}_3)_2\text{C}$), 173.7 (s, $\text{C}=\text{O}$); *m/z* (NH_3 , CI): 261 ($\text{M}+\text{NH}_4^+$, 100%). (Found C, 44.45; H, 5.06; N, 17.06. $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_5$ requires C, 44.45; H, 5.39; N, 17.28%).

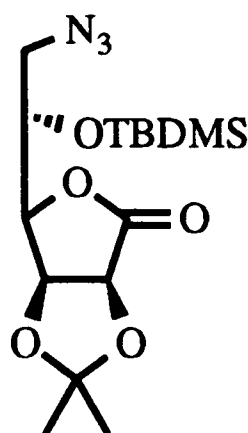
Method 2.

6-Bromo-6-deoxy-2,3-*O*-isopropylidene-*L*-gulono-1,4-lactone (2.28) (1.317 g, 4.7 mmol) was dissolved in *N,N*-dimethylformamide (10 ml) and sodium azide (914 mg, 14 mmol, 3 mol equivalents) was added. The reaction was stirred at 60 °C for 4 h, and then the solvent was evaporated and the residue partitioned between ethyl acetate (30 ml) and water (30 ml). The aqueous layer was washed with ethyl acetate (30 ml) and then the combined organic layers were washed with brine (30 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (ethyl acetate:hexane, 1:3 then 1:1) to give *6-azido-6-deoxy-2,3-O-isopropylidene-L-gulono-1,4-lactone* (2.27) (1.089 g, 4.5 mmol, 95% yield) as a clear oil, identical to the product reported above.

6-Bromo-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.28).

2,3-*O*-Isopropylidene-L-gulono-1,4-lactone (2.25) (1.186g, 5.44 mmol) and carbon tetrabromide (2.17 g, 6.53 mmol, 1.2 mol equivalents) were dissolved in dry tetrahydrofuran (20 ml) and the solution cooled to 0 °C. Triphenylphosphine (1.85 g, 7.07 mmol, 1.3 mol. equivalents) in tetrahydrofuran (2 x 5 ml) was added and the reaction was stirred in the dark under nitrogen. After 2 h, the reaction was warmed to room temperature and stirring was continued for a further 14 h. At this time, t.l.c. (ethyl acetate:hexane, 2:1) showed no starting material (R_f 0.1) and one product (R_f 0.5). The reaction mixture was diluted with diethyl ether (40 ml) and filtered through a Celite plug. The solvents were evaporated and the residue purified by flash chromatography (ethyl acetate:hexane, 1:4 then 1:3 then 1:2 then 1:1) to give 6-bromo-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.28) (1.005 g, 3.6 mmol, 66% yield) as a white solid. M.p. 87.5-88.5 °C (diethyl ether/hexane) (lit.²⁰ 86-87 °C (diethyl ether/hexane)); $[\alpha]_D^{24}$ +51.7 (c , 1.0 in CHCl_3) (lit.²⁰ $[\alpha]_D^{20}$ +52.9 (c , 1.25 in CHCl_3); δ_H (CDCl_3): 1.40, 1.48 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 3.11 (1H, d, OH, J 4.1 Hz), 3.67-3.69 (2H, m, H-6, H-6'), 4.24 (1H, dt, H-5, J 10.9 Hz, J 4.1 Hz), 4.66 (1H, dd, H-3, J 6.7 Hz, J 2.8 Hz), 4.87-4.94 (2H, m, H-2, H-4).

6-Azido-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.29).



Method 1.

6-Azido-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.27) (1.089 g, 4.5 mmol) and imidazole (915 mg, 13.4 mmol, 3 mol equivalents) were dissolved in dry *N,N*-dimethylformamide (2 ml) and *tert*-butyldimethylsilyl chloride (946 mg, 6.3 mmol, 1.4 mol equivalents) in dry *N,N*-dimethylformamide (2 x 3 ml) was added. The reaction mixture was stirred at 60 °C for 4 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.2) and one product (R_f 0.6). The solvents were evaporated and the residue partitioned between ethyl acetate (30 ml) and dilute aqueous hydrochloric acid (30 ml). The aqueous layer was washed with ethyl acetate (30 ml) and the combined organic layers were then washed with brine (30 ml), dried (magnesium sulphate), filtered and evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:4 then 1:2) to give 6-azido-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.29) (1.417 g, 4.0 mmol, 88% yield) as a white solid. M.p. 86-87 °C (hexane); $[\alpha]_D^{24} +22.7$ (c , 1.0 in CHCl_3); ν_{max} (KBr): 2104 (N_3), 1781 ($\text{C}=\text{O}$) cm^{-1} ; δ_{H} (CDCl_3): 0.15, 0.17, (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{Si}$), 0.94 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.38, 1.46 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 3.35 (1H, dd, H-6, $J_{6,6'}$ 13.1 Hz, $J_{6,5}$ 3.1 Hz), 3.58 (1H, dd, H-6', $J_{6',6}$ 13.2 Hz, $J_{6',5}$ 2.9 Hz), 4.18 (1H, dt, H-5, J 8.2 Hz, J 3.0 Hz), 4.55 (1H, dd, H-4, $J_{4,5}$ 8.2 Hz, $J_{4,3}$ 3.3 Hz), 4.74 (1H, dd, H-3, $J_{3,2}$ 5.1 Hz, $J_{3,4}$ 3.4 Hz), 4.83 (1H, d, H-2, $J_{2,3}$ 5.2 Hz); δ_{C} (CDCl_3): -5.2, -4.9 (2 x q, $(\text{CH}_3)_2\text{Si}$), 17.8 (s, $(\text{CH}_3)_3\text{C}$), 25.5 (q, $(\text{CH}_3)_3\text{C}$), 25.8, 26.6 (2 x q,

(CH_3) $_2\text{C}$), 53.5 (t, C-6), 71.6, 75.7, 76.4, 80.7 (4 x d, C-2, C-3, C-4, C-5), 114.3 (s, (CH_3) $_2\text{C}$), 173.6 (s, C=O); m/z (CI, NH_3): 375 ($\text{M}+\text{NH}_4^+$, 76%), 358 ($\text{M}+\text{H}^+$, 6%), 330 ($\text{M}+\text{H}^+-\text{N}_2$, 100%). (Found C, 50.64; H, 7.59; N, 11.63. $\text{C}_{15}\text{H}_{27}\text{N}_3\text{O}_5\text{Si}$ requires C, 50.40; H, 7.61; N, 11.75%).

Method 2.

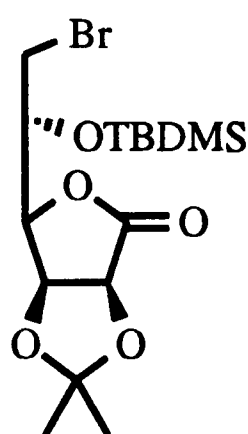
6-Bromo-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.30) (1.164 g, 2.95 mmol) was dissolved in *N,N*-dimethylformamide (10 ml) and sodium azide (575 mg, 8.84 mmol, 3 mol equivalents) was added. The reaction was stirred at 60 °C for 2.5 h at which time t.l.c. (ethyl acetate:hexane, 1:2, eluted twice) showed no starting material (R_f 0.65) and one product (R_f 0.7). The solvents were evaporated and the residue partitioned between ethyl acetate (70 ml) and water (60 ml). The organic layer was washed with brine (60 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:5 then 1:3) to give 6-azido-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.29) (990 mg, 2.77 mmol, 94% yield) as a white solid, identical to the product reported above.

Method 3.

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-6-*O*-methanesulphonyl-L-gulono-1,4-lactone (2.32) (88 mg, 0.21 mmol) was dissolved in *N,N*-dimethylformamide (2 ml) and sodium azide (42 mg, 0.64 mmol, 3 mol equivalents) was added. The reaction was stirred at 60 °C for 6 h at which time t.l.c. (diethyl ether:hexane, 4:1) showed no starting material (R_f 0.2) and one product (R_f 0.5). The solvents were evaporated and the residue partitioned between ethyl acetate (10 ml) and water (10 ml). The organic layer was washed

with brine (10 ml), dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:5 then 1:2) to give 6-azido-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.29) (64 mg, 0.18 mmol, 85% yield) as a white solid, identical to the product reported above.

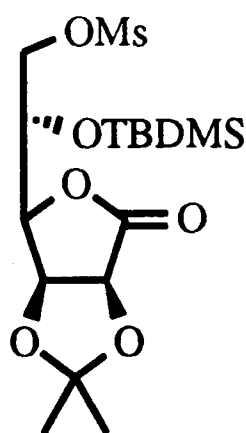
6-Bromo-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.30).



6-Bromo-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.28) (2.62 g, 9.3 mmol) and imidazole (1.90 g, 28 mmol, 3 mol equivalents) were dissolved in dry *N,N*-dimethylformamide (15 ml) and *tert*-butyldimethylsilyl chloride (1.83 g, 12.1 mmol, 1.3 mol equivalents) in dry *N,N*-dimethylformamide (2 x 7 ml) was added. The reaction mixture was stirred at 50 °C for 5 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.2) and one product (R_f 0.5). The solvents were evaporated and the residue partitioned between ethyl acetate (60 ml) and dilute aqueous hydrochloric acid (40 ml). The aqueous layer was washed with ethyl acetate (40 ml) and then the combined organic layers were washed with brine (40 ml), dried (magnesium sulphate), filtered and evaporated. The residue was purified by flash chromatography (diethyl ether:hexane, 1:4 then 1:1, then diethyl ether) to give 6-bromo-5-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.30) (2.96 g, 7.5 mmol, 80% yield) as a white solid. M.p. 138-140 °C (diethyl ether/hexane); $[\alpha]_D^{24} +60.7$ (c, 1.0 in

CHCl₃); $\nu_{\max}$ (KBr): 1789 (C=O) cm⁻¹; δ_{H} (CDCl₃): 0.13, 0.15 (2 x 3H, 2 x s, (CH₃)₂Si), 0.93 (9H, s, (CH₃)₃C), 1.40, 1.47 (2 x 3H, 2 x s, (CH₃)₂C), 3.52 (1H, dd, H-6, $J_{6,6'}$ 11.2 Hz, $J_{6,5}$ 2.9 Hz), 3.74 (1H, dd, H-6', $J_{6',6}$ 11.3 Hz, $J_{6',5}$ 3.0 Hz), 4.14 (1H, dt, H-5, J 7.9 Hz, J 2.9 Hz), 4.54 (1H, dd, J 8.0 Hz, J 2.6 Hz), 4.80-4.86 (2H, m); δ_{C} (CDCl₃): -4.9, -4.6 (2 x q, (CH₃)₂Si), 18.1 (s, (CH₃)₃C), 25.7 (q, (CH₃)₃C), 26.0, 26.8 (2 x q, (CH₃)₂C), 35.4 (t, C-6), 69.9, 75.4, 76.4, 81.9 (4 x d, C-2, C-3, C-4, C-5), 114.3 (s, (CH₃)₂C), 173.4 (s, C=O); m/z (NH₃, CI): 414 (M+NH₄⁺, 30%), 412 (M+NH₄⁺, 35%), 397 (M+H⁺, 28%), 395 (M+H⁺, 29%), 129 (100%). (Found C, 45.68; H, 7.16. C₁₅H₂₇BrO₅Si requires C, 45.57; H, 6.88%).

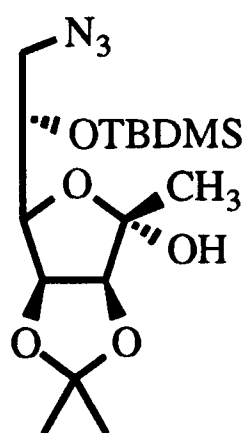
5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-6-*O*-methanesulphonyl-L-gulono-1,4-lactone (2.32).



5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (2.31) (143 mg, 0.43 mmol) and 4-(*N,N*-dimethylamino)-pyridine (5 mg, 0.04 mmol, 0.1 mol equivalents) were dissolved in dry pyridine (2 ml) and the solution was cooled to 0 °C. Methanesulphonyl chloride (50 μ l, 0.65 mmol, 1.5 mol equivalents) was added and the reaction mixture was stirred at 0 °C for 45 min at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.4) and one product (R_f 0.35). The solvent was evaporated and the residue partitioned between ethyl acetate (15 ml) and dilute aqueous hydrochloric acid (10 ml). The organic layer was washed with brine (10 ml), dried (magnesium sulphate), filtered

and evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:1 then diethyl ether) to give *5-O-tert-butyltrimethylsilyl-2,3-O-isopropylidene-6-O-methanesulphonyl-L-gulono-1,4-lactone* (2.32) (134 mg, 0.33 mmol, 76% yield) as a white solid. M.p. 131-132 °C (diethyl ether/hexane); $[\alpha]_D^{24} +40.0$ (c, 1.0 in CHCl₃); $\nu_{\max}$ (KBr): 1775 (C=O), 1360 (SO₂) cm⁻¹; δ_H (CDCl₃, 500 MHz): 0.15, 0.17 (2 x 3H, 2 x s, (CH₃)₂Si), 0.93 (9H, s, (CH₃)₃C), 1.41, 1.48 (2 x 3H, 2 x s, (CH₃)₂C), 3.10 (3H, s, CH₃S), 4.23 (1H, dt, H-5, *J* 8.2 Hz, *J* 2.9 Hz), 4.40 (1H, dd, *J* 11.1 Hz, *J* 2.5 Hz), 4.48-4.51 (2H, m), 4.83-4.85 (2H, m); δ_C (CDCl₃): -4.9, -4.8 (2 x q, (CH₃)₂Si), 18.1 (s, (CH₃)₃C), 25.7 (q, (CH₃)₃C), 26.0, 26.8 (2 x q, (CH₃)₂C), 37.6 (q, CH₃S), 70.8 (t, C-6), 70.1, 75.4, 76.4, 79.6 (4 x d, C-2, C-3, C-4, C-5), 114.3 (s, (CH₃)₂C), 173.2 (s, C=O); *m/z* (NH₃, CI): 428 (M+NH₄⁺, 100%), 411 (M+H⁺, 8%), 315 (53%). (Found C, 46.58; H, 7.26. C₁₆H₃₀O₈SSi requires C, 46.81; H, 7.37%).

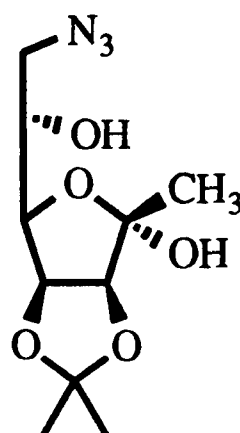
7-Azido-6-O-tert-butyltrimethylsilyl-1,7-dideoxy-3,4-O-isopropylidene-β-L-gulo-heptulofuranose (2.33).



6-Azido-5-O-tert-butyltrimethylsilyl-6-deoxy-2,3-O-isopropylidene-L-gulono-1,4-lactone (2.29) (918 mg, 2.57 mmol) was dissolved in dry tetrahydrofuran (10 ml) and the solution cooled to -70 °C. Methyllithium (1.4 M in diethyl ether, 2.2 ml, 3.08 mmol, 1.2 mol equivalents) was added and the reaction mixture was stirred under nitrogen for 5 min by which time t.l.c. (diethyl ether) showed complete conversion of the starting material (*R_f* 0.5) to a single product (*R_f* 0.6).

The reaction was quenched by the addition of saturated aqueous ammonium chloride solution and the solvents were evaporated. The crude residue was partitioned between brine (40 ml) and diethyl ether (40 ml). The aqueous layer was washed with a further portion of diethyl ether (40 ml). The organic extracts were combined, dried (magnesium sulphate), filtered and the solvent evaporated. The crude product was purified by flash chromatography (diethyl ether:hexane, 1:5 then 1:3 then 1:1) to give *7-azido-6-O-tert-butyltrimethylsilyl-1,7-dideoxy-3,4-O-isopropylidene-β-L-gulo-heptulofuranose* (2.33) (855 mg, 2.29 mmol, 89% yield) as a colourless oil. $[\alpha]_{\text{D}}^{21}$ -3.2 (*c*, 2.7 in CHCl_3); ν_{max} (neat): 3436 (OH), 2103 (N_3) cm^{-1} ; δ_{H} (CDCl_3): 0.12 (6H, s, $(\text{CH}_3)_2\text{Si}$), 0.93 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.29, 1.43, 1.50 (3 x 3H, 3 x s, 3 x CH_3), 3.25 (1H, dd, H-7, $J_{7,7'}$ 12.7 Hz, $J_{7,6}$ 3.7 Hz), 3.52 (1H, dd, H-7', $J_{7',7}$ 12.8 Hz, $J_{7',6}$ 2.1 Hz), 4.04-4.16 (2H, m, H-5, H-6), 4.46 (1H, d, H-3, $J_{3,4}$ 6.0 Hz), 4.70 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.2 Hz); δ_{C} (CDCl_3): -5.2, -4.5 (2 x q, $(\text{CH}_3)_2\text{Si}$), 18.4 (s, $(\text{CH}_3)_3\text{C}$), 25.7 (q, $(\text{CH}_3)_3\text{C}$), 22.4, 24.7, 26.1 (3 x q, C-1, $(\text{CH}_3)_2\text{C}$), 54.2 (t, C-7), 71.7, 80.1, 80.4, 85.9 (4 x d, C-3, C-4, C-5, C-6), 104.8 (s, C-2), 112.7 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 373 ($\text{M}+\text{NH}_4^+-\text{H}_2\text{O}$, 5%), 346 ($\text{M}+\text{NH}_4^+-\text{H}_2\text{O}-\text{N}_2$, 27%), 328 ($\text{M}+\text{H}^+-\text{H}_2\text{O}-\text{N}_2$, 100%). (Found C, 51.51; H, 8.11; N, 11.15. $\text{C}_{16}\text{H}_{31}\text{N}_3\text{O}_5\text{Si}$ requires C, 51.45; H, 8.37; N, 11.25%).

7-Azido-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.34).



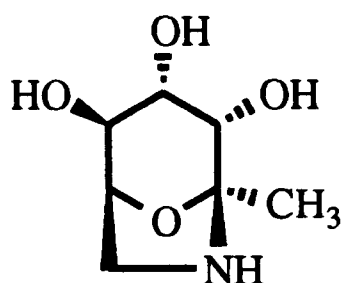
Method 1

7-Azido-6-*O*-*tert*-butyldimethylsilyl-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.33) (1.274 g, 3.4 mmol) was dissolved in dry tetrahydrofuran (15 ml) under nitrogen at room temperature and tetra-*n*-butylammonium fluoride (1.0 M in tetrahydrofuran, 5.1 ml, 5.1 mmol, 1.5 mol equivalents) was added. After stirring for 1.5 h, t.l.c. (ethyl acetate:hexane, 1:2) showed a trace of starting material (R_f 0.6) and one product (R_f 0.2). The reaction mixture was preabsorbed onto silica and purified by flash chromatography (ethyl acetate:hexane, 1:5 then 1:2 then 1:1 then 2:1) to give 7-azido-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.34) (798 mg, 3.1 mmol, 91% yield) as a white crystalline solid. M.p. 91-93 °C (diethyl ether/hexane); $[\alpha]_D^{21} +4.4$ (c , 1.0 in CHCl_3); ν_{max} (KBr): 3350 (OH), 2099 (N_3) cm^{-1} ; δ_{H} (CDCl_3): 1.32, 1.48, 1.56 (3 x 3H, 3 x s, 3 x CH_3), 3.47 (1H, dd, H-7, $J_{7,7'}$ 12.5 Hz, $J_{7,6}$ 5.6 Hz), 3.49 (1H, dd, H-7', $J_{7',7}$ 12.7 Hz, $J_{7',6}$ 3.5 Hz), 4.08-4.20 (2H, m, H-5, H-6), 4.49 (1H, d, H-3, $J_{3,4}$ 6.0 Hz), 4.80 (1H, dd, H-4, $J_{4,3}$ 5.8 Hz, $J_{4,5}$ 3.5 Hz); δ_{C} (CDCl_3): 22.2, 24.5, 25.9 (3 x q, C-1, $(\text{CH}_3)_2\text{C}$), 53.1 (t, C-7), 70.0, 79.0, 80.6, 85.7 (4 x d, C-3, C-4, C-5, C-6), 105.0 (s, C-2), 112.8 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 277 ($\text{M}+\text{NH}_4^+$, 10%), 259 ($\text{M}+\text{NH}_4^+-\text{H}_2\text{O}$, 22%), 216 (100%). (Found C, 46.39; H, 6.48; N, 15.92. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_5$ requires C, 46.33; H, 6.61; N, 16.21%).

Method 2

7-Azido-6-*O*-*tert*-butyldimethylsilyl-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.33) (116 mg, 0.31 mmol) was stirred at room temperature in acetic acid (1.4 ml) and water (0.6 ml) for 48 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.6) and one product (R_f 0.2). The solvents were evaporated and the residue co-evaporated with toluene (3 x 1 ml). The crude product was purified by flash chromatography (ethyl acetate:hexane, 1:4 then 1:2) to give 7-azido-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.34) (58 mg, 0.22 mmol, 72% yield) as a white crystalline solid, identical to the material reported above.

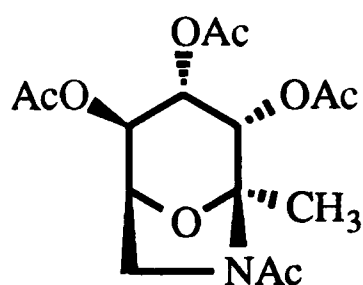
1,2,7-Trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.4).



7-Azido-1,7-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (2.34) (439 mg, 1.7 mmol) was stirred with Dowex 50W-X8(H) ion exchange resin (*ca.* 1 ml) in water (5 ml) at room temperature for 48 h. At this time t.l.c. (ethyl acetate) showed no starting material (R_f 0.7) and one product (R_f 0.1). The solution was filtered to remove the resin and the filtrate was stirred vigorously with palladium black (catalytic amount) under an atmosphere of hydrogen. After 6 h t.l.c. (ethyl acetate) showed no starting material (R_f 0.2) and a baseline spot (ninhydrin active). The reaction mixture was filtered through a Celite plug, eluting with water. The solution was freeze dried to give a crude product which was purified by ion exchange chromatography (Dowex 50W-X8(H)) resin, eluted with water

then 0.5 M aqueous ammonia) to give *1,2,7-trideoxy-2,7-imino-β-L-gulo-heptulopyranose* (2.4) (254 mg, 1.45 mmol, 85% yield) as a pale brown, highly hygroscopic foam. $[\alpha]_{\text{D}}^{21}$ -59.0 (*c*, 1.1 in H₂O); ν_{max} (KBr): 3402 (OH, NH) cm⁻¹; δ_{H} (D₂O, 500 MHz, pH 9): 1.51 (3H, s, CH₃), 2.95 (1H, d, H-7, $J_{7,7'}$ 10.8 Hz), 3.10 (1H, dd, H-7', $J_{7',7}$ 10.7 Hz, $J_{7',6}$ 6.5 Hz), 3.60 (1H, d, H-3, $J_{3,4}$ 4.3 Hz), 3.65 (1H, dd, H-4, $J_{4,5}$ 9.3 Hz, $J_{4,3}$ 4.4 Hz), 3.77 (1H, dd, H-5, $J_{5,4}$ 9.4 Hz, $J_{5,6}$ 3.9 Hz), 4.38 (1H, dd, H-6, $J_{6,7'}$ 6.2 Hz, $J_{6,5}$ 4.0 Hz); δ_{C} (D₂O, 125 MHz, pH 9): 20.7 (q, C-1), 44.8 (t, C-7), 70.7, 70.8, 75.1, 76.5 (4 x d, C-3, C-4, C-5, C-6), 96.0 (s, C-2); m/z (NH₃, CI): 176 (M+H⁺, 100%).

N-Acetyl-3,4,5-tri-*O*-acetyl-1,2,7-trideoxy-2,7-imino-β-*L*-gulo-heptulopyranose (2.36).



Method 1.

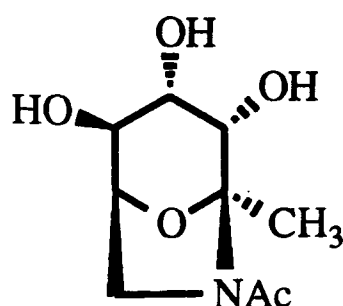
1,2,7-Trideoxy-2,7-imino-β-L-gulo-heptulopyranose (2.4) (94 mg, 0.54 mmol) was dissolved in dry pyridine (1 ml) at room temperature and acetic anhydride (0.25 ml) was added. After stirring for 3.5 h t.l.c. (ethyl acetate) showed conversion of the starting material (baseline) to a single product (R_f 0.3). The solvents were evaporated and the residue partitioned between ethyl acetate (10 ml) and dilute aqueous hydrochloric acid (10 ml). The aqueous phase was washed with ethyl acetate (10 ml) and the combined organic phases were dried (magnesium sulphate), filtered and evaporated. The crude product was purified by flash chromatography (ethyl acetate:hexane, 4:1 then ethyl acetate) to give *N-acetyl-3,4,5-tri-O-acetyl-1,2,7-trideoxy-2,7-imino-β-L-gulo-heptulopyranose* (2.36) (147 mg, 0.43 mmol, 79% yield) as a white solid. M.p. 134-135 °C;

$[\alpha]_{\text{D}}^{24}$ -63.2 (*c*, 1.1 in CHCl_3); ν_{max} (KBr): 1751, 1662 ($\text{C}=\text{O}$) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz + COSY): 1.69 (3H, s, C-1), 1.97, 2.06, 2.14, 2.16 (4 x 3H, 4 x s, 3 x CH_3CO_2 , CH_3CON), 3.64 (1H, d, H-7, $J_{7,7'}$ 9.4 Hz), 3.68 (1H, dd, H-7', $J_{7,7}$ 9.4 Hz, $J_{7',6}$ 5.4 Hz), 4.56 (1H, t, H-6, J 4.8 Hz), 5.16 (1H, dd, H-4, $J_{4,5}$ 9.8 Hz, $J_{4,3}$ 4.6 Hz), 5.23 (1H, dd, H-5, $J_{5,4}$ 9.8 Hz, $J_{5,6}$ 4.4 Hz), 5.64 (1H, d, H-3, $J_{3,4}$ 4.6 Hz); δ_{C} (CDCl_3): 19.1, 20.5, 20.6, 20.7, 20.8 (5 x q, C-1, 3 x CH_3CO_2 , CH_3CON), 46.8 (t, C-7), 67.2, 68.8, 69.8, 71.9 (4 x d, C-3, C-4, C-5, C-6), 93.2 (s, C-2), 167.7, 169.4, 169.8 (3 x s, 4 x $\text{C}=\text{O}$); m/z (NH_3 , CI): 361 ($\text{M}+\text{NH}_4^+$, 100%), 344 ($\text{M}+\text{H}^+$, 90%). (Found C, 52.46; H, 5.98; N, 4.29. $\text{C}_{15}\text{H}_{21}\text{NO}_8$ requires C, 52.48; H, 6.17; N, 4.08%).

Method 2.

1,2,7-Trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.4) (43 mg, 0.25 mmol) was dissolved in acetic acid (1 ml) at room temperature under nitrogen and acetic anhydride (1 ml) and concentrated sulphuric acid (1 drop) were added. After stirring for 30 h t.l.c. (ethyl acetate) showed conversion of the starting material (baseline) to a single product (R_f 0.3). The solvents were evaporated and the residue was co-evaporated with toluene (3 x 1 ml). The crude product was purified by flash chromatography (ethyl acetate) to give *N*-acetyl-3,4,5-tri-*O*-acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.36) (39 mg, 0.11 mmol, 46% yield) as a white solid, identical to the material prepared above.

N-Acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.37).



Method 1.

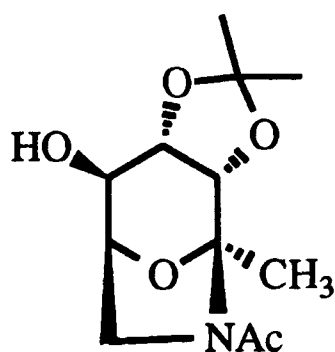
1,2,7-Trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.4) (88 mg, 0.5 mmol) was dissolved in dry methanol (2 ml) at room temperature and acetic anhydride (71 μ l, 0.75 mmol, 1.5 mol equivalents) was added. After stirring for 0.75 h t.l.c. (methanol:dichloromethane, 1:2) showed conversion of the starting material (R_f 0.2) to a single product (R_f 0.5). The solvents were evaporated and the crude residue purified by flash chromatography (methanol:ethyl acetate, 1:10) to give *N*-acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.37) (83 mg, 0.38 mmol, 76% yield) as a white solid. M.p. 178-180 $^{\circ}$ C; $[\alpha]_D^{24}$ -73.5 (c , 1.15 in EtOH); $\nu_{\max}$ (KBr): 3369 (OH), 1641, 1620 (C=O) cm^{-1} ; δ_H (D_2O , 500 MHz + COSY): 1.65 (3H, s, CH_3), 2.07 (3H, s, CH_3CON), 3.55 (1H, dd, H-4, $J_{4,5}$ 9.4 Hz, $J_{4,3}$ 4.6 Hz), 3.64 (1H, d, H-7, $J_{7,7'}$ 10.2 Hz), 3.69 (1H, dd, H-7', $J_{7',7}$ 10.1 Hz, $J_{7',6}$ 5.5 Hz), 3.83 (1H, dd, H-4, $J_{5,4}$ 9.5 Hz, $J_{5,6}$ 4.5 Hz), 4.02 (1H, d, H-3, $J_{3,4}$ 4.6 Hz), 4.41 (1H, t, H-6, J 5.1 Hz); δ_H (d_6 -DMSO, 500 MHz): 1.59 (3H, s, CH_3), 1.96 (3H, s, CH_3CON), 3.27 (1H, ddd, H-4 or H-5, J 9.1 Hz, J_{OH} 6.9 Hz, J 4.6 Hz), 3.45 (1H, dd, H-7, $J_{7,7'}$ 9.4 Hz, $J_{7,6}$ 5.7 Hz), 3.51 (1H, d, H-7', $J_{7',7}$ 9.4 Hz), 3.58 (1H, dt, H-4 or H-5, J 9.3 Hz, J 4.7 Hz, J_{OH} 4.7 Hz), 3.80 (1H, dd, H-3, $J_{3,\text{OH}}$ 5.8 Hz, $J_{3,4}$ 4.9 Hz), 4.14 (1H, t, H-6, J 5.1 Hz), 4.44 (1H, d, OH-4 or OH-5, J 7.0 Hz, D_2O exchanges), 4.70 (1H, d, OH-3, $J_{\text{OH},3}$ 6.1 Hz, D_2O exchanges), 5.06 (1H, d, OH-4 or OH-5, J 5.2 Hz, D_2O exchanges); δ_C (D_2O): 20.0 (q, C-1), 24.1 (q, CH_3CON), 47.6 (t, C-7), 70.1, 71.0, 71.8, 74.5 (4 x d, C-3, C-4, C-5, C-6), 96.2 (s, C-2), 172.9 (s, C=O); m/z (NH_3 , CI): 218 ($\text{M}+\text{H}^+$, 100%).

(Found C, 50.00; H, 6.97; N, 6.24. $C_9H_{15}NO_5$ requires C, 49.76; H, 6.96; N, 6.45%).

Method 2.

N-Acetyl-3,4,5-tri-*O*-acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.36) (60 mg, 0.17 mmol) was dissolved in dry methanol (2 ml) and triethylamine (24 μ l, 0.17 mmol, 1 mol equivalent) was added. The reaction was stirred at room temperature for 20 h at which time t.l.c. (ethyl acetate) showed no starting material (R_f 0.3) and one product (baseline). The solvents were evaporated to give *N*-acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.37) (36 mg, 0.17 mmol, quantitative crude yield) as a white solid, identical to the material reported above.

N-Acetyl-1,2,7-trideoxy-2,7-imino-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.38).



N-Acetyl-1,2,7-trideoxy-2,7-imino- β -L-*gulo*-heptulopyranose (2.37) (72 mg, 0.33 mmol) and a catalytic amount of DL-10-camphorsulphonic acid were stirred in acetone (4 ml) at room temperature. After 24 h, t.l.c. (methanol:ethyl acetate, 1:10) showed no starting material (R_f 0.1) and one product (R_f 0.3). The reaction was quenched by the addition of solid sodium hydrogen carbonate and the solvents were evaporated. The crude product was purified by column chromatography (ethyl acetate) to give *N*-acetyl-1,2,7-trideoxy-2,7-imino-3,4-*O*-

isopropylidene-β-L-gulo-heptulopyranose (2.38) (73 mg, 0.28 mmol, 86% yield) as a white solid. M.p. 134-136 °C (diethyl ether/hexane); $[\alpha]_{\text{D}}^{22}$ -108.3 (c, 1.0 in CHCl₃); ν_{max} (KBr): 3849 (OH), 1668 (C=O) cm⁻¹; δ_{H} (CDCl₃, 500 MHz): 1.32, 1.52 (2 x 3H, 2 x s, (CH₃)₂C), 1.84 (3H, s, C-1), 2.08 (3H, s, CH₃CON), 2.98 (1H, br s, OH), 3.58 (1H, dd, *J* 9.4 Hz, *J* 5.7 Hz), 3.61 (1H, dd, *J* 9.3 Hz, *J* 1.0 Hz), 3.96-3.99 (2H, m), 4.41-4.42 (1H, m), 4.51-4.54 (1H, m); δ_{C} (CDCl₃): 19.5, 23.9, 26.3, 28.0 (4 x q, C-1, (CH₃)₂C, CH₃CON), 45.6 (t, C-7), 71.1, 74.3, 75.6, 78.8 (4 x d, C-3, C-4, C-5, C-6), 92.5 (s, C-2), 112.0 (s, (CH₃)₂C), 172.9 (s, C=O); *m/z* (NH₃, CI): 258 (M+H⁺, 100%). (Found C, 55.93; H, 7.67; N, 5.39. C₁₂H₁₉NO₅ requires C, 56.02; H, 7.44; N, 5.44%).

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22. This compound was kindly supplied by David Hyett; see David Hyett, Part II Thesis, Oxford University, 1994.
23. K. Onodera and S. Kitaoka, *J. Org. Chem.*, **1960**, *25*, 1332; J. E. Hodge and B. F. Moy, *J. Org. Chem.*, **1963**, *28*, 2784.
24. The enzyme inhibition studies were performed by Dr. B. G. Winchester, Institute of Child Health, University of London, 31 Guilford Street, London.

Chapter 3

The Synthesis of a Novel Bicyclic Mimic of α -L-Homofuconojirimycin.

3.1 Introduction.

The synthesis of two bicyclic mimics of α -L-fucose (1.1), (2.1)¹ and (2.4), has been reported in chapter 2. Both compounds were found to be stable (Figure 1).

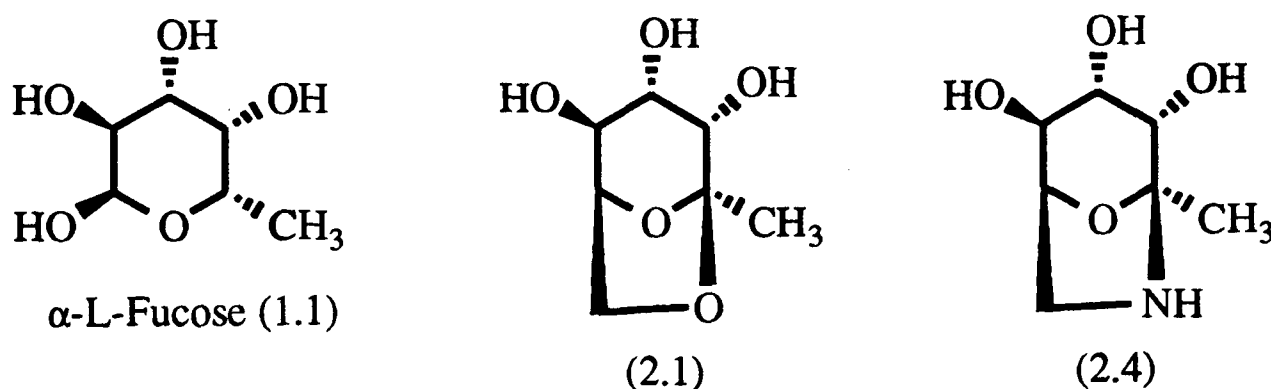


Figure 1

A third bicyclic system (3.1) can be viewed as a mimic of both α -L-fucose (1.1) and of the known fucosidase inhibitor α -L-homofuconojirimycin (1.6)² (Figure 2). It was envisaged that such a framework would be accessible via the literature route to α -L-homofuconojirimycin (1.6) itself.^{2,3}

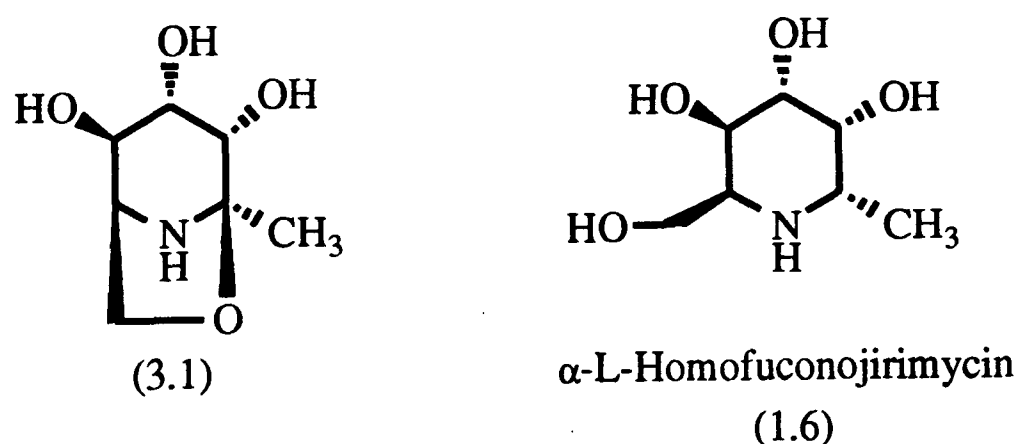
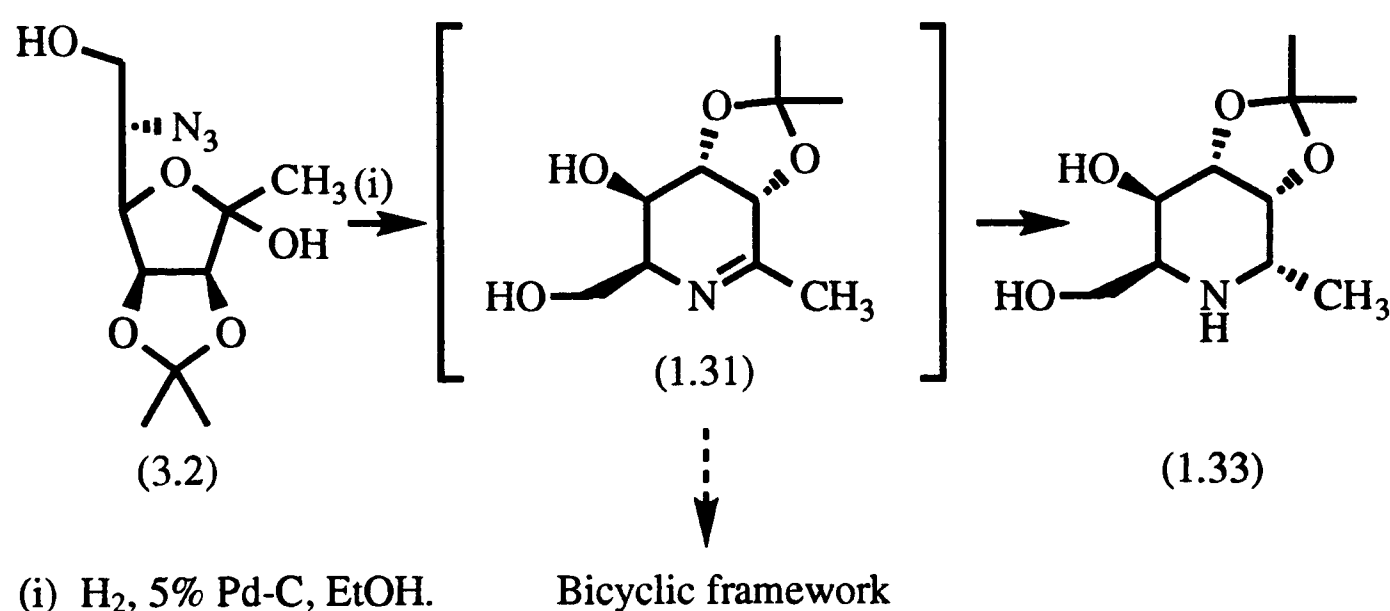


Figure 2

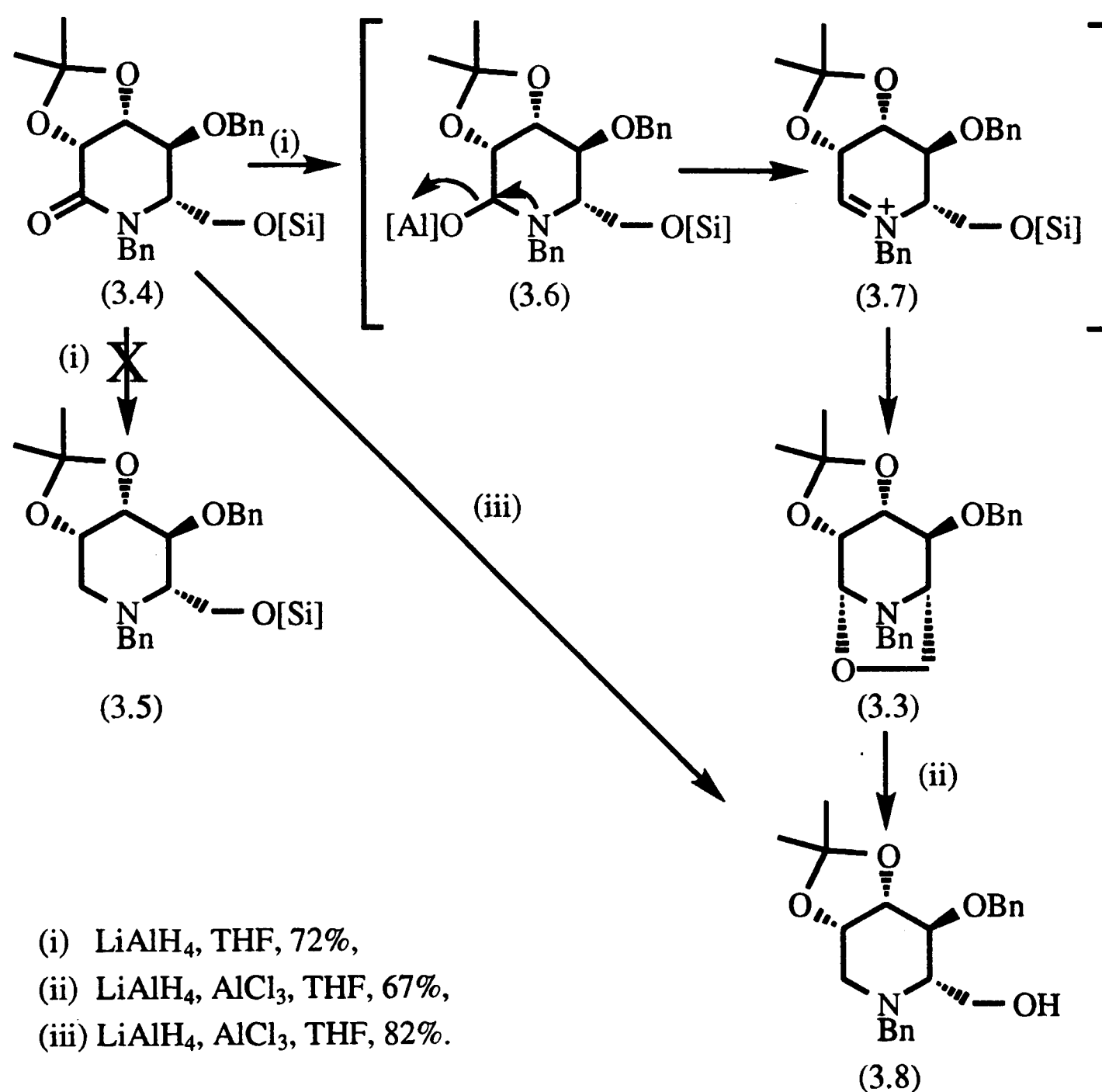
The target system (3.1) is at the imine oxidation level. The catalytic hydrogenation of the azide (3.2) proceeded via the imine (1.31) to give the protected α -L-homofuconojirimycin derivative (1.33).² If this reduction could be stopped at the intermediate stage, the imine (1.31) might form the desired bicyclic framework (Scheme 1). Such imines are not usually isolated from reductive aminations,⁴ but there have been reports of cases where it was possible to stop the reductions at the imine stage.⁵



Scheme 1

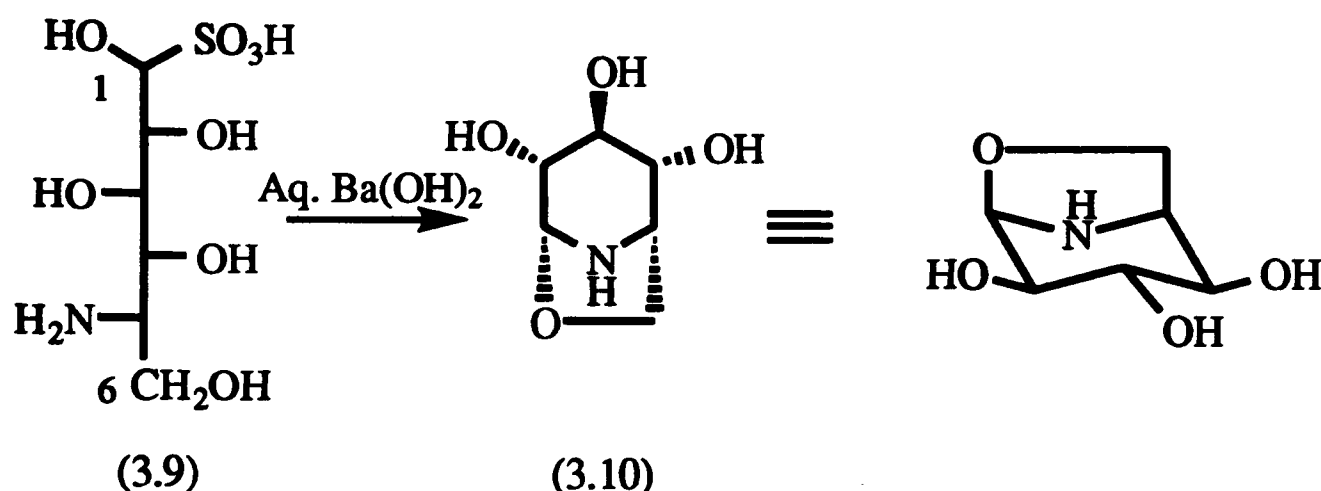
A similar bicyclic hemiaminal (3.3) with L-mannose stereochemistry has been reported in the literature and was found to be stable.⁶ An attempted reduction of the lactam (3.4) to the tertiary amine (3.5) using lithium aluminium hydride gave the bicyclic structure (3.3) rather than the expected product (3.5) (Scheme 2). It was postulated that the reaction proceeded via hydride addition to the carbonyl group of the lactam (3.4) to give a tetrahedral adduct (3.6) which underwent fragmentation to give an iminium ion (3.7), or its equivalent. This was then trapped intramolecularly by the oxygen at the primary position, resulting in the observed product (3.3). Treatment of either the bicyclic system (3.3) or the

lactam (3.4) with lithium aluminium hydride in the presence of aluminium trichloride furnished the desired tertiary amine (3.8)



Scheme 2

5-Amino-5-deoxy-L-idose-1-sulphonic acid (3.9) formed a similar bicyclic anhydro structure (3.10) under alkaline conditions.⁷ In this case, the ease of cyclisation was attributed to the fact that all the hydroxyl groups can adopt an equatorial configuration in the bicyclic system (3.10) (Scheme 3).



Scheme 3

A number of five-ring sugar imines have been reported in the literature. Their behaviour in aqueous media seems to depend on their structure. Although none has been reported to form an intramolecular hemiaminal, some show a tendency to hydrate or to dimerise in solution. Nectrisine (3.11) was isolated from a fungus, *Nectria lucida* F-4490, and its structure was confirmed by a stereocontrolled synthesis from D-glucose.⁸ It is a potent inhibitor of α -glucosidase and α -mannosidase, and also acts as an immunomodulator (Figure 3).⁹

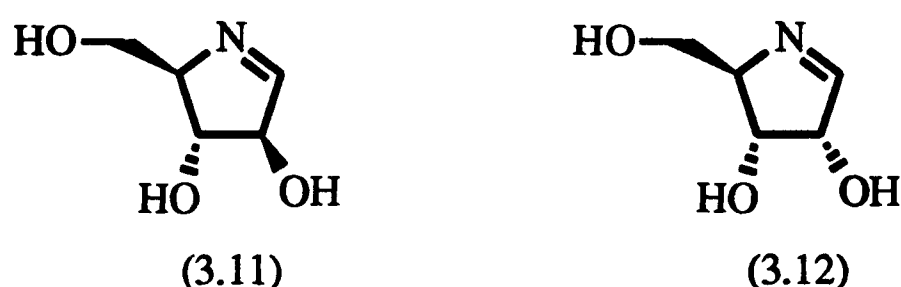
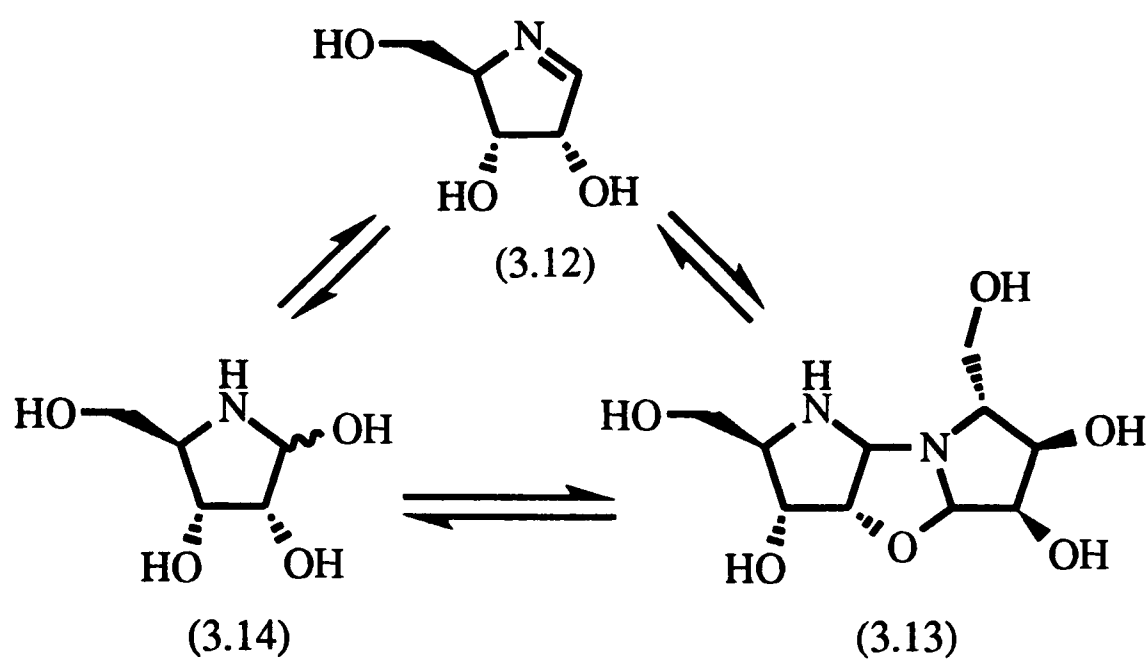


Figure 3

The epimeric compound (3.12), a potent α -glucosidase inhibitor, has been synthesised and it is reported to exist in solution as one component of a complex equilibrium mixture which includes the dimerised (3.13) and hydrated (3.14) forms (Scheme 4).¹⁰ This is in contrast to nectrisine (3.11) itself which appears to exist solely as the imino form.



Scheme 4

Both 4-amino-4-deoxy-glucose (3.15) and 4-amino-4-deoxy-galactose (3.16) have also been reported to exist in equilibrium mixtures which include the imines, the hydrated and the dimerised forms (Figure 4).¹¹

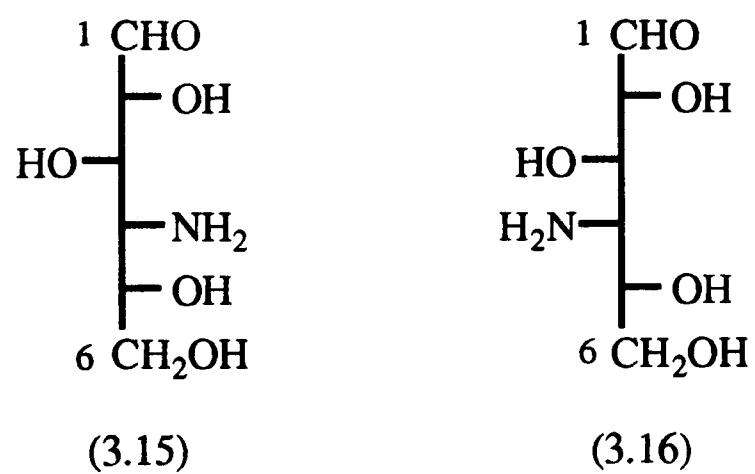
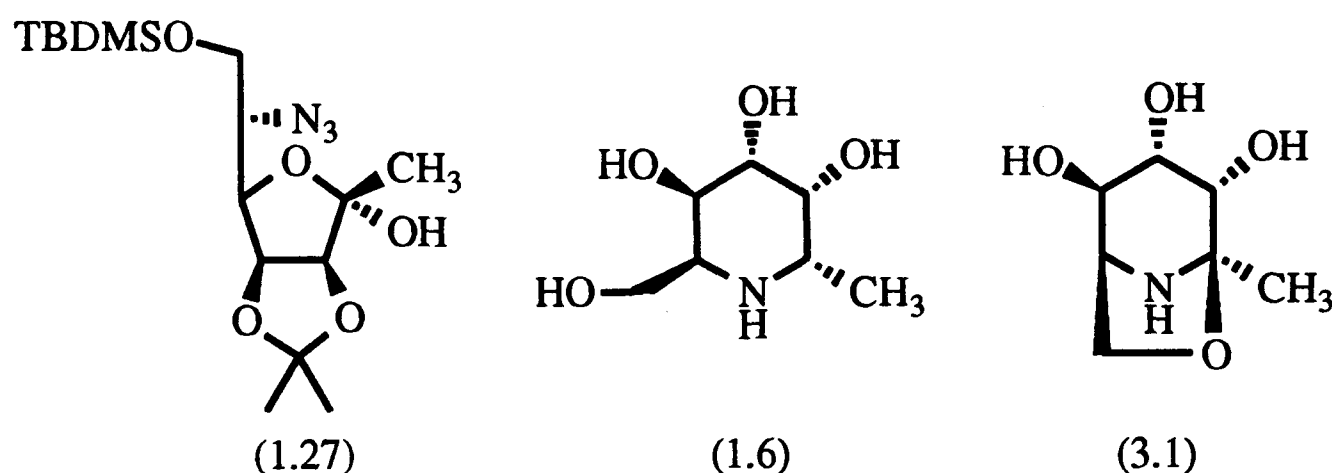


Figure 4

3.2 Results and Discussion.

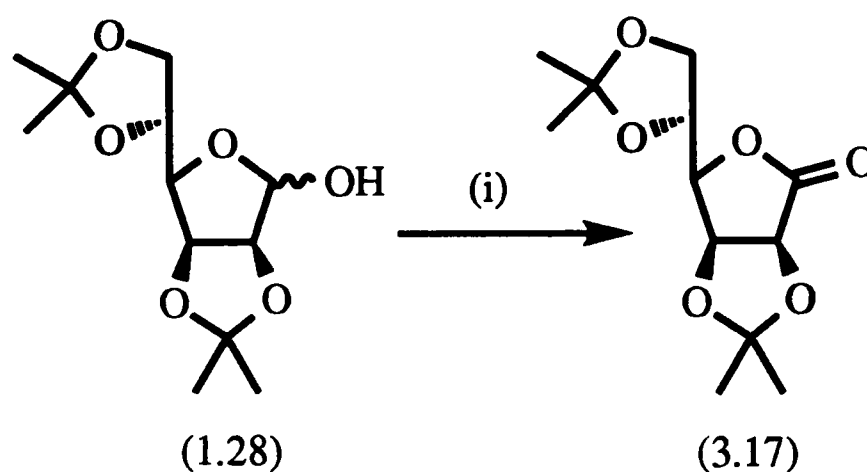
The work will be discussed in the following four parts:

- i. The synthesis of 6-azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27).
- ii. The synthesis of α -L-homofuconojirimycin (1.6).
- iii. The synthesis of the target system (3.1).
- iv. The evaluation of α -L-homofuconojirimycin (1.6) and the target system (3.1) as glycosidase inhibitors.



i. The synthesis of 6-azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27).

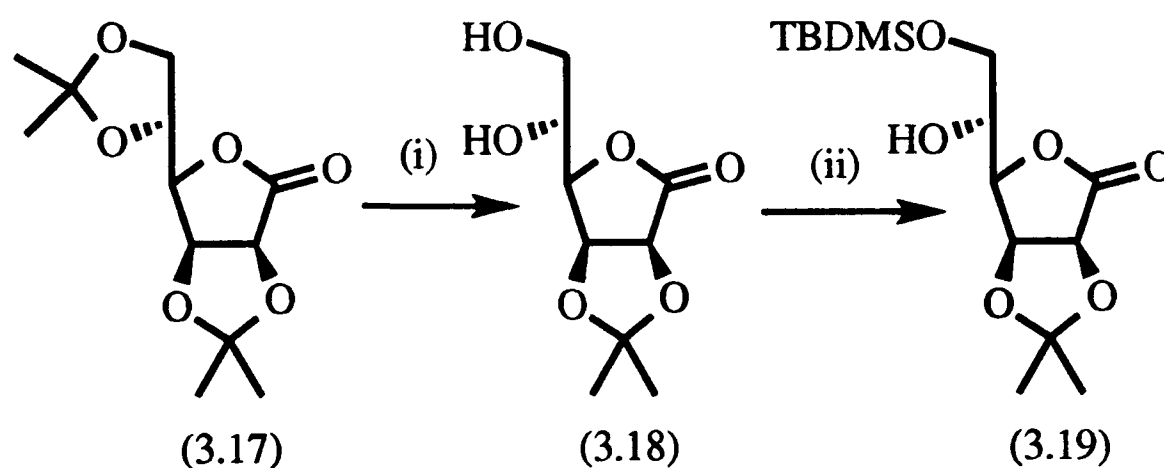
6-Azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27) was synthesised following the literature route.³ The starting material was the readily available diacetone of D-mannose (1.28). This was oxidised to the lactone (3.17)¹² in an 88% yield using pyridinium chlorochromate in the presence of molecular sieves (Scheme 5). This reaction can easily be performed on a 25 g scale and the product purified by recrystallisation.



(i) PCC, DCM, molecular sieves, 88%.

Scheme 5

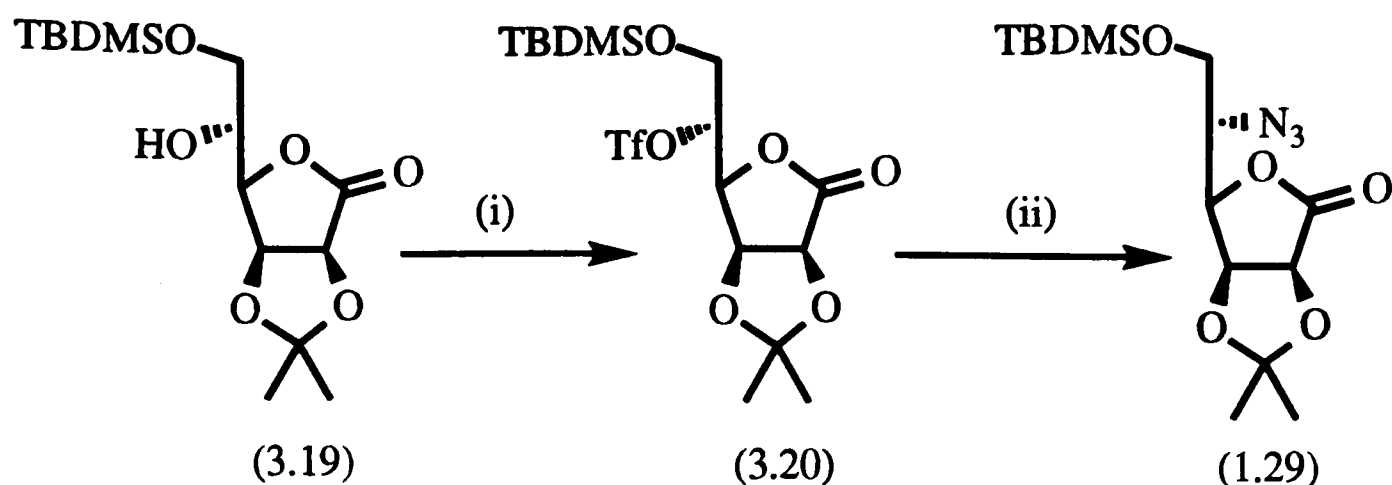
Selective removal of the primary acetonide on hydrolysis in aqueous acetic acid gave the diol (3.18).¹³ The primary hydroxyl group was protected as its silyl ether (3.19) on treatment with *tert*-butyldimethylsilyl chloride and imidazole in *N,N*-dimethylformamide at -10 °C (Scheme 6).



(i) AcOH, H₂O, 77%, (ii) TBDMS-Cl, imidazole, DMF, -10 °C, 87%.

Scheme 6

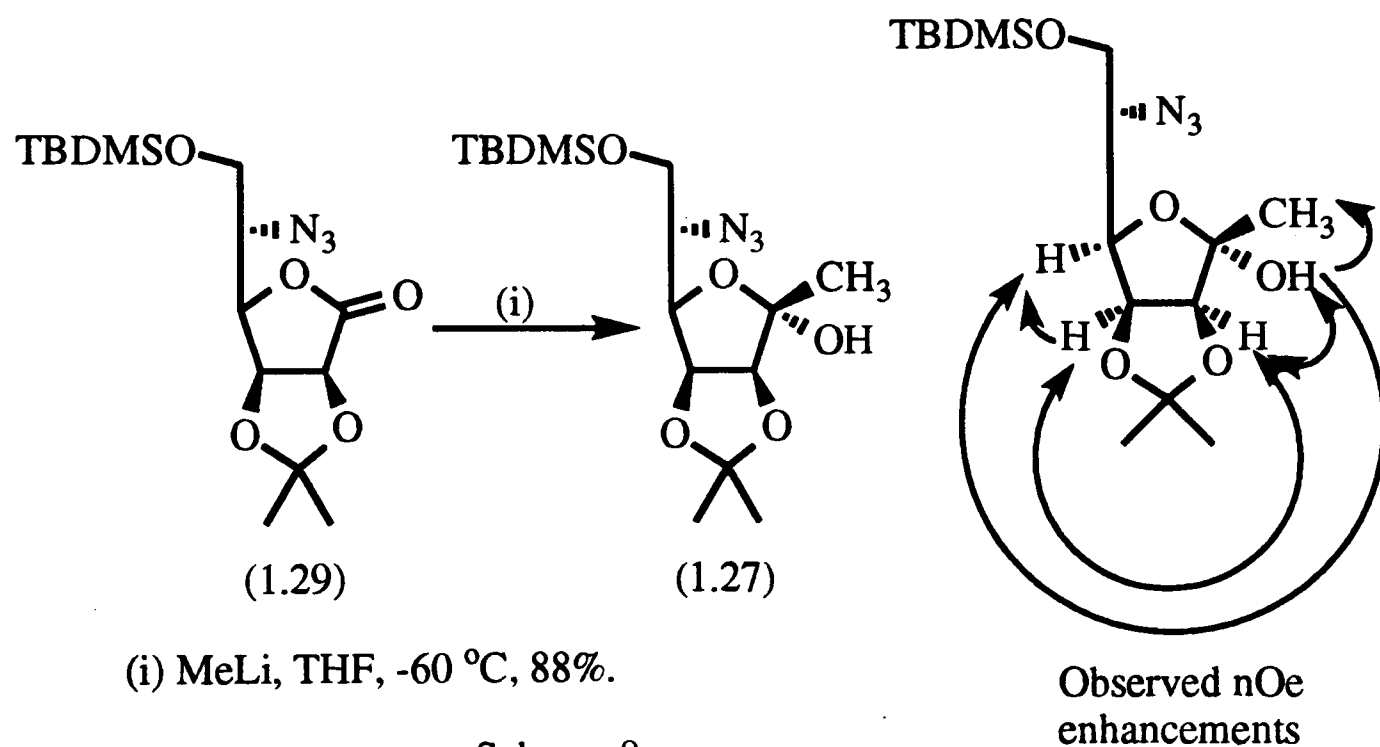
This left only the C-5 position unprotected. Introduction of nitrogen at this position, with inversion of configuration, was achieved via azide displacement of the triflate ester of compound (3.19). The triflate (3.20) was not purified but was used crude in the azide displacement reaction (Scheme 7).



(i) Tf_2O , py, DCM, $-30\text{ }^\circ\text{C}$, (ii) NaN_3 , DMF, 62% over 2 steps.

Scheme 7

The addition of one equivalent of methyllithium to the lactone carbonyl group of compound (1.29) then gave the keto-sugar (1.27) as a single anomer (Scheme 8).



(i) MeLi , THF, $-60\text{ }^\circ\text{C}$, 88%.

Scheme 8

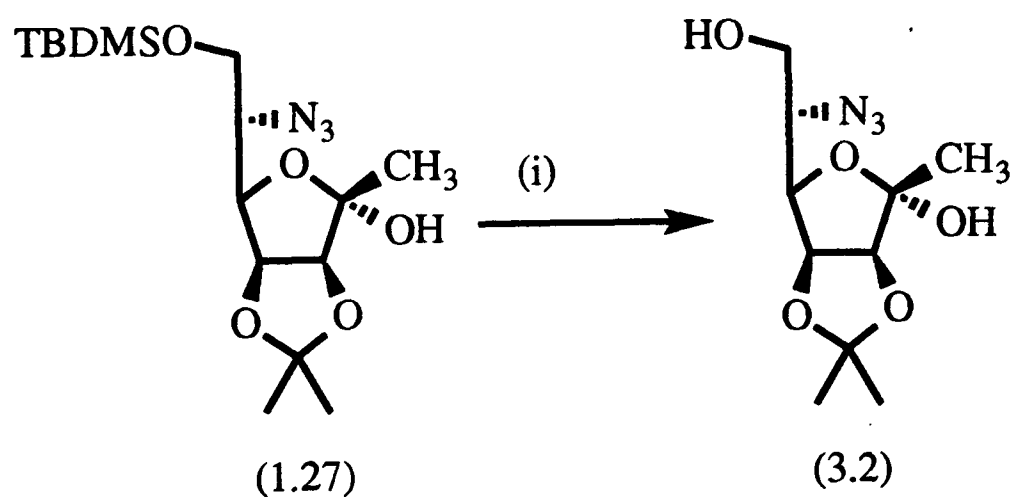
The anomeric configuration of this compound was not reported in the literature.³ Equilibrium nOe experiments were consistent with the β -configuration, as shown in Scheme 8. In the proton NMR spectrum in d_6 -DMSO, large nOe enhancements between the hydroxyl proton and the ring protons were observed, indicating that they were on the same face of the furanose ring. The product is therefore that of formal addition to the more hindered face of

the lactone. This result is in agreement with those observed for the addition of methyllithium to a number of other lactones reported in this thesis.

The keto-sugar (1.27) was therefore synthesised in an overall yield of 32% over six steps from diacetone mannose (1.28).

ii. The synthesis of α -L-homofuconojoirimycin (1.6).

The keto-sugar (1.27) was then converted into α -L-homofuconojoirimycin (1.6) as reported in the literature.² The silyl protecting group was removed in quantitative yield using tetra-*n*-butylammonium fluoride in tetrahydrofuran (Scheme 9). The product was a single compound which appeared to have remained in the furanose form. The anomeric configuration was assigned by comparison to the keto-sugar (1.27).

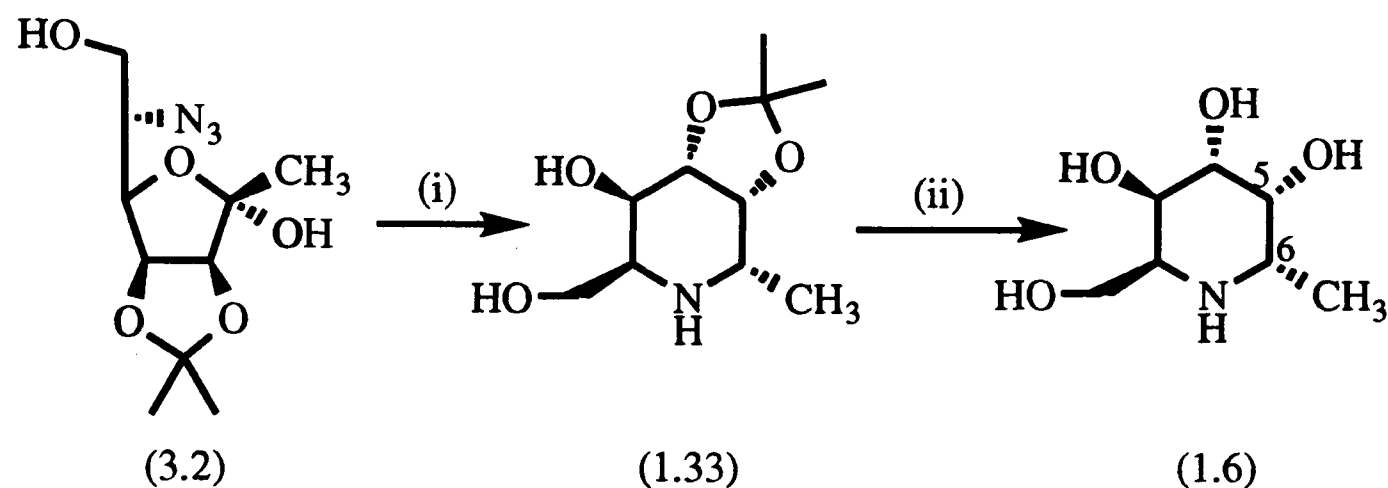


(i) TBAF, THF, quant.

Scheme 9

The product (3.2) was then reduced by catalytic hydrogenation using palladium on carbon as the catalyst. The reduction was slow, requiring three days to go to completion. As reported in the literature, the product of the reduction was the

α -L-homofuconojirimycin derivative (1.33) (Scheme 10).² The reduction was very clean and gave only one product by proton NMR.



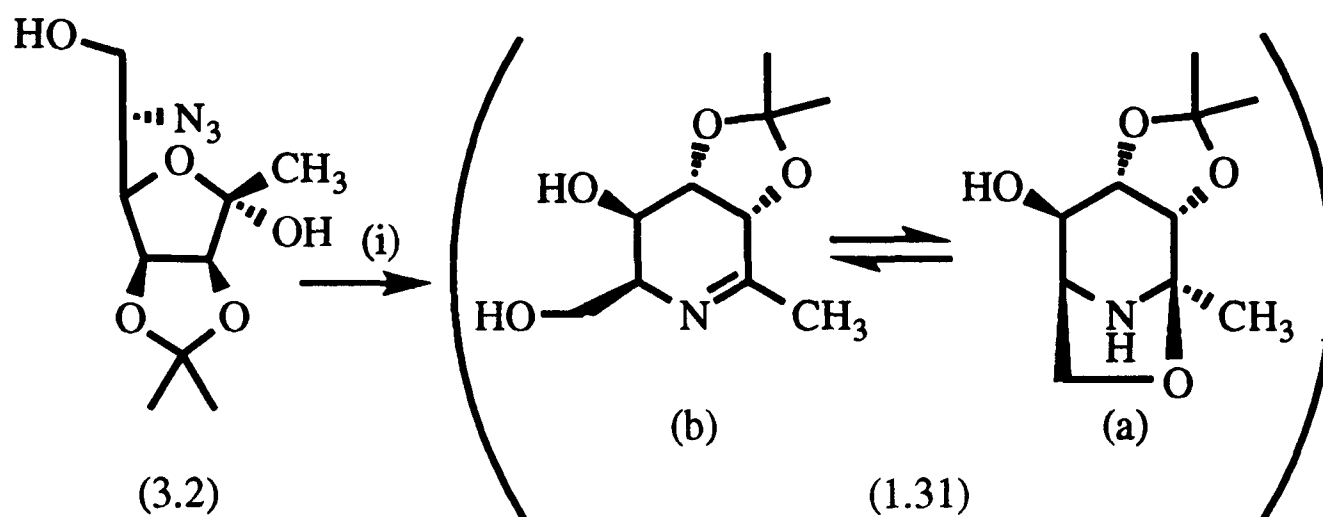
(i) H₂, Pd-C, EtOH, 3 days, quant.,

(ii) aq. TFA then ion exchange chromatography, 89%.

Scheme 10

The acetonide protecting group was removed on hydrolysis with aqueous trifluoroacetic acid. Following purification by ion exchange chromatography, the free base was obtained in 89% yield. The proton NMR spectrum of the product showed one compound, and was identical to spectra of the literature compound.¹⁴ A coupling constant of only 1.4 Hz between H-5 and H-6 indicated that the methyl group and the C-5 hydroxyl group were indeed *cis* to each other in the product. This is in comparison to a value for $J_{5,6}$ of 10.3 Hz reported for the epimeric compound (1.9) where the two groups are *trans* to one another.³

The reduction of the azide (3.2) was repeated, in the hope that it could be stopped at the intermediate imine stage. The reduction was worked up after 1.5 h, as soon as, judged by t.l.c, all the original starting material had been consumed. The product was indeed the imine (1.31b) which appeared to exist in equilibrium with the bicyclic hemiaminal (1.31a) (Scheme 11).

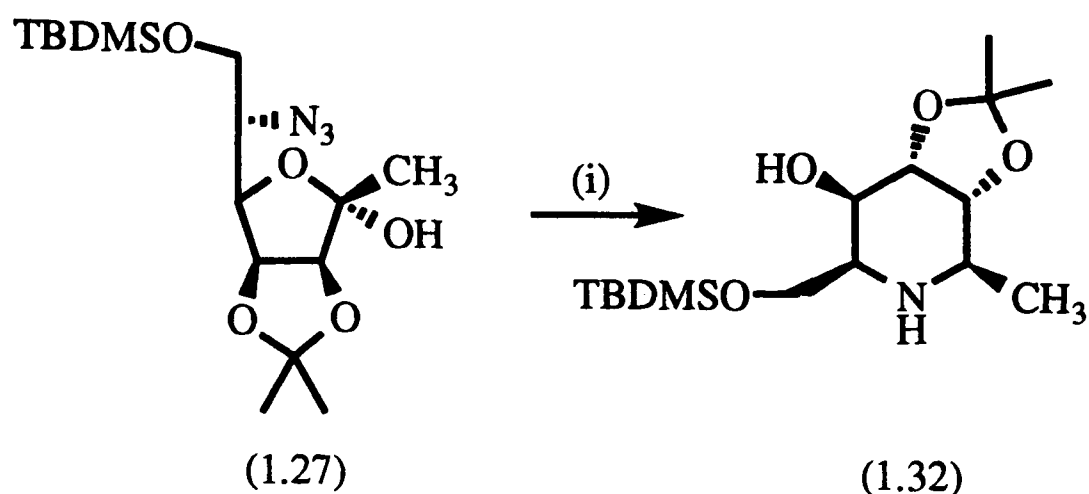


(i) H_2 , Pd-C, EtOH, 1.5 h, 90%.

Scheme 11

The proton and carbon NMR spectra of this mixture were complicated, but only two components were observed in a range of different solvents (CD_3CN , $\text{d}_6\text{-DMSO}$, $\text{d}_5\text{-py}$ and D_2O), and the number and type of signal were consistent with the equilibrium drawn above (Appendix 2). The position of this equilibrium was highly solvent dependent. In acetonitrile and water the bicyclic form (1.31a) was predominant, whilst in dimethylsulphoxide both forms were present in roughly equal amounts. In the proton NMR spectrum in $\text{d}_6\text{-DMSO}$, signals for one amine and three hydroxyl protons were observed. Deuterium exchange experiments and a COSY spectrum allowed full assignment of the proton spectrum of both components in $\text{d}_6\text{-DMSO}$. No sign of any hydration or dimerisation was observed by NMR. The equilibrium mixture (1.31) was hydrogenated over Adam's catalyst for a further three days to give a mixture of products, the major component of which, as judged by NMR, was the protected $\alpha\text{-L-HFJ}$ derivative (1.33). This confirmed that the imine mixture (1.31) was indeed an intermediate in the reduction of the azide (3.2) to the $\alpha\text{-L-HFJ}$ derivative (1.33).

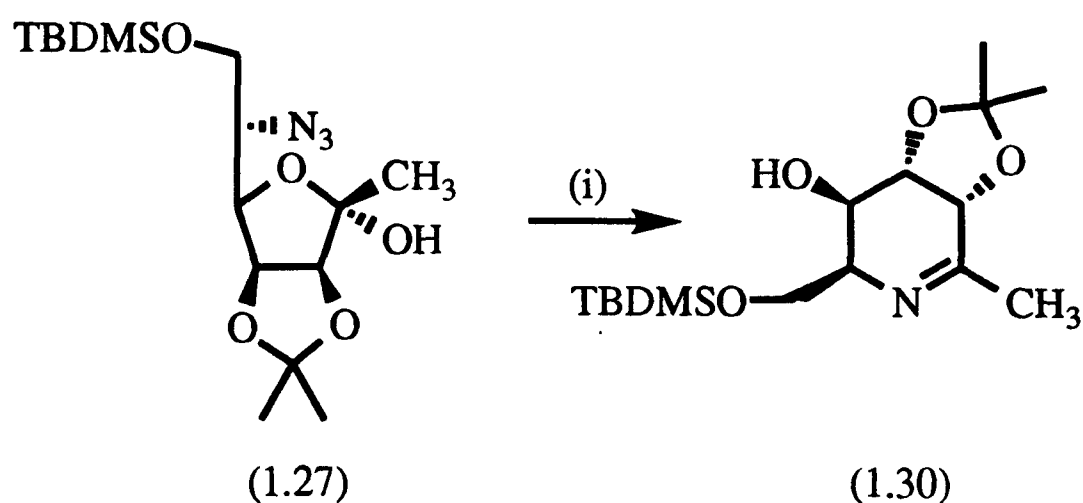
Further confirmation that the reduction of the azide (3.2) had indeed been halted at the imine stage was achieved by synthesising the imine mixture (1.31) via an alternative route. It had been reported that reduction of the keto-sugar (1.27) gave a derivative of 6-epi- $\alpha\text{-L}$ -homofuconojirimycin (1.32) (Scheme 12).³



(i) H₂, Pt₂O, EtOAc, 42 h, 47%.

Scheme 12

This reduction was slow, requiring 42 hours and the use of Adam's catalyst to reach completion. However, when the reduction was performed using palladium black as the catalyst and was stopped after five hours, as soon as all the original starting material had been consumed, the product isolated was the imine (1.30).

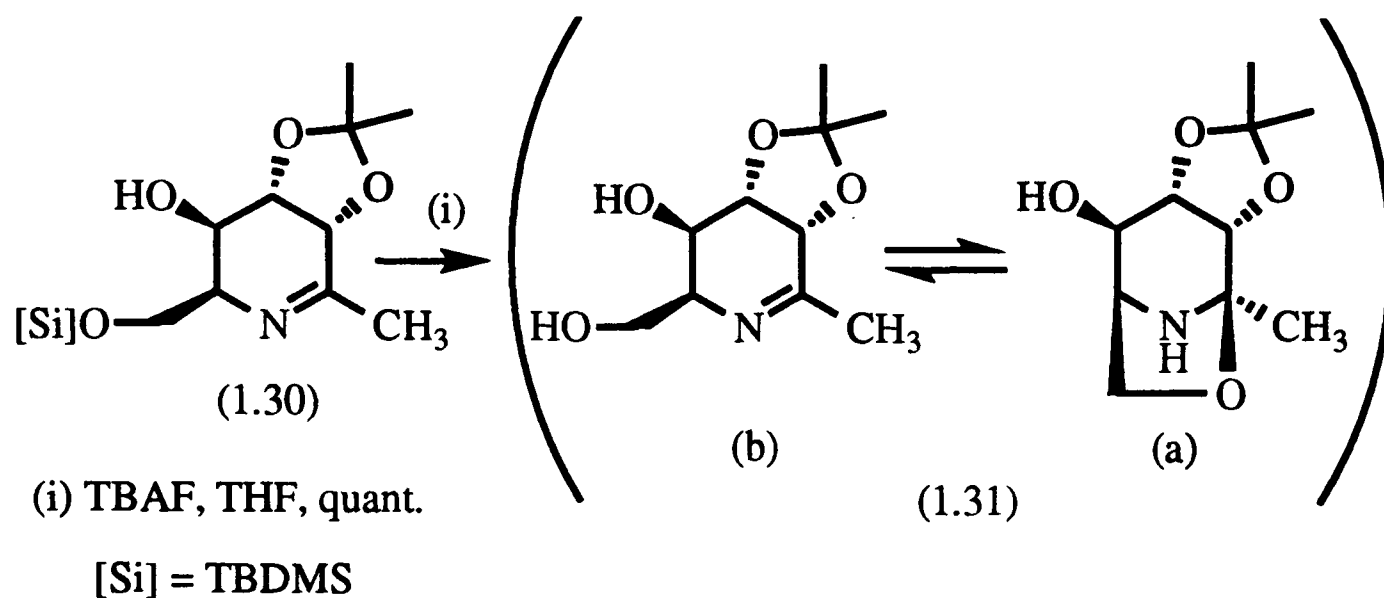


(i) H₂, Pd-black, EtOAc, 5 h, 69%.

Scheme 13

This compound (1.30) was a white crystalline solid, and all the physical data was consistent with the structure drawn. The silyl protecting group was removed by treatment with tetra-*n*-butylammonium fluoride in tetrahydrofuran. The product from this deprotection was the same equilibrium mixture of the imine (1.31b) and

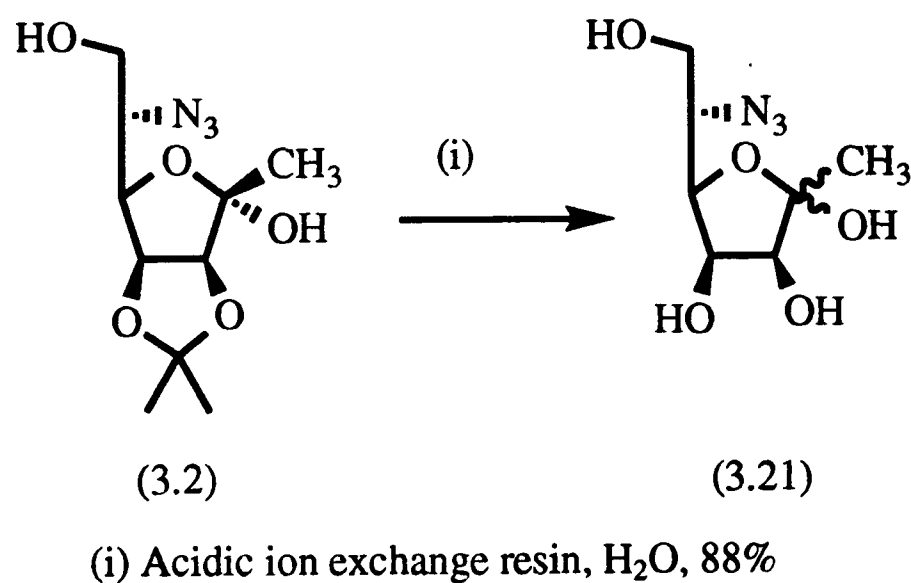
the bicyclic hemiaminal (1.31a) as was afforded by the reduction of the azide (3.2) (Scheme 14).



Scheme 14

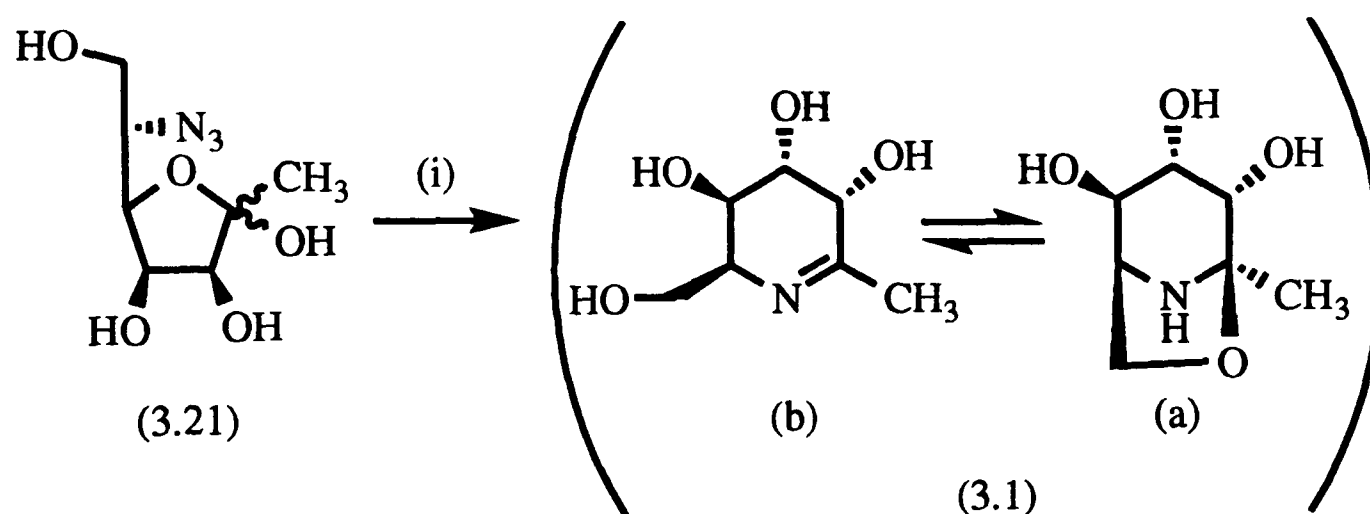
iii. The synthesis of the target system (3.1).

Attempts to remove the acetonide from the equilibrium mixture (1.31) to form the target (3.1) were unsuccessful. It was therefore decided to remove the acetonide prior to the reduction step. The acetonide protecting group was removed from the azide (3.2) by stirring in water with acidic ion exchange resin (Scheme 15).



Scheme 15

The product (3.21) was not very stable and appeared to exist as a mixture of open chain and cyclic forms. Reduction of the azido-tetrol (3.21) by catalytic hydrogenation over palladium on carbon in ethanol gave the imine (3.1b) which was in equilibrium with the bicyclic hemiaminal form (3.1a) (Scheme 16). The reduction was worked up after 1.25 h, as soon as, judged by t.l.c, all the starting material had been consumed.



(i) H_2 , Pd-C, EtOH, 69%.

Scheme 16

In contrast to the protected compound (1.31), the bicyclic form (3.1a) was the major component in both water and dimethylsulphoxide. A solution of the imine in water was at pH 9, indicating that at least some of the bicyclic amine form must be present. Unfortunately, the compound (3.1) was unstable, decomposing under both acidic and basic conditions or after prolonged periods at room temperature.

iv. The evaluation of α -L-homofuconojirimycin (1.6) and the target system (3.1) as glycosidase inhibitors.

The effect of the target compound (3.1) and of α -L-homofuconojirimycin (1.6) on a range of human liver glycosidases was investigated, and the results are shown in the table below.¹⁵ The target compound (3.1) was presumably present as an equilibrium mixture of the imine (3.1b) and the bicyclic (3.1a) forms. The data show the percentage inhibition of the enzymes at a 1×10^{-3} M concentration of the amino sugars. As has previously been reported in the literature, α -L-HFJ (1.6) is a potent and selective inhibitor of α -fucosidase. The K_i value was determined and was found to be 1×10^{-8} M, in agreement with the values reported for α -L-HFJ (1.6) against the α -fucosidases from both the bovine epididymus and from human neutrophils.² In contrast, the target system (3.1) showed no activity against α -fucosidase, and no significant activity against any of the other glycosidases. This result at least confirms that there was no contamination of the target compound by HFJ (1.6), which could potentially have been formed by over-reduction of the azide (3.21). Even a small contamination by such a powerful inhibitor would have lead to the observation of some α -fucosidase activity in the enzyme assay.

Inhibition (%) at 1 mM

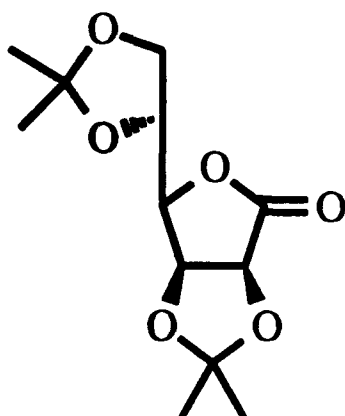
(A negative value signifies inhibition)

Enzyme	(3.1)	α-L-HFJ (1.6)
α-mannosidase pH 4	-10	-26
α-mannosidase pH 6.5	+4	-6
β-mannosidase	+2	+1
α-glucosidase	+61	-1
β-glucosidase	-27	+28
α-galactosidase	+27	-4
β-galactosidase	+9	-37
α-fucosidase	+4	-97
β-hexosaminidase	-12	-6
β-glucuronidase	-16	+10
β-xylosidase	-56	-4
α-arabinosidase	-2	-16

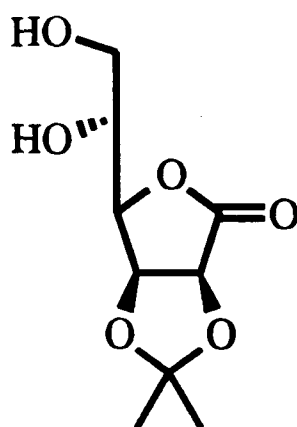
3.3 Experimental

For the general experimental details see page 46.

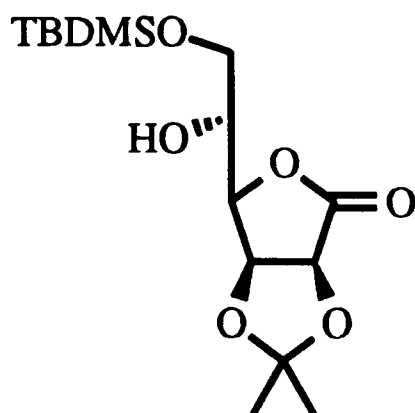
2,3;5,6-Di-*O*-isopropylidene-D-mannono-1,4-lactone (3.17).



Dried powdered molecular sieves (4Å, c. 50 g) and pyridinium chlorochromate (83.3 g, 386 mmol, 4 mol equivalents) were stirred in dry dichloromethane (200 ml) at 0 °C under nitrogen. 2,3;5,6-Di-*O*-isopropylidene-D-mannofuranose (1.28) (25.15 g, 96.6 mmol) in further dry dichloromethane (2 x 50 ml) was added dropwise over a period of 10 min and the reaction mixture was allowed to warm to room temperature. After 3.5 h t.l.c. (diethyl ether) showed complete conversion of the starting material (R_f 0.6) to one major product (R_f 0.5). The reaction mixture was diluted with diethyl ether (450 ml) and filtered through a silica plug topped with Celite, eluting with further diethyl ether. The combined diethyl ether washings were evaporated to give a white solid which was purified by recrystallisation (diethyl ether) to give 2,3;5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (3.17) (21.94 g, 85 mmol, 88% yield). M.p. 125-126 °C (diethyl ether) [lit.¹² 124-126 °C]; $[\alpha]_D^{20}$ +51.9 (c , 1.0 in CHCl_3) [lit.¹² +50.3 (c , 1.0 in CHCl_3); δ_H (CDCl_3): 1.40, 1.41, 1.48, 1.49 (4 x 3H, 4 x s, 2 x $(\text{CH}_3)_2\text{C}$), 4.08 (1H, dd, H-6, $J_{6,6'}$ 9.2 Hz, $J_{6,5}$ 3.7 Hz), 4.16 (1H, dd, H-6', $J_{6',6}$ 9.2 Hz, $J_{6',5}$ 5.4 Hz), 4.37 (1H, dd, H-4, $J_{4,5}$ 8.1 Hz, $J_{4,3}$ 3.0 Hz), 4.45 (1H, m, H-5), 4.84 (1H, d, H-2, $J_{2,3}$ 5.3 Hz), 4.89 (1H, dd, H-3, $J_{3,2}$ 5.3 Hz, $J_{3,4}$ 3.0 Hz).

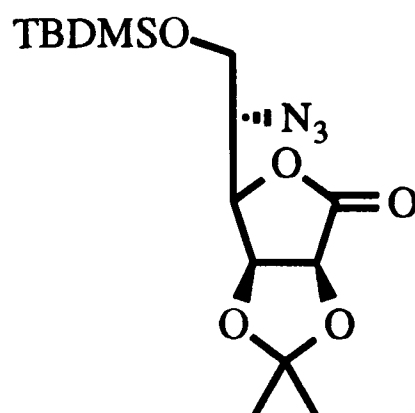
2,3-*O*-Isopropylidene-D-mannono-1,4-lactone (3.18).

2,3;5,6-Di-*O*-isopropylidene-D-mannono-1,4-lactone (3.17) (9.06 g, 35.1 mmol) was stirred at room temperature in acetic acid (140 ml) and water (60 ml) for 21 h when t.l.c. (ethyl acetate:hexane, 2:1) showed a trace of starting material (R_f 0.7) and one major product (R_f 0.1). The solvents were removed and the residue co-evaporated with toluene (3 x 40 ml) to give, after purification by flash chromatography (ethyl acetate), 2,3-*O*-isopropylidene-D-mannono-1,4-lactone (3.18) (5.88 g, 27.0 mmol, 77% yield) as a white crystalline solid. M.p. 104-105 °C (ethanol) [lit.¹³ 130-131 °C (ethanol)]; $[\alpha]_D^{20}$ +69.0 (c , 1.05 in CH_3CN); δ_{H} (CD_3CN): 1.37, 1.39 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.90-2.95 (1H, m, OH, D_2O exchanges), 3.24-3.31 (1H, m, OH, D_2O exchanges), 3.55 (1H, dd, H-6, $J_{6,6'}$ 11.8 Hz, $J_{6,5}$ 4.6 Hz), 3.70 (1H, dd, H-6', $J_{6',6}$ 11.8 Hz, $J_{6',5}$ 2.7 Hz), 3.79-3.88 (1H, m, H-5), 4.43 (1H, dd, J 9.2 Hz, J 3.0 Hz), 4.86-4.93 (2H, m).

6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-D-mannono-1,4-lactone (3.19).

2,3-*O*-Isopropylidene-D-mannono-1,4-lactone (3.18) (5.58 g, 25.6 mmol) and imidazole (4.36 g, 64 mmol, 2.5 mol equivalents) were dissolved in dry *N,N*-dimethylformamide (50 ml) and cooled to -10 °C under nitrogen. A solution of *tert*-butyldimethylsilyl chloride (4.63 g, 30.7 mmol, 1.2 mol equivalents) in dry *N,N*-dimethylformamide (4 x 10 ml) was added and the reaction mixture was allowed to warm to room temperature. After 0.5 h, t.l.c. (ethyl acetate:hexane, 1:1) showed no starting material (baseline) and one major product (R_f 0.5). The reaction was quenched by the addition of a few drops of water and the solvents were evaporated. The crude residue was dissolved in ethyl acetate (150 ml) and washed with water (2 x 100 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. Purification of the residue by flash chromatography (ethyl acetate:hexane, 1:4 then 1:3 then 1:1) gave 6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-mannono-1,4-lactone (3.19) (7.41 g, 22.4 mmol, 87% yield) as a colourless oil. $[\alpha]_D^{20} +58.3$ (c , 1.15 in CHCl_3) [lit.³ +51.6 (c , 1.33 in CHCl_3)]; δ_H (CDCl_3): 0.11 (6H, s, $(\text{CH}_3)_2\text{Si}$), 0.92 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.45, 1.50 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.74 (1H, br d, OH, J 6.8 Hz, D_2O exchanges), 3.81 (1H, dd, H-6, $J_{6,6'}$ 10.1 Hz, $J_{6,5}$ 3.4 Hz), 3.90 (1H, dd, H-6', $J_{6',6}$ 10.4 Hz, $J_{6',5}$ 3.0 Hz), 3.97-4.15 (1H, m, H-5), 4.41 (1H, dd, H-4, $J_{4,5}$ 8.9 Hz, $J_{4,3}$ 3.0 Hz), 4.86 (1H, d, H-2, $J_{2,3}$ 5.2 Hz), 4.97 (1H, dd, H-3, $J_{3,2}$ 5.2 Hz, $J_{3,4}$ 3.4 Hz).

5-Azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-L-gulonono-1,4-lactone (1.29).

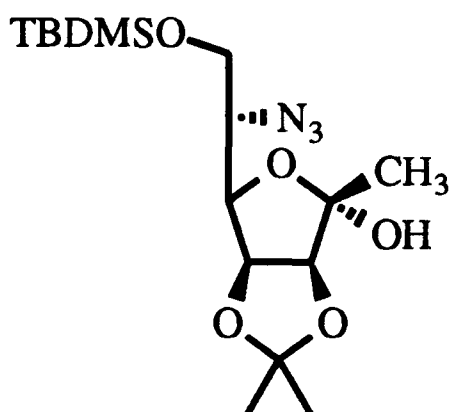


6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-D-mannono-1,4-lactone (3.19) (7.41 g, 22.4 mmol) was dissolved in dry dichloromethane (100 ml) and the solution cooled to -35 °C under nitrogen. Pyridine (5.2 ml, 67.2 mmol, 3 mol equivalents) was added followed by trifluoromethanesulphonic anhydride (5.65 ml, 33.6 mmol, 1.5 mol equivalents) and the reaction was stirred at -30 °C. After 1 h, t.l.c. (ethyl acetate:hexane, 1:2) showed no starting material (R_f 0.3) and one product (R_f 0.5). The reaction mixture was diluted with dichloromethane (50 ml) and washed with dilute aqueous hydrochloric acid (100 ml) and water (2 x 100 ml), dried (magnesium sulphate), filtered and evaporated to give 6-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-5-*O*-trifluoromethanesulphonyl-D-mannono-1,4-lactone (3.20) as a clear oil, which was used crude without further purification. δ_H (CDCl₃): 0.09 (6H, s, (CH₃)₂Si), 0.90 (9H, s, (CH₃)₃C), 1.41, 1.49 (2 x 3H, 2 x s, (CH₃)₂C), 3.96 (1H, dd, H-6, $J_{6,6'}$ 12.6 Hz, $J_{6,5}$ 3.4 Hz), 4.14 (1H, dd, H-6', $J_{6',6}$ 12.6 Hz, $J_{6',5}$ 2.2 Hz), 4.80-4.89 (3H, m, H-2, H-3, H-4), 5.17 (1H, ddd, H-5, $J_{5,4}$ 8.2 Hz, $J_{5,6}$ 3.2 Hz, $J_{5,6'}$ 2.3 Hz).

The crude triflate (3.20) was dissolved in *N,N*-dimethylformamide (100 ml) and sodium azide (4.37 g, 67.2 mmol, 3 mol equivalents) was added. The reaction was stirred at room temperature for 4 h at which time t.l.c. (ethyl acetate:hexane, 1:2) showed no starting material (R_f 0.5) and one major product (R_f 0.4). The solvent was evaporated and the residue partitioned between ethyl acetate (100 ml) and water (100 ml). The organic layer was dried (magnesium sulphate), filtered

and evaporated. Purification of the residue by flash chromatography (ethyl acetate:hexane, 1:10 then 1:5) gave 5-azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (1.29) (4.95 g, 13.9 mmol, 62% yield over 2 steps) as a colourless gum. $[\alpha]_D^{20} +81.0$ (*c*, 1.05 in CHCl_3) [lit.³ +78.5 (*c*, 1.56 in CHCl_3)]; δ_{H} (CDCl_3): 0.11, 0.12 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{Si}$), 0.92 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.41, 1.49 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 3.73 (1H, ddd, H-5, $J_{5,4}$ 8.7 Hz, $J_{5,6'}$ 4.0 Hz, $J_{5,6}$ 3.1 Hz), 3.91 (1H, dd, H-6, $J_{6,6'}$ 11.0 Hz, $J_{6,5}$ 3.1 Hz), 3.95 (1H, dd, H-6', $J_{6',6}$ 11.0 Hz, $J_{6',5}$ 4.0 Hz), 4.62 (1H, dd, H-4, $J_{4,5}$ 8.8 Hz, $J_{4,3}$ 3.4 Hz), 4.78 (1H, dd, H-3, $J_{3,2}$ 5.2 Hz, $J_{3,4}$ 3.4 Hz), 4.88 (1H, d, H-2, $J_{2,3}$ 5.2 Hz).

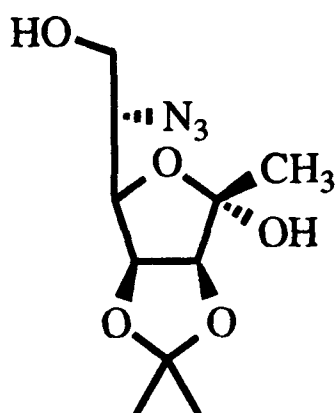
6-Azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-gulo-heptulofuranose (1.27).



Methylolithium (1.4 M in diethyl ether, 10.9 ml, 15.3 mmol, 1.1 mol equivalents) was added to a stirred solution of 5-azido-6-*O*-*tert*-butyldimethylsilyl-5-deoxy-2,3-*O*-isopropylidene-L-gulono-1,4-lactone (1.29) (4.945 g, 13.9 mmol) in dry tetrahydrofuran (50 ml) at $-60\text{ }^{\circ}\text{C}$ under nitrogen. After 5 min, t.l.c. (ethyl acetate:hexane, 1:2, eluted twice) showed no starting material (R_f 0.75) and one product (R_f 0.8). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (10 ml) and allowed to warm to room temperature. The solvents were evaporated and the residue partitioned between diethyl ether (50 ml) and water (50 ml). The aqueous layer was washed with diethyl ether (50 ml). The combined organic extracts were dried (magnesium sulphate), filtered

and evaporated to give a residue which was purified by flash chromatography (diethyl ether:hexane, 1:3 then 1:2 then 1:1) to give 6-azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27) (4.581 g, 12.3 mmol, 88% yield) as a gum. $[\alpha]_{\text{D}}^{20} +24.4$ (*c*, 1.05 in CHCl_3); δ_{H} (CDCl_3): 0.11 (6H, s, $(\text{CH}_3)_2\text{Si}$), 0.92 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.31, 1.47, 1.56 (3 x 3H, 3 x s, 3 x CH_3C), 3.64 (1H, ddd, H-6, $J_{6,5}$ 9.3 Hz, $J_{6,7}$ 5.2 Hz, $J_{6,7'}$ 3.0 Hz), 3.83 (1H, dd, H-7, $J_{7,7'}$ 10.7 Hz, $J_{7,6}$ 5.2 Hz), 3.94 (1H, dd, H-7', $J_{7,7}$ 10.7 Hz, $J_{7',6}$ 2.9 Hz), 4.17 (1H, dd, H-5, $J_{5,6}$ 9.3 Hz, $J_{5,4}$ 3.6 Hz), 4.48 (1H, d, H-3, $J_{3,4}$ 6.0 Hz), 4.72 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.7 Hz); δ_{H} (d_6 -DMSO, 400 MHz), 0.10 (6H, s, $(\text{CH}_3)_2\text{Si}$), 0.91 (9H, s, $(\text{CH}_3)_3\text{C}$), 1.27, 1.37, 1.39 (3 x 3H, 3 x s, 3 x CH_3C), 3.52 (1H, ddd, H-6, $J_{6,5}$ 9 Hz, $J_{6,7}$ 6 Hz, $J_{6,7'}$ 2.5 Hz), 3.76 (1H, dd, H-7, $J_{7,7'}$ 11 Hz, $J_{7,6}$ 6 Hz), 3.89 (1H, dd, H-7', $J_{7',7}$ 11 Hz, $J_{7',6}$ 2.5 Hz), 4.03 (1H, dd, H-5, $J_{5,6}$ 9 Hz, $J_{5,4}$ 4 Hz), 4.33 (1H, d, H-3, $J_{3,4}$ 6 Hz), 4.72 (1H, dd, H-4, $J_{4,3}$ 6 Hz, $J_{4,5}$ 4 Hz), 6.11 (1H, s, OH).

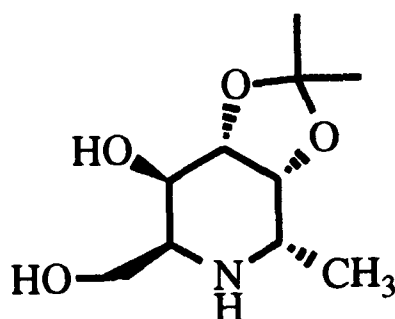
6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (3.2).



6-Azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27) (1.297 g, 3.5 mmol) was dissolved in dry tetrahydrofuran (10 ml) under nitrogen at room temperature and tetra-*n*-butylammonium fluoride (1.0 M in tetrahydrofuran, 4.55 ml, 4.55 mmol, 1.3 mol equivalents) was added. After stirring for 1 h, t.l.c. (ethyl acetate:hexane, 1:2) showed no starting material

(R_f 0.5) and one product (R_f 0.1). The reaction mixture was preabsorbed onto silica and purified by flash chromatography (ethyl acetate:hexane, 1:1) to give *6-azido-1,6-dideoxy-3,4-O-isopropylidene-β-L-gulo-heptulofuranose* (3.2) (923 mg, 0.35 mmol, quantitative yield) as an oil. $[\alpha]_D^{20} +25.6$ (c , 1.0 in CHCl_3); ν_{max} (neat): 3436 (br, OH), 2099 (N_3) cm^{-1} ; δ_{H} (CDCl_3): 1.32, 1.49, 1.56 (3 x 3H, 3 x s, 3 x CH_3), 3.70-3.76 (1H, m), 3.82-3.88 (2H, m), 4.20 (1H, dd, J 9.0 Hz, J 3.7 Hz), 4.48 (1H, d, H-3, $J_{3,4}$ 5.9 Hz), 4.77 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.7 Hz); δ_{C} (CDCl_3): 22.2, 24.6, 25.9 (3 x q, C-1, $(\text{CH}_3)_2\text{C}$), 62.1 (t, C-7), 63.3, 78.6, 80.3, 85.7 (4 x d, C-3, C-4, C-5, C-6), 105.2 (s, C-2), 113.0 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 277 ($\text{M}+\text{NH}_4^+$, 3%), 259 ($\text{M}+\text{NH}_4^+-\text{H}_2\text{O}$, 27%), 242 ($\text{M}+\text{H}^+-\text{H}_2\text{O}$, 100%), 202 ($\text{M}+\text{H}^+-\text{acetone}$, 14%). (Found C, 46.10; H, 6.77; N, 16.50. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_5$ requires C, 46.33; H, 6.61; N, 16.21%).

2,6,7-Trideoxy-2,6-imino-4,5-*O*-isopropylidene-L-glycero-D-gluco-heptitol
(1.33).



Method 1.

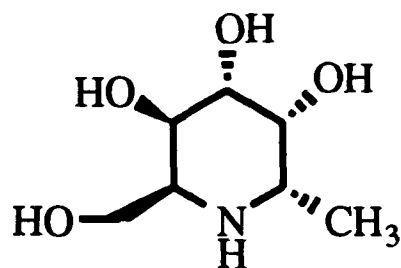
6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene-β-*L*-gulo-heptulofuranose (3.2) (115 mg, 0.44 mmol) and 10% palladium on carbon (20% by weight, ca. 25 mg) were stirred in ethanol (5 ml) under an atmosphere of hydrogen for 3 days. The reaction mixture was filtered and the solvent evaporated to give 2,6,7-trideoxy-2,6-imino-4,5-*O*-isopropylidene-*L*-glycero-*D*-gluco-heptitol (1.33) (96 mg, 0.44 mmol, quantitative crude yield), which was used without further purification.

M.p. 104-106 °C; ν_{max} (KBr): 3318 (OH, NH) cm^{-1} ; δ_{H} (d_6 -DMSO, 500 MHz): 1.00 (3H, d, C-7, 3J 6.8 Hz), 1.25, 1.38 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.96 (1H, dt, H-2, J 6.9 Hz, J 3.5 Hz), 3.19 (1H, dq, H-6, J 6.7 Hz, $J_{6,5}$ 1.5 Hz), 3.33 (1H, dd, H-1, $J_{1,1'}$ 10.5 Hz, $J_{1,2}$ 7.2 Hz), 3.41 (1H, dd, H-1', $J_{1',1}$ 10.6 Hz, $J_{1',2}$ 6.7 Hz), 3.66 (1H, t, H-3, J 3.3 Hz), 4.01-4.05 (2H, m, H-4, H-5); δ_{C} (D_2O , pH 9): 18.0 (q, C-7), 25.2, 27.4 (2 x q, $(\text{CH}_3)_2\text{C}$), 45.1, 54.4 (2 x d, C-2, C-6), 60.6 (t, C-1), 69.1, 76.8, 77.2 (3 x d, C-3, C-4, C-5), 110.7 (s, $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 218 ($\text{M}+\text{H}^+$, 100%).

Method 2.

2,7-Anhydro-1,2,6-trideoxy-2,6-imino-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (1.31) (69 mg, 0.32 mmol) and platinum IV oxide (catalytic amount) were stirred in ethanol (1 ml) under an atmosphere of hydrogen for 3 days. Magnesium sulphate was added and the reaction mixture was filtered through a Celite plug. The solvent was evaporated to give a mixture of products (69 mg), the major component of which was 2,6,7-trideoxy-2,6-imino-4,5-*O*-isopropylidene-L-*glycero*-D-*gluco*-heptitol (1.33), as judged by proton and carbon NMR.

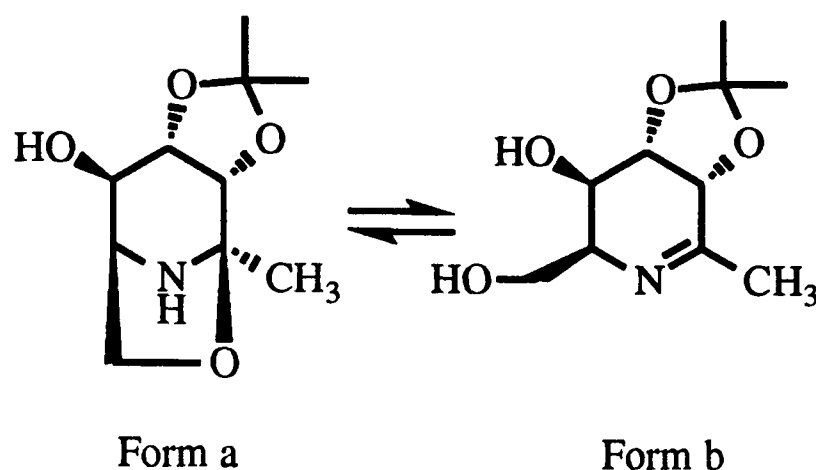
2,6,7-Trideoxy-2,6-imino-L-glycero-D-gluco-heptitol (α -L-homofuconojirimycin)
(1.6).



2,6,7-Trideoxy-2,6-imino-4,5-*O*-isopropylidene-L-glycero-D-gluco-heptitol

(1.33) (76 mg, 0.35 mmol) was stirred in trifluoroacetic acid (2 ml) and water (1 ml) at room temperature for 2 h. The solvents were evaporated and the residue co-evaporated with toluene (2 x 5 ml). The crude product was purified by ion exchange chromatography (Dowex 50W-X8(H) resin, eluted with 0.5 M aqueous ammonia solution) to give 2,6,7-trideoxy-2,6-imino-L-glycero-D-gluco-heptitol (α -L-homofuconojirimycin) (1.6) (55 mg, 0.31 mmol, 89% yield) as a brown solid. M.p. 39-48 °C (lit.² 160-170 °C (dec.)); $[\alpha]_{\text{D}}^{25}$ -69.7 (*c*, 0.53 in H₂O) (lit.² $[\alpha]_{\text{D}}^{20}$ -28 (*c*, 1.0 in CHCl₃)); δ_{H} (D₂O, pH 9, 500 MHz): 1.04 (3H, d, C-7, ³*J* 6.8 Hz), 3.04 (1H, dq, H-6, *J* 6.6 Hz, *J*_{6,5} 1.4 Hz), 3.19 (1H, dt, H-2, *J* 8.0 Hz, *J* 6.0 Hz), 3.61 (1H, dd, H-4, *J*_{4,3} 10.0 Hz, *J*_{4,5} 3.3 Hz), 3.70-3.73 (3H, m, H-1, H-1', H-5), 3.90 (1H, dd, H-3, *J*_{3,4} 10.0 Hz, *J*_{3,2} 5.9 Hz); δ_{C} (D₂O, pH 9): 17.1 (q, C-7), 48.5, 57.9 (2 x d, C-2, C-6), 58.0 (t, C-1), 69.0, 72.1, 73.0 (3 x d, C-3, C-4, C-5). A small portion was dissolved in water (2 ml) and the pH adjusted to 1 by the dropwise addition of dilute aqueous hydrochloric acid. The solution was then freeze-dried to give the hydrochloride salt of 2,6,7-trideoxy-2,6-imino-L-glycero-D-gluco-heptitol (α -L-homofuconojirimycin, hydrochloride salt). M.p. 181-185 °C (dec.); $[\alpha]_{\text{D}}^{25}$ -59.8 (*c*, 0.86 in H₂O); δ_{H} (D₂O, pH 3, 500 MHz): 1.30 (3H, d, C-7, ³*J* 6.7 Hz), 3.60 (1H, dq, H-6, *J* 6.7 Hz, *J*_{6,5} 1.9 Hz), 3.66-3.70 (1H, m, H-2), 3.76 (1H, dd, *J* 9.5 Hz, *J* 3.2 Hz), 3.81 (1H, dd, *J* 12.6 Hz, *J* 10.2 Hz), 3.92-3.96 (2H, m), 4.08 (1H, dd, *J* 9.5 Hz, *J* 5.7 Hz); δ_{C} (D₂O, pH 3): 14.7 (q, C-7), 51.1, 57.2 (2 x d, C-2, C-6), 56.2 (t, C-1), 66.8, 70.1, 70.6 (3 x d, C-3, C-4, C-5).

2,7-Anhydro-1,2,6-trideoxy-2,6-imino-3,4-*O*-isopropylidene- β -L-gulo-heptulopyranose (1.31).



Method 1.

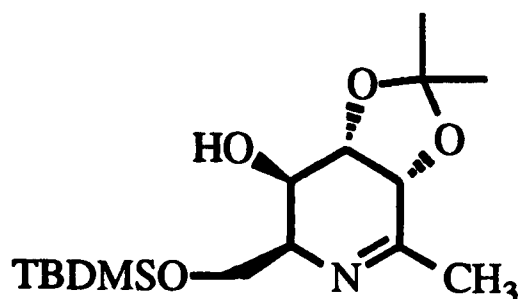
6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-gulo-heptulofuranose (3.2) (124 mg, 0.48 mmol) and 10% palladium on carbon (20% by weight, *ca.* 25 mg) were stirred vigorously in ethanol (5 ml) under an atmosphere of hydrogen at room temperature. After 1.5 h t.l.c. (diethyl ether) showed no starting material (R_f 0.5) and a product (baseline). The catalyst was removed by filtration and the solvent was evaporated to give 2,7-anhydro-1,2,6-trideoxy-2,6-imino-3,4-*O*-isopropylidene- β -L-gulo-heptulopyranose (1.31) (93 mg, 0.43 mmol, 90% crude yield), as a colourless oil, which existed as an equilibrium mixture of two forms (a and b above). $[\alpha]_D^{25}$ -22.6 (*c.* 1.28 in CH_3CN); ν_{max} (neat): 3437 (OH) cm^{-1} ; δ_{H} (d_6 -DMSO, 500 MHz + COSY): Form a; 1.23, 1.34, 1.37 (3 x 3H, 3 x s, 3 x CH_3), 3.15 (1H, br s, NH, D_2O exchanges), 3.28 (1H, dd, H-7, $J_{7,7'}$ 7.0 Hz, $J_{7,6}$ 5.6 Hz), 3.38 (1H, t, H-6, J 4.7 Hz), 3.57-3.58 (1H, m, H-5), 3.66 (1H, d, H-7', $J_{7',7}$ 7.3 Hz), 3.81-3.84 (2H, m, H-3, H-4), 5.13 (1H, d, OH-5, J 4.2 Hz): Form b; 1.27, 1.30 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 1.95 (3H, d, C-1, 3J 1.9 Hz), 3.32-3.33 (1H, m, H-6), 3.54 (1H, dd, H-7, $J_{7,7'}$ 10.6 Hz, $J_{7,6}$ 7.5 Hz), 3.70 (1H, dd, H-7', $J_{7',7}$ 10.5 Hz, $J_{7',6}$ 4.9 Hz), 3.75 (1H, dd, H-5, $J_{5,4}$ 4.7 Hz, $J_{5,6}$ 3.3 Hz), 4.23 (1H, dd, H-4, $J_{4,3}$ 6.5 Hz, $J_{4,5}$ 4.8 Hz), 4.29 (1H, d, H-3, $J_{3,4}$ 6.5 Hz), 4.54

(1H, t, OH-7, *J* 5.1 Hz, D₂O exchanges), 5.01 (1H, d, OH-5, *J* 4.6 Hz, D₂O exchanges); δ_C (CD₃CN, 125 MHz): Form a (major component); 21.6, 26.5, 28.3 (3 x q, C-1, (CH₃)₂C), 61.0 (d, C-6), 65.1 (t, C-7), 74.3, 80.2, 80.8 (3 x d, C-3, C-4, C-5), 94.4 (s, C-2), 111.2 (s, (CH₃)₂C): Form b (minor component); 24.3, 25.6, 27.2 (3 x q, C-1, (CH₃)₂C), 59.3 (d, C-6), 63.7 (t, C-7), 68.3, 72.6, 75.8 (3 x d, C-3, C-4, C-5), 110.2 (s, (CH₃)₂C), 168.0 (s, C=N); *m/z* (NH₃, CI): 216 (M+H⁺, 100%). (Found MH⁺ 216.1233471. Calculated for C₁₀H₁₈NO₄ 216.123583).

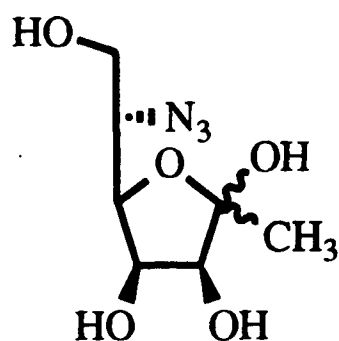
Method 2.

(2*S*,3*R*,4*S*,5*R*)-2-(*O*-*tert*-Butyldimethylsilyloxymethyl)-4,5-*O*-isopropylidene-6-methyl-2,3,4,5-tetrahydropyridine-3,4,5-triol (1.30) (340 mg, 1.03 mmol) was dissolved in dry tetrahydrofuran (3 ml) at room temperature and tetra-*n*-butylammonium fluoride (1.0 M in tetrahydrofuran, 1.14 ml, 1.14 mmol, 1.1 mol equivalents) was added. The reaction was stirred for 0.5 h, at which time t.l.c. (ethyl acetate:hexane, 1:2) showed no starting material (*R_f* 0.3) and one product (baseline). The reaction mixture was preabsorbed directly onto silica and purified by flash chromatography (ethyl acetate then methanol:ethyl acetate, 1:20) to give 2,7-anhydro-1,2,6-trideoxy-2,6-imino-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (1.31) (219 mg, 1.02 mmol, 99% yield) as a colourless oil, identical to the material prepared above.

(2*S*,3*R*,4*S*,5*R*)-2-(*O*-*tert*-Butyldimethylsilyloxymethyl)-4,5-*O*-isopropylidene-6-methyl-2,3,4,5-tetrahydropyridine-3,4,5-triol (1.30).

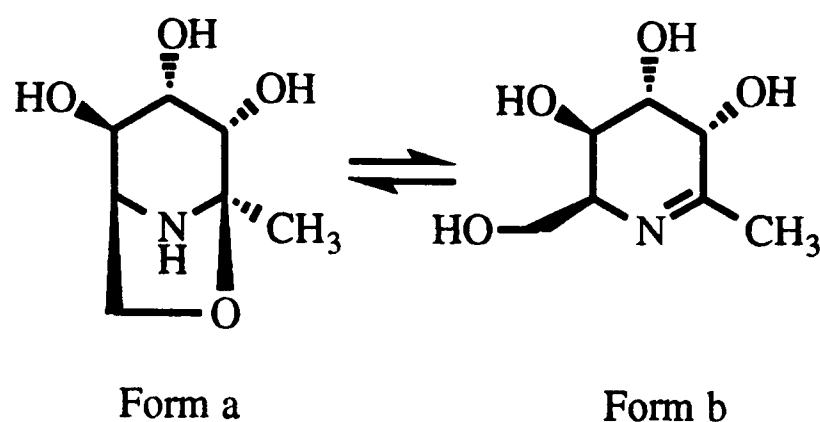


6-Azido-7-*O*-*tert*-butyldimethylsilyl-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulofuranose (1.27) (593 mg, 1.59 mmol) and palladium black (catalytic amount) were stirred in ethyl acetate (6 ml) under an atmosphere of hydrogen at room temperature. After 5 h, t.l.c. (ethyl acetate:hexane, 1:2) showed no starting material (R_f 0.5) and one product (R_f 0.1). Magnesium sulphate was added and the reaction mixture filtered through a Celite pad, eluting with ethyl acetate. The solvent was evaporated and the residue purified by flash chromatography (ethyl acetate:hexane, 1:1 then 2:1) to give (2*S*,3*R*,4*S*,5*R*)-2-(*O*-*tert*-butyldimethylsilyloxymethyl)-4,5-*O*-isopropylidene-6-methyl-2,3,4,5-tetrahydropyridine-3,4,5-triol (1.30) (361 mg, 1.1 mmol, 69% yield) as a white solid. M.p. 94-96 °C (hexane); $[\alpha]_D^{20}$ -48.4 (c , 1.0 in CHCl_3); ν_{max} (KBr): 3120 (OH), 1672 (C=N) cm^{-1} ; δ_{H} (CDCl_3): 0.10, 0.11 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{Si}$), 0.89 (9H, s, $(\text{CH}_3)_3\text{C}$) 1.36, 1.39 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 2.13 (3H, d, CH_3 , 3J 2.0 Hz), 3.51 (1H, br s), 4.15-4.17 (3H, m), 4.31-4.36 (2H, m); δ_{C} (CDCl_3): -5.7, -5.6 (2 x q, $(\text{CH}_3)_2\text{Si}$), 18.0 (s, $(\text{CH}_3)_3\text{C}$), 23.9, 25.4, 26.9 (3 x q, CH_3 , $(\text{CH}_3)_2\text{C}$), 25.7 (q, $(\text{CH}_3)_3\text{C}$), 66.0 (t, CH_2), 57.1, 69.1, 71.9, 74.5 (4 x d, C-2, C-3, C-4, C-5), 109.8 (s, $(\text{CH}_3)_2\text{C}$), 168.6 (s, C=N); m/z (NH_3 , CI): 330 ($\text{M}+\text{H}^+$, 100%), 272 ($\text{M}+\text{H}^+$ -acetone, 28%). (Found C, 58.25; H, 9.58; N, 4.23. $\text{C}_{16}\text{H}_{31}\text{NO}_4\text{Si}$ requires C, 58.32; H, 9.48; N, 4.25%).

6-Azido-1,6-dideoxy-L-gulo-heptulose (3.21).

6-Azido-1,6-dideoxy-3,4-*O*-isopropylidene- β -L-gulo-heptulofuranose (3.2) (431 mg, 1.66 mmol) was dissolved in water and Dowex 50W-8X(H) ion exchange resin (*ca.* 2 ml) was added. The reaction mixture was stirred at room temperature for 4 days at which time t.l.c. (methanol:ethyl acetate, 1:10) showed no starting material (R_f 0.8) and one product (R_f 0.4). The reaction mixture was filtered and the solution freeze-dried to give 6-azido-1,6-dideoxy-L-gulo-heptulose (3.21) (319 mg, 1.46 mmol, 88% yield) as a white solid, which existed as an equilibrium mixture of open chain and closed ring forms. The compound was used crude without further purification. A small sample was recrystallised for characterisation. M.p. 106-107 °C (ethyl acetate/hexane); $[\alpha]_D^{25} +2.2 \rightarrow +41.5$ (24 h) (*c.* 1.0 in EtOH); $\nu_{\max}$ (KBr): 3408 (OH), 2120 (N₃) cm⁻¹; $\nu_{\max}$ (DMSO): 3316, 3262, 3229 (OH), 2138, 2095 (N₃), 1787 (C=O) cm⁻¹; δ_H (D₂O): 1.26 (2.25 H, s, CH₃), 2.12 (0.25 H, s, CH₃), 3.35-4.18 (6H, m); δ_C (D₂O): 23.0, 24.0, 27.9 (3 x q, C-1), 61.7, 62.0 (2 x t, C-7), 63.8, 65.6, 66.1, 70.5, 71.5, 71.9, 72.4, 75.7, 77.1, 77.9, 78.8, 79.1 (12 x d, C-2, C-3, C-4, C-5), 103.3, 106.1 (2 x s, C-2), 215.3 (s, C=O); m/z (NH₃, CI): 237 (M+NH₄⁺, 6%), 216 (22%), 174 (M+H⁺-N₂-H₂O, 32%), 140 (100%). (Found C, 38.37; H, 6.04; N, 18.95. C₇H₁₃N₃O₅ requires C, 38.36; H, 5.98; N, 19.17%).

2,7-Anhydro-1,2,6-trideoxy-2,6-imino- β -L-*gulo*-heptulopyranose (3.1).



6-Azido-1,6-dideoxy-L-*gulo*-heptulose (3.21) (82 mg, 0.37 mmol) and 10% palladium on carbon (20% by weight, *ca.* 16 mg) were stirred in ethanol (4 ml) under an atmosphere of hydrogen for 1.25 h, when t.l.c. (methanol:ethyl acetate, 1:10) showed no starting material (R_f 0.3) and one product (baseline). The reaction mixture was filtered and the solvent evaporated to give 2,7-anhydro-1,2,6-trideoxy-2,6-imino- β -L-*gulo*-heptulopyranose (3.1) (45 mg, 0.26 mmol, 69% yield) as an unstable yellow solid. M.p. 99-102 °C (dec.); $[\alpha]_D^{25}$ -8.9 (*c*, 0.94 in H₂O); $\nu_{\max}$ (KBr): 3401, 3256 (OH), 1722 (weak, C=N) cm⁻¹; δ_H (D₂O): 1.26 (2.8H, s, CH₃), 1.85 (0.2H, d, *J* 1.7 Hz), 3.30-3.49 (4H, m), 3.52-3.57 (1H, m), 3.70 (1H, d, *J* 8.0 Hz); δ_C (D₂O, 125 MHz, pH 9): Form a (major component); 21.0 (q C-1), 66.2 (t, C-7), 59.8, 71.8, 72.7, 74.4 (4 x d, C-3, C-4, C-5, C-6), 97.6 (s, C-2); Form b (minor component); 19.8 (q, C-1), 67.6 (t, C-7), 60.1, 73.7, 77.0 (3 x d, C-3, C-4, C-5, C-6); *m/z* (NH₃, CI): 176 (M+H⁺, 44%), 158 (M+H⁺-H₂O, 89%), 140 (M+H⁺-2H₂O, 100%).

3.4 References.

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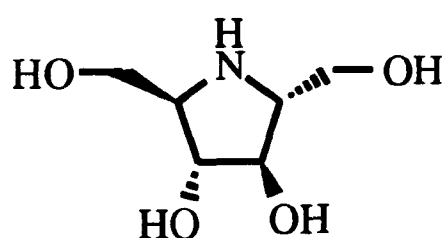
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14. Copies of the proton NMR spectra of the original samples of α -L-homofuconojirimycin were kindly provided by Glaxo Research and Development, Greenford, Middlesex.
15. The enzyme inhibition studies were performed by Dr. B. G. Winchester, Institute of Child Health, University of London, 31 Guilford Street, London.

Chapter 4

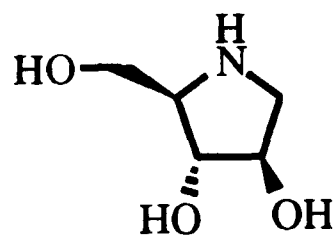
The Synthesis of Two New Pyrrolidine Amino Sugars.

4.1 Introduction.

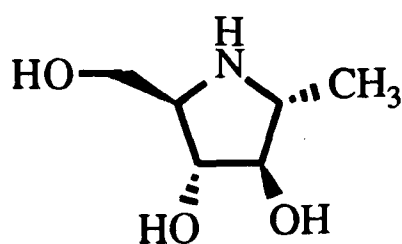
In contrast to the large number of polyhydroxylated piperidine natural products so far reported, there have been relatively few polyhydroxylated pyrrolidines isolated from nature.¹ In fact, only four simple polyhydroxylated pyrrolidines have so far been isolated from plants, although a large number have been produced synthetically (Figure 1).



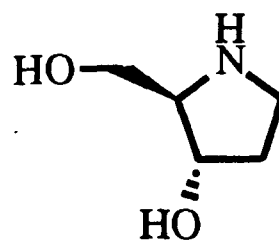
DMDP
(4.1)



DAB1
(4.2)



6-Deoxy-DMDP
(1.14)



CYB3
(4.3)

Figure 1

DMDP (4.1)² strongly inhibits human liver α - and β -D-glucosidase and β -D-mannosidase.³ DAB1 (4.3)⁴ is a powerful inhibitor of a range of α -D-glucosidases,⁵ and also shows activity against some mouse gut

disaccharidases.⁶ Both of these compounds have also been demonstrated to display insect antifeedant activity.⁷ As yet, no significant biological activity has been reported for CYB3 (4.3).⁸ The fourth compound, 6-deoxy-DMDP (1.14),⁹ has already been mentioned in chapter 1 in its role as a fucosidase inhibitor (K_i 2.2×10^{-5} M).¹⁰

DMDP (4.1) is numbered as a pyrrolidine derivative, that is the nitrogen is numbered 1 and the ring carbons 2 to 5. DMDP stands for 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydropyrrolidine. The compound (1.14) can therefore be regarded as 6-deoxy-DMDP. Alternatively, as the pattern of chiral centres in the molecule corresponds to D-mannose (1.10), it can also be named as a mannitol derivative, 1,2,5-trideoxy-2,5-imino-D-mannitol.

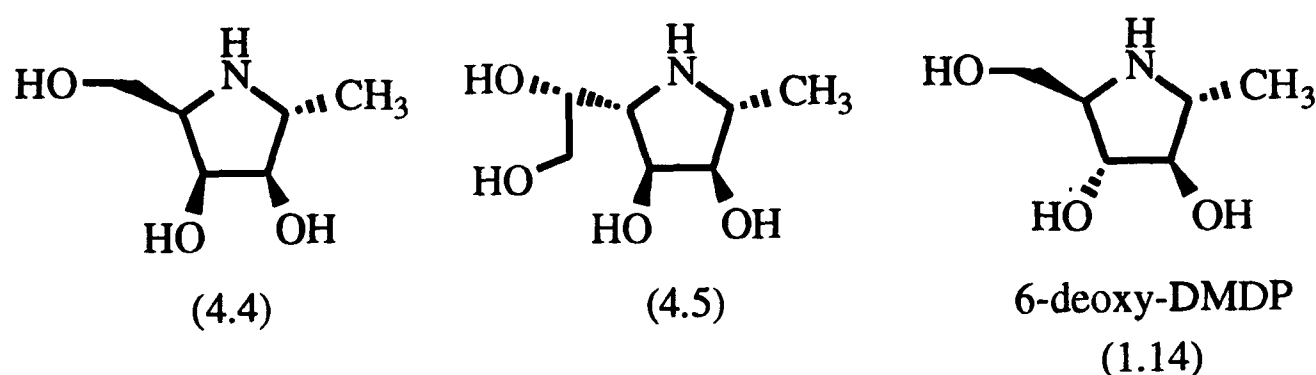


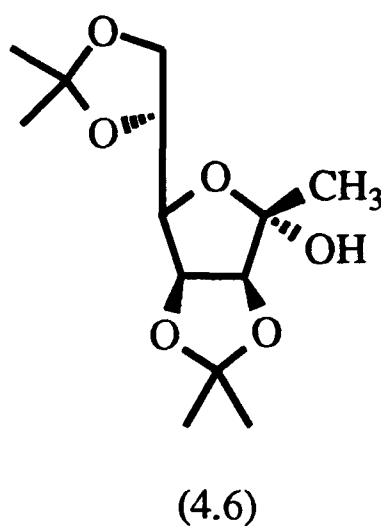
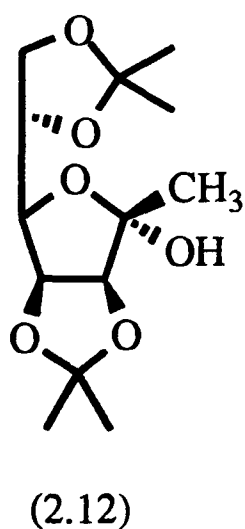
Figure 2

Some of the intermediates utilized in the synthesis of a previous target, (2.1)¹¹ in chapter 2, were also used to synthesise two new pyrrolidine amino sugars (4.4) and (4.5), analogues of 6-deoxy-DMDP (1.14) (Figure 2). The synthesis of 6-deoxy-DMDP (1.14) itself, and of a number of its stereoisomers, was discussed in chapter 1.^{9,10}

4.2 Results and Discussion.

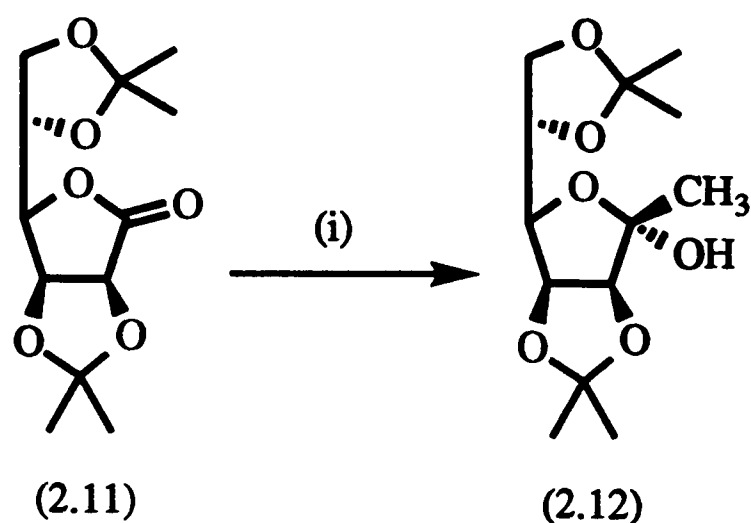
The work will be discussed in the following four parts:

- i. Investigations into the reduction of 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -*L*-*gulo*-heptulofuranose (2.12).
- ii. The synthesis of the amino sugars (4.4) and (4.5).
- iii. Investigations into the reduction of 1-deoxy-3,4;6,7-di-*O*-isopropylidene- α -*D*-*manno*-heptulofuranose (4.6).
- iv. The evaluation of the amino sugars (4.4) and (4.5) as glycosidase inhibitors.



i. Investigations into the reduction of 1-deoxy-3,4;6,7-di-*O*-isopropylidene- β -*L*-*gulo*-heptulofuranose (2.12).

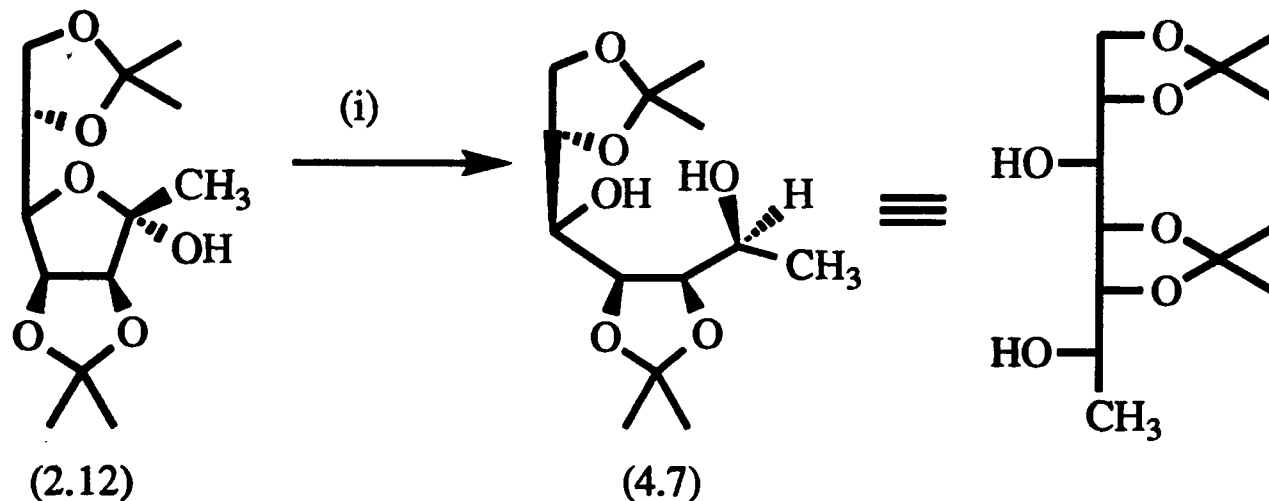
The keto-sugar (2.12) was the only product of the addition of methyllithium to the diacetonide of *L*-gulono-1,4-lactone (2.11) (Scheme 1).¹¹



(i) MeLi, THF, -70 °C, 89%.

Scheme 1

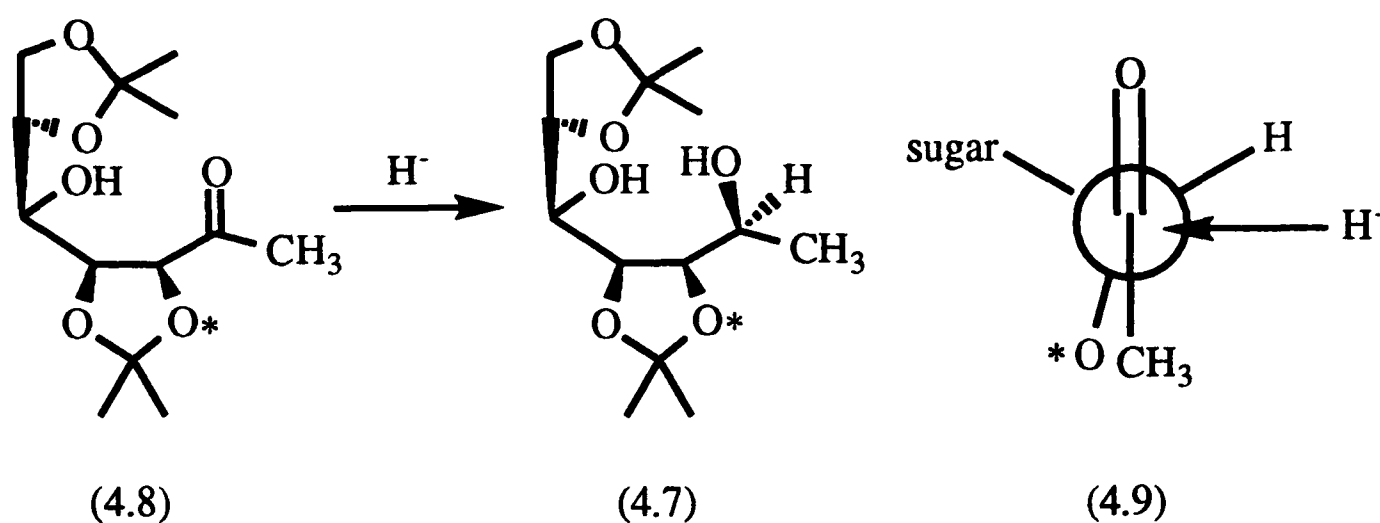
This keto-sugar was reduced by sodium borohydride in ethanol to give a single product, the heptitol (4.7). None of the other possible epimeric product was observed (Scheme 2). This heptitol (4.7) was the starting material for the synthesis of the two amino sugars (4.4) and (4.5).



(i) NaBH₄, EtOH, 89%.

Scheme 2

The reduction must proceed via the open chain ketone (4.8). The stereochemistry of the reduction¹² is that which is predicted by Cram's¹³ rule, where the electronegative α -oxygen substituent in the transition state (4.9) is *trans* to the carbonyl group.¹⁴ The hydride ion then approaches from the less hindered face of the ketone (Scheme 3).



Scheme 3

Alternatively, a Felkin¹⁵ type transition state (4.10) may be invoked to explain the stereochemistry of the reduction. The reaction is postulated to go via the conformation (4.10) which has the most electronegative α -substituent perpendicular to the carbonyl group and which also minimises the other steric interactions. Attack of the hydride ion is again from the less hindered face of the carbonyl group (Figure 3).

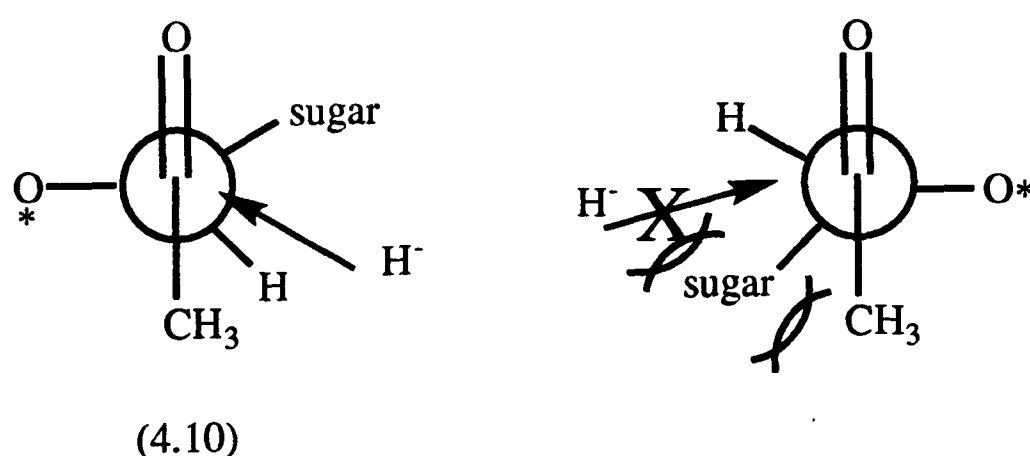
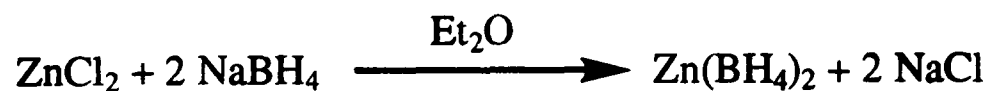


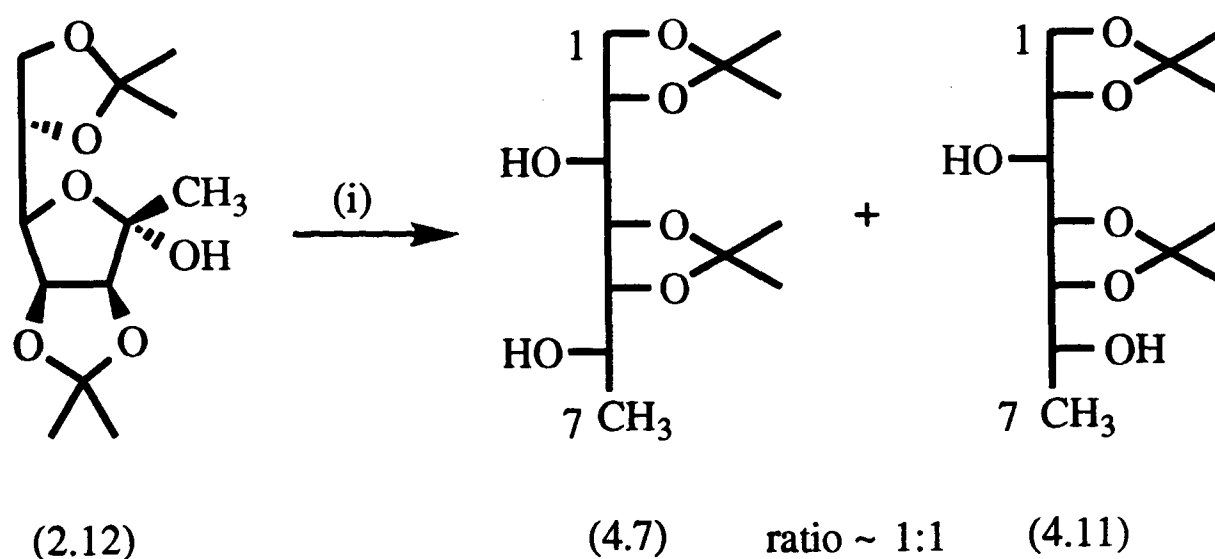
Figure 3

In both the above models, α -chelation was not invoked, as this would lead to the alternative, unobserved product. For comparison, the same reduction was attempted using zinc borohydride as the reducing agent. Zinc is a highly chelating metal ion, and reductions with zinc borohydride often exhibit chelation control.¹² The zinc borohydride was synthesised as a solution in diethyl ether following a literature procedure (Scheme 4).¹⁶



Scheme 4

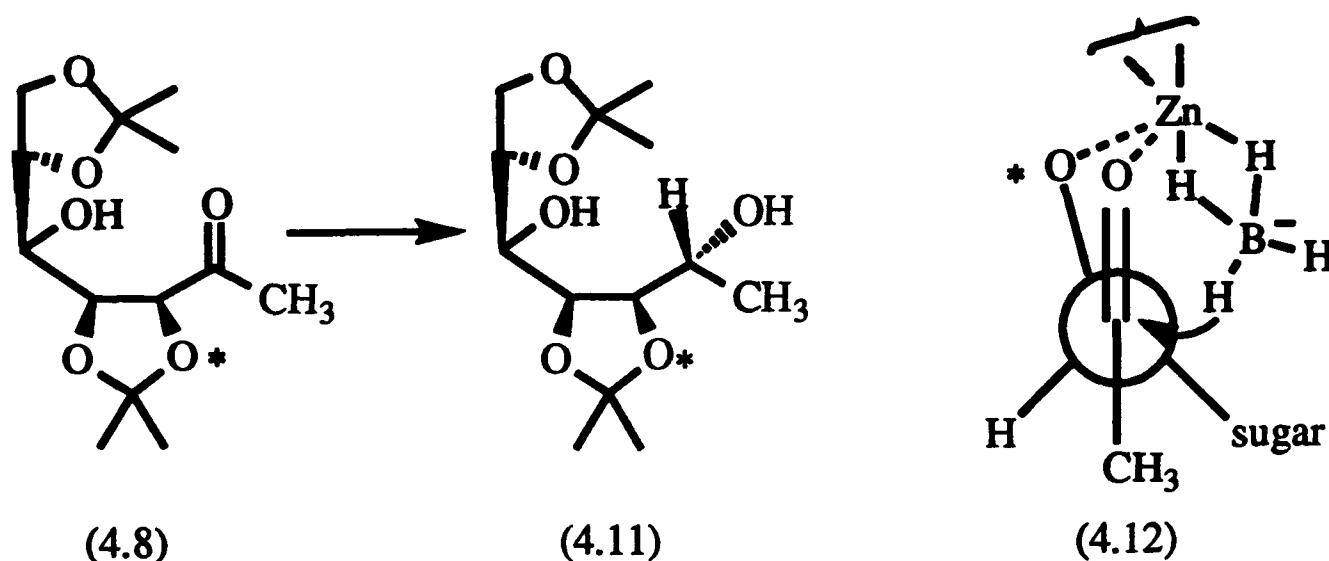
When the keto-sugar (2.12) was reduced using the zinc borohydride solution, a mixture of two products (4.7) and (4.11) was obtained (Scheme 5).



(i) $\text{Zn(BH}_4)_2$, Et_2O , 64% combined yield.

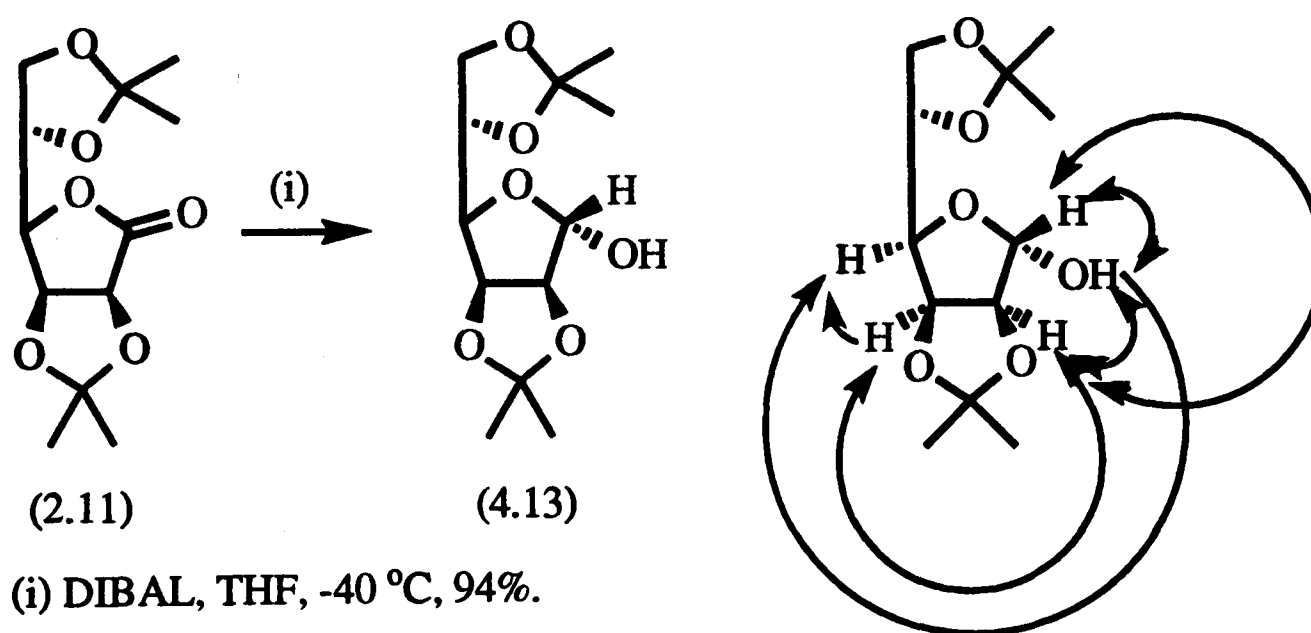
Scheme 5

From the proton NMR of the product mixture, it appeared that they were present in a ratio of approximately 1:1. They were difficult to separate by flash chromatography but a small amount of each was obtained pure. Both were heptitols and the more polar product was identical to the product (4.7) of the sodium borohydride reduction. The less polar product was therefore assigned as its C-6 epimer (4.11). This was the expected product if an α -chelated transition state (4.12) was involved (Scheme 6).¹²



Scheme 6

The addition of methyllithium to furanose sugars is well documented in the literature, and such reactions are usually highly stereoselective.^{17,18,19} It was therefore of interest to investigate whether the addition of methyllithium to diacetone-L-gulose (4.13) would give the same product (4.7) as the sodium borohydride reduction above. The diacetone of L-gulono-1,4-lactone (2.11) was reduced by treatment with diisobutylaluminium hydride in tetrahydrofuran at $-40\text{ }^{\circ}\text{C}$ to give the lactol (4.13)²⁰ in 94% yield (Scheme 7).



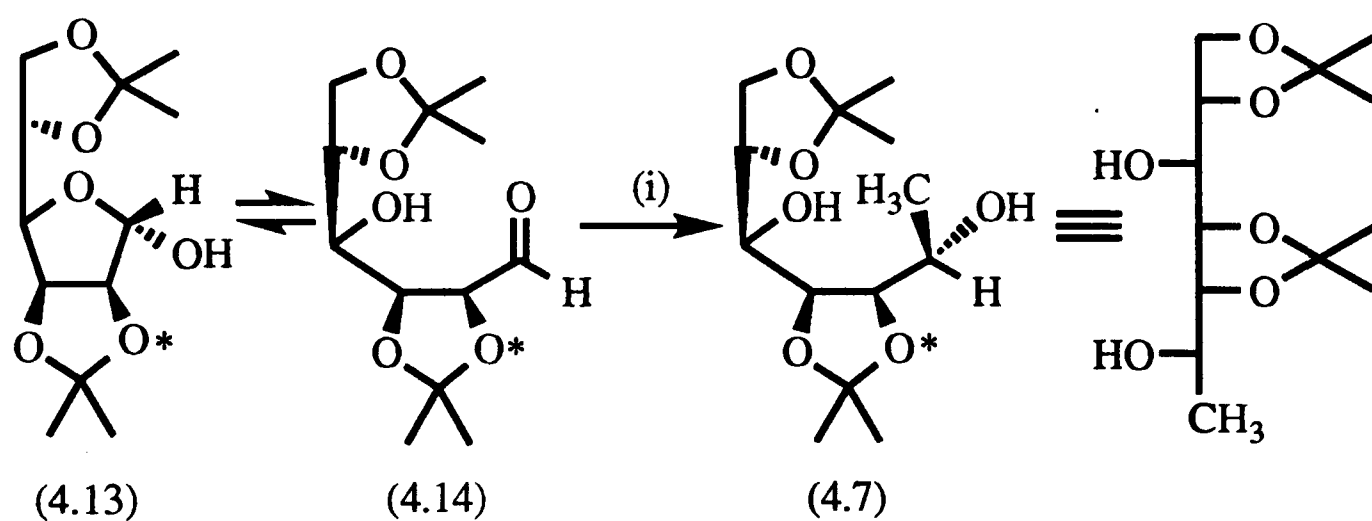
Scheme 7

Observed nOe enhancements

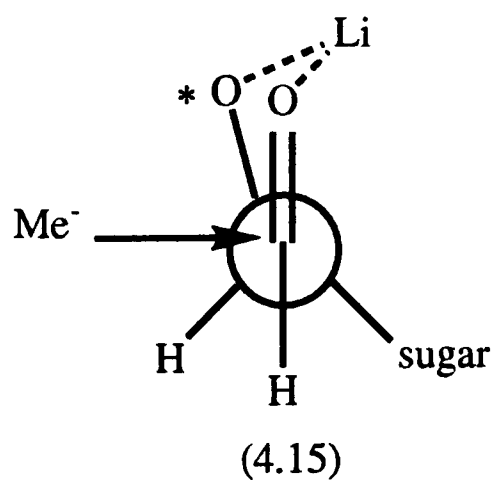
Only one anomer is produced in this reaction and its configuration was assigned as β - on the basis of equilibrium nOe measurements in d_6 -DMSO. As for the keto-sugars (1.27), (2.12) and (2.13), large nOe enhancements between the

hydroxyl proton and the ring protons were observed, implying that they were all on the same face of the furanose ring. The product is therefore that of formal attack of hydride ion onto the more hindered face of the lactone carbonyl group.

Diacetone-L-gulose (4.13) was reacted with methyllithium in tetrahydrofuran at 0 °C. Two equivalents of the methyllithium were required as the first one acted as a base and deprotonated the free hydroxyl group in the starting material. The reaction did not go to completion and some starting material (28%) was recovered. The product (4.7) was the same compound as that obtained from the sodium borohydride reduction of the keto-sugar (2.12) above. The reaction must go via the open chain aldehyde (4.14) and the product is that of *syn* addition, as expected from a Cram type α -chelated transition state (4.15) (Scheme 8).¹³

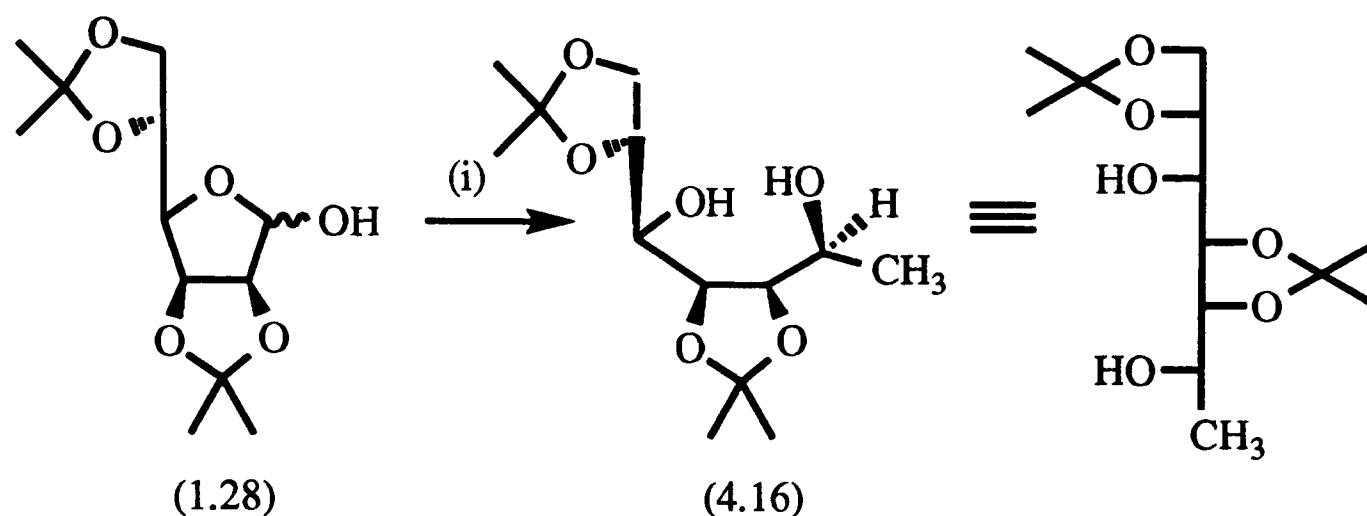


(i) MeLi, THF, 0 °C, 53%.



Scheme 8

This result agrees with that reported for the addition of methyllithium to diacetone mannose (1.28), in which the *syn* product (4.16) was the only one observed (Scheme 9).¹⁸



(i) MeLi, THF, 0 °C, 99%.

Scheme 9

It therefore makes no difference as to the heptitol obtained which way round the reduction and the methyllithium addition are performed on the diacetone of L-gulono-1,4-lactone (2.11).

ii. The synthesis of the amino sugars (4.4) and (4.5).

Fischer projections of the heptitol (4.7) and of the target (4.5) are shown below. It can be seen that introduction of nitrogen to link C-3 and C-6 of the heptitol, with inversion of configuration at both centres, will give the target framework (Figure 4). Both targets (4.4) and (4.5) may then be formed by elaboration of this framework.

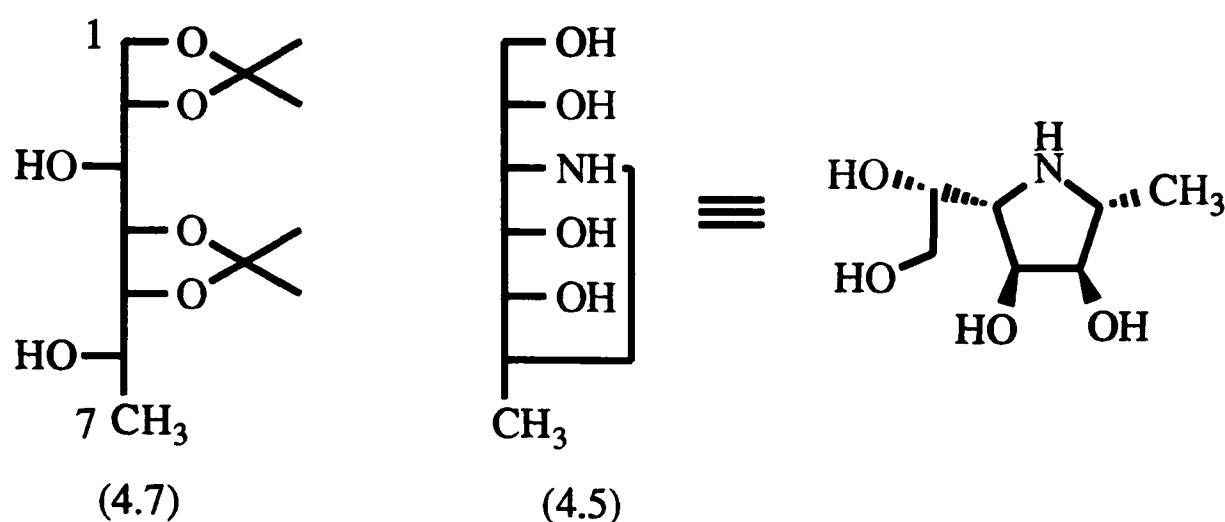
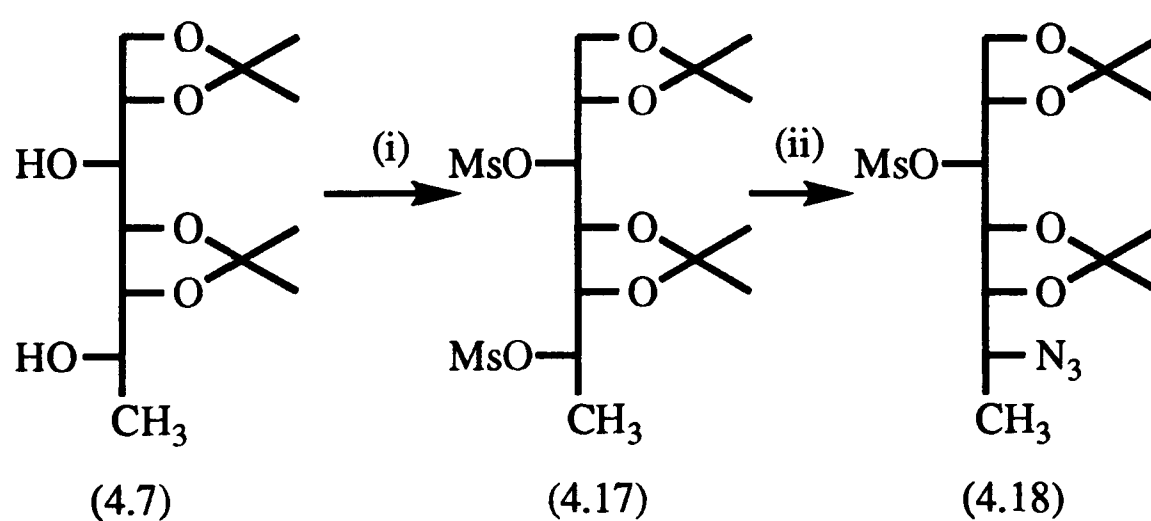


Figure 4

As C-3 and C-6 were the only unprotected hydroxyl groups in the heptitol (4.7), they were converted directly into leaving groups.²¹ Reaction with excess mesyl chloride in pyridine with a catalytic amount of 4-(*N,N*-dimethylamino)-pyridine at 0 °C gave the dimesylate (4.17) in 80% yield. Warming the dimesylate (4.17) in *N,N*-dimethylformamide with one equivalent of sodium azide led to displacement of only the C-6 mesylate group (Scheme 10).

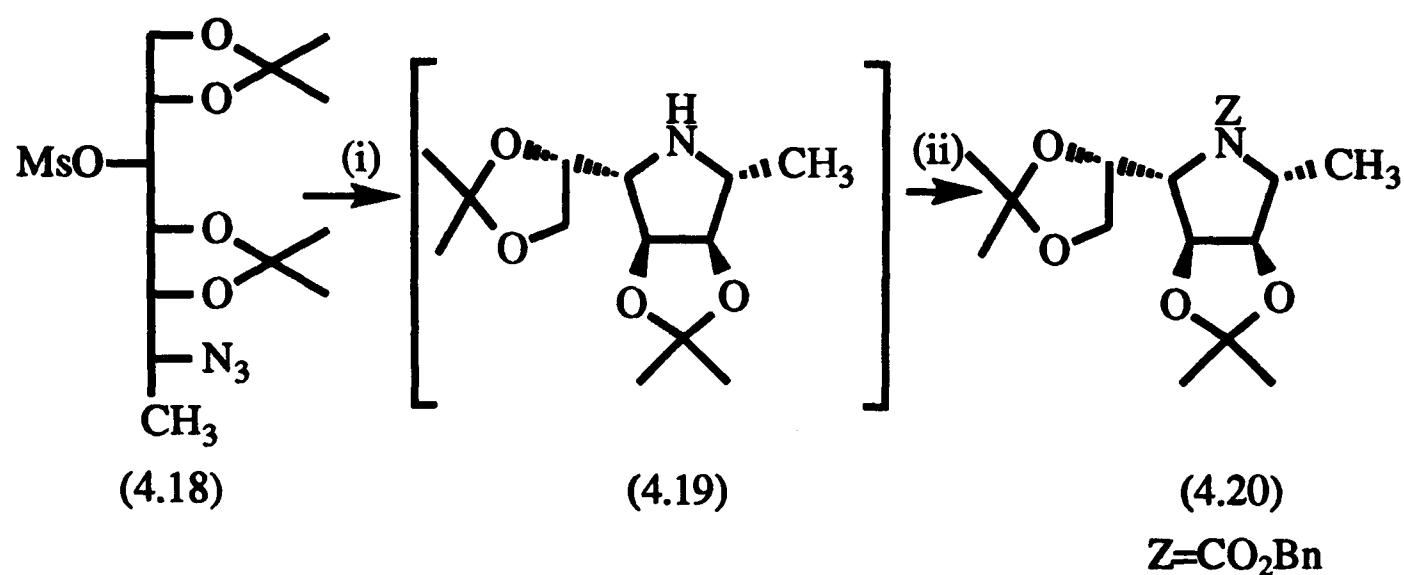


(i) MsCl, DMAP, py, 0 °C, 80%, (ii) NaN₃, DMF, 80 °C, 65%.

Scheme 10

The azide functionality in compound (4.18) was reduced to an amine by catalytic hydrogenation over palladium black in ethanol. The resultant primary amine cyclised onto the remaining mesylate group *in situ* to give a secondary amine (4.19). One equivalent of sodium acetate was added to the reduction to neutralise the methanesulphonic acid thus generated. The secondary amine (4.19) was not

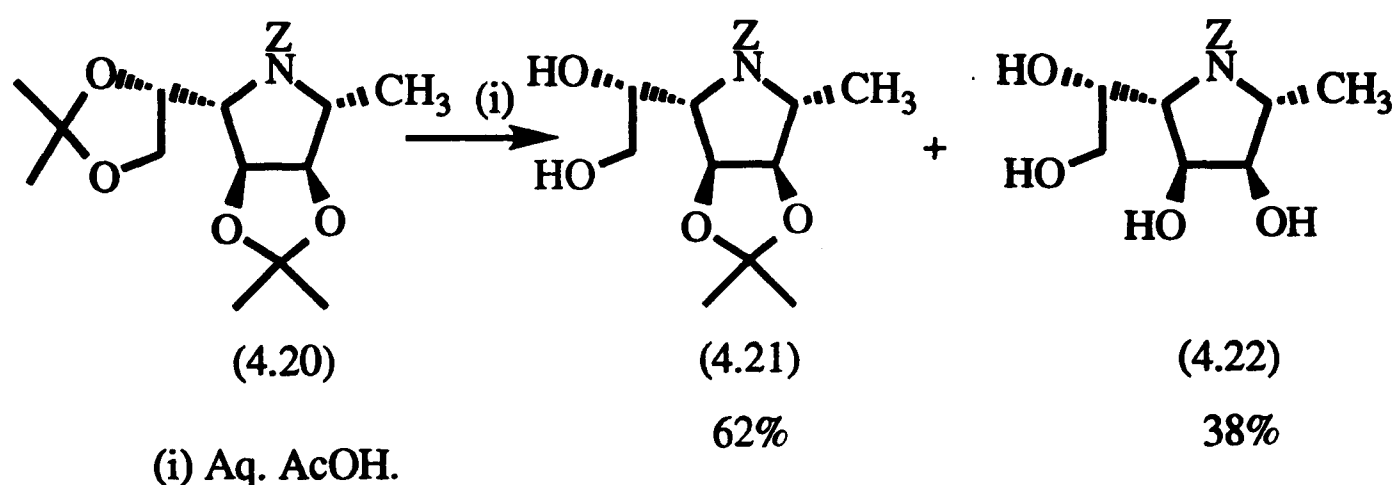
isolated but was immediately protected as its Z (Z=CO₂Bn) derivative (4.20) to facilitate handling and purification (Scheme 11). However, the Z group complicated the NMR spectra of the protected compounds. Restricted rotation about the amide bond gave rise to signals for two conformers in the NMR of a number of the protected compounds.²²



(i) H₂, Pd-black, NaOAc, EtOH, (ii) ZCl, Et₂O, aq. NaHCO₃, 78% over 2 steps.

Scheme 11

The target framework had therefore been synthesised and it now required elaboration to the two amino sugars. Hydrolysis of the fully protected amine (4.20) with aqueous acetic acid gave a mixture of the diol (4.21) and the tetrol (4.22). These were readily separated by flash chromatography (Scheme 12).



Scheme 12

The structure of the tetrol (4.22) was confirmed by single crystal X-ray analysis (Figure 5, Appendix 3).²³ This in turn confirmed the structure of the heptitol (4.7), and thus the stereochemistry of the sodium borohydride reduction of the keto-sugar (2.12).

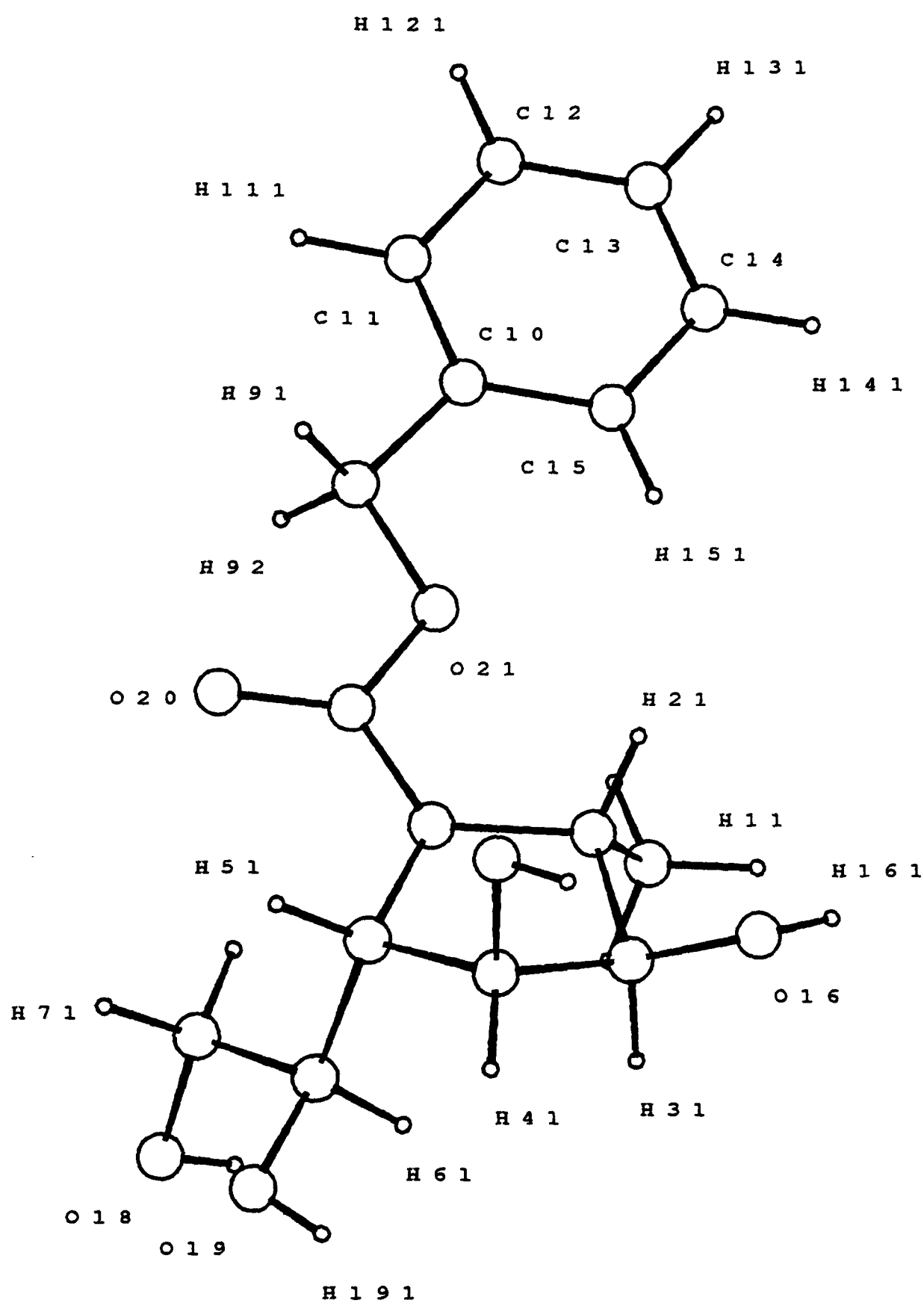
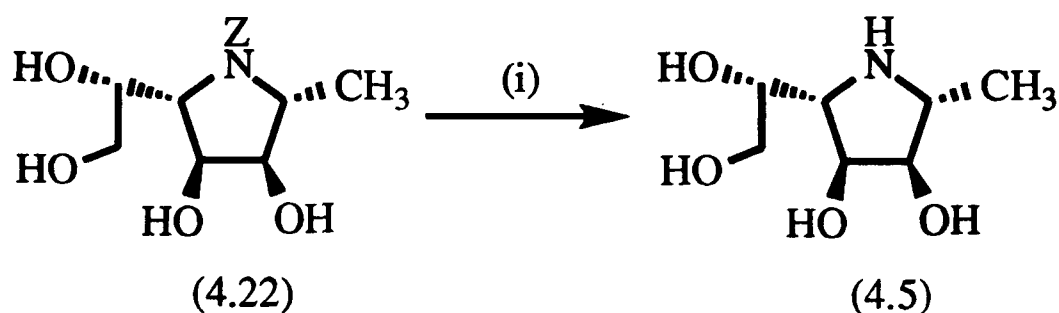


Figure 5

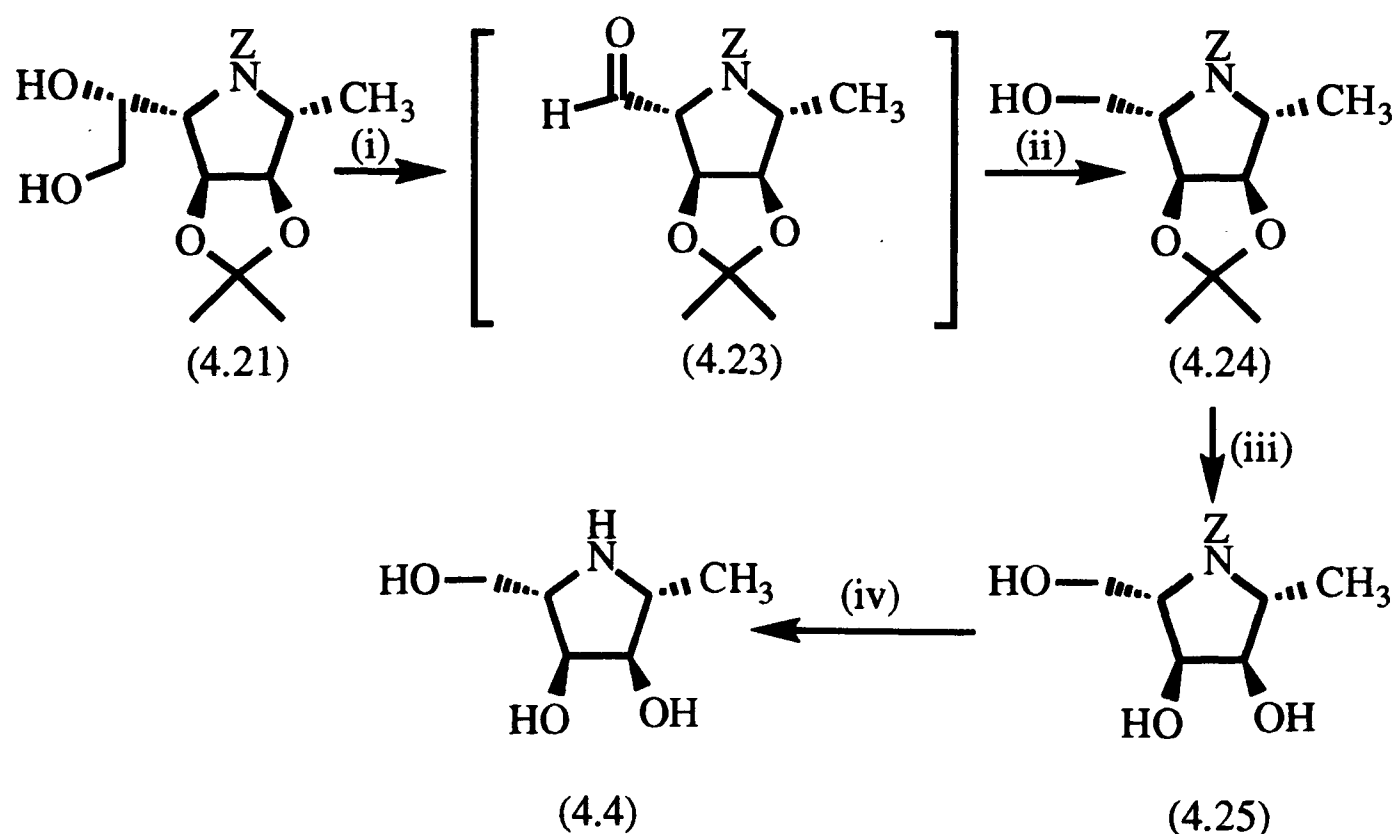
The Z protecting group was removed from the tetrol (4.22) in quantitative yield by catalytic hydrogenation to give the first target amino sugar (4.5) (Scheme 13).



(i) H₂, Pd-black, EtOH, quant.

Scheme 13

Further elaboration of the diol (4.21) was required to give the second target compound (4.4). The exposed diol unit was cleaved with periodic acid in tetrahydrofuran to give an aldehyde (4.23). This aldehyde was not isolated but was reduced immediately with sodium borohydride in ethanol to give the alcohol (4.24). The remaining acetonide was hydrolysed with aqueous trifluoroacetic in 65% yield, and the Z protecting group removed by catalytic hydrogenation to give the second target amino sugar (4.4) (Scheme 14).



(i) H₅IO₆, THF, (ii) NaBH₄, EtOH, 94% over 2 steps,
(iii) aq. TFA, 65%, (iv) H₂, Pd-black, EtOH, quant.

Scheme 14

iii. Investigations into the reduction of 1-deoxy-3,4:6,7-di-*O*-isopropylidene- α -D-manno-heptulofuranose (4.6).

It was originally envisaged that similar chemistry to that described above could be carried out on the system derived from diacetone mannose (1.28), to yield a third pyrrolidine amino sugar (4.26). As D-mannose is epimeric to L-gulose at C-5, the target would be epimeric to that (4.5) derived from L-gulonolactone (2.10) (Figure 6).

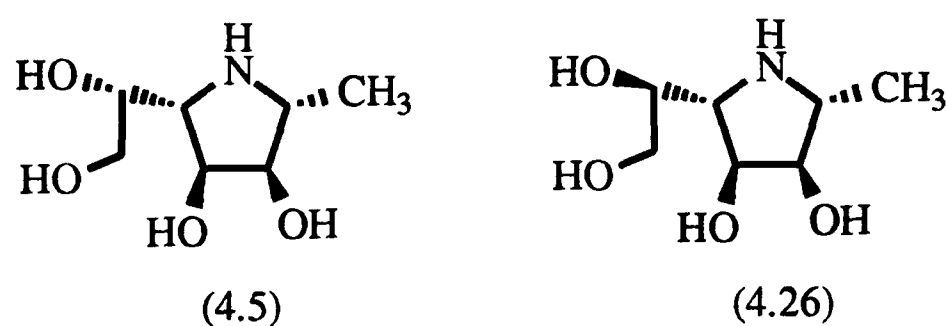
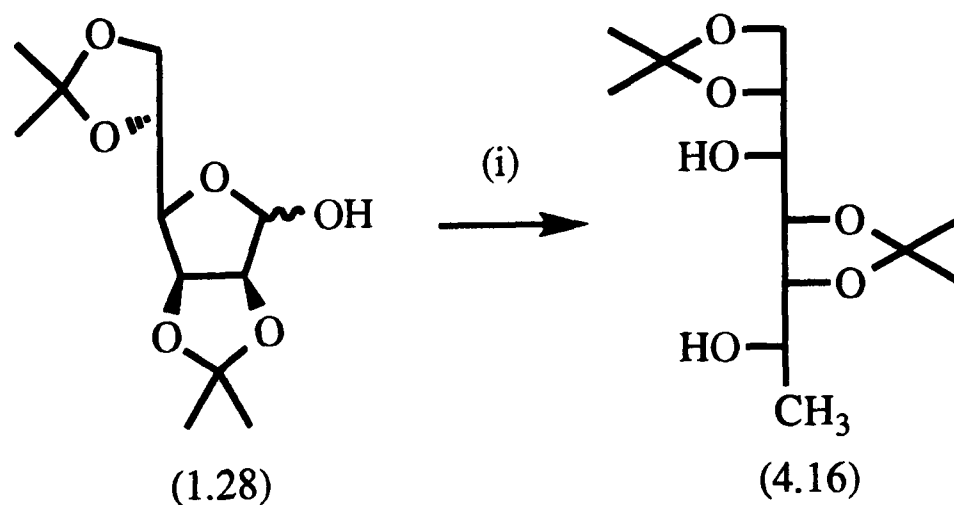


Figure 6

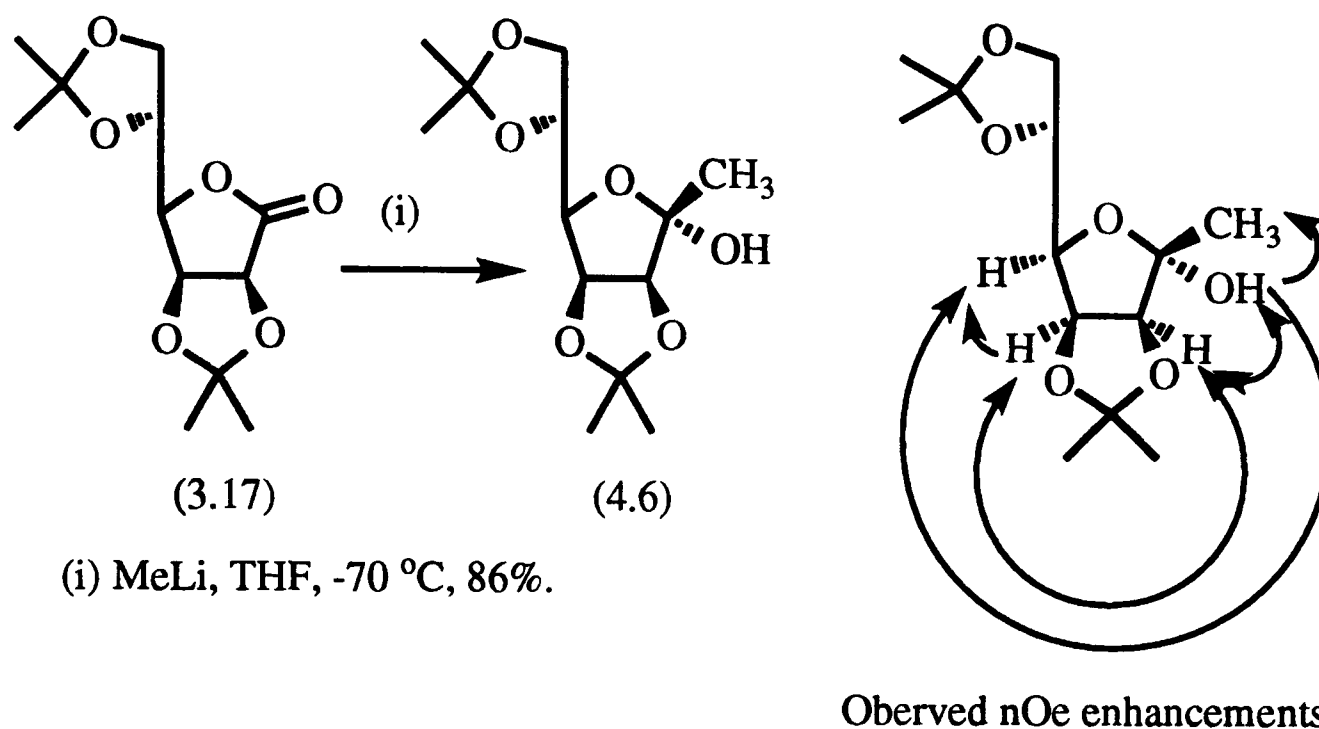
As reported in the literature,^{17,18} the addition of methyllithium to diacetone mannose (1.28) gave one major product, the heptitol (4.16).



(i) MeLi, THF, 0 °C, 67%.

Scheme 15

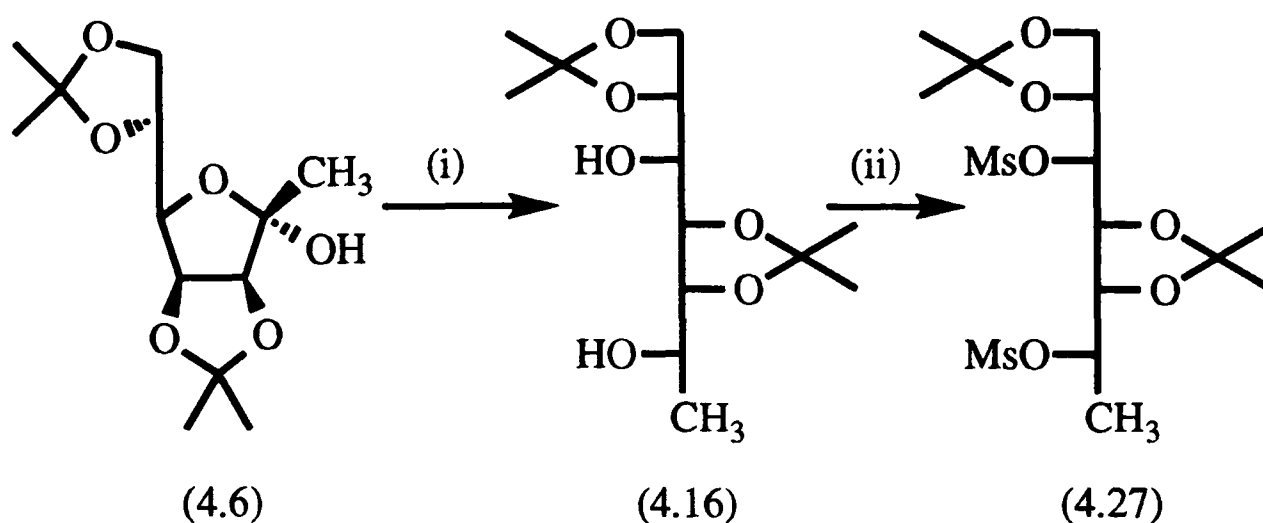
This heptitol could also be formed via the keto-sugar (4.6), in a route analogous to that employed for the amino sugars (4.4) and (4.5). Methyl lithium was added to the diacetone of D-mannonolactone (3.17)²⁴ to give the keto-sugar (4.6)²⁵ (Scheme 16).



Scheme 16

As in the L-gulonolactone case (2.12), only one anomer was isolated from the reaction and its configuration was determined by equilibrium nOe measurements. In the proton NMR in d_6 -DMSO, large nOe enhancements were observed between the hydroxyl proton and the ring protons, indicating that they were on the same face of the furanose ring. This reaction had been reported,²⁵ on the basis of hydrogen bonding observed between the anomeric hydroxyl group and the vicinal oxygen, as giving the other anomer. However, the alternative configuration is not consistent with the observed nOe results. The product therefore appears to be that of formal attack from the more hindered face of the lactone carbonyl group. This result was in agreement with the results observed for the addition of methyl lithium to a number of other lactones reported in this thesis.

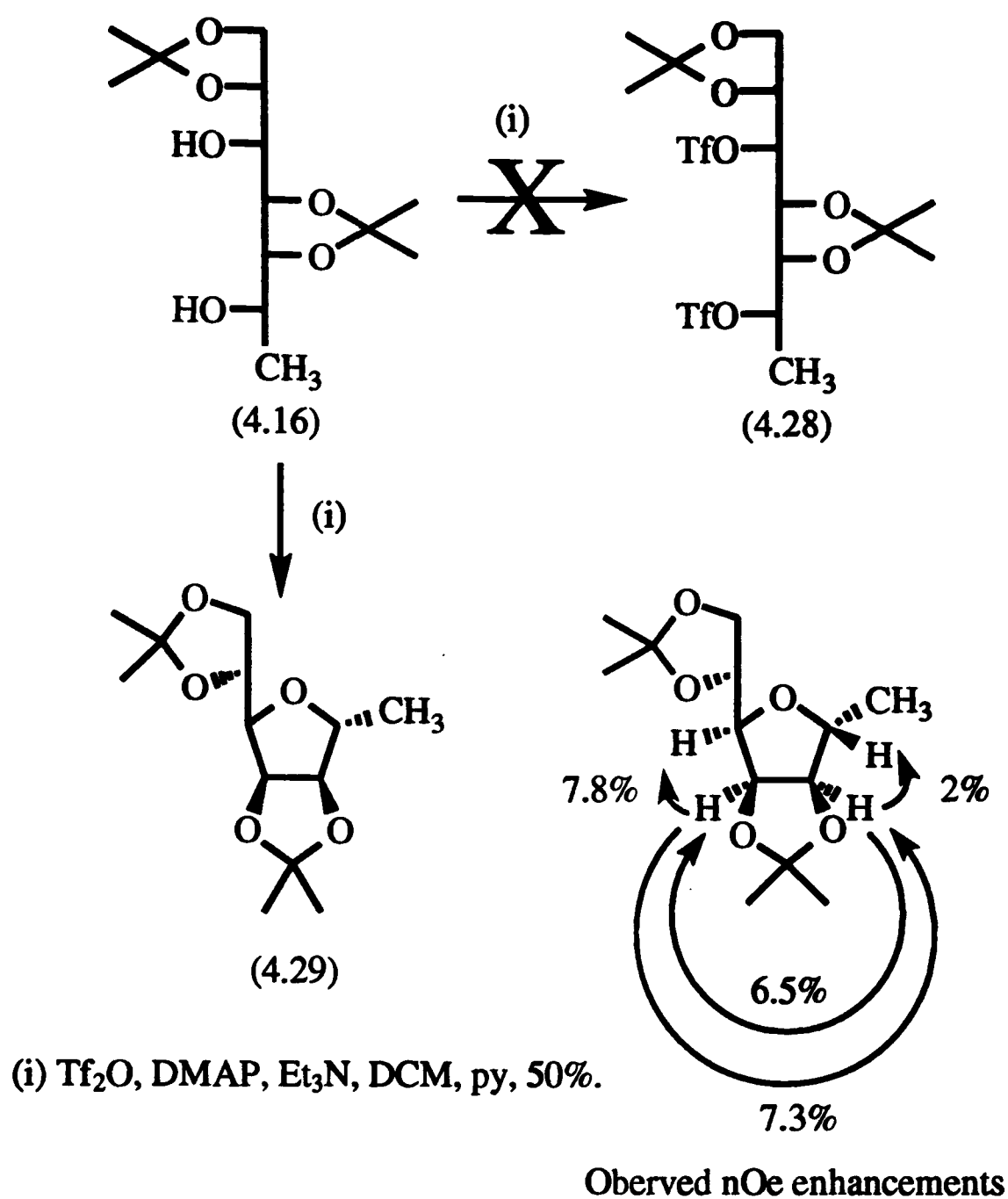
The keto-sugar (4.6) was reduced by sodium borohydride in ethanol to give a heptitol (4.16) which was identical to that produced by the addition of methyl lithium to diacetone mannose (1.28) (Scheme 17).



(i) NaBH_4 , EtOH, 71%, (ii) MsCl, Et_3N , DMAP, DCM, 72%.

Scheme 17

Both the free hydroxyl groups in the heptitol (4.16) were converted to leaving groups on treatment with mesyl chloride, 4-(*N,N*-dimethylamino)-pyridine and triethylamine in dichloromethane. However, attempts to displace one or both of these mesylate groups from the dimesylate (4.27) with nitrogen nucleophiles were unsuccessful. Heating with sodium azide in dimethylformamide or in butanone lead to either recovery of the starting material (4.27) or decomposition. Heating of the dimesylate (4.27) with benzylamine caused complete decomposition. As the mesylate groups appeared to be resistant to nucleophilic displacement, attempts were made to synthesise the ditriflate (4.28), as triflates are much better leaving groups than mesylates. However, the only product isolated from the attempted triflation was the tetrahydrofuran derivative (4.29), even when a large excess of triflic anhydride was employed. This was also the product if the heptitol (4.16) was treated with only one equivalent of triflic anhydride. It arises from formation of the triflate ester at the hydroxyl group α - to the methyl group, followed by intramolecular nucleophilic displacement by the remaining free hydroxyl group to give the tetrahydrofuran (4.29). The stereochemistry was confirmed by equilibrium nOe measurements, which indicated that the methyl group was *trans* to the acetonide and the side chain (Scheme 18).



Scheme 18

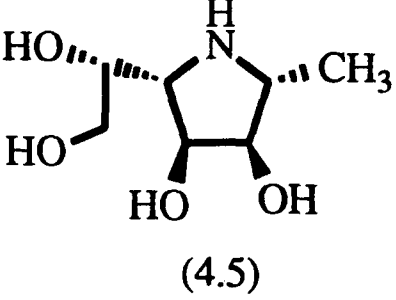
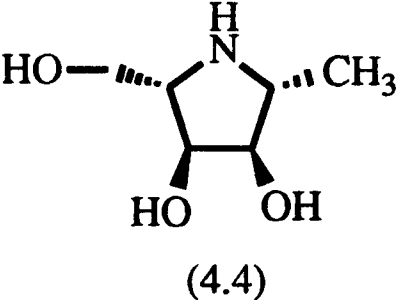
It was therefore concluded that the synthesis of a third amino sugar via this route was not practicable.

iv. Evaluation of the amino sugars (4.4) and (4.5) as glycosidase inhibitors.

The effect of the target compounds (4.4) and (4.5) on a range of human liver glycosidases was investigated, and the results are shown in the table below.²⁶ The data show the percentage inhibition of the enzymes at a 1×10^{-3} M concentration of the amino sugars. As can be seen, both compounds do exhibit some activity against α -fucosidase. However, they are not very potent inhibitors

nor very selective, with both compounds exhibiting some β -galactosidase inhibition in addition to their α -fucosidase activity.

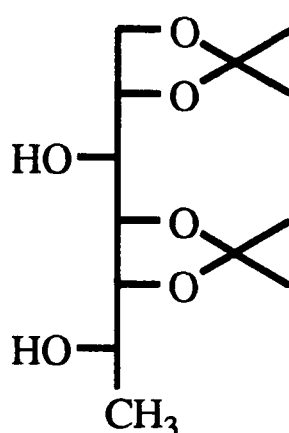
Inhibition (%) at 1 mM
(a negative value signifies inhibition)

Enzyme	 (4.5)	 (4.4)
α -mannosidase pH 4	-3	-19
α -mannosidase pH 6.5	-17	-14
β -mannosidase	+2	+7
α -glucosidase	+6	+14
β -glucosidase	+32	+37
α -galactosidase	+4	-2
β -galactosidase	-49	-41
α -fucosidase	-66	-55
β -hexosaminidase	+25	+50
β -glucuronidase	-1	+12
β -xylosidase	+3	-9
α -arabinosidase	+2	-25

4.3 Experimental.

For the general experimental details see page 46.

7-Deoxy-1,2;4,5-di-*O*-isopropylidene-*L*-glycero-*D*-gluco-heptitol (4.7).



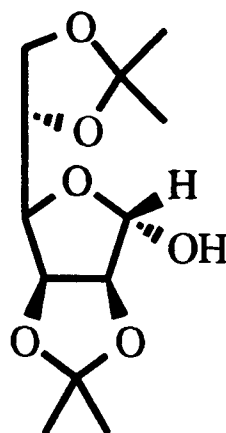
Method 1.

1-Deoxy-3,4;6,7-di-*O*-isopropylidene-*L*-*gulo*-heptulofuranose (2.12) (2.36 g, 8.6 mmol) was dissolved in ethanol (26 ml) at room temperature under nitrogen. Sodium borohydride (489 mg, 12.9 mmol, 1.5 mol equivalents) was added and the reaction was left to stir for 16 h by which time t.l.c. (diethyl ether) showed no starting material (R_f 0.3) and one major product (R_f 0.25). The reaction was quenched by the addition of ammonium chloride, stirred until effervescence ceased and then filtered through a short pad of Celite, eluting with ethyl acetate. The solvents were evaporated and the crude residue purified by flash chromatography (diethyl ether:hexane, 4:1 then diethyl ether) to yield 7-*deoxy*-1,2;4,5-di-*O*-isopropylidene-*L*-glycero-*D*-gluco-heptitol (4.7) (2.09 g, 7.6 mmol, 89% yield) as a colourless gum. $[\alpha]_D^{20}$ -8.2 (c , 1.35 in CHCl_3); ν_{max} (neat): 3401 (br, OH) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.30 (3H, d, C-7, 3J 6.4 Hz), 1.39 (6H, s, 2 x CH_3), 1.46, 1.57 (2 x 3H, 2 x s, 2 x CH_3), 3.81 (1H, dd, H-3, $J_{3,2}$ 6.4 Hz, $J_{3,4}$ 1.6 Hz), 3.87 (1H, dd, H-1, $J_{1,1'}$ 8.4 Hz, $J_{1,2}$ 6.7 Hz), 4.02 (1H, dd, H-5,

$J_{5,4}$ 7.2 Hz, $J_{5,6}$ 2.8 Hz), 4.06 (1H, dq, H-6, J 6.4 Hz, $J_{6,5}$ 2.7 Hz), 4.08 (1H, dd, H-1', $J_{1',1}$ 8.3 Hz, $J_{1',2}$ 6.5 Hz), 4.10 (1H, dd, H-4, $J_{4,5}$ 7.2 Hz, $J_{4,3}$ 1.6 Hz), 4.31 (1H, q, H-2, J 6.5 Hz); δ_C (CDCl₃): 20.5 (q, C-7), 24.8, 25.1, 26.2, 26.5 (4 x q, 2 x (CH₃)₂C), 65.9 (t, C-1), 65.4, 69.8, 75.8, 77.0, 80.9 (5 x d, C-2, C-3, C-4, C-5, C-6), 108.8, 109.7 (2 x s, 2 x (CH₃)₂C); m/z (NH₃, CI): 294 (M+NH₄⁺, 22%), 277 (M+H⁺, 77%), 219 (M+H⁺-acetone, 79%), 201 (M+NH₄⁺-acetone, 100%). (Found C, 56.59; H, 9.04. C₁₂H₂₄O₆ requires C, 56.51; H, 8.75%).

Method 2.

2,3;5,6-Di-*O*-isopropylidene- β -L-gulofuranose (4.13) (164 mg, 0.63 mmol) was dissolved in tetrahydrofuran (3 ml) and cooled to -50 °C. Methyllithium (1.4 M in diethyl ether, 1.04 ml, 1.45 mmol, 2.3 mol equivalents) was added and the reaction was allowed to warm to 0 °C over 2 h. After a further 6 h stirring at 0 °C, t.l.c. (diethyl ether) showed some starting material (R_f 0.4) and one product (R_f 0.3). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution and the solvents were evaporated. The residue was partitioned between water (30 ml) and ethyl acetate (50 ml). The aqueous layer was washed with ethyl acetate (2 x 30 ml) and the combined ethyl acetate extracts were dried (magnesium sulphate), filtered and evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:1 then 4:1 then diethyl ether) to give 7-deoxy-1,2;4,5-di-*O*-isopropylidene-L-*glycero*-D-*gluco*-heptitol (4.7) (93 mg, 0.34 mmol, 53% yield) as a clear gum, identical to that reported above. Some starting material (46 mg, 0.17 mmol, 28%) was also recovered.

2,3;5,6-Di-*O*-isopropylidene- β -L-gulofuranose (4.13).

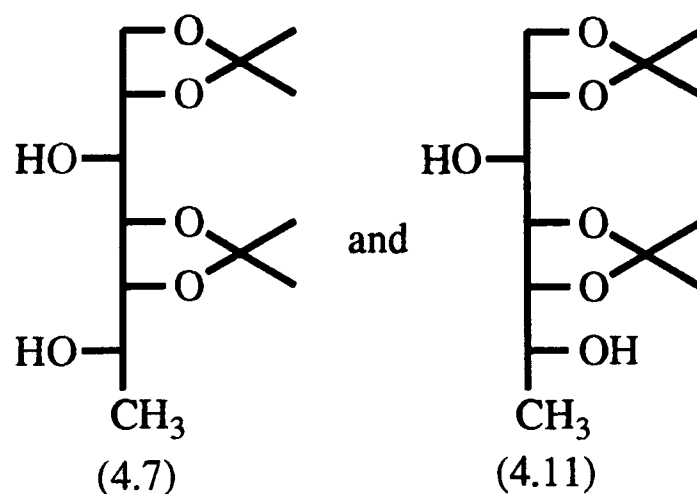
2,3;5,6-Di-*O*-isopropylidene-L-gulono-1,4-lactone (2.11) (311 mg, 1.2 mmol) was dissolved in tetrahydrofuran (5 ml) and the solution cooled to -40 °C. Diisobutylaluminium hydride (1.5 M in toluene, 1.0 ml, 1.45 mmol, 2 mol equivalents) was added dropwise and the reaction mixture was stirred at -40 °C. After 0.5 h, t.l.c. (diethyl ether:hexane, 4:1) showed no starting material (R_f 0.2) and a single product (R_f 0.3). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (2 ml). The reaction mixture was filtered through a silica plug topped with Celite, eluting with ethyl acetate. The solvents were evaporated and the resulting crude product was purified by flash chromatography (diethyl ether:hexane, 1:1) to give 2,3;5,6-di-*O*-isopropylidene- β -L-gulofuranose (4.13) (292 mg, 1.12 mmol, 94% yield) as a white crystalline solid. M.p. 116-118 °C (diethyl ether/hexane) [lit.²⁰ 119-120 °C (diethyl ether)]; $[\alpha]_D^{25} +1.36$ (c , 1.03 in CHCl_3) [lit.²⁰ +0.44, (c , 0.68 in CHCl_3)]; δ_H (CDCl_3): 1.29, 1.39 (2 x s, 2 x 3H, 2 x CH_3), 1.45 (s, 6H, 2 x CH_3), 3.72 (1H, dd, H-6, $J_{6,6'}$ 8.2 Hz, $J_{6,5}$ 7.2 Hz), 4.13 (1H, dd, H-4, $J_{4,5}$ 8.4 Hz, $J_{4,3}$ 3.6 Hz), 4.22 (1H, dd, H-6', $J_{6',6}$ 8.3 Hz, $J_{6',5}$ 6.5 Hz), 4.31-4.43 (1H, m, H-5), 4.63 (1H, d, H-2, $J_{2,3}$ 6.0 Hz), 4.70 (1H, dd, H-3, $J_{3,2}$ 5.9 Hz, $J_{3,4}$ 3.7 Hz), 5.46 (1H, s, H-1); δ_H (d_6 -DMSO, 250 MHz): 1.21, 1.27, 1.32, 1.35 (4 x s, 4 x 3H, 2 x $(\text{CH}_3)_2\text{C}$), 3.75 (1H, t, H-6, J 7.5 Hz), 3.95 (1H, dd, H-4, $J_{4,5}$ 7.5 Hz, $J_{4,3}$ 3.8 Hz), 4.10 (1H, dd, H-6', $J_{6',6}$ 8.1 Hz, $J_{6',5}$ 6.9 Hz), 4.15 (1H, q, H-5, J 7.5 Hz), 4.47 (1H, d, H-2,

$J_{2,3}$ 6.6 Hz), 4.76 (1H, dd, H-3, $J_{3,2}$ 6.3 Hz, $J_{3,4}$ 3.8 Hz), 5.19 (1H, s, H-1), 6.42 (1H, s, OH, D₂O exchanges).

Preparation of zinc borohydride-diethyl ether solution.

A solution of zinc borohydride in diethyl ether was prepared according to a literature procedure.¹⁶ Anhydrous zinc chloride (c. 5 g) was fused four times *in vacuo* and then freshly distilled diethyl ether (50 ml) was added at room temperature under nitrogen. The resulting suspension was heated at reflux for 2 h and then allowed to settle. The supernatant saturated solution of zinc chloride in diethyl ether (20 ml) was added to a stirred suspension of sodium borohydride (1.0 g, 26.5 mmol) in dry diethyl ether (75 ml) and the mixture stirred for 2 days under nitrogen at room temperature. The supernatant solution was then used for reduction.

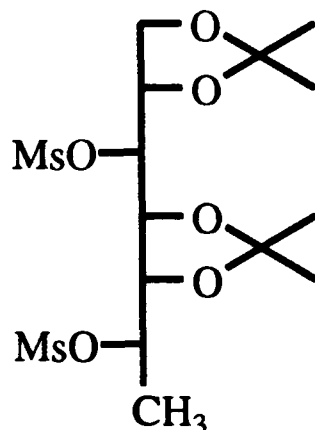
7-Deoxy-1,2;4,5-di-*O*-isopropylidene-L-glycero-D-gluco-heptitol (4.7) and 7-deoxy-1,2;4,5-di-*O*-isopropylidene-D-glycero-D-gluco-heptitol (4.11).



A solution of zinc borohydride in diethyl ether (20 ml) was added to 1-deoxy-3,4;6,7-di-*O*-isopropylidene-L-*gulo*-heptulofuranose (2.12) (475 mg, 1.7 mmol)

under nitrogen at room temperature. The reaction was stirred for 28 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.4) and two products (R_f 0.25 and R_f 0.3). The reaction was quenched by the dropwise addition of saturated aqueous ammonium chloride solution until effervescence ceased. The reaction mixture was filtered through a Celite plug, eluting with ethyl acetate. The solvents were evaporated and the residue purified by flash chromatography (diethyl ether:hexane, 1:1 then 2:1 then diethyl ether) to give a mixture of 7-deoxy-1,2;4,5-di-*O*-isopropylidene-L-glycero-D-gluco-heptitol (4.7) and 7-deoxy-1,2;4,5-di-*O*-isopropylidene-D-glycero-D-gluco-heptitol (4.11) (302 mg, 1.09 mmol, 64% combined yield) in a ratio of approximately 1:1, as judged by NMR. A small amount of each epimer was separated by further flash chromatography for characterisation. The more polar product, (R_f 0.25), was identical to the product (4.7) of the sodium borohydride reduction of the same starting material. The less polar product (R_f 0.3) was 7-deoxy-1,2;4,5-di-*O*-isopropylidene-D-glycero-D-gluco-heptitol (4.11), a white solid. M.p. 59-61 °C; $[\alpha]_D^{25}$ -25.2 (*c*, 0.65 in CHCl_3); ν_{max} (neat): 3436 (br, OH) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.30 (3H, d, C-7, 3J 6.4 Hz), 1.35, 1.38, 1.45, 1.47 (4 x 3H, 4 x s, 2 x $(\text{CH}_3)_2\text{C}$), 2.91, 2.99 (2x br s, 2 x OH, D_2O exchange), 3.80 (1H, t, J 7.6 Hz), 3.85 (1H, dd, J 8.6 Hz, J 6.0 Hz), 3.94- 4.02 (1H, m), 4.05 (1H, dd, J 5.9 Hz, J 2.4 Hz), 4.06 (1H, dd, J 8.2 Hz, J 6.5 Hz), 4.09-4.13 (1H, m), 4.31 (1H, q, H-2, J 6.7 Hz); δ_{C} (CDCl_3): 20.8 (q, C-7), 25.2, 25.3, 26.6, 27.2 (4 x q, 2 x $(\text{CH}_3)_2\text{C}$), 66.2 (t, C-1), 65.8, 69.8, 76.5, 77.0, 81.3 (5 x d, C-2, C-3, C-4, C-5, C-6), 108.4, 109.7 (2 x s, 2 x $(\text{CH}_3)_2\text{C}$); m/z (NH_3 , CI): 294 ($\text{M}+\text{NH}_4^+$, 8%), 277 ($\text{M}+\text{H}^+$, 100%), 219 ($\text{M}+\text{H}^+$ -acetone, 100%), 201 ($\text{M}+\text{H}^+$ - H_2O -acetone, 59%). (Found C, 56.46; H, 8.43. $\text{C}_{12}\text{H}_{24}\text{O}_6$ requires C, 56.51; H, 8.75%).

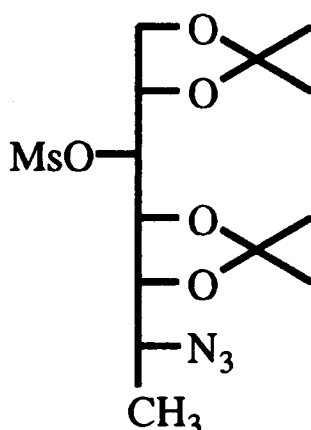
7-Deoxy-1,2;4,5-di-O-isopropylidene-3,6-di-O-methanesulphonyl-L-glycero-D-gluc
heptitol (4.17).



7-Deoxy-1,2;4,5-di-O-isopropylidene-L-glycero-D-gluc heptitol (4.7) (2.088 g, 7.6 mmol) and 4-(*N,N*-dimethylamino)-pyridine (185 mg, 1.5 mmol, 0.2 mol equivalents) were dissolved in pyridine (15 ml) and cooled to 0 °C. Methanesulphonyl chloride (2.3 ml, 34.4 mmol, 4 mol equivalents) was added dropwise and the reaction was left to stir for 1.25 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.25) and one major product (R_f 0.4). The solvents were evaporated and the crude residue partitioned between diethyl ether (60 ml) and water (40 ml). The aqueous layer was washed with diethyl ether (2 x 50 ml) and the organic extracts were combined, dried (magnesium sulphate), filtered and evaporated. The product was purified by flash chromatography (diethyl ether:hexane, 1:1 then 2:1 then 3:1 then diethyl ether) to give 7-deoxy-1,2;4,5-di-O-isopropylidene-3,6-di-O-methanesulphonyl-L-glycero-D-gluc heptitol (4.17) (2.623 g, 6.07 mmol, 80% yield) as a white solid. M.p. 38-40 °C; $[\alpha]_D^{20} +4.2$ (*c*, 1.2 in CH₃CN); $\nu_{\max}$ (neat): 1353 (SO₂) cm⁻¹; δ_H (CDCl₃, 500 MHz): 1.38, 1.40, 1.46, 1.54 (4 x 3H, 4 x s, 2 x (CH₃)₂C), 1.53 (3H, d, C-7, 3J 6.4 Hz), 3.13, 3.19 (2 x 3H, 2 x s, 2 x CH₃S), 3.99 (1H, dd, H-1, $J_{1,1'}$ 8.9 Hz, $J_{1,2}$ 6.5 Hz), 4.17 (1H, dd, H-1', $J_{1',1}$ 8.9 Hz, $J_{1',2}$ 6.8 Hz), 4.18 (1H, dd, H-5, $J_{5,4}$ 6.2 Hz, $J_{5,6}$ 4.9 Hz), 4.35 (1H, t, H-4, J 6.3 Hz), 4.40 (1H, dt, H-2, J 6.6 Hz, $J_{2,3}$ 4.8 Hz), 4.99 (1H, dd, H-3, $J_{3,4}$ 6.4 Hz, $J_{3,2}$ 4.8 Hz), 5.09 (1H, dq, H-6, J 6.4 Hz, $J_{6,5}$ 4.8 Hz); δ_C (CDCl₃): 18.4 (q, C-7), 25.1, 25.4, 25.9, 26.1 (4 x q, 2 x (CH₃)₂C), 38.4, 39.0 (2 x q, 2 x CH₃S), 65.5 (t, C-1), 74.6, 75.0, 75.4, 78.9 (4 x d,

C-2, C-3, C-4, C-5, C-6), 109.9, 110.5 (2 x s, 2 x (CH₃)₂C); *m/z* (NH₃, CI): 450 (M+NH₄⁺, 100%), 433 (M+H⁺, 32%). (Found C, 41.82; H, 6.43. C₁₅H₂₈O₁₀S₂ requires C, 41.66; H, 6.53%).

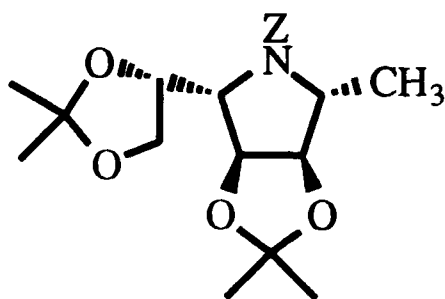
6-Azido-6,7-dideoxy-1,2:4,5-di-*O*-isopropylidene-3-*O*-methanesulphonyl-*D*-glycero-*D*-gluco-heptitol (4.18).



7-Deoxy-1,2:4,5-di-*O*-isopropylidene-3,6-di-*O*-methanesulphonyl-*L*-glycero-*D*-gluco-heptitol (4.17) (2.30 g, 5.3 mmol) and sodium azide (415 mg, 7.4 mmol, 1.2 mol equivalents) were dissolved in dry *N,N*-dimethylformamide (16 ml) under nitrogen. The reaction mixture was stirred at 80 °C for 55 h at which time t.l.c. (diethyl ether:hexane, 4:1) showed no starting material (*R_f* 0.2) and one major product (*R_f* 0.5). The reaction mixture was allowed to cool to room temperature and was then partitioned between ethyl acetate (150 ml) and water (80 ml). The aqueous layer was washed with further ethyl acetate (2 x 80 ml) and the combined organic layers were dried (magnesium sulphate), filtered and evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:4 then 1:3 then 1:2) to give 6-azido-6,7-dideoxy-1,2:4,5-di-*O*-isopropylidene-3-*O*-methanesulphonyl-*D*-glycero-*D*-gluco-heptitol (4.18) (1.378 g, 3.6 mmol, 65% yield) as a colourless gum. [α]_D²⁰ -42.2 (*c*, 1.05 in CH₃CN); ν_{max} (neat): 2101 (N₃), 1357 (SO₂) cm⁻¹; δ_{H} (CDCl₃, 500 MHz): 1.36, 1.39, 1.49, 1.50 (4 x 3H, 4 x s, 2 x (CH₃)₂C), 1.47 (3H, d, C-7, ³*J* 6.2 Hz), 3.19 (3H, s, CH₃S), 3.89 (1H, dd,

H-5, $J_{5,6}$ 9.9 Hz, $J_{5,4}$ 5.8 Hz), 3.91 (1H, dd, H-1, $J_{1,1'}$ 8.6 Hz, $J_{1,2}$ 6.9 Hz), 3.97 (1H, dq, H-6, $J_{6,5}$ 9.9 Hz, $J_{6,2}$ 6.2 Hz), 4.15 (1H, dd, H-1', $J_{1',1}$ 8.6 Hz, $J_{1',2}$ 6.6 Hz), 4.18 (1H, dd, H-4, $J_{4,5}$ 5.8 Hz, $J_{4,3}$ 4.1 Hz), 4.50 (1H, q, H-2, $J_{2,3}$ 6.6 Hz), 4.95 (1H, dd, H-3, $J_{3,2}$ 6.2 Hz, $J_{3,4}$ 4.1 Hz); δ_C (CDCl₃): 16.6 (q, C-7), 25.4, 25.5, 26.0, 26.6 (4 x q, 2 x (CH₃)₂C), 39.4 (q, CH₃S), 54.3 (d, C-6), 65.7 (t, C-1), 75.3, 75.8, 79.1, 80.0 (4 x d, C-2, C-3, C-4, C-5), 109.8, 110.4 (2 x s, 2 x (CH₃)₂C); m/z (NH₃, CI): 397 (M+NH₄⁺, 1%), 380 (M+H⁺, 1%), 352 (M+H⁺-N₂, 50%), 256 (100%). (Found C, 44.63; H, 6.96; N, 10.77%. C₁₄H₂₅N₃O₇S requires C, 44.32; H, 6.64; N, 11.07%).

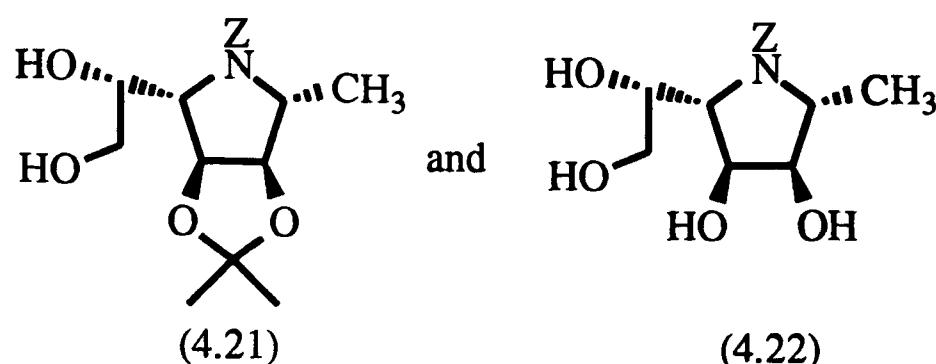
N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4:6,7-di-*O*-isopropylidene-L-glycero-L-*allo*-heptitol (4.20).



6-Azido-6,7-dideoxy-1,2;4,5-di-*O*-isopropylidene-3-*O*-methanesulphonyl-D-glycero-D-*gluco*-heptitol (4.18) (1.447g, 3.8 mmol), sodium acetate (376 mg, 4.6 mmol, 1.2 mol equivalents) and palladium black (catalytic amount) were stirred in ethanol (20 ml) under an atmosphere of hydrogen at room temperature. After 16 h t.l.c. (diethyl ether) showed no starting material (R_f 0.5) and one product (R_f 0.1). A small amount of magnesium sulphate was added and the reaction mixture was filtered through a Celite plug. The solvents were evaporated and the crude residue, a brown oil, was redissolved in a 3:2 mixture of diethyl ether and saturated aqueous sodium hydrogen carbonate solution (15 ml). Benzyl chloroformate (0.8 ml, 5.7 mmol, 1.5 mol equivalents) was added and the

reaction was stirred vigorously at room temperature for 0.5 h by which time t.l.c. showed no starting material (R_f 0.1) and one major product (R_f 0.6). The organic phase was separated and the aqueous layer was washed with diethyl ether (4 x 5 ml). The organic extracts were combined, dried (magnesium sulphate), filtered and evaporated. Purification by flash chromatography (diethyl ether:hexane, 1:4 then 1:3 then 1:2) gave *N-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4;6,7-di-O-isopropylidene-L-glycero-L-allo-heptitol* (4.20) (1.154 g, 2.95 mmol, 78% yield over two steps) as an oil. $[\alpha]_D^{20} +46.6$ (c , 1.55 in CHCl_3); ν_{max} (neat): 1699 (C=O) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.32 (6H, s, 2 x CH_3), 1.33 (3H, d, C-1, 3J 5.4 Hz), 1.44, 1.45 (2 x 3H, 2 x s, 2 x CH_3), 3.88 (1H, br s), 4.12-4.23 (3H, m), 4.37 (1H, dd, J 3.8 Hz, J 1.4 Hz), 4.40 (1H, br s), 4.75 (1H, d, J 5.6 Hz), 5.17 (2H, s, CH_2Ph), 7.30-7.38 (5H, m, ArCH); δ_{C} (CDCl_3): 18.6, 19.1 (2 x q, C-1), 24.5, 25.1, 26.1, 27.1 (4 x q, 2 x $(\text{CH}_3)_2\text{C}$), 66.6, 70.0 (2 x t, C-7, CH_2Ph), 60.7, 61.4, 64.8, 66.3, 74.8, 75.6, 79.8, 80.7, 85.5, 86.1 (10 x d, C-2, C-3, C-4, C-5, C-6), 109.8, 111.8 (2 x s, 2 x $(\text{CH}_3)_2\text{C}$), 127.8, 128.2, 128.7 (3 x d, ArCH), 136.8 (s, ArC), 154.6, 155.1 (2 x s, C=O); m/z (NH_3 , CI): 392 ($\text{M}+\text{H}^+$, 100%), 334 ($\text{M}+\text{H}^+$ -acetone, 34%), 91 (C_7H_7^+ , 67%). (Found C, 64.37; H, 7.42; N, 3.95%. $\text{C}_{21}\text{H}_{29}\text{NO}_6$ requires C, 64.43; H, 7.47%; N, 3.58%).

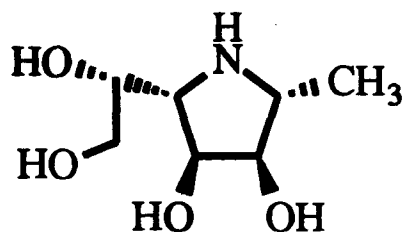
N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-*O*-isopropylidene-*L*-glycero-*L*-allo-heptitol (4.21) and *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-*L*-glycero-*L*-allo-heptitol (4.22).



N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4,6,7-di-*O*-isopropylidene-*L*-glycero-*L*-allo-heptitol (4.20) (224 mg, 0.57 mmol) was dissolved in a mixture of acetic acid (1 ml) and water (1 ml) and the reaction was stirred at room temperature. After 24 h t.l.c. (diethyl ether) showed no starting material (R_f 0.8) and two products (R_f 0.1 and baseline). The solvents were evaporated and the residue was co-evaporated with toluene (3 x 1 ml). Purification by flash chromatography (ethyl acetate:hexane, 1:3 then 1:2 then 2:1, then ethyl acetate, then methanol:ethyl acetate, 1:20) gave *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-*O*-isopropylidene-*L*-glycero-*L*-allo-heptitol (4.21) (124 mg, 0.35 mmol, 62% yield) as a white solid (R_f 0.1). M.p. 103-104 °C (ethyl acetate/hexane); $[\alpha]_D^{20} +13.0$ (c, 0.2 in CHCl_3); ν_{max} (KBr): 3488 (OH), 1683 (C=O) cm^{-1} ; δ_{H} (CDCl_3): 1.17 (3H, d, C-1, J 7.1 Hz), 1.32, 1.44 (2 x 3H, 2 x s, $(\text{CH}_3)_2\text{C}$), 3.29-3.34 (1H, m), 3.60 (2H, s), 4.00 (1H, br d, J 10.0 Hz), 4.23 (1H, q, J 6.7 Hz), 4.42 (1H, d, J 5.6 Hz), 4.90 (1H, d, J 5.6 Hz), 5.19 (2H, s, CH_2Ph), 7.32-7.36 (5H, m, ArCH); δ_{C} (CDCl_3): 19.9 (q, C-1), 24.9, 26.9 (2 x q, $(\text{CH}_3)_2\text{C}$), 62.3, 67.6 (2 x t, C-7, CH_2Ph), 60.7, 65.9, 72.3, 82.3, 85.4 (5 x d, C-2, C-3, C-4, C-5, C-6), 112.0 (s, $(\text{CH}_3)_2\text{C}$), 127.8, 128.0, 128.8 (3 x d, ArCH), 136.4 (s, ArC), 156.7 (s, C=O); m/z (NH_3 , CI): 352 ($\text{M}+\text{H}^+$, 91%), 91 (C_7H_7^+ , 100%). (Found C, 61.31; H, 6.98; N, 3.76%. $\text{C}_{18}\text{H}_{25}\text{NO}_6$ requires C, 61.52; H, 7.17%; N, 3.99%),

and *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-*L*-glycero-*L*-allo-heptitol (4.22) (68 mg, 0.22 mmol, 38% yield) as a white solid (baseline). M.p. 133-134 °C (methanol/ethyl acetate); $[\alpha]_D^{20} +25.1$ (c, 1.2 in CH₃CN); $\nu_{\max}$ (KBr): 3439 (OH), 1654 (C=O) cm⁻¹; δ_H (CDCl₃): 1.02 (3H, d, C-1, 3J 6.7 Hz), 3.35-3.62 (4H, m), 3.74 (1H, t, J 3.9 Hz), 3.83-3.85 (1H, m), 4.13 (1H, t, J 4.4 Hz), 4.94 (2H, s, CH₂Ph), 7.17-7.22 (5H, m, ArCH); δ_C (CD₃OD): 17.0, 17.7 (2 x q, C-1), 59.2, 59.4, 66.0, 70.3, 70.8, 76.6 (6 x d, C-2, C-3, C-4, C-5, C-6), 63.5, 66.8 (2 x t, C-7, CH₂Ph), 127.7, 127.9, 128.4 (3 x d, ArCH), 136.8 (s, ArC), 156.4, 157.0 (2 x s, C=O); m/z (NH₃, CI): 312 (M+H⁺, 66%), 91 (C₇H₇⁺, 100%). (Found C, 57.96; H, 6.87; N, 4.20%. C₁₅H₂₁NO₆ requires C, 57.87; H, 6.80; N, 4.50%).

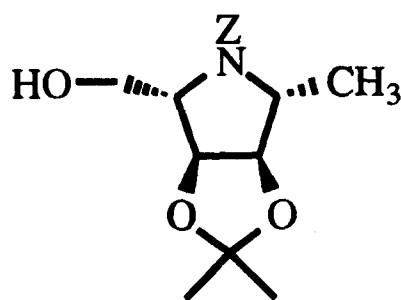
1,2,5-Trideoxy-2,5-imino-*L*-glycero-*L*-allo-heptitol (4.5).



N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-*L*-glycero-*L*-allo-heptitol (4.22) (133 mg, 0.43 mmol) and palladium black (catalytic amount) were stirred in ethanol (2 ml) under an atmosphere of hydrogen. After 5 h t.l.c. (methanol:ethyl acetate, 1:9) showed no starting material (R_f 0.1) and a baseline spot (ninhydrin active). Magnesium sulphate was added and the reaction mixture was filtered through a Celite plug, eluting with ethanol. The solvent was evaporated to give 1,2,5-trideoxy-2,5-imino-*L*-glycero-*L*-allo-heptitol (4.5) (77 mg, 0.43 mmol, quantitative crude yield) as a pale brown solid. A small portion was further purified by ion exchange chromatography (CG 400 (OH⁻ form), water elution) to give a white solid. M.p. 144-146 °C; $[\alpha]_D^{20} +13.7$ (c, 0.65 in H₂O); $\nu_{\max}$ (KBr):

3471, 3392 (OH, NH) cm^{-1} ; δ_{H} (D_2O , 500 MHz, pH 9): 1.09 (3H, d, CH_3 , 3J 6.7 Hz), 2.84-2.88 (2H, m), 3.39 (1H, dd, J 8.0 Hz, J 6.3 Hz), 3.49 (1H, dd, J 12.6 Hz, J 8.1 Hz), 3.59-3.63 (2H, m), 3.97 (1H, dd, J 6.3 Hz, J 4.5 Hz); δ_{C} (D_2O , pH 9): 17.6 (q, C-1), 57.9, 66.4, 72.7, 73.3, 78.5 (5 x d, C-2, C-3, C-4, C-5, C-6), 64.7 (t, C-7); m/z (NH_3 , DCI): 178 ($\text{M}+\text{H}^+$, 100%). (Found C, 47.26; H, 8.59; N, 7.76%. $\text{C}_7\text{H}_{15}\text{NO}_4$ requires C, 47.45; H, 8.53; N, 7.90%).

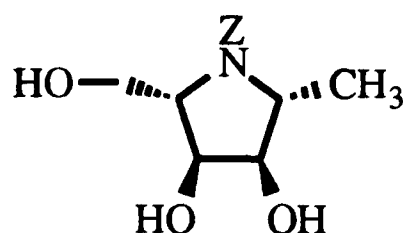
N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-*O*-isopropylidene-*L*-allitol
(4.24).



N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-*O*-isopropylidene-*L*-glycero-*L*-allo-heptitol (4.21) (143 mg, 0.37 mmol) was dissolved in tetrahydrofuran (2 ml) at room temperature under nitrogen and periodic acid (100 mg, 0.44 mmol, 1.2 mol equivalents) was added. After stirring for 5 min a white precipitate had appeared and t.l.c. (ethyl acetate:hexane, 2:1) showed no starting material (R_f 0.3) and a product which streaked (R_f 0.4-0.6). The reaction mixture was filtered through a Celite plug, eluting with ethyl acetate. The solvents were evaporated and the crude residue was redissolved in ethanol (2 ml). Sodium borohydride (17 mg, 0.44 mmol, 1.2 mol equivalents) was added and the reaction was stirred at room temperature for 20 min, when t.l.c.(ethyl acetate:hexane, 2:1) showed one product (R_f 0.5). The reaction was quenched by the addition of ammonium chloride and was stirred until effervescence ceased. The reaction mixture was filtered through a Celite plug, eluting with ethanol. The solvent was evaporated and the residue purified by flash chromatography (ethyl acetate:hexane, 1:1) to

give *N-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-O-isopropylidene-L-allitol* (4.24) (112 mg, 0.35 mmol, 94% yield) as a colourless oil. $[\alpha]_{\text{D}}^{20} +12.3$ (*c*, 0.4 in CHCl_3); ν_{max} (neat): 3452 (OH), 1682 (C=O) cm^{-1} ; δ_{H} (CDCl_3): 1.25 (3H, d, C-1, 3J 6.7 Hz), 1.31, 1.46 (2 x s, 2 x 3H, $(\text{CH}_3)_2\text{C}$), 3.72 (2H, br s), 4.10-4.22 (2H, m), 4.36 (1H, d, J 5.6 Hz), 4.64 (1H, br s), 4.94 (2H, s, CH_2Ph), 7.24-7.44 (5H, m, ArCH); δ_{C} (CDCl_3): 19.5 (q, C-1), 25.2, 27.2 (2 x q, $(\text{CH}_3)_2\text{C}$), 60.3, 67.0, 81.5, 85.3 (4 x d, C-2, C-3, C-4, C-5), 63.8, 67.1 (2 x t, C-6, CH_2Ph), 111.8 (s, $(\text{CH}_3)_2\text{C}$), 127.6, 128.0, 128.5 (3 x d, ArCH), 136.5 (s, ArC), 155.0 (s, C=O); m/z (NH_3 , CI): 322 ($\text{M}+\text{H}^+$, 100%), 91 (C_7H_7^+ , 86%). (Found C, 63.43; H, 7.44; N, 4.53%. $\text{C}_{17}\text{H}_{23}\text{NO}_5$ requires C, 63.54; H, 7.21; N, 4.36%).

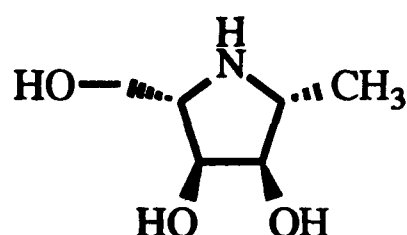
N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-*L*-allitol (4.25).



N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-3,4-*O*-isopropylidene-*L*-allitol (4.24) (88 mg, 0.27 mmol) was dissolved in a mixture of trifluoroacetic acid (1 ml) and water (1 ml) and the reaction mixture was stirred at room temperature. After 1 h, t.l.c. (ethyl acetate/hexane, 2:1) showed no starting material (R_f 0.4) and one product (baseline). The solvents were evaporated and the residue was co-evaporated with toluene (3 x 1 ml). Purification by flash chromatography (ethyl acetate:hexane, 4:1 then ethyl acetate) gave *N-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-allitol* (4.25) (49 mg, 0.17 mmol, 65% yield) as a colourless oil. $[\alpha]_{\text{D}}^{20} +10.8$ (*c*, 1.25 in CH_3CN); ν_{max} (neat): 3418 (OH), 1674 (C=O) cm^{-1} ; δ_{H} (CD_3CN): 1.18 (3H, d, C-1, J 6.8 Hz), 3.40-3.76 (5H, m), 4.04-4.08 (1H, m), 5.10 (2H, s, CH_2Ph), 7.30-7.39 (5H, m, ArCH); δ_{C} (CD_3CN): 18.4, 19.1 (2 x q,

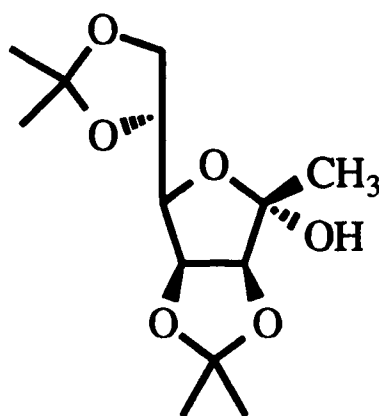
C-1), 60.6, 66.3, 72.5, 76.8 (4 x d, C-2, C-3, C-4, C-5), 61.5, 62.8, 67.4 (3 x t, C-6, CH₂Ph), 128.6, 128.8, 129.4 (3 x d, ArCH), 138.1 (s, ArC), 156.3, 157.2 (2 x s, C=O); *m/z* (NH₃, CI): 282 (M+H⁺, 100%), 91 (C₇H₇⁺, 67%). (Found MH⁺ 282.133786. Calculated for C₁₄H₂₀NO₅ 282.134148).

1,2,5-Trideoxy-2,5-imino-L-allitol (4.4).



N-Benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-allitol (4.25) (89 mg, 0.32 mmol) and palladium black (catalytic amount) were stirred in ethanol (2 ml) under an atmosphere of hydrogen. After 1.5 h t.l.c. (methanol:ethyl acetate, 1:9) showed no starting material (*R_f* 0.2) and a baseline spot (ninhydrin active). Magnesium sulphate was added and the reaction mixture was filtered through a Celite plug, eluting with ethanol. The solvent was evaporated to give 1,2,5-trideoxy-2,5-imino-L-allitol (4.4) (47 mg, 0.32 mmol, quantitative crude yield) as a pale brown solid. A small portion was further purified by ion exchange chromatography (CG 400 (OH⁻ form), water elution) to give a white solid. M.p. 111-113 °C; [α]_D²⁵ -5.1 (c, 1.58 in EtOH); *v*_{max} (KBr): 3402 (OH, NH) cm⁻¹; δ_H (D₂O, 500 MHz, pH 9): 1.08 (3H, d, CH₃, *J* 6.5 Hz), 2.88 (1H, qu, H-2, *J* 6.7 Hz), 2.93 (1H, q, H-5, *J* 5.4 Hz), 3.44 (1H, t, H-3, *J* 6.8 Hz), 3.53 (1H, dd, H-6, *J*_{6,6'} 11.6 Hz, *J*_{6,5} 5.8 Hz), 3.58 (1H, dd, H-6', *J*_{6',6} 11.6 Hz, *J*_{6',5} 5.0 Hz); δ_C (D₂O, pH 9): 18.2 (q, C-1), 58.4, 65.8, 73.5, 78.5 (4 x d, C-2, C-3, C-4, C-5), 63.4 (t, C-6); *m/z* (NH₃, CI): 148 (M+H⁺, 100%). (Found MH⁺ 148.097334. Calculated for C₆H₁₄NO₃ 148.097368).

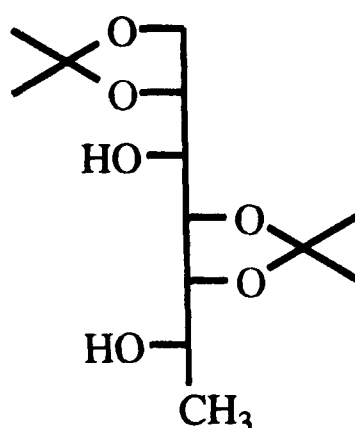
1-Deoxy-3,4;6,7-di-*O*-isopropylidene- α -D-manno-heptulofuranose (4.6).



2,3;5,6-Di-*O*-isopropylidene-D-mannono-1,4-lactone (3.17) (477 mg, 1.8 mmol) was dissolved in dry tetrahydrofuran (15 ml) and the solution cooled to -70 °C. Methyllithium (1.4 M in diethyl ether, 1.5 ml, 1.1 mol equivalents) was added and the reaction mixture was stirred under nitrogen for 10 min at which time t.l.c. (diethyl ether:hexane, 4:1) showed complete conversion of the starting material (R_f 0.2) to a single product (R_f 0.3). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (1 ml) and the solvents were evaporated. The crude residue was partitioned between water (30 ml) and diethyl ether (50 ml). The aqueous layer was washed with a further portion of diethyl ether (50 ml). The organic extracts were combined, dried (magnesium sulphate), filtered and the solvent evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 1:1) to give 1-deoxy-3,4;6,7-di-*O*-isopropylidene- α -D-manno-heptulofuranose (4.6) (422 mg, 1.5 mmol, 86% yield) as a white solid. M.p. 103-104.5 °C (diethyl ether/hexane) (lit.²⁵ 102 °C); $[\alpha]_D^{20}$ +6.5 (*c*, 1.0 in CHCl₃) (lit.²⁵ $[\alpha]_D^{23}$ +8.33); $\nu_{\max}$ (KBr): 3413 (br, OH) cm⁻¹; δ_H (CDCl₃, 500 MHz): 1.34, 1.38, 1.45, 1.48, 1.50 (5 x 3H, 5 x s, 5 x CH₃), 4.00 (1H, dd, H-7, $J_{7,7'}$ 8.6 Hz, $J_{7,6}$ 4.8 Hz), 4.08 (1H, dd, H-7', $J_{7,7}$ 8.6 Hz, $J_{7',6}$ 6.3 Hz), 4.11 (1H, dd, H-5, $J_{5,6}$ 7.4 Hz, $J_{5,4}$ 3.8 Hz), 4.38 (1H, ddd, H-6, $J_{6,5}$ 7.4 Hz, $J_{6,7'}$ 6.3 Hz, $J_{6,7}$ 4.8 Hz), 4.46 (1H, d, H-3, $J_{3,4}$ 5.9 Hz), 4.84 (1H, dd, H-4, $J_{4,3}$ 5.9 Hz, $J_{4,5}$ 3.8 Hz); δ_H (d₆-DMSO, 400 MHz): 1.25, 1.28, 1.33, 1.34, 1.37

(5 x 3H, 5 x s, 5 x CH₃), 3.81 (1H, dd, H-7, $J_{7,7'}$ 8 Hz, $J_{7,6}$ 6 Hz), 3.97 (1H, dd, H-5, $J_{5,6}$ 6 Hz, $J_{5,4}$ 4 Hz), 3.98 (1H, dd, H-7', $J_{7',7}$ 8 Hz, $J_{7',6}$ 6 Hz), 4.24 (1H, q, H-6, J 6 Hz), 4.29 (1H, d, H-3, $J_{3,4}$ 6 Hz), 4.73 (1H, dd, H-4, $J_{4,3}$ 6 Hz, $J_{4,5}$ 4 Hz), 6.02 (1H, s, OH); δ_C (CDCl₃): 22.1 (q, C-1), 24.4, 25.0, 25.8, 26.8 (4 x q, 2 x (CH₃)₂C), 66.6 (t, C-7), 73.3, 77.8, 80.5, 85.5 (4 x d, C-3, C-4, C-5, C-6), 105.4 (s, C-2), 109.2, 112.8 (2 x s, 2 x C(CH₃)₂); m/z (NH₃, CI): 292 (M+NH₄⁺, 4%), 275 (M+H⁺, 15%), 257 (M+H⁺-H₂O, 70%), 199 (100%). (Found C, 57.13; H, 7.92. C₁₃H₂₂O₆ requires C, 56.92; H, 8.08%).

7-Deoxy-1,2:4,5-di-*O*-isopropylidene-*L*-glycero-*D*-manno-heptitol (4.16).



Method 1.

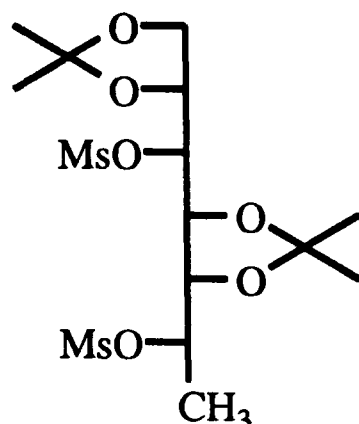
2,3;5,6-Di-*O*-isopropylidene-*D*-mannofuranose (1.28) (112 mg, 0.43 mmol) was dissolved in dry tetrahydrofuran (2 ml) and cooled to -50 °C. Methyllithium (1.4 M in diethyl ether, 1.5 ml, 0.95 mmol, 2.2 mol equivalents) was added and the reaction was allowed to warm to 0 °C over 2 h. After a further 6 h stirring at 0 °C, t.l.c. (diethyl ether) showed a trace of starting material (R_f 0.4) and one major product (R_f 0.3). The reaction was quenched by the addition of saturated aqueous ammonium chloride solution and the solvents were evaporated. The residue was partitioned between water (10 ml) and ethyl acetate (15 ml). The aqueous layer was washed with ethyl acetate (15 ml) and the combined ethyl

acetate extracts were dried (magnesium sulphate), filtered and evaporated. The crude residue was purified by flash chromatography (diethyl ether:hexane, 2:1 then 3:1) to give 7-deoxy-1,2;4,5-di-*O*-isopropylidene-*L*-glycero-*D*-manno-heptitol (4.16) (79 mg, 0.29 mmol, 67% yield) as a clear gum. $[\alpha]_D^{25}$ -8.6 (*c*, 1.03 in CHCl₃); δ_H (CDCl₃): 1.32 (3H, d, C-7, 3J 6.3 Hz), 1.35 (3H, s, CH₃), 1.40 (6H, s, 2 x CH₃), 1.54 (3H, s, CH₃), 3.60 (1H, d, *J* 6.7 Hz), 4.02-4.13 (5H, m), 4.38 (1H, d, *J* 7.4 Hz).

Method 2.

1-Deoxy-3,4;6,7-di-*O*-isopropylidene- α -*D*-manno-heptulofuranose (4.6) (1.282 g, 4.96 mmol) was dissolved in ethanol (7 ml) at room temperature under nitrogen. Sodium borohydride (282 mg, 7.44 mmol, 1.5 mol equivalents) was added and the reaction was left to stir for 0.5 h by which time t.l.c. (diethyl ether) showed no starting material (*R_f* 0.6) and one major product (*R_f* 0.4). The reaction was quenched by the addition of ammonium chloride, stirred until effervescence ceased and then filtered through a short pad of Celite, eluting with ethyl acetate. The solvents were evaporated and the crude residue purified by flash chromatography (diethyl ether:hexane, 1:1 then 2:1 then diethyl ether) to yield 7-deoxy-1,2;4,5-di-*O*-isopropylidene-*L*-glycero-*D*-manno-heptitol (4.16) (977 mg, 3.53 mmol, 71% yield) as a colourless gum, identical to the material produced above.

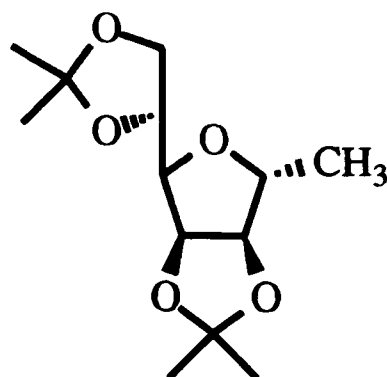
7-Deoxy-1,2;4,5-di-O-isopropylidene-3,6-di-O-methanesulphonyl-L-glycero-D-manno-heptitol (4.27).



7-Deoxy-1,2;4,5-di-O-isopropylidene-L-glycero-D-manno-heptitol (4.16) (489 mg, 1.75 mmol) and 4-(*N,N*-dimethylamino)-pyridine (214 mg, 1.75 mmol, 1 mol equivalent) were dissolved in dry dichloromethane (5 ml) at room temperature under nitrogen. Triethylamine (0.98 ml, 7 mmol, 4 mol equivalents) and methanesulphonyl chloride (0.54 ml, 7 mmol, 4 mol equivalents) were added and the reaction was left to stir for 0.5 h at which time t.l.c. (diethyl ether) showed no starting material (R_f 0.3) and one major product (R_f 0.4). The reaction mixture was diluted with dichloromethane (25 ml) and washed with dilute aqueous hydrochloric acid (10 ml) and brine (10 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. The product was purified by flash chromatography (diethyl ether:hexane, 1:1 then diethyl ether) to give 7-deoxy-1,2;4,5-di-O-isopropylidene-3,6-di-O-methanesulphonyl-L-glycero-D-manno-heptitol (4.27) (544 mg, 1.26 mmol, 72 % yield) as a white foam. $[\alpha]_D^{25} +18.4$ (c, 1.03 in CHCl_3); ν_{max} (neat): 1357, 1332 (SO_2) cm^{-1} ; δ_{H} (CDCl_3 , 500 MHz): 1.37, 1.41, 1.45, 1.54 (4 x 3H, 4 x s, 2 x $(\text{CH}_3)_2\text{C}$), 1.50 (3H, d, C-7, 3J 6.4 Hz), 3.11, 3.18 (2 x 3H, 2 x s, 2 x CH_3S), 4.07 (1H, dd, H-1, $J_{1,1'}$ 8.6 Hz, $J_{1,2}$ 7.2 Hz), 4.14-4.17 (1H, m), 4.18 (1H, dd, H-5, $J_{5,4}$ 6.1 Hz, $J_{5,6}$ 4.5 Hz), 4.23 (1H, dd, H-1', $J_{1',1}$ 8.6 Hz, $J_{1',2}$ 5.9 Hz), 4.37 (1H, t, J 6.3 Hz), 4.99 (1H, t, J 6.9 Hz), 5.27 (1H, dq, H-6, J 6.4 Hz, $J_{6,5}$ 4.6 Hz); δ_{C} (CDCl_3): 18.3 (q, C-7), 25.3, 25.6, 26.0 (3 x q, 2 x $(\text{CH}_3)_2\text{C}$), 38.9, 39.1 (2 x q, 2 x CH_3S), 67.3 (t, C-1), 75.1, 75.9, 76.4, 77.1, 77.7 (5 x d, C-2, C-3, C-4, C-5, C-6), 109.6, 110.3 (2 x s, 2 x $(\text{CH}_3)_2\text{C}$);

m/z (NH_3 , CI): 450 ($\text{M}+\text{NH}_4^+$, 100%). (Found C, 41.42; H, 6.58. $\text{C}_{15}\text{H}_{28}\text{O}_{10}\text{S}_2$ requires C, 41.66; H, 6.53%).

3,6-Anhydro-7-deoxy-1,2;4,5-di-*O*-isopropylidene-*D*-glycero-*D*-manno-heptitol (4.29).



7-Deoxy-1,2;4,5-di-*O*-isopropylidene-*L*-glycero-*D*-manno-heptitol (4.16) (163 mg, 0.6 mmol) and 4-(*N*, *N*-dimethylamino)-pyridine (72 mg, 0.6 mmol, 1 mol equivalent) were dissolved in dry dichloromethane (3 ml) under nitrogen and cooled to 0 °C. Triethylamine (247 μl , 1.8 mmol, 3 mol equivalents) and trifluoromethanesulphonyl chloride (120 μl , 0.7 mmol, 1.2 mol equivalents) were added and the reaction was stirred at 0 °C for 0.5 h and then allowed to warm to room temperature. After stirring for a further 3.5 h at room temperature t.l.c. (diethyl ether:hexane, 4:1) showed some starting material (R_f 0.2) and one major product (R_f 0.5). The reaction mixture was diluted with dichloromethane (15 ml) and washed with dilute aqueous hydrochloric acid (10 ml) and brine (10 ml). The organic layer was dried (magnesium sulphate), filtered and evaporated. The product was purified by flash chromatography (diethyl ether:hexane, 1:8 then 1:6 then 1:2 then diethyl ether) to give 3,6-anhydro-7-deoxy-1,2;4,5-di-*O*-isopropylidene-*D*-glycero-*D*-manno-heptitol (4.29) (77 mg, 0.3 mmol, 50% yield) as a colourless oil. $[\alpha]_D^{25}$ -44.4 (c , 0.85 in CHCl_3); ν_{max} (neat): 2986, 2937 (CH stretch) cm^{-1} ; δ_{H} (CDCl_3): 1.11 (3H, d, C-7, J 7.0 Hz), 1.31, 1.35, 1.43, 1.47 (4 x 3H, 4 x s, 2 x $(\text{CH}_3)_2\text{C}$), 3.78 (1H, dd, H-3, $J_{3,2}$ 7.6 Hz, $J_{3,4}$ 3.8 Hz), 4.00 (1H, dd, H-1, $J_{1,1'}$ 8.6 Hz, $J_{1,2}$ 4.8 Hz), 4.07 (1H, dd, H-1', $J_{1',1}$ 8.4 Hz, $J_{1',2}$ 4.8 Hz),

4.23 (1H, q, H-6, J 6.9 Hz), 4.32-4.42 (1H, m, H-2), 4.46 (1H, d, H-5, $J_{5,4}$ 6.0 Hz), 4.76 (1H, dd, H-4, $J_{4,5}$ 6.0 Hz, $J_{4,3}$ 3.8 Hz); δ_C (CDCl₃): 16.8 (q, C-7), 24.5, 25.1, 26.0, 26.9 (4 x q, 2 x (CH₃)₂C), 66.8 (t, C-1), 73.4, 79.7, 80.0, 80.8, 86.2 (5 x d, C-2, C-3, C-4, C-5, C-6), 109.0, 112.4 (2 x s, 2 x (CH₃)₂C); m/z (NH₃, CI): 276 (M+NH₄⁺, 35%), 259 (M+H⁺, 100%), 201 (M+H⁺-acetone, 56%). (Found C, 60.52; H, 8.31. C₁₃H₂₂O₅ requires C, 60.45; H, 8.58%). Some starting material (54 mg, 0.2 mmol, 33%) was also recovered.

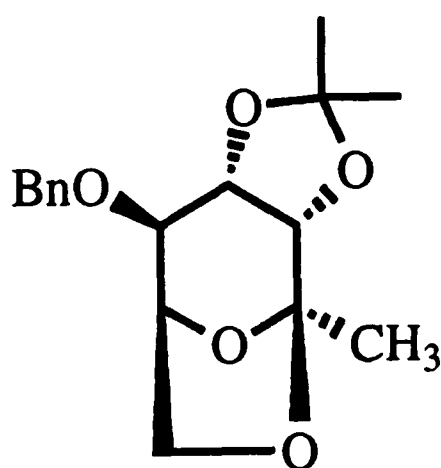
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26. The enzyme inhibition studies were performed by Dr. B. G. Winchester, Institute of Child Health, University of London, 31 Guilford Street, London.

Appendix 1

X-Ray Crystal Structure Analysis of 2,7-anhydro-5-*O*-benzyl-1-deoxy-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (2.19)



X-Ray Crystal Structure Analysis of 2,7-anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19).

The structure of 2,7-anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19) (crystallised from methanol/water) was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to $\theta = 72^\circ$ (Cu-K α radiation). The data were corrected for absorption, Lorentz and polarisation effects. All calculations were carried out on a Microvax 3800 computer using SHELXS-86¹ for direct methods and CRYSTALS² for all other calculations. Atomic scattering factors were taken from International Tables.³ The coordinates of all non-hydrogen atoms were given by SHELXS-86. The hydrogen atoms were placed geometrically. The structures were refined by full-matrix least-squares with isotropic temperature factors for the hydrogen atoms and anisotropic temperature factors for all other atoms. The models were refined almost to convergence. The data were refined using Chebyshev weighting schemes (Tukey & Prince)⁴ to give a final value of $R = 4.20\%$.

Crystal Data for 2,7-anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19).

Molecular formula	C ₁₇ H ₂₂ O ₅	Formula weight	306.3
Crystal system	Monoclinic		
a/Å	5.509(9)	$\alpha/^\circ$	90
b/Å	14.236(3)	$\beta/^\circ$	103.5(1)
c/Å	10.773(7)	$\gamma/^\circ$	90
Z	2		
Space group	P12 ₁ 1	vol / Å ³	806.5
Linear absorption coeff. μ/cm^{-1}	6.5	D _c /g cm ⁻³	1.20
Crystal size /mm	0.8 x 0.35 x 0.10		
Reflections for lattice parameters	25		

Data collection.

$h_{\min}$ -1 $h_{\max}$ 6
 $k_{\min}$ -1 $k_{\max}$ 17
 $l_{\min}$ -13 $l_{\max}$ 13
X-radiation $\lambda = 1.5418 \text{ \AA}$ Cu-K α
 θ min., max. / $^\circ$ 21.35, 30.38 (for lattice parameters)
 ω -scan parameters: A, B ($^\circ$) (A + B tan θ) A = 1.0 B = 0.15
Horizontal aperture parameters: A, B (mm) (A + B tan θ) A = 4 B = 0
Scan speed/ $^\circ \text{ min}^{-1}$ 1.3 (min.) to 5.9 (max.)
Total data 2347 (measured)
Total observed data 1518 for [I>n σ (I)] where n = 3
Absorption correction: min. 1.00, max 1.13 Merging R 2.37%

Refinement.

Robust least squares refinements done using CRYSTALS.
Weighting Scheme type Tukey & Prince Weights 4.80, 4.59, 1.41
Extinction parameter 31.7 (2)
Maximum residual electron density/ e $\text{\AA}^{-3}$ 0.03
Final R 4.20% R_w 4.58 %

Atomic co-ordinates for non-hydrogen atoms of 2,7-anhydro-5-O-benzyl-1-deoxy-3,4-O-isopropylidene- β -L-gulo-heptulopyranose (2.19), and the equivalent isotropic values.

Atom	x/a	y/b	z/c	equiv?	U(iso)
O(18)	0.2700(3)	0.8350(2)	0.6471(2)		0.0511
O(19)	0.3152(4)	1.0219(2)	0.7647(2)		0.0542
O(20)	0.6552(4)	0.8016(2)	0.7680(2)		0.0616
O(21)	0.6280(4)	0.9280(2)	0.4292(2)		0.0541
O(22)	0.5388(4)	1.0862(2)	0.6304(2)		0.0576
C(1)	0.3246(8)	0.8327(3)	0.8781(3)		0.0651
C(2)	0.4361(5)	0.8580(2)	0.7657(3)		0.0509
C(3)	0.5199(5)	0.9588(2)	0.7658(3)		0.0491
C(4)	0.6026(5)	0.9886(2)	0.6430(3)		0.0469
C(5)	0.4774(5)	0.9331(2)	0.5224(3)		0.0477
C(6)	0.4288(5)	0.8323(2)	0.5563(3)		0.0517
C(7)	0.6550(6)	0.7810(2)	0.6352(3)		0.0583
C(9)	0.1503(7)	1.1566(3)	0.6406(5)		0.0732
C(10)	0.526(1)	1.1690(3)	0.8254(5)		0.0824
C(11)	0.6369(6)	1.0148(2)	0.3656(3)		0.0592
C(13)	0.8955(6)	1.0898(2)	0.2315(3)		0.0602
C(14)	1.0347(7)	1.0887(3)	0.1376(3)		0.0654
C(15)	1.0802(7)	1.0063(3)	0.0831(3)		0.0667
C(16)	0.9848(9)	0.9238(3)	0.1191(4)		0.0750
C(17)	0.8439(7)	0.9245(3)	0.2129(4)		0.0660
C(12)	0.7974	1.0081	0.2683		0.0506
C(8)	0.3825	1.1093	0.7179		0.0538

Atom	x/a	y/b	z/c	U(iso)
H(11)	0.411(7)	0.853(3)	0.957(4)	0.0709
H(12)	0.155(8)	0.864(3)	0.858(3)	0.0709
H(13)	0.290(7)	0.768(3)	0.876(3)	0.0709
H(31)	0.653(6)	0.967(2)	0.849(3)	0.0497
H(41)	0.780(6)	0.979(2)	0.655(3)	0.0492
H(51)	0.314(6)	0.966(2)	0.481(3)	0.0507
H(61)	0.360(6)	0.799(2)	0.478(3)	0.0568
H(71)	0.810(6)	0.807(3)	0.609(3)	0.0627
H(72)	0.631(6)	0.713(3)	0.624(3)	0.0627
H(91)	0.186(7)	1.223(3)	0.605(4)	0.0764
H(92)	0.065(8)	1.171(3)	0.703(4)	0.0764
H(93)	0.067(7)	1.116(3)	0.560(4)	0.0764
H(101)	0.568(8)	1.229(3)	0.780(4)	0.0841
H(102)	0.460(8)	1.188(3)	0.894(4)	0.0841
H(103)	0.675(8)	1.134(3)	0.877(4)	0.0841
H(111)	0.689(6)	1.066(3)	0.436(3)	0.0626
H(112)	0.458(7)	1.027(3)	0.310(3)	0.0626
H(131)	0.879(6)	1.154(3)	0.277(3)	0.0616
H(141)	1.108(7)	1.150(3)	0.125(3)	0.0693
H(151)	1.149(7)	1.007(3)	0.009(3)	0.0732
H(161)	1.012(8)	0.865(3)	0.073(4)	0.0876
H(171)	0.778(7)	0.868(3)	0.242(4)	0.0746

Atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
O(18)	0.0432(9)	0.067(1)	0.061(1)	-0.0096(9)	0.0252(8)	-0.0139(9)
O(19)	0.059(1)	0.051(1)	0.072(1)	-0.0002(9)	0.0363(9)	0.0029(8)
O(20)	0.056(1)	0.062(1)	0.073(1)	0.004(1)	0.0216(9)	0.0104(9)
O(21)	0.057(1)	0.063(1)	0.062(1)	0.0042(9)	0.0328(9)	0.0061(9)
O(22)	0.063(1)	0.052(1)	0.081(1)	0.001(1)	0.040(1)	-0.0021(9)
C(1)	0.083(2)	0.066(2)	0.065(2)	0.003(2)	0.037(2)	-0.005(2)
C(2)	0.052(1)	0.052(1)	0.055(1)	-0.001(1)	0.021(1)	0.000(1)
C(3)	0.043(1)	0.055(1)	0.051(1)	-0.003(1)	0.013(1)	-0.001(1)
C(4)	0.038(1)	0.050(1)	0.062(1)	-0.002(1)	0.020(1)	-0.003(1)
C(5)	0.039(1)	0.061(2)	0.055(1)	-0.002(1)	0.021(1)	0.001(1)
C(6)	0.051(1)	0.062(2)	0.058(1)	-0.012(1)	0.027(1)	-0.011(1)
C(7)	0.060(2)	0.051(2)	0.081(2)	-0.002(1)	0.034(1)	0.002(1)
C(9)	0.055(2)	0.076(2)	0.108(3)	0.017(2)	0.029(2)	0.008(2)
C(10)	0.111(3)	0.065(2)	0.083(2)	-0.018(2)	0.022(2)	-0.009(2)
C(11)	0.063(2)	0.064(2)	0.060(1)	0.005(1)	0.027(1)	0.007(1)
C(13)	0.068(2)	0.056(2)	0.062(2)	0.002(1)	0.022(1)	0.005(1)
C(14)	0.074(2)	0.064(2)	0.070(2)	0.006(1)	0.030(2)	-0.010(2)
C(15)	0.070(2)	0.080(2)	0.069(2)	-0.002(2)	0.037(2)	-0.006(2)
C(16)	0.111(3)	0.066(2)	0.092(2)	-0.014(2)	0.063(2)	-0.007(2)
C(17)	0.090(2)	0.058(2)	0.080(2)	-0.007(2)	0.047(2)	-0.015(2)
C(12)	0.0490	0.0579	0.0479	0.0031	0.0149	0.0029
C(8)	0.0551	0.0513	0.0671	-0.0029	0.0285	0.0011

Bond Angles, with hydrogens excluded.

Pivot Atoms??	Angle / °
C(6) - O(18) - C(2)	103.3(2)
C(8) - O(19) - C(3)	106.4(2)
C(7) - O(20) - C(2)	106.7(2)
C(11) - O(21) - C(5)	112.0(2)
C(8) - O(22) - C(4)	109.4(2)
O(20) - C(2) - O(18)	104.6(2)
C(1) - C(2) - O(18)	110.4(2)
C(1) - C(2) - O(20)	110.4(3)
C(3) - C(2) - O(18)	110.9(2)
C(3) - C(2) - O(20)	105.9(2)
C(3) - C(2) - C(1)	114.1(2)
C(2) - C(3) - O(19)	110.8(2)
C(4) - C(3) - O(19)	102.0(2)
C(4) - C(3) - C(2)	114.5(2)
C(3) - C(4) - O(22)	103.4(2)
C(5) - C(4) - O(22)	111.9(2)
C(5) - C(4) - C(3)	113.5(2)
C(4) - C(5) - O(21)	112.4(2)
C(6) - C(5) - O(21)	106.1(2)
C(6) - C(5) - C(4)	111.4(2)
C(5) - C(6) - O(18)	107.5(2)
C(7) - C(6) - O(18)	101.2(2)
C(7) - C(6) - C(5)	114.7(2)
C(6) - C(7) - O(20)	104.7(2)
C(12) - C(11) - O(21)	111.1(2)
C(12) - C(13) - C(14)	120.8(3)
C(15) - C(14) - C(13)	120.0(3)
C(16) - C(15) - C(14)	120.0(3)
C(17) - C(16) - C(15)	120.1(3)
C(12) - C(17) - C(16)	119.9(3)
C(13) - C(12) - C(11)	118.1(2)
C(17) - C(12) - C(11)	122.8(2)
C(17) - C(12) - C(13)	119.1(2)
O(22) - C(8) - O(19)	105.5(1)
C(9) - C(8) - O(19)	108.8(2)
C(9) - C(8) - O(22)	108.2(2)
C(10) - C(8) - O(19)	111.7(2)
C(10) - C(8) - O(22)	109.2(2)
C(10) - C(8) - C(9)	113.1(3)

Bond Lengths

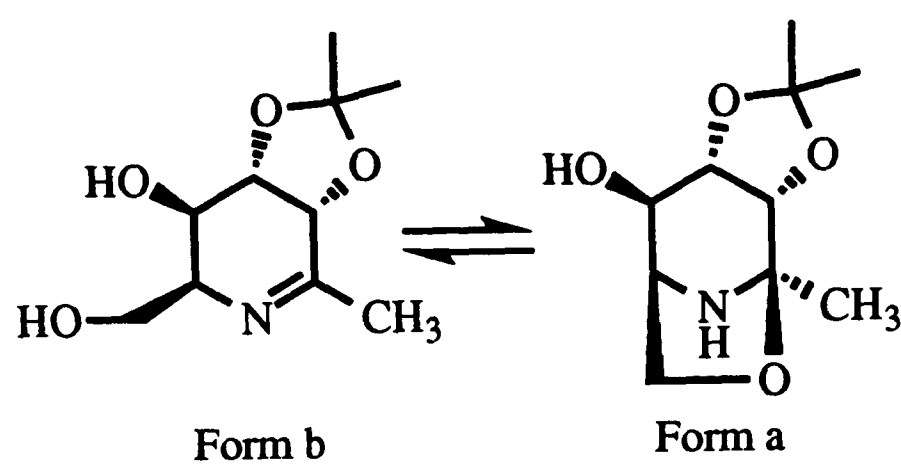
O(18) - C(2)	1.408(3)
O(18) - C(6)	1.442(3)
O(19) - C(3)	1.440(3)
O(19) - C(8)	1.421(2)
O(20) - C(2)	1.445(3)
O(20) - C(7)	1.434(4)
O(21) - C(5)	1.431(3)
O(21) - C(11)	1.412(4)
O(22) - C(4)	1.431(4)
O(22) - C(8)	1.441(2)
C(1) - C(2)	1.503(4)
C(2) - C(3)	1.508(4)
C(3) - C(4)	1.533(4)
C(4) - C(5)	1.522(4)
C(5) - C(6)	1.517(4)
C(6) - C(7)	1.516(5)
C(9) - C(8)	1.507(4)
C(10) - C(8)	1.491(4)
C(11) - C(12)	1.508(3)
C(13) - C(14)	1.388(4)
C(13) - C(12)	1.376(3)
C(14) - C(15)	1.357(5)
C(15) - C(16)	1.376(5)
C(16) - C(17)	1.395(5)
C(17) - C(12)	1.376(4)

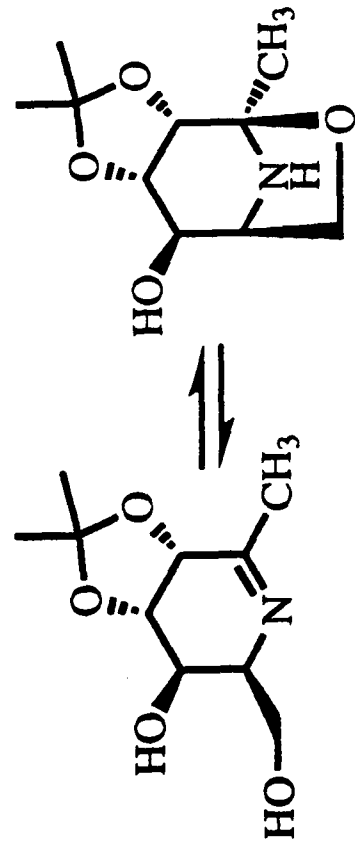
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1. G. M. Sheldrick, Crystallographic Computing 3, Ed. G. M. Sheldrick, C. Kruger and R. Goddard, Oxford University Press, Oxford, 1985.
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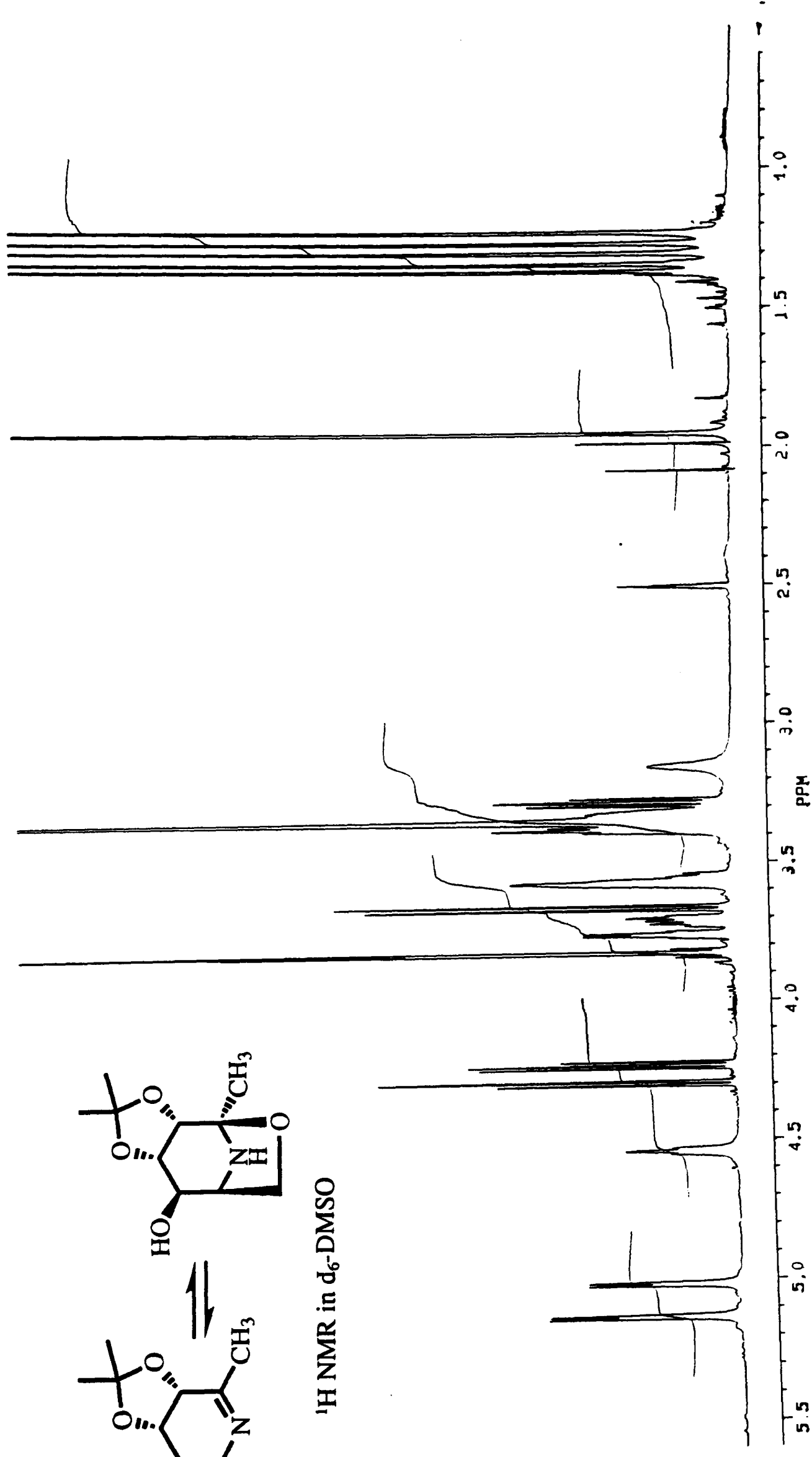
Appendix 2

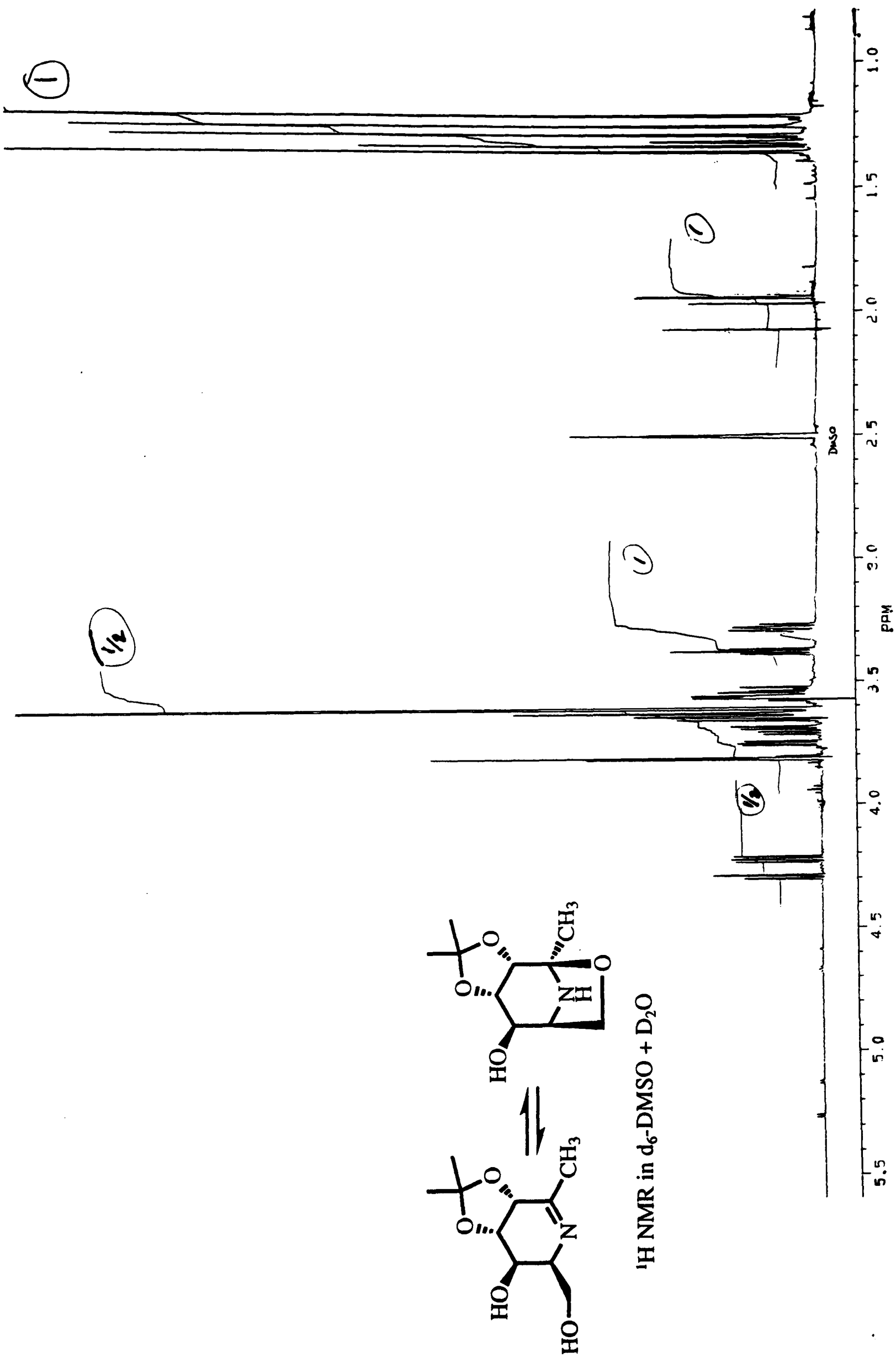
^1H and ^{13}C NMR spectra of 2,7-anhydro-1,2,6-trideoxy-2,6-imino-3,4-*O*-isopropylidene- β -L-*gulo*-heptulopyranose (1.31)

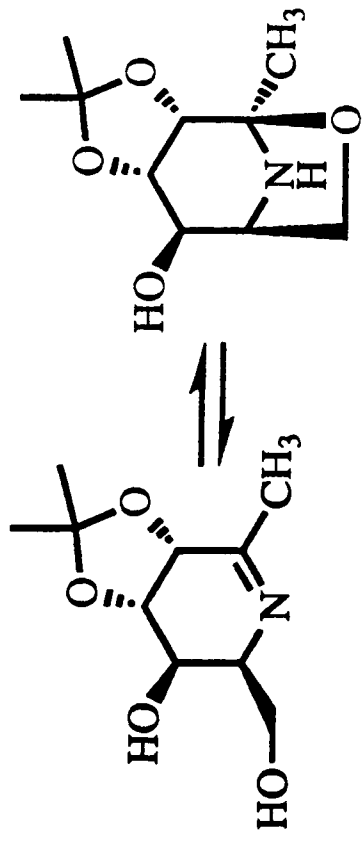




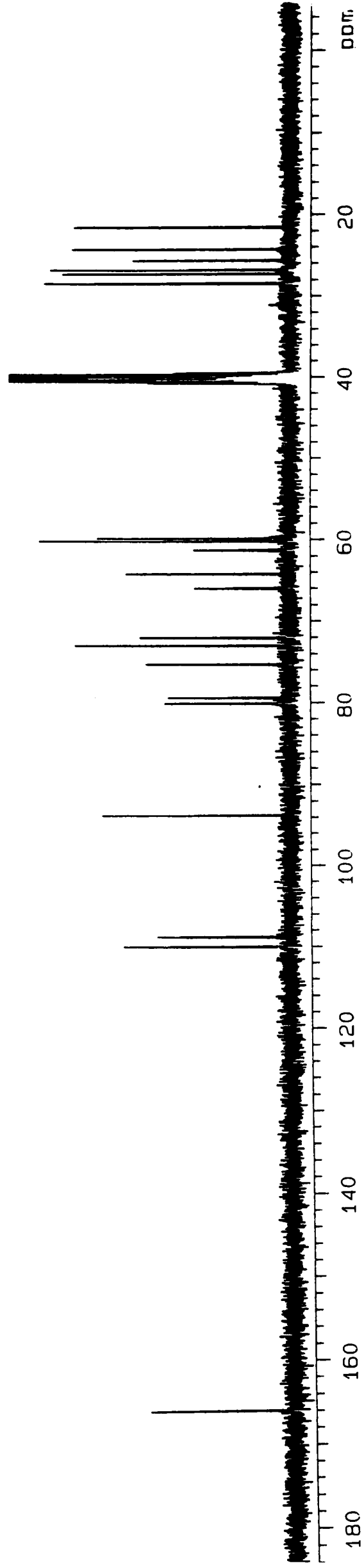
¹H NMR in d₆-DMSO

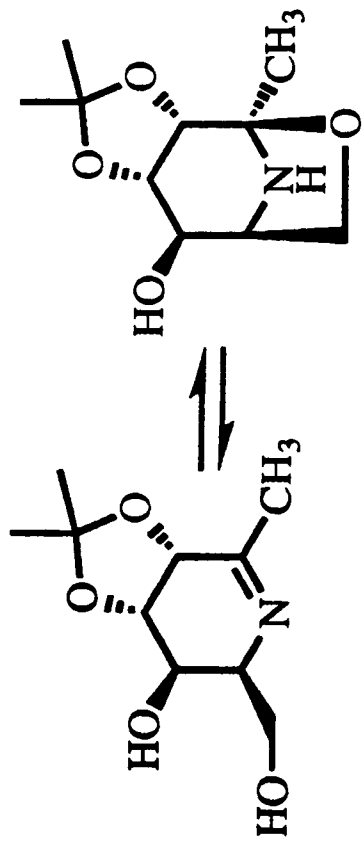




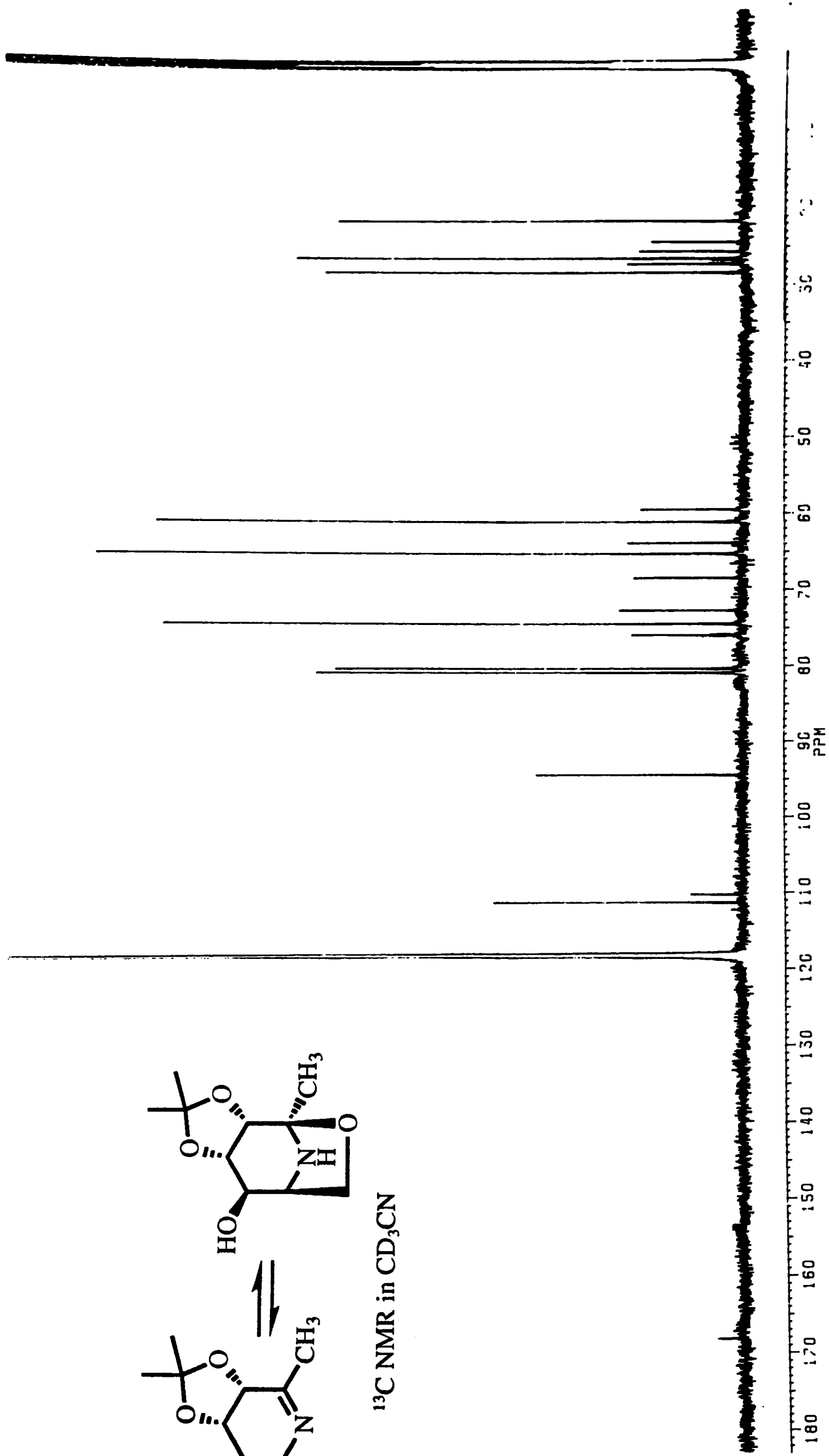


¹³C NMR in d₆-DMSO



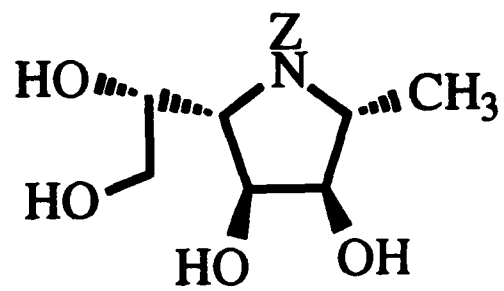


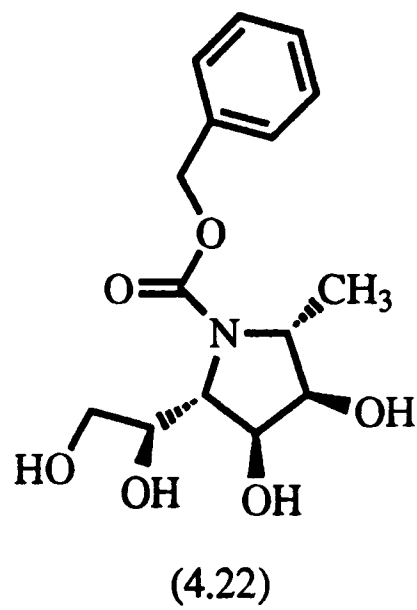
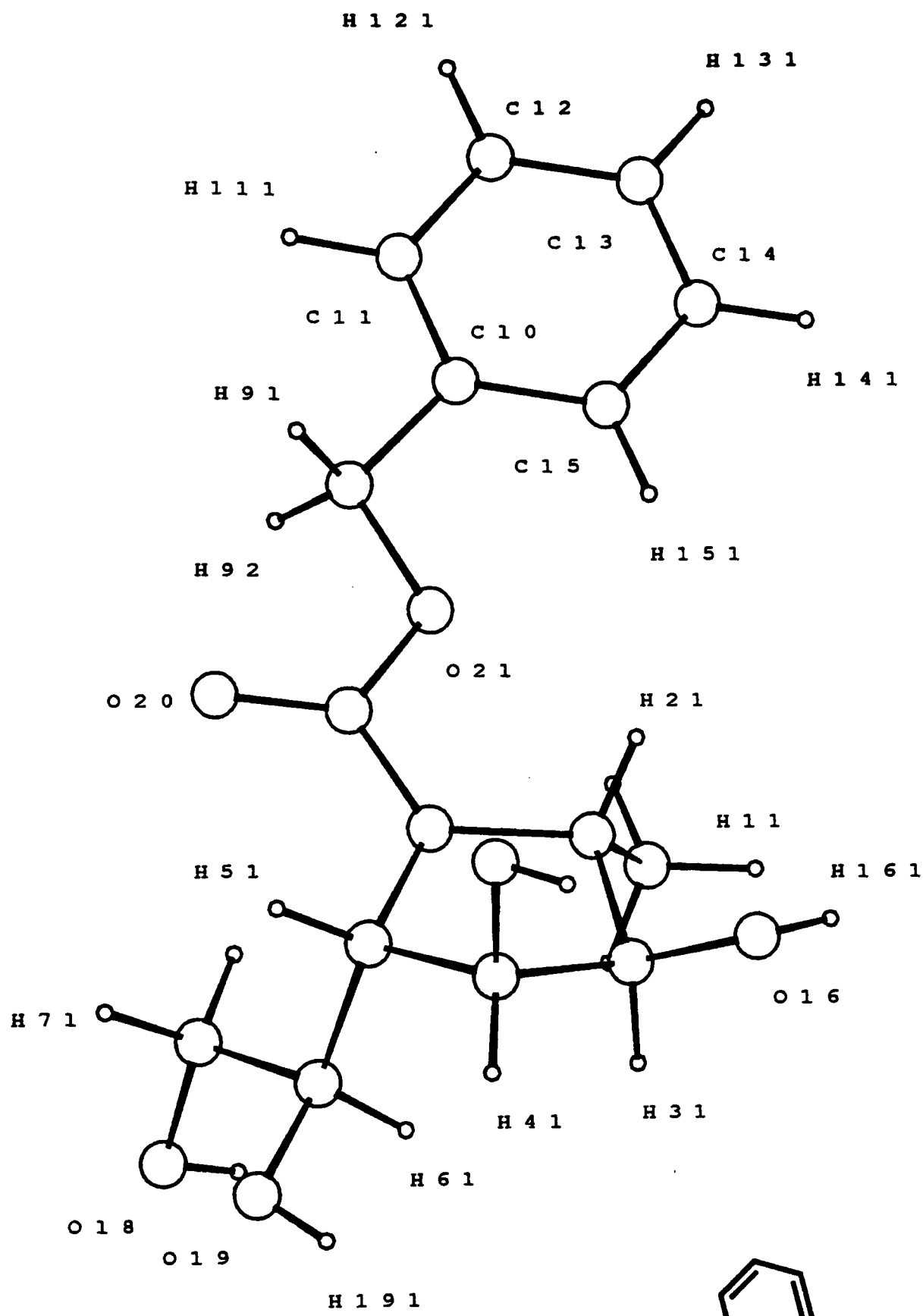
^{13}C NMR in CD_3CN



Appendix 3

X-Ray Crystal Structure Analysis of *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-*glycero*-L-*allo*-heptitol (4.22)





X-Ray Crystal Structure Analysis of *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-glycero-L-allo-heptitol (4.22).

The structure of *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-glycero-L-allo-heptitol (4.22) (crystallised from methanol/ethyl acetate) was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to $\theta = 30^{\circ}$ (Mo-K α radiation). The data were corrected for Lorentz and polarisation effects. All calculations were carried out on a Microvax 3800 computer using SHELXS-86¹ for direct methods and CRYSTALS² for all other calculations. Atomic scattering factors were taken from International Tables.³ The coordinates of all non-hydrogen atoms were given by SHELXS-86. The hydrogen atoms were placed geometrically except for the hydroxyl protons which were found from the Fourier difference maps. The structures were refined by full-matrix least-squares with isotropic temperature factors for the hydrogen atoms and anisotropic temperature factors for all other atoms. The models were refined almost to convergence. The data were refined using Chebyshev weighting schemes (Tukey & Prince)⁴ to give a final R value of 4.1%.

Crystal data for *N*-benzyloxycarbonyl-1,2,5-trideoxy-2,5-imino-L-glycero-L-allo-heptitol (4.22).

Molecular formula	C ₁₅ H ₂₁ NO ₆	Formula weight	311.3
Crystal system	Orthorhombic	Space group	P2 ₁ 2 ₁ 2 ₁
a (Å)	7.032(2)	a/ ^o	90
b (Å)	7.130(2)	b/ ^o	90
c (Å)	30.399(10)	c/ ^o	90
V (Å ³)	1524.2	D _c /g cm ⁻³	1.35
Z	4		
Reflections for lattice parameters	24		
Linear absorption coeff. μ (cm ⁻¹)	0.98		
θ range for lattice parameters (^o)	13.08-13.85		
Crystal size (mm)	0.4 x 0.4 x 0.2		

Data collection.

$h_{\min}$	-1	$h_{\max}$	10		
$k_{\min}$	-1	$k_{\max}$	10		
$l_{\min}$	-1	$l_{\max}$	42		
Total data collected	4953	Total unique data	2101		
Total observed data	3559	Criterion for observed	$I > 3\sigma$		

Refinement.

Sheldrick merging R_{factors}	1.64%		
No. of parameters	220		
Ratio of data : parameters	9.6		
Weighting scheme	Tukey & Prince		
$W = \text{weight} \times [1 - (\delta F / 6\sigma F)^2]^2$			
$(\Delta\rho)_{\min} (\text{e}\text{\AA}^{-3})$	-1.55	$(\Delta\rho)_{\max} (\text{e}\text{\AA}^{-3})$	+0.6
Final R_{factor}	0.041	Final R_w	0.054

Fractional atomic co-ordinates and equivalent isotropic temperature factors

Atom	x/a	y/b	z/c	U(iso)
O(1)	0.9888(2)	-0.3201(2)	0.17946(5)	0.0352
O(2)	0.8316(2)	0.4224(1)	0.12134(4)	0.0315
O(3)	0.8090(2)	0.1809(2)	0.07336(3)	0.0307
O(4)	1.1439(2)	0.0263(2)	0.19534(4)	0.0294
O(5)	0.6767(2)	0.2310(2)	0.26141(3)	0.0291
O(6)	0.3410(2)	0.3580(2)	0.21435(4)	0.0324
N(7)	0.8049(2)	0.1205(2)	0.14515(4)	0.0237
C(8)	0.8626(2)	-0.1669(2)	0.18134(5)	0.0257
C(9)	0.8351(2)	0.1644(2)	0.19190(4)	0.0213
C(10)	0.6440(2)	0.1888(2)	0.21618(4)	0.0224
C(11)	0.8139(2)	-0.0829(2)	0.13609(5)	0.0256
C(12)	0.8078(3)	0.3164(2)	0.03836(5)	0.0318
C(13)	0.8174(2)	0.2544(2)	0.11421(5)	0.0250
C(14)	0.5280(2)	0.3478(2)	0.19659(5)	0.0282
C(15)	0.8028(2)	0.2139(2)	-0.00496(5)	0.0318
C(16)	0.6277(3)	-0.1582(3)	0.11749(7)	0.0387
C(17)	0.7868(3)	0.0428(4)	-0.08741(6)	0.0468
C(18)	0.7840(3)	0.2342(4)	-0.08408(6)	0.0448
C(19)	0.9500(2)	-0.0043(2)	0.20770(4)	0.0227
C(20)	0.7991(4)	-0.0642(3)	-0.04972(8)	0.0526
C(21)	0.7923(3)	0.3200(3)	-0.04304(6)	0.0412
C(22)	0.8072(3)	0.0205(3)	-0.00822(6)	0.0434

Fractional atomic co-ordinates and equivalent isotropic temperature factors.

Atom	x/a	y/b	z/c	U(iso)
H(11)	0.943(4)	-0.406(3)	0.1621(7)	0.041(6)
H(14)	1.206(4)	-0.068(4)	0.2054(8)	0.050(7)
H(15)	0.700(4)	0.134(4)	0.2742(8)	0.040(6)
H(16)	0.284(4)	0.264(4)	0.2040(8)	0.044(7)
H(18)	0.7445	-0.1921	0.1974	0.0500
H(91)	0.9038	0.2858	0.1962	0.0215
H(101)	0.5708	0.0688	0.2153	0.0227
H(111)	0.9096	-0.1119	0.1128	0.0267
H(121)	0.9251	0.3953	0.0400	0.0318
H(122)	0.6930	0.3983	0.0411	0.0318
H(141)	0.5948	0.4688	0.2027	0.0286
H(142)	0.5185	0.3284	0.1641	0.0286
H(161)	0.6402	-0.2957	0.1119	0.0439
H(162)	0.5230	-0.1358	0.1391	0.0439
H(163)	0.5979	-0.0923	0.0893	0.0439
H(171)	0.7799	-0.0189	-0.1169	0.0490
H(181)	0.7760	0.3126	-0.1113	0.0450
H(191)	0.9549	-0.0261	0.2401	0.0230
H(201)	0.8023	-0.2040	-0.0521	0.0551
H(211)	0.7906	0.4598	-0.0410	0.0413
H(221)	0.8161	-0.0583	0.0189	0.0438

Interatomic distances (Å)

O(1) - C(8)	1.410(2)
O(2) - C(13)	1.223(2)
O(3) - C(12)	1.439(2)
O(3) - C(13)	1.350(2)
O(4) - C(19)	1.432(2)
O(5) - C(10)	1.427(2)
O(6) - C(14)	1.424(2)
N(7) - C(9)	1.472(2)
N(7) - C(11)	1.479(2)
N(7) - C(13)	1.344(2)
C(8) - C(11)	1.540(2)
C(8) - C(19)	1.539(2)
C(9) - C(10)	1.544(2)
C(9) - C(19)	1.528(2)
C(10) - C(14)	1.519(2)
C(11) - C(16)	1.525(2)
C(12) - C(15)	1.508(2)
C(15) - C(21)	1.386(2)
C(15) - C(22)	1.384(3)
C(17) - C(18)	1.370(4)
C(17) - C(20)	1.380(3)
C(18) - C(21)	1.392(3)
C(20) - C(22)	1.401(3)

Interatomic angles (°).

C(13) - O(3) - C(12)	114.8(1)
C(11) - N(7) - C(9)	112.5(1)
C(13) - N(7) - C(9)	121.0(1)
C(13) - N(7) - C(11)	124.4(1)
C(11) - C(8) - O(1)	113.9(1)
C(19) - C(8) - O(1)	110.8(1)
C(19) - C(8) - C(11)	105.2(1)
C(10) - C(9) - N(7)	111.1(1)
C(19) - C(9) - N(7)	102.3(1)
C(19) - C(9) - C(10)	113.5(1)
C(9) - C(10) - O(5)	110.2(1)
C(14) - C(10) - O(5)	107.9(1)
C(14) - C(10) - C(9)	111.4(1)
C(8) - C(11) - N(7)	103.0(1)
C(16) - C(11) - N(7)	112.2(1)
C(16) - C(11) - C(8)	112.7(1)
C(15) - C(12) - O(3)	108.7(1)
O(3) - C(13) - O(2)	123.2(1)
N(7) - C(13) - O(2)	125.4(1)
N(7) - C(13) - O(3)	111.4(1)
C(10) - C(14) - O(6)	112.7(1)
C(21) - C(15) - C(12)	117.8(2)
C(22) - C(15) - C(12)	123.1(2)
C(22) - C(15) - C(21)	119.1(2)
C(20) - C(17) - C(18)	119.4(2)
C(21) - C(18) - C(17)	120.3(2)
C(8) - C(19) - O(4)	111.0(1)
C(9) - C(19) - O(4)	107.5(1)
C(9) - C(19) - C(8)	102.6(1)
C(22) - C(20) - C(17)	120.8(2)
C(18) - C(21) - C(15)	120.8(2)
C(20) - C(22) - C(15)	119.6(2)

Final anisotropic temperature factors.

Atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
O(1)	0.0542(8)	0.0222(5)	0.0533(7)	-0.0097(5)	-0.0237(6)	0.0129(5)
O(2)	0.0464(7)	0.0228(5)	0.0297(5)	-0.0014(4)	-0.0011(5)	-0.0010(5)
O(3)	0.0469(6)	0.0277(5)	0.0224(4)	-0.0022(4)	-0.0008(4)	0.0001(5)
O(4)	0.0226(5)	0.0266(5)	0.0425(6)	-0.0008(4)	-0.0021(4)	0.0016(4)
O(5)	0.0375(6)	0.0289(5)	0.0227(5)	-0.0010(4)	-0.0017(4)	0.0001(5)
O(6)	0.0248(5)	0.0307(5)	0.0501(7)	-0.0109(5)	0.0036(5)	0.0034(5)
N(7)	0.0308(6)	0.0191(5)	0.0233(5)	-0.0026(4)	-0.0024(4)	0.0012(4)
C(8)	0.0292(6)	0.0185(5)	0.0338(6)	-0.0020(5)	-0.0076(6)	0.0006(5)
C(9)	0.0234(5)	0.0190(5)	0.0223(5)	-0.0026(4)	-0.0028(4)	-0.0003(5)
C(10)	0.0233(6)	0.0208(5)	0.0240(6)	-0.0032(5)	-0.0016(5)	-0.0007(5)
C(11)	0.0328(7)	0.0192(5)	0.0302(6)	-0.0047(5)	-0.0090(6)	0.0021(5)
C(12)	0.0409(8)	0.0320(7)	0.0246(6)	0.0004(5)	-0.0013(6)	0.0006(7)
C(13)	0.0262(6)	0.0246(6)	0.0246(6)	-0.0027(5)	-0.0005(5)	0.0013(5)
C(14)	0.0259(6)	0.0281(6)	0.0320(6)	0.0004(6)	-0.0002(5)	0.0052(6)
C(15)	0.0290(7)	0.0422(9)	0.0264(7)	-0.0032(6)	0.0007(6)	-0.0001(7)
C(16)	0.049(1)	0.0304(8)	0.056(1)	-0.0013(7)	-0.0279(9)	-0.0063(8)
C(17)	0.046(1)	0.077(1)	0.0333(9)	-0.0172(9)	0.0039(8)	0.000(1)
C(18)	0.048(1)	0.076(1)	0.0253(8)	0.0022(8)	0.0028(7)	-0.002(1)
C(19)	0.0228(6)	0.0197(5)	0.0270(6)	-0.0021(5)	-0.0044(5)	-0.0000(5)
C(20)	0.068(1)	0.051(1)	0.048(1)	-0.0161(9)	0.006(1)	0.004(1)
C(21)	0.0471(9)	0.049(1)	0.0304(8)	0.0019(7)	0.0020(7)	0.0024(9)
C(22)	0.056(1)	0.0412(9)	0.0365(8)	-0.0036(7)	0.0014(8)	0.0063(9)

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2. D. J. Watkin, J. R. Carruthers and P. W. Betteridge, CRYSTALS User Guide, Chemical Crystallography Laboratory, University of Oxford, 1985.
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