THE EFFICIENT SYNTHESIS OF 1-DEOXYMANNOJIRIMYCIN AND ITS DERIVATIVES

BY

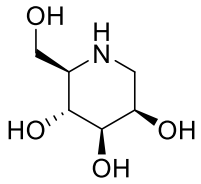
BENJAMIN MARK MANDINKA DEEBLE

A thesis
submitted to the Victoria University of Wellington
In fulfilment of the requirements for the degree of
Masters in Chemistry.

Victoria University of Wellington
2016

Abstract

Azasugars are structural analogues of carbohydrates whereby the oxygen in the heterocyclic ring is substituted for a nitrogen. These carbohydrates are an important class of compounds with medicinal bioactivities and have shown potential for the treatment of diabetes, viral-infection, cancers, and lysosomal storage diseases. 1-deoxymannojirimycin (DMJ), is a mannosidase inhibiting azasugar which has shown anti-cancer and anti-viral activity. There has been significant effort put towards developing methodology to produce this compound and libraries of its derivatives.



1-deoxymannojirimycin

This thesis presents the synthesis of DMJ and a selection of its derivatives via an efficient 4 step methodology from a carbohydrate starting material, exploiting chemo and regioselective reactions to allow for a total synthesis with minimal use of protecting groups. The synthesis of DMJ, using the methodology developed herein, surpasses published syntheses in efficiency. This synthetic strategy was then used for the preparation of *N*-functionalised DMJ derivatives without the requirement of additional synthetic steps. To illustrate the versatility of this methodology, a selection of derivatives incorporating different functionalities have been synthesised.

Acknowledgements

First and foremost, I would like to thank my supervisors Dr Mattie Timmer and Dr Bridget Stocker. Your continued guidance, support, encouragement and patience is as impressive as the knowledge of your field you both continually display. Your guidance has substantially advanced my abilities as chemist, a scientist and has instilled skills and a work ethic which will serve me throughout my future.

I would like to thank the members of the immunoglycomics group past and present. Mention must go to Alex Hunt-Painter, whose initial work was the genesis for this project, a fact he is fond of reminding me. Your advice in matters both in and outside of the lab has been greatly appreciated. To the other members of the group who have been present for various parts of this journey, Amy, Amy Jr., Cij, Rhia, Jessie, Jaime, Kris, Krystel, Hillary, Janice, Billy, Thomas and Stefan, your companionship, enthusiasm and willingness to assist me has been invaluable over the last two and a bit years. Kia Kaha.

To my parents Mark and Helen, and my brother Gabriel, I thank you for your ongoing support in this and in all my endeavours, be they great or small. Knowing I only need ask, or make the trip home across town and receive anything I need has been a continued comfort throughout this work.

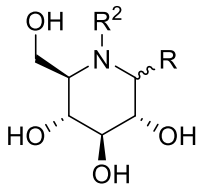
To my flatmates, I thank you for the help you gave me, the encouragement when the pressure built up, the willingness to entertain me in my free time and the patience to put up with many late-night arrivals when I was working.

Table of Contents

1. Introduction

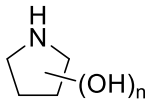
1.1. Azasugars

Azasugars are structural analogues of carbohydrates whereby the oxygen in the heterocyclic ring is substituted for a nitrogen. Azasugars exhibit a range of biological activities and accordingly have found wide interest in drug development, with their potential use for the therapeutic treatment of viral infection, cancer, diabetes and the rare but highly fatal glycosphingolipid storage diseases.[1,2] There are several classes of azasugar (Figure 1), namely the piperidines (6 membered ring), the pyrrolidines (5- membered), pyrrolizidines (1,2-fused-5,5-bicycles), indolizines (1,2-fused-5,6 bicyclic rings) and the nortropanes (5,6-bridged bicycles). The biological activity of azasugars is, in part, due to their ability to mimic the transition state of enzymatic glycoside hydrolysis and thus inhibit glycosidase enzymes. In addition to their inhibitory activity, azasugars have been explored for use as molecular chaperones[3] and immuno-modulators.[4] To this end, several azasugars are currently implemented as established clinical drugs such as glycet (miglitol **2a**) for type II diabetes and zaveska (miglistat **2b**) for Gaucher's disease.[3]

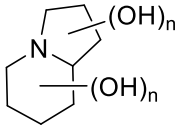


Piperidine

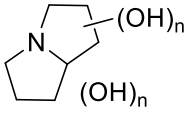
1a R = H R^2 = H 1-deoxynojirimycin
1b R = OH R^2 = H nojirimycin
2a R = H R^2 = $(CH_2)_2OH$ miglistol
2b R = H R^2 = $(CH_2)_3CH_3$ miglistat



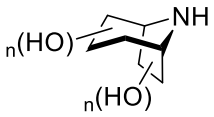
Pyrrolidines



Indolizines



Pyrrolizidines



Nortropanes

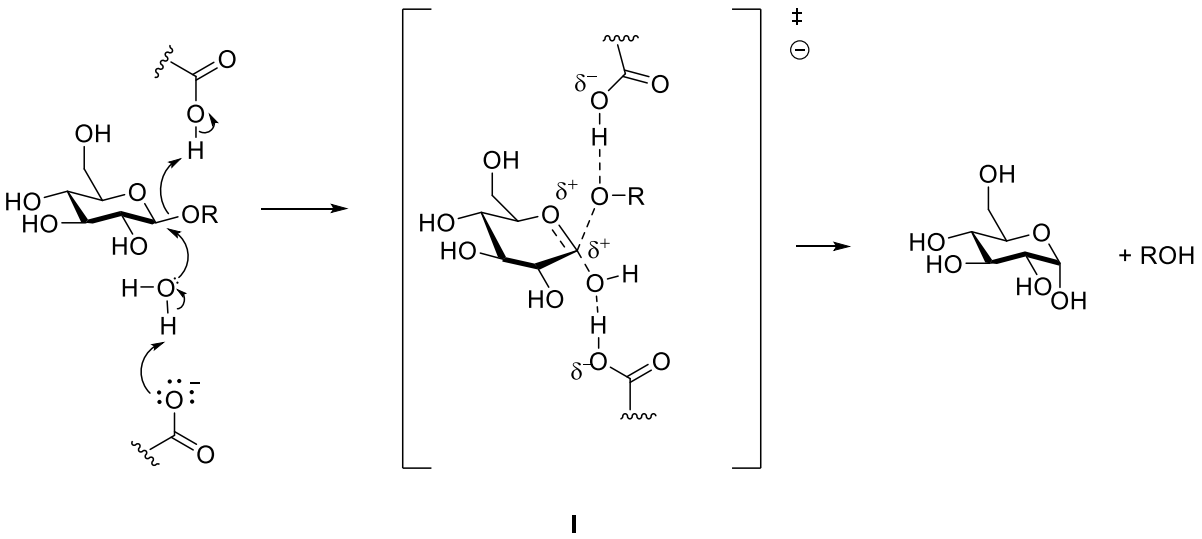
Figure 1.1. Classes of azasugar

The investigation of azasugars and their biochemistry commenced in the 1960s with the isolation of the first azasugar, nojirimycin (**1b**), from *Streptomyces reseochromogenes* and the subsequent identification of its anti-microbial activity.[5] This was soon followed by the synthesis[6] and isolation[7] of 1-deoxynojirrimycin (DNJ) (**1a**) which was found to inhibit glycosidase hydrolase enzymes.[8] The potent bioactivity of DNJ ignited interest in azasugar research, with a focus on developing analogues of a large range of carbohydrates.[9] Indeed, a better understanding of azasugar bioactivity led to a rapid advancement in the interest and productivity of research into azasugar synthesis, with a focus on the synthesis of a variety of original and complex imminosugars alongside the development of effective methodology for synthesising large imminosugar libraries.[10]

1.1.1. Enzyme Inhibition

One aspect of interest in azasugar biochemistry is the ability of this class of compound to act as inhibitors of glycosidase hydrolase enzymes. Glycosidase enzymes catalyse the hydrolysis of the glycosidic linkages within polysaccharides, thereby breaking the carbohydrate down into its component monosaccharide sugars. Cleavage is initiated by two carboxylate residues on the enzyme located on opposite sides in the active site, one of which is protonated and the other not. These two residues act as a protonating acid and a nucleophilic base, respectively. Hydrolysis can result in the inversion or retention of the stereochemistry at the anomeric centre.[11]

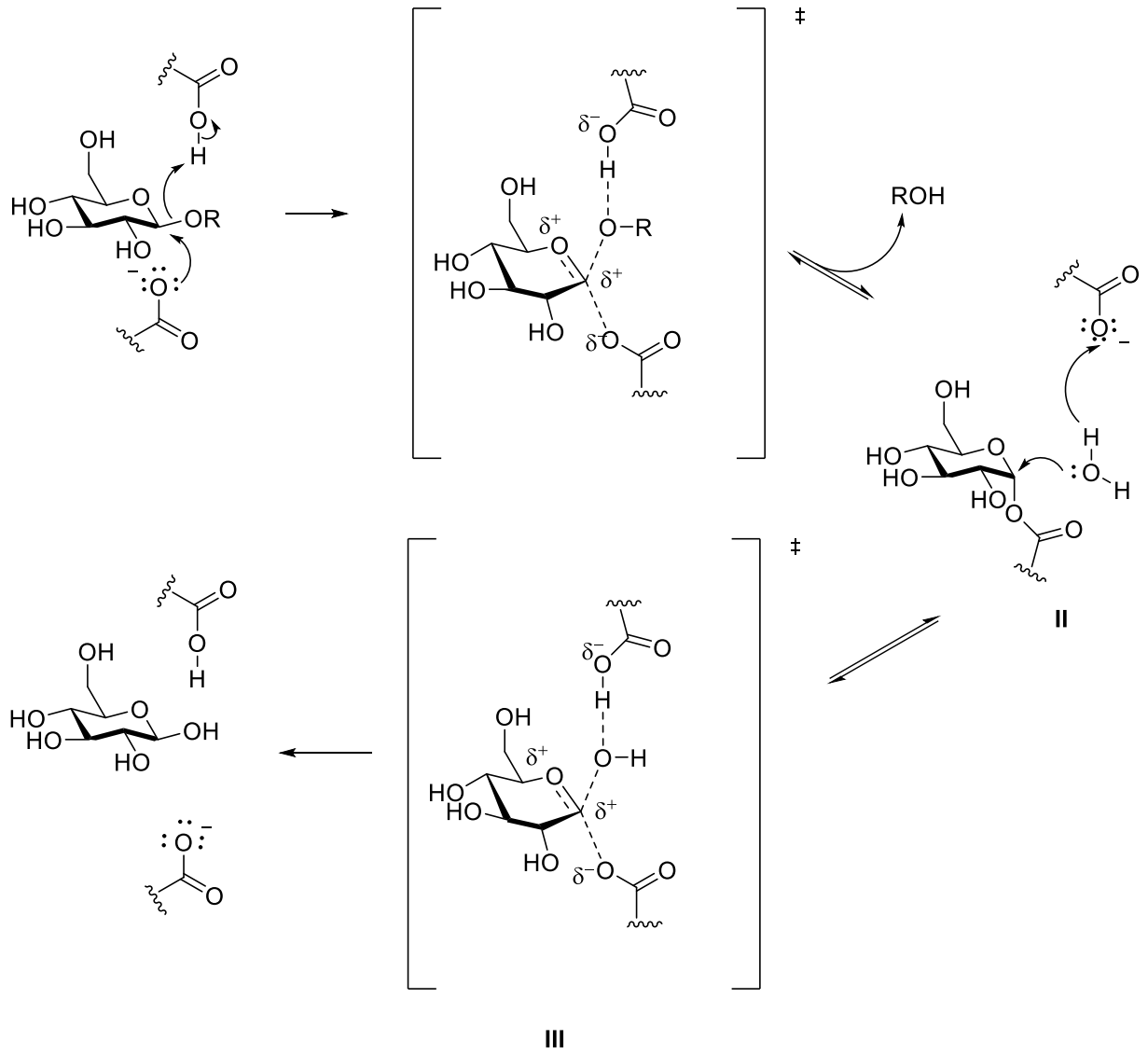
In the inversion mechanism (Scheme 1.1) the deprotonated carboxylate acts as a general base to deprotonate the incoming water nucleophile, which attacks the anomeric carbon. The other carboxylate acts as an acidic residue to protonate the leaving group (R = sugar), thereby forming a positive charge on the glycosidic oxygen in the transition state and making leaving more favourable. Moreover, the ring oxygen donates negative charge to stabilise the accumulating positive charge at the carbon resulting in the formation of an oxocarbenium ion in the transition state (**I**). The observed inversion results from the nucleophilic attack occurring from the opposite face to that of the glycosidic linkage and proceeding through a single transition state in the manner of an S_N2-like reaction.[9]



I

Scheme 1.1. The inversion mechanism of enzymatic hydrolysis

In the retention mechanism (Scheme 1.2) the acidic carboxylate residue acts in the same manner as in the inversion mechanism by protonating the glycosidic oxygen. However, the deprotonated carboxylate acts as the initial nucleophile in a substitution reaction to form a covalently bound glycosyl-enzyme intermediate (**II**), via an oxocarbenium ion transition state. Subsequent nucleophilic attack then occurs at the opposite face, i.e. on the same face as the original glycosidic linkage. The deprotonated carboxylate group in the enzyme's active site can then deprotonate the incoming nucleophile (H_2O) thereby initiating the hydrolytic breakdown of the intermediate and subsequent hemi-acetal formation via the substitution and departure of the carboxylate through a second oxo-carbenium ion transition state (**III**).[9] In sum, this leads to the hydrolysed monosaccharide.



Scheme 1.2. The retention mechanism of enzymatic hydrolysis.

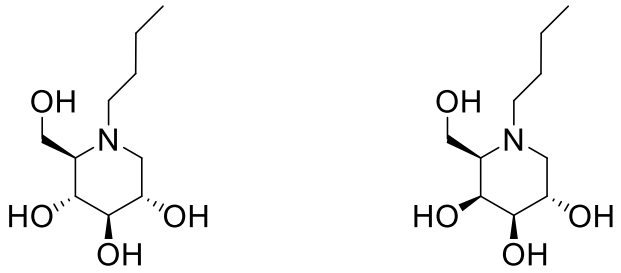
Azasugars are able to competitively inhibit the glycosidase enzyme by binding to the active site of the hydrolase via their ability to mimic the oxocarbenium ion transition state(s). At physiological pH (pH = 7.4), the azasugar nitrogen becomes protonated forming a charged species. The charge and conformation of the azasugar mimics the charge and conformation of the oxocarbenium transition state of glycosidic hydrolysis (Figure 1.2).[12,13] Accordingly, this

enzymatic inhibition is an effective way by which to treat a number of diseases. The most pertinent example of the therapeutic activity of azasugars in this manner is the control of glucose release for the treatment of type II diabetes. Here, the azasugar can be used to slow the release of glucose monomers, allowing the patient to better control blood sugar levels through a non-insulin dependent mechanism.[14] Another target is the processing of oligosacchides into glycoproteins and glycosphingolipids, which can be used to alter N-glycan and O-glycan biosynthesis for cancer treatment[15-17] and prevent viral glycosidase interaction of cell surface glycoproteins.[18-21]

Figure 1.2. Protonated azasugars mimic the charge and conformation oxocarbenium ion.

1.1.2. Molecular Chaperone use in Protein Folding

Azasugars have also found therapeutic use for the treatment of various protein miss-folding disorders. The correct folding of the primary amino acid sequence of a protein is essential to form the desired tertiary and quaternary structure required for its function. Genetic mutation can induce incorrect folding and hence alter enzymatic functions. To prevent the release of dysfunctional proteins in the body, miss-folded proteins are not released from the endoplasmic reticulum (ER).[22] This can induce a deficiency in proteins that are essential to various enzymatic processes and result in the accumulation of intermediate substrates, which in turn can result in significant morbidity and sometimes mortality. In particular, the accumulation of intermediate substrates can result in defective lysosmal function and lysosomal storage diseases such as Gaucher's disease and Fabry's disease.[23] If the nature of the folding defect is minor, such as a point defect, small molecules such as azasugars are able to act in the manner of molecular chaperones and assist in the folding of the protein without being involved in its function. Chemical chaperones act as active site inhibitors, binding to the active site and stabilising the protein in a conformation resembling that of the correctly folded protein. This allows the protein to be released from the ER and behave with an activity resembling that of the native enzyme.[23,24] The azasugars *N*-butyl-DNJ (**2b**) and *N*-butyl-deoxygalactojirimycin (**3**) have shown successful chaperone activity targeting the mutant enzymes involved in Gaucher's[25] disease and Fabry's[24,26] disease, respectively (Figure 1.3).

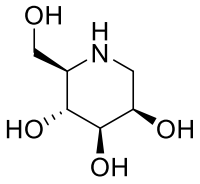


2b **3**

Figure 1.3. *N*-butyl-DNJ **2b** and *N*-butyl-DGJ **3** with application for treatment of lysosomal storage disease.

1.1.3. 1-Deoxymannojirimycin

1-Deoxymannojirimycin (DMJ) (**4a**) (Figure 1.4) is an azasugar with *manno* stereochemistry configuration, and is therefore able to inhibit mannosidases. DMJ was first isolated in 1979 from the legumes *Lonchocarpus sericeus* and *L. costaricensis,*[27] and first synthesised in 1982 from a mannofurano-lactone.[28]



4a

Figure 1.4. 1-Deoxymannojirimycin

The investigation of the bioactive properties of DMJ began in 1984 when DMJ was first shown to be a mannosidase inhibitor.[29] This inhibitory activity has since been shown to interfere with the processing of a number of mannose associated N-glycans and proteins. For example, the alteration of glycoproteins by the inhibition of golgi α-mannosidases can be used to prevent the cleavage of terminal mannose monomers, a key step in the processing of glycoprotein oligosaccharides.[30] Exploration of this biological activity, has since led to the investigation into a number of potential medicinal applications. One avenue of interest is anti-viral inhibition. Here, increasing the number of high mannose N-glycans through the use of DMJ as a mannosidase inhibitor has been shown to increase the binding of viruses to C-type lectins (such as DC-SIGN involved in antigen presentation and the activation of the immune response). This activity can be exploited to aid the immune response to HIV and leukaemia.[31] In addition to promoting inhibitory activity, it has also been shown that DMJ may partially reverse phenotypic resistance with mutant

strains of the HIV virus.[18] α-Mannosidase inhibition in this manner has also been shown to have direct implications for anti-cancer treatment. Here, the prevention of mannose cleavage from N-linked oligosaccharides may prevent the processing of dysfunctional proteins and the accumulation of these proteins then induces endoplasmic reticulum stress leading to cell apoptosis.[15] The rapid metabolism of cancer cells[32] leads to rapid uptake of the mannosidase inhibitor and thus causes selective cell death. In preliminary trials this therapeutic approach has shown a reduction in tumour growth and metastasis, with limited side effects from adverse lysosomal inhibition in non-cancer tissue.[33] The change to cell surface oligosaccharide composition has been found to inhibit melanoma cell invasion in a dose-dependent manner.[16] DMJ treatment of human colon cancer cell lines has also been shown to competitively inhibit the uptake of D-[2-^{3}H]mannose in a dose-dependent and reversible manner.[34] In 2009 multivalent effects in glycosidase inhibition with azasugar clusters were first observed, sparking a new avenue of azasugar research. Here, a significant increase in bioactivity has been shown for the multivalent azasugar clusters over the corresponding monovalent ligand.[35] The observation of this multivalent activity has renewed interest in the medicinal potential of DMJ as a mannosidase inhibitor.[36]

The ubiquitous nature of glycosidase hydrolysis and corresponding potential for inhibitory targets has resulted in an ongoing expansion of the potential applications for azasugar bioactivity and, in turn, the bioactivity of DMJ and its derivatives. The established anti-viral and anti-cancer activity, and the ongoing discovery of new therapeutic targets has sustained continual interest into developing syntheses to prepare DMJ. As more examples of potent inhibitory activity

emerge, and new avenues research such as multivalent therapy develops, there is an increasing mandate to find an efficient synthetic methodology to produce DMJ and its derivatives.

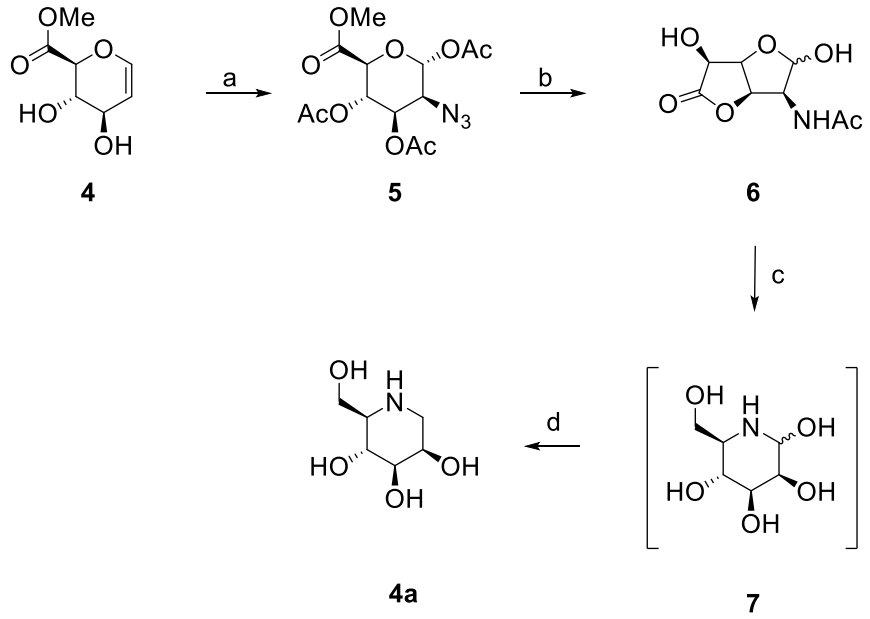
1.2. Synthetic Routes to the Production of 1-Deoxymannojirimycin

There are two general synthetic approaches that have been employed for the synthesis of 1-deoxymannonjirimycin (DMJ, **4a**). The first synthetic approach involves the use of chiral-pool starting materials, typically employing carbohydrates as the chiral material. In the second approach, the piperidine scaffold is constructed by metathesis and a series of asymmetric reactions. These syntheses have different advantages in terms of efficiency, scalability and the ease with which DMJ analogues can be prepared, and some strategies simply serving as a means to explore the scope of new synthetic methodology. As the focus of this project is the development of an efficient and low-cost synthesis of DMJ (**4a**), those synthetic strategies which are highly efficient are of greater interest, however, it is also interesting to provide an overview of the different approaches to the synthesis of DMJ to date. Accordingly, an overview of the previous syntheses of DMJ is presented below.

1.2.1. Using a Pre-existing Chiral Scaffold with Protected Hydroxyls

The first synthesis of 1-deoxymannojirimycin (**4a**) was published in 1982 by Lonngren and co-workers.[28] Key in the synthesis was the installation of the nitrogen functionality via an azide which is subsequently reduced. This approach has proved to be popular for many subsequent syntheses of DMJ. In Lonngren and co-workers' synthesis, azidonitration and acetylation of methyl 2,6-anhydro-5-deoxy-D-lyxo-hex-5-enonate (**4**) afforded the azide **5** which was then subjected to acid hydrolysis followed by hydrogenation to produce **6** (Scheme 1.3).[37] Reduction of this lactone **6** with sodium borohydride was reported to have generated hemiaminal **7** *in situ*

(not isolated), [28] which then afforded DMJ following acidification (**4a**) as its hydroacetate salt in 7 steps in an overall 3.5% yield.

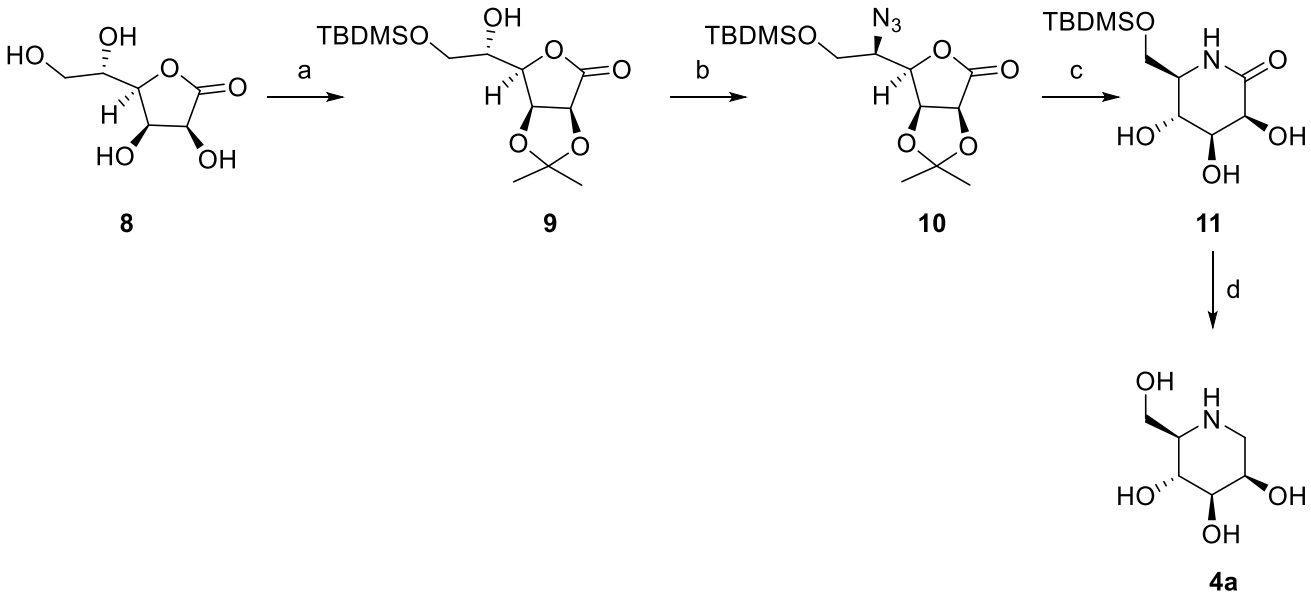


Scheme 1.3. First synthesis of DMJ by Longrenn and co-workers.[28,37]

Reagents and Conditions: a) $(NH_4)_2Ce(NO_3)_6$, NaN_3, MeCN (6%); (b) (i) HCl; (ii) H_2, Pd/C, Ac_2O/MeOH (80%); (c) $NaBH_4$, H_2O; d) AcOH (72%) 2 steps;

Other early chiral pool syntheses also focused on the use of lactones as chiral starting materials, illustrated by the strategy pioneered by Fleet and co-workers (Scheme 1.4).[38] Here, the lactone functionality is used to afford the deoxygenation at the first position via selective reduction of an amide. The synthesis commenced with the installation of an isopropylidine group across C2 and C3, and a silyl protecting group at C6 of the 1,4-gulolactone (**8**), which then allowed for the selective installation of an azide at C5 to give azido-lactone **10**. Palladium catalysed hydrogenation in methanol allowed for cleavage of the isopropylidene protecting group and reduction of the azide to the corresponding amine, which was subsequently used in reductive

amination, in a single step to produce the lactam (**11**). Reduction of the amide using borane dimethyl sulphide in THF then gave DMJ in 25% overall yield over 8 steps.[38]

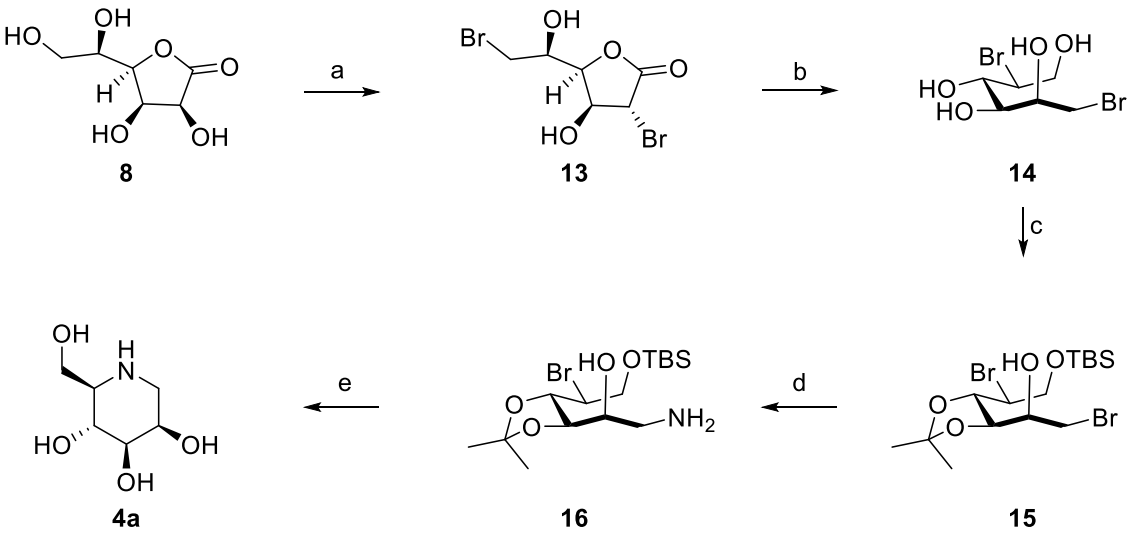


Scheme 1.4. Lactone starting material strategy used by Fleet.[38]

(a) (i) acetone, dimethoxypropane, pTsOH (cat), N_2; (ii) $AcOH/H_2O$; (iii) imidazole, TBDMSCl, DMF, N_2, 45%; (b) (i) pyradine, trifluoromethanesulfonic anhydride, CH_2Cl_2, N_2, -40°C; (ii) NaN_3, DMF, N_2, 76%; (c) H_2, Pd/C, MeOH, 91%; (d) (i) BMS, THF, N_2; (ii) TFA/H_2O 80%.

In a similar synthesis of DMJ (**4a**) employing lactone **8**, bromination of the 2- and 6- positions afforded the dibrominated product **13**, which was reduced to produce iditol **14** (Scheme 1.5). Protection of the C1- C3- and C4- hydroxyls then gave **15**, which, under basic conditions, selectively formed an epoxide from the bromine at the C6-position and its adjacent hydroxyl, thereby allowing for the introduction of ammonia in a single step to give amine **16**. Nucleophilic intramolecular attack of the amine onto the bromine then leads to cyclisation and the formation of the piperidine ring. Finally, removal of the isopropylidene protecting group, afforded DMJ (**4a**)

in an overall 29% yield in 9 steps from D-mannose. Key in the approach was the use of a 1,2-isopropylidene group in the protection of the C3- and C4- positions to avoid the formation of the 4, 5-protected isomer. This synthesis is particularly notable in that the use of azides can be avoided.[39]

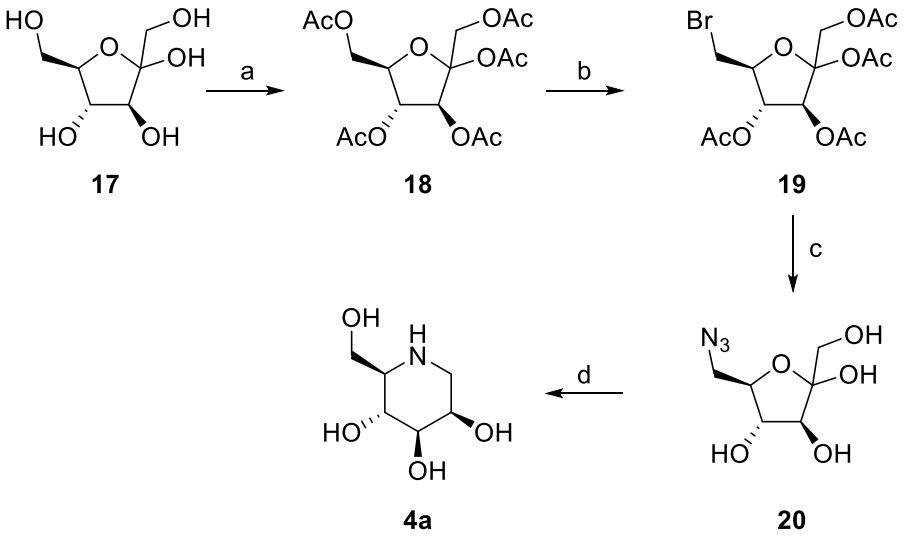


Scheme 1.5. Synthesis from a lactone via a 2,6 dibrominated intermediate.

Reagents and conditions: (a) HBr, acetic acid; (b) $NaBH_4$, MeOH, amberlite-H^+ resin, H_2O, 55% over two steps; (c) (i) acetone; (ii) TBDMSCl, pyridine; (d) NH_3 in MeOH, rt, 80% over 3 steps; (e) (i) NaOAc, $MeNO_2$, reflux, 74%; (ii) 4 N HCl, MeOH, 95%.

In perhaps one of the more elegant syntheses of DMJ (**4a**), which has since been further optimised by others, Furneaux and co-workers used the inexpensive α-D-fructose (**17**) as a starting material to synthesise DMJ in 5 steps and in a 25% yield.[40] Use of this synthetic strategy required the installation of an azide functionality at the 6-position by first protecting the hydroxyls with acetyl groups and then installing a tosyl derivative, 2,4,6,-triisopropylbenzenesulfonyl, at the 6 position for subsequent substitution with an azide. The

azide could then be used in a reductive cyclisation which when deprotected gave DMJ.[40] Wrodnigg and co-workers later modified this synthesis to simplify the protecting group strategy (Scheme 1.6). Acetylation of D-fructose (**17**) to give the per-acetylated product **18** followed by selective installation of the bromine at the 6-position (**19**) then allowed for the rapid synthesis of DMJ in 27% yield. Moreover, the compounds **18** and **19** could be isolated by crystallisation, thereby improving the yield.[41]

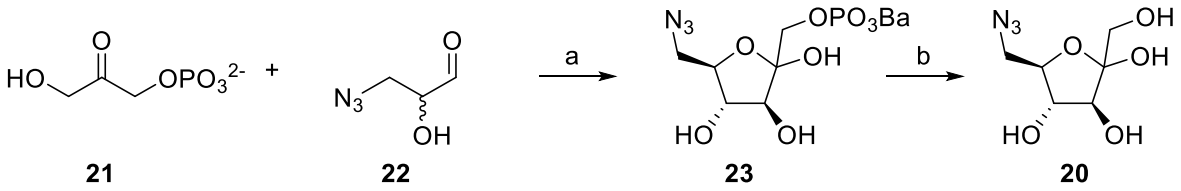


Scheme 1.6. Synthesis of DMJ from D-fructose [41]

Reagents and Conditions: (a) Ac_2O, H_2SO_4, 60%; (b) PPh_3Br_2, pyridine, CH_2Cl_2, reflux, 90%; (c) (i) NaOMe, MeOH, 0 °C; (ii) NaN_3, DMF, rt, 65%; (d) H_2, Pd/C rt, 70%;

Enzymatic synthesis has also been investigated as an alternative protecting group free strategy to obtain the 6-azido-fructofuranose precursor **20** (Scheme 1.7).[42] Here, aldolase is used to catalyse the reaction between dihydroxyacetone phosphate **21** and (*R,S*)-3-azido-2-hydroxypropanal **22** to give the azide **23** with a barium phosphate at C-1. The phosphate which could be easily removed by phosphatase to yield the key intermediate **20**. Producing the

precursor **20** in this manner afforded the synthesis of DMJ in 36% total yield over 3 steps (Scheme 1.7).[42]

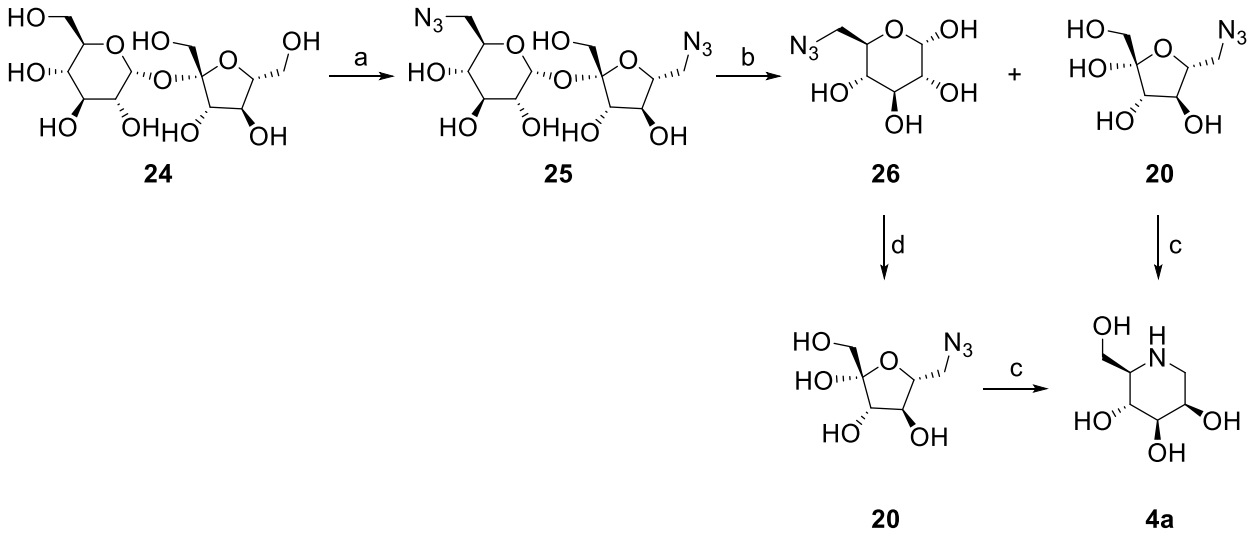


21 **22** **23** **20**

Scheme 1.7. Synthesis of DMJ from D-fructose

Reagents and Conditions: (a) aldolase (EC 4.1.2.13), 25 °C, $BaCl_2.2H_2O$, 70%; b) Phosphatase, 80%;

The synthetic strategies of Furneaux[40] and Wrodnigg[41] have also been adapted for the use of the disaccharide sucrose (**24**) as an alternative substrate and thereby exploiting the potential for the synthesis of two azasugar molecules from a single equivalent of staring material (Scheme 1.8). To this end, azides are installed at the C6 positions of the monosaccharide units in the sucrose **24** to give the diazido intermediate **25**. Hydrolysis of the 6,6'-diazide into its monosaccharide components produces the desired azido-fructofuranose **20** and its *gluco* epimer **26**. The *gluco* monosaccharide can be biochemically transformed into its fructose equivalent by the enzyme glucose isomerase, an enzyme utilised on an industrial scale in sugar conversion. This additional fructose intermediate allows for an increase in total yields to 35%.[43] This synthetic strategy also represents an example of a synthetic strategy which does not require the use of protecting groups.

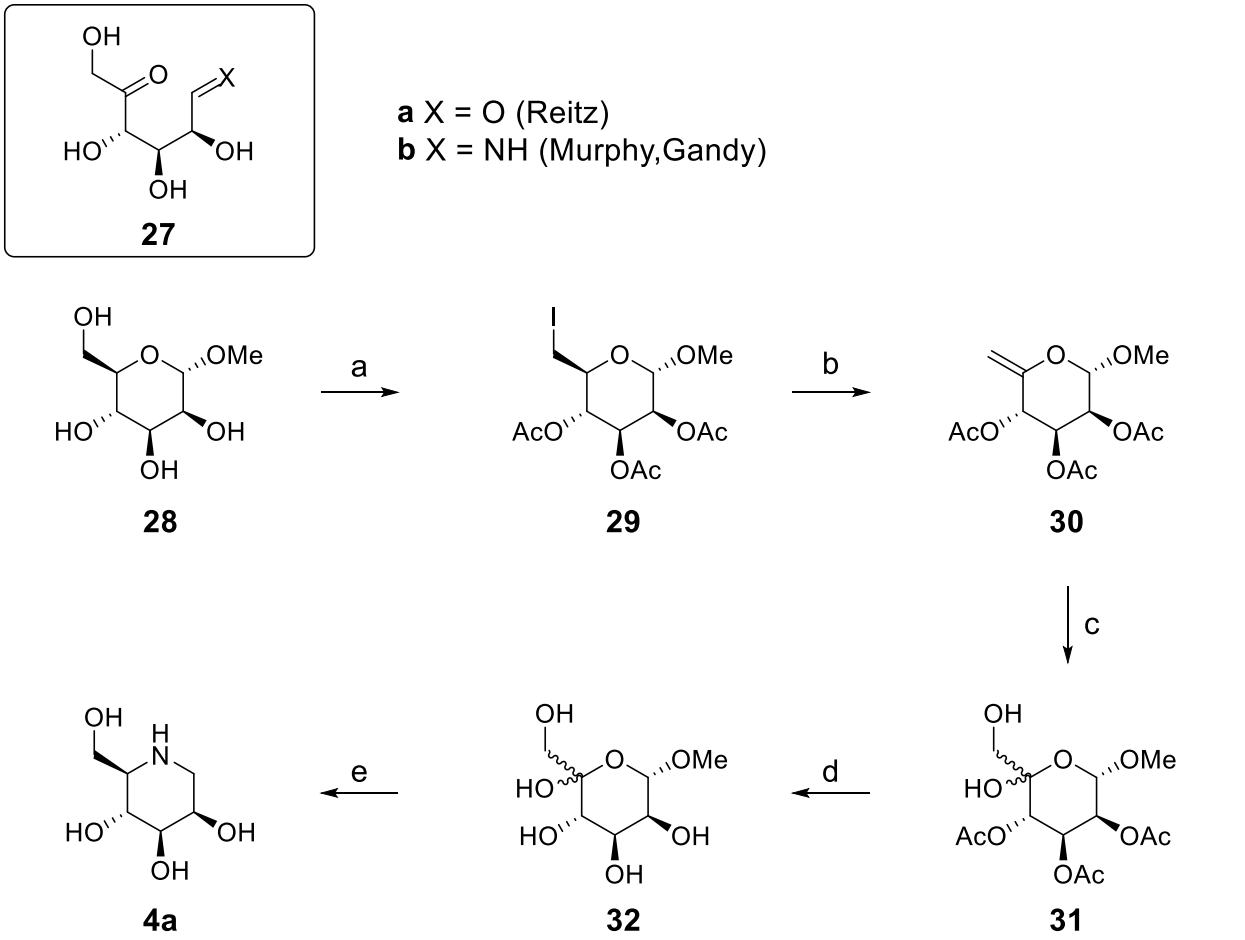


Scheme 1.8. Using sucrose as a chiral starting material

Reagents and Conditions (a) (i) PPh_3, CCl_4, pyridine, (ii) NaN_3, DMF, 57%; (b) Amberlite IR 120 (H^+) 63% (51% gluco, 49% *manno*) (c) H_2, Pd/C 78%; (d)(i) glucose isomerase (E.C. 5.3.1.5) (ii) H_2, Pd/C, 8% over two steps;

Another motif which has been explored is a dicarbonyl intermediate **27** which can be utilised for double reductive amination with intramolecular cyclisation to produce DMJ (Scheme 1.9). Baxter and Reitz pioneered the use of this dicarbonyl intermediate in a double reductive amination of 5-keto-D-mannose to produce DMJ in 10% overall yield,[44] the dicarbonyl can also be prepared via an uloside derivative of methyl mannopyranoside**.** In this latter work, acetylation of methyl mannopyranoside **28** allowed for the selective iodination of the primary hydroxyl to produce **29**. Upon treatment of **29** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), deprotonation occurred at the C4 position to produce alkene **30**, which in turn underwent dihydroxylation to give the protected uloside **31**. Deprotection of the acetyls in **31** then afforded **32,** which could undergo reductive amination (via **27)** to obtain DMJ (**4a**) in 5 steps and in a yield

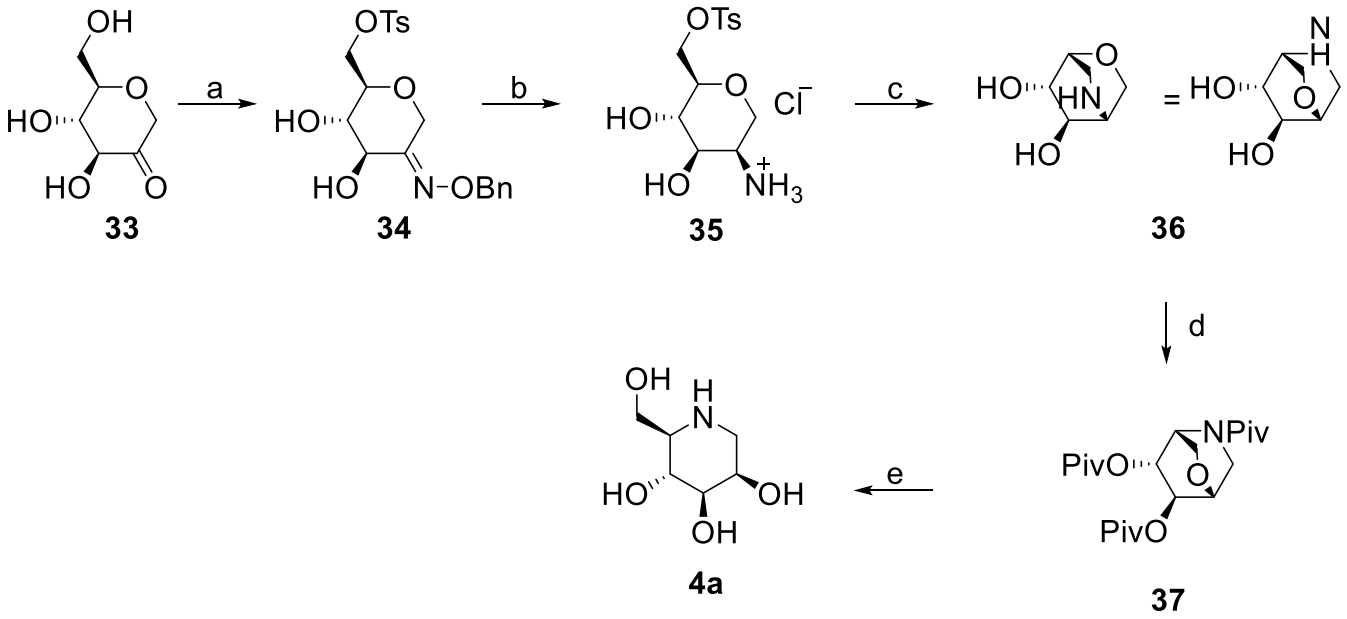
of 32% from methyl α-D-mannopyranoside. Murphy *et al.* proposed an adaption of this strategy in which the methoxide in **30** was substituted with an azide group prior to the reductive amination, and the hydroxylation was then performed via epoxidation, this allowed for the synthesis of DMJ via formation of the keto-imine **27b** in 9 steps. The keto-imine intermediate may have a potential for use when developing efficient syntheses of azasugar oligosaccharides but for the synthesis of DMJ alone, the route is lengthy and did not advance the total yield beyond 10%.[45]



Scheme 1.9. The use of ulosides to produce a dicarbonyl intermediate *en route* to the synthesis of DMJ

Reagents and Conditions (a) (i) I_2, Ph_3P, imidazole, PhMe; (ii) Ac_2O, pyridine, 82%; (b) DBU, THF, 91%; (c) 3-Chloroperbenzoic acid, CH_2Cl_2, BnOH; (d) NaOMe, MeOH, 74% over two steps; (e) NH_4OAc, $Pd(OH)_2/C$, H_2, MeOH:H_2O (15:1) 61%;

Another approach undertaken by Maier *et al*. to synthesise DMJ from anhydro-D-fructose **33** involved formation of a pyran intermediate (Scheme 1.10). The transformation of the ketone in **33** into an oxime and subsequent tosylation of the 6-hydroxy afforded **34**. Reduction of the oxime in **34** using catalytic palladium also resulted in the cleavage of the benzyl group to give the HCl salt of the amine **35**. Deprotonation of the amine with triethylamine **35** then allowed for the nucleophilic substitution of the tosylate to afford the bicyclic pyran 2,6-anhydro-1-deoxymannojirimycin **36**. To allow for the cleavage of the pyran-ether bond, global protection was achieved using pivaloyl groups to give **37**. Pivaloyl was selected as the protecting functionality over a more typical protecting group such as an acetyl, to allow for the use of tribromoborate in the subsequent cleavage reaction, as pivaloyl does not coordinate to the borate. After BBr_3 was used to induce the cleavage of the pyran, global deprotection gave DMJ (**4a**). While the pyran-ether (**36**) could be obtained from the oxime (**34**) in a one pot reaction via palladium catalysed hydrogenation and subsequent base mediated cyclisation, it was found that isolation of the HCl salt of the amine (**35**) before the nucleophilic attack allowed for a quantitative yield in the subsequent cyclisation reaction. In this manner DMJ was produced in an overall yield of 35% (7 steps) or 27% (6 steps).[46]

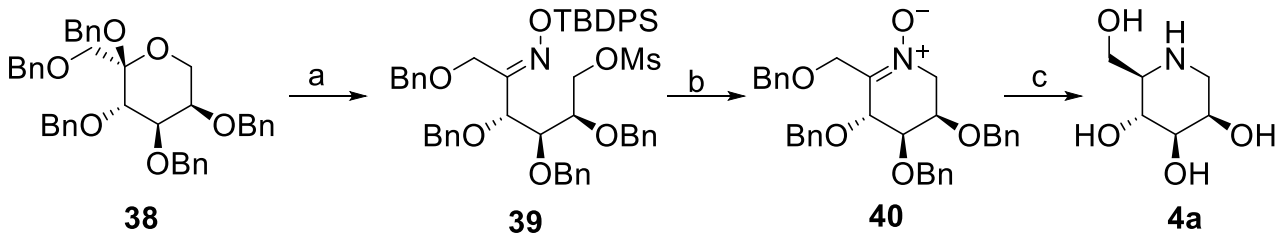


Scheme 1.10.
Utilisation of a pyran-ether

Reagents and Conditions: (a) (i) $NH_2OBn{\cdot}HCl$, KOH, r.t., EtOH, 91%; (ii) TsCl, Pyridine -20 °C, 77%; (b) H_2, Pd/C, MeOH/HCl, 30 bar, r.t., 81%; (c) Et_3N, DMF, r.t., quant; (d) PivCl, DMAP, Pyridine, r.t., 93%; (e) i) BBr_3, $CHCl_3$, then MeOH, r.t.; ii) NaOMe, MeOH, r.t., 67% (2 steps).

Cyclic nitrones and other *N*-oxy species have also been investigated as potential intermediates for the synthesis of azasugars. For example, the treatment of per-benzylated glycoside **38** with $TBDPSONH_2$ leads to ring opening and formation of the silylated oxime, which was followed by the introduction of a mesyl ester at the primary hydroxyl to give **39** (Scheme 1.11).[47] Substitution of the mesylate followed by deprotection of the silyl ether afforded the nitrone **40** in the *manno* configuration and in a 47% yield over 3 steps. The formation of the *E*- and *Z*-isomers of the oxime **39**, however decreased the yield by 40%, as the *Z*-isomer was unable to undergo cyclisation.

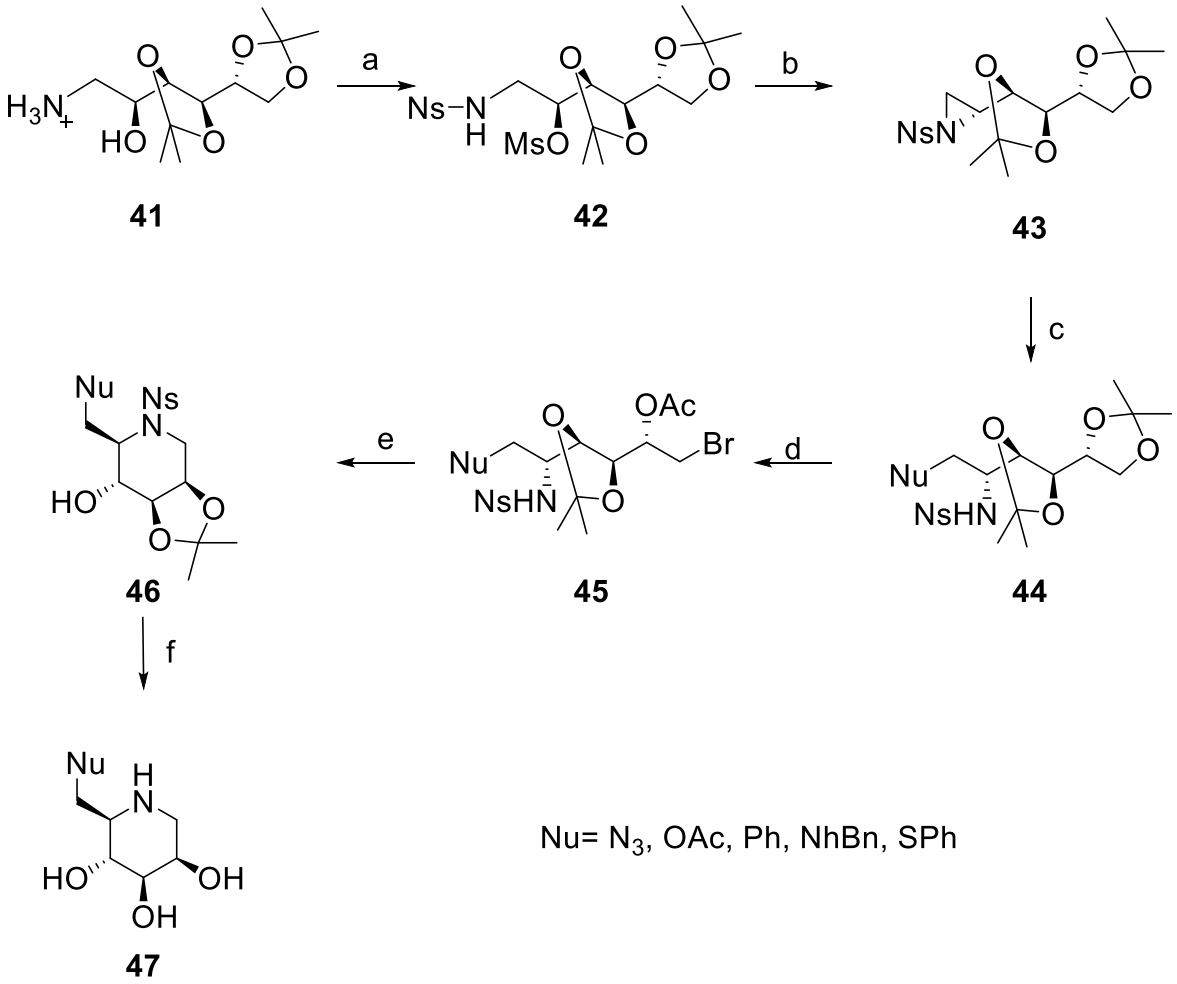
Catalytic hydrogenation with palladium on carbon then afforded DMJ (**4a**) in 39% yield from **38**.[47] The formation of azasugars using an aldo-pyranose in place of the keto-furanose as the starting material has been explored to allow for derivatisation at C5. Using mannose in this manner allowed for the synthesis of DMJ in a 31% overall yield.[48]



Scheme 1.11. Using nitrones as an intermediate

Reagents and Conditions: (a) (i) $TBDPSONH_2$, PPTS (cat.), toluene, 100 °C; (ii) MsCl, Et_3N, CH_2Cl_2, 86%, *E*:*Z* = 6:4 (2 steps); (b) TBAF/SiO_2, THF, 92%; (c) H_2, Pd/C, HCl-MeOH, 83%.

Mao *et al*. utilised the 3,4:5,6-diisopropylidene derivative of 1-amino-1-deoxy-D-glucitol to synthesise derivatives of DMJ.[49] Installation of a p-nitro-benzenesuflonyl (nosyl, Ns) group on the amine followed by mesylation of the hydroxyl at the C2 position afforded **42** (Scheme 1.12). Intramolecular displacement of the mesylate then led to the formation of the the aziridine **43**, which could then be attacked by a variety of nucleophiles (e.g., N_3, Ph, NHBn, SPh) to give various substituted derivatives **44**. Removal of the terminal isopropylidene then allowed for bromination of the terminal carbon and, to ensure cyclisation did not occur at the C5 position, acetylation of the remaining free hydroxyl was undertaken to give **45**. Substitution of the bromine in a nucleophilic cyclisation afforded the piperidine scaffold **46,** which could be deprotected to give the DMJ analogues **47** in total yields of up to 28% over 9 steps.[49]



Scheme 1.12. Incorporation of derivatives at the C6-position

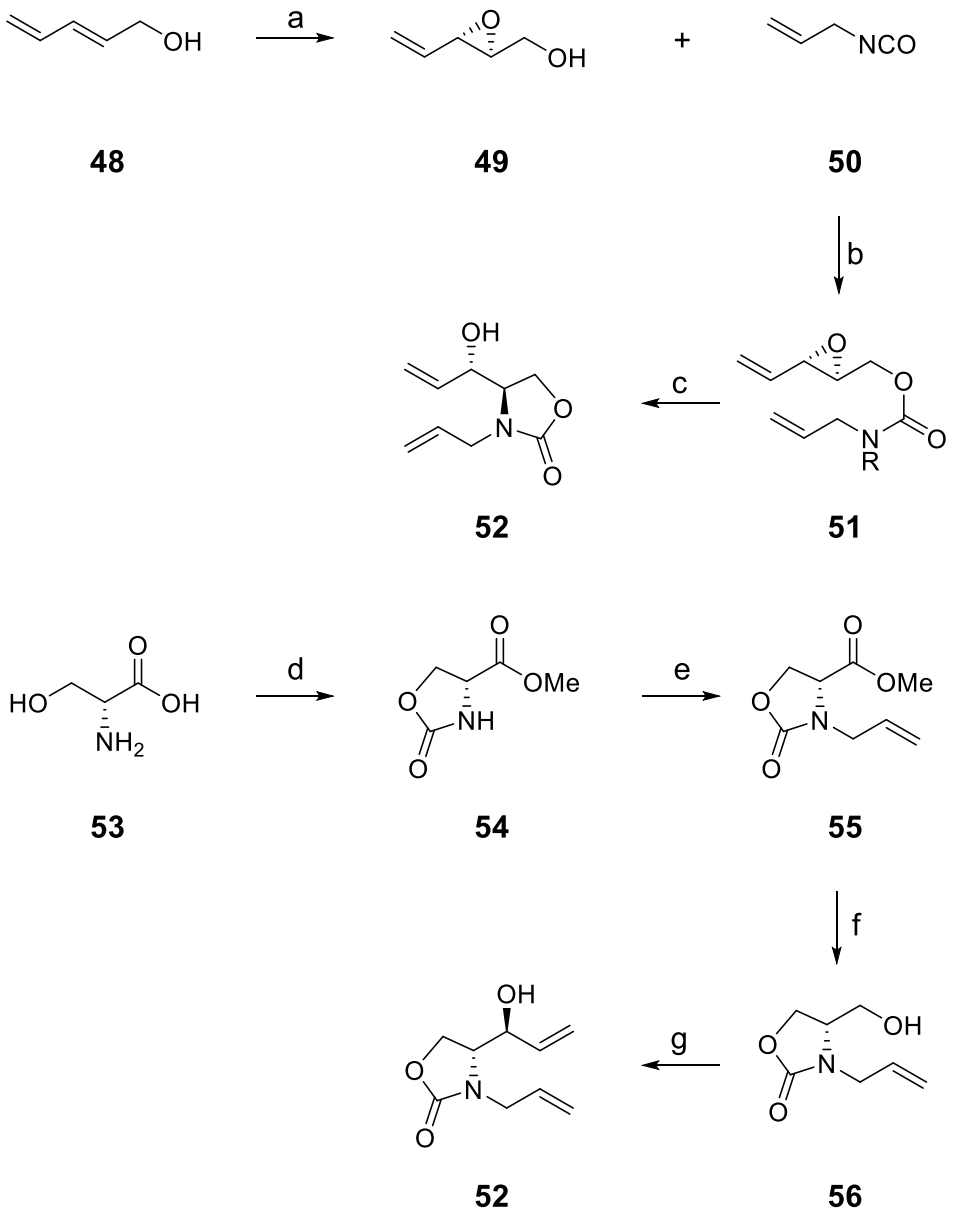
Reagents and conditions: (a) Na_2CO_3 (aq. sat. solution)/CH_2Cl_2 (1.5:1), 1.5 equiv. p-NsCl, 20°C; CH_2Cl_2/pyridine (4:1), 1.5 equiv. MsCl, 0–20 °C, 89%; (b) 2 equiv. NaH, THF, 20 °C, 74%; (c) nucleophile, e.g NaN_3, DMF 98%; (d) (i) Dowex 50X8-200, 9:1/ MeOH/H_2O, rt, 72%; (ii) Ph_3P, CBr_4, THF, rt; (iii) CH_2Cl_2/pyridine (4:1), Ac_2O (4 equiv.); (e) CH_2Cl_2, excess of DMAP, 67% (4 steps); (f) (i) MeOH, K_2CO_3; (ii) PhSH, K_2CO_3 MeCN/DMSO (49:1), 50°C; (iii) sat. HCl/MeOH 59%;

1.2.3. *In situ* Aza-heterocycle Construction

An alternative approach is to assemble the piperidine ring as part of the synthetic methodology. In such syntheses, the molecular scaffold is constructed from an alkylamine starting material and asymmetric catalysis is used to generate the desired stereochemistry. While individual reactions often produce high yields, the loss of yield associated with a high number of steps often contributes to a reduced total yield when constructing the azasugar scaffold in a piecewise manner. The general nature and focus of these syntheses appears to be the expansion of synthetic methodology and divergent syntheses, rather than the expedient synthesis of the desired molecule.

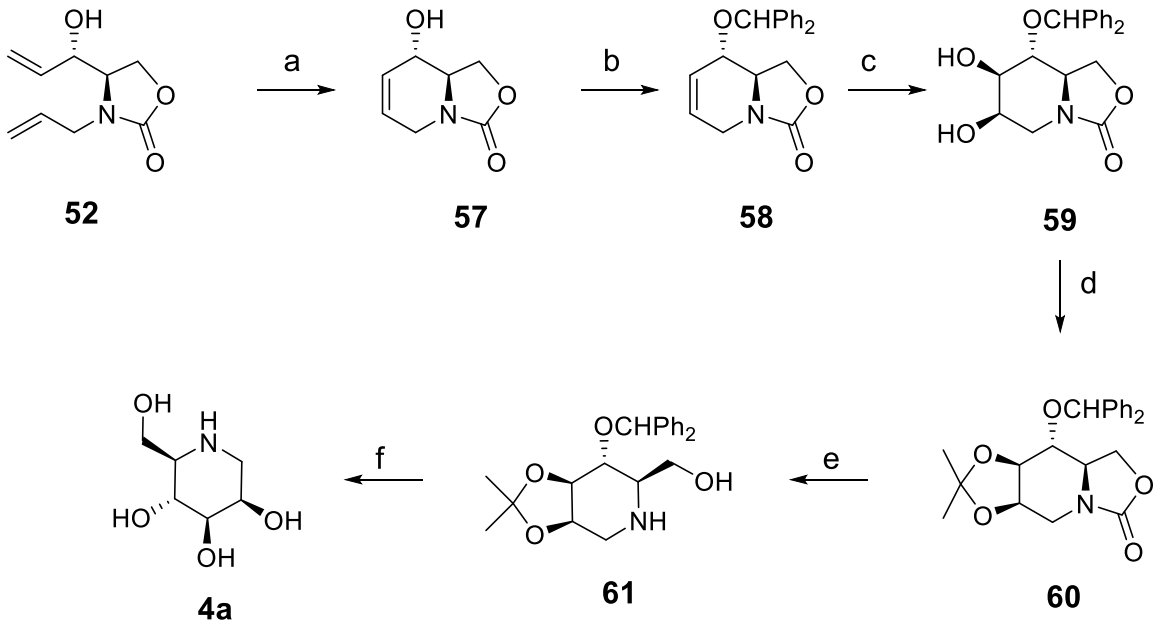
A prominent feature of many syntheses involving the construction of the piperidine ring is the use of a carbamate motif to afford *in situ* protection, and to introduce amine and 6-hydroxy functionalities of the target compounds. Indeed, such a strategy was first explored via the nucleophilic substitution of an allylic mesylate with a carbamate to form a bicyclic piperidine ring. This allowed for DMJ to be synthesised in 20% total yields from the previously prepared chiral staring material 4-methoxycarbonyloxazolidinone.[50] In 1999 Martin *et al*. illustrated that a carbamate-ring closing metathesis (RCM) strategy could be used for the development of an azasugar precursor (Scheme 1.13). 2,4-Pentadienol **48** was epoxidised at the allylic position to the epoxy-alcohol **49** which added to isocyanate **50** to form carbamate **51**. Intramolecular attack of the epoxide by the nitrogen atom of the amide then afforded oxaziladone diene **52** in 4 steps and in a 54% yield.[51] Subramanian *et al.* later used a similar strategy toward the synthesis of diene **52**, however, here D-serine (**53**) was used as a starting material. Accordingly, D-serine was

converted to the acid chloride then reacted with triphosgene to form the oxazilidone **54**. A bromo-alkene was then used to install the alkene chain of the desired length. For the synthesis of DMJ this involved treatment with allyl bromide to give alkene **55**. Reduction with sodium borohydride then gave the alcohol **56**. Swern oxidation to an aldehyde followed by the addition of vinyl magnesium bromide gave the diene **52**. While producing diene **52** in this manner allowed for a more divergent synthesis as different ring sizes were possible, the overall yield for the synthesis of DMJ was only a modest 11%.[52] The strategy by Martin *et al.* however, allowed for the synthesis of DMJ in 9 steps from **49** and a 28% yield (Schemes 1.13 and 1.14). To complete the synthesis, RCM with Grubbs' catalyst ($Cl_2(PCy_3)_2Ru{=}CHPh$) afforded piperidine **57**, and was followed by protection of the hydroxyl with a benzyl group (→ **58**). Dihydroxylation was affected by osmium tetroxide with the anti-selectivity dictated by the alkoxy group to give diol **59**. The hydroxyls were then protected with an isopropylidine group to give the fully protected intermediate **60**. The carbamate was subsequently hydrolysised (→**61**), and the isopropylidine group removed to give DMJ (**4a**). [53]



Scheme 1.13. Formation of the diene intermediate **52**

Reagents and Conditions: (a) ^{t}BuOOH, L-(+)-DIPT, $Ti(PrO)_4$ CH_2Cl_2 (b) Et_2O, 59% (2 steps); (c) NaN_3, TMS, THF; (d) (i) $SOCl_2$, MeOH, reflux; (ii) K_2CO_3, triphosgene, H_2O/toluene, 86% (2 steps); (e) NaH, allyl bromide, DMF, 65%; (f) $NaBH_4$, MeOH, 74%; (g) (i) DMSO, $(COCl)_2$, $CH2Cl_2$, $EtN(i\text{-}Pr)_2$; (ii) vinylmagnesium bromide, CH_2Cl_2, 53% (2 steps).

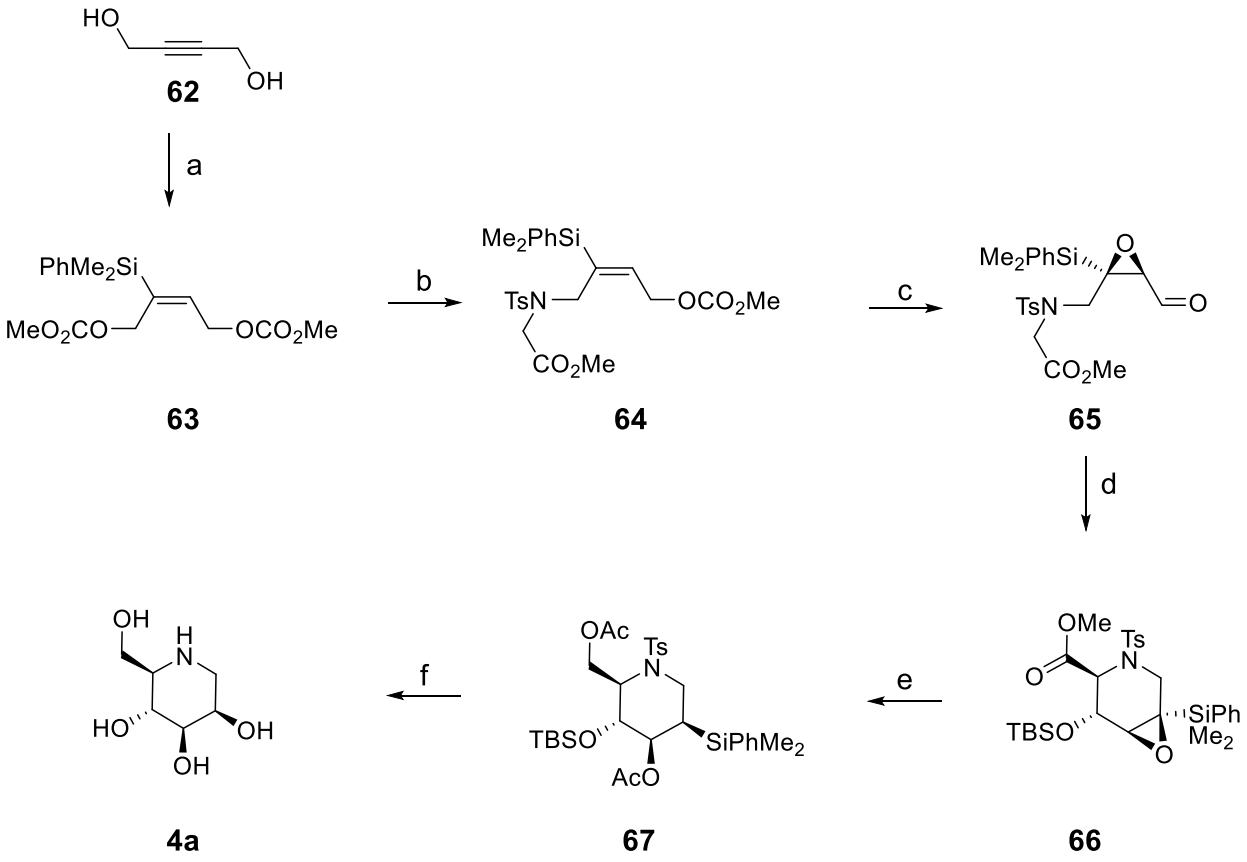


Scheme 1.14. Utilisation of carbamates as key intermediates *en route* to the synthesis of DMJ

Reagents and Conditions (a) Grubbs' catalyst ($Cl_2(PCy_3)_2Ru=CHPh$), CH_2Cl_2, 98%; (b) NaHMDS, THF, 89%; (c) OsO_4, NMO, Acetone/H_2O, 96%; (d) dimethoxyisopropyladine, acetone, pTsOH (cat.), 88%; (e) NaOH, MeOH/H_2O, 90%; (f) HCl, THF, 91%.

Functionalised expoxysilanes have also been investigated as precursors for DMJ, and could be used to produce the piperidine ring of DMJ (**4a**) via a stereo-selective aldol reaction (Scheme 1.15). Here, silylation of butyne-diol **62** gave silylalkene **63**, which could undergo palladium catalysed amination to introduce the amine to give **64**. Treatment of **64** with potassium carbonate afforded the deprotection of the carbonate to an alcohol which could be oxidised to give the aldehyde which could undergo asymmetric epoxidation of the alkene with *m*-CPBA to produce epoxisilane **65**. Base-mediated cyclisation of **65** produced the piperidine ring, which following the protection of the free hydroxyl afforded **66**. Reductive opening of the epoxide and acetylation of the resulting hydroxyl produced the protected azasugar **67** in the *manno*

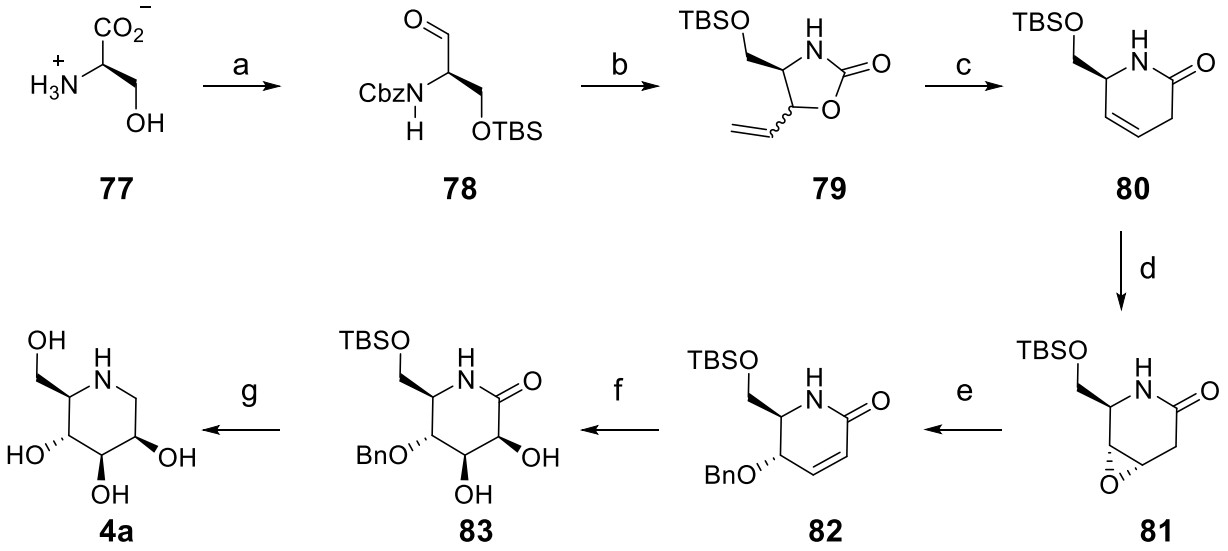
configuration. Tamao-Fleming oxidation of the silyl group followed by a series of deprotection steps to afford DMJ (**4a**) in a 28% overall yield. Poor yields in the epoxide opening step and the extensive deprotection Scheme required to obtain DMJ severely limited the efficiency of this synthesis.[54] Performing the epoxidation via the asymmetric Sharpless reaction showed no significant change in the total yield.[55]



Scheme 1.15. Exploiting epoxysilane intermediates.

Reagents and Conditions: (a) (i) $ClCO_2Me$; (ii) $HSiMe_2Ph$, $H_2PtCl_6.6H_2O$ 95%; (b) (i) TsGlyMe, NEt_3 $^{i}PrOH$, 55 ^{o}C; (ii) $Pd(OAc)_2$, dppe, 71%; (c) (i) MeOH, K_2CO_3; (ii) *m*-CPBA; (iii) IBX, DMSO 85%; d) (i) DBU, THF; (ii) TBDMSCl, imidazole, DMF, 88%; (e) (i) $LiAlH_4$, Et_2O (ii) Ac_2O, DMAP, NEt_3, 78%; (f) (i) $Hg(OAc)_2$, AcOOH/AcOH; (ii) TBAF; (iii) AcO_2; (iv) HCl, reflux 65% (2 steps).

The final strategy to be discussed for the *de novo* synthesis of the chiral scaffold of DMJ (**4a**) involves lactam formation as a key step. Here, the lactam carbonyl can be deoxygenated to afford the deoxygenated position at C1 or used to install the hydroxymethyl group at C5. Accordingly, D-serine (**77**) could be transformed to aldehyde **78** in a four step method published by Pederson,[56] and the alkene functional group added via Grignard reaction with vinylmagnesium bromide. Cyclisation mediated by potassium *tert*-butoxide then gave the vinyloxazolidin-2-one **79** from which the lactam **80** could be synthesised via a palladium catalysed carbonylation. The C4 hydroxyl was then introduced via epoxide **81**, which was subjected to base mediated elimination followed by benzyl protection of the remaining alcohol to give alkene **82**. Finally dihydroxylation of alkene **82** afforded the azasugar lactam **83**, which could be selectively reduced with $LiAlH_4$ and deprotected to produce DMJ (**4a**) in a 12% overall yield.[57] An alternative strategy is to use the lactam carbonyl for the installation of the hydroxymethyl group at C5 through palladium mediated cabonylation via the formation of enol phosphates. A more elaborate protection strategy was required for this strategy which limited overall yields to 5%.[58] Unfortunately, in both strategies utilising a lactam piperidine, the formation of an epimeric mixture during the installation of the hydroxyl at C4 significantly reduced the yield of DMJ. [57,58]



Scheme 1.16. Exploiting the lactam at the deoxygenated position

(a) (i) CbzCl, K_2CO_3; (ii) TBDMSCl; (iii) $Ca(BH_4)_2$; (iii) $(COCl)_2$, DMSO, Et_3N 79%; (b) (i) $H_2CCHMgBr$, THF, -78°C to r.t; (ii) KOtBu, THF, 75%; (c) $PdCl_2(PPh_3)_2$, CO (65 atm), EtOH, 60°C, 81%; (d) Oxone (5 equiv.), $NaHCO_3$ acetone/H_2O, 95%; (e) (i) DBU (2 equiv.) CH_2Cl_2, reflux; (ii) NaH (2 equiv.), DMF, BnBr, 0°C to rt, 60%; (f) OsO_4 (7 mol%), NMO (3 equiv.), ^tBuOH, rt, 3 h, 89%; (g) (i) $LiAlH_4$ (5 equiv.), Et_2O; (ii) 20 Bu_4NF (1.5 equiv.), THF, rt,; (iii) H_2, Pd/C, EtOH, HCl, 68%.

1.3. Efficient synthesis

Imperative to the design of the synthesis of any commercially desirable material is the concept of efficiency. To this end, it is essential that the target product is prepared in a high overall yield in a minimum number of steps, and from a inexpensive, commercially available material. Parallel to the innate economic desire for efficiency is the idea of "green chemistry" and the environmentally driven desire to minimise waste. While the main goal of green chemistry is "to reduce or eliminate substances hazardous to the human health and the environment",[59] improving reaction efficiency and waste prevention are also key principles, as is the measurement of green metrics. In particular the metrics of atom economy, defined as the number of atoms in reactants which end up in the final product,[60] and the concept of E-factor, which assesses the mass of waste per mass of desired product,[61] are pertinent to the efficient synthesis of materials. Recently there has been an increasing uptake of green chemistry approaches within the pharmaceutical industry.[62,63]

As previously illustrated, while there are some very elegant syntheses of DMJ (**4a**), apart from the enzymatic route, which can be difficult to scale up, all reported strategies to date require the use of protecting groups to achieve chemoselectivity. Several strategies also require the use of expensive metal catalysts, and are not amenable to rapid derivatisation for the preparation of DMJ analogues. As exemplified by the commercial success of DNJ derivatives Miglitol and Miglistat, [3,14] and the improvement in viral inhibition afforded by a long alkyl chain and benzylated derivatives of DNJ against Hepatitis and HIV, respectively,[64-66] derivatisation is highly

desirable for the development of improved azasugar drug candidates. Therefore, the development of an efficient synthesis of DMJ and its derivatives is highly sought after.

1.3.1. Protecting Group Free Synthesis

One way to improve the efficiency of the synthesis of DMJ and derivatives is to develop a Protecting Group Free (PGF) route. PGF methodologies are often avoided in carbohydrate synthesis due to the inherent difficulties in controlling regioselectivity, associated with there being multiple hydroxyl groups in carbohydrate scaffolds. While protecting group strategies have allowed for significant advancement in the synthesis of complex organic molecules, the use of protecting groups increases the number of steps required (both in the incorporation and removal of the protecting group), which in turn increases the cost of additional reagents and solvents, and also leads to a loss of yield. Accordingly, there is significant incentive to develop a PGF synthesis of DMJ and derivatives thereof.

To date there have been several PGF syntheses of pyrrolidine and piperidine azasugars by the Stocker/Timmer group.[67-69] To achieve the desired synthetic transformation, two new reaction methodologies were developed: the first being the Vasella Reductive Amination on unprotected iodoglycosides, and the second, a novel carbamate annulation. Herein, a new PGF strategy will be developed for the synthesis of piperidines. This strategy will employ a regioselective cyclisation from a suitably functionalised iodosugar.

1.4. Project aims

1.4.1. The Efficient Synthesis of DMJ and Derivatives

The aim of this Master's project is to develop efficient PGF methodology for the synthesis of the azasugar 1-deoxymannojrrimycin (DMJ) from a readily available carbohydrate starting material. The synthetic strategy will be developed so that *N*-functionalised DMJ can be prepared without the requirement of additional synthetic steps. A group of 9 derivatives have been chosen to both explore how different *N*-substituents effect the biological activity of the derivatives and also, to explore how versatile the proposed methodology is for analogue synthesis (Figure 1.5). It is hypothesised that the synthetic strategy may also be adaptable to the synthesis of piperidines with different stereochemistry through the use of different carbohydrate starting materials. If the synthesis of the DMJ derivatives is readily achieved within the timeframe of the project, the aims of this project will be expanded to assess the synthesis of other desirable piperidine azasugars.

1.4.2. Derivative targets

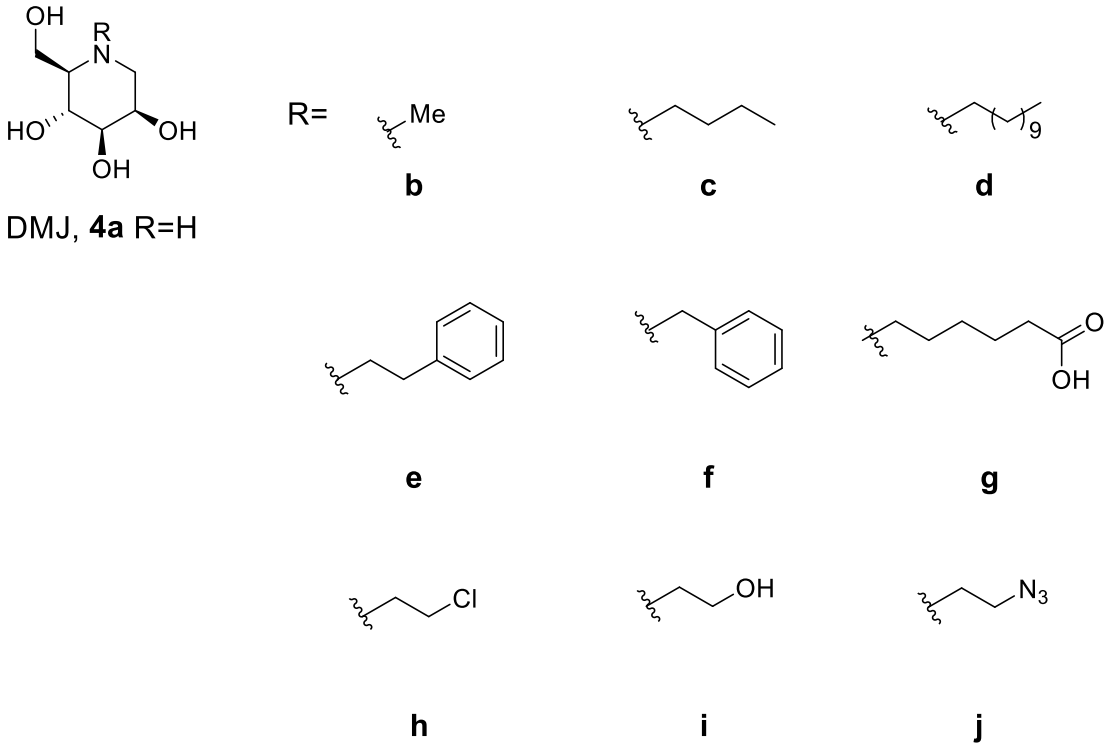


Figure 1.5.
Target derivatives

1.4.2.1. Alkylated DMJ derivatives

The alkylation of azasugars to introduce a lipophilic component to the sugar mimic has led to compounds with selective anti-viral properties, as well as inhibitors of sugar processing enzymes other than glycosidases.[70] For example, the inhibition of enzymes such as transferases is enhanced by the incorporation of a lipophilic alkyl group on the azasugar, such alkylated azasugars assist in the prevention of lysosomal glycosphingolipid accumulation and can be used to treat lysosomal storages diseases, including Gaucher's disease.[3] *N*-Methyl-DMJ (**4b**) is interesting in this respect, as it is primarily known as a glycosidase inhibitor for mannosidases,

but additionally has shown potent anti-HIV activity.[71,72] Synthesis of *N*-methyl-DMJ has been accomplished via the alkylation of DMJ and a better route to this type of compound is therefore desirable.[73-75]

N-Butyl-DMJ (**4c**) is the C2-epimer of the well-known glucosidase inhibitor *N*-butyl-DNJ, used to treat Gaucher's disease.[76] *N*-butyl-DMJ has been synthesised from DMJ in 60% yield via N-alkylation.[77] The synthesis of *N*-butyl-DMJ can also be incorporated into the formation of the piperidine ring of the DNJ analogue which can be converted to the DMJ epimer.[78] The synthesis of *N*-butyl-DMJ has also been achieved as part of a divergent bioconversion synthesis to produce a number of azasugar derivatives.[79] No biological testing has been reported for this derivative.

The alkylated targets will also be extended to the synthesis of a number of extended alkyl chain derivatives (**4d**). The synthesis of nonyl derivatives has been accomplished via the formation of a DNJ precursor which can be converted to DMJ through oxidation followed by reduction with L-selectride in 74% yield,[78] in a method which would be applicable for a variety of different alkyl chain lengths dictated by the alkyl amine utilised. Derivatives of deoxygalatonojirimycin (DGJ) and DNJ with an alkyl chain length of eight carbons or greater have shown pronounced antiviral activity,[20] with the nonyl derivatives active against hepatitis B virus[64] and hepatitis C virus.[65] Interaction with membrane proteins is suggested to be the target of this antiviral activity rather than glycosidase enzyme inhibition, which is decreased for these derivatives in comparison to those with shorter alkyl chains.[65] Little investigation has been undertaken for alkylated deoxyazasugars besides the methyl, butyl and nonyl derivatives described.

1.4.2.2. Aromatic derivatives

Following the synthesis of the alkylated derivatives (**4b-d**), the next series of targets concerns the inclusion of more sterically demanding functionalities. The primary interest in the synthesis of these derivatives is to test the tolerance of the synthetic methodology towards incorporating more sterically demanding amines in the reductive amination step of the reaction sequence (*vide infra*). In addition, aromatic-DMJ derivatives (**4e** and **4f**), are of interest as sugar mimics that supress protein synthases and have been identified as anti-viral targets with potential for HIV inhibition.[66]

The syntheses of derivatives **4e** and **4f** has not been well explored, and while the benzyl derivative **4f** has been synthesised for DMJ previously as part of a series of derivatives[79] and is obtainable via benzylation of DMJ,[80] **4e** represents a novel target. Analogues of **4e**, and **4f** have been produced for the *gluco* configuration (DNJ) via the double reductive amination of the dicarbonyl 5-keto-D-glucose in 73% and 70% yields respectively (24%, 23% from 1,2-*O*-isopropylidene-α-D-glucofuranose).[44]

1.4.2.3. Bi-functional derivatives

The synthesis of various bifunctional derivatives of DMJ represents a dual interest. The incorporation of additional reactive functionalities during the course of the azasugar synthesis is highly desirable as it allows for the direct synthesis of DMJ derivatives with functional handles. Furthermore, many of the chosen functionalities can also serve as chemoselective handles for subsequent bio-orthogonal reactions. These functionalised DMJ-derivatives (**4g-l**) are also of

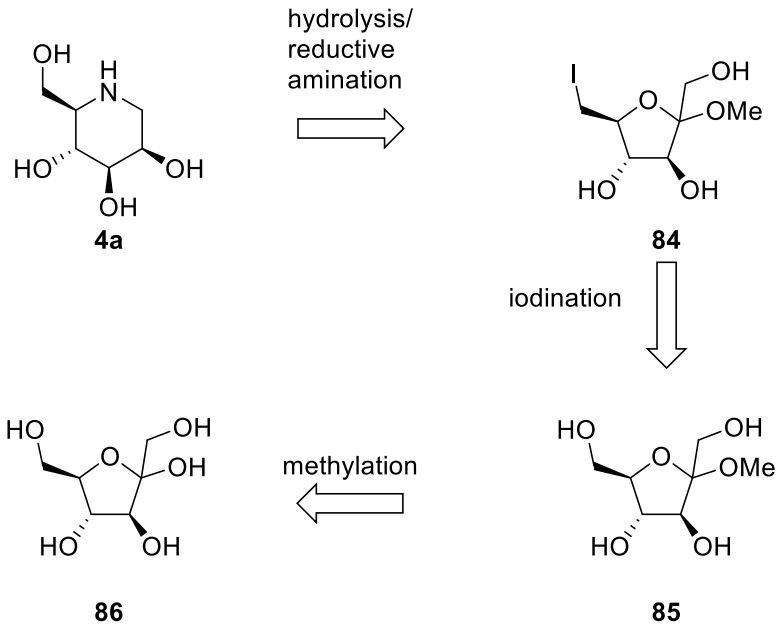
interest as they will allow for investigations into the tolerance of the reductive amination reaction when a secondary reactive functionality is present on the amine (*vide infra*).

N-5-Carboxypentyl-DMJ (**4g**) is a previously synthesised derivative which has been used as a chromatography agent for the isolation and characterisation of mannosidases.[74] The synthesis of this derivative is achieved from DMJ by the addition of methyl 5-formylvalerate and subsequent hydrolysis of the methoxy group.[81] Accordingly, the rapid synthesis of carboxyl-functionalised DMJ (**4g**) is an important target. The chloroethyl derivative (**4h**) however, represents a novel target which is not naturally isolated and has no published synthesis, while *N*-hydroxyethyl-1-deoxymannojirimycin (**4i**) is a stereochemical isomer of the *gluco* derivative known as miglitol which is used in the clinical treatment of type II diabetes. Both the DNJ and DMJ analogues have been synthesised in an 85% yield from their respective parent azasugars using 2-benzyloxyethanal followed by reduction with H_2, Pd/C.[77]

Finally, the azido derivative (**4l**) is a novel derivative for all azasugars. The azide functionality is desirable to install as it is a small chemoselective group that can be subsequently used for bioorthogonal reactions, such as click chemistry[82] and in Staudinger ligations.[83]

1.5. Synthetic strategy

1.5.1. Retrosynthesis of 1-deoxymannojirimycin



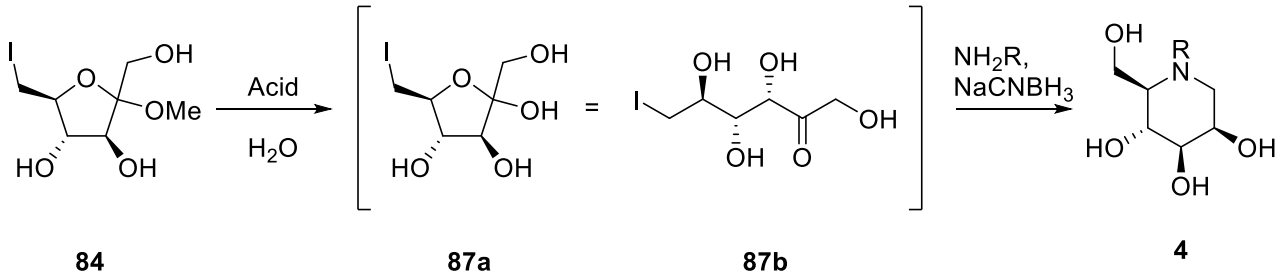
Scheme 1.17.
Synthetic strategy for the synthesis of 1-deoxymannojirimycin

The synthetic strategy which will be utilised for the production of 1-deoxymannojirimycin and its derivatives is above in Scheme 1.17. It is anticipated that DMJ (**4a**) can be synthesised in 4 steps and without an extensive protecting group scheme. To this end, deoxymannojirimycin **4a** could be formed via the reductive amination and substitution of the iodine functionality of substituted D-fructose **84**, where the reductive amination reaction is of significant historical precedent.[1,39,40,84-86] The *in situ* hydrolysis of the methyoxy group which is key to this synthetic step, however, is of little precedent. Methyl-iodoglycoside **84**, can in turn be prepared via the selective iodination of the 6-position of methyl glycoside **85**. Here, the methoxy group is used to ensure the parent sugar, D-fructose **86**, remains in its furanose form, thereby preventing ring

opening. In the furanose configuration, iodination is favoured at the least sterically hindered 6-position. Finally, the stereochemistry at C2, C3 and C4 of DMJ is obtained by using the readily available and inexpensive ketose monosaccharide D-fructose (**86**). The stereochemistry at C5 of DMJ adopts the favoured equatorial orientation such that reduction stereoselectively occurs at the axial position.[44,87]

1.5.2. Reductive amination

The reductive amination step allows for the incorporation of various substituted amines to produce DMJ derivatives directly. This is a significant advantage over other DMJ syntheses whereby the nitrogen functionality is introduce into in the sugar by way of an azide group before being reduced to the amine to induce cyclisation.[39,40] Furthermore, by incorporating the amine functionality directly into the synthesis, this allows for the rapid synthesis of *N*-alkylated DMJ derivatives without additional steps. To prepare the iodo-sugar **87a**, which is amenable to the reductive amination, the anomeric methoxy group must first be hydrolysed via the treatment of intermediate **84** with strong acid (Scheme 1.18). Following the removal of the methoxy group the carbohydrate now exists in equilibrium between the open chain (ketose) and ring (hemiacetal) forms. The ketose sugar is then subjected to nucleophilic attack by and amine to form an imine which can be subsequently cyclised *in situ* and reduced, via $NaCNBH_3$, and to afford DMJ (**4**). Moreover, the removal of the methoxy group and reductive amination can occur in one pot. In addition to the ease of derivatisation it is theorised that the reaction methodology may also be translatable to different sugar scaffolds.

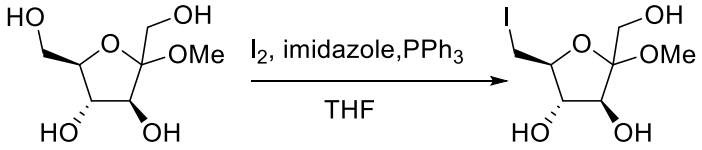


84 **87a** **87b** **4**

Scheme 1.18. Reductive amination methyl 6-deoxy-6-iododructofuranoside to produce DMJ

1.5.3. Iodination

Alongside the versatility of the reductive amination the ability to selectively install an iodine in high yields with minimal protection presents a significant improvement on published azasugar syntheses. The iodination of the alcohol is afforded through a mild Appel reaction using triphenylphosphine and imidazole, a reaction which has been utilised for a variety of alcohols.[88,89] similar reactions to this have been applied to the iodination of primary hydroxyls using triphenylphosphine and *N*-iodosuccinimide.[90]



86 **85**

Scheme 1.19. Selective iodination of the 6-position

1.6. Ethics Statement

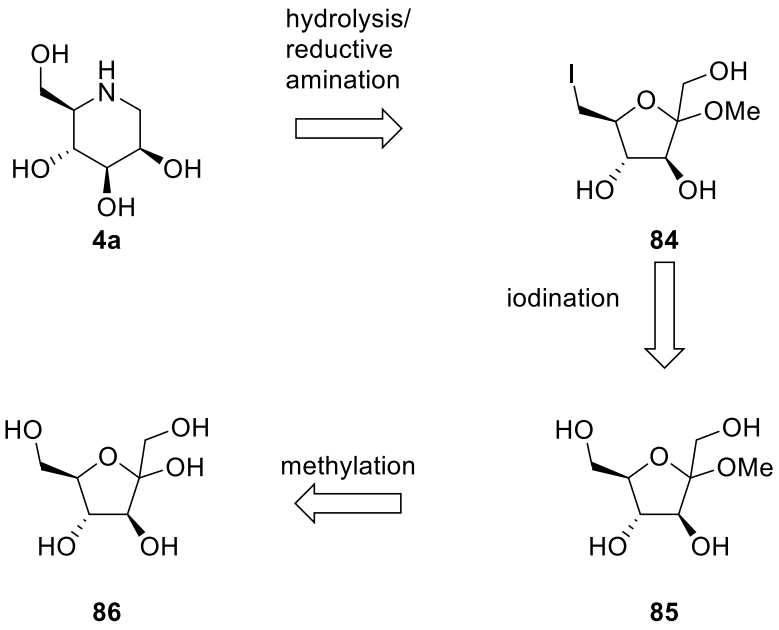
There is no ethical approval required for the undertaking of this project, nor any foreseeable cultural, social or legal impediments to the successful completion of the research.

2. Results and Discussion

2.1. The Synthesis of 1-Deoxymannojirimycin (DMJ) (4a)

2.1.1. Retrosynthesis

To synthesise DMJ (**4a**), a retrosynthetic strategy was proposed whereby the target azasugar could be synthesised in four steps thereby minimising the need for extensive protecting group methodology (Scheme 2.1). To this end, it was envisioned that DMJ (**4a**) could be formed via reductive amination of methyl-6-deoxy-6-iodofructofuranosides (**84**). This methyl iodo-glycoside (**84**) could, in turn, be prepared via the selective iodination of the corresponding methyl glycoside (**85**). In this instance, the methoxy group is introduced to ensure the parent sugar, D-fructose (**86**), remains in its furanose form to prevent ring opening while also sterically disfavouring reaction of the primary hydroxyl at the 1-position during the iodination step. Fisher glycosidation of D-fructose is a common method to access furanosides as reported in the literature,[91] while selective iodination at the 6-position of furanosides has been utilised for several protecting group free syntheses of azasugars and the functionalisation of other sugar scaffolds.[69,92]



Scheme 2.1. Synthetic strategy for the synthesis of 1-deoxymannojirimycin

2.1.2. Synthesis

In order to synthesise DMJ (**4a**), D-fructose **86** was first converted into its methyl furanoside via well-established Fischer glycosidation methodology (Scheme 2.2).[93] The reaction was followed using TLC analysis, which showed complete conversion to the methyl glycoside of the starting materials to higher running products after 30 minutes. The reaction was then quenched via the addition of ammonia solution (28% (aq), v:v) to prevent the formation of the thermodynamically favoured methyl pyranoside.[91] The resulting ammonium sulfate was removed by filtration to give the α- and β-glycosides (**85**) in a [6:4] ratio. Silica gel column chromatography was then used to attempt to remove any pyranose present. While full separation was not achieved, the remaining pyranose was contributed to less than 5% of the glycoside product, and yields of the desired furanose product were attainable in excess of 90%.

The successful formation of the desired methyl glycosides was confirmed by the presence of high intensity resonances, assigned to the methyl group carbons, in the ^{13}C NMR (δ_C = 48.8 [β-OMe], δ_C= 48.8 [α-OMe]) and the corresponding singlets in the ^{1}H spectrum (δ_H = 3.32 [β-OMe], δ_H=3.29 [α-OMe]). The formation of the desired furanose forms were confirmed by comparing the chemical shifts of the carbons at the 5-position, which is strongly dependent on whether the sugar is in the pyranose or furanose form.[91] For example, in the furanose **85** the ^{13}C resonances attributed to C-5 were found further downfield (δ_C = 83.3 [C-5$_\alpha$], δ_C = 81.1 [C-5$_\beta$]), than the remainder of the hydroxylated ring carbons, which are situated between 76-74 ppm. In the pyranose form, the ^{13}C resonance of C-5 would exhibit a similar shift to the other carbons reflecting alcohol substitution as opposed to ether substitution of C5 in the furanose. Furthermore, a weak correlation was observed in the HMBC spectrum between C-2 (δ_C = 103.8 [C-2$_\beta$], δ_C = 108.2 [C-2$_\alpha$]), and H5 (δ_H = 3.83 [H-5$_\beta$], δ_H = 3.93-3.90 [5$_\alpha$]), representative of 3-bond correlations via the ring oxygens, thereby proving furanose form (Figure 2.1).

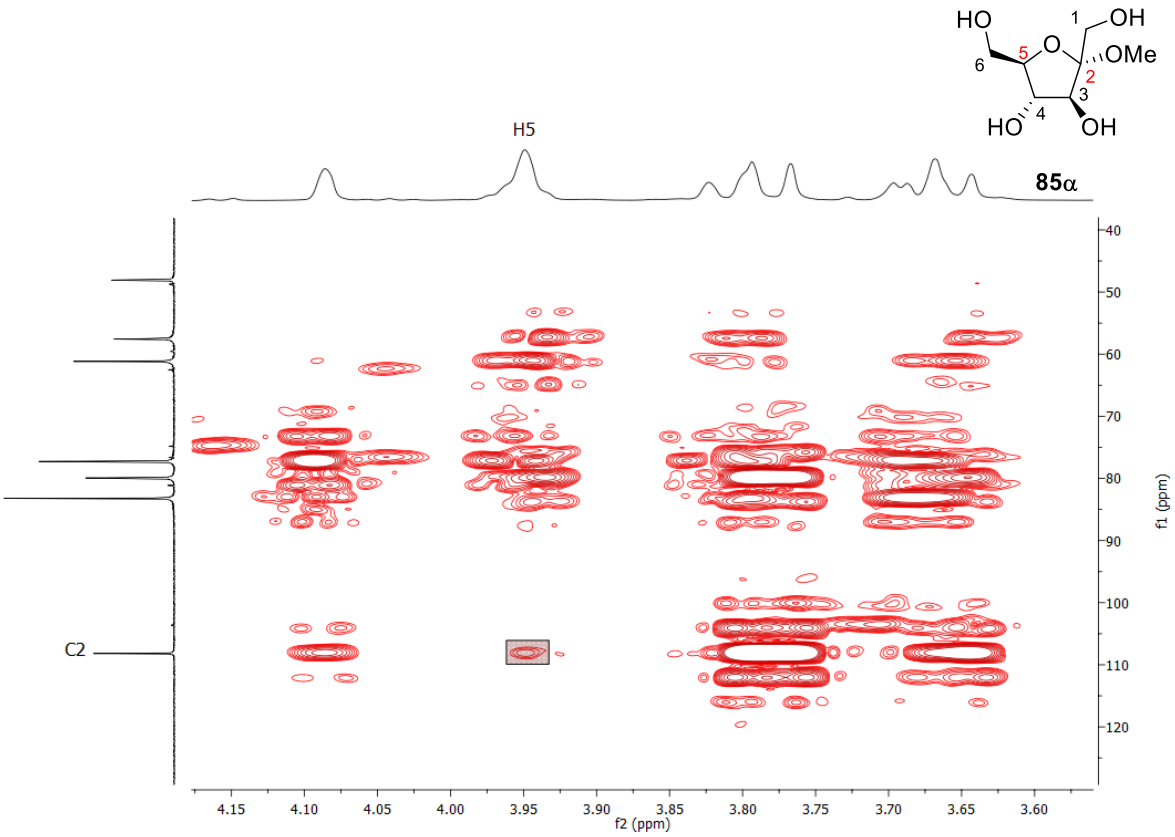
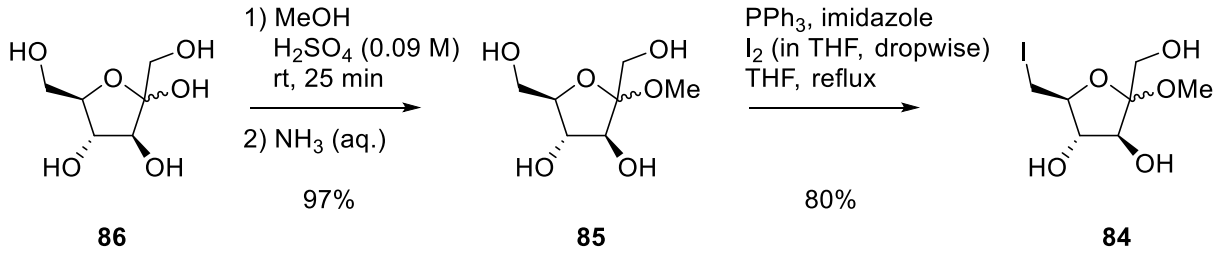


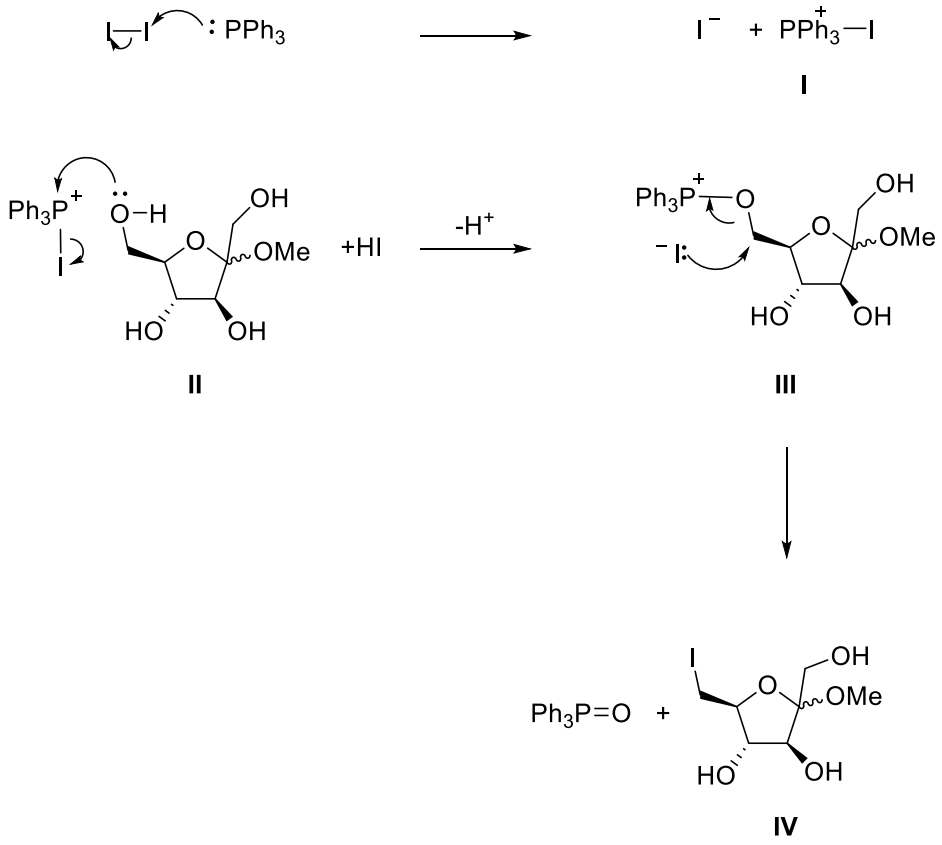
Figure 2.1. HMBC of the α anomer of fructoside **85** illustrating correlation of H5 to C2

Next, methyl glycosides **85** were treated with triphenylphosphine, imidazole, and iodine to install an iodide at the 6-position, which gave iodide **84** in excellent (80%) yield.



Scheme 2.2. Synthesis of methyl 6-deoxy-6-iodo-D-fructoside.

The selective instalment of the iodine at the 6-position was achieved using an Appel reaction,[88] which favours reaction of primary hydroxyls. Here, a triphenylphosphonium iodide intermediate **I** is generated by reacting triphenylphosphine with iodine. The phosphonium iodide then reacts with in **II** to form the phosphonium glycoside **III**. With the alcohol now transformed into a very good leaving group, nucleophilic displacement with iodine, and elimination of triphenylphosphine oxide leads to the formation of the iodide product (Scheme 2.3). Selectivity towards primary hydroxyls is afforded by steric and torsional constraints which prevent the approach of the triphenylphosphonium intermediate to react with secondary hydroxyls. Moreover, the installation of a methoxy group at the anomeric position is used to sterically hinder the approach of the triphenylphosphonium iodide intermediate and thus prevent reaction at the 1-position (Figure 2.2). To this end, the reaction affords selective iodination at the desired 6-position without requiring further protecting groups. Unfortunately, however, generation of HI as a by-product resulted in the continual increase in acidity which led to product degradation and other undesired side reactions over time. Accordingly, 2.5 equivalents of imidazole were required to neutralise the reaction mixture. The use of greater than three equivalents of imidazole did not improve the reaction yield and only complicated the subsequent purification steps. It is important to note that dropwise addition of iodine in THF was required to ensure the selective formation of the desired products, and to control the rate of HI formation. When iodine was added too quickly, degradation of both the staring materials and products was observed by TLC analysis.



Scheme 2.3. Mechanism of Iodination

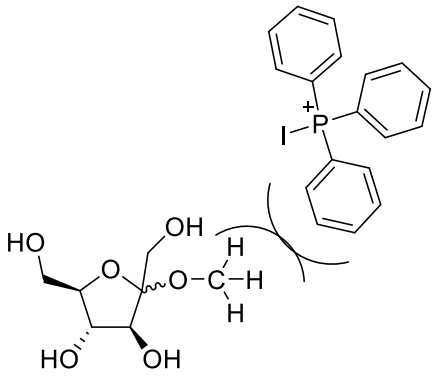
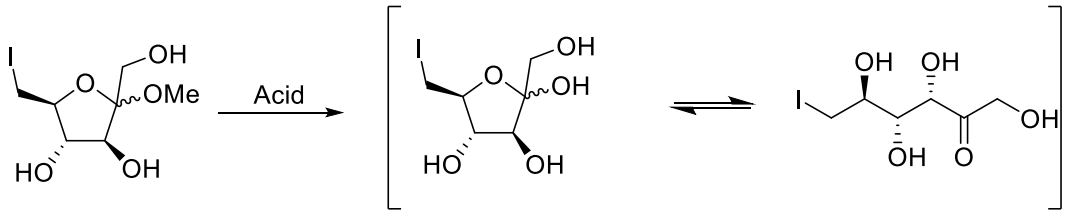


Figure 2.2. Steric prevention of iodination at the 1-position

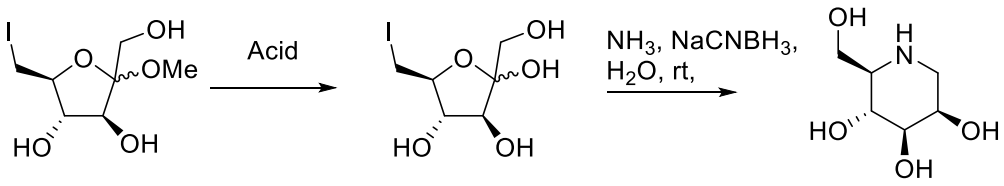
Having successfully prepared the methyl iodo-glycosides (**84**), hydrolysis of the methyl glycosides

was then undertaken to give 6-deoxy-6-iodofructose, which exists in an equilibrium between its furanose (**87a**), and open chain forms (**87b**) (Scheme 2.4). Initial attempts to isolate this product proved unsuccessful, with significant loss of material. Accordingly, a two-step one-pot process, which incorporates both the hydrolysis of the methyl glycoside and the reductive amination steps, was envisioned (Scheme 2.5). First it was sought to develop reaction conditions which would afford the hydrolysed product without complications and which allowed for the amine nucleophile and reducing agent to be added in the same pot in a subsequent step. The initial protocol for the hydrolysis involved the treatment of methyl iodoglycosides **84** with 0.09 M H_2SO_4 in water (entry 1a, table 1). However, this methodol proved to be inconsistent, with approximate yields ranging between 60-90%, and the presence of varying amounts of unreacted starting material. In an attempt to fully hydrolyse the methyl iodoglycosides **84**, the pressure of the reaction mixture was reduced to drive the reaction to completion by the removal of any methanol liberated during the reaction. This approach also yielded inconsistent results (entry 1b) and product degradation was observed when the reaction mixture was concentrated *in vacuo*. Next, a series of acids, including acetic acid, trifluoroacetic acid, and hydrochloric acid were trialled in attempts to find a more robust demethylation procedure.



84 **87a** **87b**

Scheme 2.4. Hydrolysis of the methyl glycoside



84 **87a** **4a**

Scheme 2.5. Proposed 2 step 1 pot synthesis of DMJ **4a** from **84**

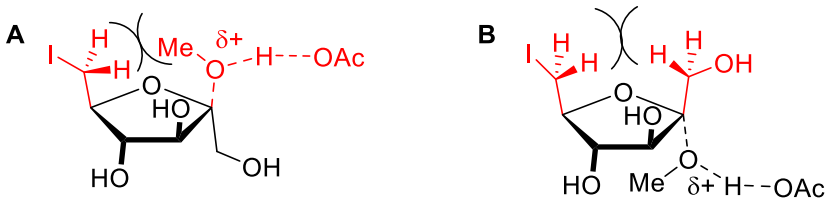
Table 1. Acidic hydrolysis of methyl 6-deoxy-6-iodo-α/β-D-fructofuranoside (87)

Entry	Acid	Concentration	Temp	Time	Conversion[a]
1a	Aq. H_2SO_4 (0.09 M)	10 mL/mmol	rt	30 min	60-90%
1b[b]	Aq. H_2SO_4 (0.09 M)	10 mL/mmol	rt	30 min	60-90%
2	AcOH:H_2O (9:1)	10 mL/mmol	rt	1 hr	10%
3	AcOH:H_2O (9:1)	20 mL/mmol	50	14 hr	60%
4	AcOH:H_2O (9:1)	20 mL/mmol	reflux	12 hr	60%
5[c]	TFA: H_2O (9:1)	10 mL/mmol	rt	1 hr	75%
6	Aq. HCl (2 M)	12 mL/mmol	rt	1 hr	100%

a Approximate conversion only based on the presence of starting material or side products by TLC. Here, the presence of stating material was compared to a sample taken of the reaction mixture prior to addition of acid using a consistent volume of solution for both spots.
b Evaporation under vacuum at 110 mbar after 30 minutes
c Multiple side products also observed

Firstly, the reaction was carried out in a mixture of acetic acid in H_2O in a 9:1 ratio under different reaction conditions (entries 2, 3 and 4). Here, acetic acid was selected as a mild acid which could be removed via azeotropic coevaporation with water without degradation of the substrate. The reaction was closely followed by TLC analysis, which consistently showed that the lower R_f anomer disappears at a much faster rate than the other. This difference in reactivity is a consequence of the difference in activation energy for the hydrolysis of the two methyl glycosides. As acetic acid is not a strong enough acid to protonate the methoxy group for

subsequent strong acid catalysed hydrolysis. Instead general acid catalysis is proposed to occur (Figure 2.3). For the β-anomer, the increase in steric bulk of the methoxy leaving group and the 1,3-steric strain this causes with the iodomethyl substituent on the furanose ring (Figure 2.3A) is envisioned to lead to an increase in the transition state energy. However, for the α-anomer the increase in size of the leaving group is not thought to have a similar impact. Instead, the change in hybridization of the anomeric carbon centre from sp^3 to sp^2, to accommodate the developing positive charge, will release the 1,3-strain caused by the interaction of iodomethyl and hydroxymethyl groups (Figure 2.3B). To this end, the lower energy transition state results in the activation energy for the α-anomer being smaller relative to the β-anomer and therefore, the observation that the α-anomer reacts at a faster rate can be explained. Nevertheless, the reaction could not be driven to completion, e.g. by heating the reaction mixture to 50 °C or reflux (entries 3 and 4, respectively), so it was decided to explore stronger acids.



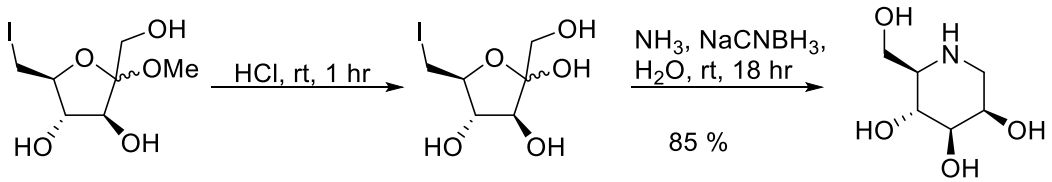
87b **87a**

Figure 2.3. 1,3-Interactions in the hydrolysis of methyl iodofructosides **84**

Next, triflouroacetic acid (TFA) was selected for its reported use in hydrolysis of glycosides (entry 5).[94] However, while the formation a significant amount of product was observed via TLC, several other unknown by-products were also formed during the course of the reaction. Consequently, the use of TFA in the demethylation reaction was not pursued further.

Hydrolysis with hydrochloric acid was then employed, which resulted in complete conversion of starting material within one hour (entry 6,). The HCl was then removed via repeated co-evaporation with water so as to avoid the formation of ammonium chloride as a by-product of the subsequent reductive amination. The formation of this azeotrope succesfully avoided any acid-catalysed degradation, as previously observed with H_2SO_4. In this way, the 6-deoxy-6-iodofructosides **87** could be generated and isolated for use in the subsequent reductive amination reaction. It is important to note that care must be taken during the work up (co-evaporation) procedure as rapid concentration results in the formation of side products, which interfered with the efficacy of the subsequent reaction.

Following acid hydrolysis using the methodology described above, the residue was re-dissolved in water and subjected to reductive amination conditions without further purification. Gratifyingly, the reaction with ammonia and sodium cyanoborohydride proceeded efficiently to form the desired azasugar DMJ (**4a**) as a single epimer in an excellent yield (85%), after purification using Dowex-H^+ and silica column chromatography (Scheme 2.6).



84 **87a** **4a**

Scheme 2.6. Synthesis of DMJ

The formation of the desired azasugar DMJ (**4a**) was confirmed by elucidation of ^1H, ^{13}C 1D NMR and COSY, HMBC and HSQC 2D NMR. In the ^1H NMR spectra (Figure 2.4)., the presence of the

two ABX systems of the CH_2's of the 1- and 6- positions, (δ_H = 4.00, $J_{6a,6b}$ = 12.8 Hz, $J_{5,6a}$ = 3.3 Hz, [H-6_a], δ_H = 3.89-3.82, [H4 and 6_b]) and (δ_H = 3.41, $J_{1a,1b}$ = 13.4 Hz, $J_{1a,2}$= 2.3 Hz, [H-1_a], δH_{1b} = 3.25 $J_{1a,1b}$ = 13.6 Hz, [H-1_b]) are indicative of the formation of the azasugar, where the unreduced product would be expected to show a single ABX system, as well as an AB. The triplet resonance identified for the 4-position is indicative of the desired stereochemistry where the large coupling constants [$J_{3,4}$ = $J_{4,5}$ =9.7 Hz] are consistent with trans-diaxial couplings with the 3- and 5-positions. HRMS analysis (ESI calcd. $[C_6H_{14}NO_4]^+$ *m/z*: 164.0917, obsd.: 164.0920) and the Correlation of the 1- position with the 5- position (δ_H= 3.25 $J_{1a,1b}$ = 13.6 Hz, [H-1_b]), (δH_5 = 3.18-3.14) observed via COSY 2D NMR further confirm the successful formation of DMJ (**4a**).

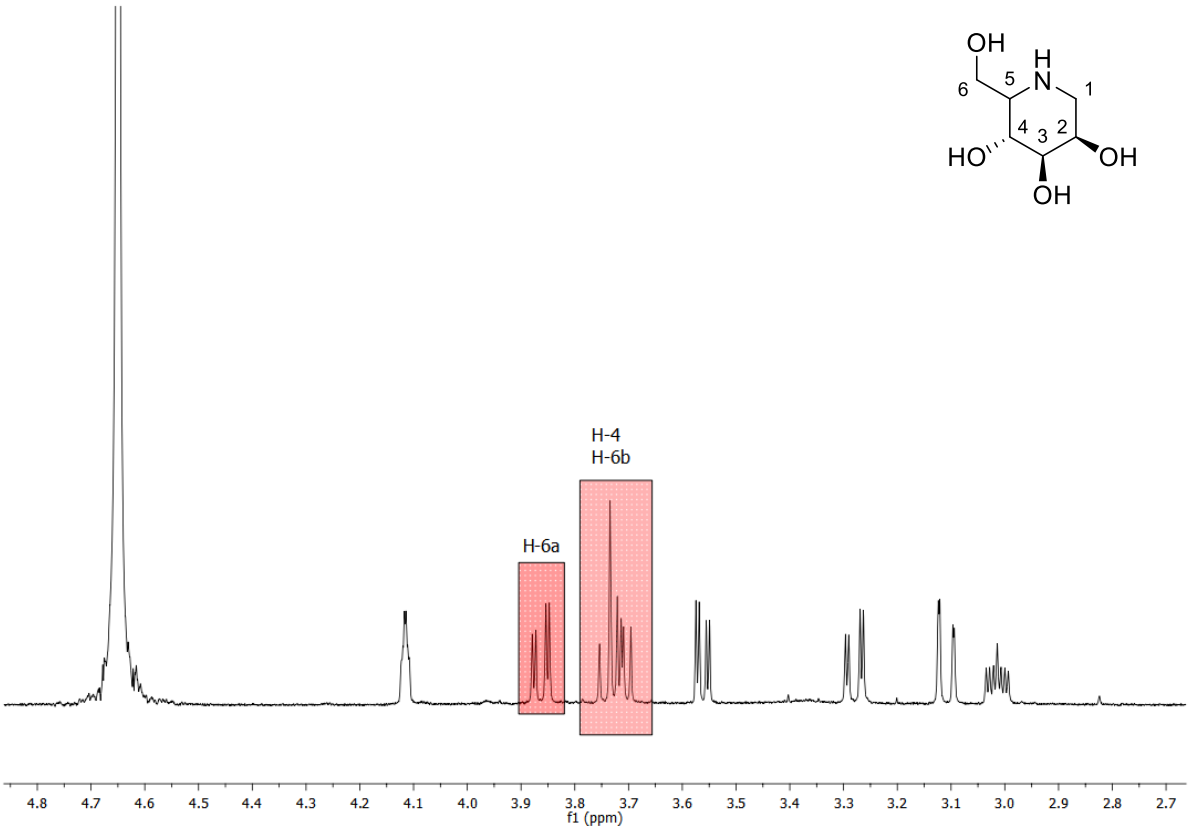
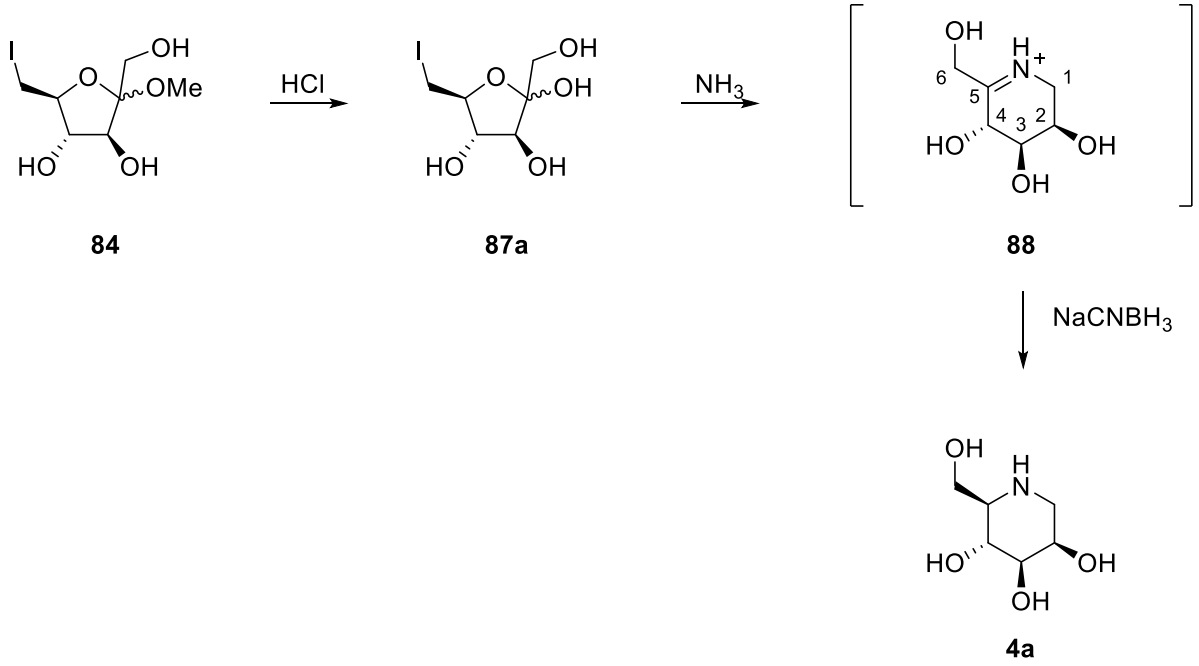


Figure 2.4. ^{1}H NMR spectrum of 1-deoxymannojirrymycin

During the reductive amination it is conceivable that epimerisation may occur at the 5- position. Fortunately, only the desired epimer DMJ (**4a**) was isolated, as evidenced by TLC and ^{1}H NMR analysis of the crude reaction mixture suggesting that the reduction was stereoselective. It is hypothesised that this reduction occurs following the formation of the proposed cyclic iminium ion intermediate (**88**) (Scheme 2.7). Reduction of cyclic imines in similar substrates have previously been reported to be stereoselective,[44,87,95,96] and would account for the stereoselectivity towards the desired D-*manno* configuration. This stereoselectivity is attributed to a mix of hydroxyl directing effects and the minimisation of torsional strain.[44,87,96] There is insufficient evidence to determine the mechanism of formation for the proposed iminium ion

(**88**). In the open chain form (Scheme 2.4), both the iodine and ketone present sites where the initial substitution can occur. It is envisioned that a primary amine such as ammonia will not be sufficiently nucleophilic to displace iodine. Indeed, previous syntheses of similar substrates, where a halide was the initial site of substitution, required more nucleophilic secondary amines or an azide.[40,41] therefore, imine formation could be followed by cyclisation to form **88**. Stereoselective reduction then affords DMJ (**4a**).



Scheme 2.7.
Proposed mechanism of the reductive amination

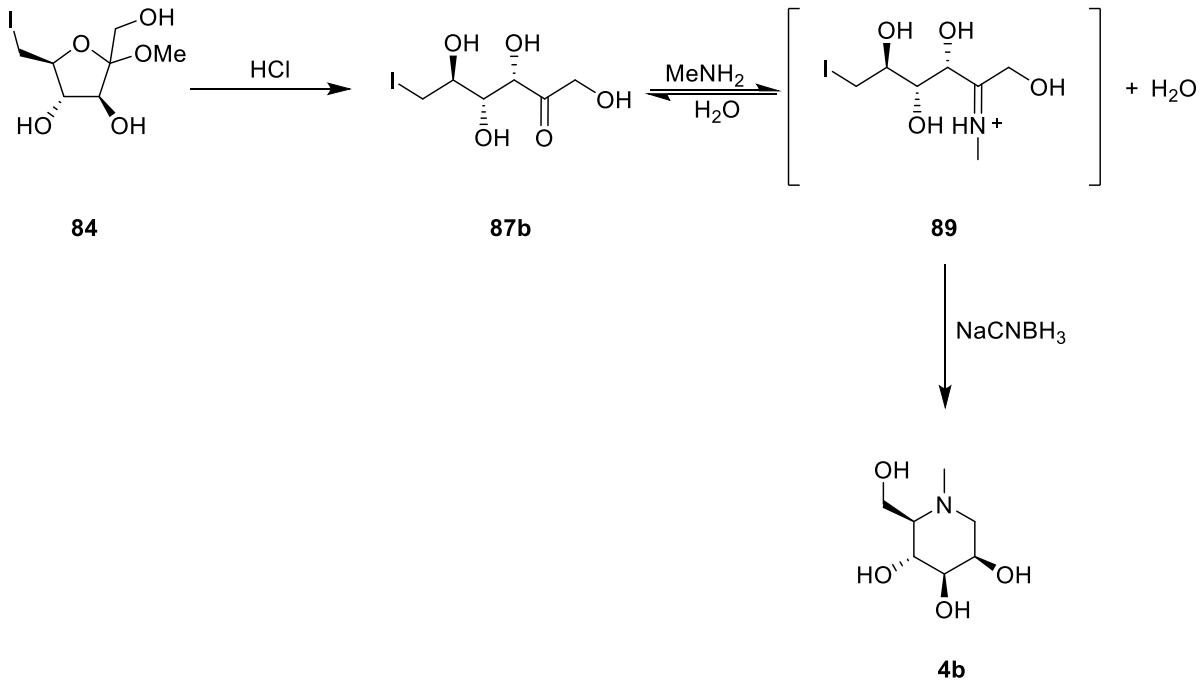
In summary, the azasugar DMJ (**4a**) was synthesised in an efficient and stereoselective four step synthesis starting from the commercially available D-fructose, with an overall yield of 61%. This represents a significant improvement on the most efficient published synthesis of DMJ which has a 28% yield over 5 steps.[41] The use of a chemoselective reaction has allowed for the selective

installation of key functionalities without requiring an extensive protecting group scheme, significantly enhancing the efficiency of the total synthesis. The reductive amination appears to be stereoselective, such that only the desired epimer is formed. It is anticipated that this methodology will be applicable to the synthesis of other derivatives through the use of different amines. It is projected that the observation of intermediates and/or side products formed with different amines may yield further information about the mechanism of the reductive amination.

2.2. The Synthesis of *N*-Methyl-DMJ

Following the successful synthesis of DMJ from fructoside **84**, it was proposed that this starting material could be used for the preparation of DMJ analogues. The first derivative selected was *N*-methyl-DMJ. While this derivative has been previously synthesised via the methylation of DMJ,[71,84] it was envisioned that the direct synthesis of *N*-methyl-DMJ from the corresponding methyl iodoglycoside **84** would lead to a shorter synthetic route for the preparation of this compound. Moreover, this would establish a proof-of-principle methodology for the preparation of other *N*-alkylated DMJ analogues.

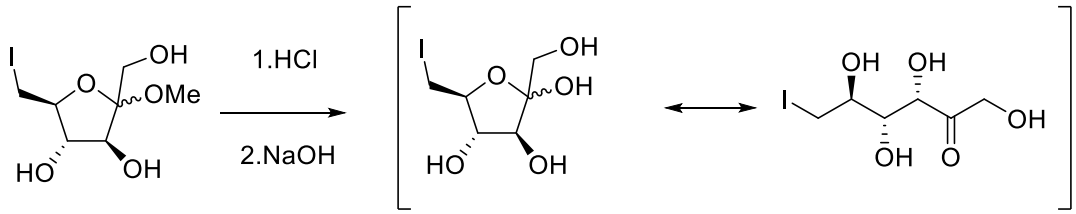
To this end, the reaction protocol used followed that derived for the aforementioned synthesis of DMJ, whereby aqueous methylamine solution (40% w/w) was used in place of ammonia (Scheme 2.8). Initially, mixed results were observed, with yields of less than 40% for the target compound. It was hypothesised that the low yield may be attributable to a loss of material during purification, and the procedure was therefore modified by the removal of the Dowex-H^+ column from the purification procedure. In order to accommodate this change, the excess sodium borohydride was removed by co-evaporation with a mixture of methanol: acetic acid: toluene (1:4:6). Unfortunately, poor yields of *N*-methyl DMJ (**4b**) were still obtained despite multiple attempts using this protocol.



Scheme 2.8. Hypothesised reductive amination of the iodoglycoside

It was hypothesised that the reduced yield of *N*-methyl-DMJ (**4b**), may be due to the competing hydrolysis of the iminium ion **89**, proposed to form *en route* to *N*-methyl-DMJ (**4b**), which may be favoured by the high concentration of water (Scheme 2.8). While this was not observed to be an apparent factor in the synthesis of DMJ, it was thought that the increased steric requirements of the alkylated amine may make cyclisation of the proposed *N*-alkylated iminium ion intermediate **89** more difficult, such that hydrolysis becomes more favourable. Accordingly, it would be desirable to perform the reaction sequence with minimal water and instead use an alternative solvent, such as ethanol. However, hydrolysis of the methyl glycoside cannot be performed in ethanol, or indeed any alcoholic solvent due to the formation of the corresponding glycoside. Consequently, attempts were made to neutralise the HCl used to form

the keto-iodide (**87b**) prior to reductive amination, to ensure a large excess of acid would not be present to catalyse glycoside formation upon the addition of an alcoholic solvent. Unfortunately, it was found that the co-evaporation methodology previously used to concentrate the iodoglycoside **87b** during the synthesis of DMJ (**4a**), did not sufficiently neutralise the reaction mixture and upon the addition of EtOH, glycosidation was observed. This necessitated further investigations into the addition of base amenable to subsequent reductive amination to the reaction mixture in order to neutralise the solution prior to addition of an alcoholic solvent. A number of bases were tried, including Dowex OH^- and triethylamine, but adverse reactions were observed via TLC. Ultimately, a dilute solution of NaOH was found to be the most suitable base for neutralisation of the reaction solution (Scheme 2.9). TLC analysis, ^1H and ^{13}C NMR of the concentrated reaction mixture confirmed the formation of the desired demethylated product **87**. Fortunately, the sodium chloride formed during neutralisation was sparingly soluble in ethanol and could be readily separated from the reaction mixture via decantation or filtration, thereby facilitating purification of the final product, *N*-methyl DMJ (**4b**).



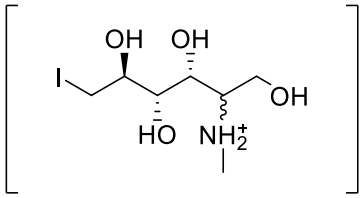
84 **87a** **87b**

Scheme 2.9. Hydrolysis of the methyl glycoside 84

Following this success, the subsequent reductive amination of **87b** was undertaken in ethanol. In order to keep the water content to a minimum, methylammonium chloride was used in place of

methylamine solution. The use of the hydrochloride salt provided a lower pH of 6 than the methylamine solution (pH 11), and could aid in imine formation, which is favoured at a pH of 6.[97,98] The catalytic action of acid was found to be beneficial towards imine formation without being too acidic to form the undesired glycoside by-products. A minimum amount of amine was used in order to simplify purification. It was found that 1.5 molar equivalents of the amine was optimal for complete conversion of the iodo glycoside **87**.

Having established the reaction conditions, **87** was dissolved in ethanol before methylammonium chloride and sodium cyanoborohydride were added to initiate the reductive amination reaction. Complete consumption of the keto-iodide and the appearance of a single product with an R_f value indicative of the desired product were observed via TLC analysis. However, when the reaction mixture was concentrated the ^{1}H NMR spectrum showed multiple products were present and subsequent attempts to purify the desired azasugar resulted in very poor yields of less than 15%. When the reaction was repeated and followed by HRMS analysis to confirm product formation, a significant peak around 306.0203 m/z was consistently observed along with a mass corresponding to the desired product at 178.1101 m/z. A separate product was also observable via TLC when the polarity of the solvent mixture was increased [(Dichloromethane: ethanol: methanol: ammonia) (28% w/w) (5:2:2:1) to (5:2:3:1), (v:v:v:v)]. This unknown material was proposed to be the 6-iodo-2-amino-glycitol **90** (calculated mass: 306.0197), which is the un-cyclised amine intermediate (Figure 2.5). However, this intermediate could not be isolated, nor identified by ^{1}H NMR in the crude reaction mixture.



90

Calcd.: 306.0197
Observed:306.0203

Figure 2.5. Proposed aminoglycitol **90** intermediate formed during the reductive amination reaction.

It was thought that the acidity of the reaction mixture would stabilise the proposed uncyclised intermediates **89** and **90**, slowing the cyclisation to the desired product. It was therefore proposed that while a slightly acidic pH favoured reductive amination, increasing the pH would favour cyclisation. To this end, the pH of the reaction mixture was increased from a pH of 6 to a pH of 10, via the addition of a methylamine solution, after which the previously observed product **90** was no longer present as gauged by TLC or HRMS. It was hoped the imine hydrolysis would not be favourable even with the presence of minimal water. Following completion of the cyclisation, the reaction material was concentrated under reduced pressure, before being treated with a methanol-acetic acid-toluene mixture [(1:4:6) (v:v:v)], to remove the excess borohydride. A ^{1}H NMR of the crude sample was then taken which confirmed the presence of the desired product. Satisfyingly, only the desired product was identifiable with no indication of the presence of alternative products or formation of the 5-epimer. After purification, the ^{1}H NMR spectrum confirmed the formation of the desired stereoisomer, where the triplet correlating to H-4 (δ_H = 3.93, t, $J_{4,3} = J_{4,5}$ = 10.1 Hz [H-4]), had two large coupling constants indicative of diaxial couplings. Furthermore, only a single AB-X system was observed for the protons at the 6 position

(δ_H= 4.04, $J_{6a,6b}$ = 12.9 Hz [H-6_a], δ_H= 3.99, $J_{6a,\ 6b}$ = 13.19 Hz, $J_{6b,\ 5}$ = 2.52 Hz [H-6_b]), whereas epimerisation of the 5-position would result in a second ABX system for these protons. It is noteworthy that only the desired stereoisomer was observed. Reduction following cyclisation is typically stereoselective,[44,87,95] however reduction prior to cyclisation, as suggested by the observation of the amine via HRMS, is not expected to be stereoselective and would likely result in the production of two epimeric azasugars.

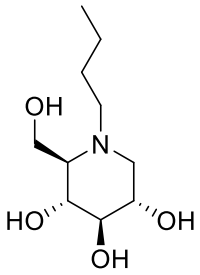
Attempts were then made to purify the product *N*-methyl-DMJ by column chromatography. Due to the high polarity of both the product and salt by-products, purification proved extremely difficult. Unfortunately only a 45% yield of *N*-methyl-DMJ (**4b**) was obtained following a single silica gel flash chromatography column. Further recovered product was difficult to isolate from the remaining methylammonium hydrochloride, which remained in excess. Moreover, the ratio of *N*-methyl-DMJ to salt appeared to become constant even after multiple purification steps by silica gel column chromatography. It was proposed that the exchange of the methylamine hydrochloride salt for the corresponding triethylamine salt would result in easier separation. Accordingly, the reaction mixture was iteratively dissolved in water and treated with triethylamine before being concentrated so as to evaporate the volatile methylamine. Gratifyingly, this greatly facilitated in the purification of the product via a single silica gel column. Accordingly, treatment with triethylamine was incorporated into the purification protocol after the removal of excess sodium borohydride via co-evaporation using a solution of the methanol: acetic acid: toluene mixture [(1:4:6), (v:v:v)].

In this way, the *N*-methyl-DMJ (**4b**) could be synthesised from methyl6-deoxy-6-iodofructoside (**84**) in a 65% yield over two steps. Thus, the overall yield for the three step synthesis of *N*-methyl-DMJ (**4b**) starting from D-fructose (**86**) is 47%. This represents an improvement on previous syntheses which require the methylation of DMJ,[71,84] a compound for which the most efficient published synthesis has a 28% yield over 5 steps.[41]

Thus, in summary, an efficient synthesis of *N*-methyl-DMJ (**4b**) was achieved in 47% yield in four steps. During the optimisation of this synthesis, two main issues were solved. First, it was determined that imine hydrolysis prevented an efficient reductive amination, which could be solved by the use of a dry solvent. Secondly, the methyl ammonium salts proved difficult to separate from the desired product. To circumvent this issue, the creude reaction mixture was co-evaporated with NEt_3 and the resulting triethyl ammonium salts were readily separated via silica gel column chromatography. These optimised conditions should therefore be amenable to the synthesis of a variety of *N*-alkylated DMJ derivatives. Moreover, it is interesting to note that the 6-iodo-2-amino glycitol **90** was observed during the synthesis of *N*-methyl-DMJ. At present it is not clear whether this product is an intermediate that is formed *en route* to *N*-methyl-DMJ, or whether it is a by-product that is not involved in the formation of the desired product. It is hoped that the syntheses of further *N*-alkylated derivatives will yield more conclusive insight towards the mechanism of reductive amination reactions on these substrates.

2.3. The Synthesis of *N*-butyl-DMJ

Having accomplished the synthesis of the first DMJ derivative, *N*-methyl-DMJ (**4b**), the synthesis of other alkylated DMJ-derivatives was then undertaken. Increasing the length of the alkyl chain on the ring nitrogen increases the lipophilicity of these compounds, which has been shown to enhance selective antiviral activity.[20] Accordingly, the synthesis of the *N*-butyl derivative was carried out. The synthesis of a butyl analogue of DMJ is of particular interest as the corresponding C2 epimer, *N*-butyl-DNJ **2b** or Miglustat (Figure 2.6), is a well-known drug used for the clinical treatment of Gauchers disease.

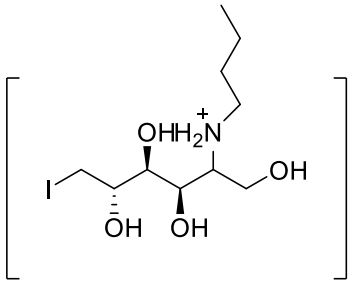


Miglustat **2b**

Figure 2.6.

To this end, the iodoglycoside **87b** was generated by following the protocol established during the aforementioned synthesis of *N*-methyl-DMJ, (Scheme 2.9). The longer alkyl chain was expected to increase the nucleophilicity of the amine, which in turn, may increase the likelihood of direct substitution of the iodine. To determine whether this was the case, the reductive amination was initially attempted without the addition of catalytic acid, which was previously used to favour imine formation from the *in situ* generated ketone before substitution of the iodine could occur. This would also mimic the synthetic strategy for the

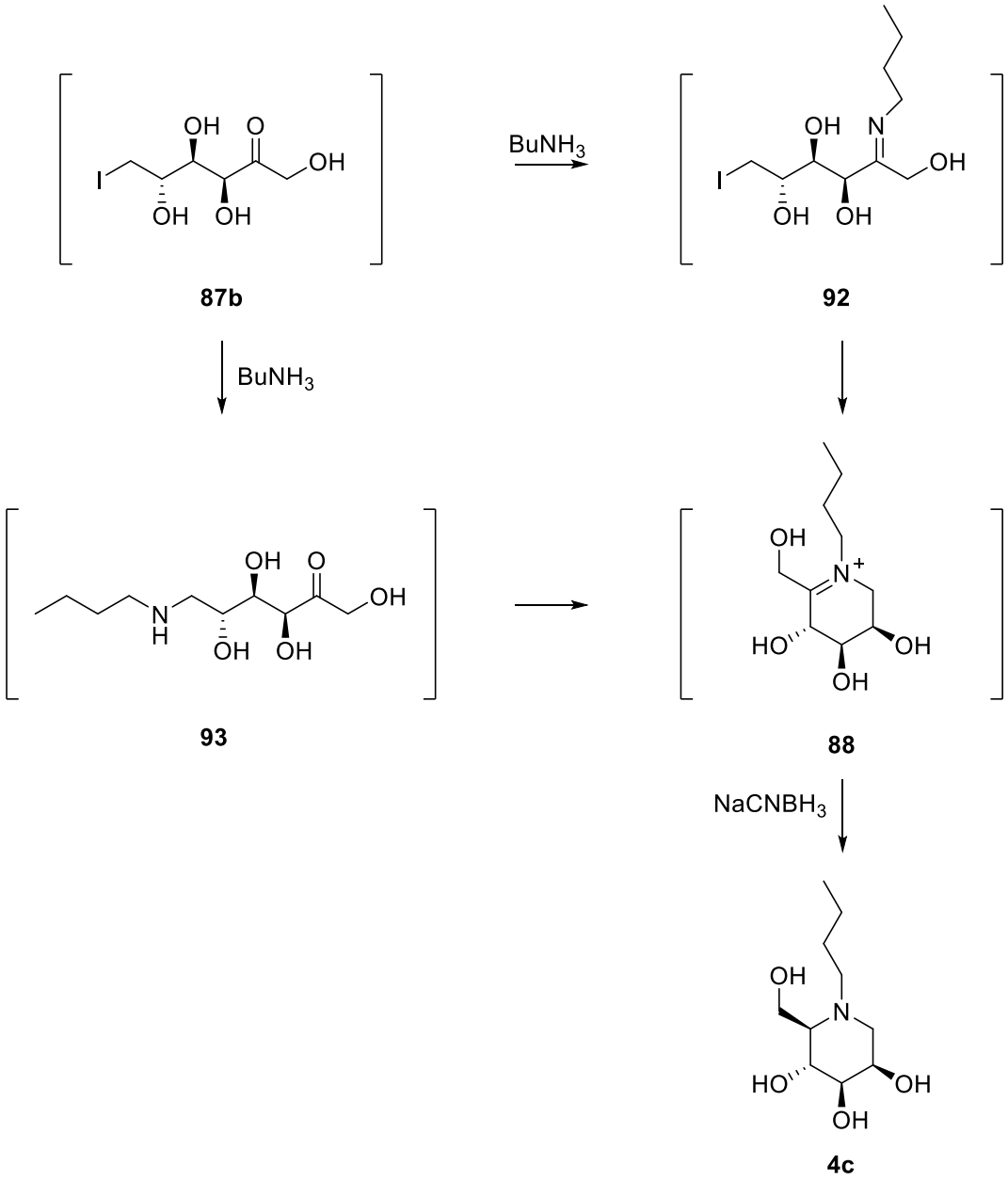
preparation of DMJ (**4a**) (see section 2.1.) Furthermore, it was hoped that performing the synthesis under basic conditions to promote iodine substitution as the initial step of reductive amination of **87b**, or alternatively, during cyclisation of the proposed iminium intermediate **92** (Scheme 2.10) would avoid the generation of the proposed 6-iodo-2-aminoglycitol side product **91** (Figure 2.7), corresponding in structure to **90** (see Figure 2.5, section 2.2).



91

Calcd.: 348.0666
Obsd.: 348.0682

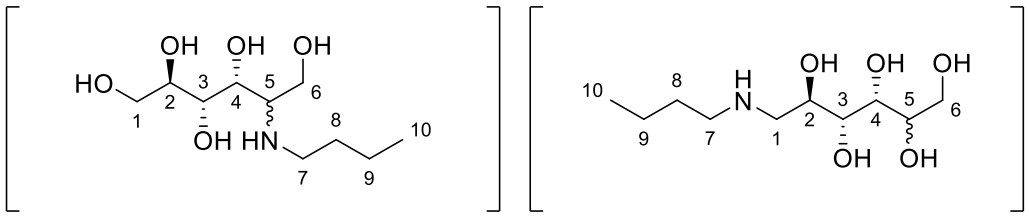
Figure 2.7.
6-iodo-2-aminoglycitol side product indicated by HRMS data



Scheme 2.10. Proposed route to 4c avoiding the formation of aminoglycitol **91**

Accordingly, iodoglycoside **87** was treated with *N*-butylamine and sodium cyanoborohydride. After 12 hours, analyses via TLC and HRMS indicated the complete consumption of iodoglycoside **87** and the generation of the desired azasugar, which was purified via co-evaporation with the aforementioned acetic acid : toluene : MeOH mixture followed by silica gel column

chromatography, as established in the synthesis of *N*-methyl-DMJ(section 2.2), to afford *N*-Butyl-DMJ in a moderate 34% yield. Along with the desired azasugar which had an R_f of 0.65, a second product with R_f of 0.40 was also isolated in a significant ~20% yield. As suggested by HRMS this product was tentatively assigned as one of two acyclic amino glycitol compounds **94a** or **94b** (Figure 2.8), with the observed mass of mass: 238.1649, corresponding to the calculated mass 238.1649 [$C_{10}H_{22}NO_5H^+$]. Analysis by ^{13}C and ^{1}H NMR indicated the formation of a minor and major product, thought to be diastereomers, in a 2:1 ratio. Here, the chemical shifts for the methylene protons assigned to the 5-position differed significantly more for the two diastereomers than the 6 position, indicating the stereocentre is closer to H5. As no ^{13}C NMR resonances were in the range that is typical for a halide substituted carbon (4-15 ppm), and the M+2 and M+4 isotopic distribution characteristic of atomic iodine was not observed via HRMS, it was tentatively suggested that substitution of the iodine in **87b** had occurred to give the second product **94b**. The downfield chemical shift observed via ^{1}H NMR for the methylene protons at H6 of this product (δ_H= 3.85 [H-6a], δ_H= 3.67 [H-6_b]) indicated that **94b** rather than **94a** had been formed. Unfortunately, the assignment of the second product could not be confirmed by 2D data as no correlation was observed between the methylene protons on either side of the nitrogen. As no more information could be obtained while the diastereomers products remained in a mixture, it was necessary to separate the two products.



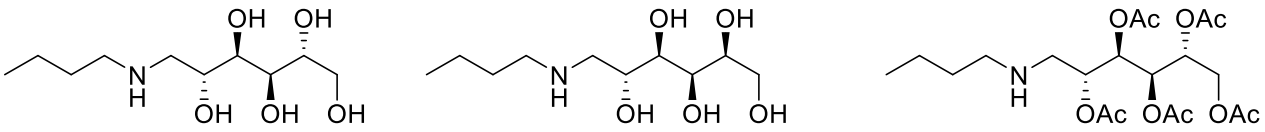
94a **94b**

Figure 2.8. Possible products according to HRMS data.

Unfortunately, however, the diastereomers were not separable by silica gel flash column chromatography. Instead, it was envisioned that acetylation would aid in the separation of the two derivatives. Additionally, acetylation could verify the configuration of the products, where a downfield shift would be observed in the ^{1}H NMR spectrum for each acetylated position in an acyclic compound, whereas in a cyclic product, the CHO involved in the ring would remain at higher field. Fortunately, the two acetylated products were easily separated via silica gel column chromatography, though the amount of the minor product isolated was very little, reducing the resolution of the resulting NMR spectra.

Elucidation of the 2D NMR data of the acetylated major product led to the confirmation that alkylation of the amine had occurred via substitution of the iodine, and the reduction of the ketone to an alcohol. The resulting downfield shift of the protons adjacent to hydroxyl groups confirmed an acyclic structure and the compound was identified as the peractylated aminoglycitol **95** derived from **94b** (Figure 2.9). This assignment was confirmed by comparison with the ^{1}H NMR data of **94b** to a series of terminal aminoglycitol products with similar stereochemistry, which had been prepared through reductive amination of aldoses.[99] Reduction of the ketone in of a carbohydrate with *fructo* stereochemistry leads to diastereomers with D-*manno* and L-*gulo* stereochemistries. Comparison of the coupling constant between H4 and H5

with that observed for the corresponding protons in a published 6-amino mannitol derivative indicated that the major isomer was in the mannose configuration.[99]



94b-1 **94b-2** **95b-1**

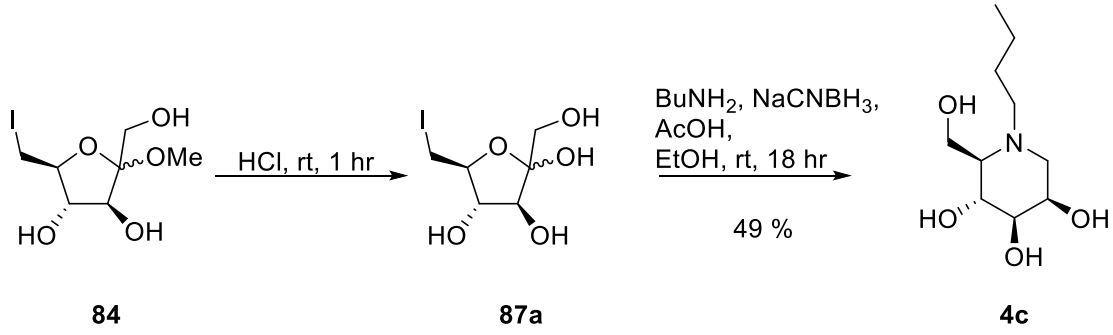
Figure 2.9. 6-aminoglycitol side products generated under basic conditions

It was unexpected that the substitution of the iodine results in formation of significant amounts of side products. The increased nucleophilicity of the resulting secondary amine was expected to increase the favourability of a subsequent nucleophilic attack on the carbonyl. This has been exploited in previous syntheses of azasugars involving intramolecular reductive amination, where the amino group is first installed through the substitution of a halide in a separate step.[40,41] While not ideal for imine formation, it is unlikely that the basic conditions prevented intramolecular attack of the carbonyl, as reductive amination was previously achieved using ammonia under basic conditions to give a high yield (section 2.1.1). Furthermore it was unlikely that the slightly higher steric bulk would be sufficient to prevent cyclisation. It therefore seems likely that the reduction of the carbonyl occured prior to alkylation of the amine via iodine substitution. While, a pH of 3 or less is normally required for reduction of a ketone with cyanoborohydride, with carbonyl reduction normally neglible under basic conditions,[100] the synthesis of the analogous 1-

butylamino-1-deoxyglucitol has been recorded as a side product of azasugar formation via reductive amination of the aldehyde of 5-keto-D-glucose under near identical conditions.[44]

With the generation of **94b** under basic conditions it was decided that the chemoselective generation of imminium ion **92** via the use of catalytic acid could be more favourable for the synthesis of the desired azasugar (Scheme 2.11). When acetic acid was added concurrently with the reductive amination reagents the 1-amino-glycitol side product **94b** could no longer be observed by HRMS. Unfortunately, a second product was observed at 238.0682. This peak corresponds to an acyclic 6-iodo-2-aminoglycitol **91** (calculated mass 348.0666 [$C_{10}H_{22}INO_4H^+$]) (Figure 2.7), a mass corresponding to a butyl substituted amine, in place of the methyl amine observed in the synthesis of the methyl derivative **4b**. The observation of a mass fitting a similar structure to that of methylamine **90**, indicates that the observation of **90** was not an artefact and could reproducibly be formed upon the reductive amination of **87** with multiple different amines. In turn, this observation further supports the hypothesis that reduction occured prior to cyclisation, with the risk of epimerisation during reduction resulting in a lower yield. Following the protocol established for the methyl derivative (**4b**), excess butylamine was added to increase the pH and induce cyclisation of the proposed aminoglycitol. Following 22 hours, the mass at *m/*z: 346.0510 was no longer observed via HRMS, whereupon the reaction mixture was concentrated and purified via the purification protocol established for **4b** (section 2.2.1) followed by co-evaporation with dilute HCl (aq) to afford the desired product *N*-Bu-DMJ (**4c**) as its HCl salt in a 42% yield. The lower polarity of *N*-butyl-DMJ compared to the methyl derivative **4b** allowed the purification to occur more smoothly. Thus *N*-Bu-DMJ (**4c**) was synthesised from methyl-

iodoglycoside **84** in a 42% yield over two steps. The overall yield for the four-step synthesis starting from the commercially available from D-fructose (**86**) is 30%, a significant advancement on the previous synthesis where **4c** was obtained in overall yield of 6% over 16 steps.[78]

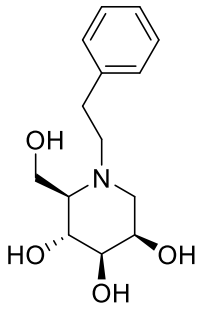


84 **87a** **4c**

Scheme 2.11. The synthesis of *N*-butyl-DMJ

2.4. The Synthesis of *N*-[2-phenylethyl]-DMJ

With the alkylated derivatives **4b** and **4c** in hand, the viability of incorporating amines substituted with aromatic groups was assessed. Aromatic-DMJ analogues such as *N*-benzyl-DMJ (**4f**), have shown to suppress protein synthases, providing potential for HIV inhibitors.[66] *N*-[2-phenylethyl]-DMJ (**4e**) (Figure 2.10) was selected as the first aromatic derivative of DMJ to be synthesised, as 2-phenylethylamine would allow for the incorporation of an aromatic group while keeping any added steric bulk away from the nucleophilic amine functionality. In addition to providing a desired bioactive functionality, the UV-active phenyl group could aid in monitoring the reaction and subsequent purification of the desired azasugar **4e**. This could, in turn, allow for the observation and isolation of side products responsible for the moderately low yields obtained in the syntheses of the methyl (**4b**) and butyl (**4c**) derivatives of DMJ when compared to yield obtained for the parent azasugar DMJ (**4a**). In addition to being the first derivative featuring aromatic functionalisation in this body of work, *N*-[2-phenylethyl]-DMJ (**4e**) was the first novel DMJ derivative to be prepared via this synthetic approach.

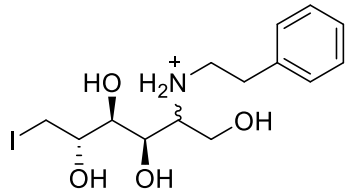


4e

Figure 2.10. *N*-[2-phenylethyl]-DMJ (**4e**)

The synthesis of the desired azasugar was conducted following the protocol established during the synthesis of the alkylated derivatives (Scheme 2.11, section 2.3.1), whereby the neutral residue of the iodoglycoside **87**, generated from the corresponding methyl glycoside **84** was subjected to reductive amination using 2-phenylethylamine and catalytic amounts of acetic acid used to generate a pH of 6 for the promotion of iminium ion formation. After complete reaction of starting material was observed via TLC analysis, HRMS analysis showed the presence of a mass corresponding to the desired product **4e** (observed mass: 268.1559 calculated mass: 268.1543 [$C_{14}H_{21}NO_4H^+$]). Furthermore, a mass matching that of an acyclic iodo-aminoglycitol product **96** (observed mass: 396. 0685, calculated mass: 396.0666 [$C_{14}H_{22}INO_4,H^+$]) was observed, which further supporting the idea that this side product was indeed being produced as seen in the syntheses of the previous derivatives **4b** and **4c** (Figure 2.11). As established in the synthesis of previous derivatives, **4b** and **4c**, excess amine was added to bring the pH to 10 to promote the cyclisation of this postulated compound to form the piperidine ring. After the acyclic side product **96** was no longer observed via HRMS, the reaction was concentrated and purification of the

product was undertaken using the previously described evaporation and chromatography protocol (see section 2.2.1).



93

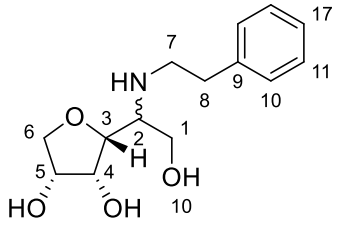
calcd: 395.0666
obsd: 395.0710

Figure 2.11. Proposed aminoglycitol according to the mass observed via HRMS

Initially, purification afforded *N*-[2-phenylethyl]-DMJ in a 20% yield. In order to improve the purification protocol, the solvent gradient used for silica gel chromatography altered to incorporate an initial nonpolar gradient of PE:EtOAc (2:1 → 1:3, v:v) before the use of a DCM:MeOH:EtOH:NH_3 (aq) gradient protocol as was used to purify the DMJ derivatives **4b** and **4c** [(DCM:MeOH:EtOH:NH_3 (aq)) (195:2:2:1→15:4:4:2, v:v:v:v)]. Despite careful elution using silica gel chromatography and repeated iterations of coevaporation with water and toluene, excess acetic acid was still present. Accordingly coevaporation with dilute hydrochloric acid was used to displace acetic acid, affording the product *N*-[2-phenylethyl]-DMJ **4e** as its HCl salt, which improved the yield to 42% over two steps.

Alongside the desired product **4e**, a secondary product was also obtained. HRMS analysis indicated this by-product had the same mass as **4e** (observed mass: 268.1559, calculated mass: 268.1543, [$C_{14}H_{21}NO_4H^+$]). However, the ^{1}H NMR spectra did not contain DMJ nor its C5 epimer 1-deoxygulojirrimycin derivatives, which were the most likely azasugar products. Correlation

between the methylenes on either side of the amine nitrogen, (δ_H= 3.28, [H-2], δ_H= 3.36 [H-7a]) (δ_C= 58.2 [C-2], δ_C= 47.4 [C-7]), were observed via COSY and HMBC 2D NMR and indicated that nucleophilic substitution by the amine occurred at C2, rather than terminal substitution of the iodine at the 6-position. The mass observed in the HRMS data (Observed mass: 268.1559) indicated some cyclisation must have occurred as all expected acyclic products were higher in mass. Importantly, a correlation between H3 and C6 (δ_H= 4.01, [H-3], δ_C= 71.0 [C-6]) was observed in the HMBC spectrum, which indicated cyclisation had occurred via substitution of the iodine by the hydroxyl at C3 to give the 5-membered furan ring **97** (Figures 2.12 and 2.13). All coupling constants between H1-H6 in the ^{1}H NMR spectrum are in the order of 5 Hz, which is consistent with the proposed structure of **97**, but did not provide any information of the absolute and relative configurations of the stereocentres. The structure of **97** is therefore based on the stereochemistry of the fructose starting material (Figure 2.12).



97

Figure 2.12. Side product **97** formed during the synthesis of *N*-[2-phenylethyl]-DMJ synthesis indicated by HRMS and NMR.

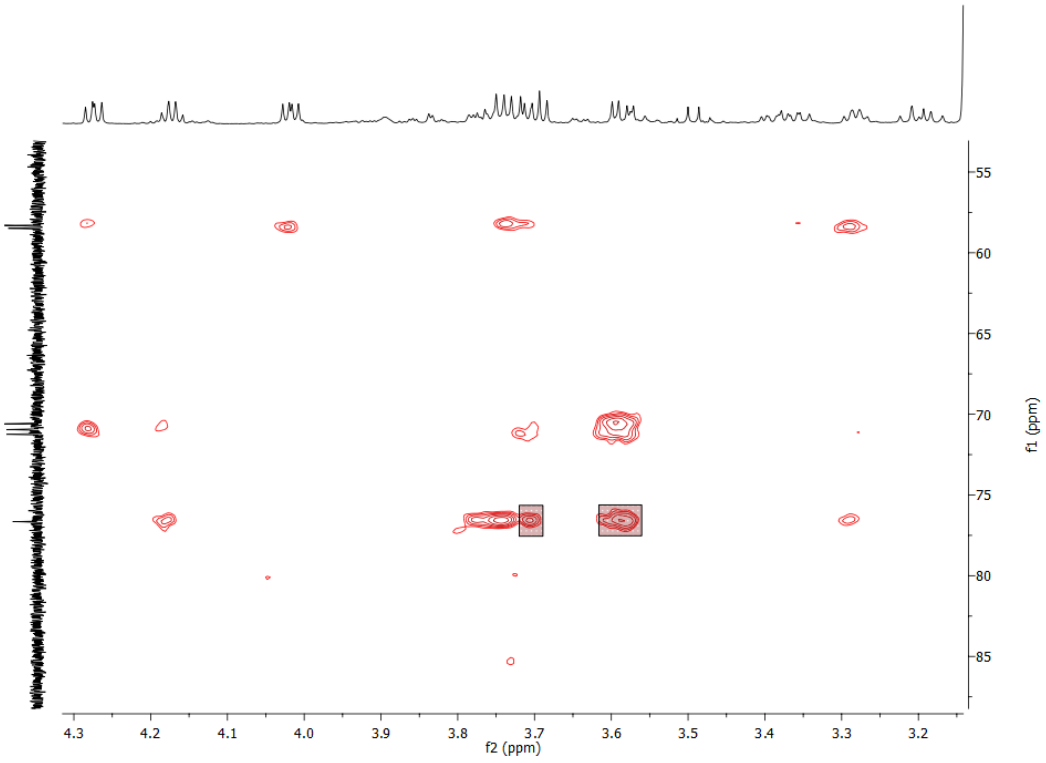
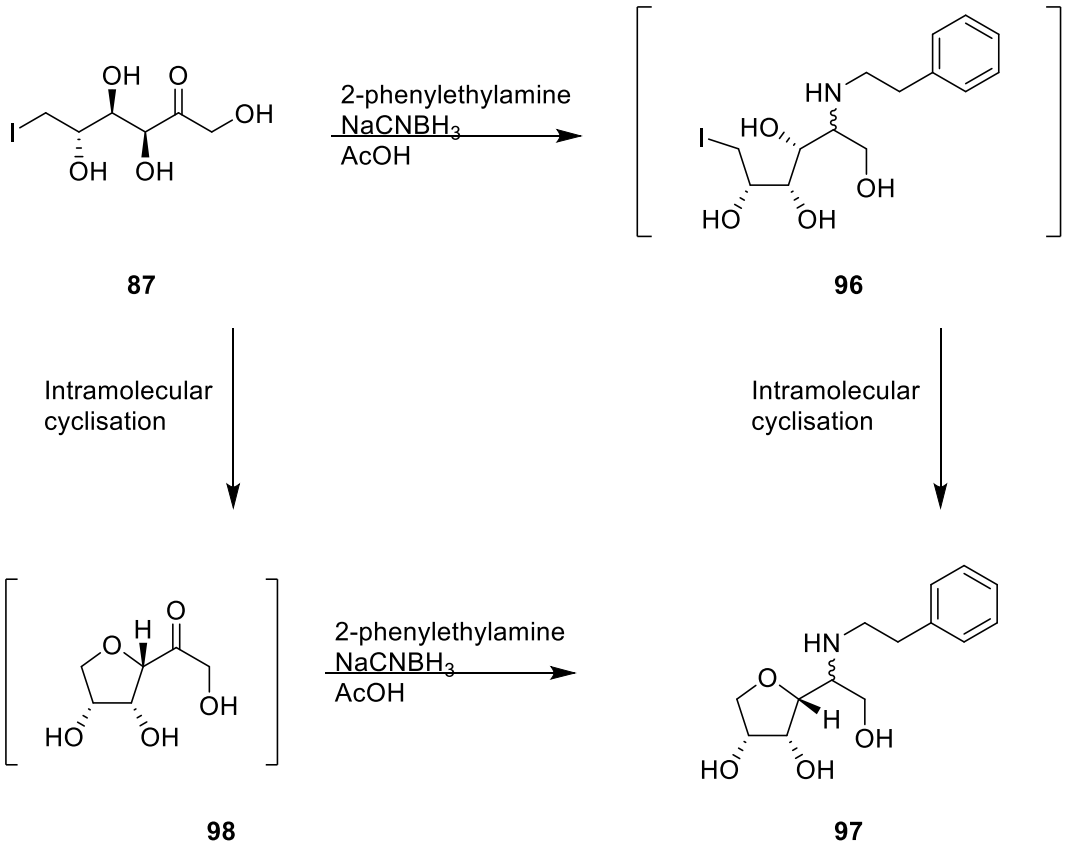


Figure 2.13. HMBC of **97** showing correlation of H6a and H6b to C3

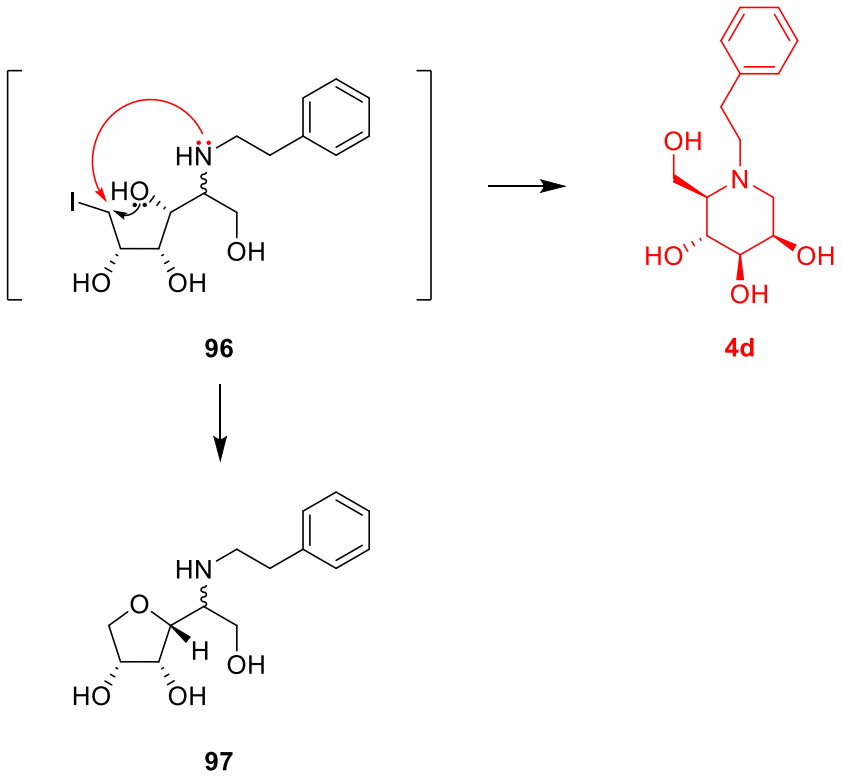
While it is envisioned that the by-product **97** could form through the reductive amination of the ketone carbonyl of **87b** with 2-phenylethylamine and subsequent intramolecular substitution of the iodine by the hydroxy substituent at C3 (Scheme 2.12), the order in which these events took place was not apparent. A mass matching that of 6-iodo-2-aminoglycitol **96** (observed mass: 396. 0685, calculated mass: 396.0666 [$C_{14}H_{22}INO_4$,H^+]) observed via HRMS could mean that the furanose **97** was formed via glycitol **96** as an intermediate, where iodine substitution occurred before the intramolecular attack from the amine took place. This may account for the observation of masses which match both the side product **97** and postulated intermediate **96** via HRMS when the reaction was performed under acidic conditions. It is envisioned that products

with analogous structures would be observed in the syntheses of other derivatives, however, none were isolated. Moreover, the secondary amine of **96** was envisioned to be significantly more nucleophilic than the hydroxyl at C3, and would react to form the azasugar in conditions favouring cyclisation (Scheme 2.4.2). This would suggest the formation of furanose **97** instead occurred via the proposed intermediate **98**, where irreversible iodine substitution prevented the formation of the piperidine ring following reductive amination of the ketone (Scheme 2.12). Ultimately, isolation of either of the proposed intermediates, followed by reaction to product **97**, is required to conclusively determine the mechanism through which this product is formed.



87 **96** **98** **97**

Scheme 2.12. Postulated routes to the formation of side product **93**



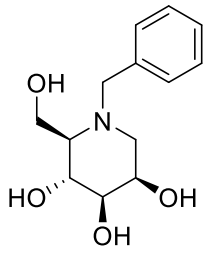
Scheme 2.13. Proposed competing nucleophilic substitutions of the iodide **96**

Further attempts at synthesising *N*-[2-phenylethyl]-DMJ **4e** improved the yield of the desired azasugar as its corresponding HCl salt to 47% over two steps from methylglycoside **84**, giving an overall yield for the four step synthesis of *N*-[2-phenylethyl]-DMJ (**4e**) from commercially available D-fructose (**86**) of 34%. This represents the first synthesis of a novel DMJ derivative using this synthetic methodology. Isolation of the side product **94** suggests a possible explanation for the moderate yields observed in the synthesis of **4e** and other DMJ derivatives (**4b** and **4c**) compared to the synthesis of the parent azasugar DMJ (**4a**). Furthermore, the furanose **97** may have been derived from the proposed intermediate 6-iodo-2-amino-glycitol **96**, which was observed via HRMS analysis for the synthesis of the 2-phenylethyl derivative **4e**. As products with

analogous structures were not been isolated during the syntheses of derivatives with other amines, the formation of the by-product **97** may be unique to the synthesis of *N*-[2-phenylethyl]-DMJ **4e** and not a consistent side product for the reductive amination of iodoglycoside **87**.

2.5. The synthesis of *N*-Benzyl-DMJ

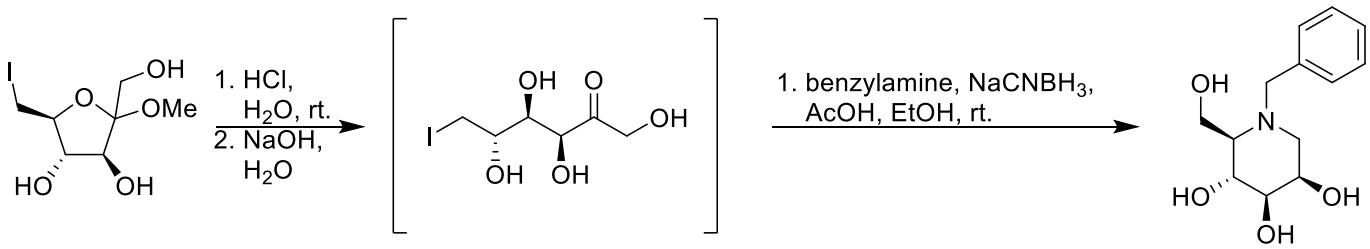
With *N*-[2-phenylethyl]-DMJ (**4e**) successfully synthesised from methyl iodoglycoside **84**, it was sought to extend the methodology to the synthesis of a more sterically hindered DMJ derivative, such as *N*-benzyl-DMJ (**4f**), (Figure 2.13). As with the 2-phenylethyl DMJ derivative **4e**, the phenyl ring of **4f** has been shown to introduce desirable bioactivity to DMJ,[66] while also providing a UV active functionality to assist in reaction monitoring and purification.



4f

Figure 2.14. *N*-Butyl-DMJ

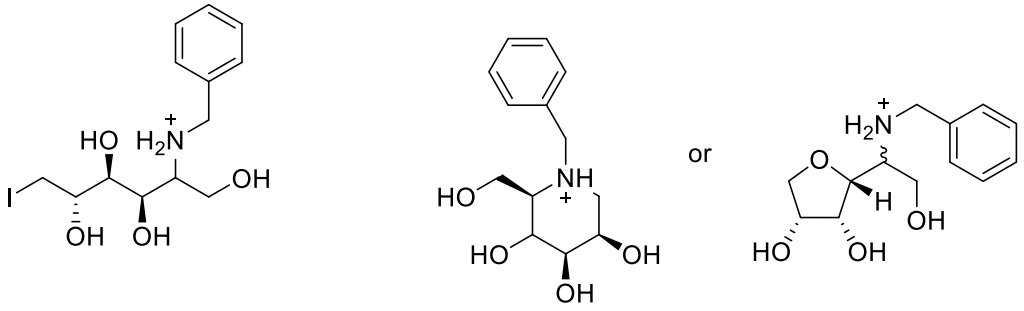
Using the previously established methodology, iodoglycoside **87** was prepared from **84** and reacted with benzylamine in the presence of a catalytic amount of acetic acid to give *N*-benzyl-DMJ (**4f**).



84 **87** **4f**

Scheme 2.14. Preparation and reductive amination of **87** to form **4f**

While it was anticipated that the increased steric bulk of benzylamine may hinder the reaction, TLC and HRMS of the reaction mixture suggested that the reaction was proceeding towards the desired azasugar product. After the iodoglycoside **87** was no longer observable via TLC, the reaction mixture was analysed by HRMS. The product observed at *m/z*: 382.0524 was tentatively assigned as 6-iodo-2-aminoglycitol **99** (calculated mass: 382.0510), a structural motif which had been observed in the previous syntheses of DMJ derivatives **4b**, **4c** and **4e**. Alongside this proposed intermediate, a product was observed at *m/z*: 254.1393 corresponded to the desired azasugar **4f** (calculated mass: 254.1387). The isolation of **97** during the synthesis of **4e** suggested that this mass may also correlate to the amine anhydride side product **100** formed from **87** via intramolecular substitution of the iodide by the hydroxyl at the 3-position and reductive amination of the ketone (calculated mass: 254.1387) (Figure 2.5). (see Figure 2.15, Schemes 2.12 and 2.13, section 2.4).



99 **4f** **100**

Calcd: 382.0510
Obsd: 382.0524

Calcd: 254.1387
Obsd: 254.1393

Figure 2.15.
Possible products indicated by via HRMS of the reaction in acidic conditions.

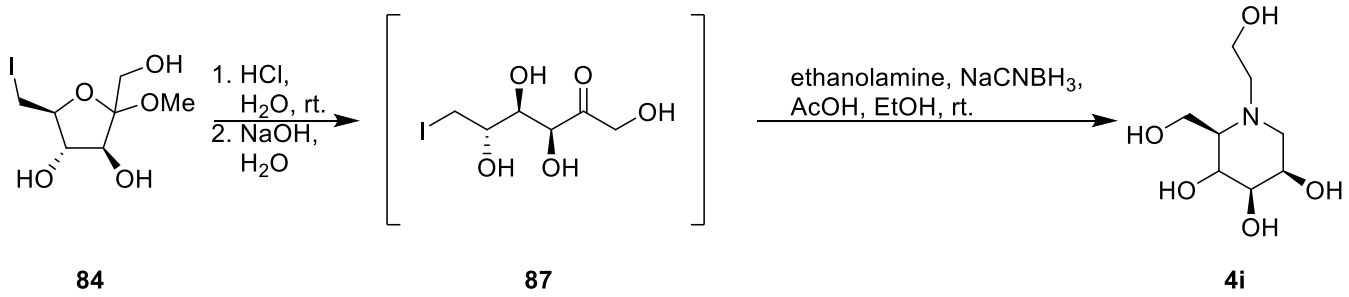
To this end, following the addition of excess amine to raise the pH and remove the proposed aminoglycitol, the reaction was concentrated and purification undertaken using the established protocol (see section 2.3.1). In order to afford greater separation between the desired product and the remaining excess benzylaimine the chromatography protocol was modified to include a gradient of lower polarity PE:EtOAc (2:1→1:3, v:v) , prior to the use of the DCM: EtOH: MeOH: NH_3 (aq) gradient [195:2:2:1→65:14:14:7, v:v:v:v). With this purification protocol, the desired azasugar was purified using a single silica gel column followed by coevaporation with dilute HCl which afforded *N*-benzyl-DMJ as its HCl salt.

In summary, *N*-benzyl-DMJ **4f** was synthesised in a yield of 32% from methyl iodoglycoside **84** resulting in an overall yield for the 4 step synthesis of (**4f**) from D-fructose of 23%. This is an advancement on the published literature where synthesis of **4f** required additional steps to functionalised DMJ.[80] While mass corresponding to iodo aminoglycoside **99** was observed via HRMS of the acidic reaction mixture, consistent with the observations the synthesis of derivatives

4b-4e, only the desired azasugar was observed via TLC, HRMS, and ^{1}H NMR of the crude reaction products or during purification. It was envisioned that the furanose **100,** analogous to **94** (section 2.4.1) may have been observed in keeping with the structural similarity of the benzylamine to 2-phenylethylamine used for the synthesis of **4e** however, this was not isolated. Additionally, it is proposed that the reduction to form the amine **99** is not stereoselective and this may have also contributed to the modest yield of the desired DMJ derivative **4f.**

2.6. The Synthesis of N-hydroxyethyl-DMJ

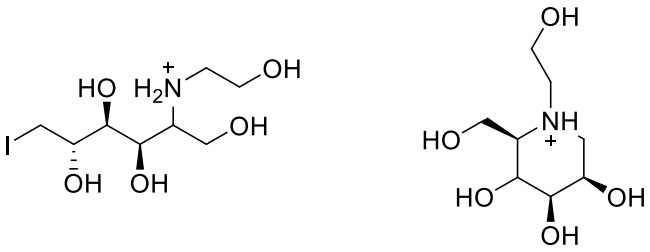
With the successful synthesis of the two aromatic derivatives **4e** and **4f** in hand, it was desired to further illustrate the chemoselectivity of this synthetic methodology through the synthesis of a derivative with a secondary reactive functional handle. In doing so, derivatives could be synthesised with reactive groups which could be used for subsequent reaction as desired, without the need for additional steps in the reactive methodology. It was proposed that 2-hydroxyethyl-DMJ **4i** could be synthesised from methyl iodoglycoside **84** using ethanolamine in the reductive amination step (Scheme 2.15). In addition to possessing a desirable functional handle, this derivative is also the C2 epimer of *N*-hydroxyethyl-DNJ, also known as migitol, which has been used in the clinical treatment of type II diabetes. As Reductive amination of **87** occurs through the substitution of a halide and a carbonyl functionality, while the alcohol substituents of the carbohydrate substrate are unreactive, it was envisioned that the use of an alcohol containing amine, rather than the corresponding halide and carboxy derivatives, would be less vulnerable to competing side reactions. Therefore, *N*-hydroxyethyl-DMJ **4i** was selected as the starting point for the synthesis of bifunctional derivatives.



84 **87** **4i**

Scheme 2.15. The synthesis of **4i** from **84**

Accordingly, iodoglycoside **87** was prepared from **84** and subjected to the previously established reductive amination conditions with ethanolamine (Scheme 2.7.1). Similar to the syntheses of previous DMJ derivatives **4a**, **4b**, **4c**, **4e** and **4f**, HRMS analysis showed masses matching the formation of the proposed aminoglycitol **101** (calculated mass: 336.0302, observed: 336.0312) and the azasugar **4i** (calculated mass: 208.1179, Obsd: 208.1184) (Figure 2.16). Excess amine was added to the reaction mixture to induce cyclisation of the proposed aminoglycitol intermediate **101**, The reaction was monitored by HRMS and TLC analysis until the aminoglycitol **101** was no longer observed, whereupon the reaction was concentrated and purified using the established protocol. Here, the increased polarity of both the excess ethanolamine and the derivative made purification more difficult than the previous derivatives. To this end, iterative silica gel chromatography was required to completely remove excess amine. In this initial synthesis, *N*-hydroxyethyl-DMJ (**4i**) was obtained as its HCl salt in a 31% yield over 2 steps from iodoglycoside **84**.



101 **4i**

Calcd.: 336.0302
Obsd.: 336.0312

Calcd.: 208.1179
Obsd.: 208.1184

Figure 2.16.
Possible products indicated by via HRMS of the reaction in acidic conditions

In order to ease purification, it was sought to minimise the amine used for the acid catalysed reductive amination. Reducing the amine concentration to 1.2 equivalents afforded a comparable rate of conversion of **87** to the initial reductive amination, and this was observed via TLC. As no corresponding product was isolated during purification, it was hoped that this may be an artefact of HRMS and not an actual representation of the reaction mixture. To this end, workup and purification of the reaction proceeded as per the initial attempts at synthesis.

In spite of the reduction in excess ethanolamine used in this reaction, purification of **4i** in good yields remained challenging, with total separation of **4i** and ethanolamine difficult. To avoid further chromatography, coevaporation with water under reduced pressure (9 mbar) at 70 ^{o}C was attempted to remove any remaining amine. As no known azeotropes for ethanolamine are reported in the literature, water was used to prevent degradation of the "dry" DMJ derivative **4i**. However, after iterative coevaporation cycles, excess ethanolamine was still observed in the ^{1}H NMR spectrum. Ultimately, purification was achieved through the use of silica gel column

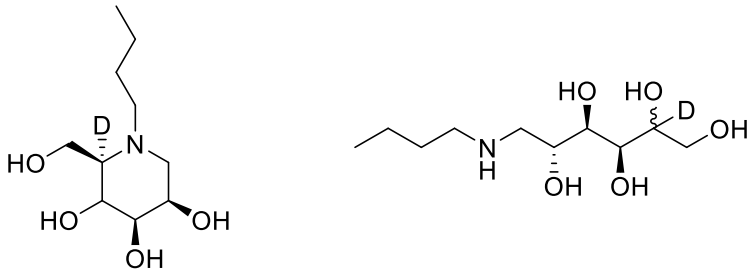
chromatography, using a constant eluent [(DCM:MeOH:EtOH:NH_3) (28 % w/w) (155:18:18:9) (v:v:v:v)] which was less polar than the amine eluted in the previous gradient chromatography [(DCM:MeOH:EtOH:NH_3) (35:6:6:3) (v:v:v:v)] , until elution of ethanolamine was observed to cease via TLC. Although the desired azasugar N-hydroxyethyl-DMJ (**4i**) was purified in a 39% yield, a further 10% yield of product (as determined via integration of ^{1}H NMR) could not be separated from ethanolamine despite numerous attempts.

N-hydroxyethyl-DMJ **4i** was synthesised from methyl glycoside **84** in a yield of 39% over two steps. Thus, the total yield of the synthesis of **4i** from D-fructose **85** was 28% over 4 steps. This is an increase over currently published synthesis with a 11% yield over 5 steps which required the functionalisation of DMJ prepared through a semi-synthetic methodology.[77] While a greater total yield may be achieved functionalisation of DMJ **4a**, with a reported synthesis using 2-benzyloxyethanal and palladium catalysed reduction obtaining **4i** from DMJ in an 85% yield,[77] the direct incorporation of the functionality during reductive amination used here creates a precedent for the efficient synthesis of bifunctional DMJ derivatives. It is envisioned that further optimisation of the general synthesis of DMJ derivatives should afford a methodology which also exceeds the total yield achieved from functionalisation of DMJ **4a**.

2.7. The Synthesis of *N*-duetero-*N*-Butyl-DMJ

In order to gain further understanding into the reductive amination reaction, synthesis of the deutero analogue of *N*-butyl-DMJ (**4c**) was carried out using sodium cyanoborodeuteride as the reducing agent. The butyl derivative **4c** was chosen due to its easy purification protocol. Moreover, during the preparation of **4c**, a side product **94b** was repeatedly isolated, and it was envisioned that the corresponding deutero side product may provide insight towards the mechanism of its formation.

Accordingly, iodoglycoside **87** was prepared from methyl glycoside **84** using the protocol previously described, and subject to reductive amination with butylamine, sodium cyanoborodeuteride and catalytic acetic acid. The procedure, workup and purification methodologies were the same as those used for **4b.** The desired deuterated DMJ-derivative **4m** was isolated in a 37% yield (Figure 2.17). In addition to **4m**, the deuterated analogue (**103**) of 6-aminoglycitol **90**, was isolated in an 3% yield (Figure 2.17). During the synthesis of **4c** the formation of **90** was only observed under basic conditions, as also previously reported in the synthesis of *N*-butyl-DNJ from a glucose ketoaldyhyde.[44] However, the formation of the deuterated analogue **103** in slightly acidic conditions (pH= 6) suggested that generation of this by-product is not pH dependent. (see section 2.3).



4m **102**

Figure 2.17.
Products resulting from the reductive amination of **87** with sodium cyanoborduteride and *N*-butylamine

Comparison of the ^{1}H, ^{13}C and HSQC NMR spectra of the deutero-analogues **4m** and **105** to that of **4c** and **90**, respectively, allowed for the site of reduction to be determined. For **4m**, NMR spectra indicated that the deuterium was bonded to C5, which confirmed that reduction had occurred at the electrophilic carbon of the imine. The C5 carbon resonance possessed a similar chemical shift as the same carbon position in **4c**, however no proton correlation was observed in the HSQC spectrum of **4m**. The assignment of the deuterated position was confirmed by comparison of the ^{1}H NMR spectra of **4c** and **4m**, where no proton resonance was observed for H5 in **4m**. Furthermore, the triplet observed in the ^{1}H NMR spectrum of **4c** was now observed as a doublet in the spectrum of **4m**, which reflected the loss of coupling of between H4 and H5. In **105**, no correlation was observed in the HSQC for C5, which has a chemical shift of δ 64.9 ppm indicating its connectivity to an alcohol. This suggested that deuteration had occurred through reduction of the ketone at C5. Comparison of the ^{1}H NMR spectra of **103** and **94b** confirmed deuteration at this position as the proton shift for H5 was absent. Moreover, a change in the multiplicities of H4 and H6a from a doublet and doublet of doublets, respectively, in the ^{1}H NMR

of **94b** to a singlet and a doublet in the ^{1}H NMR of the deuterated product **105** reflected the absence of coupling between H4 and H5 and H6a and H5. To this end, through the use of a deuterated reducing agent it was determined that reduction during the formation of DMJ derivatives occurred as anticipated at the electrophilic imine. Additionally, it was confirmed that the formation of the acyclic aminoglycitol **94b** likely occurred through reduction of the ketone.

3. Conclusions and Future prospects

3.1. Conclusions

In this Masters Project, the azasugar DMJ (**4a**) was synthesised from the inexpensive carbohydrate D-fructose in a total yield of 65% using highly efficient four step, three pot synthetic methodology. This methodology surpasses recorded total syntheses of this azasugar in both efficiency and overall yield. Key to this synthesis was the use of an anomeric methoxy group which afforded selectivity to the iodination of the 6-position via Appel reaction, and could be subsequently hydrolysed to give the keto-iodo-glycoside **87** amenable to reductive amination. This allowed for the synthesis of the desired azasugar without the need for an extensive protecting group scheme.

This synthetic methodology was also used for the generation of DMJ derivatives through the incorporation of functionalised amines in the reductive amination step. Synthesis of these derivatives was achieved without the need for any additional reactive steps with alterations to the workup of **87** and the workup of DMJ derivatives being the only significant modification to the synthetic procedure. The incorporation of a variety of amines to synthesise the corresponding azasugars gave derivatives **4b**, **4c**, **4e**, **4f**, **4i** and **4m** in modest to good yields, often surpassing the efficiency of the reported synthetic procedure. The synthesis of DMJ derivatives with differing functionalities, including the novel derivative **4e** has begun to illustrate the versatility of this synthetic strategy towards the synthesis of a diverse library of DMJ derivatives. Moreover, the synthesis of **4i** creates a precedent for the synthesis of derivatives containing secondary reactive functionalities without significant change to the reactive procedure.

Lower yield was observed for reductive amination with substituted amines compared to the synthesis of the parent azasugar DMJ. Masses corresponding to 6-iodo-2-amino-glycitols with the relevant functionalised amine **90**, **92**, **96**, **99** and **101** were observed via HRMS of the acidic reaction mixture for each derivative suggesting reduction was occurring before cyclisation. As observed in the reductive amination of D-fructose, reduction of the imine prior to cyclisation is not stereoselective and instead results in epimerisation at C5. It is envisioned that the formation of these proposed acyclic derivatives may be responsible for the lower yields for the synthesis of derivatives. Moreover, this would also explain epimerisation recorded in the formation of DMJ derivatives not seen in the corresponding DNJ derivatives.[44] The formation of 6-amino-glycitols such as **94b**, the side product of the butyl derivative **4c**, or furanose side products such as **97**, isolated as a side product of the synthesis of 2-phenylethyl derivative **4e**, are also envisioned to be possible avenues of side product formation in the synthesis of DMJ derivatives.

3.2. Future prospects.

3.2.1. Improving the synthesis of the reductive amination step.

The primary future prospect for this research is the improvement of the reductive amination yield for the synthesis of derivatives. While the synthetic methodology used to synthesise DMJ derivatives **4b**, **4c**, **4e**, **4f**, **4i** and **4m** eclipses previous syntheses in overall efficiency, published reductive aminations in the synthesis of azasugar derivatives imply that a significantly higher yield could be obtained.[44,87]

Important to optimisation of this synthetic strategy is the isolation of the proposed 6-iodo-2-amino-glycitol intermediate or a product derived thereof. With the time constraints of this project exacerbated by the difficulty in purification for earlier derivatives, priority was given to the synthesis of new derivatives to illustrate the versatility of this synthetic methodology. Compound **97** is the only side product isolated which could be derived from the proposed 6-iodo-2-amino glycitol, in this case **96**, however, more substantial evidence is required to confirm this. While a protected *galacto* analogue has previously been synthesised and isolated,[101] to our knowledge no examples of unprotected 6-iodo-2-amino-glycitols have been reported in the literature. It may be that the unprotected substrates are not stable enough to be isolated and cyclise or degrade during concentration. It is envisioned that reductive amination with a sterically hindered secondary amine may prevent cyclisation of the 6-iodo-2-amino-glycitol, however, care must be taken to prevent substitution of the iodine as observed with the formation of **94b**.

3.2.2. Expanding the library of DMJ derivatives

Several derivatives have been selected as initial targets for the creation of an expanded library of DMJ derivatives (Figure 2.18). Two bifunctional derivatives, *N*-carboxypentyl-DMJ (**4g**) and *N*-chloroethyl-DMJ (**4h**), were attempted to be synthesised during this Masters project. Masses representative of both compounds and their corresponding proposed amino-glycitol derivatives were observed via HRMS, however successful workup and purification of these compounds proved difficult and was ultimately not able to be achieved in the time constraints of this project. Expanding on the synthesis of *N*-Me-DMJ (**4a**) and *N*-Bu-DMJ (**4c**), the preparation of derivatives incorporating longer alkyl chains, for example, *N*-nonyl-DMJ (**4d**), are also of interest. Here, the longer alkyl chain affords interaction with membrane proteins which can be exploited for anti-viral activity. The corresponding DNJ and DGJ derivatives have shown activity against hepatitis B virus and hepatitis C virus. Finally, the novel azido derivative (**4l**) and propargyl derivative (**4n**)are of interest as derivatives with bioorthogonal handles that can be chemoselectively functionalised by click chemistry.[82] As reduction of azides and alkynes with sodium borohydride requires the presence of either a metal catalyst,[102] chemoselective reduction of the imine with the milder and more selective sodium cyanoborohydride should be achievable without reducing the desired functional handle.

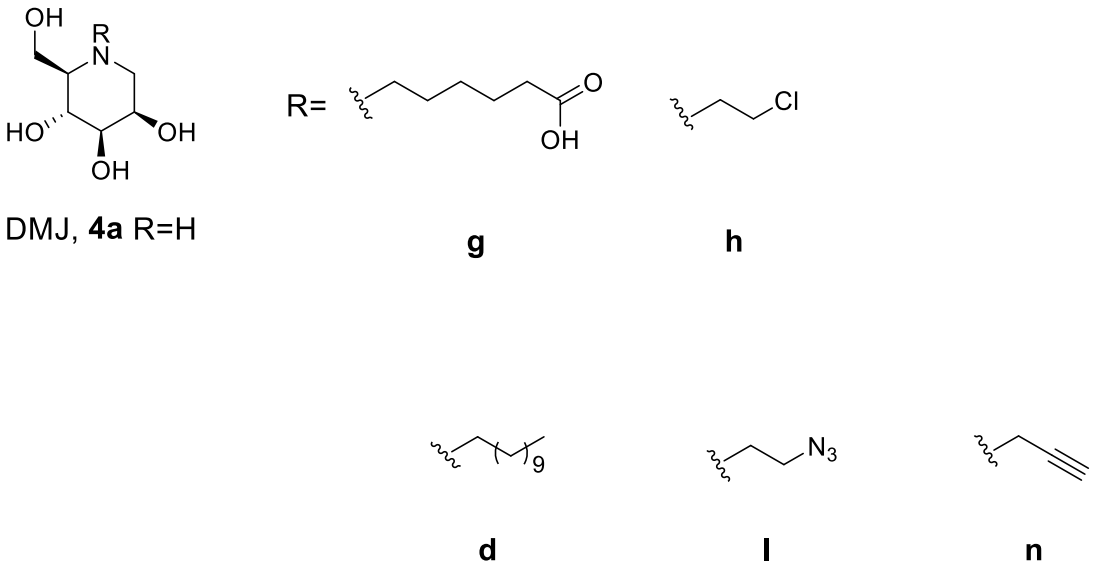


Figure 2.18.
Proposed future targets for the creation of library of DMJ derivatives

It is also anticipated that the synthetic strategy may be used for the synthesis of azasugars with different stereochemistries. To this end, deoxyxylojirrimycin (DXJ, **106**) has already been synthesised during preliminary work, although optimisation is still required. The most interesting target for this avenue of development is the synthesis of DNJ (**1a**), which mimics the glucose stereochemistry and therefore is foreseen to have numerous bioactive targets.

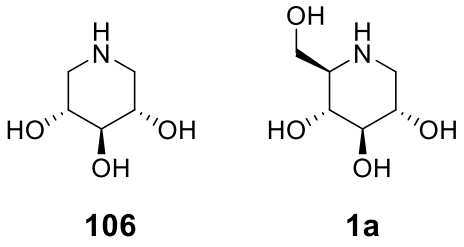


Figure 2.19.
DXJ (**106**) and DNJ (**1a**), future targets for efficient azasugar synthesis.

In addition to monosaccharide mimicking azasugars, recent discovery of multivalent activity against hydrolase enzymes has led to interest in the synthesis multivalent DMJ scaffolds. While

bifunctional derivatives such as **4n** or **4l** possess biorthogonal handles, which could be used to attach the azasugar to a desired scaffold or linker molecule, it is anticipated that the use of a scaffold with multiple terminal amines, could be used in a reductive amination with iodoglycoside **87** to synthesise a multivalent azasugar directly.

4. Experimental

4.1. General Experimental

Unless otherwise stated, all reactions were performed under an open to air atmosphere. Prior to use, THF (Chemsolute) was distilled from Na wire and benzopheneone. Water was distilled using a RiOs Millipore system prior to use. D-Fructose (Riedel –De Haen Ag Seelze-Hannover), H_2SO_4 (Panreac), MeOH, ETOH, DCM, Pet. Et and EtOAc (Fischer), Toluene (Roth), Ammomnia (28% in H_2O) (Panreac), PPh_3 (Acros), Imidazole (Apollo Scientific), Iodine (resublimed) (Scichem), HCl (Chemsolute), $NaCNBH_3$ (Chem-Impex), $NaCNBD_3$ (Cambridge Isotope Laboratory), Acetic acid (Panreac), methylamine (40% w/w) (Riedel- De Haen- Ag Seelze Hanover), methylammonium hydrochloride (BDH), triethylamine (Panreac), 2-phenylethylamine (Hopkin and Williams), *N*-butylamine (Ajax), benzylamine (Aldrich), ethanolamine (M&B), Dowex H^+ 50 WX8 (Supelco), $CDCl_3$ (Aldrich) and D_2O were all used as received.

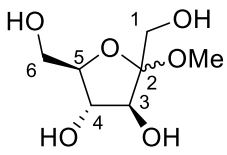
All solvents were removed *via* evaporation at reduced pressure. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets (0.30 mm; Polygram SIL G/UV254) with detection dipping in a solution of 5% H_2SO_4 in EtOH followed by charring at ~150^oC or coating in a solution of 5% ninhydrin in EtOH followed by heating. Detection was also afforded using UV light (365 nm). Column Chromatography was performed using Pure Science silica gel (40-60 µm), HP-20 (Supelco) or Dowex H^+ (Supelco) ion exchange beads where noted. High resolution mass spectra were recorded on a Waters Q-TOF premier^{TM} Tandem Mass spectrometer using positive electro-spray ionisation. Optical Rotations were recorded on a Perkin-Elmer 241 polarimeter at 589 (sodium D-line). Infrared spectra were recorded as thin films using a Bruker FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are

reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 20 ^{o}C in D_2O unless otherwise stated using a Varian INOVA operating at 500 MHz. Chemical Shifts are given in ppm (δ) relative to residual solvent peak. NMR assignments were made using ^{1}H and ^{13}C 1D and COSY HSQC and HMBC 2D experiments.

Purification:

For compounds **4b**, **4c**, **4e**, **4f**, **4i**, and **4m**, a general purification procedure was used involving coevaporation with a mixture of methanol: acetic acid: toluene (1:4:6) followed by silica gel column chromatography, with alterations and additional purification as stated.

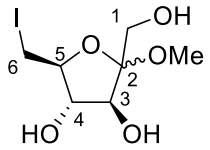
Methyl-D-fructoside (85)



To a solution of D-fructose (**84**) (1.01 g, 5.59 mmol) in MeOH (200 mL) conc. H_2SO_4 (1 mL) was added and was stirred at room temperature. After 30 minutes, ammonia (5 mL, 28% v/v water) was added dropwise until pH= 7. The resulting white precipitate was removed via filtration and the filtrate was concentrated and purified using silica gel column chromatography (PE:EtOAc 1:3 →0:1, EtOAc:MeOH 99:1, v:v) to give the title compound as a clear oil (1.0350g, 5.33 mmol, 95.0% (α furanose:β furanose:β pyranose, 38:57:5).

$[\alpha]_D^{22.9}$ = +4 (MeOH, c = 10 mg/mL); IR (film, MeOH): 3296, 2946, 2834, 1449, 1016.9 cm^{-1}; ^1H NMR (500MHz, D_2O): δ 4.14 (d, $J_{3,4}$ = 8.26 Hz, 1H, H-3β), 4.07 (d, $J_{3,4}$ = 2.93 Hz, 1H, H-3α), 4.02 (t, $J_{3,4}$ = $J_{4,5}$ = 7.07 Hz, 1H, H-4β), 3.93-3.90 (m, 2H, H-4α and 5α), 3.83 (dd, $J_{4,5}$ = 6.86, $J_{5,6a}$ = 2.74 Hz, 1H, H-5β), 3.81-3.78 (m, 1H, H-6αa), 3.78-3.76 (m, 1H, H-6βa), 3.76 (d, $J_{1a,1b}$ = 12.50 Hz, 1H, H-1αa), 3.70 (d, $J_{1a,1b}$ = 12.57 Hz, 1H, H-1βa), 3.67-3.64 (m, 1H, H-6αb), 3.64 (d, $J_{1a,1b}$ = 12.12 Hz, 1H, H-1αb), 3.64-3.63 (m, 1H, H-6βb), 3.61 (d, $J_{1a,1b}$ = 12.12 Hz, 1H, H-1βb), 3.32 (s, 3H, OMe), 3.29 (s, 3H, OMe), ^{13}C NMR (125 MHz D_2O): δ 108.2 (C-2α), 103.8 (C-2β), 83.3 (C-5α), 81.1 (C-5β), 80.0 (C-3α), 77.3 (C-4α), 76.0 (C-3β), 75.0 (C-4β), 62.1 (C-6β), 61.3 (C-6α), 59.6 (C-1β), 57.6 (C-1α), 48.8 (OMe_β), 48.8 (OMe_α) HRMS (ESI) calcd for $[C_7H_{14}NaO_6]^+$ *m/z* 217.0679, found 217.0679.

Methyl-6-deoxy,6-iodo-D-fructoside. (84)



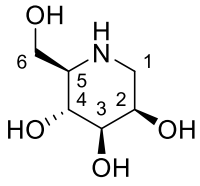
methyl-D-fructoside (**85**) (1.7557 g, 9.0 mmol) was dissolved in Tetrahydrafuran (THF) (45 mL) and stirred at room temperature, triphenylphosphine (3.557 g, 13.6 mmol) and imidazole (1.2300 g, 18.1 mmol) were added and stirred under reflux. Iodine (3.421 g, 13.6 mmol) was dissolved in THF (22.5 mL) and added dropwise to the reaction mixture at a rate of 1 drop every 5 seconds. As the concentration of iodine in the reaction mixture increased the reaction mixture became golden yellow and then light brown. When the reaction was light brown the dropwise addition of iodine ceased, followed by the addition of triphenylphosphine (1.1783 g 4.4 mmol) turning the reaction mixture colourless. Addition of the iodine solution was then resumed until the iodine was fully consumed, and the resulting golden yellow solution was stirred under reflux for an hour. After the reaction cooled, MeOH (30 mL) was added turning reaction mixture transparent, before it was concentrated under reduced pressure. Purification by silica gel flash column chromatography (PE: EtOAC, 4:1 to 1:2), followed by reverse phase HP^{20} column chromatography (water: MeOH 1:0 to 4:1), gave the named product **84** as a colourless oil, (2.23 g, 7.3 mmol, 80 %)

$[\alpha]_D^{22.9}$ = 47 (MeOH, c = 10 mg/mL); IR (film, MeOH): 3362, 2943, 1346, 1060, 1014 cm^{-1}

^1H NMR (500 MHz D_2O): δ 4.17 (d, $J_{3,4}$ = 8.14 Hz, 1H, H-3β), 4.13 (d, $J_{3,4}$ = 14.99 Hz, 1H, H-3α), 4.04 (t, $J_{3,4} = J_{4,5}$ = 7.48 Hz, 1H, H-4β), 3.91-3.86 (m,2H, H-4α and 5α), 3.83 (m, 1H, H-5β), 3.76 (d, $J_{1\alpha a,\ 1\alpha b}$ = 12.72 Hz, 1H, H-1αa), 3.69 (d, $J_{1\beta a,\ 1\beta b}$ = 12.17 Hz, 1H, H-1βa), 3.63 (d, $J_{1\alpha b}$, $1_{\alpha a}$ = 12.66 Hz,

1H, H-$1_{\alpha b}$), 3.63 (d, *J* = 12.39Hz, 1H, H-$1_{\beta b}$), 3. 50 (d, $J_{5\beta,6\beta a}$ = 4.64 Hz, 1H, H-$6_{\beta a}$), 3.46 (m, 1H, H-$6_{\alpha a}$), 3.39 (m, 1H, H$6_{\beta b}$), 3.36-3.33 (m, 1H, H-$6_{\alpha b}$), 3 .33 (s, 3H, OMe_{β}), 3.29 (s, 3H, OMe_{α}), ^{13}C NMR (125 MHz, D_2O): δ 108.2 (C-2_{α}), 103.9 (C-2_{β}), 81.8 (C-5_{α}), 80. 9 (C-4_{α}), 80.4 (C-3_{α}), 80.1 (C-5_{β}), 78.8 (C-4_{β}), 77.1 (C-3_{β}), 59.5 (C-1_{β}), 57.8 (C-1_{α}), 49.4 (OMe_{β}), 48.3 (OMe_{α}), 7.1 (C-6_{β}), 5.4 (C-6_{α}); HRMS (ESI) calcd for $C_7H_{13}IO_5$ *m/z* 303.9808, found 303.9820.

1-Deoxymannojirimicin (4a)



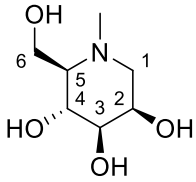
Methyl-6-deoxy,6-Iodo-D-fructoside **84** (0.0631 g 0.2 mmol) was dissolved in H_2SO_4 (2.07 mL 0.09 M) in water. The reaction was stirred at room temperature for 1 hr after which complete disappearance of the starting material was observed by TLC analysis. Ammonia solution (0.179 mL, 28% v/v in H_2O) was then added followed by $NaCNBH_3$ (0.0521 g 0.8 mmol). The reaction was then stirred for 3 days at rt. before being concentrated. The product was then isolated using Dowex H^+ ion exchange chromatography (ammonia in water 3% to 28% v/v) followed by purification using silica gel flash chromatography DCM:MeOH:EtOH:NH_3 aq (28%) 15:2:2:1 to 5:2:2:1, v:v:v:v) , to give the named product **4a** as an clear oil. (0.0290 g, 0. 17 mmol, 85 %). The spectral data matched that previously reported. [57]

$[\alpha]_D^{22.9}$ = 16 (MeOH, c = 10 mg/mL); IR (film, MeOH): 3363, 2958, 2807, 1594, 1399 cm^{-1} ^{1}H NMR (500MHz D_2O):δ4.25 (brs, 1H, H-2) 4.00 (dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 3.3 Hz, 1H, H- *H-6a*), 3.89-3.82 (d, $J_{6a,6b}$ = 12.6 Hz, 1H, H-6_b), 3.83 (t, $J_{3,4} = J_{4,5}$ =9.7 Hz, 1H, H-4), 3.69 (dd, $J_{3,4}$ = 9.8 Hz, $J_{3,2}$ = 2.84 Hz, 1H, H-3), 3.41 (dd $J_{1a,1b}$ = 13.4 Hz, $J_{1a,2}$= 2.3 Hz, 1H, H-1_a), 3.25 (d, $J_{1a,1b}$ = 13.6 Hz,

1H, H-1_b), 3.18-3.14 (m, 1H, H-5), ^{13}C NMR (125MHz D_2O), δ 72.3 (C-2), 65.8 (C-4), 65.6 (C-3), 60.3 (C-5), 58.0 (C-6), 47.4 (C-1)

HRMS (ESI) calcd for $[C_6H_{14}NO_4]^+$ *m/z* 164.0917, found 164.0920.

***N*-Methyl-1-Deoxymannojirimicin.**



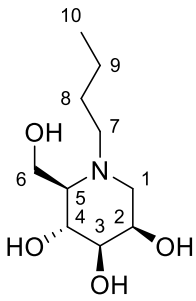
4b

Methyl-6-deoxy-6-iodo-D-fructoside (0.110 g, 4.6 mmol) was dissolved in aqueous HCl (1.5 M, 4.34 mL). The reaction was stirred at rt for 1 hr, after which complete conversion of the starting material to a lower running spot was observed by TLC analysis. The reaction was neutralised by the dropwise addition of NaOH in water (1.5 M), before it was concentrated in *vaccuo*, and the residue taken up in EtOH. The insoluble salt was removed from the solution via decantation and the solution concentrated to a clear oil. The oil was then redissolved in EtOH (1.77 mL) to which methylammonium hydrochloride (0.0394 g, 5.3 mmol) followed by sodium cyanoborohydride (0.089 g, 14 mmol) were added. The reaction was then stirred for 18 hr at rt, after which the solution was subjected to methylamine (0.90 mL, 10 mmol, 40% w/w) and left to stir for a 16 hr when the reaction was deemed to be complete by TLC and HRMS analysis. The solution was concentrated and coevaporated with methanol:acetic acid:toluene (2 mL, 1:4:6, v:v:v). The solution was then iteratively coevaporated with water (2 mL) and triethylamine (1 mL). The residue was then purified using silica gel flash chromatography (100% DCM- DCM: EtOH: MeOH: NH_3 aq. (28% w/w), 5:2:2:1, v:v:v:v), to give *N*-methyl-1-deoxymannojirimicin (**4b**) as a clear oil, (0.041 g, 0.23 mmol, 65%). $[\alpha]_D^{22.9}$ = -3° (MeOH, c = 1); IR (film, MeOH): 3285, 3258, 2838, 2804, 1399 cm^{-1}

^{1}H NMR (500 MHz D_2O): δ 4.17 (m, 1H, H-2), 4.04 (d, $J_{6a,6b}$ = 12.9 Hz, 1H, H-6a), 3.99 (dd, $J_{6a,6b}$ =

13.2, $J_{6b,5}$ = 2.5 Hz, 1H, $H6_b$), 3.93 (t, $J_{4,3} = J_{4,5}$ = 10.0 Hz, 1H, H4), 3.79 (dd, $J_{3,4}$ = 9.74, $J_{3,2}$ = 3.3 Hz, 1H, H-3), 3.41 (d, $J_{1a,1b,}$= 12.6 Hz, 1H, H-1_a), 3.24 (d, $J_{1a,1b}$ = 12.6 Hz, 1H, H-1_b), 3.18-3.14 (m, 1H, H-2), 2.84 (m, 4H, H-5 and NMe); ^{13}C NMR (125MHz, D_2O) δ 72.8 (C-3), 67.8 (C-5), 66.0 (C-2), 65.1 (C-4), 58.65 (C-1), 54.8 (C-6) 40.0 (NMe); HRMS (ESI) calcd for $[C_7H_{16}NO_4]^+$ *m/z* 178.1074, found 178.1077

***N*-butyl-1-Deoxymannojirimicin (4c).**

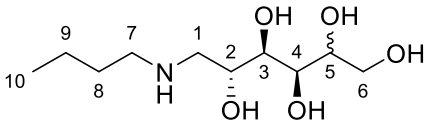


4c

Methyl-6-deoxy-6-iodo-D-fructoside (0.0683g, 0.22 mmol) was dissolved in aqueous HCl (2 M, 2.69 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.15 mL) to which *N*- butylamine (0.05 mL, 0.46 mmol) and AcOH (0.02 mL) followed by sodium cyanoborohydride (0.0564 g, 0.89 mmol) were added. The reaction was then stirred for 22 hrs at rt after which the solution was subject to *N*-butylamine (0.05 mL 0.46 mmol) and left to stir at rt for 18 hrs when the reaction was deemed to be complete by TLC and HRMS analysis. The reaction mixture was then concentrated under reduced pressure, before the residue was coevaporated with methanol: acetic acid: toluene (2 mL, 1:4:6 v:v:v). The residue was then purified using silica gel flash chromatography (100% DCM- DCM: EtOH: MeOH: NH_3 (28% w:w), 5:2:2:1, v:v:v:v), to give *N*-butyl-1-Deoxymannojirimicin as its HCl salt as

a clear oil. (0.0239g, 0.094 mmol, 42 %) $[\alpha]_D^{22.9}$ = -1° (MeOH, c = 0.1); IR (film, MeOH): 3277, 3197, 2920, 1656,1103, cm^{-1} ^{1}H NMR (500 MHz D_2O): δ 4.25 (m, 1H, H-2), 4.09 (dd, $J_{6a,6b}$ = 13.4 Hz, $J_{6a,5}$ = 1.80 Hz, 1H, H-6a), 3.99 (dd, $J_{6a,6b}$ = 13.1 Hz, $J_{6b,5}$ = 2.61 Hz, 1H, $H6_b$), 3.98 (t, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, 1H, H4), 3.69 (dd, $J_{3,4}$ = 9.7, $J_{3,2}$ = 3.3 Hz, 1H, H-3), 3.50 (dd, $J_{1a,1b,}$= 13.2 Hz, $J_{1a,1b,}$=3.1 Hz, 1H, H-1_a), 3.43 (d, $J_{1a,1b}$ = 13.3 Hz, 1H, H-1_b), 3.29 (t, $J_{7,8}$ = 8.6 Hz, 2H, H-7), 3.13 (app. d, $J_{4,5,}$=10.4 Hz, 1H, H-5), 1.73-1.64 (m, 2H, H-8), 1.38 (qd, $J_{9,10}$ =7.45 Hz, $J_{8,9}$ = 1.55 Hz, 2H, H-9), 0.93 (t, $J_{9,10}$ = 7.4 Hz, 3H, H-10); ^{13}C NMR (125MHz, D_2O) δ 72.0 (C-3), 65.5 (C-5), 65.4 (C-2), 65.0 (C-4), 54.7 (C-1), 54.1 (C-6) 52.8 (C-7), 23.7 (C-8), 19.2 (C-9), 12.7 (C-10); HRMS (ESI) calcd for $[C_7H_{16}NO_4]^+$ *m/z* 219.1465, found 219.1467

6-deoxy-.6-butylamino-D-mannitol (94b-1) and 6-deoxy-.6-butylamino-L-gulitol (94b-2).

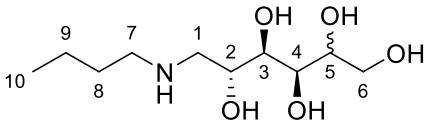


6-deoxy-.6-butylamino-D-mannitol (**94b-1**) and 6-deoxy-.6-butylamino-L-gulitol were isolated as side products during a strategy initially used to prepare **4c**. The protocol used was as follows: Methyl-6-deoxy-6-iodo-D-fructoside (0.1294g, 0.42 mmol) was dissolved in aqueous HCl (2 M, 5.1 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (2.12 mL) to which *N*- butylamine (0.082 mL, 0.85 mmol) and sodium cyanoborohydride (0.1117g, 1.7 mmol) were added. The mixture was stirred at rt for 18 hr before the solvent was removed under reduced

pressure. The residue was then coevaporated with a mixture of methanol: acetic acid: toluene (2x 2 mL, 1:4:6 v:v:v) and purified using silica gel flash chromatography (100% DCM- DCM: EtOH: MeOH: ammonia (25% w:w), 5:2:2:1, v:v:v:v) to give **4c** as a clear oil (g, mmol, %) and a diastereomeric mixture of **94b-1** and **94b-2** also a clear oil (approximate yield 0.0238g, mmol, 23.6%).

$[\alpha]_D^{22.9}$ = -1° (MeOH, c = 1); IR (film, MeOH): 3314, 2943, 2381, 1448, 1021 cm^{-1} ^1H NMR (500 MHz D_2O): δ 4.01 (m, 1H, H-2), 3.85 (d, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 2.6 Hz, 1H, H-6a), 3.79 (d, $J_{2,3}$ = 8.5 Hz, 1H, H3), 3.76 (d, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 9.0 Hz, 1H, H-4), 3.67 (dd, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6b}$ = 5.7 Hz, 1H, $H6_b$), 3.43 (dd, $J_{1a,1b}$= 12.9 Hz, $J_{1a,2}$ = 2.6 Hz, 1H, H-1_a), 3.12 (d, $J_{1a,1b}$ = 12.9 Hz, $J_{1b,2}$ = 5.7 Hz, 1H, H-1_b), 3.18-3.14 (m, 1H, H-7), 1.69 (app q, $J_{7,8}$ = 7.6 Hz, 2H, H-8), 1.38 (app sex, $J_{9,10}$ =7.45 Hz, 2H, H-9), (t, $J_{9,10}$ = 7.4 Hz, 3H, H-10); ^{13}C NMR (125MHz, D_2O) δ 70.9 (C-3), 70.6 (C-5), 69.3 (C-4), 66.5 (C-2), 63,1 (C-1), 50.4 (C-6), 47.6 (C-7), 23.3 (C-8), 19.1 (C-9), 12.7 (C-10); HRMS (ESI) calcd for $[C_{10}H_{22}NO_5H^+]$ *m/z* 238.1649, found 238.1649

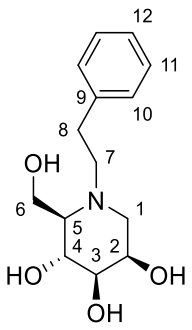
Per acetylated 6-deoxy-.6-butylamino-D-mannitol (94b-1) and 6-deoxy-.6-butylamino-L-gulitol (94b-2).



6-deoxy-.6-butylamino-D-mannitol (**94b-1**) and 6-deoxy-.6-butylamino-L-gulitol were (0.079 g, 0.33 mmol) were subject to a mixture of acetic anhydride (1.11 mL) and pyridine (0.55 mL) and left at rt for 3 days before the solvent was removed under reduced pressure and the resulting residue purified using silica gel flash chromatography (PE:EtOAC 1:0 to 1:4, v:v) to give per acetylated **94b-1** in a 2:1 ratio as a clear oil (yield not obtained)

^{1}H NMR (500 MHz D_2O): δ 5.44 (d, 1H, H-4) 3.85 (d, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 2.6 Hz, 1H, H-6a) 3.67 (dd, $J_{6a,6b}$ = 11.7, $J_{5,6b}$ = 5.7 Hz, 1H, H-6_b) 3.79 (d, $J_{2,3}$ = 8.5 Hz, 1H, H-3) 3.76 (d, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 9.0 Hz, 1H, H-4) 3.43 (dd, $J_{1a,1b}$= 12.9 Hz, $J_{1a,2}$ = 2.6 Hz, 1H, H-1_a) 3.12 (d, $J_{1a,1b}$ = 12.9 Hz, $J_{1b,2}$ = 5.7 Hz, 1H, H-1_b) 3.18-3.14 (m, 1H, H-7) 1.69 (app q, $J_{7,8}$ = 7.6 Hz, 2H, H-8), 1.38 (app sex, $J_{9,10}$ =7.45 Hz, 2H, H-9), (t, $J_{9,10}$ = 7.4 Hz, 3H, H-10); ^{13}C NMR (125MHz D_2O) δ 70.9 (C-3), 70.6 (C-5), 69.3 (C-4), 66.5 (C-2), 63,1 (C-1), 50.4 (C-6) 47.6 (C-7), 23.3 (C-8), 19.1 (C-9), 12.7 (C-10); HRMS (ESI) calcd for $[C_7H_{16}NO_4]^+$ *m/z* 178.1074, found 178.1077

_N_-[2-phenylethyl]-1-Deoxymannojirimicin (4e)



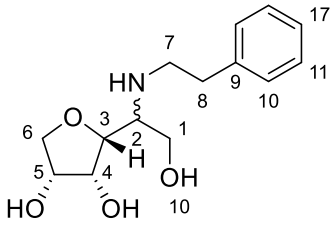
4e

Methyl-6-deoxy-6-iodo-D-fructoside (0.0885 g, 0.29 mmol) was dissolved in aqueous HCl (2 M, 3.49 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.45 mL) to which 2-phenylethylamine (0.07mL, 0.58 mmol) and AcOH (0.02 mL) followed by sodium cyanoborohydride (0.0732 g, 0.11 mmol) were added. The reaction was then stirred for 25 hrs at rt before being subject to additional 2-phenylethylamine (0.07mL, 0.58 mmol). The reaction was stirred at rt for a further 15 hrs when the proposed intermediate **93** was no longer observed by TLC and HRMS analysis. The solution was then concentrated and the

resulting residue was then coevaporated with methanol: acetic acid: toluene (2 mL, 1:4:6 v:v:v). The residue was then purified using silica gel flash chromatography (DCM: EtOH: MeOH: NH_3 (28% w:w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)), followed by co-evaporation with dilute HCl (0.12 M, 2 mL) to give *N*-[2-phenylethyl]-1-Deoxymannojirimicin (**4e**) as it's HCl salt as a clear oil. (0.042g, 0.14 mmol, 47%)

$[\alpha]_D^{22.9}$ = -6° (MeOH, c = 0.1); IR (film, MeOH): cm^{-1}: 3347, 3153, 3048, 2969, 1737, 1407, 1080 cm^{-1} ^{1}H NMR (500 MHz D_2O): δ 7.42 (d, $J_{10,11}$ = 7.5 Hz, 2H, H-10), 7.35 (d, $J_{6a,6b}$ = 6.5 Hz, 3H, H-10 and 12), 4.27 (brs, 1H, H-2), 4.10 (d, $J_{6a,6b}$ = 13.6 Hz, 1H, H-6a) 4.04 (d, $J_{6a,6b}$ = 13.6, 1H, $H6_b$), 3.98 (t, $J_{4,3} = J_{4,5}$ = 9.9 Hz, 1H, H4) 3.73 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 3.65 (d, $J_{1a,1b}$,= 13.1 Hz, 1H, $H\text{-}1_a$), 3.54 (m, 2H, H-7) 3.52 (d, $J_{1a,1b}$ = 12.9 Hz, 1H, $H\text{-}1_b$) 3.25 (d, $J_{4,5}$ = 10.5 Hz, 1H, H-5), 3.17-3.07 (m, 2H, H-8); ^{13}C NMR (125MHz, D_2O) δ 136.9 (C-10), 129.1 (C-12), 129.0 (C-11), 128.8 (C-9), 72.01 (C-3), 65.7 (C-5), 65.4 (C-2), 65.1 (C-4), 55.0 (C-1), 54.2 (C-6) 53.7 (C-7), 28.3 (C-8); HRMS (ESI) calcd for $[C_{14}H_{21}NO_4H^+]$).*m/z* 268.1543, found 268.1559

[2-phenylethylamino]-3,6-anhydro-2-deoxy-glycitol

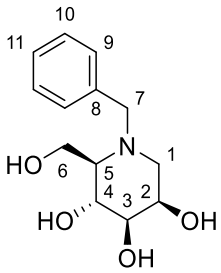


97

97 was originally isolated as a side product during a strategy used to initially prepare **4e**. The protocol used was as follows: Methyl 6-deoxy-6-iodo-D-fructoside (0.0527 g, 0.17 mmol) was dissolved in aqueous HCl (2 M, 2.08 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl

salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (0.86 mL) to which 2-phenylethylamine (0.04 mL, 0.35 mmol) and AcOH (0.015 mL) followed by sodium cyanoborohydride (0.0436 g, 0.69 mmol) were added. The reaction was then stirred for 21 hrs at rt before being subject to additional 2-phenylethylamine (0.04 mL, 0.35 mmol). The reaction was stirred at rt for a further 13 hrs when the proposed intermediate **93** was no longer observed by TLC and HRMS analysis. then the solvent removed under reduced pressure. The residue was then co-evaporated with methanol: acetic acid: toluene (2x 2 mL, 1:4:6 v:v:v) then purified using silica gel flash chromatography (DCM: EtOH: MeOH: ammonia (25% w/w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)) followed by Dowex H^+ column chromatography (1%-28% NH_3 (aq), v:v) to give **97** as a clear oil (not fully purified) ^{1}H NMR (500 MHz D_2O): δ 4.27 (m, $J_{4,3}$ =4.7 Hz, $J_{4,5}$ = 6.0 Hz, 1H, H-4), 4.16 (app q , $J_{5,4}$ = $J_{5,6a}$ = 4.6 Hz, 1H, H-5), 4.01 (dd, $J_{3,4}$= 6.0 Hz, $J_{3,6}$ = 4.3 Hz, 1H, H-3), 3.73 (dd, $J_{1a,1b}$= 12.2 Hz, $J_{1,2}$ =6.1 Hz, 1H, H-1_a), 3.70 (dd, $J_{6a,6b}$ = 9.8, $J_{6a,5}$ = 4.3 Hz, 1H, H-6a), 3.58 (dd, $J_{6a,6b}$ = 9.8 Hz, $J_{6b,5}$ = 5.1 Hz, 1H, H-6b), 3.36 (m, 1H, H-7a), 3.28 (app q, $J_{1a,2}$ = 5.0 Hz, 1H, H-2), 3.19 (dd, $J_{7a,7b}$ = 12.4 Hz, $J_{7,8}$ = 7.7 Hz, 1H, H-7b) 2.89 (m, 2H, H-8); ^{13}C NMR (125MHz, D_2O) δ 136.6 (C-9), 128.9 (C-12) 128.8 (C-) 76.4 (C-3), 71.1 (C-4), 71.0 (C-6), 70.6 (C-5), 58.5 (C-1), 58.2 (C-2), 47.4 (C-7), 32.4 (C-8); HRMS (ESI) calcd [$C_{14}H_{21}NO_4H^+$]).*m/z* 268.1543, found 268.1559

***N*-benzyl-1-Deoxymannojirimicin**



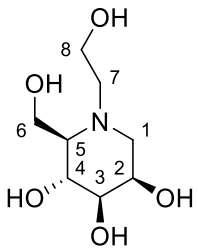
4f

Methyl-6-deoxy-6-iodo-D-fructoside (0.0846 g, 0.28 mmol) was dissolved in aqueous HCl (2 M, 3.3 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (3 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.39 mL) to which benzylamine (0.04 mL, 0.34 mmol) and AcOH (0.02 mL) followed by sodium cyanoborohydride (0.0697 g, 1.12 mmol) were added. The reaction was then stirred for 16 hrs at rt before being subject to additional benzylamine (0.06 mL, 0.56 mmol). The reaction was stirred at rt for a further 6 hrs when the proposed intermediate **99** was no longer observed by TLC and HRMS analysis. The solution was then concentrated and the resulting residue was then coevaporated with methanol: acetic acid: toluene (2x 1.5 mL, 1:4:6 v:v:v). The residue was 99then purified using silica gel flash chromatography (DCM: EtOH: MeOH: NH_3 (28% w:w), 195:2:2:1 to 46:18:18:9, (v:v:v:v)), followed by co-evaporation with dilute HCl (0.12 M, 2mL), to give *N*-benzyl-1-Deoxymannojirimicin (**4f**) as yellow oil. (0.0227 g, 0.09 mmol, 32 %)

$[\alpha]_D^{22.9}$ = -9° (MeOH, c = 1); IR (film, MeOH): 3254, 3222, 2888, 2173, 1457, 1078 cm^{-1} ^1H NMR (500 MHz D_2O): δ 4.70 (brs, 5H, H-9-11), 4.70 (d, $J_{7a,7b}$ = 13.3 Hz, 1H, H-7a), 4.32 (dd, $J_{7a,7b}$ = 13.3 Hz, 1H, $H7_b$), 4.30 (d, $J_{6a,6b}$ = 13.5 Hz, 1H, H-6a) 4.23 (dd, $J_{6a,6b}$ = 13.5, 1H, $H6_b$), 4.13 (m, 1H, H-2), 4.01 (t, $J_{4,3} = J_{4,5}$ = 10.0 Hz, 1H, H4), 3.61 (dd, $J_{3,4}$ = 9.7, $J_{3,2}$ = 2.7 Hz, 1H, H-3), 3.35 (d, $J_{1a,1b}$= 13.2

Hz, 1H, H-1_a), 3.22 (d, $J_{1a,1b}$ = 12.57 Hz, 1H, H-1_b) 3.14 (app. d, $J_{4,5}$ = 10.1 Hz, 1H, H-5); ^{13}C NMR (125MHz, D_2O) δ 131.7 (C-9), 130.3 (C-11), 129.3 (C-10), 127.7 (C-8), 71.9 (C-3), 66.3 (C-2), 65.1 (C-4 and 5), 56.9 (C-7), 54.5 (C-1), 54.3 (C-6) ; HRMS (ESI) calcd for $[C_{13}H_{20}NO_4]^+$ *m/z* 254.1387, found 254.1393

_N_-hydroxyethyl-1-Deoxymannojirimicin.

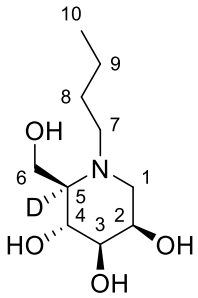


4k

Methyl-6-deoxy-6-iodo-D-fructoside (0.0682 g, 0.22 mmol) was dissolved in aqueous HCl (2 M, 2.69 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.12 mL) to which ethanolamine (0.02 mL, 0.27 mmol) and AcOH (0.015 mL) followed by sodium cyanoborohydride (g, 14mmol) were added. The reaction was then stirred for 15 hrs at rt before being subject to additional ethanolamine (0.02 mL, 0.27 mmol). The reaction was stirred at rt for a further 6.5 hrs when the proposed intermediate **101** was no longer observed by TLC and HRMS analysis. The solution was then concentrated and the resulting residue was then coevaporated with methanol: acetic acid: toluene (2x 1.5 mL, 1:4:6 v:v:v). The residue was then purified using silica gel flash chromatography (100% DCM- DCM: EtOH: MeOH: NH_3 (28% w:w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)) and (DCM:MeOH:EtOH:NH_3 (28% w:w) (155:18:18:9) (v:v:v:v)) to give *N*-hydroxyethyl-1-Deoxymannojirimicin (**4f**) as a clear oil. (0.0217 g, mmol, 39%)

[α] $_D^{22.9}$ = -5° (MeOH, c = 1); IR (film, MeOH): 3314, 2942, 2831, 1448, 1020 cm^{-1} ^{1}H NMR (500 MHz D_2O): δ 4.26 (br s, 1H, H-2), 4.10 (d, $J_{6a,6b}$ = 13.5 Hz, 1H, H-6a) 4.06 (d, $J_{6a,6b}$ = 13.5, 1H, $H6_b$), 4.00 (m, 1H, H4), 3.99 (t, $J_{7,8}$ = 6.5 Hz, 2H, H4), 3.74 (dd, $J_{3,4}$ = 9.5, $J_{3,2}$ = 2.5 Hz, 1H, H-3) 3.66 (d, $J_{1a,1b}$= 13.5 Hz, 1H, $H\text{-}1_a$), 3.49 (d, $J_{1a,1b}$ = 13.5 Hz, 1H, $H\text{-}1_b$), 3.61-3.58 (m, 1H, H-7a), 3.40 (d, $J_{1a,1b}$ = 13.5 Hz, 1H, $H\text{-}7_b$), 3.26 (d, $J_{1a,1b}$ = 8.0 Hz, 1H, H-2); ^{13}C NMR (125MHz, D_2O) δ 71.9 (C-3), 66.5 (C-5), 665.4 (C-2), 65.2 (C-4), 55.3 (C-1), 54.9 (C-6 or 7 or 8) 54.2 (C-6 or 7 or 8); HRMS (ESI) calcd for $[C_8H_{18}NO_5]^+$ *m/z* 208.1179, found 208.1184

_N_-butyl-5-deutero-1-Deoxymannojirimicin.



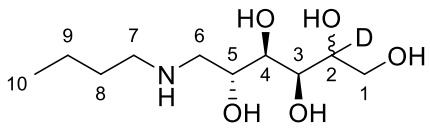
4k

Methyl 6-deoxy-6-iodo-D-fructoside (0.0940 g, 0.31 mmol) was dissolved in aqueous HCl (2 M, 3.71 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.54 mL) to which *N*-Butylamine (0.06 mL, 0.62 mmol) and AcOH (0.03 mL) followed by sodium cyanoborohydride (0.081 g, 1.24 mmol) were added. The reaction was then stirred for 9 hrs at rt before being subject to additional *N*-Butylamine (0.06 mL, 0.62 mmol). The reaction was stirred at rt for a further 7 hrs when the proposed intermediate **102** was no longer observed by TLC and HRMS analysis. The solution was then concentrated and the resulting residue was then coevaporated with methanol: acetic acid: toluene (2x 1.5 mL, 1:4:6 v:v:v). The residue was then

purified using silica gel flash chromatography (DCM: EtOH: MeOH: NH_3 (28% w:w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)), followed by co-evaporation with dilute HCl (0.12 M, 2mL), to give *N*-butyl-5-deutero-1-Deoxymannojirimicin (**4i**) as its HCl salt a clear oil. (0.0293 g, 0.14 mmol, 37%)

$[\alpha]_D^{22.9}$ = -3° (MeOH, c = 1); IR (film, MeOH): 3355, 2958, 2873, 1458, 1096, 1057 cm^{-1}; ^1H NMR (500 MHz D_2O): δ 4.07 (br s, 1H, H-2), 3.97 (d, $J_{6a,6b}$ = 13.1 Hz, 1H, H-6a), 3.93 (d, $J_{6a,6b}$ = 13.1 Hz, 1H, $H6_b$), 3.87 (d, $J_{4,3}$ = 10.0 Hz, 1H, H4), 3.62 (dd, $J_{3,4}$ = 9.6, $J_{3,2}$ = 3.4 Hz, 1H, H-3), 3.33 (dd, $J_{1a,1b,}$= 13.4 Hz, $J_{1a,1b,}$=3.0 Hz, 1H, H-1_a), 3.09 (d, $J_{1a,1b}$ = 13.3 Hz, 1H, H-1_b), 3.08 (m, 2H, H-7), 1.89-1.58 (m, 2H, H-8), 1.34 (app. sex, $J_{9,10}$ =7.7 Hz, 2H, H-9), 0.92 (t, $J_{9,10}$ = 7.4 Hz, 3H, H-10); ^{13}C NMR (125MHz, D_2O) δ 72.8 (C-3), 66.2 (C-2), 65.8 (C-4), 64.9 (C-5), 55.3 (C-1), 54.5 (C-6) 52.5 (C-7), 24.3 (C-8), 19.5 (C-9), 12.8 (C-10); HRMS (ESI) calcd for $[C_{10}H_{21}DNO_4]^+$ *m/z* 221.1606, found 221.1601

6-deoxy-6-butylamino-2-deutero-D-mannitol and 6-deoxy-6-butylamino-2-deutero-L-gulitol



Methyl 6-deoxy-6-iodo-D-fructoside (0.0940 g, 0.31 mmol) was dissolved in aqueous HCl (2 M, 3.71 mL). The reaction was stirred at rt for 1.5 hrs, after which complete conversion of the starting material to a lower running spot was observed by TLC. The reaction was brought to pH 7 by the dropwise addition of aqueous NaOH (1.5 M) and concentrated under reduced pressure, before being taken up in EtOH (4 mL). The insoluble NaCl salt was removed from the solution via filtration and the remaining

solution was concentrated to a clear oil. The oil was then redissolved in EtOH (1.54 mL) to which *N*-Butylamine (0.06 mL, 0.62 mmol) and AcOH (0.03 mL) followed by sodium cyanoborohydride (0.081 g, 1.24 mmol) were added. The reaction was then stirred for 9 hrs at rt before being subject to additional *N*-Butylamine (0.06 mL, 0.62 mmol). The reaction was stirred at rt for a further 7 hrs when the proposed intermediate **102** was no longer observed by TLC and HRMS analysis. The solution was then concentrated and the resulting residue was then coevaporated with methanol: acetic acid: toluene (2x 1.5 mL, 1:4:6 v:v:v). The residue was then purified using silica gel flash chromatography (DCM: EtOH: MeOH: NH_3 (28% w:w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)), followed by co-evaporation with dilute HCl (0.12 M, 2mL and further silica gel flash chromatography (DCM: EtOH: MeOH: ammonia (25% w/w), 195:2:2:1 to 5:2:2:1, (v:v:v:v)) to give a diastereomeric mixture of **103a** and **103b** [2:1] as their corresponding HCl salts in a clear oil (0.0025g, 0.01 mmol, 3 %).

$[\alpha]_D^{22.9}$ = -5° (MeOH, c = 1); IR (film, MeOH): 3338, 2922, 1425, 1073 cm^{-1}; ^1H NMR (500 MHz D_2O): δ 4.00 (m, 1H, H-2), 3.70 (d, $J_{6a,6b}$ = 11.88 Hz, 1H, H-6a), 3.65 (dd, $J_{2,3}$ = 8.30 Hz, $J_{3,4}$ = 1.16 Hz, 1H, H-3), 3.61 (s, 1H, H-4), 3.52 (d, $J_{6a,6b}$ = 11.9 Hz, 1H, $H6_b$), 3.28 (dd, $J_{1a,1b}$ = 13.1 Hz, $J_{1a,2}$ = 3.0 Hz, 1H, $H\text{-}1_a$), 3.12 (d, $J_{1a,1b}$ = 12.9 Hz, $J_{1b,2}$ = 5.7 Hz, 1H, $H\text{-}1_b$), 3.18-3.14 (m, 1H, H-7) 1.54 (app quin, $J_{7,8}$ = 7.5 Hz, 2H, H-8), 1.25 (app sex, $J_{9,10}$ =7.5 Hz, 2H, H-9), 0.78 (t, $J_{9,10}$ = 7.4 Hz, 3H, H-10); ^{13}C NMR (125MHz D_2O) δ 71.5 (C-5), 70.9 (C-3), 68.7 (C-4), 66.3 (C-2), 62.9 (C-1), 50.3 (C-6) 47.5 (C-7), 27.3 (C-8), 19.1 (C-9), 12.7 (C-10); HRMS (ESI) calcd for $[C_{10}H_{22}DNO_5]^+$ *m/z* 238.1639, found 238.1643

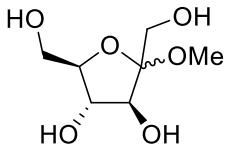
5. References

(1) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Glycobiology* **2005**, *15*, 43r.
(2) O.R. Martin, P. C. E. *Iminosugars: Recent Insights into Their Bioactivity and Potential as Therapeutic Agents*; 5 ed., 2003; Vol. 3.
(3) Benito, J. M.; Garcia Fernandez, J. M.; Ortiz Mellet, C. *Expert Opin Ther Pat* **2011**, *21*, 885.
(4) Olden, K.; Breton, P.; Grzegorzewski, K.; Yasuda, Y.; Gause, B. L.; Oredipe, O. A.; Newton, S. A.; White, S. L. *Pharmacol. Ther* **1991**, *50*, 285.
(5) Inouye, S.; Tsuruoka, T.; Nida, T. *J. Antibiot* **1966**, *19*, 288.
(6) Paulsen, H. *Angew.Chem. Int. Ed.* **1966**, *5*, 495.
(7) Yagi M., K. T., Aoyagi Y., Murai H *Nippon Nogeikagaku Kaishi* **1976**, *50*, 571.
(8) Schmidt, D. D.; Frommer, W.; Muller, L.; Truscheit, E. *Naturwissenschaften* **1979**, *66*, 584.
(9) Stütz, A. E. *Iminosugars as glycosidase inhibitors : nojirimycin and beyond*; Wiley-VCH: Weinheim ; Cambridge, 1998.
(10) Compain, P. M. O. R.; John Wiley and Sons: Chichester, West Sussex, England; Hoboken, NJ, 2007.
(11) Rempel, B. P.; Withers, S. G. *Glycobiology* **2008**, *18*, 570.
(12) Varrot, A.; Tarling, C. A.; Macdonald, J. M.; Stick, R. V.; Zechel, D. L.; Withers, S. G.; Davies, G. J. *J. Am. Chem. Soc.* **2003**, *125*, 7496.
(13) Borges de Melo, E.; da Silveira Gomes, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277.
(14) Campbell, L. K.; Baker, D. E.; Campbell, R. K. *Ann. Pharm.* **2000**, *34*, 1291.
(15) Lu, Y.; Xu, Y.-Y.; Fan, K.-Y.; Shen, Z.-H. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 221.
(16) Sefter, R. E. B.; Seftor, E. A.; Grimes, W. J.; Liotta, L. A.; Stetler-Stevenson, W. G.; Welch, D. R.; Hendrix, M. J. C. *Melanoma Research* **1991**, *1*, 43.
(17) J. E.; Wrodnigg, *et al.*, G. J. *Proc. Nat. Acad. Sci.* **2012**, *109*, 781.
(18) Balzarini, J. *FEBS Lett.* **2007**, *581*, 2060.
(19) Boeshagen, H.; Junge, B.; Kinast, G.; Schueller, M.; Stoltefuss, J.; Paessens, A.; Bayer A.-G., Fed. Rep. Ger. . 1989, p 18 pp.
(20) Durantel, D.; Branza-Nichita, N.; Carrouée-Durantel, S.; Butters, T. D.; Dwek, R. A.; Zitzmann, N. *Journal of Virology* **2001**, *75*, 8987.
(21) Elbein, A. D.; Legler, G.; Tlusty, A.; McDowell, W.; Schwarz, R. *Arch. Biochem. Biophys.* **1984**, *235*, 579.
(22) Berg, J. M.; Tymoczko, J. L.; Stryer, L.; Freeman and Company: New York: 2002.
(23) Schulze, H.; Sandhoff, K. *Biochim. Biophys. Acta (BBA) - Molecular and Cell Biology of Lipids* **2014**, *1841*, 799.
(24) Ishii, S. *Proceedings of the Japan Academy, Series B* **2012**, *88*, 18.
(25) Sawkar, A. R.; Cheng, W.-C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. *Proceedings of the National Academy of Sciences* **2002**, *99*, 15428.
(26) Fan, J.-Q.; Ishii, S.; Asano, N.; Suzuki, Y. *Nat Med* **1999**, *5*, 112.
(27) Fellows, L. E.; Bell, E. A.; Lynn, D. G.; Pilkiewicz, F.; Miura, I.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1979**, 977.
(28) Leontein, K.; Lindberg, B.; Lönngren, J. *Acta Chemica Scandinavia B* **1982**, *36*, 515.
(29) Fuhrmann, U.; Bause, E.; Legler, G.; Ploegh, H. *Nature* **1984**, *307*, 755.
(30) Tanaka, Y.; Kato, J.; Kohara, M.; Galinski, M. S. *Antiviral Res.* **2006**, *72*, 1.

(31) Lin, G.; Simmons, G.; Pöhlmann, S.; Baribaud, F.; Ni, H.; Leslie, G. J.; Haggarty, B. S.; Bates, P.; Weissman, D.; Hoxie, J. A.; Doms, R. W. *J. Virol.* **2003**, *77*, 1337.
(32) Cairns, R. A.; Harris, I. S.; Mak, T. W. *Nat Rev Cancer* **2011**, *11*, 85.
(33) Jakob, C. A.; Burda, P.; Roth, J.; Aebi, M. *J. Cell Biol.* **1998**, *142*, 1223.
(34) Ogier-Denis, E.; Trugnan, G.; Sapin, C.; Aubery, M.; Codogno, P. *J. Biol. Chem.* **1990**, *265*, 5366.
(35) Gouin, S. G. *Chem. Eur. J.* **2014**, *20*, 11616.
(36) Stauffert, F.; Lepage, M. L.; Pichon, M. M.; Hazelard, D.; Bodlenner, A.; Compain, P. *Synthesis*.
(37) Darakas, E.; Hultberg, H.; Leontein, K.; Lönngren, J. *Carbohydr. Res.* **1982**, *103*, 176.
(38) Fleet, G. W. J.; Ramsden, N. G.; Witty, D. R. *Tetrahedron.* **1989**, *45*, 319.
(39) Song, X.; Hollingsworth, R. I. *Tetrahedron Lett.* **2007**, *48*, 3115.
(40) Furneaux, R. H.; Tyler, P. C.; Whitehouse, L. A. *Tetrahedron Lett.* **1993**, *34*, 3613.
(41) Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Carbohydr. Res.* **2002**, *337*, 183.
(42) Ziegler, T.; Straub, A.; Effenberger, F. *Angew Chem. Int. Ed.* **1988**, *27*, 716.
(43) de Raadt, A.; Stütz, A. E. *Tetrahedron Lett.* **1992**, *33*, 189.
(44) Baxter, E. W.; Reitz, A. B. *J. Org. Chem.* **1994**, *59*, 3175.
(45) McDonnell, C.; Cronin, L.; O'Brie, J. L.; Murphy, P. V.*J Org. Chem* **2004**, *69*, 3565.
(46) Maier, P.; Andersen, S. M.; Lundt, I. *Synthesis* **2006**, *2006*, 827.
(47) Racine, E.; Bello, C.; Gerber-Lemaire, S.; Vogel, P.; Py, S. *J. Org. Chem.* **2009**, *74*, 1766.
(48) Chan, T.-H.; Chang, Y.-F.; Hsu, J.-J.; Cheng, W.-C. *J Org. Chem. Eur.* **2010**, *2010*, 5555.
(49) Mao, H.; Joly, G. J.; Peeters, K.; Hoornaert, G. J.; Compernolle, F. *Tetrahedron* **2001**, *57*, 6955.
(50) Asano, K.; Hakogi, T.; Iwama, S.; Katsumura, S. *Chem. Commun.* **1999**, 41.
(51) Martín, R.; Moyano, A.; Pericàs, M. A.; Riera, A. *Org. Lett.* **2000**, *2*, 93.
(52) Subramanian, T.; Lin, C.-C.; Lin, C.-C. *Tetrahedron Letters* **2001**, *42*, 4079.
(53) Martín, R.; Murruzzu, C.; Pericàs, M. A.; Riera, A. *J Org. Chem* **2005**, *70*, 2325.
(54) Boglio, C.; Stahlke, S.; Thorimbert, S.; Malacria, M. *Org. Lett.* **2005**, *7*, 4851.
(55) Lamas, M.-C.; Malacria, M.; Thorimbert, S. *Eur. J. Org. Chem.* **2011**, *2011*, 2777.
(56) Kang, M.; Park, J.; Konradi, A. W.; Pedersen, S. F. *J Org. Chem* **1996**, *61*, 5528.
(57) Knight, J. G.; Tchabanenko, K. *Tetrahedron* **2003**, *59*, 281.
(58) Scarpi, D.; Bartali, L.; Casini, A.; Occhiato, E. G. *Eur. J. Org. Chem.* **2012**, *2012*, 2597.
(59) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press, 2000.
(60) Trost, B. *Science* **1991**, *254*, 1471.
(61) Sheldon, R. A. *Green Chemistry* **2007**, *9*, 1273.
(62) Dunn, P. J. *Chem. Soc. Rev* **2012**, *41*, 1452.
(63) Sheldon, R. A. *Chem. Soc. Rev.* **2012**, *41*, 1437.
(64) Mehta, A.; Conyers, B.; Tyrrell, D. L. J.; Walters, K.-A.; Tipples, G. A.; Dwek, R. A.; Block, T. M. *Antimicrob. Agents Chemother.* **2002**, *46*, 4004.
(65) Pavlović, D.; Neville, D. C. A.; Argaud, O.; Blumberg, B.; Dwek, R. A.; Fischer, W. B.; Zitzmann, N. *Proceedings of the National Academy of Sciences* **2003**, *100*, 6104.
(66) Huang, R.; Dietsch, E.; Lockhof, O.; Schüller, M.; Reutter, W. *FEBS Lett.* **1991**, *291*, 199.
(67) Dangerfield, E. M.; Plunkett, C. H.; Stocker, B. L.; Timmer, M. S. M. *Molecules* **2009**, *14*, 5298.
(68) Stocker, B. L.; Win-Mason, A. L.; Timmer, M. S. M. *Carbohydr. Res.* **2012**, *356*, 163.
(69) Corkran, H. M.; Munneke, S.; Dangerfield, E. M.; Stocker, B. L.; Timmer, M. S. M. *The Journal of Organic Chemistry* **2013**, *78*, 9791.
(70) Compain, P.; Martin, O. R. *Iminosugars: From Synthesis to Therapeutic Applications*; Wiley, 2007.
(71) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128.

(72) Makeev, G. M.; Kumskov, M. I. *Mendeleev Commun.* **1996**, *6*, 27.
(73) Lopes, C. C.; Lopes, R. S. C.; Matos, C. R. R.; Brazil . 2000, p 56 pp.
(74) Schweden, J.; Legler, G.; Bause, E. *Eur. J. Biochem.* **1986**, *157*, 563.
(75) Kinast, G.; Bayer A.-G., Fed. Rep. Ger. . 1986, p 37 pp.
(76) Cox, T. M.; Aerts, J. M. F. G.; Andria, G.; Beck, M.; et al. *Journal of Inherited Metabolic Disease* **2003**, *26*, 513.
(77) Concia, A. L.; Lozano, C.; Castillo, J. A.; Parella, T.; Joglar, J.; Clapés, P. *Chem. Eur. J.* **2009**, *15*, 3808.
(78) Boucheron, C.; Compain, P.; Martin, O. R. *Tetrahedron Letters* **2006**, *47*, 3081.
(79) Grabner, R. W.; Landis, B. H.; Rutter, R. J.; Scaros, M. G.; Monsanto Co., USA; G.D. Searle and Co. . 1993, p 47 pp.
(80) van den Broek, L. A. G. M.; Vermaas, D. J.; Heskamp, B. M.; van Boeckel, C. A. A.; Tan, M. C. A. A.; Bolscher, J. G. M.; Ploegh, H. L.; van Kemenade, F. J.; de Goede, R. E. Y.; Miedema, F. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 82.
(81) Bernotas, R. C.; Ganem, B. *Biochem. J.* **1990**, *270*, 539.
(82) Wilkinson, B. L.; Bornaghi, L. F.; Lopez, M.; Healy, P. C.; Poulsen, S.-A.; Houston, T. A. *Aust. J. Chem.* **2010**, *63*, 821.
(83) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.
(84) Fleet, G. W. J.; Ramsden, N. G.; Witty, D. R. *Tetrahedron* **1989**, *45*, 319.
(85) Barnes, N. A.; Brooker, A. T.; Godfrey, S. M.; Mallender, P. R.; Pritchard, R. G.; Sadler, M. *Eur. J. Org. Chem.* **2008**, 1019.
(86) O'Brien, J. L.; Tosin, M.; Murphy, P. V. *Org. Lett.* **2001**, *3*, 3353.
(87) Dhavale, D. D.; Saha, N. N.; Desai, V. N. *J.Org. Chem* **1997**, *62*, 7482.
(88) Appel, R. *Angew. Chem. Int. Ed.* **1975**, *14*, 801.
(89) Anilkumar, G.; Nambu, H.; Kita, Y. *Organic Process Research & Development* **2002**, *6*, 190.
(90) Hanessian, S.; Ponpipom, M. M.; Lavallee, P. *Carbohydr Res* **1972**, *24*, 45.
(91) Nurminen, E.; Poijärvi, P.; Koskua, K.; Hovinen, J. *J. Chem. Educ.* **2007**, *84*, 1480.
(92) Stocker, B. L.; Dangerfield, E. M.; Win-Mason, A. L.; Haslett, G. W.; Timmer, M. S. *European J.Org. Chem* **2010**, *2010*, 1615.
(93) Duker, J. M.; Serianni, A. S. *Carbohydrate Research* **1993**, *249*, 281.
(94) Izquierdo, I.; Plaza, M. T.; Asenjo, R.; Rodríguez, M. *Tetrahedron: Asym.* **1995**, *6*, 1117.
(95) Pino-González, M. S.; Assiego, C. *Tetrahedron: Asym* **2005**, *16*, 199.
(96) Look, G. C.; Fotsch, C. H.; Wong, C. H. *Acc. Chem. Res.* **1993**, *26*, 182.
(97) Sayer, J. M.; Peskin, M.; Jencks, W. P. *J. Am. Chem. Soc.* **1973**, *95*, 4277.
(98) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
(99) Corkran, H. M.; Dangerfield, E. M.; Haslett, G. W.; Stocker, B. L.; Timmer, M. S. M. *Biorg. Med. Chem.* **2016**, *24*, 693.
(100) Clinton, F. *Synthesis* **1975**, *3*, 135.
(101) Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 2661.
(102) Tran, A. T.; Huynh, V. A.; Friz, E. M.; Whitney, S. K.; Cordes, D. B. *Tetrahedron Lett.* **2009**, *50*, 1817.

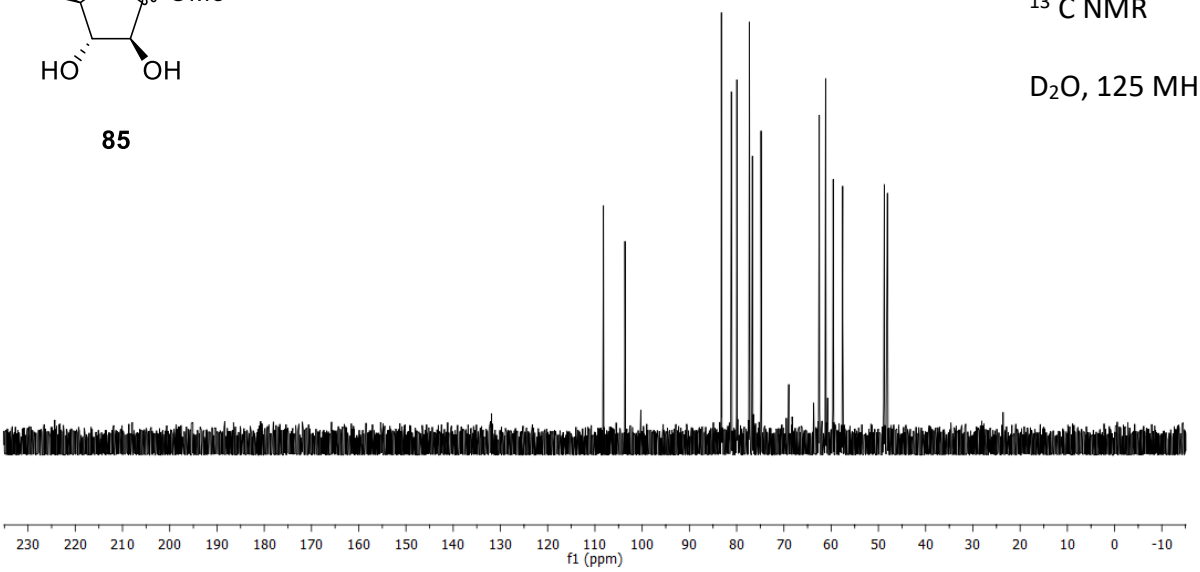
6. Appendix



85

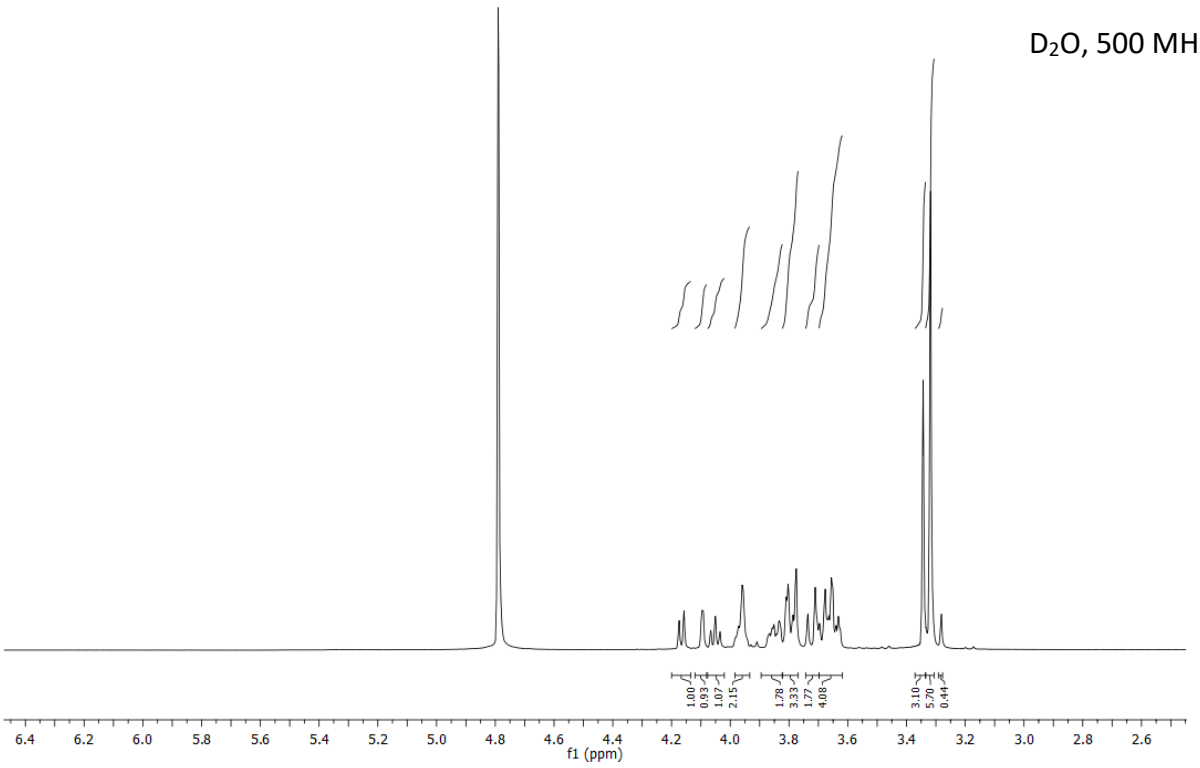
^{13} C NMR

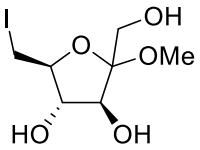
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

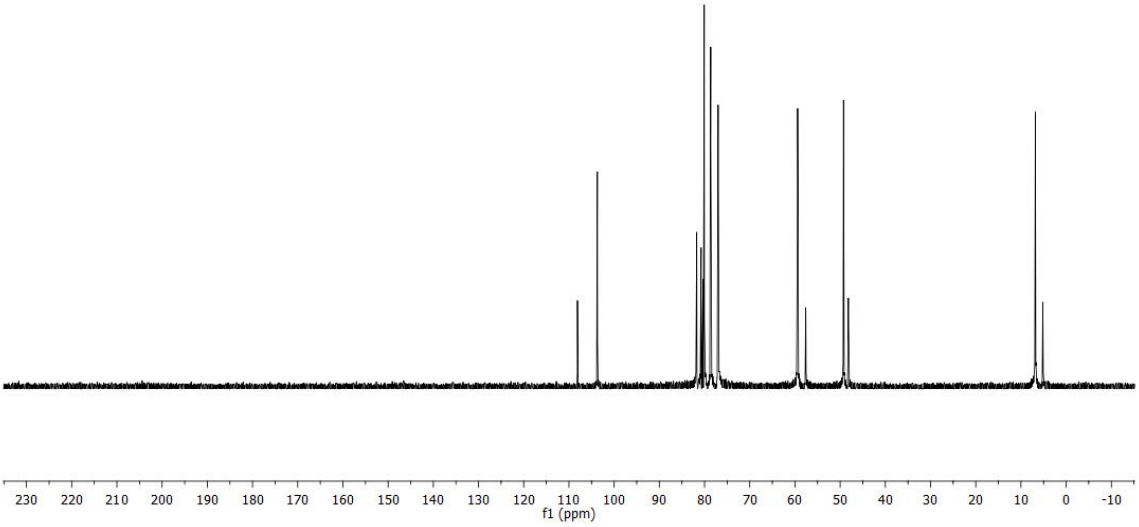




84

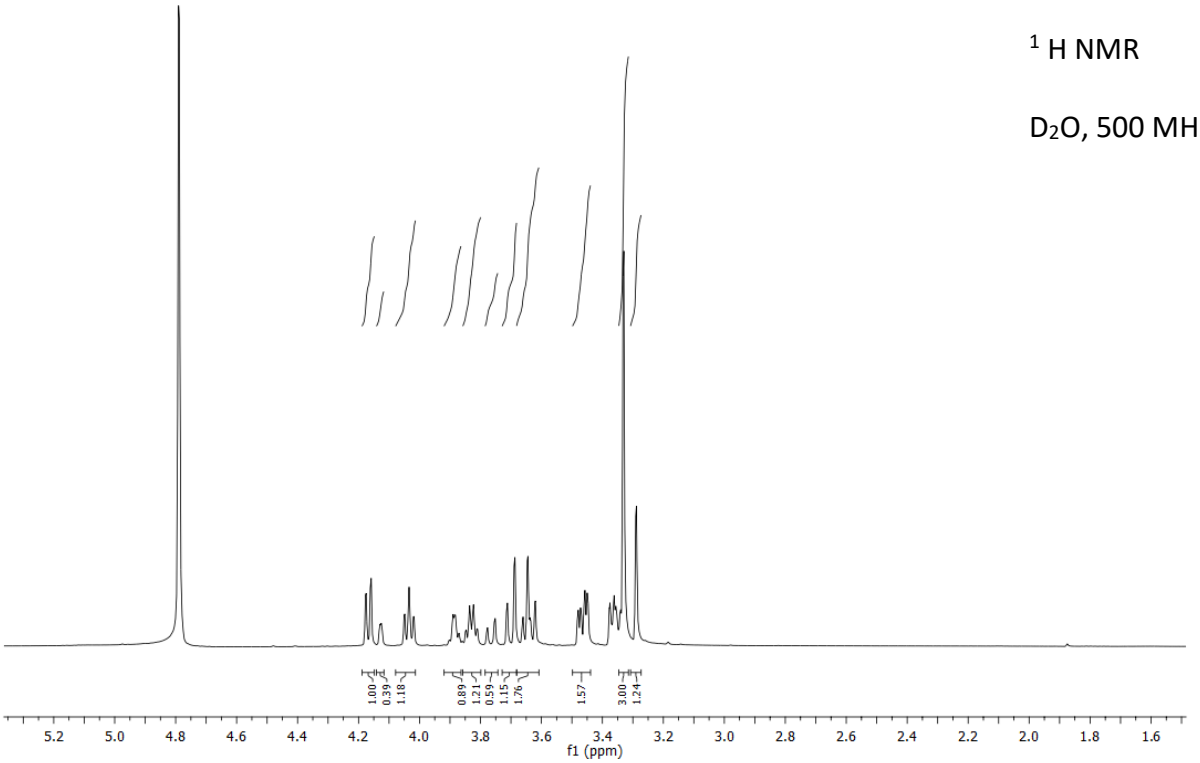
^{13} C NMR

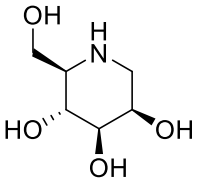
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

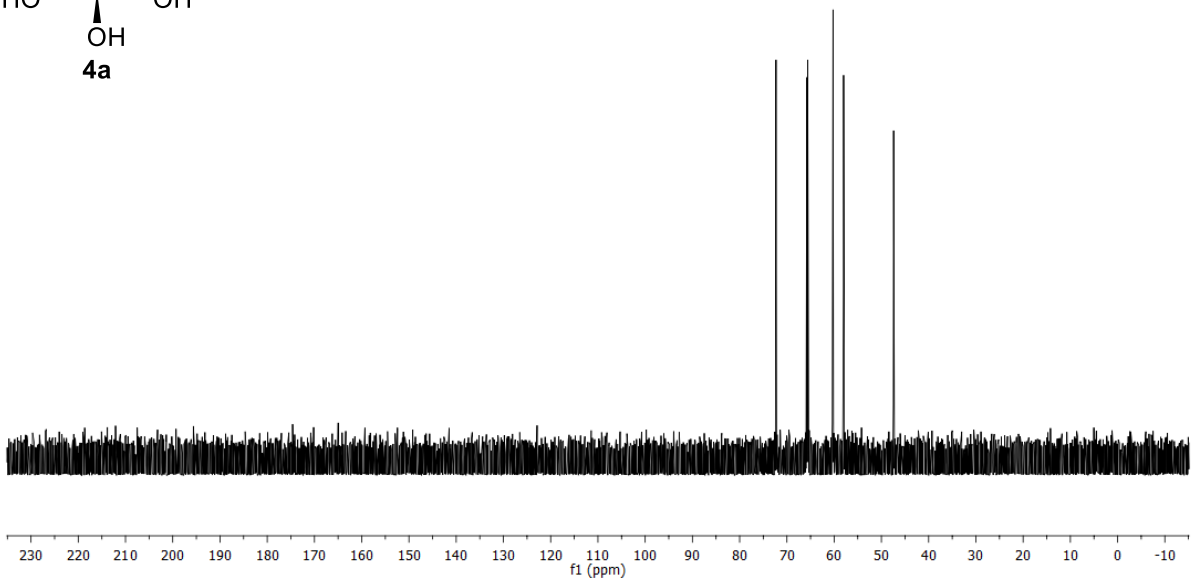




4a

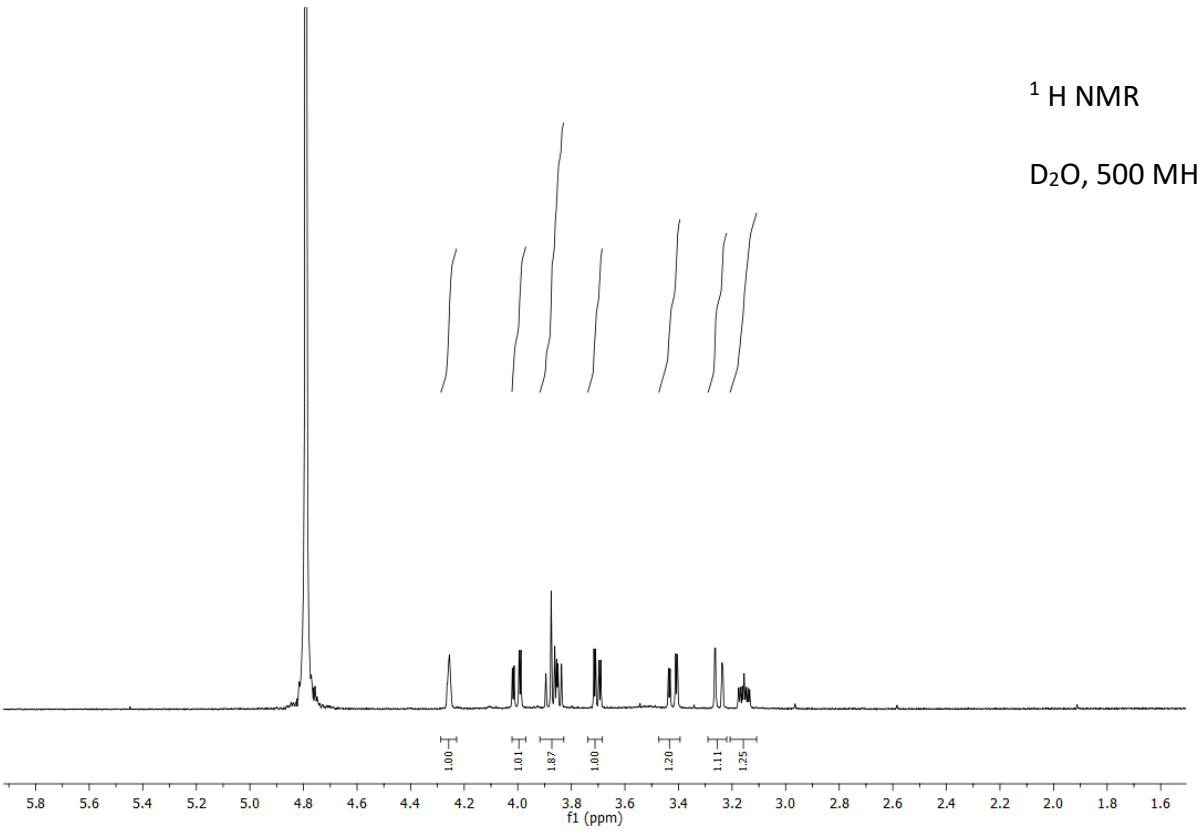
^{13} C NMR

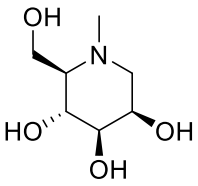
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

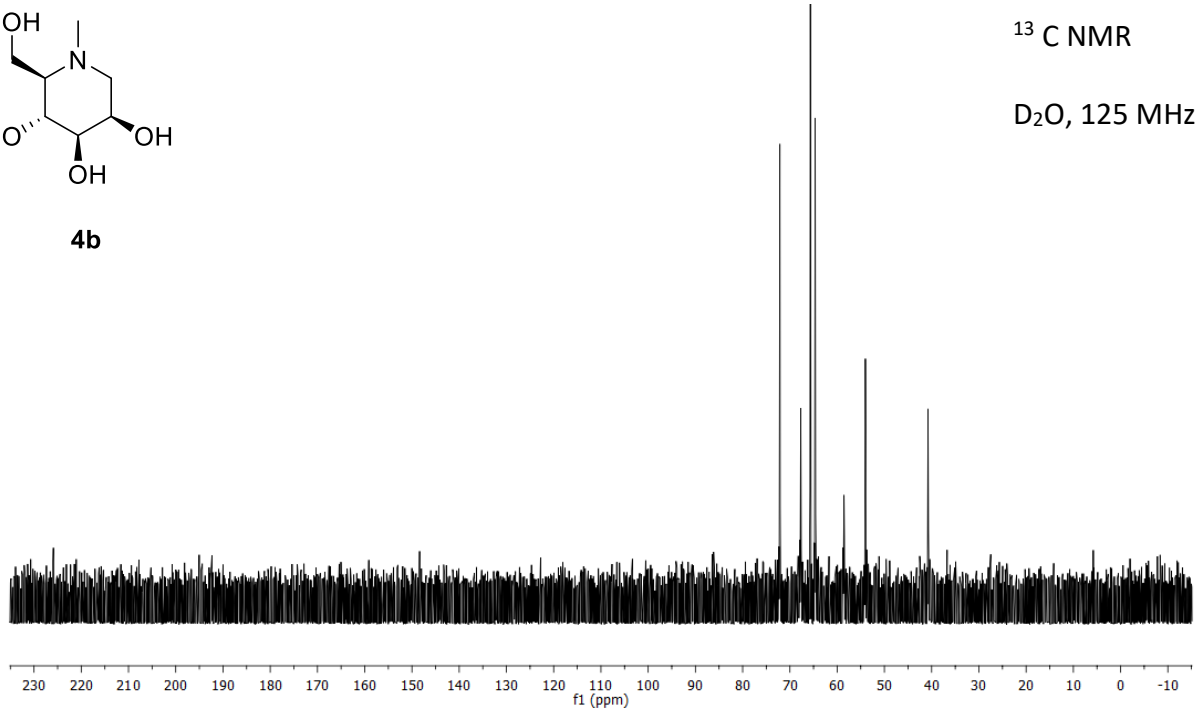




4b

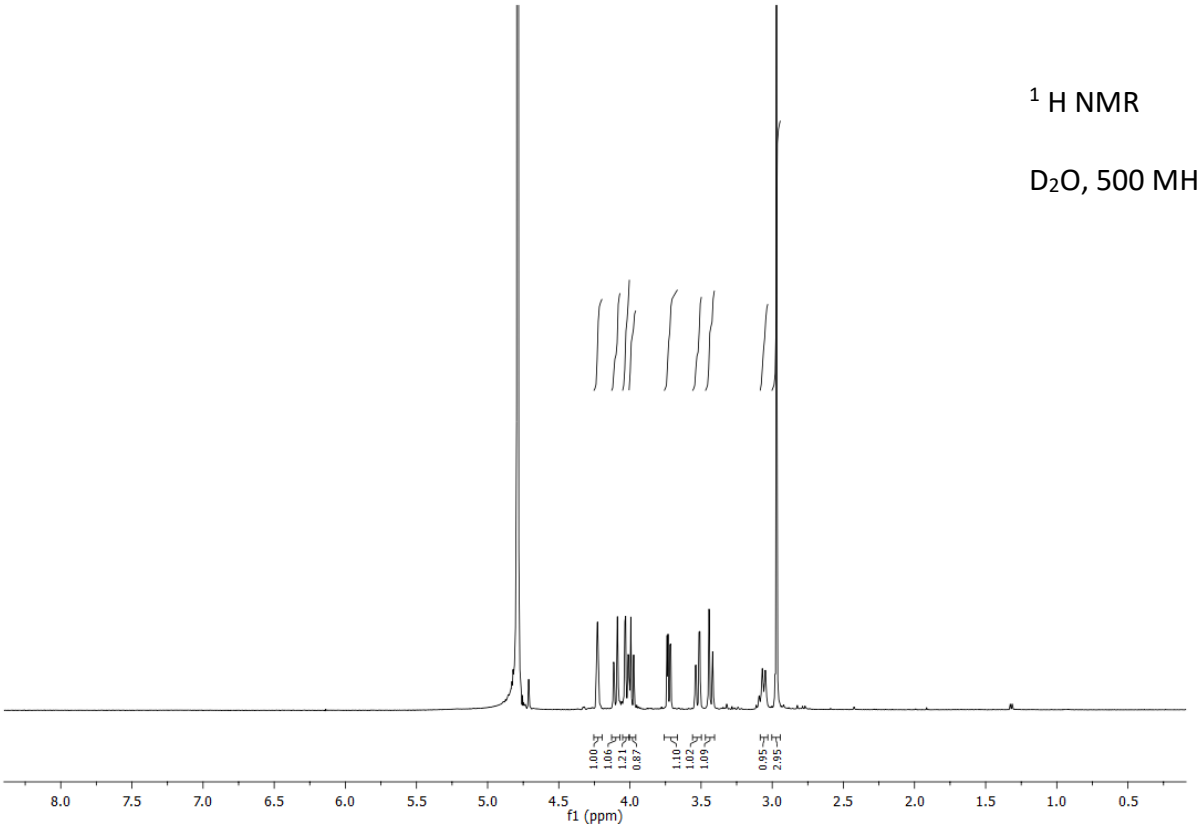
^{13} C NMR

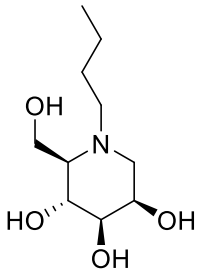
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

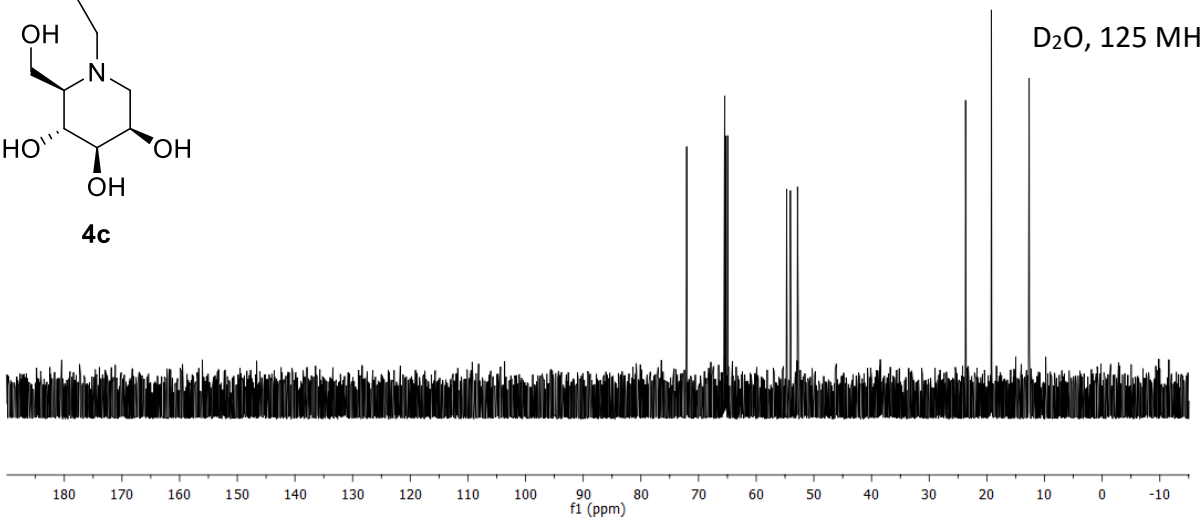




4c

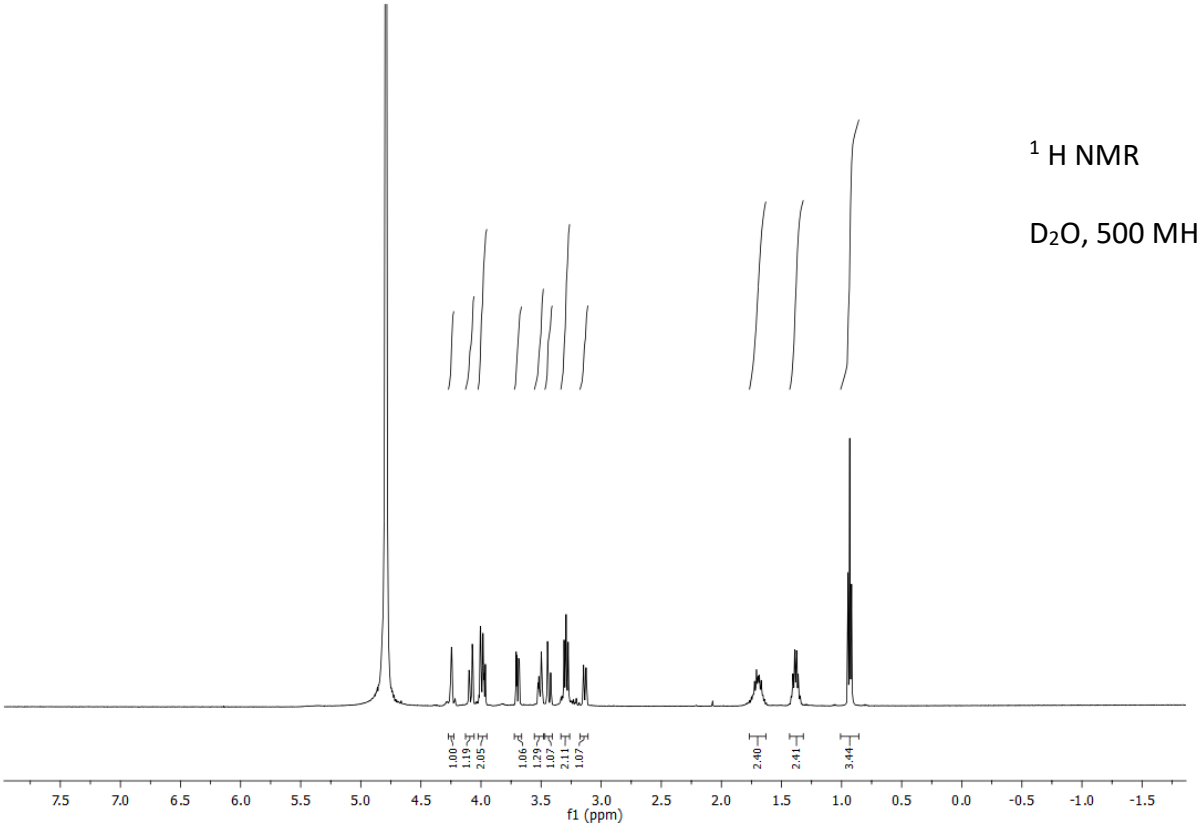
^{13} C NMR

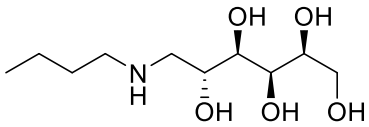
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

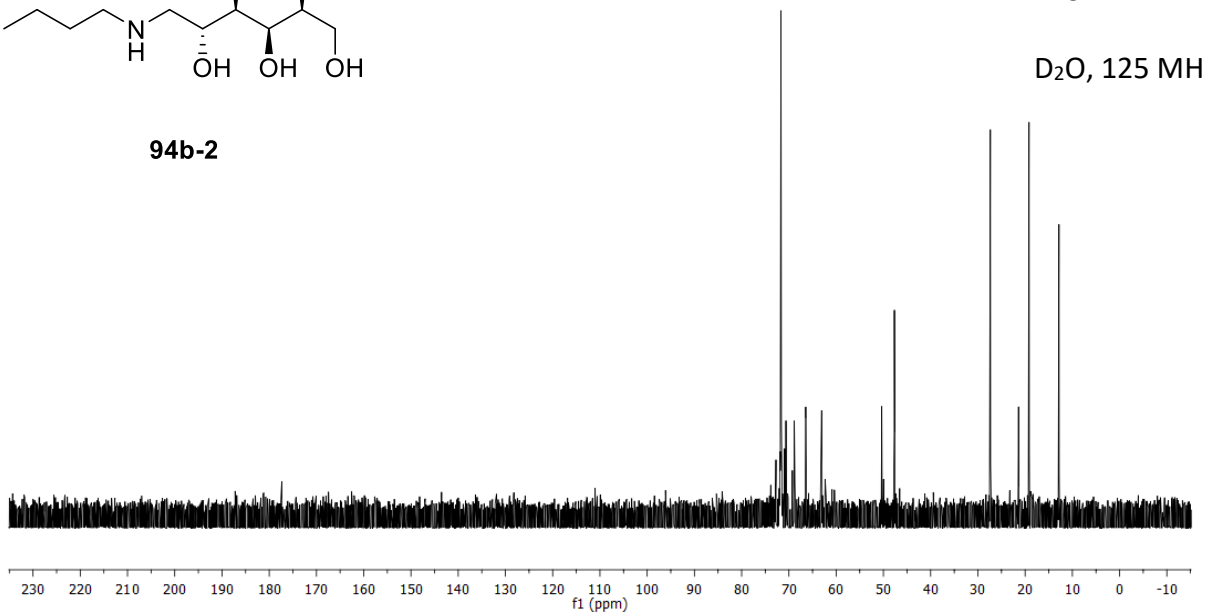




94b-2

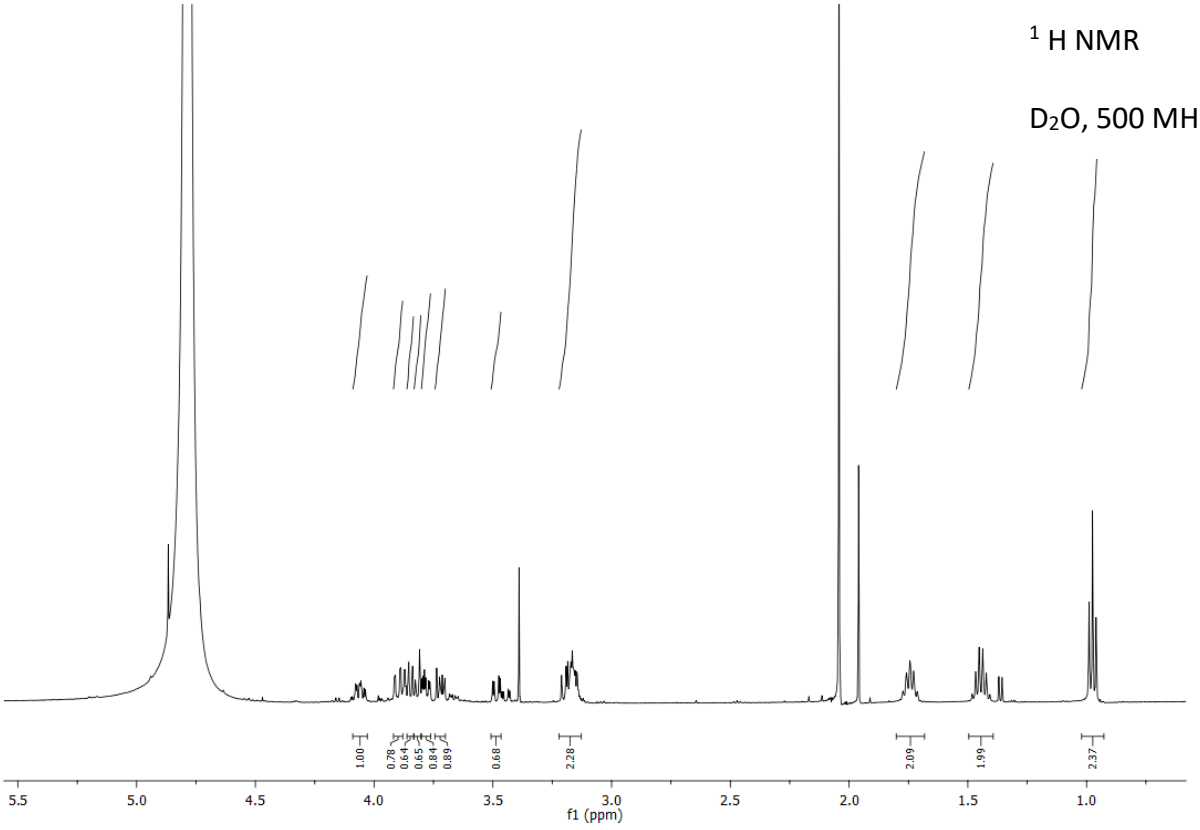
^{13} C NMR

D_2O, 125 MHz



^{1} H NMR

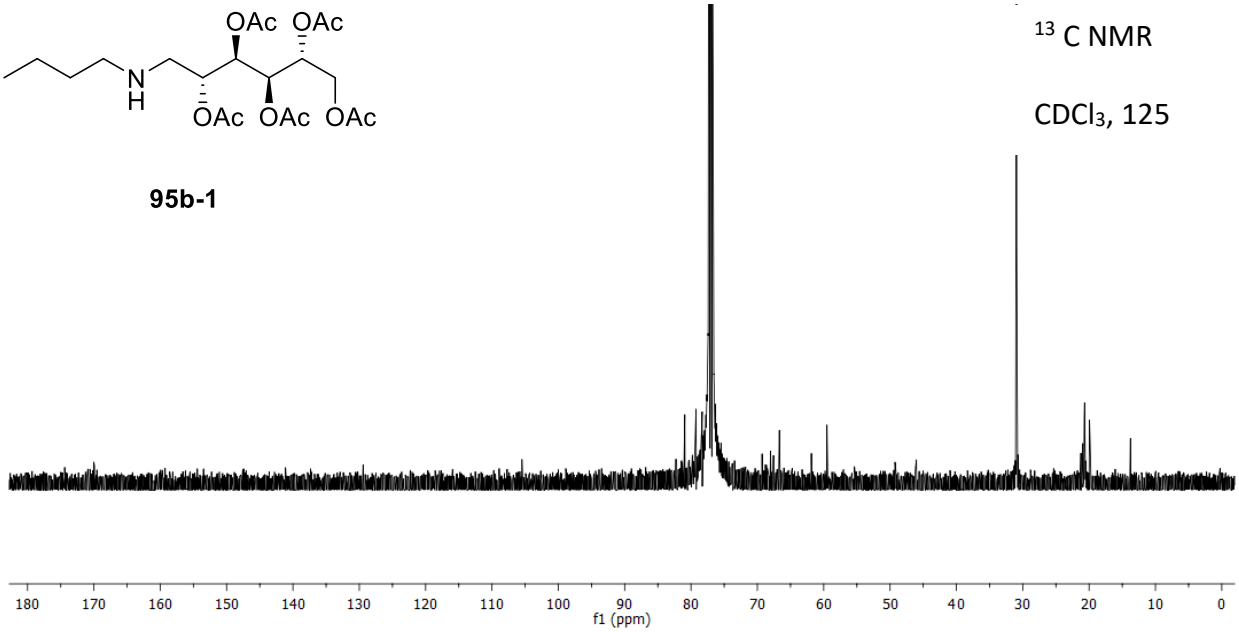
D_2O, 500 MHz



95b-1

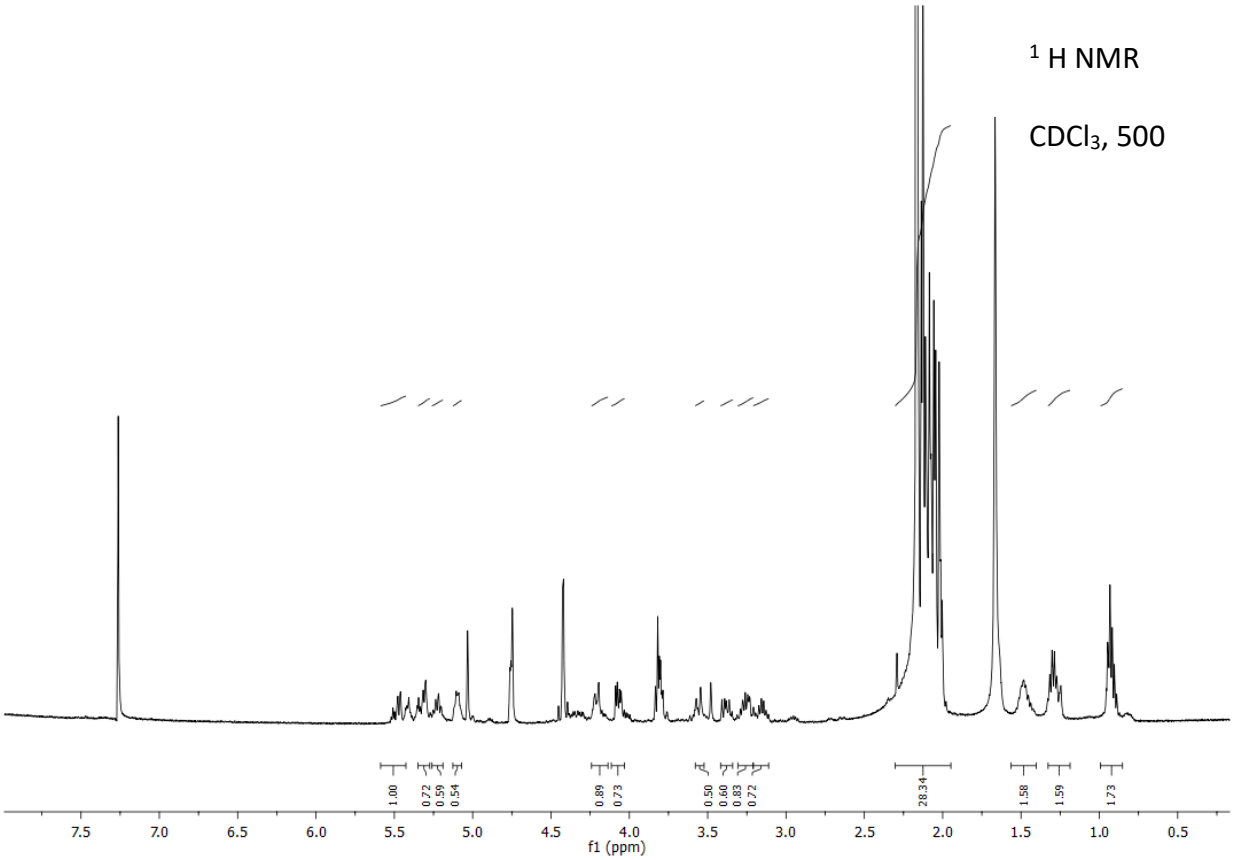
^{13} C NMR

$CDCl_3$, 125



^{1} H NMR

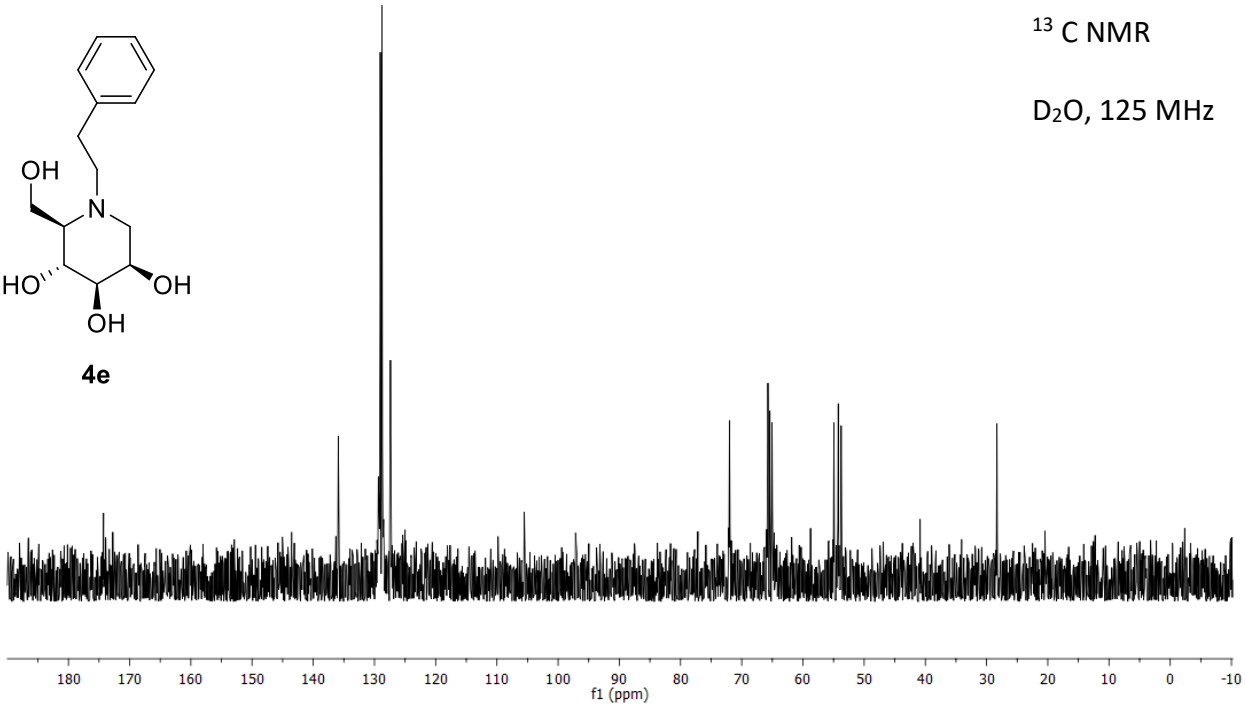
$CDCl_3$, 500



4e

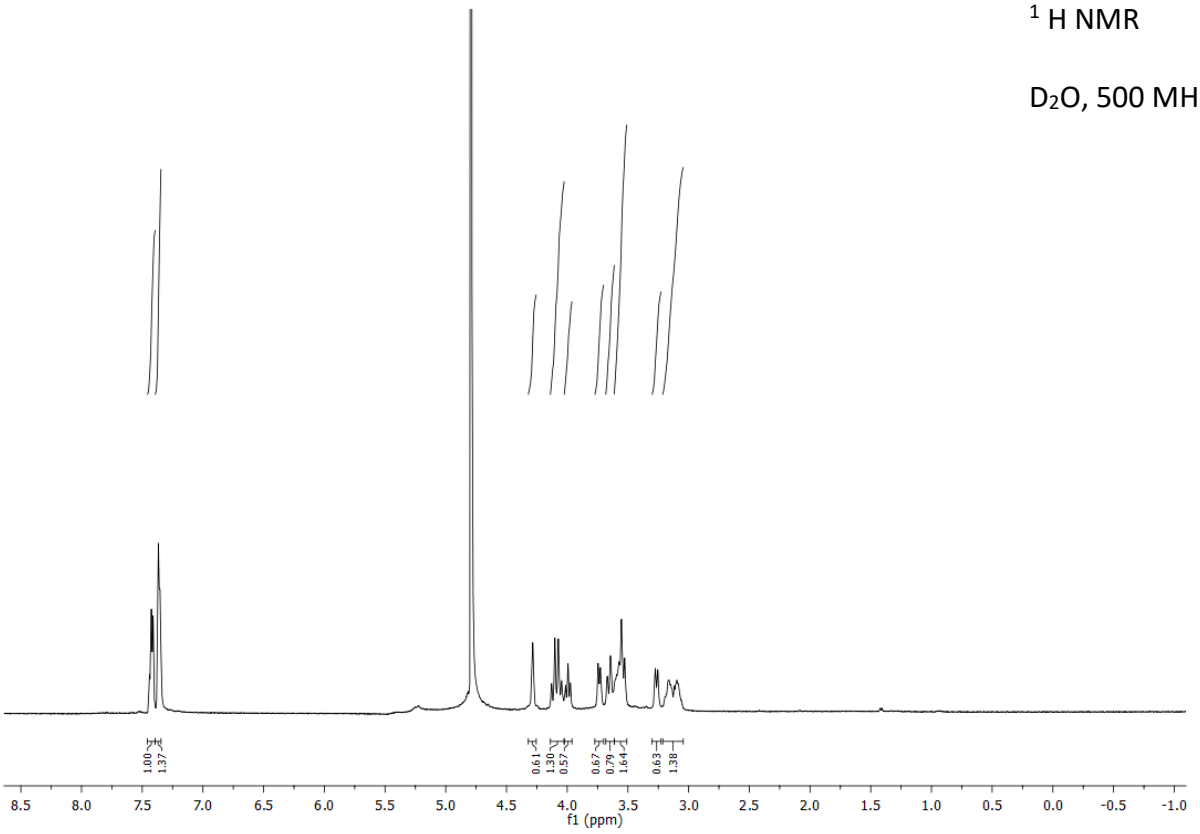
^{13} C NMR

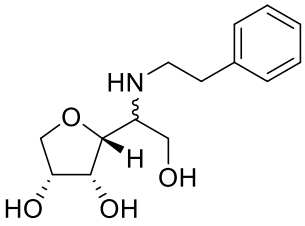
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

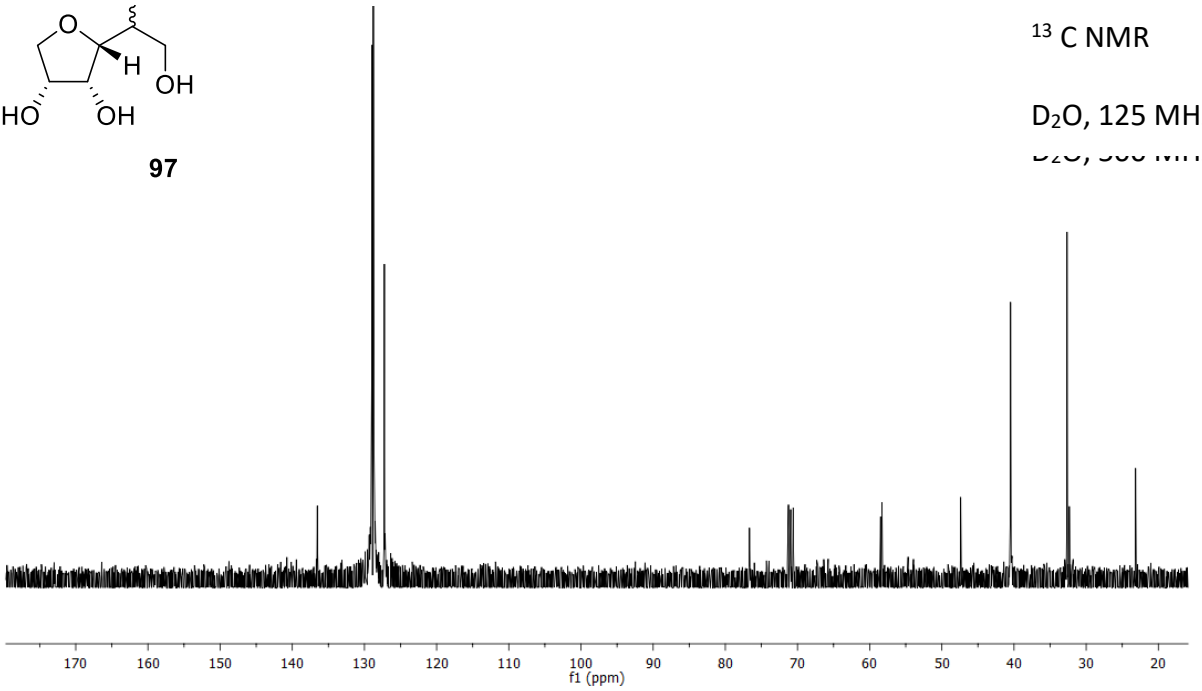




97

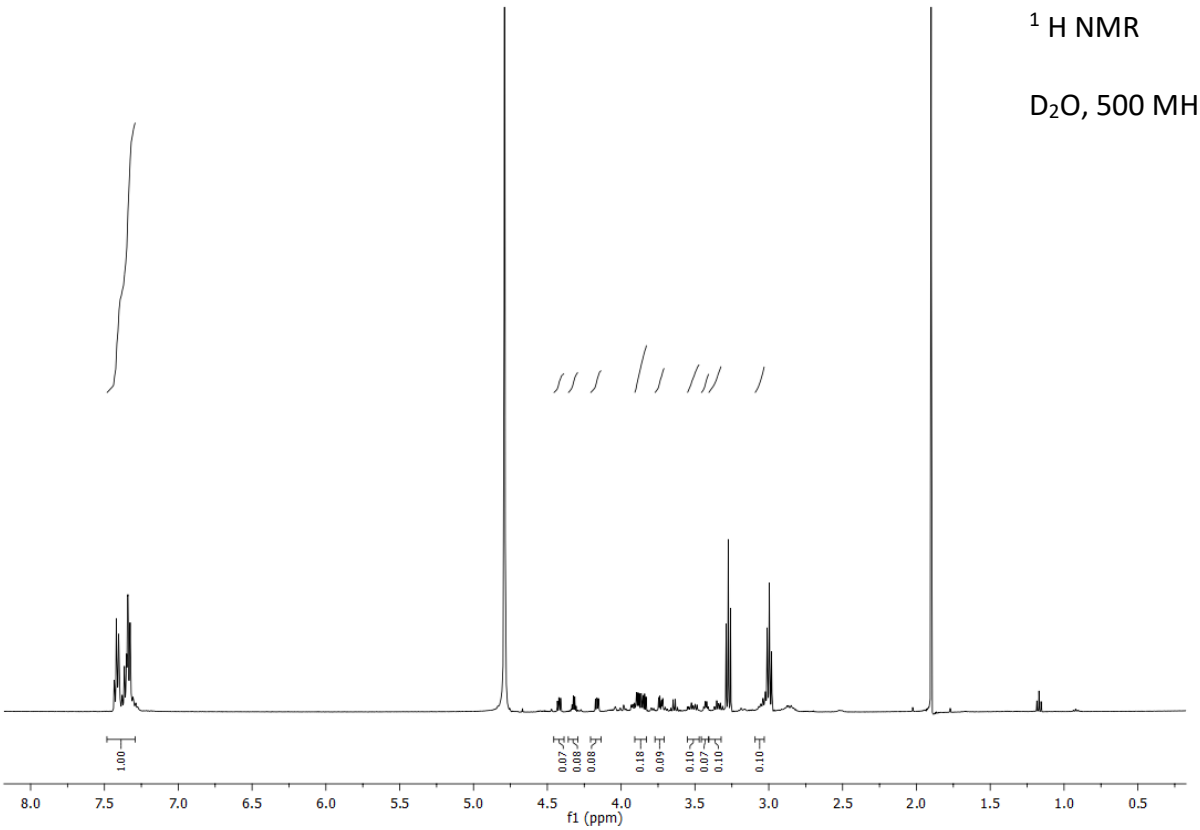
^{13} C NMR

D_2O, 125 MHz



^{1} H NMR

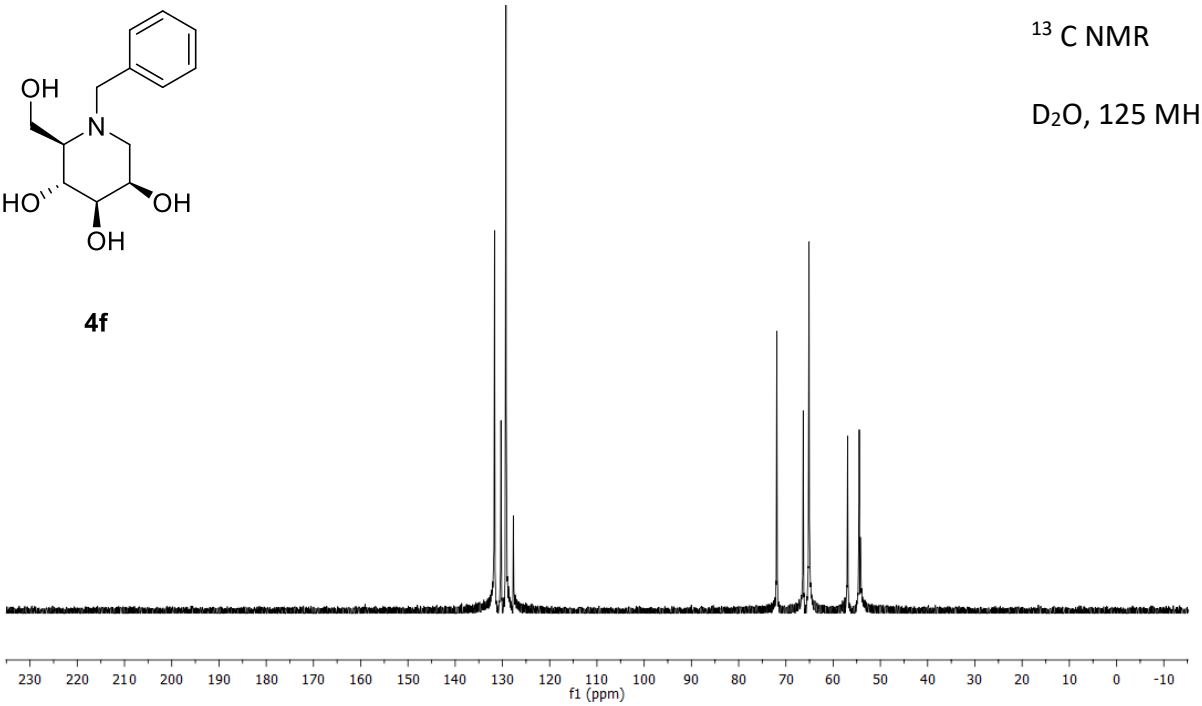
D_2O, 500 MHz



4f

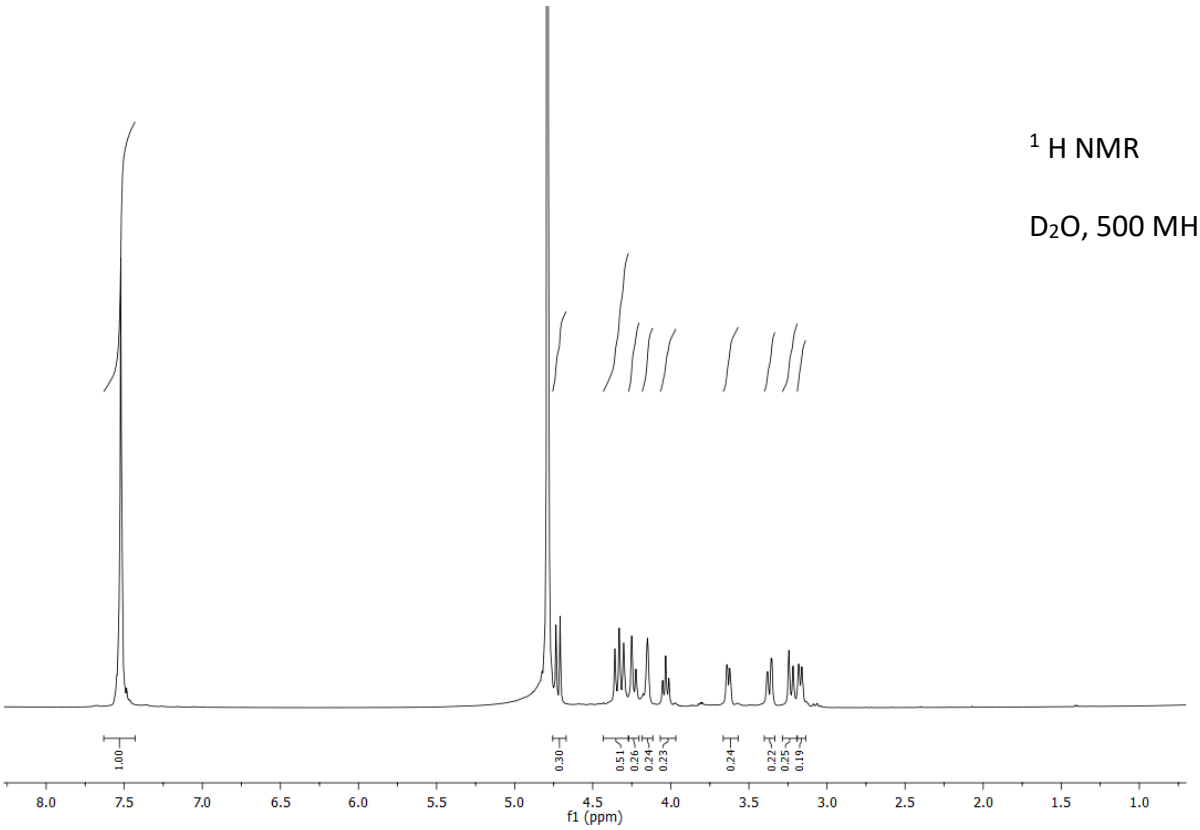
^{13} C NMR

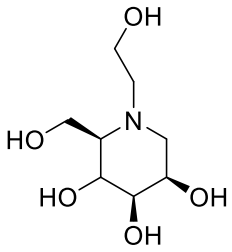
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

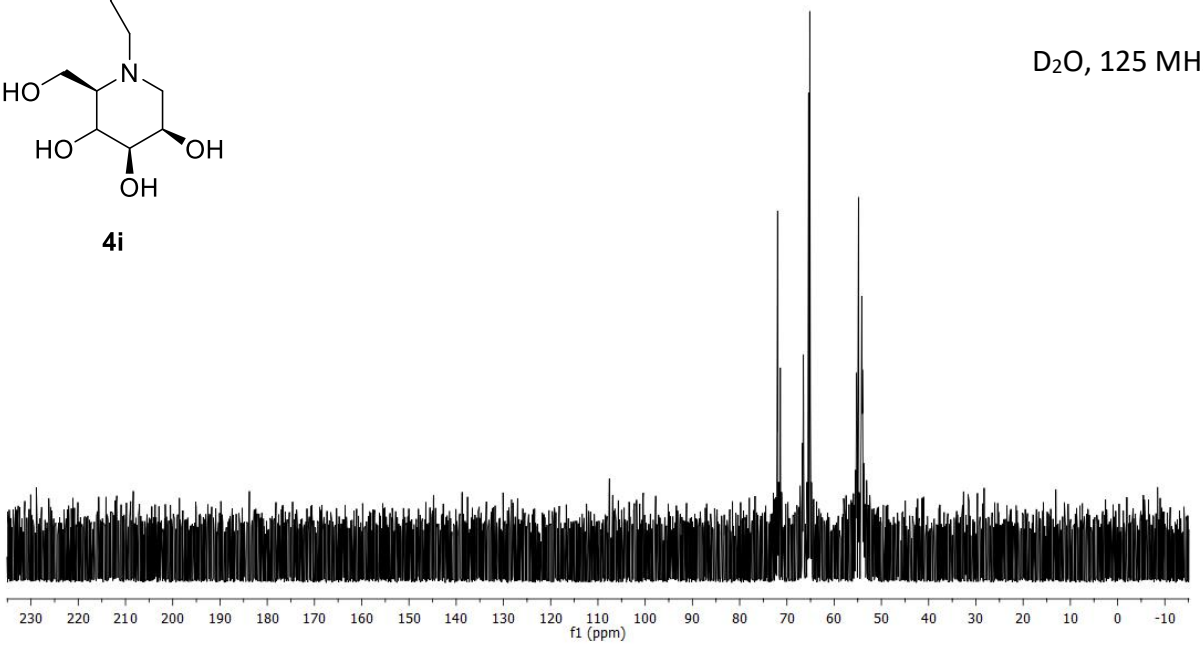




4i

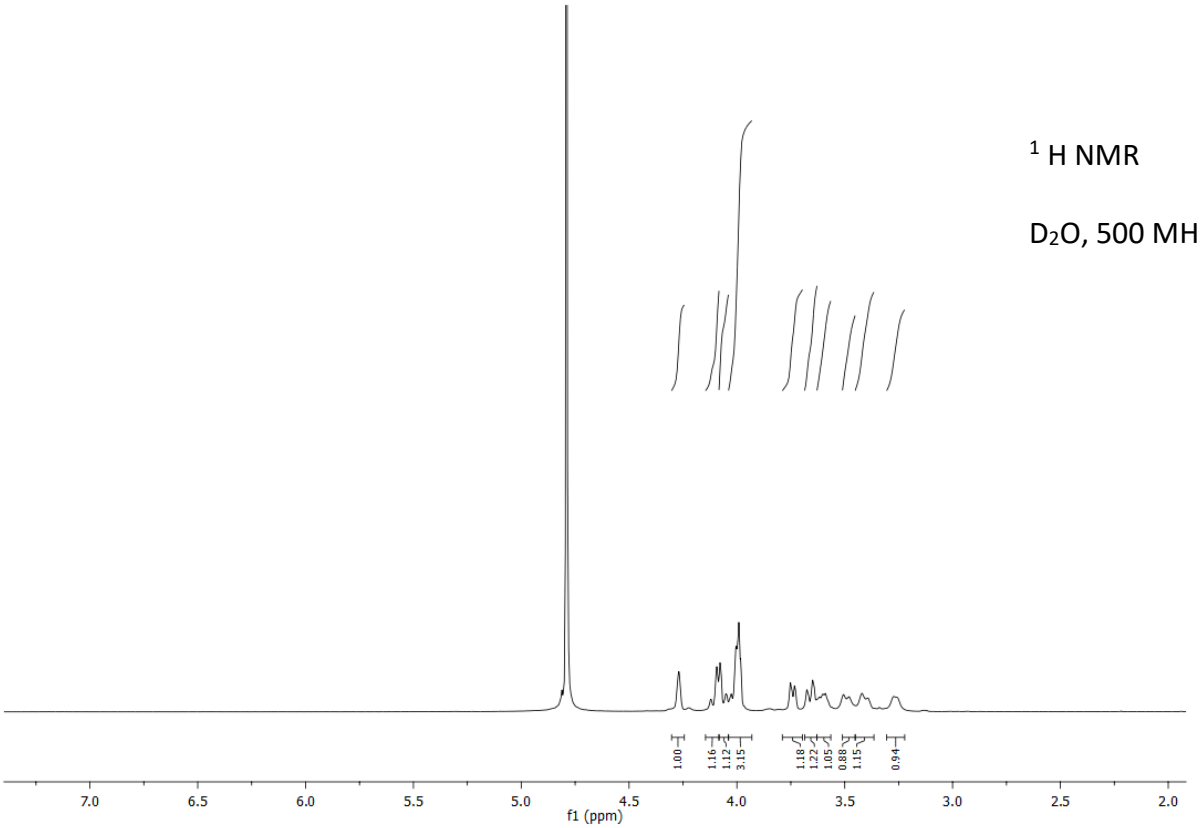
^{13} C NMR

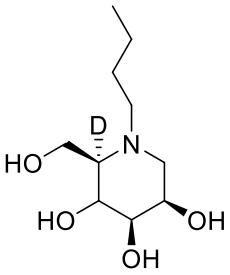
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

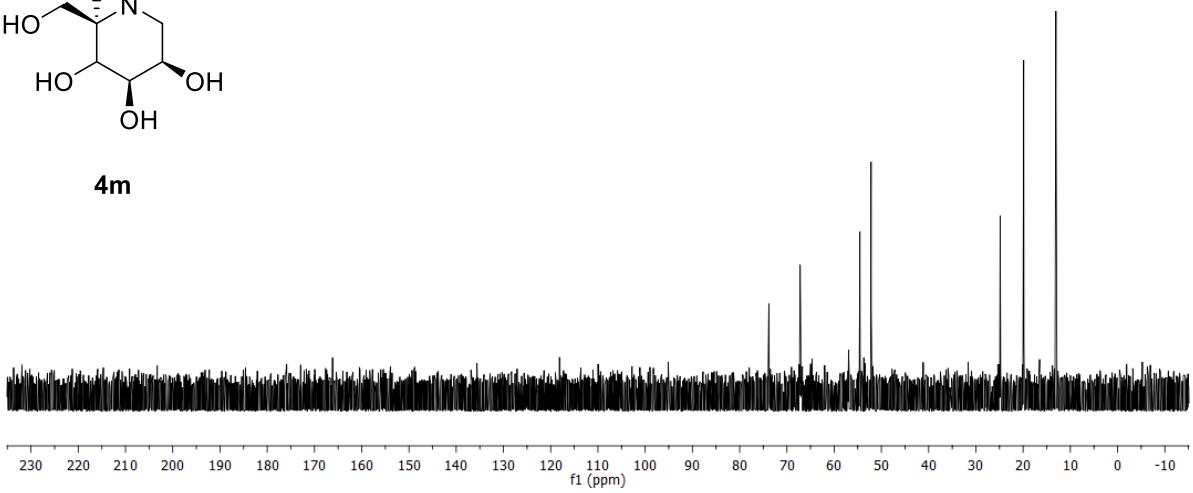




4m

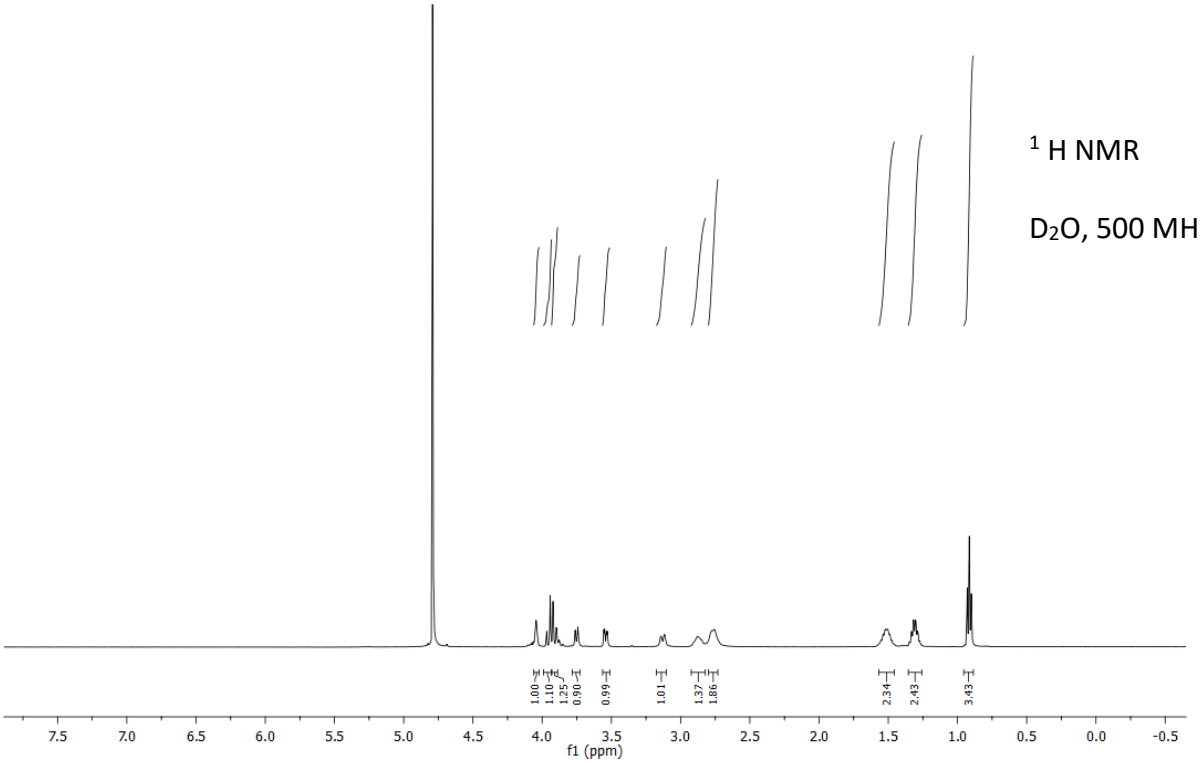
^{13} C NMR

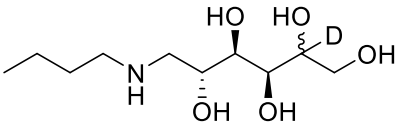
D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz

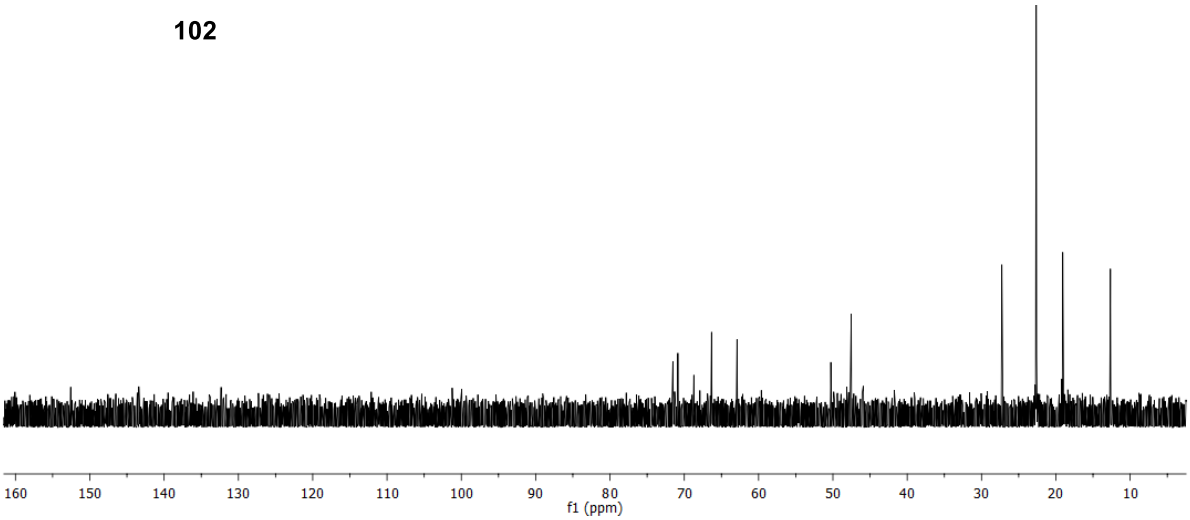




102

^{13} C NMR

D_2O, 125 MHz



^{1} H NMR

D_2O, 500 MHz