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Synthesis of 1,5-Dideoxy-3-O-(α-D-mannopyranosyl)-1,5-imino-D-mannitol and 1,5-Dideoxy-3-O-(α-D-glucopyranosyl)-1,5-imino-D-mannitol: Powerful Inhibitors of Endomannosidase

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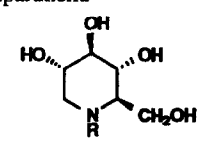
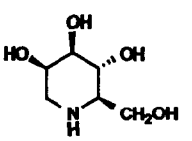
Abstract: The synthesis and conformations of 3-O-methylDMJ, 3-O-(α-D-glucopyranosyl)-DMJ (Glcα1,3DMJ), and 3-O-(α-D-mannopyranosyl)-DMJ (Manα1,3DMJ) are described. Manα1,3DMJ and Glcα1,3DMJ are shown to be very powerful inhibitors of an endomannosidase. The potential use of these compounds in both probing the pathways of N-linked glycoprotein processing and in the chemotherapy of some viral diseases is discussed.

Polyhydroxylated mono- and bicyclic nitrogen heterocycles, in which nitrogen is substituted for oxygen in the carbohydrate ring, have provided an important class of glycosidase inhibitors;[1] the properties of these aminosugar glycosidase inhibitors has maintained interest in the further isolation of new natural products,[2] such as 6-deoxyDMDP[3] and in the design of analogues with better specificity for individual hydrolases.[4] Although there are many potential applications of such materials,[5] one area in which such compounds have made a major contribution has been in the investigation of the processing of N-linked oligosaccharides.[6] The major route for N-linked oligosaccharide biosynthesis in most cell types is *via* the sequential hydrolysis of a cotranslationally transferred, triglucosylated oligosaccharide by exoglycosidases of the intracellular membrane compartment.



1 R = H
2 R = *n*-Bu



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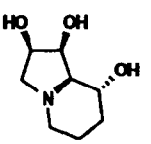

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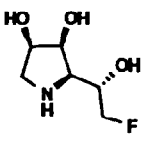

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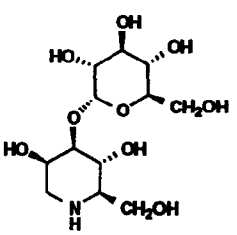

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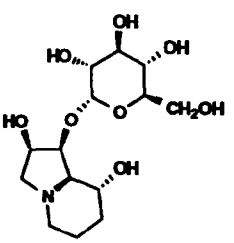

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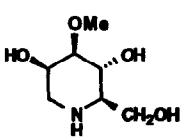

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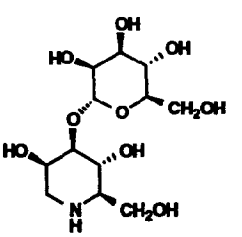

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Oligosaccharide maturation to a high mannose, hybrid, or complex type, requires that the glucose units are removed by α-glucosidase I which hydrolyses the terminal α−1,2 linked glucose residue, and α-

glucosidase II which removes the inner two α–1,3 linked glucose residues; this initiates the processing which ultimately leads to mature complex and hybrid type N-linked oligosaccharides. The initial removal of glucose units may be inhibited by the use of basic nitrogen mimics such as deoxynojirimycin (1), an inhibitor of both glucosidase I and glucosidase II. Deoxymannojirimycin (DMJ) (3), although a rather poor inhibitor of many α-mannosidases, is a powerful and specific inhibitor of mannosidase I but has little effect on mannosidase II; in contrast, swainsonine (4) and simpler synthetic analogues of mannofuranose, such as the 6-deoxy-6-fluoromannofuranose analogue (5),[7] are inhibitors of mannosidase (II). All these mimics (1-5) of glucose or mannose inhibit only exoglycosidases; no reports of their inhibition of endoglycosidases have appeared.

However, cells that are grown in the presence of α-glucosidase inhibitors[8] or which are mutationally negative for these enzymes[9,10] retain the ability to form fully matured glycans by invoking a glucosidase-independent pathway that uses a Golgi located endo-α-mannosidase.[11] This apparently constitutive enzyme converts Glc_3-, Glc_2-, and $Glc_1Man_9GlcNAc_2$ oligosaccharides to a $Man_8GlcNAc_2$ isomer and is not inhibited by a number of compounds that are potent exoglycosidase inhibitors, such as castanospermine, deoxynojirimycin (1), bromoconduritol, DMJ (3) or swainsonine (4).[12]

Since the endomannosidase cleaves Glcα1-3Man, it was postulated that a suitable inhibitor would be a disaccharide possessing a glucose unit α1-3 linked to a mannose analogue such as DMJ or swainsonine, since there is no evidence to indicate whether the nature of the active site of the endomannosidase more closely resembles a mannopyranose or mannofuranose. Accordingly it was decided to synthesise the azadisaccharides Glcα1,3DMJ (6) and Glcα1,3swainsonine (7). Very recently, Spiro and co-workers[13] reported that *in vitro*, Glcα1,3DMJ (6) was the most potent endomannosidase inhibitor among a number of 3-*O*-alkylated DMJ derivatives; any modification to the structure by alkylation or removal of the hydroxyl groups in (6) reduced the inhibition of endomannosidase. The chemical synthesis of (6) has not yet been published.

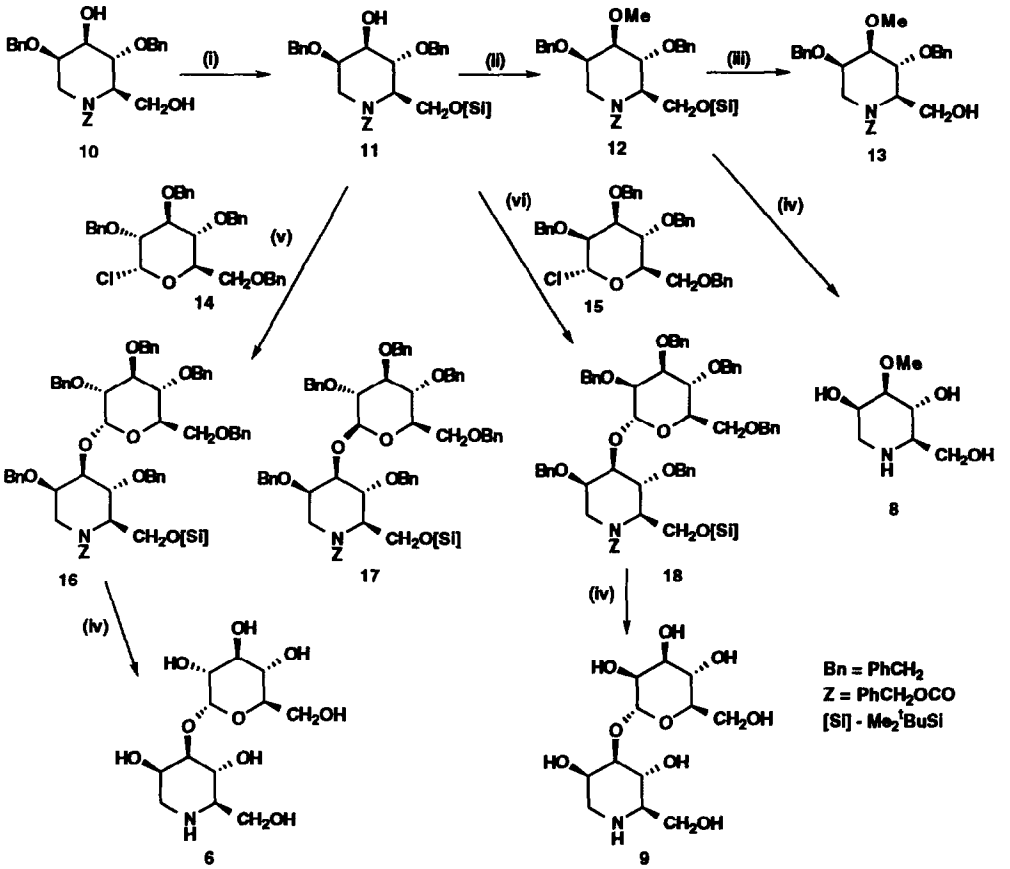
This[13] has prompted us to publish our work on the synthesis of such compounds and this paper describes the synthesis of Glcα1,3DMJ (6), Manα1,3DMJ (9) and 3-*O*-methylDMJ (8). We confirm Spiro's observation that Glcα1,3DMJ (6) is indeed a powerful endomannosidase inhibitor; the previously unreported mannose analogue (9) is also a good inhibitor of endomannosidase.

The availability of Glcα1,3DMJ (6) and Manα1,3DMJ (9) not only will allow further biochemical dissection of the N-linked glycoprotein processing pathway but has potential in augmenting α-glucosidase inhibitor-mediated therapies for infectious agents. For example, HIV may be rendered non-infectious by the formation of completely glucosylated N-linked oligosaccharides. Because Golgi endomannosidase is capable of acting as a salvage pathway, coadministration of (6) or (9) with castanospermine,[14] butylDNJ (2)[15] and other naturally occurring[16] and synthetic[17] glucosidase inhibitors may provide a potentially better therapeutic strategy for the treatment of such diseases than would use of a glucosidase I inhibitor by itself.

Synthesis. Probably the most convenient chemical synthesis of DMJ itself is that from L-gulonolactone which allows the ready preparation of multigram quantities.[18] However there is no modification to this synthesis which would give access to 3-*O*-alkylated DMJ derivatives such as (6) (8) and (9), since a protected derivative of DMJ with only the C-3 hydroxyl group free is required. An alternative synthesis of DMJ from glucose[19] has been reported in which the derivative (10) had been prepared on a several gram scale. Reaction of the diol (10) [Scheme] with *tert*-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole at -20°C resulted in the selective protection of the primary hydroxyl function to afford (11) in 93%

yield; (11) is a suitable intermediate for the synthesis of the target since only the C-3 hydroxyl group of DMJ remains unprotected.

Reaction of (11) with methyl iodide and potassium hydroxide in tetrahydrofuran in the presence of a catalytic quantity of 18-crown-6 resulted in efficient methylation to give (12) in 73% yield. The silyl protecting group could be removed from (12) by treatment with *n*-tetrabutylammonium fluoride in tetrahydrofuran to give (13) in 57% yield. However, attempts to remove the benzyl protecting groups in (13) by hydrogenation under a variety of conditions gave mixtures of products. In contrast, hydrogenolysis of (12) in ethanol in the presence of palladium black and a trace of concentrated aqueous hydrochloric acid lead to clean removal of the silyl and all the benzyl protecting groups to give 3-*O*-methylDMJ (8) in 89% yield.



Scheme. (i) $Me_2{}^tBuSiCl$, Imidazole, DMF (ii) MeI, KOH, 18-crown-6, THF (iii) Bu_4NF, THF (iv) H_2, Pd black, EtOH, HCl (v) (14), CH_2Cl_2, CF_3SO_3Ag (vi) (15), CH_2Cl_2, CF_3SO_3Ag

For the synthesis of the azadisaccharide targets (6) and (9), the protected glucopyranosyl chloride (14)[20] and mannopyranosyl chloride (15)[21] were prepared from tetrabenzylglucose and tetrabenzylmannose, respectively by minor modification of the literature procedures. Reaction of (14) with (11) in dichloromethane in the presence of 2,4,6-trimethylpyridine, molecular sieve and silver triflate gave both the separable α- (16) and β- (17) coupled products in 35% and 5% yields, respectively. The mannopyranosyl chloride (15) reacted

with (11) under similar conditions to give the α-linked disaccharide (18) in 22% yield. Again, all the protecting groups could be removed from (16) by hydrogenolysis in ethanol in the presence of palladium black and a trace of concentrated aqueous hydrochloric acid to afford Glcα1,3DMJ (6) in 92% yield; under these conditions, the anomeric linkage was not affected and this may be that acid hydrolysis of the acetal link is protected by protonation of the amine derived from removal of the carbamate protecting group. The protecting groups were removed similarly from (18) to give Manα1,3DMJ (9) in 91% yield.

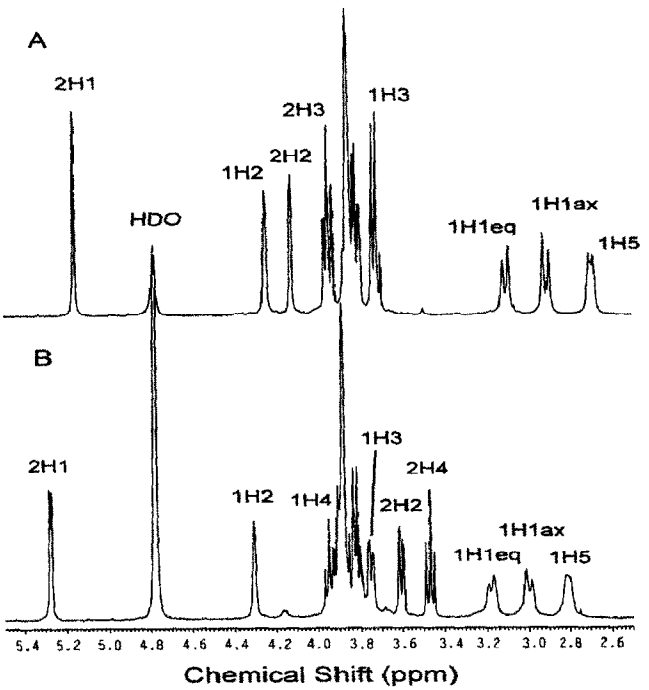


Figure 1. 1D ^{1}H NMR spectrum of A - Manα1,3DMJ (9) and B- Glcα1,3DMJ (6) in ^2H_2O, recorded at 500 MHz and a temperature of 30°C. The assignments shown are based on analysis of the coupling constants obtained from the 1D spectrum and coupling patterns from the 2D COSY spectrum. The spin-systems identified from the coupling patterns are labelled 1 and 2 in each case.

NMR Analysis of Glcα1,3DMJ (6).

Assignment and determination of anomericity. The 1D ^{1}H NMR spectrum of Glcα1,3DMJ (6) is shown in figure 1B. From the peak intensities it is clear that the sample contains a single major product of approximately 95% purity. The nearly complete assignment (except for the stereospecific assignments of the H6 and H6' of spin-system 1, and the assignments of H6 and H6' of spin-system 2) of the spectrum was obtained from

analysis of the coupling patterns obtained from the 2D COSY spectrum and the coupling constants obtained from the 1D spectrum (see experimental details). This is fully consistent with a D-glucopyranose ring in the $^4C_1$ conformation (spin-system 2), modified at the reducing terminus, and a DMJ ring in the $^4C_1$ conformation (spin-system 1). The glucose H1-H2 coupling constant of 3.75 Hz indicates that the D-glucose is in exclusively the α anomeric configuration.

Linkage analysis. Linkage information was obtained from the 2D NOESY spectrum (figure 2). At short mixing times (100 ms), a very strong NOE is observed between the glucose H1 (2H1) resonance and the DMJ H3 (1H3) resonance and an additional cross-linkage NOE is observed between the 2H5 and 1H2 resonances. At long mixing time (400 ms), a very weak NOE is observed between the 2H1 and 1H2 resonances (not shown in figure 2). This pattern of NOEs is only consistent with a 1,3 linkage between the two components (shown in figure 4).

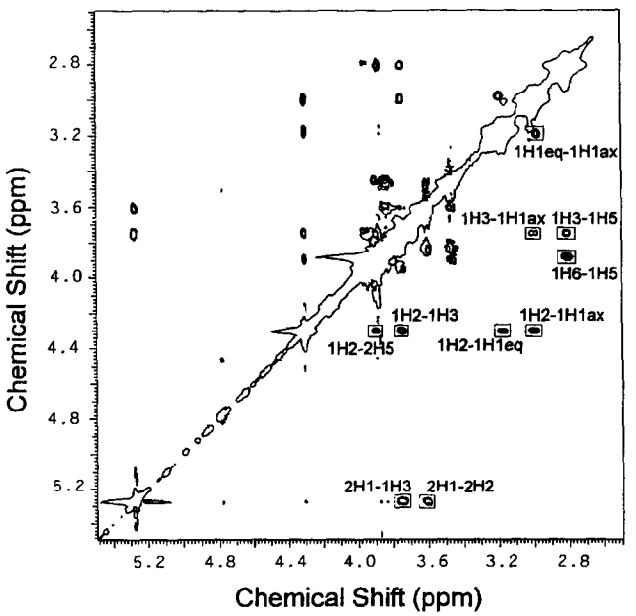


Figure 2. 2D NOESY spectrum of Glcα1,3DMJ (6) in ^2H_2O using a 400 ms mixing time, recorded at 500 MHz and a temperature of 30C (1D spectrum is shown in figure 1B). The spectrum is phased to give negative diagonal peaks. Negative peaks are plotted at a single contour level whilst positive peaks are plotted at four contour levels. Both intra- and inter-residue NOEs are labelled.

Linkage conformation. The linkage conformation of Glcα1,3DMJ was determined by measuring NOE build-up rates for the cross-linkage NOEs and using the two-spin approximation to determine distances between the protons involved (see Experimental). This gives a distance of 2.2Å to 2.4Å for the 2H1-1H3 NOE (using the 2H1-2H2 distance of 2.42Å as the internal calibration) and a 2.6Å to 3.0Å distance for the 1H2-2H5 NOE

(using the 1H2-1H3 distance of 2.45Å as the internal calibration). In addition, conformational constraints can be obtained in this case from the absence of NOEs.[22] In particular, the absence of a 2H1-1H2 and a 2H1-1H4 NOE in the 200 ms mixing time NOESY was taken as a minimum distance constraint of 3Å for these proton pairs. These conformational constraints can be summarised in a torsion angle map (figure 3). This shows the regions of conformational space (defined by the glycosidic torsion angles) consistent with the determined proton-proton distances. As can be seen, there are two major regions consistent with all the NOE data, H1-C1-O3'-C3' = -45 / C1-O3'-C3'-H3' = 38 (conformation shown in figure 4) and H1-C1-O3'-C3' = -40 / C1-O3'-C3'-H3' = -10. Neither of these conformations lead to unfavourable steric interactions. The observation of a very weak 2H1-1H2 NOE in the long mixing time NOESY is consistent with the first conformation (shown in figure 4) rather than the second, as long as the effects of spin-diffusion can be ignored. Of course, the NMR data observed is an average over all possible conformations on the 10^{-6} s time scale and thus if the compound adopts several conformations in equilibrium, the conformers given above will only represent these averages. However, it is interesting to note that the average NMR conformation of the glycosidic linkage [and that of Manα1,3DMJ (9), see below] is significantly different from the Manα1,3Man linkage in $Man_9GlcNAc_2$.[23]

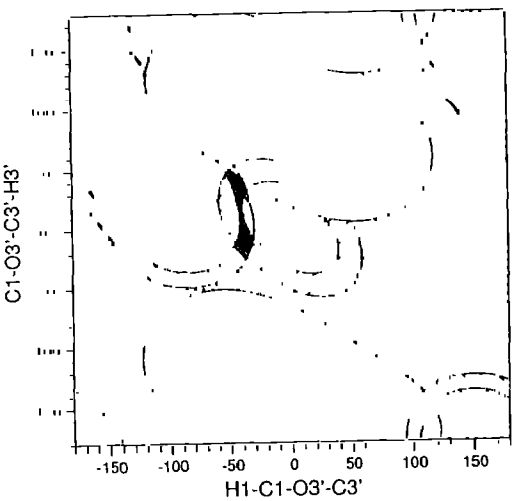


Figure 3. Torsion angle map for the α1,3 linkage of (6) showing the observed NOE data (the 400 ms NOESY is shown in figure 2). The shaded areas give the regions of torsion angle space consistent with the absence of the 2H1-1H2 and 2H1-1H4 NOEs (assuming a 3Å minimum distance). The diagonal shaded area {////} gives the region of torsion angle space consistent with a 2H1-1H3 distance of 2.2Å to 2.4Å and the diagonal shaded area {\\\\} gives the region consistent with a 2H5-1H2 distance of 2.6Å to 3.0Å. The dark shaded area gives the regions of torsion angle space consistent with all the observed NOE data.

NMR Analysis of Manα1,3DMJ (9).

Linkage analysis and conformation. The 1D ^1H NMR spectrum of Manα1,3DMJ (9) is shown in figure 1A. From peak intensities and assignments, it is clear that (9) consists of a $^4C_1$ D-mannopyranose ring in the α anomeric configuration linked to a $^4C_1$ DMJ ring (purity approximately 95%). The pattern of inter-residue NOEs at 400 ms mixing time is identical to that observed for compound (6), indicating a 1,3 linkage. The

NOE build-up curves have not been determined for (9); however the relative intensities of the NOEs in the 400 ms mixing time NOESY are very close to those obtained for compound (6), suggesting a similar average conformation.

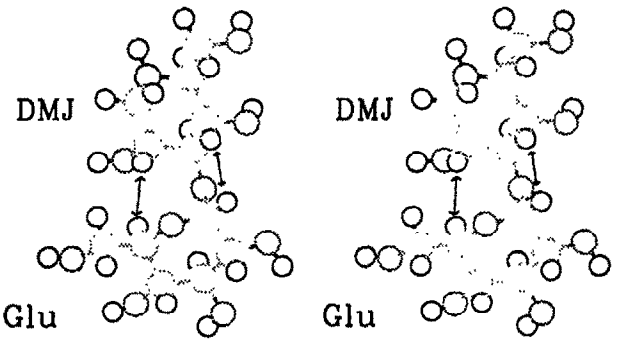


Figure 4. Stereodiagram of Glcα1,3DMJ (6) in one of the conformations consistent with all the available NOE data (H1-C1-O3'-C3' = -45 / C1-O3'-C3'-H3' = 38). The arrows show the observed cross-linkage NOEs.

Endomannosidase Inhibition. Glcα1,3DMJ (6) was found to inhibit cleavage of $Glc_3Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ (Figure 5) with an IC_{50} of 5.6 μM (concentration of the compound at which 50% inhibition of enzyme activity occurred). This figure is in good agreement with that of previously published data[13] which used a more favorable enzyme substrate, $Glc_1Man_9GlcNAc$.[12] Significant inhibition of endomannosidase activity was also obtained with Manα1,3DMJ (9), (IC_{50}=25.1μM) and also confirms that modifications at the glycosyl C-2 result in decreased potency.[12] Other mannose analogues had little or no effect on endomannosidase activity. In similar assays to those performed in Fig 5, 3-*O*-methyl-DMJ (8) was partially inhibitory (IC_{50}=660μM) and both DMJ(3) and butylDMJ were only effective at high concentrations (82% and 91% of control activity at 6mM respectively).

These results confirm and extend previous work describing the effects of disaccharide inhibitors on endomannosidase activity.[13] The finding here that Manα1,3DMJ is a potent inhibitor of the enzyme and is not expected to be hydrolysed by either α-glucosidases or α-mannosidases may provide the means with which to probe for the role of the endomannosidase catalysed pathway in N-linked oligosaccharide biosynthesis in the absence of α-glucosidase inhibitors.

Although Glcα1,3DMJ (6) and Manα1,3DMJ (9) are good inhibitors of endomannosidase, it is still not clear whether even better inhibition would be obtained with glucose or mannose analogues of swainsonine or other mannofuranose aminosugar structures. For chemotherapeutic applications, there are significant synthetic challenges in the design of non-hydrolysable disaccharide equivalents of these materials.

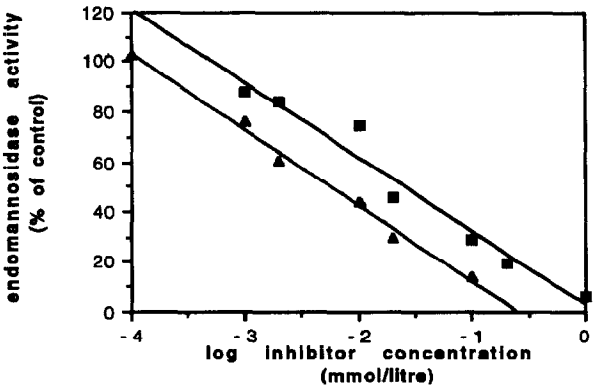


Figure 5. Effect of Glcα1,3DMJ (▲) and Manα1,3DMJ (■) on endomannosidase activity. Golgi membranes (50 μg protein) were incubated with ^{14}C-labelled $Glc_3Man_9GlcNAc_2$ (3750 dpm) in the presence of different amounts of inhibitor as shown. Endomannosidase released Glc_3Man_1 was recovered by lectin affinity chromatography as described in the text. Enzyme activity is expressed as a percentage of control reactions that contained no inhibitor.

Experimental. Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (at 200 MHz) or Bruker AM 500 (at 500 MHz) spectrometers. ^{13}C Nuclear magnetic resonance spectra (δ_C) were recorded on a Varian Gemini 200 (50 MHz). Multiplicities were assigned using a DEPT sequence. All nuclear magnetic resonance spectra were recorded on the Varian Gemini 200 at the relevant frequency, unless otherwise stated. All chemical shifts are quoted on the δ-scale using residual solvent as an internal standard. ^{13}C Spectra run in D_2O had 1,4-dioxane added as an internal standard. Infra red spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrometer. Mass spectra were recorded on VG 20-250, ZAB 1F, or TRIO-1 GCMS (DB-5 column) spectrometers using desorption chemical ionisation (NH_3, DCI) or chemical ionisation (NH_3, CI), as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis services of the Dyson Perrins laboratory. Thin layer chromatography was carried out on aluminium sheets coated with 60 F_{254} silica or glass plates coated with silica blend GF_{254}. Plates were developed using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid, or 0.5 % ninhydrin in methanol for amines. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Dry column chromatography was carried out using Merck Kieselgel 60H. Solvents and commercially available reagents were dried and purified before use according to standard procedures; dichloromethane was refluxed over and distilled from calcium hydride; pyridine was distilled from, and stored over, potassium hydroxide; tetrahydrofuran was distilled, under nitrogen, from a solution dried in the presence of benzophenone. *N,N*-Dimethylformamide was distilled under reduced pressure from calcium hydride. Hexane was distilled at 68°C, and ethyl acetate at 78°C, before use to remove involatile fractions. All solvents were removed *in vacuo*. Molecular sieves (powdered) were activated by flaming at atmospheric pressure for 30 minutes, then cooled under nitrogen before use. 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (10) was prepared as previously described.[19]

2,3,4,6-Tetra-O-benzyl glucose and 2,3,4,6-tetra-O-benzyl mannose were purchased from the Sigma Chemical Company and converted, respectively, into 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (14) and 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl chloride (15) were prepared by minor modifications of the literature procedures.[20,21] For the NMR analysis of the azadisaccharides (6) and (9), 500 MHz ^1H NMR spectra were run on a Varian Unity 500 spectrometer, with a probe temperature of 30°C. One-dimensional (1D) and phase-sensitive two-dimensional (2D) COSY spectra were recorded in ^2H_2O on all products (see experimental details). Phase-sensitive 2D NOESY spectra on Glcα1,3DMJ (6) were recorded with mixing times of 100 ms, 150 ms, 200 ms and 400 ms, without any random variation of the mixing times. Absolute peak volumes were measured from the phase-sensitive data set. Any cross-peaks due to scalar coupling are anti-phase and thus will have an absolute volume of zero. They will therefore effect the shape of the resultant dipolar cross-peak but not its volume integral. NOE build up curves were plotted for all cross-peaks of interest. These were linear in the mixing time range 0 to 200 ms but spin-diffusion was observed at 400 ms mixing time. Inter-proton distances were then determined using the two-spin approximation in the linear regions of the NOE build-up curves and using intra-residue proton-proton distances as calibration.[24] Spectral noise was estimated by measuring the volume integrals of regions of the base-line around the cross-peaks, leading to errors of approximately 0.1 to 0.2 Å in the calculated distances. A NOESY spectrum with a 400 ms mixing time was also recorded on Manα1,3DMJ (9). 1D spectra are presented without prior mathematical manipulation before the Fourier transform. Unshifted cosine-bell window functions were used in both domains when processing NOESY spectra. Molecular modelling was performed on a Silicon Graphics Personal IRIS workstation using INSIGHT and DISCOVER software (Biosym. Technologies Inc.). For the endomannosidase inhibition assay, rat liver Golgi membranes (50μg protein) were purified[25] as a source of endomannosidase and were preincubated with 1 mM deoxynojirimycin (1), and 10mM EDTA for 30 min at 2°C to inhibit α-glucosidases and α-mannosidases. Endomannosidase inhibitors were added at varying concentrations and preincubated for a further 30 min. The reaction was started by the addition of radiolabelled oligosaccharide substrate, [^{14}C-*glc*] $Glc_3Man_9GlcNAc_2$ (3,750 dpm), prepared[26] from metabolically labelled bovine thyroid micromes and incubation allowed to proceed for 14h at 37°C. The reaction was stopped by protein precipitation with ice cold PCA/PTA and the soluble reactants separated by chromatography using a concanavalin A-Sepharose column equilibrated with 20mM TrisHCl buffer, pH 8.0, containing 1mM $CaCl_2$ and 1mM $MnCl_2$. Endomannosidase released, radiolabelled Glc_3Man_1 eluting in the non-bound fraction from the affinity matrix was measured by scintillation counting. Examination of the reaction products from these digestions by thin layer chromatography[10] confirmed that only [^{14}C-*glc*] labelled Glc_3Man_1 was released using the assay conditions described above.

2,4-Di-O-benzyl-N-benzyloxycarbonyl-6-O-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-D-mannitol (11) A solution of 2,4-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (10) (1.05g, 2.2 mmol) in dry *N,N*-dimethylformamide (10 ml) was cooled to -20° C and treated with *tert* -butyldimethylsilylchloride (437 mg, 2.9 mmol, 1.3 equivalents) and imidazole (449 mg, 6.6mmol, 3 equivalents). After 75 minutes t.l.c. (ethyl actetate - hexane 1:1) showed no starting material (R_f 0.15) and one product (R_f 0.8). The reaction was quenched by the addition of a few drops of water, the solvent removed and the residue partitioned between water (30 ml) and dichloromethane (30 ml). The aqueous layer was extracted with dichloromethane (3 x 30 ml) and the combined organic extracts dried ($MgSO_4$), filtered and evaporated. Purification by flash

chromatography (ether-hexane 1:3) gave *2,4-di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-D-mannitol (11)* (1.21g, 2.05 mmol, 93 %) as a colourless oil. $[\alpha]_D^{25}$ -17.7 (*c*, 1.09 in $CHCl_3$); ν_{max} (film) 3468 (OH), 1700 (C=O) cm^{-1}; δ_H ($CDCl_3$) 7.21-7.39 (15H, m, H-Ph), 5.15 (2H, ABq, CH_2-Z, *J* 12.5 Hz), 3.77-4.32 (11H, m), 3.01-3.11 (1H, m), 0.87 (9H, s, $C(CH_3)_3$), 0.01, 0.02 (6H, 2 x s, CH_3). δ_C ($CDCl_3$) 156.7, 156.1 (2 x s, C=O), 138.2, 138.1, 137.0 (3 x s, C-Ph), 128.6, 128.1, 128.0, 127.7 (4 x d, HC-Ph), 75.6, 75.4 (2 x d, C-2), 72.6 (d, C-3), 71.4 (t, CH_2-Ph), 68.2, 68.0 (2 x d, C-4), 67.3 (t, CH_2-Z), 61.3, 60.9 (2 x t, C-1), 55.4, 54.4 (2 x d, C-5), 38.5, 37.5 (2 x t, C-6), 25.8 (q, $C(\underline{C}H_3)_3$), 18.1 (s, $\underline{C}(CH_3)_3$), -5.6 (q, $Si(CH_3)_2$). *m/z* : 91 ($C_7H_7^+$, 100%), 592 ($M+NH_4^+-H_2O$, 44%). (Found C, 69.15; H, 7.81; N, 2.32. $C_{34}H_{45}NO_6Si$ requires C, 69.00; H, 7.66; N, 2.37 %).

2,4-Di-O-benzyl-N-benzyloxycarbonyl-6-O-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-O-methyl-D-mannitol (12) A solution of 2,4-di-*O* -benzyl-*N*-benzyloxycarbonyl-6-*O-tert* -butyldimethylsilyl-1,5-dideoxy-1,5-imino-D-mannitol (11) (93 mg, 0.16 mmol) in dry tetrahydrofuran (2 ml) was treated with methyl iodide (11.2 µl, 0.18 mmol, 1.1 equivalents), potassium hydroxide (16.1 mg, 0.29 mmol), and 18-crown-6 (1.7 mg, catalytic amount). The mixture was stirred at room temperature for 4 hours by which time t.l.c. (ether-hexane 1:2) showed no starting material (R_f 0.3) and one product (R_f 0.5). The solvent was removed and the residue dissolved in dichloromethane (40 ml). The dichloromethane layer was washed with water (3 x 40 ml), brine (40 ml), dried ($MgSO_4$), filtered and evaporated. Purification by flash chromatography (ether-hexane 1:5) gave *2,4-di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-O-methyl-D-mannitol (12)* (71 mg, 0.12 mmol, 73 %) as a colourless oil. $[\alpha]_D^{20}$ -30.6 (*c*, 1.12 in $CHCl_3$); ν_{max} (film) 1700 (C=O) cm^{-1}; δ_H ($CDCl_3$) 7.20-7.36 (15H, m, H-Ph), 5.15, 5.13 (2H, 2 x s, CH_2-Z), 4.71-4.38 (5H, m), 4.29 (1H, dd, *J* 5.0 Hz, *J* 12.9 Hz), 4.08-3.60 (5H, m), 3.41, 3.39 (3H, 2 x s, CH_3-O), 3.05 (1H, q, *J* 11.6 Hz), 0.87, 0.86 (9H, 2 x s, $(CH_3)_3C$), 0.05, 0.03, 0.00, -0.01 (6H, 2 x s, CH_3Si); δ_C ($CDCl_3$) 156.2, 155.8 (2 x s, C=O), 138.3, 137.9, 136.7 (3 x s, C-Ph), 128.3, 127.8, 127.6, 127.4 (4 x d, HC-Ph), 67.2 (t, CH_2-Z), 60.3, 59.9 (2 x t, C-1), 58.9 (q, CH_3-O), 55.4, 54.4, (2 x d, C-5), 38.9, 39.4 (2 x t, C-6), 25.8 (q, $(\underline{C}H_3)_3C$), 18.1 (s, $(CH_3)_3\underline{C}$), -5.5, -5.6 (2 x q, $(CH_3)_2Si$); *m/z* (FAB+) 91 ($C_7H_7^+$, 100 %), 606 ($M+H^+$, 2 %). (Found C, 69.37; H 8.12; N 2.20. $C_{35}H_{47}NO_6Si$ requires C, 69.39; H,7.82; N, 2.31 %).

2,4-Di-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-3-O-methyl-D-mannitol (13) A solution of 2,4-di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O-tert*-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-*O*-methyl-D-mannitol (12) (58 mg, 0.10 mmol) in dry tetrahydrofuran (2ml) was treated with tetra-*N*-butylammonium fluoride (1.0 M solution in tetrahydrofuran, 42 µl, 0.14 mmol). The reaction was stirred at room temperature for 3 hours by which time t.l.c (ether-hexane 1:2) showed no starting material (R_f 0.4) and one product (R_f 0.05). The reaction mixture was partitioned between ether (30 ml) and water (30 ml). The ether layer was washed with brine (30 ml), dried ($MgSO_4$), filtered and evaporated. Purification by flash chromatography gave *2,4-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-3-*O*-methyl-D-mannitol (13)* (27 mg, 0.055 mmol, 57%) as a colourless oil, $[\alpha]_D^{25}$ -37.7 (*c*, 1.30 in $CHCl_3$), ν_{max} (film) 3295 (OH), 1600 (C=O) cm^{-1}. δ_H ($CDCl_3$) 7.21-6.92 (15H, m-Ph), 5.06 (2H, ABq, CH_2-Z, $J_{H,H'}$ 12.5 Hz), 4.50 (dt, 1H, *J* 1.9 Hz, *J* 6.8 Hz), 4.46 (d, 1H, H-6, $J_{6,6'}$ 11.8 Hz), 4.34 (2H, ABq, *J* 12.0 Hz), 4.24 (1H, d, *J* 11.8 Hz), 4.23 (1H, dd), 3.82 (1H, ddd, *J* 2.8 Hz, *J* 4.8 Hz, *J* 10.7 Hz), 3.77 (1H, d, *J* 7.0 Hz), 3.71 (1H, d, *J* 6.8 Hz), 3.66 (1H, dd, *J* 2.0 Hz, *J* 4.0 Hz), 3.52 (1H, t, *J* 3.1 Hz), 3.28 (1H, dd, *J* 10.7 Hz, *J* 13.0 Hz), 3.25 (3H,

s, CH_3); δ_C ($CDCl_3$) 156.5 (s, C=O), 138.3, 137.8, 136.7 (3 x s, C-Ph), 128.5, 128.41, 128.38, 127.8, 127.7, 127.6 (7 x d, HC-Ph), 77.8, 74.8, 73.0 (3 x d, C-2, C-3, C-4), 71.6, 71.5, 67.4, 61.4 (4 x t, 3 x CH_2-Ph, C-1, C-6), 59.4 (q, CH_3), 55.8 (d, C-5); *m/z* (CI, NH_3): 384 (M-OBn-H_2O+NH_4^+, 100%), 492 ($M+H^+$, 18%). Found C, 71.14; H, 6.87; *N*, 3.00. $C_{29}H_{33}NO_6$ requires C, 70.86; H, 6.77; N, 2.85 %).

1,5-Dideoxy-1,5-imino-3-O-methyl-D-mannitol [3-O-Me-DMJ] (8) 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*tert*-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-*O*-methyl-D-mannitol (12) (43 mg, 0.070 mmol) was dissolved in ethanol (1 ml) and added to a pre-reduced mixture of palladium black (5 mg) and ethanol (2 ml) under hydrogen. Concentrated hydrochloric acid (0.5 ml) was added and the mixture was stirred at room temperature for 4 days by which time t.l.c. (ether-hexane 1:2) showed no starting material (R_f 0.5) and one product (R_f 0.0). The reaction mixture was filtered through Celite and the solvent removed to give *1,5-dideoxy-1,5-imino-3-*O*-methyl-D-mannitol (8)* (11 mg, 0.062 mmol, 89 %) as a pale yellow oil. $[\alpha]_D^{20}$ -18.9 (*c*, 0.53 in water); δ_H (D_2O) 4.57 (s, 1H, H-1), 4.06 (dd, 1H, H-6, $J_{6,5}$ 3.1 Hz, $J_{6,6'}$ 12.5 Hz), 3.97 (t, 1H, H-4, *J* 10.3 Hz), 3.91 (dd, 1H, H-6', $J_{6',5}$ 6.6 Hz, $J_{6',6}$ 12.5 Hz), 3.53 (s, 3H, CH_3), 3.54 (d, 1H, H-2, *J* 3.7 Hz), 3.46 (dd, 1H, H-3, $J_{3,2}$ 2.9 Hz, $J_{3,4}$ 9.5 Hz), 3.30 (d, 1H, H-1, *J* 13.8 Hz), 3.25 (ddd, 1H, H-5, $J_{5,4}$ 9.6 Hz, $J_{5,6}$ 3.1 Hz, $J_{5,6'\ 6,6}$ 6.6 Hz); δ_C (D_2O/Dioxan) 82.8 (d, C-3), 66.1, 62.8 (2 x d, C-2, C-4), 61.5 (d, C-5), 59.2 (t, C-6), 58.0 (q, CH_3), 48.5 (t, C-1); *m/z* (electrospray) 178 ($M+H^+$, 100%).

2,3,4,6-Tetra-O -benzyl-α-D-glucopyranosyl chloride (14) 2,3,4,6-Tetra-*O*-benzyl-α-D-glucose (1.20 g, 2.2 mmol) was added to a freshly prepared solution of *N,N*-dimethylchloroforminium chloride (0.7 ml *N,N*-dimethylformamide, 4.3 ml thionyl chloride). The reaction was stirred at room temperature for 3.5 hours by which time t.l.c. (ether-hexane 1:3) showed no starting material (R_f 0.05) and one product (R_f 0.35). The solvent was evaporated and the residue co-evaporated with toluene (3 x 2ml). Purification by flash chromatography (ether-hexane 1:4) gave 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (14) (1.025 g, 83%) as a colourless oil. $[\alpha]_D^{25}$ +71.3 (*c*, 1.1 in $CHCl_3$) [Lit.[20] +62 (*c*, 1 in $CHCl_3$)]; δ_H ($CDCl_3$) 7.27-7.39 (18H, m, H-Ph), 7.16-7.18 (2H, m, H-Ph), 6.09 (1H, d, H-1, $J_{1,2}$ 3.8 Hz), 4.99, 4.53 (2H, ABq, CH_2-Ph, $J_{H,H'}$ 10.9 Hz), 4.86, 4.84 (2H, ABq, CH_2-Ph, $J_{H,H'}$ 8.8 Hz), 4.75, 4.72 (2H, ABq, CH_2-Ph, $J_{H,H'}$ 11.8 Hz), 4.59, 4.48 (2H, ABq, CH_2-Ph, $J_{H,H'}$ 12.1 Hz), 4.11 (1H, dt, H-5, $J_{5,6}$ 2.6 Hz, $J_{4,5}$ 10.2 Hz), 4.05 (1H, t, H-4, *J* 9.2 Hz), 3.79 (1H, dd, H-6, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 11.0 Hz), 3.75 (2H, m, H-2, H-3), 3.67 (1H, dd, H-6', $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 11.0 Hz).

2,4-Di-O-benzyl-N-benzyloxycarbonyl-6-O-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-O-(2,3,4,6-tetra-O-benzyl-αβ-D-glucopyranosyl)-D-mannitol (16) and (17). 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*tert*-butyldimethylsilyl-1,5-dideoxy-1,5-imino-D-mannitol (11) (320 mg, 0.54 mmol) was dissolved in dry dichloromethane (3ml). 2,3,4,6-Tetra-*O* -benzyl-α-D-glucopyranosyl chloride (14) (347 mg, 0.62 mmol, 1.15 equivalents), 2,4,6-trimethylpyridine (142 µl, 1.08 mmol, 2 equivalents) and dry 3Å molecular sieves (0.2 g) were added. The reaction was cooled to -60°C under nitrogen in the dark and silver trifluoromethanesulphonate (277 mg, 1.08 mmol, 2 equivalents) was added. The reaction was allowed to warm to -20°C over 3 hours by which time t.l.c. (ether-hexane 1:2, 2 elutions) showed very little of either starting material (R_f 0.55, 0.65) and one main product (R_f 0.4). The reaction was stirred in the light at room temperature for 10 minutes to precipitate silver salts. The solution was filtered through Celite, washed with 2M aqueous hydrochloric acid (20 ml), brine (20 ml), dried ($MgSO_4$), filtered, and evaporated. Purification

by flash chromatography (ether-hexane 1:6) gave both anomers of *2,4-di-O-benzyl-N-benzyloxycarbonyl-6-O-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosyl)-D-mannitol* (α-anomer (16), 208 mg, 0.19 mmol; 35%; β-anomer (17), 28 mg, 0.025 mmol, 5%) as colourless oils. *α-anomer (16)*, R_f 0.25 (ether-hexane 1:2);.$[\alpha]_D^{20}$ +27.9 (*c*, 0.76 in $CHCl_3$); ν_{max} (film) 1699 (C=O) cm^{-1}; δ_H ($CDCl_3$) 7.28-6.99 (35H, m, H-Ph), 6.00 (1H, d, H-1, $J_{1,2}$ 3.7 Hz), 5.15-2.94 (28H, m), 0.73 (9H, s, $(CH_3)_3C$), -0.12, -0.16 (6H, 2 x s, CH_3-Si); δ_C ($CDCl_3$) 157.0 (s, C=O), 139.2, 138.7, 138.5, 138.4, 138.3, 138.1, 138.0, 137.1 (8 x s, 7 x C-Ph), 128.7-127.6 (HC-Ph), 97.8 (d, C-1), 82.4, 79.8, 77.6, 75.4 (4 x d, C-2, C-3, C-4, C-5), 75.8, 75.0, 73.8, 73.5, 71.5, 70.9, 67.7, 67.3 (8 x t, 6 x CH2-Ph, CH2-Z, C-6), 74.4, 72.5, 70.3 (3 x d, C'-2, C'-3, C'-4,), 60.8, 60.5 (2 x t, C'-1), 56.0 (d, C'-5), 37.5 (t, C'-6), 25.8 (q, $(\underline{C}H_3)_3C$), 18.0 (s, $(CH_3)_3\underline{C}$), -5.3, -5.4 (2 x q, CH_3-Si); *m/z* (matrix assisted laser desorption) 1137 ($M+Na^+$); (Found C, 73.22; H, 7.11; N, 1.17. $C_{68}H_{79}NO_{11}Si$ requires C,73.29; H, 7.14; N, 1.26 %). *β-anomer (17)*, R_f 0.35 (ether-hexane 1:2); $[\alpha]_D^{20}$ -4.8 (*c*, 0.90 in $CHCl_3$); ν_{max} (film) 1699 (C=O) cm^{-1}; δ_H ($CDCl_3$);δ_C ($CDCl_3$) 156.4 (s, C=O), 138.7, 138.6, 138.25, 138.20, 136.7 (7 x s, 7 x C-Ph), 128.5-127.4 (HC-Ph), 103.4 (d, C-1), 84.7, 82.3, 78.0,75.3, (4 x d, C-2, C-3, C-4, C-5), 75.6, 75.0, 74.9, 74.8, 73.5, 71.3, 69.1, 67.3 (8 x t, 6 x CH_2-Ph, CH_2-Z), 75.3, 72.5, 68.2 (3 x d, C'-2, C'-3, C'-4), 65.8, 65.5 (2 x t, C-6), 61.2, 60.9 (2 x t, C'-1), 55.4, 54.4 (2 x d, C'-5), 38.6, 37.6 (2 x t, C'-6), 25.8 (q, $(\underline{C}H_3)_3C$), 18.2 (s, $(CH_3)_3\underline{C}$), -5.5 (q, 2 x CH_3-Si); *m/z* (matrix assisted laser desorption) 1137 ($M+Na^+$).

1,5-Dideoxy-3-O-(α-D-glucopyranosyl)-1,5-imino-D-mannitol [Glcα1-3DMJ] (6) 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-D-mannitol (16) (145 mg, 0.13 mmol) was dissolved in ethanol (1 ml) and added to a pre-reduced mixture of palladium black (5 mg) and ethanol (2 ml) under hydrogen. 0.05 ml concentrated hydrochloric acid was added and the mixture was stirred at room temperature for 16 days by which time t.l.c. (ether-hexane 1:2) showed no starting material (R_f 0.25) and one product (R_f 0.0). The reaction mixture was filtered through Celite and the solvent removed. The crude product was purified by ion exchange chromatography (Amberlite AG-50, H^+ form, eluting with 1M NH_3 (aq)) and the solvent removed. Co-evaporation with water gave *1,5-dideoxy-3-O-(α-D-glucopyranosyl)-1,5-imino-D-mannitol (6)* (39 mg, 0.12 mmol, 92 %).as a colourless oil. $[\alpha]_D^{20}$ +49.6 (*c*, 0.55 in water); δ_H (D_2O) 5.28 (1H, s, 2H1), 4.30 (1H, s, 1H2), 3.95 (1H, d, 1H4, *J* 10.3 Hz), 3.79-3.91 (6H, m, 1H6, 1H6', 2H3, 2H5, 2H6, 2H6'), 3.76 (1H, d, 1H3, *J* 9.5 Hz), 3.61 (1H, dd, 2H2, *J* 3.7 Hz, *J* 9.5 Hz), 3.47 (1H, t, 2H4, *J* 9.5 Hz), 3.18 (1H, d, $1H1_{eq}$, $J_{1,1'}$ 13.2 Hz), 3.00 (1H, d, $1H1_{ax}$, $J_{1,1'}$ 13.9 Hz), 2.82 (1H, bs, 1H5); δ_C (D_2O/dioxane) 101.3 (d, C-1), 80.9, 73.6, 72.4, 70.4 (4 x d, C-2, C-3, C-4, C-5), 73.2 (d, C'-2), 66.4, 65.9 (2 x d, C'-3, C'-4), 61.4 (t, C-6), 61.0 (d, C'-5), 58.7 (t, C'-6), 48.2 (t, C'-1); *m/z* (electrospray) 326 ($M+H^+$, 100%).

2,3,4,6-Tetra-O -benzyl-α-D-mannopyranosyl chloride (15). 2,3,4,6-Tetra-*O* -benzyl-α-D-mannose (380 mg, 0.70 mmol) was added to a freshly prepared solution of *N,N*-dimethylchloroforminium chloride (0.2 ml *N,N*-dimethylformamide, 1.4 ml thionyl chloride). The reaction was stirred at room temperature for 20 hours by which time t.l.c. (ether-hexane 1:2) showed no starting material (R_f 0.1) and one product (R_f 0.55). The solvent was evaporated and the residue co-evaporated with toluene (3 x 2ml). Purification by flash chromatography (ether-hexane 1:5) gave 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl chloride (15) (221 mg, 0.40 mmol, 57%) as a colourless oil. $[\alpha]_D^{25}$ +76.4 (*c*, 0.99 in $CHCl_3$); δ_H ($CDCl_3$) 7.40-7.23 (20H, m, H-Ph), 6.17 (1H, bs, H-1), 4.96 (1H, d, CH_2-Ph, $J_{H,H'}$ 10.7 Hz), 4.77-4.54 (7H, m, CH_2-Ph), 4.23-4.09

(3H, m), 3.94-3.78 (3H, m); δ_C ($CDCl_3$) 138.5, 138.3, 137.9 (3 x s, C-Ph), 128.7, 128.6, 128.1, 127.8 (4 x d, HC-Ph), 91.7 (d, C-1) [lit.[21] δ_C ($CDCl_3$) 91.7 (d, C-1)], 75.4, 73.5, 73.0, 72.6, 68.3 (5 x t, C-6, CH_2-Ph), 78.4, 77.8, 74.7, 74.1 (4 x d, C-2, C-3, C-4, C-5).

2,4-Di-O-benzyl-N-benzyloxycarbonyl-6-O-tert-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-D-mannitol (18) 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*tert*-butyldimethylsilyl-1,5-dideoxy-1,5-imino-D-mannitol (11) (203 mg, 0.34 mmol) was dissolved in dry dichloromethane (6ml). 2,3,4,6-Tetra-*O* -benzyl-α-D-mannopyranosyl chloride (15) (221 mg, 0.40 mmol, 1.15 equivalents), 2,4,6-trimethylpyridine (91 µl, 0.69 mmol, 2 equivalents) and dry 3Å molecular sieves (0.2 g) were added. The reaction was cooled to -60°C under nitrogen in the dark and silver trifluoromethanesulphonate (177 mg, 0.69 mmol, 2 equivalents) was added. The reaction was allowed to warm to -20°C over 1 hour and stood at -20°C for 20 hours, by which time t.l.c. (ether-hexane 1:2, 2 elutions) showed very little of either starting material (R_f 0.55, 0.35) and one main product (R_f 0.5). The reaction was stirred in the light at room temperature for 10 minutes to precipitate silver salts. The solution was filtered through Celite, washed with 2M aqueous hydrochloric acid (10 ml), brine (10 ml), dried ($MgSO_4$), filtered, and evaporated. Purification by flash chromatography (ether-hexane 1:6) gave *2,4-di*-O-*benzyl*-N-*benzyloxycarbonyl-6*-O-tert-*butyldimethylsilyl-1,5-dideoxy-1,5-imino-3*-O-*(2,3,4,6-tetra*-O-*benzyl-α-D-mannopyranosyl)-D-mannitol (18)* (83 mg, 0.07 mmol, 22 %, 33% yield accounting for recovered starting material) as a colourless oil. $[\alpha]_D^{20}$ +24.2 (*c*, 1.17 in $CHCl_3$); ν_{max} (film) 1702 cm^{-1}; δ_H ($CDCl_3$) 7.37-7.15 (35H, m, H-Ph), 5.18-3.47 (29H, m), 0.86 (9H, s, $(CH_3)_3C$), -0.01, -0.04 (6H, 2 x s,CH_3-Si); δ_C ($CDCl_3$) 156.5 (s, C=O), 139.0, 138.6, 138.4, 137.9, 136.8 (5 x s, C-Ph), 96.4 (d, C-1), 80.2, 77.6, 75.2, 74.7 (4 x d, C-2, C-3, C-4, C-5), 75.0, 73.4, 72.8, 72.4, 71.3, 71.2, 68.7, 67.5 (8 x t, 6 x CH_2-Ph, CH_2-Z, C-6), 71.9, 71.8, 71.7 (3 x d, C'-2, C'-3, C'-4), 60.4 (t, C'-1), 55.6 (d, C'-5), 38.1 (t, C'-6), 25.8 (q, $(\underline{C}H_3)_3C$), 18.0 (s, $(CH_3)_3\underline{C}$), -5.3 (q, CH_3-Si); *m/z* (matrix assisted laser desorption) 1137 ($M+Na^+$).

1,5-Dideoxy-3-O-(α-D-mannopyranosyl)-1,5-imino-D-mannitol [Manα1-3DMJ] (9) 2,4-Di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*tert*-butyldimethylsilyl-1,5-dideoxy-1,5-imino-3-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-D-mannitol (9) (82 mg, 0.074 mmol) was dissolved in ethanol (1 ml) and added to a pre-reduced mixture of palladium black (5 mg) and ethanol (2 ml) under hydrogen. Concentrated hydrochloric acid (0.05 ml) was added and the mixture was stirred at room temperature for 5 days by which time t.l.c. (ether-hexane 1:1) showed no starting material (R_f 0.5) and one product (R_f 0.0). The reaction mixture was filtered through Celite and the solvent removed to give *1,5-dideoxy-3*-O-*(α-D-mannopyranosyl)-1,5-imino-D-mannitol (9)* (22 mg, 0.067 mmol, 91 %) as a pale yellow oil. $[\alpha]_D^{20}$ +27.8 (*c*, 0.40 in water); δ_H (D_2O) 5.16 (1H, s, 2H1), 4.25 (1H, bs, 1H2), 4.12 (1H, bs, 2H2), 3.93 (1H, m, 2H3), 3.71-3.89 (3H, m, 1H4, 1H6, 1H6'), 3.72 (1H, m, 1H3), 3.10 (1H, d, 1H1eq, $J_{1,1'}$ 13.5 Hz), 2.91 (1H, d, 1H1ax, $J_{1,1'}$ 13.5 Hz), 2.69 (1H, m, 1H5); δ_C (D_2O/Dioxan) 103.1 (d, C-1), 80.6, 74.2, 71.0, 70.7, 67.4 (5 x d, C-2, C-3, C-4, C-5, C'-2), 66.2, 65.8 (2 x d, C'-3, C'-4), 61.7 (t, C-6), 61.0 (d, C'-5), 58.7 (t, C'-6), 48.2 (t, C'-1); *m/z* (electrospray) 326 ($M+H^+$, 100%).

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REFERENCES

1. Winchester, B., Fleet, G. W. J., *Glycobiology*, 1992, **2**, 199.
2. Fellows, L. E., Kite, G. C., Nash, R. J., Simmonds, M. S. J., Scofield, A., in Poulton, J. E., Romeo, J. T., Conn, E. E. (Eds.) *Nitrogen Metabolism of Plants*, Clarendon Press, Oxford, pp. 271, **1992**.
3. Molyneux, R. J., Pan, Y. T., Tropea, J. E., Elbein, A. D., Lawyer, C. H., Hughes, D. J., Fleet, G. W. J., *J. Nat. Prod.*, 1993, in press.
4. Mysercough, P. M., Fairbanks, A. J., Jones, A. H., Choi, S.-S., Fleet, G. W. J., Al-Daher, S. S., Cenci di Bello, I., Winchester, B.,*Tetrahedron*, 1992, **48**, 10177; Bruce, I.,Fleet, G. W. J., Cenci di Bello I., Winchester, B.,*Tetrahedron*, 1992, **48**, 10191 and references cited therein.
5. Collyer, C. A., Goldberg, J. D., Viehmann, H., Blow, D. M., Ramsden, N. G., Fleet, G. W. J., Montgomery, F. J., Grice, P., *Biochemistry*, 1992, **31**, 12211.
6. Kornfeld, R., Kornfeld, S., *Annu. Rev. Biochem.* 1985, **54**, 631; Schwarz, R. T., *Behring Inst. Mitt.*, 1991, **89**, 198.
7. Winchester, B., Al-Daher, S., Carpenter, N. C., Cenci di Bello, I., Choi, S. S., Fairbanks, A. J., Fleet, G. W. J., *Biochem. J.*, 1993, **290**, 743.
8. Moore, S.E.H., Spiro, R.G.,*J. Biol. Chem.*, 1990, **265**, 13104.
9. Fujimoto, K., Kornfeld, R.,*J. Biol. Chem.*, 1991,**266**, 3571.
10. Moore, S.E.H., Spiro, R.G.,*J. Biol. Chem.*, 1992, **267**, 8443.
11. Lubas, W.A., Spiro, R.G., *J. Biol. Chem.*, 1987**262**, 3775.
12. Lubas, W.A., Spiro, R.G., *J. Biol. Chem.*, 1988, **263**, 3990.
13. Hiraizum, S., Spohr, U., Spiro, R.G., *J. Biol. Chem.*, 1993, **268**, 9927.
14. Walker, B. D., Kowalski, M., Goh, W. C., Kozarsky, K., Krieger, M., Rosen, C., Rohrschneider, L., Haseltine, W. A., Sodroski, J., *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 8120.
15. Karpas, A., Fleet, G.W.J., Dwek, R.A., Petursson, S., Namgoong, S.K., Ramsden, N.G., Jacob, G.S., Rademacher, T.W., *Proc. Nat. Acad. Sci USA*, 1988, **85**, 9229.
16. Tyms, A. S., Berrie, E. M., Ryder, T. A., Nash, R. J., Hegarty, M. P., Taylor, D. L., Mobberley, M. A., Davis, J. M., Bell, E. A., Jeffries, D. J., Taylor-Robinson, D., Fellows, L. E., *Lancet*, **1987**, 1025; Sunkara, P. S., Taylor, D. L., Kang, M. S., Bowlin, T. L., Liu, P. S., Tyms, A. S., Sjoerdsma, A., *Lancet*, **1989**, 1206.
17. 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. X., Witty, D. R., Jacob, G. S., Rademacher, T. W., *FEBS Lett.*, 1988, **237**, 128; Behling, J. R., Campbell, A. L., Babiak, K. A., Ng, J. S., Medich, J., Farid, P., Fleet, G. W. J.,*Tetrahedron*, 1993, **49**, 3359.
18. Fleet, G. W. J., Ramsden, N. G., Witty, D. R.,*Tetrahedron Lett.*, 1988, **29**, 2871; Fleet, G. W. J., Ramsden, N. G., Witty, D. R.,*Tetrahedron*, 1989, **45**, 319.
19. Fleet, G. W. J., Ramsden, N. G., Witty, D. R., *Tetrahedron*, 1989, **45**, 327.
20. Grob, V. D., Squires, T. G., Vercellotti, J. R., *Carbohydr. Res.*, 1969, **10**, 595; Leroux, J., Perlin, A. S., *Carbohydr. Res.*, 1978, **67**, 163.
21. Tamura, J., Horito, S., Yoshimura, J., Hashimoto, H., *Carbohydr. Res.*, 1990, **207**, 153.
22. Wormald, M. R., Edge, C. J., *Carbohydr.. Res.*, 1993, in press.
23. Wooten, E. W., Edge, C. J., Bazzo, R., Dwek, R. A., Rademacher, T. W., *Carbohydr. Res.*, 1990, **203**, 13.
24. Homans, S. W., Dwek, R. A., Rademacher, T. W., *Biochemistry*, 1987, **26**, 6553.
25. Tulsiani, D. R. P., Opheim, D. J., Touster, O., *J. Biol. Chem.*, 1977, **252**, 3277.
26. Grinna, L. S., Robbins, P. W., *J. Biol. Chem.*, 1979, **254**, 8814.