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# Synthesis of an eight carbon homologue of α-homomannojirimycin via a bicyclic aminolactone

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**Abstract**

A thermally induced intramolecular 1,3-dipolar cycloaddition of an azidoester and subsequent sodium cyanoborohydride reduction of the resulting bicyclic vinylogous urethane to give a bicyclic aminolactone allows access to an eight carbon homologue of α-homomannojirimycin which is a weak fucosidase inhibitor. Intermediates with both an α- and β-amino acid moiety are described and may be useful for incorporation of homopipecolic acids into novel peptide libraries. 

## 1. Introduction

Many highly oxygenated alkaloids, which can be viewed as mimics of individual sugars, have been isolated from a wide range of both exotic and common plants.[1,2] The leaves[3] and bulbs[4] of hyacinths contain a wide range of pyrrolidine alkaloids including DAB **1** and DMDP **2**, both of which occur in many disparate species of plants.[5] DMDP, which has also been isolated from *Streptomyces*,[6] is the major alkaloid present at all growth stages of *Hyacinthoides non-scripta* and is found at high concentrations in the leaves, seed pods and bulbs. Among the minor components isolated from *Hyacinthus orientalis*, two new alkaloids **3** and **5** (or their mirror images) were isolated[4] and provide the first examples of such alkaloids with a 2-hydroxyethyl side chain; both **3** and **5** have interesting properties as glycosidase inhibitors and thus are still recognised by some enzymes as sugar mimics.[4] Such compounds may be viewed as a homologous series where introduction of a hydroxymethyl substituent in DAB **1** gives either DMDP **2** or DGDP **4**; addition of a hydroxyethyl substituent in DAB (or insertion of a $CH_2$ group into **2** or **4**, respectively) would give either of the two epimers **3** or **5**.

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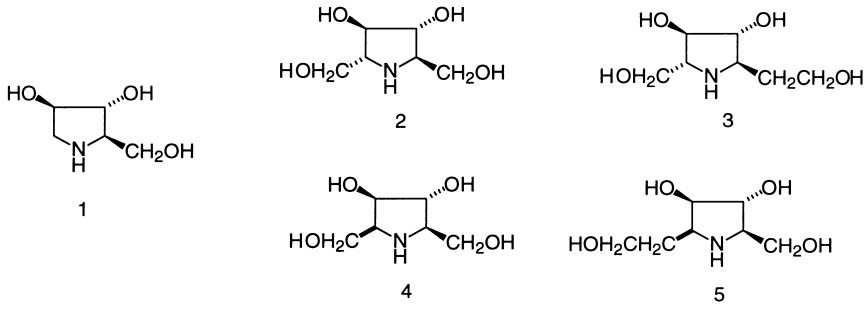
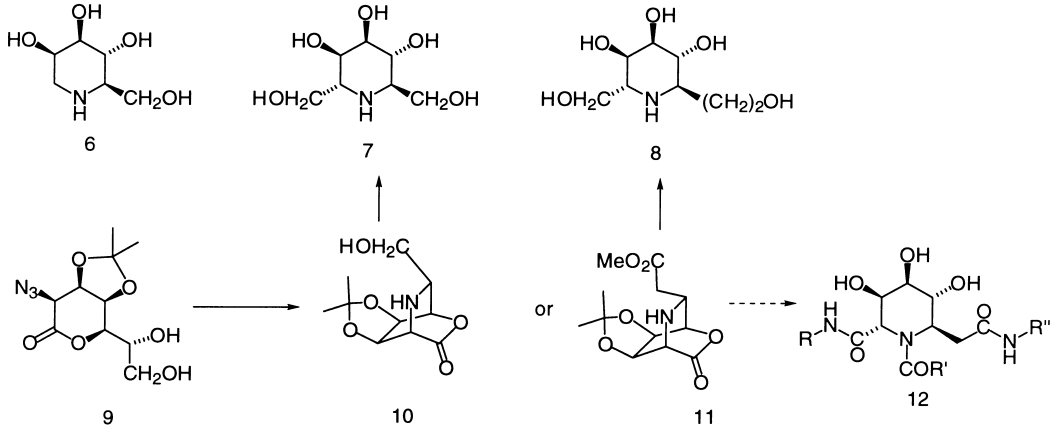



A similar series of sugar mimics could be derived from a piperidine; thus the natural products DMJ **6** and α-HMJ **7** are related by introduction of an α-hydroxymethyl group; further insertion of a methylene unit would give the eight carbon analogue of DMJ **8** and may provide a new class of bioactive pyranose mimics.




α-HMJ **7** has been synthesised from the bicyclic aminolactone **10** derived from the diol **9**.[7] This paper reports the synthesis of the homologous lactone **11** and its conversion to the mannose (or perhaps fucose) analogue **8** and reports preliminary investigation of the properties of **8** as a glycosidase inhibitor. Additionally, **11** possesses both α- and β-amino acid functionality and is therefore of interest as similar bicyclic lactones have been shown to be potential divergent intermediates for the introduction of hydroxylated prolines[8] into amide libraries and pipecolic acids into amide libraries.

## 2. Results and discussion

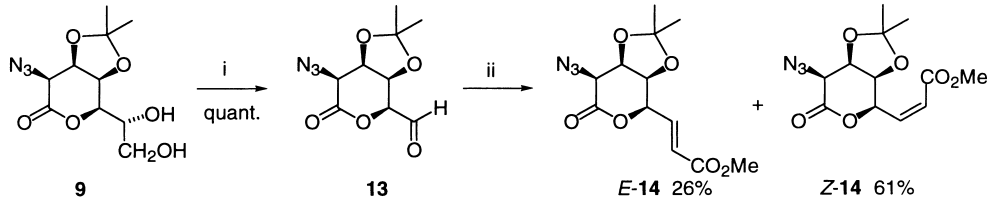
The key steps in the synthesis of the eight carbon deoxymannojirimycin analogue **8** are:

(i) the formation of the six membered piperidine ring by an intramolecular 1,3-dipolar cycloaddition reaction of the azidoesters **14**; and

(ii) a stereo- and chemoselective reduction of the vinylogous carbamate **15** by sodium cyanoborohydride.

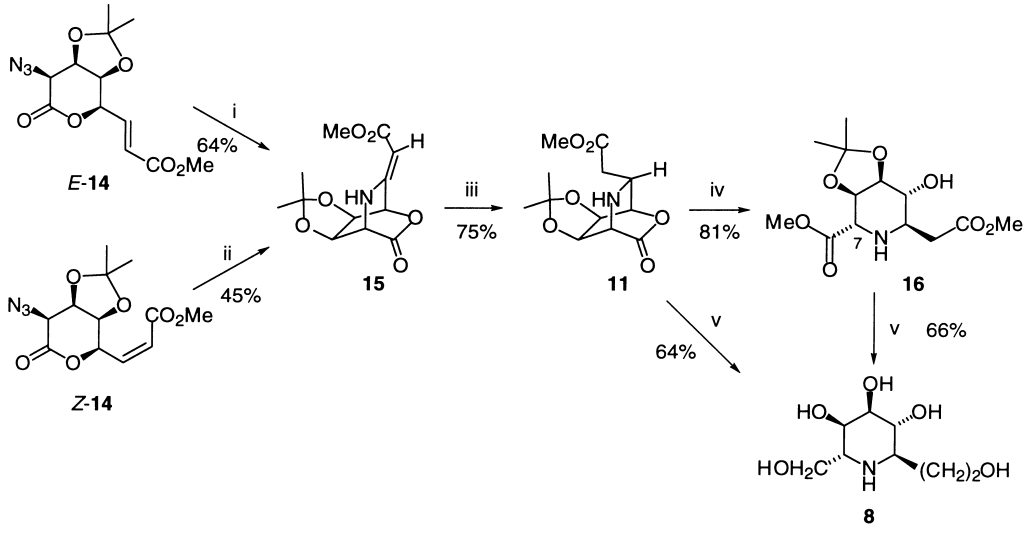
Cleavage of the vicinal diol unit in **9**[9] with periodic acid in THF afforded the unstable aldehyde **5** in quantitative yield; subsequent treatment of the aldehyde **5** with methoxycarbonylmethylene(triphenyl)-phosphorane in toluene gave the isomeric enoates *E*-**14** and *Z*-**14** required for the 1,3-dipolar cyclisation in yields of 26 and 61%, respectively (Scheme 1).

The intramolecular 1,3-dipolar cycloadditions of open-chain azido-enoates give a range of products with the outcome depending on the conditions of the reaction and specific structural features of the starting material.[10] The triazolines initially formed[11] may fragment to β-amino-α-diazoesters which can




Scheme 1. (i) $H_5IO_6$, THF; (ii) $Ph_3PCHCO_2Me$, toluene

further lose nitrogen to afford vinylogous urethanes.[12] When a solution of *E*-enoate *E*-**14** in toluene was heated to 100°C for 20 h, the *Z*-bicyclic vinylogous urethane **15** was isolated in 64% yield (Scheme 2); neither an intermediate triazoline nor a diazoester was isolated from the reaction mixture.




Scheme 2. (i) Toluene, 100°C; (ii) xylenes, 140°C; (iii) $NaBH_3CN$, AcOH; (iv) NaOAc, MeOH, Δ; (v) $LiBHEt_3$, THF, −60°C, then HCl, MeOH

Characteristic features of the $^1H$ and $^{13}C$ NMR spectra provided strong evidence for the proposed structure of **15**. The $^1H$ NMR spectrum indicated the presence of a broad signal for the NH-group at δ 8.10, and a singlet for the vinylic proton at δ 4.80. In addition, the $^{13}C$ NMR spectrum contained the olefinic carbons at δ 82.6 and 151.1. These signals are consistent with those observed for previously reported vinylogous urethane systems. The double bond geometry of this system was assigned as being *Z*, based on a significant NOE enhancement observed between H-2 and H-4 (Fig. 1). The formation of the *Z*-vinylogous urethane **15** under these conditions is consistent with observations reported for similar systems.[9,11]

Under the same conditions, the *Z*-enoate *Z*-**14** reacted very slowly to give the same bicyclic vinylogous urethane **15**, with prolonged reaction times resulting in low yields of **15** due to decomposition of product

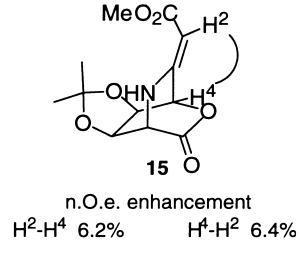



Figure 1.

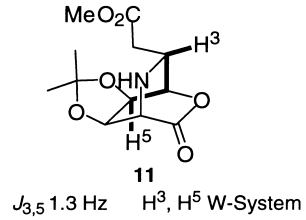
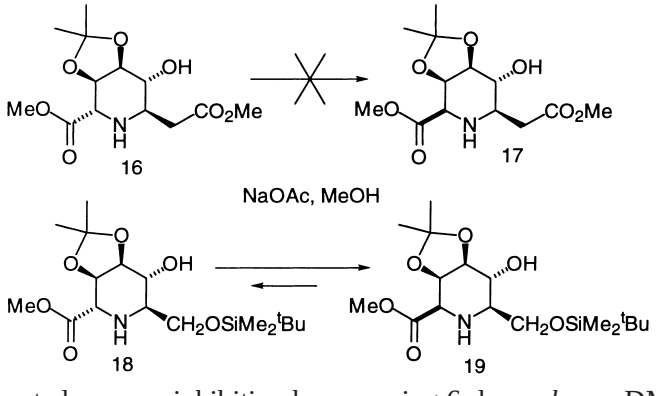



Figure 2.

and starting material. However, heating a solution of the *Z*-enoate *Z*-**14** in a mixture of xylenes at 140°C for 1 h afforded vinylogous urethane **15** in 45% yield.

All attempts at catalytic hydrogenation of the vinylogous urethane **15** were unsuccessful. Conditions for hydride reduction of the double bond have to be found under which there is no reduction of the reactive lactone carbonyl and no ring opening of the lactone by nucleophilic attack by solvent; sodium cyanoborohydride in acetic acid as a reagent satisfied those criteria and afforded the bicyclic amino-lactone **11** as a single stereoisomer in 75% yield. The configuration at C-3 was assigned based on the observed four-bond coupling between H-3 and H-5 ($^4J_{3,5}$ 1.3 Hz) in the $^1$H NMR spectrum due to their W-arrangement within the rigid [2.2.2] bicyclic system (Fig. 2). The high stereoselectivity of this reduction step is due to hydride delivery from the least hindered face of the tautomeric bicyclic iminium ion, i.e. opposite to the isopropylidene protecting group. This result is consistent with the high facial selectivity observed for the reduction of related bicyclic imines.[7,13]

Treatment of the bicyclic amino-lactone **11** in the presence of sodium acetate in methanol under reflux afforded a single diester **16** in 81% yield. Reduction of both the aminolactone **11** and the diester **16** with Super-Hydride® in THF at −60°C, with subsequent removal of the isopropylidene protecting group with methanolic hydrogen chloride, gave the eight carbon DMJ analogue **8** in yields of 64 and 66%, respectively. There was no evidence for any epimerisation of **16** to the diequatorial ester **17** which would have allowed access to an additional set of homopipecolic acid analogues. This is somewhat surprising since the mannose analogue **18** undergoes a very efficient isomerisation to the more thermodynamically stable isomer **19**; a similar isomerisation has also been observed in rhamnose analogues of **18** and **19**.[8,13] Both the amino-lactone **11** and the diester **16** contain both α- and β-amino acid functionality and are potentially useful intermediates for incorporation of pipecolic and homopipecolic acid moieties into amide libraries.




On the basis of the reported enzyme inhibition by occurring 6-deoxy-*homo*-DMDP **3**,[4] it would be difficult to predict which carbohydrate metabolising enzymes might be affected by DMJ homologues such as **8**, which was accordingly assayed against — and was inactive against — the following glycosidases: α-glucosidase (yeast, rice), β-glucosidase (almond), α-galactosidase (*Aspergillus niger*), β-galactosidase (bovine liver, *A. niger*, *Escherichia coli*), α-mannosidase (jack bean) and α-rhamnosidase (*Penicillium decumbens*). However, **8** displayed weak competitive inhibition against α-fucosidase (bovine kidney,

25% at 70 μM) and α-galactosidase (green coffee bean, $IC_{50}$ 52 μM). The inhibition of α-fucosidase and lack of inhibition of α-mannosidase by **8** is similar to the behaviour of other aza-D-mannopyranose analogues.[14,15]

In summary, this paper describes the stereoselective synthesis of an eight carbon homologue of α-homomannojirimycin, **8**, based on piperidine ring formation via 1,3-dipolar cycloaddition of azido-enoates to form a vinylogous urethane and subsequent sodium cyanoborohydride reduction. The azasugar **8** was found to inhibit an α-fucosidase and an α-galactosidase, and may be a framework for incorporation into larger structures in the search for more selective and potent inhibitors of carbohydrate processing enzymes. Two intermediates **11** and **16** having α- and β-amino acids may be useful intermediates for incorporation into potentially interesting structures providing 'core structures' of inhibitors of carbohydrate processing enzymes.

## 3. Experimental

### 3.1. General

THF was distilled from sodium before use and hexane refers to petroleum ether boiling in the range 60–80°C, distilled before use. All other solvents were used as supplied (AR or HPLC grade). Super-Hydride® refers to lithium triethylborohydride. Other reagents were used as supplied. TLC was performed on aluminium or plastic sheets coated with silica gel 60 $F_{254}$ visualisation being effected using 0.2% w/v cerium(IV) sulphate and 5% ammonium molybdate in 2 M sulphuric acid. Column chromatography was performed on Sorbsil C 60 and ion-exchange chromatography was performed on Amberlite IR-120 ($H^+$ form). Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with a path length of 1 dm, concentrations are quoted in g/100 ml. $^1$H NMR spectra were recorded on either a Bruker AM 500 or AMX 500 spectrometer (500 MHz) and $^{13}$C NMR spectra were recorded on a Bruker AC 200 spectrometer (50 MHz); chemical shifts (δ) are quoted in ppm and coupling constants ($J$) in Hz; residual signals from solvents were used as internal reference and $^{13}$C NMR spectra in $D_2O$ were referenced to 1,4-dioxane (δ 67.4). IR spectra were recorded on a Perkin–Elmer Paragon 1000 spectrophotometer using either thin films on NaCl plates (film) or KBr discs (KBr). Low resolution mass spectra were recorded on either a VG MASS LAB 20-250 using chemical ionisation (CI, $NH_3$) or a VG Platform using atmospheric pressure chemical ionisation (APCI). High resolution mass spectra were recorded on a VG Autospec spectrometer. Elemental analyses were carried out by the microanalysis service of the Dyson Perrins Laboratory. The diol **9** was prepared as previously described.[9]

### 3.2. 5-Deoxy-5-azido-3,4-O-isopropylidene-D-altrurono-2,6-lactone **13**

Periodic acid (3.87 g, 14.2 mmol) was added to a stirred solution of diol **9** (3.88 g, 14.2 mmol) in THF (40 ml). After 3 min TLC (ethyl acetate) indicated the complete conversion of the starting material ($R_f$ 0.3) to a single product ($R_f$ 0.6). The reaction mixture was pre-adsorbed onto silica and purified by flash chromatography (ethyl acetate) on silica to afford the aldehyde **13** as a colourless, unstable gum (3.41 g, quant.). $\delta_H$ (200 MHz; $CDCl_3$) 1.37, 1.50 [6H, 2s, $C(CH_3)_2$], 3.90 (1H, d, $J_{6,7}$ 2.3, H-7), 4.52 (1H, m), 4.88–4.97 (3H, m), 9.70 (1H, s, C*H*O).

### 3.3. (E)-Methyl 7-azido-2,3,7-trideoxy-5,6-O-isopropylidene-D-altro-oct-2-enaro-4,8-lactone E-**14** and (Z)-methyl 7-azido-2,3,7-trideoxy-5,6-O-isopropylidene-D-altro-oct-2-enaro-4,8-lactone Z-**14**

Methoxycarbonylmethylene(triphenyl)phosphorane (4.75 g, 14.4 mmol) was added to a solution of aldehyde **13** (3.41 g, 14.1 mmol) in toluene (40 ml) at room temperature. The reaction mixture was stirred for 5 h and TLC (ethyl acetate) indicated the conversion of the starting material ($R_f$ 0.6) to two materials ($R_f$ 0.80 and 0.85). The solvent was removed in vacuo and the residue purified by flash chromatography on silica (25% ethyl acetate/hexane) to afford *Z*-enoate *Z*-**14** ($R_f$ 0.85) as a white crystalline solid (2.54 g, 61%); mp 117–118°C; $[\alpha]_D^{20}$ −3.7 (*c* 1.02 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 1.34, 1.48 [6H, 2s, $C(CH_3)_2$], 3.75 (3H, s, $CO_2CH_3$), 3.96 (1H, d, $J_{6,7}$ 3.3, H-7), 4.80 (1H, dd, $J_{5,6}$ 7.7, $J_{4,5}$ 1.7, H-5), 4.87 (1H, dd, $J_{5,6}$ 7.7, $J_{6,7}$ 3.3, H-6), 5.87 (1H, brd, H-4), 6.04 (1H, dd, $J_{2,3}$ 11.6, $J_{2,4}$ 1.5, H-2), 6.38 (1H, dd, $J_{3,4}$ 6.9, $J_{2,3}$ 11.6, H-3); $\delta_C$ (50 MHz; $CDCl_3$) 24.0, 25.6 [2q, $C(CH_3)_2$], 51.8 (q, $CO_2CH_3$), 59.1 (d, C-7), 74.0, 75.2, 75.7 (3d, C-4, C-5, C-6), 111.2 [s, $C(CH_3)_2$], 121.5, 142.6 (2d, C-2, C-3), 166.1, 166.8 (2s, C-1, C-8); $\nu_{max}$(KBr)/$cm^{-1}$ 2122 (s, $N_3$), 1772 (s, C=O, lactone), 1707 (s, C=O, ester); *m/z* (CI, $NH_3$) 315 ($MNH_4^+$, 81%), 298 ($MH^+$, 21%); Found C: 48.56, H: 5.22, N: 14.17, $C_{12}H_{15}N_3O_6$ requires C: 48.49, H: 5.09, N: 14.14%; and *E*-enoate *E*-**14** ($R_f$ 0.80) as a white crystalline solid (1.10 g, 26%); mp 140–142°C; $[\alpha]_D^{20}$ +73.7 (*c* 1.3 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 1.37, 1.47 [6H, 2s, $C(CH_3)_2$], 3.79 (3H, s, $CO_2CH_3$), 3.89 (1H, d, $J_{6,7}$ 3.4, H-7), 4.59 (1H, dd, $J_{5,6}$ 7.6, $J_{4,5}$ 1.6, H-5), 4.83 (1H, m, H-4), 4.88 (1H, dd, $J_{5,6}$ 7.6, $J_{6,7}$ 3.4, H-6), 6.26 (1H, dd, $J_{2,3}$ 15.7, $J_{2,4}$ 1.7, H-2), 6.90 (1H, dd, $J_{3,4}$ 5.0, $J_{2,3}$ 15.7, H-3); $\delta_C$ (50 MHz; $CDCl_3$) 24.1, 25.6 [2q, $C(CH_3)_2$], 51.9 (q, $CO_2CH_3$), 59.4 (d, C-7), 74.6, 75.6, (3d, C-4, C-5, C-6), 111.7 [s, $C(CH_3)_2$], 124.5, 138.6 (2d, C-2, C-3), 166.2, 166.4 (2s, C-1, C-8); $\nu_{max}$(KBr)/$cm^{-1}$ 2122 (s, $N_3$), 1757 (s, C=O, lactone), 1717 (s, C=O, ester); *m/z* (CI, $NH_3$) 315 ($MNH_4^+$, 82%), 298 ($MH^+$, 45%), 270 ($MH–N_2^+$, 100%); Found C: 48.78, H: 5.06, N: 14.25, $C_{12}H_{15}N_3O_6$ requires C: 48.49, H: 5.09, N: 14.14%.

### 3.4. (Z)-Methyl 2,3,7-trideoxy-3,7-imino-5,6-O-isopropylidene-D-altro-oct-2-enaro-4,8-lactone **15**

*Method 1:* A solution of (*E*)-enoate *E*-**14** (900 mg, 3.03 mmol) in toluene (10 ml) was stirred at 100°C for 18 h. TLC (50% ethyl acetate/hexane) indicated the conversion of the starting material ($R_f$ 0.5) to a major product ($R_f$ 0.6). The solvent was removed in vacuo and the residue subjected to flash chromatography on silica (20% ethyl acetate/hexane) to afford the vinylogous urethane **15** as a white solid (520 mg, 64%); mp 161–163°C; $[\alpha]_D^{23}$ +63.1 (*c* 0.64 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 1.36, 1.42 [6H, 2s, $C(CH_3)_2$], 3.69 (3H, s, $CO_2CH_3$), 4.27 (1H, dd, $J_{6,7}$ 2.9, $J_{7,NH}$ 5.0, H-7), 4.58 (1H, dd, $J_{6,7}$ 2.9, $J_{5,6}$ 7.4, H-6), 4.65 (1H, dd, $J_{5,6}$ 7.4, $J_{4,5}$ 4.3, H-5), 4.90 (1H, s, H-2), 4.91 (1H, dd, $J_{4,NH}$ 2.0, $J_{4,5}$ 4.3, H-4), 8.10 (1H, br m, NH); $\delta_C$ (50 MHz; $CDCl_3$) 25.4, 25.9 [2q, $C(CH_3)_2$], 50.7 (q, $CO_2CH_3$), 54.0 (d, C-7), 73.0, 73.2, 77.9 (3d, C-4, C-5, C-6), 86.2 (d, C-2), 115.2 [s, $C(CH_3)_2$], 151.1 (s, C-3), 167.1, 169.5 (2s, C-1, C-8); $\nu_{max}$(KBr)/$cm^{-1}$ 3367 (s, NH), 1805 (s, C=O, lactone), 1668 (s, C=O, ester), 1625 (s, C=C); *m/z* (CI, $NH_3$) 270 ($MH^+$, 100%); Found C: 53.84, H: 5.27, N: 5.12, $C_{12}H_{15}NO_6$ requires C: 53.53, H: 5.62, N: 5.20%.

*Method 2:* A solution of (*Z*)-enoate *Z*-**14** (1.38 g, 4.64 mmol) in xylenes (10 ml) was stirred at 140°C for 1 h. The solvent was removed in vacuo and the residue subjected to flash chromatography on silica (20% ethyl acetate/hexane) to afford the vinylogous urethane **15** as a white solid (562 mg, 45%), identical to the material described above.

### 3.5. Methyl 2,3,7-trideoxy-3,7-imino-5,6-O-isopropylidene-D-glycero-D-manno-octaro-4,8-lactone **11**

A stirred solution of sodium cyanoborohydride (109 mg, 1.74 mmol) in acetic acid (3 ml) was treated with a solution of vinylogous urethane **15** (360 mg, 1.34 mmol) in acetic acid (3 ml). After 5 min, TLC

(1:1, ethyl acetate:hexane) indicated the complete conversion of the starting material ($R_f$ 0.7) to a major product ($R_f$ 0.4). The solvent was removed in vacuo and the residue subjected to flash chromatography on silica (40% ethyl acetate/hexane) to afford the bicyclic amino-lactone **11** as a white solid (267 mg, 75%); mp 136–137°C; $[\alpha]_D^{23}$ +21.4 (*c* 0.74 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 1.39, 1.67 [6H, 2s, C(C$H_3$)$_2$], 2.74 (1H, dd, $J_{2,3}$ 4.7, $J_{2,2'}$ 16.9, H-2), 3.06 (1H, dd, $J_{2',3}$ 9.5, $J_{2,2'}$ 16.9, H-2′), 3.71 (3H, s, $CO_2CH_3$), 3.76 (1H, d, $J_{6,7}$ 2.9, H-7), 3.82 (1H, m, H-3), 4.44 (1H, dd, $J_{6,7}$ 2.9, $J_{5,6}$ 8.2, H-6), 4.56 (1H, ddd, $J_{5,6}$ 8.2, $J_{4,5}$ 4.5, $J_{3,5}$ 1.3, H-5), 4.71 (1H, dd, $J_{4,5}$ 4.5, $J_{3,4}$ 2.2, H-4); $\delta_C$ (50 MHz; $CDCl_3$) 24.0, 25.0 [2q, C($CH_3$)$_2$], 37.3 (t, C-2), 51.9 (q, $CO_2CH_3$), 51.7, 54.5 (2d, C-3, C-7), 71.9, 73.2, 75.8 (3d, C-4, C-5, C-6), 113.9 [s, C($CH_3$)$_2$], 170.2, 171.2 (2s, C-1, C-8); $\nu_{max}$(KBr)/cm$^{-1}$ 3350 (br, NH), 1778 (s, C=O, lactone), 1738 (s, C=O, ester); *m/z* (APCI$^+$) 272 (MH$^+$, 100%); Found C: 53.18, H: 6.25, N: 5.18, $C_{12}H_{17}NO_6$ requires C: 53.13, H: 6.31, N: 5.16%.

### 3.6. *Dimethyl 2,3,7-trideoxy-3,7-imino-5,6-*O*-isopropylidene-*D*-glycero-*D*-manno-octarate* ***16***

A solution of bicyclic amino-lactone **11** (260 mg, 0.95 mmol) and sodium acetate (79 mg, 0.95 mmol) in methanol (5 ml) was stirred at reflux for 1.5 h. TLC (60% ethyl acetate/hexane) indicated the conversion of the starting material ($R_f$ 0.6) to a major product ($R_f$ 0.2) and the solvent was removed in vacuo. The residue was subjected to flash chromatography on silica (80% ethyl acetate/hexane) to afford the diester **16** as a colourless oil (235 mg, 81%). $[\alpha]_D^{23}$ −36.1 (*c* 0.71 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 1.40, 1.55 [6H, 2s, C(C$H_3$)$_2$], 2.49 (1H, dd, $J_{2,3}$ 8.5, $J_{2,2'}$ 16.2, H-2), 2.84 (1H, dd, $J_{2',3}$ 3.7, $J_{2,2'}$ 16.2, H-2′), 2.97 (1H, m, H-3), 3.53 (1H, m, H-4), 3.71, 3.80 (6H, 2s, 2$CO_2CH_3$), 3.98 (1H, d, $J_{6,7}$ 2.8, H-7), 4.05 (1H, dd, $J_{5,6}$ 5.5, $J_{4,5}$ 6.7, H-5), 4.55 (1H, dd, $J_{6,7}$ 2.8, $J_{5,6}$ 5.5, H-6); $\delta_C$ (50 MHz; $CDCl_3$) 26.2, 28.1 [2q, C($CH_3$)$_2$], 36.0 (t, C-2), 51.8, 51.9 (2q, 2$CO_2CH_3$), 52.5, 56.9 (2d, C-3, C-7), 73.4, 73.7, 79.0 (3d, C-4, C-5, C-6), 109.3, [s, C($CH_3$)$_2$], 171.7, 172.5 (2s, C-1, C-8); $\nu_{max}$(KBr)/cm$^{-1}$ 3330 (br, OH, NH), 1736 (s, C=O); *m/z* (CI, $NH_3$) 304 (MH$^+$, 100%); HRMS *m/z* (+ve Electrospray) 304.1393 (MH$^+$) $C_{13}H_{22}NO_7$ requires 304.1396.

### 3.7. *2,3,7-Trideoxy-3,7-imino-*D*-glycero-*D*-manno-octitol* ***8***

*Method 1:* Super-Hydride® (1.0 M solution in THF, 3.3 ml, 3.3 mmol) was added to a stirred solution of bicyclic amino-lactone **11** (150 mg, 0.55 mmol) in THF (5 ml) at −60°C. The solution was allowed to reach room temperature and the reaction mixture was quenched by the addition of methanol (1 ml) and the solvent removed in vacuo. The residue was redissolved in 3% methanolic hydrochloric acid (3 ml) and left standing for 24 h. The solvent was removed in vacuo and the residue purified by ion-exchange chromatography (Amberlite IR-120, $H^+$ form: eluting with 1.0 M aqueous ammonium hydroxide) to afford the azasugar **8** as a hygroscopic foam (73 mg, 64%). $[\alpha]_D^{23}$ +3.2 (*c* 0.80 in $H_2O$); $\delta_H$ (500 MHz; $D_2O$) 1.54 (1H, m, H-2), 1.98 (1H, m, H-2′), 2.65 (1H, ddd, *J* 3.5, *J* 9.0 H-3), 3.00 (1H, ddd, H-7), 3.41 (1H, dd, *J* 9.2, H-4), 3.55–3.70 (5H, m, H-1, H-1′, H-5, H-8, H-8′), 3.93 (1H, m, H-6); $\delta_C$ (50 MHz; $D_2O$) 34.5 (t, C-2), 53.2, 59.2 (2d, C-3, C-7), 60.1 (2t, C-1, C-8), 69.8, 72.5, 72.9 (3d, C-4, C-5, C-6); $\nu_{max}$(KBr)/cm$^{-1}$ 3330 (br, OH, NH); *m/z* (APCI$^+$) 208 (MH$^+$, 100%); HRMS *m/z* (CI$^+$) 208.1182 (MH$^+$) $C_8H_{18}NO_5$ requires 208.1185.

*Method 2:* Super-Hydride® (1.0 M solution in THF, 2.0 ml, 2.0 mmol) was added to a stirred solution of diester **16** (100 mg, 0.33 mmol) in THF (3 ml) at −60°C. The solution was allowed to reach room temperature and the reaction mixture was quenched by the addition of methanol (1 ml) and the solvent removed in vacuo. The residue was redissolved in 3% methanolic hydrochloric acid (3 ml) and left standing for 24 h. The solvent was removed in vacuo and the residue purified by ion-exchange

chromatography (Amberlite IR-120, $H^+$ form: eluting with 1.0 M aqueous ammonium hydroxide) to afford azasugar **8** as a hygroscopic foam (45 mg, 66%). Material identical to that described above.

### *3.8. Enzyme assays*

Activity against a range of commercially available glycosidases (Sigma) was assayed at a microtitre scale at the pH optimum for each enzyme. The incubation mixture was 20 μl of enzyme solution (10 μg/ml), 20 μl of inhibitor solution and 100 μl of 5 mM of the appropriate *p*-nitrophenyl-glycopyranoside substrate. Enzyme and inhibitor were pre-incubated for 15 min at 30°C before starting the reaction by addition of the substrate. The reaction was quenched after a period of 10 min by the addition of 160 μl glycine solution (1 M, pH 10.4) and absorbance measured at 405 nm. $K_i$ and/or $IC_{50}$ values were determined by Lineweaver–Burk analysis. The enzymes used were α-glucosidase (brewer's yeast and rice), α-fucosidase (bovine kidney), β-glucosidase (almond emulsin), α-galactosidase (*A. niger*), β-galactosidase (*E. coli, A. niger* and bovine liver), α-mannosidase (jack bean) and naringinase (*Penicillium decumbens*).

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