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Intermediates for incorporation of tetrahydroxypipecolic acid analogues of α- and β-D-mannopyranose into combinatorial libraries: unexpected nanomolar-range hexosaminidase inhibitors. Synthesis of α- and β-homomannojirimycin

John P. Shilvock,[a] Robert J. Nash,[b] Janet D. Lloyd,[b] Ana L. Winters,[b] Naoki Asano [c] and George W. J. Fleet [a,*]

[a] *Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QY, UK*
[b] *Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK*
[c] *Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa 920-11, Japan*

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Abstract

Homoazasugars have the distinction as a class of natural products in that most of them have been synthesised before they were isolated. Syntheses of α-**1** and β-homomannojirimycin **2** rely on the stereoselective and chemoselective sodium cyanoborohydride reduction of a [2.2.2] bicyclic imino lactone (**6**) to give a single [2.2.2] bicyclic amino-lactone (**7**). Methanolysis of **7** under basic conditions is accompanied by efficient epimerisation of the first formed α-amino-ester (**8**) to the more stable β-amino-ester (**9**) in which the 2,6-substitutents are equatorial. Both **7** and **9** are suitable intermediates for the incorporation of tetrahydroxypipecolic acid derivatives into combinatorial libraries containing α- and β-C-glycosyl analogues of aza-D-mannopyranose, respectively. Methylamides derived from **7** and **9** are shown to be specific and potent inhibitors of two β-N-acetylglucosaminidases but have no effect on an α-N-acetylgalactosaminidase. The synthesis of α-**14** and β-**17** manno-pipecolic acids is also reported.

1. Introduction

Highly hydroxylated pipecolic acids provide an opportunity for the incorporation of a pyranose carbohydrate motif into amide libraries to produce diverse and novel compounds for biological evaluation. The natural[1] distribution and biological properties[2] of oxygenated pipecolic acids have attracted a number of approaches to their synthesis.[3] Modified pipecolic acids, as well as prolines, have the potential of

* Corresponding author.

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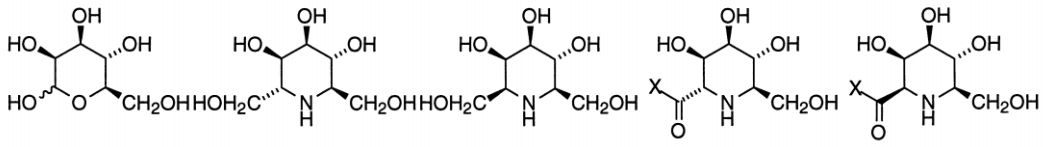
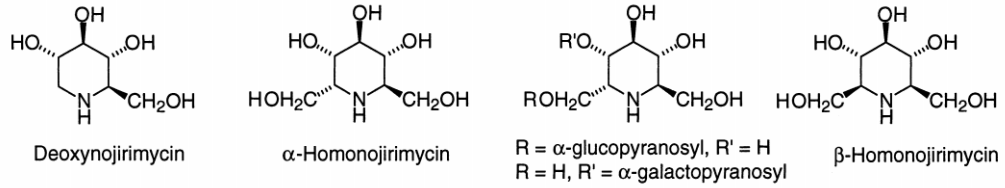
inducing secondary structure in short sequences.[4,5] Both bi- and mono-cyclic lactones — with relatively reactive carbonyl groups — are flexible intermediates and allow the introduction of polyhydroxylated proline analogues for such studies.[6]



This paper reports the synthesis of a bicyclic amino lactone (**7**) which may be used to introduce a tetrahydroxypipecolic acid moiety (which corresponds to an α-C-mannopyranoside mimic) into amide libraries; the formation of **7** relies on the efficient reduction of the bicyclic iminolactone by sodium cyanoborohydride in acetic acid — conditions under which there are no nucleophiles present to cause lactone ring opening. Ring opening of the reactive lactone **7** gives the ring opened methyl ester **8** which undergoes an unanticipated but efficient epimerisation to **9** and should allow the generation of libraries of β-C-mannopyranoside mimics. Reduction of **7** and **9** gives easy access to homoazasugars such as α-**1** and β-homomannojirimycin **2**, while treatment with nucleophilic amines gives **3** and **4**, respectively. This paper also reports that **3** and **4** [where X=NHMe] are specific and potent inhibitors of some N-acetylglucosaminidases; this result was completely unanticipated and augurs well for the likely value of such libraries providing new leads for biological activity.

Naturally occurring[7] and synthetic[8] polyhydroxylated nitrogen heterocycles (azasugars) such as deoxynojirimycin have received much attention as selective inhibitors of glycosidases and glycosyltransferases. Homoazasugars with an additional anomeric hydroxymethyl such as α-HMJ **1** and β-HMJ **2**, as well as some glycosides, have recently been shown to be in popular cultivated plants such as Hyacinths[9] and Aglaonema[10] and are likely to be widespread in nature. For the most part, the synthesis[11] of homoazasugars (2,6-imino-heptitols) has preceded their isolation as natural products; both **1**[12,13] and **2**[14,15] were synthesised prior to their isolation. Before the isolation of α-homonojirimycin (HNJ) as a natural product,[16] Liu[17] had shown that an additional carbon substituent at the anomeric position of DNJ might provide additional potency and specificity so that α-homonojirimycin (HNJ) and its 7-O-β-glucopyranosyl derivative (MDL 25,637) might have significant advantages over deoxynojirimycin for the treatment of late-onset diabetes. α-HNJ inhibited α-glucosidases and trehalase to a similar extent to DNJ but had no inhibitory activity to other glycosidases, thus showing a greater selectivity than DNJ. MDL 25,637 is an effective inhibitor of rat intestinal glucohydrolases in vitro and in vivo, and is more effective against glycoprotein processing α-glucosidase II than α-glucosidase I;[18] MDL 25,637 is also a potent inhibitor of pig kidney trehalase.[19] MDL 25,637 and the 5-α-galactoderivative of HNJ[20] have also been isolated as natural products and it is very probable that such compounds are defences for plants. It is likely that these alkaloids reduce the nutritional value of plants to predators and additionally interfere with other essential processes involving glycosidases and transferases. β-Homonojirimycin[21,22] in contrast does not inhibit β-glucosidases, or indeed any other glycosidase, to any significant extent.[10]



The first synthesis[12] of α-HMJ **1** involved a direct but rather inefficient reduction of the iminolactone **6**. This paper describes the efficient reduction of **6** by sodium cyanoborohydride in acetic acid to the bicyclic aminolactone **7** which on further reduction gives access to α-HMJ **1** while reaction of **7** with methylamine allows isolation of the α-amide **13**. Reaction of **7** with methanol in the presence of sodium acetate as base forms the α-methyl ester **8** first, which is efficiently epimerised to the β-methyl ester **9**, which allows the preparation of the β-amide **16**. Thus C-aza-mannose moieties should be easily introduced into libraries by the use of the intermediates **7** and **9**. Surprisingly, screening of the amides **13** and **16** against a panel of glycosidases showed that they are excellent and specific inhibitors of β-N-acetylglucosaminidases, respectively; **7** and **9** may thus provide access to libraries of hexosaminidase inhibitors. The synthesis and characterisation of α-**14** and β-**17** manno-pipecolic acids is also reported.

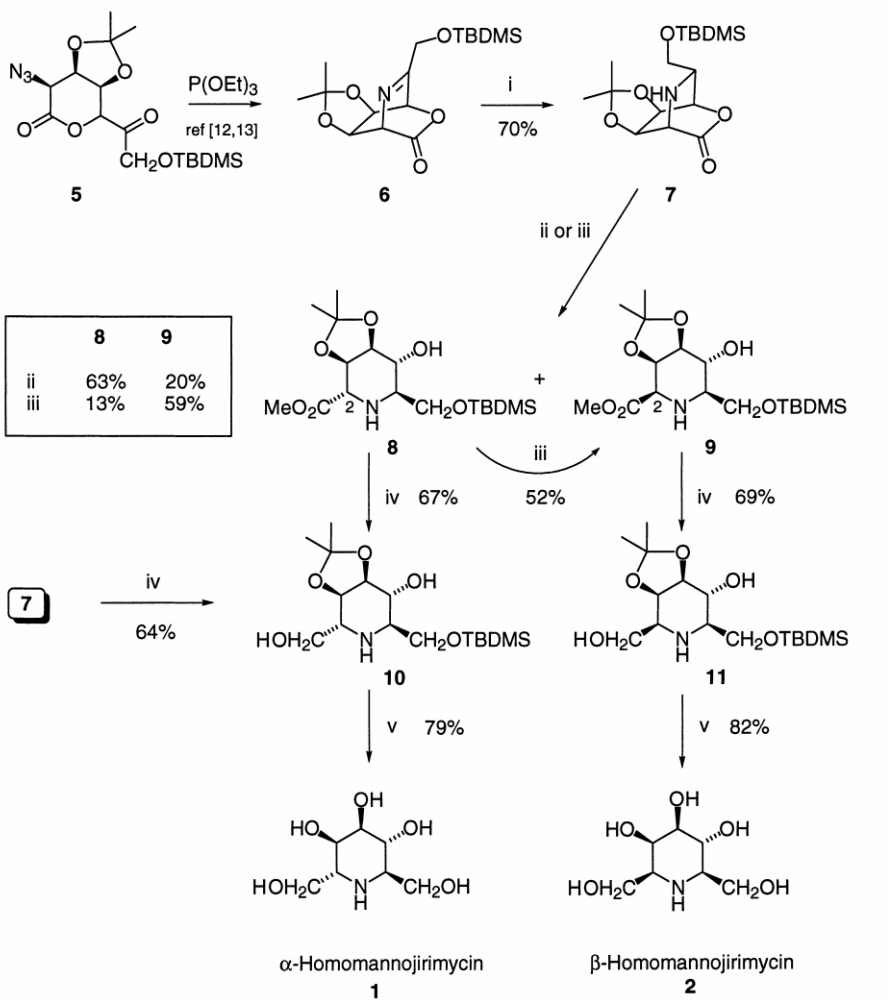
2. Synthesis

The original synthesis of α-homomannojirimycin **1** relied on reduction of the azido-ketone **5** by phosphite with a concomitant aza-Wittig reaction, resulting in formation of a bicyclic imine lactone **6** (Scheme 1). Subsequent treatment of **6** with lithium aluminium hydride resulted in stereoselective reduction of the imine and reductive ring opening of the lactone to give **10** in relatively low yield. Chemoselective reduction of the imine functionality in **6** would allow the isolation of **7** which should prove a suitable intermediate for incorporation of polyhydroxylated pipecolic acids into amide libraries. The bicyclic lactone **7** contains the required piperidine ring with an unprotected nitrogen (capable of elaboration by electrophilic attack) together with the carbonyl group of the lactone which is very sensitive to nucleophilic ring opening.

All attempts at conversion of **6** to give **7** by hydrogenation were unsuccessful; it is necessary to use a selective hydride reducing agent in a solvent which will not induce ring opening of the lactone. When the bicyclic imine **6** was treated with sodium cyanoborohydride in acetic acid, an efficient and highly chemo- and stereoselective reduction afforded the bicyclic amino-lactone **7** in 70% yield (Scheme 1). The assignment of the configuration at C-6 in **7** was based on the observed four bond coupling between H-4 and H-6 ($^4J_{4,6}$ 1.4 Hz) in the ^{1}H NMR spectrum, indicative of a W-system within the rigid bicyclic framework (Fig. 1). None of the known[13] C-6 epimer **7a** was observable in the ^{1}H NMR spectrum of the crude reaction mixture; hydride delivery occurs solely from the least hindered face of the imine **6**.

The relatively unstable lactone **7** is highly susceptible to nucleophilic attack. The lactone **7** with sodium acetate in methanol gave initial ring opening to the α-amino-ester **8** accompanied by subsequent epimerisation to the β-ester **9** in isolated yields of 63% and 20%, respectively. Treatment of **7** under more basic conditions with sodium carbonate in methanol afforded the two esters **8** and **9** in yields of 13% and 59%. It is thus clear that, while this reaction needs to be carefully monitored, suitable conditions will allow isolation of either **8** or **9** in good yield. The initial product **8** of ring opening of the lactone **7** has an axial ester group, while **9** has a thermodynamically stable 2,6-diequatorially substituted piperidine ring (Fig. 2). The relative ease of epimerisation of the ester **8** and the lack of fragmentation of the intermediate anion are noteworthy.

Reduction of either the bicyclic lactone **7** or the α-ester **8** with Super-Hydride® in THF at −60°C afforded **10** in yields of 64% and 67%, respectively. Subsequent deprotection of **10** with aqueous hydrochloric acid afforded α-homomannojirimycin **1** in 79% yield. Similarly, reduction of β-ester **9** with Super-Hydride® in THF at −60°C gave **11** in 69% yield which, on treatment with aqueous hydrochloric acid, gave β-homomannojirimycin **2** in 82% yield. The correlation of **8** and **9** with the known natural products **1** and **2** firmly establishes the configurations of the anomeric esters.



Scheme 1. (i) $NaBH_3CN$, AcOH, (ii) NaOAc, MeOH, Δ, (iii) Na_2CO_3, MeOH, Δ, (iv) $LiBHEt_3$, THF, −60°C, (v) HCl (aq)

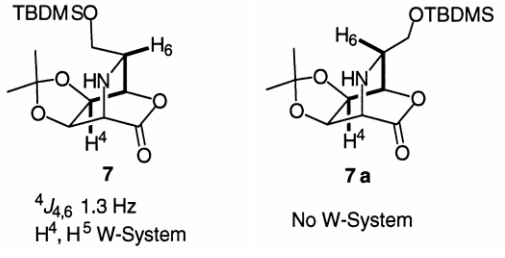


Fig. 1.

The bicyclic amino-lactone **7** should provide a potential divergent intermediate for the generation of libraries containing α-linked C-glycosyl azasugar mimics of D-mannopyranose for incorporation into larger structures. Treatment of bicyclic amino-lactone **7** with methylamine in THF afforded the methyl amide **12** in 93% yield (Scheme 2), and subsequent removal of the protecting groups using methanolic hydrochloric acid afforded **13** in 86% yield. Since the lactone **7** is highly reactive towards nucleophiles, this procedure should allow the incorporation of an α-manno-pipecolic acid moiety into

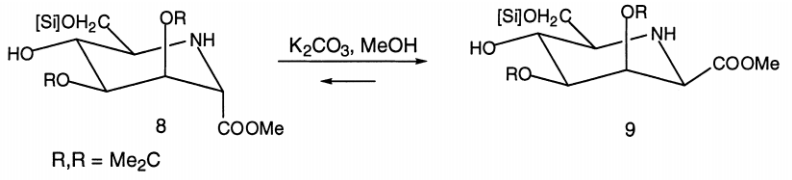
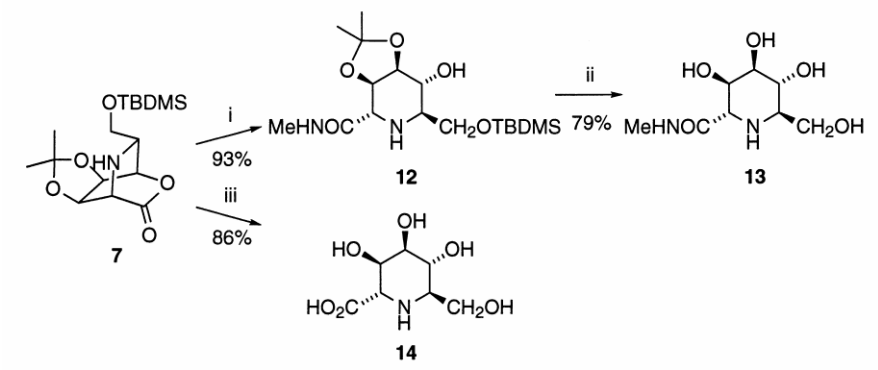


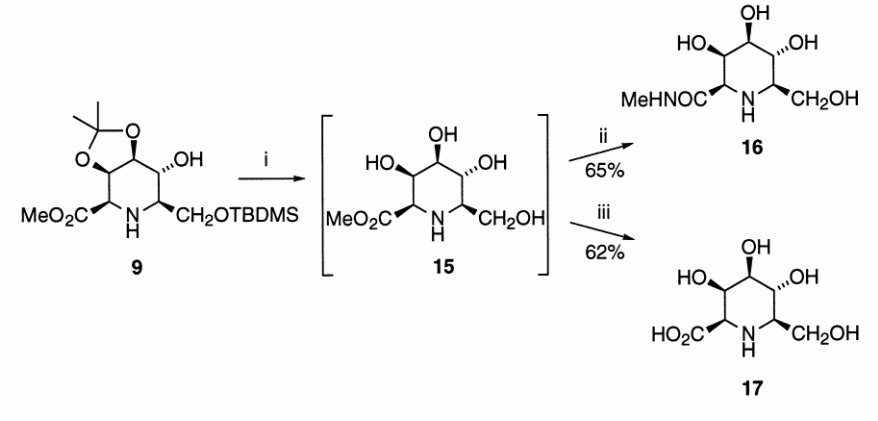
Fig. 2.

amide assemblies. The free manno-pipecolic acid **14** itself was obtained directly in 79% yield by the treatment of **7** with aqueous trifluoroacetic acid.



Scheme 2. (i) $MeNH_2$, THF, (ii) HCl, MeOH, (iii) TFA (aq)

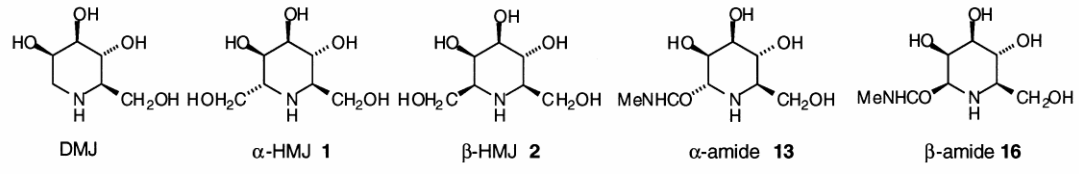
Similarly, the β-ester **9** might be used to incorporate β-linked C-glycosyl azasugar mimics of D-mannopyranose into larger structures. The use of the β-ester **9** has been investigated (Scheme 3) by initial removal of the protecting groups using aqueous trifluoroacetic acid to give the ester **15**; subsequent treatment of **15** with an excess of methylamine in methanol afforded the methyl amide **16** in 65% yield over the two steps. Although the methyl ester **15** is less reactive than the lactone **7** to nucleophilic attack, treatment of **15** with primary amines should thus provide access to amide libraries containing a β-manno-pipecolic acid fragment. α-manno-Pipecolic acid **17** was formed in 62% yield overall by hydrolysis of ester **15** with aqueous sodium hydroxide and subsequent neutralisation with hydrochloric acid.



Scheme 3. (i) TFA (aq), (ii) $MeNH_2$, THF, (iii) NaOH (aq), then HCl (aq)

3. Glycosidase inhibition

The most common natural occurring homoazasugar HNJ is a potent and fairly specific inhibitor of various α-glucosidases (see earlier). α-HMJ **1** is a weak inhibitor of human liver α-mannosidases, and has much the same potency and specificity towards human liver α-mannosidases as does DMJ, although α-HMJ is more selective in regard to its lack of inhibition of other human glycosidases. DMJ is usually a more potent inibitor of α-fucosidases than of α-mannosidases,[23,24] and β-HMJ **2** is a potent inhibitor of bovine epididymis α-L-fucosidase with a K_i of 0.45 μM. Neither **1** nor **2** inhibited either α- or β-mannosidases from a number of other sources but were both good inhibitors of rat digestive α-glucosidases.[10]



The amides **13** and **16** were screened against a range of glycosidases, including α-glucosidase (brewer's yeast and rice), α-fucosidase (human placenta and bovine kidney), β-glucosidase (Almond emulsin), α-galactosidase (green coffee beans and *Aspergillus niger*), β-galactosidase (*E. coli*, *A. niger* and bovine liver), α-mannosidase (jack bean) and naringinase (*Penicillium decumbens*) to see if there was any significant recognition of either L-fucose or D-mannose. The α-amide (**13**) was not inhibitory to any of the glycosidases at 650 μM; at the same concentration, the β-amide (**16**) was a weak inhibitor of yeast α-glucosidase (IC_{50} 220 μM) but not rice α-glucosidase, and also weakly inhibited α-galactosidase from green coffee beans (IC_{50} 90 μM). The pipecolic acid (**17**) was weakly inhibitory (40%) only to the β-glucosidase at 690 μM.

However, both the α- **13** and the β-amide **16** showed strong competitive inhibition of two β-N-acetylglucosaminidases. Against human placenta β-N-acetylglucosaminidase, using the p-nitrophenyl glycoside as substrate, the K_i for the β-amide (**16**) is 39 μM and for the α-amide (**13**) is 0.01 μM. The IC_{50} for inhibition of bovine liver β-N-acetylglucosaminidase for the two compounds were similar to the IC_{50} for the human placenta enzyme, being 11 μM for **16** and 0.09 μM for **13**. In contrast there was no inhibition of α-N-acetylgalactosaminidase from chicken liver by either compound.

In summary, this paper reports the extension of the original synthetic methodology used to access α-homomannojirimycin, via the synthesis of a key intermediate bicyclic amino-lactone bearing a D-mannose configuration. This material was shown to be of use in the generation of α- and β-C-glycosyl analogues of aza-D-mannopyranose. The unanticipated hexosaminidase inhibition by the amides **13** and **16** indicates that unexpected biological activities may arise from carbohydrate mimics which might be generated by combinatorial library procedures. Such materials as the bicyclic lactone (**7**) and open chain pipecolic acid ester (**9**) may provide valuable 'core structures' for incorporation into new classes of inhibitors of carbohydrate processing enzymes. Further synthetic and modelling studies in attempts to rationalise and optimise the hexosaminidase inhibition are in progress.[25]

4. Experimental

4.1. 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 hertz; 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 analysis was carried out by the microanalysis service of the Dyson Perrins Laboratory. The bicyclic imine **6** was prepared as previously described.[13]

*4.2. 7-O-*tert*-Butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-talo-heptono-1,5-lactone **7***

A solution of sodium cyanoborohydride (512 mg, 8.20 mmol) in acetic acid (10 ml) was treated with a solution of the unstable bicyclic imine **6** (2.14 g, 6.27 mmol) in acetic acid (10 ml) at room temperature. TLC (30% ethyl acetate:hexane) indicated the complete conversion of the starting material (R_f 0.5) to a major product (R_f 0.3). Toluene (60 ml) was added to the solution and the mixture concentrated in vacuo. The residue was subjected to flash chromatography on silica (30% ethyl acetate:hexane) to afford bicyclic amino-lactone **7** as a colourless oil (1.50 g, 70%). $[\alpha]_D^{25}$ −1.6 (*c* 1.91; $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 0.07 (6H, s, Si(C*H*$_3$)$_2$), 0.90 (9H, s, SiC(C*H*$_3$)$_3$), 1.38, 1.63 (6H, 2s, C(C*H*$_3$)$_2$), 3.48 (1H, dddd, $J_{4,6}$=1.4, $J_{5,6}$=2.2, $J_{6,7}$=6.5, $J_{6,7'}$=7.9, H-6), 3.78 (1H, d, $J_{2,3}$=2.9, H-2), 3.79 (1*H*, dd, $J_{6,7}$=6.5, $J_{7,7'}$=9.7, H-7), 4.04 (1*H*, dd, $J_{6,7'}$=7.9, $J_{7,7'}$=9.7, H-7′), 4.42 (1H, dd, $J_{2,3}$=2.9, $J_{3,4}$=8.2, H-3), 4.55 (1H, ddd, $J_{3,4}$=8.2, $J_{4,5}$=4.5, $J_{4,6}$=1.4, H-4), 4.81 (1H, dd, $J_{5,6}$=2.2, $J_{4,5}$=4.5, H-5); δ_C (50 MHz; $CDCl_3$) −5.4 (q, Si(*C*H$_3$)$_2$), 18.2 (s, Si*C*(CH$_3$)$_3$), 23.9, 24.8 (2q, C(*C*H$_3$)$_2$), 25.8 (q, SiC(*C*H$_3$)$_3$), 54.5, 57.8 (2d, C-2, C-6), 63.5 (t, C-7), 71.9, 73.0, 74.4 (3d, C-3, C-4, C-5), 113.6 (s, *C*(CH$_3$)$_2$), 170.8 (s, C-1); ν_{max} (film)/cm^{-1} 3352 (br, NH), 1773 (s, C=O); *m/z* (CI, NH_3) 344 (MH^+, 100%); HRMS *m/z* (CI^+). Found 344.1896 (MH^+), $C_{16}H_{30}NO_6Si$ requires 344.1893.

*4.3. Methyl 7-O-*tert*-butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-talo-heptonate **8** and methyl 7-O-*tert*-butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-galacto-heptonate **9***

4.3.1. Method 1

A solution of bicyclic amino-lactone (**7**) (450 mg, 1.31 mmol) and sodium acetate (119 mg, 1.46 mmol) in methanol (10 ml) was stirred at reflux for 6 h. TLC (50% ethyl acetate:hexane) indicated the complete conversion of the starting material (R_f 0.8) into two major products (R_f 0.5 and 0.2) and the reaction mixture was concentrated in vacuo. The residue was subjected to flash chromatography on silica (gradient elution: 25–60% ethyl acetate:hexane) to afford ester **8**, first eluted, as a colourless oil (310 mg, 63%). $[\alpha]_D^{25}$ −35.8 (*c* 2.27; $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 0.09, 0.11 (6H, 2s, Si(C*H*$_3$)$_2$), 0.91 (9H,

s, SiC(CH_3)$_3$), 1.37, 1.51 (6H, 2s, C(CH_3)$_2$), 2.50 (1H, ddd, $J_{5,6}$=10.0, $J_{6,7}$=5.0, $J_{6,7'}$=4.3, H-6), 3.64 (1H, dd, $J_{4,5}$=7.2, $J_{5,6}$=10.0, H-5), 3.78 (1H, dd, $J_{6,7}$=5.0, $J_{7,7'}$=9.9, H-7), 3.80 (3H, s, CO_2CH_3), 3.86 (1H, d, $J_{2,3}$=3.0, H-2), 3.95 (1H, dd, $J_{6,7'}$=4.3, $J_{7,7'}$=9.9, H-7′), 4.02 (1H, dd, $J_{3,4}$=5.2, $J_{4,5}$=7.2, H-4), 4.43 (1H, dd, $J_{3,4}$=5.2, $J_{2,3}$=3.0, H-3); δ_C (50 MHz; $CDCl_3$) −5.5 (q, Si(*C*H$_3$)$_2$), 18.1 (s, Si*C*(CH$_3$)$_3$), 25.7 (q, SiC(*C*H$_3$)$_3$), 26.3, 28.1 (2q, C(*C*H$_3$)$_2$), 52.4 (q, O(*C*H$_3$), 55.5, 56.9 (2d, C-2, C-6), 63.4 (t, C-7), 72.0, 73.8, 79.4 (3d, C-3, C-4, C-5), 109.2, (s, C(CH$_3$)$_2$), 171.8 (s, C-1); ν_{max} (film)/cm^{-1} 3458 (br, NH, OH), 1737 (s, C=O); *m/z* (APCI$^+$) 376 (MH$^+$, 100%); HRMS *m/z* (CI$^+$). Found 376.2154 (MH$^+$), $C_{17}H_{34}NO_6Si$ requires 376.2155, and ester **9**, second eluted, as a white solid (102 mg, 20%). M.p. 97–99°C with phase change at 81–83°C (diethyl ether:hexane); $[\alpha]_D^{25}$ −34.3 (*c* 0.47; *C*HCl_3); δ_H (500 MHz; $CDCl_3$) 0.08 (6H, s, Si(CH_3)$_2$), 0.91 (9H, s, SiC(CH_3)$_3$), 1.41, 1.55 (6H, 2s, C(CH_3)$_2$), 2.65 (1H, ddd, $J_{5,6}$=9.6, $J_{6,7}$=6.2, $J_{6,7'}$=4.6, H-6), 3.26 (1H, dd, $J_{4,5}$=7.2, $J_{5,6}$=9.6, H-5), 3.74 (1H, dd, $J_{6,7}$=6.2, $J_{7,7'}$=9.8, H-7), 3.78 (3H, s, CO_2CH_3), 3.82 (1H, dd, $J_{6,7'}$=4.6, $J_{7,7'}$=9.8, H-7′), 4.02 (1H, d, $J_{2,3}$=2.6, H-2), 4.06 (1H, dd, $J_{3,4}$=5.5, $J_{4,5}$=7.2, H-4), 4.56 (1H, dd, $J_{3,4}$=5.5, $J_{2,3}$=2.6, H-3); δ_C (50 MHz; $CDCl_3$) −5.5, −4.9 (2q, Si(*C*H$_3$)$_2$), 18.1 (s, Si*C*(CH$_3$)$_3$), 25.8 (q, SiC(*C*H$_3$)$_3$), 26.5, 28.1 (2q, C(*C*H$_3$)$_2$), 52.3 (q, CO_2CH_3), 57.4, 58.0 (2d, C-2, C-6), 63.4 (t, C-7), 71.8, 76.4, 81.0 (3d, C-3, C-4, C-5), 109.9 (s, *C*(CH$_3$)$_2$), 170.1 (s, C-1); ν_{max} (film)/cm^{-1} 3459 (br, NH, OH), 1755 (s, C=O); *m/z* (APCI$^+$) 376 (MH$^+$, 100%); HRMS *m/z* (CI$^+$). Found 376.2150 (MH$^+$), $C_{17}H_{34}NO_6Si$ requires 376.2155.

4.3.2. Method 2

A solution of bicyclic amino-lactone **7** (500 mg, 1.46 mmol) and sodium carbonate (159 mg, 1.46 mmol) in methanol (10 ml) was stirred at reflux for 20 min. TLC (50% ethyl acetate:hexane) indicated the complete conversion of the starting material (R_f 0.8) into two major products (R_f 0.5 and 0.2) and the reaction mixture was concentrated in vacuo. The residue was subjected to flash chromatography on silica (gradient elution: 25–60% ethyl acetate:hexane) to afford ester **8**, first eluted, as a colourless oil (71 mg, 13%) and ester **9**, second eluted, as a white solid (323 mg, 59%), both materials possessing identical data to that listed in Method 1.

*4.4. Methyl 7-O-*tert*-butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-galacto-heptonate **9***

A solution of ester **8** (100 mg, 0.30 mmol) and sodium carbonate (10 mg, 94 mmol) in methanol (3 ml) was stirred at 70°C for 3 h. TLC (ethyl acetate) indicated the presence of starting material (R_f 0.8) and a major product (R_f 0.5). The solvent was removed in vacuo and the residue subjected to flash chromatography on silica (70% ethyl acetate:hexane) to afford the starting material **8**, first eluted, as a colourless oil (21 mg, 21%) and ester **9**, second eluted, as a white solid (52 mg, 52%), the material identical to that listed above.

*4.5. 1-O-*tert*-Butyldimethylsilyl-2,6-dideoxy-2,6-imino-4,5-O-isopropylidene-*D*-glycero-*D*-manno-heptitol **10***

4.5.1. Method 1

Super-Hydride® (1.0 M solution in THF, 0.64 ml, 0.64 mmol) was added to a stirred solution of bicyclic amino-lactone **7** (100 mg, 0.29 mmol) in THF (3 ml) at −20°C and the reaction mixture allowed to warm to room temperature over 30 min. TLC (60% ethyl acetate:hexane) indicated the complete conversion of the starting material (R_f 0.9) to a major product (R_f 0.2) and saturated ammonium chloride solution (5 drops) was added. The mixture was concentrated in vacuo and the residue subjected to flash

chromatography on silica (ethyl acetate) to afford **10** as a white solid (65 mg, 64%). M.p. 163–165°C, lit.[13] 165–166°C; $[\alpha]_D^{23}$ −26.8 (*c* 0.37; $CHCl_3$), lit.[13] $[\alpha]_D^{20}$ −28.5 (*c* 1.0; $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 0.09 (6H, 2s, $Si(CH_3)_2$), 0.91 (9H, s, $SiC(CH_3)_3$), 1.37, 1.52 (6H, 2s, $C(CH_3)_2$), 2.60 (1H, m, H-2), 3.30 (1H, ddd, $J_{2,3}$=9.9, $J_{1,2}$=3.1, $J_{1',2}$=5.0, H-2), 3.49 (1H, dd, H-3), 3.63–3.66 (2H, m), 3.73 (1H, dd, *J*=7.4, *J*=9.4), 3.95–4.02 (3H, m).

4.5.2. Method 2

Super-Hydride® (1.0 M solution in THF, 0.36 ml, 0.36 mmol) was added to a stirred solution of ester **8** (50 mg, 0.13 mmol) in THF (2 ml) at −30°C and the reaction mixture allowed to warm to room temperature over 30 min. TLC (ethyl acetate) indicated the complete conversion of the starting material (R_f 0.8) to a major product (R_f 0.2) and saturated ammonium chloride solution (5 drops) was added. The mixture was concentrated in vacuo and the residue subjected to flash chromatography on silica (ethyl acetate) to afford **10** as a white solid (31 mg, 67%). Material identical to that described in Method 1.

*4.6. 7-O-*tert*-Butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-galacto-heptitol **11***

Super-Hydride® (1.0 M solution in THF, 1.1 ml, 1.1 mmol) was added to a stirred solution of ester **9** in THF (5 ml) at −60°C and the reaction mixture allowed to warm to room temperature over 30 min. TLC (ethyl acetate) indicated the complete conversion of starting material (R_f 0.5) to a major product (R_f 0.3) and saturated ammonium chloride solution (5 drops) was added. The mixture was concentrated in vacuo and the residue subjected to flash chromatography on silica (ethyl acetate) to afford **11** as an amorphous solid (95 mg, 69%). $[\alpha]_D^{21}$ −55.9 (*c* 0.89; $CHCl_3$), lit. $[\alpha]_D$ −6.5 (*c* 0.80; $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 0.09, 0.10 (6H, 2s, $Si(CH_3)_2$), 0.91 (9H, s, $SiC(CH_3)_3$), 1.38, 1.53 (6H, 2s, $C(CH_3)_2$), 2.50 (1H, ddd, $J_{5,6}$=10.1, $J_{6,7}$=4.5, $J_{6,7'}$=5.8, H-6), 3.09 (1H, ddd, $J_{2,3}$=2.8, $J_{1,2}$=5.0, $J_{1',2}$=4.1, H-2), 3.63 (1H, dd, $J_{4,5}$=7.4, $J_{5,6}$=10.1, H-5), 3.74 (1H, dd, $J_{6,7}$=5.0, $J_{7,7'}$=9.9, H-7), 3.81 (1H, dd, $J_{1,2}$=4.5, $J_{1,1'}$=11.3, H-1), 3.86 (1H, dd, $J_{1',2}$=5.8, $J_{1,1'}$=11.3, H-1′), 3.94 (1H, dd, $J_{6,7'}$=4.1, $J_{7,7'}$=9.9, H-7′), 4.00 (1H, dd, $J_{3,4}$=5.2, $J_{4,5}$=7.4, H-4), 4.18 (1H, dd, $J_{3,4}$=5.2, $J_{2,3}$=2.8, H-3)

*4.7. 2,6-Dideoxy-2,6-imino-*D*-glycero-*D*-manno-heptitol **1** (α-homomannojirimycin)*

A solution of **10** (50 mg, 0.14 mmol) in hydrochloric acid (2.0 M, 5 ml) was stirred at room temperature for 2 h. TLC (20% methanol:ethyl acetate) indicated the complete conversion of the starting material (R_f 0.8) to a single product (R_f 0.2). The solution was concentrated in vacuo and the residue subjected to ion-exchange chromatography (Amberlite IR-120, H^+ form: eluting with 1.0 M ammonium hydroxide) to afford **1** as a hygroscopic solid (22 mg, 79%). $[\alpha]_D^{24}$ +6.8 (*c* 0.65; H_2O), lit.[13] $[\alpha]_D^{20}$ +7.45 (*c* 0.55; H_2O); δ_H (500 MHz; D_2O) 2.75 (1H, m, H-2), 3.14 (1H, m, H-6), 3.59 (6H, m), 3.96 (1H, m, H-5).

*4.8. 2,6-Dideoxy-2,6-imino-*D*-glycero-*D*-galacto-heptitol **2** (β-homomannojirimycin)*

A solution of **11** (70 mg, 0.20 mmol) in hydrochloric acid (2.0 M, 5 ml) was stirred at room temperature for 2 h. TLC (20% methanol:ethyl acetate) indicated the complete conversion of the starting material (R_f 0.8) to a single product (R_f 0.2). The solution was concentrated in vacuo and the residue subjected to ion-exchange chromatography (Amberlite IR-120, H^+ form: eluting with 1.0 M ammonium hydroxide) to afford **2** as a hygroscopic solid (32 mg, 82%). $[\alpha]_D^{24}$ −5.1 (*c* 0.75; MeOH), lit.[14] $[\alpha]_D^{20}$ −4.3 (*c* 1.3; MeOH); δ_H (500 MHz; CD_3OD) 2.50 (1H, ddd, $J_{5,6}$=9.8, $J_{6,7}$=3.0, $J_{6,7'}$=6.0, H-6), 2.76, (1H, m,

$J_{2,3}$=1.3, H-2), 3.36 (1H, dd, $J_{4,5}$=9.4, $J_{3,4}$=3.1, H-4), 3.53 (1H, dd, $J_{4,5}$=9.4, $J_{5,6}$=9.8, H-5), 3.65 (2H, m, H-1, H-1′), 3.67 (1H, $J_{7,7'}$=11.0, $J_{6,7'}$=6.0, H-7′), 3.84 (1H, $J_{7,7'}$=11.0, $J_{6,7}$=3.0, H-7), 3.87 (1H, dd, $J_{2,3}$=1.3, $J_{3,4}$=3.1, H-3).

*4.9. Methyl 7-O-*tert*-butyldimethylsilyl-2,6-dideoxy-2,6-imino-3,4-O-isopropylidene-*D*-glycero-*D*-talo-heptonamide **12***

Methylamine (33% w/w solution in industrial methylated spirit, 0.2 ml, 1.75 mmol) was added to a solution bicyclic amino-lactone **7** (200 mg, 0.58 mmol) in dry THF (3 ml) and the mixture stirred at room temperature for 2 h. TLC (ethyl acetate) indicated the complete conversion of the starting material (R_f 0.9) to a single product (R_f 0.5) and the reaction mixture was concentrated in vacuo. The residue was subjected to flash chromatography on silica (80% ethyl acetate/hexane) to afford methyl amide **12** as a colourless foam (203 mg, 93%). $[\alpha]_D^{23}$ −47.0 (*c* 1.53; $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 0.09 (6H, s, Si(CH_3)$_2$), 0.91 (9H, s, SiC(CH_3)$_3$), 1.42, 1.52 (6H, 2s, C(CH_3)$_2$), 2.35 (1H, ddd, $J_{5,6}$=10.0, $J_{6,7}$=3.4, $J_{6,7'}$=3.2, H-6), 2.35 (1H, br m, OH/NH), 2.84 (3H, d, *J*=5.0, CONHCH_3), 3.64 (1H, dd, $J_{4,5}$=7.8, $J_{5,6}$=10.0, H-5), 3.69 (1H, dd, $J_{6,7}$=3.4, $J_{7,7'}$=10.0, H-7), 3.84 (1H, m, H-2), 3.93 (1H, dd, $J_{6,7'}$=4.3, $J_{7,7'}$=9.9, H-7′), 3.95 (1H, m, H-4), 4.93 (1H, dd, $J_{3,4}$=5.3, $J_{2,3}$=1.9, H-3), 7.37 (1H, m, CON*H*CH$_3$); δ_C (50 MHz; $CDCl_3$) −5.5 (q, Si(*C*H$_3$)$_2$), 18.2 (s, Si*C*(CH$_3$)$_3$), 25.8 (q, SiC(*C*H$_3$)$_3$), 26.0 (q, CONH*C*H$_3$), 26.4, 28.2 (2q, C(*C*H$_3$)$_2$), 55.0, 56.9 (2d, C-2, C-6), 61.9 (t, C-7), 69.8, 73.9, 79.4 (3d, C-3, C-4, C-5), 108.5, (s, *C*(CH$_3$)$_2$), 170.6 (s, C-1); ν_{max} (KBr)/cm^{-1} 3353 (br, NH, OH), 1665 (s, amide I), 1530 (m, amide II); *m/z* (APCI$^+$) 375 (MH$^+$, 100%). Found C: 54.52, H: 9.40, N: 7.39, $C_{17}H_{34}N_2O_5Si$ requires C: 54.51, H: 9.15, N: 7.48%.

*4.10. Methyl 2,6-dideoxy-2,6-imino-*D*-glycero-*D*-talo-heptonamide **13***

A solution of methyl amide **12** (160 mg, 0.43 mmol) in 3% methanolic hydrochloric acid was stirred at room temperature for 24 h. The solution was concentrated in vacuo (co-evaporation with methanol, 3×10 ml) and the residue purified by ion-exchange chromatography (Amberlite IR-120, H$^+$ form: eluting with 1.0 M ammonium hydroxide) to afford **13** as a hygroscopic foam (81 mg, 86%). $[\alpha]_D^{22}$ −10.0 (*c* 0.66; H_2O, pH 9); δ_H (500 MHz; D_2O, pH 9) 2.61 (1H, ddd, $J_{5,6}$=9.5, $J_{6,7}$=3.1, $J_{6,7'}$=5.6, H-6), 2.84 (3H, s, CONHCH_3), 3.45 (1H, dd, $J_{4,5}$=9.5, $J_{3,4}$=3.0, H-4), 3.56 (1H, dd, $J_{4,5}$=$J_{5,6}$=9.5, H-5), 3.67 (1H, d, $J_{2,3}$=2.9, H-2), 3.71 (1H, dd, $J_{6,7'}$=5.7, $J_{7,7'}$=11.6, H-7′), 3.79 (1H, dd, $J_{6,7}$=3.1, $J_{7,7'}$=11.6, H-7), 4.40 (1H, dd, $J_{3,4}$=$J_{2,3}$=2.9, H-3); δ_C (50 MHz; D_2O, pH 9) 26.4 (q, CONH*C*H$_3$), 58.2, 61.7 (2d, C-2, C-6), 61.0 (t, C-7), 68.5, 70.4, 73.0 (3d, C-3, C-4, C-5), 173.5 (s, C-1); ν_{max} (KBr)/cm^{-1} 3400 (br, NH, OH), 1654 (s, amide I), 1535 (m, amide II); *m/z* (APCI$^+$) 221 (MH$^+$, 100%); HRMS *m/z* (CI$^+$). Found 221.1131 (MH$^+$), $C_8H_{17}N_2O_5$ requires 221.1137.

*4.11. 2,6-Dideoxy-2,6-imino-*D*-glycero-*D*-talo-heptonic acid **14***

A solution of bicyclic amino-lactone **7** (150 mg, 0.44 mmol) in 50% aqueous trifluoroacetic acid (4 ml) was stirred at room temperature for 18 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 pyridine) to afford pipecolic acid **14** as a hygroscopic foam (71 mg, 79%). $[\alpha]_D^{22}$ +9.8 (*c* 0.54; H_2O); δ_H (500 MHz; D_2O) 3.30 (1H, ddd, $J_{5,6}$=10.6, $J_{6,7}$=3.1, $J_{6,7'}$=6.2, H-6), 3.43 (1H, dd, $J_{4,5}$=9.6, $J_{3,4}$=2.9, H-4), 3.70 (1H, dd, $J_{4,5}$=9.6, $J_{5,6}$=10.6, H-5), 3.73 (1H, dd, $J_{6,7'}$=6.2, $J_{7,7'}$=12.4, H-7′), 3.86 (1H, dd, $J_{6,7}$=3.1, $J_{7,7'}$=12.4, H-7), 3.89 (1H, d, $J_{2,3}$=2.9, H-2), 4.40 (1H, dd, $J_{3,4}$=3.0, $J_{2,3}$=2.9, H-3); δ_C (50 MHz; D_2O, pH 9) 58.2, 62.3 (2d, C-2, C-6), 58.9 (t, C-7), 66.0, 69.5, 71.4 (3d, C-3, C-4, C-5), 170.7 (s, C-1); ν_{max}

(KBr)/cm^{-1} 3400 (br, NH, OH), 1625 (s, C=O); *m/z* (−ve electrospray) 106 (M−H$^+$, 100%); HRMS *m/z* (CI$^+$). Found 208.0827 (MH$^+$), $C_7H_{14}NO_6$ requires 208.0821.

4.12. *Methyl 2,6-dideoxy-2,6-imino*-D-*glycero*-D-*galacto-heptonamide* ***16***

A solution of ester **9** (50 mg, 0.13 mmol) in 50% aqueous trifluoroacetic acid (2 ml) was stirred at room temperature for 18 h. The solvent was removed in vacuo by co-evaporation with water (×3) and the residue dissolved in methanol (2 ml). The solution was treated with methylamine (33% w/w solution in industrial methylated spirit, 1 ml, 8.6 mmol) and stirred for 5 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 ammonium hydroxide) to afford methyl amide **16** as a hygroscopic foam (19 mg, 65%). $[\alpha]_D^{22}$ +16.4 (*c* 0.72; H_2O, pH 9); δ_H (500 MHz; D_2O, pH 9) 2.56 (1H, ddd, $J_{5,6}$=9.5, $J_{6,7}$=3.1, $J_{6,7''}$=5.1, H-6), 2.76 (3H, s, CONHC*H*$_3$), 3.50 (1H, m, H-2), 3.55 (1H, dd, $J_{4,5}$=9.7, $J_{5,6}$=9.5, H-5), 3.60 (1H, dd, $J_{4,5}$=9.7 $J_{3,4}$=2.9, H-4), 3.74 (1H, dd, $J_{6,7}$=5.2, $J_{7,7'}$=11.7, H-7), 3.79 (1H, dd, $J_{6,7'}$=3.1, $J_{7,7'}$=11.7, H-7′), 4.18 (1H, dd, $J_{3,4}$=2.9, $J_{2,3}$=1.6, H-3); δ_C (50 MHz; D_2O, pH 9) 26.5 (q, CONH*C*H$_3$), 60.2 (d, C-6), 61.5 (d and t, C-2, C-7), 68.6, 71.2, 75.4 (3d, C-3, C-4, C-5), 173.5 (s, C-1); ν_{max} (KBr)/cm^{-1} 3410 (br, NH, OH), 1648 (s, amide I), 1560 (m, amide II); *m/z* (APCI$^+$) 221 (MH$^+$, 100%); HRMS *m/z* (CI$^+$). Found 221.1138 (MH$^+$), $C_8H_{17}N_2O_5$ requires 221.1137.

4.13. *2,6-Dideoxy-2,6-imino*-D-*glycero*-D-*galacto-heptonic acid* ***17***

A solution of ester **9** (50 mg, 0.13 mmol) in 50% aqueous trifluoroacetic acid (2 ml) was stirred at room temperature for 18 h. The solvent was removed in vacuo by co-evaporation with water (×3) and the residue redissolved in water (2 ml) and treated with sodium hydroxide solution (0.5 M, 0.5 ml) and left standing for 2 h. The solution was taken to pH 8 by the addition of 2 M hydrochloric acid and the solvent removed in vacuo. The residue was purified by ion-exchange chromatography (Amberlite IR-120, H$^+$ form: eluting with 1.0 M aqueous pyridine) to afford pipecolic acid (**17**) as a hygroscopic foam (17 mg, 62%). $[\alpha]_D^{22}$ +18.7 (*c* 0.63; H_2O); δ_H (500 MHz; D_2O) 3.15 (1H, ddd, $J_{5,6}$=10.2, $J_{6,7}$=3.2, $J_{6,7'}$=6.6, H-6), 3.73 (1H, dd, $J_{4,5}$=9.6, $J_{3,4}$=3.0, H-4), 3.86 (2H, m, H-2, H-5), 3.89 (1H, dd, $J_{6,7}$=3.2, $J_{7,7'}$=12.6, H-7), 4.02 (1H, dd, $J_{6,7'}$=6.2, $J_{7,7'}$=12.4, H-7′), 4.45 (1H, dd, $J_{3,4}$=3.0, $J_{2,3}$=1.6, H-3); δ_C (50 MHz; D_2O, pH 9) 59.0, 62.4 (2d, C-2, C-6), 59.9 (t, C-7), 66.5, 69.3, 73.9 (3d, C-3, C-4, C-5), 171.8 (s, C-1); ν_{max} (KBr)/cm^{-1} 3400 (br, NH, OH), 1625 (s, C=O); *m/z* (APCI$^+$) 208 (MH$^+$, 100%); HRMS *m/z* (CI$^+$). Found 208.0825 (MH$^+$), $C_7H_{14}NO_6$ requires 208.0821.

4.14. *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 range of enzymes used is listed in the discussion. The incubation mixture was 20 μl of enzyme solution (approx. 0.1 unit per ml), 20 μl of inhibitor solution and 100 μl of 5 mM of the appropriate p-nitrophenyl-glycopyranoside substrate made up in 50 mM phosphate citrate buffer. Enzyme and inhibitor were pre-incubated for 15 min at 30°C (those extracted from animal tissues which were evaluated at 37°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 (0.4 M, pH 10.4) and absorbance measured at 405 nm. K_i values were determined for inhibitors of β-N-acetylglucosaminidase (human placenta) with substrate concentrations ranging from 0.18 to 1.8 mM using Lineweaver–Burk analysis.

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