Synthesis of homorhamnojirimycins and related trihydroxypipecolic acid derivatives *via* divergent bicyclic amino lactone intermediates: Inhibition of naringinase (L-rhamnosidase) and dTDP-rhamnose biosynthesis

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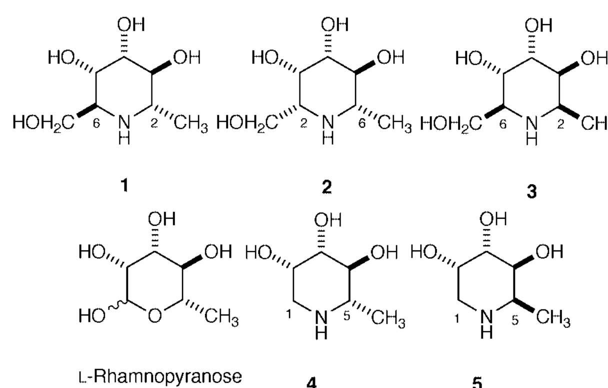
A series of homorhamnojirimycins and related compounds are prepared from two epimeric [2.2.2] bicyclic amino lactones **6** and **7** *via* the 2-azidoheptono-1,5-lactone **8**, itself derived from L-rhamnose. Aminolysis and deprotection of the bicyclic lactones provides an efficient route to trihydroxypipecolic acid amide analogues of 5-*epi*-L-rhamnopyranose **12a**–**d** and L-rhamnopyranose **14a**–**d**. Some of the L-rhamnopyranose analogues display inhibitory activity against naringinase (L-rhamnosidase) and dTDP-rhamnose biosynthesis and are potentially useful as tools for investigating cell wall biosynthesis of *Mycobacterium tuberculosis*, the causative agent of tuberculosis. The synthesis of other homoiminosugar analogues including *epi*-homorhamnojirimycin (HRJ) **3** is also reported. Methanolysis of the bicyclic lactone **7** possessing a configuration corresponding to α-L-rhamnopyranose under basic conditions affords both α- and β-methyl 2,6-iminoheptonates **16** and **17**. Reduction and subsequent deprotection affords the 2,6-iminoheptitols, α-homorhamnojirimycin (α-HRJ) **1** and β-homorhamnojirimycin (β-HRJ) **2**, potent inhibitors of L-rhamnosidase and α-galactosidase, respectively. The crystal-structure determination of the bicyclic lactone **7** is also reported.

Introduction

Synthetic and naturally occurring ‘nitrogen-in-the-ring’ analogues of pyranoses and furanoses are frequently found to be potent inhibitors of many carbohydrate-processing enzymes involved in important biological systems.[1] This has resulted in the use of deoxynojirimycin derivatives and analogues as investigative tools for probing the role played by such enzymes in carbohydrate metabolism.[2] The potential applications for this class of small but highly functionalized molecules have generated much interest in their synthesis and evaluation as glycosidase inhibitors.[3,4]

Homonojirimycin-type analogues, such as the homorhamnojirimycins **1** and **2**, are an important subclass of iminosugar. The presence of the additional α- or β-hydroxymethyl group often leads to an increase in either their potency and/or specificity as inhibitors. Many synthetic[5] and natural[6] homoiminosugars have been reported.

Iminosugars bearing hydroxy groups with a particular configuration corresponding to a carbohydrate epitope are generally inhibitors of the hydrolase for the associated parent sugar. One important exception is the lack of naringinase (L-rhamnosidase) inhibition by deoxyrhamnojirimycin (DRJ) **4**.[7,8] In contrast, 5-*epi*-DRJ **5**[9,10] was found to be a potent inhibitor of naringinase (K_i 1.0 μM). This observation has been rationalized using molecular modelling studies.[11] This paper describes the synthesis and evaluation of α- and β-homorhamnojirimycin (HRJ) **1** and **2** as analogues of L-rhamnopyranose, and *epi*-HRJ **3** as an analogue of 5-*epi*-L-rhamnopyranose. Other *C*-glycosyl derivatives of **4** and **5** containing amide and carboxylic acid substituents[12] are also evaluated as inhibitors to investigate the effects of structural variations on inhibitor potency and specificity.



L-Rhamnopyranose occurs in a disaccharide linker between the arabinogalactan polysaccharide and peptidoglycan regions of the cell wall of *Mycobacterium tuberculosis*,[13] the causative agent of tuberculosis. Mimics of L-rhamnose which are able to act as inhibitors either of thymidine diphosphate–(dTDP)–rhamnose biosynthesis from dTDP–glucose or of incorporation of L-rhamnose into the mycobacterial cell wall may have

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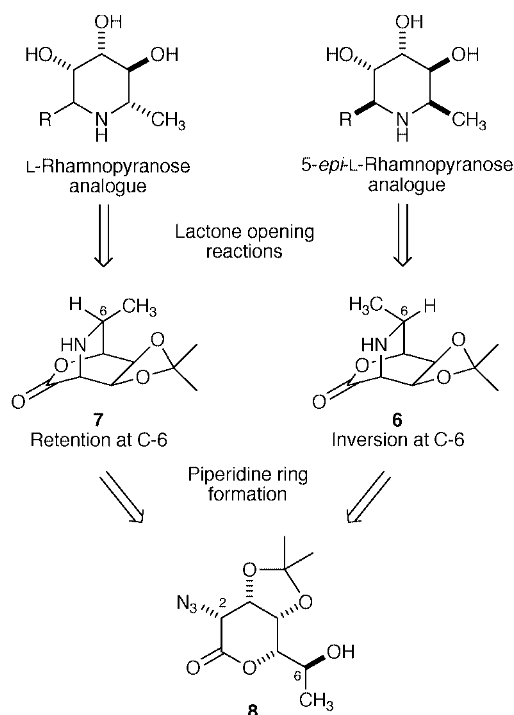
potential as novel carbohydrate-based[14] chemotherapeutic agents for the treatment of mycobacterial infection. A demonstration of any correlation of inhibition of naringinase with inhibition of dTDP–rhamnose biosynthesis may provide a strategy for the design of biologically active rhamnose analogues.

Results and discussion

Synthesis

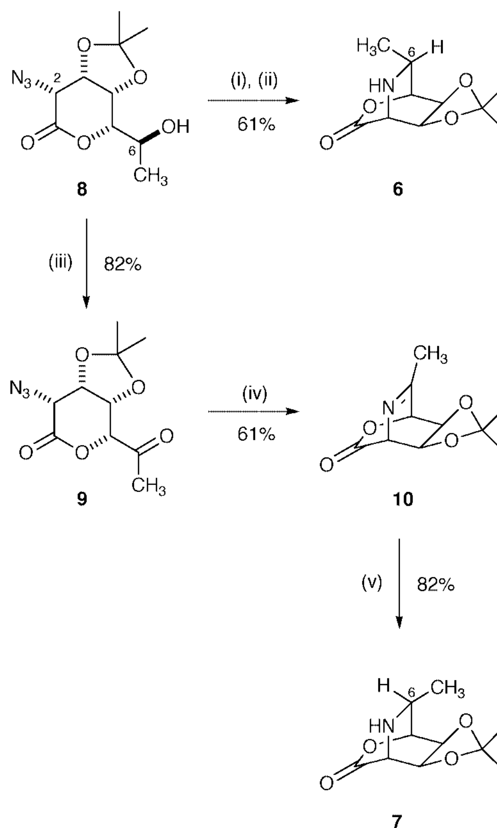
Aza-*C*-glycosyl analogues of both L-rhamnopyranose and 5-*epi*-L-rhamnopyranose were synthesized *via* lactone-opening reactions of two C-6 epimeric [2.2.2] bicyclic amino lactones **6** and **7**. Both **6** and **7** were accessible from the protected, L-rhamnose-derived 2-azidoheptono-1,5-lactone **8**[15] by piperidine-ring formation with either inversion or retention of configuration at C-6, respectively (Scheme 1). Part of this work has been reported previously as a communication.[10]



Scheme 1 Synthetic approach.

The synthesis of bicyclic lactone **6**, the precursor to analogues of 5-*epi*-L-rhamnopyranose, required the formation of the piperidine ring with inversion of configuration at C-6 (Scheme 2). Esterification of the C-6 hydroxy group in **8** with trifluoromethanesulfonic anhydride gave the azido triflate ester; subsequent hydrogenation of the azido group gave an intermediate amine which underwent spontaneous cyclization to afford the [2.2.2] bicyclic amino lactone **6** in 61% yield from **8**. The bicyclic lactone **7**, precursor to L-rhamnopyranose analogues, was accessible by formation of the piperidine ring with retention of configuration at C-6. Oxidation of 2-azido lactone **8** using PCC in dichloromethane afforded the C-6 ketone **9** in 82% yield. Subsequent intramolecular aza-Wittig reaction[16] upon treatment of **9** with triethyl phosphite gave the bicyclic imine **10** in 61% yield. A highly stereoselective reduction of the imine functionality in **10** with sodium cyanoborohydride in acetic acid gave the amino lactone **7** in 82% yield, in which the *O*-isopropylidene protecting group shielded one face of the imine from hydride delivery; none of the C-6 epimeric material **6** was observed.

Evidence that the formation of the new stereogenic centre at C-6 in **7** had proceeded with overall retention of configuration



Scheme 2 *Reagents and conditions*: (i) Tf_2O, pyridine, CH_2Cl_2, −20 °C; (ii) H_2, Pd, NaOAc, EtOAc; (iii) PCC, 4 Å molecular sieve, CH_2Cl_2; (iv) $P(OEt)_3$, THF, reflux; (v) $NaBH_3CN$, AcOH.

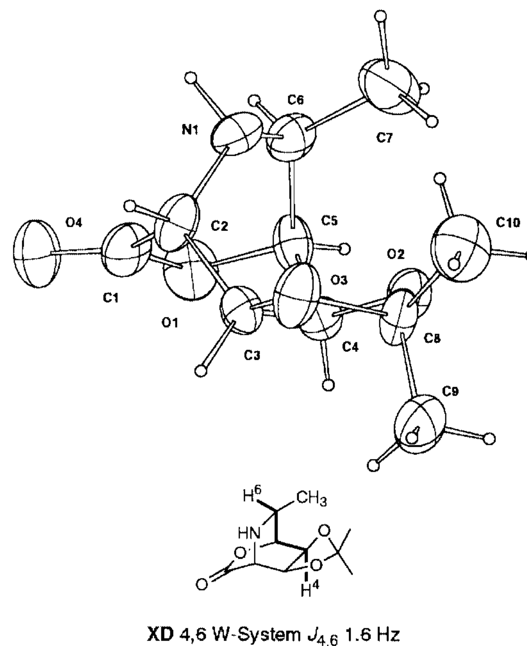
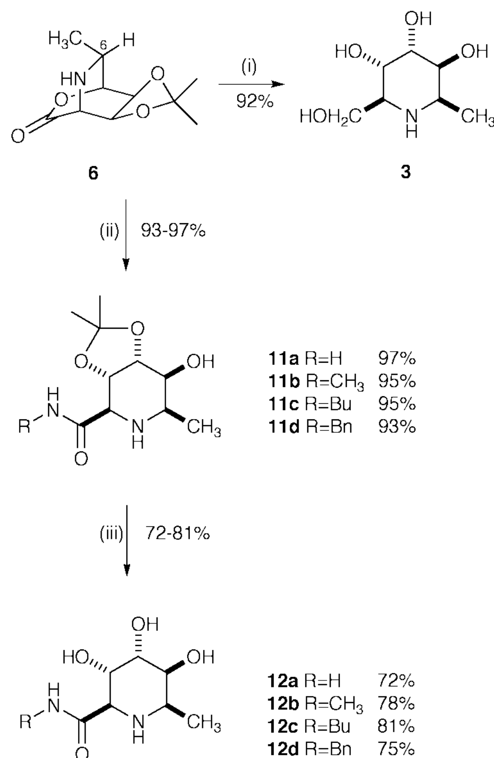


Fig. 1 ORTEP representation of X-ray crystal structure of bicyclic lactone **7** with H-4, H-6 W-system.

was provided by the additional splittings in the resonances of H-4 and H-6 in the 500 MHz ^1H NMR spectrum. Small four-bond couplings ($^4J_{4,6}$ 1.4 Hz) were observed as a result of these hydrogen atoms being locked in a W-arrangement within the rigid bicyclic system. X-Ray crystallographic analysis firmly established the C-6 configuration of **7** (Fig. 1).

The bicyclic lactone **6** was used to generate a series of imino-*C*-glycosyl analogues of 5-*epi*-L-rhamnopyranose by nucleophilic ring-opening reactions (Scheme 3). Reduction of the



Scheme 3 *Reagents and conditions*: (i) $LiBH_4$, THF, then H^+; (ii) RNH_2, THF; (iii) HCl, MeOH.

lactone with lithium borohydride in THF, followed by deprotection by treatment with acid, produced *epi*-homorhamnojirimycin **3** in 92% overall yield. In addition, treatment of **6** with ammonia or a variety of primary amines in THF afforded the 3,4-*O*-protected imino-*C*-glycosyl amides **11a–d** in excellent yields (93–97%). Removal of the *O*-isopropylidene protection using methanolic hydrogen chloride and subsequent purification by ion-exchange chromatography on acidic resin afforded imino-*C*-glycosyl amides **12a–d** in good yields (72–81%).

Similarly, bicyclic amino lactone **7** was used to access α-imino-*C*-glycosyl analogues of α-L-rhamnopyranose. Reduction of **7** with Super-Hydride® with subsequent deprotection in methanolic hydrogen chloride afforded α-homorhamnojirimycin (α-HRJ) **1** in 80% yield (Scheme 4). Reaction of **7** with ammonia or a variety of primary amines in THF gave the 3,4-*O*-protected amides **13a–d** in excellent yields (85–93%). Subsequent deprotection in methanolic hydrogen chloride and purification on acidic ion-exchange resin afforded the L-rhamnose-configured amides **14a–d** in good yields (79–86%). Additionally, lactone hydrolysis of **7** with aqueous sodium hydroxide followed by deprotection with aqueous HCl and ion-exchange chromatography gave the trihydroxypipecolic acid **15** in 90% yield.

A common feature in the 500 MHz ^{1}H NMR spectra of all the 5-*epi*-rhamnose analogues synthesized (**12a–d**) was the magnitude of the H-2, H-3 coupling constant, which was found to be $J_{2,3}$ 10.4 Hz in D_2O at pH 8–9 (Fig. 2). This suggested that these materials exist in an approximate chair conformation with the C-2 and C-6 substituents in a *cis*-diequatorial arrangement, resulting in a *trans*-diaxial relationship for H-2 and H-3. These findings are consistent with the ^{1}H NMR spectral data for 5-*epi*-deoxyrhamnojirimycin **5**, where no *trans*-diaxial couplings are observed for ring protons H-2 to H-5 (note differing numbering).[10,11] For the rhamnose analogues **14a–d**, *trans*-diaxial couplings were observed between H-4 and H-5, and between H-5 and H-6 (9.2–9.5 Hz) (Fig. 2). It is likely, therefore, that an approximate chair conformation is adopted with the methyl group in an equatorial

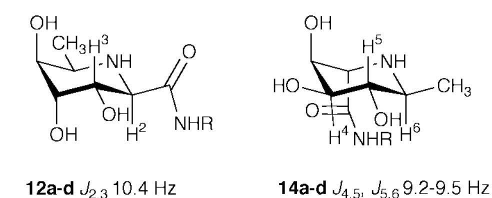
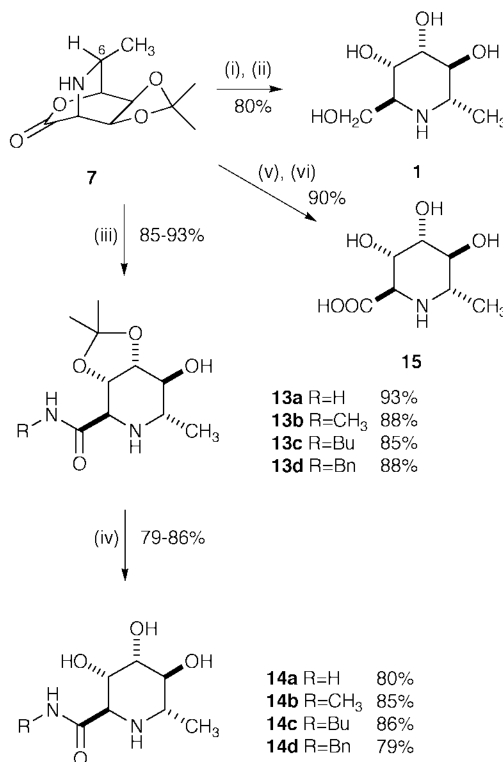


Fig. 2 *trans*-Diaxial couplings in compounds **12a–d** and **14a–d**.



Scheme 4 *Reagents and conditions*: (i) $LiBEt_3H$, THF; (ii) HCl, MeOH; (iii) RNH_2, THF; (iv) HCl, MeOH; (v) NaOH (aq); (vi) HCl (aq).

position and the α-amide group in an axial orientation, consistent with axial hydrogen atoms H-4, H-5 and H-6. Similar observations were also made for the 3,4-*O*-protected materials **13a–d**.

Treatment of the lactone of **7** with sodium acetate in methanol at reflux initially produced the axial α-ester **16**, which underwent subsequent epimerization at C-2 to the thermodynamically more stable β-2,6-*cis*-diequatorial substituted piperidine **17** (Scheme 5). The two separable epimeric methyl esters **16** (isolated in 30–34% yield) and **17** (isolated in 45–53% yield) were obtained in ratios depending on reaction conditions. Under these reaction conditions **16** afforded a 1 : 1.8 mixture of **16** and **17**, as judged by inspection of the ^{1}H NMR spectrum of the crude material. Analogous results have been observed in a related D-mannose-derived system.[12b] The α-ester **16** and β-ester **17** were respectively converted to α-HRJ **1** (homorhamnojirimycin) in 71% yield (identical to the material obtained *via* reduction of **7**) and β-HRJ **2** in 63% yield using Super-Hydride® in THF at room temperature followed by deprotection with methanolic hydrogen chloride.

Both α-HRJ **1** and β-HRJ **2** appear to exist in approximate chair conformations, based on inspection of their 500 MHz ^{1}H NMR spectra in D_2O at pH 8–9 (Fig. 3). The appearance of *trans*-diaxial coupling constants (J 9.4–9.7 Hz) for the three adjacent axial hydrogen atoms H-2, H-3 and H-4 in α-HRJ **1** and H-4, H-5 and H-6 in β-HRJ **2** is consistent with an equatorial methyl group in both cases and a hydroxymethyl group which is axially orientated in **1** and equatorial in **2**. These

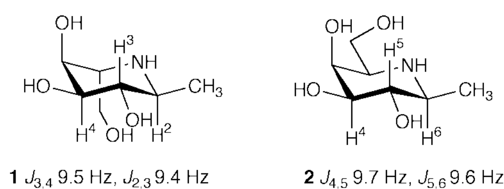
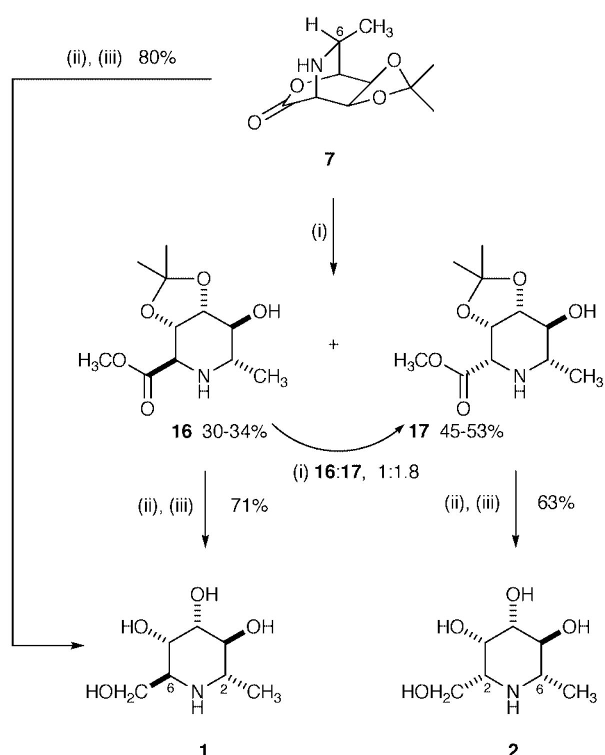


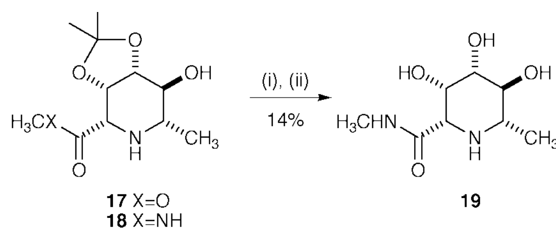
Fig. 3 *trans*-Diaxial couplings in compounds **1** and **2**.



Scheme 5 *Reagents and conditions*: (i) NaOAc, MeOH, reflux; (ii) $LiBEt_3H$, THF; (iii) HCl, MeOH.

observations parallel those made for the parent methyl esters **16** and **17** as well as the α-amides **14a**–**d**.

The β-ester **17** allowed access to β-methylamide **19** for further structure–activity relationship studies. Treatment of the β-ester **17** with an excess of methylamine in methanol at reflux afforded the protected β-methylamide **18** in 18% yield and subsequent deprotection with methanolic hydrogen chloride gave **19** in 80% yield (Scheme 6).



Scheme 6 *Reagents and conditions*: (i) CH_3NH_2, MeOH, reflux; (ii) HCl, MeOH.

In summary, the synthesis of a series of imino-*C*-glycoside analogues of 5-*epi*-rhamnose and L-rhamnose was achieved from the L-rhamnose-derived key intermediate bicyclic lactones **6** and **7**, thus demonstrating their potential for generating libraries of analogues for screening for biological activity.

Enzyme-inhibition studies

The set of compounds synthesized were assayed against a variety of glycosidases including rabbit gut activities of α-glucosidase, β-glucosidase and rabbit liver homogenate activities of β-glucosidase and β-galactosidase, in addition to α-glucosidase (Brewers' yeast), β-glucosidase (almond emulsin), α-galactosidase (green coffee bean), β-galactosidase (Jack bean), α-mannosidase (Jack bean), β-*N*-acetylglucosaminidase (bovine and Jack bean), α-fucosidase (bovine epididymis) and naringinase (*Penicillium decumbens*). The naringinase inhibition data are summarized in Table 1.

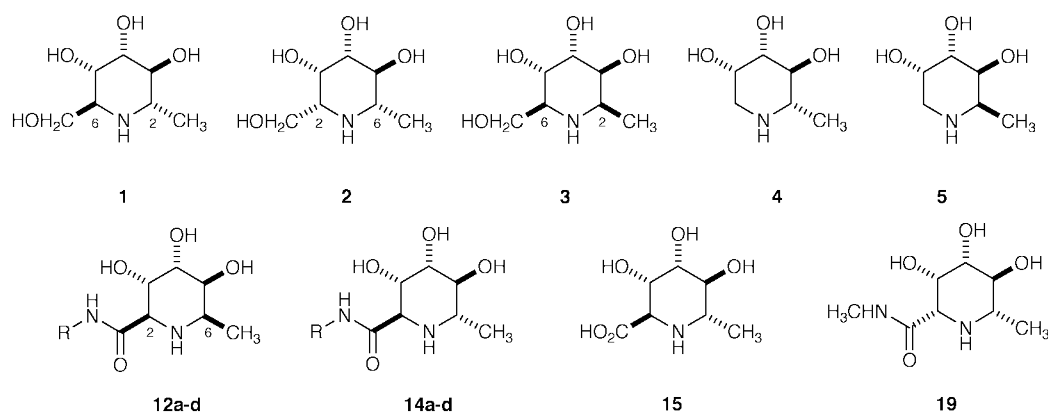
The analogues of 5-*epi*-rhamnose, **12a**–**d** and **3**, did not display any significant inhibition of naringinase. Conversely, the α-amide analogues of L-rhamnopyranose, **14a**–**d**, were found to be potent and selective competitive inhibitors of naringinase (IC_{50} – **14a** R = H 30 μM; **14b** R = CH_3 21 μM; **14c** R = Bu 104 μM; **14d** R = Bn 100 μM). The trihydroxypipecolic acid **15** did not cause any inhibition of the enzymes used. α-HRJ **1** was a potent inhibitor of naringinase (IC_{50} 15 μM; K_i 5.3 μM), although weak inhibition of some other enzymes was observed at high concentrations, this is comparable with nariginase inhibition by 5-*epi*-DRJ **5** (IC_{50} 5 μM; K_i 1 μM).[10] The β-epimer β-HRJ **2** was, by contrast, a far weaker inhibitor of naringinase (IC_{50} 750 μM), as was the β-methylamide **19** (IC_{50} 350 μM). Importantly, β-HRJ **2** was found to be a potent and selective inhibitor of α-galactosidase (green coffee bean) (K_i 0.31 μM), although it caused no inhibition of β-galactosidase (Jack bean). This may be rationalized since **2** may also be viewed as the β-1-methyl derivative of deoxygalactonojirimycin (DGJ) **20**, DGJ itself being a potent inhibitor of α- and β-galactosidase[17] (Fig. 4).

These studies indicate, therefore, that imino-*C*-glycosyl analogues of 5-*epi*-rhamnopyranose are not generally able to inhibit naringinase, whereas the α-imino-*C*-glycosyl analogues of L-rhamnopyranose are potent and selective inhibitors. However, the β-aza-*C*-glycosyl analogues of L-rhamnopyranose appear to lose their ability to inhibit naringinase. These observations are in contrast to the behaviour of DRJ **4** and 5-*epi*-DRJ **5** and provide further examples of the changes in potency and selectivity resulting from incorporation of *C*-glycosyl substituents into deoxyiminosugar structures. The behaviour of β-HRJ **2** acting as an α-galactosidase inhibitor serves to illustrate that the three-dimensional configuration of the carbohydrate epitope is a major factor in determining specificity for the associated hydrolase. Molecular modelling studies are currently in progress in order to rationalize this behaviour.

Some of the compounds (**4**, **5**, **12a**–**d**, and **14a**–**d**) were also assayed for inhibition of dTDP-L-rhamnose biosynthesis. The biosynthesis of dTDP-L-rhamnose from dTDP-D-glucose has been elucidated for a number of bacteria other than *Mycobacterium* and the conversion involves three enzymes (dTDP-D-glucose-4,6-dehydratase, dTDP-4-keto-6-deoxy-D-glucose-3,5-epimerase and dTDP-4-keto-L-rhamnose reductase).[18] The results of these assays (Table 1) indicated that DRJ **4** and the 5-*epi*-rhamnopyranose analogues **12a**–**d** caused no inhibition of dTDP-L-rhamnose biosynthesis, although 5-*epi*-rhamnopyranose analogues 5-*epi*-DRJ **5** and *epi*-HRJ **3** were found to be weak inhibitors [**5** 21% (at 0.1 mM) and **3** 22% (at 0.5 mM)]. Of the rhamnopyranose analogues **14a**–**d**, the butylamide **14c** and the benzylamide **14d** displayed no inhibition; however, the methyl amide **14b** caused weak inhibition [39% (at 0.1 mM)] and the primary amide **14a** was markedly more potent [69% (at 10 μM)]. The β-amide **19** was also found to be a weak inhibitor [18% (at 0.1 mM)]. To the best of our knowledge, these results represent the first set of compounds to display inhibition of dTDP-L-rhamnose biosynthesis.

This work provides some indication that naringinase inhibition correlates with the ability to inhibit dTDP-L-rhamnose biosynthesis. This is best demonstrated by comparing the behaviour of the deoxyiminosugars DRJ **4** and 5-*epi*-DRJ **5**, where the 5-*epi*-rhamnopyranose analogue is an inhibitor, and the homologous amides **12a**–**d**, **14a** and **14b**, where the L-rhamnopyranose analogues are inhibitors.

Table 1



Compound	Inhibition of naringinase (IC_{50} or K_i)	Inhibition of other glycosidases[a]	Inhibition of dTDP-L-rhamnose biosynthesis[a]
1	IC_{50} 15 µM K_i 5.3 µM	α-mannosidase (Jack bean) 40% α-galactosidase (green coffee bean) 46% β-galactosidase (*E. coli*) 35% (0.8 mM)	NT
2	IC_{50} 750 µM	α-galactosidase (green coffee bean) IC_{50} 0.34 µM, K_i 0.31 µM	NT
3	IC_{50} 850 µM	NI	22% (0.5 mM)
4	NI	NI	NI
5	IC_{50} 5 µM, K_i 1 µM	β-glucosidase (almond) 60% (130 µM)	21% (0.1 mM)
12a–d	NI	NI	NI
14a	IC_{50} 30 µM	NI	69% (10 µM)
14b	IC_{50} 21 µM	NI	39% (0.1 mM)
14c	IC_{50} 104 µM	NI	NI
14d	IC_{50} 100 µM	α-mannosidase (Jack bean) 30% (0.7 mM)	NI
15	NI	NI	NT
19	IC_{50} 350 µM	NI	18% (0.1 mM)

[a] Figures in parentheses refer to inhibitor concentrations used. NI – No inhibition at concentrations of approximately 0.1 mM. NT – Not tested.

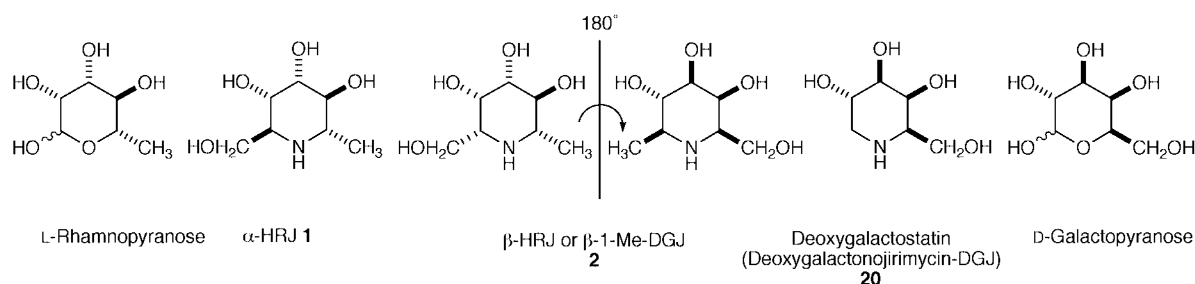


Fig. 4 Structural relationship of compound **2** to L-rhamnopyranose and D-galactopyranose.

Conclusions

This paper describes the efficient synthesis of a series of homonojirimycin analogues of 5-*epi*-rhamnopyranose and both α- and β-rhamnopyranose from bicyclic amino lactone divergent intermediates, accessible from L-rhamnose. Biological assays indicate that imino-*C*-glycosyl analogues of α-rhamnopyranose are potent and selective inhibitors of naringinase whereas the β-rhamnopyranose and 5-*epi*-rhamnopyranose analogues lose their activity. Further studies into the inhibition of dTDP-L-rhamnose biosynthesis indicate that there may be correlation between the inhibition of naringinase and the multienzyme dTDP-L-rhamnose biosynthesis.

Experimental

THF was distilled under an atmosphere of dry nitrogen from sodium benzophenone ketyl; dichloromethane and pyridine were distilled from calcium hydride; hexane refers to the fraction of petroleum ether which boils in the range 60–80 °C and was re-distilled before use. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reactions were performed under an atmosphere of nitrogen or argon maintained by an inflated balloon. Butyllithium was used as a solution in hexanes at the molarity stated; Super-Hydride® (lithium triethylborohydride) and TBAF were used as solutions in THF at the molarity stated; hydrogenations were performed using an atmosphere of hydrogen gas maintained by an inflated balloon. All other reagents were used as supplied, without prior purification. Flash chromatography was performed on Sorbsil C60, and ion-exchange chromatography was performed on Amberlite IR-120 (H^+). Mps were recorded on a Kofler hot block and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (^{1}H: 200 MHz and ^{13}C: 50.3 MHz) or Bruker AM 500 or AMX 500 (^{1}H: 500 MHz and ^{13}C: 125.8 MHz) spectrometer for samples in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hz. Residual signals from the solvents were used as an internal reference and ^{13}C NMR spectra in D_2O were referenced to 1,4-dioxane (δ_C 67.4). ^{13}C Multiplicity was assigned using a DEPT sequence. IR spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform,

or Perkin-Elmer Paragon 1000 spectrophotometer using either thin films on NaCl plates (film) or KBr discs (KBr) as stated. For clarity, only the salient, characteristic peaks are quoted. Low-resolution mass spectra (*m/z*) were recorded on VG MASS LAB 20-250, BIO Q, VG Platform, or VG Autospec spectrometers, and high-resolution mass spectra (HRMS *m/z*) on a VG Autospec spectrometer. Techniques used were chemical ionization (CI, NH_3), desorption chemical ionization (DCI, NH_3), electrospray, electron impact (EI) or atmospheric pressure chemical ionization (APCI) using partial purification by HPLC with methanol : acetonitrile : water (40 : 40 : 20) as eluent, as stated. Specific optical rotations $[\alpha]_D$ are quoted in 10^{-1} deg cm^2 g^{-1} and were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in 10^{-2} g cm^{-3}. Elemental analyses were performed by the microanalysis service of the Dyson Perrins Laboratory.

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-D-*glycero*-L-*talo*-heptono-1,5-lactone 6

Trifluoromethanesulfonic anhydride (1.7 cm^3, 10.1 mmol) was added to a solution of azido lactone **8** (2.0 g, 7.8 mmol) in dichloromethane (50 cm^3) containing pyridine (1.9 cm^3, 23.4 mmol) at −20 °C. After stirring for 10 min, water (5 cm^3) was added and the mixture allowed to warm to room temperature. The solution was filtered through silica, eluting with ethyl acetate, and the filtrate concentrated *in vacuo* to afford the crude azido triflate. The material was dissolved in ethyl acetate (50 cm^3) and the solution stirred under an atmosphere of hydrogen in the presence of palladium black (150 mg) and sodium acetate (1.9 g, 23.1 mmol) for 16 h. The mixture was filtered through Celite and the filtrate concentrated *in vacuo*. The residue was purified by chromatography on silica (ethyl acetate–hexane, 1 : 1) to afford the bicyclic amino lactone **6** as a white crystalline solid (1.01 g, 61%), mp 96–98 °C; $[\alpha]_D^{21}$ +24.8 (*c* 1.0 in $CHCl_3$) (Found: C, 56.55; H, 7.15; N, 6.65. $C_{10}H_{15}NO_4$ requires C, 56.3; H, 7.1; N, 6.6%); ν_{max}(KBr)/cm^{-1} 3391 (br, NH), 1775 (s, C=O); δ_H(500 MHz; $CDCl_3$) 1.17 (3H, d, *J* 6.8, H_3-7), 1.41, 1.61 (6H, 2d, $C(CH_3)_2$), 3.61 (1H, q, $J_{6,7}$ 6.8, H-6), 3.70 (1H, d, $J_{2,3}$ 3.2, H-2), 4.37–4.39 (1H, m), 4.46–4.48 (2H, m); δ_C(50 MHz; CD_3CN) 19.5, 24.2, 25.4 (3q, $CH_3(CH_2)_3$, C-7), 42.6, 54.5 (2d, C-2, C-6), 71.8, 72.1, 79.3 (3d, C-3, C-4, C-5), 113.7 (s, $C(CH_3)_2$), 171.7 (s, C-1); *m/z* (CI, NH_3) 214 (MH^+, 100%).

2-Azido-2,7-dideoxy-3,4-*O*-isopropylidene-L-*talo*-hept-6-ulosono-1,5-lactone 9

Pyridinium chlorochromate (25.5 g, 118 mmol) was added to a solution of azido lactone **8** (10.12 g, 39.4 mmol) in dichloromethane (300 cm^3) containing activated 4 Å molecular sieve (26 g) at room temperature. The mixture was stirred for 2 h and subsequently filtered through a column of silica topped by a layer of Celite, eluting with diethyl ether. The residual layer was removed and stirred in diethyl ether for 20 min and the mixture re-filtered. This process was repeated and the combined filtrate concentrated *in vacuo*. The residue was purified by chromatography on silica (ethyl acetate–hexane, 1 : 1) to afford the azido ketone **9** as a white crystalline solid (8.2 g, 82%), mp 122–123 °C (decomp.); $[\alpha]_D^{21}$ −9.2 (*c* 1.0 in $CHCl_3$) (Found: C, 46.9; H, 4.9; N, 16.3. $C_{10}H_{13}N_3O_5$ requires C, 47.1; H, 5.1; N, 16.5%); ν_{max}(KBr)/cm^{-1} 2117 (s, N_3), 1763, 1732 (s, C=O); δ_H(500 MHz; $CDCl_3$) 1.36, 1.50 (6H, 2d, $C(CH_3)_2$), 2.39 (3H, s, H_3-7), 3.77 (1H, d, *J* 2.8, H-2 or H-5), 4.47 (1H, d, *J* 1.5, H-5 or H-2), 4.85–4.85 (2H, m, H-3, H-4); δ_C(50 MHz; $CDCl_3$) 24.1, 25.6, 28.0 (3q, $C(CH_3)_2$, C-7), 59.6 (d, C-2), 74.5, 75.1, 80.5 (3d, C-3, C-4, C-5), 111.7 (s, $C(CH_3)_2$), 166.0 (s, C-1), 203.6 (s, C-6); *m/z* (DCI, NH_3) 212 (100%), 228 ($MH^+ - N_2$, 70), 273 (MNH_4^+, 20).

2-Amino-*N*,6-anhydro-2,7-dideoxy-3,4-*O*-isopropylidene-L-*talo*-hept-2-ulosono-1,5-lactone 10

Triethyl phosphite (8.4 cm^3, 48 mmol) was added to a solution of the azido ketone **9** (6.2 g, 24 mmol) in THF (100 cm^3) and the mixture was stirred at reflux for 4 h. The solvent was removed *in vacuo* and the residue purified by chromatography on silica (ethyl acetate–hexane, 2 : 3) to afford the bicyclic imine **10** as a white crystalline solid (3.13 g, 61%), mp 183–185 °C; $[\alpha]_D^{21}$ −167.3 (*c* 1.0 in $CHCl_3$) (Found: C, 56.9; H, 6.25; N, 6.4. $C_{10}H_{13}NO_4$ requires C, 56.9; H, 6.2; N, 6.6%); ν_{max}(KBr)/cm^{-1} 1786 (s, C=O), 1648 (C=N); δ_H(500 MHz; $CDCl_3$) 1.32, 1.37 (6H, 2d, $C(CH_3)_2$), 2.32 (3H, s, H_3-7), 4.61 (1H, dd, *J* 2.6, 7.0, H-3 or H-4), 4.67 (1H, dd, *J* 4.2, 7.0, H-4 or H-3), 5.10 (1H, d, *J* 4.2, H-2 or H-5), 5.13 (1H, d, *J* 2.6, H-5 or H-2); δ_C(50 MHz; $CDCl_3$) 23.9, 24.8, 25.3 (3q, $C(CH_3)_2$, C-7), 62.1 (d, C-2), 72.8, 74.2, 75.2 (3d, C-3, C-4, C-5), 114.4 (s, $C(CH_3)_2$), 168.1, 175.2 (2s, C-1, C-6); *m/z* (EI) 100 (100%), 211 (M^+, 15).

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*talo*-heptono-1,5-lactone 7

A solution of sodium cyanoborohydride (245 mg, 3.9 mmol) in acetic acid (5 cm^3) was treated with a solution of bicyclic imine **10** (785 mg, 3.72 mmol) in acetic acid (15 cm^3) at room temperature. The mixture was stirred for 30 min, subsequently concentrated *in vacuo* and the residue subjected to flash chromatography on silica (ethyl acetate–hexane, 1 : 1) to afford bicyclic lactone **7** as a white solid (660 mg, 82%), mp 118–119 °C; $[\alpha]_D^{21}$ +32.8 (*c* 1.34 in $CHCl_3$) (Found: C, 56.5; H, 7.3; N, 6.7. $C_{10}H_{15}NO_4$ requires C, 56.3; H, 7.1; N, 6.6%); ν_{max}(KBr)/cm^{-1} 3357 (br, NH), 1782 (s, C=O); δ_H(500 MHz; $CDCl_3$) 1.39 (3H, s, $C(CH_3)_2$), 1.44 (3H, d, $J_{6,7}$ 7.1, H_3-7), 1.65 (3H, s, $C(CH_3)_2$), 3.47 (1H, ddq, $J_{4,6}$ 1.6, $J_{5,6}$ 1.8, $J_{6,7}$ 7.1, H-6), 3.75 (1H, d, $J_{2,3}$ 2.6, H-2), 4.41 (1H, dd, $J_{2,3}$ 2.6, $J_{3,4}$ 8.2, H-3), 4.53 (1H, dd, $J_{5,6}$ 1.8, $J_{4,5}$ 4.4, H-5), 4.59 (1H, ddd, $J_{4,6}$ 1.6, $J_{4,5}$ 4.4, $J_{3,4}$ 8.2, H-4); δ_C(50 MHz; $CDCl_3$) 17.7, 23.8, 24.9 (3q, C-7, $C(CH_3)_2$), 51.7, 54.9 (2d, C-2, C-6), 72.5, 72.9, 77.3 (3d, C-3, C-4, C-5), 113.7 (s, $C(CH_3)_2$), 170.6 (s, C-1); *m/z* (CI, NH_3) 214 (MH^+, 100%). Repeating the reaction using bicyclic imine **10** (2.50 g, 11.8 mmol) and sodium cyanoborohydride (740 mg, 11.8 mmol) in acetic acid afforded the bicyclic amino lactone **7** (1.76 g, 70%), identical to the material listed above.

Crystal data for 7.[19,20] $C_{10}H_{15}NO_4$, M = 213.23. Crystallizes from mixtures of dichloromethane–hexane as clear, colourless blocks. Monoclinic, a = 9.1220(15), b = 20.847(3), c = 5.6640(5) Å, α = 90°, β = 92.048(9)°, γ = 90°, V = 1076.4 Å^3. Space group $P1\,2_1\,1$. 2 molecules per asymmetric unit. Z = 4. μ = 0.10 mm^{-1}. T(K) 295. No. of reflections measured: 8413. No. of independent reflections: 1320. R_{int} 0.02. CCDC reference number 207/381. See http://www.rsc.org/suppdata/p1/1999/2735 for crystallographic files in .cif format.

General procedure 1 – lactone-opening reactions

A solution of the requisite bicyclic amino lactone (**6** or **7**) (1 equivalent) in THF (approx. 5 cm^3 mmol^{-1}) was treated with the requisite amine (1.2 equivalents – large excess) and the mixture stirred at room temperature until completion (0.5–18 h), as monitored by TLC. The solvent was removed *in vacuo* and the residue purified by column chromatography on silica (ethyl acetate–hexane or methanol–ethyl acetate).

General prodedure 2 – deprotections

A solution of the requisite protected material in 1% methanolic hydrogen chloride (approx. 20 cm^3 mmol^{-1}) was stirred at room temperature for 18–24 h and subsequently concentrated *in vacuo* (with further co-evaporation with methanol, three times). The residue was purified by ion-exchange chromatography on Amberlite (IR 120, H^+ form) (1.0 M ammonium hydroxide as eluent).

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-D-*glycero*-L-*talo*-heptonamide 11a

Amide **11a** was prepared according to general procedure 1 using bicyclic lactone **6** (150 mg, 0.70 mmol) and conc. ammonia solution (0.5 cm^3). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a hygroscopic foam (157 mg, 97%), $[\alpha]_D^{23}$ −98.2 (*c* 1.53 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3317 (br, NH, OH), 1679 (s, Amide I), 1611 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.21 (3H, d, $J_{6,7}$ 6.6, H_3-7), 1.40, 1.55 (6H, 2s, $C(CH_3)_2$), 3.11 (1H, dq, $J_{5,6}$ 1.5, $J_{6,7}$ 6.6, H-6), 3.32 (1H, d, $J_{2,3}$ 9.2, H-2), 3.76 (1H, br m, H-5), 4.16 (1H, dd, $J_{3,4}$ 2.5, $J_{2,3}$ 9.2, H-3), 4.32 (1H, dd, $J_{3,4}$ 2.5, $J_{4,5}$ 4.8, H-4), 5.68, 6.58 (2H, 2br m, $CONH_2$); δ_C(50 MHz; $CDCl_3$) 16.6 (q, C-7), 26.1, 28.2 (2q, $C(CH_3)_2$), 51.3 (d, C-6), 61.2 (d, C-2), 68.8, 72.7, 77.7 (3d, C-3, C-4, C-5), 109.8 (s, $C(CH_3)_2$), 174.2 (s, C-1); *m*/*z* (CI, NH_3) 231 (MH^+, 100%); (CI^+) [Found: 231.1339 (MH^+). $C_{10}H_{19}N_2O_4$ requires *m*/*z*, 231.1345].

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-*N*-methyl-D-*glycero*-L-*talo*-heptonamide 11b

Amide **11b** was prepared according to general procedure 1 using bicyclic lactone **6** (150 mg, 0.70 mmol) and methylamine (33% w/w solution in industrial methylated spirit; 0.11 cm^3, 0.91 mmol). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a hygroscopic foam (163 mg, 95%), $[\alpha]_D^{22}$ −93.7 (*c* 2.15 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3310 (br, NH, OH), 1656 (s, Amide I), 1551 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.21 (3H, d, $J_{6,7}$ 6.6, H_3-7), 1.40, 1.55 (6H, 2s, $C(CH_3)_2$), 2.85 (3H, d, *J* 4.8, $NHCH_3$), 3.10 (1H, dq, $J_{5,6}$ 1.5, $J_{6,7}$ 6.6, H-6), 3.27 (1H, d, $J_{2,3}$ 9.1, H-2), 3.75 (1H, br m, H-5), 4.16 (1H, dd, $J_{3,4}$ 4.9, $J_{2,3}$ 9.1, H-3), 4.31 (1H, dd, $J_{3,4}$ 4.9, $J_{4,5}$ 2.5, H-4), 6.52 (1H, br m, CON*H*); δ_C(50 MHz; $CDCl_3$) 16.6 (q, C-7), 26.0, 26.1, 28.2 (3q, NCH_3, $C(CH_3)_2$), 51.2 (d, C-6), 61.4 (d, C-2), 69.8, 72.8, 77.7 (3d, C-3, C-4, C-5), 109.8 (s, $C(CH_3)_2$), 172.1 (s, C-1); *m*/*z* (CI, NH_3) 245 (MH^+, 100%); (CI^+) [Found: 245.1494 (MH^+). $C_{11}H_{21}N_2O_4$ *m*/*z*, requires 245.1501].

***N*-Butyl-2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-D-*glycero*-L-*talo*-heptonamide 11c**

Amide **11c** was prepared according to general procedure 1 using bicyclic lactone **6** (100 mg, 0.47 mmol) and butylamine (0.06 cm^3, 0.6 mmol). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a white solid (123 mg, 95%), mp 106–107 °C; $[\alpha]_D^{22}$ −85.7 (*c* 1.02 in $CHCl_3$) (Found: C, 58.6; H, 9.4; N, 9.65. $C_{14}H_{26}N_2O_4$ requires C, 58.7; H, 9.15; N, 9.8%); ν_{max}(KBr)/cm^{-1} 3308 (br, NH, OH), 1652 (s, Amide I), 1547 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 0.93 (3H, t, *J* 7.3, $CH_3(CH_2)_3$), 1.21 (3H, d, $J_{6,7}$ 6.6, H_3-7), 1.35 (2H, m, CH_2), 1.40 (3H, s, $C(CH_3)_2$), 1.51 (2H, m, CH_2), 1.55 (3H, s, $C(CH_3)_2$), 3.10 (1H, dq, $J_{5,6}$ 1.4, $J_{6,7}$ 6.6, H-6), 3.27 (1H, d, $J_{2,3}$ 9.2, H-2), 3.23–3.34 (2H, m, CH_2NH_2), 3.75 (1H, br m, H-5), 4.16 (1H, dd, $J_{3,4}$ 4.8, $J_{2,3}$ 9.2, H-3), 4.31 (1H, dd, $J_{3,4}$ 4.8, $J_{4,5}$ 2.6, H-4), 6.58 (1H, br m, CON*H*); δ_C(50 MHz; $CDCl_3$) 13.7, 16.6 (2q, $CH_3(CH_2)_3$, C-7), 20.0 (t, CH_2), 26.1, 28.2 (2q, $C(CH_3)_2$), 31.6, 38.9 (2t, $(CH_2)_2$), 51.3 (d, C-6), 61.3 (d, C-2), 68.9, 73.0, 77.8 (3d, C-3, C-4, C-5), 109.8 (s, $C(CH_3)_2$), 171.5 (s, C-1); *m*/*z* (CI, NH_3) 287 (MH^+, 100%).

***N*-Benzyl-2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-D-*glycero*-L-*talo*-heptonamide 11d**

Amide **11d** was prepared according to general procedure 1 using bicyclic lactone **6** (100 mg, 0.47 mmol) and benzylamine (0.07 cm^3, 0.6 mmol). Purification of the crude material using column chromatography (ethyl acetate) afforded the title compound as a white solid (140 mg, 93%), mp 109–110 °C; $[\alpha]_D^{22}$ −74.0 (*c* 1.03 in $CHCl_3$) (Found: C, 63.9; H, 7.8; N, 8.6. $C_{17}H_{24}N_2O_4$ requires C, 63.7; H, 7.55; N, 8.7%); ν_{max}(KBr)/cm^{-1} 3316 (br, NH, OH), 1657 (s, Amide I), 1536 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.22 (3H, d, $J_{6,7}$ 6.6, H_3-7), 1.38, 1.54 (6H, 2s, $C(CH_3)_2$), 3.12 (1H, dq, $J_{5,6}$ 1.5, $J_{6,7}$ 6.6, H-6), 3.35 (1H, d, $J_{2,3}$ 9.1, H-2), 3.76 (1H, m, H-5), 4.21 (1H, dd, $J_{3,4}$ 4.9, $J_{2,3}$ 9.1, H-3), 4.32 (1H, dd, $J_{3,4}$ 4.9, $J_{4,5}$ 2.5, H-4), 4.50 (2H, ABX system, *J* 5.9, 15.0, $PhCH_2NH$), 6.95 (1H, br m, CON*H*), 7.26–7.36 (5H, m, Ar*H*); δ_C(50 MHz; $CDCl_3$) 16.6 (q, C-7), 26.1, 28.3 (2q, $C(CH_3)_2$), 43.1 (t, $PhCH_2$), 51.3 (d, C-6), 61.5 (d, C-2), 68.8, 72.8, 77.7 (3d, C-3, C-4, C-5), 109.9 (s, $C(CH_3)_2$), 127.3, 128.6 (3d, $C_{ortho,\ meta,\ para}$), 138.2 (s, C_{ipso}), 171.7 (s, C-1); *m*/*z* (CI, NH_3) 186 (100%), 321 (MH^+, 73).

2,6,7-Trideoxy-2,6-imino-D-*glycero*-L-*talo*-heptonamide 12a

Amide **11a** (80 mg, 0.35 mmol) was deprotected according to general procedure 2 to afford the title compound as a hygroscopic foam (48 mg, 72%), $[\alpha]_D^{23}$ −45.0 (*c* 1.22 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3418 (br, NH, OH), 1674 (s, Amide I), 1630 (sh, Amide II); δ_H(500 MHz; D_2O, pH 8) 1.09 (3H, d, $J_{6,7}$ 6.9, H_3-7), 3.07 (1H, dq, $J_{5,6}$ 1.4, $J_{6,7}$ 6.9, H-6), 3.44 (1H, d, $J_{2,3}$ 10.4, H-2), 3.69 (1H, dd, $J_{4,5}$ 3.9, $J_{5,6}$ 1.4, H-5), 3.82 (1H, dd, $J_{3,4}$ 3.1, $J_{2,3}$ 10.4, H-3), 4.01 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 3.9, H-4); δ_C(50 MHz; D_2O, pH 8) 16.3 (q, C-7), 48.2 (d, C-6), 58.9 (d, C-2), 67.4, 71.6, 72.8 (3d, C-3, C-4, C-5), 176.4 (s, C-1); *m*/*z* (Electrospray) 191 (MH^+, 100%); (CI^+) [Found: 191.1034 (MH^+). $C_7H_{15}N_2O_4$ requires *m*/*z*, 191.1032].

2,6,7-Trideoxy-2,6-imino-*N*-methyl-D-*glycero*-L-*talo*-heptonamide 12b

Amide **11b** (80 mg, 0.33 mmol) was deprotected according to general procedure 2 to afford the title compound as a hygroscopic foam (52 mg, 78%), $[\alpha]_D^{23}$ −49.3 (*c* 1.04 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3377 (br, NH, OH), 1636 (s, Amide I), 1586 (m, Amide II); δ_H(500 MHz; D_2O, pH 8) 1.05 (3H, d, $J_{6,7}$ 6.8, H_3-7), 2.75 (3H, s, $NHCH_3$), 3.06 (1H, dq, $J_{5,6}$ 1.6, $J_{6,7}$ 6.8, H-6), 3.36 (1H, d, $J_{2,3}$ 10.4, H-2), 3.69 (1H, $J_{5,6}$ 1.6, $J_{4,5}$ 3.9, H-5), 3.83 (1H, dd, $J_{3,4}$ 3.1, $J_{2,3}$ 10.4, H-3), 4.00 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 3.9, H-4); δ_C(50 MHz; D_2O, pH 8) 16.4 (q, C-7), 26.5 (q, NCH_3), 48.1 (d, C-6), 59.6 (d, C-2), 69.5, 71.7, 73.0 (3d, C-3, C-4, C-5), 109.8, 174.4 (s, C-1); *m*/*z* (Electrospray) 205 (MH^+, 100%); (CI^+) [Found: 205.1184 (MH^+). $C_8H_{17}N_2O_4$ requires *m*/*z*, 205.1188].

***N*-Butyl-2,6,7-trideoxy-2,6-imino-D-*glycero*-L-*talo*-heptonamide 12c**

Amide **11c** (100 mg, 0.41 mmol) was deprotected according to general procedure 2 to afford the title compound as a hygroscopic foam (70 mg, 81%), $[\alpha]_D^{23}$ −37.9 (*c* 0.98 in H_2O, pH 8); ν_{max}(film)/cm^{-1} 3425 (br, NH, OH), 1642 (s, Amide I), 1567 (m, Amide II); δ_H(500 MHz; D_2O, pH 8) 0.85 (3H, t, *J* 7.3, $CH_3(CH_2)_3$), 1.04 (3H, d, $J_{6,7}$ 6.9, H_3-7), 1.29 (2H, m, CH_2), 1.46 (2H, m, CH_2), 3.04 (1H, dq, $J_{5,6}$ 1.4, $J_{6,7}$ 6.9, H-6), 3.19 (2H, m, CH_2NH_2), 3.33 (1H, d, $J_{2,3}$ 10.4, H-2), 3.68 (1H, dd, $J_{5,6}$ 1.4, $J_{4,5}$ 3.9, H-5), 3.81 (1H, dd, $J_{3,4}$ 3.1, $J_{2,3}$ 10.4, H-3), 3.99 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 3.9, H-4); δ_C(50 MHz; D_2O, pH 8) 13.6, 16.3 (2q, $CH_3(CH_2)_3$, C-7), 20.0, 31.0, 39.8 (3t, $(CH_2)_3$), 48.0 (d, C-6), 59.6 (d, C-2), 67.4, 71.7, 72.9 (3d, C-3, C-4, C-5), 173.5 (s, C-1); *m*/*z* (CI, NH_3) 247 (MH^+, 100%); (CI^+) [Found: 247.1663 (MH^+). $C_{11}H_{23}N_2O_4$ requires *m*/*z*, 247.1658].

***N*-Benzyl-2,6,7-trideoxy-2,6-imino-D-*glycero*-L-*talo*-heptonamide 12d**

Amide **11d** (100 mg, 0.31 mmol) was deprotected according to general procedure 2 to afford the title compound as a foam (54 mg, 75%), $[\alpha]_D^{23}$ −44.4 (*c* 0.99 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3439 (br, NH, OH), 1650 (s, Amide I), 1555 (m, Amide II); δ_H(500 MHz; D_2O, pH 8) 1.05 (3H, d, $J_{6,7}$ 6.9, H_3-7), 3.05 (1H, dq, $J_{5,6}$ 1.5, $J_{6,7}$ 6.9, H-6), 3.43 (1H, d, $J_{2,3}$ 10.4, H-2), 3.69 (1H,

dd, $J_{5,6}$ 1.5, $J_{4,5}$ 3.9, H-5), 3.85 (1H, dd, $J_{3,4}$ 3.1, $J_{2,3}$ 10.4, H-3), 4.01 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 3.9, H-4), 4.40, 4.44 (2H, AB system, *J* 15.3, PhC*H*$_2$NH), 7.31–7.41 (5H, m, Ar*H*); δ_C(125 MHz; D_2O, pH 8) 16.1 (q, C-7), 43.4 (t, Ph*C*H$_2$), 47.7 (d, C-6), 59.4 (d, C-2), 67.3, 71.5, 72.7 (3d, C-3, C-4, C-5), 127.6, 127.9, 129.2 (3d, C$_{ortho,\ meta,\ para}$), 138.1 (s, C$_{ipso}$), 173.7 (s, C-1); *m/z* (CI, NH_3) 281 (MH^+, 100%); (CI^+) [Found: 281.1504 (MH^+). $C_{14}H_{21}N_2O_4$ requires *m/z*, 281.1501].

1,2,6-Trideoxy-2,6-imino-L-*glycero*-L-*gluco*-heptitol 3

A solution of lithium borohydride (30 mg) in THF (4 cm^3) was added to a solution of bicyclic lactone **6** (197 mg, 0.92 mmol) in THF (5 cm^3) at 0 °C. The mixture was stirred for 30 min and allowed to warm to room temperature. After stirring for a further 1 h, the mixture was treated with 2.0 M aqueous hydrochloric acid until the solution reached pH 2. The solvent was removed *in vacuo* and the residue purified by ion-exchange chromatography on Amberlite (IR 120, H^+ form) (1.0 M ammonium hydroxide) to afford *epi*-HRJ **3** as a hygroscopic solid (150 mg, 92%), $[a]_D^{25}$ −38.1 (*c* 0.9 in H_2O, pH 9); ν_{max}(film)/cm^{-1} 3401 (br, NH, OH); δ_H(500 MHz; D_2O) 1.11 (3H, d, $J_{1,2}$ 6.8, H$_3$-1), 2.93 (1H, dt, *J* 4.0, 10.7, H-6), 3.20 (1H, q, $J_{1,2}$ 6.8, H-2), 3.71–3.75 (3H, m), 3.77 (1H, dd, $J_{4,5}$ 3.1, $J_{5,6}$ 10.7, H-5), 3.98 (1H, t, *J* 3.5); δ_C(50 MHz; D_2O) 15.9 (q, C-1), 61.0 (t, C-7), 49.3, 55.9 (2d, C-2, C-6), 65.4, 71.4, 72.5 (3d, C-3, C-4, C-5); *m/z* (DCI) 178 (MH^+, 100%); (+ve Electrospray) [Found: 178.1082 (MH^+). $C_7H_{15}NO_4$ requires *m/z*, 178.1079].

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*talo*-heptonamide 13a

Amide **13a** was prepared according to general procedure 1 using bicyclic lactone **7** (100 mg, 0.47 mmol) and conc. ammonia solution (1 cm^3). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a white solid (101 mg, 93%), $[a]_D^{23}$ +75.3 (*c* 0.80 in $CHCl_3$) (Found: C, 52.4; H, 7.8; N, 12.4. $C_{10}H_{18}N_2O_4$ requires C, 52.2; H, 7.9; N, 12.2%); ν_{max}(KBr)/cm^{-1} 3437, 3319 (br, NH, OH), 1678 (s, Amide I), 1611 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.22 (3H, d, $J_{6,7}$ 6.5, H$_3$-7), 1.41, 1.54 (6H, 2s, C(C*H*$_3$)$_2$), 2.53 (1H, dq, $J_{5,6}$ 9.4, $J_{6,7}$ 6.5, H-6), 3.27 (1H, dd, $J_{4,5}$ 7.1, $J_{5,6}$ 9.4, H-5), 3.83 (1H, d, $J_{2,3}$ 2.3, H-2), 3.97 (1H, dd, $J_{3,4}$ 5.3, $J_{4,5}$ 7.1, H-4), 4.87 (1H, dd, $J_{3,4}$ 5.3, $J_{2,3}$ 2.3, H-3), 5.59, 7.24 (2H, 2br m, CON*H*$_2$); δ_C(50 MHz; $CDCl_3$) 14.1 (q, C-7), 18.0, 21.0 (2q, C(*C*H$_3$)$_2$), 50.4 (d, C-6), 56.8 (d, C-2), 73.7, 75.7, 78.9 (3d, C-3, C-4, C-5), 108.7 (s, *C*(CH$_3$)$_2$), 173.7 (s, C-1); *m/z* (+ve Electrospray) 231 (MH^+, 100%).

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-*N*-methyl-L-*glycero*-L-*talo*-heptonamide 13b

Amide **13b** was prepared according to general procedure 1 using bicyclic lactone **7** (120 mg, 0.56 mmol) and methylamine (33% w/w solution in industrial methylated spirit, 0.085 cm^3, 0.73 mmol). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a colourless oil (120 mg, 88%), $[a]_D^{23}$ +59.2 (*c* 1.20 in $CHCl_3$) (Found: C, 54.3; H, 8.4; N, 11.35. $C_{11}H_{20}N_2O_4$ requires C, 54.1; H, 8.25; N, 11.5%); ν_{max}(film)/cm^{-1} 3354 (br, NH, OH), 1660 (s, Amide I), 1531 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.20 (3H, d, $J_{6,7}$ 6.5, H$_3$-7), 1.40, 1.51 (6H, 2s, C(C*H*$_3$)$_2$), 2.41 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.5, H-6), 2.84 (3H, d, *J* 5.0, NHC*H*$_3$), 3.21 (1H, dd, $J_{4,5}$ 7.3, $J_{5,6}$ 9.6, H-5), 3.77 (1H, m, H-2), 3.88 (1H, dd, $J_{3,4}$ 5.3, $J_{4,5}$ 7.3, H-4), 4.91 (1H, dd, $J_{3,4}$ 5.3, $J_{2,3}$ 2.0, H-3), 7.35 (1H, br m, CON*H*); δ_C(50 MHz; $CDCl_3$) 18.1 (q, C-7), 26.1, 26.3, 28.2 (3q, N*C*H$_3$, C(*C*H$_3$)$_2$), 50.1 (d, C-6), 56.9 (d, C-2), 74.0, 75.9, 78.8 (3d, C-3, C-4, C-5), 108.6 (s, *C*(CH$_3$)$_2$), 170.7 (s, C-1); *m/z* (CI, NH_3) 245 (MH^+, 100%).

N-Butyl-2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*talo*-heptonamide 13c

Amide **13c** was prepared according to general procedure 1 using bicyclic lactone **7** (100 mg, 0.47 mmol) and butylamine (0.06 cm^3, 0.6 mmol). Purification of the crude material using column chromatography (methanol–ethyl acetate, 1:13) afforded the title compound as a colourless oil (114 mg, 85%), $[a]_D^{24}$ +34.1 (*c* 2.01 in $CHCl_3$) (Found: C, 58.9; H, 9.2; N, 9.9. $C_{14}H_{26}N_2O_4$ requires C, 58.7; H, 9.15; N, 9.8%); ν_{max}(film)/cm^{-1} 3334 (br, NH, OH), 1661 (s, Amide I), 1525 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 0.93 (3H, t, *J* 7.3, C*H*$_3$(CH$_2$)$_3$), 1.19 (3H, d, $J_{6,7}$ 6.5, H$_3$-7), 1.32–1.38 (2H, m, C*H*$_2$), 1.39 (3H, s, C(C*H*$_3$)$_2$), 1.46–1.52 (2H, m, C*H*$_2$), 1.51 (3H, s, C(C*H*$_3$)$_2$), 2.39 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.5, H-6), 3.18–3.34 (2H, m, C*H*$_2$NH$_2$), 3.21 (1H, dd, $J_{4,5}$ 7.3, $J_{5,6}$ 9.6, H-5), 3.77 (1H, d, $J_{2,3}$ 1.8, H-2), 3.88 (1H, dd, $J_{3,4}$ 5.2, $J_{4,5}$ 7.3, H-4), 4.92 (1H, dd, $J_{3,4}$ 5.2, $J_{2,3}$ 1.8, H-3), 7.39 (1H, br m, CON*H*); δ_C(50 MHz; $CDCl_3$) 13.7, 18.1 (2q, *C*H$_3$(CH$_2$)$_3$, C-7), 20.0 (t, *C*H$_2$), 26.3, 28.2 (2q, C(*C*H$_3$)$_2$), 31.5, 38.9 (2t, (*C*H$_2$)$_2$), 50.2 (d, C-6), 56.9 (d, C-2), 74.1, 75.9, 78.9 (3d, C-3, C-4, C-5), 108.5 (s, *C*(CH$_3$)$_2$), 169.9 (s, C-1); *m/z* (CI, NH_3) 287 (MH^+, 100%).

N-Benzyl-2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*talo*-heptonamide 13d

Amide **13d** was prepared according to general procedure 1 using bicyclic lactone **7** (100 mg, 0.47 mmol) and benzylamine (0.06 cm^3, 0.56 mmol). Purification of the crude material using column chromatography (ethyl acetate–hexane, 4:1) afforded the title compound as a colourless oil (132 mg, 88%), $[a]_D^{24}$ +32.4 (*c* 2.29 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3338 (br, NH, OH), 1667 (s, Amide I), 1587 (m, Amide II); δ_H(500 MHz; $CDCl_3$) 1.17 (3H, d, $J_{6,7}$ 6.5, H$_3$-7), 1.41, 1.53 (6H, 2s, C(CH$_3$)$_2$), 2.43 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.5, H-6), 3.23 (1H, dd, $J_{4,5}$ 7.3, $J_{5,6}$ 9.6, H-5), 3.85 (1H, d, $J_{2,3}$ 1.9, H-2), 3.92 (1H, dd, $J_{3,4}$ 5.2, $J_{4,5}$ 7.3, H-4), 4.48 (2H, ABX system, *J* 5.9, 6.0, 15.0, PhC*H*$_2$NH), 4.97 (1H, dd, $J_{3,4}$ 5.2, $J_{2,3}$ 1.9, H-3), 7.25–7.37 (5H, m, Ar*H*), 7.72 (1H, br m, CON*H*); δ_C(50 MHz; $CDCl_3$) 17.9 (q, C-7), 26.3, 28.2 (2q, C(*C*H$_3$)$_2$), 43.3 (t, Ph*C*H$_2$), 50.3 (d, C-6), 57.1 (d, C-2), 74.1, 76.0, 79.0 (3d, C-3, C-4, C-5), 108.8 (s, *C*(CH$_3$)$_2$), 127.7, 128.9 (3d, C$_{ortho,\ meta,\ para}$), 138.3 (s, C$_{ipso}$), 170.5 (s, C-1); *m/z* (CI, NH_3) 321 (MH^+, 100%); (CI^+) [Found: 321.1814 (MH^+). $C_{17}H_{25}N_2O_4$ requires *m/z*, 321.1814].

2,6,7-Trideoxy-2,6-imino-L-*glycero*-L-*talo*-heptonamide 14a

Amide **13a** (80 mg, 0.34 mmol) was deprotected according to general procedure 2 to afford the title compound as a foam (53 mg, 80%), $[a]_D^{23}$ +35.6 (*c* 1.02 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3421 (br, NH, OH), 1666 (s, Amide); δ_H(500 MHz; D_2O, pH 8) 1.17 (3H, d, $J_{6,7}$ 6.5, H$_3$-7), 2.69 (1H, dq, $J_{5,6}$ 9.2, $J_{6,7}$ 6.5, H-6), 3.33 (1H, dd, $J_{4,5}$ 9.5, $J_{5,6}$ 9.2, H-5), 3.46 (1H, dd, $J_{3,4}$ 3.0, $J_{4,5}$ 9.5, H-4), 3.68 (1H, d, $J_{2,3}$ 2.6, H-2), 4.38 (1H, m, H-3); δ_C(50 MHz; D_2O, pH 8) 18.0 (q, C-7), 52.8 (d, C-6), 61.2 (d, C-2), 70.6, 72.8, 73.9 (3d, C-3, C-4, C-5), 176.3 (s, C-1); *m/z* (+ve Electrospray) 191 (MH^+, 100%); (CI^+) [Found: 191.1040 (MH^+). $C_7H_{15}N_2O_4$ requires *m/z*, 191.1032].

2,6,7-Trideoxy-2,6-imino-*N*-methyl-L-*glycero*-L-*talo*-heptonamide 14b

Amide **13b** (80 mg, 0.33 mmol) was deprotected according to general procedure 2 to afford the title compound as a hygroscopic foam (57 mg, 85%), $[a]_D^{23}$ +28.0 (*c* 1.09 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3348 (br, NH, OH), 1651 (s, Amide I), 1538 (m, Amide II); δ_H(500 MHz; D_2O, pH 8) 1.16 (3H, d, $J_{6,7}$ 6.4, H$_3$-7), 2.61 (1H, dq, $J_{5,6}$ 9.2, $J_{6,7}$ 6.4, H-6), 2.74 (3H, s, NC*H*$_3$), 3.32 (1H, dd, $J_{4,5}$ 9.4, $J_{5,6}$ 9.2, H-5), 3.43 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 9.4, H-4), 3.61 (1H, d, $J_{2,3}$ 2.8, H-2), 4.38 (1H, $J_{2,3}$ 2.8, $J_{3,4}$ 3.1, H-3); δ_C(50 MHz; D_2O, pH 8) 18.0 (q, C-7), 26.5 (q, N*C*H$_3$), 52.7 (d, C-6), 61.3 (d, C-2), 70.7, 72.8, 73.9 (3d, C-3, C-4, C-5), 173.7 (s,

C-1); *m/z* (Electrospray) 205 (MH^+, 100%); (CI^+) [Found: 205.1193 (MH^+). $C_8H_{17}N_2O_4$ requires *m/z*, 205.1188].

N-Butyl-2,6,7-trideoxy-2,6-imino-L-*glycero*-L-*talo*-heptonamide 14c

Amide **13c** (80 mg, 0.28 mmol) was deprotected according to general procedure 2 to afford the title compound as a foam (59 mg, 86%), $[\alpha]_D^{23}$ +11.4 (*c* 1.02 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3400 (br, NH, OH), 1648 (s, Amide I), 1534 (m, Amide II); δ_H(500 MHz; D_2O, pH 8) 0.87 (3H, t, *J* 7.4, $CH_3(CH_2)_3$), 1.16 (3H, d, $J_{6,7}$ 6.4, H_3-7), 1.25–1.32 (2H, m, CH_2), 1.44–1.49 (2H, m, CH_2), 2.62 (1H, dq, $J_{5,6}$ 9.2, $J_{6,7}$ 6.4, H-6), 3.13–3.28 (2H, m, CH_2NH_2), 3.32 (1H, dd, $J_{5,6}$ 9.2, $J_{4,5}$ 9.5, H-5), 3.43 (1H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 9.5, H-4), 3.62 (1H, d, $J_{2,3}$ 2.8, H-2), 4.38 (1H, dd, $J_{3,4}$ 3.1, $J_{2,3}$ 2.8, H-3); δ_C(50 MHz; D_2O, pH 8) 13.8, 17.9 (2q, $CH_3(CH_2)_3$, C-7), 20.3, 31.4, 39.7 (3t, $(CH_2)_3$), 52.8 (d, C-6), 61.4 (d, C-2), 70.7, 72.8, 73.8 (3d, C-3, C-4, C-5), 172.9 (s, C-1); *m/z* (−ve Electrospray) 245 ($M - H^+$, 100%); (CI^+) [Found: 247.1663 (MH^+). $C_{11}H_{23}N_2O_4$ requires *m/z*, 247.1658].

N-Benzyl-2,6,7-trideoxy-2,6-imino-L-*glycero*-L-*talo*-heptonamide 14d

Amide **13d** (80 mg, 0.25 mmol) was deprotected according to general procedure 2 to afford the title compound as a colourless foam (55 mg, 79%), $[\alpha]_D^{23}$ +3.8 (*c* 2.03 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3431 (br, NH, OH), 1643 (s, Amide I), 1586 (m, Amide II); δ_H(200 MHz; D_2O, pH 8) 0.99 (3H, d, $J_{6,7}$ 6.3, H_3-7), 2.80 (1H, dq, $J_{5,6}$ 9.4, $J_{6,7}$ 6.3, H-6), 3.22 (2H, m, H-4, H-5), 3.59 (1H, d, $J_{2,3}$ 2.9, H-3), 4.22 (3H, m, H-3, PhCH_2NH), 7.10–7.29 (5H, m, Ar*H*); δ_C(50 MHz; D_2O, pH 8) 17.8 (q, C-7), 43.7 (t, PhCH_2), 53.1, 61.4 (2d, C-2, C-6), 70.6, 72.8, 73.6 (3d, C-3, C-4, C-5), 128.0, 128.3, 129.7 (3d, $C_{ortho,\ meta,\ para}$), 138.9 (s, C_{ipso}), 172.8 (s, C-1); *m/z* (−ve Electrospray) 279 ($M - H^+$, 100%); (CI^+) [Found: 281.1493 (MH^+). $C_{14}H_{21}N_2O_4$ requires *m/z*, 281.1501].

2,6,7-Trideoxy-2,6-imino-L-*glycero*-L-*talo*-heptonic acid 15

Sodium hydroxide solution (0.5 M (aq); 1.4 cm^3, 0.70 mmol) was added to a suspension of lactone **7** (150 mg, 0.70 mmol) in water (5 cm^3) at room temperature. After 5 min the solvent was removed *in vacuo*. The residue was dissolved in hydrochloric acid (2.0 M; 5 cm^3) and the solution left for 24 h at room temperature with subsequent removal of solvent *in vacuo*. The residue was re-dissolved in water and evaporated to dryness (×3), to give a residue, which was subjected to ion-exchange chromatography [Amberlite IR-120, H^+ form: eluting with 1.0 M aq. pyridine] to give pipecolic acid **15** as a hygroscopic solid (121 mg, 90%), $[\alpha]_D^{23}$ +6.6 (*c* 1.24 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3380 (br, NH, OH), 1622 (s, C=O); δ_H(500 MHz; D_2O, pH 8) 1.47 (3H, d, $J_{6,7}$ 6.5, H_3-7), 3.46 (1H, dq, $J_{5,6}$ 10.3, $J_{6,7}$ 6.5, H-6), 3.56 (1H, dd, $J_{4,5}$ 9.6, $J_{3,4}$ 2.9, H-4), 3.70 (1H, dd, $J_{5,6}$ 10.3, $J_{4,5}$ 9.6, H-5), 4.06 (1H, d, $J_{2,3}$ 2.9, H-2), 4.56 (1H, dd, $J_{2,3}$ 2.9, $J_{3,4}$ 2.9, H-3); δ_C(50 MHz; D_2O, pH 8) 15.4 (q, C-7), 53.6 (d, C-6), 62.5 (d, C-2), 69.5, 70.6, 71.4 (3d, C-3, C-4, C-5), 170.6 (s, C-1); *m/z* (−ve Electrospray) 190 ($M - H^+$, 100%); (CI^+) [Found: 192.0873 (MH^+). $C_7H_{14}NO_5$ requires *m/z*, 192.0872].

Methyl 2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*talo*-heptonate 16 and methyl 2,6,7-trideoxy-2,6-imino-3,4-*O*-isopropylidene-L-*glycero*-L-*galacto*-heptonate 17

A solution of bicyclic lactone **7** (150 mg, 0.70 mmol) and sodium acetate (57 mg, 0.70 mmol) in methanol (10 cm^3) was stirred at reflux for 6 h. The reaction mixture was concentrated *in vacuo* and the crude material subjected to column chromatography (ethyl acetate–hexane, 9:1) to afford the methyl ester **16**, first eluted, as a white crystalline solid (52 mg, 30%), mp 128–129 °C; $[\alpha]_D^{24}$ −23.7 (*c* 0.60 in $CHCl_3$) (Found: C, 54.0; H, 7.8; N, 5.55. $C_{11}H_{19}NO_5$ requires C, 53.9; H, 7.8; N, 5.7%); ν_{max}(KBr)/cm^{-1} 3328 (br, NH, OH), 1725 (s, C=O); δ_H(500 MHz; $CDCl_3$) 1.23 (3H, d, $J_{6,7}$ 6.3, H_3-7), 1.41, 1.56 (6H, 2s, $C(CH_3)_2$), 2.63 (1H, dq, $J_{5,6}$ 9.2, $J_{6,7}$ 6.3, H-6), 3.36 (1H, $J_{4,5}$ 7.1, $J_{5,6}$ 9.2, H-5), 3.79 (3H, s, CO_2CH_3), 4.01 (1H, d, $J_{2,3}$ 2.3, H-2), 4.32 (1H, dd, $J_{3,4}$ 5.4, $J_{4,5}$ 7.1, H-4), 4.61 (1H, dd, $J_{3,4}$ 5.4, $J_{2,3}$ 2.3, H-3); δ_C(50 MHz; $CDCl_3$) 18.0 (q, C-7), 26.2, 28.1 (2q, $C(CH_3)_2$), 50.5 (d, C-6), 52.5 (q, OCH_3), 57.1 (d, C-2), 73.9, 75.8, 79.2 (3d, C-3, C-4, C-5), 109.0, (s, $C(CH_3)_2$), 172.0 (s, C-1); *m/z* ($APCI^+$) 246 (MH^+, 100%); and methyl ester **17**, second eluted, as a white solid (92 mg, 53%), mp 175–176 °C; $[\alpha]_D^{23}$ +44.9 (*c* 0.81 in $CHCl_3$) (Found: C, 54.1; H, 8.0; N, 5.7%); ν_{max}(KBr)/cm^{-1} 3212 (br, NH, OH), 1750 (s, C=O); δ_H(500 MHz; $CDCl_3$) 1.27 (3H, d, $J_{6,7}$ 6.3, H_3-7), 1.37, 1.52 (6H, 2s, $C(CH_3)_2$), 2.52 (1H, dq, $J_{5,6}$ 9.8, $J_{6,7}$ 6.3, H-6), 3.26 (1H, $J_{4,5}$ 7.3, $J_{5,6}$ 9.8, H-5), 3.80 (3H, s, CO_2CH_3), 3.87 (1H, d, $J_{2,3}$ 3.0, H-2), 3.96 (1H, dd, $J_{3,4}$ 5.2, $J_{4,5}$ 7.3, H-4), 4.45 (1H, dd, $J_{3,4}$ 5.2, $J_{2,3}$ 3.0, H-3); δ_C(50 MHz; $CDCl_3$) 18.1 (q, C-7), 26.2, 28.0 (2q, $C(CH_3)_2$), 52.3 (q, CO_2CH_3), 52.9 (d, C-6), 58.2 (d, C-2), 74.4, 76.2, 80.7 (3d, C-3, C-4, C-5), 110.0 (s, $C(CH_3)_2$), 170.4 (s, C-1); *m/z* ($APCI^+$) 246 (MH^+, 55%). Repeating the reaction using bicyclic lactone **7** (700 mg, 3.28 mmol) and sodium acetate (270 mg, 3.28 mmol) in methanol (20 cm^3) at reflux for 2.5 h afforded methyl ester **16** (273 mg, 34%) and methyl ester **17** (362 mg, 45%), identical to the materials detailed above.

1,2,6-Trideoxy-2,6-imino-L-*glycero*-L-*manno*-heptitol 1 (α-HRJ)

Method 1. Super-Hydride® (1.0 M in THF; 1.31 cm^3, 1.31 mmol) was added to a stirred solution of bicyclic lactone **7** (140 mg, 0.66 mmol) in THF (5 cm^3) at room temperature. After 5 min, the reaction mixture was diluted with ethyl acetate (20 cm^3) and extracted with water (2 × 20 cm^3). The combined aqueous layers were concentrated *in vacuo* and the residue dissolved in 1% methanolic hydrogen chloride (10 cm^3). The solution was left at room temperature for 18 h, the solvent was removed *in vacuo* and the residue purified by ion-exchange chromatography on Amberlite (IR-120, H^+ form) (1.0 M ammonium hydroxide) to give α-HRJ **1** as a colourless hygroscopic oil (93 mg, 80%), $[\alpha]_D^{25}$ +15.0 (*c* 0.9 in H_2O, pH 8); ν_{max}(film)/cm^{-1} 3350 (br, OH, NH); δ_H(500 MHz; D_2O, pH 8) 1.05 (3H, d, $J_{1,2}$ 6.3, H_3-1), 2.59 (1H, dq, $J_{2,3}$ 9.4, $J_{1,2}$ 6.3, H-2), 2.96 (1H, ddd, $J_{6,7}$ 7.7, $J_{6,7'}$ 7.1, $J_{5,6}$ 2.3, H-6), 3.26 (1H, dd, $J_{2,3}$ 9.4, $J_{3,4}$ 9.5, H-3), 3.51 (1H, dd, $J_{3,4}$ 9.5, $J_{4,5}$ 3.3, H-4), 3.56 (1H, dd, $J_{7,7'}$ 11.7, $J_{6,7'}$ 7.1, H-7′), 3.62 (1H, dd, $J_{7,7'}$ 11.7, $J_{6,7}$ 7.7, H-7), 3.91 (1H, dd, $J_{5,6}$ 2.3, $J_{4,5}$ 3.3, H-5); δ_C(50 MHz; D_2O, pH 8) 17.2 (q, C-1), 51.2 (d, C-2), 59.3 (t, C-7), 59.6 (d, C-6), 69.3, 71.8, 73.6 (3d, C-3, C-4, C-5); *m/z* (DCI, NH_3) 178 (MH^+, 100%); (+ve Electrospray) [Found: 178.1087 (MH^+). $C_7H_{16}NO_4$ requires *m/z*, 178.1079].

Method 2. Super-Hydride® (1.0 M in THF, 0.35 cm^3, 0.35 mmol) was added to a stirred solution of methyl ester **16** (40 mg, 0.16 mmol) in THF (5 cm^3) at room temperature. After 5 min, the reaction mixture was diluted with ethyl acetate (20 cm^3) and extracted with water (2 × 20 cm^3). The combined aqueous layers were concentrated *in vacuo*, the residue dissolved in 1% methanolic hydrogen chloride (5 cm^3) and the solution was left at room temperature for 18 h. The solvent was removed *in vacuo* and the residue subjected to ion-exchange chromatography on Amberlite (IR-120, H^+ form) (1.0 M ammonium hydroxide) to give α-HRJ **1** as a colourless hygroscopic oil (20 mg, 71%). Material identical to that described above.

2,6,7-Trideoxy-2,6-imino-L-*glycero*-L-*galacto*-heptitol 2 (β-HRJ)

Super-Hydride® (1.0 M in THF; 0.54 cm^3, 0.54 mmol) was added to a stirred solution of methyl ester **17** (60 mg, 0.24 mmol) in THF (5 cm^3) at −20 °C. After 5 min, methanol (1 cm^3) was added and the solution was concentrated *in vacuo*. The residue was dissolved in 1% methanolic hydrogen chloride

(5 cm^3). The solution was left at room temperature for 18 h, and the solvent removed *in vacuo*. The residue was purified by ion-exchange chromatography on Amberlite (IR 120, H^+ form) (1.0 M ammonium hydroxide) to give β-HRJ **2** as a colourless hygroscopic oil (27 mg, 63%), $[a]_D^{24}$ +12.1 (*c* 0.95 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3256 (br, NH, OH); δ_H(500 MHz; D_2O, pH 8) 1.07 (3H, d, $J_{6,7}$ 6.4, H_3-7), 2.45 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.4, H-6), 2.73 (1H, ddd, $J_{1,2}$ 6.9, $J_{1',2}$ 6.7, $J_{2,3}$ 1.3, H-2), 3.21 (1H, dd, $J_{5,6}$ 9.6, $J_{4,5}$ 9.7, H-5), 3.40 (1H, dd, $J_{4,5}$ 9.7, $J_{3,4}$ 3.2, H-4), 3.49 (1H, dd, $J_{1,1'}$ 11.2, $J_{1',2}$ 6.7, H-1′), 3.53 (1H, dd, $J_{1,1'}$ 11.2, $J_{1,2}$ 6.9, H-1), 3.90 (1H, dd, $J_{2,3}$ 1.3, $J_{3,4}$ 3.2, H-3); δ_C(50 MHz; D_2O, pH 8) 17.7 (q, C-7), 55.6, 58.9 (2d, C-2, C-6), 61.9 (t, C-1), 69.8, 74.5, 75.4 (3d, C-3, C-4, C-5); *m*/*z* ($APCI^-$) 176 (M − H^+, 100%); (CI^+) [Found: 178.1084 (MH^+). $C_7H_{16}NO_4$ *m*/*z*, requires 178.1079].

2,6,7-Trideoxy-2,6-imino-3,4-*O*-isopropylidene-*N*-methyl-L-*glycero*-L-*galacto*-heptonamide 18

Methylamine (33% w/w solution in industrial methylated spirit, 0.47 cm^3, 4.10 mmol) was added to a solution of methyl ester **17** (100 mg, 0.41 mmol) in methanol (5 cm^3). The solution was stirred at reflux for 18 h. The solvent was removed *in vacuo* and the residue purified by column chromatography (methanol–ethyl acetate, 1 : 9) to afford amide **18** (second eluted, R_f 0.2) as a white crystalline solid (18 mg, 18%), mp 192–195 °C; $[a]_D^{22}$ +2.6 (*c* 1.03 in $CHCl_3$) (Found: C, 54.3; H, 8.5; N, 11.4. $C_{11}H_{20}N_2O_4$ requires C, 54.1; H, 8.25; N, 11.5%); ν_{max}(KBr)/cm^{-1} 3360 (br, NH, OH), 1665 (s, Amide I), 1540 (m, Amide II); δ_H(500 MHz; CD_3OD) 1.19 (3H, d, $J_{6,7}$ 6.4, H_3-7), 1.32, 1.47 (6H, 2s, $C(CH_3)_2$), 2.44 (1H, dq, $J_{5,6}$ 9.7, $J_{6,7}$ 6.4, H-6), 2.77 (3H, s, $NHCH_3$), 3.17 (1H, dd, $J_{4,5}$ 7.4, $J_{5,6}$ 9.7, H-5), 3.65 (1H, d, $J_{2,3}$ 3.0, H-2), 3.90 (1H, dd, $J_{3,4}$ 5.2, $J_{4,5}$ 7.4, H-4), 4.44 (1H, dd, $J_{3,4}$ 5.2, $J_{2,3}$ 3.0, H-3); δ_C(50 MHz; CD_3OD) 18.4 (q, C-7), 26.2, 26.6, 28.4 (3q, NCH_3, $C(CH_3)_2$), 55.1 (d, C-6), 60.4 (d, C-2), 75.8, 77.0, 81.8 (3d, C-3, C-4, C-5), 110.6 (s, $C(CH_3)_2$), 172.4 (s, C-1); *m*/*z* ($APCI^+$) 245 (MH^+, 100%).

2,6,7-Trideoxy-2,6-imino-*N*-methyl-L-*glycero*-L-*galacto*-heptonamide 19

Amide **18** (10 mg, 41 μmol) was deprotected according to general procedure 2 to afford the title compound as a hygroscopic foam (6.6 mg, 80%), $[a]_D^{23}$ −5.7 (*c* 0.66 in H_2O, pH 8); ν_{max}(KBr)/cm^{-1} 3330 (br, NH, OH), 1655 (s, Amide I), 1524 (m, Amide II); δ_H(200 MHz; D_2O, pH 8) 0.97 (3H, d, $J_{6,7}$ 6.4, H_3-7), 2.36 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.4, H-6), 2.49 (3H, s, NCH_3), 3.32 (1H, t, *J* 9.6, H-5), 3.30–3.35 (2H, m), 3.95 (1H, m); δ_C(50 MHz; D_2O, pH 8) 17.3 (q, C-7), 26.5 (q, NCH_3), 54.9 (d, C-6), 61.4 (d, C-2), 71.2, 73.5, 74.7 (3d, C-3, C-4, C-5), 172.6 (s, C-1); *m*/*z* (+ve Electrospray) 205 (MH^+, 100%); (CI^+) [Found: 205.1186 (MH^+). $C_8H_{17}N_2O_4$ requires *m*/*z*, 205.1188].

Biological assays

Glycosidases. Glycosidase assays were carried out in microtitre plates. For screening assays using enzymes from Sigma Chemicals Ltd, the incubation mixture consisted of 0.020 cm^3 of enzyme solution (approx. 0.1 unit per cm^3), 0.020 cm^3 of inhibitor solution and 0.10 cm^3 of a 5 mM solution of the appropriate substrate (*p*-nitrophenyl glycoside) made up in 50 mM phosphate buffer at the optimum pH for the enzyme. Glycosidase assays using rabbit liver and gut preparations were carried out using *p*-nitrophenyl glycosides as previously described.[21] The assays using enzymes from Sigma were incubated at 30 °C and those using rabbit tissue homogenates were incubated at 37 °C. K_i-Values were determined for compounds inhibitory to naringinase using Lineweaver–Burk analysis and substrate concentrations ranging from 0.18 to 1.8 mM.

Galactosidase assays for 2. Enzymes were purified as described[22] and assayed using the corresponding *p*-nitrophenyl glycoside. Coffee bean α-galactosidase was assayed using 0.8 mM (for IC_{50} values) or 0.06–0.4 mM (for K_i-values) *p*-nitrophenyl α-galactopyranoside in 0.1 M citrate–phosphate buffer,[23] pH 6.0 for 30 min at 37 °C in the presence of tert compound (0–0.75 μM). The reaction was stopped with 0.5 M sodium carbonate and the absorbance measured at 400 nm. Inhibition constants were calculated from the slope of inhibitor concentration *versus* activity (IC_{50}) or from Lineweaver–Burk plots (K_i). Jack bean β-galactosidase was assayed using 0.5 mM *p*-nitrophenyl β-galactopyranoside in 0.1 M citrate phosphate buffer, pH 3.8 for 30 min at 37 °C in the presence of 0–0.9 mM tert compound. The reaction was stopped, absorbance measured and the data plotted as described above.

dTDP-L-rhamnose biosynthesis assays. The inhibition of the conversion of dTDP-D-glucose to dTDP-L-rhamnose was assessed using an enzyme incubation method based on that described by Glaser and Kornfeld[23] which incorporated the potential inhibitors, followed by acid hydrolysis to release the monosaccharides from the thymidine diphosphate nucleotides. The free sugars were subsequently analysed and quantified as their trimethylsilyl derivatives by GLC-MS.

The biosynthesis of dTDP-L-rhamnose from dTDP-D-glucose involves three enzymes (dTDP-D-glucose-4,6-dehydratase, dTDP-4-keto-6-deoxy-D-glucose-3,5-epimerase and dTDP-4-keto-L-rhamnose reductase.[18] These were presumed to be present in a cell-free extract of *M. smegmatis* prepared in 50 mM Tris-HCl buffer, pH 8.0, containing 10 mM $MgCl_2$ and 1 mM EDTA. The reaction mixture contained 0.20 cm^3 of the crude enzyme preparation, 0.010 cm^3 of a solution of dTDP-D-glucose substrate in buffer (1 mM final concentration in assay), 0.050 cm^3 of a solution of NAD (3 mM), 0.075 cm^3 of a solution of NADPH (3 mM), 0.020 cm^3 of a solution of α-oxoglutarate (3 mM), 0.020 cm^3 of a solution of L-cysteine (3 mM), 0.075 cm^3 of buffer and 0.050 cm^3 of inhibitor solution in H_2O. All reagents were obtained from Sigma Chemicals Ltd. The reaction mixture was incubated for 3 h at 37 °C before termination by the addition of 1 cm^3 of 0.5 M HCl and heating at 100 °C for 15 min. Pinitol (0.050 cm^3 of a 1 mg cm^{-3} stock solution = 50 μg) was added to each sample at this stage as the internal standard for the purposes of the GLC-MS analyses. All samples were freeze-dried and then made up to 0.5 cm^3 with H_2O. Aliquots (0.10 cm^3) were removed and freeze-dried in preparation for GLC-MS analysis. Trimethylsilyl derivatives of the free sugars present in the samples were produced for GLC-MS analysis using Sigma Sil A (0.050 cm^3 per sample). The GLC system was a Perkin-Elmer Autosystem 8000 fitted with a BPX5 column (25 m × 0.22 mm i.d. × 0.25 μm, SGE Ltd). The temperature programme started at 180 °C and rose to 250 °C at 10 °C min^{-1}. Electron-impact mass spectrometry of the column effluent was carried out using a Perkin-Elmer Q-Mass 910 Benchtop Mass Spectrometer operated at 70 eV with a mass detection range of 100–400 Da.

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