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Synthesis of, and Lack of Inhibition of a Rhamnosidase by, Both Enantiomers of Deoxyrhamnojirimycin and Rhamnonolactam: β-Mannosidase inhibition by δ-lactams

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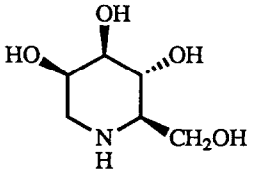
Abstract: Synthesis of both enantiomers of deoxyrhamnojirimycin and rhamnonolactam from D- and L-gulonolactones are described. The effects as inhibitors of the enantiomeric deoxyrhamnojirimycins and rhamnonolactams on human liver glycosidases are compared with deoxymannojirimycin and mannonolactam. No significant inhibition of the activity of naringinase (an α-L-rhamnosidase) was caused by any of these compounds.



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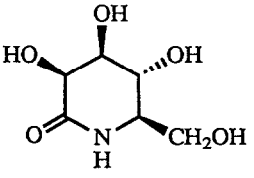

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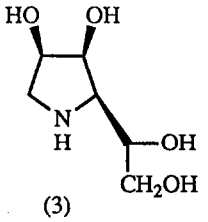

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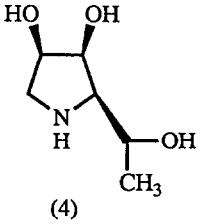

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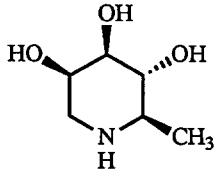

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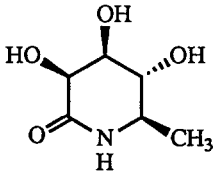

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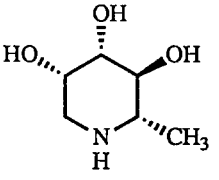

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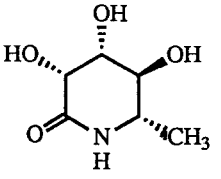
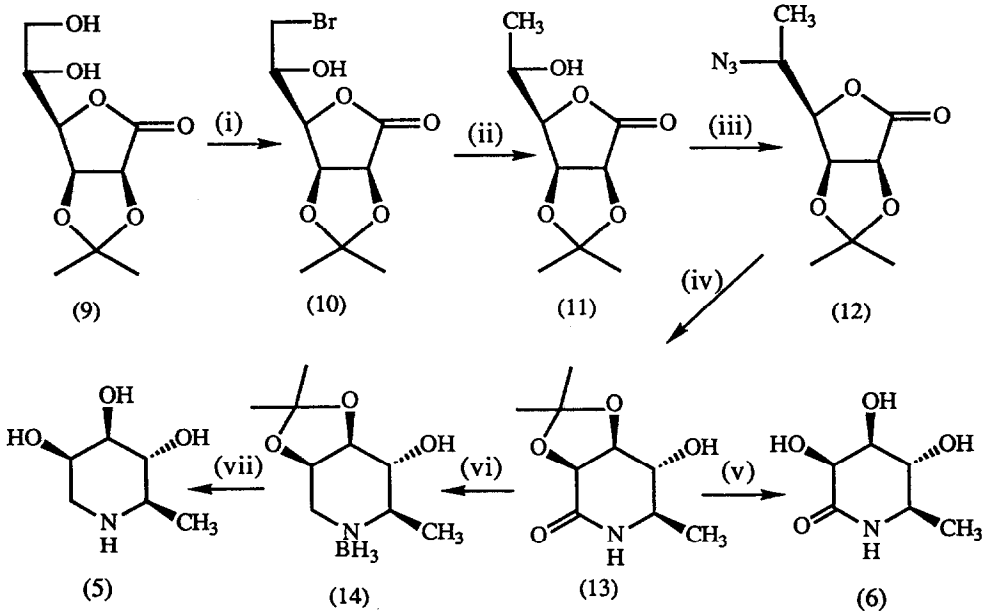


(8)

In general, removal of the anomeric hydroxyl group from a pyranose sugar and replacement of the ring oxygen by a nitrogen atom reliably produces compounds which are effective inhibitors of the corresponding glycosidases.[1] For example, deoxymannojirimycin (1), isolated from *Lonchocarpus sericeus*,[2] is a potent inhibitor of glycoprotein processing mannosidase I[3,4] and a bovine α-L-fucosidase.[5] The corresponding lactam,

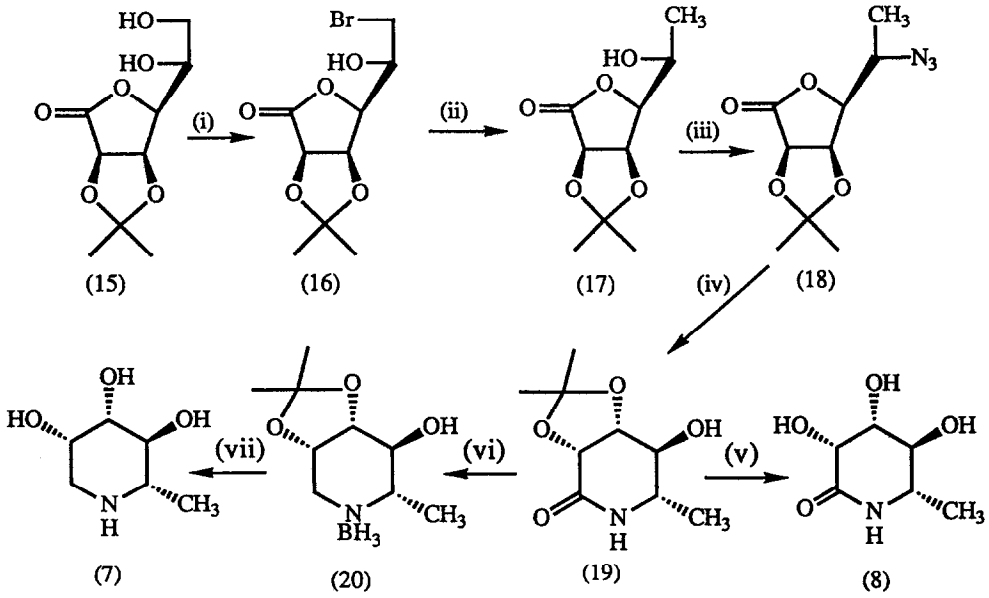
D-mannonolactam (2), is a powerful inhibitor of human lysosomal and rat epididymal α-mannosidases, and of apricot β-glucosidase.[6] Many mannopyranosidases are also inhibited by nitrogen analogues of mannofuranose. Thus, 1,4-dideoxy-1,4-iminomannitol (3)[7] is a strong inhibitor of many mannosidases[8] including a mannosidase of glycoprotein processing.[9] Also, the 6-deoxyderivative (4) is an excellent inhibitor of human liver[10] and other mannosidases.[11] Rhamnose (6-deoxymannose) residues are widely found intracellularly and extracellularly in plant tissues. The most common form found within the cell is as a component of a glycoside such as flavanoids, phenols, sterols, and coumarins;[12] rhamnose is found typically as a disaccharide conjugate either as an α or β rhamnopyranoside. The degradation of these glycosides is accomplished by a stepwise removal of monosaccharides by exoglycosidases. The aglycones are usually physiologically inactive compounds compared to the glycoside. These exoglycosidases would be an ideal system to test the stereospecificity of inhibition by rhamnose analogues. Rhamnose is also found in extracellular polysaccharides of plants either as a component of cell walls or root mucilage; α-L-rhamnose is a major constituent of rhamnogalacturonan I[13] and is found in α- and β- linkages in rhamnogalacturonan II.[14] If specific exorhamnosidases or endorhamnosidases could be assayed using the polymers as substrates, the use of rhamnose analogues to inhibit the enzyme may provide a useful tool for probing the structure of cell walls. This paper reports the synthesis of the D-deoxyrhamnojirimycin (5), D-rhamnono-1,5-lactam (6), L-deoxyrhamnojirimycin (7) and L-rhamnono-1,5-lactam (8); the effects of these compounds on human liver glycosidases are reported and compared with the effects of (1) and (2). The effects on naringinase - a readily available exo-L-rhamonosidase[15] - are also described. D-Deoxyrhamnojirimycin (5) and its enantiomer (7) have recently been prepared by a sequence involving a key enzymatic aldol reaction, though no results of inhibition of glycosidases were described.[16] The inhibition of human liver mannosidases by (5) has been reported.[20]



SCHEME 1 (i) Ph_3P,CBr_4, THF (ii) H_2, Et_3N, 10% Pd on C, EtOH (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; then NaN_3, DMF (iv) H_2, 10% Pd on C, MeOH (v) CF_3COOH/H_2O (2:1) (vi) $Me_2S:BH_3$ (vii) aq. conc. HCl

Synthesis Efficient syntheses of both enantiomers of deoxymannojirimycin (1) and mannonolactam (2) from the enantiomers of the monoacetonides of gulonolactone (9) and (15) have been reported;[17] for the synthesis of the 6-deoxy analogues (5) - (8), an identical strategy was employed save that deoxygenation - rather than protection - of the C-6 primary hydroxyl is required. A wide range of deoxygenation techniques of primary alcohols are applicable to sugar derivatives but bromination, followed by hydrogenolysis of the C-Br bond,[18] was chosen as the most convenient.

Thus, for the synthesis of the D-deoxyrhamnojorimycin (5) and D-rhamnonolactam (6), the monoacetonide from L-gulonolactone (9) was stirred in tetrahydrofuran with carbon tetrabromide and triphenyl phosphine to give the 6-bromo derivative (10) in 86% yield (Scheme 1). Hydrogenolysis of (10) in ethanol with 10% palladium on carbon in the presence of excess triethylamine gave the corresponding 6-deoxy compound (11) in 72% yield; in the absence of the triethylamine, hydrogen bromide accumulates and poisons the catalyst. Esterification of the unprotected hydroxyl function in (11) with trifluoromethanesulphonic anhydride and pyridine in dichloromethane, followed by displacement of the resulting triflate with sodium azide in dimethylformamide, afforded the azide (12) in 66% yield. Hydrogenation of the azide (12) in methanol in the presence of 10% palladium on carbon caused reduction to the corresponding amine which underwent spontaneous cyclisation to the protected 6-deoxy-mannono-γ-lactam (13) in 89% yield [36% overall yield from (9)]. Subsequent deprotection by hydrolysis of the acetonide with aqueous trifluoroacetic acid gave D-rhamnonolactam (6) in 75% yield. Reduction of the lactam (13) with borane:dimethyl sulphide in tetrahydrofuran gave the amine borane adduct (14); treatment of (14) with hydrochloric acid caused both decomposition of the borane complex and removal of the acetonide to give D-deoxyrhamnojirimycin (5) as the hydrochloride, in 75% yield from (13).



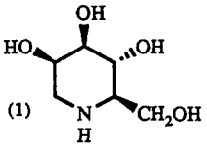
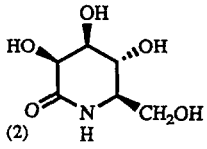
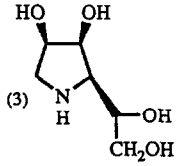
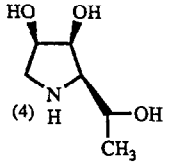
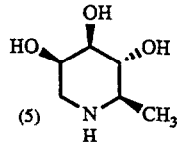
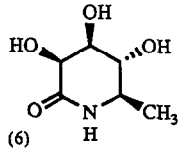
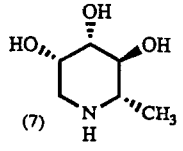
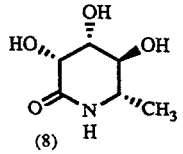
SCHEME 2 (i) Ph_3P,CBr_4, THF (ii) H_2, Et_3N, 10% Pd on C, EtOH (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; then NaN_3, DMF (iv) H_2, 10% Pd on C, MeOH (v) CF_3COOH/H_2O (2:1) (vi) $Me_2S:BH_3$ (vii) aq. conc. HCl

L-Deoxyrhamnojirimycin (7) and L-rhamnonolactam (8) were prepared by an identical sequence from the monoacetonide from D-gulonolactone (15). Thus bromination of the D-2,3-O-acetonide (15) with triphenylphosphine-carbon tetrabromide afforded the bromide (16) [85% yield] which, on hydrogenolysis, gave the 6-deoxy compound (17) in 91% yield (Scheme 2). Triflation and azide displacement of the unprotected hydroxyl group in (17) yielded the azide (18) (76% yield) which on hydrogenation afforded the protected lactam (19) [94% yield; 56% overall yield from (15)] as the required divergent intermediate. Acid hydrolysis of (19) gave L-rhamnono-δ-lactam (8) in 71% yield; borane reduction of (19) gave the borane adduct (20) which on treatment with acid afforded L-deoxyrhamnojirimycin (7) in 84% yield.

Effects on Glycosidases The inhibitory effects of DMJ (1), mannonolactam (2), D-deoxyrhamnojirimycin (5) and its enantiomer (7), and the corresponding lactams (6) and (8) towards a panel of human liver glycosidases have been studied (Table). Deoxymannojirimycin (1) and D-deoxyrhamnojirimycin (5) are inhibitors of both lysosomal α-mannosidase and α-fucosidase; this is not surprising as they both contain the minimum structural features necessary for the inhibition of the two enzymes by polyhydroxylated piperidines, namely the correct relative stereochemistry at C2, C3 and C4.[8,19,20] α-Mannosidase is inhibited more strongly by DMJ (1) than by the 6-deoxycompound (5), presumably because the interaction of the C6 hydroxyl group in (1) with the active site of the α-mannosidase contributes significant binding interactions. The corresponding mannofuranose nitrogen analogues 1,4-dideoxy-1,4-imino-D-mannitol (DIM) (3) [of DMJ (1)] and the 6-deoxy derivative (4) [of D-deoxyrhamnojirimycin (5)] are far more potent inhibitors of lysosomal mannosidase with K_i values of 13μM and 1.3 μM, respectively; this is consistent with previous observations showing that lysosomal α-mannosidase is more susceptible to inhibition by pyrrolidine rather than piperidine analogues of mannose.[8] There is thus a marked contrast in that the 6-deoxy analogue in the furanose case (4) is a stronger inhibitor of lysosomal α-mannosidase, whereas the 6-deoxy analogue of the pyranose form of mannose is a weaker inhibitor than the compound with a 6-hydroxyl group. Both the piperidine analogues (1) and (5) are more potent inhibitors of α-fucosidase than of α-mannosidase; in this case, the greater inhibition of fucosidase by DMJ (1) with the hydroxymethyl group than by D-deoxyrhamnojirimycin (5) with a methyl group is presumably attributable to the benefit of having an oxygen in the anomeric position of a fucose analogue. The mannofuranose analogues (3) and (4) are only very weak inhibitors of α-L-fucosidase [24% and 21% respectively at 1mM] because the structural basis[20] for the inhibition of α-fucosidase by mannose analogues is not retained in the furanose derivatives.

The mannonolactam (2) and D-rhamnonolactam (6) are better inhibitors of α-mannosidase than DMJ (1) and D-deoxyrhamnojirimycin (5) respectively; again, the lactam (2) with the C6 hydroxyl group is a more powerful inhibitor than the correpsonding deoxy compound (6). It may therefore be reasonably surmised that the half-chair conformation of the lactams resembles more closely the structure of the transition state[21] for the enzymic cleavage of the anomeric mannopyranoside. Neither of the lactams (2) or (6) show any significant inhibition of α-fucosidase, even though they both have the required relative stereochemistry at C2, C3 and C4. Examination of these structures, when viewed as L-fucose analogues, indicates that the conformation of the lactams would be different from the conformation of the putative carbonium ion intermediate formed in the hydrolysis of α-L-fucopyranosides. L-Fuconolactam-δ-lactam is also only a relatively weak inhibitor of α-L-fucosidase [Ki 400 μM].[22] Thus, no lactams have yet been found to cause significant inhibition of α-L-fucosidase. However, the lactams - especially mannonolactam (2) - are good inhibitors of lysosomal β-mannosidase. The inhibition of both α- and β-mannosidases is unusual since all the very potent inhibitors of α-

Table: Effect of compounds on human liver glycosidases and α-rhamnosidase % inhibition at 1mM

Compound	Lysosomal α-mannosidase	Lysosomal α-fucosidase	Other human liver glycosidase	α-Rhamnosidase (naringinase)
 (1)	58 (K_i 7.5 x 10^{-4}M)	92 (K_i 5 x 10^{-6}M)	N-acetyl-β-D-hexoseaminidase, 54	not determined
 (2)	94 (I_{50} 5 x 10^{-5}M)	0	cytosolic β-glucosidase, 98 (I_{50} 5 x 10^{-5}M) β-mannosidase, 98 (I_{50} 1 x 10^{-5}M)	12
 (3)	89 (K_i 1.3 x 10^{-5}M)	24	β-galactosidase, 73 α-arabinosidase, 66	not determined
 (4)	100 (K_i 1.3 x 10^{-6}M)	21	β-galactosidase, 65 α-arabinosidase, 91	not determined
 (5)	55 (I_{50} 1 x 10^{-3}M)	90 (K_i 7 x 10^{-5}M)	-	10
 (6)	86 (I_{50} 1 x 10^{-4}M)	0	cytosolic β-glucosidase, 67 (I_{50} 5 x 10^{-5}M) β-mannosidase, 40	3
 (7)	19	0	-	0
 (8)	0	0	-	0

mannosidases - such as swainsonine - fail to inhibit β-mannosidase.[8] These results may be taken to imply that for the human liver enzymes the hydrolysis of both α- and β-mannopyranosides involves a common intermediate with a conformation similar to that of the δ-lactams. The only other aminosugar that shows significant inhibition of β-mannosidase is DMDP [2,5-dideoxy-2,5-imino-D-mannitol].[23] The structural requirement for significant inhibition of β-mannosidase by amino sugars must be very stringent since 1-C-β-methyldeoxymannojirimycin does not cause significant β-mannosidase inhibition.[19] The lactams also inhibit the broad specificity cytosolic β-glucosidase, which also hydrolyses β-galactosides, β-xylosides and α-arabinosides. The conformation of the lactams may thus mimic the pyrilium cationic intermediates involved in the enzymic hydrolysis; the sensitivity to the stereochemistry at C2 of the sugar appears to be lost at the transition state since both mannosidases and β-glucosidase are significantly inhibited. In contrast neither of these lactams causes any significant inhibition of α-glucosidases.

Neither L-deoxyrhamnojirimycin (7) or the corresponding lactam (8) showed any significant inhibition of any glycosidases; the structures lack the key stereochemical features at C2, C3 and C4 required for either mannosidase or fucosidase inhibition. L-Deoxyrhamnojirimycin (7) can be viewed as an analogue of β-D-galactose, but it did not inhibit β-D-galactosidase; the presence of the 1-β-methyl substituent may prevent strong binding to the active site.

Almost invariably, polyhydroxylated piperidine analogues of glycopyranosides cause inhibition of the corresponding glycosidases. However, none of the compounds in this paper inhibited naringinase, an exo-α-rhamnosidase of plant origin, suggesting that the mechanism of this enzyme may be different from that of other glycosidases. Rhamnose itself is only a very weak competitive inhibitor of naringinase [Ki 1.2 mM].[15] Perhaps recognition of the aglycone moiety prunin (4', 5,7- trihydroxyflavanone-7-β-glucoside) in the case of the natural substrate, naringin, is important to the active site of the enzyme.

This paper for the first time has elucidated structural features in regard to the comparative inhibition of glycosidase activity (α- and β-mannosidases) by piperidine, pyrrolidine and δ-lactam analogues of the corresponding 1-deoxy- and 1,6-dideoxy-aminosugars.

Acknowledgments

This work has been supported by SERC and Monsanto/ G D Searle

Experimental

General Procedures. Melting points were recorded on a Kofler hot block. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (at 200 MHz), Bruker WH 300 (300 MHz), or Bruker AM 500 (500 MHz) spectrometers. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz) or a Bruker 250 (62.9 MHz) spectrometer. Multiplicities were assigned using DEPT sequence on the Gemini and by off resonance decoupling on the Bruker. Spectra run in D_2O were referenced to methanol as an internal standard. All chemical shifts are quoted on the δ-scale. Infra-red spectra were recorded on a Perkin-Elmer 297, or on a Perkin-Elmer 781 spectrophotometer. Mass spectra were recorded on VG Micromass 30F, ZAB 1F, Masslab 20-250 or Trio-1 GCMS (DB-5 column) spectrometers using desorption chemical ionisation (NH_3 DCI), electron impact (EI), chemical ionisation (NH_3 CI) and fast atom bombardment (FAB) techniques, as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations were given in g/100 ml. Hydrogenations were run under an atmosphere of

hydrogen gas maintained by inflated balloon. Microanalyses were performed by the microanalysis service of the Dyson-Perrins laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica. Plates were developed using either 5% v/v concentrated sulphuric acid in methanol, 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid and 0.5% ninhydrin in methanol. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Ion-exchange chromatography was performed using Dowex 50W-X8 (H) and Sigma CG 400 (Cl) resins. The resins were washed with 2M hydrochloric acid and 2M sodium hydroxide solution respectively, and subsequently with distilled water, prior to use. Solvents and commercially available reagents were dried and purified before use according to standard procedures; dichloromethane was refluxed over and distilled from calcium hydride, *N,N*-dimethylformamide was distilled under reduced pressure from calcium hydride, methanol was distilled from magnesium methoxide, pyridine was distilled from, and stored over, potassium hydroxide and tetrahydrofuran was distilled from a purple solution of sodium benzophenone ketyl immediately before use. Hexane was distilled at 68 °C before use to remove involatile fractions. 2,3-*O*-Isopropylidene-L-gulono-1,4-lactone (9)[24] and 2,3-*O*-isopropylidene-D-gulono-1,4-lactone (15)[25] were prepared from L- and D-gulonolactones [purchased from the Sigma Chemical Company] as reported previously. Human liver glycosidases were assayed in the absence and presence [1mM] of each of the compounds using the appropriate buffered 4-methylumbelliferyl-glycosides as substrates, as described previously.[19] Naringinase was assayed using *p*-nitrophenyl α-L-rhamnoside (1.75 mM) as substrate, according to Romero.[15]

6-Bromo-6-deoxy-2,3-O-isopropylidene-L-gulono-γ-lactone (10). 2,3-O-Isopropylidene-L-gulono-γ-lactone (9) (10.0 g, 45.9 mmol) was dissolved in tetrahydrofuran (150 ml) and cooled to 0°C under nitrogen with stirring. Carbon tetrabromide (14.8 g, 44.7 mmol) and triphenyl phosphine (15.26 g, 58.2 mmol) were added and the reaction allowed to warm to room temperature over 2 h and then stirred at room temperature for 10 h when t.l.c. (ethyl acetate) revealed no starting material (R_f 0.4) and one product (R_f 0.9). The reaction was diluted with ether (150 ml), filtered and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (ether/hexane 2:1), followed by recrystallisation from ether/hexane, to yield *6-bromo-6-deoxy-2,3-O-isopropylidene-L-gulono-γ-lactone (10)* as white needles (9.37 g, 72%), m.p. 86-87°C. (Found: C, 38.36; H, 4.49%. $C_9H_{13}O_5Br$ requires C, 38.46; H, 4.66%). $[\alpha]_D^{20}$ +52.9 (*c*, 1.25 in $CHCl_3$). ν_{max} (KBr disc): 3300 cm^{-1} (br, OH), 1790 cm^{-1} (C=O). m/z (CI NH_3): 300 and 298 ($M+NH_4^+$, 100%). δ_H ($CDCl_3$): 4.90 (1H, s), 4.66 (1H, dd), 4.24 (1H, m), 3.68 (1H, d), 1.50, 1.42 (2 x 3H, 2 x s, $C(CH_3)_2$), 1.44 (2H, m). δ_C ($CDCl_3$): 173.0 (s, C-1), 114.8 (s, $C(CH_3)_2$), 80.2, 76.4, 75.9, 69.5 (4 x d, C-2, C-3, C-4, C-5), 33.6 (t, C-6), 26.7, 25.8 (2 x q, $C(CH_3)_2$).

6-Deoxy-2,3-O-isopropylidene-L-gulono-γ-lactone (11). The bromolactone (10) (8.72 g, 31.0 mmol) was dissolved in ethanol (50 ml). Triethylamine (5 ml) and palladium on carbon (10%, ~50 mg) were added and the reaction was stirred under an atmosphere of hydrogen for 3 h when t.l.c. (ethyl acetate) showed no starting material (R_f 0.9) and one product (R_f 0.7). The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was recrystallised from ethyl acetate to yield *6-deoxy-2,3-O-isopropylidene-L-gulono-γ-lactone (11)* as a white solid, (5.39 g, 86%), m.p. 142-144°C. (Found: C, 53.59; H, 7.21%. $C_9H_{14}O_5$ requires C, 53.46; H, 6.98%). $[\alpha]_D^{20}$ +64.1 (*c*, 1.2 in EtOH). ν_{max} (KBr disc): 3300 cm^{-1} (br, OH), 1790 cm^{-1} (C=O). m/z (CI NH_3): 220 ($M+NH_4^+$, 100%). δ_H (CD_3OD): 4.92 (1H, d), 4.82 (1H, d),

4.27 (1H, dd), 4.04 (1H, m), 1.39, 1.35 (2 x 3H, 2 x s, $C(CH_3)_2$), 1.25 (3H, d). δ_C (CD_3OD): 176.4 (s, C-1), 114.4 (s, $C(CH_3)_2$), 84.9, 77.6, 77.2, 67.0 (4 x d, C-2, C-3, C-4, C-5). 26.5, 25.3 (2 x q, $C(CH_3)_2$), 17.8 (q, C-6).

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-D-mannono-γ-lactone (12). The 6-deoxylactone (11) (5.39 g, 26.7 mmol) was dissolved in a mixture of dichloromethane (75 ml) and pyridine (20 ml) and cooled under dry nitrogen to -20°C. Trifluoromethanesulphonic anhydride (4.95 ml, 29.4 mmol) was added and the reaction stirred for 1 h when t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (R_f 0.2) and one product (R_f 0.8). The reaction mixture was washed sequentially with 50 ml aliquots of 2M hydrochloric acid, water and brine, dried (magnesium sulphate) and the solvents removed under reduced pressure. The resulting crude triflate was dissolved in dimethylformamide (50 ml) and stirred at room temperature with sodium azide (5.21 g, 80.1 mmol) for 24 h when t.l.c. (ethyl acetate/hexane 1:2) revealed one product (R_f 0.7). Solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and washed with brine (3 x 100 ml). The organic layer was dried (magnesium sulphate) and the solvents removed under reduced pressure. Purification by flash column chromatography (ethyl acetate/hexane 1:3) and recrystallisation from ether gave *5-azido-5,6-dideoxy-2,3-O-isopropylidene-D-mannono-γ-lactone (12)* as a white solid, (4.01 g, 66%), m.p. 70-72°C. (Found: C, 47.68; H, 5.64; N, 18.52%. $C_9H_{13}O_4N_3$ requires C, 47.57; H, 5.77; N; 18.49%). $[\alpha]_D^{20}$ +2.64 (*c*, 1.1 in $CHCl_3$). ν_{max} (KBr disc): 2100 cm^{-1} (N_3), 1760 cm^{-1} (C=O). m/z (CI NH_3): 245 ($M+NH_4^+$,100%). δ_H ($CDCl_3$): 4.86 (2H, m), 4.10 (1H, dd), 3.94 (1H, m), 1.46 (3H, d), 1.50, 1.44 (2 x 3H, 2 x s, $C(CH_3)_2$). δ_C ($CDCl_3$): 173.5 (s, C-1), 114.5 (s, $C(CH_3)_2$), 80.3, 76.1, 75.7 (3 x d, C-2, C-3, C-4), 55.2 (d, C-5), 26.6, 25.8 (2 x q, $C(CH_3)_2$), 16.9 (q, C-6).

6-Deoxy-1,2-O-isopropylidene-D-mannono-δ-lactam (13). A solution of the azidolactone (12) (4.00 g, 16.8 mmol) in freshly distilled methanol (50 ml) was stirred in the presence of 10% palladium on carbon (~50 mg) under an atmosphere of hydrogen for 5 h when t.l.c. (ethyl acetate/hexane 1:2) showed baseline material only and t.l.c. (10% methanol in ethyl acetate) showed one major product (R_f 0.6). The reaction mixture was filtered through Celite and the solvents removed under reduced pressure; the residue was purified by flash column chromatography (10% ethanol in ethyl acetate) to afford *6-deoxy-1,2-O-isopropylidene-D-mannono-δ-lactam (13),* (3.0 g, 89%) as a hydroscopic foam. (Found: C, 53.73; H, 7.86; N, 6.47%. $C_9H_{15}O_4N$ requires C, 53.72; H, 7.51; N, 6.96%). $[\alpha]_D^{20}$ -17.22 (*c*, 0.76 in $CHCl_3$). ν_{max} (film): 3300 cm^{-1} (br, OH), 1670 cm^{-1} (C=O). m/z (GCMS CI NH_3): (202, $M+H^+$, 100%). δ_H ($CDCl_3$): 6.58 (1H, s, br), 4.61 (1H, d, J 7.9 Hz), 4.29 (1H, d, J 7.7 Hz), 3.38 (2H, m), 1.52, 1.41 (2 x 3H, 2 x s, $C(CH_3)_2$), 1.34 (3H, d, J 6.2 Hz). δ_C ($CDCl_3$): 169.7 (s, C-1), 110.7 (s, $C(CH_3)_2$), 78.8, 74.3, 72.9 (3 x d, C-2, C-3, C-4), 49.5 (d, C-5), 29.8, 24.7 (2 x q, $C(CH_3)_2$), 17.4 (q, C-6).

6-Deoxy-D-mannono-δ-lactam [D-Rhamnonolactam] (6) .The protected mannono-δ-lactam (13) (274 mg, 1.36 mmol) was dissolved in trifluoroacetic acid (4 ml) and water (2 ml) was added. The reaction was left to stand for 2 h when t.l.c. (10% methanol in ethyl acetate) revealed no starting material (R_f 0.6) and one product (R_f 0.1). The solvents were removed under reduced pressure and toluene (2 x 5 ml) was distilled from the residue. The resulting solid was dissolved in the minimum quantity of hot ethanol and twice the volume of ethyl acetate was added to yield *D-rhamnonolactam (6),* a white crystalline solid (166 mg, 75%), m.p. 164-166°C. (Found: C,

44.68; H, 7.05; N, 8.62%. $C_6H_{11}O_4N$ requires C, 44.72; H, 6.88; N, 8.69%). $[\alpha]_D^{20}$ -16.6 (*c*, 0.27 in H_2O). m/z (DCI NH_3): 162 ($M+H^+$, 100%). δ_H (D_2O): 4.18 (1H, d, J 4.2 Hz), 3.84 (1H, dd, J 4.2 and 5.7 Hz), 3.42 (1H, dd, J 5.7 and 7.9 Hz), 3.17 (1H, dd, J 6.8 Hz), 1.15 (3H, d, J 6.5 Hz). δ_C (D_2O): 174.5 (s, C-1), 74.5, 74.1, 69.8 (3 x d, C-2, C-3, C-4), 52.2 (d, C-5), 19.0 (q, C-6).

1,5-Imino-1,5,6-trideoxy-D-mannitol hydrochloride [D-Deoxyrhamnojirimycin hydrochloride] (5). Borane:dimethylsulphide complex (10M, 0.5 ml) was added to a soltuion of the protected D-mannono-δ-lactam (13) (424 mg, 2.15 mmol) in dry tetrahydrofuran (10 ml). The reaction was stirred for 12 h under nitrogen. Methanol was then added until effervescence ceased. Solvents were removed under reduced pressure and methanol (2 x 10 ml) was distilled off the residue, which was dissolved in ethanol (5 ml). A few drops of concentrated aqueous hydrochloric acid were added and the reaction mixture was stored at -20°C for 12 h. The crystals so obtained were filtered and washed with ethanol followed by ether to afford *D-deoxyrhamnojirimycin hydrochloride (5)* as a white solid, (244 mg, 75%), m.p. 247-248°C (decomp). (Found: C, 39.40; H, 7.83; N, 7.38%. $C_6H_{14}O_3NCl$ requires C, 39.24; H, 7.68; N, 7.63%). $[\alpha]_D^{20}$ -35.7 (*c*, 0.60 in H_2O). m/z (DCI NH_3): 148 ($M+H^+$). δ_H (D_2O): 4.06 (1H, m), 3.47 (2H, m), 3.18 (1H, m), 3.02 (1H, m), 2.92 (1H, m), 1.23 (3H, d, J 6.5 Hz). δ_C (D_2O): 73.4, 72.3, 67.2 (3 x d, C-2, C-3, C-4), 56.4 (d, C-5), 48.7 (t, C-1), 15.5 (q, C-6).

6-Bromo-6-deoxy-2,3-O-isopropylidene-D-gulono-γ-lactone (16). 2,3-O-Isopropylidene-D-gulono-γ-lactone (15) (7.20 g, 33.0 mmol) was dissolved in tetrahydrofuran (100 ml) and cooled to 0°C under nitrogen with stirring. Carbon tetrabromide (12.60 g, 38.0 mmol) and triphenyl phosphine (12.98 g, 49.5 mmol) were added and the reaction allowed to warm to room temperature over 2h and then stirred at room temperature for 10h when t.l.c. (ethyl acetate) revealed no starting material (R_f 0.4) and one product (R_f 0.9). The reaction was diluted with ether (100 ml), filtered and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (ether/hexane 2:1) followed by recrystallisation from ether/hexane to yield *6-bromo-6-deoxy-2,3-O-isopropylidene-D-gulono-γ-lactone (16)* as white needles (8.59 g, 85%). m.p. 86-87°C. $[\alpha]_D^{20}$ -53.4 (*c*, 1.0 in $CHCl_3$), identical in all other respects to the enantiomer (10) above.

6-Deoxy-2,3-O-isopropylidene-D-gulono-γ-lactone (17). The bromolactone (16) (8.59 g, 28.7 mmol) was dissolved in ethanol (50 ml). Triethylamine (5 ml) and palladium on carbon (10%, ~50 mg) were added and the reaction was stirred under an atmosphere of hydrogen for 3 h when t.l.c. (ethyl acetate) showed no starting material (R_f 0.9) and one product (R_f 0.7). The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was recrystallised from ethyl acetate to yield *6-deoxy-2,3-O-isopropylidene-D-gulono-γ-lactone (17)* as a white solid (5.28 g, 91%). m.p. 142-144°C, $[\alpha]_D^{20}$ -62.5 (*c*, 1.5 in EtOH), identical in all other respects to the enantiomer (11) above.

5-Azido-5,6-dideoxy-2,3-O-isopropylidene-L-mannono-γ-lactone (18). The deoxylactone (17) (4.97 g, 24.6 mmol) was dissolved in dichloromethane (50 ml) and cooled under dry nitrogen to -40°C. Pyridine (2.98 ml, 36.9 mmol) and trifluoromethanesulphonic anhydride (27.1 ml, 45.6 mmol) were added and the reaction stirred for 1 h when t.l.c. (ethyl acetate/hexane 1:1) showed no starting material (R_f 0.2) and one product (R_f 0.8). The reaction mixture was washed with 50 ml aliquots of 2M hydrochloric acid, water and brine, dried (magnesium

sulphate) and the solvents removed under reduced pressure. The crude triflate was dissolved in dimethylformamide (50 ml) and stirred at room temperature with sodium azide (4.80 g, 73.8 mmol) for 24 h when t.l.c. (ethyl acetate/hexane 1:2) revealed one product (R_f 0.7). Solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and washed with brine (3 x 100 ml). The organic layer was dried (magnesium sulphate) and the solvents removed under reduced pressure. Purification by flash column chromatography (ethyl acetate/hexane 1:3) and recrystallisation from ether gave *5-azido-5,6-dideoxy-2,3-O-isopropylidene-L-mannono-γ-lactone (18)* as a white solid (4.43 g, 76%), m.p. 70-72°C, $[\alpha]_D^{20}$ -4.64 (*c*, 0.8 in $CHCl_3$) identical in all other respects to the enantiomer (12) above.

6-Deoxy-1,2-O-isopropylidene-L-mannono-δ-lactam (19). The azide (18) (3.20 g, 13.5 mmol) was dissolved in freshly distilled methanol (50 ml) and 10% palladium on carbon (~50 mg) was added. The reaction was stirred under an atmosphere of hydrogen for 5 h when t.l.c. (ethyl acetate/hexane 1:2) showed baseline material only and t.l.c. (10% methanol in ethyl acetate) showed one major product (R_f 0.6). The reaction mixture was filtered through Celite and the solvents removed under reduced pressure. The residue was purified by flash column chromatography (10% ethanol in ethyl acetate) to yield *6-deoxy-1,2-O-isoproylidene-L-mannono-δ-lactam (19)* as a hydroscopic foam, (2.57 g, 94%), $[\alpha]_D^{20}$ +17.4 (*c*, 0.34 in $CHCl_3$), identical in all other respects to the enantiomer (13) above.

6-Deoxy-L-mannono-δ-lactam [L-Rhamnonolactam] (8). The lactam (19) (250 mg, 1.24 mmol) was dissolved in trifluoroacetic acid (4 ml) and water (2 ml) was added. The reaction was left to stand for 2 h when t.l.c. (10% methanol in ethyl acetate) revealed no starting material (R_f 0.6) and one product (R_f 0.1). The solvents were removed under reduced pressure and toluene (2 x 5 ml) was distilled from the residue. The resulting solid was dissolved in the minimum quantity of hot ethanol and twice the volume of ethyl acetate was added to afford, after crystallisation, *L-rhamnonolactam (8)* as a white crystalline solid (141 mg, 71%), $[\alpha]_D^{20}$ +16.8 (*c*, 0.31 in H_2O), identical in all other respects to the enantiomer (6) above.

1,5-Imino-1,5,6-trideoxy-L-mannitol hydrochloride [L-Deoxyrhamnojirimycin hydrochloride] (7). The protected L-mannono-δ-lactam (19) (237 mg, 1.2 mmol) was dissolved in dry tetrahydrofuran (5 ml) and borane:dimethylsulphide complex (10M, 0.5 ml) was added. The reaction was stirred for 12 h under nitrogen. Methanol was added until effervescence had ceased. Solvents were removed under reduced pressure and triturated with methanol (2 x 10 ml). The residue was dissolved in ethanol (5 ml) and a few drops of concentrated aqueous hydrochloric acid were added. The reaction was stored at -20°C for 12 h and the crystals so obtained were filtered and washed successively with ethanol and ether to yield *L-deoxyrhamnojirimycin hydrochloride (7)* as a white solid (177 mg, 84%), $[\alpha]_D^{20}$ +37.5 (*c*, 0.67 in H_2O), identical in all other respects to the enantiomer (5) above.

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