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A PRACTICAL SYNTHESIS OF DEOXYMANNOJIRIMYCIN AND OF (2S,3R,4R,5R)-3,4,5-TRIHYDROXYPIPECOLIC ACID FROM D-GLUCOSE

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Deoxymannojirimycin [1,5-dideoxy-1,5-imino-D-mannitol] may be prepared in moderate amounts in an overall yield of 35% in ten steps from diacetone glucose; the key step is formation of the piperidine ring by intramolecular nucleophilic displacement of a triflate at C-2 of a methyl glucofuranoside by a nitrogen function at C-6, irrespective of the anomeric configuration of the sugar. A synthesis of (2S,3R,4R,5R)-3,4,5-trihydroxypipecolic acid is reported.

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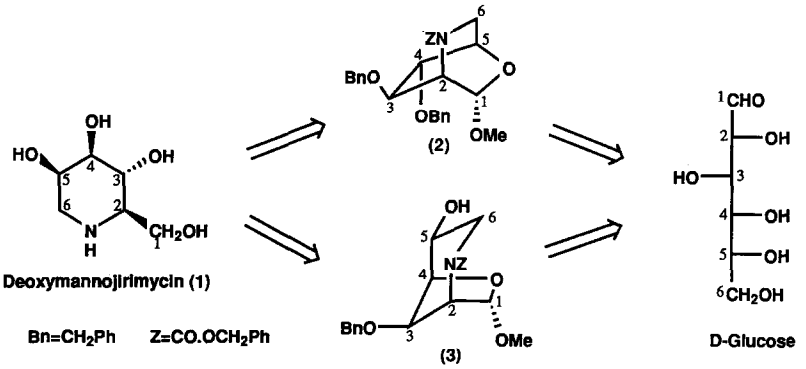
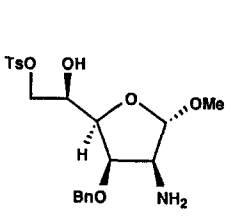
Deoxymannojirimycin (1) is an inhibitor of a bovine α-L-fucosidase and of mannosidase I of glycoprotein processing, and aside from any potential therapeutic value, is a useful tool for the study of biochemical pathways;[1] although several syntheses of deoxymannojirimycin have been reported,[2] there is a need for practical syntheses of such a compound on a moderate scale. The conversion of D-glucose into deoxymannojirimycin (1) requires the joining of C-6 and C-2 by nitrogen with inversion of configuration at C-2, and the reduction of aldehyde function at C-1 to an alcohol. The piperidine ring can be constructed while the anomeric position is protected either as a pyranoside or a furanoside. The formation of the bicyclic amine (2) from a pyranoside is slow, both by cyclisation of a 6-amino-2-O-trifluoromethanesulphonyl-glucopyranoside and by cyclisation of a 2-amino-6-O-tosyl-mannopyranoside; additionally, the acid hydrolysis of the [2.2.2]-acetal is very slow.[3] Accordingly, in practical terms, the approach via a protected pyranoside can only be used for the preparation of very small amounts of material.



In contrast, the protected bicyclic amine (3) may readily be prepared by cyclisation of the amine (4); also, the furanoside acetal (3) undergoes rapid hydrolysis with aqueous acid and thus (3) has been used as a divergent intermediate for the synthesis of a number of piperidine analogues of sugars.[4,5] The amine (4) is derived from the azidomannofuranoside (5) which is formed cleanly and in high yield by reaction of sodium azide with the α-triflate (6α); however, the β-triflate (6β) does not undergo nucleophilic displacement reaction of triflate in good yield.



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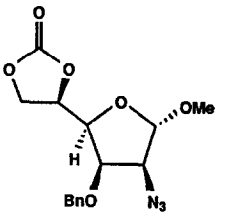

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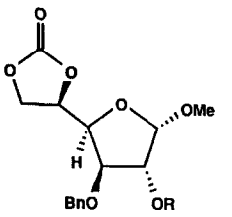
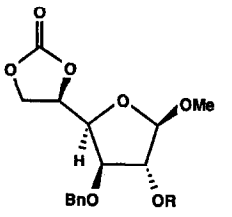
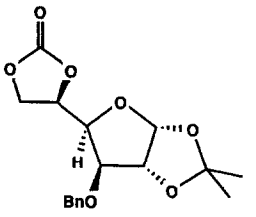


(6 α) R=SO_2CF_3
(7 α) R=H

Ts=4-Me-$C_6H_4SO_2$



(6 β) R=SO_2CF_3
(7 β) R=H

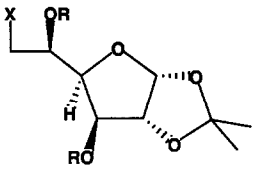


(8)

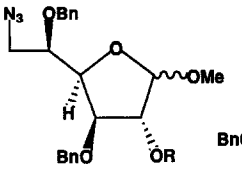
So, it is necessary to separate the anomeric precursors (7α) and (7β). In the preparation of these anomers from the acetonide (8), the unwanted (7β) is the major product and the chromatographic separation of large amounts of the required α-anomer (7α) is time-consuming, so that this is a serious disadvantage in the synthesis of large amounts of the key bicyclic intermediate (3).[6] In general, intermolecular nucleophilic displacements of 2-O-trifluoromethane sulphonates occur smoothly with furanosides where the anomeric group is cis- to the leaving group, but give very poor yields where the anomeric group is trans- to the leaving group.[7] This paper describes the synthesis of deoxymannojirimycin (1) in an overall yield of 35% from diacetone glucose by a procedure in which the formation of the piperidine ring is achieved by efficient intramolecular displacement of 2-O-trifluoromethane sulphonate by a nitrogen substituent at C-6 of the sugar which takes place with either anomer; the synthesis of (2S,3R,4R,5R)-3,4,5-trihydroxypipecolic acid (20) is also reported.

The conversion of 60 g of diacetone glucose into 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (11) in an overall yield of 84% was achieved by minor modifications of literature procedures. Hydrolysis of diacetone glucose with aqueous acetic acid gave monoacetone glucose (9) which was selectively esterified with p-toluenesulphonyl chloride[8] to give (10) [92% yield from diacetone glucose]. Reaction of the tosylate with sodium azide in dimethylformamide gave the azido diol (11)[9] [92% yield]. The two free hydroxyl groups in (11) were converted into the corresponding crystalline dibenzyl ether (12) by treatment with sodium hydride and benzyl bromide in the presence of tetrabutylammonium iodide [68% yield]. Reaction of the acetonide (12) with methanolic hydrogen chloride formed a mixture of the methyl furanosides (13; α:β ratio 4:3) in a yield of 96%. Treatment of the furanosides (13αβ) with trifluoromethane sulphonic anhydride in dichloromethane in the presence of pyridine afforded the triflates (14αβ) [97% yield]. Pure samples of the different anomers of (13) and (14) may be isolated by flash chromatography, but there is no advantage or necessity to separate any of the anomers in this sequence. The assignment of the anomeric configurations is based on the magnitude of the coupling constants between the anomeric proton and the proton on adjacent carbon ($J_{H-1,H-2}$); in the α-series where the hydrogens are cis to each other, the coupling constant (4.5 - 4.7 Hz) is much greater than in the β-series (0.7 - 1.3 Hz).[6]

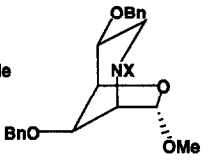
Attempted reduction of the azides (14) by hydrogenation in the presence of a variety of palladium catalysts resulted in partial removal of the benzyl protecting groups. Reaction of the anomeric azides (14αß) with triphenylphosphine, followed by treatment with aqueous potassium carbonate, caused reduction of the azide function to the corresponding amine and <u>in situ</u> cyclisation to the bicyclic amines (15αß) which were converted to the fully protected carbamates (16αß) [87% yield from (14)]. This transformation was also performed on each individual anomer of (14); both (14α) and (14ß) underwent efficient cyclisation to the bicyclic amines (16α) and (16ß) respectively.



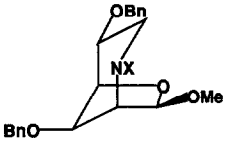
(9) X=OH; R=H
(10) X=OTs; R=H
(11) X=N_3; R=H
(12) X=N_3; R=Bn



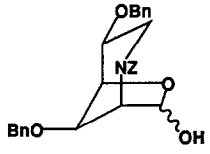
(13 αβ) R=H
(14 αβ) R=SO_2CF_3



(15 α) X=H
(16 α) X=Z



(15 β) X=H
(16 β) X=Z



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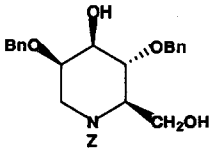

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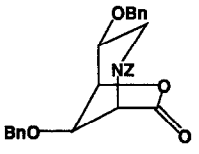

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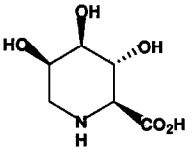

(20)

Hydrolysis of the furanoside acetals (16αß) with aqueous trifluoroacetic acid gave the lactol (17) [85% yield] which on reduction with sodium borohydride in aqueous ethanol afforded the diol (18) [94% yield]. Hydrogenolysis of (18) in acetic acid in the presence of palladium black caused removal of both the benzyl and benzyloxycarbonyl protecting groups to give deoxymannojirimycin (1), readily crystallised as the hydrochloride from methanol-ether, in 95% yield [35% overall yield from diacetone glucose].

The lactol (17) provides a convenient precursor for the synthesis of the highly functionalised amino acid, (2S,3R,4R,5R)-3,4,5-trihydroxypipecolic acid (20). Thus oxidation of (17) with bromine water in the presence of barium carbonate gave the bicyclic lactone (19) which on hydrogenolysis in aqueous acetic acid in the presence of palladium black gave (2S,3R,4R,5R)-3,4,5-trihydroxypipecolic acid (20) [84% yield from (17)].

In summary, this paper reports a practical synthesis of deoxymannojirimycin from glucose in an overall yield of 35%. The accompanying paper describes a practical synthesis of deoxymannojirimycin from L-gulonolactone.

Experimental

M.p.s were recorded on a Kofler block. Infra red spectra were recorded on Perkin Elmer 297 or 781 spectrophotometers; unless otherwise stated, infra red spectra of solids were obtained in $CHCl_3$ solution and those of syrups, as thin films. ^1H NMR spectra were run at 300 MHz on a Bruker WH 300 spectrometer; ^{13}C NMR were recorded on a Bruker AM 250 (62.9 MHz) spectrometer. All NMR spectra were obtained using deuteriochloroform as solvent unless otherwise stated; for ^{13}C NMR spectra in D_2O, 1,4-dioxan (δ 67.6) was used as an internal standard. Mass spectra were recorded on a VG Micromass 16F or 30F spectrometers, using the desorption chemical ionisation technique (DCI NH_3) unless otherwise stated. Optical rotations were measured on a Perkin Elmer 241 polarimeter; concentrations are given in g / 100 ml. Microanalyses were performed by the microanalytical service of the Dyson Perrins laboratory. TLC was performed on glass plates coated with silica gel blend 41 or on aluminium sheets pre-coated with Merck silica gel $60F_{254}$, and compounds were visualised with sprays of 5% v/v concentrated sulphuric acid in methanol, 5% w/v ninhydrin in ethanol or a solution of 0.2% w/v Ceric sulphate and 5% ammonium molybdate in 2M sulphuric acid. Flash chromatography was carried out using Merck Kieselgel 60, 230-400 mesh and dry column chromatography using Merck Kieselguhr 60H. The solvent system CMAW refers to a mixture of chloroform, methanol, acetic acid and water in ratio 60:30:3:5. The following ion exchange resins were utilised: Aldrich Chemical Company 50x 8-100, Sigma CG 120 (fine mesh) Na^+ form, Sigma CG 400 Cl^- form. The acid resin was used in the H^+ form, eluting with 0.5 M NH_3 solution in the cases of amino alcohols and 0.5 M pyridine solution for amino acids. The basic resin was used as the OH^- form, with water as eluent and used only for purification of amino alcohols. Solutions in organic solvents were dried with anhydrous sodium sulphate unless stated otherwise, and solvents were removed under reduced pressure.

1,2-O-Isopropylidene-6-O-p-toluenesulphonyl-α-D-glucofuranose (10). A solution of the 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (9) (60.0 g, Aldrich) in a 2:1 mixture of acetic acid and water (500 ml) was left to stand at room temperature for six hours then 4°C for 12 hours. The solvents were removed to give a pale yellow solid. Purification by recrystallisation from methanol/ether gave 1,2-O-isopropylidene-α-D-glucofuranose, (44.8 g, 93.2%), m.p. 158°-159°C [lit.[10] 160°-161°C]; $[\alpha]_D^{20}$ -11.4° (c, 2.79 in water) [lit.[10] $[\alpha]_D^{19}$ -13° (c, 1 in water)]. A portion of this material (31.8 g, 144 mmol) was dissolved in dry pyridine (250 ml) and cooled to -14°C. A solution of freshly recrystallised p-toluenesulphonyl chloride (28.9 g, 152 mmol), in dry pyridine (70 ml), was added and the mixture left to stand at -14°C for 12 hours. The solvent was evaporated and the residue dissolved in dichloromethane (100 ml), washed with 0.5M HCl (50 ml) and saturated sodium bicarbonate (50 ml), dried, filtered and evaporated to yield 1,2-O-isopropylidene-6-O-p-toluenesulphonyl-α-D-glucofuranose (53.0 g, 98%) as a pale yellow solid. A small quantity was recrystallised (ether-hexane) to give colourless crystals, m.p. 100-101°C [lit.[8] 99.5-100.2°C], $[\alpha]_D^{20}$ -9.7° (c, 1.03 in chloroform) [lit.[8] $[\alpha]_D^{20}$ -9.0° (c, 1.0 in chloroform)]; ^1H δ NMR 1.30, 1.46 (6H, 2 x s, acetonide); 2.45 (3H, s, CH_3-Ar); 3.3 (2H, bs, OH); 4.02, 4.10 (2H, 2 x dd, H-6,6', $J_{H,H'}$ 7.5 Hz, $J_{5,6}$ 2.8 Hz, $J_{5',6}$ 6.0 Hz); 4.18 (1H, ddd, H-5); 4.29 (1H, dd, H-4, $J_{4,5}$ 10.0 Hz, $J_{3,4}$ 2.6 Hz); 4.35 (1H, d, H-3); 4.51 (1H, d, H-2, $J_{1,2}$ 3.6 Hz); 5.89 (1H, d, H-1); 7.35, 7.80 (4H, 2 x d, H-Ar, $J_{o,m}$ 8.2 Hz). m/z : 392 ($M+NH_4^+$, 100%), 162 (77%), 220 (70%).

6-Azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (11). Sodium azide (28.2 g, 434 mmol) was added to a solution of crude 1,2-O-isopropylidene-6-O-p-

toluenesulphonyl-α-D-glucofuranose (10) (53.0 g) in dry N,N-dimethylformamide (500 ml) and the mixture stirred at 40°C for 15 hours. The solvent was removed and the residue dissolved in dichloromethane (200 ml), washed with water (3 x 50 ml) then brine (25 ml), dried, filtered and concentrated to a viscous oil. Purification by crystallisation (ether-hexane), and flash chromatography of the mother liquors, gave 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose, (32.1 g, 92%), m.p. 104°-106°C [lit.[11] 107.5°-108.5°C], $[\alpha]_D^{20}$ -17.9° (c, 1.1 in chloroform) [lit.[11] $[\alpha]_D^{25}$ -11° (c, 1 H_2O)]; ν_{max} ($CHCl_3$) 3430 (OH), 3010, 2100 (N_3), 1070; ^1H NMR δ 1.33, 1.50 (6H, 2 x s, acetonide); 3.0, 3.2 (2H, 2 x bs, 2 x OH); 3.55, 3.63 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 12.8 Hz, $J_{5,6}$ 6.3 Hz, $J_{5,6'}$ 3.6 Hz); 4.05 (1H, dd, H-4, $J_{4,5}$ 6.7 Hz, $J_{3,4}$ 2.7 Hz); 4.16 (1H, ddd, H-5); 4.37 (1H,d, H-3); 4.54 (1H, d, H-2, $J_{1,2}$ 3.6 Hz); 5.96 (1H, d, H-1). m/z (CI NH_3) : 206 (100%), 218 ($M+H-N_2^+$ 85%), 263 ($M+NH_4^+$, 80%)

6-Azido-3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (12). A solution of 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (11) (29.9 g, 122 mmol) in dry tetrahydrofuran (250 ml) was added dropwise to a stirred suspension of sodium hydride (50% dispersion in oil, 13.0 g, 274 mmol) and tetrabutylammonium iodide (2.25 g, 6.10 mmol) in dry tetrahydrofuran (250 ml). The mixture was stirred at 35°C for 18 hours with benzyl bromide (41.0 ml, 45.6 g, 268 mmol). The reaction was quenched with methanol (20 ml), stirred for a further 2 hours then filtered through celite and concentrated to an orange oil. Purification by flash chromatography (hexane to ether-hexane 1:3) afforded 6-azido-3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose, (35.4 g, 68%), m.p. 50°-51°C, $[\alpha]_D^{20}$ -66.7° (c, 1.10 in chloroform); ν_{max} ($CHCl_3$) 2920, 2000 (N_3), 1385, 1375, 1075 and 700 cm^{-1}; ^1H NMR δ 1.33, 1.51 (6H, 2 x s, acetonide); 3.45, 3.68 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 13.2 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6'}$ 2.6 Hz); 4.02 (1H, ddd, H-5); 4.13 (1H, d, H-3, $J_{3,4}$ 3.1 Hz); 4.29 (1H, dd, $J_{4,5}$ 9.0 Hz); 4.50 (1H, d, H-2, $J_{1,2}$ 3.8 Hz); 4.46-4.73 (4H, m, CH_2Ph); 5.90 (1H, d, H-1); 7.26-7.40 (10H, m, H-Ph). ^{13}C NMR δ 26.06, 26.53 (2 x q, acetonide-Me); 52.01 (t, C-6), 71.58, 73.57 (2 x t, 2 x CH_2Ph); 75.26, 79.20 (2 x d, C-3, C-5); 81.59, 81.67 (2 x d, C-2, C-4); 105.07 (d, C-1); 112.09 (s, acetonide); 127.39, 127.50, 127.64, 127.78, 128.11, 128.32 (6 x d, HC-Ph); 137.42, 137.97 (2 x s, C-Ph). m/z : 91 (100%), 398 ($M-N_2+H^+$, 50%), 443 ($M+NH_4^+$, 9%). (Found C, 65.11; H, 6.48; N, 10.22. $C_{23}H_{27}N_3O_5$ requires C, 64.92; H, 6.40; N, 9.88).

Methyl 6-Azido-3,5-di-O-benzyl-6-deoxy-D-glucofuranoside (13αβ). 6-Azido-3,5-di-O-benzyl-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (12) (39.9 g, 93.8 mmol) was dissolved in methanolic hydrogen chloride (400 ml, 1M in HCl) and stood at room temperature for 12 hours. The solution was neutralised by the addition of excess anhydrous sodium carbonate, filtered and evaporated. The crude material was purified by dry column chromatography (ether-hexane 1:3 to 1:1) to give a 4:3 α:β mixture of methyl 6-azido-3,5-di-O-benzyl-6-deoxy-D-glucofuranoside (35.8 g, 95.5%) as a pale orange oil. A small quantity of the anomeric mixture was separated by flash chromatography to give colourless oils.

α-anomer, R_f 0.3 (ether-hexane 1:1); $[\alpha]_D^{20}$ +10.6° (c, 1.28 in chloroform); ν_{max} (film) 3520 (OH), 2930, 2100 (N_3), 1455, 1125 and 735 cm^{-1}; ^1H NMR δ 2.9 (1H, bs, OH); 3.43, 3.63 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 13.5 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6'}$ 2.7 Hz); 3.50 (3H, s, CH_3); 3.97-4.02 (1H, m, H-5); 4.07 (1H, dd, H-2, $J_{1,2}$ 4.6 Hz, $J_{2,3}$ 2.5 Hz); 4.27 (1H, dd, H-3); 4.31 (1H, dd, H-4, $J_{3,4}$ 4.5 Hz, $J_{4,5}$ 8.0 Hz); 4.50, 4.54, (2H, 2 x bs, CH_2Ph); 4.68, 4.73 (2H, 2 x d, CH_2Ph, $J_{H,H'}$ 11.7 Hz); 7.26-7.35 (10H, m, H-Ph). ^{13}C NMR δ 51.95 (t, C-6); 56.17 (q, Me); 72.18, 72.63 (2 x t, CH_2Ph); 76.44,

78.49 (2 x d, C-3, C-5); 80.12 (d, C-4); 82.70 (d, C-2); 110.18 (d, C-1); 127.95, 128.13, 128.59, 128.67, 128.68 (5 x d, HC-Ph); 137.81, 138.13 (2 x s, C-Ph). m/z : 91 (100%); 372 ($M-N_2+H^+$, 70%); 400 ($M+H^+$, 10%). (Found C, 62.75; H, 6.38; N, 10.16. $C_{21}H_{25}N_3O_5$ requires C, 63.14; H, 6.30; N, 10.51).

β-anomer, R_f 0.2 (ether-hexane 1:1): $[\alpha]_D^{20}$ -82.2° (c, 1.12 in chloroform); ν_{max} (film) 3430 (OH), 2930, 2100 (N_3), 1455, 1110 and 740 cm^{-1}; ^1H NMR δ 3.41 (3H, s, CH_3); 3.47, 3.70 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 13.1 Hz, $J_{5,6}$ 8.5 Hz, $J_{5,6'}$ 2.6 Hz); 3.99 (1H, dd, H-3, $J_{2,3}$ 1.5 Hz, $J_{3,4}$ 5.0 Hz); 4.06 (1H, ddd, H-5); 4,23 (1H, bs, H-2); 4.40 (1H, dd, H-4, $J_{4,5}$ 8.5 Hz); 4.51, 4.68 (2H, 2 x d, CH_2, $J_{H,H'}$ 6.1 Hz); 4.55, 4.65 (2H, 2 x d, CH_2, $J_{H,H'}$ 6.7 Hz); 4.82 (1H, d, H-1, $J_{1,2}$ 0.7 Hz); 7.26-7.35 (10H, m, H-Ph). ^{13}C NMR δ 51.95 (t, C-6); 55.83 (q, Me); 71.52, 72.47 (2 x t, 2 x CH_2Ph); 75.52, 76.14, 77.64 (3 x d, C-3, C-4, C-5); 83.61 (d, C-2); 102.07 (d, C-1); 127.80, 127.94, 128.53 (3 x d, HC-Ph), 137.76, 138.13 (2 x s, C-Ph). m/z : 91 (100%), 372 ($M-N_2+H^+$, 20%), 400 ($M+H^+$, 5%). (Found C, 62.85; H, 6.45; N, 10.17. $C_{21}H_{25}N_3O_5$ requires C, 63.14; H, 6.30; N, 10.51).

Methyl 6-azido-3,5-di-O-benzyl-6-deoxy-2-O-trifluoromethanesulphonyl-D-glucofuranoside (14αβ). A 4:3 mixture of the α and β anomers of methyl 6-azido-3,5-di-O-benzyl-6-deoxy-D-glucofuranoside (13αβ) (35.8 g, 89.8 mmol) in dry dichloromethane was cooled to -50°C under nitrogen, and treated successively with pyridine (15.8 ml, 198 mmol) and triflic anhydride (16.6 ml, 99 mmol). The solution was allowed to warm to 0°C over 90 minutes. Methanol (5 ml) was added and the solution warmed to room temperature. The solvent was removed and the residue dissolved in ether (200 ml) and filtered. The ether layer was washed with 0.1M HCl (50 ml) then brine (50 ml), dried filtered and evaporated to give methyl 6-azido-3,5-di-O-benzyl-6-deoxy-2-O-trifluoromethane-sulphonyl-D-glucofuranoside (46.3 g, 97%) as a pale yellow oil. A small quantity was purified by flash chromatography (ether-hexane 1:5).

α-anomer, m.p. 47°-49°C, R_f 0.58 (ether-hexane 1:3); $[\alpha]_D^{20}$ +12.4° (c, 0.96 in chloroform); ν_{max} ($CHCl_3$) 2910, 2105 (N_3), 1420, 1220, 1155, 910, 735 & 700 cm^{-1}; ^1H NMR δ 3.42, 3.64 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 13.2 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6'}$ 2.9 Hz); 3.48 (3H, s, CH_3); 3.96 (1H, ddd, H-5); 4.36 (1H, dd, H-4, $J_{4,5}$ 7.6 Hz, $J_{4,3}$ 5.5 Hz); 4.41 (1H, dd, H-3, $J_{2,3}$ 2.9 Hz); 4.50 (2H, dd, CH_2Ph, $J_{H,H'}$ 12.9 Hz); 4.67 (2H, 2 x d, CH_2Ph, $J_{H,H'}$ 8.7 Hz); 5.07 (1H, dd, H-2, $J_{1,2}$ 4.33 Hz); 7.23-7.36 (10H, m, H-Ph). ^{13}C NMR δ 51.19 (t, C-6); 56.17 (q, Me); 72.45, 72.73 (2 x t, 2 x CH_2Ph); 75.75, 76.49 (2 x d, C-3, C-5); 80.37 (d, C-4); 87.65 (d, C-2); 100.45 (d, C-1); 123.01 (q, CF_3); 127.89, 128.03, 128.54, 128.71, 128.88 (d, HC-Ph); 136.68, 137.84 (2 x s, C-Ph). m/z (CI NH_3) : 171 (100%), 205 (70%), 354 (70%), 504 ($M-N_2+H^+$, 40%), 549 ($M+NH_4^+$, 30%). (Found C, 50.02; H, 4.73; N, 7.60. $C_{22}H_{24}NO_7SF_3$ requires C, 49.72; H, 4.52; N, 7.90).

β-anomer, a clear oil, R_f 0.63 (ether-hexane 1:3); $[\alpha]_D^{20}$ -53.5° (c, 0.94 in chloroform); ν_{max} (film) 2920, 2105 (N_3), 1420, 1250, 1215, 1150, 1030, 1070 & 960, 740, and 700 cm^{-1}; ^1H NMR δ 1.91 (1H, bs, NH); 2.82, 3.12 (1H, dd, H-6,6', $J_{6,6'}$ 13.5 Hz, $J_{5,6}$ 4.0 Hz, $J_{5,6'}$ 6.4 Hz); 3.17 (1H, d, H-2, $J_{2,3}$ 3.5); 3.41 (3H, s, CH_3); 3.69 (1H, dd, H-4, $J_{3,4}$ 6.1 Hz, $J_{4,5}$ 9.6 Hz, 4.22 (1H, dd, H-3); 4.43-4.58 (5H, m, H-5, CH_2Ph); 5.02 (1H, s, H-1); 7.29-7.36 (10H, m, H-Ph). m/z : 91 (100%), 504 ($M-N_2+H^+$, 40%), 549 ($M+NH_4^+$, 30%).

Methyl 3,5-Di-O-benzyl-2,6-dideoxy-2,6-imino-D-mannofuranoside (15αβ). A solution of the anomeric mixture of methyl 6-azido-3,5-di-O-benzyl-6-deoxy-2-O-trifluoromethane-sulphonyl-D-glucofuranosides (14αβ), (α:β 4:3) (46.3 g, 87.1 mmol) in dichloromethane (300 ml), was stirred with triphenylphosphine (24.0 g, 91.5 mmol)

in a water bath at room temperature for 30 minutes. The solution was then refluxed for two hours, after which IR spectroscopy showed no azide peak (2105 cm^{-1}). The mixture was cooled and stirred vigorously with saturated potassium carbonate solution (80 ml) for 48 hours. The organic layer was separated and the aqueous phase was extracted with dichloromethane (3 x 50 ml). The combined organic phase was dried, filtered and evaporated to give a pale brown oil, used directly in the next step. A small quantity was purified by flash chromatography (dichloromethane-ethanol 30:1) giving methyl 3,5-Di-O-benzyl-2,6-dideoxy-2,6-imino-D-mannofuranoside. α-anomer, m.p. 69°-70°C, R_f 0.45 (5% ethanol in dichloromethane); $[\alpha]_D^{20}$ 73.0° (c, 1.03 in chloroform); ν_{max} ($CHCl_3$) 3005, 2930, 1455, 1085, 1140, 950 and 700 cm^{-1}; ^1H NMR δ 1.83 (1H, bs, NH); 2.82, 3.12 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 13.5 Hz, $J_{5,6}$ 9.5 Hz, $J_{5,6'}$ 3.5 Hz); 3.17 (1H, d, H-2, $J_{1,2}$ 2,3 Hz); 3.41 (3H, s, CH_3); 3.69 (1H, dd, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 6.6 Hz); 4.22 (1H, dd, H-3); 4.43-4.58 (4H, 2 x dd, CH_2Ph); 4.48 (1H, ddd, H-5); 5.02 (1H, s, H-1); 7.24-7.35 (10H, m, H-Ph). ^{13}C NMR δ 44.30 (t, C-6); 55.47 (q, Me); 58.17 (d, C-2); 70.93, 72.51 (2 x t, CH_2Ph); 71.71 (d, C-4); 75.97 (d, C-3); 79.34 (d, C-5); 104.91 (d, C-1); 127.97, 128.12, 128.36, 128.64, 128.82 (5 x d, HC-Ph); 137.69, 138.50 (2 x s, C-Ph). m/z : 356 ($M+H^+$, 100%), 91 (85%), 204 (70%). (Found C, 70.53; H, 6.78; N, 3.43. $C_{21}H_{25}NO_4$ requires C, 70.96; H, 7.09; N, 3.94).

β-anomer, a colourless oil, R_f 0.40 (5% ethanol in dichloromethane); $[\alpha]_D^{20}$ -18.2° (c, 0.72 in chloroform); ν_{max} ($CHCl_3$) 3010, 2930, 1450, 1360, 1120, 1085, 1020 and 965 cm^{-1}; ^1H NMR δ 1.93 (1H, bs, NH); 3.09, 3.27 (2H, 2 x dd, H-6,6', $J_{6,6'}$ 2.3 Hz, $J_{5,6}$ 6.7 Hz, $J_{5,6'}$ 9.6 Hz); 3.20 (1H, dd, H-2, $J_{1,2}$ 3.0 Hz, $J_{2,3}$ 3.4 Hz); 3.55 (3H, s, CH_3); 3.72 (5H, dd, H-5); 3.96 (1H, dd, H-3, $J_{3,4}$ 6.0 Hz); 4.38 (1H, d, H-4); 4.46, 4.54 (2H, 2 x d, CH_2Ph, $J_{H,H'}$ 11.8 Hz); 4.53 (2H, s, CH_2Ph); 5.04 (1H, d, H-1, $J_{1,2}$ 3.0 Hz); 7.29-7.36 (10H, m, H-Ph). ^{13}C NMR δ 44.30 (t, C-6); 55.47 (q, Me); 56.89 (d, C-2); 70.76, 71.27 (2 x t, 2 x CH_2Ph); 71.88 (d, C-4); 77.00, 77.24 (2 x d, C-5, C-3); 103.28 (d, C-1); 127.80, 127.98, 128.27, 128.53, 128.71 (5 x d, HC-Ph); 137.44, 138.67 (2 x s, C-Ph). m/z : 356 ($M+H^+$, 100%), 91 (25%), 204 (10%). (Found C, 70.66; H, 7.01; N, 3.76. $C_{21}H_{25}NO_4$ requires C, 70.96; H, 7.09; N, 3.94).

Methyl 3,5-Di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannofuranoside (16αβ). The crude amine mixture (15αβ) (α:β 4:3) was dissolved in a 3:2 mixture of diethyl ether and saturated sodium bicarbonate solution (450 ml). This was treated with benzyl chloroformate (18.5 ml, 131 mmol) and stirred at room temperature for 18 hours. The ether layer was separated and the aqueous phase extracted with ether (4 x 50 ml). The combined organic phase was dried, filtered and evaporated. Benzyl alcohol was removed by distillation (80°C, 0.05mmHg). Purification by flash chromatography (ether-hexane 1:5 to 1:2) gave methyl 3,5-Di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannofuranoside as a partially separated 4:3 α:β mixture of anomers (37.5 g, 87% over two steps).

α-anomer, a clear oil, R_f 0.55 (ether-hexane 1:2); $[\alpha]_D^{20}$ 29.7 (c 0.17 in chloroform); ν_{max} (film) 3030, 1690, 1450, 1330,1230, 1100, 1040, 735 & 700 cm^{-1}; ^1H NMR δ 2.98-3.08 (1H, m, H-6); 3.40, 3.42 (3H, m, CH_3); 3.87-3.95 (1H, m, H-6'); 4.20-4.80 (8H, m, H-2, H-3, H-4, H-5, H-Ph); 5.01, 5.08 (2H, 2 x d, CH_2-Z, $J_{H,H'}$ 3.3 Hz); 5.13 (1H, s, H-1); 7.12-7.42 (15H, m, H-Ph). ^{13}C NMR δ 43.25 (t, C-6); 56.29, 56.96 (2 x q, Me); 63.70, 63.89 (2 x d, C-2); 67.48, 67.78 (2 x t, CH_2-Z); 69.02, 69.40 (2 x t, CH_2-Ph), 71.55 (t, CH_2Ph); 72.44 (2 x d, C-4); 75.49, 75.56, 76.12, 76.42 (4 x d, C-3, C-5); 103.38, 103.78 (2 x d, C-1); 127.32, 127.87, 128.00, 128.13, 128.28, 128.62 (6 x d, HC-Ph); 136.33, 136.64, 137.69, 137.88, 138.10 (5 x s, C-Ph); 155.59 (s, C=O). m/z : 91 (100%), 108 (48%), 294 (15%), 490 ($M+H^+$, 6%). m/z : 91 (100%), 108 (45%), 356 (12%), 490 ($M+H^+$, 8%). (Found C, 70.98; H,

6.62; N, 3.12. $C_{29}H_{31}N_{O6}$ requires C, 71.15; H, 6.38; N, 2.86).
β-anomer, a clear oil, R_f 0.30 (ether-hexane 1:2); $[\alpha]_D^{20}$ -50.2 (c, 1.00 in chloroform); ν_{max} (film) 2960, 1695, 1430, 1320, 1220, 1130, 950, 870 & 705 cm^{-1}; ^1H NMR δ 3.37-3.46 (1H, m, H-6); 3.51 (3H, s, CH_3); 3.83-4.06 (2H, m, H-2, H-6'); 4.21-4.88 (7H, m, H-3, H-4, H-5, CH_2-Ph); 5.06-5.31 (3H, m, CH_2-Z, H-1); 7.12-7.37 (15H, m, H-Ph). ^{13}C NMR δ 43.77, 43.94 (2 x t, C-6); 53.55, 54.07 (2 x q, Me); 56.68, 57.79 (2 x d, C-2); 67.29, 67.45 (2 x t, CH_2-Z); 70.14, 70.65, 71.17, 71.31 (4 x t, CH_2Ph); 71.60, 71.85 (2 x d, C-4); 74.99, 75.09 77.28 (3 x d, C-3, C-5); 102.39, 102.71 (2 x d, C-1); 127.53, 127.76, 127.97, 128.18, 128.51, 128.60 (6 x d, HC-Ph); 136.65, 136.78, 137.18, 137.36, 138.30 (5 x s, C-Ph); 156.37 (s, C=O). (Found C, 71.27; H, 6.34; N, 2.64. $C_{29}H_{31}N_{O6}$ requires C, 71.15; H, 6.38; N, 2.86).

3,5-Di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannose (17). A 1:2 mixture of the α and β anomers of methyl 3,5-di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannofuranoside (16αβ) (12.0 g 24.5 mmol) was dissolved in a 1:2:1 mixture of water, 1,4-dioxan and trifluoroacetic acid (80 ml). After 24 hours, water (300 ml) and sodium acetate (20 g) were added, and the product extracted into dichloromethane (4 x 100 ml). The combined organics layers were dried and evaporated then purified by flash chromatography to give 3,5-di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannose (9.95 g, 85%) as a clear oil, $[\alpha]_D^{20}$ +22.0 (c, 0.90 in chloroform); ν_{max} (film) 3420, 1670 (C=O), 1455, 1425, 1220, 1195 and 740 cm^{-1}; ^1H NMR δ 2.6 (2H, bs, OH, H-6); 2.95-3.20 (1H, m, H-6'); 3.78-3.95 (1H, m, H-2); 4.12-4.79 (8H, m, H-3, H-4, H-5, CH_2Ph); 5.03-5.46 (2H, m, CH_2-Z); 5.47 (0.6H, s, H-1); 7.16-7.37 (15H, m, H-Ph); 9.53, 9.54 (0.4H, 2 x s, H-1). m/z : 91 (100%), 108 (40%), 476 ($M+H^+$, 4%).

The pure α-anomer (16α) reacted similarly; the furanoside (720 mg, 1.48 mmol) afforded the title compound (17) (610 mg, 89%) under the same reaction conditions.

2,4-Di-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (18). A solution of 3,5-di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannose (17) (9.95 g, 20.8 mmol) in ethanol (90 ml) was treated with a suspension of sodium borohydride (1.51 g, 40 mmol) in 1:1 ethanol-water (10 ml). After 20 minutes, the reaction was quenched with excess NH_4Cl, the solvent removed and the residue partitioned between water (50 ml) and dichloromethane (50 ml). The aqueous layer was extracted with dichloromethane (3 x 50 ml) and the combined organics dried, filtered and evaporated to give 2,4-Di-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (9.39 g, 94%) as a clear oil; $[\alpha]_D^{20}$ -22.7° (c, 0.89 in chloroform); ν_{max} (film) 3420 (OH), 1670, 1455, 1430, 1340, 1230 and 1070 cm^{-1}; ^1H NMR δ (broadened due to rotamers) 1.7, 2.7 (2H, 2 x bs, 2 x OH); 2.8 (1H, bs, H-1); 3.2 (1H, bs, H-1'); 3.3 (1H, bs, H-5); 3.75-4.75 (9H, m, H-2, H-3, H-4, H-6,6', CH_2Ph); 5.18 (2H, s, CH_2-Z); 7.17-7.40 (15H, m, H-Ph). m/z (CI NH_3) : 370 ($M-BnO^+$, 100%), 91 (77%), 250 (50%), 478 ($M+H^+$ 25%). ^{13}C NMR δ 53.35 (t, C-1); 55.11 (t, C-6); 61.42 (d, C-5); 67.49 (t, CH_2-Z); 71.38 (d, C-4); 76.01, 77.19 (2 x d, C-3, C-5); 127.97, 128.19, 128.62, 128.70 (4 x d, HC-Ph); 136.58, 137.76, 137.81 (3 x s, C-Ph); 156.63 (s, C=O). (Found C, 69.92; H, 6.69; N, 3.02. $C_{28}H_{31}NO_6$ requires C, 70.42; H, 6.54; N, 2.93).

Deoxymannojirimycin, [1,5-Dideoxy-1,5-imino-D-mannitol] (1). 2,4-Di-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (18) (9.39 g, 19.6 mmol) was dissolved in acetic acid (50 ml) and stirred with palladium black (1 g), under hydrogen at atmospheric pressure, for 18 hours. The reaction mixture was filtered through celite and the solvent removed. Purification by flash chromatography

(CMAW), ion exchange chromatography, acidification with dil. HCl and crystallisation from methanol-ether gave deoxymannojirimycin, (3.67 g, 94.5%) as the hydrochloride salt, a white crystalline solid, m.p. 186°-188°C dec., $[\alpha]_D^{20}$ -13.8° (c, 1.1 in water); ^{1}H NMR (D_2O) δ 3.01 (1H, ddd, H-5, $J_{5,4}$ 10.2 Hz, $J_{5,6}$ 6.8 Hz, $J_{5,6'}$ 3.3 Hz); 3.10 (1H, dd, H-1, $J_{1,2}$ 1.4 Hz, $J_{1,1'}$ 13.6 Hz); 3.27 (1H, dd, H-1', $J_{1',2}$ 3.1 Hz); 3.54 (1H, dd, H-3, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.0 Hz); 3.69 (1H, dd, H-6, $J_{6,6'}$ 12.6 Hz); 3.72 (1H, t, H-4); 4.10 (1H, ddd, H-2). ^{13}C NMR δ (D_2O) 48.25 (t, C-1); 58.81 (t, C-6); 61.08 (d, C-5); 66.45, 66.60 (d, C-3,4); 73.14 (d, C-2). m/z (CI NH_3) : 164 ($M+H^+$, 100%), 110 (30%), 128 (25%).

3,5-Di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannono-1,4-lactone (19) A solution of 3,5-di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannose (17) (510 mg, 1.074mmol) in a 3:1 mixture of 1,4-dioxan and water was treated with barium carbonate (618 mg, 3.22 mmol) and bromine (67 l, 1.34 mmol). The mixture was stirred at room temperature for 36 hours. TLC (ethyl acetate-hexane 1:1) showed no starting material (R_f 0.45) and two products (R_fs 0.65 and 0.10). The bromine was destroyed by the dropwise addition of 1M aqueous sodium thiosulphate and the solution acidified with 2M HCl. Colloidal sulphur was removed by centrifugation and the supernatent extracted with ethyl acetate (4 x 25 ml), dried, filtered and evaporated to give a pale yellow oil (485 mg), a 2:1 mixture of the lactone and ring opened acid, used directly in the next step. A small quantity of lactone was purified by flash chromatography to give 3,5-di-O-benzyl-N-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-D-mannono-1,4-lactone as a clear oil, R_f 0.65 (ethyl acetate-hexane 1:1); $[\alpha]_D^{20}$ -61.3° (c, 1.06 in chloroform); ν_{max} (film) 3030, 2880, 1795 (lactone-CO), 1700 (Z-CO), 1425, 1365, 1325, 1295, 1230, 1165, 1110, 1030, 975, 760 and 700 cm^{-1}; ^{1}H NMR δ 2.96-3.08 (1H, m, H-6); 4.03-4.17 (2H, m, H-2, H-6); 4.32-4.81 (7H, m, H-3, H-4, H-5, CH_2Ph); 5.01-5.20 (2H, m, CH_2-Z); 7.13-7.35 (15H, m, H-Ph). m/z : 91 (100%); 186 (20%), 341 (20%), 431 (12%), 491 ($M+NH_4^+$, 12%).

(2S, 3R, 4R, 5R)-3,4,5-Trihydroxypipecolic acid (20). The crude mixture of acid and lactone (19) (457 mg) was dissolved in acetic acid (8 ml) and water (4 ml) then stirred under hydrogen with palladium black (110 mg). After 48 hours, the solvent was removed and the crude material purified by flash chromatography (CMAW) and ion exchange to yield (2S, 3R, 4R, 5R)-3,4,5-Trihydroxypipecolic acid, monohydrate (164 mg 2 steps: 84%), a white crystalline solid, m.p. 154°-156°C dec. [lit.[5] hydroscopic oil], $[\alpha]_D^{20}$ -28.3° (c, 0.64 in water) [lit.[5] -13.8° (c, 0.21 in water)]; ν_{max} (KBr disc) 3430, 3010, 1600 (CO), 1370, 1400 and 1085 cm^{-1}; ^{1}H NMR δ (D_2O) 3.07 (1H, dd, H-6, $J_{6,6'}$ 13.3 Hz, $J_{5,6}$ 2.2 Hz); 3.28 (1H, d, H-6', $J_{5,6'}$ 4.6 Hz); 3.38 (1H, d, H-2, $J_{2,3}$ 9.1 Hz); 3.61 (1H, dd, H-4, $J_{3,4}$ 8.6 Hz, $J_{4,5}$ 3.1 Hz); 3.96 (1H, t, H-3); 4.07 (1H, ddd, H-5). ^{13}C NMR δ D_2O 46.42 (t, C-6); 61.39 (d, 2-C); 65.93, 68.53, 72.44 (3 x d, C-3,4,5); 172.19 (s, C-1). m/z : 98 (100%), 96 (95%), 114 (85%), 178 ($M+H^+$ 57%). (Found C, 36.75; H, 6.91; N, 7.11. $C_6H_{11}NO_5.H_2O$ requires C, 36.92; H, 6.71; N, 7.18).

References

1. S. V. Evans. L. E. Fellows, T. K. M. Shing and G. W. J. Fleet, Phytochemistry, 1985, 24, 1953; U. Fuhrmann, E. Bause, G. Legler and H. Ploegh, Nature, 1984, 307, 755.

2. L. E. Fellows and G. W. J. Fleet, Alkaloidal Glycosidase Inhibitors from Plants, Chap. 13 in Natural Product Isolation, (ed. G. H. Wagman and R. Cooper), p.540-560, Elsevier, 1988.

3. G. W. J. Fleet, M.J. Gough and T.K.M. Shing, Tetetrahedron Lett., 1984, 25, 4029.

4. G. W. J. Fleet and P. W. Smith, Tetrahedron Lett., 1985, **26**, 1469.
5. G. W. J. Fleet and P. W. Smith, Tetrahedron 1987, **47**, 979.
6. G. W. J. Fleet L. E. Fellows and P. W. Smith, Tetrahedron, 1987, **43**, 971.
7. G. W. J. Fleet and S. K. Namgoong, unpublished results.
8. 1. G. Mogel and A. M. Yurkevich, Zh. Obsihikhim, 1969, **39**, 1882.
9. F. D. Cramer, Methods in Carbohydrate Chemistry I, (ed. R. L. Whister and M. L. Wolfrom), p.242, Academic Press, 1962.
10. O. Th. Schmidt, Methods in Carbohydrate Chemistry II, (ed. R. L. Whister and M. L. Wolfrom), p.322, Academic Press, 1963.
11. T. Tsuchiya, T. Miyahe, S. Keyeyanca and S. Umezawa, Tetrahedron Lett, 1981, **22**, 1413.

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