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SYNTHESIS OF DEOXYMANNOJIRIMYCIN FAGOMINE DEOXYNOJIRIMYCIN 2-ACETAMIDO-1,5-IMINO-1,2,5-TRIDEOXY-D-MANNITOL 2-ACETAMIDO-1,5-IMINO-1,2,5-TRIDEOXY-D-GLUCITOL 2S,3R,4R,5R-TRIHYDROXYPIPECOLIC ACID AND 2S,3R,4R,5S-TRIHYDROXYPIPECOLIC ACID FROM METHYL 3-O-BENZYL-2,6-DIDEOXY-2,6-IMINO-α-D-MANNOFURANOSIDE

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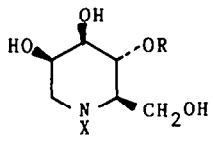
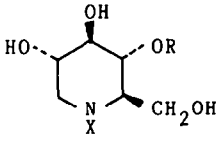
The value of methyl 3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside as a divergent intermediate for the preparation of polyhydroxylated piperidines is illustrated by the synthesis of deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol), fagomine (1,5-imino-1,2,5-trideoxy-D-arabino-hexitol), deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol), 2-acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol, 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol, 2S,3R,4R,5R-trihydroxypipecolic acid and 2S,3R,4R,5S-trihydroxypipecolic acid.

Several synthetic[1,2] and naturally[3] occurring 1,5-dideoxy-1,5-iminohexitols - analogues of pyranoses in which the ring oxygen has been replaced by nitrogen and the anomeric hydroxyl group replaced by hydrogen - have been shown to be potent and specific inhibitors of glycosidase enzymes from a variety of sources. Deoxymannojirimycin (1), isolated from Lonchocarpus sp.,[4] is a moderate inhibitor of several α-mannosidases[5,6] and a good inhibitor of mammalian α-fucosidase.[7] Deoxynojirimycin (3), first prepared by hydrogenation of a Streptomyces product nojirimycin[8] isolated from mulberries,[9] is an inhibitor of a number of glucosidases.[10,11] Fagomine, the corresponding analogue of 2-deoxyglucose, has been found as the free base (5) in Japanese buckwheat[12] and also as a glucoside in Xanthocercis zambesiaca;[13] although fagomine had no inhibitory effect on glycosidases from various sources, it inhibits α-glucosidase activity in mouse gut.[11] The glucuronic acid analogue (7), isolated from Baphia racemosa,[14] has been shown to be an inhibitor of human liver β-D-glucuronidase.[15] The synthetic compound (8), the corresponding analogue of N-acetylglucosamine, is a potent and specific inhibitor of a number of β-N-acetylglucosaminidases, whereas the manno-epimer (10) shows no inhibition of a wide range of glycosidases.[2] All these polyfunctionalised piperidines, together with the mannuronic acid analogue (12), have in common the stereochemistry of the substituents at C-2, C-3 and C-4 of the mannofuranoside (13); they vary in the oxidation level of the substituent derived from C-1 and in the functional group or stereochemistry of the substituent at C-5. The benzyl (14) and tert-butyl (15) carbamates of methyl 3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside, in which only the C-5 hydroxyl group is unprotected, may be efficiently synthesised from methyl 2-azido-3-O-benzyl-2-deoxy-α-D-mannofuranoside[16] and are suitable divergent intermediates for all these 1,5-dideoxy-1,5-iminohexitols. Some of this work has been published in preliminary form.[2,17]

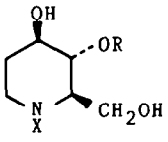
The stereochemistry in the piperidine ring of the bicyclic carbamate (14) is correct for the synthesis of deoxymannojirimycin (1). Thus hydrolysis of the furanoside ring in (14) with aqueous trifluoroacetic acid followed by reduction of the resulting lactol with sodium borohydride gave (2) [81% yield]; subsequent removal of the protecting groups by hydrogenolysis formed deoxymannojirimycin (1) [68% yield; 55% yield from (14)]. It is noteworthy that in the final deprotection in this and the following sequences that care must be exercised in the choice of solvent; if the hydrogenolysis is conducted in methanol, substantial formation of N-



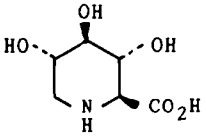
(1) R = X = H
(2) R = Bn; X = Z



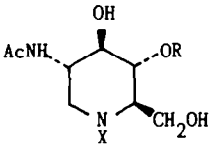
(3) R = X = H
(4) R = Bn; X = Z



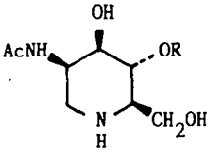
(5) R = X = H
(6) R = Bn; X = Z



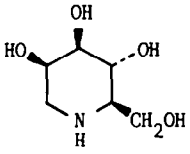
(7)



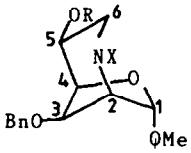
(8) R = X = H
(9) R = Bn; X = Z



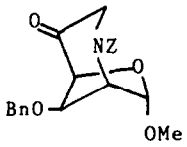
(10) R = H
(11) R = Bn



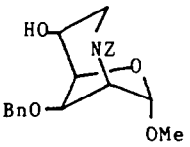
(12)



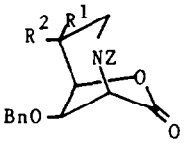
(13) X = R = H
(14) X = Z; R = H
(15) X = BOC; R = H
(16) X = Z; R = PhO.CS
(17) X = Z; R = CF_3SO_2
(18) X = BOC; R = CF_3SO_2



(19)

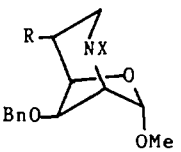


(20)

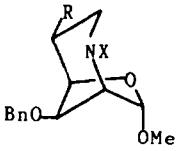


(21) R_1 = OH; R^2 = H
(22) R^1 = H; R^2 = OH

Bn = $PhCH_2$; Z = $PhCH_2OCO$
Ac = CH_3CO; BOC = tBuOCO



(23) X = Z; R = H
(24) X = Z; R = N_3
(25) X = Z; R = AcNH
(26) X = BOC; R = N_3



(27) X = Z; R = N_3
(28) X = BOC; R = N_3
(29) X = BOC; R = AcNH

methylated products are observed. Several other syntheses of deoxymannojirimycin have been reported.[18]

The trihydroxypipecolic acid (12) requires oxidation (rather than the reduction in the synthesis of deoxymannojirimycin) of the lactol formed on hydrolysis of (14); oxidation of the lactol with bromine in aqueous dioxan buffered with barium carbonate[19] gave the corresponding lactone (22) [93% yield from (14)]. Subsequent removal of the protecting groups by hydrogenolysis gave the mannuronic acid analogue (12) in quantitative yield; this short sequence provides an efficient synthesis of this previously unknown trihydroxypipecolic acid.

Inversion of the free hydroxyl group in the bicyclic lactam (14) is necessary for the synthesis of deoxynojirimycin (3). Oxidation of (14) with pyridinium chlorochromate in the presence of powdered molecular sieve[20] gave the ketone (19) which on reduction with sodium borohydride gave exclusive reduction to the inverted alcohol (20) [78% from (14)]; attack from the more hindered face to give (14) would require the development of a 1,3-diaxial interaction of the incoming hydride nucleophile with the O-benzyl group. Sequential hydrolysis and borohydride reduction of (20) gave the protected iminoglucitol (4) [65% yield], from which the protecting groups were removed by hydrogenolysis to give deoxynojirimycin (3) [quantitative yield], identical to an authentic sample; a number of other syntheses of (3) have been reported.[21]

Hydrolysis of the lactam (20), followed by bromine water oxidation, gave the lactone (22) in 19% yield; this low yield is in contrast to the high yield of the epimeric lactone (21) and may be due to the relative instability of (22) in which four substituents are axial. Hydrogenation of (21), followed by purification by ion exchange chromatography, gave the glucuronic acid analogue (7) identical to an authentic sample of the natural product. A more efficient synthesis of (7) has recently been described;[22] an alternative synthesis from glucose has been reported.[23]

The synthesis of fagomine (5) from the key bicyclic intermediate (14) requires removal of the free hydroxyl group. Treatment of (14) with phenylchlorothionocarbonate and dimethylaminopyridine in acetonitrile gave the thionocarbonate (16) which with tributyltin hydride underwent the Barton deoxygenation[24] to give (23); the deoxygenated material (23) was contaminated with tin containing compounds which could not be removed at this stage. Acid hydrolysis of the crude acetal (23), followed by borohydride reduction of the resulting lactol, gave the diol (6) in 23% yield from (14). A more efficient procedure for the removal of the hydroxyl group in (14) involved esterification of the alcohol with trifluoromethanesulphonic anhydride followed by reduction of the triflate (17) with lithium triethylborohydride; some loss of the benzyloxycarbonyl protecting group occurred under these conditions, so that the crude reaction mixture was treated with benzyl chloroformate to give (23) in 78% yield. Removal of the protecting groups in the diol (6) by hydrogenolysis followed by purification by ion exchange chromatography gave fagomine (5) [74% yield] identical with an authentic sample.

Preparation of the analogues of N-acetylglucosamine (8) and N-acetylmannosamine (10) require replacement of the hydroxyl group in (14) by a nitrogen function with, respectively, inversion and retention of configuration. The trifluoromethanesulphonyl ester (17), in which the amine function is protected as the benzyl carbamate, was treated with sodium azide in dimethylformamide to give the inverted azide (24) in 36% yield, together with the azide (27) [20% yield] in which the configuration had been retained during the displacement reaction; some material (approximately 15%) arising from competing elimination of triflic acid was also obtained. Selective reduction of the azide (24) with sodium hydrogen telluride[25] gave, after acetylation, the secondary amide (25) [92% yield]; subsequent acid

hydrolysis of (25) followed by borohydride reduction gave (9) from which the protecting groups were removed by hydrogenolysis to give 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (8) [82% yield]. When an identical sequence of reactions was performed on the epimeric azide (27), 2-acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol (10) was obtained [53% yield from (27)]. In contrast, the triflate (18) derived from the tert-butyloxycarbonyl derivative (15) reacted with sodium azide to give the inverted azide (26) as the minor product [15% yield] and the azide (28) in which configuration had been retained in the displacement as the major product [42% yield]. Reduction of azide (28) by hydrogenation, followed by acetylation gave the amide (29) in 96% yield. Treatment of (29) with aqueous trifluoroacetic acid resulted in hydrolysis of the methyl furanoside, together with simultaneous removal of the tert-butyloxycarbonyl protecting group; subsequent borohydride reduction gave (11) from which the benzyl group was removed by hydrogenolysis to give (10) identical to that prepared by the alternative sequence [52% yield from (29)]. Due to the hydrolytic removal of the tert-butyloxycarbonyl protecting group, the later intermediates in this sequence are very polar and more difficult to purify than the intermediates in the sequence using benzyloxycarbonyl protection (where the protecting group withstands the conditions necessary for the acid hydrolysis of the furanoside). Neighbouring group participation by carbamates in nucleophilic substitution reactions is well known;[26] however, the difference in the relative amounts of retained and inverted azides for the two different triflates (17) and (18) is noteworthy. In this case, the tert-butyloxycarbonyl group is a more effective neighbouring group than the benzyloxycarbonyl group.

The stereochemistry of the epimeric N-acetyl hexosamine analogues (8) and (10) are readily recognised by the appearance in the ^{1}H NMR spectra of the three protons on C-1 and C-5. The pattern for these protons in the N-acetylglucosamine analogue (8) is identical to that for the corresponding protons in deoxynojirimycin (3); a different characteristic pattern for the corresponding protons in the N-acetylmannosamine analogue (10) and in deoxymannojirimycin (1) is also found. Furthermore, the coupling constants between the protons attached to C-1 and the proton attached to C-2 are highly informative; in the compounds (8) and (3) with the gluco-configuration one large and one small coupling indicate that the proton attached to C-2 is in an axial position, whereas the two small coupling constants observed in the manno-compounds (10) and (1) demonstrate that the C-2 proton is equatorial [Table].

TABLE: Coupling Constants in Hz Between Protons Attached to C-1 and C-2

	J_{1e1a}	J_{1e2}	J_{1a2}
Deoxynojirimycin (3)	12.3	5.1	10.9
2-Acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (8)	12.6	4.9	11.5
Deoxymannojirimycin (1)	13.7	2.4	2.4
2-Acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol (10)	14.4	2.6	1.4

In summary, this paper demonstrates the value of methyl 3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (13) as a divergent intermediate for the preparation of polyhydroxylated piperidines.[27]

Experimental

M.p.s were recorded on a Kofler block. Infra red spectra were recorded on Perkin-Elmer 297, 310, 781 or Pye-Unicam SP3-200 spectrophotometers; unless otherwise stated, infra red spectra of solids were obtained in $CHCl_3$ solution and of syrups were obtained as thin films. ^{1}H NMR spectra were run at 300 MHz on a Bruker WH 300 spectrometer (500 MHz on a Bruker AM 500 spectrometer); ^{13}C NMR were recorded on a Bruker AM 250 (62.9 MHz) or a Bruker AM 500 (125.0 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 the internal standard. Mass spectra were recorded on VG Micromass 16F or 30F spectrometers, using the desorption chemical ionisation (DCI, NH_3) technique 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 services of the Dyson Perrins Laboratory. TLC was performed on glass plates coated with silica gel Blend 41 or on aluminum sheets pre-coated with Merck silica gel $60F_{254}$, and compounds were visualised with a spray of 5% v/v concentrated sulphuric acid in methanol or 5% dodecamolybdophosphoric acid in ethanol, or 5% w/v ninhydrin in ethanol. Flash chromatography was carried out using Merck Kieselgel 60, 230-400 mesh and dry column chromatography using Merck Kieselguhr 60H. 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. Solutions in organic solvents were dried with anhydrous sodium sulphate; solvents were removed under reduced pressure. The benzyl (14) and tert-butyl (15) carbamates of methyl 3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside were prepared as described in the preceding paper;[16] authentic samples of the natural products (1), (3), (5) and (7) were isolated at the Royal Botanic Gardens at Kew.[4,13,14]

N-Benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-mannitol (2). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (14) (98 mg, 0.25 mmol) was dissolved in 50% aqueous trifluoroacetic acid and stirred at room temperature for 10 min. The solvent was removed and the resulting syrup dissolved in ethanol (2 ml). A solution of sodium borohydride (20 mg, 0.5 mmol) in water (0.5 ml) was added, with stirring, at 0°C and the mixture was slowly allowed to warm to room temperature over 30 min. The unreacted sodium borohydride was destroyed by the addition of excess ammonium chloride and the solvent was removed. The solid residue was suspended in chloroform (10 ml), filtered and the chloroform was dried and evaporated to a syrup which was purified by flash chromatography (5% ethanol in chloroform) to give N-benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-mannitol, (77 mg, 81%), m.p 138-140°C, $[\alpha]_D^{20}$ -22.3° (c, 0.3 in MeOH); ν_{max} 3300, 1675 cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 5.2-3.8 (9H, m); 3.3-3.2 (3H, m, H-1,1',4); 2.9 (3H, br s, OH). m/z : 297 ($M + NH_4^+$-$PhCH_2OH$), 280 ($M + H^+$-$PhCH_2OH$, 100%).

Deoxymannojirimycin (1,5-Dideoxy-1,5-imino-D-mannitol) (1). N-Benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-mannitol (2) (66 mg, 0.17 mmol) was dissolved in ethanol (1 ml) and hydrogenated with a catalyst of 10% palladium hydroxide on charcoal (10 mg). The Z group was removed in a few minutes, but the removal of the benzyl ether was only complete after 36 h. The catalyst was filtered and the solvent evaporated to give a brown syrup which was purified by ion exchange chromatography (Sigma CG 120 H^+ form, elute with aqueous ammonia) to afford deoxymannojirimycin, (19 mg, 68%), an amorphous solid, m.p 185-187°C, $[\alpha]_D^{20}$ -27.1° (c, 0.14 in H_2O) [lit[18] 186°C, $[\alpha]_D^{20}$ -34.0° (c, 0.3 in MeOH)]; ^1H NMR (D_2O) δ a) free base: 3.81 (1H, m, H-2); 3.58 (2H, d, H-6,6'); 3.39 (2H, m, H-3,4); 2.81 (1H, dd, H-1, $J_{11'}$ 14.4 Hz, J_{12} 2.6 Hz); 2.57 (1H, dd, H-1', $J_{1'2}$ 1.4Hz); 2.29 (1H, m, H-5). b) hydrochloride salt: 3.97 (1H, m, H-2); 3.72 (1H, dd, H-6, $J_{66'}$ 12.3 Hz, J_{56} 3.2 Hz); 3.62 (1H, dd, H-6'); 3.58 (1H, t, H-4); 3.45 (1H, dd, H-3, J_{34} 9.5 Hz, J_{23} 3.0 Hz); 3.07 (1H, dd, H-1, $J_{11'}$ 13.9 Hz, J_{12} 2.9 Hz); 2.87 (1H, dd, H-1', $J_{1'2}$ 1.3 Hz); 2.72 (1H, m, H-5). m/z : 164 ($M + H^+$, 100%), 132 ($M + H^+$-MeOH), 110. This synthetic material was identical spectroscopically to an authentic sample.

Methyl N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-β-L-gulofuranoside (20). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (15) (0.82 g, 2.04 mmol) was dissolved in dry dichloromethane (8 ml) and stirred at room temperature with pyridinium chlorochromate (1.1 g, 2.5 equiv) and powdered molecular sieve (1 g). After 2 h, the solution was diluted with ether (10 ml) and filtered through a silica plug (eluted with ether). The ether was then evaporated to give the crude ketone (19) as a colourless syrup. To a solution of (19) in in ethanol (10 ml), sodium borohydride (75 mg, 1 molar equivalent) was added at 0°C and the reaction mixture was then maintained at this temperature for 1 h. Ammonium chloride (1 g, excess) was added and the solvent removed. The residue was partitioned between chloroform (20 ml) and water (20 ml). The chloroform layer was dried and evaporated to a syrup which was purified by flash chromatography (ethyl acetate : hexane, 1:3) to give methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-β-L-gulofuranoside, (0.63 g, 78%), as a colourless syrup, $[\alpha]_D^{20}$ +6.1° (c, 0.96 in $CHCl_3$); ν_{max} 3500 (sharp), 1690 cm^{-1}; ^1H NMR δ 7.5-7.3 (10H, m, ArH); 7.1 (1H, br d, OH); 5.21 (2H, ABq, $PhCH_2$); 5.01 (1H, s, H-1); 4.67 (2H, ABq, $PhCH_2$); 4.90-3.51 (6H, m); 3.39, 3.38 (3H, 2s, CH_3O). m/z (CI, NH_3) : 417 (M + NH_4^+), 400 (M + H^+, 100%). (Found C, 66.41; H, 6.45; N, 3.32. $C_{22}H_{25}NO_6$ requires C, 66.17; H, 6.27; N, 3.51).

N-Benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (4). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-β-L-gulofuranoside (20) (0.12 g, 0.31 mmol) was dissolved in 50% aqueous trifluoroacetic acid (5 ml) and left at room temperature for 30 min. The solvent was removed and the lactol dissolved in aqueous ethanol (1:4, 5 ml). Sodium borohydride (11 mg, 1 molar equivalent) was added and the solution stirred at room temperature for 15 min. The solvent was evaporated and the residue taken up into ethyl acetate (10 ml) and filtered. Evaporation and purification by flash chromatography (5% methanol in chloroform) gave N-benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-glucitol, (79 mg, 65%), a clear syrup, $[\alpha]_D^{20}$ -16.8° (c, 0.15 in MeOH); ν_{max} 3400, 1675 cm^{-1}; ^1H NMR δ 7.3-7.1 (10H, m, ArH); 4.7-4.4 (4H, m, $PhCH_2$); 4.3-3.1 (11H, m). m/z (CI, NH_3) : 388 (M + H^+), 280 (M + H^+-$PhCH_2OH$).

Deoxynojirimycin [1,5-Dideoxy-1,5-imino-D-glucitol] (3). N-Benzyloxycarbonyl-4-O-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (4) (75 mg, 0.19 mmol) was dissolved in glacial acetic acid (5 ml) and hydrogenated in the presence of palladium black (10 mg). After 48 h, the catalyst was removed by filtration and the solvent evaporated. Purification of the residue by ion exchange chromatography (Sigma CG 400 OH^- form, then CG 120 H^+ form eluted with aqueous ammonia) gave deoxynojirimycin, (30 mg, 100%), as the free base, which was crystallised as the hydrochloride salt (by neutralisation of an aqueous solution of (3) with dilute hydrochloric acid and subsequently freeze drying the neutral solution). Hydrochloride salt of (3), m.p 203°C (lit[28] 206°C); ^1H NMR (D_2O) δ a) free base: 3.66 (1H, dd, H-6, $J_{66'}$ 11.8 Hz, J_{56} 3.0 Hz); 3.47 (1H, dd, H-6', $J_{56'}$ 6.2 Hz); 3.34 (1H, ddd, H-2); 3.16 (1H, t); 3.08 (1H, t, H-3,4); 2.97 (1H, dd, H-1e, J_{1e1a} 12.3 Hz, J_{1e2} 5.1 Hz); 2.42 (1H, ddd, H-5); 2.32 (1H, dd, H-1a, J_{1a2} 10.9 Hz). b) hydrochloride salt: 3.9 (1H, dd, H-6); 3.8 (1H, dd, H-6'); 3.7 (1H, ddd, H-2); 3.4 (3H, m, H-1e,3,4); 3.05 (1H, ddd, H-5); 2.9 (1H, dd, H-1a). m/z (CI, NH_3) : 164 (M + H^+, 100%). This synthetic material was identical spectroscopically to an authentic sample.

N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-D-mannurono-1,4-lactone (21). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (14) (0.1 g, 0.25 mmol) was dissolved in 50% aqueous trifluoroacetic acid (3 ml) at room

temperature. After 15 min the solvent was removed and the crude syrup dissolved in dioxane : water (3:1, 4 ml), containing barium carbonate (0.12 g, 3 equiv). The suspension was cooled to 0°C and bromine (0.016 ml, 1.25 equiv) was added dropwise. The solution was slowly allowed to warm to room temperature and stirred for 24 h in the dark. Excess bromine was destroyed by dropwise addition of sodium thiosulphate solution (1M aq) until no red colour was visible. The precipitated sulphur was removed by centrifugation and the resulting clear solution diluted with ethyl acetate (10 ml). The phases were separated and the aqueous phase was extracted with further ethyl acetate (2 x 10 ml). The organic extracts were combined, dried and evaporated to a syrup which was purified by flash chromatography (ethyl acetate hexane, 1:1) to give N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-D-mannurono-1,4-lactone, (89 mg, 93%), a syrup, $[\alpha]_D^{20}$ -72.3° (c, 0.24 in $CHCl_3$); ν_{max} 3400 (br), 1770, 1700 cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 5.1-4.5 (7H, m); 3.9-3.7 (3H, m, H-2,6,6'); 3.2 (1H, br s, OH). m/z : 401 ($M + NH_4^+$), 340, 250. (Found C, 65.98; H, 5.73; N, 3.42. $C_{21}H_{21}NO_6$ requires C, 65.80; H, 5.48; N, 3.66%).

(2S,3R,4R,5R)-3,4,5-Trihydroxypipecolic acid (12). N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-D-mannurono-1,4-lactone (21) (62 mg, 0.16 mmol) was dissolved in acetic acid : water (2:1) (2 ml) and hydrogenated with palladium black (8 mg). After 48 h the catalyst was filtered and the solvent evaporated. Purification by ion exchange chromatography (Aldrich 50X 8-100, H^+ form, elute with aqueous pyridine) gave (2S,3R,4R,5R)-3,4,5-trihydroxypipecolic acid, (28 mg, 100%), a hygroscopic gum, $[\alpha]_D^{20}$ -13.8° (c, 0.21 in H_2O); ν_{max} 3500-3300, 1730, 1620 (w) cm^{-1}; ^1H NMR (D_2O) δ 4.00 (1H, dt, H-5); 3.84 (1H, t, H-3); 3.54 (1H, dd, H-4, J_{34} 8.9 Hz, J_{45} 3.1 Hz); 3.21 (1H, d, H-2, J_{23} 9.2 Hz); 3.15 (1H, dd, H-6, $J_{66'}$ 13.6 Hz, J_{56} 4.0 Hz); 2.93 (1H , dd, H-6', $J_{56'}$ 2.0 Hz). m/z : 178 ($M + H^+$, 100%), 160, 132, 124.

N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-L-gulono-1,4-lactone (22). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-β-L-gulofuranoside (20) (82 mg, 0.21 mmol) was converted into N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-L-gulono-1,4-lactone, (15 mg, 19%) by an identical procedure to that described above for the preparation of (21) from (14); ν_{max} 1790, 1700 cm^{-1}. ^1H NMR δ 7.4 (10H, m, ArH); 5.3-3.5 (11H, m). m/z : 401 ($M + NH_4^+$, 100%), 340.

(2S,3R,4R,5S)-3,4,5-Trihydroxypipecolic acid (7). N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-L-gulono-1,4-lactone (22) (9 mg, 0.023 mmol) was hydrogenated in 50% aqueous acetic acid (1 ml) in the presence of palladium black (5 mg). After 24 h, the catalyst was removed by filtration and the solvent evaporated. Purification of the residue by ion exchange chromatography (Aldrich 50X 8-100, H^+ form, elute with aqueous pyridine) gave (2S,3R,4R,5S) 3,4,5-trihydroxypipecolic acid (7), (3 mg, 72%), m.p 222-226°C (lit[14] 228-230°C); ^1H NMR (D_2O) δ 3.7 (1H, ddd, H-5); 3.55 (1H, dd, H-3); 3.3 (3H, m, H-2,4,6e); 2.9 (1H, dd, H-6a). m/z : 178 ($M + H^+$), 124 (100%). This synthetic material was identical spectroscopically to an authentic sample.

Methyl N-Benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-5-O-phenyloxythiocarbonyl-α-D-mannofuranoside (16). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (14) (0.27 g, 0.68 mmol) and 4-dimethylamino pyridine (0.22 g, 4 equivs) were dissolved in dry acetonitrile (3 ml) and stirred at room temperature, under nitrogen, with phenyl chlorothionocarbonate (0.08 ml, 1.2 equivs) for 24 h. The solution was washed with water (5 ml), dried and evaporated to a syrup which was purified by flash chromatography (ether : hexane, 1:3) to afford methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-5-O-

phenyloxythiocarbonyl-α-D-mannofuranoside, (0.34 g, 91%), a syrup, $[\alpha]_D^{20}$ +34.7° (c, 0.57 in $CHCl_3$); ν_{max} 1700 cm^{-1}; ^1H NMR δ 7.4-7.1 (15H, m, ArH); 5.68 (1H, m, H-5); 5.08 (1H, s, H-1); 5.20-4.20 (8H, m); 3.43 (3H, d, CH_3O); 3.23 (1H, m, H-6). m/z : 536 ($M + H^+$), 91 (100%).

Methyl N-Benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (23). (a) By hydride displacement of triflate. Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (14) (0.15 g, 0.38 mmol) was dissolved in dry dichloromethane (5 ml) containing pyridine (0.06 ml, 2 equiv) and stirred at -30°C under nitrogen with trifluoromethanesulphonic anhydride (0.076 ml, 1.1 equiv). After 1 h, the solution was washed with water (5 ml), dried and evaporated to a syrup containing the crude triflate (17) which was dissolved in THF (5 ml) and stirred at room temperature under nitrogen with lithium triethyl borohydride (1M solution in THF, 0.76 ml). After 6 h, t.l.c (ether : hexane, 1:1) showed loss of the triflate (R_f 0.9) and appearance of the deoxygenated material (R_f 0.5) together with baseline products. The solvent was evaporated and the residue dissolved in ethyl acetate : saturated aqueous sodium bicarbonate solution (5:2, 10 ml). An excess of benzyl chloroformate was added and the solution stirred vigorously for 30 min. The layers were separated and the organic layer was dried and evaporated to a syrup which was purified by flash chromatography to afford methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside, (0.112 g, 78%), a mobile oil, $[\alpha]_D^{20}$ +39.5° (c, 0.41 in $CHCl_3$); ν_{max} 1700 cm^{-1}; ^1H NMR δ 7.4-7.2 (10H, m, ArH); 5.15 (1H, s, H-1); 5.1-4.0 (6H, m); 3.3 (3H, d, CH_3O); 3.1 (1H, m, H-6'); 2.1-1.4 (4H, m). m/z : 384 ($M + H^+$), 250 ($M + NH_4^+ - CO_2 - PhCH_2O$, 100%). (b) By Barton deoxygenation. A solution of methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-5-O-phenyloxythiocarbonyl-α-D-mannofuranoside (16) (183mg, 0.33 mmol) in dry toluene (5 ml) with azobisisobutyronitrile (10.8 mg, 0.2 equiv) was degassed with nitrogen and then treated with tri-n-butyltin hydride (0.13 ml, 1.5 equiv). The solution was heated at 75°C for 24 h. The reaction mixture was cooled, washed with water (5 ml) and dried; removal of the solvent gave a residue which was purified by flash chromatography (ether : hexane, 1 : 4) to yield methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (23) (96 mg, 76% yield); this sample of (23) prepared by the Barton deoxygenation was contaminated with tin-containing residues.

N-Benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-arabino-hexitol (6). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (23) (99 mg, 0.28 mmol) was dissolved in 50% aqueous trifluoroacetic acid (3 ml). After 30 min the solvent was evaporated and the crude syrup dissolved in ethanol (5 ml). Sodium borohydride (10 mg) was added and the mixture stirred at room temperature for 1 h. The solvent was removed and the residue partitioned between water and chloroform. The chloroform was evaporated and the residue was purified by flash chromatography (ethyl acetate : hexane, 1:2) to give N-benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-arabino-hexitol, (55 mg, 58%), a syrup, $[\alpha]_D^{20}$ -46.1° (c, 0.17 in $CHCl_3$); ν_{max} 3400, 1670 cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 5.1 (2H, s, $PhCH_2$); 4.8 (2H, ABq, $PhCH_2$); 4.3 (1H, br m, H-3); 4.0-3.5 (6H, m); 2.9 (1H, br s, OH); 2.1 (1H, m, H-2); 1.5 (1H, br s, OH); 1.4 (1H, dd, H-2'). m/z : 372 ($M + H^+$), 328 ($M + H^+ - CO_2$), 264 (100%).

Fagomine [1,5-imino-1,2,5-trideoxy-D-arabino-hexitol] (5). N-Benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-arabino-hexitol (6) (25 mg, 0.067 mmol) was dissolved in ethanol (5 ml) and stirred under hydrogen with 10% palladium hydroxide on charcoal (5 mg) at atmospheric pressure. After 12 h the catalyst was filtered and

the solvent evaporated. Purification by ion exchange chromatography (Sigma CG 120, H^+ form, elute with aqueous ammonia) afforded fagomine (5) (8 mg, 74%) as the free base, m.p 178-182°C, $[\alpha]_D^{20}$ +21.6° (c, 0.36 in H_2O) [lit[12,13] m.p. 180-184°C), $[\alpha]_D^{20}$ +24.7° (c, 0.4 in H_2O)]; ^1H NMR (D_2O) δ 3.68 (1H, dd, H-6, J_{66}, 11.6 Hz, J_{56} 2.9 Hz); 3.46 (1H, dd, H-6', J_{56}, 6.6 Hz); 3.37 (1H, m, H-3); 2.99 (1H, t, H-4); 2.83 (1H, ddd, H-1, J_{11}, 12.8 Hz); 2.45 (1H, ddd, H-1'); 2.4 (1H, m, H-5); 1.81 (1H, ddd, H-2); 1.28 (1H, ddd, H-2'). m/z : 148 ($M + NH_4^+$, 100%), 130, 116. This synthetic material was identical spectroscopically to an authentic sample.

Methyl 5-Azido-N-Benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (27) and Methyl 5-Azido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (24). Methyl N-benzyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (14) (1.6 g, 4.01 mmol) was dissolved in dichloromethane (20 ml) containing pyridine (0.68 ml, 2 equivs) and stirred at -30°C under nitrogen, with trifluoromethanesulphonic anhydride (0.94 ml, 1.4 equivs). After 30 min the solution was washed with water (20 ml) and evaporated to a syrup containing the crude triflate (17). This was not purified but dissolved in DMF (20 ml) and stirred at 60°C with sodium azide (0.3 g, 1.15 equiv). After 24 h, the solvent was removed and the residue partitioned between chloroform (25 ml) and water (25 ml). The organic layer was dried and evaporated. Purification of the residue by flash chromatography (ether : hexane, 1:4) gave three major products. The first was the azide with retention of configuration: methyl 5-azido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (27), (0.39 g, 23%), a syrup, ν_{max} 2100, 1700 cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 5.2-3.5 (11H, m); 3.4 (3H, s, CH_3O). m/z (FD) : 424 (M^+). The second product was a product produced from elimination of the triflate (17) (170 mg, 11%); ν_{max} 1700 (s), 1630 (w) cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 7.1, 6.9 (2H, 2d, H-5,6); 5.1-4.1 (8H, m); 3.3 (3H, s, CH_3O). m/z : 382 ($M + H^+$, 100%), 338. The third product eluted was the inverted azide: methyl 5-azido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (24), (0.6 g, 36%), a syrup, $[\alpha]_D^{20}$ +5.9° (c, 0.68 in $CHCl_3$); ν_{max} 2105, 1700, 1450, 1420 cm^{-1}; ^1H NMR δ 7.37-7.29 (10H, m, ArH); 5.2-3.8 (9H, m); 4.88, 4.82 (1H, 2s, H-1); 3.74, 3.65 (1H, 2m, H-5); 3.37, 3.32 (3H, 2s, CH_3O). m/z : 442 ($M + NH_4^+$), 397 (100%). (Found C, 61.97; H, 5.60; N, 12.97. $C_{22}H_{24}N_4O_5$ requires C, 62.26; H, 5.66; N, 13.21%).

Methyl 5-Acetamido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (25). Powdered tellurium (0.31 g, 2.5 equivs) and sodium borohydride (0.22 g, 6 equivs) were refluxed in ethanol (20 ml) under an atmosphere of nitrogen until all the tellurium had dissolved and a cherry red solution was obtained (2 h). The solution was cooled to room temperature and a solution of methyl 5-azido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (24) (0.42 g, 0.98 mmol) in ether (20 ml) was injected slowly with stirring. The solution immediately lost its red colour and tellurium was precipitated with evolution of nitrogen. After 10 min, the reaction vessel was opened to the atmosphere to destroy excess reagent and then the solution was filtered through celite. The solvent was evaporated and the crude amine dissolved in pyridine (8 ml). Acetic anhydride (0.46 ml, 5 equivs) was added to the resulting solution and the reaction mixture stirred at room temperature for 2 h. The solvent was evaporated and purification of the residue by flash chromatography (ethyl acetate : hexane, 3:1) gave methyl 5-acetamido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside, (0.4 g, 92%), a syrup, $[\alpha]_D^{20}$ +88.0° (c, 0.41 in $CHCl_3$); ν_{max} 3410 (sharp), 1700, 1680, 1510, 1425 cm^{-1}; ^1H NMR δ 7.4-7.3 (10H, m, ArH); 7.0 (1H, m, NH); 5.1 (2H, ABq, $PhCH_2$); 5.05 (1H, s, H-1); 4.7 (2H. ABq, $PhCH_2$); 5.1-3.7 (6H, m);

3.4 (3H, s, CH_3O); 1.8 (3H, s, CH_3CO). m/z : 441 ($M + H^+$, 100%). (Found C, 65.41; H, 6.37; N, 6.42. $C_{24}H_{28}N_2O_6$ requires C, 65.45; N, 6.36; N, 6.36%).

2-Acetamido-N-benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol (9). Methyl 5-acetamido-N-benzyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (25) (0.39 g, 0.89 mmol) was dissolved in 50% aqueous trifluoroacetic acid (4 ml) and stirred at room temperature for 15 min. The solvent was removed and the resulting syrup dissolved in ethanol (4 ml). A solution of sodium borohydride (33 mg, 1 molar equivalent) in water (1 ml) was added and the mixture stirred at room temperature for 10 min. Ammonium chloride (0.5 g) was added, the solvent evaporated and the residue extracted into ethyl acetate (3 x 20 ml). The organic extracts were filtered, combined, dried and evaporated to a syrup which was purified by flash chromatography (2% ethanol in ethyl acetate) to give 2-acetamido-N-benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol, (0.31 g, 80%), a syrup, $[\alpha]_D^{20}$ +9.6° (c, 0.23 in $CHCl_3$); ν_{max} 3400 (br), 1670, 1530 cm^{-1}; ^1H NMR δ 7.5-7.4 (10H, m, ArH); 6.8 (1H, br d, NH); 5.2-3.6 (12H, m); 2.9 (2H, br s, OH); 1.4 (3H, s, CH_3CO). m/z : 429 ($M + H^+$), 338 ($M + NH_4^+$-$PhCH_2OH$), 321 ($M + H^+$-$PhCH_2OH$, 100%). (Found C, 64.2; H, 6.7; N, 6.9. $C_{23}H_{28}N_2O_6$ requires C, 64.5; H, 6.5; N, 6.5%).

2-Acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (8). 2-Acetamido-N-benzyloxycarbonyl-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-glucitol (9) (0.31 g, 0.71 mmol) was dissolved in acetic acid (5 ml) and stirred under an atmosphere of hydrogen with palladium black (40 mg). After 24 h the catalyst was removed by filtration and the solvent evaporated. Purification of the residue by ion exchange chromatography (Sigma CG 400 OH^- form, then Aldrich 50X 8-100, H^+ form, eluted with aqueous ammonia) gave 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol, (0.15 g, 100%), a colourless solid, m.p 227-228°C (decomp), $[\alpha]_D^{20}$ +35.0° (c, 0.3 in H_2O); ν_{max} (KBr) 3400 (br), 3280 (sharp), 1640, 1560 cm^{-1}; ^1H NMR (D_2O) δ 3.64 (1H, dd, H-6, $J_{66'}$ 11.7 Hz, J_{56} 3.0 Hz); 3.55 (1H, dt, H-2); 3.47 (1H, dd, H-6', $J_{56'}$ 6.0 Hz); 3.21 (1H, t); 3.21 (1H, t, H-3,4); 2.88 (1H, dd, H-1e, J_{1e1a} 12.6 Hz, J_{1e2} 4.9 Hz); 2.37 (1H, ddd, H-5, J_{45} 9.3 Hz); 2.25 (1H, dd, H-1a, J_{1a2} 11.5 Hz); 1.82 (3H, s, CH_3CO). ^{13}C NMR (D_2O) δ 22.77 (q, CH_3); 47.64 (t, C-1); 52.88 (d, C-5); 61.15 (d, C-2); 61.95 (t, C-6); 72.68 (d), 76.54 (d, C-3,4); 170 (s, $CH_3\underline{C}O$). m/z : 205 ($M + H^+$, 100%), 145. (Found C, 47.1; H, 8.0; N, 13.4. $C_8H_{16}N_2O_4$ requires C, 47.1; H, 7.8; N, 13.7%).

Methyl 5-Azido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (28) and Methyl 5-Azido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (26). Methyl N-tert-butyloxycarbonyl-3-O-benzyl-2,6-dideoxy-2,6-imino-α-D-mannofuranoside (15) (0.38 g, 1.04 mmol) was dissolved in dichloromethane (10 ml) containing pyridine (0.17 ml, 2 equiv) and stirred at -30°C under nitrogen with trifluoromethanesulphonic anhydride (0.22 ml, 1.2 equiv). After 30 min the solution was washed with water (10 ml) and concentrated to a syrup containing the triflate (18). The triflate was not purified but dissolved in DMF (10 ml) and stirred at 70°C with sodium azide (0.1 g, 1.5 equivs). After 48 h the solvent was removed and the residue partitioned betweeen chloroform (20 ml) and water (20 ml). The chloroform layer was dried and evaporated to a syrup. Purification by flash chromatography (ether : hexane, 1:4) gave two azide containing products. The first product eluted was the azide with retention of configuration: methyl 5-azido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (28), (0.17 g, 42%), a syrup, $[\alpha]_D^{20}$ +44.7° (c, 1.33 in $CHCl_3$); ν_{max} 2095, 1695 cm^{-1}; ^1H NMR δ 7.4-7.3 (5H, m, ArH); 5.0 (1H, d, H-1); 4.9-4.3 (6H, m);

3.9 (1H, m, H-5); 3.4 (3H, s, CH_3O); 3.0 (1H, m, H-6); 1.5 (9H, d, tBu). m/z : 408 (M + NH_4^+), 391 (M +H^+, 100%). The second product was the azide with inversion of configuration: methyl 5-azido-N-tert-butyloxycarbonyl-5-azido-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-L-gulofuranoside (26), (35 mg, 15%); ν_{max} 2110, 1690 cm^{-1}; ^1H NMR δ 7.4-7.3 (5H, m, ArH); 4.9-4.2 (6H, m); 3.4 (3H, s, CH_3O); 3.3-3.2 (3H, m); 1.5 (9H, d, tBu). m/z : 363 (M + H^+-N_2), 356 (M + NH_4^+-butene), 248 (100%).

Methyl 5-Acetamido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (29). Methyl 5-azido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (28) (0.18 g, 0.46 mmol) was hydrogenated at atmospheric pressure in the presence of palladium black (20 mg) in ethanol (5 ml). After 90 min the catalyst was removed by filtration and the solvent evaporated. The resulting syrup was dissolved in pyridine (2 ml) and stirred at room temperature with acetic anhydride (0.1 ml, 2.3 equivs). After 2 h the solvent was evaporated and the residue purified by flash chromatography (ethyl acetate : hexane, 2:1) to give methyl 5-acetamido-N-tert-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside, (0.18 g, 96%), m.p 109-110°C, $[\alpha]_D^{20}$ +15.9° (c, 1.07 in $CHCl_3$). ν_{max} 3400 (br), 1690, 1650, 1540 cm^{-1}. ^1H NMR δ 7.4-7.3 (5H, m, ArH); 5.7 (1H, br s, NH); 4.95 (1H, s, H-1); 4.9-4.2 (7H, m); 3.4 (3H, s, CH_3O); 2.8 (1H, m, H-6); 2.0 (3H, s, CH_3CO); 1.5 (9H, d, tBu). m/z : 351 (M + H^+-butene, 100%), 307 (Found C, 61.7; H, 7.6; N, 6.54. $C_{21}H_{30}N_2O_6$ requires C, 62.07; H, 7.4; N, 6.90%).

2-Acetamido-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-mannitol (11). Methyl 5-acetamido-N-t-butyloxycarbonyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-α-D-mannofuranoside (29) (58 mg, 0.14 mmol) was dissolved in 50% aqueous trifluoroacetic acid (2 ml). After 5 min, the solvent was removed and the crude syrup dissolved in ethanol (2 ml). A solution of sodium borohydride (14 mg, 0.38 mmol) in water (0.5 ml) was added at 0°C and the solution was stirred for 15 min. Ammonium chloride (0.1 g) was added, the solvent was evaporated and the residue was taken up into 50% ethanol/chloroform (10 ml) and filtered. The solvent was removed and the residue purified by flash chromatography (ethanol : chloroform, 1:1) to afford 2-acetamido-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-mannitol, (40 mg, 97%), a syrup, $[\alpha]_D^{20}$ +11.0° (c, 0.37 in MeOH); ^1H NMR (D_2O) δ 7.4-7.3 (5H, m, ArH); 4.7 (2H, m, $PhCH_2$); 4.3-3.8 (5H, m); 3.3-3.1 (3H, m, H-1,1',5); 2.0 (3H, s, CH_3CO). m/z : 295 (M + H^+).

2-Acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol (10). 2-Acetamido-4-O-benzyl-1,5-imino-1,2,5-trideoxy-D-mannitol (11) (32 mg, 0.11 mmol) was hydrogenated in ethanol (10 ml) in the presence of 10% palladium hydroxide on charcoal (10 mg). After 24 h, the catalyst was removed by filtration and the solvent evaporated. Purification of the residue by ion exchange chromatography (Sigma CG 400, OH^- form, then CG 120, H^+ form, elute with aqueous ammonia) produced 2-acetamido-1,5-imino-1,2,5-trideoxy-D-mannitol, (22 mg, 100%), m.p 203-207°C, $[\alpha]_D^{20}$ -53.3° (c, 0.12 in H_2O). ν_{max} (KBr) 3450, 3350, 3300, 3150 (br), 1660, 1550 cm^{-1}; ^1H NMR (D_2O) δ 4.05 (1H, dt, H-2, $J_{12}=J_{1'2}$ 2.4 Hz, J_{23} 4.7 Hz); 3.64 (1H, dd, H-6, $J_{66'}$ 11.7 Hz, J_{56} 3.0 Hz); 3.55 (1H, dd, H-3, J_{34} 9.7 Hz); 3.54 (1H, dd, H-6', $J_{56'}$ 5.9 Hz); 3.29 (1H, t, H-4); 2.84 (1H, dd, H-1, $J_{11'}$ 13.7 Hz); 2.63 (1H, dd, H-1'); 2.36 (1H, ddd, H-5); 1.9 (3H, s, CH_3CO). ^{13}C NMR (D_2O) δ 22.08 (q, $\underline{C}H_3CO$); 46.52 (t, C-1); 50.54 (d, C-5); 60.74 (d, C-2); 61.01 (t, C-6); 68.70, 73.03 (2d, C-3,4); 174.65 (s, $CH_3\underline{C}O$). m/z : 217 (impurity), 205 (M + H^+, 100%). (Found C, 47.2; H, 8.1; N, 13.0. $C_8H_{16}N_2O_4$ requires C, 47.1; H, 7.8; N, 13.7%).

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