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POLYHYDROXYLATED PYRROLIDINES FROM SUGAR LACTONES: SYNTHESIS OF 1,4-DIDEOXY-1,4-IMINO-D-GLUCITOL FROM D-GALACTONOLACTONE AND SYNTHESES OF 1,4-DIDEOXY-1,4-IMINO-D-ALLITOL, 1,4-DIDEOXY-1,4-IMINO-D-RIBITOL, AND (2S,3R,4S)-3,4-DIHYDROXYPROLINE FROM D-GULONOLACTONE

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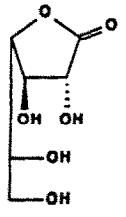
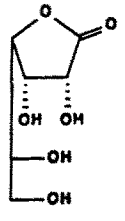
The use of readily available sugar lactones in the synthesis of polyhydroxylated pyrrolidines is illustrated by the preparation of the glucosidase inhibitor 1,4-dideoxy-1,4-imino-D-glucitol from D-galactonolactone and by the conversion of D-gulonolactone into 1,4-dideoxy-1,4-imino-D-allitol, 1,4-dideoxy-1,4-imino-D-ribitol, and (2S,3R,4S)-3,4-dihydroxyproline.

Polyhydroxylated pyrrolidines, piperidines and octahydroindolizines provide an extensive class of powerful and specific glycosidase inhibitors.[1] Castanospermine, deoxynojirimycin and dihydroxymethyldihydroxypyrrolidine have been demonstrated to inhibit human immunodeficiency virus synctium formation and virus replication[2] and such compounds may have potential as antiretroviral agents.[3] Also swainsonine[4] and simple open chain hydroxylated pyrrolidines[5] have potential as agents for stimulation of the immune response. The effects of these compounds may arise from their properties as specific glycosidase inhibitors. 1,4-Dideoxy-1,4-imino-D-mannitol, the azafuranose analogue of mannose, is a powerful α-mannosidase inhibitor[6,7] and 1,4-dideoxy-1,4-imino-D-glucitol (3) is a glucosidase inhibitor.[8] Several other hydroxylated pyrrolidines are glycosidase inhibitors[9] and for these and other reasons there is considerable interest in the synthesis of polyhydroxylated pyrrolidines.[10] Carbohydrates are an attractive group of starting materials for the synthesis of such highly functionalised compounds and although other strategies have been used,[11] the commonest method for the synthesis of hydroxylated pyrrolidines has been the joining together of C-1 and C-4 of a sugar. It is clearly desirable to devise short sequences for the synthesis of such compounds,[12] and readily available sugar lactones (in which C-1 and C-4 are protected as the lactone function) provide suitable starting materials for short sequences for the preparation of highly functionalised chiral pyrrolidines. Several such lactones are commercially available[13] and this paper describes the use of D-galactonolactone (1) in the synthesis of 1,4-dideoxy-1,4-imino-D-glucitol (3), and of D-gulonolactone (2) in the syntheses of 1,4-dideoxy-1,4-imino-D-allitol (4), 1,4-dideoxy-1,4-imino-D-ribitol (5), and (2S,3R,4S)-3,4-dihydroxyproline (6); a synthesis of (3) from glucitol has been reported.[8]



(1)



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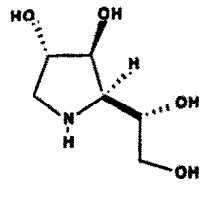

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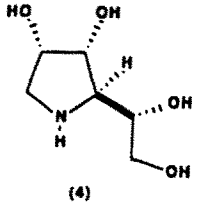

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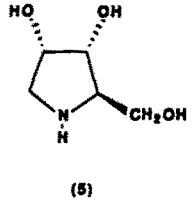

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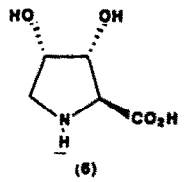

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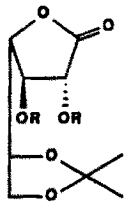
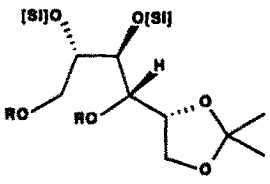
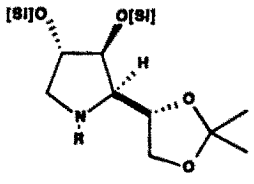


(7) R = H
(8) R = [Si]

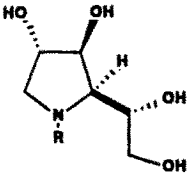
[Si] = Si^tBuMe_2



(9) R = H
(10) R = SO_2Me



(11) R = CH_2Ph
(12) R = H

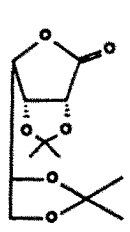


(3) R = H
(13) R = CH_2Ph

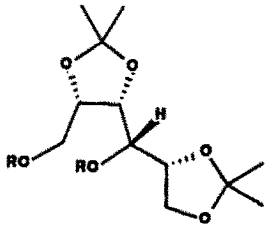
For the preparation of 1,4-dideoxy-1,4-imino-D-glucitol (3), D-galactonolactone (1) was converted into the acetonide (7) as previously described[14] in quantitative yield; subsequent treatment of (7) with excess tert-butyldimethylsilyl chloride gave the fully protected lactone (8) [79% yield from (1)]. Reduction of (8) with lithium aluminum hydride gave a mixture of products arising from migration of the silyl protecting groups during the reduction; however, reaction of (8) with lithium borohydride in tetrahydrofuran cleanly gave the diol (9) [96% yield]. Esterification of the hydroxyl groups in (9) with methanesulphonyl chloride gave the dimesylate (10) [53% yield] which with benzylamine underwent a highly efficient cyclisation to give the protected pyrrolidine (11) [92% yield]. Hydrogenolysis of the benzyl group in (11) catalysed by 10% palladium on charcoal gave (12) [86% yield]; hydrolysis of (12) with aqueous trifluoroacetic acid gave 1,4-dideoxy-1,4-imino-D-glucitol (3) [94% yield] with spectroscopic properties consistent with those previously reported; the synthesis of (3) from D-glucitol required two inversion of the configuration at C-4.[8] The overall yield from D-galactonolactone in this seven step synthesis of the α-glucosidase inhibitor (3) is 30%. Removal of the silyl and isopropylidene protecting groups from the fully protected pyrrolidine by acid hydrolysis gave the N-benzyliminoglucitol (13) in 80% yield.

1,4-Dideoxy-1,4-imino-D-allitol (4) was prepared in six steps from D-gulonolactone (2)[15] in an overall yield of 48%. Treatment of D-gulonolactone with acetone and dimethoxypropane as previously described[16] gave the di-O-isopropylidene derivative (14) [85% yield] which on reduction by lithium aluminum hydride afforded the diol (15) in 87% yield. The dimesylate (16), formed in quantitative yield by the esterification of the hydroxyl groups in (15) with methanesulphonyl chloride, underwent an efficient cyclisation with benzylamine to give the protected pyrrolidine (17) [77% yield]. Hydrogenolytic removal of the benzyl group in (17) gave (18) [94% yield] and subsequent hydrolysis of (18) with aqueous trifluoroacetic acid gave 1,4-dideoxy-1,4-imino-D-allitol (4) [96% yield]. Treatment of the fully protected N-benzylpyrrolidine (17) with aqueous trifluoroacetic acid caused loss of both isopropylidene protecting groups to give (19) [96% yield]; however, hydrolysis of (17) with 80% aqueous acetic acid resulted in selective hydrolysis of the sidechain acetonide to give the diol (20) in 93% yield. Periodate oxidation of the diol (20), followed by reduction of the resulting aldehyde with sodium borohydride, afforded the imino-D-ribitol derivative (23) in 71% yield. Hydrolysis of the acetonide gave the N-benzylamine (25) [86% yield], whereas hydrogenation of (23) to remove the benzyl protecting group followed by acid hydrolysis gave 1,4-dideoxy-1,4-imino-D-ribitol (5) in 78% yield. The spectroscopic properties and other analytical data of the D-ribitol derivatives (5), (23) and (25) were identical, save for the specific rotation, to those found for the corresponding L-ribitol compounds reported in the accompanying paper.[7] Also, removal of the N-benzyl group in (20) by

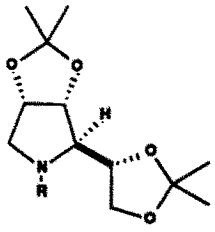
hydrogenolysis gave (21) which on treatment with di-tert-butyl dicarbonate gave (22); periodate cleavage of the the diol in (22) followed by oxidation of the resulting aldehyde by sodium chlorite in the presence of cyclohexene led to the formation of the protected proline (24). Reaction of (24) with aqueous trifluoroacetic acid caused removal of both the tert-butyloxycarbonyl and isopropylidene protecting groups to give (2S,3R,4S)-3,4-dihydroxyproline (6); the spectral properties of (6) - other than specific rotation - were identical to those of an authentic sample of the enantiomer (2R,3S,4R)-3,4-dihydroxyproline,[17] the structure of which has been established by X-ray crystallography.[18] These transformations firmly establish the structures of the hydroxylated pyrroldines described in this and the accompanying paper, confirming that the cyclisation to the pyrrolidines occurs with inversion of configuration at C-4 of the sugar.



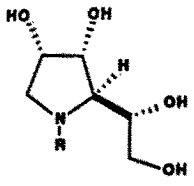
(14)



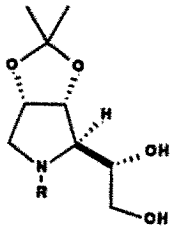
(15) R = H
(16) R = SO_2Me



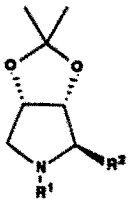
(17) R = CH_2Ph
(18) R = H



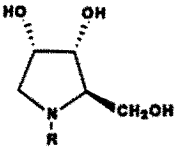
(4) R = H
(19) R = CH_2Ph



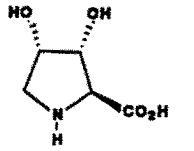
(20) R = CH_2Ph
(21) R = H
(22) R = $CO_2{}^tBu$



(23) R^1 = CH_2Ph R^2 = CH_2OH
(24) R^1 = $CO_2{}^tBu$ R^2 = CO_2H



(5) R = H
(25) R = CH_2Ph



(6)

This paper illustrates the potential of commercially available sugar lactones as starting materials for the synthesis of a wide range of polyhydroxlyated pyrrolidines; it is noteworthy that both D-gulonolactone (2) and L-gulonolactone are readily available. The biological properties of these hydroxylated pyrrolidines, including evaluation of their effects on the AIDS virus,[19] will be reported elsewhere.[20]

Experimental

M.p.s were recorded on a Kofler block. Infra red spectra were recorded on a Perkin-Elmer 297 spectrophotometer. ^1H NMR spectra were run at 300 MHz on a Bruker WH 300 spectrometer (500 MHz on a Bruker AM 500 spectrometer); ^{13}C NMR spectra were recorded on a Bruker AM 250 (at 62.9 MHz) or on a Bruker AM 500 (at 125 MHz) spectrometer. For ^{13}C NMR spectra in D_2O, 1,4-dioxane (δ 67.6) was used as the internal standard. Mass spectra were recorded on VG Micromass ZAB 1F or MM 30F spectrometers; in order to obtain satisfactory mass spectra for the majority of compounds reported in this paper it was necessary to use ACE, DCI or FAB techniques. Microanalyses were performed by the microanalytical services of the Dyson Perrins Laboratory. TLC was performed on glass plates coated with silia gel Blend 41, and compounds were visualised with a spray of 5% v/v sulphuric acid in methanol or a

solution of 5% dodecamolybdophosphoric acid in methanol. Flash chromatography was carried out using Merck Kieselgel 60, 230-400 mesh. Unless otherwise stated, all organic solvents were dried before use; tetrahydrofuran was distilled from a solution dried with sodium in the presence of benzophenone under dry nitrogen. D-Galactonolactone and D-gulonolactone were obtained from the Sigma Chemical Company and were used without purification.

5,6-O-Isopropylidene-D-galactonolactone (7) was prepared by modification of the previously described procedure.[14] D-Galactonolactone (1) (5.92 g, 33.3 mmol) was suspended in a mixture of acetone (135 ml) and 2,2-dimethoxypropane (15 ml); the reaction mixture was acidified to pH 3 with p-toluenesulphonic acid, stirred at room temperature for 2 hours and then neutralised by addition of anhydrous sodium carbonate and filtered. The filtrate was evaporated to give an oil which was dissolved in ethyl acetate (100 ml), washed with saturated aqueous sodium bicarbonate (100 ml), dried (magnesium sulphate) filtered and the solvent removed to give, after purification by flash chromatography (3:1, ethyl acetate:hexane), 5,6-O-isopropylidene-D-galactonolactone (7), (7.1 g, 100%), syrup, $[\alpha]_D^{20}$ -47.7° (c, 2.14 in Me_2CO) [lit.[14] $[\alpha]_D^{20}$ -42° (c, 2.0 in Me_2CO)]. ν_{max} (neat) 3600-3200 (OH), 1780 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.41 (3H, s, Me), 1.44 (3H, s, Me), 3.03 (1H, br, OH), 3.48 (1H, br, OH), 4.02 (1H, dd, J 6.8 and 8.7 Hz), 4.16 (1H, dd, J 6.8 and 8.7 Hz), 4.22 (1H, dd, J 3.3 and 6.9 Hz), 4.39 (1H, dt, J 3.2 Hz), 4.43 (2H, m).

2,3-Bis-(O-tert-butyldimethylsilyl)-5,6-O-isopropylidene-D-galactonolactone (8). A solution of the acetonide of D-galactonolactone (7) (2.50 g, 11.5 mmol) in dimethylformamide (10 ml) was added to a stirred solution of tert-butyldimethylsilyl chloride (5.97 g, 39.6 mmol) and imidazole (4.23 g, 62.2 mmol) in dimethylformamide (20 ml) under nitrogen at room temperature. After 2 days, the solvent was removed and the residue dissolved in chloroform (100 ml). The solution was washed with water (2 x 100 ml), dried (magnesium sulphate) and the solvent removed to give, after purification by flash chromatography (4:1, hexane:ethyl acetate), 2,3-bis-(O-tert-butyldimethylsilyl)-5,6-O-isopropylidene-D-galactonolactone (8), (8.1 g, 79%), m.p. 131°-132°C, $[\alpha]_D^{20}$ -7.0° (c, 0.95 in $CHCl_3$). ν_{max} (KBr) 1800 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.14 (3H, s, SiMe), 0.16 (6H, s, 2 x SiMe), 0.22 (3H, s, Si Me), 0.91 (9H, s, t-Bu), 0.95 (9H, s, t-Bu), 1.38 (3H, s, Me), 1.41 (3H, s, Me), 3.97 (2H, m), 4.09 (1H, dd, J 6.6 and 8.0 Hz), 4.26 (1H, m), 4.37 (2H, m). m/z (ACE, NH_3): 464 ($M+NH_4^+$, 87%), 447 ($M+H^+$, 67%). (Found C, 56.50; H, 9.42. $C_{21}H_{42}O_6Si_2$ requires C, 56.31; H, 9.37%).

2,3-Bis-(O-tert-butyldimethylsilyl)-5,6-O-isopropylidene-D-galactitol (9). A solution of lithium borohydride in tetrahydrofuran (2.0 M, 8.0 ml) was added to a solution of the silylated lactone (8) (2.00 g, 4.48 mmol) in tetrahydrofuran (10 ml) under nitrogen at room temperature. After 6 h, the excess hydride was destroyed by addtion of saturated aqueous ammonium chloride (10 ml) and the reaction mixture was partitioned between brine (60 ml) and ethyl acetate (100 ml). The organic layer was dried (magnesium sulphate), filtered and the solvent removed in vacuo to give a syrup which was purified by flash chromatography (5:1, hexane: ethyl acetate) to afford 2,3-bis-(O-tert-butyldimethylsilyl)-5,6-O-isopropylidene-D-galactitol (9), (1.94 g, 96%), a colourless oil, $[\alpha]_D^{20}$ -34.3° (c, 1.94 in $CHCl_3$) . ν_{max} (neat) 3600-3200 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.13 (3H, s, SiMe), 0.14 (3H, s, SiMe), 0.15 (3H, s, SiMe), 0.17 (3H, s, SiMe), 0.89 (9H, s, t-Bu), 0.92 (9H, s, t-Bu), 1.36 (3H, s, Me), 1.42 (3H, s, Me), 3.65 (2H, m), 3.87 (3H, m), 4.02 (2H, m), 4.23 (1H, dt, J 2.3 Hz). m/z (ACE, NH_3): 451 ($M+H^+$, 100%), 393 (53%).

2,3-Bis-(O-tert-butyldimethylsilyl)-1,4-bis-O-(methanesulphonyl)-5,6-O-isopropylidene-D-galactitol (10). Methanesulphonyl chloride (2.5 ml, 32.0 mmol) and a catalytic amount of 4,4-dimethylaminopyridine (0.20 g) were added to a stirred solution of the diol (9) (3.60 g, 8.0 mmol) in pyridine (20 ml) and the reaction mixture stirred for 2 h at 0°C. The pyridine was evaporated to give a residue which was dissolved in chloroform (100 ml); the chloroform solution was washed with water, dried (magnesium sulphate) and the solvent removed to give an oil which was purified by flash chromatography (1:2, ethyl acetate:hexane) to afford 2,3-bis-(O-tert-butyldimethylsilyl)-1,4-bis-(O-methanesulphonyl)-5,6-O-isopropylidene-D-galactitol (10), oil, (2.58 g, 53% yield), $[\alpha]_D^{20}$ -24.8° (c, 0.77 in $CHCl_3$). ν_{max} (KBr) 1350 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.12 (3H, s, SiMe), 0.14 (3H, s, SiMe), 0.16 (3H, s, SiMe), 0.19 (3H, s, Si Me), 0.93 (9H, s, t-Bu), 0.94 (9H, s, t-Bu), 1.34 (3H, s, Me), 1.47 (3H, s, Me), 3.00 (3H, s, $MeSO_2$), 3.13 (3H, s, $MeSO_2$), 3.74 (2H, m), 4.05 (1H, m), 4.11 (1H, dd, J 6.4 and 8.7 Hz), 4.32 (1H, dd, J 8.1 and 10.5 Hz), 4.60 (2H, m), 4.71 (1H, dd, J 2.6 and 9.1 Hz). m/z (ACE, NH_3): 624 ($M+NH_4^+$, 50%), 101 (100%).

N-Benzyl-2,3-bis-(O-tert-butyldimethylsilyl)-1,4-dideoxy-5,6-O-isopropylidene-1,4-imino-D-glucitol (11). The silylated dimesylate (10) (2.58 g, 4.3 mmol) in benzylamine (10 ml) was warmed at 80°-90°C for 60 h. The reaction mixture was then partitioned between brine (50 ml) and chloroform (100 ml). The organic layer was washed with water (100 ml), dried (magnesium sulphate) and the solvent removed to give, after purification by flash chromatography (20:1, hexane:ethyl acetate), N-benzyl-2,3-bis-(O-tert-butyldimethylsilyl)-1,4-dideoxy-5,6-O-isopropylidene-1,4-imino-D-glucitol (11), m.p. 69°-70°C, (2.05 g, 92% yield), $[\alpha]_D^{20}$ +6.4° (c, 0.87 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ 0.02 (3H, s, SiMe), 0.06 (3H, s, SiMe), 0.10 (3H, s, SiMe), 0.11 (3H, s, Si Me), 0.90 (9H, s, t-Bu), 0.91 (9H, s, t-Bu), 1.35 (3H, s, Me), 1.39 (3H, s, Me), 2.31 (1H, dd, H-1, $J_{1,1'}$ 11.0 Hz, $J_{1,2}$ 3.2 Hz), 3.06 (1H, dd, H-1', $J_{1',2}$ 4.0 Hz), 3.46 (1H, dd, H-4, $J_{3,4}$ 5.8 Hz, $J_{4,5}$ 3.3 Hz), 3.68 (1H of AB, $PhCH_2$, J_{AB} 13.6 Hz), 3.85 (1H, dd, H-2, $J_{2,3}$ 2.9 Hz), 4.03 (1H, dd, H-6, $J_{6,6'}$ 7.5 Hz, $J_{5,6}$ 6.7 Hz), 4.10 (1H, dd, H-3, $J_{3,4}$ 5.8 Hz), 4.19 (1H, dd, H-6', $J_{5,6'}$ 7.5 Hz), 4.26 (1H of AB, $PhCH_2$), 4.36 (1H, dt, H-5), 7.29 (5H, m, ArH). ^{13}C NMR ($CDCl_3$) δ -4.09 (q), -4.59 (q), -4.55 (q), 18.82 (s), 18.83 (s), 24.80 (q), 25.84 (q), 25.88 (q), 26.51 (q), 58.50 (t), 62.39 (t), 66.54 (t), 66.89 (d), 77.00 (d), 77.76 (d), 79.62 (d), 107.42 (s), 126.55 (d), 128.18 (d), 128.43 (d), 140.90 (s). m/z (DCI, NH_3): 522 ($M+H^+$, 40%), 420 (100%). (Found C, 64.65; H, 9.88; N, 2.63. $C_{28}H_{51}NO_4Si_2$ requires C, 64.49; H, 9.79%; N, 2.69).

2,3-Bis-(O-tert-butyldimethylsilyl)-1,4-dideoxy-5,6-O-isopropylidene-1,4-imino-D-glucitol (12). The tertiary benzylamine (11) (550 mg, 1.06 mmol) in ethanol (20 ml) was stirred under an atmosphere of hydrogen in the presence of 10% palladium on charcoal (300 mg) at room temperature for 3 h. The reaction mixture was filtered through celite to remove the catalyst and the solvent then removed to give, after purification by flash chromatography (1:1. ethyl acetate:hexane), 2,3-bis-(O-tert-butyldimethylsilyl)-1,4-dideoxy-5,6-O-isopropylidene-1,4-imino-D-glucitol (12), (390 mg, 86%), oil, $[\alpha]_D^{20}$ +16.2° (c, 0.84 in $CHCl_3$), ν_{max} (neat) 3380 (w, NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.06 (3H, s, SiMe), 0.08 (3H, s, SiMe), 0.11 (6H, s, 2 x SiMe), 0.88 (9H, s, t-Bu), 0.89 (9H, s, t-Bu), 1.33 (3H, s, Me), 1.42 (3H, s, Me), 2.72 (1H, d, H-1, $J_{1,1'}$ 11.8 Hz), 3.16 (1H, dd, H-1', $J_{1',2}$ 3.7 Hz), 3.27 (1H, dd, H-4, $J_{3,4}$ 3.6 Hz, $J_{4,5}$ 7.8 Hz), 3.85 (1H, dd, H-6, $J_{6,6'}$ 7.8 Hz, $J_{5,6}$ 6.5 Hz), 3.95 (1H, d, H-2), 3.99 (1H, d, H-3), 4.08 (1H, dd, H-6', $J_{5,6'}$ 6.2 Hz), 4.15 (1H, m, H-5). ^{13}C NMR ($CDCl_3$) δ -4.99 (q), -4.66 (q), 18.66 (s), 18.71 (s), 25.43 (q), 25.76 (q), 26.86 (q), 53.80 (t), 63.52 (d), 68.13 (t), 74.50 (d), 78.25 (d), 78.61 (d), 108.49 (s). m/z (CI, NH_3): 432 ($M+H^+$, 100%), 330 (60%).

1,4-Dideoxy-1,4-imino-D-glucitol (3). The silylated acetonide (12) (343 mg, 0.80 mmol) in 50% aqueous trifluoroacetic acid (8 ml) was stirred at 50°C for 24 h. The solvent was removed; the resulting trifluoroacetate salt was neutralised with dilute aqueous sodium hydroxide and purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give 1,4-dideoxy-1,4-imino-D-glucitol (3), (122 mg, 94%), m.p. 194°-196°C, $[\alpha]_D^{20}$ -10.1° (c, 0.43 in H_2O) [lit.[8] m.p. 200°-202°C, $[\alpha]_D^{20}$ -11° (H_2O)]; ν_{max} (KBr) 3600-3200 (br, NH and OH) cm^{-1}. ^1H NMR (D_2O) δ 2.69 (1H, d, H-1, $J_{1,1'}$ 12.8 Hz), 3.14 (1H, dd, H-4, J 3.6 and 9.5 Hz), 3.20 (1H, dd, H-1', $J_{1',2}$ 4.9 Hz), 3.43 (1H, dd, H-6, $J_{6,6'}$ 12.0 Hz, $J_{5,6}$ 6.5 Hz), 3.59 (1H, dd, H-6', $J_{5,6'}$ 3.2 Hz), 3.70 (1H, m, H-5), 4.03 (2H, m, H-2, H-3). ^{13}C NMR (D_2O) δ 52.45 (t), 61.91 (d), 65.33 (t), 71.09 (d), 77.59 (d), 77.70 (d). m/z (ACE, NH_3): 164 ($M+H^+$, 100%). (Found: C, 44.01; H, 8.24; N, 8.27. $C_6H_{13}NO_4$ requires C, 44.17; H, 7.98; N, 8.59%.).

The free base (3) was dissolved in water (5 ml) and the solution was adjusted to pH 4 with dilute aqueous hydrochloric acid to afford, after freeze drying, 1,4-dideoxy-1,4-imino-D-glucitol hydrochloride, (99.7 mg, 100%), m.p. 143°-144°C, $[\alpha]_D^{20}$ -28.1° (c, 0.42 in H_2O) [lit.[8] m.p. 140°-142°C, $[\alpha]_D^{20}$ -27° (H_2O)]; ν_{max} (KBr) 3600-3000 (br, NH and OH) cm^{-1}. ^1H NMR (D_2O) δ 3.14 (1H, d, H-1, $J_{1,1'}$ 13.1 Hz), 3.59 (4H, m, H-1', H-4. H-6, H-6'), 3.96 (1H, m, H-5), 4.21 (2H, m, H-2, H-3). ^{13}C NMR (D_2O) δ 52.49 (t), 63.27 (d), 64.32 (t), 67.81 (d), 74.63 (d), 75.51 (d). m/z (FAB, Gly/MeOH): 164 ($M+H^+$, 100%). (Found: C, 35.81; H, 7.12; N, 6.89. $C_6H_{14}ClNO_4$ requires C, 36.09; H, 7.02; N, 7.02%.).

N-Benzyl-1,4-dideoxy-1,4-imino-D-glucitol Hydrochloride (13). The fully protected amine (11) (224 mg, 0.43 mmol) was stirred at 50°C in 50% aqueous trifluoroacetic acid (10 ml) for 18 h. The solvent was removed and the residue purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give N-benzyl-1,4-dideoxy-1,4-imino-D-glucitol (13); the free base (13) was dissolved in water (5 ml) and the solution was adjusted to pH 4 with dilute aqueous hydrochloric acid to afford, after freeze drying, N-benzyl-1,4-dideoxy-1,4-imino-D-glucitol hydrochloride, (99 mg, 80%), a very hygroscopic solid, $[\alpha]_D^{20}$ -31.9° (c, 0.68 in H_2O). ^1H NMR (D_2O) δ 3.27 (1H, d, H-1, $J_{1,1'}$ 13.2 Hz), 3.60 (3H, br s, H-4, H-6, H-6'), 3.71 (1H, dd, H-1', $J_{1',2}$ 4.2 Hz), 3.79 (1H, m, H-5), 4.16 (1H, d, H-3), 4.31 (2H, m, H-2 and 1H of CH_2), 4.46 (1H of AB, $PhCH_2$, J_{AB} 13.0 Hz), and 7.37 (5H, m, ArH). ^{13}C NMR (D_2O) δ 59.87 (t), 61.39 (t), 63.56 (t), 68.77 (d), 69.95 (d), 74.78 (d), 76.85 (d), 130.47 (d), 131.39 (d), 131.91 (d). m/z (ACE, NH_3): 254 ($M+H^+$, 100%), 192 (62%).

2,3:5,6-Di-O-isopropylidene-D-gulonolactone (14). D-Gulonolactone (2) (3.0 g, 16.8 mmol) was suspended in acetone (80 ml) and 2,2-dimethoxypropane (16 ml); the reaction mixture was acidified to pH 3 with p-toluenesulphonic acid, stirred at room temperature for 2 days and then neutralised by addition of anhydrous sodium carbonate and filtered. The filtrate was evaporated to give an oil which was dissolved in ethyl acetate (100 ml), washed with saturated aqueous sodium bicarbonate (100 ml), dried (magnesium sulphate) filtered and the solvent removed to give 2,3:5,6-di-O-isopropylidene-D-gulonolactone (14), (3.7 g, 85%), m.p. 155°C (from ethyl acetate) $[\alpha]_D^{20}$ -76.6° (c, 1.99 in $CHCl_3$) [lit.[16] m.p. 150°-151°C, $[\alpha]_D^{20}$ -67.8° (c, 4.16 in $CHCl_3$)]. ν_{max} (KBr) 1790 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.39 (3H, s, Me), 1.41 (3H, s, Me), 1.48 (3H, s, Me), 1.49 (3H, s, Me), 3.84 (1H, m), 4.24 (1H, m), 4.45 (2H, m), 4.75 (1H, dd, J 3.4 and 5.7 Hz), 4.85 (1H, d, J 5.6 Hz). m/z (ACE, NH_3): 259 ($M+H^+$, 100%), 243 ($M-Me^+$, 67%), 201 (80%). (Found C, 55.92; H, 7.19. $C_{12}H_{18}O_6$ requires C, 55.81; H, 6.98%).

2,3:5,6-Di-O-isopropylidene-D-gulitol (15). The lactone (14) (2.00 g, 7.69 mmol) was added to a stirred solution of lithium aluminum hydride (292 mg, 7.69 mmol) in 8:1 tetrahydrofuran:ether (90 ml) at room temperature. After 30 min, the excess hydride was destroyed by addtion of water (2 ml) and the reaction mixture was partitioned between brine (50 ml) and ethyl acetate (120 ml). The organic layer was dried (magnesium sulphate), filtered and the solvent removed *in vacuo* to give a syrup which was recrystallised from ether to give 2,3:5,6-di-O-isopropylidene-D-gulitol (15), (1.95 g, 87%), m.p. 73°-75°C (from ether) $[\alpha]_D^{20}$ +11.3° (c, 1.80 in $CHCl_3$). ν_{max} (KBr) 3500-3100 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.39 (6H, s, 2 x Me), 1.46 (3H, s, Me), 1.53 (3H, s, Me), 3.83 (4H, m), 4.11 (2H, m), 4.28 (2H, m). m/z (ACE, NH_3): 280 ($M+NH_4^+$, 80%), 263 ($M+H^+$, 100%), 247 ($M-Me^+$, 53%). (Found C, 55.10; H, 8.71. $C_{12}H_{22}O_6$ requires C, 54.96; H, 8.40%).

1,4-Bis(methanesulphonyl)-2,3:5,6-di-O-isopropylidene-D-gulitol (16). Methane sulphonyl chloride (3.5 ml, 45.8 mmol) and a catalytic amount of 4,4-dimethylamino-pyridine (0.28 g) were added to a stirred solution of the diol (15) (3.00 g, 11.5 mmol) in pyridine (20 ml) and the reaction mixture stirred for 2 h at room temperature. The pyridine was evaporated to give a residue which was dissolved in chloroform (100 ml); the chloroform solution was washed with water, dried (magnesium sulphate) and the solvent removed to give an oil which was purified by flash chromatography (2:1, ethyl acetate:hexane) to afford 1,4-bis(methanesulphonyl)-2,3:5,6-di-O-isopropylidene-D-gulitol (16), colourless oil, (4.77 g, 100% yield), $[\alpha]_D^{20}$ -7.3° (c, 1.82 in $CHCl_3$). ν_{max} (KBr) 1350 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.36 (3H, s, Me), 1.38 (3H, s, Me), 1.45 (3H, s, Me), 1.51 (3H, s, Me), 3.08 (3H, s, $MeSO_2$), 3.17 (3H, s, $MeSO_2$), 3.99 (1H, dd, J 6.5 and 8.9 Hz), 4.14 (1H, dd, J 6.7 and 8.9 Hz), 4.41 (5H, m), 4.82 (1H, dd, J 4.8 and 6.6 Hz). m/z (DCI, NH_3): 436 ($M+NH_4^+$, 100%), 419 ($M+H^+$, 65%).

N-Benzyl-1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-allitol (17). The dimesylate (16) (4.77 g, 11.4 mmol) in benzylamine (10 ml) was warmed at 60°-70°C for 60 h. The reaction mixture was then dissolved in brine (50 ml) and extracted with chloroform (2 x 50 ml). The combined organic extracts were washed with water (2 x 50 ml), dried (magnesium sulphate) and the solvent removed to give, after purification by flash chromatography, (2:3, ether:hexane), N-benzyl-1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-allitol (17), colourless oil, (2.92 g, 77% yield), $[\alpha]_D^{20}$ -12.2° (c, 1.07 in $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.34 (6H, s, 2 x Me), 1.42 (3H, s, Me), 1.57 (3H, s, Me), 2.82 (1H, dd, H-1, $J_{1,1'}$ 11.7 Hz, $J_{1,2}$ 2.3 Hz), 3.03 (1H, dd, H-4, $J_{3,4}$ 1.4 Hz, $J_{4,5}$ 5.0 Hz), 3.12 (1H, dd, H-1', $J_{1',2}$ 4.7 Hz), 3.70 (1H, dd, H-6, $J_{6,6'}$ 8.2 Hz, $J_{5,6}$ 6.8 Hz), 3.84 (1H of AB, $PhCH_2$, J_{AB} 13.2 Hz), 4.03 (1H, dd, H-6', $J_{5,6'}$ 6.6 Hz), 4.07 (1H of AB, $PhCH_2$), 4.18 (1H, dt, H-5), 4.70 (2H, m, H-3, H-4), 7.32 (5H, m, ArH). ^{13}C NMR ($CDCl_3$) δ 24.53 (q), 24.96 (q), 26.48 (q), 27.00 (q), 58.50 (t), 58.77 (t), 67.14 (t), 70.16 (d), 74.00 (d), 80.21 (d), 82.05 (d), 109.38 (s), 112.02 (s), 127.03 (d), 128.25 (d), 128.82 (d), 139.04 (s). m/z (ACE, NH_3): 334 ($M+H^+$, 100%), 232 (90%). (Found C, 68.46; H, 8.16; N, 4.07. $C_{19}H_{27}NO_4$ requires C, 68.47; H, 8.11; N, 4.20%).

1,4-Dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-allitol (18). The cyclised tertiary amine (17) (320 mg, 0.96 mmol) in ethanol (20 ml) was stirred under an atmosphere of hydrogen in the presence of 10% palladium on charcoal (150 mg) at room temperature for 2 h. The reaction mixture was filtered through celite to remove the catalyst and the solvent then removed to give, after purification by flash

chromatography (ethyl acetate), 1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-allitol (18), (219 mg, 94%), yellow oil, $[\alpha]_D^{20}$ +34.1° (c, 0.41 in $CHCl_3$), ν_{max} (neat) 3325 (w, NH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.34 (6H, s, 2 x Me), 1.44 (3H, s, Me), 1.47 (3H, s, Me), 2.88. (1H, dd, H-1, $J_{1,1'}$ 13.6 Hz, $J_{1,2}$ 3.9 Hz), 3.04 (1H, d, H-1'), 3.12 (1H, d, H-4, $J_{4,5}$ 7.8Hz), 3.84 (1H, dd, H-6, $J_{6,6'}$ 8.1 Hz, $J_{5,6}$ 5.7 Hz), 3.94 (1H, dd, H-5), 4.11 (1H, dd, H-6', $J_{5,6'}$ 6.2 Hz), 4.72 (1H, dd, H-2, $J_{2,3}$ 5.7 Hz), 4.78 (1H, d, H-3). m/z (CI, NH_3): 244 ($M+H^+$, 100%), 142 (63%).

1,4-Dideoxy-1,4-imino-D-allitol (4). The acetonide (18) (146 mg, 0.60 mmol) in 50% aqueous trifluoroacetic acid (8 ml) was stirred at room temperature for 14 h. The solvent was removed; the resulting trifluoroacetate salt was neutralised with dilute aqueous sodium hydroxide and purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give 1,4-dideoxy-1,4-imino-D-allitol (4); the free base (4) was dissolved in water (5 ml) and the solution was adjusted to pH 4 with dilute aqueous hydrochloric acid to afford, after freeze drying, 1,4-dideoxy-1,4-imino-D-allitol hydrochloride, (117 mg, 98%), m.p. 110°-111°C, $[\alpha]_D^{20}$ +29.4° (c, 0.53 in H_2O), ν_{max} (KBr) 3600-3200 (br, NH and OH) cm^{-1}. ^1H NMR (D_2O) δ 3.20 (1H, dd, H-1, $J_{1,1'}$ 12.8 Hz, $J_{1,2}$ 2.0 Hz), 3.29 (1H, dd, H-1', $J_{1',2}$ 3.7 Hz), 3.51 (1H, dd, H-4, $J_{3,4}$ 8.2 Hz, $J_{4,5}$ 3.5 Hz), 3.57 (2H, m, H-6, H-6'), 3.97 (1H, dt, H-5), 4.20 (1H, dt, H-2, $J_{2,3}$ 4.2 Hz), 4.26 (1H, dd, H-3). ^{13}C NMR (D_2O) δ 50.93 (t), 62.75 (d), 63.32 (t), 69.44 (d), 70.85 (d), 71.12 (d). m/z (FAB, Gly/MeOH): 164 ($M+H^+$, 100%).

N-Benzyl-1,4-dideoxy-1,4-imino-D-allitol Hydrochloride (19). The fully protected amine (17) (208 mg, 0.62 mmol) was stirred at room temperature in 50% aqueous trifluoroacetic acid (10 ml) for 24 h. The solvent was removed; the resulting trifluoroacetate salt was neutralised with dilute aqueous sodium hydroxide and the residue purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give N-benzyl-1,4-dideoxy-1,4-imino-D-allitol (11) , (150 mg, 96%), as a syrup; the free base (11) was dissolved in water (5 ml) and the solution was adjusted to pH 4 with dilute aqueous hydrochloric acid to afford, after freeze drying, N-benzyl-1,4-dideoxy-1,4-imino-D-allitol hydrochloride, a very hygroscopic solid, $[\alpha]_D^{20}$ +23.1° (c, 0.72 in H_2O), ^1H NMR (D_2O) δ 3.28 (2H, m), 3.40 (2H, d, J 6.0 Hz), 3.51 (1H, dd, J 4.3 and 12.8 Hz), 3.58 (1H, dd, J 2.5 and 6.3 Hz), 4.24 (2H, m), 4.42 (1H, s) and 7.37 (5H, m, ArH). ^{13}C NMR (D_2O) δ 58.42 (t), 62.36 (t), 63.01 (t), 69.07 (d), 70.25 (d), 70.70 (d), 71.44 (d), 130.29 (s), 130.56 (d), 131.52 (d), 131.93 (d). m/z (DCI, NH_3): 254 ($M+H^+$, 100%), 192 (77%).

N-Benzyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (20). The fully protected amine (17) (1.51 g, 4.53 mmol) was stirred at 50°C in 80% aqueous acetic acid (20 ml) for 48 h. The solvent was removed and the residue purified by flash chromatography (ethyl acetate) to give N-benzyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (20), (1.24 g, 93%), pale yellow oil, $[\alpha]_D^{20}$ -48.2° (c, 2.01 in $CHCl_3$), ν_{max} (film) 3600-3200 (br, OH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.33 (3H, s, Me), 1.54 (3H, s, Me), 2.69 (1H, dd, H-1, $J_{1,1'}$ 11.2 Hz, $J_{1,2}$ 3.9 Hz), 2.91 (1H, t, H-4, $J_{3,4} = J_{4,5} = 3.7$ Hz), 3.31 (1H, dd, H-1', $J_{1',2}$ 5.9 Hz), 3.61 (1H of AB, $PhCH_2$, J_{AB} 12.8 Hz), 3.68 (1H, dd, $J_{6,6'}$ 11.5 Hz, $J_{5,6}$ 5.3 Hz), 3.79 (1H, dd, H-6', $J_{5,6'}$ 6.3 Hz), 3.94 (1H, dt, H-5), 4.11 (1H of AB, $PhCH_2$), 4.63 (1H, dt, H-2, $J_{2,3}$ 6.7 Hz), 4.75 (1H, dd, H-3), 7.33 (5H, m, ArH). ^{13}C NMR ($CDCl_3$) δ 24.53 (q), 27.01 (q), 58.26 (t), 58.66 (t), 64.36 (t), 66.23 (d), 71.38 (d), 78.62 (d), 80.24 (d), 112.72 (s), 127.71 (d), 128.56 (d), 129.19 (d), 136.77 (s). m/z (CI, NH_3): 294 ($M+H^+$, 100%), 232 (60%).

N-Benzyl-1,4-dideoxy-1,4-imino-2,3-isopropylidene-D-ribitol (23). Sodium periodate (1.53 g, 7.17 mmol) was added to a stirred solution of N-benzyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (20) (700 mg, 2.39 mmol) in 5:1 ethanol:water (24 ml) at room temperature; when tlc showed all the starting material had been consumed after 20 min, the reaction mixture was treated with sodium borohydride (207 mg, 5.46 mmol) and stirred at 0°C for a further 30 min. The excess hydride was then decomposed by addition of excess solid ammonium chloride and the solvent removed to give, after purification by flash chromatography (ethyl acetate:hexane, 3:1), N-benzyl-1,4-dideoxy-1,4-imino-2,3-isopropylidene-D-ribitol (23), (444 mg, 71%), yellow oil, $[\alpha]_D^{20}$ -45.9° (c, 1.0 in $CHCl_3$) [lit. for L-enantiomer $[\alpha]_D^{20}$ +45.7° (c, 1.0 in $CHCl_3$)]. ν_{max} (film) 3600-3200 (br, OH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.33 (3H, s, Me), 1.55 (3H, s, Me), 2.65 (1H, dd, H-1, $J_{1,1'}$ 10.7 Hz, $J_{1,2}$ 3.8 Hz), 2.98 (1H, br dd, H-4), 3.22 (1H, dd, H-1', $J_{1',2}$ 5.7 Hz), 3.58 (1H, dd, H-5, $J_{5,5'}$ 11.2 Hz, $J_{4,5}$ 3.6 Hz), 3.61 (1H of AB, $PhCH_2$, J_{AB} 13.0 Hz), 3.71 (1H, dd, H-5', $J_{4,5'}$ 3.8 Hz), 3.99 (1H of AB, $PhCH_2$), 4.61 (2H, m, H-2, H-3), 7.31 (5H, m, ArH). ^{13}C NMR ($CDCl_3$) δ 24.80 (q), 27.18 (q), 58.12 (t), 58.44 (t), 59.57 (t), 69.88 (d), 78.43 (d), 82.50 (d), 112.58 (s), 127.25 (d), 128.37 (d), 128.64 (d), 138.27 (s). m/z (DCI, NH_3): 264 ($M+H^+$, 100%), 232 (36%). (Found C, 68.62; H, 8.27; N, 5.21. $C_{15}H_{21}NO_3$ requires C, 68.44; H, 7.98%; N, 5.32). The ^1H and ^{13}C NMR spectra of (23) are superimposable on those of the L-enantiomer of (23) reported in the accompanying paper.[7]

1,4-Dideoxy-1,4-imino-D-ribitol (5). The protected ribitol (23) (256 mg, 0.97 mmol) in ethanol (10 ml) was stirred under an atmosphere of hydrogen in the presence of catalyst of 10% palladium on charcoal (120 mg) at room temperature for 2h. The reaction mixture was filtered through celite and the solvent removed; the crude product [^1H NMR showed that the benzyl group had been completely removed] was dissolved in 50% aqueous trifluoroacetic acid and the solution was allowed to stand at room temperature for 24 h. The solvent was removed; the resulting trifluoroacetate salt was neutralised with dilute aqueous sodium hydroxide and purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give 1,4-dideoxy-1,4-imino-D-ribitol (5); the free base (5) was dissolved in water (5 ml) and the solution was adjusted to pH 4 with dilute aqueous hydrochloric acid to afford, after freeze drying, 1,4-dideoxy-1,4-imino-D-ribitol hydrochloride, (129 mg, 78%), m.p. 128°-132°C, $[\alpha]_D^{20}$ +57.6° (c, 0.59 in H_2O) [data for L-enantiomer of (5) m.p. 126°-131°C, $[\alpha]_D^{20}$ -59.0° (c, 0.59 in H_2O)]; ν_{max} (film) 3600-3200 (br, NH and OH) cm^{-1}. ^1H NMR (D_2O) δ 3.22 (1H, dd, H-1, $J_{1,1'}$ 13.0 Hz, $J_{1,2}$ 2.0 Hz), 3.33 (1H, dd, H-1', $J_{1',2}$ 4.0 Hz), 3.46 (1H, m, H-4), 3.66 (1H, dd, H-5, $J_{5,5'}$ 12.6 Hz, $J_{4,5}$ 6.0 Hz), 3.81 (1H, dd, H-5', $J_{4,5'}$ 3.5 Hz), 4.04 (1H, dd, H-3, $J_{2,3}$ 4.1 Hz, $J_{3,4}$ 8.5 Hz), 4.22 (1H, dt, H-2). ^{13}C NMR (D_2O) δ 50.66 (t), 59.04 (t), 62.86 (d), 70.42 (d), 72.17 (d). m/z (DCI, NH_3): 134 ($M+H^+$, 100%), 102 (18%). (Found: C, 34.93; H, 7.40; N, 8.05. $C_5H_{12}ClNO_3$ requires C, 35.19; H, 7.04; N, 8.21%). The ^1H and ^{13}C NMR spectra of (5) are superimposable on those of the L-enantiomer of (5) reported in the accompanying paper.[7]

N-Benzyl-1,4-dideoxy-1,4-imino-D-ribitol (25). The acetonide (23) (96 mg, 0.37 mmol) in 50% aqueous trifluoroacetic acid (6 ml) was stirred at room temperature for 20 h. The solvent was removed; the resulting trifluoroacetate salt was neutralised with dilute aqueous sodium hydroxide and purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give N-benzyl-1,4-dideoxy-1,4-imino-D-ribitol (25), (72 mg, 86%), a very hygroscopic solid, $[\alpha]_D^{20}$ -37.3° (c, 0.49 in H_2O) [data for L-enantiomer of (25) $[\alpha]_D^{20}$ +33.0° (c, 0.32

in H_2O)]; ^1H NMR (D_2O) δ 2.37 (1H, dd, H-1, $J_{1,1'}$ 10.0 Hz, $J_{1,2}$ 8.0 Hz), 2.59 (1H, q, H-4), 2.87 (1H, dd, H-1', $J_{1',2}$ 6.0 Hz), 3.35 (2H, m, H-5), 3.44 (1H of AB, $PhCH_2$, J_{AB} 12.4 Hz), 3.78 (2H, m, H-3 and 1H of $PhCH_2$), 3.88 (1H, dt, H-2), 7.2 (5H, m, ArH). ^{13}C NMR (D_2O) δ 57.65 (t), 60.42 (t), 62.25 (t), 70.02 (d), 71.52 (d), 73.86 (d), 128.85 (d), 129.62 (d), 131.06 (d), 137.97 (s). m/z (DCI, NH_3): 224 ($M+H^+$, 57%), 192 (100%). The ^1H and ^{13}C NMR spectra of (25) are superimposable on those of the L-enantiomer of (25) reported in the accompanying paper.[7]

N-tert-Butyloxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (22). The diol (20) (140 mg, 0.48 mmol) in ethanol (10 ml) was stirred under an atmosphere of hydrogen in the presence of 10% palladium on charcoal (70 mg) at room temperature for 2 h. The reaction mixture was filtered through celite and the solvent removed *in vacuo* to give 1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (21), (90 mg, 93%), a syrup which without further purification was dissolved in pyridine (2 ml) and treated with di-*tert*-butyl dicarbonate (106 mg, 0.48 mmol) at room temperature. The reaction mixture was then allowed to stand for 2.5 h, the pyridine was evaporated and the residue dissolved in ethyl acetate (50 ml); the ethyl acetate solution was washed with brine (2 x 50 ml), dried (magnesium sulphate), filtered and the solvent removed. The crude product was purified by flash chromatography (ethyl acetate) to give N-tert-Butyloxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-allitol (22), (100 mg, 75%), a colourless oil which slowly crystallised on standing at room temperature for several days, m.p. 73°-74°C, $[\alpha]_D^{20}$ -33.5° (*c*, 0.17 in $CHCl_3$); ν_{max} 3600-3100 (OH), 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.34 (3H, s, Me), 1.46 (3H, s, Me), 1.48 (9H, s, *tert*-butyl); the remainder of the proton spectrum was complicated due to the existence of rotamers. m/z (ACE, NH_3): 304 ($M+H^+$, 90%), 248 (63%), 204 (100%).

(2S,3R,4S)-N-tert-Butyloxycarbonyl-3,4-dihydroxy-3,4-O-isopropylidene-proline (24). The diol (22) (64 mg, 0.21 mmol) in 5:2 ethanol:water (2.8 ml) was stirred with sodium periodate (123 mg, 0.57 mmol) at room temperature. After 10 min, the reaction mixture was filtered and the solvent removed *in vacuo*; the crude aldehyde so obtained was dissolved in *tert*-butanol (3 ml) and treated with cyclohexene (0.2 ml), followed by a solution of sodium chlorite ($NaClO_2$) (190mg, 2.1 mmol) and potassium dihydrogen phosphate (286 mg, 2.1 mmol) in water (2 ml). The reaction mixture was stirred overnight at room temperature and then evaporated to dryness; the residue was partitioned between ethyl acetate (30 ml) and water (5 ml). The organic layer was dried (magnesium sulphate) and the solvent removed to give, after purification by flash chromatography, (2S,3R,4S)-N-tert-butyloxycarbonyl-3,4-dihydroxy-3,4-O-isopropylidene-proline (24), (45 mg, 75%), syrup, ^1H NMR ($CHCl_3$) δ 1.34 (3H, s, Me), 1.44 (3H, s, Me), 1.50 (9H, s, *tert*-butyl); the remainder of the proton spectrum was complicated due to the existence of rotamers. m/z (DCI, NH_3): 288 ($M+H^+$, 17%), 249 (20%), 232 (17%), 216 (20%), 188 (100%).

(2S,3R,4S)-3,4-Dihydroxy-proline (6). The protected amino acid (24) (43 mg, 0.15 mmol) was stirred in 80% aqueous trifluoroacetic acid (5 ml) for 23 h at room temperature. The solvent was removed and the residue purified by ion exchange chromatography (Dowex 50 x, 8-100, H^+ form, eluted with 0.5 molar aqueous ammonium hydroxide) to give (2S,3R,4S)-3,4-dihydroxy-proline (6), (18 mg, 81%), a white solid, decomposes at 240°-250°C, $[\alpha]_D^{20}$ +7.5° (*c*, 0.16 in H_2O). m/z (FAB$^+$): 148 ($M+H^+$, 100%). [data for (2R,3S,4R)-3,4-dihydroxy-proline, the enantiomer of (6), decomposes without melting at 247°C, $[\alpha]^{20}$ -6.8° (*c*, 0.43 in H_2O)]. The ^1H NMR spectrum of (6) is superimposable on that of an authentic sample of the enantiomer, (2R,3S,4R)-3,4-dihydroxy-proline.[18,19]

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