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THE SYNTHESIS OF POLYHYDROXYLATED AMINO ACIDS FROM GLUCURONOLACTONE: ENANTIOSPECIFIC SYNTHESES OF 2S,3R,4R,5S-TRIHYDROXYPIPECOLIC ACID, 2R,3R,4R,5S-TRIHYDROXYPIPECOLIC ACID AND 2R,3R,4R-DIHYDROXYPROLINE

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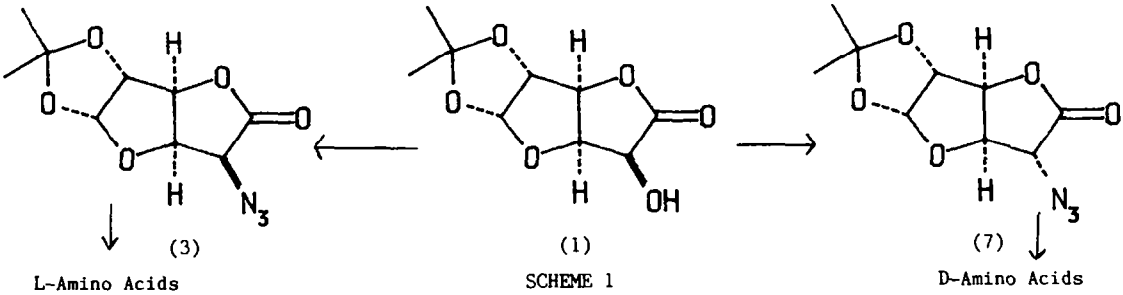
The potential of D-glucuronolactone for the synthesis of polyhydroxylated amino acids is illustrated by the enantiospecific syntheses of 2S,3R,4R,5S-trihydroxypipecolic acid, 2R,3R,4R,5S-trihydroxypipecolic acid and 2R,3R,4R-dihydroxyproline.

Sugar lactones, which may be converted efficiently to derivatives in which only the hydroxyl group adjacent to the lactone carbonyl function is unprotected, are suitable candidates for divergent syntheses of polyfunctionalised amino acids; nucleophilic displacement of a leaving group α to the carbonyl function is relatively facile and the lactone function removes the necessity of an oxidative step for the generation of the carboxylic acid. The need for protection of functional groups in this approach to amino acids is minimal in comparison to many syntheses of amino acids from carbohydrates. D-Ribonolactone may readily be converted into intermediates suitable for the synthesis of D-amino acids such as 2R,3R,4R-dihydroxyproline.[1,2] D-Glucuronolactone, a cheap chiral starting material, reacts efficiently with acetone[3] to give the acetonide (1) in 80% yield on a 30 g scale in which only the C-5 OH is unprotected. Replacement of the C-5 OH group in (1) by azide with inversion of configuration would lead to the ido-azide (7), a suitable precursor for the synthesis of polyhydroxylated D-amino acids, whereas introduction of azide with overall retention of configuration to give the gluco-azide (3) would provide an intermediate for elaboration to L-amino acids [Scheme].



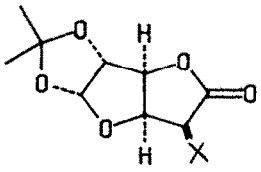
SCHEME 1

Treatment of (1) with triphenylphosphine - diethyl azodicarboxylate - hydrazoic acid[4] gives an epimeric mixture of the azides (3) and (7) in the ratio of 2:1; however, this procedure is not convenient for the preparation of substantial

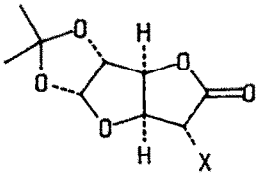
quantities of either azide, since the epimers have to be separated by careful column chromatography and the use of hydrazoic acid on a large scale should be avoided.
This paper describes the efficient synthesis of both azides (3) and (7) from (1), and the conversion of the gluco-azide (3) into the L-amino acid 2S,3R,4R,5S-trihydroxypipecolic acid (13), a naturally occurring nitrogen analogue of glucuronic acid, isolated from the seeds of Baphia racemosa,[5] which has been shown to be a glucuronidase inhibitor;[6] this paper also reports the elaboration of the ido-azide (7) into the D-amino acids 2R,3R,4R,5S-trihydroxypipecolic acid (15), the corresponding epimeric nitrogen analogue of iduronic acid, and 2R,3R,4R-dihydroxyproline (16), the enantiomer of which has been isolated from diatom cell walls.[7,8,9] The accompanying paper[10] describes the synthesis from the ido-azide (7) of 2S,4S-2-amino-4-hydroxyacids; a preliminary account of this work has been published.[11]

The conversion of (1) into the ido-azide (7) requires the introduction of azide with a single inversion of configuration. Treatment of the isopropylidine glucuronolactone (1) with trifluoromethanesulphonic anhydride in dichloromethane in the presence of pyridine give the corresponding triflate ester (2)[12] in quantitative yield; the triflate (2) is kinetically stable and easily handled, an analytical sample being purified by recrystallisation from ethanol. When the triflate (2) in dimethylformamide is treated with a suspension of sodium azide at -20°C, a smooth nucleophilic displacement reaction occurs leading to the ido-azide (7) in 83% yield, with no concurrent formation of the epimeric gluco-azide (3); palladium catalysed hydrogenation of the azide function in (7), followed by protection of the resulting amine by reaction with benzyl chloroformate, gave the carbamate (8)[13] in 75% yield [62% yield from (1)]. Both the azide (7) and the carbamate (8) are easily crystallised compounds and no column purification is necessary in the preparation of 10 g amounts of these key intermediates for the synthesis of D-amino acids.
The isopropylidene protecting groups may be removed from both the azide (7) and the carbamate (8) by hydrolysis with aqueous trifluoroacetic acid to give the corresponding lactols (9) and (10) in respective yields of 82% and 70%. Hydrogenation of the azidolactol (9) in aqueous acetic acid in the presence of palladium black leads to hydrolysis of the lactone ring and intramolecular reductive amination to give, after purification by ion exchange and flash chromatography, 2R,3R,4R,5S-trihydroxypipecolic acid (15) [32% from (9); 22% from (1)]; the ido configuration of (15) is indicated by the small coupling constant (J, 2.5 Hz) between H-2 (on the carbon adjacent to the carboxyl group) and H-3 in the ^{1}H NMR of (15) while the ^{13}C NMR of (15) is similar to, but significantly different from, the epimeric trihydroxypipecolic acid (13). A small amount of 2S,4S,5S-dihydroxypipecolic acid[14] (14) was also isolated from the reaction mixture; a possible pathway for the formation of (14) involves initial elimination of water from (9) to give (17) which would undergo addition of hydrogen from the least hindered side to give (18) with the correct stereochemistry for the formation of (14). Hydrogenation of the carbamate (10) under similar conditions also produces the trihydroxypipecolic acid (15).

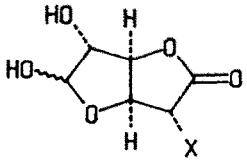
Periodate oxidation of azidolactol (9),followed by hydrogenation of the resulting unstable aldehyde (11), gives 2R,3R,4R-dihydroxyproline in 15% yield from (7) [12% from (1)]; although the yield in this reaction is low, the sequence for the preparation is short and moderate amounts of (16) may be prepared by this route.
For the synthesis of the gluco-carbamate (4) as an intermediate for the preparation of the L-amino acid (13), introduction of nitrogen at C-5 of (1) with overall retention of configuration is required. Thus treatment of the triflate (2) with



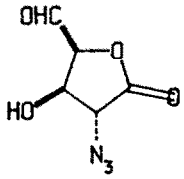
(1) X=OH (2) X=OSO_2CF_3
(3) X=N_3 (4) X=NHZ



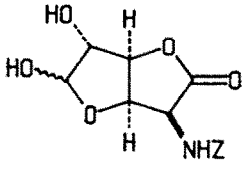
(5) X=OH (6) X=OSO_2CF_3
(7) X=N_3 (8) X=NHZ



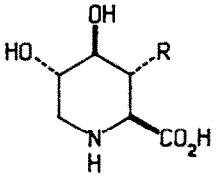
(9) X=N_3
(10) X=NHZ



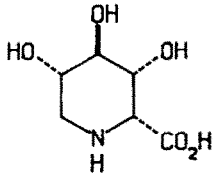
(11)



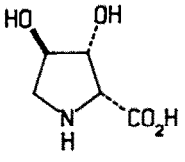
(12)
Z=$COOCH_2Ph$



(13) R=OH (14) R=H

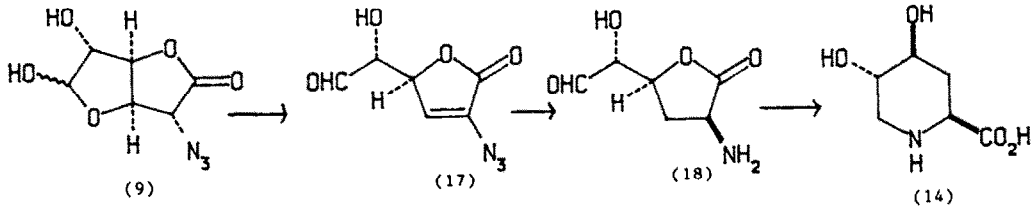


(15)



(16)

sodium trifluoroacetate, followed by methanolysis of the trifluoroacetate ester, according to literature procedures,[12] gives the ido alcohol (5) in 78% yield.



(9) (17) (18) (14)

SCHEME 2

Esterification of the hydroxyl group in (5) with trifluoromethanesulphonic anhydride to give (6), followed by treatment with sodium azide in dimethylformamide gave the gluco-azide (3); palladium catalysed hydrogenation of (3) followed by reaction of the resulting amine with benzylchloroformate gave the gluco-carbamate (4) in 44% overall yield from (5). Hydrolysis of (4) with aqueous trifluoroacetic acid caused removal of the isopropylidene protecting group to give the lactol (12) [81% yield] which on hydrogenation in the presence of palladium black formed 2S,3R,4R,5S-trihydroxypipecolic acid (13) [59% yield], together with 5% of the dihydroxypipecolic acid (14); the formation of (14) in this sequence may be accounted for by a similar pathway to that for the formation of (14) from (9). A synthesis of 2S,3R,4R,5S-trihydroxypipecolic acid (13) from glucose has also been reported.[15]

In summary, this paper reports the preparation of intermediates from D-glucuronolactone which are convenient for conversion to polyhydroxylated amino acids. The ability of 2S,3R,4R,5S-trihydroxypipecolic acid (13) to inhibit glucuronidase activity has been reported; the potential of the iduronic acid analogue 2R,3R,4R,5S-trihydroxypipecolic acid (16) to inhibit glucuronidase and / or iduronidase activity is currently under investigation.[16]

Experimental

M.p.s were recorded on a Kofler block. Infra red spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{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-dioxane (δ 67.6) was used as the internal standard. Mass spectra were recorded on VG Micromass 16 F spectrometer; in order to obtain satisfactory mass spectra for these highly polar compounds, it was generally necessary to use ACE, DCI or FAB techniques. Microanalyses were performed by the microanalytical services of the Dyson Perrins Laboratory, other than analysis for sulphur and fluorine which were determined by the analytical services of the chemistry department of Manchester University. TLC was performed on glass plates coated with silica gel Blend 41, and compounds were visualised with a spray of 5% v/v sulphuric acid in methanol, 5% dodecamolybdophosphoric acid in methanol, or 0.5% ninhydrin in methanol. Flash chromatography was carried out using Merck Kieselgel 60, 230-400 mesh. Dowex 50x 8-100 ion exchange resin was obtained from the Aldrich Chemical Company. α-D-Glucuronolactone was obtained from the Aldrich Chemical Company and used without purification.

1,2-O-Isopropylidene-α-D-glucuronolactone (1) was prepared by the method of Kitihara et al.[3] Concentrated sulphuric acid (24 ml) was added dropwise to a stirred suspension of α-D-glucuronolactone (30.0 g, 0.17 mol) in acetone (750 ml) at room temperature; the reaction mixture was then stirred for a further 4 h. The resulting clear yellow solution was neutralised with solid sodium bicarbonate, filtered and the solvent removed *in vacuo*. The solid residue was dissolved in ethyl acetate (500 ml), washed with brine (100 ml) and the solution dried (sodium sulphate). After removal of the solvent, the residue was recrystallised to give 1,2-O-isopropylidene-α-D-glucuronolactone (1), (30.3 g, 82%), m.p. 120.5-121.5°C (from toluene), $[\alpha]_D^{20}$ +52.5° (*c*, 1.95 in $CHCl_3$) [lit.[3] m.p. 140-142°C (from benzene)]; ν_{max} (KBr) 3440 (OH), and 1774 (C=O) cm^{-1}; ^{1}H NMR δ 1.34 (3H, s, Me), 1,52 (3H, s, Me), 2.89 (1H, d, OH, J 9.4 Hz), 4.52 (1H, dd, 5-H, J 4.4 and 9.4 Hz), 4.82-4.84 (2H, m, 2-H and 3-H), 4.97 (1H, dd, 4-H, J 2.8 and 4.4 Hz), and 5.99 (1H, d, 1-H, J 3.5 Hz); ^{13}C NMR δ 26.6 (q, Me), 27.0 (q, Me), 71.7 (d), 82.1 (d), 82.4 (d), 85.1 (d) 106.1 (d), 113.2 (s), and 174.7 (s, C=O); *m/z* (DCI, NH_3) 234 (100%, $M+NH_4^+$), 217 (10%, $M+H^+$) and 201 (40%, $M-Me^+$) (Found C, 50.4; H, 5.7. $C_9H_{12}O_6$ requires C, 50.0; H, 5.6%).

1,2-O-Isopropylidene-5-O-trifluoromethanesulphonyl-α-D-glucuronolactone(2). Pyridine (10 ml) and trifluoromethane sulphonic anhydride (8.5 ml, 0.051 mol) was added to a solution of 1,2-O-isopropylidene-α-D-glucuronolactone (10.0 g, 0.0463 mol) in dichloromethane (100 ml) at -40°C, and the reaction stirred at this temperature for a further 1.5 h. Additional dichloromethane (200 ml) was added and the reaction mixture washed with 5% aqueous hydrochloric acid (3 x 80 ml), water (3 x 80 ml) and dried (sodium sulphate) to give, after removal of solvent, 1,2-O-isopropylidene-5-O-trifluoromethanesulphonyl-α-D-glucuronolactone, (16.0 g, 99%), m.p. 160°C (from ethanol), $[\alpha]_D^{20}$ +49.2° (*c*, 1.28 in $CHCl_3$) [lit.[12] m.p. 149.5°C]; ν_{max} (KBr) 1785 (C=O) cm^{-1}; ^{1}H NMR δ 1.36 (3H, s, Me), 1.54 (3H, s, Me), 4.86 (1H, d, 2-H, J 3.5 Hz), 4.93 (1H, d, 3-H, J 2.8 Hz), 5.07 (1H, dd, 4-H, J 2.8 and 4.3 Hz), 5.42 (1H, d, 5-H, J 4.2 Hz), and 6.05 (1H, d, 1-H, J 3.6 Hz); ^{13}C NMR δ 26.4 (q, Me), 26.8 (q, Me), 76.4 (d), 78.2 (d), 82.3 (d), 82.4 (d), 107.0 (d), 113.0 (s), 117 (q, not proton decoupled, CF_3) and 165.9 (s, C=O); *m/z* (DCI, NH_3) 366 (100%, $M+NH_4^+$), and 333 (50%, $M-Me^+$) (Found C, 34.8; H, 3.2; F, 16.9; S, 9.5. $C_{10}H_{11}F_3O_8S$ requires C, 34.5; H, 3.2; F, 16.4; S, 9.2%).

5-Azido-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone (7). Sodium azide (3.0 g, 46.1 mmol) was added in one portion to a solution of 1,2-O-isopropylidene-5-O-trifluoromethanesulphonyl-α-D-glucuronolactone (16.0 g, 46.0 mmol) in dimethylformamide (20 ml) at -20°C and the reaction mixture stirred for 2.5 h. Water (20 ml) and ethyl acetate (350 ml) were then added and the organic layer subsequently washed with water (3 x 80 ml), brine (2 x 50 ml) and then dried (sodium sulphate). The volume of the reaction mixture was reduced to about 50 ml and hexane (5 ml) was added; the resulting solution was left to stand overnight at 0°C and 5-azido-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone, (9.2 g, 83%), was collected by filtration, m.p. 114-116°C , $[\alpha]_D^{20}$ +243° (c, 1.1 in $CHCl_3$) [lit.[4] m.p. 115°C, $[\alpha]^{20}$ +233° (c, 1.03 in $CHCl_3$)]; ν_{max} (KBr) 2120 (N_3) and 1790 (C=O) cm^{-1}; ^1H NMR δ 1.34 (3H, s, Me), 1.50 (3H, s, Me), 4.24 (1H, s, 5-H), 4.64 (1H, d, J 3.0 Hz), 4.82 (1H, d, J 3.5 Hz), 4.94 (1H, d, J 3.4 Hz), and 5.93 (1H, d, 1-H, J 3.7 Hz); ^{13}C NMR δ 26.5 (q, Me), 27.8 (q, Me), 61.4 (d), 81.1 (d), 82.0 (d), 84.9 (d), 106.3 (d), 113.4 (s), and 170.5 (s, C=O); m/z (DCI, NH_3) 259 (100%, $M+NH_4^+$), and 226 (80%, $M-Me^+$) (Found C, 44.9; H, 4.5; N, 17.3. $C_9H_{11}N_3O_5$ requires C, 44.8; H, 4.6; N, 17.4%).

5-(N-Benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone (8). A solution of 5-azido-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone (9.0 g, 37.3 mmol) in ethyl acetate (180 ml) was stirred in an atmosphere of hydrogen in the presence of 10% palladium on carbon (2 g) for 6 h at room temperature. The catalysts was then removed by filtration through celite, and the celite washed with ethyl acetate (2 x 40 ml). The ethyl acetate solution was cooled to 0°C and treated with saturated sodium bicarbonate solution (100 ml) and benzyl chloroformate (8 ml, 56 mmol), and the two phase mixture stirred at room temperature for 30 min. The organic layer was then separated and the aqueous layer extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were then washed with brine and dried (sodium sulphate) and the reaction mixture concentrated (to approximately 30 ml). Ether (5 ml) was then added and the solution was left overnight at 0°C; collection of the resulting crystals by filtration gave 5-(N-Benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone, (9.8 g, 75%), m.p. 135-140°C , $[\alpha]_D^{20}$ +83.6° (c, 0.58 in $CHCl_3$) [lit.[13] $[\alpha]_D^{20}$ +85.5 (c, 0.40 in $CHCl_3$)]; ν_{max} (Nujol) 3360 (NH) 1775 (C=O) and 1710 (C=O) cm^{-1}; ^1H NMR δ 1.33 (3H, s, Me), 1.52 (3H, s, Me), 3.88 (1H, d, 5-H, J 7.3 Hz), 4.8 (2H, m), 5.12 (2H, br s, benzyl CH_2), 5.24 (1H, br s), 5.58 (1H, br s, NH), 5.95 (1H, d, 1-H, J 4.0 Hz)and 7.37 (5H, m, ArH); ^{13}C NMR δ 26.6 (q, Me), 27.1 (q, Me), 57.1 (d), 67.9 (t), 82.4 (d), 83.6 (d) 85.8 (d), 105.9 (d), 112.9 (s), 128.2 (d), 128.5 (d), 128.6 (d), 135.6 (s), 155.9 (s) and 173.2 (s, C=O); m/z (DCI, NH_3) 367 (100%, $M+NH_4^+$), and 91 (50%, $C_7H_7^+$) (Found C, 58.5; H, 5.5; N, 3.9. $C_{17}H_{19}NO_7$ requires C, 58.5; H, 5.4; N, 4.0%).

5-Azido-5-deoxy-L-iduronolactone (9). A solution of 5-azido-5-deoxy-1,2-O-isopropylidene-ß-L-iduronolactone (1.0 g, 4.14 mmol) in trifluoracetic acid - water (3:1, 8 ml) was stirred at room temperature for 3.5 h. The solvent was removed *in vacuo* and the resulting residue recrystallised from ethyl acetate to give 5-azido-5-deoxy-L-iduronolactone, (0.68 g, 82%), m.p. 119-121°C , $[\alpha]_D^{20}$ +192° (c, 0.47 in EtOAc); ν_{max} (KBr) 3340 (OH) 2110 (N_3) and 1790 (C=O) cm^{-1}; ^1H NMR (d_6-acetone) δ 3.0 (br s, OH), 4.26 (1H, s, 5-H), 4.49 (1H, d, 2-H, J 0.9 Hz), 4.75 (1H, dd, 3-H, J 0.9 and 5.9 Hz), 4.95 (1H, d, 4-H, J 5.9 Hz) and 5.37 (1H, s, 1-H); ^{13}C NMR (d_6-DMSO) δ 64.9 (d), 78.4 (d), 83.0 (d), 87.6 (d), 104.5 (d), and 174.1 (s, C=O); m/z (DCI, NH_3) 219 (100%, $M+NH_4^+$), and 201 (20%, M^+) (Found C, 35.9; H, 3.4; N, 20.7. $C_6H_7N_3O_5$ requires C, 35.8; H, 3.5; N, 20.9%).

5-(N-Benzyloxycarbonyl)amino-5-deoxy-L-iduronolactone (10). A solution of 5-(N-benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (1.0 g, 2.86 mmol) in trifluoroacetic acid - water (3:4, 14 ml) was stirred at room temperature for 14h. The solvent was removed *in vacuo* and the resulting residue purified by flash chromatography to give 5-(N-benzyloxycarbonyl)amino-5-deoxy-L-iduronolactone, (0.62 g, 70%), m.p. 149-151°C; ν_{max} (film) 3440 (NH and OH), 1785 (C=O) and 1680 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 3.95 (1H, m, 5-H), 4.03 (br s, OH), 4.7 - 5.0 (3H, m, 2-H, 3-H and 4-H), 5.06 (2H, s, benzyl CH_2), 5.18 (1H, br s, 1-H), 7.35 (5H, m, ArH), and 8.31 (1H, d, NH, J 7.9 Hz); ^{13}C NMR (d_6-DMSO) δ 58.2 (d), 66.3 (t), 77.2 (d), 83.6 (d) 86.0 (d), 103.0 (d), 128.0 (d), 128.2 (d), 128.6 (d), 130.7 (s), 156.1 (s) and 174.5 (s, C=O); m/z (DCI, NH_3) 327 (70%, $M+NH_4^+$), 309 (50%, M^+) and 265 (100%).

2R,3R,4R,5S-Trihydroxypipecolic acid (15). A suspension of 5-azido-5-deoxy-L-iduronolactone (0.35 g, 1.74 mmol) in 10% acetic acid - water (50 ml) was stirred in an atmosphere of hydrogen in the presence of palladium black (0.17 g) at room temperature for 3 days. The mixture was filtered through celite and the solvent removed to give a colourless residue which was dissolved in water (5 ml), applied to an ion exchange resin column (Dowex-50, H+) and eluted with 1M aqueous pyridine. The solution was then freeze dried and the residue further purified by flash chromatography (2% water in ethanol) to give 2R,3R,4R,5S-trihydroxypipecolic acid, (108 mg, 32%), m.p. 198-202°C (dec), $[\alpha]_D^{20}$ +34° (*c*, 0.35 in H_2O) ; ν_{max} (KBr) 3600-2500 (br) and 1625 (C=O) cm^{-1}, ^1H NMR (D_2O) δ 3.15 (1H, dd, 6-H, J 2.7 and 13.6 Hz)), 3.20 (1H, dd, 6-H', J, 2.6 and 13.6 Hz), 3.76 (1H, d, 2-H, J 2.5 Hz), 3.81 (1H, m), 3.85 (1H, t, J 3.7 Hz), and 4.08 (1H, m); ^{13}C NMR (D_2O) δ 45.9 (t, CH_2N), 59.1 (d, CHN), 67.1 (d, CHOH), 68.9 (d, CHOH), 69.7 (d, CHOH) and 171.6 (s, C=O); m/z (FAB) 178 (100%, $M+H^+$) (Found C, 36.4; H, 6.9; N, 6.9. $C_6H_{11}NO_5.H_2O$ requires C, 36.9; H, 6.7; N, 7.2%).

A small amount of 2S,4S,5S-dihydroxypipecolic acid (14), identical to an authentic sample, was also isolated from the reaction mixture. Hydrogenation of 5-(N-benzyloxycarbonyl)amino-5-deoxy-L-iduronolactone under the same conditions also gave 2R,3R,4R,5S-trihydroxypipecolic acid.

2R,3R,4R-Dihydroxyproline (16). A solution of 5-azido-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (1.0 g, 4.14 mmol) in trifluoracetic acid - water (3:1, 8 ml) was stirred at room temperature for 3.5 h. The solvent was removed *in vacuo* and the resulting residue was dissolved in ethanol - water (2:1, 30 ml). The solution was treated with sodium periodate (1.8 g, 8.4 mmol); the reaction mixture was then stirred at room temperature for 25 min, and extracted with ether (300 ml); the organic extracts were washed with brine, dried (sodium sulphate) and the solvent removed to give the aldehyde (11) as a colourless oil; without further purification, the aldehyde (11) in water (80 ml) was stirred in an atmosphere of hydrogen in the presence of a palladium black (300 mg) at room temperature for 4 days. The mixture was filtered through celite and the solvent removed; the residue which was dissolved in water (5 ml), applied to an ion exchange resin column (Dowex-50, H+) and eluted with 1M aqueous pyridine. The solution was then freeze dried and the residue further purified by flash chromatography (2% water in ethanol) to give 2R,3R,4R-dihydroxyproline (16), (90 mg, 15%), m.p. 247-254°C (dec), $[\alpha]_D^{20}$ +63.4° (*c*, 0.35 in H_2O) ν_{max} (KBr) 3360 (OH and NH) and 1630 (C=O) cm^{-1}; ^1H NMR (D_2O) δ 3.21 (1H, d, 5-H, J 12.8 Hz)), 3.54 (1H, dd, 5-H', J, 3.4 and 12.8 Hz), 4.22 (1H, d, J 4.0 Hz), 4.25 (1H, d, J 3.4 Hz), and 4.30 (1H, d, J 3.8 Hz); ^{13}C NMR (D_2O) δ 52.0 (t, CH_2N), 66.3 (d, CHN), 75.9 (d, CHOH), 76.4 (d, CHOH), and 171.6 (s, C=O); m/z

(DCI, NH_3) 148 (100%, $M+H^+$), and 104 (40%, $M-COOH^+$) (Found C, 40.5; H, 6.3; N, 9.2. $C_5H_9NO_4$ requires C, 40.8; H, 6.1; N, 9.5%). For the enantiomer, 2S,3S,4S-dihydroxyproline, previously reported physical properties are:[8,9] m.p. 262°C, $[\alpha]_D^{20}$ +63° (c, 0.8 in H_2O)•.

1,2-O-Isopropylidene-β-L-iduronolactone (5) was prepared by reaction of 1,2-O-isopropylidene-5-O-trifluoromethanesulphonyl-α-D-glucuronolactone (2) with sodium trifluoroacetate in dimethylformamide, followed by methanolysis, in 78% yield as previously described.[12]

5-(N-Benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-α-D-glucuronolactone (4). To a solution of 1,2-O-isoproplyidene-β-L-iduronolactone (5) (3.0 g, 13.9 mmol) in dichloromethane (100 ml) at -30°C, pyridine (3 ml) and trifluoromethane sulphonic anhydride (2.8 ml, 16.7 mmol) were added and the resulting reaction mixture was stirred for 3 h. The solution was then diluted with dichloromethane (200 ml) and then the reaction mixture was washed with saturated sodium bicarbonate (2 x 50 ml), water (2 x 80 ml) and brine (2 x 50 ml) and then dried (sodium sulphate). Removal of solvent gave the triflate (6) which, without any further purification, was dissolved in dimethylformamide; to this solution at -20°C, sodium azide (0.9 g, 13.8 mmol) was added and the reaction mixture was then stirred for 1 h. The solvent was removed *in vacuo* and the residue dissolved in chloroform (300 ml); the solution was washed with water (2 x 80 ml) and brine (50 ml) and the solvent removed to give 5-azido-5-deoxy-1,2-O-isopropylidene-α-D-glucuronolactone (3). The crude azide (3) in ethyl acetate (50 ml) was stirred in an atmosphere of hydrogen in the presence of 10% palladium on charcoal (0.5 g) for 5 h. The catalyst was removed from the reaction mixture by filtration through celite; the solution then treated with saturated aqueous sodium bicarbonate (30 ml) and benzyl chloroformate (3 ml, 21 mmol) at 0°C. The reaction was stirred for 3 h, the aqueous layer was extracted with ethyl acetate and combined with the organic layer, which was then washed with water (2 x 80 ml) and brine (50 ml) and dried (sodium sulphate). The solvent was removed and the residue purified by flash chromatography (hexane - ether) to give 5-(N-benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-α-D-glucuronolactone, [2.14 g, 44% yield from (5)], m.p. 142-144°C, $[\alpha]_D^{20}$ +40.4° (c, 0.9 in $CHCl_3$); ν_{max} (KBr) 3380 (NH), 1785 (C=O) and 1715 (C=O) cm^{-1}; ^1H NMR δ 1.33 (3H, s, Me), 1.50 (3H, s, Me), 3.88 (1H, d, 5-H, J 7.3 Hz)], 4.85 (3H, m, 2-H, 4-H and 5-H), 4.94 (1H, dd, 3-H, J 3.0 and 4.1 Hz), 5.14 (2H, s, benzyl CH_2), 5.50 (1H, br, NH), 5.91 (1H, d, 1-H, J 3.7 Hz) and 7.35 (5H, m, ArH); ^{13}C NMR δ 26.5 (q, Me), 26.8 (q, Me), 54.5 (d), 67.6 (t), 77.8 (d), 82.1 (d) 82.8 (d), 106.5 (d), 113.5 (s), 128.1 (d), 128.3 (d), 128.5 (d), 135.8 (s), 156.0 (s) and 172.0 (s, C=O); m/z (DCI, NH_3) 367 (100%, $M+NH_4^+$) (Found C, 58.2; H, 5.5; N, 3.7. $C_{17}H_{19}NO_7$ requires C, 58.5; H, 5.4; N, 4.0%).

5-(N-Benzyloxycarbonyl)amino-5-deoxy-D-glucuronolactone (12). A solution of 5-(N-benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-α-D-glucuronolactone (1.0 g, 2.86 mmol) in trifluoroacetic acid - water (3:2, 10 ml) was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the resulting residue purified by flash chromatography (ether) to give 5-(N-benzyloxycarbonyl)amino-5-deoxy-D-glucuronolactone, (0.72 g, 81%), m.p. 177-180°C, $[\alpha]_D^{20}$ +3.5° (c, 0.95 in DMSO); ν_{max} (film) 3600 - 3200 (OH, NH), 1788, 1772 (C=O) and 1695 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO) δ 3.4 (br s, OH), 3.99 (1H, s, 2-H), 4.8 (3H, m, 3-H, 4-H and 5-H), 5.10 (2H, s, benzyl CH_2), 5.18 (1H, br s, 1-H), 6.27 (1H, br s, OH), 6.75 (1H, br s, NH), and 7.38 (5H, m, ArH); ^{13}C NMR (d_6-DMSO) δ 53.0 (d), 66.4 (t), 76.2 (d), 77.4 (d) 84.5 (d), 102.9 (d), 128.3 (2 x d), 128.6 (d), 136.7 (s), 156.1 (s) and

173.8 (s, C=O); m/z (DCI, NH_3) 327 (70%, $M+NH_4^+$), 309 (60%, M^+) and 265 (100%) (Found C, 54.4; H, 5.2; N, 4.5. $C_{14}H_{15}NO_7$ requires C, 54.4; H, 4.9; N, 4.5%).

2S,3R,4R,5S-Trihydroxypipecolic acid (13). A suspension of 5-(N-benzyloxycarbonyl)-amino-5-deoxy-D-glucuronolactone, (0.31 g, 1.0 mmol) in 10% aqueous acetic acid (15 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (0.1 g) at room temperature for 3 days. The reaction mixture was filtered through celite and the solvent removed to give a colourless residue which was dissolved in water (5 ml) and applied to an ion exchange column (Dowex 50, H^+) and eluted with 1M aqueous pyridine. The solution was then freeze dried and the residue further purified by flash chromatography (ethanol, then 2% water in ethanol) to give 2S,4S,5S-dihydroxypipecolic acid (14) (8 mg, 5%) identical to an authentic sample, 2S,3R,4R,5S-trihydroxypipecolic acid, (105 mg, 59%), m.p. 228-232°C , $[\alpha]_D^{20}$ +14.1° (c, 0.3 in H_2O) [lit.[5] m.p. 228-230°C, $[\alpha]_D^{20}$ +18.3° (c, 1.0 in H_2O)]; ν_{max} (KBr) 3700-2500 (br NH and OH) and 1630 (C=O) cm^{-1}; ^1H NMR (D_2O) δ 2.80 (1H, dd, 6-H, J 10.9 and 12.6 Hz)), 3.35 (1H, dd, 6-H', J 5.9 and 12.6 Hz), 3.38 (1H, d, 2-H, J 10.0 Hz), 3.39 (1H, dd, 4-H, J 7.5 and 9.0 Hz), 3.57 (1H, dd, 3-H, J 9.2 and 10.1 Hz), and 3.66 (1H, ddd, 5-H, J 5.1, 9.2 and 11.1 Hz); ^{13}C NMR (D_2O) δ 46.0 (t, CH_2N), 61.9 (d, CHN), 67.8 (d, CHOH), 71.0 (d, CHOH), 76.5 (d, CHOH) and 172.5 (s, C=O); m/z (FAB) 178 (100%, $M+H^+$).

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