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SYNTHESIS OF 2S,4S,5S-DIHYDROXYPIPECOLIC ACID AND BULGECININE [2S,4S,5R-4-HYDROXY-5-(HYDROXYMETHYL)PROLINE] FROM D-GLUCURONOLACTONE; A STRATEGY FOR THE SYNTHESIS OF 2S,4S-4-HYDROXY-α-AMINO ACIDS

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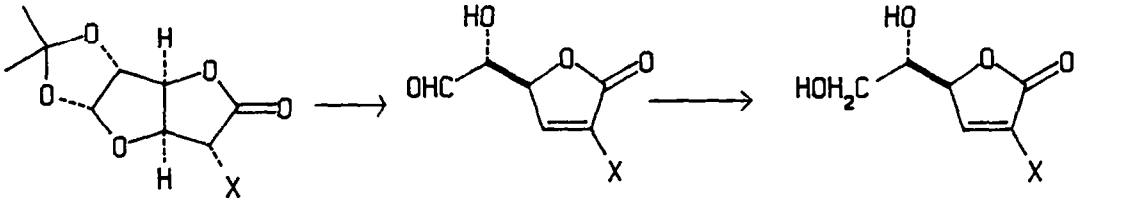
The efficient synthesis of 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (4) from D-glucuronolactone is described; the potential of (4) as a divergent intermediate for the synthesis of 2S,4S-4-hydroxy-α-amino acids is illustrated by the conversion of (4) to 2S,4S,5S-dihydroxypipecolic acid and bulgecinine [2S,4S,5R-4-Hydroxy-5-(hydroxymethyl)-proline].

The preceding paper[1] describes the synthesis of some polyhydroxylated amino acids from the readily available[2] isopropylidene derivative of D-glucuronolactone (1); this paper describes the efficient conversion of the protected ido-amine (2), easily prepared from (1) via the azide (3) in an overall yield of 63%,[1] to 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (4). The potential of (4) as a powerful divergent intermediate for the preparation of 2S,4S-4-hydroxy-L-amino acids is illustrated by the synthesis of 2S,4S,5S-dihydroxypipecolic acid (14), isolated from the leaves of *Derris eliptica*[3], and of bulgecinine [2S,4S,5R-4-hydroxy-5-(hydroxymethyl)proline] (15)[4], a constituent of the bulgecin glycopeptide antibiotics.[5,6,7] A preliminary report of this work has been published;[8] a synthesis of bulgecinine from glucose has been reported.[9]



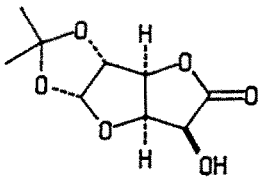
(2) X = NHZ
(3) X = N_3

(25) X = NHZ
(26) X = N_3

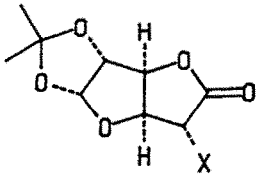
(4) X=NHZ
(27) X = N_3

SCHEME 1

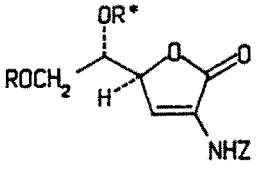
Treatment of the protected amine (2) with methoxide in methanol induced fragmentation to the unstable aldehyde (25) [Scheme 1]; subsequent reduction *in situ* by sodium borohydride afforded the crystalline diol (4) in 91% yield; there is no epimerisation at C-5 during this transformation as judged by ^{1}H NMR spectrum of (4) in which the proton attached to C-4 appears as a sharp double doublet at 5.16. The elimination reaction occurs readily and the reaction could be performed conveniently on a 5-10 g scale. A similar sodium carbonate-induced fragmentation of the azide (3) led to the formation of the aldehyde (26) which was reduced by sodium borohydride to



(1)

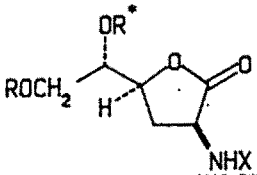


(2) X=NHZ (3) $X=N_3$

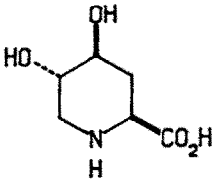


(4) R=R*=H (5) R,R*=Me_2C
(6) R=SO_2Me; R*=H (7) R=TBDMS; R*=H

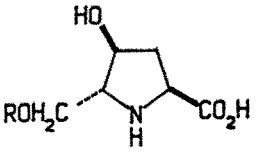
Z=$COOCH_2Ph$ TBDMS=$SiMe_2Bu^t$



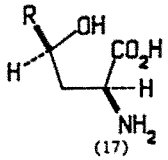
(8)R,R*=Me_2C; X=H (9)R,R*=Me_2C;X=Z
(10)R=SO_2Me; R*=X=H (11)R=TBDMS;R*=H;X=Z
(12)R=TBDMS: R*=SO_2Me; X=Z
(13)R=TBDMS; R*=SO_2Me; X=H



(14)



(15) R=H (16) R=TBDMS



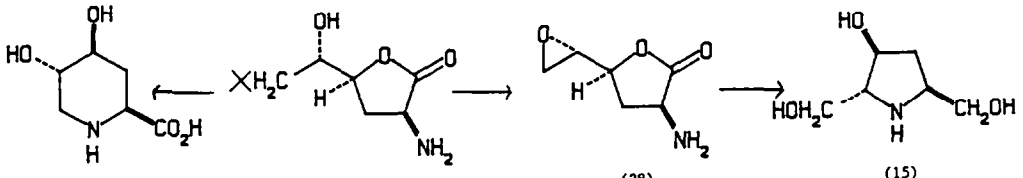
(17)

(18)R=Me (19)R=CH_2NH_2
(20)R=$CH_2CH_2NH_2$
(21)R=CH_2OH (22)R=CH_2CH_2COOH
(23)R=COOH (24)R=$CONH_2$

give (27) in 52% yield. However, the azides (26) and (27) are considerably less stable than the corresponding carbamates (25) and (4); it is more convenient to handle the carbamates rather than the azides on anything other than a small scale. Treatment of the diol (4) with 2,2-dimethoxypropane and toluene sulphonic acid gave the corresponding acetonide (5) in 97% yield; hydrogenation of the acetonide (5) in the presence of palladium black in ethyl acetate formed the amine (8) as the sole product which was reprotected as the benzyloxycarbonyl derivative (9). A number of other derivatives of the diol (4) undergo similar reduction to allow a practical and efficient sequence which establishes the correct stereochemistry at C-2, together with deoxygenation at C-3, of glucuronolactone providing suitable intermediates for the preparation of 2S,4S-4-hydroxy-α-amino acids of the general structure (17). There are a number of examples of naturally occurring amino acids of this type[10] which might be efficiently prepared using such a pathway including 2S,4S-4-hydroxynorvaline (18),[11] 2S,4S-4-hydroxyornithine (19),[12] 2S,4S-4-hydroxylysine (20),[13] 2S,4S-2-amino-4,5-dihydroxypentanoic acid (21),[14] 2S,4S-2-amino-4-hydroxypimelic acid (22),[15] 2S,4S-4-hydroxyglutamic acid (23)[16] and 2S,4S-4-hydroxyglutamine (24).[17]

The potential of the diol (4) as an intermediate for such syntheses is illustrated by the preparation of 2S,4S,5S-dihydroxypipecolic acid (14) and of bulgecinine (15) which are derived from (4) by cyclisation of the nitrogen function onto C-1 or C-2 of the original sugar, respectively. Selective esterification of diol (4) with methane sulphonyl chloride gave the primary mesylate (6).

Hydrogenation of (6) in ethyl acetate in the presence of palladium black takes place from the less hindered side to produce a single diastereomeric amino mesylate (10), which due to its lack of stability was characterised mainly by ^{1}H NMR. Treatment of the amino mesylate (10) with ethanolic potassium hydroxide gave the required pipecolic acid (14) (73% yield) contaminated with a small amount of bulgecinine (15) (7% yield); the ratio of (14) and (15) in the crude product may be estimated by ^{1}H NMR. A possible pathway [Scheme 2] for the formation of bulgecinine in this reaction



SCHEME 2

involves the initial formation of the epoxide (28) which would undergo intramolecular cyclisation by nitrogen with a 5-exo-tet process preferred to a competing 7-endo-tet process.[18] The crude pipecolic acid may be purified as the crystalline hydrochloride of (14) [25% from isopropylidene glucuronolactone (1)], with identical spectroscopic properties (^{1}H NMR, i.r., mass spec) to those of an authentic sample[19] and in agreement with previously reported data for the compound.

Protection of the primary hydroxyl group in (4) with tert-butyldimethylsilyl chloride gave the crystalline silyl ether (7) (91% yield); subsequent hydrogenation of (7) in ethyl acetate in the presence of palladium black, followed by reprotection of the amine by reaction with benzyl chloroformate, gave (11) as a crystalline compound in 90% yield; this hydrogenation is also highly stereoselective since ^{1}H NMR again indicates the presence of only one diastereomer. Reaction of (11) with methane sulphonyl chloride and pyridine in the presence of a catalytic amount of 4-(dimethylamino)pyridine gave the corresponding mesylate (12) (98% yield), also as a crystalline solid. Removal of the carbamate protecting group by hydrogenolysis gave the amino mesylate (13) which, with ethanolic potassium hydroxide under conditions which were successful for the cyclisation to dihydroxypipecolic acid (14), gave a complex mixture of products. However, (13) cleanly cyclises to the silylated amino acid (16) in the presence of ethanolic sodium bicarbonate solution; the silyl ether protecting group was removed by mild acid treatment to give bulgecinine (15) with identical spectroscopic properties (^{13}C and ^{1}H NMR, i.r., mass spec) with an authentic sample[20] and in agreement with previously reported data for the compound. In summary, this paper describes the synthesis of suitable intermediates for a general approach to 2S,4S-4-hydroxy-α-amino acids.[21]

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. 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 or 5% dodecamolybdophosphoric acid in methanol, or 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. 5-(N-Benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (2) and 5-azido-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (3) were prepared from D-glucuronolactone in overall respective yields of 63% and 83%.[1]

4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (4). To a solution of 5-(N-benzyloxycarbonyl)amino-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (2) (4.22 g, 12.66 mmol) in dry methanol (300 ml) at -10°C, a solution of sodium methoxide in methanol (14.0 ml, 1.0 M in methoxide) was added dropwise over 2 min. After the addition was complete, sodium borohydride (600 mg, 15.9 mmol) was added in one portion and the reaction mixture was stirred at 0°C for 10 min. The reaction was then quenched by addition of phosphate buffer (pH 7, 1.0 M, 80 ml) and the solvent was evaporated *in vacuo*. The residue was treated with water (30 ml) and then extracted with ethyl acetate (5 x 75 ml); the combined organic extracts were then dried (magnesium sulphate) and the solvent removed. The residue was recrystallised from chloroform to give 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide, (3.3 g, 91%), m.p. 148-150°C , $[\alpha]_D^{20}$ +54.7° (*c*, 0.17 in EtOH); ν_{max} (KBr) 3600-3360 (br OH), 3330 (NH), 1752 (C=O) 1695 (C=O) and 1666 (C=C) cm^{-1}; ^1H NMR δ 2.0 (1H, br, OH), 2.45 (1H, br, OH), 3.75 - 3.90 (3H, m, 5-H and 6-H), 5.16 (1H, dd, 4-H, J 2.0 and 4.7 Hz), 5.22 (2H, s, benzyl CH_2), 6.99 (1H, br s, NH), 7.12 (1H, br s, 3-H), and 7.38 (5H, m, ArH); ^{13}C NMR (CD_3OD) δ 64.0 (t), 68.3 (t), 73.0 (d), 83.0 (d), 126.5 (d), 128.8 (d), 129.1 (d), 129.3 (d), 129.5 (d), 137.6 (s), 155.5 (s) and 170.7 (s, C=O); *m/z* (DCI, NH_3) 311 (100%, $M+NH_4^+$), 294 (80%, $M+H^+$), 108 (55%, $PhCH_2OH^+$) and 91 (80%, $C_7H_7^+$) (Found C, 57.4; H, 5.1; N, 4.6. $C_{14}H_{15}NO_6$ requires C, 57.3; H, 5.2; N, 4.8%). This experiment was also performed on twice this scale resulting in the same yield.

4S,5S-2-Azido-5,6-dihydroxyhex-2-en-4-olide (27). Anhydrous sodium carbonate (1.02 g, 9.62 mmol) was added in one portion to a solution of 5-azido-5-deoxy-1,2-O-isopropylidene-β-L-iduronolactone (3) (3.04 g, 8.46 mmol) in dry methanol (50 ml) at 0°C and the reaction stirred for 3 min. Sodium borohydride (0.24 g, 6.34 mmol) was then added and the mixture stirred at 0°C for a further 20 min. The reaction was then quenched with water (10 ml) and evaporated to dryness *in vacuo*. The residue was dissolved in the minimum amount of acetone and filtered through celite; the filter cake was washed with ethyl acetate (200 ml) and the combined organic extracts evaporated to give 4S,5S-2-azido-5,6-dihydroxyhex-2-en-4-olide, (0.81 g, 52%), as an oil; ^1H NMR δ 2.10 - 2.90 (2H, br s, OH), 3.72 - 3.90 (3H, m) 5.07 - 5.17 (1H, br s, 4-H) and 6.60 - 6.67 (1H, br s, 3-H). This compound is relatively unstable and decomposes over a day at room temperature.

4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxy-5,6-O-isopropylidene-hex-2-en-4-olide (5). A mixture of 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (3.0 g, 10.2 mmol) and toluenesulphonic acid (5 mg) in 2,2-dimethoxypropane (80 ml) was stirred at 20°C for 30 h, after which the solvent was removed and the residue purified by flash chromatography to give 4S,5S-2-(N-benzyloxycarbonyl)amino-

5,6-dihydroxy-5,6-O-isopropylidene-hex-2-en-4-olide, (3.2 g, 95%), m.p. 110-111°C (from ether), $[\alpha]_D^{20}$ +24.3° (c, 0.12 in $CHCl_3$); ν_{max} (KBr) 3340 (NH), 1765 (C=O), 1690 (C=O) and 1662 (C=C) cm^{-1}; ^1H NMR δ 1.36 (3H, s, Me), 1.44 (3H, s, Me), 3.86 (1H, m, 5-H or 6-H), 4.10 (1H, m, 5-H or 6-H), 4.24 (1H, m, 5-H or 6-H), 5.06 (1H, dd, 4-H, J 1.8 and 4.4 Hz), 5.22 (2H, s, benzyl CH_2), 6.98 (1H, br, NH), 7.07 (1H, br, 3-H), and 7.38 (5H, m, ArH); m/z (DCI, NH_3) 351 (80%, $M+NH_4^+$), 334 (100%, $M+H^+$), 243 (60%, $M+H-C_7H_7^+$) and 91 (40%, $C_7H_7^+$) (Found C, 61.4; H, 5.9; N, 4.1. $C_{17}H_{19}NO_6$ requires C, 61.3; H, 5.7; N, 4.2%).

2S,4S,5S-2-Amino-5,6-dihydroxy-5,6-O-isopropylidene-hexan-4-olide (8). 4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxy-5,6-O-isopropylidene-hex-2-en-4-olide (5) (12 mg, 0.036 mmol) was hydrogenated in ethyl acetate (3 ml) in the presence of palladium black (5 mg) at room temperature for 24 h. The reaction mixture was filtered through a pack of celite (3 g) and the filter cake washed thoroughly with ethyl acetate (2 x 20 ml). The solvent was removed to give 2S,4S,5S-2-amino-5,6-dihydroxy-5,6-O-isopropylidene-hexan-4-olide, (7 mg, 97%), as an oil; ^1H NMR δ 1.38 (3H, s, Me), 1.43 (3H, s, Me), 1.98 (1H, ddd, 3-H, J 8.9, 9.7 and 12.8 Hz), 2.26 - 2.41 (2H, br, NH_2), 2.52 (1H, ddd, 3-H', J 6.5, 9.7 and 12.9 Hz),3.75 (1H, t, 2-H, J 9.5 Hz), 3.92 (1H, dd, 6-H, J 6.6 and 8.5 Hz), 4.10 (1H, dd, 6-H', J 6.8 and 8.5 Hz), 4.21 (1H, dt, 5-H , J 3.6 and 6.6 Hz), and 4.43 (1H, ddd, 4-H, J 3.6, 6.6 and 8.8 Hz); m/z (DCI, NH_3) 202 (100%, $M+H^+$).

2S,4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxy-5,6-O-isopropylidene-hexan-4-olide (9). A solution of 2S,4S,5S-2-amino-5,6-dihydroxy-5,6-O-isopropylidene-hex-2-en-4-olide (5)(0.50 g, 1.50 mmol) in ethyl acetate (20 ml) was hydrogenated for 24 h in the presence of palladium black (140 mg); the catalyst was removed by filtration. The filtrate was cooled to 0°C, treated with saturated aqueous sodium bicarbonate (20 ml) and benzylchloroformate (0.25 ml, 1.8 mmol), and the reaction mixture stirred at 0°C for 15 min. The organic layer was separated and the aqueous layer extracted with ethyl acetate (50 ml); the combined organic extracts were washed with brine and dried (sodium sulphate) and the solvent removed to form a residue which was purified by flash chromatography (ethyl acetate - hexane, 1:1) to give 2S,4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxy-5,6-O-isopropylidene-hexan-4-olide, (360 mg, 72%), m.p. 102-104, $[\alpha]_D^{20}$ +18.5° (c, 0.26 in $CHCl_3$); ν_{max} (KBr) 3340 (NH), 1765 (C=O), 1690 (C=O) and 1662 (C=C) cm^{-1}; ^1H NMR δ 1.38 (3H, s, Me), 1.43 (3H, s, Me), 2.08 (1H, dt, 3-H, J 9.8 and 12.8 Hz), 2.75 (1H, m, 3-H'), 3.9 - 4.6 (5H, m, 2-H, 4-H, 5-H and 6-H), 5.14 (2H, br s, benzyl CH_2), 5.50 (1H, br, NH), and 7.37 (5H, m, ArH); ^{13}C NMR δ 25.3 (q, Me), 26.0 (q, Me), 31.7 (t, C-3), 50.5 (d), 65.0 (t), 67.3 (t), 76.1 (d), 76.2 (d), 110.4 (s), 128.1 (d), 128.2 (d), 128.5 (d), 135.9 (s), 155.9 (s) and 173.8 (s, C=O); m/z (DCI, NH_3) 353 (30%, $M+NH_4^+$), 336 (30%, $M+H^+$), 292 (60%, $M-C_3H_7^+$), 245 (60%, $M+H-C_7H_7^+$) and 228 (100%, $M-C_7H_7O^+$) (Found C, 60.7; H, 6.2; N, 4.0. $C_{17}H_{21}NO_6$ requires C, 60.9; H, 6.3; N, 4.2%).

4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxy-6-O-methanesulphonyl-hex-2-en-4-olide (6). A solution of 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (4) (586 mg, 2.00 mmol) and methane sulphonyl chloride (0.186 ml, 2.20 mmol) in pyridine (8 ml) were stirred at -15°C for 30 min. The reaction mixture was then poured into water (20 ml) and extracted with dichloromethane (2 x 30 ml). The combined organic extracts were washed with 5% aqueous hydrochloric acid (50 ml) and dried (magnesium sulphate); after removal of the solvent, the residue was purified by flash chromatography (ethyl acetate - hexane, 2:1) to give, after recrystallisation from chloroform, needles of 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxy-6-O-methanesulphonyl-hex-2-en-4-olide, (542 mg, 80%), m.p. 139-140°C,

$[\alpha]_D^{20}$ +21.7° (c, 0.51 in $CHCl_3$); ν_{max} (KBr) 3500-3250 (OH), 3120 (NH), 1735 (C=O) 1720 (C=O), 1660 (C=O), 1350 (SO_2), and 1170 (SO_2) cm^{-1}; ^1H NMR δ 2.49 (1H, br, OH), 3.11 (3H, s, CH_3SO_2), 4.12 (1H, m, 5-H), 4.38 (2H, m, 6-H), 5.18 (1H, dd, 4-H, J 2.0 and 4.1 Hz), 5.23 (2H, s, benzyl CH_2), 7.02 (1H, br, NH), 7.10 (1H, m, 3-H), and 7.39 (5H, m, ArH); m/z (DCI, NH_3) 389 (100%, $M+NH_4^+$), 372 (25%, $M+H^+$), 108 (40%, $PhCH_2OH^+$) and 91 (45%, $C_7H_7^+$) (Found C, 48.5; H, 4.6; N, 3.8. $C_{15}H_{17}NO_8S$ requires C, 48.5; H, 4.6; N, 3.8%).

Further elution of the column with ethyl acetate allowed recovery of the starting diol (4), (48 mg).

2S,4S,5S-Dihydroxypipecolic Acid Hydrochloride (14). 4S,5S-2-(N-Benzyloxycarbonyl)-amino-5,6-dihydroxy-6-O-methanesulphonyl-hex-2-en-4-olide (6) (380 mg, 1.02 mmol) in ethyl acetate (50 ml) and pyridine (1 ml) was stirred in an atmosphere of hydrogen in the presence of palladium black (200 mg) at 20°C for 4h. The reaction mixture was filtered through celite (10 g) and the filter cake washed with ethyl acetate (2 x 20 ml). Removal of solvent gave the saturated aminomesylate (10) as an oil, ^1H NMR δ 1.5 - 1.8 (3H, br, OH and NH_2), 2.07 (1H, dt, 3-H, J 7.9 and 13.2 Hz), 2.69 (1H, dt, 3-H', J 8.2 and 13.2 Hz), 3.10 (3H, s, CH_3SO_2), 3.82 (1H, t, 2-H, J 8.1 Hz), 3.97 (1H, m, 5-H), 4.36 (2H, m, 6-H), and 4.64 (1H, dt, 4-H, J 2.7 and 7.1 Hz). This compound (10) was then treated with potassium hydroxide (1.2 ml, 2M in water, 2.4 mmol) in ethanol - water (1:1, 40 ml) at 20°C for 1 h. The pH of the reaction was then adjusted to 7 and the solvent evaporated; the residue was purified by ion exchange chromatography (Dowex 50, H^+ form, eluted with 1M aqueous pyridine) to give, after freeze drying, a mixture of 2S,4S,5S-dihydroxypipecolic acid (14) and bulgecinine (15) (134 mg, 81%) in the ratio of 11 to 1 as judged by ^1H NMR. The free amino acid (14) has the following ^1H NMR (D_2O) δ 1.56 (1H, ddd, 3-H_{ax}, J 10.7, 12.6 and 14.1 Hz), 2,36 (1H, dt, 3-H_{eq}, J 3.8 and 14.1 Hz), 2.72 (1H, dd, 6-H_{ax}, J 10.7 and 12.5 Hz), 3.36 (1H, dd, 6-H_{eq}, J 4.5 and 12.6 Hz), and 3.50 - 3.63 (3H, m, 2-H, 4-H and 5-H). This amino acid mixture was dissolved in aqueous hydrochloric acid (1 ml, 2M, 2.0 mmol) and the water removed *in vacuo*; the residue crystallised on standing to give the hydrochloride salt of 2S,4S,5S-dihydroxypipecolic acid (14), [113 mg], as cubes, m.p. 195-196°C , $[\alpha]_D^{20}$ +24.4° (c, 0.64 in 2M aqueous HCl) [lit.[3] $[\alpha]_D^{20}$ +19.5° (c, 0.15 in 2M aqueous HCl)]; ν_{max} (KBr) 3600-3250 (OH), and 1740 (C=O) cm^{-1}; ^1H NMR (D_2O) δ 1.71 (1H, dt, 3-H_{ax}, J 10.5 and 14.3 Hz), 2.41 (1H, dt, 3-H_{eq}, J 3.8 and 14.2 Hz), 2.79 (1H, dd, 6-H_{ax}, J 9.3 and 12.7 Hz), 3.42 (1H, dd, 6-H_{eq}, J 4.1 and 12.9 Hz), 3.55 - 3.69 (2H, m, 4-H and 5-H), and 3.90 (1H, dd, 2-H, J 3.9 and 11.0 Hz); m/z (FAB) 162 (100%, $M+H^+$), 75 (65%), and 57 (30%) (Found C, 36.5; H, 6.3; N, 6.9. $C_6H_{12}NO_4Cl$ requires C, 36.5; H, 6.1; N, 7.1%). The ^1H NMR of a sample of the authentic hydrochloride[19] of (14) was superimposable on that of this synthetic material.

4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxy-6-O-tert-butyldimethylsilyl-hex-2-en-4-olide (7). 4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxyhex-2-en-4-olide (4) (939 mg, 3.2 mmol) was dissolved in a mixture of dimethylformamide (6 ml) and dichloromethane (20 ml) and treated with dimethylaminopyridine (15 mg, 0.12 mmol), triethylamine (0.5 ml) and tert-butyldimethylsilyl chloride (540 mg, 3.58 mmol); the reaction mixture was stirred at 20°C for 12 h, then poured into water (20 ml) and extracted with dichloromethane (3 x 50 ml). The combined organic extracts were washed with water (50 ml), dried (magnesium sulphate) and the solvent removed to leave a residue which was recrystallised from toluene - petroleum ether (b.p. 40-60°C) to give white needles of 4S,5S-2-(N-benzyloxycarbonyl)amino-5,6-dihydroxy-6-O-tert-butyldimethylsilyl-hex-2-en-4-olide, (1.19 g, 91%), m.p. 108.5-110°C , $[\alpha]_D^{20}$ +27.2° (c, 0.75 in $CHCl_3$); ν_{max} (KBr) 3600-3400 (br), 3305 (NH), 1760 (C=O), 1730

(C=O) and 1660 (C=C) cm^{-1}; ^1H NMR δ 0.09, 0.10 (6H, 2s, $SiMe_2$), 0.91 (9H, s, t-Bu), 2.36 (1H, br, OH), 3.70 - 3.85 (3H, m, 5-H and 6-H), 5.17 (1H, dd, 4-H, J 1.8 and 4.1 Hz), 5.22 (2H, s, benzyl CH_2), 6.99 (1H, br, NH), 7.14 (1H, br, 3-H), and 7.38 (5H, m, ArH); ^{13}C NMR δ -5.6 (q, Me_2Si), 18.1 (s, Me_3CCSi), 25.7 (q, Me), 63.2 (t), 67.7 (t), 72.0 (d), 81.6 (d), 124.0 (d), 126.7 (d), 128.1 (d), 128.4 (d), 128.5 (d), 135.3 (s), 153.0 (s) and 169.0 (s, C=O); m/z (DCI, NH_3) 425 (45%, $M+NH_4^+$), 408 (100%, $M+H^+$), 108 (35%, $PhCH_2OH^+$) and 91 (90%, $C_7H_7^+$) (Found C, 59.1; H, 7.2; N, 3.4. $C_{20}H_{29}NO_6Si$ requires C, 59.0; H, 7.2; N, 3.4%).

2S,4S,5S-2-(N-Benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-hexan-4-olide (11). 4S,5S-2-(N-Benzyloxycarbonyl)amino-5,6-dihydroxy-6-O-tert-butyl-dimethylsilyl-hex-2-en-4-olide (7) (777 mg, 1.91 mmol) was hydrogenated in ethyl acetate (60 ml) and pyridine (1 ml) in the presence of palladium black (250 mg) at room temperature for 24 h. The reaction mixture was filtered through a pack of celite (10 g) and the filter cake washed with ethyl acetate (2 x 20 ml). The combined organic extracts were cooled to 0°C and treated with benzyl chloroformate (341 mg, 2.00 mmol) and saturated aqueous sodium bicarbonate solution (10 ml) and the resulting two phase system stirred at 0°C for 1 h. The organic layer was separated and the aqueous layer extracted with ethyl acetate (20 ml); the combined organic extracts were dried (magnesium sulphate) and the solvent removed to form a residue which was purified by flash chromatography (ether - petroleum ether b.p. 40-60°C, 2:1) to give, after crystallisation from carbon tetrachloride, needles of 2S,4S,5S-2-(N-Benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-hexan-4-olide, (706 mg, 90%), m.p. 99-101°C, $[\alpha]_D^{20}$ +16.4° (c, 0.34 in $CHCl_3$); ν_{max} (KBr) 3600 - 3350 (OH), 3300 (NH), 1765 (C=O), and 1700 (C=O) cm^{-1}; ^1H NMR δ 0.09 (6H, s, $SiMe_2$), 0.91 (9H, s, t-Bu), 2.21 (1H, dt, 3-H, J 9.5 and 13.0 Hz), 2.58 (1H, br, OH), 2.73 (1H, m, 3-H'), 3.68 - 4.63 (5H, m, 2-H, 4-H, 5-H and 6-H), 5.14 (2H,br s, benzyl CH_2), 5.57 (1H, br, NH), and 7.35 (5H, m, ArH); ^{13}C NMR δ -5.5 (q, Me_2Si), 18.1 (s, Me_3CCSi), 25.8 (q, Me), 30.8 (t, C-3), 50.7 (d), 63.2 (t), 67.1 (t), 72.2 (d), 77.0 (d), 127.99 (d), 128.03 (d), 128.4 (d), 136.0 (s), 156.2 (s) and 174.9 (s, C=O); m/z (ACE, NH_3) 427 (20%, $M+NH_4^+$), 410 (30%, $M+H^+$), 366 (50%), 319 (30%), 108 (40%, $PhCH_2OH^+$) and 91 (100%, $C_7H_7^+$) (Found C, 58.8; H, 7.8; N, 3.4. $C_{20}H_{31}NO_6Si$ requires C, 58.7; H, 7.6; N, 3.4%).

2S,4S,5S-2-(N-Benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-5-O-methanesulphonyl-hexan-4-olide (12). 2S,4S,5S-2-(N-Benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-hexan-4-olide (11) (482 mg, 1.18 mmol), 4-dimethyl-amino-pyridine (50 mg, 0.41 mmol) and methane sulphonyl chloride (172 mg, 1.50 mmol) were stirred in pyridine (10 ml) at room temperature for 14 h. The reaction mixture was poured into 5% aqueous hydrochloric acid (20 ml) and extracted with dichloromethane (3 x 20 ml); the combined organic extracts were washed with 5% aqueous hydrochloric acid (50 ml) and brine (20 ml) and dried (magnesium sulphate). The residue obtained after removal of the solvent was recrystallised from carbon tetrachloride to give prisms of 2S,4S,5S-2-(N-benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-5-O-methanesulphonyl-hexan-4-olide, (565 mg, 98%), m.p. 113-114°C, $[\alpha]_D^{20}$ +22.1° (c, 0.43 in $CHCl_3$); ν_{max} (KBr) 3340 (NH), 1770 (C=O), 1710 (C=O), 1330 and 1175 (SO_2) cm^{-1}; ^1H NMR δ 0.10, 0.12 (6H, 2s, $SiMe_2$), 1.57 (9H, s, t-Bu), 2.20 (1H, m, 3-H), 2.87 (1H, m, 3-H'), 3.14 (3H, s, $MeSO_2$), 3.94 (2H, m, 6-H), 4.48 - 4.82 (3H, m, 2-H, 4-H, and 5-H), 5.15 (2H,br s, benzyl CH_2), 5.40 (1H, br, NH), and 7.39 (5H, m, ArH); m/z (DCI, NH_3) 505 (100%, $M+NH_4^+$), 397 (90%), 354 (60%), 108 (40%, $PhCH_2OH^+$) and 91 (30%, $C_7H_7^+$) (Found C, 51.5; H, 6.9; N, 2.8. $C_{21}H_{33}NO_8SSi$ requires C, 51.7; H, 6.8; N, 2.9%).

Bulgecinine [2S,4S,5R-4-hydroxy-5-(hydroxymethyl)proline (15). 2S,4S,5S-2-(N-Benzyloxycarbonyl)amino-6-O-tert-butyldimethylsilyl-5,6-dihydroxy-5-O-methanesulphonylhexan-4-olide (12) (1.20 g, 2.46 mmol) in ethyl acetate (40 ml) and ethanol (10 ml) was hydrogenated using a catalyst of palladium black at room temperature for 48 h. The solution was filtered through celite (10 g) and the filter cake washed with ethyl acetate (3 x 50 ml); the organic extracts were combined, dried (magnesium sulphate) and the solvents removed to give the aminomesylate (13) as an oil, ^{1}H NMR δ 0.11, 0.12 (6H, 2s, $SiMe_2$), 0.91 (9H, s, t-Bu), 1.8 - 2.8 (2H, br, NH_2), 2.05 (1H, m, 3-H), 2.76 (1H, m, 3-H'), 3.16 (3H, s, $MeSO_2$), 3.92 (3H, m, 2-H and 6-H), and 4.64 - 4.73 (2H, m, 4-H, and 5-H); this compound (13) was dissolved in ethanol (100 ml) and stirred with saturated aqueous sodium bicarbonate (10 ml) at room temperature for 24 h. The solvent was evaporated *in vacuo* and the residue treated with 5% aqueous hydrochloric acid (15 ml) in tetrahydrofuran (50 ml) for 4 h in order to remove the silyl ether protecting group. The pH of the solution was adjusted to 7, and the reaction mixture freeze dried; the residue was purified by ion exchange chromatography (Dowex 50, H^+ form, elution with 1M aqueous pyridine) to give bulgecinine (15), (220 mg, 56% yield), as an oil which slowly crystallised as needles on standing in 30% aqueous ethanol (2 ml), m.p. 188-192°C , $[\alpha]_D^{20}$ -15.6° (c, 0.53 in water) [lit.[4] m.p. 183°C , $[\alpha]_D^{20}$ -13.1° (c, 0.95 in water)]; ν_{max} (KBr) 3500-2700 (br, NH and OH), and 1630 (C=O) cm^{-1}; ^{1}H NMR (D_2O) δ 1.97 (1H, ddd, 3-H, J 5.2, 6.5 and 13.8 Hz), 2.49 (1H, ddd, 3-H', J 5.8, 9.1 and 13.8 Hz), 3.53 - 3.75 (3H, m, 5-H and CH_2O), 4.02 (1H, dd, 2-H, J 6.6 and 9.1 Hz), and 4.20 (1H, ddd, 4-H, J 4.6, 5.1 and 5.4 Hz); ^{13}C NMR (D_2O) δ 37.1 (t), 58.7 (t), 60.0 (d), 67.5 (d), 71.2 (d), and 174.7 (s); m/z (DCI, NH_3) 162 (100%, $M+H^+$). The literature[4] values for ^{13}C NMR (D_2O) of bulgecinine are δ 37.3 (t), 58.8 (t), 60.1 (d), 67.6 (d), 71.3 (d), and 174.4 (s); also the ^{1}H NMR spectrum of synthetic bulgecinine (16) is superimposable on the ^{1}H NMR spectrum of an authentic sample of bulgecinine.[20]

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