Synthesis of δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-valine † and some Carbon-13 and Nitrogen-15 Labelled Isotopomers

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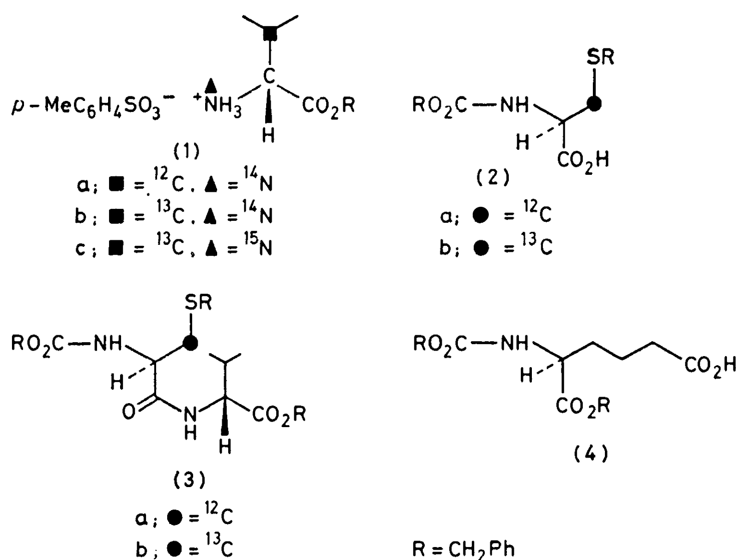
δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-valine (7a), δ-(L-α-aminoadipoyl)-L-[3-^{13}C]cysteinyl-D-valine (7b), δ-(L-α-aminoadipoyl)-L-[3-^{13}C]cysteinyl-D-[3-^{13}C]valine (7c), and δ-(L-α-aminoadipoyl)-L-[3-^{13}C]cysteinyl-D-[3-^{13}C, ^{15}N]valine (7d) were prepared by conventional means using benzyl-based protective groups.

As part of a research programme designed to study the cell-free conversion of the tripeptide δ-(L-aminoadipoyl)-L-cysteinyl-D-valine into isopenicillin N by means of ^{13}C n.m.r. spectroscopy we required several labelled isotopomers of the tripeptide. Three syntheses of this peptide have been reported [1-3] all of which differ in the type of protective groups used and, consequently, in the number and nature of the subsequent deprotection steps used to give the free peptide. We describe herein two alternative syntheses of this peptide which involve only benzyl-based protective groups, and a final deprotection step with sodium in liquid ammonia which removes all protective groups in one step. Both syntheses proceed *via* the fully protected tripeptide (5) but differ in the sequence and reagents of the peptide coupling steps. In Method A, which was used to prepare (7a) and the singly labelled peptide (7b), synthesis was *via* the protected dipeptides (3); in Method B, which was used to prepare (7a), (7c), and (7d), synthesis was *via* the partially protected dipeptides (6).

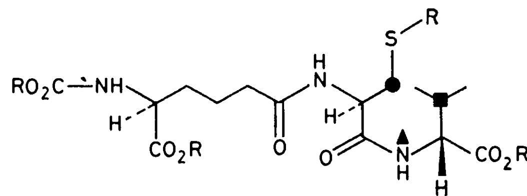
The synthesis of the fully protected tripeptide (5a) by Method A is now described. Coupling of the tosyl ammonium salt of D-valine benzyl ester [4] (1a) with the *NS*-protected L-cysteine derivative [5] (2a) by means of hydroxybenzotriazole and dicyclohexylcarbodi-imide [6] gave the fully protected dipeptide (3a). Selective removal of the *N*-benzyloxycarbonyl group with hydrogen bromide in acetic acid and treatment with triethylamine gave the free amine which was coupled with the derivative [7] (4) of L-α-aminoadipic acid (again with hydroxybenzotriazole and dicyclohexylcarbodi-imide) to give the fully protected tripeptide (5a) in 40% overall yield from (2a). The singly labelled isotopomer (5b) was prepared in a similar manner from (2b). The position and extent (92 atom % ^{13}C) of labelling was confirmed with n.m.r. spectroscopy. The three-spin system of the protons at C-2 and C-3 of the cysteine residue in (5a) gave rise to an ABX pattern in the ^{1}H n.m.r. spectrum, whilst the same protons in (5b) gave a pattern characteristic of the ABM part of an ABMX system.



The synthesis of the fully protected tripeptide (5a) by Method B is now described. Treatment of the derivative (4) of L-α-aminoadipic acid with isobutyl chloroformate in the presence of triethylamine gave a mixed anhydride which was coupled with *S*-benzyl-L-cysteine to give the partially protected dipeptide (6a). Reaction of crude (6a) with the ammonium tosylate of D-valine benzyl ester (1a) in the presence of triethylamine and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline gave the fully protected tripeptide (5a), which was identical with the

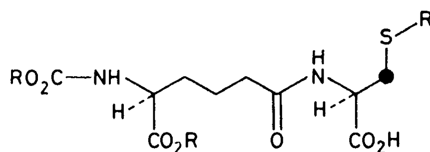
† α-Aminoadipoyl = 5-amino-5-carboxypentanoyl throughout.

product obtained from Method A. The overall yield was 46% from the D-valine derivative (1a). In a similar manner, reaction of *S*-benzyl-L-[3-^{13}C]cysteine with (4) gave the ^{13}C-labelled derivative (6b). Reaction of this with the derivatives (1b) and (1c) of labelled D-valine gave the fully protected tripeptide isotopomers (5c) and (5d) respectively.



(5)

a; ● = ^{12}C, ■ = ^{12}C, ▲ = ^{14}N
b; ● = ^{13}C, ■ = ^{12}C, ▲ = ^{14}N
c; ● = ^{13}C, ■ = ^{13}C, ▲ = ^{14}N
d; ● = ^{13}C, ■ = ^{13}C, ▲ = ^{15}N

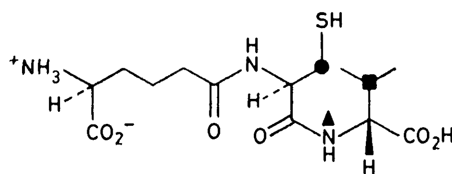


(6)

a; ● = ^{12}C
b; ● = ^{13}C

R = CH$_2$Ph

Treatment of the fully protected tripeptide (5a) with sodium in liquid ammonia removed all protective groups and gave the free tripeptide (7a) which was separated as



(7)

a; ● = ^{12}C, ■ = ^{12}C, ▲ = ^{14}N
b; ● = ^{13}C, ■ = ^{12}C, ▲ = ^{14}N
c; ● = ^{13}C, ■ = ^{13}C, ▲ = ^{14}N
d; ● = ^{13}C, ■ = ^{13}C, ▲ = ^{15}N

the mercury complex. Decomposition of this complex with hydrogen sulphide gave, in 98% yield, crude tripeptide which appeared homogeneous by ^{1}H and ^{13}C n.m.r. spectroscopy, but was shown by combustion analysis to contain *ca.* 30% ash. Final purification of the product was by gel filtration chromatography. Deprotection of the fully protected tripeptide isomers (5b), (5c), and (5d) gave the corresponding tripeptides (7b), (7c), and (7d).

EXPERIMENTAL

DL-[3,3′-^{13}C$_2$]Cystine was prepared from ^{13}C-paraformaldehyde (92 atom %, Merck Sharp and Dohme) using the method described [8] for the preparation of the ^{14}C-isotopomer. The labelled cystine was converted [9] into *S*-benzyl-DL-[3-^{13}C]cysteine and resolved [10] *via* the *N*-acetyl derivative with hog kidney acylase to give *S*-benzyl-L-[3-^{13}C]cysteine. This was converted into *N-benzyloxycarbonyl-S-benzyl-L-[3-^{13}C]cysteine* (2b) using the literature procedure.[5]

DL-[3-^{13}C]Valine and DL-[3-^{13}C,^{15}N]valine were prepared from [^{13}C]methanol (99 atom %, Prochem) and $^{15}NH_4Cl$ (97 atom %, Prochem) using the sequence of Whaley *et al.*[11] Resolution [12] with hog kidney acylase gave the corresponding D-antipodes which on subsequent reaction with benzyl alcohol gave the ammonium tosylate salts of *D-[3-^{13}C]-valine benzyl ester* (1b) and *D-[3-^{13}C,^{15}N]valine benzyl ester* (1c).

N-Benzyloxycarbonyl-L-α-aminoadipic Acid α-Benzyl Ester (4).—*N*-Benzyloxycarbonyl-L-α-aminoadipic acid [7] (0.295 g, 1.0 mmol) was dissolved in methanol (1.0 cm^3) and treated with caesium carbonate (0.163 g, 0.5 mmol) in water (1.0 cm^3). The solution was evaporated to dryness and the residue re-evaporated twice from dimethylformamide (DMF) (2.0 cm^3) at 45 °C to give a white solid. A solution of this solid in DMF (2.0 cm^3) was treated with freshly distilled benzyl bromide (0.205 g, 1.2 mmol) and the mixture stored at 50 °C for 30 min and then allowed to cool. The mixture was diluted with water (10 cm^3) and then extracted with ether (10 cm^3). The ether solution was washed with 0.01 mol dm^{-3} aqueous $NaHCO_3$ (3 × 5 cm^3) and saturated aqueous NaCl (5 cm^3), and then dried. Evaporation of the solvent and the excess of benzyl bromide gave a colourless gum which crystallised from benzene to give *N-benzyloxycarbonyl-L-α-aminoadipic acid α-benzyl* ester (4) (0.154 g, 40%), m.p. 90—92 °C; $[\alpha]_D^{25}$ −13.3° (*c* 2, acetone) (lit.,[7] m.p. 92—94 °C, $[\alpha]_D^{25}$ −14° under identical conditions, and +13.5° for the D-antipode); ^{1}H n.m.r. (90 MHz, $CDCl_3$): δ 1.4—2.0 (4 H, m, β- and γ-methylene protons), 2.30 (2 H, bt, *J ca.* 6.5 Hz, δ-methylene protons), 4.4 (1 H, bm, α-*H*), 5.08 (2 H, s, OC*H*$_2$Ph), 5.14 (2 H, s, OC*H*$_2$Ph), 5.4 (1 H, bs, exchanges with D_2O, NH), 7.30 (10 H, s, aromatic protons), and 9.2 (1 H, bs, exchanges with D_2O, CO_2H).

N-Benzyloxycarbonyl-S-benzyl-L-cysteinyl-D-valine Benzyl Ester (3a) *and N-Benzyloxycarbonyl-S-benzyl-L-[3-^{13}C]cysteinyl-D-valine Benzyl Ester* (3b).—*N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteine [5] (2a) (0.35 g, 1.0 mmol), 1-hydroxybenzotriazole (0.14 g, 1.0 mmol), and dicyclohexylcarbodiimide (0.21 g, 1.0 mmol) were mixed at 0 °C in chloroform (20 cm^3) and the mixture stirred at 0 °C for 1 h, then at room temperature for 1 h. A solution of the ammonium tosylate salt of D-valine benzyl ester (1a) (0.38 g, 1.0 mmol) and triethylamine (0.15 cm^3, 1.0 mmol) in chloroform (10 cm^3) was added and the mixture was evaporated to give a white solid. The solid was chromatographed on silica gel. Elution with ethyl acetate gave the crude product (0.51 g, 95%) which crystallised from ethyl acetate–pentane to give *N-benzyloxycarbonyl-S-benzyl-L-cysteinyl-D-valine benzyl ester* (3a) as a white solid (0.35 g, 65%), m.p. 99.5—101 °C; $[\alpha]_D^{20}$ −15.0 (*c* 1, acetone) (Found: C, 67.2; H, 6.4; N, 5.3. $C_{30}H_{34}N_2O_5S$ requires C, 67.4; H, 6.4; N, 5.25%); ^{1}H n.m.r. (300 MHz, $CDCl_3$): δ 0.82 (3 H, d, *J* 7.0 Hz, C*H*$_3$CH), 0.89 (3 H, d, *J* 6.8 Hz, C*H*$_3$CH), 2.18 (1 H, m, C*H*CH$_3$), 2.74, 2.88 (2 H, AB part of ABX with δ_A 2.74, δ_B 2.88, J_{AB} 14.1 Hz, J_{AX} 7.1 Hz, J_{BX} 5.5 Hz, β-H's of cys),

3.74 (2 H, s, PhCH_2S), 4.29 (1 H, bm, α-H cys), 4.57 (1 H, dd, *J* 4.6 and 8.8 Hz collapses to d *J* 4.6 Hz after D_2O exchange, α-H val), 5.12 (2 H, s, PhCH_2O), 5.13, 5.19 (2 H, AB_q with J_{AB} 12.2 Hz, PhCH_2O), 5.58 (1 H, bd, exchanges with D_2O, N*H*), 6.63 (1 H, bd, *J* 8.6 Hz, exchanges with D_2O, NH), and 7.18—7.48 (15 H, m, aromatic protons); ^{13}C n.m.r. (22.63 MHz, $CDCl_3$): 17.6 (q, CH_3), 19.0 (q, CH_3), 31.3 (d, β-C val), 34.0 (t, β-C cys), 36.5 (t, SCH_2Ph), 54.3 (d, α-C), 57.3 (d, α-C), 67.1 (t, 2 × OCH_2Ph), 127.2—137.9 (complex, aromatic carbon atoms), 156.0 (s, *C*O), 170.2 (s, *C*O), and 171.4 (s, *C*O).

The above procedure was repeated with *N*-benzyloxycarbonyl-*S*-benzyl-L-[3-^{13}C]cysteine (2b) to give N-*benzyloxycarbonyl*-S-*benzyl*-L-[3-^{13}C]*cysteinyl*-D-*valine benzyl ester* (3b) (58%, m.p. 99.0—100.5 °C). The ^{1}H n.m.r. (300 MHz) spectrum was essentially identical with that of the unlabelled isotopomer except for the extra coupling, $^1J(^1H,^{13}C)$ 142 Hz, experienced by the β-protons of the cysteinyl residue. The labelled C-atom showed a ^{13}C chemical shift of 33.8 p.p.m.

N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-*cysteinyl*-D-*valine Benzyl Ester* (5a) *and* N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]*cysteinyl*-D-*valine Benzyl Ester* (5b).—*Method A*. *N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-D-valine benzyl ester (3a) (0.267 g, 0.50 mmol) was treated with 45% hydrogen bromide in acetic acid (1 cm^3) for 15 min at room temperature, and the solution evaporated under reduced pressure at 35 °C. The residual gum was triturated with ether–pentane to give an off-white, gummy solid (0.255 g) which was dissolved in a mixture of chloroform (5 cm^3) and triethylamine (0.5 cm^3). The solution was evaporated to give a white solid which was dissolved in chloroform (5 cm^3) to give a solution of the amine component. *N*-Benzyloxycarbonyl-L-α-aminoadipic acid α-benzyl ester (4) (0.193 g, 0.50 mmol), 1-hydroxybenzotriazole (0.068 g, 0.50 mmol), and dicyclohexylcarbodi-imide (0.103 g, 0.50 mmol) were mixed at 0 °C in chloroform (10 cm^3) and the mixture stirred at 0 °C for 1 h, then at room temperature for 1 h. The solution of the amine component was added and the mixture evaporated to give a white solid. Chromatography of the solid on silica gel and elution with ethyl acetate gave the crude product as a solid (0.303 g, 79%) which crystallised from methanol to give N-*benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-*cysteinyl*-D-*valine benzyl ester* (5a) (0.237 g, 62%), m.p. 114—115.5 °C; $[\alpha]_D^{20}$ −18.2° (*c* 1, acetone) (Found: C, 67.15; H, 6.35; N, 5.6. $C_{43}H_{49}N_3O_8S$ requires C, 67.25; H, 6.45; N, 5.45%); field desorption mass spec. *m/e* 768 (*M* + 1), 677 (*M* − 90); $\nu_{max.}$ 3 380, 1 735, and 1 685 cm^{-1}; ^{1}H n.m.r. (300 MHz, $CDCl_3$), δ 0.83 (3 H, d, *J* 6.8 Hz, CH_3CH), 0.89 (3 H, d, *J* 6.8 Hz, CH_3CH), 1.62 (3 H, m, β-H and γ-H's of α-AAA), 1.81 (1 H, m, β-H of α-AAA), 2.13 (3 H, m, β-H of val and δ-H's of α-AAA), 2.67, 2.84 (2 H, AB part of ABX with δ_A 2.67, δ_B 2.84, J_{AB} 14.1 Hz, J_{AX} 5.8 Hz, J_{BX} 7.2 Hz, β-H's of cys), 3.76 (2 H, s, SCH_2Ph), 4.35—4.60 (3 H, complex, 3 × α-H's), 5.06 and 5.16 (2 H, ABq, J_{AB} 12.2 Hz, OCH_2Ph), 5.10 (2 H, s, OCH_2 Ph), 5.16 (2 H, s, OCH_2-Ph), 5.60 (1 H, d, *J* 7.9 Hz, N*H*), 6.26 (1 H, d, *J* 7.2 N*H*), 6.76 (1 H, d, *J* 9.0 Hz, N*H*), and 7.20—7.45 (20 H, complex m, aromatic protons); ^{13}C n.m.r. (22.64 MHz, $CDCl_3$), δ 17.6 (q, CH_3), 19.0 (q, CH_3), 21.5 (t, γ-C of α-AAA), 31.1 (d, β-C of val), 31.6 (t, β-C of α-AAA), 33.4 (t, β-C of cys), 35.3 (t, δ-C of α-AAA), 36.5 (t, SCH_2Ph), 52.2 (d, α-C), 53.8 (d, α-C), 57.4 (d, α-C), 67.1 (t, 3 × OCH_2Ph), 127.2—138.1 (complex, aromatic carbon atoms), 156.2 (s, CO), 170.4 (s, CO), 171.4 (s, CO), 172.2 (s, CO), and 172.6 (s, CO).

The preparation was repeated using *N*-benzyloxycarbonyl-*S*-benzyl-L-[3-^{13}C]cysteinyl-D-valine benzyl ester (3b) to give N-*benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]*cysteinyl*-D-*valine benzyl ester* (5b) (63%), m.p. 114—115.5 °C. The ^{1}H n.m.r. spectrum was identical with that of the unlabelled isotopomer except for the presence of further coupling to the β-protons of the cysteinyl residue, $^1J(^1H,^{13}C)$ 142 Hz, and to the benzyl protons of the *S*-benzyl group, $^3J(^1H,^{13}C)$ 4.1 Hz. The labelled β-C of the cysteinyl residue showed a ^{13}C chemical shift of δ 33.2 p.p.m.

N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-*cysteine* (6a) *and* N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]*cysteine* (6b).—Dry triethylamine (0.086 cm^3, 0.62 mmol) was added to a solution of *N*-benzyloxycarbonyl-L-α-aminoadipic acid α-benzyl ester (4) (0.238 g, 0.62 mmol) in dry freshly distilled tetrahydrofuran (6 cm^3) under N_2, and the mixture was cooled to −15 °C. After the mixture had been stirred at −15 °C for 15 min, isobutyl chloroformate (0.080 cm^3, 0.62 mmol) was added and the mixture was stirred at −15 °C for 30 min. A solution of *S*-benzyl-L-cysteine (0.131 g, 0.62 mmol; Aldrich) in water (6 cm^3) and triethylamine (0.120 cm^3) was cooled to 0 °C and added in one portion to the cold, vigorously stirred solution of the mixed anhydride. The mixture was stirred at room temperature for 50 min, then diluted with water (10 cm^3) and washed with ether (2 × 10 cm^3). The aqueous phase was layered with ethyl acetate (20 cm^3), and stirred and acidified to Congo Red with aqueous HCl (1 mol dm^{-3}). The organic phase was washed with saturated aqueous NaCl (10 cm^3), dried, and evaporated to give crude N-*benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-*cysteine* (6a) as a foam (0.247 g, 69%); ^{1}H n.m.r. (300 MHz, $CDCl_2$), δ 1.8 (2 H, bm, γ-H's of α-AAA), 1.9 (2 H, bm, β-H's of α-AAA), 2.2 (2 H, bt, *J* 7 Hz, δ-H's of α-AAA), 2.9 (2 H, AB part of ABX, β-H's of cys), 3.7 (2 H, s, SCH_2Ph), 4.5 (1 H, bt, *J* 7 Hz, α-H of α-AAA), 4.8 (1 H, X part of ABX, α-H of cys), 5.1 (4 H, 2 × ABq, 2 × PhCH_2O), 5.8 (1 H, d, *J* 7 Hz, N*H*), 6.8 (1 H, d, *J* 7 Hz, N*H*), and 7.3 (15 H, m, aromatic protons). The crude extract was used without further purification in subsequent reactions.

The preparation was repeated with *S*-benzyl-L-[3-^{13}C]-cysteine to give N-*benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]*cysteine* (6b) (75%) as a foam. The ^{1}H n.m.r. spectrum was identical with that of (6a) except that the ABX pattern ascribed to the α- and β-protons of the cysteine residue appeared as the ABM part of an ABMX pattern with $^1J(^1H,^{13}C)$ 141 Hz.

N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-*cysteinyl*-D-*valine Benzyl Ester* (5a), N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]-*cysteinyl*-D-[3-^{13}C]*valine Benzyl Ester* (5c), *and* N-*Benzyloxycarbonyl-α-benzyl-δ-*(L-*α-aminoadipoyl*)-S-*benzyl*-L-[3-^{13}C]-*cysteinyl*-D-[3-^{13}C,^{15}N]*valine Benzyl Ester* (5d).—*Method B*. To a solution of *N*-benzyloxycarbonyl-α-benzyl-δ-(L-α-aminoadipoyl)-*S*-benzyl-L-cysteine (6a) (0.233 g, 0.40 mmol) in dry CH_2Cl_2 (10 cm^3) was added the ammonium tosylate salt of D-valine benzyl ester (1a) (0.142 g, 0.40 mmol) followed by triethylamine (0.055 cm^3, 0.40 mmol) and solid 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (0.099 g, 0.40 mmol). The solution was stirred at room temperature under N_2 for 15 h and then evaporated to dryness under

reduced pressure. The residue was dissolved in ethyl acetate (20 cm^3) and this solution washed with saturated aqueous $NaHCO_3$ (15 cm^3), aqueous HCl (1 mol dm^{-3}; 15 cm^3), and saturated aqueous NaCl (15 cm^3) and dried. Evaporation of the solvent under reduced pressure gave a foam which was kept under high vacuum for several hours to give a waxy solid (0.254 g, 83%) which crystallised from methanol to give *N-benzyloxycarbonyl-α-benzyl-δ-(*L*-α-aminoadipoyl)-S-benzyl-*L*-cysteinyl-*D*-valine benzyl ester* (5a) (0.142 g, 46%), m.p. 116—117 °C. The ^1H n.m.r. spectrum of the product was identical with that of the fully protected tripeptide prepared by Method A.

The preparation was repeated with (6b) and (1b) to give *N-benzyloxycarbonyl-α-benzyl-δ-(*L*-α-aminoadipoyl)-S-benzyl-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C]valine benzyl ester* (5c) (0.152 g, 79%) which crystallised from methanol as a white solid (0.115 g, 60%), m.p. 113—115 °C; ^1H n.m.r. (300 MHz, $CDCl_3$), δ 0.76 [3 H, dd, *J* 6.8 Hz, $^2J(^1H,^{13}C)$ 4.2 Hz, $CH_3{}^{13}CH$], 8.82 [3 H, dd, *J* 6.8 Hz, $^2J(^1H,^{13}C)$ 6.8 Hz, $CH_3{}^{13}CH$], 1.6 (3 H, bm β-H and γ-H's of α-AAA), 1.8 (1 H, bm, β-H of α-AAA), 2.05 [1 H, dd sept, *J* 6.8, 8.2 and $^1J(^1H,^{13}C)$ 127 Hz, β-H of val], 2.1 (2 H, bm, δ-H's of α-AAA), 2.7 and 2.8 (2 H, AB part of ABMX $\delta_A = 2.7$, δ_B 2.8, J_{AB} 13.9 Hz, J_{AM} 7.4 Hz, J_{BM} 5.6, J_{AX} 141.7, and J_{BX} 142.4 Hz, β-H's of cys), 3.7 [2 H, d, $^3J(^1H,^{13}C)$ 4.0 Hz, SCH_2Ph], 4.4 (1 H, bm, α-H of α-AAA), 4.5 (2 H, m, two overlapping X parts of ABX and ABMX, α-H's of val and cys), 5.01 and 5.08 (2 H, ABq with δ_A 5.01, δ_B 5.08, J_{AB} 12.4 Hz, OCH_2Ph), 5.02 (2 H, s, OCH_2Ph), 5.08 (2 H, s, OCH_2Ph), 5.6 (1 H, d, *J* 8.3 Hz, NH), 6.1 (1 H, d, *J* 7.4 Hz, NH), 6.7 (1 H, d, *J* 8.6 Hz, NH), and 7.3 (20 H, m, aromatic protons).

The preparation was repeated with (6b) and (1c) to give *N-benzyloxycarbonyl-α-benzyl-δ-(*L*-α-aminoadipoyl)-S-benzyl-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C,^{15}N]valine benzyl ester* (5d) which crystallised from methanol as a white solid (0.139 g, 45%), m.p. 112—114 °C. The ^1H n.m.r. spectrum was identical with that of compound (5c) except for the extra coupling experienced by the NH]-[$^1J(^1H,^{15}N)$ *ca.* 91.4 Hz] and 2-H-protons of the valine residue.

*δ-(*L*-α-Aminoadipoyl)-*L*-cysteinyl-*D*-valine* (7a), *δ-(*L*-α-Aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-valine* (7b), *δ-(*L*-α-Aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C]valine* (7c), *and δ-(*L*-Aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C,^{15}N]valine* (7d).—Liquid ammonia (20 cm^3) was distilled under argon from sodium metal onto the fully protected tripeptide (5a) (0.200 g, 0.26 mmol). Freshly cut sodium metal was added in very small pieces to the magnetically stirred mixture at −33 °C until the blue colour persisted for 10 min. The mixture was decolourised with a little solid ammonium sulphate, then the ammonia was allowed to evaporate to dryness under a stream of argon. The residue was mixed with aqueous H_2SO_4 (0.05 mol dm^{-3}, 30 cm^3), and the mixture was filtered. The precipitate was washed with aqueous H_2SO_4 (0.05 mol dm^{-3}, 10 cm^3), and to the combined filtrate and washings Hopkins reagent [13] (1 cm^3) was added in drops. The white precipitate was collected by centrifugation and washed with boiled distilled water (3 × 20 cm^3). The washed solid was suspended in boiled distilled water (20 cm^3) and hydrogen sulphide passed through for 30 min. The black mixture was filtered through Celite and the insoluble precipitate washed with boiled distilled water (20 cm^3). The combined filtrate and washings were degassed on a rotary evaporator to remove hydrogen sulphide, and then freeze-dried to give crude δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (7a) as a white powder (0.093 g, 98%) (Found: C, 35.3; H, 6.2; N, 8.7. Calc. for $C_{14}H_{25}N_3O_6S{\cdot}3H_2O$: C, 40.3; H, 7.5; N, 10.05%), ^1H n.m.r. (300 MHz, D_2O), δ 0.76 (3 H, d, *J* 6.8 Hz, CH_3CH), 0.78 (3 H, d, *J* 6.8 Hz, CH_3CH), 1.42—1.88 [4 H, complex, β-H's and γ-H's of α-AAA], 1.93—2.11 (1 H, m, β-H of val), 2.23 (2 H, m, δ-H's of α-AAA), 2.70 and 2.75 (2 H AB part of ABX with δ_A 2.70, δ_B 2.75, J_{AB} 14.1, J_{AX} 7.2, J_{BX} 5.9 Hz, β-H's of cys), 3.77 (1 H, m, α-H of α-AAA), 4.10 (1 H, d, *J* 5.9 Hz, α-H of val), 4.38 (1 H, X, part of ABX with J_{AX} 6.7 and J_{BX} 6.1 Hz, α-H of cys); ^{13}C n.m.r. (22.64 MHz, D_2O), δ 18.0 ($CHCH_3$), 19.2 ($CHCH_3$), 21.5 (γ-C of α-AAA), 26.1 (β-C of cys), 30.0 and 30.7 ($CHCH_3$, and β-C of α-AAA), 35.2 (δ-C of α-AAA), 53.9, 56.4, 59.3 (3 × α-C), and 172.9, 173.5, 175.8, and 176.5 (4 × CO).

The preparation was repeated with (5b) to give *δ-(*L*-α-aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-valine* (7b) as a white powder (0.095 g, 100%) (Found: C, 32.7; H, 5.8; N, 8.15; sulphated ash 28.4; $C_{13}{}^{13}CH_{25}N_3O_6S$ with 28.4% of material containing no C, H, or N requires C, 33.0; H, 4.95; N, 8.25%); ^1H n.m.r. identical with that of the unlabelled isotopomer except for the presence of further coupling to the β-protons of the cysteinyl residue, $^2J(^1H,^{13}C)$ *ca.* 142 Hz and to the α-proton of the cysteinyl residue $^2J(^1H,^{13}C)$ 6 Hz; some unlabelled material was apparent but from the integration the extent of ^{13}C labelling was 92%. The labelled β-C for the cysteinyl residue showed a ^{13}C chemical shift of δ 26.3 p.p.m.

The preparation was repeated with (5c) to give *δ-(*L*-α-aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C]valine* (7c); ^1H n.m.r. identical with that of the singly labelled isotopomer described above except for the presence of further coupling of the ^{13}C atom of the valine with the valine β-proton $^1J(^1H,^{13}C)$ *ca.* 160, with the valine α-proton $^2J(^1H,^{13}C)$ *ca.* 5.3, and with the valine γ-protons $^2J(^1H,^{13}C)$ *ca.* 4.4 Hz. The labelled β-C of the cysteinyl residue showed a ^{13}C chemical shift of δ 26.2 p.p.m.; the labelled β-C of the valine residue showed a shift of δ 30.8 p.p.m.

The preparation was repeated with (5d) to give *δ-(*L*-α-aminoadipoyl)-*L*-[3-^{13}C]cysteinyl-*D*-[3-^{13}C,^{15}N]valine* (7d). The ^1H n.m.r. spectrum was identical with that of the doubly labelled isotopomer described above except for the presence of further coupling of the ^{15}N atom of the valine with the valine α-proton $^2J(^1H,^{15}N)$ *ca.* 5 Hz.

*Further Purification of δ-(*L*-α-Aminoadipoyl)-*L*-cysteinyl-*D*-valine* (7a).—Sephadex G-25 (40 cm^3) was soaked overnight in a mixture of propan-2-ol–boiled distilled water (70 : 30, v/v) to give approximately 80 cm^3 of gel which was packed into a column (30 × 2 cm). Crude tripeptide (0.35 g) was dissolved in water (3 cm^3), the pH changed from 1.8 to 3.5 using ammonia (6 mol dm^{-3}), and the solution made up to 70% in propan-2-ol. The mixture was filtered, then applied to the above column and the product eluted with 70% propan-2-ol, with the automatic collection of fractions of 16 cm^3. Fractions were examined by electrophoresis at pH 4.5 for 30 min to locate the tripeptide (mobility 9.5 cm) and the tripeptide disulphide (mobility 12 cm), and with nitric acid–barium hydroxide to locate the sulphate. Fractions 6—18 contained the tripeptide, 20—35 contained the disulphide, and 41—52 contained the sulphate. Fractions 13—16 were combined (volume 64 cm^3), washed with ether (640 cm^3), and the ether ' back-extracted ' with water (6.4 cm^3). The combined aqueous solutions were evacuated to remove ether, then freeze-dried to give 0.1 g of white powder. This was taken up in water and freeze-dried (ten times) to remove propan-2-ol and give δ-(L-α-aminoadipoyl)-

L-cysteinyl D-valine (7a) (Found: C, 40.2; H, 7.1; N, 10.25. Calc. for $C_{14}H_{25}N_3O_6S \cdot 3H_2O$: C, 40.3; H, 7.5; N, 10.05%); ^1H n.m.r. (300 MHz, H_2O) δ 0.86 (3 H, d, *J* 6.6 Hz, CH_3CH), 0.90 (3 H, d, *J* 6.6 Hz, CH_3CH), 1.5—2.0 (4 H, complex, β-H's and γ-H's of α-AAA), 2.0—2.2 (1 H, m, $CHCH_3$), 2.38 (2 H, m, δ-H's of α-AAA), 2.8—3.0 (2 H, AB part of ABX, β-H's of cys), 3.73 (1 H, m, α-H of α-AAA), 4.12 (1 H, complex, α-H of val), 4.53 (1 H, complex, α-H of cys), 7.80 (1 H, d, *J* 8.5 Hz, NH), and 8.35 (1 H, d, *J* 7.4 Hz, NH); traces of other NH doublets in this region possibly arose from different conformations of the tripeptide.

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