

Synthesis of δ -(L- α -Amino adipyl)-L-cysteinyl-D-valine and δ -(L- α -Amino adipyl)-L-cysteinyl-D-valylglycine

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An efficient synthesis of δ -(L- α -amino adipyl)-L-cysteinyl-D-valine (ACV; 1) and δ -(L- α -amino adipyl)-L-cysteinyl-D-valylglycine (ACVG; 2) via the protected tripeptide *N*-benzyloxycarbonyl-1-(*p*-nitrobenzyl)- δ -(L- α -amino adipyl)-*S*-benzyl-L-cysteinyl-D-valine, benzhydryl ester (3) is described.

In the course of studies on the biosynthesis of the penicillins and their acyclic precursor,¹ δ -(L- α -amino adipyl)-L-cysteinyl-D-valine (ACV; 1),† we required an efficient synthesis of the tripeptide which could be utilised for the preparation of ¹³C labelled isotopomers. In addition we wished to prepare δ -(L- α -amino adipyl)-L-cysteinyl-D-valylglycine (ACVG; 2), for biosynthetic studies. A tetrapeptide with this sequence of amino-acids but of undefined stereochemistry has been isolated from a β -lactam producing *Cephalosporium* sp.² but its significance in penicillin biosynthesis is as yet unknown.

Five syntheses of the ACV tripeptide have been reported previously in the literature³⁻⁶ each of which utilise different strategies and/or protecting groups. Two distinct routes have been employed involving elaboration from either the valine carboxy³⁻⁶ or the amino-terminus.⁶ Since it was our intention to prepare isotopomers labelled in the valine residue the overall yield from D-valine was of paramount importance and necessitated an approach using the second strategy. We report here a synthesis of ACV (1) by a route employing benzyl-based and benzhydryl-based protecting groups which offers the advantage of removal of the protecting groups by reduction with sodium in liquid ammonia⁶ or selective deprotection of the valine carboxy-group to afford, in a single step, the acid (4) as the intermediate for the synthesis of ACVG (2).

Treatment of *N*-benzyloxycarbonyl-L- α -amino adipic acid⁷ (6a) with *p*-nitrobenzyl bromide and one equivalent of triethylamine in dimethylformamide afforded the ester (6b) in 50% yield. Coupling of this derivative with *S*-benzyl-L-cysteine, benzhydryl ester⁸ (7) was carried out with 1-hydroxybenzotriazole and dicyclohexylcarbodi-imide⁹ to give the fully protected dipeptide (8a). Removal of the benzhydryl protecting group was achieved in virtually quantitative yield by mild acid hydrolysis and the resulting dipeptide acid (8b) coupled with D-valine benzhydryl ester using 1-hydroxybenzotriazole and dicyclohexylcarbodi-imide to afford the fully protected tripeptide (3) in 22% overall yield from (6a).

Reduction of the protected ACV (3) with sodium in liquid ammonia gave the unprotected tripeptide (1). Isolation as the corresponding sulphide followed by regeneration with hydrogen sulphide afforded ACV in apparently 77% yield. However elemental analysis of the freeze-dried product showed it to contain ca. 4% inorganic contaminants.⁶ The product was shown to be a 3:1 mixture of (1) and the corresponding disulphide (9) by Ellman's procedure¹⁰ and by paper electrophoresis. To circumvent the inhomogeneity introduced by the above procedure the crude product from the reduction was oxidised by passing air through an aqueous solution and the resultant disulphide⁵ (9) isolated by ion exchange chromatography. The product obtained in this manner, in 96% yield from (3), was homogeneous by paper

electrophoresis, h.p.l.c., and t.l.c. and proved free of inorganic contaminants. Confirmation that the product was the desired disulphide (9) was obtained from the ¹³C n.m.r. spectrum which showed a resonance at 38.5 p.p.m. corresponding to C-3 of the cystine residue. In contrast the spectrum of the monomeric tripeptide showed a peak at 26.1 p.p.m.⁶ for C-3 of cysteine. Oxidation of (9) with performic acid¹¹ afforded the sulphonic acid (11) which proved to be chromatographically and spectroscopically identical with material prepared from ACV isolated from *Cephalosporium acremonium* N-2.¹

With the protected tripeptide (3) in hand, synthesis of ACVG (2) could now be carried out in a straightforward manner. Deprotection of the valine carboxy-group by mild acid hydrolysis afforded the acid (4). Coupling of (4) with glycine benzyl ester using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole as above gave only poor yields of the desired product, the major product being the corresponding *N*-acyl urea.¹² In contrast, treatment of (4) with ethyl chloroformate in the presence of pyridine to afford a mixed anhydride and subsequent reaction of the anhydride with glycine benzyl ester gave the desired product (5) in 46% yield from (3). Sodium-liquid ammonia reduction of (5) followed by oxidation and purification of the product by ion-exchange chromatography as described for (9) above gave the tetrapeptide disulphide (10).

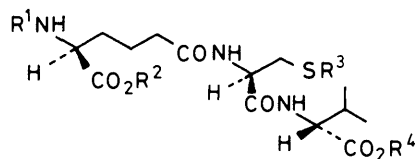
The disulphides (9) and (10) were quantitatively reduced to the respective monomers (1) and (2) with an excess of ethanethiol. The overall yields of ACV (ACVG) from D-valine and *S*-benzyl-L-cysteine by the route described were 68 (24%) and 43 (15%) respectively, which compare favourably with yields achieved in previous syntheses.³⁻⁶

Experimental

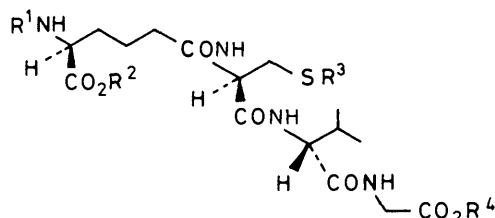
Electrophoresis was carried out at 50 V/cm on Whatman 3MM paper using the apparatus described by Michl,¹³ pH 2.1 and 3.5 buffers were prepared as described by Ambler.¹⁴ Merck silica G60 (70-230 mesh) was used for column chromatography and preparative t.l.c. was carried out on 200 × 200 × 1 mm layers of Merck 60GF254 silica. Thiol determinations were carried out using Ellman's procedure.¹⁰ L- α -Amino adipic acid [α]_D²⁰ +7.4° (c 2.0, 5M-HCl) (lit.,¹⁵ [α]_D +25°) was purchased from Sigma Chemical Co. Inc. Solvents were purified and dried by standard procedures and organic extracts typically dried over MgSO₄ or Na₂SO₄. N.m.r. spectra were recorded on Varian EM360, Bruker WM300, or Bruker WM360 spectrometers. Mass spectra (e.i.) were recorded on an AEI MS901 and a Kratos MS50 using a FAB source.

p-Nitrobenzyl *N*-Benzyloxycarbonyl-L- α -amino adipate (6b).—*N*-Benzyloxycarbonyl-L- α -amino adipic acid⁷ (2.065 g, 7.00 mmol) was dissolved in dimethylformamide (5 cm³),

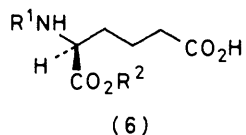
† α -Amino adipyl = 5-amino-5-carboxypentanoyl



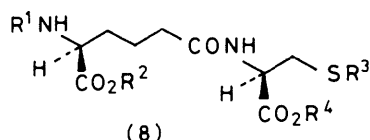
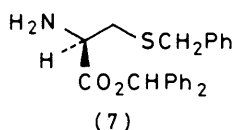
- (1) $R^1 = R^2 = R^3 = R^4 = H$
 (3) $R^1 = PhCH_2OCO$, $R^2 = p-NO_2C_6H_4CH_2$
 $R^3 = PhCH_2$, $R^4 = Ph_2CH_2$
 (4) $R^1 = PhCH_2OCO$, $R^2 = p-NO_2C_6H_4CH_2$
 $R^3 = PhCH_2$, $R^4 = H$



- (2) $R^1 = R^2 = R^3 = R^4 = H$
 (5) $R^1 = PhCH_2OCO$, $R^2 = p-NO_2C_6H_4CH_2$
 $R^3 = PhCH_2$, $R^4 = PhCH_2$

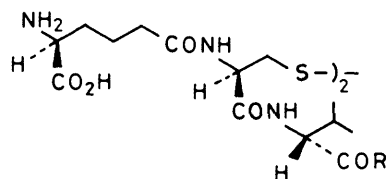


- a: $R^1 = PhCH_2OCO$, $R^2 = H$
 b: $R^1 = PhCH_2OCO$,
 $R^2 = p-NO_2C_6H_4CH_2$

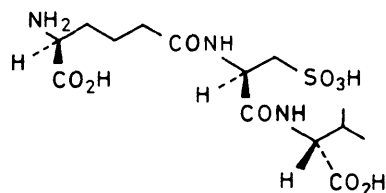


- a: $R^1 = PhCH_2OCO$, $R^2 = p-NO_2C_6H_4CH_2$
 $R^3 = PhCH_2$, $R^4 = Ph_2CH$
 b: $R^1 = PhCH_2OCO$, $R^2 = p-NO_2C_6H_4CH_2$
 $R^3 = PhCH_2$, $R^4 = H$

triethylamine (1.02 cm³, 7.35 mmol) added, and the solution cooled to 0 °C. To the stirred solution, *p*-nitrobenzyl bromide (1.588 g, 7.35 mmol) was added in aliquots during 5 h. The reaction mixture was allowed to come to room temperature overnight, after which saturated aqueous NaCl (25 cm³) was added; the mixture was then adjusted to pH 1 with 5M-HCl and extracted with EtOAc (30 cm³ × 4). The extracts were evaporated to yield a yellow oil which was chromatographed on silica (200 g) with EtOAc-*n*-hexane (7:3) as eluant to afford (6b) which crystallised from EtOAc-*n*-hexane (1.22 g, 50%), m.p. 101–104 °C; $[\alpha]_D^{20} - 8.01^\circ$ (*c* 2.0, acetone) (Found: C, 58.4; H, 5.1; N, 6.45. C₂₁H₂₂N₂O₈ requires C, 58.60; H, 5.15; N, 6.51%), δ (60 MHz, CDCl₃) 1.45–2.05 (4 H, m, 3, 4-H), 2.37 (2 H, t, *J* 5.5 Hz, 5-H), 4.18–4.70 (1 H, m, 2-H), 5.12 and 5.23 (4 H, 2s, benzyl-H), 5.4 (1 H, m, NH), 7.32 (5 H, s, ArH), 7.43 and 8.13 (4 H, 2 d, *J* 9 Hz, ArH),



- (9) $R = OH$
 (10) $R = NHCH_2CO_2H$



(11)

8.75 (1 H, s, CO₂H); *m/z* 430 (*M*⁺), 306, and 250.1091 (C₁₃H₁₆NO₄ requires 250.1079), 206.1168 (C₁₂H₁₆NO₂ requires 206.1181).

N-Benzylloxycarbonyl-1-(*p*-nitrobenzyl)- δ -(*L*- α -aminoadipyl)-*S*-benzyl-*L*-cysteine, Benzhydryl Ester (8a).—*S*-Benzyl-*L*-cysteine (0.422 g, 2 mmol) was converted into the corresponding benzhydryl ester as previously described.⁸ The crude product was added to a solution of (6b) (0.88 g, 2.05 mmol) and 1-hydroxybenzotriazole hydrate (0.284 g, 2.1 mmol) in EtOAc (10 cm³). To the stirred solution, dicyclohexylcarbodi-imide (0.433 g, 2.1 mmol) in EtOAc (5 cm³) was added and the mixture stirred overnight. After addition of oxalic acid dihydrate (13 mg) the solution was stirred for 1 h, filtered, and the filtrate washed successively with aqueous HCl (1M; 30 cm³), water (30 cm³), and saturated aqueous NaHCO₃ (30 cm³), water (30 cm³) and saturated NaCl (30 cm³). Evaporation afforded an oil which was chromatographed on silica (150 g), with EtOAc-*n*-hexane (6:4) as eluant, to give (8a) which was crystallised from EtOAc-*n*-hexane (1.01 g, 64%), m.p. 122–127 °C; $[\alpha]_D^{20} - 5.64^\circ$ (*c* 1.0, CH₂Cl₂) (Found: C, 66.65; H, 5.6; N, 5.1. C₄₄H₄₃N₃O₉S requires C, 66.90; H, 5.49; N, 5.32%), δ (360 MHz, CDCl₃) 1.68–1.90br (4 H, m, aminoadipyl 3,4-H), 2.15–2.26br (2 H, m, aminoadipyl 5-H), 2.85 (2 H, AB of ABX δ_A 2.91, δ_B 2.79, *J* 13.9, 4.7, 6.4 Hz, *cys* 3-H), 3.58 (2 H, AB; δ_A 3.62, δ_B 3.54, *J* 13.4 Hz, SCH₂Ph), 4.41 (1 H, m, aminoadipyl 2-H), 4.90 (1 H, M of ABMX, *J* 4.7, 6.4, 7.9 Hz, *cys* 2-H), 5.09 (2 H, AB; δ_A 5.12, δ_B 5.06, *J* 12.2 Hz; OCH₂Ar), 5.21 (2 H, AB, δ_A 5.24, δ_B 5.19, *J* 13.6 Hz, OCH₂Ar), 5.47 (1 H, d, *J* 7.9 Hz, NH), 6.19 (1 H, d, *J* 7.8 Hz, NH), 6.86 (1 H, s, CHPh₂), 7.18–7.41 (20 H, m, ArH), 7.46 and 8.17 (4 H, 2 d, *J* 8.5 Hz, ArH); *m/z* 698 (*M* – 91), 622 (*M* – 167), 167, and 91.

N-Benzylloxycarbonyl-1-(*p*-nitrobenzyl)- δ -(*L*- α -aminoadipyl)-*S*-benzyl-*L*-cysteinyl-*D*-valine, Benzhydryl Ester (3).—The protected dipeptide (8a) (0.59 g, 0.75 mmol) was dissolved in 0.2M HCl-nitromethane (19 cm³) and the solution stirred at room temperature for 5 h; it was then evaporated to give an oil which was subjected to column chromatography on silica (30 g), with EtOAc-*n*-hexane-HOAc (90:10:0.4) as eluant to afford the free acid (8b) (0.46 g, 98%) as a colourless foam.

The acid (8b) was added to a solution of 1-hydroxybenzotriazole hydrate (0.11 g, 0.83 mmol) and *D*-valine, benzhydryl ester [prepared from *D*-valine (97 mg, 0.83 mmol) as described⁸] in EtOAc (7.5 cm³). Dicyclohexylcarbodi-imide

(0.17 g) in EtOAc (5 cm³) was added, the reaction mixture stirred overnight, treated with oxalic acid, and washed as described above. Evaporation of the organic fraction afforded crude (3) which was purified by column chromatography on silica (30 g), with EtOAc–*n*-hexane (6:4) as eluant, and crystallised from CH₂Cl₂–ether–*n*-hexane as colourless needles (0.47 g, 71%), m.p. 77–80 °C remelting at 133–135 °C; $[\alpha]_{436}^{20} -11.9^\circ$ (*c* 1.0, acetone) (Found: C, 66.4; H, 5.8; N, 6.0. C₄₉H₅₂N₄O₁₀S requires C, 66.20; H, 5.90; N, 6.30%); δ (300 MHz, CDCl₃) 0.75 (3 H, dd, *J* 6.9, 1.1 Hz, val 4-H), 0.87 (3 H, d, *J* 7.0 Hz, val 4-H), 1.68–1.85 (4 H, m, amino adipyl 3, 4-H), 2.10–2.26 (3 H, m, amino adipyl 5-H, val 3-H), 2.74 (2 H, AB of ABMX; δ_A 2.84, δ_B 2.68, *J* 14.0, 7.0, 6.1, 2.1, 1.8 Hz, *cys* 3-H), 3.72 (2 H, s, SCH₂Ph), 4.36–4.41 (1 H, m, amino adipyl 2-H), 4.55–4.66 (2 H, m, *cys* 2-H, val 2-H), 5.09 (2 H, AB; δ_A 5.11, δ_B 5.06, *J* 11.9 Hz, OCH₂Ar), 5.20 (2 H, AB; δ_A 5.23, δ_B 5.17, *J* 13.7 Hz, OCH₂Ar), 5.74 (1 H, dd, *J* 4.1, 7.8 Hz, NH), 6.50 (1 H, dd, *J* 7.3, 4.3 Hz, NH), 6.87 (1 H, s, CHPh₂), 6.95 (1 H, m, NH), 7.17–7.37br (20 H, m, ArH), 7.43 (2 H, m, ArH), and 8.16 (2 H, d, *J*, 8.9 Hz, ArH); *m/z* 797 (*M* – 91), 752, 721, 167, and 91.

N-Benzoyloxycarbonyl-1-(*p*-nitrobenzyl)- δ -(*L*- α -amino adipyl)-*S*-benzyl-*L*-cysteinyl-*D*-valylglycine, Benzyl Ester (5). The benzhydryl ester (3) (0.2 g, 0.23 mmol) was hydrolysed to the free acid (4) (0.16 g) as above and the product dissolved in dry THF (3 cm³). Pyridine (20.0 μ l, 0.25 mmol) was added, the solution cooled to 0 °C and a solution of ethyl chloroformate (23.8 μ l, 0.25 mmol) in THF (2 cm³) added in one portion. The solution was stirred for 15 min at 0 °C when a solution of glycine benzyl ester, toluene-*p*-sulphonic acid salt (83.6 mg) and pyridine (20.0 μ l) in CH₂Cl₂ (3 cm³) was added and the mixture allowed to warm to room temperature overnight. After evaporation of the solvent the residue was dissolved in EtOAc (15 cm³), and the solution washed successively with aqueous HCl (1M; 10 cm³), water (10 cm³), and saturated aqueous NaCl (10 cm³) and then dried, and evaporated. The residue was subjected to preparative t.l.c. on silica, with CH₂Cl₂–MeOH (19:1) as eluant, and the purified protected ACVG crystallised from CH₂Cl₂–EtOH–*n*-hexane as a microcrystalline solid (90 mg, 46%), m.p. 155–158 °C, $[\alpha]_{D}^{20} -7.4^\circ$ [*c* 1.0, CH₂Cl₂–MeOH (1:1)] (Found: C, 61.85; H, 6.15; N, 7.8. C₄₅H₅₁N₅O₁₁S requires C, 62.13; H, 5.91; N, 8.05%); δ (300 MHz, CDCl₃: CD₃OD (1:1)) 0.88 and 0.91 (6 H, 2 d, *J* 6.8 Hz, val 4-H), 1.66–1.85br (4 H, m, amino adipyl 3, 4-H), 2.13–2.23br (3 H, m, amino adipyl 5-H, val 3-H), 2.68 (2 H, AB of ABX; δ_A 2.74, δ_B 2.63, *J* 13.8, 7.4, 6.5 Hz, *cys* 3-H), 3.69 (2 H, s, SCH₂Ph), 4.24, 4.30, 4.46 (3 H, 3 m, 3 \times 2-H), 5.06 (2 H, AB; δ_A 5.09, δ_B 5.03, *J* 12.2 Hz, OCH₂Ar), 5.11 (2 H, s, OCH₂Ar), 5.20 (2 H, AB; δ_A 5.23, δ_B 5.17, *J* 13.5 Hz, OCH₂Ar), 7.20–7.35 (15 H, m, ArH), and 7.45 and 8.15 (4 H, 2 d, *J* 8.6 Hz, ArH).

δ -(*L*- α -Amino adipyl)-*L*-cysteinyl-*D*-valine (ACV; 1).—Typically the protected tripeptide (3) (100 mg, 0.12 mmol) was stirred in liquid NH₃ under N₂ and small pieces of freshly cut Na added until the blue colour persisted for 5 min. Solid NH₄OAc (*ca.* 100 mg) was added, the NH₃ evaporated under a stream of dry N₂, and the residue desiccated over H₂SO₄. The product was isolated as follows.

Procedure A. The residue was dissolved in 5% (v/v) aqueous HOAc (5 cm³), the solution filtered and a 10% (w/v) solution of Hg(OAc)₂ in 5% (v/v) aqueous HOAc added slowly to the filtrate to afford a precipitate of the tripeptide sulphide. The precipitate was separated by centrifugation and washed sequentially with degassed water (5 cm³ \times 4), MeOH (5 cm³ \times 2), and ether (5 cm³). The dried residue was resus-

pended in degassed water (2 cm³) and a stream of H₂S passed through the suspension for 15 min. After separation by centrifugation the pellet of HgS was washed with water (1 cm³) and the combined supernatant liquid and washings filtered through Celite. The filtrate was degassed *in vacuo* and lyophilized to afford a mixture of (1) and the corresponding disulphide (9) as a colourless powder (31.5 mg, 77%) (Found: C, 44.75; H, 6.85; N, 11.1; ash, 4%. C₁₄H₂₅N₃O₆S requires C, 46.30; H, 6.87; N, 11.57%). Free thiol 75%, *m/z* (FABS) 364 [monomer (*M* + 1)].

Procedure B. The residue was dissolved in 5% (v/v) aqueous HOAc (5 cm³), extracted with ether (2 cm³ \times 2), and the aqueous layer lyophilized. The residue was dissolved in water (5 cm³), the pH adjusted to 8, and the solution aerated for 2 h. The solution was freeze dried, the residue dissolved in water (100 μ l) and subjected to ion exchange chromatography on Biorad AG 50 \times 2 resin (200–400 mesh, H⁺, 18 \times 1.5 cm) with a water–1M-pyridine gradient as eluant. Lyophilization of the ninhydrin positive fractions gave ACV disulphide as a colourless powder (39 mg, 96%), m.p. 200–203 °C (decomp.), $[\alpha]_{D}^{20} -9.5^\circ$ (*c* 2.0, 2M-HCl) (lit.,³ $[\alpha]_{D} -9.5^\circ$; lit.,⁵ $[\alpha]_{D} -11.0^\circ$) (Found: C, 46.2; H, 6.35; N, 11.0. C₂₈H₄₈N₆O₁₂S₂ requires C, 46.40; H, 6.67; N, 11.59%); δ (300 MHz, D₂O) 0.27 (3 H, d, *J*, 6.9 Hz, val 4-H), 0.32 (3 H, d, *J*, 8.5 Hz, val 4-H), 1.06–1.27br (4 H, m, amino adipyl 3,4-H), 1.54br (1 H, m, val 3-H), 1.76br (2 H, m, amino adipyl 5-H), 2.40 (2 H, AB, *cys* 3-H), 3.18 (1 H, m, amino adipyl 2-H), and 3.58 (1 H, m, val 2-H); δ_C (75 MHz, D₂O) 17.14 and 18.52 (val 4-C), 20.70 (amino adipyl 4-C), 29.57 and 30.07 (val and amino adipyl 3-C), 34.56 (amino adipyl 5-C), 38.49 (*cys* 3-C), 52.43, 59.51, and 59.77 (3 \times 2-C); *m/z* (FAB) 725 (*M* + 1) and 364.

δ -(*L*- α -Amino adipyl)-*L*-cysteinyl-*D*-valylglycine (ACVG; 2).—Deprotection of the tetrapeptide derivative (5) (54 mg, 0.06 mmol) was carried out in a manner identical with that of (3) above. Isolation using procedure B afforded ACVG as its dimer (10), a colourless powder (19.4 mg, 74%), m.p. 230–235 °C (decomp.) (Found: C, 46.05; H, 6.2; N, 12.3. C₃₂H₅₄N₈O₁₄S₂ requires C, 45.8; H, 6.5; N, 13.35%); δ (300 MHz, D₂O) 0.25 and 0.28 (6 H, 2 d, *J* 7.5 Hz, val 4-H), 1.10br (4 H, m, amino adipyl 3,4-H), 1.48br (1 H, m, val 3-H), 1.63–1.72 (2 H, m, amino adipyl 5-H), 2.40 (2 H, AB of ABX; δ_A 2.48, δ_B 2.33, *J* 13.9, 7.4, 5.8 Hz, *cys* 3-H), 3.07 (1 H, t, *J* 5.6 Hz, amino adipyl 2-H), 3.20 (2 H, s, gly 2-H), and 3.54 (1 H, m, val 2-H); *m/z* (FAB) 839 (*M* + 1) and 421.

Quantitative reduction of (10) to the monomer (2) was carried out by brief treatment with 5% (v/v) aqueous ethanethiol. The freeze-dried residue was homogeneous by electrophoresis and t.l.c. Reduction of (9) was carried out in a similar manner.

Acknowledgements

One of us (G. A. T.) acknowledges the support of the Science Faculty of Edinburgh University through the Sir David Baxter Scholarship. We thank Dr. R. P. Ambler of the Molecular Biology Department, Edinburgh University, for kind permission to use his electrophoresis apparatus, and Dr. D. Hazelby of Kratos Analytical Instruments Ltd., Manchester, for the FAB spectra.

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Received 29th July 1982; Paper 2/1309