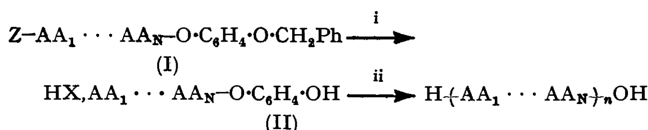
Sequential Polypeptides. Part V.[1,2] The Use of Monoesters of Catechol in the Synthesis of Sequential Polypeptides with Amino- or Carboxy-side-chains

By R. D. Cowell and J. H. Jones,* The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

Benzyloxycarbonylpeptide 2-benzyloxyphenyl esters with side-chain protection based on t-butyl alcohol can be deprotected without difficulty by hydrogenolysis in acetic acid, provided sulphur-containing reagents are avoided at all previous stages. The resulting peptide 2-hydroxyphenyl ester acetate salts are not isolated but are immediately polymerised by means of triethylamine in dimethyl sulphoxide. Hydrogenolysis of *N*(α)-benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-L-alanine 2-benzyloxyphenyl ester and polymerisation of the active ester derivative thus obtained gave a protected polymer from which poly(L-lysyl-L-alanyl-L-alanine) was obtained after trifluoroacetic acid treatment, dialysis, and lyophilisation. Poly(L-glutamyl-L-alanyl-L-alanine) was similarly prepared.

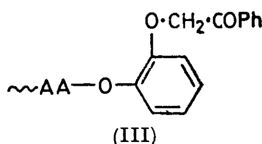
It has been shown[3] that simultaneous removal of the protecting groups of a benzyloxycarbonylpeptide 2-benzyloxyphenyl ester (I) followed by polymerisation of the resulting peptide 2-hydroxyphenyl ester salt (II) with triethylamine in dimethyl sulphoxide is a convenient and racemisation-free[3,4] route to sequential polypeptides (see Scheme 1). In our earlier work,[3]



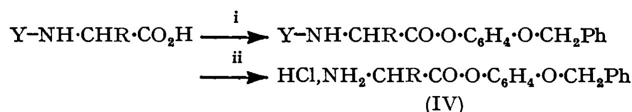
AA = An amino-acid residue; X = an anion; $\cdot C_6H_4\cdot O\cdot CH_2Ph$ = 2-benzyloxyphenyl; $\cdot C_6H_4\cdot OH$ = 2-hydroxyphenyl; Z = benzyloxycarbonyl. Conditions: i, HBr–AcOH or H_2–Pd; ii, Et_3N–Me_2SO.

Scheme 1

hydrogenolysis proved less satisfactory than acidolysis for the deprotection of (I) so that our preferred procedure was not applicable to cases with functional side-chains masked by means of protecting groups which could be removed under mild conditions after polymerisation. A recently outlined modification[4] of our method employs peptide esters of mono-*O*-phenacylcatechol (III): since the phenacyl group can be selectively removed in the presence of protecting groups based on t-butyl alcohol this extension could be used in conjunction with acid-labile side-chain protection for the synthesis of sequential polypeptides.



We now ascribe our previous difficulties in hydrogenations of the benzyloxyphenyl esters (I) to the presence of traces of catalyst poisons derived from sulphur-containing reagents used in preceding stages. Subsequent experience has shown that, providing such reagents are avoided at all stages of the preparations of (I), then hydrogenolysis proceeds smoothly. The most probable source of the previous difficulty was the preparation of the intermediate amino-acid benzyloxyphenyl ester hydrochlorides (IV) *via* 2-nitrophenylsulphenyl derivatives [Scheme 2(*a*)]. The ester salts (IV) were conveniently obtained in this way but were on occasion difficult to obtain free from yellow impurities, possibly because of some attack on the aromatic rings by the 2-nitrophenylsulphenyl chloride formed during deprotection. The obvious alternative procedure *via* the corresponding t-butoxycarbonyl derivative [Scheme 2(*b*)] has now been found to be a superior method of preparing the ester salts (IV). When the ester salts



Y = (*a*) Nps, (*b*) Boc. Conditions: i, $HO\cdot C_6H_4\cdot O\cdot CH_2Ph$, mixed carbonic anhydride; ii, HCl–Et_2O or EtOAc.

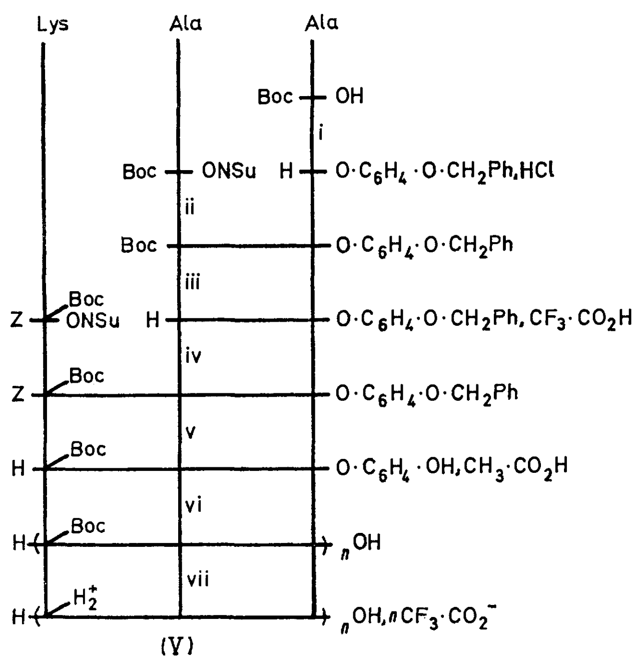
Scheme 2

(IV) made in this way are used, the benzyloxycarbonylpeptide 2-benzyloxyphenyl esters subsequently obtained can be hydrogenolysed without difficulty.

This improvement has enabled us to extend our original method to the preparation of sequential polypeptides with amino- or carboxy-side-chains. Poly(lysylalanylalanine) (V) was synthesised as outlined in Scheme 3 and obtained after exhaustive dialysis and lyophilisation as a white fluffy powder which gave satisfactory elemental and amino-acid analyses and had the expected spectroscopic properties. Gel chromatography on a calibrated column of Bio-Gel P100 which was swollen and eluted with phenol–acetic acid–water[5] gave the elution profile shown in Figure 1. Since a small proportion of the applied material emerged at the void volume (molecular weight > *ca.* 30,000), the approach previously

[1] Part IV, R. Fairweather and J. H. Jones, *J.C.S. Perkin I*, 1972, 1908.
[2] Preliminary communication, R. D. Cowell and J. H. Jones, *Chem. Comm.*, 1971, 1009.
[3] R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.
[4] Y. Trudelle, *Chem. Comm.*, 1971, 639.
[5] A. Pusztai and W. B. Watt, *Biochim. Biophys. Acta*, 1970, **214**, 463.

applied [6,7] in similar cases for calculation of molecular weight averages from the results of gel chromatography could in this instance only give a partial distribution



Conditions: i, $HO{\cdot}C_6H_4{\cdot}O{\cdot}CH_2Ph$, carbonic mixed anhydride, then HCl–EtOAC; ii, Et_3N–DMF; iii, 90% $CF_3{\cdot}CO_2H$; iv, Et_3N–DMF; v, H_2–Pd; vi, Et_3N–Me_2SO; vii, 90% $CF_3{\cdot}CO_2H$.
Here and throughout this paper all amino-acid residues are L and abbreviated nomenclature follows the relevant Tentative Rules of the IUPAC–IUB Commission on Biochemical nomenclature which are reprinted in *Specialist Periodical Reports on Amino-acids, Peptides and Proteins*, 1970, **2**, ch. 5.

SCHEME 3

curve (see Figure 2) and minimum values for the molecular weight: these were 6700 and 11,100 for the number and weight average molecular weights respectively. Shortly after this work was completed and submitted for preliminary publication we learned that Yaron and his colleagues [8] had also synthesised poly-(lysylalanylalanine), by a route quite different to that described here. Since Yaron's group have made an exhaustive study of the conformational properties of their fractionated preparation, we have made no more than a cursory investigation of our polydisperse preparation.

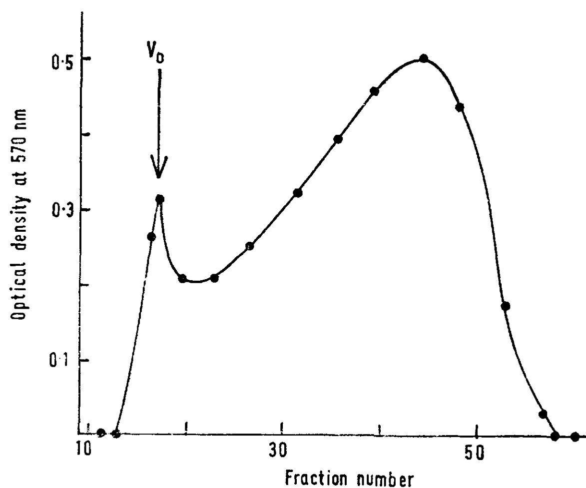


FIGURE 1 Elution profile of polymer (V) on the calibrated Bio-Gel P100 column. Optical densities at 570 nm were measured after total alkaline hydrolysis of each fraction and reaction with ninhydrin

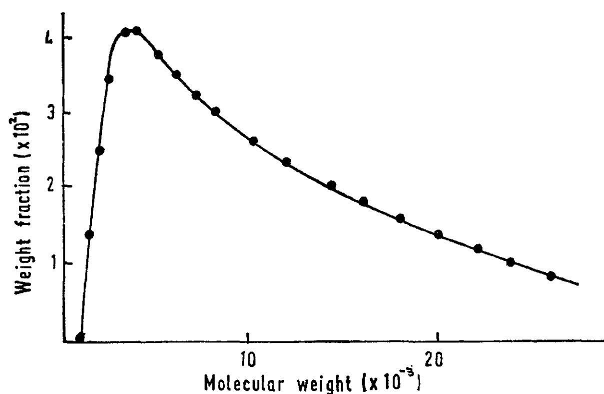


FIGURE 2 Differential molecular weight distribution curve of polymer (V)

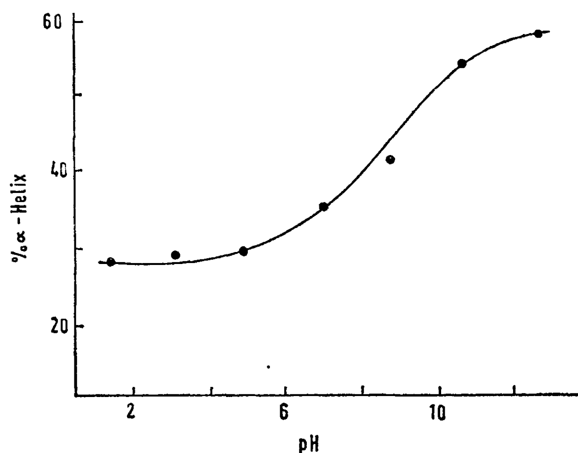


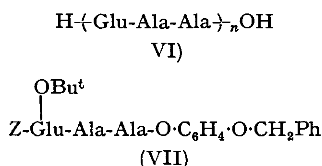
FIGURE 3 Variation of helix content of polymer (V) with pH in buffers of constant ionic strength 0·1

Our results confirm the conclusion of Yaron's group that the alanine residues in (V) stabilise the α-helical conformation of the fully protonated polymer. The variation of helix content (calculated from the ellipticity at 222 nm using the ellipticities of the random coil and α-helical forms of poly-L-glutamic acid at this wavelength [9] as limiting values) with pH in buffers of constant ionic strength 0·1 is shown in Figure 3. It can be seen

[6] R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1814.
[7] R. Fairweather, J. H. Jones, and J. K. Wilcox, *J. Chromatog.*, 1972, **67**, 157.
[8] A. Yaron, N. Turkeltaub, and A. Berger, *Israel. J. Chem.*, 1970, **8** (Supplement), 180p.
[9] J. T. Yang in ' A Laboratory Manual of Analytical Methods of Protein Chemistry,' eds. P. Alexander and H. P. Lundgren, Pergamon, 1969, p. 57.

that the minimum at 222 nm which is characteristic of the α-helix persists even under highly acidic conditions: poly-L-lysine is only able to adopt an α-helical conformation at high pH values.[10] A possible interpretation of the curve in Figure 3 is that a proportion of the polydisperse material is of too low a molecular weight to form α-helices at all, a proportion of intermediate molecular weight is able to form α-helices but these collapse below a critical pH depending on the molecular weight, and the remainder of high molecular weight is able to adopt and maintain an α-helical conformation even when fully protonated.

Poly-(L-glutamyl-L-alanyl-L-alanine) (VI) was synthesised *via* the crystalline intermediate (VII) by a route which was analogous to that shown in Scheme 3. In



this case the polymer obtained after dialysis and lyophilisation was insoluble in acidic media and its molecular weight could therefore not be determined by gel chromatography in the system used for (V). A rough estimate of the molecular weight can however be made from viscosity measurements, since DeTar and his colleagues [11] have correlated viscosity and molecular weight for some comparable polydisperse polytripeptides obtained by the *p*-nitrophenyl ester method. They found that an intrinsic viscosity of 0·15—0·20 dl g^{-1} (in dichloroacetic acid) generally corresponds to a weight average molecular weight of between *ca.* 2500 and *ca.* 6000 (determined in the ultracentrifuge): the intrinsic viscosity of (VI) in dichloroacetic acid was 0·16 dl g^{-1}, which suggests that the weight average molecular weight falls in this range. Despite this low apparent molecular weight, the c.d. spectra of (VI) in buffers of ionic strength 0·1 showed a minimum at 222 nm of intensity corresponding to 10—20% α-helix which persisted even at pH 11: conditions under which poly-L-glutamic acid is unable to adopt an α-helical conformation.[10]

At pH 7 both poly-L-glutamic acid and poly-L-lysine show typical random coil c.d. spectra, but when these homopolymers are mixed in equimolecular proportions the spectrum undergoes radical changes and becomes that typical of a β-structure.[12] When a similar experiment was performed with solutions of (V) and (VI) in pH 7 buffers, the resulting solution had a c.d. spectrum which was exactly as expected from the independent contributions of (V) and (VI).

Immunological studies of (V) and (VI) are in hand: preliminary studies show that both are antigenic in guinea pigs.[13]

EXPERIMENTAL

The general instructions given in Parts II [14] and III [6] apply. The gel chromatography system used for molecular weight determination and its operation are described in ref. 6. In calculating the concentrations of polymer solutions, no correction for residual solvent was made. The buffers of constant ionic strength 0·1 were those recommended by Miller and Golder.[15]

L-*Alanine 2-Benzyloxyphenyl Ester Hydrochloride*.—A solution of ethyl chloroformate (1·08 g, 10 mmol) in chloroform (10 ml) was added during 6 min to a stirred solution of t-butoxycarbonyl-L-alanine [16] (1·89 g, 10 mmol) and triethylamine (1·01 g, 10 mmol) in chloroform (15 ml) at −6°. After 5 min a solution of 2-benzyloxyphenol [3] (2·00 g, 10 mmol) and triethylamine (1·01 g, 10 mmol) in chloroform (10 ml) was added during 6 min with stirring at −6°. This temperature was maintained for 30 min and then the solution was set aside at room temperature for 6 h. After removal of the chloroform, the residue was distributed between ethyl acetate (50 ml) and water (50 ml). The organic phase was washed with 10% citric acid (1 × 40 ml), saturated sodium hydrogen carbonate (1 × 40 ml), and water (1 × 40 ml), and dried. Evaporation gave an oil (2·68 g), TLC-1, major spot, R_F 0·60, TLC-2, major spot, R_F 0·90. A solution of hydrogen chloride in ethyl acetate (2·5 ml, 3N) was added to a solution of this oil in ether (25 ml). After 16 h, the precipitate was isolated to give ester hydrochloride as white needles (1·95 g, 64% based on t-butoxycarbonyl-L-alanine), m.p. 164—171°, $[\alpha]_D^{20}$ −15·7° (*c* 1, chloroform) {lit.,[3] m.p. 160—170°, $[\alpha]_D^{20}$ −14·6° (*c* 1, chloroform)}.

t-Butoxycarbonyl-L-*alanyl*-L-*alanine 2-Benzyloxyphenyl Ester*.—Triethylamine (0·37 ml, 2·65 mmol) and t-butoxycarbonyl-L-alanine succinimido ester [17] (0·758 g, 2·65 mmol) were added to a stirred solution of L-alanine 2-benzyloxyphenyl ester hydrochloride (0·815 g, 2·65 mmol) in dimethylformamide (5 ml) at room temperature. The mixture was set aside for 5 h and then diluted with ethyl acetate (60 ml) and water (20 ml). The organic layer was washed with 10% citric acid (1 × 25 ml), saturated sodium hydrogen carbonate (1 × 25 ml), and water (2 × 25 ml), and dried. Evaporation gave an oil which solidified on trituration with light petroleum. Recrystallisation from ether–light petroleum gave *protected dipeptide* as white needles (1·01 g, 86%), m.p. 100—102°, $[\alpha]_D^{20}$ −49·6° (*c* 1, chloroform), TLC-1 R_F 0·59, TLC-4 R_F 0·74, $\nu_{max.}$ ($CHCl_3$) 1670—1720 (partially superimposed bands) and 1765 cm^{-1}, τ ($CDCl_3$) 2·5—3·5 (10H, singlet at 2·67 on complex, aromatic protons and peptide NH), 4·7—5·5 (4H, singlet and 4·98 on complex, O·CH_2Ph, urethane NH, and NH·C*H*·CO), 5·5—6·0 (1H, complex, NH·C*H*·CO), and 8·4—8·8 (15H, singlet at 8·58 superimposed on complex, CMe_3 and CH*Me*) (Found: C, 62·25; H, 6·8; N, 6·2. $C_{24}H_{30}N_2O_6$ requires C, 65·2; H, 6·8; N, 6·3%).

[10] G. D. Fasman in 'Poly-α-Amino Acids,' ed. G. D. Fasman, Marcel Dekker, New York, 1967, ch. 11 and literature therein cited.
[11] D. F. DeTar, F. F. Rogers, jun., and H. Bach, *J. Amer. Chem. Soc.*, 1967, **89**, 3039.
[12] G. G. Hammes and S. E. Schullery, *Biochemistry*, 1968, **7**, 3882.
[13] P. Brown and L. E. Glynn, unpublished results.
[14] R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1809.
[15] G. L. Miller and R. H. Golder, *Arch. Biochem.*, 1950, **29**, 420.
[16] E. Schnabel, *Annalen*, 1967, **702**, 188.
[17] G. W. Anderson, J. E. Zimmermann, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1964, **86**, 1839.

N(α)-*Benzyloxycarbonyl*-N(ε)-*t*-*butoxycarbonyl*-L-*lysine Succinimido Ester*.—This was prepared by the method of Otsuka *et al.*[18] on a 50 mmol scale, except that dioxan–ethyl acetate (1 : 1) was used as solvent for the reaction, which was performed at 0°. The crude product was recrystallised from propan-2-ol–light petroleum to give the ester (72%), m.p. 98—99°, $[\alpha]_D^{20}$ −16·8° (*c* 1·7, dioxan), TLC-1 R_F 0·55, τ ($CDCl_3$) 2·67 (5H, s, ArH), 4·38 (1H, d, *J* 7 Hz, CO·N*H*·CH), 4·90 (2H, s, O·CH_2Ph), 5·1—5·5 (2H, complex, NH·C*H*·CO and CH_2·N*H*·CO), 6·7—7·8 (2H, complex, NH·CH_2·CH_2), 7·22 (4H, s, CO·CH_2·CH_2·CO), and 7·8—8·6 (15H, singlet at 8·60 on complex, CMe_3 and CH·[CH_2]$_3$·CH_2) (Found: C, 57·6; H, 6·5; N, 8·7. Calc. for $C_{23}H_{31}N_3O_8$: C, 57·9; H, 6·5; N, 8·8%) {lit.,[18] m.p. 94·5—95·5°, $[\alpha]_D^{20}$ −14·8° (*c* 1·77, dioxan)}.

N(α)-*Benzyloxycarbonyl*-N(ε)-*t*-*butoxycarbonyl*-L-*lysyl*-L-*alanyl*-L-*alanine* 2-*Benzyloxyphenyl Ester*.—t-Butoxycarbonyl-L-alanyl-L-alanine 2-benzyloxyphenyl ester (1·33 g, 3 mmol) was dissolved in 90% aqueous trifluoroacetic acid (4·0 ml). After 1 h, the mixture was evaporated and the residue was dried first by repeated addition of benzene and distillation, and then at 20° and 0·1 mmHg to give a colourless glass. Triethylamine (0·42 ml, 3 mmol) and *N*(α)-benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysine succinimido ester (1·44 g, 3 mmol) were added to a stirred solution of this glass in dimethylformamide (4 ml). After 18 h, the mixture was diluted with ethyl acetate (150 ml) and water (50 ml). The organic layer was washed with 10% citric acid (1 × 50 ml), saturated sodium hydrogen carbonate (1 × 50 ml), and water (1 × 50 ml), and dried. Evaporation gave a solid which was recrystallised from chloroform–light petroleum to give *protected tripeptide* as needles (1·65 g, 79%), m.p. 164—166°, $[\alpha]_D^{20}$ −55·7° (*c* 1, chloroform), TLC-4 R_F 0·81; TLC-10 R_F 0·91, $\nu_{max.}$ ($CHCl_3$) 1650—1730br and 1765 cm^{-1}, τ ($CDCl_3$) 2·5—3·3 (16H, complex, ArH and peptide NH), 4·25 (1H, d, *J* 8 Hz, urethane NH), 4·8—5·0 (4H, s at 4·90 and 4·96, O·CH_2Ph), 5·1—6·0 (3H, complex, α-protons), 6·8—7·2 (2H, complex, CH_2·CH_2·NH), and 8·0—9·0 (21H, s at 8·58 on complex, CH·[CH_2]$_3$·CH_2, CH*Me*, and CMe_3) (Found: C, 64·7; H, 6·5; N, 7·7. $C_{38}H_{48}N_4O_9$ requires C, 64·8; H, 6·8; N, 8·0%).

Poly-(L-*lysyl*-L-*alanyl*-L-*alanine*) *Trifluoroacetate* (V).—A solution of the preceding tripeptide derivative (1·20 g, 1·7 mmol) was hydrogenated in glacial acetic acid (25 ml) over 10% palladium–charcoal (1·20 g) for 3 h. The solution was filtered through Celite, and the residue after evaporation was dried at 20° and 0·1 mmHg to give a crisp white foam. Triethylamine (0·48 ml, 3·4 mmol) was added to a stirred solution of this foam in dimethyl sulphoxide (1·5 ml). After 4 days during which the mixture set to a stiff paste, ethanol (15 ml) was added and the precipitate was collected by centrifugation and washed by decantation with ethanol (3 × 10 ml) and ether (3 × 25 ml) to give crude protected polymer (254 mg, 40%). A solution of this material in 90% aqueous trifluoroacetic acid was set aside for 1 h at room temperature. Ether (25 ml) was added and the flocculent precipitate was isolated by centrifugation and washed by decantation with ether (4 × 25 ml) to give crude deprotected polymer (238 mg, 36·5%) as a pale buff powder, which was dissolved in water (20 ml) and dialysed against water (4 l) for 24 h with water changes after 3, 6, 12, and 20 h. Lyophilisation and drying at 20° and 0·1 mmHg for 24 h gave *deprotected polymer* as a fluffy white powder (99 mg, 16%), m.p. 175—190° (decomp.), $[\alpha]_D^{20}$ −89°, $[\alpha]_{578}^{20}$ −92°, $[\alpha]_{546}^{20}$ −103°, $[\alpha]_{436}^{20}$ −187° (*c* 0·10, buffer pH 7·0), $[\alpha]_D^{20}$ −61°, $[\alpha]_{578}^{20}$ −66°, $[\alpha]_{546}^{20}$ −74°, $[\alpha]_{436}^{20}$, −133° (*c* 0·10, buffer pH 10·7), $[\alpha]_D^{20}$ −76°, $[\alpha]_{578}^{20}$ −81°, $[\alpha]_{546}^{20}$ −90°, $[\alpha]_{436}^{20}$ −175° (*c* 0·10, buffer pH 3·1), $\nu_{max.}$ (KBr disc) 1650br and 3300br cm^{-1}, τ (CF_3·CO_2H) 1·8—3·6 (6H, broad overlapping bands, peptide NH and NH_3^+), 4·6—6·0 (3H, broad, α-protons), 6·2—7·2 (2H, broad CH_2·CH_2·NH_3^+), 7·3—9·0 (12H, broad, CH·[CH_2]$_3$·CH_2 and CH*Me*), η_{sp}/c 0·265 dl g^{-1} (*c* 0·63, dichloroacetic acid), amino-acid analysis: Ala 2·00, Lys 0·91, c.d., $[\theta]_{222}$ −1·10 × 10^4 (min.) (*c* 0·1, buffer pH 4·8), $[\theta]_{222}$ −2·26 × 10^4 (min.) (*c* 0·1, NaOH pH 12·7), $[\theta]_{222}$ −1·20 × 10^4 (min.) (*c* 0·1, buffer pH 3·1), $[\theta]_{222}$ −1·32 × 10^4 (min.) (*c* 0·1, buffer pH 7·0), $[\theta]_{222}$ −1·45 × 10^4 (min.) (*c* 0·1, buffer pH 8·8), $[\theta]_{222}$ −2·13 × 10^4 (min.) (*c* 0·1, buffer pH 10·7), and $[\theta]_{222}$ −1·12 × 10^4 (min.) (*c* 0·1, HCl pH 1·4), $\bar{M}_n$, 6700, $\bar{M}_w$ 11,100 (minimum values) by gel chromatography (Found: C, 41·9; H, 6·35; N, 13·8. $C_{14}H_{23}F_3N_4O_5$·$1H_2O$ requires C, 41·8; H, 6·2; N, 13·9%).

N-*Benzyloxycarbonyl*-γ-*t*-*butyl*-L-*glutamyl*-L-*alanyl*-L-*alanine* 2-*Benzyloxyphenyl Ester* (VII).—t-Butoxycarbonyl-L-alanyl-L-alanine 2-benzyloxyphenyl ester (0·663 g, 1·5 mmol) was treated with 90% trifluoroacetic acid as described above. Triethylamine (0·21 ml, 1·5 mmol) and *N*-benzyloxycarbonyl-α-succinimido-γ-t-butyl-L-glutamate[19] (0·650 g, 1·5 mmol) were added to a stirred solution of the resulting glass in dimethylformamide (2 ml). After 18 h, the mixture was diluted with ethyl acetate (100 ml) and water (50 ml). The organic layer was washed with 10% citric acid (1 × 50 ml), saturated sodium hydrogen carbonate (1 × 50 ml), and water (1 × 50 ml), and dried. Evaporation gave a solid which was recrystallised from ethyl acetate–light petroleum to give *protected tripeptide* as needles (0·785 g, 79%), m.p. 165—168°, $[\alpha]_D^{20}$ −14·2° (*c* 1, chloroform), TLC-1 R_F 0·58, TLC-2 R_F 0·73, $\nu_{max.}$ ($CHCl_3$) 1660—1730 (superimposed bands) and 1765 cm^{-1}, τ ($CDCl_3$) 2·5—3·4 (16H, complex, ArH and peptide NH), 4·08 (1H, d, *J* 8 Hz, urethane NH), 4·8—6·0 (7H, s at 4·93 and 4·99 on broad band, O·CH_2Ph and α-protons), 7·4—6·3 (4H, complex, CH·CH_2·CH_2·CO), and 8·45—8·90 (15H, s at 8·60 on complex, CH*Me* and CMe_3) (Found: C, 65·2; H, 6·5; N, 6·4. $C_{36}H_{43}N_3O_9$ requires C, 65·3; H, 6·5; N, 6·4%).

Poly-(L-*glutamyl*-L-*alanyl*-L-*alanine*) (VI).—A solution of the preceding tripeptide derivative (890 mg, 1·35 mmol) in glacial acetic acid (25 ml) was hydrogenated over 10% palladium–charcoal (890 mg) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried at 20° and 0·1 mmHg to give a crisp white foam. Triethylamine (0·38 ml, 2·7 mmol) was added to the stirred solution of this foam in dimethyl sulphoxide (1·5 ml). The mixture set to a rigid gel during 2 days. After a further 2 days, the mixture was triturated with ethanol (10 ml). The solid was collected by centrifugation, and washed with ethanol (3 × 10 ml) and ether (3 × 25 ml) to give crude protected polymer as a buff powder (285 mg, 65%).

A solution of this polymer in 90% aqueous trifluoroacetic acid was left for 1 h at room temperature. Ether (25 ml) was added and the flocculent precipitate was isolated by centrifugation. This was washed with further portions of ether (4 × 25 ml), to give crude deprotected polymer as a pale buff powder (227 mg, 62%).

[18] H. Otsuka, K. Inouye, M. Kanayama, and F. Shinozaki, *Bull. Chem. Soc. Japan*, 1966, **39**, 882.
[19] R. Zabel and H. Zahn, *Z. Naturforsch.*, 1965, **20***B*, 650.

A solution of this polymer in aqueous sodium hydroxide (20 ml, 0·5N) was dialysed against water (4 l) for 6 h (water changes every 1 h). Adjustment of the pH to 3 by addition of hydrochloric acid, followed by further dialysis against water for 24 h (water changes after 1, 3, 6, 12, and 20 h) gave, after lyophilisation and drying at 20° and 0·1 mmHg for 24 h, *deprotected polymer* as a fluffy white solid (97 mg, 31%), m.p. 250—260°, $[\alpha]_D^{20}$ −141°, $[\alpha]_{578}^{20}$ −149°, $[\alpha]_{546}^{20}$ −170°, $[\alpha]_{436}^{20}$ −274° (*c* 0·10, buffer, pH 7·0), $[\alpha]_D^{20}$ −131°, $[\alpha]_{578}^{20}$ −142°, $[\alpha]_{546}^{20}$ −160°, $[\alpha]_{436}^{20}$ −261° (*c* 0·10, buffer, pH 10·7), $\nu_{max.}$ (KBr disc) 1650br, 1720br, and 3300br cm^{-1}, τ ($CF_3 \cdot CO_2H$) 1·6—3·0 (3H, br, peptide NH), 4·6—6·0 (3H, br, α-protons), 6·8—8·0 (4H, complex, $CH \cdot CH_2 \cdot CH_2 \cdot CO$), and 8·6 (6H, br, CH*Me*), $[\eta]$ 0·16 dl g^{-1}, amino-acid analysis: Ala 2·00, Glu 1·09, c.d. $[\theta]_{225}$ $-1{\cdot}5 \times 10^3$ (min.) (*c* 0·1, buffer pH 10·7), $[\theta]_{225}$ $-1{\cdot}4 \times 10^3$ (min.) (*c* 0·1, buffer pH 7·0), and $[\theta]_{222}$ $-5{\cdot}3 \times 10^3$ (min.) (*c* 0·1, buffer pH 4·8) (Found: C, 44·9; H, 6·3; N, 13·9%; C : N 3·23 : 1. $C_{11}H_{17}N_3O_8,1{\cdot}4H_2O$ requires C, 44·6; H, 6·7; N, 14·2%; C : N 3·14 : 1).

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