Sequential Polypeptides. Part IV.[1] The Synthesis of Poly-(L-alanyl-glycyl-L-proline) and its Stereoisomers

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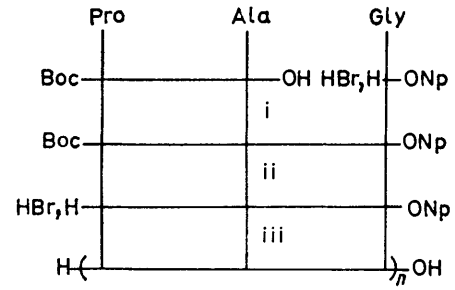
Several alternative routes to sequential polypeptides containing repeating glycyl-L-prolyl-L-alanyl sequences have been examined. The most satisfactory involved treatment of L-alanylglycyl-L-proline pentachlorophenyl ester hydrobromide with tertiary base in dimethyl sulphoxide: polydisperse poly-(L-alanylglycyl-L-proline) was obtained, after dialysis, in 50—70% yield (number and weight average molecular weight *ca.* 9000 and *ca.* 12,000—14,000, respectively). Analogous routes were employed for the synthesis of polydisperse preparations of poly-(D-alanylglycyl-D-proline), poly-(D-alanylglycyl-L-proline), and poly-(L-alanylglycyl-D-proline): the D–L and L–D polymers were obtained in lower yield and were of lower molecular weight than were the L–L and D–D polymers.

THE sequential collagen model poly-(glycyl-L-prolyl-L-alanine), prepared as described in Part I,[2] is antigenic in guinea pigs and shows some immunological cross reactivity with collagen;[3] similar results have been reported for poly-(L-prolylglycyl-L-proline).[4] We are currently engaged in the synthesis of an extensive series of sequential polypeptide collagen models for an investigation of the relationship between structure and immunological properties in this class of polypeptide. In this connection we required a satisfactory route to polytripeptides comprising the repeating sequence glycyl-L-prolyl-L-alanyl which could be modified with maximal use of common intermediates for the synthesis of closely related analogues.

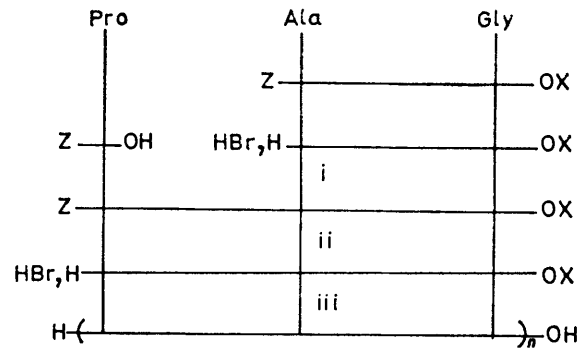
The general procedure for the preparation of polytripeptides which has given the most consistently satisfactory results involves the treatment of tripeptide active ester salts with tertiary bases, usually in concentrated solution in a dipolar aprotic solvent.[5] A sequential polytripeptide with the desired sequence glycyl-L-prolyl-L-alanyl could in principle be prepared by any one of the following three routes: through a monomer of this sequence (' route 1 ' in subsequent discussion) or of sequence L-prolyl-L-alanylglycine (route 2) or of sequence L-alanylglycyl-L-proline (route 3). The sequences produced by the three routes are identical apart from a few residues at the termini. Route 1 is a satisfactory method[2,6] of preparing poly-(glycyl-L-prolyl-L-alanine) which carries little risk of racemisation at the optically active *C*-terminal alanine residue if activation is brought about by formation of a 2-hydroxyphenyl ester.[2] However this route [2] does not lend itself to modification such that common intermediates could be used in the preparation of a series of analogues. We therefore turned our attention to routes 2 and 3, neither of which carries any significant risk of racemisation, providing the tripeptide monomers are prepared by procedures which are known to preclude it.

Blout and his colleagues [7] have described the version of route 2 which is shown in Scheme 1. As Blout and his associates recognise,[7] such an approach is not entirely free of the danger of racemisation although none was in their case detected, since the activation of a dipeptide with *C*-terminal L-alanine is involved. As we were anxious to avoid this danger we have examined some variants of route 2 (Schemes 2 and 3) in which the



SCHEME 1 † Conditions: i, mixed carbonic anhydride; ii, HBr–AcOH; iii, Et_3N–Me_2SO

† Abbreviations for amino-acid residues and their mode of use throughout this paper follow the relevant Tentative Rules of the I.U.P.A.C.-I.U.B. Combined Commission on Biochemical Nomenclature, reprinted in ' Specialist Periodical Reports, Amino-acids, Peptides, and Proteins,' The Chemical Society, 1970, **2**, ch. 5.

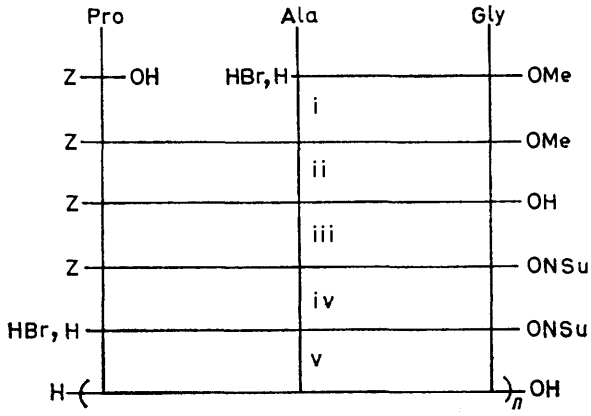


SCHEME 2 (a) X = Np, (b) X = Pcp. Conditions: i, mixed pivalic anhydride; ii, HBr–AcOH; iii, (a) Et_3N–Me_2SO, (b) *N*-methylmorpholine–Me_2SO

L-alanyl-glycine peptide bond of the monomer was formed with benzyloxycarbonyl-L-alanine as carboxy-

[1] Part III, R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1814.
[2] R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.
[3] P. Brown and L. E. Glynn, unpublished results.
[4] F. Borek, J. Kurtz, and M. Sela, *Biochim. Biophys. Acta*, 1969, **188**, 314.
[5] For recent references to the synthesis and study of sequential polypeptides, see J. H. Jones, ' Specialist Periodical Reports on Amino-acids, Peptides and Proteins,' The Chemical Society, 1969, **1**, 174; 1970, **2**, 143; 1971, **3**, 219.
[6] P. J. Oriel and E. R. Blout, *J. Amer. Chem. Soc.*, 1966, **88**, 2035.
[7] F. R. Brown tert., A. di Corata, G. P. Lorenzi, and E. R. Blout, *J. Mol. Biol.*, 1972, **63**, 85.

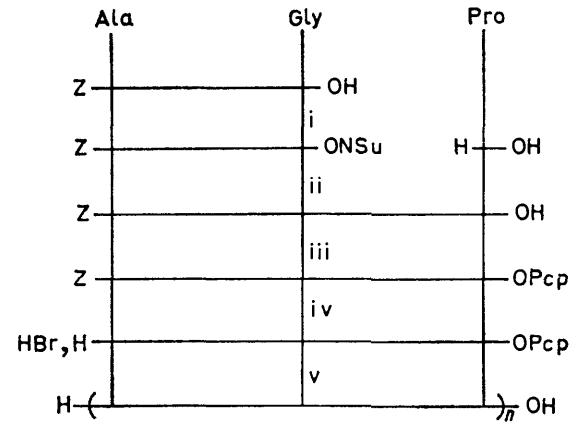
component. All three methods gave reasonable (50—70%) crude yields but the yield after dialysis was low in all three cases and the polymer was not of high molecular weight as judged by viscosity determinations.* In the case of our 4-nitrophenyl ester route



SCHEME 3 Conditions: i, dicyclohexylcarbodi-imide or mixed pivalic anhydride method; ii, OH^-; iii, mixed pivalic anhydride method; iv, HBr–AcOH (salt not characterised); v, *N*-methylmorpholine–Me_2SO

[Scheme 2 (a)] this unsatisfactory outcome was no doubt due, in part at least, to the fact that we obtained the crucial tripeptide 4-nitrophenyl ester hydrobromide as a hygroscopic material which could not be purified, and rigorous purification at this stage is probably important: [8] Blout and his colleagues, whose similar synthesis (Scheme 1) proceeded *via* the identical intermediate were able to purify it by reprecipitation. The poor performance of our succinimido ester route (Scheme 3) was also unexpected since some highly successful instances of polymerisations through succinimido esters have been described [9,10] recently: here also the difficulty is probably the fact that the tripeptide succinimido ester hydrobromide was obtained as a hygroscopic solid which was of necessity used without purification. The pentachlorophenyl ester route [Scheme 2 (b)] was not subject to difficulties of this kind, since the tripeptide pentachlorophenyl ester hydrobromide was obtained in a pure condition. The route was nevertheless still unsatisfactory, in contrast to the numerous successful applications of pentachlorophenyl esters in sequential polypeptide synthesis which have been recorded.[11] The most likely major cause of the poor yields and low molecular weights obtained is steric interference with the polycondensation by the *N*-terminal proline residue. Only a few comparable cases of active ester polymerisation through *N*-terminal proline have been reported,[7,12-14] but in one [13] of these the yield was low relative to similarly conducted reactions lacking this feature and in two others [12,14] the apparent molecular weight was low. Sporadic notes [15] of steric hindrance problems in active ester couplings with proline or other *N*-substituted amino-acids in the *N*-terminal position of the nucleophile appear in the literature, but the implication of the absence of reports to the contrary is that no special steric problems are associated with carboxy-components which have *C*-terminal proline. We therefore turned our attention to route 3.

The version of route 3 shown in Scheme 4 proved convenient for the synthesis of poly-(L-alanylglycyl-L-proline). The conversion of the tripeptide acid into its pentachlorophenyl ester was performed by a mixed carbonic anhydride procedure, or, in similar yield but more simply, by use of pentachlorophenyl trichloroacetate.[16] The same acyltripeptide pentachlorophenyl ester could also be prepared by a ' backing-off ' procedure involving the coupling of benzyloxycarbonyl-L-alanylglycine with L-proline pentachlorophenyl ester hydrobromide. The tripeptide active ester salt was



SCHEME 4 Conditions: i, dicyclohexylcarbodi-imide–HONSu; ii, $Me_2N{\cdot}CHO$–Et_3N; iii, mixed carbonic anhydride method or $CCl_3{\cdot}CO{\cdot}OPcp$–$Et_3N$–EtOAc; iv, HBr–AcOH; v, *N*-methylmorpholine–Me_2SO

treated with tertiary base in concentrated dimethyl sulphoxide solution and the polymer was isolated in the

* We find viscosity measurements useful as a rough but quick method of obtaining an indication of the range within which the molecular weight falls. Our experience is that sequential polypeptides related to collagen with weight average molecular weights as indicated by gel chromatography or ultracentrifugation of *ca.* 5000 and below generally have reduced specific viscosities, at 0·5—1% concentration in dichloroacetic acid, of below *ca.* 0·2 dl g^{-1}, whereas a value of 0·3 dl g^{-1} or greater generally corresponds to a weight average of more than *ca.* 10,000.

[8] D. F. DeTar, in ' Peptides,' eds. H. C. Beyerman, A. van de Linde, and W. Maassen van den Brink, North Holland, Amsterdam, 1967, p. 125.

[9] *E.g.*, D. M. Segal, *J. Mol. Biol.*, 1969, **43**, 497; P. M. Hardy, H. N. Rydon, and R. C. Thompson, *J.C.S. Perkin I*, 1972, 5.

[10] G. P. Lorenzi, B. B. Doyle, and E. R. Blout, *Biochemistry*, 1971, **10**, 3046.

[11] *E.g.*, B. J. Johnson, *J. Chem. Soc.* (*C*), 1969, 1412.

[12] A. M. Tamburro, A. Scatturin, and F. Marchiori, *Gazzetta*, 1968, **98**, 638.

[13] F. H. C. Stewart, *Austral. J. Chem.*, 1965, **18**, 887.

[14] F. H. C. Stewart, *Austral. J. Chem.*, 1969, **22**, 1291.

[15] *E.g.*, P. A. Jacquenod, *Chimia* (*Switz.*), 1960, **14**, 373; F. H. C. Stewart, *Austral. J. Chem.*, 1969, **24**, 2451; D. W. Russel, quoted by J. Rudinger in ' Peptides,' ed. G. T. Young, Pergamon, London, 1963, p. 133.

[16] M. Fujino and C. Hatanaka, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 929.

usual way and purified by exhaustive dialysis. On two separate occasions, 50 and 70% yields of poly-(L-alanylglycyl-L-proline) preparations were obtained with weight average molecular weights, determined by gel chromatography,[17] of 12,000 and 14,000 respectively. A similarly satisfactory yield and molecular weight were obtained by use of precisely the same procedure in the synthesis of poly-(D-alanylglycyl-D-proline).

Difficulties were encountered when methods analogous to those used for the L–L and D–D polymers were applied to syntheses of poly-(D-alanylglycyl-L-proline) and poly-(L-alanylglycyl-D-proline). Whereas the pivalic anhydride method had been satisfactory for the preparation of benzyloxycarbonyl-L-alanylglycyl-L-proline pentachlorophenyl ester by ' backing off,' in attempted preparations of the corresponding L–D derivative the same conditions gave pivaloylproline pentachlorophenyl ester as the major and only isolable product. The required D–L and L–D active esters were therefore prepared from the corresponding D–L and L–D tripeptide acids, when no special problems were encountered, and were subjected to the sequence of reactions shown in Scheme 4. Unfortunately the polymerisation conditions which provided high molecular weight L–L and D–D polymer preparations in high yield were much less successful with the L–D and D–L polymers. In both cases the yields and weight average molecular weights obtained by gel filtration [17] after exhaustive dialysis were consistently 30—40% and *ca.* 3000—4000, respectively. It seems unlikely that this markedly different experience is attributable to a configurational effect on reactivity since it appears from the few fragmentary studies of asymmetric synthesis in peptide bond formation which have been reported (*e.g.* involving mixed carbonic anhydride,[18] dicyclohexylcarbodiimide,[19] or azide couplings [20]) that D–L or L–D peptides are formed more easily than L–L or D–D peptides.* A more likely explanation is that the L–D and D–L peptide active esters have a higher propensity to cyclise and thereby to give lower polymer yields and average molecular weights than their L–L and D–D isomers: there have been numerous examples [22] of cyclisations which were more difficult with configurationally uniform peptides than with the corresponding diastereoisomers.

EXPERIMENTAL

The general instructions given in Parts II [23] and III [1] apply. In calculating the concentrations of polymer solutions no correction was made for residual solvent in the polymer preparations. The rotatory properties of solutions of sequential collagen models can be critically dependent on the previous history of the solution and difficult to reproduce precisely because of slow changes in ordered structure (see *e.g.* ref. 7): specific rotations are only reported here for polymers where this was not the case. The gel chromatography system used for molecular weight determination has been described fully elsewhere.[17] The absolute values for carbon and nitrogen which were obtained for the polymers were low owing to tenacious retention of water (*cf.* ref. 8), but their purity was otherwise good as judged by C : N ratio and the fact that in no case were impurity absorptions detected in the n.m.r. spectrum.

Benzyloxycarbonyl-L-prolyl-L-alanylglycine 4-Nitrophenyl Ester.—Pivaloyl chloride (0·48 g, 4 mmol) was added to a stirred solution of benzyloxycarbonyl-L-proline (1 g, 4 mmol) and triethylamine (0·4 g, 4 mmol) in chloroform (15 ml) at 0°. After 10 min L-alanylglycine 4-nitrophenyl ester hydrobromide [13] (1·4 g, 4 mmol) was added, followed immediately by triethylamine (0·8 g, 8 mmol). The mixture was left at 0° for 30 min and then at 20° for 5 h, at the end of which period a clear solution had been obtained. The solvent was removed and the residue was distributed between ethyl acetate and water. The organic layer was washed with 10% sodium carbonate, 2N-hydrochloric acid, and brine, and dried. Evaporation left a white solid which was recrystallised from ethyl acetate–light petroleum to give *protected tripeptide 4-nitrophenyl ester* (1·30 g, 65%), m.p. 159—161°, $[\alpha]_D^{20}$ −53·5° (*c* 2 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1760, 1725, and 1640 cm^{-1}; τ ($CDCl_3$) 1·73 (2H, low-field doublet of AB quartet *J* 9 Hz, protons *ortho* to NO_2), 2·36 (1H, complex, N*H*·CH_2), 2·55—2·70 (7H, complex, remainder of aromatic protons), 2·92 (1H, complex, N*H*·CHMe), 4·85 (2H, s, PhCH_2), 5·25—5·92 (4H, complex, all α-CH), 6·44 (2H, complex, N·CH_2), 7·70—8·27 (4H, complex, CH_2·CH_2·CH), and 8·63 (3H, d, *J* 7 Hz, CH_3·CH) (Found: C, 57·5; H, 5·35; N, 11·1. $C_{24}H_{26}N_4O_8$ requires C, 57·8; H, 5·3; N, 11·2%).

Benzyloxycarbonyl-L-alanylglycine Pentachlorophenyl Ester.—Pivaloyl chloride (24·1 g, 0·2 mol) was added during 5 min to a vigorously stirred solution of benzyloxycarbonyl-L-alanylglycine [24] (56 g, 0·2 mol) and triethylamine (20·2 g, 0·2 mol) in chloroform (500 ml) at 0°. After a further 5 min a solution of pentachlorophenol (53·3 g, 0·2 mol) and triethylamine (20·2 g, 0·2 mol) in chloroform (250 ml) was added during 5 min. The product began to separate out after 15 min and after 75 min stirring was no longer possible. The mixture was then left at 20° for 18 h. The solid was filtered off and washed with chloroform (1 l). The filtrate and combined washings were washed with water, 10% sodium carbonate, 2N-hydrochloric acid, and brine, and dried. Evaporation left a white solid which was extracted with hot methanol and then combined with the residue from the filtration to give *acyldipeptide pentachlorophenyl ester* (60·3 g, 58%), m.p. 209—211°, $[\alpha]_D^{20}$ −23·4° (*c* 0·9 in CF_3·CO_2H), $\nu_{max.}$ (Nujol) 1780, 1690, and 1650 cm^{-1}; τ (CF_3·CO_2H) 2·1 (2H, complex, both NH), 2·65 (5H, s, aromatic protons), 4·75 (2H, s, PhCH_2), 5·4 (3H, complex, all α-CH), and 8·45 (3H, d, *J* 6 Hz, CH·CH_3)

* The examples cited are model di- or tri-peptide syntheses. It is conceivable that macromolecular factors would militate in the opposite sense and be more favourable to the formation of an all-L polymer (*cf.* in *N*-carboxyanhydride polymerisation [21]).

[17] R. Fairweather, J. H. Jones, and J. K. Wilcox, *J. Chromatog.*, 1972, **67**, 157.

[18] H. Herlinger, H. Klaimann, and I. Ugi, *Annalen*, 1967, **706**, 37.

[19] O. Cervinka and J. Budilova, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2383.

[20] L. Otvos, I. Tömösközi, and T. Mohacsi, *Tetrahedron Letters*, 1970, 1995.

[21] R. D. Lundberg and P. Doty, *J. Amer. Chem. Soc.*, 1957, **79**, 3961; M. Idelsen and E. R. Blout, *ibid.*, 1958, **80**, 2387.

[22] E. Schröder and K. Lübke, ' The Peptides,' Academic Press, New York, 1965, vol. 1, p. 274 and references cited there.

[23] Part II, R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1809.

[24] F. Weygand and W. Steglich, *Chem. Ber.*, 1960, **93**, 2983.

(Found: C, 43·4; H, 2·85; Cl, 33·8; N, 5·4. $C_{19}H_{15}Cl_5N_2O_5$ requires C, 43·2; H, 2·9; Cl, 33·6; N, 5·3%).

L-*Alanylglycine Pentachlorophenyl Ester Hydrobromide.*—Benzyloxycarbonyl-L-alanylglycine pentachlorophenyl ester (60·0 g, 0·114 mol) was suspended in acetic acid (150 ml) at 100° and hydrogen bromide in acetic acid (5·6N; 100 ml) was added. The mixture solidified after 3 min. It was kept at 100° for 10 min and then at 20° for 1 h. Ether was added and the solid was filtered off and washed with more ether (1 l). Recrystallisation from methanol–ether gave *dipeptide active ester hydrobromide* (45·1 g, 84%), m.p. 224—225° (decomp), $[\alpha]_D^{20}$ −1·16° (*c* 0·86 in MeOH), $\nu_{max.}$ (Nujol) 1770 and 1670 cm^{-1}; τ [$(CD_3)_2SO$] 0·8 (1H, t, *J* 6 Hz NH), 1·75br (3H, $\overset{+}{N}H_3$), 5·55 (2H, d, *J* 6 Hz, CH_2), 6·0br (1H, C*H*Me), and 8·55 (3H, d, *J* 6 Hz, CH_3) (Found: C, 28·1; H, 1·9; Br, 16·7; Cl, 37·6; N, 5·9. $C_{11}H_{10}BrCl_5N_2O_3$ requires C, 27·8; H, 2·1; Br, 16·8; Cl, 37·3; N, 5·9%).

Benzyloxycarbonyl-L-prolyl-L-alanylglycine Pentachlorophenyl Ester.—Pivaloyl chloride (1·2 g, 0·01 mol) was added to a solution of benzyloxycarbonyl-L-proline (2·49 g, 0·01 mol) and triethylamine (1·01 g, 0·01 mol) in chloroform (25 ml) at 0°. After 5 min a suspension of L-alanylglycine pentachlorophenyl ester hydrobromide (4·75 g, 0·01 mol) in chloroform (75 ml) was added, followed immediately by triethylamine (2·02 g, 0·02 mol). A clear solution was obtained after 5 min but product began to separate out after 15 min. The mixture was left at 0° for 30 min and then then at 16° for 18 h to give a solid mass. Chloroform (50 ml) was added and the mixture was warmed to give a solution which was washed quickly with water, 10% sodium carbonate, N-hydrochloric acid, and brine, and dried. Removal of solvent gave a gel which was reprecipitated from solution in ethyl acetate by addition of light petroleum to give *acyltripeptide active ester* (4·05 g, 65%), m.p. 208—210°, $[\alpha]_D^{20}$ −38·0° (*c* 0·96 in $CHCl_3$), $\nu_{max.}$ (Nujol) 1770, 1710, and 1625 cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 2·0—2·4 (2H, complex, both NH), 2·6 (5H, s, aromatic protons), 4·75 (2H, s, PhCH_2), 5·1—5·6 (4H, complex, all α-CH), 6·3 (2H, complex, N·CH_2·CH_2), 7·4—8·2 (4H, complex, N·CH_2·CH_2·CH_2), and 8·55 (3H, d, *J* 6 Hz, CH·CH_3) (Found: C, 45·9; H, 3·75; Cl, 28·0; N, 6·8. $C_{24}H_{22}Cl_5N_3O_6$ requires C, 46·1; H, 3·5; Cl, 28·8; N, 6·7%).

L-*Prolyl-L-alanylglycine Pentachlorophenyl Ester Hydrobromide.*—A solution of benzyloxycarbonyl-L-prolyl-L-alanylglycine pentachlorophenyl ester (3·75 g, 6 mmol) in hydrogen bromide in acetic acid (4N; 6 ml) was set aside at room temperature for 1 h. Ether (80 ml) was added and the resulting oil was triturated with ether to give a solid which was washed with ether (500 ml) by decantation. Recrystallisation from methanol–ether gave *tripeptide active ester hydrobronide* (3·3 g, 96%), m.p. 178—180° (decomp.) $[\alpha]_D^{20}$ −56·0° (*c* 1·1 in MeOH), $\nu_{max.}$ (Nujol) 1790 and 1685 cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 1·4—2·4 (4H, complex, $\overset{+}{N}H_2$ and both NH), 5·05br (2H, α-CH of proline and alanine), 5·30br (2H, s, CH_2·CO), 6·23 (2H, complex, $H_2\overset{+}{N}{\cdot}CH_2$), 7·0—7·9 (4H, complex, $CH_2{\cdot}CH_2{\cdot}$CH), and 8·32 (3H, d, *J* 6 Hz, CH·CH_3) (Found: C, 33·8; H, 3·2; Br, 14·3; Cl, 30·6; N, 7·25. $C_{16}H_{17}BrCl_5N_3O_4$ requires C, 33·6; H, 3·0; Br, 14·0; Cl, 31·0; N, 7·3%).

Benzyloxycarbonyl-L-prolyl-L-alanylglycine Methyl Ester.—*Method* (*a*). A solution of dicyclohexylcarbodi-imide (6·18 g, 30 mmol) in chloroform (20 ml) was added to a stirred solution of benzyloxycarbonyl-L-proline (7·5 g, 30 mmol), L-alanylglycine methyl ester hydrobromide [25] (7·23 g, 30 mmol), and triethylamine (3·03 g, 30 mmol) in chloroform (40 ml) at 0°. The mixture was stirred at 0° for 0·5 h and then at room temperature for 14 h. After filtration, the solvent was evaporated and the residue was extracted with ethyl acetate. The filtered extract was washed and dried in the usual manner and the solvent was removed to yield a colourless oil which crystallised on trituration with light petroleum. Recrystallisation from ethyl acetate–light petroleum gave benzyloxycarbonyl-L-prolyl-L-alanylglycine methyl ester (8·1 g, 70%), m.p. 124·5—125·5°, $[\alpha]_D^{20}$ −79·0° (*c* 2 in EtOH) {lit.,[26] m.p. 133—134·5°, $[\alpha]_D^{20}$ −81·0° (*c* 2 in EtOH); the published m.p. may be a misprint}.

Method (*b*). Pivaloyl chloride (1·2 g, 10 mmol) was added to a stirred solution of benzyloxycarbonyl-L-proline (2·5 g, 10 mmol) and triethylamine (1·01 g, 10 mmol) in chloroform at 0°. After 5 min a mixture of L-alanylglycine methyl ester hydrobromide [25] (2·4 g, 10 mmol) and triethylamine (2·02 g, 20 mmol) in chloroform (25 ml) was added. The resulting solution was stirred at 0° for 0·5 h and then at 20° for 2 h. The residue obtained on evaporation was distributed between ethyl acetate and water, and the organic layer was washed and dried in the usual manner. Evaporation gave a colourless oil which crystallised on trituration with light petroleum. Recrystallisation from ethyl acetate–light petroleum afforded benzyloxycarbonyl-L-prolyl-L-alanylglycine methyl ester (2·65 g, 68%) identical with that obtained by method (*a*).

Benzyloxycarbonyl-L-prolyl-L-alanylglycine.—A solution of benzyloxycarbonyl-L-prolyl-L-alanylglycine methyl ester (7·82 g, 20 mmol) in methanol (50 ml) was treated with N-sodium hydroxide (25 ml) and the mixture was stirred at room temperature for 0·5 h. After acidification to pH 7 with 2N-hydrochloric acid the methanol was removed by evaporation and the residue was acidified to pH 2 with 2N-hydrochloric acid. The oil which separated was extracted into ethyl acetate and the organic layer was extracted with 10% sodium carbonate. Acidification of the aqueous layer reprecipitated an oil which was again extracted with ethyl acetate. The organic layer was washed with water and brine, and dried. Evaporation gave a solid which was recrystallised from ethyl acetate–light petroleum to give *acyltripeptide* (5·5 g, 73%), m.p. 101—103°, $[\alpha]_D^{20}$ −72·6° (*c* 1·9 in EtOH), $\nu_{max.}$ (Nujol) 1750 and 1660br cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 2·05—2·70 (7H, singlet at 2·58 superimposed on complex band, aromatic protons and both NH), 4·70 (2H, s, PhCH_2), 5·0—5·5 (2H, complex, α-CH of proline and alanine), 5·77 (2H, complex, NH·CH_2), 6·24 (2H, complex, N·CH_2), 7·4—8·1 (4H, complex, $CH{\cdot}CH_2{\cdot}$CH), and 8·56 (3H, d, *J* 7 Hz, $CH_3{\cdot}$CH) (Found: O, 57·1; H, 6·3; N, 10·75. $C_{18}H_{23}N_3O_6$ requires C, 57·3; H, 6·1; N, 11·1%).

Benzyloxycarbonyl-L-prolyl-L-alanylglycine Succinimido Ester.—Pivaloyl chloride (1·2 g, 10 mmol) was added to a stirred solution of benzyloxycarbonyl-L-prolyl-L-alanylglycine (3·77 g, 10 mmol) and triethylamine (1·01 g, 10 mmol) in chloroform (20 ml). After 5 min a solution of *N*-hydroxysuccinimide (1·15 g, 10 mmol) and triethylamine (1·01 g, 10 mmol) in chloroform (15 ml) was added and the mixture was left at 0° for 0·5 h and then at 18° for 2 h.

[25] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

[26] R. Walter and V. du Vigneaud, *Biochemistry*, 1966, **5**, 3720.

The solution was then washed and dried in the usual manner and the solvent was evaporated to give an oil which crystallised slowly on trituration with light petroleum. Recrystallisation from propan-2-ol–light petroleum gave *acyltripeptide active ester* (3·98 g, 84%), m.p. 98—100°, $[\alpha]_D^{20}$ −71·5° (*c* 1·2 in EtOH), $\nu_{max.}$ ($CHCl_3$) 1825, 1790, 1745, and 1685br cm^{-1}; τ ($CDCl_3$) 2·2—3·15 (7H, s at 2·61 superimposed on complex band, aromatic protons and both NH), 4·85 (2H, s, PhCH_2), 5·25—6·0 (4H, complex, all α-CH), 6·44 (2H, complex, N·CH_2), 7·21 (4H, s, CO·CH_2·CH_2·CO), 7·7—8·3 (4H, complex, CH_2·CH_2·CH), and 8·65 (3H, d, *J* 7 Hz, CH_3·CH) (Found: C, 55·6; H, 5·5; N, 11·45. $C_{22}H_{26}N_4O_8$ requires C, 55·7; H, 5·5; N, 11·8%).

Poly-(L-*prolyl*-L-*alanylglycine*).—(a) via *the Pentachlorophenyl ester*. *N*-Methylmorpholine (2·5 ml, 10 mmol) was added to a stirred solution of L-prolyl-L-alanylglycine pentachlorophenyl ester hydrobromide (2·365 g, 5 mmol) in dimethyl sulphoxide (2 ml). After 6 days the rigid mass was triturated with ethanol (50 ml) and the crude polymer was collected by centrifugation and washed with ethanol (3 × 50 ml) and ether (2 × 50 ml). It was dissolved in 50% acetic acid (50 ml) and the solution was dialysed against water (4 l) for 24 h, with changes of water every 8 h. Lyophilisation gave a fluffy white solid which was dried to constant weight at 70° and 0·1 mmHg to give poly-(L-prolyl-L-alanylglycine) (0·384 g, 34%), $\nu_{max.}$ (KBr) 1650br cm^{-1}, $\eta_{sp.}/c$ 0·23 dl g^{-1} (*c* 0·9 in $CHCl_2$·CO_2H); τ (CF_3·CO_2H) 1·8—2·4 (2H, complex, both NH), 4·9—5·9 (4H, complex, all α-CH), 5·9—6·4 (2H, complex, N·CH_2), 7·2—8·0 (4H, complex, CH_2·CH_2·CH), and 8·40br (3H, s, CH_3·CH) [Found: C, 47·2; H, 6·45; N, 17·3%; C/N 2·74. Calc. for $(C_{10}H_{15}N_3O_3)_n$: C, 53·3; H, 6·7; N, 18·7%; C/N 2·85].

(b) via *the Succinimido ester*. A solution of benzyloxycarbonyl-L-prolyl-L-alanylglycine succinimido ester (1·0 g, 2·1 mmol) in 4N-hydrogen bromide in acetic acid (2 ml) was set aside at room temperature for 1 h. Ether (80 ml) was added and the mixture was triturated to give a white solid which was washed with ether (10 × 50 ml) by decantation and dried. The resulting hygroscopic solid was dissolved in dimethyl sulphoxide (1 ml) and *N*-methylmorpholine (1 ml, 9 mmol) was added to the stirred solution. After 4 days the rigid mass was triturated with ethanol (50 ml) and the crude polymer was isolated and purified as described in (a). Lyophilisation, followed by drying to constant weight at 80° and 0·1 mmHg, gave polymer as a fluffy white solid (0·074 g, 16%) $\eta_{sp.}/c$ 0·17 dl g^{-1} (*c* 1 in $CHCl_2$·CO_2H) (Found: C, 47·8; H, 6·4; N, 16·4%; C/N 2·91).

(c) via *the 4-Nitrophenyl ester*. Benzyloxycarbonyl-L-prolyl-L-alanylglycine 4-nitrophenyl ester (1·0 g, 2·0 mmol) was treated with hydrogen bromide in acetic acid and the resulting hygroscopic hydrobromide was treated as described for the corresponding succinimido ester. Lyophilisation gave polymer (0·121 g, 22%) as a fluffy white solid, $\eta_{sp.}/c$ 0·21 dl g^{-1} (*c* 1 in $CHCl_2$·CO_2H) (Found: C, 47·4; H, 6·3; N, 16·9%; C/N 2·8).

*Benzyloxycarbonyl-*L-*alanylglycine Succinimido Ester*.—Dicyclohexylcarbodi-imide (4·12 g, 20 mmol) dissolved in dioxan (200 ml) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycine [24] (5·60 g, 20 mmol) and *N*-hydroxysuccinimide (2·30 g, 20 mmol) in dioxan at 5°. After 0·5 h at 5° the temperature was allowed to rise to room temperature during 4 h, and solvent was removed after filtration. The residual oil crystallised on trituration with ether. Recrystallisation from propan-2-ol gave *acyldipeptide active ester* (6·96 g, 92%), m.p. 128—129°, $[\alpha]_D^{20}$ −18·6° (*c* 1·1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1830, 1795, 1750, and 1700br cm^{-1}; τ ($CDCl_3$) 2·55—2·9 (6H, singlet at 2·66 superimposed on complex band, aromatic protons and NH·CH_2), 4·24 (1H, d, *J* 8 Hz, urethane NH), 4·91 (2H, s, PhCH_2), 5·69 (3H, complex, all α-CH), 7·25 (4H, s, CO·CH_2·CH_2·CO), and 8·64 (3H, d, *J* 8 Hz, CH_3·CH) (Found: C, 54·3; H, 5·1; N, 11·0. $C_{17}H_{19}N_3O_7$ requires C, 54·1; H, 5·1; N, 11·1%).

*Benzyloxycarbonyl-*L-*alanylglycyl-*L-*proline*.—L-Proline (4·6 g, 0·04 mol) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycine succinimido ester (15·08 g, 0·04 mol) and triethylamine (5·6 ml, 0·04 mol) in dimethylformamide (40 ml) at 20°. The clear solution which was obtained after 5 min was set aside for a further 16 h at 20° and then the solvent was evaporated. Water (20 ml) was added to the residue and the aqueous solution was acidified to pH 2 with 2N-hydrochloric acid. The resulting oil was extracted into chloroform, and the combined organic extracts were washed with water and brine, and dried. Evaporation gave a gel which was recrystallised from ethyl acetate–light petroleum to give *acyltripeptide* (14·75 g, 98%), m.p. 117—120°, $[\alpha]_D^{20}$ −53·3° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1720br cm^{-1}; τ ($CDCl_3$) 1·45br (1H, s, CO_2H), 2·40br (1H, s, NH·CH_2), 2·63 (5H, s, aromatic protons), 3·95br (1H, urethane NH), 4·86 (2H, s, PhCH_2), 5·3—6·1 (4H, complex, all α-CH), 6·25—6·7 (2H, complex, N·CH_2), 7·6—8·3 (4H, complex, CH_2·CH_2·CH), and 8·57 (3H, d, *J* 7 Hz, CH_3·CH) (Found: C, 56·9; H, 6·3; N, 10·8. $C_{18}H_{23}N_3O_6$ requires C, 57·3; H, 6·1; N, 11·1%).

L-*Proline Pentachlorophenyl Ester Hydrobromide*.—A solution of benzyloxycarbonyl-L-proline pentachlorophenyl ester [27] (3·1 g, 6·25 mmol) in 4N-hydrogen bromide in acetic acid (6·5 ml) was set aside at room temperature for 1 h. Ether (80 ml) was added and the white precipitate was washed with ether (6 × 50 ml) by decantation. Recrystallisation from methanol gave *active ester hydrobromide* (2·55 g, 92%), m.p. 209—210° (decomp.), $[\alpha]_D^{20}$ +14·9° (*c* 1·1 in MeOH), $\nu_{max.}$ (Nujol) 1790 cm^{-1} (Found: C, 29·8; H, 2·1; Br, 18·1; Cl, 39·7; N, 2·95. $C_{11}H_9BrCl_5NO_2$ requires C, 29·7; H, 2·0; Br, 18·0; Cl, 39·9; N, 3·2%).

*Benzyloxycarbonyl-*L-*alanylglycyl-*L-*proline Pentachlorophenyl Ester*.—*Method* (*a*). Pivaloyl chloride (0·6 g, 5 mmol) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycine [24] (1·4 g, 5 mmol) and triethylamine (0·5 g, 5 mmol) in chloroform (10 ml) at 0°. After 5 min a suspension of L-proline pentachlorophenyl ester hydrobromide (2·22 g, 5 mmol) in chloroform (20 ml) was added, followed by triethylamine (1 g, 10 mmol). The resulting clear solution was left at 0° for 0·5 h and then at 20° for 2 h. The residue obtained on evaporation was distributed between ethyl acetate and water and the organic layer was washed with 10% sodium carbonate, water, N-hydrochloric acid, and brine, and dried. Evaporation gave an oil which crystallised slowly on addition of ethyl acetate (5 ml). Recrystallisation from ethyl acetate gave white crystals of *acyltripeptide active ester* (1·90 g, 61%), m.p. 157—158°, $[\alpha]_D^{20}$ −55·6° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1786, 1720, and 1655 cm^{-1}; τ ($CDCl_3$) 2·63 (5H, s, aromatic protons), 2·87br (1H, NH·CH_2), 4·35 (1H, complex, urethane NH), 4·97 (2H, s, PhCH_2), 5·07 (1H, complex, α-CH

[27] J. Kovacs, M. Q. Ceprini, C. A. Dupraz, and G. N. Schmit, *J. Org. Chem.*, 1967, **32**, 3696.

of proline), 5·63 (1H, complex, α-CH of alanine), 5·90br (2H, NH·CH_2), 6·2—6·6 (2H, complex, CH_2·N), 7·4—8·1 (4H, complex, CH_2·CH_2·CH), and 8·62 (3H, d, J 8 Hz, CH_3·CH) (Found: C, 46·0; H, 3·6; Cl, 28·15; N, 6·6. $C_{24}H_{22}Cl_5N_3O_6$ requires C, 46·1; H, 3·5; Cl, 28·3; N, 6·7%). On concentration of the liquors from the foregoing recrystallisation, long white needles of *pivaloyl-L-proline pentachlorophenyl ester* (266 mg) were deposited; m.p. 152—153°, $[\alpha]_D^{20}$ −4·7° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1790 and 1627 cm^{-1}; τ ($CDCl_3$) 5·09 (1H, complex, α-CH of proline), 6·17 (2H, complex, CH_2·N), 7·55—8·1 (4H, complex, CH_2·CH_2·CH), and 8·70 (9H, s, Me_3C) (Found: C, 43·0; H, 3·5; Cl, 39·4; N, 3·0. $C_{16}H_{16}Cl_3$ requires C, 43·0; H, 3·6; Cl, 39·6; N, 3·1%).

A mixed carbonic anhydride reaction performed with ethyl chloroformate and the acyltripeptide in the usual way gave the acyltripeptide active ester (66% yield), m.p. 156—159°, $[\alpha]_D^{20}$ −55·5 (*c* 1 in $CHCl_3$).

Method (*b*). A solution of pentachlorophenyl trichloroacetate [16] (8·6 g, 20 mmol) in ethyl acetate (30 ml) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycyl-L-proline (7·54 g, 20 mmol) and triethylamine (2·8 ml, 20 mmol) in ethyl acetate (20 ml) at room temperature. After 16 h ethyl acetate (100 ml) was added and the mixture was warmed to give a clear solution which was washed quickly with water, 10% sodium carbonate, water, N-hydrochloric acid, and brine, and dried. Evaporation gave an oil which crystallised on trituration with ethyl acetate (5 ml). Recrystallisation from ethyl acetate gave product (8·63 g, 69%), m.p. 160—161°, $[\alpha]_D^{20}$ −55·5° (*c* 1 in $CHCl_3$).

L-Alanylglycyl-L-proline Pentachlorophenyl Ester Hydrobromide.—Hydrogen bromide in acetic acid (5·6; 10 ml) was added to a solution of benzyloxycarbonyl-L-alanylglycyl-L-proline pentachlorophenyl ester (6·25 g, 10 mmol) in acetic acid (10 ml) at room temperature. After 1 h ether (100 ml) was added and the precipitate was filtered off and washed with ether (500 ml). Recrystallisation from propan-2-ol–ether gave *tripeptide active ester hydrobromide* (5·38 g, 94%), m.p. 159—161°, $[\alpha]_D^{20}$ −56·2° (*c* 1·1 in MeOH), $\nu_{max.}$ (Nujol) 1785, 1680, and 1650br cm^{-1}; τ (CF_3·CO_2H) 1·65br (1H, NH·CH_2), 2·5br (3H, $\overset{+}{N}H_3$), 4·76 (1H, complex, α-CH of proline), 5·0—5·7 (3H, complex, α-CH of alanine and glycine), 5·8—6·3 (2H, complex, N·CH_2·CH_2), 7·0—7·9 (4H, complex, CH_2·CH_2·CH), and 8·11br (3H, s, CH_3·CH) (Found: C, 33·6; H, 3·1; Br, 14·15; Cl, 30·8; N, 7·1. $C_{16}H_{17}BrCl_5N_3O_4$ requires C, 33·6; H, 3·0; Br, 14·0; Cl, 31·0; N, 7·3%).

Poly-(L-alanylglycyl-L-proline).— *N*-Methylmorpholine (0·4 ml, 3·75 mmol) was added to a stirred solution of L-alanylglycyl-L-proline pentachlorophenyl ester hydrobromide (1·15 g, 2 mmol) in dimethyl sulphoxide (1 ml) at room temperature. After 3 days the resulting stiff paste was triturated with ethanol (50 ml) and the insoluble material was collected by centrifugation. It was washed with ethanol (4 × 50 ml) and ether (2 × 50 ml), and dried to give crude polymer (0·325 g, 78%) as a pale grey powder. This was dissolved in 50% acetic acid (20 ml) and the solution was dialysed against water (4 l) for 30 h, with changes of water every 10 h. Lyophilisation, followed by drying to constant weight at 100° and 0·1 mmHg gave poly-(L-alanylglycyl-L-proline) (0·225 g, 50%) as a fluffy white solid $\nu_{max.}$ (KBr) 1650br cm^{-1}, $\eta_{sp.}/c$ 0·34 dl g^{-1} (*c* 1 in $CHCl_2$·CO_2H), $\bar{M}_n$ 9000, $\bar{M}_w$ 12,000 (by gel chromatography [17]); τ (CF_3·CO_2H) 1·8—2·4 (2H, complex, both NH), 4·9—5·9 (4H, complex, all α-CH), 5·9—6·4 (2H, complex, N·CH_2), 7·2—8·0 (4H, complex, CH_2·CH_2·CH), and 8·40br (3H, s, CH_3·CH). Amino-acid analysis: Ala 1·00; Gly 0·96; Pro 0·94 [Found: C, 48·2; H, 6·5; N, 16·9%; C/N 2·85. Calc. for $(C_{10}H_{15}N_3O_3)_n$: C, 53·3; H, 6·7; N, 18·7%; C/N 2·85]. The preparation was repeated on an 8 mmol scale; purified polymer was obtained in 70% yield; $\eta_{sp.}/c$ 0·38 dl g^{-1} (*c* 1 in $CHCl_2$·CO_2H), $\bar{M}_n$ 9000, $\bar{M}_w$ 14,000 (by gel chromatography [17]). Amino-acid analysis: Ala 1·00; Gly 0·97; Pro 0·97 (Found: C, 47·3; H, 7·1; N, 16·7%; C/N 2·84).

Benzyloxycarbonyl-D-alanine S-Phenyl Thioester.—This was prepared from benzyloxycarbonyl-D-alanine [28] by the method previously used [24] for the L-isomer, on a 50 mmol scale in 91% yield; m.p. 82—83·5°, $[\alpha]_D^{20}$ +34·8° (*c* 2·1 in EtOH) {lit.,[24] m.p. 84·5°, $[\alpha]_D^{23}$ −39·4° (*c* 2·06 in EtOH) for the L-isomer}, $\nu_{max.}$ ($CHCl_3$) 1725 and 1710 cm^{-1}; τ ($CDCl_3$) 2·63 (10H, 2 superimposed singlets, aromatic protons), 4·4—4·7 (1H, complex, NH), 4·84 (2H, s, PhCH_2), 5·25—5·65 (1H, complex, CH·CO), and 8·56 (3H, d, J 7 Hz, CH_3·CH) (Found: C, 64·6; H, 5·7; N, 4·7; S, 9·95. $C_{17}H_{17}NO_3S$ requires C, 64·7; H, 5·4; N, 4·7; S, 10·2%).

Benzyloxycarbonyl-D-alanylglycine.—This compound was prepared from the foregoing thioester on a 40 mmol scale, by the method described [24] for the L-isomer; yield 75%, m.p. 125—127°, $[\alpha]_D^{20}$ +16·0° (*c* 2·7 in EtOH) {lit.,[24] m.p. 127—128°, $[\alpha]_D^{25}$ −17·0° (*c* 2·65 in EtOH) [24] for the L-isomer; lit.,[29] m.p. 103—104° for the D-isomer}; $\nu_{max.}$ ($CHCl_3$) 1720 and 1675 cm^{-1}; τ (CF_3·CO_2H) 2·1—2·4 (2H, complex, both NH), 2·58 (5H, s, aromatic protons), 4·70 (2H, s, PhCH_2), 5·2—5·4 (1H, complex, CHMe), 5·73br (2H, s, NH·CH_2), and 8·44 (3H, d, J 6 Hz, CH_3·CH) (Found: C, 55·3; H, 5·65; N, 9·7. Calc. for $C_{13}H_{16}N_2O_5$: C, 55·7; H, 5·75; N, 10·0%).

Benzyloxycarbonyl-D-proline.—This compound was prepared by resolution [30] of the DL-form. Recrystallisation from ether–light petroleum afforded benzyloxycarbonyl-D-proline, m.p. 75—77°, $[\alpha]_D^{20}$ +57·6° (*c* 5·1 in AcOH), $[\alpha]_D^{20}$ +39·5° (*c* 2 in EtOH) (Found: C, 62·3; H, 6·0; N, 5·5. Calc. for $C_{13}H_{15}NO_4$: C, 62·6; H, 6·1; N, 5·6%). Vogler and Lanz [30] report m.p. 76—77°, $[\alpha]_D^{23}$ +61·2° (*c* 5·3 in AcOH); a sample of the L-isomer prepared from L-proline of natural origin in this laboratory had m.p. 76—77°, $[\alpha]_D^{20}$ −57·5° (*c* 5 in AcOH) (Found: C, 62·4; H, 6·1; N, 5·6%). Berger *et al.*[31] quote m.p. 76—77°, $[\alpha]_D^{20}$ −61·7° (*c* 5·3 in AcOH), and Grassmann and Wünsch [32] give m.p. 77°, $[\alpha]_D^{20}$ −60·5° ± 0·5° (*c* 2 in AcOH), $[\alpha]_D^{20}$ −40·6° ± 0·5° (*c* 2 in EtOH).

Benzyloxycarbonyl-D-proline Pentachlorophenyl Ester.—A solution of dicyclohexylcarbodi-imide (10·3 g, 50 mmol) in ethyl acetate (25 ml) was added during 5 min to a stirred solution of benzyloxycarbonyl-D-proline (12·5 g, 50 mmol) and pentachlorophenol (13·3 g, 50 mmol) in ethyl acetate (75 ml) at −10°. After 2 h at −10° and 3 h at 20° the mixture was filtered and the filtrate was evaporated to yield an oil which crystallised when mixed with light petroleum and left overnight. Recrystallisation from

[28] M. Hunt and V. du Vigneaud, *J. Biol. Chem.*, 1938, **124**, 699.
[29] M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider, and H. Schleich, *J. Biol. Chem.*, 1935, **109**, 325.
[30] K. Vogler and P. Lanz, *Helv. Chim. Acta*, 1966, **49**, 1348.
[31] A. Berger, J. Kurtz, and E. Katchalski, *J. Amer. Chem. Soc.*, 1954, **76**, 5552.
[32] W. Grassman and E. Wünsch, *Chem. Ber.*, 1958, **91**, 462.

ethanol gave *benzyloxycarbonyl-D-proline pentachlorophenyl ester* (21·7 g, 87%), m.p. 93—95°, $[\alpha]_D^{20}$ +46·5° (*c* 1 in $Me_2N{\cdot}CHO$) {lit.,[27] m.p. 93—95·5°, $[\alpha]_D^{21}$ −47·7° (*c* 1·01 in $Me_2N{\cdot}CHO$) for the L-isomer}; $\nu_{max.}$ ($CHCl_3$) 1893 and 1705 cm^{-1}; τ ($CDCl_3$) 2·62 (5H, s, aromatic protons), 4·84 (2H, complex, $PhCH_2$), 5·20 (1H, complex, α-CH), 6·1—6·6 (2H, complex, $N{\cdot}CH_2$), and 7·4—8·15 (4H, complex, $CH_2{\cdot}CH{\cdot}CH$) (Found: C, 46·0; H, 2·95; Cl, 35·3; N, 2·45. $C_{19}H_{14}Cl_5NO_4$ requires C, 45·85; H, 2·8; Cl, 35·6; N, 2·8%).

D-Proline Pentachlorophenyl Ester Hydrobromide.—This compound was prepared in 90% yield as described for the L-isomer. Recrystallisation from methanol–ether gave *D-proline pentachlorophenyl ester hydrobromide* as plates, m.p. 209—210° (decomp.), $[\alpha]_D^{20}$ −15·4° (*c* 1·1 in MeOH), $\nu_{max.}$ (Nujol) 1788 cm^{-1} (Found: C, 30·0; H, 2·3; Br, 17·9; Cl, 40·3; N, 3·2. $C_{11}H_9BrCl_5NO_2$ requires C, 29·7; H, 2·0; Br, 18·0; Cl, 39·9; N, 3·2%).

Benzyloxycarbonyl-D-alanylglycyl-D-proline Pentachlorophenyl Ester.—This was synthesised on a 10 mmol scale from benzyloxycarbonyl-D-alanylglycine and D-proline pentachlorophenyl ester hydrobromide *via* the pivalic mixed anhydride as described for the enantiomer; yield 57%, m.p. 158—159°, $[\alpha]_D^{20}$ +55·6° (*c* 1 in $CHCl_3$); spectroscopic properties as detailed for the enantiomer (Found: C, 46·3; H, 3·5; Cl, 28·05; N, 6·4. $C_{24}H_{22}Cl_5N_3O_6$ requires C, 46·1; H, 3·5; Cl, 28·3; N, 6·7%). On concentration of the mother liquors from the recrystallisation, a few crystals of *pivaloyl-D-proline pentachlorophenyl ester* were deposited; m.p. 153—156°, $[\alpha]_D^{20}$ +4·6° (*c* 1 in $CHCl_3$); spectroscopic properties as detailed for the L-isomer (Found: C, 43·2; H, 3·6; Cl, 39·7; N, 3·0. $C_{16}H_{16}Cl_5NO_3$ requires C, 43·0; H, 3·6; Cl, 39·6; N, 3·1%).

D-Alanylglycyl-D-proline Pentachlorophenyl Ester Hydrobromide.—This compound was prepared as described for the enantiomer; yield 91%, m.p. 160—162°, $[\alpha]_D^{20}$ +56·2° (*c* 1·1 in MeOH); spectroscopic properties as detailed for the enantomer (Found: C, 33·9; H, 3·1; Br, 13·8; Cl, 30·6; N, 7·2. $C_{16}H_{17}BrCl_5N_3O_4$ requires C, 33·6; H, 3·0; Br, 14·0; Cl, 31·0; N, 7·3%).

Poly-(D-alanylglycyl-D-proline).—This was prepared as described for the enantiomer, and was obtained after lyophilisation in 53% yield; $\nu_{max.}$ (KBr) 1650br cm^{-1}; $\eta_{sp.}/c$ 0·34 dl g^{-1} (*c* 1·26 in $CHCl_2{\cdot}CO_2H$); $\bar{M}_n$ 8900, $\bar{M}_w$ 12,700 (by gel chromatography [17]); n.m.r. spectrum ($CF_3{\cdot}CO_2H$) as detailed for the enantiomer. Amino-acid analysis: Ala 1·00; Gly 0·97; Pro 1·02 [Found: C, 44·7; H, 6·1; N, 15·2%; C/N 2·85. $(C_{10}H_{15}N_3O_3)_n$ requires C, 53·3; H, 6·7; N, 18·7%; C/N 2·85].

Attempted Preparation of Benzyloxycarbonyl-L-alanylglycyl-D-proline Pentachlorophenyl Ester by the 'Backing-off' Method.—Pivaloyl chloride (0·12 g, 1 mmol) was added to a stirred solution of benzyloxycarbonyl-L-alanylglycine [24] (0·28 g, 1 mmol) and triethylamine (0·1 g, 1 mmol) in chloroform (10 ml) at 0°. After 10 min, D-proline pentachlorophenyl ester hydrobromide (0·45 g, 1 mmol) was added, followed immediately by triethylamine (0·2 g, 2 mmol). The solution was left at 0° for 0·5 h and overnight at 20°, and was then washed and dried in the usual manner. Evaporation left an oil which crystallised on addition of ethyl acetate. Recrystallisation from ethyl acetate gave chromatographically pure long needles of pivaloyl-D-proline pentachlorophenyl ester (0·29 g, 64%), identified by comparison with an authentic sample. T.l.c. of the mother liquors and comparison with authentic material obtained later showed them to contain some of the required product, but none could be isolated.

Benzyloxycarbonyl-L-alanylglycyl-D-proline.— Triethylamine (2·8 ml, 20 mmol) was added to a stirred suspension of D-proline [30] (2·3 g, 20 mmol) in a solution of benzyloxycarbonyl-L-alanylglycine succinimido ester (7·54 g, 20 mmol) in dimethylformamide (35 ml) at room temperature. The clear solution which was obtained after 5 min was left overnight and then the solvent was evaporated. The residue was dissolved in water and the aqueous solution was acidified to pH 2 with 2N-hydrochloric acid. The oil which separated was extracted into chloroform, washed with water and brine, and dried. Evaporation left an oil which crystallised on addition of ethyl acetate (5 ml). Recrystallisation from ethyl acetate gave long needles of *acyltripeptide* (7·09 g, 94%), m.p. 161—163°, $[\alpha]_D^{20}$ +34·6° (*c* 1 in MeOH), $\nu_{max.}$ ($CHCl_3$) 1730, 1695, and 1670 cm^{-1}; τ ($CDCl_3$) 0·95br (1H, CO_2H), 2·05br (1H, $NH{\cdot}CH_2$), 2·62 (5H, s, aromatic protons), 3·8—4·2 (1H, complex, urethane NH), 4·86 (2H, s, $PhCH_2$), 5·4—6·15 (4H, complex, all α-CH), 6·2—6·7 (2H, complex, $N{\cdot}CH_2$), 7·89 (4H, complex, $CH_2{\cdot}CH_2{\cdot}CH$), and 8·68 (3H, d, *J* 8 Hz, $CH_3{\cdot}CH$) (Found: C, 57·5; H, 6·1; N, 11·0. $C_{18}H_{23}N_3O_6$ requires C, 57·3; H, 6·1; N, 11·1%). Ethanol was also a satisfactory solvent for the reaction, giving yields of 80—90%.

Benzyloxycarbonyl-L-alanylglycyl-D-proline Pentachlorophenyl Ester.—A solution of ethyl chloroformate (2·02 g, 18·6 mmol) in ethyl acetate (10 ml) was added during 3 min to a stirred solution of benzyloxycarbonyl-L-alanylglycyl-D-proline (7·02 g, 18·6 mmol) and triethylamine (1·88 g, 18·6 mmol) in ethyl acetate (120 ml) at −10°. After 15 min a solution of pentachlorophenol (5 g, 18·8 mmol) and triethylamine (1·88 g, 18·6 mmol) in ethyl acetate (25 ml) was added during 5 min. The solution was left at −10° for 0·5 h and was then allowed to attain room temperature during 3·5 h. It was washed with water, 10% sodium carbonate, water, 2N-hydrochloric acid, and brine, and dried. Evaporation left an oil which solidified alowly on trituration with light petroleum. Reprecipitation from chloroform–light petroleum gave *acyltripeptide active ester* as an amorphous solid (10·63 g, 91·5%), m.p. 97—100° (with softening above 80°), $[\alpha]_D^{20}$ +49·1° (*c* 1 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1790, 1715, and 1660 cm^{-1}; τ ($CDCl_3$) 2·68 (5H, s, aromatic protons), 2·90 (1H, complex, $NH{\cdot}CH_2$), 4·37 (1H, d, *J* 8 Hz, urethane NH), 4·90 (2H, s, $PhCH_2$), 5·09 (1H, complex, α-CH of proline), 5·68 (1H, complex, α-CH of alanine), 5·94 (2H, complex, $NH{\cdot}CH_2$), 6·26—6·60 (2H, complex, $N{\cdot}CH_2$), 7·4—8·1 (4H, complex, $CH_2{\cdot}CH_2{\cdot}CH$), and 8·63 (3H, d, *J* 7 Hz, $CH{\cdot}CH$) (Found: C, 46·4; H, 3·8; Cl, 27·9; N, 6·6. $C_{24}H_{22}Cl_5N_3O_6$ requires C, 46·1; H, 3·5; Cl, 28·3; N, 6·7%).

L-Alanylglycyl-D-proline Pentachlorophenyl Ester Hydrobromide.—Hydrogen bromide in acetic acid (5·6N; 3·5 ml) was added to a solution of benzyloxycarbonyl-L-alanylglycyl-D-proline pentachlorophenyl ester (3·15 g, 5 mmol) in acetic acid (1·5 ml). The mixture, which solidified after 15 min, was left at room temperature for 1 h and was then triturated with ether (100 ml). The resulting solid was filtered off and was washed with ether (500 ml), giving an extremely hygroscopic off-white solid. This was dried (KOH) at 50° and 0·1 mmHg for 24 h and was then recrystallised from propan-2-ol–ether. Further drying (KOH) at 60° and 0·1 mmHg for 48 h gave *tripeptide active ester hydrobromide* as a white powder (2·62 g, 91·5%),

softening at 160° (decomp.), $[\alpha]_D^{20}$ +80·0° (*c* 1 in MeOH), $\nu_{max.}$ (Nujol) 1790 and 1650br cm^{-1}; τ ($CF_3 \cdot CO_2H$) 1·70br (1H, $NH \cdot CH_2$), 2·60br (3H, $\overset{+}{N}H_3$), 4·80br (1H, α-CH of proline), 5·1—5·8 (3H, complex, α-CH of alanine and glycine), 5·85—6·3 (2H, complex, $N \cdot CH_2$), 7·1—7·8 (4H, complex, $CH_2 \cdot CH_2 \cdot CH$), and 8·14br (3H, s, $CH_3 \cdot CH$) (Found: C, 33·25; H, 3·1; Br, 14·1; Cl, 31·1; N, 7·3. $C_{16}H_{17}BrCl_5N_3O_4$ requires C, 33·6; H, 3·0; Br, 14·0; Cl, 31·0; N, 7·3%).

*Poly-(*L*-alanylglycyl-*D*-proline).*— *N*-Methylmorpholine (0·44 ml, 4 mmol) was added to a stirred solution of L-alanylglycyl-D-proline pentachlorophenyl ester hydrobromide (1·15 g, 2 mmol) in dimethyl sulphoxide (1 ml) at room temperature. After 4 days the solid mass was triturated with water (50 ml) and the precipitated pentachlorophenol was extracted with ether (2 × 20 ml). The aqueous solution was dialysed against water (4 l) for 27 h, changing the water every 9 h. Lyophilisation, followed by drying to constant weight at 100° and 0·1 mmHg afforded *polymer* (0·186 g, 41·5%) as a buff-coloured fluffy solid, m.p. 200—210°, $[\alpha]_D^{20}$ +49·5°, $[\alpha]_{578}^{20}$ +51·3°, $[\alpha]_{546}^{20}$ +57·5°, $[\alpha]_{436}^{20}$ +91·4°, $[\alpha]_{365}^{20}$ +174° (*c* 0·011 in H_2O); $\nu_{max.}$ (KBr) 1650br cm^{-1}; $\eta_{sp.}/c$ 0·18 dl g^{-1} (*c* 0·9 in $CHCl_2 \cdot CO_2H$); $\bar{M}_n$ 2200, $\bar{M}_w$ 3700 (by gel chromatography [17]); τ ($CF_3 \cdot CO_2H$) 1·95br (2H, both NH), 4·8—5·8 (4H, complex, all α-CH), 6·12 (2H, complex, $N \cdot CH_2$), 7·3—8·1 (4H, complex, $CH_2 \cdot CH_2 \cdot CH$), and 8·41br (3H, d, $CH_3 \cdot CH$). Amino-acid analysis: Ala 1·00; Gly 0·96; Pro 0·96 [Found: C, 46·9; H, 6·25; N, 15·8%; C/N 2·96. $(C_{10}H_{15}N_3O_3)_n$ requires C, 53·3; H, 6·7; N, 18·7%; C/N 2·85]. Four further preparations on the same scale all gave material in similar yields after dialysis with $\eta_{sp.}/c$ 0·17—0·21 dl g^{-1} (*c* 1 in $CHCl_2 \cdot CO_2H$).

*Benzyloxycarbonyl-*D*-alanylglycine Succinimido Ester.*— This was prepared as described for the L-isomer in 69% yield; m.p. 127—129°, $[\alpha]_D^{20}$ +18·9° (*c* 1 in $CHCl_3$); spectroscopic properties as detailed for the L-isomer (Found: C, 54·0; H, 5·3; N, 11·3. $C_{17}H_{19}N_3O_7$ requires C, 54·1; H, 5·1; N, 11·1%).

*Benzyloxycarbonyl-*D*-alanylglycyl-*L*-proline.*—This was prepared as described for the enantiomer; yield 89%, m.p. 161—163°, $[\alpha]_D^{20}$ −34·8° (*c* 1·1 in MeOH); spectroscopic properties as detailed for the enantiomer (Found: C, 57·5; H, 6·1; N, 11·1. $C_{18}H_{23}N_3O_6$ requires C, 57·3; H, 6·1; 11·1%).

*Benzyloxycarbonyl-*D*-alanylglycyl-*L*-proline Pentachlorophenyl Ester.*—This was prepared as described for the enantiomer; yield 92%, m.p. 98—101° (with softening above 85°), $[\alpha]_D^{20}$ −49·5° (*c* 1 in $CHCl_3$); spectroscopic properties as detailed for the enantomer (Found: C, 46·5; H, 3·8; Cl, 28·0, N, 6·6. $C_{24}H_{22}Cl_5N_3O_6$ requires C, 46·1; H, 3·5; Cl, 28·3; N, 6·7%).

*D-Alanylglycyl-*L*-proline Pentachlorophenyl Ester Hydrobromide.*—This was prepared as described for the enantiomer; yield 95%, softening at 158° (decomp.) $[\alpha]_D^{20}$ −79·2° (*c* 1 in MeOH); spectroscopic properties as detailed for the enantiomer (Found: C, 33·6; H, 3·2; Br, 13·8; Cl, 30·7; N, 7·35. $C_{16}H_{17}BrCl_5N_3O_4$ requires C, 33·6; H, 3·0; Br, 14·0; Cl, 31·0; N, 7·3%).

*Poly-(*D*-alanylglycyl-*L*-proline).*—This was prepared on a 2 mmol scale as described for the enantiomer and was obtained as a fluffy white solid (0·130 g, 30%), $[\alpha]_D^{20}$ −59·4°, $[\alpha]_{578}^{20}$ −62·2°, $[\alpha]_{546}^{20}$ −70·8°, $[\alpha]_{436}^{20}$ −118°, $[\alpha]_{365}^{20}$ −206° (*c* 0·021 in H_2O), $\nu_{max.}$ (KBr) 1650br cm^{-1}; $\eta_{sp.}/c$ 0·18 dl g^{-1} (*c* 0·73 in $CHCl_2 \cdot CO_2H$), $\bar{M}_n$ 2550, $\bar{M}_w$ 4300 (by gel chromatography [17]); n.m.r. spectrum ($CF_3 \cdot CO_2H$) as detailed for the enantiomer. Amino-acid analysis: Ala 1·00; Gly 1·03; Pro 1·04 [Found: C, 47·5; H, 6·5; N, 16·3%; C/N 2·91. $(C_{10}H_{15}N_3O_3)_n$ requires C, 53·3; H, 6·7; N, 18·7%, C/N 2·85].

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