Sequential Polypeptides. Part III.[1] The Synthesis of Two Polyheptapeptides with Functional Side-chains

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Poly(leucylglutamyl-lysylalanylglutamylserylglycine) (I) and poly(leucylglutamyl-lysylalanylglutamylalanylglycine) (II) have been synthesised *via* the corresponding 1-(biphenyl-4-yl)-1-methylethoxycarbonylheptapeptide acids which had their side-chains protected by t-butoxycarbonyl, t-butyl ester, and t-butyl ether groups. The *C*-terminal carboxy-group was activated with bis-4-nitrophenyl sulphite: selective acidolysis of the *N*(α)-protecting group followed by treatment with triethylamine in dimethyl sulphoxide gave a polymer which was deprived of its side-chain protecting groups by treatment with trifluoroacetic acid. After dialysis the weight average molecular weight of peptide (I) was found, by gel chromatography, to be 11,000 and that of peptide (II) to be 6000.

THERE are three principal classes of fibrous protein: (i) the collagens, (ii) those with β-structures such as the silk fibroin of *Bombyx mori*, and (iii) those with α-helical structures. Much of the primary structure of collagen can be written as $(Gly\text{-}X\text{-}Y)_n$ where X is an imino-acid and Y is variable, and simple synthetic polypeptides with invariable repeating tripeptide sequences of this type have been employed as collagen models in diverse studies. These include conformational investigations in solution [2] and in the solid state,[3] work with enzymes for which collagen is the natural substrate,[4] and immunological experiments.[5] Similarly, the suggestion that

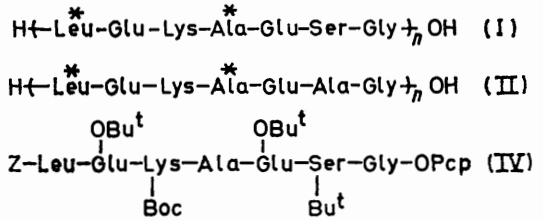
[1] Part II, R. D. Cowell and J. H. Jones, preceding paper.
[2] *E.g.* F. R. Brown, tert., A. J. Hopfinger, and E. R. Blout, *J. Mol. Biol.*, 1972, **63**, 101.
[3] *E.g.* D. M. Segal, W. Traub, and A. Yonath, *J. Mol. Biol.*, 1969, **43**, 519.
[4] *E.g.* (*a*) D. J. Prockop, K. Juva, and J. Engel, *Z. physiol. Chem.*, 1967, **348**, 553; (*b*) E. Adams, S. Antoine, and A. Goldstein, *Biochem. Biophys. Acta*, 1969, **185**, 251.
[5] F. Borek, J. Kurtz, and M. Sela, *Biochim. Biophys. Acta*, 1969, **188**, 314.

much of the sequence of *Bombyx mori* silk fibroin can be represented by $(Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser\text{-}Gly)_n$ has been corroborated by *X*-ray measurements [6] on a synthetic sequential hexapeptide with this sequence.

The proposition that the α-helical fibrous proteins also have sequences which embody some regular repetition is, however, less firmly established. Crick [7] and Pauling and Corey [8] independently pointed out in the early nineteen fifties that the *X*-ray diffraction patterns of this class of protein could be interpreted as indicative of a structure in which α-helices were interwoven in multi-strand rope arrangements, and the possibility of regularly repeating sequences was discussed. In 1963, consideration of the *X*-ray diffraction pattern and amino-acid composition of the α-helical molluscan catch muscle protein paramyosin led Cohen and Holmes [9] to propose for this protein a structure comprising two α-helical coils coiled one about the other, with a primary sequence with leucine at every seventh position and alanine three residues removed from every leucine residue. With these sequential constraints it was possible to construct a model such that the leucine residues of each helical strand fitted into cavities left by the alanine residues of the other, so that the interior of the coiled coil was composed of interlocking hydrophobic side-chains. Most of the remaining amino-acids present were acidic, basic, or hydroxylic: these could be placed in the remaining positions of the repeating heptapeptide sequence with the result that the exterior of the coiled coil was highly hydrophilic.

However, the detailed interpretation of the *X*-ray diffraction patterns obtained from this class of fibrous protein is a matter of current controversy,[10] and no amino-acid sequence results are available to support the idea of sequential periodicity. One possible approach to the problems of this area is through studies of the *X*-ray diffraction patterns and other properties of sequential polypeptides designed as models of the α-helical proteins. At the instigation of Dr. A. Miller, Department of Molecular Biophysics, Oxford, we have therefore embarked upon the synthesis and characterisation of a series of such models primarily for this purpose, and now describe our initial efforts in this direction.

The design of our first two models, the polyheptapeptides (I) and (II), was based largely on the amino-acid composition of paramyosin [11] and the structure proposed by Cohen and Holmes [9] for this protein. The leucine and alanine residues marked with an asterisk in structures (I) and (II) are disposed to meet the requirements of the proposed paramyosin structure, and the relative proportions of the different types of residue are not grossly different from the relative proportions in paramyosin, except that paramyosin has a much lower glycine content than the model. The amino-acid composition of peptide (I) is similar to that of the α-protein prekeratin,[12] which has a higher proportion of glycine but less alanine than paramyosin. We were obliged



to choose glycine as the *C*-terminal residue of the monomeric unit to ensure that racemisation could not intervene in the final stages of our synthesis: at the time this work was initiated, other work [13] on the racemisation-free preparation of sequential polypeptides composed entirely of optically active amino-acids and having functional side-chains was still at a preliminary stage.

The synthesis of peptide (I) from the protected hexapeptide acid [1] (III) is shown in the Scheme. This synthesis follows current practice in the sequential polypeptide field in that polymerisation is achieved *via* a peptide active ester: we chose side-chain protection based on derivatives of t-butyl alcohol in order to maximise the solubility of the heptapeptide active ester and to enable exposure of the functional groups by mild acidolysis after polymerisation. This choice would have been compatible with the use of a benzyloxycarbonyl group for the *N*-terminal α-amino-group of a fully protected activated monomer such as (IV). However, selective α-amino-deprotection of the monomer (IV) prior to polymerisation would have required catalytic hydrogenolysis. This strategy has been used in the synthesis of a polytripeptide,[14] but the report that it was unsatisfactory in a polytetrapeptide synthesis [15] discouraged us from applying the method in our case. We therefore selected the 1-(biphenyl-4-yl)-1-methylethoxycarbonyl group [16] for the *N*-terminal protection of our monomer, since this group can be removed by extremely mild acidolysis with complete selectivity in the presence of t-butyl-containing protective groups. The only step in the synthesis shown in the Scheme which calls for special comment is the use of bis-4-nitrophenyl sulphite [17] for the preparation of the activated heptapeptide (V): all other methods of preparing an activated ester of the protected heptapeptide (VI) which were investigated (dicyclohexylcarbodi-imide, mixed pivalic, and mixed

[6] F. H. C. Stewart, *Austral. J. Chem.*, 1966, **19**, 489; R. D. B. Fraser, T. P. MacRae, and F. H. C. Stewart, *J. Mol. Biol.*, 1966, **19**, 580.
[7] F. H. C. Crick, *Nature*, 1952, **170**, 882; *Acta Cryst.*, 1953, **6**, 689.
[8] L. Pauling and R. B. Corey, *Nature*, 1953, **171**, 59.
[9] C. Cohen and K. C. Holmes, *J. Mol. Biol.*, 1963, **6**, 423.
[10] D. A. D. Parry, *J. Theor. Biol.*, 1969, **24**, 73, and references therein.
[11] S. Seifter and P. M. Gallop in, ' The Proteins,' vol. IV, ed. H. Neurath, Academic Press, New York, 1966, p. 410.
[12] H. P. Baden and A. M. Gifford, *Biochem. Biophys. Acta*, 1970, **221**, 674.
[13] R. D. Cowell and J. H. Jones, *Chem. Comm.*, 1971, 1009.
[14] B. J. Johnson, *J. Chem. Soc.* (*C*), 1967, 2638.
[15] B. J. Johnson, *J. Chem. Soc.* (*C*), 1969, 1412.
[16] P. Sieber and B. Iselin, *Helv. Chim. Acta*, 1968, **51**, 622.
[17] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373; B. Iselin and R. Schwyzer, *ibid.*, 1960, **43**, 1760.

carbonic anhydride methods) gave complex mixtures from which reasonably pure material could not be isolated. Bis-4-nitrophenyl sulphite, on the other hand, gave essentially pure protected heptapeptide 4-nitrophenyl ester, which is in line with several recently described examples [18] where this reagent has been found useful for the preparation of complex protected peptide 4-nitrophenyl esters.

The deprotected polymer (I) was obtained after dialysis and lyophilisation as a fluffy white powder which gave satisfactory results in elemental and amino-acid analyses and showed the expected spectroscopic properties.

Gel chromatography on a calibrated column of Bio-Gel P-100, swollen and eluted with phenol–acetic acid–water (1 : 1 : 1 w/v/v),[19] was used for molecular weight determination. It has been reported [19] that use of this system gives an excellent linear relationship between elution volume and the logarithm of molecular weight for diverse peptides and proteins in the molecular weight range 1000—40,000, which can be expressed by equation (1). We checked this relationship using a set of synthetic

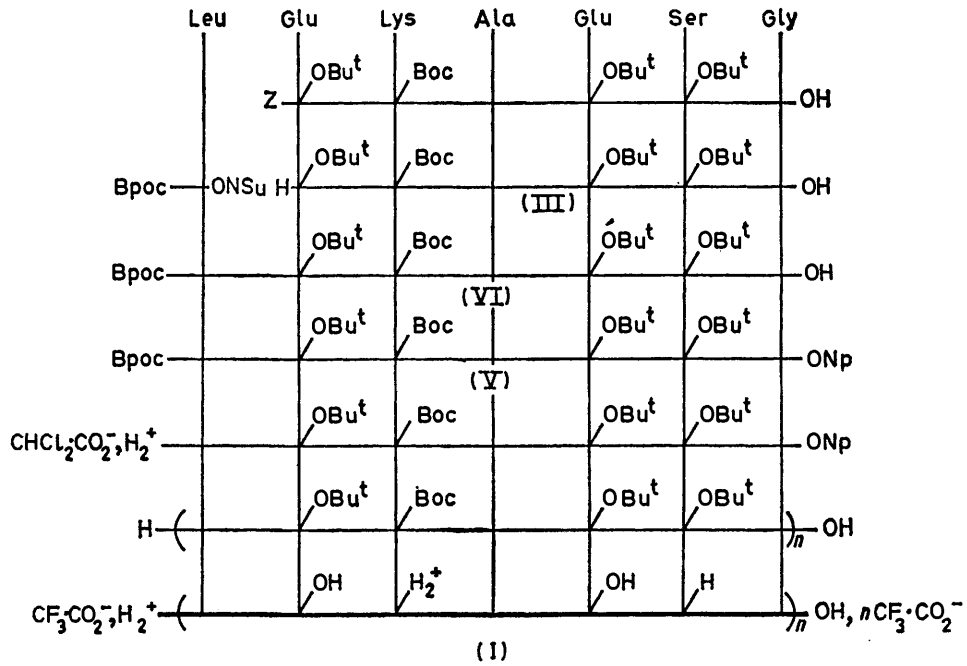
$$\log_{10} M = \{5{\cdot}13 - (0{\cdot}53 \pm 0{\cdot}013)V_e/V_o\} \pm 0{\cdot}059 \quad (1)$$

peptides and natural proteins which spanned the molecular weight distribution of our polymer. In agreement with published [19] data, we obtained an excellent linear correlation of elution volume with the logarithm of molecular weight, which is shown in Figure 1 since some of our calibrants were different. In quantitative terms our correlation corresponds to equation (2), which differs only slightly from that in the literature. A

$$\log_{10} M = 5{\cdot}17 - 0{\cdot}68V_e/V_o \quad (2)$$

sample of the polymer (I) was applied to the calibrated column, and, by use of an approach which is described in detail for a similar case elsewhere,[20] the differential molecular weight distribution curve shown in Figure 2 was derived from the elution profile, and the number and weight average molecular weights were found to be 6000 and 11,000, respectively.

The polymer (II) was prepared by a procedure precisely analogous to that shown in the Scheme with similar results, except that in this case the number and weight average molecular weights (3000 and 6000, respectively) were disappointingly low.



SCHEME Abbreviations follow the Tentative Rules in I.U.P.A.C. Information Bulletin No. 26. Bpoc = 1-(biphenyl-4-yl)-1-methylethoxycarbonyl; NSu = succinimido

Immunological and physicochemical investigations of peptides (I) and (II) and fractionated preparations of these materials are in hand. Preliminary c.d. studies of the polydisperse preparations described here indicate in both cases the presence of α-helix in acidic aqueous buffer solutions of ionic strength 0·1, as judged by the observation of the characteristic minima at 208 and 222 nm. By use of the ellipticities of the random coil and α-helical forms of poly-L-glutamic acid at 222 nm as limiting values [21] the proportion of peptide bonds present in α-helical conformations at pH 3 is shown to be *ca.* 40% for (I) and *ca.* 20% for (II); at neutral and alkaline pH both polymers had less negative ellipticities

[18] *E.g.* (*a*) J. Meienhofer, *J. Amer. Chem. Soc.*, 1970, **92**, 3771; (*b*) O. Abe and N. Izumiya, *Bull. Chem. Soc. Japan*, 1970, **43**, 1202, and earlier papers in the same series.

[19] A. Pusztai and W. B. Watt, *Biochim. Biophys. Acta*, 1970, **214**, 463.

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

[21] J. T. Yang in ' A Laboratory Manual of Analytical Methods in Protein Chemistry,' eds. P. Alexander and H. P. Lundgren, Pergamon, London, 1969, vol. 5, p. 57.

at 222 nm and in the case of peptide (I) the minimum shifted to *ca.* 225 nm. If we assume that comparison with poly-L-glutamic acid is soundly based, these low

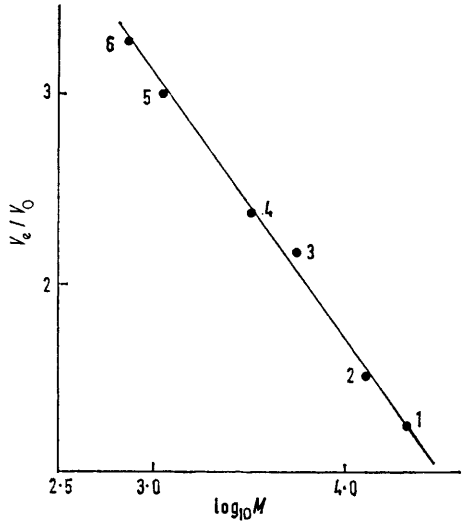


FIGURE 1 Calibration of the column of Bio-Gel P-100 swollen in phenol–water–acetic acid (1 : 1 : 1 w/v/v). The calibrants are: 1, trypsin; 2, ribonuclease A; 3, insulin; 4, synacthen ® 5, [5,8-β-cyclohexylalanine]-bradykinin; 6, leucylglutamyllysylalanylglutamylserylglycine

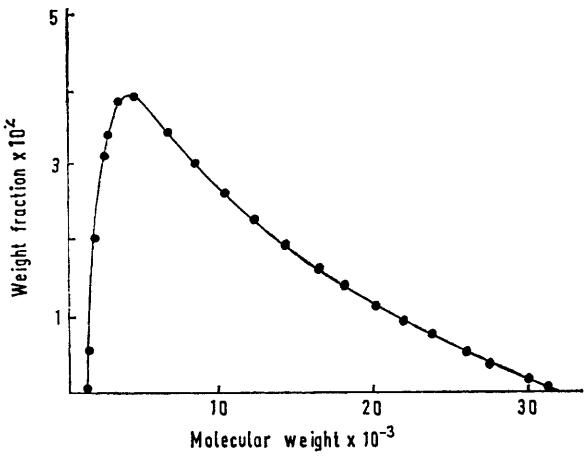


FIGURE 2 Differential molecular weight distritubtion curve for polymer (I)

values for α-helix content could indicate that only short portions of the peptide chain are able to adopt an α-helical conformation—in the case of (I), which has contiguous serine and glycine residues at intervals along the peptide chain, this would be consistent with the view [22] that such residues do not favour α-helix formation. Alternatively the low helix content of these polydisperse preparations may indicate that only material above a critical molecular weight range is able to adopt an α-helical conformation. We currently incline to the latter view in agreement with the conclusions of a recent study [23] on poly(tyrosylalanylglutamic acid) in which it was found that the critical molecular weight for the onset of α-helix formation was in the region of 5000.

[22] D. E. Goldsack, *Biopolymers*, 1969, **7**, 299, and references therein.

EXPERIMENTAL

The general instructions given in Part II [1] apply. Dimethyl sulphoxide was dried over calcium hydride and fractionally distilled. Viscosities were determined with an Ubbelohde viscometer (British Standard number BS/IP/MSL/2) at 25 ± 0·1°. C.d. spectra were recorded on a Jouan Dichrograph II (1, 0·5, or 0·1 mm cells at 22°); mean residue ellipticities are given in deg cm^2 $dmol^{-1}$ and no correction for refractive index was applied. The buffer solutions were made up as recommended by Miller and Golder: [24] these were all of ionic strength 0·1 and had nominal pH values of 3·1, 7·0, and 10·7. In calculating the concentration of polymer solutions no correction was applied for residual solvent in the polymer preparations.

Gel Chromatography.—Bio-Gel P-100 (control number 46173, 100—200 mesh) was allowed to swell for 2 weeks in phenol–acetic acid–water (1 : 1 : 1 w/v/v) under nitrogen at room temperature; no further swelling was observed after 2 weeks. After removal of the smallest particles by decantation a column of dimensions 70 × 1·2 cm was prepared and stabilised by the passage of several bed volumes of solvent. The column was operated at room temperature with a flow rate of 1·5 ml h^{-1}: 1 ml fractions were collected and the eluate was monitored by testing with ninhydrin after alkaline hydrolysis. For calibration, samples of peptides or proteins (1—5 mg in 0·2 ml) of known molecular weight were applied to the column: the void volume, determined with bovine serum γ-globulin, was 18·5 ml. The results of the calibration and the names of the calibrants are given in Figure 1. For molecular weight distribution analysis 10—20 mg of polypeptide dissolved in 0·5 ml was applied. The molecular weight distributions and averages were derived from the elution profile as described in detail elsewhere [20] for a similar system and application.

1-(*Biphenyl-4-yl*)-1-*methylethoxycarbonyl-L-leucine* 2,4,5-*Trichlorophenyl Ester.*—A solution of dicyclohexylcarbodiimide (1·25 g, 6 mmol) in ethyl acetate (5 ml) was added during 10 min to a stirred solution of 1-(biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucine [16] (2·22 g, 6 mmol) and 2,4,5-trichlorophenol (1·19 g, 6 mmol) in ethyl acetate (10 ml) at 0°. The mixture was held at this temperature overnight. Urea (1·29 g, 96%) was filtered off and the solution was evaporated to give an oil which solidified on trituration with light petroleum. Recrystallisation from ether–light petroleum gave *active ester* as white needles (2·45 g, 75%), m.p. 109—110°, $[\alpha]_D^{20}$ −35·5° (*c* 1 in $CHCl_3$); TLC-2 R_F 0·85; $\nu_{max.}$ ($CHCl_3$) 1720 and 1775 cm^{-1}; τ ($CDCl_3$) 2·3—2·85 (11H, complex, aromatic), 4·85br (1H, d, *J* 8 Hz, CO·N*H*·CH), 5·2—5·9 (1H, complex, NH·C*H*·CO), 7·95—8·40 (9H, s at 8·16 on complex, CH·CH_2·C*H*Me_2 and C_6H_4·CMe_2·O), and 9·02 (6H, d, *J* 6 Hz, CHMe_2) (Found: C, 61·5; H, 5·3; Cl, 19·3; N, 2·75. $C_{28}H_{28}Cl_3NO_4$ requires C, 61·3; H, 5·1; Cl, 19·4; N, 2·5%).

1-(*Biphenyl-4-yl*)-1-*methylethoxycarbonyl-L-leucyl-γ-t-butyl-L-glutamyl-N(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-O-t-butyl-L-serylglycine* (VI).—A solution of benzyloxycarbonyl-γ-t-butyl-L-glutamyl-*N*(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-*O*-t-butyl-L-serylglycine [1] (III) (1·88 g, 1·84 mmol) in 80% aqueous acetic acid (25 ml) was hydrogenated over 10% palladium–charcoal (0·40 g) for 3 h. The solution was filtered through Celite

[23] B. Schechter, I. Schechter, J. Ramachandran, A. Conway-Jacobs, and M. Sela, *European J. Biochem.*, 1971, **20**, 301.
[24] G. L. Miller and R. H. Golder, *Arch. Biochem.*, 1950, **29**, 420.

and evaporated. The residue was dried by repeated addition of benzene followed each time by distillation to give a white solid. 1-(Biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (1·42 g, 2·02 mmol) and triethylamine (0·26 ml, 1·85 mmol) were added to a stirred solution of this solid in dimethylformamide (15 ml). After 96 h the mixture was poured into 5% citric acid (50 ml). The resulting precipitate was immediately collected by centrifugation, washed with water (5 × 50 ml), and dried by repeated addition of benzene followed each time by evaporation. The crude product was triturated with ether (3 × 30 ml) and then light petroleum (1 × 30 ml) to give *protected heptapeptide acid* as a white solid (1·88 g, 82%), m.p. 220° (decomp.), $[\alpha]_D^{20}$ −15·4° (*c* 1·1 in C_5H_5N); TLC-7 R_F 0·97, TLC-10 R_F 0·50; amino-acid analysis Ala 1·00, Glu 2·03, Gly 1·00, Leu 0·94, Lys 0·95, Ser 0·89 (Found: C, 61·0; H, 7·9; N, 9·2. $C_{63}H_{98}N_8O_{17}$ requires C, 61·1; H, 7·9; N, 9·0%).

1-(*Biphenyl-4-yl*)-1-*methylethoxycarbonyl*-L-*leucyl*-γ-*t-butyl*-L-*glutamyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-O-*t-butyl*-L-*serylglycine* 4-*Nitrophenyl Ester* (V).—Bis-4-nitrophenyl sulphite [17] (3·94 g, 12·2 mmol) was added to a stirred solution of the preceding heptapeptide derivative (1·50 g, 1·22 mmol) in dimethylformamide–pyridine (1 : 4; 10 ml). After 21 h ether–light petroleum (4 : 1; 50 ml) was added. The gel which separated was washed with further portions of this solvent (20 × 30 ml) and dried to give *protected heptapeptide 4-nitrophenyl ester* as a buff powder (1·60 g, 96%), m.p. 175° (decomp.), $\nu_{max.}$ (KBr) 1620—1640br, 1690—1750br, and 1780 cm^{-1}; TLC-2 R_F 0·44, TLC-4 R_F 0·87 (Found: C, 60·9; H, 7·2; N, 9·4. $C_{69}H_{99}N_9O_{19}$ requires C, 61·1; H, 7·3; N, 9·3%); 4-nitrophenyl ester content [25] 96 and 98% (two separate determinations).

Poly-(L-*leucyl*-L-*glutamyl*-L-*lysyl*-L-*alanyl*-L-*glutamyl*-L-*serylglycine*) *Trifluoroacetate* (I).—The preceding heptapeptide active ester (1·45 g, 1·07 mmol) was dissolved in acetic acid–dichloroacetic acid (6 : 1; 7 ml) and the solution was kept for 1 h at room temp. Ether (50 ml) was added and the resulting precipitate was collected by centrifugation and washed with further portions of ether (6 × 30 ml) to give heptapeptide 4-nitrophenyl ester dichloroacetate as a pale buff powder (1·26 g, 94%). Triethylamine (0·25 ml, 1·8 mmol) was added to a stirred solution of the dichloroacetate salt (1·1 g, 0·88 mmol) in dimethyl sulphoxide (4 ml). The solution became more viscous during 3 days. After a further 2 days the mixture was diluted with ethanol (20 ml); the resulting precipitate was collected by centrifugation and washed with ethanol (2 × 20 ml) and ether (3 × 25 ml) to give crude protected polymer as an off-white powder (536 mg, 62%). This polymer was dissolved in 90% aqueous trifluoroacetic acid (5 ml) and stored at room temp. for 1 h. Ether (30 ml) was added and the precipitate was collected by centrifugation and washed with ether (3 × 40 ml) to give crude deprotected polymer as a white powder (416 mg, 57%). A solution of this polymer in trifluoroacetic acid (1 ml) was diluted with water (9 ml), filtered, and dialysed against water (4 l) for 24 h (water changes after 1, 2, 3, 5, 10, and 12 h). Lyophilisation and drying at 20° and 0·1 mmHg for 24 h gave purified *poly-heptapeptide trifluoroacetate* as a fluffy white powder (245 mg, 34%), m.p. 240° (decomp.), $[\alpha]_D^{20}$ −91°, $[\alpha]_{578}^{20}$ −96°, $[\alpha]_{546}^{20}$ −110° (*c* 0·13 in aqueous buffer pH 7·0); $[\alpha]_D^{20}$ −63°, $[\alpha]_{578}^{20}$ −66°, $[\alpha]_{546}^{20}$ −73° (*c* 0·15 in aqueous buffer pH 3·1); $[\alpha]_D^{20}$ −85°, $[\alpha]_{578}^{20}$ −89°, $[\alpha]_{546}^{20}$ −102° (*c* 0·14 in aqueous buffer pH 10·7); $\nu_{max.}$ (KBr) 1580br, 1675br, and 3350br cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 2·1br (7H, peptide NH), 3·2br (3H, NH_3^+), 4·6—6·1 (10H, s at 5·25 and 5·8, α-protons and $CH{\cdot}CH_2{\cdot}OH$), 6·7br (2H, $CH_2{\cdot}CH_2{\cdot}NH_3^+$), 7·0—8·8 (22H, broad s at 7·25 and 7·7 on complex, other protons), and 9·0 (6H, s, $CHMe_2$), η_{sp}/c 0·25 dl g^{-1} (*c* 0·48 in $Cl_2CH{\cdot}CO_2H$); amino-acid analysis Ala 1·01, Glu 2·18, Gly 1·00, Leu 0·96, Lys 0·98, Ser 0·91; c.d. $[\theta]_{225}$ −1300 (min.) (*c* 0·1 in pH 7·0 buffer); $[\theta]_{222}$ −12,000 (min.) (*c* 0·1 in pH 10·7 buffer); $[\theta]_{225}$ −1300 (min.) (*c* 0·1 in pH 3·1 buffer); $\bar{M}_n$ 6200; $\bar{M}_w$ 11,100 (Found: C, 46·3; H, 6·6; N, 13·8. $C_{32}H_{51}F_3N_8O_{14}$ requires C, 46·4; H, 6·2; N, 13·5%).

L-*Leucyl*-L-*glutamyl*-L-*lysyl*-L-*alanyl*-L-*glutamyl*-L-*serylglycine Bistrifluoroacetate*.—Aqueous trifluoroacetic acid (10%; 4 ml) was added to 1-(biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucyl-γ-t-butyl-L-glutamyl-N(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-O-t-butyl-L-serylglycine (248 mg, 0·2 mmol) at room temp. After 50 min the mixture was evaporated and the residue dried by repeated addition of benzene followed each time by distillation to give a colourless gel. This was precipitated from methanol with ether to give *heptapeptide bistrifluoroacetate* as a white powder (137 mg, 72%), m.p. 210—214° (decomp.), $[\alpha]_D^{20}$ −29·5° (*c* 0·8 in $Cl_2CH{\cdot}CO_2H$); TLC-11 R_F 0·74 (Found: C, 42·4; H, 6·1; N, 11·8. $C_{34}H_{54}F_6N_8O_{17}$ requires C, 42·5; H, 5·6; N, 11·7%).

1-(*Biphenyl-4-yl*)-1-*methylethoxycarbonyl*-L-*leucyl*-γ-*t-butyl*-L-*glutamyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-L-*alanylglycine*.—A solution of *N*-benzyloxycarbonyl-γ-t-butyl-L-glutamyl-N(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine [1] (1·42 g, 1·5 mmol) in 80% aqueous acetic acid (40 ml) was hydrogenated over 10% palladium–charcoal (0·30 g) for 3·5 h. The solution was filtered through Celite and evaporated. The residue was dried by repeated addition of benzene followed each time by evaporation to give a white solid. Triethylamine (0·21 ml, 1·5 mmol) and 1-(biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (0·81 g, 1·65 mmol) were added to a stirred suspension of this solid in dimethylformamide (15 ml) and water (0·3 ml) at room temp. After 3 days the solution was diluted with ethyl acetate (750 ml) and washed with 10% citric acid (1 × 100 ml) and then water until the washings were neutral. Evaporation, then repeated addition of benzene followed each time by evaporation gave a colourless gel, which was washed by decantation with ether (3 × 50 ml) and dried at 20° and 0·1 mmHg to give *protected heptapeptide acid* (1·62 g, 92%) as an amorphous white solid, m.p. 230° (decomp.), $[\alpha]_D^{20}$ −4·7° (*c* 1·2 in C_5H_5N); TLC-7 R_F 0·83, TLC-10 R_F 0·61; amino-acid analysis Ala 2·00, Glu 2·03, Gly 1·00, Leu 0·94, Lys 0·95 (Found: C, 60·45; H, 7·8; N, 9·3. $C_{59}H_{90}N_8O_{16}$ requires C, 60·7; H, 7·8; N, 9·6%).

1-(*Biphenyl-4-yl*)-1-*methylethoxycarbonyl*-L-*leucyl*-γ-*t-butyl*-L-*glutamyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-L-*alanylglycine* 4-*Nitrophenyl Ester*.—Bis-4-nitrophenyl sulphite (308 mg 1·0 mmol) was added to a stirred solution of 1-(biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucyl-γ-butyl-L-glutamyl-N(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine (120 mg, 0·1 mmol) in dimethylformamide–pyridine (1 : 4; 1·5 ml). After 18 h ether–light petroleum (4 : 1; 25 ml) was added. The gel which separated was washed with further portions of this solvent mixture (20 × 20 ml), and dried to give

protected heptapeptide 4-nitrophenyl ester as a buff solid (122 mg, 94%), m.p. 155° (decomp.), $\nu_{max.}$ (KBr) 1620—1640br, 1690—1750br, and 1780 cm^{-1} (Found: C, 59·0; H, 6·4; N, 8·9. $C_{65}H_{93}O_{18}$ requires C, 60·5; H, 7·3; N, 9·8%); 4-nitrophenyl ester content,[25] 97 and 101% (two separate determinations).

Poly-(L-leucyl-L-glutamyl-L-lysyl-L-alanyl-L-glutamyl-L-alanylglycine) Trifluoroacetate.—1-(Biphenyl-4-yl)-1-methylethoxycarbonyl-L-leucyl-γ-t-butyl-L-glutamyl-*N*(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine 4-nitrophenyl ester (1·50 g, 1·16 mmol) was dissolved in acetic acid–dichloroacetic acid (6 : 1; 7 ml) and the solution was kept for 1 h at room temp. Ether (50 ml) was added and the resulting precipitate was collected by centrifugation and then washed with further portions of ether (6 × 30 ml) to give heptapeptide 4-nitrophenyl ester dichloroacetate as a buff powder (1·34 g, 97%), TLF-10 R_F 0·54. Triethylamine (0·24 ml, 1·68 mmol) was added to a stirred solution of the dichloroacetate salt (1·00 g, 0·84 mmol) in dimethyl sulphoxide (2·0 ml). After 24 h the mixture had set to a rigid gel. After 5 days this gel was triturated with ethanol (20 ml), collected by centrifugation, and then washed with ethanol (3 × 20 ml) and ether (3 × 30 ml) to give crude protected polyheptapeptide as a buff powder (270 mg, 37%). This polymer was dissolved in 90% aqueous trifluoroacetic acid (3 ml) and left for 1 h at room temp. Ether (20 ml) was added and the flocculent precipitate was collected by centrifugation and washed with ether (3 × 30 ml) to give crude deprotected polymer as a buff powder (232 mg, 34%). A solution of this polymer in trifluoroacetic acid (1 ml) was diluted with water (10 ml), filtered, and dialysed against water (4 l) for 24 h (water changes after 1, 2, 3, 6, 9, 12, and 20 h). Lyophilisation and drying at 20° and 0·1 mmHg for 24 h gave purified *polyheptapeptide trifluoroacetate* as a fluffy buff powder (126 mg, 15·5%), m.p. 260° (decomp.); $[\alpha]_D^{20}$ −74°, $[\alpha]_{578}^{20}$ −78°, $[\alpha]_{546}^{20}$ −90° (*c* 0·09 in aqueous buffer pH 7·0); $[\alpha]_D^{20}$ −80°, $[\alpha]_{578}^{20}$ −88°, $[\alpha]_{546}^{20}$ −95° (*c* 0·10 in aqueous buffer pH 10·7); $[\alpha]_D^{20}$ −68°, $[\alpha]_{578}^{20}$ −72°, $[\alpha]_{546}^{20}$ −81° (*c* 0·10 in aqueous buffer pH 3·1); $\nu_{max.}$ (KBr) 1650br, 1640—1740br, and 3320br cm^{-1}; τ ($CF_3{\cdot}CO_2H$) 2·1br (7H, peptide NH), 3·1vbr (3H, NH_3^+), 5·2br (6H, NH·C*H*·CO), 5·65br (2H, NH·C*H*·CO), 6·7br (2H, $CH_2{\cdot}CH_2{\cdot}NH_3^+$), 7·1—8·7 (23H, broad peaks at 7·25 and 7·70 on complex, other protons), and 9·0 (6H, s, $CHMe_2$); η_{sp}/c 0·17 dl g^{-1} (*c* 0·61 in $HCCl_2{\cdot}CO_2H$); amino-acid analysis Ala 1·90, Glu 2·03, Gly 1·00, Leu 0·87, Lys 1·04; c.d.: $[\theta]_{222}$ −2600 (min.) (*c* 0·1 in aqueous buffer pH 7·0); $[\theta]_{222}$ −3500 (min.) (*c* 0·1 in aqueous buffer pH 3·1); $[\theta]_{222}$ −8000 (min.) (*c* 0·1 in aqueous buffer pH 10·7); $\bar{M}_n$ 3000; $\bar{M}_w$ 5000 (Found: C, 44·4; H, 6·2; N, 12·6. $C_{32}H_{51}F_3N_8O_{13},3H_2O$ requires C, 44·3; H, 6·6; N, 12·9%).

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[25] N. Mitsuyasu, S. Matsuura, M. Waki, M. Ohno, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Japan*, 1970, **43**, 1829.