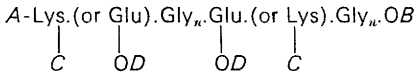
Polypeptides. Part XIV.[1] The Synthesis of Some Oligopeptides Containing Lysine and Glutamic Acid Residues

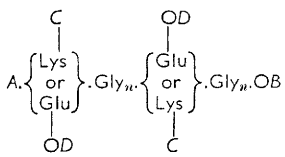
By A. T. Moore, H. N. Rydon, and M. J. Smithers

Several oligopeptides of the general formula



have been synthesised; their projected use for polymerisation experiments and for " doubling " imposes limitations on the protecting groups which can be used, since these must be selectively removable. In two cases, it was not found possible to remove an *N*-formyl group without breaking peptide linkages, while in these and a further case, selective removal of other protecting groups was prevented by the great lability of side-chain benzyl ester groups towards alkali. Finally, four protected oligopeptides of the required type were synthesised, using benzyloxycarbonyl for α-*N*-protection, t-butyl ester for ω-*C*-protection, and trifluoroacetyl and methyl ester for the protection of lysine and glutamic acid side-chains, respectively. Three of these were converted into side-chain protected peptides suitable for polymerisation and into the free peptides, and two of them, by a " doubling " procedure into the octa- and dodeca-peptides.

THIS Paper is concerned with the synthesis of some side-chain protected oligopeptides, containing lysine and glutamic acid residues, required for polymerisation



(I)

studies, which have been reported elsewhere.[2] In addition to using these oligopeptides as monomers in polymerisation experiments we also wished to convert them into larger peptides by a " doubling " procedure; this imposed rather severe restrictions on the type of protecting groups which could be used. Suitable protected oligopeptides for our purpose were of the general type, (I), in which the protecting groups *A*, *B*, *C*, and *D* are such that they can be selectively removed, *A* and *B* separately first, followed by *C* and *D* simultaneously. The six possible combinations of widely-used protecting groups classified according to the procedures (acid hydrolysis, H^+; alkaline hydrolysis, OH^-; hydrogenolysis, H_2) required for their removal are as follows:*

	A	*B*	*C*	*D*
(*a*)	$Z(H_2)$	$Me,Et(OH^-)$	$H{\cdot}CO,BOC(H^+)$	$Bu^t(H^+)$
(*b*)	$Z(H_2)$	$Bu^t(H^+)$	$TFA(OH^-)$	$Me,Et(OH^-)$
(*c*)	$H{\cdot}CO,TRI,BOC(H^+)$	$Me,Et(OH^-)$	$Z(H_2)$	$BZL(H_2)$
(*d*)	$H{\cdot}CO,TRI,BOC(H^+)$	$BZL(H_2)$	$TFA(OH^-)$	$Me,Et(OH^-)$
(*e*)	$TFA(OH^-)$	$Bu^t(H^+)$	$Z(H_2)$	$BZL(H_2)$
(*f*)	$TFA(OH^-)$	$BZL(H_2)$	$H{\cdot}CO,BOC(H^+)$	$Bu^t(H^+)$

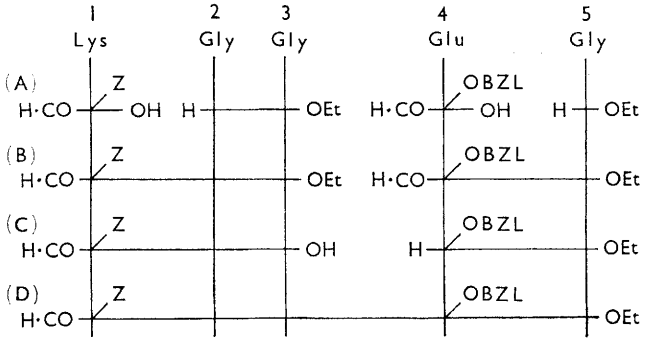
* Here, and elsewhere, the abbreviations used for protecting groups and amino-acid residues are those recommended by the Committee on Nomenclature of the Fifth European Peptide Symposium.[3]

[1] Part XIII, M. J. S. A. Amaral, G. C. Barrett, H. N. Rydon, and J. E. Willett, *J. Chem. Soc.* (*C*), 1966, 807.

[2] A. T. Moore and H. N. Rydon, *Acta Chim. Acad. Sci. Hung.*, 1965, **44**, 103.

[3] *Proc. Fifth European Peptide Symposium*, 1963, 261.

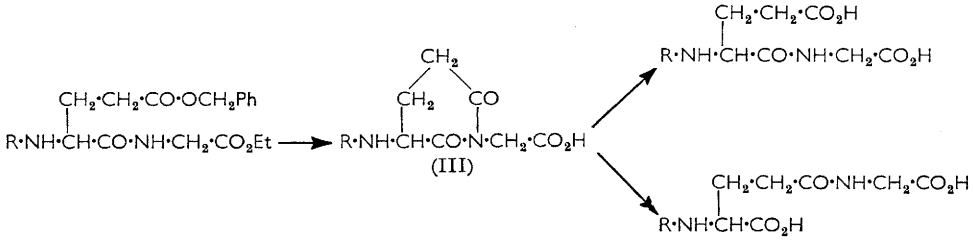
Of these possibilities, method (*c*) (with A = H·CO– and B = Et) seemed the most promising, since the key intermediates, α-*N*-formyl-ε-*N*-benzyloxycarbonyl-L-lysine [4] (IIA1) and γ-benzyl α-*N*-formyl-L-glutamate [5] (IIA4), were known, only well-tried protecting groups were involved and there could be no danger of transpeptidation in the final removal of the side-chain protecting groups (benzyloxycarbonyl and benzyl ester) by catalytic hydrogenolysis. Accordingly, the protected pentapeptide (IID) was synthesised as follows:



(II)

at room temperature left the protected pentapeptide almost unaffected, while raising the temperature to 100° resulted in considerable rupture of peptide linkages; there was no such difficulty in the case of the more soluble protected dipeptide (IIB4–5), from which the formyl group was easily removed. Attempts to remove the *C*-terminal ethyl ester group from (IID) were likewise unsuccessful, treatment with one equivalent of sodium hydroxide in either ethanol or dimethylformamide giving a mixture of two products, both of which were shown spectroscopically to have lost the side-chain benzyl ester group; the protected dipeptide (IIB4–5) behaved similarly. It is clear that, in both cases, there is rapid conversion into the imide (III) and subsequent transpeptidation.[8]

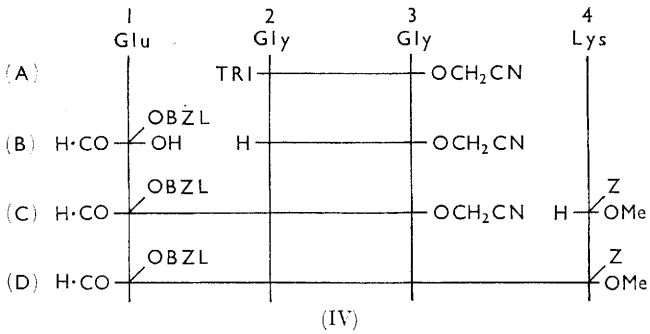
Since the ethyl ester group had been successfully removed from the protected tripeptide (IIB1–3) and the *N*-formyl group from the protected dipeptide (IIB4–5) it seemed worth while interchanging the positions of the lysine and glutamic acid residues. We accordingly synthesised the protected tetrapeptide (IVD) according to the following Scheme, in which the strategy of Goodman and Stueben is used,[9] the cyanomethyl ester



(III)

Dicyclohexylcarbodi-imide [6] was used for all the couplings except the last (IIC1–3 + IIC4–5), for which bis-*o*-phenylene pyrophosphite [7] was superior as coupling agent, largely owing to the difficulty of separating the protected pentapeptide (IID) from the dicyclohexylurea with which it co-precipitated during the coupling reaction. The insolubility of (IID) also prevented us from finding suitable conditions for the selective removal of the *N*-formyl group; treatment with solutions of hydrogen chloride in acetic acid, phenol, methanol or ethanol

group [10] being used first for *C*-protection and then, finally, as an activated ester for coupling:



(IV)

Dicyclohexylcarbodi-imide was used for the coupling (IVB1) + (IVB2–3). Unfortunately, in this case, too, it was not found possible to remove the *N*- and *C*-protecting groups selectively from the final protected tetrapeptide, removal of the *N*-formyl group being accompanied by extensive rupture of peptide linkages and treatment with cold alkali leading to loss of the side-chain benzyl ester group and to other changes.

Two variants of method (*c*) thus having failed, we next turned our attention to method (*e*), the only disadvantages of which were the novelty, at the time (1959—60), of t-butyl esters for *C*-protection [11] and the fact that the

[4] D. E. Wolf, J. Valiant, R. L. Peck, and K. Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 2002; K. Hofmann, E. Stutz, G. Spühler, H. Yajima, and E. T. Schwartz, *ibid.*, 1960, **82**, 3727.

[5] B. A. Borek and H. Waelsch, *J. Biol. Chem.*, 1953, **205**, 459.

[6] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.

[7] P. C. Crofts, J. H. H. Markes, and H. N. Rydon, *J. Chem. Soc.*, 1958, 4250; 1959, 3610.

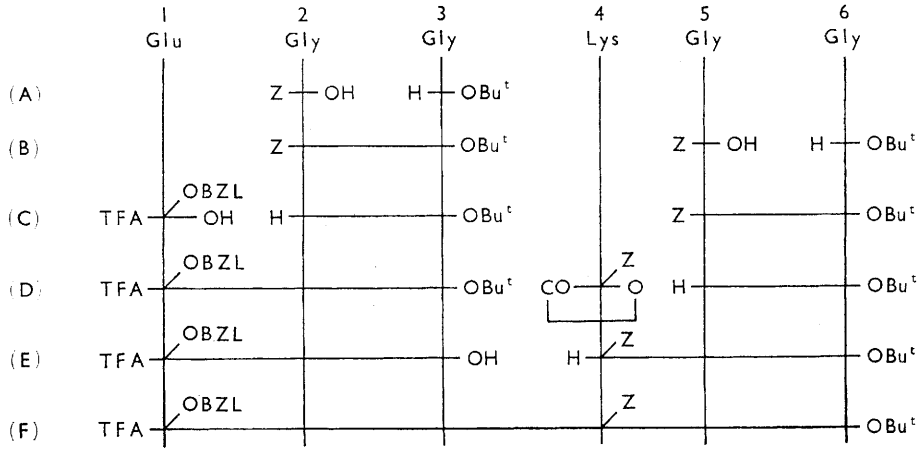
[8] Cf. E. Sondheimer and R. W. Holley, *J. Amer. Chem. Soc.*, 1954, **76**, 2467; A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 1955, 259; S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Amer. Chem. Soc.*, 1962, **84**, 2421; A. A. Adler, G. D. Fasman, and E. R. Blout, *ibid.*, 1963, **85**, 90; R. W. Hanson and H. N. Rydon, *J. Chem. Soc.*, 1964, 836.

[9] M. Goodwin and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

[10] R. Schwyzer, B. Iselin, W. Rittel, and P. Sieber, *Helv. Chim. Acta*, 1956, **39**, 872.

[11] R. W. Roeske, *Chem. and Ind.*, 1959, 1121; G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359; A. Vollmar and M. S. Dunn, *J. Org. Chem.*, 1960, **25**, 387.

key starting material, γ-benzyl *N*-trifluoroacetyl-L-glutamate (VC1), was unknown. A fully protected hexapeptide of the required type (VF) was readily synthesised by this method, as follows:

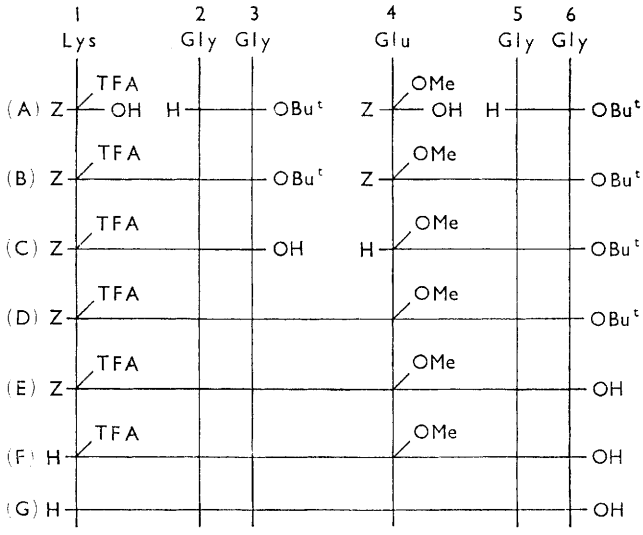


(V)

All the couplings, except one, were carried out with the aid of dicyclohexylcarbodi-imide. The exception was that, (VD4 + VD5–6), in which the lysine residue was introduced by means of the α-*N*-carboxy-anhydride [12] of ε-*N*-benzyloxycarbonyl-L-lysine. This little used procedure [13] can be of real value since no α-*N*-protecting group is required and the coupling product is immediately ready for use as the amino-component for a further coupling; a number of other similar couplings are described in the Experimental section; the main drawbacks of the method are the variability of yield, due to the great tendency of *N*-carboxy-anhydrides to polymerise, and the necessity to work at low temperature. Glycyl-glycine t-butyl ester (VC2–3) is most conveniently prepared, as shown, by coupling *N*-benzyloxycarbonylglycine with glycine t-butyl ester [14] followed by catalytic hydrogenolysis of the product. This synthesis, too, proved unsatisfactory for our purposes, again owing to the lability towards alkali of the γ-benzyl ester grouping in the final product (VF); all attempts to remove the *N*-trifluoroacetyl group selectively from (VF) failed owing to simultaneous, or prior, removal of the benzyl group under a variety of alkaline conditions. However, the free hexapeptide, L-glutamyl-diglycycl-L-lysyl-glycyl-glycine, was prepared from (VF) by catalytic hydrogenolysis, followed by treatment of the product first with aqueous hydrochloric acid and then with aqueous ammonia.

Of the remaining methods for the synthesis of oligopeptides of the required type, the most promising appeared to be method (*b*), which, however, suffered from the serious danger of transpeptidation [8] accompanying the removal of the side-chain trifluoroacetyl and methyl ester groups by treatment with alkali in the final stage of the synthesis. However, Bruckner and his co-workers had shown [15] that such transpeptidation can be avoided by carrying out the alkaline hydrolysis in the presence of added cupric ions; under such conditions the NH group of the peptide linkage involving the glutamic acid residue is involved in complexing the copper and thus not available for imide formation. We confirmed these findings by showing that the alkaline hydrolysis of γ-benzyl *N*-trifluoroacetyl-L-glutamyl-glycyl-glycine (V; E1–3) gave only one product (α-peptide) in the presence of added cupric ions, but two (α- and γ-peptides) in their absence. In the event, method (*b*) proved entirely satisfactory and was used for the successful synthesis of four oligopeptides of the required type.

The most convenient synthesis of a suitable hexapeptide (VIG) is the following, which was carried through several times on a moderately large scale:



(VI)

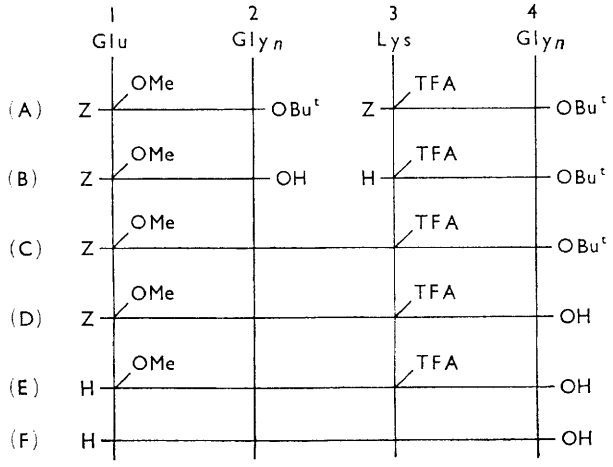
Of the two key starting materials, (VIA1) and (VIA4), the latter was already known,[16] while the former was

[12] M. Bergmann, L. Zervas, and W. F. Ross, *J. Biol. Chem.*, 1935, **111**, 245.
[13] J. L. Bailey, *J. Chem. Soc.*, 1950, 3461.
[14] A. T. Moore and H. N. Rydon, *Org. Synth.*, 1965, **45**, 47.
[15] V. Bruckner, A. Kótai, and K. Kovács, *Acta Chim. Acad. Sci. Hung.*, 1959, **21**, 427.
[16] W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 1950, 3239.

readily prepared by direct partial trifluoroacetylation of lysine, followed by treatment of the resulting ε-*N*-trifluoroacetyl derivative [17] with benzyl chloroformate. The coupling reactions were carried out with dicyclohexylcarbodi-imide. No difficulty was encountered in the selective removal of the t-butyl ester group from (VIB1–3) by heating with toluene-*p*-sulphonic acid in benzene under reflux or of the *N*-benzyloxycarbonyl group from (VIB4–6) by catalytic hydrogenolysis over palladised charcoal in slightly aqueous t-butyl alcohol. Treatment of the fully protected hexapeptide (VID) with toluene-*p*-sulphonic acid in dioxan removed the t-butyl ester group selectively to give the partially protected hexapeptide (VIE), from which the *N*-benzyloxycarbonyl group was removed by catalytic hydrogenolysis. Treatment of the resulting side-chain protected hexapeptide (VIF) with cold aqueous alkali removed the side-chain protecting groups, but gave a mixture of products owing to transpeptidation; alkaline hydrolysis in the pressure of cupric ions, however, gave only one product, the required α-peptide, L-lysyl-diglycyl-L-glutamyl-glycyl-glycine, (VIG). Barium hydroxide is greatly superior to sodium hydroxide in such alkaline hydrolyses, use of the latter giving products from which it is extremely difficult to remove the last traces of inorganic matter.

Replacement, in Scheme (VI), of glycyl-glycine t-butyl ester by glycine t-butyl ester led to the tetrapeptide L-lysyl-glycyl-L-glutamyl-glycine by way of analogous intermediates; this synthesis, too, was repeated several times on a moderately large scale. In this series both dicyclo-hexylcarbodi-imide and bis-*o*-phenylene pyrophosphite [7] were used for the couplings, the former being marginally superior to the latter; the use of the *p*-nitrophenyl ester method [18] for the final coupling was also investigated but offered no advantage, the product being difficult to free from the last traces of *p*-nitrophenol.

For polymerisation experiments, it is the separation of the lysine and glutamic acid residues, rather than the order in which they occur, which is important and we accordingly prepared tetra- and hexa-peptides analogous to (VIG), but with these two residues transposed, as follows:

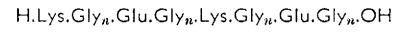


(VII)

The starting materials were already available from the work on Scheme (VI) and the methods used were the same as those used in that scheme. The synthesis was taken through to the free tetrapeptide, L-glutamyl-glycyl-L-lysyl-glycine, (VIIF; $n = 1$), but only to the fully protected stage (VIIC; $n = 2$) in the case of the hexapeptide. This procedure was marginally less convenient than that of Scheme (VI) and was accordingly less fully studied.

For comparison with products obtained [2] by polymerisation of the side-chain protected hexapeptide, (VIF), and the analogous tetrapeptide, we required the corresponding dodeca- and octa-peptides; these were synthesised by a "doubling" procedure. The fully protected hexapeptide, (VID), was catalytically hydrogenolysed and the resulting *C*-protected hexapeptide, with a free α-amino-group, coupled, using dicyclohexylcarbodi-imide or bis-*o*-phenylene pyrophosphite, with the *N*-protected hexapeptide (VIE). The resulting fully-protected dodecapeptide, (VIII; $n = 2$), was converted, by the procedures used for converting (VID) into (VIG), into the free dodecapeptide,

(VIII)



(IX)

(IX; $n = 2$). The corresponding protected (VIII; $n = 1$) and free (IX; $n = 1$) octapeptides were prepared similarly from the tetrapeptide analogue of (VIF).

The preparation and properties of a number of other peptides and derivatives of L-glutamic acid and L-lysine are recorded in the Experimental section.

EXPERIMENTAL

All evaporations and concentrations were carried out under reduced pressure. Extracts were dried over magnesium or sodium sulphate. Unless otherwise stated all solvents were anhydrous; light petroleum refers to the fraction b. p. 40—60°; acetic acid used for determinations of [α] was 95%. Catalytic hydrogenations were carried out at room temperature at 3—5 atmospheres.

The purity of most products was confirmed chromatographically. The absence of racemisation in the peptide syntheses was established by complete acid hydrolysis of the end product, and of important intermediates, followed by comparison of the optical rotation of the hydrolysate with a similarly treated control mixture of amino-acids.

Unless otherwise stated melting points are uncorrected; corrected melting points were determined directly on a Kofler hot-stage apparatus.

Coupling Procedures.—(a) *With dicyclohexylcarbodi-imide.* (i) In methylene dichloride or chloroform. The carbodi-imide (10% excess) was added to a solution, at 0°, of the two reactants in equimolecular proportions. After 1 hr. at

[17] E. E. Schallenberg and M. Calvin, *J. Amer. Chem. Soc.*, 1955, **77**, 2779.
[18] M. Bodanszky, *Nature*, 1955, **175**, 685; *Acta Chim. Acad. Sci. Hung.*, 1956, **10**, 335.

0° and 24—48 hr. at room temperature, a little acetic acid was added and the precipitated dicyclohexylurea removed by filtration after 30 min. at 0°. The filtrate was washed successively with cold N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water, and dried. The solvent was then removed and the residue taken up in acetone and kept at 0° overnight. A further quantity of dicyclohexylurea was then removed by filtration and the filtrate evaporated to dryness and the residue crystallised from a suitable solvent.

(ii) In pyridine. The coupling was conducted as in (i). After removal of the dicyclohexylurea the filtrate was evaporated to dryness. The residue was then taken up in chloroform and the solution washed and further worked up as in procedure (i).

(*b*) *With bis-o-phenylene pyrophosphite.* The pyrophosphite (10% excess) was added to a solution of the two reactants, in equimolecular proportions, in pyridine. The mixture was heated, under a calcium chloride guard-tube, on a boiling-water bath for 30 min. After cooling, the mixture was poured into 3—4 volumes of ice–water, stirred and set aside at 0° overnight. The product was isolated by filtration, if solid, or by decantation, if a gum, and triturated successively with cold N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water. It was then dried in a vacuum desiccator and recrystallised from a suitable solvent.

Derivatives of Lysyl-diglycyl-glutamyl-glycine (*Scheme II*). —α-N-*Formyl*-ε-N-*benzyloxycarbonyl*-L-*lysyl-glycyl-glycine*, (IIC1–3). ε-*N*-Benzyloxycarbonyl-L-lysine[19] (27·6 g.) was kept at room temperature for 20 hr. with 98% formic acid (170 ml.) and acetic anhydride (20 ml.). Water (50 ml.) was then added and the mixture evaporated to dryness. The gummy residue was dissolved in ethanol; cautious addition of water precipitated a solid (29·4 g.) which was crystallised from aqueous ethanol, giving α-*N*-formyl-ε-*N*-benzyloxycarbonyl-L-lysine, (IIA1) (21·8 g., 72%), m. p. 80—82°, $[\alpha]_D^{20}$ +9·2° (*c* 0·5 in ethanol) (Found: C, 58·85; H, 6·8; N, 9·0. Calc. for $C_{15}H_{20}N_2O_5$: C, 58·4; H, 6·5; N, 9·1%) (Hofmann *et al.*[4] give p. m. 74—78°, $[\alpha]_D^{24}$ +9·6°).

This derivative (12·40 g.) was coupled with glycylglycine ethyl ester (from the hydrochloride,[20] 7·88 g., and triethylamine, 5·55 ml.) in methylene dichloride (250 ml.), using dicyclohexylcarbodi-imide (9·04 g.). Recrystallisation of the product from ethanol–ether gave α-N-*formyl*-ε-N-*benzyl oxycarbonyl*-L-*lysyl-glycyl-glycine ethyl ester*, (IIB1–3), (11·50 g., 64%), m. p. 122—124°, $[\alpha]_D^{20}$ −11·2° (*c* 1·3 in chloroform) (Found: N, 12·45. $C_{21}H_{30}N_4O_7$ requires N, 12·45%). Aqueous N-sodium hydroxide (22 ml.) was added to a solution of this ester (9·01 g.) in ethanol (100 ml.). After 1 hr. at room temperature, N-hydrochloric acid (22 ml.) was added and the mixture evaporated to dryness. Two recrystallisations of the residue from ethanol–ether (charcoal) gave the N-*protected tripeptide*, (IIC1–3) (6·10 g., 72%), m. p. 110—112°, $[\alpha]_D^{19}$ −0·7° (*c* 1·7 in ethanol) (Found: C, 50·4; H, 6·2; N, 13·1. $C_{19}H_{26}N_4O_7$, 1·5 H_2O requires C, 50·8; H, 6·5; N, 12·5%).

γ-Benzyl L-*glutamyl-glycine ethyl ester*, (IIC4–5). γ-Benzyl *N*-formyl-L-glutamate[5] (13·30 g.) and glycine ethyl ester (from the hydrochloride, 6·95 g., and triethylamine, 6·95 ml.) were condensed in chloroform (250 ml.) with dicyclohexylcarbodi-imide (11·30 g.). Two recrystallisations of the product from acetone–light petroleum gave *γ-benzyl N-formyl*-L-*glutamyl-glycine ethyl ester*, (IIB4–5) (10·56 g., 60%), m. p. 105—107°, $[\alpha]_D^{21}$ −13·5° (*c* 2·1 in chloroform) (Found: C, 58·6; H, 6·4; N, 7·7. $C_{17}H_{22}N_2O_6$ requires C, 58·3; H, 6·3; N, 8·0%); in nine other experiments the yields ranged from 40—80%. This compound (7·00 g.) was heated under reflux for 10 min. with a mixture of acetone (40 ml.) and methanolic N-hydrogen chloride (20 ml.). Addition of water to the evaporated product precipitated unchanged starting material (2·38 g.) which was recovered by filtration and used in further preparations. The filtrate was basified with saturated aqueous sodium hydrogen carbonate and extracted with chloroform. Evaporation of the dried extract gave the required ester (IIC4–5) (3·50 g., 82%) as an uncrystallisable gum. After a few days at room temperature a crystalline solid was deposited; recrystallisation of this from ethanol gave *benzyl 2,5-dioxopiperazine-3-propionate*, m. p. 207—209°, $[\alpha]_D^{24}$ −8·9° (*c* 1·5 in acetic acid) (Found: C, 60·4; H, 5·8; N, 10·4. $C_{14}H_{16}N_2O_4$ requires C, 60·8; H, 5·8; N, 10·1%).

ε-N-*Benzyloxycarbonyl*-α-N-*formyl*-L-*lysyl-diglycyl-γ-benzyl*-L-*glutamyl-glycine ethyl ester*, (IID). ε-*N*-Benzyloxycarbonyl-α-*N*-formyl-L-lysyl-glycyl-glycine (3·02 g.) and γ-benzyl L-glutamyl-glycine ethyl ester (2·45 g.) were coupled, in pyridine, with bis-*o*-phenylene pyrophosphite (2·45 g.). Several precipitations of the crude, solid, product (2·75 g., 50%) from dimethylformamide with ether gave the pure *protected pentapeptide*, (IID), m. p. 190—192°, $[\alpha]_D^{21}$ −7·2° (*c* 1·4 in dimethylformamide) (Found: C, 57·7; H, 6·5; N, 11·05. $C_{35}H_{46}N_6O_{11}$ requires C, 57·8; H, 6·4; N, 11·6%).

A similar condensation of γ-benzyl L-glutamylglycine ethyl ester and *N*-benzyloxycarbonyl-glycyl-glycine gave N-*benzyloxycarbonyl-diglycyl-γ-benzyl*-L-*glutamyl-glycine ethyl ester* (42% yield), m. p. 136—138° (after recrystallisation from aqueous acetone), $[\alpha]_D^{22}$ −4·4° (*c* 1·9 in chloroform) (Found: C, 59·4; H, 5·8; N, 9·95. $C_{28}H_{39}N_4O_9$ requires C, 58·9; H, 6·0; N, 9·8%).

Derivatives of Glutamyl-diglycyl-lysine (*Scheme IV*).—*γ-Benzyl N-formyl*-L-*glutamyl-glycyl-glycine cyanomethyl ester*, (IVC1–3). 1·2 N-Hydrogen chloride in ethyl acetate (196 ml.) was added to a solution of *N*-tritylglycyl-glycine cyanomethyl ester[10] (31·2 g.) in acetonitrile (210 ml.) heated under reflux. After heating for 15 min. under reflux, the mixture was cooled and kept at 0° for 12 hr. Recrystallisation of the crystalline precipitate (12·3 g., 79%) from ethanol gave *glycyl-glycine cyanomethyl ester hydrochloride*, m. p. 156—158° (Found: C, 35·4; H, 5·1; N, 20·5. $C_6H_{10}ClN_3O_3$ requires C, 34·7; H, 4·85; N, 20·2%). The free ester (from the hydrochloride, 2·08 g. and triethylamine, 1·39 ml.) was condensed with γ-benzyl *N*-formyl-L-glutamate (2·66 g.) in methylene dichloride (50 ml.) with the aid of dicyclohexylcarbodi-imide (2·06 g.). The product was triturated with ether and the ether-insoluble fraction crystallised from ethyl acetate–ether, giving the *protected tripeptide*, (IVC1–3) (1·40 g., 34%), m. p. 56—58°, $[\alpha]_D^{21}$ −2·5° (*c* 1·5 in dimethylformamide) (Found: C, 54·7; H, 5·5; N, 13·6. $C_{19}H_{22}N_4O_7$ requires C, 54·6; H, 5·3; N, 13·4%). Recrystallisation of the ether-soluble material from aqueous ethanol gave N-(N-*formyl-γ-benzyl*-L-*glutamyl*)-NN′-*dicyclohexylurea* (0·99 g., 21%), m. p. (corr.) 144°, $[\alpha]_D^{22}$ +24·4° (*c* 1·7 in ethanol) (Found: C, 66·2; H, 8·0; N, 8·85. $C_{26}H_{37}N_3O_5$ requires C, 66·2; H, 7·9; N, 8·9%).

[19] A. Neuberger and F. Sanger, *Biochem. J.*, 1943, **37**, 515.
[20] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.

γ-Benzyl N-*formyl*-L-*glutamyl-diglycyl*-ε-N-*benzyloxycarbonyl*-L-*lysine methyl ester*, (IVD). The above tripeptide cyanomethyl ester (IVC1–3) (6·27 g.), in tetrahydrofuran (15 ml.), was added to a filtered solution prepared from ε-*N*-benzyloxycarbonyl-L-lysine methyl ester hydrochloride [12] (4·95 g.), triethylamine (2·08 ml.) and tetrahydrofuran (15 ml.). After the addition of two drops of acetic acid, the mixture was kept at 60° for 6 hr. and then at room temperature for 24 hr. Precipitation with ethyl acetate, followed by crystallisation from dimethylformamide–ether gave the *protected tetrapeptide* (IVD) (2·18 g., 31%), m. p. (corr.) 171—172°, $[\alpha]_D^{20}$ −8·8° (*c* 1·6 in dimethylformamide) (Found: C, 58·6; H, 6·3; N, 10·65. $C_{32}H_{41}N_5O_{10}$ requires C, 58·6; H, 6·3; N, 10·7%).

Glutamyl-diglycyl-lysyl-glycyl-glycine and Derivatives (*Scheme V*).—*Glycyl-glycine t-butyl ester*, (VC2–3). Glycine t-butyl ester [14] (66 g.) and *N*-benzyloxycarbonylglycine [20] (105 g.) were coupled, with the aid of dicyclohexylcarbodi-imide (103 g.), in methylene dichloride (800 ml.). The product (157 g., 95%), an uncrystallisable gum, was hydrogenated in methanol (300 ml.) over 5% palladised charcoal (3 g.). After filtration through Kieselguhr, 2*N*-methanolic hydrogen chloride (1 equivalent) was added, followed by ether (1 l.). The product, which separated overnight at 0°, was collected by filtration and crystallised from ethanol–ether, giving the *ester hydrochloride* (38·9 g., 70%), m. p. 179—180° (Found: C, 42·7; H, 7·4; N, 12·2. $C_8H_{17}ClN_2O_3$ requires C, 42·8; H, 7·6; N, 12·5%); yields in several other preparations varied from 50—80%. Replacement, in the preparation, of phosphorous acid for hydrogen chloride gave the *phosphite*, m. p. (corr.) 148—149° from methanol (Found: C, 35·9; H, 7·3; N, 10·3. $C_8H_{19}N_2O_6P$ requires C, 35·6; H, 7·1; N, 10·4%). The hydrochloride (22·4 g.) was shaken with 2N-sodium hydroxide (75 ml.) and chloroform (200 ml.). The aqueous layer was extracted with more chloroform (2 × 100 ml.) and the combined chloroform solutions washed with water (25 ml.), dried and distilled, giving the *free ester* (VC2–3) (11·4 g., 61%), b. p. 114—116°/0·1 mm., n_D^{22} 1·4661 (Found: N, 15·0. $C_8H_{16}N_2O_3$ requires N, 14·9%); the ester was unstable at room temperature, a crystalline precipitate of 2,5-dioxopiperazine filling the oil after a week.

γ-Benzyl N-*trifluoroacetyl*-L-*glutamyl-glycyl-glycine* (VE1–3). Phenyl trifluoroacetate [21] (11·4 g.) and γ-benzyl L-glutamate [22] (11·9 g.) were stirred together in phenol (28 g.) for 2 hr. at 100°. Evaporation, trituration of the residue with light petroleum and crystallisation from carbon tetrachloride yielded *γ-benzyl* N-*trifluoroacetyl*-L-*glutamate* (VC1) (15·0 g., 91%), m. p. (corr.) 98°, $[\alpha]_D^{19}$ −29·6° (*c* 2·3 in dimethylformamide) (Found: N, 4·25. $C_{14}H_{14}F_3NO_5$ requires N, 4·2%); the *dicyclohexylamine salt*, prepared in ether and crystallised from ethyl acetate–light petroleum, had m. p. 139—141°, $[\alpha]_D^{18}$ +13·2° (*c* 2·0 in dimethylformamide) (Found: C, 61·3; H, 7·1; N, 5·7. $C_{26}H_{37}F_3N_2O_5$ requires C, 60·8; H, 7·3; N, 5·4%).

The above ester (VC1) (6·68 g.) and glycyl-glycine t-butyl ester (from the hydrochloride, 4·4 8g., and triethylamine, 2·78 g.) were coupled in methylene dichloride (100 ml.), using dicyclohexylcarbodi-imide (4·53 g.). Recrystallisation of the product from ethyl acetate–light petroleum gave *γ-benzyl* N-*trifluoroacetyl*-L-*glutamyl-glycyl-glycine* t-*butyl ester* (VD1—3) (6·15 g., 61%), m. p. (corr.) 104°, $[\alpha]_D^{19}$ −2·1° (*c* 2·4 in acetic acid), +2·1° (*c* 2·2 in chloroform) (Found: N, 8·4. $C_{22}H_{28}F_3N_3O_7$ requires N, 8·35%). This ester (6·10 g.), in benzene (50 ml.), was kept for 24 hr. with a saturated solution of hydrogen chloride in benzene (200 ml.). The gum which separated was isolated by decantation, triturated with light petroleum and recrystallised from ethyl acetate–light petroleum, giving *γ-benzyl* N-*trifluoroacetyl*-L-*glutamyl-glycyl-glycine* (VE1–3) (5·15 g., 95%), m. p. 130–132°, $[\alpha]_D^{20}$ −4·8° (*c* 1·7 in acetic acid (Found: N, 9·2. $C_{18}H_{20}F_3N_3O_7$ requires N, 9·4%). Hydrogenation over 5% palladised charcoal in 60% aqueous t-butyl alcohol gave N-*trifluoroacetyl*-L-*glutamyl-glycyl-glycine* (86% yield), m. p. (corr.) 137° from ethyl acetate–light petroleum, $[\alpha]_D^{19}$ −5·4° (*c* 2·0 in water) (Found: C, 36·6; H, 4·25; N, 11·7. $C_{11}H_{14}F_3N_3O_7$ requires C, 37·0; H, 3·95; N, 11·8%).

A similar reaction sequence, using glycine t-butyl ester in place of glycyl-glycine t-butyl ester gave *γ-benzyl* N-*trifluoroacetyl*-L-*glutamyl-glycine t-butyl ester* (76% yield) m. p. (corr.) 94° from ethyl acetate–light petroleum), $[\alpha]_D^{19}$ −18·7° (*c* 2·3 in acetic acid) (Found: C, 53·9; H, 5·8; N, 6·3. $C_{20}H_{25}F_3N_2O_6$ requires C, 53·8; H, 5·65; N, 6·3%) and *γ-benzyl* N-*trifluoroacetyl*-L-*glutamyl-glycine* (92% yield), m. p. (corr.) 118—119° from ethyl acetate–light petroleum, $[\alpha]_D^{19}$ −13·6° (*c* 0·9 in acetic acid) (Found: N, 7·2%. $C_{16}H_{17}F_3N_2O_6$ requires N, 7·2%).

ε-N-*Benzyloxycarbonyl*-L-*lysyl-glycyl-glycine t-butyl ester*, (VE4–6). A solution of freshly-prepared ε-*N*-benzyloxycarbonyl-L-lysine α-*N*-carboxy-anhydide [12] (3·06 g.) in methylene dichloride (50 ml.) was cooled to −70° and added to a solution, in the same solvent (30 ml.) at the same temperature, of glycyl-glycine t-butyl ester hydrochloride (2·24 g.) and triethylamine (2·78 ml.). After 1 hr. at −70° and 12 hr. at room temperature, the solution was washed successively with water, saturated aqueous sodium hydrogen carbonate, and water, dried, and evaporated. Crystallisation of the product (3·79 g., 84%) from chloroform gave the *protected tripeptide*, m. p. (corr.) 76°, $[\alpha]_D^{19}$ +8·4° (*c* 1·7 in acetic acid) (Found: C, 59·3; H, 7·3; N, 12·1. $C_{22}H_{34}N_4O_6$ requires C, 58·6; H, 7·6; N, 12·4%).

γ-Benzyl N-*trifluoroacetyl*-L-*glutamyl-diglycyl*-ε-N-*benzyloxycarbonyl*-L-*lysyl-glycyl-glycine t-butyl ester* (VF). The *N*-protected tripeptide (VE1–3) (890 mg.) and the *C*-protected tripeptide (VE4–6) (900 mg.) were coupled with the aid of dicyclohexylcarbodi-imide (470 mg.) in pyridine (15 ml.). The resulting *protected hexapeptide* (500 mg., 57%), recrystallised from ethanol–ether, had m. p. (corr.) 165—166°, $[\alpha]_D^{21}$ −8·2° (*c* 2·0 in dimethylformamide) (Found: C, 56·55; H, 6·0; N, 10·9. $C_{40}H_{52}F_3N_7O_{12}$ requires C, 56·6; H, 6·0; N, 11·1%).

L-*Glutamyl-diglycyl*-L-*lysyl-glycyl-glycine*. The above protected hexapeptide (3·5 g.) was hydrogenated in 95% aqueous t-butyl alcohol over 5% palladised charcoal (0·5 g.). Filtration, followed by evaporation and precipitation from ethanol with ether gave a hygroscopic powder (2·42 g.) which resisted final purification. This was kept at room temperature for 12 hr. in N-hydrochloric acid (25 ml.). Evaporation and trituration of the residue with isopropyl alcohol gave a solid which was dissolved in a mixture of water (12 ml.) and aqueous ammonia (*d* 0·880, 12 ml.). After 2 hr. at room temperature and a few minutes at 60°, the solution was evaporated to dryness. The residue, in water (50 ml.), was shaken mechanically for 1 hr. with

[21] L. Benoiton, H. N. Rydon, and J. E. Willett, *Chem. and Ind.*, 1960, 1060.

[22] S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, 1958, **41**, 1852.

Zeo-Karb 225. Elution of the material adsorbed on the resin with a mixture of water (25 ml.) and aqueous ammonia (*d* 0·880, 25 ml.), followed by evaporation of the eluate and crystallisation from aqueous ethanol gave the free *hexapeptide* (750 mg., 40%), m. p. (corr.) 178—182° (decomp.), $[\alpha]_D^{24}$ −11·8° (*c* 1·2 in N-HCl), −11·2° (*c* 0·8 in acetic acid) (Found: C, 45·2; H, 7·2; N, 19·1. $C_{19}H_{33}N_7O_9$ requires C, 45·3; H, 6·6; N, 19·4%).

Lysyl-diglycyl-glutamyl-glycyl-glycine and Derivatives; (Scheme VI).—α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysine*, (VIA1). Ethyl thioltrifluoroacetate [23] (10 ml.) was added to a solution of L-lysine monohydrochloride (9·15 g.) in N-sodium hydroxide (50 ml.) and the mixture shaken mechanically for 12 hr. The reaction mixture was cooled in ice and the solid product (7·15 g., 59%), m. p. 255—257°, collected by filtration. Recrystallisation from aqueous ethanol gave ε-N-*trifluoroacetyl*-L-*lysine*, m. p. 260—262° (decomp.), $[\alpha]_D^{20}$ +17·5° (*c* 2·1 in acetic acid) (Found: C, 39·8; H, 5·3; N, 11·5. $C_8H_{13}F_3N_2O_3$ requires C, 39·7; H, 5·4; N, 11·6%). This compound (300 g.) was suspended in water (2750 ml.) containing sodium hydrogen carbonate (230 g.), the mixture was cooled to 0° and stirred mechanically while benzyl chloroformate (330 g.) was added dropwise over 3 hr. Stirring was continued, at room temperature, for a further 6 hr., after which the solution was extracted with ether (3 × 500 ml.), acidified (pH 4) with concentrated hydrochloric acid and again extracted with ether (3 × 500 ml.). The latter extracts were dried and evaporated and the residue triturated with light petroleum and recrystallised from ethyl acetate–light petroleum, affording the required *derivative* (VIA1) (352 g., 75%), m. p. 90—91° $[\alpha]_D^{21}$ −2·0° (*c* 4·2 in acetic acid), −7·0° (*c* 2·3 in dimethylformamide) (Found: N, 7·2. $C_{16}H_{10}F_3N_2O_5$ requires N, 7·45%).

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-glycine t-butyl ester*, (VIB1–3). The above derivative (VIA1) (37·6 g.) and glycyl-glycine t-butyl ester (from the hydrochloride, 22·5 g., and triethylamine, 10·1 g.) in methylene dichloride (300 ml.) were coupled with the aid of dicyclohexylcarbodi-imide (22·6 g.). Crystallisation of the product from ethyl acetate–light petroleum gave the *protected tripeptide* (VIB1–3) (38·7 g., 71%), m. p. 107—108°, $[\alpha]_D^{20}$ −6·1° (*c* 2·3 in acetic acid) (Found: C, 53·1; H, 6·4; N, 10·1. $C_{24}H_{33}F_3N_4O_7$ requires C, 52·7; H, 6·1; N, 10·25%).

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-glycine*, (VIC1–3). The above peptide ester (VIB1–3) (5·46 g.) was heated under reflux for 30 min. with anhydrous toluene-*p*-sulphonic acid [24] (0·5 g.) in benzene (50 ml.). After cooling, the benzene was removed, by decantation, from the deposited gummy solid, which was then taken up in saturated aqueous sodium hydrogen carbonate. After an extraction with ethyl acetate, the solution was acidified and thrice extracted with this solvent. Washing with water, drying, evaporation and crystallisation from ethyl acetate–light petroleum gave the *protected tripeptide* (VIC1–3) (3·99 g., 81%), m. p. 108—109°, $[\alpha]_D^{18}$ −6·4° (*c* 1·9 in acetic acid) (Found: C, 48·5; H, 5·0; N, 11·0. $C_{26}H_{25}F_3N_4O_7$ requires C, 49·0; H, 5·1; N, 11·4%).

Hydrogenation of this protected tripeptide (7·21 g.) in 50% aqueous ethanol (100 ml.) over 5% palladised charcoal (1·25 g.) gave, on working up as usual, followed by crystallisation from aqueous ethanol, ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-glycine* (3·38 g., 64%), m. p. (corr.) 226—228° (decomp.), $[\alpha]_D^{20}$ +28·2° (*c* 2·3 in acetic acid) (Found: C, 40·1; H, 5·8; N, 15·4. $C_{12}H_{19}F_3N_4O_5$ requires C, 40·5; H, 5·4; N, 15·7%); removal of the trifluoroacetyl group with aqueous ammonia gave L-lysyl-glycycl-glycine as a chromatographically homogeneous, uncrystallisable gum.

γ-Methyl N-*benzyloxycarbonyl*-L-*glutamyl-glycyl-glycine t-butyl ester*, (VIB4–6). γ-Methyl *N*-benzyloxycarbonyl-L-glutamate [16] (29·5 g.) and glycyl-glycine t-butyl ester (from the hydrochloride, 22·5 g., and triethylamine, 10·1 g.) were coupled in methylene dichloride (250 ml.) with the aid of dicyclohexylcarbodi-imide (22·6 g.). The product (39·6 g., 85%) was a chromatographically homogeneous gum which could not be induced to crystallise.

γ-Methyl L-*glutamyl-glycyl-glycine t-butyl ester*, (VIC4–6). The above derivative (12·1 g.) was hydrogenated over 5% palladised charcoal (1·0 g.) in 95% aqueous t-butyl alcohol (100 ml.). Recrystallisation of the product, isolated as usual with the addition of hydrogen chloride (1 equivalent), from chloroform–light petroleum gave the *tripeptide ester hydrochloride* (7·7 g., 80%), m. p. (corr.) 96°, $[\alpha]_D^{24}$ +6·0° (*c* 1·7 in acetic acid) (Found: C, 45·7; H, 7·2; N, 11·2. $C_{14}H_{26}ClN_3O_6$ requires C, 45·7; H, 7·1; N, 11·4%).

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-diglycyl-γ-methyl*-L-*glutamyl-glycyl-glycine t-butyl ester*, (VID). The *N*-protected tripeptide (VIC1–3) (4·99 g.) and the *C*-protected tripeptide (VIC4–6) (as the hydrochloride, 3·67 g.) were coupled, in pyridine (20 ml.), using dicyclohexylcarbodi-imide (2·26 g.). Recrystallisation of the product from ethyl acetate–light petroleum gave the *protected hexapeptide* (4·40 g., 55%), m. p. (corr.) 100°, $[\alpha]_D^{20}$ −8·0° (*c* 2·0 in acetic acid), −7·6° (*c* 2·0 in dimethylformamide) (Found: C, 50·3; H, 6·6; N, 12·0. $C_{34}H_{48}F_3N_7O_{12}$ requires C, 50·8; H, 6·0; N, 12·2%).

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-diglycyl-γ-methyl*-L-*glutamyl-glycyl-glycine*, (VIE). The above t-butyl ester (VID) (32·2 g.) was heated under reflux for 1 hr. with anhydrous toluene-*p*-sulphonic acid (4·0 g.) in dioxan (400 ml.). The solution was evaporated to dryness and the residue was taken up in saturated aqueous sodium hydrogen carbonate. The solution was extracted with chloroform, acidified and re-extracted with ethyl acetate. Evaporation of the dried ethyl acetate extract, followed by concentration, precipitation with light petroleum, and crystallisation from ethanol–ether gave the *protected hexapeptide* (16·0 g., 53%), m. p. (corr.) 93—95°, $[\alpha]_D^{20}$ −8·5° (*c* 2·0 in acetic acid) (Found: C, 47·7; H, 5·6; N, 13·1. $C_{30}H_{40}F_3N_7O_{12}$ requires C, 48·2; 5·8; N, 13·1%).

ε-N-*Trifluoroacetyl*-L-*lysyl-diglycyl-γ-methyl*-L-*glutamyl-glycyl-glycine*, (VIF). The hexapeptide (VIE) (15·0 g.) was hydrogenated in 60% aqueous t-butyl alcohol (150 ml.) over 5% palladised charoal (1·5 g.). Crystallisation of the product from methanol–ether gave the *protected hexapeptide* (9·7 g., 78%), m. p. (corr.) 169—171° (decomp.), $[\alpha]_D^{21}$ +14·4° (*c* 2·1 in acetic acid) (Found: N, 15·3. $C_{22}H_{34}F_3N_7O_{10}$ requires N, 15·9%).

L-*Lysyl-diglycyl*-L-*glutamyl-glycyl-glycine*, (VIG). The hexapeptide (VIF) (153 mg.) was suspended in a solution of cupric sulphate pentahydrate (125 mg.) in water (10 ml.). 0·3N-Barium hydroxide (20·0 ml.) was added and the mixture shaken at room temp. for 12 hr. An exact equivalent of 0·1N-sulphuric acid was then added and the precipitated barium sulphate removed by filtration; the

[23] M. Hauptschein, C. S. Stokes, and E. A. Nodiff, *J. Amer. Chem. Soc.*, 1952, **74**, 4005.

[24] J. M. Theobald, M. W. Williams, and G. T. Young, *J. Chem. Soc.*, 1963, 1927.

filtrate was then saturated with hydrogen sulphide and the precipitated cupric sulphide removed by filtration through Kieselguhr. Addition of a few drops of aqueous ammonia and evaporation gave the *free hexapeptide* (87 mg., 69%); after removal of traces of inorganic matter by electrolytic de-salting, followed by lyophilisation, the product had m. p. (corr.) 190—192° (decomp.), $[\alpha]_D^{20}$ +13·2° (*c* 2·2 in acetic acid) (Found: C, 45·3; H, 7·1; N, 19·1. $C_{19}H_{33}N_7O_9$ requires C, 45·3; H, 6·6; N, 19·5%).

Lysyl-glycyl-glutamyl-glycine and Derivatives.—α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine t-butyl ester.* α-*N*-Benzyloxycarbonyl-ε-*N*-trifluoroacetyl-L-lysine (37·6 g.) and glycine t-butyl ester (13·1 g.) were coupled in methylene dichloride (250 ml.) with the aid of dicyclohexylcarbodi-imide (22·6 g.). Recrystallisation of the product from ethyl acetate–light petroleum gave the *protected dipeptide* (40 g., 81%), m. p. (corr.) 92°, $[\alpha]_D^{19}$ −9·5° (*c* 1·3 in acetic acid) (Found: C, 53·6; H, 6·2; N, 8·4. $C_{22}H_{30}F_3N_3O_6$ requires C, 54·0; H, 6·1; N, 8·6%). A coupling, on one-tenth of the above scale, using bis-*o*-phenylene pyrophosphite (3·27 g.) in pyridine (10 ml.) gave the same product, m. p. (corr.) 89—91°, $[\alpha]_D^{19}$ −9·0°, in 74% yield.

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine.* The above dipeptide ester (4·89 g.) was heated under reflux for 1 hr. with anhydrous toluene-*p*-sulphonic acid (0·30 g.) in benzene (30 ml.). The product was isolated in the usual manner and crystallised from ethyl acetate–light petroleum, affording the *protected dipeptide* (3·30 g., 76%), m. p. (corr.) 96—98°, $[\alpha]_D^{20}$ −9·0 (*c* 2·2 in acetic acid) (Found: N, 9·5. $C_{18}H_{22}F_3N_3O_6$ requires N, 9·7%).

Hydrogenation of this protected dipeptide (8·66 g.) in 50% aqueous ethanol (100 ml.) over 5% palladised charcoal (1·25 g.), followed by crystallisation of the product from aqueous ethanol gave ε-N-*trifluoroacetyl*-L-*lysyl-glycine* (4·01 g., 67%), m. p. (corr.) 246—248° (decomp.), $[\alpha]_D^{20}$ +25·5° (*c* 2·3 in acetic acid) (Found: C, 40·0; H, 5·6; N, 14·3. $C_{10}H_{16}F_3N_3O_4$ requires C, 40·1; H, 5·4; N, 14·0%). Removal of the trifluoroacetyl group with aqueous ammonia gave L-lysyl-glycine as an uncrystallisable, but chromatographically homogeneous, gum.

γ-Methyl L-*glutamyl-glycine t-butyl ester.* γ-Methyl *N*-benzyloxycarbonyl-L-glutamate (29·5 g.) and glycine t-butyl ester (13·1 g.) were coupled in methylene dichloride (250 ml.) with the aid of dicyclohexylcarbodi-imide (22·6 g.). Crystallisation of the product from ethyl acetate–light petroleum gave *γ-methyl* N-*benzyloxycarbonyl*-L-*glutamyl-glycine* t-*butyl ester* (33·0 g., 80%), m. p. 69—70°, $[\alpha]_D^{20}$ −13·6° (*c* 2·4 in acetic acid) (Found: C, 58·7; H, 7·0; N, 6·9. $C_{20}H_{28}N_2O_7$ requires C, 58·8; H, 6·9; N, 6·9%). Coupling of the same compounds, on one-tenth the scale, using bis-*o*-phenylene pyrophosphite (3·27 g.) in pyridine (10 ml.) gave the same compound, m. p. 59—60°, $[\alpha]_D^{19}$ −12·5° in 66% yield.

This *N*-protected dipeptide (20·0 g.) was hydrogenated over 5% palladised charcoal (2·0 g.) in 95% aqueous t-butyl alcohol. The filtered solution was treated with 1 equivalent of N-hydrochloric acid. Evaporation, followed by solution in water (100 ml.), extraction with chloroform, and re-evaporation of the aqueous phase, gave the protected dipeptide hydrochloride (11·5 g., 75%) as a gum which, although chromatographically homogeneous, could not be induced to crystallise.

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-γ-methyl*-L-*glutamyl-glycine t-butyl ester.* (i) The above *C*-protected dipeptide hydrochloride (31·1 g.) and α-*N*-benzyloxycarbonyl-ε-*N*-trifluoroacetyl-L-lysyl-glycine (43·3 g.) were coupled, in pyridine (300 ml.), with the aid of dicyclohexylcarbodi-imide (22·6 g.). Crystallisation of the product from ethyl acetate–light petroleum gave the *protected tetrapeptide* (44·8 g., 65%), m. p. (corr.) 86—88°, $[\alpha]_D^{20}$ −10·0° (*c* 2·2 in acetic acid) (Found: C, 52·2; H, 6·1; N, 10·5. $C_{30}H_{42}F_3N_5O_{10}$ requires C, 52·2; H, 6·1; N, 10·2%).

(ii) A similar coupling, on one-twentieth the scale, using bis-*o*-phenylene pyrophosphite (1·63 g.) in pyridine (10 ml.), gave the same compound, m. p. 83—85°, $[\alpha]_D^{19}$ −9·0°, in 60% yield.

(iii) α-*N*-Benzyloxycarbonyl-ε-*N*-trifluoroacetyl-L-lysyl-glycine (2·16 g.) and di-*p*-nitrophenyl sulphite [25] (1·80 g.) were heated under reflux for 3 hr. in ethyl acetate (15 ml.), containing pyridine (0·8 ml.). After cooling, the solution was washed successively with water, N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water, dried and evaporated. Recrystallisation of the residue from ethyl acetate–light petroleum gave α-N-*benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine* p-*nitrophenyl ester* (1·80 g., 70%), m. p. (corr.) 159—160°, $[\alpha]_D^{20}$ −16·3° (*c* 2·2 in acetic acid) (Found: C, 52·3; H, 4·8; N, 9·8. $C_{24}H_{25}F_3N_4O_8$ requires C, 52·9; H, 4·5; N, 10·1%). This ester (2·77 g.) and γ-methyl L-glutamyl-glycine t-butyl ester hydrochloride (1·55 g.) were kept at room temperature in pyridine (10 ml.) for 3 days. The solution was then evaporated and the residue dissolved in chloroform and washed successively with ice-cold N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water. Drying, evaporation, and trituration with light petroleum, followed by three recrystallisations from ethyl acetate–light petroleum gave the protected tetrapeptide (2·44 g., 71%), m. p. (corr.) 85—87°, $[\alpha]_D^{20}$ −8·8° (*c* 2·2 in acetic acid).

ε-N-*Trifluoroacetyl*-L-*lysyl-glycyl-γ-methyl*-L-*glutamyl-glycine.* The above fully-protected tetrapeptide (27·6 g.) was heated under reflux for 1 hr. with anhydrous toluene-*p*-sulphonic acid (1·6 g.) in dioxan (160 ml.). The reaction was worked up in the usual way and the product recrystallised from ethyl acetate–light petroleum to give α-N-*benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-γ-methyl*-L-*glutamyl-glycine* (8·0 g., 64%), m. p. (corr.) 90—92°, $[\alpha]_D^{19}$ −9·5° (*c* 2·6 in acetic acid) (Found: C, 49·1; H, 5·7; N, 10·5. $C_{26}H_{34}F_3N_5O_{10}$ requires C, 49·2; H, 5·4; N, 11·1%). Hydrogenation in the usual manner gave the *side-chain protected tetrapeptide* (75% yield), m. p. (corr.) 163—165° from ethanol–ether, $[\alpha]_D^{21}$ +16·0° (*c* 2·0 in acetic acid) (Found: C, 43·0; H, 5·8; N, 13·7. $C_{18}H_{28}F_3N_5O_8$ requires C, 43·3; H, 5·6; N, 14·0%).

L-*Lysyl-glycyl*-L-*glutamyl-glycine.* The above side-chain protected tetrapeptide (125 mg.) was suspended in water (5 ml.) containing cupric sulphate pentahydrate (125 mg.). 0·3 N-Barium hydroxide (20·0 ml.) was added and the mixture shaken at room temperature for 12 hr. Working up as described for the analogous hexapeptide gave the *tetrapeptide* (62 mg., 63%), which after electrolytic de-salting and lyophilisation, had m. p. (corr.) 192—194° (decomp.), $[\alpha]_D^{19}$ +17·9° (*c* 2·2 in acetic acid) (Found: C, 42·4; H, 7·3; N, 17·3. $C_{15}H_{27}N_5O_7,2H_2O$ requires C, 42·4; H, 7·3; N, 16·5%).

Glutamyl-glycyl-lysyl-glycine and Derivatives (*Scheme VII;* n = 1).—*γ-Methyl* N-*benzyloxycarbonyl*-L-*glutamyl-glycine,* (VIIB1–2; n = 1). The corresponding t-buyl

[25] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373.

ester (4·08 g.) was heated under reflux for 1 hr. with anhydrous toluene-*p*-sulphonic acid (0·30 g.) in benzene (30 ml.). Working up in the usual manner, followed by crystallisation from ethyl acetate–light petroleum, gave the *dipeptide* (2·72 g., 77%), m. p. (corr.) 56—58° (Found: C, 53·9; H, 5·8; N, 8·3. $C_{16}H_{20}N_2O_7$ requires C, 54·6; H, 5·7; N, 8·0%); the *dicyclohexylamine salt*, prepared in ethanol, had m. p. 148°, from ethanol–light petroleum, $[\alpha]_D^{20}$ −13·0° (*c* 2·0 in acetic acid) (Found: N, 7·8. $C_{28}H_{43}N_3O_7$ requires N, 7·9%). Hydrogenation of the protected dipeptide (7·04 g.) over 5% palladised charcoal (1·5 g.) in 50% aqueous ethanol (150 ml.), followed by crystallisation of the product from aqueous ethanol, gave *γ-methyl* L-*glutamyl-glycine* (2·15 g., 49%), m. p. (corr.) 158—160°, $[\alpha]_D^{21}$ +35·6° (*c* 2·0 in acetic acid) (Found: C, 43·4; H, 6·4; N, 12·4. $C_8H_{14}N_2O_5$ requires C, 44·0; H, 6·4; N, 12·8%); saponification with aqueous barium hydroxide, containing cupric sulphate, in the usual manner gave L-glutamyl-glycine (61% yield), m. p. 215—218° (lit.,[26] m. p. 226°).

ε-N-*Trifluoroacetyl*-L-*lysyl-glycine t-butyl ester*, (VIIB3–4; n = 1). The corresponding α-*N*-benzyloxycarbonyl derivative (6·20 g.) was hydrogenated over 5% palladised charcoal (0·7 g.) in 95% aqueous t-butyl alcohol. Working up as usual, with the addition of an equivalent of hydrogen chloride, followed by recrystallisation from ethanol–ether, gave the very hygroscopic *hydrochloride* (3·51 g., 71%), m. p. 71—76°, $[\alpha]_D^{20}$ +16·9° (*c* 2·3 in acetic acid) (Found: C, 43·2; H, 6·6. $C_{14}H_{25}ClF_3N_3O_4$ requires C, 42·9; H, 6·4%).

γ-Methyl N-*benzyloxycarbonyl*-L-*glutamyl-glycyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine t-butyl ester*, (VIIC; n = 1). The above hydrochloride (4·40 g.) and γ-methyl *N*-benzyloxycarbonyl-L-glutamyl-glycine (4·00 g.) were coupled in pyridine (15 ml.) with the aid of dicyclohexylcarbodi-imide (2·59 g.). Recrystallisation of the product from ethyl acetate–light petroleum gave the *protected tetrapeptide* (5·11 g., 65%), m. p. (corr.) 96—98°, $[\alpha]_D^{21}$ −23·1° (*c* 2·5 in acetic acid) (Found: C, 53·0; H, 6·2; N, 9·8. $C_{30}H_{42}F_3N_5O_{10}$ requires C, 52·3; H, 6·1; N, 10·2%).

γ-Methyl N-*benzyloxycarbonyl*-L-*glutamyl-glycyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine*, (VIID; n = 1). The above fully protected tetrapeptide (4·50 g.) was heated under reflux for 1 hr. with toluene-*p*-sulphonic acid (0·25 g.) in dioxan (25 ml.). Working up in the usual manner, followed by crystallisation from ethyl acetate–light petroleum, gave the N-*protected tetrapeptide* (2·83 g., 69%), m. p. (corr.) 109—111°, $[\alpha]_D^{21}$ −18·6° (*c* 2·6 in acetic acid) (Found: C, 49·1; H, 5·7; N, 10·8. $C_{26}H_{34}F_3N_5O_{10}$ requires C, 49·3; H, 5·4; N, 11·1%).

γ-Methyl L-*glutamyl-glycyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycine*, (VIIE; n = 1). Hydrogenation of the above benzyloxycarbonyl derivative (2·00 g.) over 5% palladised charcoal (0·7 g.) in 60% aqueous t-butyl alcohol (20 ml.), followed by crystallisation from ethanol–ether gave the *protected tetrapeptide* (1·12 g., 71%), m. p. (corr.) 158—160°, $[\alpha]_D^{21}$ −7·0° (*c* 2·0 in acetic acid) (Found: C, 42·8; H, 5·8; N, 13·5. $C_{18}H_{28}F_3N_5O_8$ requires C, 43·3; H, 5·6; N, 14·0%).

L-*Glutamyl-glycyl*-L-*lysyl-glycine*, (VIIF; n = 1). The above side-chain protected tetrapeptide (130 mg.), in water (6 ml.) containing cupric sulphate pentahydrate (260 mg.), was shaken for 12 hr. at room temperature with 0·3N-barium hydroxide (22·0 ml.). Working up in the usual manner, followed by elecrolytic de-salting and lyophilisation, gave the *tetrapeptide* (41 mg., 41%), m. p. (corr.) 170—172° (decomp.) (Found: C, 45·8; H, 7·0; N, 17·6. $C_{15}H_{27}N_5O_7$ requires C, 46·3; H, 6·9; N, 18·0%).

Derivatives of Glutamyl-diglycyl-lysyl-glycyl-glycine (*Scheme VII;* n = 2).—*γ-Methyl* N-*benzyloxycarbonyl*-L-*glutamyl-glycyl-glycine*, (VIIB1–2; n = 2). The corresponding t-butyl ester (8·4 g.) was kept for 24 hr. at room temperature in benzene (60 ml.) saturated with hydrogen chloride. Working up in the usual manner and treatment of the uncrystallisable product with dicyclohexylamine in ethyl acetate gave the *dicyclohexylamine salt* (6·33 g., 60%), m. p. 151—153°, from ethyl acetate–ether, $[\alpha]_D^{23}$ −5·3° (*c* 3·22 in acetic acid) (Found: N, 9·4. $C_{30}H_{46}N_4O_8$ requires N, 9·5%).

This salt (2·00 g.), in ethyl acetate (50 ml.), was shaken with 10% aqueous sulphuric acid (10 ml.); the phases were separated and the aqueous phase extracted with more ethyl acetate (20 ml.). Evaporation of the washed and dried combined ethyl acetate solutions, followed by hydrogenation over 5% palladised charcoal (0·7 g.) in 50% aqueous ethanol (25 ml.) gave *γ-methyl* L-*glutamyl-glycyl-glycine* (0·81 g., 80%), m. p. (corr.) 169—171°, from aqueous ethanol, $[\alpha]_D^{20}$ +40·3° (*c* 2·0 in acetic acid) (Found: C, 43·2; H, 6·3; N, 15·4. $C_{10}H_{17}N_3O_6$ requires C, 43·6; H, 6·2; N, 15·3%). Saponification, as usual, with aqueous barium hydroxide in the presence of cupric sulphate, gave L-glutamyl-glycyl-glycine (70% yield), m. p. 159—161° (lit.,[27] m. p. 160—162°).

ε-N-*Trifluoroacetyl*-L-*lysyl-glycyl-glycine t-butyl ester*, (VIIB3–4; n = 2). The corresponding α-*N*-benzyloxycarbonyl derivative (1·37 g.) was hydrogenated over 5% palladised charcoal (0·30 g.) in 95% t-butyl alcohol (20 ml.). Working up as usual, with the addition of 1 equivalent of hydrogen chloride gave the *hydrochloride* (0·91 g., 79%), m. p. 163—164°, from methanol–ether, $[\alpha]_D^{20}$ +26·4° (*c* 2·1 in acetic acid) (Found: C, 42·45; H, 5·9; N, 12·4. $C_{16}H_{28}ClF_3N_4O_5$ requires C, 42·8; H, 6·3; N, 12·5%).

γ-Methyl N-*benzyloxycarbonyl*-L-*glutamyl-diglycyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-glycine t-butyl ester*, (VIIC; n = 2). The above hydrochloride (3·10 g.) and γ-methyl *N*-benzyloxycarbonyl-L-glutamyl-glycyl-glycine (2·84 g.) were coupled, using dicyclohexylcarbodi-imide (1·57 g.), in pyridine (20 ml.). Crystallisation of the product from aqueous ethanol gave the *protected hexapeptide* (2·30 g., 42%), m. p. (corr.) 139—141°, $[\alpha]_D^{23}$ −8·7° (*c* 3·7 in dimethylformamide) (Found: C, 51·1; H, 6·4; N, 12·1. $C_{34}H_{48}F_3N_7O_{12}$ requires C, 50·8; H, 6·0; N, 12·2%).

Lysyl-glycyl-glutamyl-glycyl-lysyl-glycyl-glutamyl-glycine and Derivatives.—ε-N-*Trifluoroacetyl*-L-*lysyl-glycyl-γ-methyl*-L-*glutamyl-glycine t-butyl ester*. The corresponding α-*N*-benzyloxycarbonyl derivative (30·0 g.) was hydrogenated over 5% palladised charcoal (1·5 g.) in 95% aqueous t-butyl alcohol (200 ml.). Working up as usual, with the addition of 1 equivalent of hydrogen chloride, followed by crystallisation from ethanol–ether, gave the *hydrochloride* (18·5 g., 72%), m. p. (corr.) 135—137°, $[\alpha]_D^{20}$ +15·0° (*c* 2·0 in acetic acid) (Found: C, 44·3; H, 6·3; N, 11·8. $C_{22}H_{37}ClF_3N_5O_8$ requires C, 44·6; H, 6·3; N, 11·4%).

α-N-*Benzyloxycarbonyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-γ-methyl*-L-*glutamyl-glycyl*-ε-N-*trifluoroacetyl*-L-*lysyl-glycyl-γ-methyl*-L-*glutamyl-glycine t-butyl ester*, (VIII; n = 1).

[26] G. Amiard, R. Heymès, and L. Velluz, *Bull. Soc. chim. France*, 1956, 97.

[27] W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 1950, 1954.

The above hydrochloride and α-*N*-benzyloxycarbonyl-ε-*N*-trifluoroacetyl-L-lysyl-glycyl-γ-methyl-L-glutamyl-glycine (6·33 g.) were condensed in pyridine (20 ml.), with the aid of dicyclohexyl-carbodi-imide (2·26 g.). Several precipitations of the product from ethanol with ether gave the *octapeptide hydrate* (5·46 g., 47%), m. p. (corr.) 147—149°, $[\alpha]_D^{22}$ −15·0° (*c* 2·2 in acetic acid) (Found: C, 49·0; H, 6·0; N, 11·4. $C_{48}H_{68}F_6N_{10}O_{17},H_2O$ requires C, 48·5; H, 5·9; N, 11·8%). Coupling of the same two compounds, on half the scale, with bis-*o*-phenylene pyrophosphite (1·63 g.) in pyridine (15 ml.) gave the same product (2·20 g., 38%), m. p. 138—140°, $[\alpha]_D^{22}$ −13·1°.

L-*Lysyl-glycyl-*L*-glutamyl-glycyl-*L*-lysyl-glycyl-*L*-glutamyl-glycine*, (IX; n = 1). The fully protected octapeptide (12·5 g.) was heated under reflux for 1 hr. with toluene-*p*-sulphonic acid (1 g.) in anhydrous dioxan (100 ml.). The solution was evaporated to dryness and the residue taken up in saturated aqueous sodium hydrogen carbonate. After filtration, the solution was acidified (pH 3·5) and the sticky precipitate repeatedly precipitated from ethanol with ether and dried in a vacuum desiccator. This product (8·0 g.) was hydrogenated in 60% aqueous t-butyl alcohol (30 ml.) over 5% palladised charcoal (1·3 g.). Repeated precipitation of the product from ethanol with ether gave ε-*trifluoroacetyl-*L*-lysyl-glycyl-γ-methyl-*L*-glutamyl-glycyl-*ε*-N-trifluoroacetyl-*L*-lysyl-glycyl-γ-methyl-*L*-glutamyl-glycine* (4·69 g., 44%), m. p. (corr.) 171—173° (decomp.), $[\alpha]_D^{20}$ +7·2° (*c* 2·3 in acetic acid) (Found: C, 42·8; H, 5·7; N, 13·6. $C_{36}H_{54}F_6N_{10}O_{15},2H_2O$ requires C, 42·5; H, 5·7; N, 13·6%).

This side-chain protected octapeptide (98 mg.) was suspended in water (5 ml.) containing cupric sulphate pentahydrate (50 mg.); 0·3N-barium hydroxide (10 ml.) was added and the mixture shaken at room temperature for 12 hr. Working up in the usual manner, followed by electrolytic de-salting and lyophilisation gave the *free octapeptide* (44 mg., 58%), m. p. (corr.) 189—191° (decomp.), $[\alpha]_D^{20}$ +10·2° (*c* 2·0 in acetic acid) (Found: C, 45·1; H, 7·0; N, 18·0. $C_{30}H_{52}N_{10}O_{13},2H_2O$ requires C, 45·2; H, 7·0; N, 17·6%).

Lysyl-diglycyl-glutamyl-diglycyl-lysyl-diglycyl-glutamyl-glycyl-glycine and Derivatives.—ε-N-*Trifluoroacetyl-*L*-lysyl-diglycyl-γ-methyl-*L*-glutamyl-glycyl-glycine t-butyl ester*. The corresponding α-*N*-benzyloxycarbonyl derivative (30·0 g.) was hydrogenated over 5% palladised charcoal (2·0 g.) in 95% aqueous t-butyl alcohol (250 ml.). Working up as usual, with the addition of 1 equivalent of hydrogen chloride gave the *peptide hydrochloride* (19·5 g., 74%), m. p. (corr.) 131—133°, from ethanol–ether, $[\alpha]_D^{20}$ +14·0° (*c* 2·0 in acetic acid) (Found: C, 44·5; H, 6·3; N, 13·9. $C_{26}H_{43}ClF_3N_7O_{10}$ requires C, 44·3; H, 6·1; N, 13·9%). Omission of the hydrogen chloride gave the *free peptide* (78% yield), m. p. (corr.) 71—73°, from ethyl acetate–ether, $[\alpha]_D^{24}$ −3·9° (*c* 2·33 in dimethylformamide) (Found: N, 14·1. $C_{26}H_{42}F_3N_7O_{10}$ requires N, 14·6%).

α-N-*Benzyloxycarbonyl-*ε*-N-trifluoroacetyl-*L*-lysyl-diglycyl-γ-methyl-*L*-glutamyl-diglycyl-*ε*-N-trifluoroacetyl-*L*-lysyl-diglycyl-γ-methyl-*L*-glutamyl-glycyl-glycine t-butyl ester*, (VIII; n = 2). The above hydrochloride (12·3 g.) and α-*N*-benzyloxycarbonyl-ε-*N*-trifluoroacetyl-L-lysyl-diglycyl-γ-methyl-L-glutamyl-glycyl-glycine (13·0 g.) were coupled in pyridine (70 ml.), with the aid of dicyclohexylcarbodi-imide (3·95 g.). Repeated precipitation of the product from dimethylformamide with ether gave the *protected dodecapeptide* (9·3 g., 38%), m. p. (corr.) 171—173°, $[\alpha]_D^{20}$ −11·5° (*c* 2·3 in acetic acid) (Found: C, 48·5; H, 5·9; N, 1·35. $C_{56}H_{80}F_6N_{14}O_{21}$ requires C, 48·1; H, 5·8; N, 14·0%). A coupling, on a smaller scale, using bis-*o*-phenylene pyrophosphite in pyridine, gave a 43% yield of a discoloured product, m. p. 169—171°, $[\alpha]_D^{20}$ −10·5°.

L-*Lysyl-diglycyl-*L*-glutamyl-diglycyl-*L*-lysyl-diglycyl-*L*-glutamyl-glycyl-glycine*, (IX; n = 2). The fully-protected dodecapeptide (13·50 g.) was heated under reflux for 1 hr. with toluene-*p*-sulphonic acid (2·0 g.) in dioxan (200 ml.). The product (8·65 g.) isolated in the usual way and repeatedly precipitated from ethanol with ether, was hydrogenated in 60% aqueous t-butyl alcohol (60 ml.) over 5% palladised charcoal (1·5 g.). Several reprecipitations of the hydrogenation product from ethanol with ether gave ε-N-*trifluoroacetyl-*L*-lysyl-diglycyl-γ-methyl-*L*-glutamyl-diglycyl-*ε*-N-trifluoroacetyl-*L*-lysyl-diglycyl-γ-methyl-*L*-glutamyl-glycyl-glycine* (4·87 g., 63%), m. p. (corr.) 189—191°, $[\alpha]_D^{21}$ 5·6° (*c* 2·0 in acetic acid) (Found: C, 43·1; H, 5·6; N, 15·7. $C_{44}H_{66}F_6N_{14}O_{19},H_2O$ requires C, 43·1; H, 5·6; N, 16·0%).

This side-chain protected dodecapeptide was hydrolysed, as described for the octapeptide, with aqueous barium hydroxide containing cupric sulphate. The resulting *free dodecapeptide* (43% yield) had m. p. (corr.) 192—194°, $[\alpha]_D^{20}$ +7·1° (*c* 2·0 in acetic acid) (Found: C, 44·1; H, 6·4; N, 18·4. $C_{38}H_{64}N_{14}O_{17},2H_2$ requires C, 44·5; H, 6·6; N, 19·1%).

Syntheses with N-*Carboxy-anhydrides*.—In all these reactions solvents and reactants were rigorously dried and glass-ware dried at 100° for 1 hr. immediately before use.

(i) Glycyl-glycine ethyl ester hydrochloride (10·9 g.), in methylene dichloride (100 ml.), containing triethylamine (14·9 ml.), was cooled to −70° and treated slowly with a solution, also at −70°, of freshly-prepared ε-*N*-benzyloxycarbonyl-L-lysine α-*N*-carboxy-anhydride [12] (16·8 g.) in methylene dichloride (150 ml.). After 1 hr. at −70° and 12 hr. at room temp., the precipitated product (16·7 g.) was collected by filtration and dissolved in chloroform (300 ml.). The solution was washed successively with water, saturated aqueous sodium hydrogen carbonate, and water, dried and evaporated. Trituration of the residue with light petroleum and recrystallisation from chloroform–light petroleum gave ε-N-*benzyloxycarbonyl-*L*-lysyl-glycyl-glycine ethyl ester* (11·6 g., 50%), m. p. (corr.) 109—110°, $[\alpha]_D^{24}$ −6·0° (*c* 2·6 in dimethylformamide) (Found: C, 56·1; H, 7·2; N, 13·0. $C_{20}H_{30}N_4O_6$ requires C, 56·85; H, 7·2; N, 13·3%).

(ii) A similar reaction using glycyl-glycine phenyl ester hydrochloride [28] (1·22 g.), methylene dichloride (35 ml.), triethylamine (1·39 ml.) and benzyloxycarbonyl-lysine carboxy-anhydride (1·54 g.) gave ε-N-*benzyloxycarbonyl-*L*-lysyl-glycyl-glycine phenyl ester* (0·78 g., 34%), m. p. (corr.) 106—108°, from chloroform–light petroleum, $[\alpha]_D^{17}$ −3·2° (*c* 2·9 in dimethylformamide) (Found: N, 11·3. $C_{24}H_{30}N_4O_6$ requires N, 11·9%).

(iii) A similar condensation using glycyl-glycine ethyl ester hydrochloride (1·0 g.), methylene dichloride (55 ml.), triethylamine (1·39 ml.) and γ-benzyl L-glutamate *N*-carboxy-anhydride [29] (1·32 g.) gave *γ-benzyl* L*-glutamyl-glycyl-glycine ethyl ester* (0·83 g., 59%), m. p. (corr.) 79° after recrystallisation from ethanol–light petroleum, $[\alpha]_D^{20}$

[28] P. Karrer and H. Heynemann, *Helv. Chim. Acta*, 1948, **31**, 398.

[29] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, 1956, **78**, 941.

$-3{\cdot}2°$ (*c* 2·8 in dimethylformamide) (Found: N, 11·0%. $C_{18}H_{25}N_3O_6$ requires N, 11·1%). This compound decomposed spontaneously, on keeping, to L-*pyroglutamyl-glycyl-glycine ethyl ester*, m. p. 128°, from ethyl acetate, $[\alpha]_D^{23}$ $-26{\cdot}6°$ (*c* 0·6 in acetic acid) (Found: N, 15·3. $C_{11}H_{18}N_3O_5$ requires N, 15·4%).

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