Sequential Polypeptides. Part II.[1] The Preparation of Two Partially Protected Hexapeptides for Use in Sequential Polypeptide Synthesis

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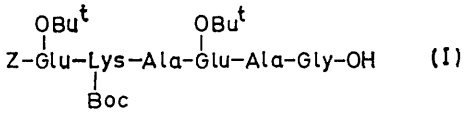
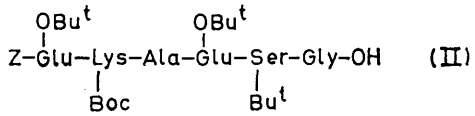
Preparations of benzyloxycarbonyl-γ-t-butyl-L-glutamyl-*N*(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine and of benzyloxycarbonyl-γ-t-butyl-L-glutamyl-*N*(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-*O*-t-butyl-L-serylglycine by stepwise active ester procedures are described.

THE protected hexapeptide acids (I) and (II) were required for further elaboration into heptapeptide monomers suitable for sequential polypeptide synthesis. Many protected intermediates similar to (I) and (II)



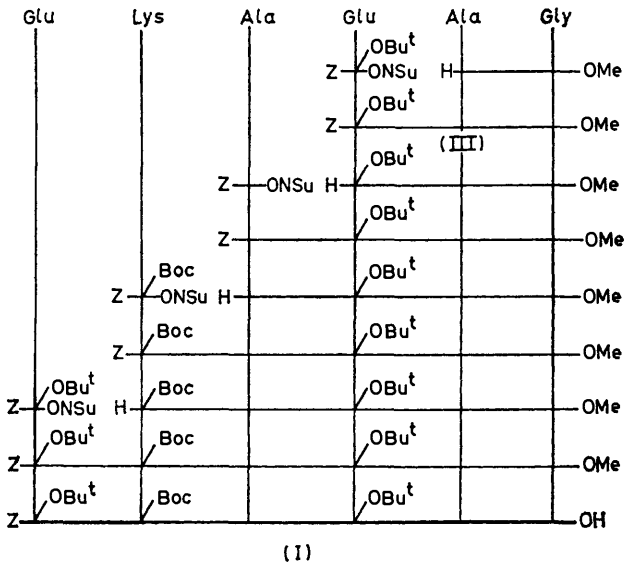
(I)



(II)

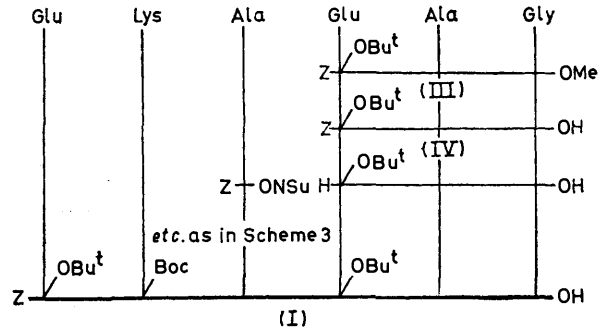
in amino-acid composition and juxtaposition of protecting groups have been encountered in the course of synthetic studies of gastrin and related peptides;[2] we have therefore based our approach on the methods which have been used with such success in that area.

Our first preparation of peptide (I) is outlined in Scheme 1. Protected amino-acid residues were added



SCHEME 1 In this and subsequent Schemes, abbreviations follow the Tentative Rules in I.U.P.A.C. Information Bulletin No. 26. NSu = succinimido

in a stepwise manner by use throughout of succinimido esters. High yields were achieved at every stage and all the fully protected intermediates were obtained in an analytically pure condition. The known protected tripeptide (III) was obtained analytically pure merely by washing procedures in organic solvents: the recorded preparation[3] *via* benzyloxycarbonyl-γ-t-butylglutamic acid pentachlorophenyl ester required column chromatography for purification. The final saponification was, however, not fully satisfactory. After extensive experimentation, conditions under which peptide (I) could be isolated in 95% yield of pure material were defined, but the conditions appeared to be critical. Since we required a route which could be scaled up with confidence we examined the possibility of avoiding this difficulty by constructing the protected peptide chain without



SCHEME 2

C-terminal protection at any stage. This proved impracticable, as attempted couplings of alanylglycine with benzyloxycarbonyl-γ-t-butylglutamic acid succinimido ester gave low yields of impure material, probably because of the feeble solubility of the dipeptide in suitable media. A compromise in which the *C*-terminal carboxy-group was blocked as its methyl ester only as far as the tripeptide level (Scheme 2) was successful. However this route was also unsuitable for scaling up since the saponification of the tripeptide methyl ester (III) was unsatisfactory: the corresponding free acid (IV) could be obtained pure by this means but, as results were not always reproducible, purification through the dicyclohexylammonium salt was necessary on some occasions. We therefore circumvented the need for saponification by employing benzyl ester *C*-terminal protection up to the tripeptide level. This route (Scheme 3) was satisfactory in every respect.

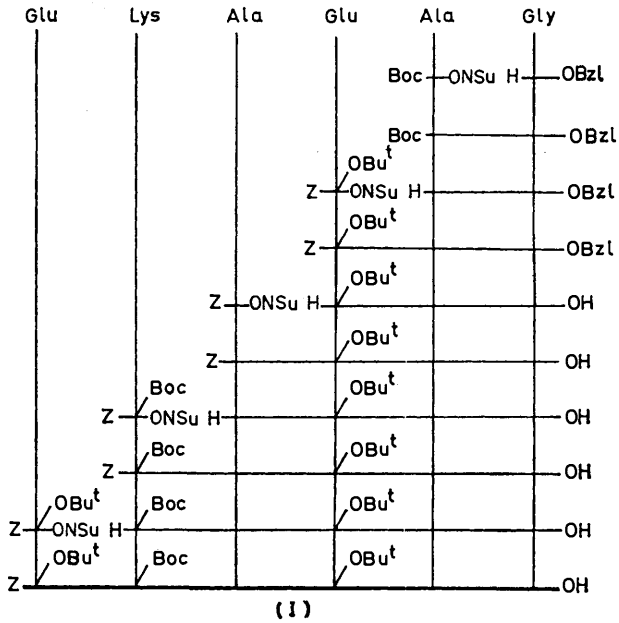
Our experience with the preparation of peptide (II) was more agreeable: in contrast to alanylglycine, *O*-t-butylserylglycine was sufficiently soluble for coupling

[1] Part I, R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.

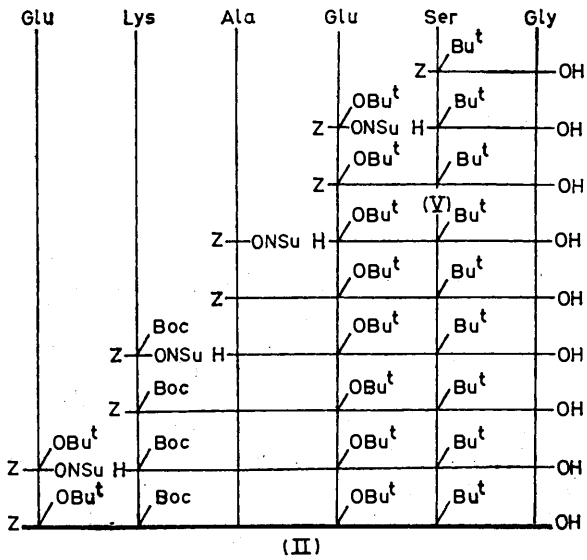
[2] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1969, 2213 and references cited therein.

[3] B. J. Johnson, *J. Chem. Soc.* (*C*), 1969, 1412.

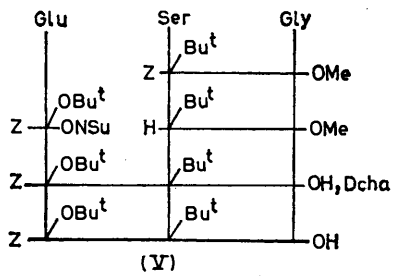
reactions so that we were able to dispense entirely with *C*-terminal protection (Scheme 4). The protected tripeptide acid (V) was also prepared by saponification of the corresponding methyl ester and purified in good overall yield *via* its dicyclohexylammonium salt (Scheme 5). This inconvenient variation had no advantage over the straightforward synthesis shown in Scheme 4, which proceeded uneventfully with high yields at every stage.



SCHEME 3



SCHEME 4



SCHEME 5 Dcha = dicyclohexylammonium salt

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus, optical rotations with a Perkin-Elmer 141 automatic polarimeter (solutions in a 1 dm cell), i.r. spectra with a Perkin-Elmer 257 spectrometer, u.v. spectra with a Carey 14M spectrometer, and n.m.r. spectra with a Perkin-Elmer R14 spectrometer operating at 100 MHz, with tetramethylsilane as internal standard (by Mrs. E. E. Richards). Amino-acid analyses were performed on a Jeol JLC-5AH analyser (by Mr. A. S. Whitworth): samples for amino-acid analyses were hydrolysed with constant boiling hydrochloric acid for 16 h at 110°.

Evaporation was performed in a rotary evaporator, and solutions in organic solvents were dried over magnesium sulphate. Ethyl acetate, acetone, acetic acid, pyridine, and benzene were of AnalaR grade. Diethyl ether was dried over sodium and distilled. Light petroleum (b.p. 40—60°) was dried over phosphorus pentoxide and distilled. Dimethylformamide was shaken with potassium hydroxide pellets for 24 h, fractionally distilled, and stored over BDH Molecular Sieve, type 4A. Dioxan was filtered through alumina immediately before use. Trifluoroacetic acid was redistilled. Triethylamine was dried over sodium and redistilled; traces of primary and secondary amine contaminants were removed by refluxing with 4-nitrophenyl acetate followed by two distillations.

Hydrogenations were performed at room temperature and atmospheric pressure.

T.l.c. was performed on Kieselgel G unbaked plates, with ninhydrin and/or chlorine starch–iodide and/or iodine vapour for detection after elution with one of the following solvent systems: ethyl acetate (TLC-1); methanol–chloroform (1 : 19) (TLC-2); n-butanol–water–acetic acid (4 : 5 : 1) (TLC-3); methanol–chloroform (1 : 1) (TLC-4); ether (TLC-5); n-butanol–water–pyridine (2 : 1 : 2) (TLC-6); pyridine–acetic acid–water (20 : 6 : 11) mixed with 1·5 volumes of ethyl acetate immediately before use (TLC-7); methanol–chloroform (1 : 3) (TLC-8); methanol (TLC-9); pyridine–acetic acid–water (20 : 6 : 11) mixed with 3 volumes of ethyl acetate immediately before use (TLC-10); methanol–chloroform (1 : 9) (TLC-11); and ethanol–water (1 : 1) (TLC-12).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-alanylglycine Methyl Ester (III).—Triethylamine (2·02 g, 20 mmol) and benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (4·82 g, 20 mmol) were added to a stirred solution of L-alanylglycine methyl ester hydrobromide [5] (8·69 g, 20 mmol) in dimethylformamide (50 ml) at 0°. The mixture was allowed to attain room temperature. After 24 h the solvent was removed and the residue was distributed between ethyl acetate (100 ml) and water (100 ml). The organic phase was washed with 10% citric acid (1 × 100 ml), saturated sodium hydrogen carbonate (1 × 100 ml), and water (1 × 100 ml), and dried. Evaporation gave the tripeptide derivative as white needles (8·80 g, 92%), m.p.

[4] R. Zabel and H. Zahn, *Z. Naturforsch.*, 1965, **20b**, 650.
[5] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

106—107°, $[\alpha]_D^{20}$ −4·8° (*c* 4·4 in Me_2N·CHO); TLC-1 R_F 0·40, TLC-2 R_F 0·54; $\nu_{max.}$ ($CHCl_3$) superimposed bands 1650—1740 cm^{-1}; τ ($CDCl_3$) 2·4—3·1 (7H, s at 2·65 superimposed on broad band, aromatic protons and peptide NH), 3·9 (1H, d, *J* 7 Hz, urethane NH), 4·89 (2H, s, CH_2Ph), 5·1—6·1 (4H, d at 6·0, *J* 5 Hz, superimposed on complex, NH·CH_2·CO and α-protons), 6·28 (3H, s, OMe), 7·4—8·2 (4H, complex, CH·CH_2·CH_2·CO), and 8·4—8·8 (12H, complex, Me_3C·O and CH*Me*) (Found: C, 57·2; H, 6·9; N, 8·8. Calc. for $C_{23}H_{33}N_3O_8$: C, 57·6; H, 6·9; N, 8·8%) {lit.,[3] m.p. 105—106°, $[\alpha]_D^{27}$ −6·1° (*c* 4·45 in Me_2N·CHO)}.

*Benzyloxycarbonyl-*L*-alanyl-γ-t-butyl-*L*-glutamyl-*L*-alanylglycine Methyl Ester.*—A solution of the preceding tripeptide derivative (3·84 g, 8 mmol) in 90% aqueous acetic acid (100 ml) was hydrogenated over 10% palladium–charcoal (0·80 g) for 2·5 h. The solution was filtered through Celite and evaporated. The resulting oil was dried first by repeated addition of benzene and evaporation, and then at 20° and 0·1 mmHg. The residue was dissolved in dimethylformamide (35 ml) and triethylamine (0·61 g, 6 mmol) and benzyloxycarbonyl-L-alanine succinimido ester [6] (2·56 g, 8 mmol) were added to the stirred solution at 20°. After 24 h the solution was evaporated and the residue was distributed between ethyl acetate (100 ml) and water (75 ml). The organic layer was washed with 10% citric acid (1 × 75 ml), saturated sodium hydrogen carbonate (1 × 75 ml), and water (1 × 75 ml), and dried. Evaporation gave *fully protected tetrapeptide* as a white solid (3·64 g, 82%), m.p. 182—184°, $[\alpha]_D^{20}$ −10·4° (*c* 1 in Me_2N·CHO); TLC-3 R_F 0·59, TLC-4 R_F 0·79 (Found: C, 56·85; H, 6·9; N, 10·0. $C_{26}H_{38}N_4O_9$ requires C, 56·7; H, 6·9; N, 10·2%).

L-Alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine Methyl Ester Acetate.—A solution of the preceding tetrapeptide derivative (3·57 g, 6·5 mmol) in 80% aqueous acetic acid (35 ml) was hydrogenated over 10% palladium–charcoal (0·70 g) for 2·5 h. The solution was filtered through Celite and evaporated. The residue was dried by repeated addition of benzene and evaporation. Trituration with ether gave *protected tetrapeptide acetate* as white needles (3·07 g, 99%), m.p. 129—131° (decomp.), $[\alpha]_D^{20}$ −14·0° (*c* 1 in AcOH); TLC-6 R_F 0·64, TLC-10 R_F 0·13 (Found: C, 50·12; H, 7·4; N, 11·6. $C_{18}H_{32}N_4O_7$,$C_2H_4O_2$ requires C, 50·4; H, 7·6; N, 11·8%).

N(α)-Benzyloxycarbonyl-N(ε)-t-butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine Methyl Ester.—Triethylamine (0·49 ml, 4·5 mmol) and *N*(α)-benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysine succinimido ester [7] (3·10 g, 6·5 mmol) were added to a stirred suspension of the preceding tetrapeptide acetate (3·02 g, 6·4 mmol) in dimethylformamide (25 ml) at 20°. After 48 h the solution was evaporated and the residue was distributed between ethyl acetate (150 ml) and water (100 ml). The organic layer was washed with 10% citric acid (1 × 100 ml), saturated sodium hydrogen carbonate (1 × 100 ml), and water (1 × 100 ml), and dried. Evaporation gave a colourless gel which on trituration with light petroleum gave *fully protected pentapeptide* as a white solid (4·64 g, 94%), m.p. 204—207°, $[\alpha]_D^{20}$ −12·6° (*c* 1 in Me_2N·CHO); TLC-2 R_F 0·36, TLC-3 R_F 0·74; amino-acid analysis Ala 1·95, Glu 1·02, Gly 1·00, Lys 0·96 (Found: C, 57·2; H, 7·4; N, 11·0. $C_{37}H_{58}N_6O_{12}$ requires C, 57·1; H, 7·5; N, 10·8%).

N(ε)-t-Butoxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine Methyl Ester Acetate Hydrate.—A solution of the preceding pentapeptide derivative (4·45 g, 5·72 mmol) in 80% aqueous acetic acid was hydrogenated over 10% palladium–charcoal (0·90 g) for 3 h. The solution was filtered through Celite and evaporated. The residual oil was dried by several additions of benzene with subsequent evaporation each time. Trituration of the residue with ether gave *protected pentapeptide acetate dihydrate* as a white solid (4·11 g, 97%), m.p. 161—165° (decomp.), $[\alpha]_D^{20}$ −15·8° (*c* 1·4 in AcOH); TLC-7 R_F 0·54, TLC-10 R_F 0·21 (Found: C, 50·5; H, 7·8; N, 11·30. $C_{29}H_{52}N_6O_{10}$,-$C_2H_4O_2$,2H_2O requires C, 50·2; H, 8·1; N, 11·35%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-N(ε)-t-butyloxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine Methyl Ester.—Triethylamine (0·56 ml, 0·40 mmol) and benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (2·5 g, 5·75 mmol) were added to a stirred solution of the preceding pentapeptide acetate dihydrate (4·10 g, 5·55 mmol) in dimethylformamide (45 ml) at 20°. After 48 h the solution was evaporated and the residue was suspended in ethyl acetate (250 ml). The suspension was washed with with 10% citric acid (1 × 100 ml), saturated sodium hydrogen carbonate (1 × 100 ml), and water (1 × 100 ml). Partial removal of the ethyl acetate followed by addition of light petroleum gave *fully protected hexapeptide* as a white solid (4·81 g, 87%), m.p. 222—225° (decomp.), $[\alpha]_D^{20}$ −12·9° (*c* 1 in Me_2N·CHO); TLC-2 R_F 0·44, TLC-9 R_F 0·74; amino-acid analysis Ala 2·00, Glu 1·97, Gly 1·05, Lys 0·98 (Found: C, 57·65; H, 7·75; N, 10·05. $C_{46}H_{73}$-N_7O_{15} requires C, 57·3; H, 7·6; N, 10·2%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-N(ε)-t-butyloxycarbonyl-L-lysyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine (I) (*Method A*).—Sodium hydroxide solution (N; 1·40 ml) was added to a stirred solution of the preceding methyl ester (193 mg, 0·2 mmol) in methanol (25 ml) at room temperature. After 1·0 h most of the methanol was removed under reduced pressure (water bath at 25°). The residue was acidified with 10% citric acid, and extracted with ethyl acetate (2 × 25 ml). The combined extracts were washed with water (4 × 25 ml) until the washings were neutral, and then evaporated. Drying by repeated addition of benzene followed by evaporation each time gave *protected hexapeptide acid* as a white solid (181 mg, 95%), m.p. 150—153°, $[\alpha]_D^{20}$ −11·4° (*c* 1 in Me_2N·CHO); TLC-7 R_F 0·75, TLC-9 R_F 0·58; amino-acid analysis Ala 2·00, Glu 1·96, Gly 1·02, Lys 0·97 (Found: C, 56·6; H, 7·35; N, 9·9. $C_{45}H_{71}N_7O_{18}$ requires C, 56·9; H, 7·5; N, 10·3%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-alanylglycine (IV).—Sodium hydroxide solution (N; 1·10 ml) was added dropwise during 5 min to a stirred solution of benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-alanylglycine methyl ester (480 mg, 1 mmol) in acetone (7 ml) at 18°. After 30 min the acetone was removed on the rotary evaporator (water bath at 30°). Water (50 ml) was added and the mixture was acidified to pH 3 with N-hydrochloric acid and extracted with ethyl acetate (1 × 25, 2 × 15 ml). The combined extracts were washed with water, dried, and evaporated. The residual oil solidified on trituration with light petroleum to give the tripeptide acid as a white solid (400 mg, 86%), m.p. 97—99°, $[\alpha]_D^{20}$ −10·3° (*c* 1 in EtOAc); TLC-3 R_F 0·69, TLC-9 R_F 0·60; τ ($CDCl_3$) 1·13 (1H, s, CO_2H), 2·2—2·8 (7H, s at 2·65 superimposed on broad band, aromatic protons and peptide NH), 3·77 (1H, d, *J* 7 Hz, urethane NH), 4·89 (2H, s, O·CH_2Ph), 5·1—6·2 (4H, d at

[6] G. W. Anderson, J. E. Zimmermann, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1964, **86**, 1839.

[7] H. Otsuka, K. Inouye, M. Kanayama, and F. Shinozaki, *Bull. Chem. Soc. Japan*, 1966, **39**, 882.

5·99, J 5 Hz, partially superimposed on complex, NH·CH_2·CO and α-protons), 7·4—8·2 (4H, complex, CH·CH_2·CH_2·CO), and 8·4—8·7 (12H, complex, Me_3C·O and CHMe) (Found: C, 56·5; H, 6·75; N, 8·95. Calc. for $C_{22}H_{31}N_3O_8$: C, 56·75; H, 6·7; N, 9·0%) {lit.,[8] m.p. 98—100°, $[\alpha]_D^{25}$ −11·2° (c 1 in EtOAc)}.

A second preparation on a 7 mmol scale under the same conditions gave product which was shown by t.l.c. to contain some unchanged methyl ester. It was dissolved in ethyl acetate (50 ml) and extracted with saturated sodium carbonate (2 × 25 ml). The aqueous solution was acidified (to pH 3) with N-hydrochloric acid and extracted with ethyl acetate (1 × 50, 2 × 25 ml). The combined extracts were washed with water, dried, and evaporated. Trituration of the residue with light petroleum gave tripeptide acid (2·68 g, 83%), identical with that just described.

A third preparation, on a 10 mmol scale, gave chromatographically impure product, but this did not contain unchanged methyl ester. The foregoing procedure did not effect purification. The impure material was dissolved in ethyl acetate (100 ml) and a solution of dicyclohexylamine (2·71 g, 15 mmol) in ethyl acetate (20 ml) was added. After 2 h at 0° the precipitate was isolated and recrystallised from methanol–ether to give *protected tripeptide acid dicyclohexylammonium salt* as a white solid (5·04 g, 78%), m.p. 175—178°, $[\alpha]_D^{20}$ −11·8° (c 1 in Me_2N·CHO) (Found: C, 62·9; H, 8·7; N, 8·6. $C_{34}H_{54}N_4O_8$ requires C, 63·2; H, 8·4; N, 8·7%). The dicyclohexylammonium salt (4·90 g, 7·59 mmol), ethyl acetate (150 ml), and 10% citric acid (150 ml) were shaken together for 30 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (100 ml). The combined extracts were washed with water, dried, and evaporated. Trituration of the residue with light petroleum gave tripeptide acid (3·14 g, 98%), identical with that already described.

Benzyloxycarbonyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine (*Method A*).—A solution of the preceding protected tripeptide acid (4·65 g, 10 mmol) in 80% aqueous acetic acid (150 ml) was hydrogenated over 10% palladium–charcoal (0·90 g) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by repeated addition of benzene and distillation to give a chromatographically pure white solid (TLC-3 R_F 0·33, TLC-6 R_F 0·58, TLC-7 R_F 0·17, TLC-9 R_F 0·48). Triethylamine (0·99 ml, 7 mmol) and benzyloxycarbonyl-L-alanine succinimido ester [6] (3·20 g, 10 mmol) were added to a stirred suspension of this solid in dimethylformamide (80 ml) and water (0·3 ml) at room temperature. After 48 h the resulting solution was evaporated and the residue was distributed between ethyl acetate (350 ml) and 10% citric acid (150 ml). The organic phase was washed with water until the washings were neutral, dried, and evaporated to give a colourless gel. Trituration with ether gave protected *tetrapeptide acid* as a white solid (4·36 g, 82%), m.p. 153—156°, $[\alpha]_D^{20}$ −8·3° (c 1 in Me_2N·CHO); TLC-7 R_F 0·65; TLC-10 R_F 0·44; amino-acid analysis Ala 2·04, Glu 0·97, Gly 1·00 (Found: C, 55·7; H, 6·7; N, 10·2. $C_{25}H_{36}N_4O_9$ requires C, 56·0; H, 6·7; N, 10·4%).

t-Butoxycarbonyl-L-alanylglycine Benzyl Ester.—Triethylamine (3·03 g, 30 mmol) and t-butoxycarbonyl-L-alanine succinimido ester [6] (8·58 g, 30 mmol) were added to a stirred solution of glycine benzyl ester toluene-p-sulphonate [9] (10·12 g, 30 mol) in dimethylformamide (2·5 ml) at 18°. After 20 h the solution was evaporated and the residue was distributed between ethyl acetate (150 ml) and water (100 ml). The organic phase was washed with 10% citric acid (1 × 100 ml), saturated sodium hydrogen carbonate (1 × 100 ml), and water (1 × 100, 1 × 50 ml), and dried. Evaporation gave a crystalline residue which was recrystallised from ethyl acetate–light petroleum to give *fully protected dipeptide* as white needles (9·25 g, 91·5%), m.p. 83—84°, $[\alpha]_D^{20}$ −23·9 (c 1 in $CHCl_3$); TLC-1 R_F 0·59; $\nu_{max.}$ 1670—1720 (partially superimposed bands) and 1745 cm^{-1}; τ ($CDCl_3$) 2·61 (5H, s, aromatic), 2·95—3·25 (1H, CO·NH·CH_2), 4·65—4·95 (3H, s at 4·80 superimposed on broad band, O·CH_2Ph and CO·NH·CH), 5·6—6·0 (3H, d, J 6 Hz, at 5·92 superimposed on complex band, NH·CH·CO and NH·CH_2·CO), and 8·40—8·75 (12H, s at 8·57 partially superimposed on d, J 7 Hz, centred at 8·64, Me_3C and CHMe) (Found: C, 60·4; H, 7·0; N, 8·3. $C_{17}H_{24}N_2O_5$ requires C, 60·7; H, 7·15; N, 8·3%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-L-alanylglycine Benzyl Ester.—The preceding dipeptide derivative (5·04 g, 15 mmol) was dissolved in 75% aqueous trifluoroacetic acid (10 ml). The solution was set aside at room temperature for 1 h and then evaporated. Water (10 ml) was added and the mixture was evaporated. The residue was dried by repeated addition of benzene followed by evaporation each time, and then at 20° and 0·1 mmHg. The residual oil was dissolved in dimethylformamide (15 ml), and triethylamine (1·51 g, 15 mmol) and benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (6·51 g, 15 mmol) were added to the stirred solution at 18°. After 24 h the solution was evaporated and the residue was distributed between ethyl acetate (150 ml) and water (100 ml). The organic phase was washed with 10% citric acid (1 × 100 ml), saturated sodium hydrogen carbonate solution (1 × 100 ml), and water (1 × 100 ml), and dried. Evaporation gave a solid which was recrystallised from ethyl acetate–light petroleum to give *protected tripeptide* as white needles (6·8 g, 82%), m.p. 120—122°, $[\alpha]_D^{20}$ −26·8° (c 1 in $CHCl_3$); TLC-1 R_F 0·48, TLC-4 R_F 0·73; $\nu_{max.}$ 1650—1760 (partially superimposed bands, maxima apparent at 1675 and 1720 cm^{-1}); τ ($CDCl_3$) 2·45—3·0 (12H, s at 2·62 superimposed on complex, aromatic protons and peptide NH), 3·98 (1H, d, J 7 Hz, urethane NH), 4·75—4·95 (4H, partially superimposed singlets at 4·83 and 4·89, O·CH_2Ph), 5·20—6·15 (4H, d at 5·96, J 6 Hz, superimposed on complex, α-protons and NH·CH_2·CO), 8·5—8·2 (4H, complex, CH·CH_2·CH_2·CO), and 8·45—8·75 (12H, s at 8·58 partially superimposed on d at 8·64, J 7 Hz, Me_3C and CHMe) (Found: C, 62·7; H, 6·7; N, 7·7. $C_{29}H_{37}N_3O_8$ requires C, 62·7; H, 6·7; N, 7·6%).

Benzyloxycarbonyl-L-alanyl-γ-t-butyl-L-glutamyl-L-alanylglycine (*Method B*).—A solution of the preceding tripeptide benzyl ester (2·78 g, 5 mmol) in 80% aqueous acetic acid (75 ml) was hydrogenated over 10% palladium–charcoal (0·60 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 distillation to give a chromatographically pure white solid (TLC-3 R_F 0·33, TLC-6 R_F 0·58, TLC-7 R_F 0·17). Triethylamine (0·51 ml, 3·6 mmol) and benzyloxycarbonyl-L-alanine succinimido ester [6] (1·60 g, 5 mmol) were added to a stirred suspension of this solid in dimethylformamide (30 ml) and water (0·15 ml) at room temperature. After 48 h the resulting solution was evaporated and the residue was distributed between ethyl acetate (300 ml) and 10% citric acid (100 ml). The

[8] B. J. Johnson, *J. Chem. Soc.* (*C*), 1967, 2638.
[9] L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, 1957, **22**, 1515.

organic phase was washed with water until the washings were neutral, dried, and evaporated to give a colourless gel. Trituration with ether gave *protected tetrapeptide acid* as a white solid (2·20 g, 84%), m.p. 153—155°, $[\alpha]_D^{20}$ −8·2° (*c* 1 in Me_2N·CHO); TLC-7 R_F 0·65, TLC-10 R_F 0·45 (Found: C, 55·6; H, 6·6; N, 10·1. $C_{25}H_{36}N_4O_9$ requires C, 56·0; H, 6·7; N, 10·4%).

N(α)-*Benzyloxycarbonyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-L-*alanylglycine*.—A solution of the preceding tetrapeptide acid (3·22 g, 6 mmol) in 80% aqueous acetic acid (100 ml) was hydrogenated over 10% palladium–charcoal for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by repeated addition of benzene followed each time by distillation to give a chromatographically pure white solid, TLC-3 R_F 0·27, TLC-6 R_F 0·63, TLC-7 R_F 0·15, TLC-9 R_F 0·50. Triethylamine (0·59 ml, 4·2 mmol) and *N*(α)-benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysine succinimido ester [7] (2·88 g, 6 mmol) were added to a stirred suspension of this solid in dimethylformamide (45 ml) and water (0·2 ml) at room temperature. After 18 h the solvent was evaporated off and the residue was suspended in ethyl acetate (2 l) and washed with 10% citric acid (1 × 0·5 l) and then water, until the washings were neutral. Evaporation, trituration with ether, and drying at 20° and 0·1 mmHg gave *protected pentapeptide acid* as a white crystalline solid (4·05 g, 89%), m.p. 189—193°, $[\alpha]_D^{20}$ +12·0° (*c* 1 in Me_2N·CHO); TLC-3 R_F 0·74, TLC-10 R_F 0·52; amino-acid analysis Ala 2·00, Glu 0·96, Gly 1·00, Lys 1·03 (Found: C, 56·35; H, 7·2; N, 10·8. $C_{36}H_{56}N_6O_{12}$ requires C, 56·55; H, 7·3; N, 11·0%).

Benzyloxycarbonyl-γ-*t-butyl*-L-*glutamyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-L-*alanylglycine* (I) (*Method B*).—A solution of the preceding pentapeptide derivative (3·85 g, 5·04 mmol) in 80% aqueous acetic acid (60 ml) was hydrogenated over 10% palladium–charcoal (0·7 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 distillation to give a chromatographically pure white solid (TLC-3 R_F 0·35, TLC-6 R_F 0·63, TLC-9 R_F 0·57). Triethylamine (0·50 ml, 3·5 mmol) and benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (2·19 g, 5·04 mmol) were added to a stirred suspension of this solid in dimethylformamide (20 ml) and water (0·3 ml) at room temperature. After 48 h the solution was evaporated and the residue was suspended in ethyl acetate (1 l) and washed with 10% citric acid (300 ml) and then water, until the washings were neutral. Evaporation of the ethyl acetate, trituration with ether, and drying at 20° and 0·1 mmHg gave *protected hexapeptide acid* as a white solid (3·91 g, 82%), m.p. 156—160°, $[\alpha]_D^{20}$ −11·6° (*c* 1 in Me_2N·CHO); TLC-3 R_F 0·81, TLC-10 R_F 0·50; amino-acid analysis Ala 2·00, Glu 1·97, Gly 1·04, Lys 0·96 (Found: C, 56·6; H, 7·4; N, 10·1. $C_{45}H_{71}N_7O_{18}$ requires C, 56·9; H, 7·5; N, 10·3%).

Benzyloxycarbonyl-γ-*t-butyl*-L-*glutamyl*-O-*t-butyl*-L-*serylglycine* (V) (*Method A*).—A solution of *N*-benzyloxycarbonyl-*O*-t-butyl-L-serylglycine [10] (4·07 g, 11·6 mmol) in 80% aqueous acetic acid (50 ml) was hydrogenated over 10% palladium–charcoal (0·80 g) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by repeated addition of benzene and distillation. Benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (5·03 g, 11·6 mmol) and triethylamine (1·62 ml, 11·6 mmol) were added to the stirred solution of this residue in dimethylformamide (10 ml). After 24 h the mixture was diluted with ethyl acetate (250 ml) and 10% citric acid (150 ml). The organic phase was washed with water until the washings were neutral, dried, and evaporated. Recrystallisation from ethyl acetate–light petroleum gave *protected tripeptide acid* (5·35 g, 86%) with physical constants identical to those described under Method B(see later).

Benzyloxycarbonyl-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-O-*t-butyl*-L-*serylglycine*.—A solution of the preceding tripeptide derivative (537 mg, 1 mmol) in 80% aqueous acetic acid (20 ml) was hydrogenated over 10% palladium–charcoal (110 mg) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by the usual method to give a white solid.

Benzyloxycarbonyl-L-alanine succinimido ester [6] (320 mg, 1 mmol) and triethylamine (0·14 ml, 1 mmol) were added to a stirred solution of this solid in dimethylformamide (2 ml). After 2 h the product was isolated and recrystallised as in the previous preparation. The *protected tetrapeptide acid* was obtained as white needles (480 mg, 79%), m.p. 145—147°, $[\alpha]_D^{20}$ +6·3° (*c* 1 in $CHCl_3$); TLC-7 R_F 0·82, TLC-10 R_F 0·53; $\nu_{max.}$ 1650—1750 cm^{-1} (overlapping bands); τ ($CDCl_3$) 2·1—2·8 (8H, s at 2·68 on complex, aromatic protons and peptide NH), 2·95 (1H, s, CO_2H), 4·18 (1H, d, *J* 7 Hz, urethane NH), 4·91 (2H, s, O·CH_2Ph), 5·2—6·7 (7H, d at 5·97, *J* 5 Hz, on complex, α-protons and CH·CH_2·O), 7·4—8·3 (4H, complex, CH_2·CH_2·CO), and 8·46—8·80 (21H, complex, CO·O·Me_3 and CMe) (Found: C, 57·0; H, 7·1; N, 9·0. $C_{29}H_{44}N_4O_{10}$ requires C, 57·2; H, 7·2; N, 9·2%).

N(α)-*Benzyloxycarbonyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-O-*t-butyl*-L-*serylglycine*.—A solution of the preceding tetrapeptide derivative (304 mg, 0·5 mmol) in 80% aqueous acetic acid (20 ml) was hydrogenated over 10 palladium–charcoal (60 mg) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by the usual method to give a white crystalline solid. *N*(α)-Benzyloxycarbonyl-*N*(ε)-t-butoxycarbonyl-L-lysine succinimido ester [7] (239 mg, 0·5 mmol) and triethylamine (0·07 ml, 0·5 mmol) were added to a stirred solution of this solid in dimethylformamide (2 ml). After 48 h the product was isolated and recrystallised as in previous preparations. The *protected pentapeptide acid* was obtained as a white solid (359 mg, 86%), m.p. 189—191°, $[\alpha]_D^{20}$ −2·5° (*c* 1 in Me_2N·CHO); TLC-7 R_F 0·78, TLC-10 R_F 0·53; τ ($CDCl_3$) 1·2—2·8 (10H, s at 2·63 on broad band, aromatic protons, peptide NH and CO_2H), 3·2—3·8 (1H, broad, CO·N*H*·CH), 4·6—5·6 (7H, s at 4·87 on complex, O·CH_2Ph and CO·N*H*·CH_2), 5·8—6·15 (2H, broad, NH·CH_2·CO), 6·15—6·65 (2H, broad, CH·CH_2·O), 6·7—7·2 (2H, broad, CH_2·CH_2·NH), 7·5—8·1 (4H, complex, CH·CH_2·CH_2·CO), and 8·1—9·0 (36H, s at 8·57 and 8·90 on complex, other protons) (Found: C, 57·6; H, 7·65; N, 10·0. $C_{40}H_{64}N_6O_{13}$ requires C, 57·4; H, 7·6; N, 10·0%).

Benzyloxycarbonyl-γ-*t-butyl*-L-*glutamyl*-N(ε)-*t-butoxycarbonyl*-L-*lysyl*-L-*alanyl*-γ-*t-butyl*-L-*glutamyl*-O-*t-butyl*-L-*serylglycine* (II).—A solution of the preceding pentapeptide derivative (2·10 g, 2·5 mmol) in 80% aqueous acetic acid (45 ml) was hydrogenated over 10% palladium–charcoal (0·40 g) for 3 h. The solution was filtered through Celite and evaporated. The residue was dried by the usual method to give a white crystalline solid. Benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (1·10 g, 2·5 mmol) and triethylamine (0·35 ml, 2·5 mmol) were added

[10] K. Poduska and M. I. Titov, *Coll. Czech. Chem. Comm.*, 1965, **30**, 1611.

to the stirred solution of this solid in dimethylformamide (15 ml). After 48 h the product was isolated as in previous preparations. Trituration with light petroleum gave *protected hexapeptide acid* as a white solid (2·40 g, 94%), m.p. 157—162°, $[\alpha]_D^{20}$ −6·9° (*c* 1 in $Me_2N{\cdot}CHO$); TLC-7 R_F 0·89, TLC-10 R_F 0·56; amino-acid analysis Ala 1·00, Glu 2·03, Gly 1·01, Lys 0·96, Ser 0·91 (Found: C, 57·2; H, 7·65; N, 9·6. $C_{49}H_{79}N_7O_{16}$ requires C, 57·6; H, 7·7; N, 9·6%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-O-t-butyl-L-seryl-glycine Methyl Ester.—A solution of *N*-benzyloxycarbonyl-*O*-t-butyl-L-serylglycine methyl ester [10] (366 mg, 1 mmol) in 80% aqueous acetic acid (15 ml) was hydrogenated over 10% palladium–charcoal (80 mg) for 3 h. The solution was filtered through Celite and evaporated. The resulting oil was dried by repeated addition of benzene followed each time by distillation, and then at 20° and 0·1 mmHg. Benzyloxycarbonyl-γ-t-butyl-L-glutamic acid succinimido ester [4] (435 mg, 1 mmol) and triethylamine (0·14 ml, 1 mmol) were added to a stirred solution of this oil in dimethylformamide (4 ml). After 18 h the mixture was distributed between ethyl acetate (100 ml) and water (50 ml). The organic layer was washed with 10% citric acid (1 × 50 ml), saturated sodium hydrogen carbonate solution (1 × 50 ml), and water (1 × 50 ml), and dried. Evaporation gave a solid which was recrystallised from ethyl acetate–light petroleum to give *fully protected tripeptide* as white needles (390 mg, 71%), m.p. 101—102°, $[\alpha]_D^{20}$ +8·6° (*c* 1 in $CHCl_3$); TLC-1 R_F 0·45, TLC-4 R_F 0·61; $\nu_{max.}$ ($CHCl_3$) 1650—1760 cm^{-1} (overlapping bands); τ ($CDCl_3$) 2·4—3·0 (7H, s at 2·64 on broad band, aromatic protons and peptide NH), 4·10 (1H, d, *J* 7 Hz, urethane NH), 4·88 (2H, s, $O{\cdot}CH_2Ph$), 5·3—6·8 (9H, d at 5·95, *J* 6 Hz, and s at 6·25 on complex, α-protons, $CH{\cdot}CH_2{\cdot}O$, and OMe), 7·4—8·2 (4H, complex, $CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO$), 8·58 (9H, s, $CO{\cdot}O{\cdot}CMe_3$), and 8·80 (9H, s, $CH_2{\cdot}O{\cdot}CMe_3$) (Found: C, 58·8; H, 7·4; N, 7·5. $C_{27}H_{41}N_3O_9$ requires C, 58·8; H, 7·4; N, 7·6%).

Benzyloxycarbonyl-γ-t-butyl-L-glutamyl-O-t-butyl-L-seryl-glycine (V) (*Method B*).—Sodium hydroxide solution (2N; 0·55 ml) was added dropwise during 5 min to a solution of the preceding methyl ester (551 mg, 1 mmol) in acetone (10 ml) at 20°. After 1 h the solution was acidified (to pH 6) with 10% aqueous citric acid and evaporated (water bath at 30°). The residue was diluted with water (50 ml), further acidified (to pH 3) and extracted with ethyl acetate (2 × 40 ml). The combined ethyl acetate extracts were washed with water until the washings were neutral, and dried. Dicyclohexylamine (200 mg, 1·1 mmol) and ether (50 ml) were added and the solution was set aside at 0°. After 6 h the precipitate was collected to give *protected tripeptide dicyclohexylammonium salt* as a white solid (6·54 mg, 91%), m.p. 84—90°, $[\alpha]_D^{20}$ −6·0° (*c* 1 in EtOH) (Found: C, 63·7; H, 8·6; N, 7·7. $C_{38}H_{62}N_4O_9$ requires C, 63·5; H, 8·6; N, 7·8%). This salt (575 mg, 0·8 mmol) was shaken with 10% citric acid (30 ml) and ethyl acetate (30 ml). The organic layer was washed with water until the washings were neutral, and dried. Evaporation gave a solid which was recrystallised from ethyl acetate–light petroleum to give *protected tripeptide acid* as white needles (408 mg, 95%), m.p. 159—162°, $[\alpha]_D^{20}$ +10·0° (*c* 1 in HCl_3); TLC-7 R_F 0·85, TLC-10 R_F 0·51; $\nu_{max.}$ ($CHCl_3$) 1650—1750 cm^{-1} (overlapping bands); τ ($CDCl_3$) 0·83 (1H, s, CO_2H), 2·4—2·7 (7H, s at 2·89 on broad band, aromatic protons and peptide NH), 3·95 (1H, d, *J* 7 Hz, urethane NH), 4·91 (2H, s, $O{\cdot}CH_2Ph$), 5·2—6·7 (6H, d at 5·98, *J* 5 Hz, on complex, α-protons and $CH{\cdot}CH_2{\cdot}O$), 7·5—8·2 (4H, complex, $CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO$) 8·59, (9H, s, $CO{\cdot}O{\cdot}CMe_3$), and 8·85 (9H, s, $CH_2{\cdot}O{\cdot}CMe_3$) (Found: C, 58·1; H, 7·3; N, 7·8. $C_{26}H_{39}N_3O_9$ requires C, 58·1; H, 7·3; N, 7·8%).

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