# Polypeptides. Part VII.[1] Variations of the Phenylalanyl Position in the *C*-Terminal Tetrapeptide Amide Sequence of the Gastrins

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The synthesis is described of analogues of L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (the *C*-terminal sequence of the gastrins) or its *N*-benzyloxycarbonyl, -t-butoxycarbonyl, or -carbamoyl derivatives wherein the phenylalanyl residue has undergone replacement by other naturally occuring amino-acyl residues or by αα-disubstituted amino-acyl residues, or has been modified by (*a*) substitution of the aromatic ring in the *p*-position, (*b*) hydrogenation of the aromatic ring, (*c*) switch of the imino-radical from the α- to the β-carbon atom, (*d*) switch of the phenyl ring from the β- to the α-carbon atom, (*e*) substitution of an hydroxy-group on the β-carbon atom, or (*f*) methylation of the imino-group.

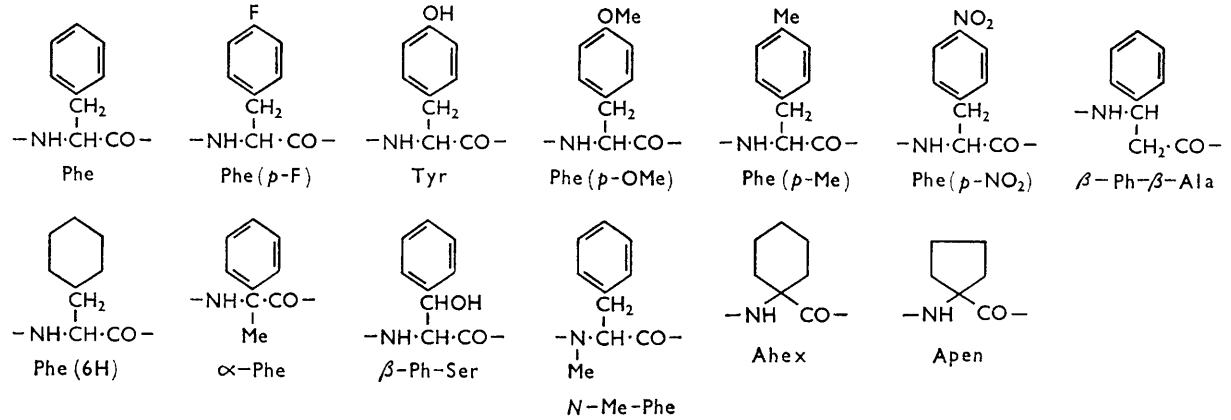
THE circumstances which prompted our investigation of structure-function relationships in the gastrin series of polypeptide hormones were outlined in Part VI [1] of this series. The present Paper gives details of the synthesis of analogues of the *C*-terminal tetrapeptide amide sequence of the gastrins, Trp·Met·Asp·Phe·$NH_2$,† wherein the phenylalanine residue only has undergone modification or replacement by other amino-acyl residues. If the variant of the phenylalanine residue is described as X, then the analogues are tetrapeptide amides of the type H·Trp·Met·Asp·X·$NH_2$, or *N*-benzyloxycarbonyl (Z), -t-butoxycarbonyl (BOC), or -carbamoyl derivatives of the type Z-, BOC-, $H_2NCO$-Trp·Met·Asp·X·$NH_2$. In two cases [X = (β-cyclohexyl)-L-alanyl or (*p*-methoxy)-L-phenylalanyl] the tetrapeptide amide analogue and all three types of acylated derivatives were prepared. There was no appreciable difference in the biological activity of the unacylated or acylated analogues (a similar situation applies to the parent tetrapeptide amide and its Z- or BOC-derivatives [3]). In general therefore, only the *N*-benzyloxycarbonyl or -t-butoxycarbonyl analogues were prepared and used to evaluate the effect of structural change on biological activity.

The analogues involved in the work arise from replacement of the phenylalanine residue by other naturally occurring amino-acyl residues (glycyl, L-alanyl, L-valyl, L-leucyl, L-glutamyl, or L-lysyl) (X = Gly, Ala, Val, Leu, Glu, or Lys), by αα-disubstituted amino-acyl residues (the residues from 1-aminocyclo-hexane- or -pentane-carboxylic acid) (X = Ahex or Apen), or by modification of the phenylalanine residue in the following ways: (*a*) substitution of the aromatic ring by a *p*-fluoro-, *p*-hydroxy-, *p*-methoxy-, *p*-methyl, or *p*-nitro-group [X = Phe(*p*-F), Tyr, Phe(*p*-OMe), Phe(*p*-Me), Phe(*p*-$NO_2$)], (*b*) hydrogenation of the aromatic ring [X = Phe(6H)], (*c*) switch of the imino-radical from the α- to the β-carbon atom (X = β-Ph-β-Ala), (*d*) switch of the phenyl ring from the β- to the α-carbon atom (X = α-Phe), (*e*) substitution of an hydroxy-group on the β-carbon atom (X = β-Ph-Ser, *erythro* and *threo* forms), or (*f*) methylation of the imino-radical (X = *N*-Me-Phe). The relationship of these structural changes is shown on p. 532.

In all except three cases, the syntheses involved the use of *N*-benzyloxycarbonyl- or -t-butoxycarbonyl-L-tryptophyl-L-methionine azide (VIa or b). Usually these azides were coupled with the appropriate dipeptide amide [V; X = Ahex, Apen, Gly, Leu, *N*-Me-Phe, α-Phe, Phe(*p*-F), Phe(*p*-Me), β-Ph-β-Ala, Val] (Scheme 1) to give *N*-benzyloxycarbonyl [VIIa; X = Ahex, Apen, Gly, *N*-Me-Phe, α-Phe, Phe(*p*-F), β-Ph-β-Ala, Val]- or -t-butoxycarbonyl [VIIb; X = Leu, Phe(*p*-Me)]-tetrapeptide amides directly. Occasionally the benzyloxycarbonyl azide (VIa) was coupled with the t-butyl ester of the appropriate dipeptide amide [XI; X = Glu($OBu^t$), Lys(BOC), Tyr] (Scheme 2), or the benzyloxycarbonyl or butoxycarbonyl azide (VIa or b) was coupled with the appropriate dipeptide methyl or ethyl ester [XVI; X = Phe(6H) or Phe(*p*-$NO_2$), R = Me; X = Phe(*p*-OMe), R = Et] (Scheme 3). *N*-Benzyloxycarbonyl-tetrapeptide amide t-butyl esters [XII; X = Glu($OBu^t$), Lys(BOC), Tyr] resulted in the former cases, and *N*-benzyloxycarbonyl- or -t-butoxycarbonyl-tetrapeptide methyl or ethyl esters [XVIIa; X = Phe(6H), R = Me; X = Phe(*p*-$NO_2$), R = Me; X =

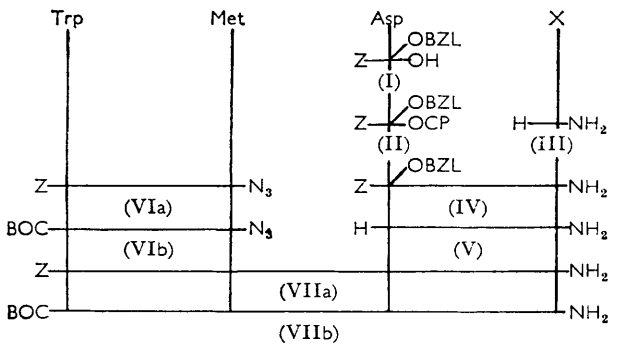
† The abbreviations for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions of the Committee on Nomenclature which reported at the Fifth European Peptide Symposium [G. T. Young (ed.), 'Peptides. Proceedings of the Fifth European Peptide Symposium,' Pergamon Press, London, 1963, p. 261] with the modifications adopted by I.U.P.A.C. (Inform. Bull., I.U.P.A.C., 1966, No. 25, p. 32). Non-standard abbreviations are explained in the footnote [a] to Tables 1—3.

[1] Part VI, H. Gregory, A. H. Laird, J. S. Morley, and J. M. Smith, preceding Paper.



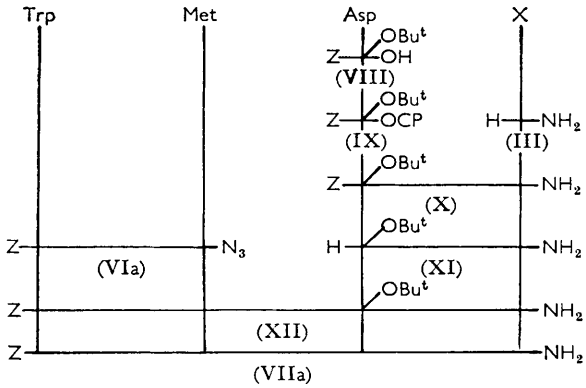

Phe(*p*-OMe), R = Et; and XVIIb; X = Phe(*p*-OMe), R = Et] in the latter cases. Two of the *N*-benzyloxycarbonyl-tetrapeptide amide t-butyl esters [XII; X = Lys(BOC) or Tyr] (Scheme 2) were smoothly cleaved with trifluoroacetic acid to give the *N*-benzyloxycarbonyl-tetrapeptide amides (VIIa; X = Lys or Tyr), but in the third case [XII; X = Glu(OBu$^t$)] a pure product could not be isolated after the cleavage. The *N*-benzyloxycarbonyl- or -t-butoxycarbonyl-tetrapeptide esters (XVIIa or b) (Scheme 3) were smoothly converted by ammonolysis to the *N*-benzyloxycarbonyl [VIIa; X = Phe(6H), Phe(*p*-OMe) or Phe(*p*-$NO_2$)]- or -t-butoxycarbonyl [VIIb; X = Phe(6H) or Phe(*p*-OMe)]-tetrapeptide amides; the latter, after treatment with hydrogen chloride in acetic acid, gave the tetrapeptide amides [VIIc; X = Phe(6H) or Phe(*p*-OMe)], which were converted to the *N*-carbamoyl tetrapeptide amides [VIId; X = Phe(6H) or Phe(*p*-OMe)] by means of potassium cyanate at pH 8.

The dipeptide amides (H-Asp·X·$NH_2$) (V) required in Scheme 1 were conveniently prepared in two stages.




Scheme 1

β-Benzyl *N*-benzyloxycarbonyl-L-aspartate (I) was first coupled with the appropriate amino-acid amide (H-X·$NH_2$) (III) by means of *NN'*-dicyclohexylcarbodi-imide or by the use of the 2,4,5-trichlorophenyl ester (II), and the resulting protected-derivative [Z-Asp(OBZL)·X·$NH_2$] (IV) was then hydrogenated; both protecting groups were thereby removed and the dipeptide amides (V) resulted in high overall yield. The dipeptide amide t-butyl esters [H-Asp(OBu$^t$)·X·$NH_2$] (XI) required in Scheme 2 were similarly prepared using β-t-butyl *N*-benzyloxycarbonyl-L-aspartate (VIII); coupling of the latter with the amino-acid amide (H-X·$NH_2$) (III) by means of *NN'*-dicyclohexylcarbodi-imide or by the use of the 2,4,5-trichlorophenyl ester (IX)

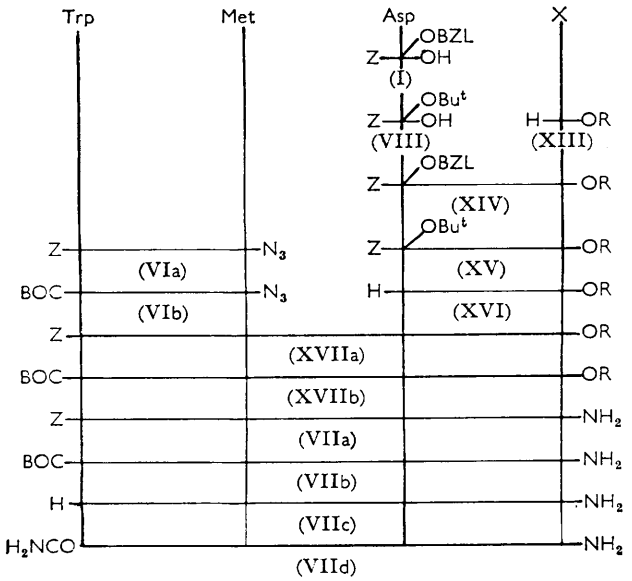



Scheme 2

gave the protected derivative [Z-Asp(OBu$^t$)·X·$NH_2$] (X), from which the benzyloxycarbonyl group was removed by hydrogenolysis. Salts of the dipeptide esters (H-Asp·X·OR) [XVI; X = Phe(6H) or Phe(*p*-$NO_2$), R = Me; X = Phe(*p*-OMe), R = Et] required in Scheme 3 were prepared by two methods. In one, β-benzyl *N*-benzyloxycarbonyl-L-aspartate (I) or its 2,4,5-trichlorophenyl ester (II) was coupled with the appropriate amino-acid methyl or ethyl ester [XIII; X = Phe(6H), R = Me; X = Phe(*p*-OMe), R = Et], and the product [XIV; X = Phe(6H), R = Me; X = Phe(*p*-OMe), R = Et] was hydrogenated in acetic acid or acetic acid containing one equivalent of hydrochloric acid. In the other, β-t-butyl *N*-benzyloxycarbonyl-L-aspartate (VIII) was coupled with the amino-acid methyl ester [XIII; X = Phe(*p*-$NO_2$), R = Me] by means of *NN'*-dicyclohexylcarbodi-imide, and the product [XV; X = Phe(*p*-$NO_2$), R = Me] was cleaved with hydrogen bromide in acetic acid.

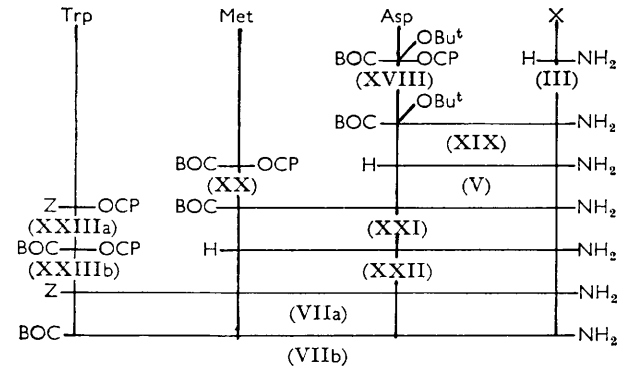
A fully stepwise method of synthesis (Scheme 4) was employed in the three exceptional cases (X = Ala, *erythro*- or *threo*-β-Ph-Ser). This alternative route was

necessary in the cases of the β-phenylserine analogues because the 2 + 2 coupling method (Scheme 1) gave unsatisfactory products, and in the case of the alanine



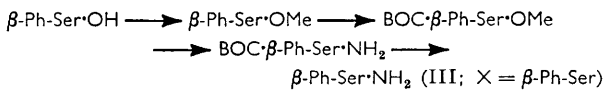

SCHEME 3

analogue because the intermediate tripeptide amide (XXII; X = Ala) was required for biological examination. L-Aspartyl-L-alanine amide (V; X = Ala) was prepared normally *via* the β-benzyl *N*-benzyloxycarbonyl-dipeptide amide (IV; X = Ala) following




SCHEME 4

the methods used in Scheme 1, but the analogous dipeptide amides containing β-phenylserine (IV; X = *erythro*- and *threo*-β-Ph-Ser) were best prepared by cleavage of the β-t-butyl *N*-t-butoxycarbonyl-dipeptide amides (XIX; X = *erythro*- and *threo*-β-Ph-Ser) [from α-2,4,5-trichlorophenyl β-t-butyl *N*-butoxycarbonyl-L-aspartate (XVIII) and the amino-acid amides (III)] with trifluoroacetic acid. Coupling of the dipeptide amides (V; X = Ala, *erythro*- and *threo*-β-Ph-Ser) with *N*-t-butoxycarbonyl-L-methionine 2,4,5-trichlorophenyl ester (XX) gave the *N*-t-butoxycarbonyl tripeptide amides (XXI; X = Ala, *erythro*- and *threo*-β-Ph-Ser) which, with trifluoroacetic acid, gave trifluoroacetates of the tripeptide amides (XXII; X = Ala, *erythro*- and *threo*-β-Ph-Ser). Finally, the tripeptide amides (XXII; X = Ala, *erythro*- and *threo*-β-Ph-Ser) were condensed with *N*-benzyloxycarbonyl- or -t-butoxycarbonyl-L-tryptophan 2,4,5-trichlorophenyl ester (XXIIIa or b) to give the *N*-benzyloxycarbonyl (VIIa; X = *erythro*- and *threo*-β-Ph-Ser)- or -t-butoxycarbonyl (VIIb; X = Ala)-tetrapeptide amides. The *erythro*- and *threo*-DL-β-phenylserine amides (III; X = β-Ph-Ser) used as starting materials in two of the syntheses were prepared as follows. *erythro*- and *threo*-DL-β-Phenylserine were esterified with methanol–hydrogen chloride, and the methyl esters were treated with *N*-t-butoxycarbonyl azide in pyridine, to give *N*-t-butoxycarbonyl-*erythro*- and-*threo*-DL-β-phenylserine methyl ester. Ammonolysis of these in methanol at room temperature afforded *N*-t-butoxycarbonyl-*erythro*- and -*threo*-DL-β-phenylserine amide. The *erythro*-isomer gave *erythro*-DL-β-phenylserine amide trifluoroacetate after treatment with 80% aqueous trifluoroacetic acid, and the *threo*-isomer gave *threo*-DL-β-phenyl serine amide hydrobromide after treatment with hydrogen bromide in acetic acid (the cleavage of the *threo*-isomer in trifluoroacetic acid or in aqueous trifluoroacetic acid was less satisfactory). These preparations are summarised below:




Details of the amino-acid, di-, tri-, and tetra-peptide derivatives involved in these syntheses are given in Tables 1—3. It should be noted (Table 1) that six DL-amino-acids [α-Phe, Phe(*p*-F), Phe(*p*-Me), β-Ph-β-Ala, *erythro*- and *threo*-β-Ph-Ser] were used as starting materials in the syntheses. In these cases the dipeptide intermediates (Table 2) are mixtures of the L,L- and L,D-isomers, the tripeptide intermediates (where applicable) (Table 3) are mixtures of the L,L,L- and L,L,D-isomers, and the tetrapeptide derivatives (Table 3) are mixtures of the L,L,L,L- and L,L,L,D-isomers. Many of the analogues described in this paper are potent stimulators of gastric acid secretion in the rat. The results have been reported and discussed elsewhere.[2]

## EXPERIMENTAL

Ascending, thin-layer chromatograms were run on Kieselgel-G with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) ($R_{FA}$), butan-1-ol–acetic acid–water–pyridine (15 : 3 : 12 : 10) ($R_{FB}$), butan-2-ol–3% ammonium hydroxide (3 : 1) ($R_{FC}$), acetonitrile–water (3 : 1) ($R_{FD}$), acetone–chloroform (1 : 1) ($R_{FE}$), ethanol–chloroform (4 : 1) ($R_{FF}$), cyclohexane–ethyl acetate (1 : 1) ($R_{FG}$), cyclohexane–ethyl acetate–methanol (1 : 1 : 1) ($R_{FH}$), or ethyl acetate ($R_{FJ}$). Descending chromatograms were run on Whatman No. 3 paper with butan-1-ol–acetic acid–water (4 : 1 : 5) ($R_{FM}$). Spots were revealed with ninhydrin, sodium hypochlorite–potassium iodide–tolidine,[3] or Ehrlich's reagent, and, in the case of thin-layer chromatography, by incorporating a

[2] J. S. Morley, *Proc. Roy. Soc.*, 1967, *B.*, in the press.
[3] S. C. Pan and J. D. Dutcher, *Analyt. Chem.*, 1956, **28**, 836.

fluorescent indicator in the thin-layer (Kieselgel GF 254, Merck) or by acid potassium permanganate [reagent prepared by dissolving potassium permanganate (100 mg.) in conc. sulphuric acid (1 ml.) and diluting the solution to 100 ml. with water]. Acid hydrolysates of peptide derivatives were prepared using 6N-hydrochloric acid (110°/16 hr.) and the amino-acid composition of the hydrolysates was determined with a Beckman-Spinco Amino Acid Analyser, model 120B. Optical rotations were determined with a Bendix NPL Automatic Polarimeter, model 143C, with Digital Converter, model 154C. Organic extracts were dried with anhydrous magnesium sulphate, and evaporations were carried out under reduced pressure in a rotary evaporator. M. p.s (uncorrected) were determined in capillary tubes in a Tottoli melting-point apparatus (manufactured by W. Büchi).

*Starting Materials*.—The following starting materials were prepared by literature methods or obtained commercially: L-alanine amide (Ala·$NH_2$), 1-aminocyclopentanecarboxylic acid (Apen),[4] γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate [Z·Glu(OBu$^t$)·OH],[5] glycine amide acetate (Gly·$NH_2$,AcOH), L-leucine amide hydrochloride (Leu·$NH_2$,HCl), *N*-methyl-L-phenylalanine (*N*-Me-Phe),[6] (*p*-fluoro)-DL-phenylalanine [Phe(*p*-F)], (*p*-methoxy)-L-phenylalanine (*O*-methyl-L-tyrosine) [Phe(*p*-OMe)],[7] (*p*-methyl)-DL-phenylalanine [Phe(*p*-Me)],[8] (*p*-nitro)-L-phenylalanine [Phe(*p*-$NO_2$)],[9] DL-3-amino-3-phenylpropionic acid (β-phenyl-β-alanine) (β-Ph-β-Ala), *erythro*- and *threo*-DL-β-phenylserine (β-Ph-Ser),[10] L-tyrosine amide hydrochloride (Tyr·$NH_2$,HCl), L-valine amide acetate (Val·$NH_2$,AcOH). 1-Aminocyclohexanecarboxylic acid (Ahex) and DL-2-amino-2-phenylpropionic acid ('α-phenylalanine') (α-Phe) were kindly supplied by Professor G. W. Kenner. (β-Cyclohexyl)-L-alanine (hexahydro-L-phenylalanine (Phe-6H) sulphate ($R_{FA}$ 0·52) (Phe had $R_{FA}$ 0·44) was prepared by hydrogenation of L-phenylalanine in 2N-sulphuric acid over a platinum catalyst at room temperature and pressure; the sulphate in hot aqueous solution was stirred with a ten-fold excess of 'De-acidite G' (acetate form) and the mixture was filtered and evaporated, to give (β-cyclohexyl)-L-alanine, decomp. 250°, $[\alpha]_D^{23}$ +16·9 (*c* 0·65 in N-hydrochloric acid) [lit.,[11] gives m. p. variable up to 324°, $[\alpha]_D^{20}$ +13·3° (*c* 4 in N-hydrochloric acid)]. $N^{\epsilon}$-t-Butoxycarbonyl-L-lysine amide acetate [Lys(BOC)·$NH_2$,AcOH], m. p. 60—62°, $[\alpha]_D^{26}$ +12·8 (*c* 1 in dimethylformamide), $R_{FA}$ 0·68, was prepared (98% yield) by hydrogenolysis of $N^{\alpha}$-benzyloxycarbonyl-$N^{\epsilon}$-t-butoxycarbonyl-L-lysine amide[12] in acetic acid over 5% palladised-charcoal at room temperature and pressure. The purity of all starting materials was checked by t.l.c. and, in the case of optically active materials, by measurements of optical rotation.

*Description of Methods* (*Tables* 1—3).—(a) *Carbobenzoxylations*. A1 A stirred solution of the amino-acid (H-X-OH) (1 mol.) in 2N-sodium hydroxide (1 mol.) was treated dropwise at 0—5° during 40—50 min. with benzyloxycarbonyl chloride (1·1 mol.) or a solution of benzyloxycarbonyl chloride (1·1 mol.) in a little acetone, and the mixture was further stirred at 20—25° until its pH did not change during 15 min. (usually 1—2 hr.). Throughout the addition and subsequent stirring a reaction pH of 10—10·5 was maintained by periodic addition of 2N-sodium hydroxide (1 mol. required) to the reaction mixture. Occasionally a sparingly-soluble sodium salt separated during the reaction; in these cases solution was maintained by the addition of water. After evaporation under reduced pressure to remove the acetone (when this was used), the mixture was added to stirred ice-cold N-hydrochloric acid (3 mol.) and the product was isolated by filtration or extraction with ethyl acetate. A2 The following conditions for the carbobenzoxylation of *N*-methyl-L-phenylalanine were found superior to those previously described.[13] A stirred solution of *N*-methyl-L-phenylalanine (17·9 g., 0·1 mole) in N-sodium hydroxide (100 ml.) and acetone (25 ml.) was treated at 55—60° during 30 min. with a solution of benzyloxycarbonyl chloride (14·2 ml., 0·1 mole) in acetone (50 ml.). During the addition, 2N-sodium hydroxide (50 ml.) was added simultaneously to maintain a pH of 11. The mixture was kept at 55—60° for 5 min. then cooled to 10° and treated with light petroleum (b. p. 60—80°) (200 ml.). The sodium salt was collected, washed with light petroleum (3 × 200 ml.), and then dissolved in warm water (300 ml.). The resulting solution was added to ice (200 g.) and conc. hydrochloric acid (20 ml.), and the mixture was extracted with ethyl acetate (3 × 300 ml.). The combined extracts were washed with water (3 × 50 ml.), dried, and evaporated. The residue crystallised rapidly under light petroleum (b. p. 60—80°) (100 ml.), to yield *N*-benzyloxycarbonyl-*N*-methyl-L-phenylalanine (21·45 g., 69%), m. p. 69—71°, $[\alpha]_D^{22}$ −69·2° (*c* 2·5 in ethyl acetate).

(b) *Mixed anhydride reactions*. B1 Triethylamine (1·4 ml., 10 mmole) followed by ethyl chloroformate (0·96 ml., 10 mmole) were added at −10° to −20° to a vigorously stirred solution of the corresponding *N*-benzyloxycarbonyl-amino-acid (Z-X-OH) (10 mmole) in dry tetrahydrofuran (20—100 ml.). The mixture was stirred at −20° for 10—20 min., saturated below −10° with dry ammonia, and then kept at room temperature overnight. After evaporation, the resulting residue was triturated with water and crystallised from the solvent indicated in Table 1. Alternatively, the residue from the evaporation was shaken with ethyl acetate and water and the organic layer was separated, washed successively with aqueous citric acid, water, N-potassium hydrogen carbonate, and water, dried, and evaporated; the resulting residue was then crystallised from the solvent indicated in Table 1. B2 The procedure was similar to that described in B1 except that the mixture was stirred at −20° for 30 min. and then at −5° for 2 hr. before the saturation with ammonia.

(c) *Active ester reactions*. C1 *N*-Benzyloxycarbonyl-*N*-methyl-L-phenylalanine (1·02 g., 3·3 mmole), 2,4,5-trichlorophenol (0·671 g., 3·4 mmoles), *NN'*-dicyclohexylcarbodiimide (0·702 g., 3·4 mmoles), and ethyl acetate (15 ml.) were kept at 4° for 5 hr. After filtration, to remove *NN'*-dicyclohexylurea, the solution was evaporated and the oily residue was dissolved at 0° in dry methanol saturated with ammonia ( 5 ml.). The solution was kept at 4° for 16 hr.,

[4] T. A. Connors and W. C. J. Ross, *J. Chem. Soc.*, 1960, 2119.
[5] J. S. Morley, *J. Chem. Soc.* (*C*), 1967, 2410.
[6] P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, 1963, **46**, 327.
[7] K. Jošt and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2345.
[8] A. L. Zhuze, K. Jošt, E. Kasafírek, and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1964, **29**, 2648.
[9] E. Erlenmeyer and A. Lipp, *Annalen*, 1883, **219**, 161.
[10] K. N. F. Shaw and S. W. Fox, *J. Amer. Chem. Soc.*, 1953, **75**, 3421.
[11] E. Waser and E. Brauchli, *Helv. Chim. Acta*, 1924, **7**, 740.
[12] E. Schnabel, *Annalen*, 1964, **674**, 218.
[13] M. Goodman and K. E. Stueben, *J. Org. Chem.*, 1962, **27**, 3409.

TABLE 1

Amino-acid derivatives [a]

| Formula [a] | X [a] | Method [b] | Yield (%) | M. p. [c] | Solvent [d] | $R_F$ [e] A | B | C | D | E | F | Analysis [f] % Found C | H | N | Formula | Required C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Z–X–OH | Ahex | A1 | 52 | 150—153° [g] | $C_6H_6$–pet | 0·89 | 0·80 | 0·43 | | 0·00 | 0·34 | 64·5 | 6·8 | 5·0 | $C_{15}H_{19}NO_4$ | 64·9 [k] | 6·9 [k] | 5·1 [k] |
| | Apen | A1 | 88 | 82—86 [h] | EtAc–pet | | 0·72 | | | 0·30 | 0·71 | 64·3 | 6·5 | 5·3 | $C_{14}H_{17}NO_4$ | 63·8 [k] | 6·5 [k] | 5·3 [k] |
| | *N*-Me-Phe | A2 | 69 | 69—71 [i] | EtAc–pet | 0·97 | 0·74 | 0·59 | | | | 68·5 | 6·0 | 4·4 | $C_{18}H_{19}NO_4$ | 69·0 [k] | 6·1 [k] | 4·5 [k] |
| | DL-α-Phe | A1 | 37 | 105—107 | $C_6H_6$–pet | 0·83 | 0·81 | 0·73 | 0·76 | | 0·72 | 68·4 | 5·7 | 4·9 | $C_{17}H_{17}NO_4$ | 68·4 [k] | 5·7 [k] | 4·7 [k] |
| | DL-Phe(*p*-F) | A1 | 89 | 124—126 | EtAc–pet | 0·75 | 0·72 | 0·44 | | | | 64·2 | 5·0 | 4·2 | $C_{17}H_{16}FNO_4$ | 64·5 | 5·1 | 4·4 |
| | DL-Phe(*p*-Me) | A1 | 90 | 125—126 | $C_6H_6$ | 0·85 | | 0·53 | | | | 68·6 | 5·9 | 4·5 | $C_{18}H_{19}NO_4$ | 69·0 | 6·1 | 4·5 |
| | DL-β-Ph-β-Ala | A1 | 78 | 123—125 | EtAc–pet | 0·96 | 0·71 | 0·48 | | | | 68·2 | 5·7 | 4·6 | $C_{17}H_{17}NO_4$ | 68·4 | 5·7 | 4·7 |
| Z–X–$NH_2$ | Ahex | B2 | 80 | 112 | EtAc–$Et_2O$ | 0·90 | 0·83 | 0·75 | | 0·41 | 0·84 | 65·5 | 7·4 | 9·7 | $C_{15}H_{20}N_2O_3$ | 65·2 | 7·3 | 10·1 |
| | Apen | B2 | 57 | 140—143 | EtAc–pet | 0·76 | 0·80 | 0·94 | | 0·35 | 0·71 | 62·3 | 7·0 | 9·8 | $C_{14}H_{18}N_2O_3,0·5H_2O$ | 62·2 | 7·1 | 10·3 |
| | Glu(OBu$^t$) | B1 | 62 | 134—135 | EtAc–pet | 0·84 | 0·85 | | 0·59 | 0·09 | 0·55 | 60·9 | 7·2 | 8·3 | $C_{17}H_{24}N_2O_5$ | 60·7 | 7·2 | 8·3 |
| | *N*-Me-Phe | B1 | 69 | 137—138 | EtOH | 0·98 | 0·82 | 0·92 | | 0·61 | 0·72 | 69·4 | 6·5 | 8·7 | $C_{18}H_{20}N_2O_3$ | 69·3 | 6·5 | 9·0 |
| | | C1 | 50 | 138—139 [l] | | | | | | | | | | | | | | |
| | DL-α-Phe | B2 | 62 | 138—140 | EtAc–pet | 0·91 | 0·88 | 0·77 | | 0·63 | 0·87 | 68·4 | 6·3 | 9·3 | $C_{17}H_{18}N_2O_3$ | 68·3 | 6·1 | 9·5 |
| | DL-Phe(*p*-F) | B1 | 82 | 175—176 | EtAc | 0·84 | 0·84 | 0·80 | | | | 64·6 | 5·5 | 8·9 | $C_{17}H_{17}FN_2O_3$ | 64·6 | 5·4 | 8·9 |
| | DL-Phe(*p*-Me) | B1 | 61 | 160—161 | EtAc–pet | 0·74 | | 0·81 | | 0·40 | 0·66 | 69·1 | 6·6 | 8·9 | $C_{18}H_{20}N_2O_3$ | 69·3 | 6·5 | 9·0 |
| | DL-β-Ph-β-Ala | B1 | 91 | 186—187 | EtAc | 0·86 | 0·84 | 0·81 | | | | 68·3 | 6·2 | 9·4 | $C_{17}H_{18}N_2O_3$ | 68·3 | 6·1 | 9·5 |
| H–X–$NH_2$ | Ahex | D2 | 67 | 100 [m] | | 0·29 [n] | 0·55 [n] | 0·59 [n] | | 0·10 [n] | 0·37 [n] | | | | | | | |
| | Apen | D2 | 75 | 93—95 [p] | | 0·40 | 0·74 | 0·73 [n] | | 0·08 | 0·32 [n] | | | | | | | |
| | Glu(OBu$^t$) | D1 | 45 | 82—85 (Ac) | EtAc | 0·48 | | 0·59 [n] | | | 0·31 | | | | | | | |
| | | | | 155—156 | | | | | | | | | | | | | | |
| | *N*-Me-Phe | D1 | 100 | 122—124 (Ac) | EtAc | 0·43 | 0·67 | 0·69 | | 0·10 | 0·43 | 67·3 | 7·9 | 15·8 | $C_{10}H_{14}N_2O$ | 67·3 | 7·9 | 15·7 |
| | DL-α-Phe | D2 | 99 | [r] | | | 0·61 [n] | 0·58 [n] | | | | | | | | | | |
| | DL-Phe(*p*-F) | D1 | 89 | 150—151 (Ac) | EtAc | 0·53 | 0·64 | 0·62 | 0·50 | | | 59·0 | 6·3 | 15·1 | $C_9H_{11}FN_2O$ | 59·3 | 6·1 | 15·4 |
| | | | | 139—140 | EtOH | | | | | | | | | | | | | |
| | DL-Phe(*p*-Me) | D1 | 90 | 137—138 (Ac) | | 0·45 | 0·66 | 0·43 | | | 0·25 | 60·6 | 7·5 | 12·1 | $C_{12}H_{18}N_2O_3$ | 60·5 | 7·6 | 11·8 |
| | DL-β-Ph-β-Ala | D1 | 92 | 133—134·5 (Ac) | MeOH–$Et_2O$ | 0·48 | 0·58 | 0·59 | 0·32 | 0·08 | 0·32 | 58·8 | 7·3 | 12·6 | $C_{11}H_{16}N_2O_3$ | 59·0 | 7·1 | 12·5 |
| | DL-β-Ph-Ser(*erythro*) | E1 | 87 | 108 (TFA) | | 0·51 | | 0·78 | | | | | | | | | | |
| | DL-β-Ph-Ser(*threo*) | F1 | 96 | (HBr) | | 0·56 | 0·10 | 0·59 | | | | | | | | | | |
| BOC–X–OMe | DL-β-Ph-Ser(*erythro*) [q] | G | 32 | 126 | EtAc–pet | $R_{FJ}$ 0·84 | | | | | | 61·2 | 6·9 | 4·9 | $C_{15}H_{21}NO_5$ | 61·0 | 7·1 | 4·8 |
| | DL-β-Ph-Ser(*threo*) [p] | G | 92 | Oil | | | | | | | | | | | | | | |
| BOC–X–$NH_2$ | DL-β-Ph-Ser(*erythro*) | H1 | 54 | 179—180 | MeOH–EtAc | 0·73 | | 0·67 | | | | 59·2 | 7·1 | 10·1 | $C_{14}H_{20}N_2O_4$ | 59·8 | 7·2 | 10·0 |
| | DL-β-Ph-Ser(*threo*) [q] | H1 | 55 | 146 | EtAc–pet | $R_{FJ}$ 0·56 | | | 0·78 | 0·42 | 0·72 | 59·8 | 7·2 | 10·2 | $C_{14}H_{20}N_2O_4$ | 59·8 | 7·2 | 10·0 |
| H–X–OMe | Phe(6H) | J2 | 86 | 151—153 (HCl) | MeOH–EtAc | | | 0·78 | 0·66 | 0·32 | 0·60 | 54·4 | 9·1 | 6·4 | $C_{10}H_{19}NO_2$,HCl | 54·2 | 9·1 | 6·3 |
| | Phe(*p*-$NO_2$) | J2 | 40 | 185—186 (HCl) [u] | MeOH–EtAc | 0·49 | | | | | | | | | | | | |
| | DL-β-Ph-Ser(*erythro*) | J1 | 94 | 176—177 d. (HCl) [v] | MeOH–$Et_2O$ | 0·54 | | 0·68 | | | | 52·2 | 6·1 | 5·9 | $C_{10}H_{12}NO_3$,HCl | 51·9 [k] | 6·1 [k] | 6·1 [k] |
| | DL-β-Ph-Ser(*threo*) | J1 | 73 | 159—160 d. (HCl) [v] | MeOH–$Et_2O$ | 0·72 | | 0·59 | 0·65 | 0·61 | 0·63 | 51·6 | 6·1 | 6·1 | $C_{10}H_{12}NO_3$,HCl | 51·9 [k] | 6·1 [k] | 6·1 [k] |
| H–X–OEt | Phe(*p*-OMe) | J3 | | 190—191 (HCl) [w] | EtOH–$Et_2O$ | | | | | | | | | | | | | |

[a] The abbreviations for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions given in the footnote on page 531; Ahex = 1-aminocyclohexanecarboxylic acid, Apen = 1-aminocyclopentanecarboxylic acid, α-Phe = 2-amino-2-phenylpropionic acid (α-phenylalanine), Phe(*p*-F, -OMe, -Me, or -$NO_2$) = (*p*-fluoro, -methoxy, -methyl, or -nitro)-phenylalanine, Phe(6H) = (β-cyclohexyl)-alanine, β-Ph-β-Ala = 3-amino-3-phenylpropionic acid (β-phenyl-β-alanine), β-Phe–Ser = β-phenylserine; unless otherwise stated, all optically active amino-acids are of the L-configuration. [b] See Experimental section for description of the methods. [c] Ac = acetate, HCl = hydrochloride, TFA = trifluoroacetate, HBr = hydrobromide, d. = with decomposition or effervescence. [d] $C_6H_6$ = benzene, pet = light petroleum (b. p. 60—80°), EtAc = ethyl acetate, cyclohex. = cyclohexane, IPE = di-isopropyl ether, DMF = dimethylformamide. [e] The methods of chromatography and an explanation of the solvent systems are given in the introduction to the experimental; unless otherwise stated, the quoting of an $R_F$ value implies the detection of one spot only by at least two of the visualisation methods described. [f] Except in the case of acetates samples for micro-analysis were usually dried at 60°/18 hr./0·1 mm. over phosphorus pentoxide. [g] P. Tailleur and L. Berlinguet, *Canad. J. Chem.*, 1961, **39**, 1309, *J. Org. Chem.*, 1962, **27**, 653, give m. p. 154—156°. [h] P. Tailleur and L. Berlinguet, *Canad. J. Chem.*, 1961, **39**, 1309, give m. p. 92—93°. [i] M. Goodman and K. C. Stueben, *J. Org. Chem.*, 1962, **27**, 3409, give m. p. 69·5—70·5°, $[\alpha]_D$ −70·3 (*c* 2·5 in ethyl acetate). [j] D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard, *J. Chem. Soc.*, 1965, 6227, give m. p. 104—106°. [k] Calculated. [l] $[\alpha]_D^{22}$ −82·2° (*c* 0·95 in ethyl acetate). [m] E. Schipper and E. Chinery, *J. Org. Chem.*, 1961, **26**, 4480, give m. p. 105—106°. [n] Minor impurities also detected. [p] T. A. Connors and W. C. T. Ross, *J. Chem. Soc.*, 1960, 2119, give m. p. 95—96°. [q] $[\alpha]_D^{22}$ +22·7° (*c* 1·01 in ethanol). [r] The m. p. of the product (a solid) was not determined; H. E. Johnson and D. G. Crosby, *J. Org. Chem.*, 1962, **27**, 798, give the hydrochloride, m. p. 266—267°. [s] The $^1H$ n.m.r. and i.r. spectra of this product were consistent with the structure shown. [t] $[\alpha]_D^{24}$ +21·3° (*c* 1 in 0·1N-hydrochloric acid). [u] M. N. Shchukina and T. P. Sycheva, *Zhur. obshchei Khim.*, 1952, **22**, 1879, give m. p. 197°. [v] K. N. F. Shaw and S. W. Fox, *J. Amer. Chem. Soc.*, 1953, **75**, 3421, give the *erythro*-isomer, m. p. 180° and the *threo*-isomer, m. p. 160°. [w] K. Jošt and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2345, give m. p. 196—198°.

TABLE 2

Dipeptide derivatives [a]

| Formula [a] | X [a] | Method [b] | Yield (%) | M. p. [c] | Solvent [d] | $R_F$ [e] A | B | C | D | E | F | Analysis [f] % Found C | H | N | Formula | Required C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Z–Asp(OBZL)–X–$NH_2$ | Ala | C2 | 74 | 180—181° | EtOH | 0·87 | 0·88 | 0·77 | 0·81 | 0·45 | 0·72 | 61·6 | 5·9 | 10·2 | $C_{22}H_{25}N_3O_6$ | 61·8 | 5·9 | 9·8 |
| | Ahex | C2 | 47 | 145—148 [g] | EtAc | 0·78 | 0·86 | 0·77 | | 0·45 | 0·77 | 64·7 | 6·3 | 8·6 | $C_{26}H_{31}N_3O_6$ | 64·8 | 6·5 | 8·7 |
| | Apen | C2 | 84 | 86—88 [h] | EtAc–pet | 0·83 | 0·82 | 0·78 | | 0·40 | 0·74 | 63·1 | 6·2 | 8·7 | $C_{25}H_{29}N_3O_6,0·5H_2O$ | 63·1 | 6·3 | 8·8 |
| | Gly | C2 | 47 | 119—121 | EtAc–pet | 0·86 | 0·89 | 0·86 | 0·70 | 0·36 | 0·72 | 61·0 | 5·6 | 10·2 | $C_{21}H_{23}N_3O_6$ | 61·0 | 5·7 | 10·7 |
| | Leu | C2 | 86 | 157—159 | EtAc | 0·87 | 0·88 | 0·70 | | 0·45 | 0·70 | 64·2 | 6·5 | 8·8 | $C_{25}H_{31}N_3O_6$ | 63·8 | 6·7 | 8·9 |
| | N-Me-Phe | K1 | 91 | 127 [i] | EtAc–cyclohex. | 0·96 | 0·95 | 0·94 | | 0·60 | 0·71 | 67·0 | 6·1 | 8·1 | $C_{29}H_{31}N_3O_6$ | 67·2 | 6·0 | 8·1 |
| | α-Phe [j] | K1 | 70 | Glass [k] | | 0·82 | 0·77 | 0·74 | | 0·56 | 0·76 | | | | | | | |
| | Phe(*p*-F) [j] | C2 | 90 | 196—198 | EtOH | 0·86 | | | 0·73 | 0·43 | 0·74 | 64·5 | 5·3 | 8·0 | $C_{28}H_{28}FN_3O_6$ | 64·5 | 5·4 | 8·1 |
| | Phe(*p*-Me) [j] | C2 | 78 | 156—159 [m] | EtOH | 0·91 | 0·88 | 0·79 | | | 0·81 | 67·4 | 6·0 | 8·0 | $C_{29}H_{31}N_3O_6$ | 67·2 | 6·0 | 8·1 |
| | β-Ph-β-Ala [j] | C2 | 85 | 152—153 | AcOH | 0·83 | | | 0·70 | 0·26 | 0·67 | 66·9 | 5·8 | 8·3 | $C_{28}H_{29}N_3O_6$ | 66·8 | 5·8 | 8·3 |
| | Val | C2 | 80 | 193—195 | EtOH | 0·95 | | 0·92 | | 0·48 | | 63·2 | 6·3 | 9·2 | $C_{24}H_{29}N_3O_6$ | 63·3 | 6·5 | 9·2 |
| Z–Asp(OBu$^t$)–X–$NH_2$ | Glu(OBu$^t$) | K2 | 99 | 102—104 | EtAc–pet | 1·0 | 1·0 | | | 0·52 | 0·75 | 56·7 | 7·1 | | $C_{25}H_{38}N_3O_9$ | 57·1 | 7·2 | |
| | Lys(BOC) | C3 | 33 | 105—111 | Aq. EtOH | | | | | 0·44 | 0·84 | 58·6 | 7·6 | 10·3 | $C_{27}H_{42}N_4O_8$ | 58·8 | 7·7 | 10·2 |
| | Tyr | C3 | 45 | 143—146 | EtAc–pet | 1·0 | 1·0 | | | 0·40 | 0·70 | 61·7 | 6·3 | 8·7 | $C_{25}H_{31}N_3O_7$ | 61·8 | 6·5 | 8·6 |
| BOC–Asp(OBu$^t$)–X–$NH_2$ | β-Ph-Ser(*erythro*) [j,l] | C7 | 74 | 80—90 d. | EtAc–pet | $R_{FJ}$ 0·70 | | | | | | 57·9 | 7·5 | | $C_{22}H_{33}N_3O_7$ | 58·4 | 7·4 | |
| | β-Ph-Ser(*threo*) [j] | C7 | 54 | Oil | | $R_{FJ}$ 0·52 [n] | | | | | | | | | | | | |
| H–Asp–X–$NH_2$ | Ala | D3 | 98 | 193 d. | EtOH–$Et_2O$ | 0·17 | 0·47 | 0·13 | 0·18 | 0·00 | 0·00 | 41·5 | 6·6 | 20·6 | $C_7H_{13}N_3O_4$ | 41·3 | 6·4 | 20·7 |
| | Ahex | D4 | 86 | Glass | | 0·30 | 0·51 | 0·25 | | 0·00 | 0·15 | | | | | | | |
| | Apen | D4 | 100 | 154—156 d. | MeOH–$Et_2O$ | 0·24 | 0·47 | 0·15 | | 0·00 | 0·04 | | | | | | | |
| | Gly | D3 | 100 | (Ac) | | 0·15 [n] | 0·33 [n] | 0·14 | 0·14 [n] | 0·02 | 0·06 | | | | | | | |
| | Leu | D4 | 40 | 146—149 | EtOH | 0·32 | 0·52 | 0·18 | | | | 48·7 | 7·8 | 17·1 | $C_{10}H_{19}N_3O_4$ | 48·9 | 7·8 | 17·2 |
| | N-Me-Phe | D4 | 100 | Oil | | 0·25 [n] | 0·53 [n] | 0·40 [n] | | 0·00 | 0·00 | | | | | | | |
| | α-Phe [j] | D4 | 88 | 127 d. [p] | MeOH–EtAc | 0·38 | 0·42 | 0·34 | | 0·00 | 0·08 | 51·4 | 6·2 | 13·4 | $C_{13}H_{17}N_3O_4,1·5H_2O$ | 51·1 | 6·5 | 13·7 |
| | Phe(*p*-F) [j] | D5 | 88 | (HCl) | | 0·35 | | 0·22 | 0·37 | | | 46·2 | 5·5 | 12·3 | $C_{13}H_{16}FN_3O_4,HCl$ | 46·7 | 5·1 | 12·6 |
| | Phe(*p*-Me) | D3 | 91 | 182—186 (Ac) | MeOH | 0·38 | 0·57 | | | | | 54·4 | 6·7 | 13·7 | $C_{14}H_{19}N_3O_4,H_2O$ | 54·0 | 6·8 | 13·5 |
| | β-Ph-β-Ala [j] | D5 | 88 | (HCl) | | 0·30 | | 0·20 | 0·27 | | | Cl, 11·0 | | | $C_{13}H_{17}N_3O_4,HCl$ | Cl, 11·2 | | |
| | β-Ph-Ser(*erythro*) [j] | E2 | 44 | (TFA) | MeOH–EtAc | 0·30 | | 0·34 | | | | | | | | | | |
| | β-Ph-Ser(*threo*) [j,l] | E2 | 25 | d. < 155 (TFA) | MeOH–EtAc | | 0·74 | 0·29 | | | | | | | | | | |
| | Val | D3 | 93 | 193—195 (Ac) | | $R_{FM}$ 0·34 | | | | | | | | | | | | |
| Z–Asp(OBZL)–X–OMe | Phe(6H) | K1 | 97 | 77–79 | IPE–pet | 1·0 | 1·0 | | | 0·80 | 0·76 | 66·5 | 6·7 | 5·5 | $C_{29}H_{36}N_2O_7$ | 66·7 | 6·6 | 5·4 |
| Z–Asp(OBu$^t$)–X–OMe | Phe(*p*-$NO_2$) | K2 | 60 | 69·5—70 | $Et_2O$–pet | 1·0 | 1·0 | | | 0·78 | 0·76 | 57·7 | 6·0 | 8·5 | $C_{26}H_{31}N_3O_9$ | 59·0 | 6·0 | 8·5 |
| Z–Asp(OBZL)–X–OEt | Phe(*p*-OMe) | C2 | 70 | 87—90 | EtAc–pet | 0·93 | 0·96 | 0·97 | 0·82 | 0·82 | 0·83 | 65·7 | 5·8 | 5·0 | $C_{30}H_{32}N_2O_8$ | 65·6 | 5·8 | 5·0 |
| H–Asp–X–OMe | Phe(6H) | D5 | 92 | d. > 80 (HCl) | | 0·57 | | | | | | | | | | | | |
| | Phe(*p*-$NO_2$) | F2 | 90 | 158—160 d. (HBr) | | 0·47 | 0·67 | 0·27 | | 0·00 | | | | | | | | |
| H–Asp–X–OEt | Phe(*p*-OMe) | D3 | 100 | 210—213 d. (Ac) | | 0·75 | | | | | | | | | | | | |

[a–f] As given in the footnotes to Table 1. [g] $[\alpha]_D^{25}$ −19·0° (*c* 1·8 in dimethylformamide). [h] $[\alpha]_D^{25}$ −52·0° (*c* 1·0 in ethanol). [i] $[\alpha]_D^{28}$ −78·9° (*c* 1·0 in dimethylformamide). [j] Mixture of L,D- and L,L-isomers. [k] $[\alpha]_D^{24}$ −9·0° (*c* 1·04 in dimethylformamide). [l] The $^1H$ n.m.r. and i.r. spectra of this product were consistent with the structure shown. [m] $[\alpha]_D^{25}$ −10·2° (*c* 1·0 in dimethylformamide). [n] Minor impurities also detected. [p] $[\alpha]_D^{28}$ +15·0° (*c* 1·0 in 10% aqueous acetic acid).

TABLE 3

Tri- and tetra-peptide derivatives [a]

| Formula [a] | X [a] | Method [b] | Yield (%) | M. p.[c] | Solvent [d] | $[\alpha]_D$ [e] | $R_F$ [e] A | B | C | D | E | F | Analysis [f] % Found C | H | N | Formula | Required C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BOC–Met–Asp–X–$NH_2$ | Ala | C4 | 84 | 179—180° d. | EtOH | | | 0·74 | 0·35 | 0·60 | 0·00 | 0·26 | 46·8 | 7·1 | 12·7 | $C_{17}H_{30}N_4O_7S$ | 46·9 | 6·9 | 12·9 |
| | β-Ph-Ser (*erythro*) [h] | C4 | 61 | 128—130 d. | $Et_2O$ [n] | | 0·67 [n] | 0·78 [n] | 0·33 [n] | | | | asp 1·02; met. 0·97; β-ph-ser 0·40 [i] | | | | | | |
| | β-Ph-Ser (*threo*) [h] | C4 | 65 | 118—120 d. | EtAc–pet | | 0·70 | 0·78 | 0·40 | | | | | | | | | | |
| H–Met–Asp–X–$NH_2$ | Ala | E3 | 94 100 | 210—211 d. (TFA) | Aq. acetone | | 0·33 | 0·55 | 0·22 | 0·23 | 0·00 | 0·16 | 43·0 | 6·7 | | $C_{12}H_{22}N_4O_5S$ | 43·1 | 6·6 | |
| | β-Ph-Ser (*erythro*) [h] | E3 | 53 | 76—78 (TFA) | MeOH–$Et_2O$ | | 0·43 | 0·62 | 0·30 | | | | 42·9 | 5·1 | 9·7 | $C_{20}H_{27}F_3O_9S,H_2O$ | 42·9 | 5·2 | 10·0 |
| | β-Ph-Ser (*threo*) [h] | E3 | 79 | d. 135 (TFA) | MeOH–EtAc | | 0·44 | 0·49 | 0·27 | | | | | | | | | | |
| Z–Trp–Met–Asp–X–$NH_2$ | Ahex | M1 | 38 | 187—190 | EtOH | −12·8° [j] | 0·96 | 0·95 | 0·88 | | | 0·78 | 60·1 | 5·9 | 12·1 | $C_{35}H_{44}N_6O_8S$ | 59·7 | 6·3 | 12·1 |
| | Apen | M1 | 56 | 123 d. | MeOH–$Et_2O$ | −29·8 | 0·70 | 0·66 | 0·72 [n] | | 0·00 | 0·05 | 55·9 | 6·0 | 11·4 | $C_{34}H_{42}N_6O_8S,2H_2O$ | 55·9 | 5·8 | 11·5 |
| | Gly | M1 | 55 | 168—170 d. | EtAc–MeOH | −20·4 [l] | | | 0·53 | | | | 56·7 | 5·5 | 13·0 | $C_{30}H_{36}N_6O_8S$ | 56·3 | 5·7 | 13·1 |
| | Lys | E4 | 98 | 140—145 d. (TFA) | $Et_2O$ [p] | −19·6 [l] | 0·43 | | 0·25 | | | | | | | | | | |
| | N-Me-Phe | M1 | 47 | 115—120 | EtAc–$Et_2O$ | −86·8 | 0·81 | 0·77 | 0·38 | | | | 59·7 | 6·2 | 10·9 | $C_{38}H_{44}N_6O_8S,H_2O$ | 59·8 | 6·0 | 11·0 |
| | α-Phe [k] | M1 | 64 | 119 | MeOH–$Et_2O$ | −34·1 | 0·88 | 0·74 | 0·72 [n] | | 0·00 | | 59·1 | 5·7 | 10·8 | $C_{37}H_{42}N_6O_8S,H_2O$ | 59·3 | 5·7 | 11·2 |
| | Phe(*p*-F) [k] | M1 | 80 | 199—200 d. | EtOH | −16·7 | 0·85 | | 0·52 | 0·58 | | | 59·1 | 5·6 | 11·1 | $C_{37}H_{41}FN_6O_8S$ | 59·3 | 5·5 | 11·2 |
| | Phe(6H) | H2 | 72 | 226—228 d. | MeOH–EtAc | −39·8 [l] | 0·88 | | 0·52 | | | | 59·7 | 6·8 | 10·8 | $C_{37}H_{48}N_6O_8S$ | 60·3 | 6·6 | 11·4 |
| | Phe(*p*-OMe) | H2 | 75 | 214—216 d. | Aq. MeO$[CH_2]_2$OH | −34·4 | 0·84 | | 0·50 | | | | 59·7 | 5·6 | 10·9 | $C_{38}H_{44}N_6O_9S$ | 60·0 | 5·8 | 11·1 |
| | Phe(*p*-$NO_2$) | H2 | 70 | 178—180 d. | EtAc–pet | −36·0 [l] | 0·96 | | 0·94 | | | | 55·8 | 5·3 | 12·9 | $C_{37}H_{41}N_7O_{10}S,H_2O$ | 56·1 | 5·4 | 12·3 |
| | β-Ph-β-Ala [k] | M1 | 85 | 233—237 | EtOH | −24·8 | 0·82 | | 0·56 | 0·55 | | | 60·7 | 5·8 | 11·6 | $C_{37}H_{42}N_6O_8S$ | 60·7 | 5·8 | 11·5 |
| | β-Ph-Ser (*erythro*) [k] | C5 | 53 | 159—162 d. | Aq. EtOH | −35·2 | 0·80 | 0·76 | 0·48 | | | | 58·9 | 5·7 | 10·7 | $C_{37}H_{42}N_6O_9S$ | 59·4 | 5·7 | 11·2 |
| | β-Ph-Ser (*threo*) [k] | C5 | 37 | 174—176 d. | Aq. EtOH | −38·4 | 0·79 | 0·76 | 0·38 | | | | 57·6 | 5·7 | 10·4 | $C_{37}H_{42}N_6O_9S,H_2O$ | 58·0 | 5·8 | 10·9 |
| | Tyr | E4 | 92 | 140—145 d. | $Et_2O$ [p] | −22·1 [l] | 0·72 | | 0·38 | | | | | | | | | | |
| | Val | M1 | 61 | 232—234 d. | Aq. MeO$[CH_2]_2$OH | −41·1 | 0·84 | | 0·50 | | | | 57·2 | 6·2 | 11·9 | $C_{33}H_{42}N_6O_8S,0·5H_2O$ | 57·3 | 6·3 | 12·1 |
| Z–Trp–Met–Asp(OBu$^t$)–X–$NH_2$ | Glu(OBu$^t$) | M1 | 75 | 135—150 d. | EtAc–pet | | | | | | | | 59·5 | 6·8 | 10·8 | $C_{41}H_{56}N_6O_{10}S$ | 59·6 | 6·8 | 10·2 |
| | Lys(BOC) | M1 | 35 | 183—185 | MeOH–EtAc–pet | −23·1 | 1·0 | | 0·92 | | 0·25 | | 59·0 | 6·8 | 11·5 | $C_{43}H_{61}N_7O_{10}S$ | 59·5 | 7·1 | 11·3 |
| | Tyr | M1 | 52 | 169—171 d. | MeOH–EtAc–pet | −27·9 | 1·0 | | 0·92 | | 0·47 | 0·74 | 61·3 | 6·2 | 9·3 | $C_{41}H_{50}N_4O_8S$ | 61·5 | 6·3 | 10·5 |
| BOC–Trp–Met–Asp–X–$NH_2$ | Ala | C6 | 74 | 202 d. | EtOH | −26·6 | 0·83 | 0·74 | 0·38 | 0·69 | 0·00 | 0·17 | 53·8 | 6·5 | 13·2 | $C_{28}H_{40}N_6O_8S$ | 54·1 | 6·5 | 13·5 |
| | Leu | M2 | 39 | 195—197 | EtOH | −37·5 | 0·79 | 0·80 | 0·38 | | | | 56·1 | 7·0 | 12·8 | $C_{31}H_{46}N_6O_8S$ | 56·1 | 7·0 | 12·7 |
| | Phe(6H) | H2 | 30 | 187 d. | MeOH–EtAc | −26·1 [m] | 0·72 | | 0·39 | | | | 57·6 | 7·6 | 12·0 | $C_{34}H_{50}N_6O_8S$ | 58·1 | 7·2 | 12·0 |
| | Phe(*p*-OMe) | H2 | 75 | 206—207 d. | Aq. DMF | −30·9 [l] | | | 0·69 | | | | 58·0 | 6·4 | 11·7 | $C_{35}H_{46}N_6O_9S$ | 57·9 | 6·4 | 11·6 |
| | Phe(*p*-Me) [k] | M2 | 38 | 190—195 | EtOH | −26·3 | 0·81 | 0·82 | 0·31 | | 0·00 | | 58·3 | 6·5 | 11·9 | $C_{35}H_{46}N_6O_8S,H_2O$ | 58·4 | 6·6 | 11·7 |
| H–Trp–Met–Asp–X–$NH_2$ | Phe(6H) | L | 90 | 160 d. (HCl) | $Et_2O$ [p] | | 0·61 | | 0·34 | | | | | | | | | | |
| | Phe(*p*-OMe) | L | 100 | 191—193 d. (HCl) | $Et_2O$ [p] | | 0·60 | | 0·52 | | | | | | | | | | |
| $H_2NCO$–Trp–Met–Asp–X–$NH_2$ | Phe(6H) | N | 70 | 223 d. | Aq. DMF | −30·2 [l] | 0·67 | | 0·39 | | | | 54·8 | 6·5 | 14·8 | $C_{30}H_{43}N_7O_7S,0·5H_2O$ | 54·4 | 6·7 | 14·8 |
| | Phe(*p*-OMe) | N | 55 | 206—207 d. | Aq. DMF | −30·8 [l] | 0·68 | | 0·35 | | | | 54·3 | 6·1 | 14·3 | $C_{31}H_{39}N_7O_8S,H_2O$ | 54·2 | 6·0 | 14·3 |
| Z–Trp–Met–Asp–X–OMe | Phe(6H) | M1 | 59 | 146—151 | MeOH–EtAc | −31·7 | 0·98 | | 0·77 | | | | 60·4 | 6·4 | 9·7 | $C_{38}H_{49}N_5O_9S$ | 60·7 | 6·6 | 9·3 |
| | Phe(*p*-$NO_2$) | M1 | 62 | 149—151 d. | EtAc–pet | −24·4 | 0·89 | | 0·59 | 0·67 | 0·55 | | 56·7 | 5·3 | 11·0 | $C_{38}H_{43}N_6O_{11}S$ | 57·6 | 5·5 | 10·6 |
| Z–Trp–Met–Asp–X–OEt | Phe(*p*-OMe) | M1 | 81 | 147—149 d. | EtAc–pet | −28·7 | 1·0 | | | 0·67 | | 0·61 | 60·6 | 5·7 | 8·9 | $C_{40}H_{47}N_5O_{10}S$ | 60·8 | 6·0 | 8·9 |
| BOC–Trp–Met–Asp–X–OEt | Phe(*p*-OMe) | M2 | 70 | 127—131 d. | MeOH–EtAc–pet | −18·6 | 1·0 | | | 0·69 | | 0·62 | 58·0 | 6·5 | 9·1 | $C_{37}H_{49}N_5O_{10}S$ | 58·5 | 6·5 | 9·3 |

[a–f] As given in the footnotes to Table 1. [g] *c* 1·0 in dimethylformamide at 24—26° unless otherwise stated. [h] Mixture of L,L,D- and L,L,L-isomers. [i] Amino-acidratios in acid hydrolysate (the β-phenylserine is partly destroyed). [j] *c* 0·7. [k] Mixture of L,L,L,D- and L,L,L,L-isomers. [l] *c* 2·0. [m] *c* 0·4. [n] Product collected after trituration with ether and used directly in the next stage of the synthesis. [p] Product collected after trituration with ether and not further characterised.

then the *amide* (0·508 g., 50%), was collected and washed with ice–cold methanol. C2 A solution of the appropriate amino-acid amide (H-X-$NH_2$), amide acetate (H-X-$NH_2$, AcOH), amide hydrochloride (H-X-$NH_2$, HCl), or ethyl ester hydrochloride (H-X-OEt, HCl) (10 mmoles), triethylamine (nil, 9, 10, and 10 mmoles, respectively) and α-2,4,5-trichlorophenyl β-benzyl *N*-benzyloxycarbonyl-L-aspartate [14] (11 mmoles) in dimethylformamide (10—40 ml.) was stirred at 4° until it failed to react with ninhydrin (1—3 days). Ice–water (~300 ml.) was then added and the product, obtained by filtration or an appropriate extraction procedure, was crystallised from the solvent indicated in Table 2. C3 The preceding method was followed except that α-2,4,5-trichlorophenyl β-t-butyl *N*-benzyloxycarbonyl-L-aspartate was used. C4 A solution of the appropriate dipeptide amide (H-Asp-X-$NH_2$) or amide trifluoroacetate (H-Asp-X-$NH_2$,TFA) (1 mmole), triethylamine (1 or 2 mmoles) and *N*-t-butoxycarbonyl-L-methionine 2,4,5-trichlorophenyl ester [15] (1 mmole) in dimethylformamide (4 ml.) was stirred at 4° for 18—48 hr. The solvent was then removed under reduced pressure and the residue was triturated with water (5 ml.) at pH 3. The product, obtained by filtration or an appropriate extraction procedure, was triturated with ether or crystallised from the solvent(s) indicated in Table 3. C5 A solution of L-methionyl-L-aspartyl-*erythro*- or -*threo*- D- and -L-β-phenylserine trifluoroacetate (42 mg., 0·08 mmole), triethylamine (0·022 ml., 0·16 mmole), and *N*-benzyloxycarbonyl-L-tryptophan 2,4,5-trichlorophenyl ester [16] (47 mg., 10% excess) in dimethylformamide (1·5 ml.) was kept at 4° for 2 days. The solution was then acidified to pH 3 at 0° with aqueous citric acid and diluted with ice–water (25 ml.). The products were isolated by extraction with ethyl acetate and recrystallised several times from the solvent indicated in Table 3. C6 A solution of L-methionyl-L-aspartyl-L-alanine amide trifluoroacetate (0·448 g., 1 mmole), triethylamine (0·28 ml., 2 mmoles), and *N*-t-butoxycarbonyl-L-tryptophan 2,4,5-trichlorophenyl ester [15] (0·483 g., 1 mmole) in dimethylformamide (7 ml.) was stirred at 4° for 2 days. After acidification of the mixture to pH 3 with aqueous citric acid, and the addition of ice–water (25 ml.) and ether (10 ml.), the solid was collected and crystallised from the solvent indicated in Table 3. C7 A solution of *erythro*- or *threo*- DL-β-phenylserine amide trifluoroacetate (46 mg., 0·16 mmole), triethylamine (0·021 ml., 0·16 mmole) and α-2,4,5-trichlorophenyl β-t-butyl *N*-t-butoxycarbonyl-L-aspartate [15] (105 mg., 0·22 mmole) in dimethylformamide (3·5 ml.) was kept at 4° for 2 days. The solution was worked-up as described in C5 and the products were crystallised from the solvent indicated in Table 1.

(d) *Hydrogenolyses.* D1 The appropriate *N*-benzyloxycarbonyl-amino-acid amide (Z-X-$NH_2$) (10 mmoles) in 90% aqueous acetic acid (25 ml.) was hydrogenated at room temperature and pressure over 5% palladised charcoal (0·5 g.) until evolution of carbon dioxide ceased (2—5 hr.). After removal of the catalyst (Kieselguhr), the solution was evaporated and the residue was dried by azeotropic distillation with benzene, to yield the acetates. Analytical samples were obtained by recrystallisation from the solvent indicated in Table 1 or by trituration with ether, and were dried at 20—25°/1 mm. over phosphorus pentoxide for 2—6 hr. (acetic acid was lost after prolonged drying at higher temperatures); in a number of cases the presence of 1 mol. of acetic acid was confirmed by $^1$H n.m.r. spectroscopy. The bases were obtained from aqueous solutions of the acetates by neutralisation with sodium hydroxide or by the use of 'De-acidite G' (acetate form). D2 The appropriate *N*-benzyloxycarbonyl-amino-acid amide (Z-X-$NH_2$) (10 mmoles) was hydrogenated as described in D1 but using 90% aqueous methanol as solvent. The residue, obtained after evaporation, was dried by azeotropic distillation with benzene, collected with ether, and used directly in the next stage of the synthesis. D3 The appropriate *N*-benzyloxycarbonyl-dipeptide benzyl ester amide [Z-Asp(OBZL)-X-$NH_2$] or diester [Z-Asp(OBZL)-X-OEt] (10 mmoles) in 90% aqueous acetic acid (25—50 ml.) was hydrogenated at room temperature and pressure over 5% palladised charcoal (1 g.) until evolution of carbon dioxide ceased (2—5 hr.) and then for an additional 2 hr. After removal of the catalyst (Kieselguhr), the solution was evaporated and the residue was dried by azeotropic distillation with benzene, to yield the acetates which were collected with ether and used directly in the next stage of the synthesis. After drying at 80°/0·1 mm/48 hr. over sodium hydroxide the acetates yielded the corresponding bases; the two analytical samples were so obtained and crystallised from the solvent indicated in Table 2. D4 The appropriate *N*-benzyloxycarbonyl-dipeptide benzyl ester amide [Z-Asp(OBZL)-X-$NH_2$] was hydrogenated as described in D3 but using 90% aqueous methanol as solvent. The residue, obtained after evaporation, was dried by azeotropic distillation with benzene and collected with ether, to yield the product which was used directly in the next stage of the synthesis or after crystallisation from the solvent indicated in Table 2. D5 The appropriate *N*-benzyloxycarbonyl-dipeptide amide benzyl ester [Z-Asp(OBZL)-X-$NH_2$] or diester [Z-Asp(OBZL)-X-OMe] (2·5 mmoles) in acetic acid (25 ml.) was treated with N-hydrochloric acid (2·5 ml.) and the solution was hydrogenated as described in D3. The residue, obtained after evaporation, was dried by azeotropic distillation with benzene and collected with ether, to yield the hydrochloride which was used directly in the next stage of the synthesis. D6 The appropriate *N*-benzyloxycarbonyl-dipeptide amide t-butyl ester [Z-Asp(OBu$^t$)-X-$NH_2$] (5 mmoles) was hydrogenated in 90% aqueous acetic acid at room temperature and pressure over 5% palladised charcoal (0·25 g.) until evolution of carbon dioxide ceased (2—5 hr.). The acetate [H-Asp(OBu$^t$)-Tyr-$NH_2$,AcOH] (100% yield, m. p. 153—155° (decomp.), $R_{FA}$ 0·69] was obtained after evaporation, and the base [H-Asp(OBu$^t$)-Glu(OBu$^t$)-$NH_2$] (95% yield), syrup, $R_{FA}$ 0·87) after evaporation and treatment of the resulting acetate with 50% aqueous potassium carbonate. D7 *N*-Benzyloxycarbonyl-(β-t-butyl)-L-aspartyl-($N^\epsilon$-t-butoxycarbonyl)-L-lysine amide (360 mg., 0·65 mmole) in methanol (150 ml.) was hydrogenated at room temperature and pressure over 5% palladised charcoal (50 mg.) for 4 hr. The filtered (Kieselguhr) solution was evaporated and the residue, $R_{FM}$ 0·75, was used directly in the next stage of the synthesis (see M1).

(e) *Trifluoracetic acid cleavages.* E1 A solution of *N*-t-butoxycarbonyl-*erythro*-DL-β-phenylserine amide (BOC-X-$NH_2$) (50 mg., 0·18 mmole) in 80% aqueous trifluoroacetic acid (2 ml.) was kept at 0° for 1 hr. and then

[14] J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc.* (*C*), 1966, 555.

[15] W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc.* (*C*), 1967, 2632.

[16] J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1609.

evaporated. The trifluoroacetate, obtained after trituration of the residue with ether, was used directly in the next stage of the synthesis. E2 A solution of *N*-t-butoxycarbonyl-(β-t-butyl)-L-aspartyl-*erythro*- or *threo*-D- and -L-β-phenylserine amide [BOC-Asp(OBu$^t$)-X-$NH_2$] (1 mmole) in anhydrous trifluoroacetic acid (5 ml.) was kept at 18—22° for 1 hr. Addition of dry ether gave the trifluoroacetates which were recrystallised from methanol–ethyl acetate. E3 A solution of the appropriate *N*-t-butoxycarbonyl-tripeptide amide (BOC-Met-Asp-X-$NH_2$) (1 mmole) in anhydrous trifluoroacetic acid (1·5—3 ml.) was kept at 20—22° for 1 hr. Addition of dry ether gave the trifluoroacetates which were collected and washed well with ether. L-Methionyl-L-aspartyl-L-alanine amide trifluoroacetate was used directly in the next stage of the synthesis; the analytical sample of the base was obtained by treating an aqueous solution of the trifluoroacetate with ' De-acidite G ' (acetate form) followed by recrystallisation of the product from aqueous acetone. In the other cases, the trifluoroacetate was recrystallised from the solvent indicated in Table 3 before use in the next stage of the synthesis. E4 A solution of the appropriate *N*-benzyloxycarbonyl-tetrapeptide amide t-butyl ester [Z-Trp-Met-Asp(OBu$^t$)-X-$NH_2$] in anhydrous trifluoroacetic acid was kept at 0° for 1 hr. and then evaporated. The products were obtained by trituration of the residue with ether.

(f) *Hydrogen bromide cleavages.* F1 A solution of *N*-t-butoxycarbonyl-*threo*-DL-β-phenylserine amide (BOC-X-$NH_2$) (100 mg., 0·44 mmole) in 5N-hydrogen bromide in acetic acid (0·2 ml.) was kept at 18—22° for 20 min. Dry ether was added and the hydrobromide was collected, washed well with ether, and the used directly in the next stage of the synthesis. F2 A solution of *N*-benzyloxycarbonyl-(β-t-butyl)-L-aspartyl-(*p*-nitro)-L-phenylalanine methyl ester [Z-Asp(OBu$^t$)-X-OMe] (390 mg.) in 5N-hydrogen bromide in acetic acid (2·0 ml.) was kept at 0° for 30 min. The solution was evaporated and the residue was triturated several times with dry ether, to yield the hydrobromide which was used directly in the next stage of the synthesis.

(g) *Preparation of* N-*t-butoxycarbonyl derivatives.* G The slurry obtained by adding an excess of saturated aqueous sodium carbonate to an ice-cold solution of *erythro*-DL-β-phenylserine methyl ester hydrochloride (H-X-OMe,HCl) (2·31 g., 10 mmoles) in the minimum amount of water was extracted with ethyl acetate and the extracts were dried and evaporated. The residue was dissolved in dry pyridine (6 ml.), *t*-butoxycarbonyl azide (2·8 mol., 20 mmoles) was added, and the solution was stirred at 18—22° for 2 days. After evaporation, the resulting residue was dissolved in ethyl acetate and the solution was washed with 10% aqueous citric acid and water, dried, and evaporated. The butoxycarbonyl derivative (1·06 g., 32%) was obtained after recrystallisation of the residue (2 g.) from ethyl acetate–light petroleum (b. p. 60—80°). The *threo*-isomer was similarly obtained as an oil.

(h) *Ammonolyses.* H1 A solution of *N*-t-butoxycarbonyl-*erythro*- or -*threo*-DL-β-phenylserine methyl ester (BOC-X-OMe) (2 mmoles) in methanol (50 ml.) saturated with ammonia was kept at 18—22° for 3 days and then evaporated. The residue was collected with light petroleum (b. p. 60—80°) and crystallised from the solvent indicated in Table 1. H2 A solution of the appropriate *N*-benzyloxycarbonyl- or -butoxycarbonyl-tetrapeptide methyl or ethyl ester (Z- or BOC-Trp-Met-Asp-X-OMe or -OEt) (0·4 mmole) in methanol (40 ml.) saturated with ammonia was kept at 0° for 2 days and then at 18—22° for 2 days. The residue, obtained after evaporation, was dissolved in dimethylformamide (10 ml.) and the pH of the solution was adjusted to 2 at 0° with 2N-hydrochloric acid. Ice–water (30 ml.) was added immediately and the precipitate was collected and recrystallised from the solvent indicated in Table 3. In the case of the butoxycarbonyl-hexahydrophenylalanine analogue [BOC-Trp-Met-Asp-Phe(6H)-$NH_2$] the requiste methyl ester was obtained as an oil by method M2 and ammonolysed directly without purification.

(j) *Esterifications.* J1 Dry hydrogen chloride was passed into a suspension of *erythro*-DL-β-phenylserine hemihydrate or *threo*-DL-β-phenylserine (3 g., 16 mmoles) in dry methanol (25 ml.) until all the solid dissolved. The solution was saturated with dry hydrogen chloride at 0° and then kept at 23—25° for 5 days. The hydrochlorides separated on the addition of dry ether. J2 Freshly distilled thionyl chloride (1·8 ml., 25 mmoles) was added during 10 min. at −10° to dry methanol (25 ml.) then the appropriate amino-acid (20 mmoles) (H-X-OH) was added. The mixture was stirred at −10° for 30 min., warmed during 30 min. until it was refluxing, and then gently boiled under reflux for 4 hr. After evaporation, the resulting residue was collected in ether and crystallised from the solvent indicated in Table 1. J3 The literature preparation [7] from L-tyrosine was followed.

(k) *Carbodi-imide couplings.* K1 Solutions of *N*-methyl-L-phenylalanine amide (1·34 g., 7·5 mmoles) in ethyl acetate (50 ml.), DL-α-phenylalanine amide (0·656 g., 4 mmoles) in acetone (5 ml.) and pyridine (3 ml.), or of methyl (β-cyclohexyl)-L-alaninate hydrochloride (1·08 g., 5 mmoles) and triethylamine (0·7 ml., 5 mmoles) in dimethylformamide (5 ml.) and chloroform (30 ml.) were prepared and treated with β-benzyl *N*-benzyloxycarbonyl-L-aspartate [14] (1 mol.) followed, at 0°, by *NN'*-dicyclohexylcarbodi-imide (1·1 mol.). The mixture was stirred at 4° for 1 day then acetic acid (2—5 drops) was added and stirring was continued at 23—25° for 30 min. After evaporation (and prior removal of dicyclohexylurea if necessary) the resulting residue was shaken with ethyl acetate and the mixture was filtered. The filtrate was washed with N-hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate and water, dried, and evaporated, to yield the products which were recrystallised from the solvent indicated in Table 2 or used directly in the next stage of the synthesis. K2 Solutions of γ-t-butyl L-isoglutaminate acetate [H-Glu(OBu$^t$)$NH_2$,AcOH] (see Table 1) (1·31 g., 5 mmoles) and triethylamine (0·7 ml., 5 mmoles) in chloroform (20 ml.) and ethyl acetate (30 ml.), or of methyl (*p*-nitro)-L-phenylalaninate [from the hydrochloride (750 mg., 2·8 mmoles)] in ethyl acetate (20 ml.) were prepared. β-t-Butyl *N*-benzyloxycarbonyl-L-aspartate [17] (1 mol.) was added and the mixture was treated as described in K1.

(l) *Cleavage with hydrogen chloride in acetic acid.* The appropriate *N*-t-butoxycarbonyl-tetrapeptide amide (BOC-Trp-Met-Asp-X-$NH_2$) (0·5 mmole) and hydrogen chloride (2·5 mmoles) in acetic acid (0·5 ml.) were stirred together at 22—24° for 1 hr. Dry ether was added and the hydrochloride was collected and washed well with ether.

(m) *Azide couplings.* M1 A solution of the appropriate dipeptide amide (H-Asp-X-$NH_2$) (or its salt), dipeptide ester (H-Asp-X-OMe or -OEt) (or its salt), or dipeptide

[17] E. Schröder and E. Klieger, *Annalen*, 1964, **673**, 208.

amide t-butyl ester [H-Asp(OBu$^t$)-X-$NH_2$] (or its salt) (1 mmole) and triethylamine (3,3 or 2 mmoles, respectively) (4,4 or 3 mmoles, respectively when salts were used) in dimethylformamide (4—20 ml.) was treated at 0° with a solution of *N*-benzyloxycarbonyl-L-tryptophyl-L-methionine azide [prepared immediately prior to use from the hydrazide (1 mmole), n-butyl nitrite (0·12 ml.) and hydrogen chloride (2 mmoles) in tetrahydrofuran as previously described [1]]. Water (0·4—2 ml.) was added and the solution was kept at 4° for 2—4 days. The mixture was acidified to pH 2 at 0° with N-hydrochloric acid and then diluted immediately with ice–water (50—150 ml.). The crude product, obtained by filtration or by appropriate extraction procedures, was purified by crystallisation from the solvent indicated in Table 3 and occasionally by preparative t.l.c. The three dipeptide amide t-butyl esters used in these syntheses were (β-t-butyl)-L-aspartyl-L-tyrosine amide acetate [H-Asp(OBu$^t$)-Tyr-$NH_2$, AcOH], (β-t-butyl)-L-aspartyl-(γ-t-butyl)-L-isoglutamine [H-Asp(OBu$^t$)-Glu(OBu$^t$)-$NH_2$], and (β-t-butyl)-L-aspartyl-($N^\epsilon$-t-butoxycarbonyl)-L-lysine amide [H-Asp(OBu$^t$)-Lys(BOC)-$NH_2$]; the preparation of these is described in D6 or D7. M2 The preceding method was followed except that *N*-t-butoxycarbonyl-L-tryptophyl-L-methionyl azide [14] was used.

(n) *Carbamoylations.* N A solution of the appropriate tetrapeptide amide hydrochloride (H-Trp-Met-Asp-X-$NH_2$, HCl) (0·2 mmole) and *N*-methylmorpholine (0·24 ml.) in dimethylformamine (1 ml.) and water (1 ml.) was adjusted to pH 8 with glacial acetic acid, and potassium cyanate (81 mg., 1 mmole) in water (0·5 ml.) was added. The mixture was stirred at 23—25° for 2 days and then acidified with hydrochloric acid and diluted with ice–water (25 ml.). The solid was collected, washed with water and crystallised from aqueous dimethylformamide.

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