Polypeptides. Part XIII.[1] Peptides related to the *C*-Terminal Tetrapeptide Sequence of the Gastrins by Complementary Reading of the Genetic Message

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Messenger RNA sequences which will code for the *C*-terminal tetrapeptide sequence (L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine) of the gastrins have been deduced. By pairing of the nucleotide bases in the conventional ' Watson–Crick ' manner in both the antiparallel and the parallel direction of reading, complementary nucleotide sequences have been obtained. The syntheses are described of derivatives of the five tetrapeptides, L-glutamyl-L-isoleucyl-L-histidyl-L-proline, L-lysyl-L-isoleucyl-L-histidyl-L-proline, L-glutamyl-L-valyl-L-histidyl-L-proline, L-lysyl-L-valyl-L-histidyl-L-proline, and L-threonyl-L-tyrosyl-L-leucyl-L-lysine, whose sequences are coded by the complementary messenger nucleotide sequences.

DURING recent years many analogues of the *C*-terminal tetrapeptide amide sequence, Trp-Met-Asp-Phe-NH_2,‡ of the gastrins have been synthesised [2] in order to gain knowledge of (*a*) the relationship between the structure of the molecule and its principal biological activity (the stimulation of gastric acid secretion), and (*b*) the structural requirements of peptide inhibitors of gastric acid secretions.[3] The major part of this work has

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‡ Abbreviations for amino-acids and peptides follow the rules in I.U.P.A.C. Information Bulletin No. 26; OCp = 2,4,5-trichlorophenoxy.

[1] Part XII, A. S. Dutta and J. S. Morley, *J. Chem. Soc.* (*C*), 1971, 2896.

[2] Parts VI—XI [see Part X, H. Gregory and J. S. Morley, *J. Chem. Soc.* (*C*), 1968, 910, for references to the earlier parts].

[3] J. S. Morley, *Fed. Proc.*, 1968, **27**, 1314.

involved single amino-acid replacements in the tetrapeptide sequence by naturally occurring or unnatural synthetic amino-acids.

In the present investigation, another approach to the problem has been made. Derivatives of peptides have been synthesised which might be expected to arise *in vivo* if the protein biosynthesis is directed by the strand of the double-stranded DNA molecule complementary to the strand which is normally utilised. That the gastrins are synthesised *in vivo* by the normal protein biosynthetic pathway is indicated by the fact that amino-acid replacements found in the sequences of gastrin obtained from different species can be accounted for by single-base changes in the genetic codons.[4] Also recent work by Yalow and Berson [5] shows the existence of ' Big Gastrin,' a polypeptide having a molecular weight of about 7000 and the same physiological properties as gastrin, and from which a smaller gastrin-like peptide is released on digestion with trypsin. This larger polypeptide may be a gastrin precursor protein.

Normally, *in vivo*, only one strand of the double-stranded DNA molecule directs the RNA polymerase-catalysed synthesis of messenger RNA; however, *in vitro*, both strands of the DNA can be copied.[6] Recently, Sugiura, Okamoto, and Takanami [7] have shown that RNA polymerase lacking σ-factor can transcribe the replicative form of phage DNA, but the ability to select the appropriate initiation sites on the template is lost and both strands are transcribed. Burch and Burwell [8] have proposed that in idiopathic auto-immunity ' the mutational events that give rise to auto-antibodies involve a spontaneous switch in the messenger-RNA transcription from the regular strand of the DNA over to the complementary, base-paired, anti-parallel strand of the Watson–Crick double-helix.'

Since the *C*-terminal tetrapeptide of the gastrins is one of the smallest peptides possessing such marked biological activity, it seemed worthwhile investigating whether peptides which would be predicted to be synthesised under the direction of the complementary DNA strand possess biological properties related to those of the gastrin tetrapeptide. More precisely, this investigation has sought to determine whether peptide analogues having this unique relationship to the gastrin tetrapeptide are able to interact in the whole animal with, for example, gastrin synthesis, gastrin release, or with the gastrin molecule itself such that the effect may be observed by inhibition or stimulation of the gastrin activities.

Determination of the ' complementary ' peptide sequence requires first the building of the messenger RNA for the gastrin *C*-terminal tetrapeptide by placing the genetic codons (nucleotide triplets) in order from the 5′- to the 3′-terminus related to the amino-acids of the peptide reading from the *N*- to the *C*-terminus. Because, however, of the degeneracy [9] of the genetic code, four messenger RNA sequences can be written. The codons for the amino-acids of the gastrin *C*-terminal tetrapeptide Trp-Met-Asp-Phe-NH_2 are Trp, UGG; Met, AUG; Asp, GAU and GAC; and Phe, UUU and UUC.* (Amidation of the tetrapeptide was not considered to be involved in the ' translation ' step of protein biosynthesis.) The four possible messenger RNA sequences therefore follow as:

5′	UGG	AUG	GAU	UUU,	3′
or	UGG	AUG	GAU	UUC,	
or	UGG	AUG	GAC	UUU,	
or	UGG	AUG	GAC	UUC.	

The complementary nucleotide sequences obtained by Watson–Crick [10] base-pairing in an antiparallel direction of reading are, respectively:

5′	AAA	AUC	CAU	CCA,	3′
or	GAA	AUC	CAU	CCA,	
or	AAA	GUC	CAU	CCA,	
or	GAA	GUC	CAU	CCA	

which on translation lead to the peptides:

	Lys-Ile-His-Pro
or	Glu-Ile-His-Pro
or	Lys-Val-His-Pro
or	Glu-Val-His-Pro

The peptide sequences which would arise by parallel reading of the base pairs were also determined. This parallel reading leads to the following nucleotide sequences:

5′	ACC	UAC	CUA	AAA	3′
or	ACC	UAC	CUA	AAG	
or	ACC	UAC	CUG	AAA	
or	ACC	UAC	CUG	AAG	

Translation of each of these messenger RNA sequences leads to the one tetrapeptide Thr-Tyr-Leu-Lys.

* Abbreviations (one-letter) for nucleic acids *etc.* follow the I.U.P.A.C.–I.U.B. recommendations (*European J. Biochem.*, 1970, **15**, 203).

[4] J. Beacham, P. H. Bentley, G. W. Kenner, J. J. Mendive, and R. C. Sheppard, Proceedings of the Eighth European Peptide Symposium, Noordwijk-on-Sea, The Netherlands, September 1966, 1967, North Holland Publishing Co., Amsterdam, p. 235.

[5] R. S. Yalow and S. A. Berson, *Gastroenterology*, 1970, **58**, 609.

[6] M. F. Singer and P. Leder, *Ann. Rev. Biochem.*, 1966, **35**, 195.

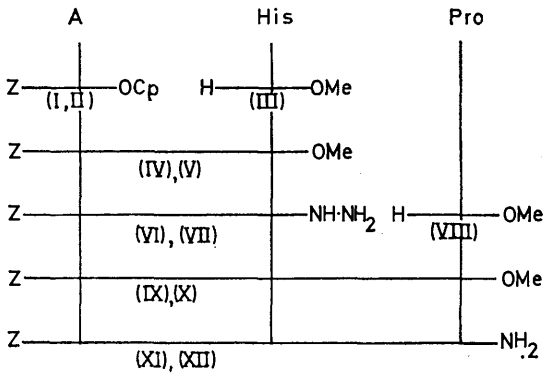
[7] M. Sugiura, T. Okamoto, and M. Takanami, *Nature*, 1970, **225**, 598.

[8] P. R. J. Burch and R. G. Burwell, *Lancet*, 1966, 767.

[9] For an explanation of the term ' degeneracy ' see F. H. C. Crick, *Progr. Nucleic Acid Res.*, 1963, **1**, 163.

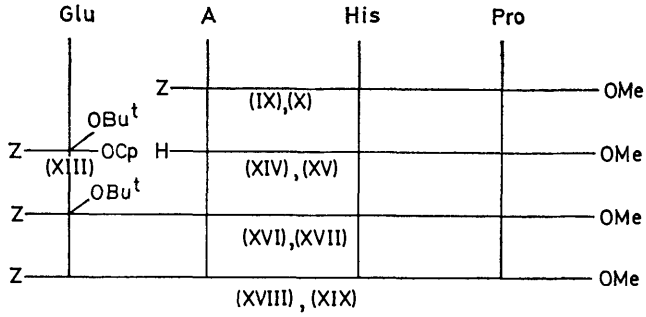
[10] J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737, 964.

The methods of synthesis used to prepare the ' complementary ' peptides derived from antiparallel reading of



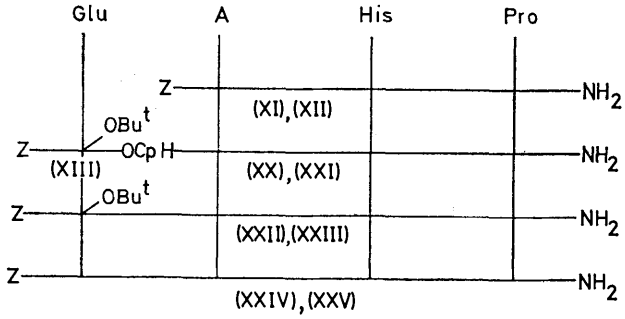
(I),(IV),(VI),(IX),(XI) : A = Ile
(II),(V),(VIII),(X),(XII) : A = Val

SCHEME 1



(IX),(XIV),(XVI),(XVIII) : A = Ile
(X),(XV),(XVII),(XIX) : A = Val

SCHEME 2



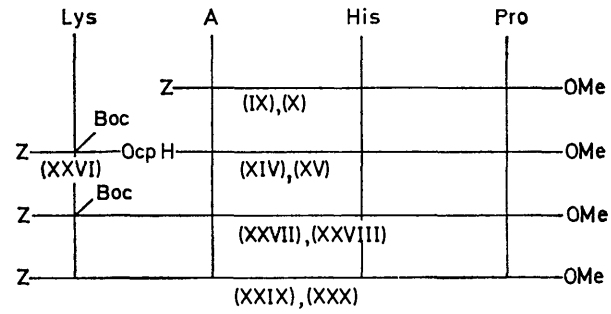
(XI),(XX),(XXII),(XXIV) : A = Ile
(XII),(XXI),(XXIII),(XXV) : A = Val

SCHEME 3

the base-paired nucleotide sequences are outlined in Schemes 1—5.

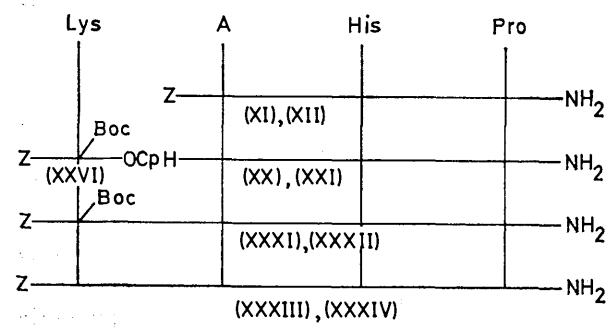
[11] H. Schwartz, F. M. Humpus, and I. H. Page, *J. Amer. Chem. Soc.*, 1957, **79**, 5697.
[12] W. Rittel, B. Iselin, H. Kappeler, B. Riniker, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 614.

The dipeptide derivatives (IV)—(VII) [11-14] and the tripeptide derivative (X) [15] have been described previously. Literature methods were used for their preparation except for (IV) and (V) which were conveniently obtained by the trichlorophenyl ester method (Scheme 1). *N*-Benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline methyl ester (IX) was prepared by a procedure similar to that described for the valyl derivative (X).[15] The tripeptide amide derivatives (XI) and (XII) were obtained in high yield by treatment of the methyl ester derivatives (IX) and (X) with methanolic ammonia. Synthesis of the tetrapeptide derivatives (XVI), (XVII), (XXII), (XXIII), (XXVII), (XXVIII), (XXXI), and (XXXII) was carried out successfully by the trichloro-



(IX),(XIV),(XXVII),(XXIX) : A = Ile
(X),(XV),(XXVIII),(XXX) : A = Val

SCHEME 4



(XI),(XX),(XXXI),(XXXIII) : A = Ile
(XII),(XXI),(XXXII),(XXXIV) : A = Val

SCHEME 5

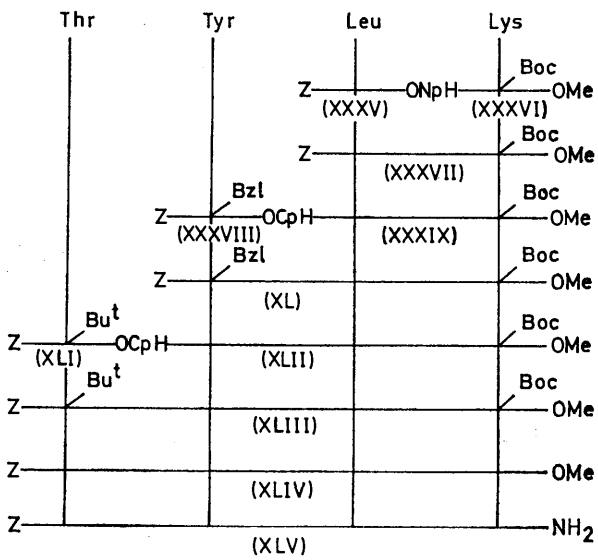
phenyl active ester method in all cases (Schemes 2—5). The benzyloxycarbonyl groups were removed quantitatively from the tripeptide derivatives (IX)—(XII) by catalytic hydrogenolysis in neutral solution and the free tripeptide esters (XIV) and (XV) or amides (XX) and (XXI) were immediately coupled with the protected

[13] R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 1958, **41**, 1273.
[14] R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 1958, **41**, 1287.
[15] St. Guttmann, *Helv. Chim. Acta*, 1961, **44**, 721.

amino-acid active ester derivatives (XIII) or (XXVI). Lower yields of two of the tetrapeptide derivatives (XVI) and (XXVII) were obtained when the pivalic acid mixed anhydride method of coupling was used. Removal of the side-chain protecting groups from the tetrapeptide derivatives with trifluoroacetic acid gave, in high yield in all cases, salts of the *N*-benzyloxycarbonyltetrapeptide methyl esters or amides (XVIII), (XIX), (XXIV), (XXV), (XXIX), (XXX), (XXXIII), and (XXXIV).

N-Benzyloxycarbonyl-L-threonyl-L-tyrosyl-L-leucyl-L-lysine methyl ester (XLIV) and the amide (XLV), derivatives of the 'complementary' peptide, derived from parallel reading of the base-paired nucleotide sequences, were synthesised in a stepwise fashion as shown in Scheme 6.

The fully protected tetrapeptide derivative (XLIII) was built up in a stepwise fashion by the active ester coupling method. *N*-Benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester (XXXV) was used in the preparation of the dipeptide derivative (XXXVII), but the trichlorophenyl esters (XXXVIII) and (XLI) were used in the syntheses of the tripeptide and tetrapeptide derivatives (XL) and (XLIII), respectively. The *N*-benzyloxycarbonyl protecting group of the dipeptide derivative



SCHEME 6

(XXXVII) and both the *N*-benzyloxycarbonyl and *O*-benzyl protecting groups of the tripeptide derivative (XL) were removed by catalytic hydrogenolysis in aqueous acetic acid.

Treatment of the fully protected tetrapeptide derivative (XLIII) with aqueous trifluoroacetic acid under nitrogen yielded *N*-benzyloxycarbonyl-L-threonyl-L-tyrosyl-L-leucyl-L-lysine methyl ester (XLIV), which was converted into the amide (XLV) with methanolic ammonia.

Investigation of the action of the tetrapeptide derivatives (XVIII), (XIX), (XXIX), (XXX), (XLIV), and (XLV) in anaesthetised rat or cat preparations showed no stimulation of gastric acid secretion. These compounds were also devoid of inhibitory action on secretion stimulated by the injection of t-butoxycarbonyl-β-alanyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (pentagastrin, Peptavlon,* I.C.I. 50,123) in similar animal preparations.

These results indicate that there is no interaction of these peptides with the receptor site at which gastrin stimulates the release of acid.

However, in experiments using conscious dogs with innervated gastric pouches stimulated to secrete by a test meal, some inhibition of gastric juice volume and acid and pepsin output has been observed with the tetrapeptide amide (XXXIII), but not with the tetrapeptide amides (XXIV), (XXV), and (XXXIV) or the tetrapeptide esters (XVIII), (XIX), (XXIX), and (XLIV).

The effect was seen after administration of the tetrapeptide (XXXIII) for periods of 3—8 days.[16]

These latter experiments were designed to demonstrate whether or not these analogues interfere with gastrin production. An explanation for an inhibitory effect being observed for one analogue only can be proposed on the assumption that the actual message used for gastrin tetrapeptide translation is UGG AUG GAU UUU. Then there is only one complementary messenger RNA for which only one peptide, Lys-Ile-His-Pro, is coded.

It is appreciated that more than this one positive biological result is required before any firm predications are made concerning the biological significance, if any, of such peptide analogues.

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–ammonium hydroxide (3%) (3 : 1) (R_{FC}), acetonitrile–water (3 : 1) (R_{FD}), acetone–chloroform (1 : 1) (R_{FE}), or ethanol–chloroform (4 : 1) (R_{FF}). Spots were revealed with ninhydrin, sodium hypochlorite–potassium iodide–tolidine (or starch),[17] or acid potassium permanganate [potassium permanganate (100 mg) dissolved in concentrated sulphuric acid (1 ml); solution diluted to 100 ml with water]. Acid hydrolysates of peptide derivatives were prepared by use of 6N-hydrochloric acid (105—110° for 16 h), and the amino-acid composition of the hydrolysates was determined with a Beckmann–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,

* Peptavlon is a registered trade mark of Imperial Chemical Industries Ltd. for pentagastrin, which is the subject of U.K. Pat. 1,042,487 (Imperial Chemical Industries Ltd.).

[16] E. L. Gerring, unpublished work.

[17] S. C. Pan and J. D. Dutcher, *Analyt. Chem.*, 1956, **28**, 836.

and evaporations were carried out under reduced pressure in a rotary evaporator. M.p.s were determined for samples in capillary tubes with a Tottoli apparatus (manufactured by W. Buchi).

Starting Materials.—The following were prepared by literature methods: L-proline methyl ester (Pro-OMe),[15] L-histidine methyl ester dihydrochloride (His-OMe,2HCl),[18] *N*-benzyloxycarbonyl-L-valine 2,4,5-trichlorophenyl ester (Z-Val-OCp),[19] *N*-benzyloxycarbonyl-L-isoleucine 2,4,5-trichlorophenyl ester (Z-Ile-OCp),[19] γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate dicyclohexylammonium salt [Z-Glu(OBu^t)-OH,DCha],[20] α-(2,4,5-trichlorophenyl) γ-t-butyl *N*-benzyloxycarbonyl-L-glutamate [Z-Glu(OBu^t)-OCp],[20] N^{α}-benzyloxycarbonyl-N^{ε}-t-butoxycarbonyl-L-lysine [Z-Lys(Boc)-OH)],[21] N^{α}-benzyloxycarbonyl-N^{ε}-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester [Z-Lys(Boc)-OCp],[21] N^{ε}-t-butoxycarbonyl-L-lysine methyl ester hydrochloride [Lys(Boc)-OMe,HCl],[22] *N*-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester (Z-Leu-ONp),[23] *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine 2,4,5-trichlorophenyl ester [Z-Tyr(Bzl)-OCp],[19] and *N*-benzyloxycarbonyl-*O*-t-butyl-L-threonine dicyclohexylammonium salt [Z-Thr(Bu^t)-OH,DCha].[24]

N-Benzyloxycarbonyl-L-isoleucyl-L-histidine Methyl Ester.—To a stirred suspension of *N*-benzyloxycarbonyl-L-isoleucine 2,4,5-trichlorophenyl ester (20 g, 45·2 mmol) and L-histidine methyl ester dihydrochloride (10·9 g, 45·2 mmol) in dimethylformamide (100 ml) at room temperature triethylamine (18·9 ml, 0·136 mol) was added slowly. The mixture was stirred at room temperature for 3 days and then added to ice–water (1 l). The resulting solid was dissolved in warm ethyl acetate (2 l) and the filtered solution was washed with aqueous 0·5N-sodium hydroxide (150 ml) and water (2 × 150 ml), dried ($MgSO_4$), and evaporated to a small volume. The dipeptide derivative (16 g, 86%) separated as a gelatinous solid, which after two recrystallisations from isopropyl alcohol–methanol–water had m.p. 181—183°, R_{FA} 0·50, R_{FB} 0·80, R_{FC} 0·70, R_{FE} 0·18, R_{FF} 0·73, $[\alpha]_D^{26}$ −44·7 ± 0·5° [*c* 0·995 in MeOH–N-HCl (1 : 1)] {lit.,[10] m.p. 186—189°, $[\alpha]_D^{25}$ −44·2° [*c* 1·0 in MeOH–N-HCl (1 : 1)]} (Found: C, 60·6; H, 7·0; N, 13·3. Calc. for $C_{21}H_{28}N_4O_5$: C, 60·6; H, 6·7; N, 13·5%).

N-Benzyloxycarbonyl-L-valyl-L-histidine Methyl Ester.—To a stirred suspension of *N*-benzyloxycarbonyl-L-valine 2,4,5-trichlorophenyl ester (20 g, 46·4 mmol) and L-histidine methyl ester dihydrochloride (11·2 g, 46·4 mmol) in dimethylformamide (100 ml), at room temperature, triethylamine (19·5 ml, 0·14 mol) was added slowly. The mixture was stirred at room temperature for 3 days and then added to ice–water (1 l). The solid was dissolved in warm ethyl acetate (1·5 l) and the solution was washed with aqueous 0·5N-sodium hydroxide (150 ml) and water (2 × 150 ml), dried ($MgSO_4$), and evaporated to a gelatinous solid. After drying, recrystallisation from ethyl alcohol afforded the dipeptide derivative (15·0 g, 80%), m.p. 163—166°, R_{FA} 0·49, R_{FB} 0·79, R_{FC} 0·71, R_{FE} 0·14, R_{FF} 0·70, $[\alpha]_D^{23·5}$ −19·2 ± 0·5° (*c* 1·9 in EtOH), {lit.,[10] m.p. 165—166°, $[\alpha]_D^{23}$ −22 ± 2° (*c* 2·04 in EtOH)} (Found: C, 59·4; H, 6·3; N, 14·0. Calc. for $C_{20}H_{26}N_4O_5$: C, 59·7; H, 6·5; N, 13·9%).

N-Benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline Methyl Ester.—To a vigorously stirred mixture of *N*-benzyloxycarbonyl-L-isoleucyl-L-histidine hydrazide[12] (6·03 g, 14·5 mmol) in N-hydrochloric acid (60 ml) and ethyl acetate (90 ml) at −5°, sodium nitrite (1·05 g, 15 mmol) was added in three portions at 30 s intervals. After a further 5 min aqueous 50% potassium carbonate (18 ml) was added, and the phases were separated. The aqueous layer was extracted with ethyl acetate (2 × 30 ml) and the extracts were dried (Na_2SO_4) and then added to L-proline methyl ester (2·02 g, 15·75 mmol) at 0°. The solution was kept at 2° for 2 days and at room temperature for 5 days. It was then washed with water (5 × 20 ml) and aqueous 5% sodium hydrogen carbonate (40 ml), dried ($MgSO_4$), and evaporated to give an oil. The *tripeptide derivative* (5·95 g, 77%), m.p. 85—90°, R_{FA} 0·41, R_{FB} 0·70, R_{FC} 0·70, R_{FE} 0·07, R_{FF} 0·68, $[\alpha]_D^{30}$ −45·2 ± 0·5° (*c* 1·48 in EtOAc), was obtained as an amorphous solid by adding ether to a solution of the oil in ethyl acetate (Found: C, 60·3; H, 6·7; N, 13·3. $C_{26}H_{35}N_5O_6$ requires C, 60·7; H, 6·8; N, 13·6%).

N-Benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline Amide.—A solution of *N*-benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline methyl ester (2·0 g, 3·9 mmol) in dry methanol (50 ml) saturated with ammonia was kept in a tightly stoppered flask at 4° for 10 days and then evaporated. Addition of ether–light petroleum (b.p. 60—80°) to a solution of the residue in ethyl acetate precipitated the *amide hemihydrate* as a white amorphous solid (1·9 g, 96%,) m.p. 118—120°, R_{FA} 0·51, R_{FB} 0·82, R_{FC} 0·78, R_{FE} 0·09, R_{FF} 0·46, $[\alpha]_D^{27}$ −30·9° ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 59·0; H, 6·7; N, 16·4. $C_{25}H_{34}N_6O_5,0·5H_2O$ requires C, 59·2; H, 6·95; N, 16·6%).

N-Benzyloxycarbonyl-L-valyl-L-histidyl-L-proline Amide.—A solution of *N*-benzyloxycarbonyl-L-valyl-L-histidyl-L-proline methyl ester[15] (2 g, 4·0 mmol) in dry methanol (50 ml) saturated with ammonia was kept in a tightly stoppered flask at 4° for 10 days and then evaporated. Addition of ether–light petroleum (b.p. 60—80°) to a solution of the residue in ethyl acetate precipitated the *amide hydrate* as a white amorphous solid (1·89, 95%), m.p. 124—125°, R_{FA} 0·47, F_{FB} 0·84, R_{FC} 0·87, R_{FE} 0·09, R_{FF} 0·44, $[\alpha]_D^{27}$ −27·0 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 58·0; H, 6·5; N, 16·6. $C_{24}H_{32}N_6O_5,H_2O$ requires C, 57·5; H, 6·8; N, 16·7%).

L-Isoleucyl-L-histidyl-L-proline Methyl Ester.—A solution of *N*-benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline methyl ester (6·0 g, 11·7 mmol) in methanol (99 ml) and water (11 ml) was hydrogenolysed at room temperature and pressure over 5% palladium–charcoal (1·5 g) for 3—5 h, and after the addition of a further 0·5 g of catalyst for a further 45 min. After removal of the catalyst, the filtrate was evaporated yielding an *oil* (4·7 g, 105%), which was used without further purification in the coupling reactions at the next stage.

L-Valyl-L-histidyl-L-proline Methyl Ester.—A solution of *N*-benzyloxycarbonyl-L-valyl-L-histidyl-L-proline methyl ester[15] (10·0 g, 20 mmol) in methanol (90 ml) and water (10 ml) was hydrogenolysed at room temperature and

[18] E. Fischer and L. H. Cone, *Annalen*, 1908, **363**, 107.
[19] J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1609.
[20] J. S. Morley, *J. Chem. Soc.* (*C*), 1967, 2410.
[21] W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc.* (*C*), 1967, 2632.
[22] R. Schwyzer and W. Rittel, *Helv. Chim. Acta*, 1961, **44**, 159.
[23] M. Bodansky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.
[24] E. Schröder, *Annalen*, 1963, **670**, 127.

pressure over 5% palladium–charcoal (1·5 g) for 3 h, and after addition of a further 0·5 g of catalyst for a further 1 h. After removal of the catalyst the solution was evaporated yielding an *oil*, R_{FA} 0·31, R_{FB} 0·50, R_{FC} 0·46, which was used without further purification in the coupling reactions at the next stage.

L-*Isoleucyl*-L-*histidyl*-L-*proline Amide*.—A solution of *N*-benzyloxycarbonyl-L-isoleucyl-L-histidyl-L-proline amide (1·75 g, 3·5 mmol) in methanol (90 ml) and water (10 ml) was hydrogenolysed at room temperature and pressure over 5% palladium–charcoal (400 mg), for 1·5 h, and after addition of a further 100 mg of catalyst for a further 1 h. After removal of the catalyst, the solution was evaporated, yielding an *oil*, R_{FA} 0·14, R_{FB} 0·62, R_{FB} 0·30, R_{FE} 0·0, R_{FF} 0·09, which was used without further purification in the coupling reactions at the next stage.

L-*Valyl*-L-*histidyl*-L-*proline Amide*.—A solution of *N*-benzyloxycarbonyl-L-valyl-L-histidyl-L-proline amide (1·75 g, 3·62 mmol) in methanol (90 ml) and water (10 ml) was hydrogenolysed at room temperature and pressure over 5% palladium–charcoal (400 mg), for 1·5 h, and after addition of a further 100 mg of catalyst for a further 1 h. After removal of the catalyst, the solution was evaporated yielding an *oil*, R_{FA} 0·1, R_{FB} 0·55, R_{FC} 0·23, R_{FE} 0·0, R_{FF} 0·06, which was used without further purification in the coupling reactions at the next stage.

N-*Benzyloxycarbonyl*-(γ-*t*-*butyl*)-L-*glutamyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Methyl Ester*.—(*a*) α-(2,4,5-Trichlorophenyl) γ-t-butyl-*N*-benzyloxycarbonyl-L-glutamate (3·0 g, 6 mmol) was dissolved in dimethylformamide (25 ml) containing L-isoleucyl-L-histidyl-L-proline methyl ester (5·9 mmol) at 0°, and the solution was kept at 2° for 2 days and at room temperature for 1 day. The gummy solid precipitated by adding the mixture to ice–water (250 ml) was extracted into ethyl acetate (1 × 150 ml; 2 × 100 ml) and the extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil. Addition of light petroleum (b.p. 60—80°) to an ethyl acetate solution of the oil yielded the *tetrapeptide derivative* as an amorphous white solid (3·22 g, 78%), m.p. 149—152°, R_{FB} 0·58, R_{FC} 0·74, R_{FE} 0·05, R_{FF} 0·66, $[\alpha]_D^{29}$ −69·3 ± 0·5° (*c* 1·17 in MeOH), amino-acid ratios in acid hydrolysate: Glu 1·02, Ile 0·96, His 1·01, Pro 1·01 (Found: C, 59·2; H, 7·5; N, 12·0. $C_{35}H_{50}N_6O_9$ requires C, 60·1; H, 7·2; N, 12·0).

(*b*) γ-t-Butyl-*N*-benzyloxycarbonyl-L-glutamic acid [from dicyclohexylammonium salt (1 mmol)] was dried by azeotropic distillation with toluene, then converted into a mixed anhydride with triethylamine (0·14 ml, 1 mmol) and pivaloyl chloride (0·12 ml, 1 mmol) in toluene (3 ml) at −5°. L-Isoleucyl-L-histidyl-L-proline methyl ester (1 mmol) in dioxan (3·4 ml) was added and the mixture was kept at room temperature for 19 h and at 60° for 3 h. Ice–water (100 ml) was then added and the oil was extracted into ethyl acetate (3 × 100 ml). Evaporation of the water-washed and then dried ($MgSO_4$) extracts gave the *tetrapeptide derivative* as an amorphous solid (from ethyl acetate–ether) (0·45 g, 59%), m.p. 150—152°, R_{FC} 0·77, R_{FE} 0·05, R_{FF} 0·70.

N-*Benzyloxycarbonyl*-(γ-*t*-*butyl*)-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*proline Methyl Ester*.—α-(2,4,5-Trichlorophenyl) γ-t-butyl-*N*-benzyloxycarbonyl-L-glutamate (0·517 g, 1 mmol) was dissolved in dimethylformamide (4·3 ml) containing L-valyl-L-proline methyl ester (1 mmol) at 0°. After 6 days at 2° and 1 day at room temperature the solution was poured into ice–water (50 ml), yielding an oil which was extracted into ethyl acetate (3 × 100 ml). The extracts were washed with water, dried ($MgSO_4$), and evaporated. Addition of light petroleum (b.p. 60—80°) to a solution of the residue in ethyl acetate gave the *tetrapeptide derivative* as an amorphous solid (0·64 g, 91%), R_{FA} 0·4, R_{FB} 0·67, R_{FC} 0·73, R_{FE} 0·1, R_{FF} 0·59, $[\alpha]_D^{28·5}$ −60·6 ± 0·5° (*c* 1·55 in MeOH), amino-acid ratios in acid hydrolysate: Glu 0·97, Val 1·04, His 1·0, Pro 0·99 (Found: C, 58·3; H, 6·95; N, 12·1. $C_{34}H_{48}N_6O_9,H_2O$ requires C, 58·2; H, 7·2; N, 11·95%).

N-*Benzyloxycarbonyl*-(γ-*t*-*butyl*)-L-*glutamyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Amide*.—α-(2,4,5-Trichlorophenyl) γ-t-butyl-*N*-benzyloxycarbonyl-L-glutamate (1·03 g, 2 mmol) was dissolved in dimethylformamide (8 ml) containing L-isoleucyl-L-histidyl-L-proline amide (1·75 mmol) and the solution was kept at 4° overnight and at room temperature for 5 days. The solid precipitated by adding the dimethylformamide solution to ice–water (30 ml) was extracted into ethyl acetate (4 × 50 ml) and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil. Addition of a solution of the oil in ethyl acetate to ether at 0° yielded the *tetrapeptide derivative* as an amorphous solid (1·02 g, 87%), m.p. 120—124°, R_{FA} 0·48, R_{FB} 0·71, R_{FC} 0·66, R_{FE} 0·0, R_{FF} 0·53, $[\alpha]_D^{27}$ −33·6 ± 5° (*c* 1·0 in Me_2N·CHO) (Found: C, 59·0; H, 7·2; N, 13·7. $C_{34}H_{49}N_7O_8,0·5H_2O$ requires C, 58·9; H, 7·3; N, 14·1%).

N-*Benzyloxycarbonyl*-(γ-*t*-*butyl*)-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*proline Amide*.—α-(2,4,5-Trichlorophenyl) γ-t-butyl-*N*-benzyloxycarbonyl-L-glutamate (1·03 g, 2 mmol) was dissolved in dimethylformamide (8 ml) containing L-valyl-L-histidyl-L-proline amide (1·81 mmol), and the solution was kept at 4° overnight and at room temperature for 5 days. The solid precipitated by adding the dimethylformamide solution to ice–water (30 ml) was extracted into ethyl acetate (4 × 50 ml), and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil. Addition of a solution of the oil in ethyl acetate to ether at 0° yielded the *tetrapeptide derivative* as an amorphous solid (1·02 g, 84%), m.p. 122—124°, R_{FA} 0·49, R_{FB} 0·71, R_{FC} 0·66, R_{FE} 0·0, R_{FF} 0·53, $[\alpha]_D^{27}$ −26·8 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 58·4; H, 7·0; N, 14·0. $C_{33}H_{47}N_7O_8,0·5H_2O$ requires C, 58·5; H, 7·1; N, 14·4%).

N^{α}-*Benzyloxycarbonyl*-(N^{ε}-*t*-*butoxycarbonyl*)-L-*lysyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Methyl Ester*.—(*a*) To a stirred solution of N^{α}-benzyloxycarbonyl-N^{ε}-butoxycarbonyl-L-lysine (0·38 g, 1 mmol) in toluene (3 ml) at −5°, triethylamine (0·14 ml, 1 mmol) was added, followed by pivaloyl chloride (0·12 ml, 1 mmol). L-Isoleucyl-L-histidyl-L-proline methyl ester (1 mmol) in dioxan (3·4 ml) was then added and the solution was kept at room temperature for 19 h and at 60° for 3 h. After the addition of ice–water (100 ml), the resulting oil was extracted into ethyl acetate (3 × 100 ml) and the extracts were washed with water, dried ($MgSO_4$), and evaporated to give the *tetrapeptide derivative* (0·54 g, 73%), as an amorphous solid [from ethyl acetate–light petroleum (b.p. 60—80°)], R_{FB} 0·78, R_{FC} 0·82, R_{FE} 0·03, R_{FF} 0·73, $[\alpha]_D^{30}$ −60·0 ± 0·5° (*c* 1·0 in MeOH), amino-acid ratios in acid hydrolysate: Lys 1·06, Ile 0·92, His 0·98, Pro 1·04 (Found: C, 58·8; H, 7·4; N, 12·8. $C_{37}H_{55}N_7O_9,H_2O$ requires C, 58·7; H, 7·5; N, 12·9%).

(*b*) N^{α}-Benzyloxycarbonyl-N^{ε}-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (3·37 g, 6 mmol) was dissolved in dimethylformamide (25 ml) containing L-isoleucyl-L-histidyl-L-proline methyl ester (5·9 mmol) at 0°. After

2 days at 2° and 1 day at room temperature the solution was poured into ice–water (250 ml). The gummy solid was extracted into ethyl acetate (1 × 150 ml; 2 × 100 ml) and the extracts were washed with water and dried ($MgSO_4$). Addition of ether–light petroleum (b.p. 60—80°) to the ethyl acetate solution yielded the *tetrapeptide derivative* as an amorphous solid (3·55 g, 81%), R_{FE} 0·05, R_{FF} 0·67, amino-acid ratios in acid hydrolysate, Lys 1·05, Ile 0·96, His 0·99, Pro 0·99.

N$^{\alpha}$-*Benzyloxycarbonyl*-(N$^{\varepsilon}$-*t-butoxycarbonyl*)-L-*lysyl*-L-*valyl*-L-*histidyl*-L-*proline Methyl Ester*.—*N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\varepsilon}$-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (0·56 g, 1 mmol) was dissolved in dimethylformamide (4·3 ml) containing L-valyl-L-histidyl-L-proline methyl ester (1 mmol) at 0°. After 6 days at 2° and 1 day at room temperature, the solution was poured into ice–water (100 ml), and the oil which separated was extracted into ethyl acetate (3 × 100 ml). Addition of light petroleum (b.p. 60—80°) to the combined, washed (H_2O), and dried ($MgSO_4$) extracts yielded the *tetrapeptide derivative* as an amorphous solid (0·66 g, 91%), R_{FA} 0·4, R_{FB} 0·69, R_{FC} 0·72, R_{FE} 0·14, R_{FF} 0·60, $[\alpha]_D^{28}$ −60·8 ± 0·5° (*c* 1·00 in MeOH), amino-acid ratios in acid hydrolysate: Lys 1·06, Val 1·01, His 0·96, Pro 1·01 (Found: C, 58·0; H, 7·3; N, 13·3. $C_{36}H_{53}N_7O_9,H_2O$ requires C, 58·0; H, 7·4; N, 13·1%).

N$^{\alpha}$-*Benzyloxycarbonyl*-(N$^{\varepsilon}$-*t-butoxycarbonyl*)-L-*lysyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Amide*.—*N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\varepsilon}$-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (1·12 g, 2 mmol) was added to a solution of L-isoleucyl-L-histidyl-L-proline amide (1·75 mmol) in dimethylformamide (8 ml) at 0°. The clear solution was kept at 4° overnight and at room temperature for 5 days, and then poured into ice–water (30 ml). The precipitated solid was extracted into ethyl acetate (4 × 50 ml) and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil. Addition of a solution of the oil in ethyl acetate to ether at 0° yielded the *tetrapeptide derivative* as an amorphous solid (1·22 g, 96%), m.p. 120—124°, R_{FA} 0·48, R_{FB} 0·71, R_{FC} 0·66, R_{FE} 0·0, R_{FF} 0·53, $[\alpha]_D^{24}$ −32·4 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 58·9; H, 7·5; N, 14·9. $C_{36}H_{54}N_8O_8,0{\cdot}5H_2O$ requires C, 58·8; H, 7·5; N, 15·2%).

N$^{\alpha}$-*Benzyloxycarbonyl*-(N$^{\varepsilon}$-*t-butoxycarbonyl*)-L-*lysyl*-L-*valyl*-L-*histidyl*-L-*proline Amide*.—*N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\varepsilon}$-t-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (1·12 g, 2 mmol) was added to a solution of L-valyl-L-histidyl-L-proline amide (1·81 mmol) in dimethylformamide (8 ml) at 0°. The clear solution was kept at 4° overnight and at room temperature for 5 days, and then poured into ice–water (30 ml). The precipitated solid was extracted into ethyl acetate (4 × 50 ml), and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil. Addition of a solution of the oil in ethyl acetate to ether at 0° yielded the *tetrapeptide derivative* as an amorphous solid (1·13 g, 88%), m.p. 122—124°, R_{FA} 0·50, R_{FB} 0·71, R_{FC} 0·66, R_{FE} 0·0, R_{FF} 0·53, $[\alpha]_D^{24}$ −29·8 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 58·1; H, 7·3; N, 15·1. $C_{35}H_{52}N_8O_8,0{\cdot}5H_2O$ requires C, 58·3; H, 7·4; N, 15·4%).

N-*Benzyloxycarbonyl*-L-*glutamyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Methyl Ester Trifluoroacetate*.—*N*-Benzyloxycarbonyl-(γ-t-butyl)-L-glutamyl-L-isoleucyl-L-histidyl-L-proline methyl ester (1·4 g, 2 mmol) was added at room temperature under nitrogen to a mixture of trifluoroacetic acid (32 ml) and water (8 ml). The solution was kept at room temperature for 5 h and then evaporated, leaving an oil. Addition of ether to a solution of the oil in ethyl acetate precipitated the *product* as a white amorphous solid (1·44 g, 96%), R_{FC} 0·30, R_{FF} 0·07, $[\alpha]_D^{26}$ −44·1 ± 0·5° (*c* 1·0 in Me_2N·CHO), amino-acid ratios in acid hydrolysate: Glu 1·00, Ile 0·96, His 0·98, Pro 1·0 (Found: C, 52·5; H, 5·65; N, 11·4. $C_{31}H_{42}N_6O_9,CF_3{\cdot}CO_2H$ requires C, 52·5; H, 5·7; N, 11·1%).

N-*Benzyloxycarbonyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*proline Methyl Ester Trifluoroacetate*.—*N*-Benzyloxycarbonyl-(γ-t-butyl)-L-glutamyl-L-valyl-L-histidyl-L-proline methyl ester (0·128 g, 0·19 mmol) was treated at room temperature for 5 h under nitrogen with a mixture of trifluoroacetic acid (3 ml) and water (1 ml), and then evaporated, leaving an oil. After dissolving in ethyl acetate and evaporating again, a solution of the residue in ethyl acetate (1 ml) was added to vigorously stirred ether (50 ml), which precipitated the *product* as an amorphous solid (0·115 g, 84%), R_{FA} 0·46, R_{FB} 0·74, R_{FC} 0·39, R_{FE} 0·0, R_{FF} 0·07, $[\alpha]_D^{26}$ −33·0 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 51·7; H, 5·8; N, 11·6. $C_{30}H_{40}N_6O_9,CF_3{\cdot}CO_2H$ requires C, 51·8; H, 5·7; N, 11·3%).

N-*Benzyloxycarbonyl*-L-*glutamyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Amide Trifluoroacetate*.—A solution of *N*-benzyloxycarbonyl-(γ-t-butyl)-L-glutamyl-L-isoleucyl-L-histidyl-L-proline amide (0·2 g, 0·29 mmol) in trifluoroacetic acid (4 ml) and water (1 ml) was kept under nitrogen at room temperature for 5 h and at 4° overnight. After evaporation, the residual oil was dissolved in ethyl acetate, and this solution was added slowly to ether at 0° to yield the *product* as a white amorphous solid (0·19 g, 89%), m.p. 125—127°, R_{FA} 0·46, R_{FB} 0·69, R_{FC} 0·32, R_{FF} 0·01, $[\alpha]_D^{24}$ −23·1 ± 0·5° (*c* 1·0 in Me_2N·CHO), amino-acid ratios in acid hydrolysate: Glu 1·0, Ile 0·93, His 0·90, Pro 1·0 (and a peak assumed to be the dipeptide Ile-His of ratio 0·1) (Found: C, 52·3; H, 5·9; N, 13·6. $C_{30}H_{41}N_7O_8,CF_3{\cdot}CO_2H$ requires C, 51·9; H, 5·9; N, 13·2%).

N-*Benzyloxycarbonyl*-L-*glutamyl*-L-*valyl*-L-*histidyl*-L-*proline Amide Trifluoroacetate*.—A solution of *N*-benzyloxycarbonyl-(γ-t-butyl)-L-glutamyl-L-valyl-L-histidyl-L-proline amide (0·2 g, 0·28 mmol) in trifluoroacetic acid (4 ml) and water (1 ml) was kept under nitrogen at room temperature for 5 h and at 4° overnight, then evaporated. The residual oil was dissolved in ethyl acetate, and this solution was added slowly to ether at 0° to yield the *product* as a white amorphous solid (0·2 g, 96%), m.p. 124—126°, R_{FA} 0·42, R_{FB} 0·67, R_{FC} 0·30, R_{FF} 0·01, $[\alpha]_D^{24}$ −19·7 ± 0·5° (*c* 1·0 in Me_2N·CHO), amino-acid ratios in acid hydrolysate (150°; 6 days): Glu 1·04, Val 1·0, His 1·0, Pro 1·0 (Found: C, 51·6; H, 5·6; N, 13·8. $C_{29}H_{39}N_7O_8,CF_3{\cdot}CO_2H$ requires C, 51·2; H, 5·55; N, 13·5%).

N$^{\alpha}$-*Benzyloxycarbonyl*-L-*lysyl*-L-*isoleucyl*-L-*histidyl*-L-*proline Methyl Ester Bistrifluoroacetate*.—*N*$^{\alpha}$-Benzyloxycarbonyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysyl-L-isoleucyl-L-histidyl-L-proline methyl ester (0·148 g, 0·2 mmol) was added at room temperature under nitrogen to a mixture of trifluoroacetic acid (3·2 ml) and water (0·8 ml). The solution was kept at room temperature for 5 h and then evaporated. Addition of ether to a solution of the residue in ethyl acetate precipitated the *product* as a deliquescent white solid (0·14 g, 80%), R_{FA} 0·32, R_{FB} 0·75, R_{FC} 0·25, R_{FD} 0·35, R_{FE} 0·0, R_{FF} 0·05, $[\alpha]_D^{26}$ −29·8 ± 0·5° (*c* 1·0 in Me_2N·CHO) (Found: C, 48·6; H, 5·3; N, 11·2.

$C_{32}H_{47}N_9O_9,2CF_3{\cdot}CO_2H$ requires C, 48·6; H, 5·7; N, 11·0%).

N$^{\alpha}$-Benzyloxycarbonyl-L-lysyl-L-valyl-L-histidyl-L-proline Methyl Ester Bistrifluoroacetate.—N$^{\alpha}$-Benzyloxycarbonyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysyl-L-valyl-L-histidyl-L-proline methyl ester (0·149 g, 0·2 mmol) was treated for 5 h at room temperature under nitrogen with a mixture of trifluoroacetic acid (3 ml) and water (1 ml), and then evaporated, leaving an oil. A solution of this in ethyl acetate was evaporated; the resulting oil in ethyl acetate (1·0 ml) was then added to vigorously stirred ether (50 ml) and the *product* was precipitated as an amorphous white solid (0·133 g, 77%), R_{FA} 0·23, R_{FB} 0·70, R_{FC} 0·34, R_{FE} 0·0, R_{FF} 0·07, $[\alpha]_D^{26}$ −29·1 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 49·2; H, 4·8; F, 12·8; N, 11·2. $C_{31}H_{45}N_7O_7,2CF_3{\cdot}CO_2H$ requires C, 49·1; H, 5·2; F, 13·3; N, 11·45%).

N$^{\alpha}$-Benzyloxycarbonyl-L-lysyl-L-isoleucyl-L-histidyl-L-proline Amide Bistrifluoroacetate.—A solution of N$^{\alpha}$-benzyloxycarbonyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysyl-L-isoleucyl-L-histidyl-L-proline amide (0·2 g, 0·27 mmol) in trifluoroacetic acid (4 ml) and water (1 ml) was kept under nitrogen at room temperature for 5 h and at 4° overnight, and was then evaporated. The residual oil was dissolved in ethyl acetate and the solution evaporated. The oil was again dissolved in ethyl acetate and added slowly to ether at 0° to yield the *product* as a white amorphous solid (0·227 g, 97%), m.p. 122—124°, R_{FA} 0·31, R_{FB} 0·60, R_{FC} 0·35, R_{FF} 0·05, $[\alpha]_D^{24}$ −20·1 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$), amino-acid ratios in acid hydrolysate (150° for 6 days): Lys 1·0, Ile 0·97, His 1·0, Pro 1·0 (Found: C, 49·1; H, 5·7; N, 13·2. $C_{31}H_{46}N_8O_6,2CF_3{\cdot}CO_2H$ requires C, 49·2; H, 5·7; N, 13·1%).

N$^{\alpha}$-Benzyloxycarbonyl-L-lysyl-L-valyl-L-histidyl-L-proline Amide Bistrifluoroacetate.—A solution of N$^{\alpha}$-benzyloxycarbonyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysyl-L-valyl-L-histidyl-L-proline amide (0·2 g, 0·28 mmol) in trifluoroacetic acid (4 ml) and water (1 ml) was kept under nitrogen at room temperature for 24 h, then evaporated. The residual oil was dissolved in ethyl acetate and the solution added slowly to ether at 0° to yield the *product* as a white amorphous solid (0·215 g, 90%), m.p. 125—126°, R_{FA} 0·31, R_{FB} 0·60, R_{FC} 0·35, R_{FF} 0·05, $[\alpha]_D^{24}$ −18·2 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 47·5; H, 5·2; N, 13·1. $C_{30}H_{44}N_8O_6,2CF_3{\cdot}CO_2H,H_2O$ requires C, 47·6; H, 5·6; N, 13·1%).

N-Benzyloxycarbonyl-L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine Methyl Ester.—Triethylamine (2·1 ml, 15 mmol) was added during 45 min to a stirred, ice-cold suspension of *N*-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester (5·8 g, 15 mmol) and N$^{\varepsilon}$-t-butoxycarbonyl-L-lysine methyl ester hydrochloride (4·5 g, 15 mmol) in dimethylformamide (50 ml). The mixture was stirred at 2° for 18 h then filtered and evaporated (0·1 mmHg) to a small volume. After the addition of ice–water, the mixture was extracted with ethyl acetate (2 × 200 ml; 1 × 100 ml) and the extracts were washed with aqueous 2N-sodium carbonate (until no further yellow colouration developed in the washings), water (2 × 50 ml), N-hydrochloric acid (2 × 50 ml), and water (2 × 50 ml), then dried ($MgSO_4$), and evaporated. Crystallisation of the residue from ethyl acetate–light petroleum (b.p. 60—80°) gave the *dipeptide derivative* (6·45 g, 85%), m.p. 76—82°, R_{FA} 0·92, R_{FB} 0·96, R_{FC} 0·93, R_{FE} 0·81, R_{FF} 0·76. Further recrystallisation, from benzene–light petroleum (b.p. 60—80°), raised the m.p. to 84·5—85°; $[\alpha]_D^{26}$ −5·6 ± 0·5° (*c* 1·0 in EtOAc) (Found: C, 61·7; H, 8·2; N, 8·2. $C_{26}H_{41}N_3O_7$ requires C, 61·6; H, 8·1; N, 8·3%).

L-Leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine Methyl Ester Acetate.—A solution of *N*-benzyloxycarbonyl-L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine methyl ester (4·5 g) in glacial acetic acid (150 ml) and water (15 ml) was hydrogenolysed at room temperature and pressure over 5% palladium–charcoal (2·0 g) for 2·75 h. After removal of the catalyst the solution was evaporated and the residue was dried by azeotropic distillation with benzene to give the acetate (an oil), R_{FA} 0·71 (trace 0·40), R_{FB} 0·79 (trace 0·73), R_{FC} 0·79 (trace 0·41), R_{FE} 0·32 (trace 0·0), R_{FF} 0·71 (trace 0·56), which was used without further purification in the next stage.

N-Benzyloxycarbonyl-(O-benzyl)-L-tyrosyl-L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine Methyl Ester.—Triethylamine (1·0 ml, 7·1 mmol) was added to a stirred, ice-cold solution of L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine methyl ester acetate (8·9 mmol) and *N*-benzyloxycarbonyl-(*O*-benzyl)-L-tyrosine 2,4,5-trichlorophenyl ester (5·55 g, 9·5 mmol) in dimethylformamide (30 ml). The mixture was stirred at 2° for 1·5 days and at room temperature for 1 day, and then poured into ice-cold ether (200 ml) and ice–water (300 ml) with stirring. The crude *tripeptide derivative* separated as a white solid (4 g), m.p. 102—108°. A further 2·13 g (total yield 90%) of crude product was obtained by evaporation of the ether solution. Recrystallisation from ethyl acetate–light petroleum (b.p. 60—80°) gave material of m.p. 117—121°, R_{FE} 0·75, $[\alpha]_D^{26}$ −12·8 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 66·1; H, 7·15; N, 7·3. $C_{42}H_{56}N_4O_9$ requires C, 66·2; H, 7·4; N, 7·3%).

L-Tyrosyl-L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine Methyl Ester Acetate.—A solution of *N*-benzyloxycarbonyl-(*O*-benzyl)-L-tyrosyl-L-leucyl-(N$^{\varepsilon}$-t-butoxycarbonyl)-L-lysine methyl ester (2·56 g, 3·5 mmol) in acetic acid (63 ml) and water (7 ml) was hydrogenolysed over 5% palladium–charcoal (0·7 g) at room temperature and pressure for 6 h. After removal of the catalyst the solution was evaporated. The residual oil was dried by azeotropic distillation with benzene and then triturated with ether, yielding the solid *acetate* (1·41 g, 67%), R_{FA} 0·80 (trace 0·53), R_{FB} 0·81 (trace 0·76), R_{FC} 0·80 (trace 0·70), R_{FE} 0·20 (trace 0·0), R_{FF} 0·68 (trace 0·51). The product was carried forward to the next stage without further purification.

N-Benzyloxycarbonyl-(O-t-butyl)-L-threonine 2,4,5-Trichlorophenyl Ester.—N-Citric acid (20 ml, 20 mmol) was added to an ice-cold suspension of *N*-benzyloxycarbonyl-(*O*-t-butyl)-L-threonine dicyclohexylammonium salt (4·9 g, 10 mmol) in ethyl acetate (30 ml) and the mixture was stirred for 30 min. The aqueous layer was extracted with ethyl acetate (2 × 30 ml), and the combined ethyl acetate solutions were washed with water (25 ml), dried ($MgSO_4$), and evaporated, to give the acid as an oil. Dicyclohexylcarbodi-imide (2·06 g, 10 mmol) was added at −10° to a vigorously stirred solution of this oil and 2,4,5-trichlorophenol (2·2 g, 11 mmol) in ethyl acetate. The mixture was stirred at −10° for 1 h and then at room temperature for 18 h. After removal of the urea, the solution was evaporated and the oily residue was dissolved in ethyl acetate (150 ml). The resulting solution was washed with N-citric acid, water, aqueous N-sodium carbonate, and water, dried ($MgSO_4$), and evaporated, to yield the *active ester* as a light brown oil, R_{FE} 0·73, R_{FF} 0·75. The i.r. spectrum showed

an activated carbonyl absorption at 1725 cm^{-1} with a shoulder at 1780 cm^{-1}.

N-*Benzyloxycarbonyl*-(O-*t-butyl*)-L-*threonyl*-L-*tyrosyl*-L-*leucyl*-(N^{ε}-*t-butoxycarbonyl*)-L-*lysine Methyl Ester*.—Triethylamine (0·03 ml, 0·22 mmol) was added to an ice-cold, stirred solution of L-tyrosyl-L-leucyl-(N^{ε}-t-butoxycarbonyl)-L-lysine methyl ester acetate (0·166 g, 0·28 mmol) and *N*-benzyloxycarbonyl-(*O*-t-butyl)-L-threonine 2,4,5-trichlorophenyl ester (0·17 g, 0·35 mmol) in dimethylformamide (1 ml). The mixture was stirred at 4° for 18 h and then at room temperature for 1·5 days. It was then poured into ice–water and the mixture was extracted with ethyl acetate (2 × 25 ml). The extracts were washed with N-hydrochloric acid (2 × 10 ml), water (3 × 10 ml), aqueous 5% sodium hydrogen carbonate (2 × 10 ml), and water (2 × 10 ml), dried ($MgSO_4$), and evaporated, yielding the *tetrapeptide derivative* as an oil which solidified under light petroleum (b.p. 60—80°); yield 0·180 g (83%), m.p. 80—85°. Recrystallisation from ethyl acetate–light petroleum (b.p. 60—80°) raised the m.p. to 105—109°; R_{FA} 0·87, R_{FB} 0·89, R_{FC} 0·88, R_{FE} 0·63, R_{FF} 0·85, $[\alpha]_D^{26}$ +0·9 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 60·7; H, 7·5; N, 8·3. $C_{43}H_{65}N_5O_{11},H_2O$ requires C, 61·0; H, 7·9; N, 8·3%).

N-*Benzyloxycarbonyl*-L-*threonyl*-L-*tyrosyl*-L-*leucyl*-L-*lysine Methyl Ester*.—*N*-Benzyloxycarbonyl-(*O*-t-butyl)-L-threonyl-L-tyrosyl-L-leucyl-(N^{ε}-t-butoxycarbonyl)-L-lysine methyl ester (1·3 g, 1·55 mmol) was dissolved in trifluoroacetic acid (24 ml) and water (6 ml) and the solution was kept at room temperature under nitrogen for 6 h. After evaporation, ethyl acetate was added and the solution was re-evaporated. Ethyl acetate was again added and the gelatinous solid which separated on cooling the solution to 0° was collected, dried, ground to a fine powder, and washed thoroughly with ethyl acetate at room temperature, yielding the pure *trifluoroacetate hydrate* (0·86 g, 71%), m.p. 171—175°, R_{FC} 0·3, R_{FE} 0·0 (Found: C, 53·6; H, 6·5; N, 9·0. $C_{34}H_{49}N_5O_9,CF_3{\cdot}CO_2H,H_2O$ requires C, 53·8; H, 6·5; N, 8·7%). The salt was shaken between 0·33M-ammonium hydroxide and ethyl acetate, and the ethyl acetate solution was washed with water and evaporated to an oil. The oil (now insoluble in dry ethyl acetate) was dissolved in a little methanol and the *free base hydrate* was precipitated by addition of ethyl acetate as an amorphous pale cream solid, m.p. 173—174°, R_{FA} 0·65, R_{FB} 0·77, R_{FC} 0·29, R_{FD} 0·71, R_{FE} 0·0, $[\alpha]_D^{26}$ −11·9 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 59·1; H, 7·5; N, 9·5. $C_{34}H_{49}N_5O_9,H_2O$ requires C, 59·2; H, 7·45; N, 10·2%).

N-*Benzyloxycarbonyl*-L-*threonyl*-L-*tyrosyl*-L-*leucyl*-L-*lysine Amide*.—A solution of the foregoing methyl ester hydrate (50 mg, 0·073 mmol) in dry methanol (2 ml) saturated with ammonia was kept in a tightly stoppered flask at room temperature for 70 h and then evaporated. Addition of ether to a solution of the residue in methanol precipitated the *amide dihydrate* as a white amorphous solid (32 mg, 64%), m.p. 172—176°, R_{FA} 0·5, R_{FB} 0·74, R_{FC} 0·23, R_{FD} 0·73, R_{FE} 0·0, $[\alpha]_D^{26}$ −1·8 ± 0·5° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 57·2; H, 7·0; N, 12·0. $C_{33}H_{48}N_6O_8,2H_2O$ requires C, 57·2; H, 7·5; N, 12·1%).

I acknowledge discussions with Dr. P. N. Edwards from which the work was conceived, and thank Mr. M. Long for technical assistance. The biological work was carried out by Dr. E. L. Gerring and his co-workers in the biology department of this Division, whom I also thank.

[2/039 *Received*, 10*th January*, 1972]