Tritiated Peptides. Part 11.[1] Synthesis of [4-^{3}H-Phe^6]-, [4-^{3}H-Phe^{11}]-, and [4-^{3}H-Phe^{6,11}]-Somatostatin and the Metabolite [des-Ala^1]-Somatostatin

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The syntheses are described of somatostatin† labelled with tritium singly in the phenylalanine residues at positions 6 and 11 and doubly at residues 6 and 11 to specific radioactivities of 15.5, 13.8, and 14.1 Cim mol^{-1}, respectively, by reductive deiodination of fully-protected precursors. Cysteine residues were protected by *S*-trityl groups and the disulphide bridge was formed by iodine oxidation of the tritiated protected precursors. The purity of the products was assessed by acidic hydrolysis, ion-exchange and high-pressure liquid chromatography, and by enzymic digestion of the products modified by reduction and aminoethylation. The synthesis of the metabolite [des-Ala^1]-somatostatin is described. The syntheses of [Phe(I)^6]-, [Phe(I)^{11}]- and [Phe(I)^{6,11}]-somatostatin are described.

CONTINUING interest in somatostatin,† the peptide with hormone release-inhibiting properties, prompted the need for a highly radioactive form of the hormone to allow study of its handling *in vivo*. The preparation of a pure sample of a high molecular-weight peptide labelled with tritium in a known position to a high specific radioactivity was first reported by us for the peptide β-corticotrophin-(1—24)-tetracosapeptide.[2] The preparation was achieved by synthesis of an iodine-substituted protected analogue of the peptide and subsequent introduction of isotope by catalytic reduction with tritium gas in an aprotic medium. The dehalogenation proceeded readily even though the peptide contained methionine, the sulphur of which might have been expected to poison the catalyst used. On the other hand, the presence of a cystine disulphide bridge in the somatostatin molecule would be expected to prevent dehalogenation, as was observed by us during development of a method for the synthesis of tritium-labelled calcitonin.[3] To circumvent the difficulties presented by the disulphide bridge, tritium was introduced into a sulphur-free fragment and the highly radioactive product was subsequently coupled to a small fragment containing the pre-formed disulphide bridge.

In the case of somatostatin, where the disulphide bridge forms a ring which includes practically all the amino-acid residues, such an approach would not be practical since two peptide bonds would need to be formed after introduction of isotope into any sulphur-free fragment. The most practical approach is that described which involves, as an experimental complication, the formation of the disulphide bridge after isotopic labelling. We achieved this by scaling down a literature method [4] and encountered no difficulties.

The preparation is described of three tritium-labelled somatostatins with label located at specific sites from fully-protected precursors containing 4-iodophenylalanine. A preliminary report of the synthesis of one of these products has been made by us.[5]

RESULTS AND DISCUSSION

The molecules for labelling were synthesised by classical procedures. For the compound containing 4-iodophenylalanine at residue 6, fragments representing the 1—4, 5—7, 8—11, and 12—14 sequences were assembled by stepwise addition and the fragments were coupled by the azide [6] or hydroxybenzotriazole-assisted carbodi-imide [7] (HOBt-DCC) procedures (Scheme 1). Two of the larger intermediates [(17) and (18)] required purification by counter-current distribution but the final product (20) could be purified by crystallisation.

The synthesis of the carboxy-terminal tripeptide sequence, Bpoc-Thr(But)-Ser(But)-Cys(Trt)-OBut (15), involved some noteworthy features. Syntheses of somatostatin published previously [8,9] used cysteine unprotected on the carboxyl function, possibly because the usual methods of forming t-butyl esters (which is the blocking group of choice) involve the use of strong acid, which is incompatible with the required protection of the other functional groups. We needed to protect all the functional groups in the molecule to minimise the number of exchangeable protons in the product (20) which might dilute the labelling medium. We synthesised *NS*-ditritylcysteine t-butyl ester by reaction of ditritylcysteine with *O*-t-butyl-*NN'*-dicyclohexylisourea. This reagent was synthesised as described by Vowinkel [10] but we found, by following the reaction course by i.r. spectroscopy, that formation of the isourea needed only 18 h reaction at room temperature, and not the 5 days stated by Vowinkel. By using a large excess of reagent to esterify ditritylcysteine, an efficient conversion to the ester was achieved. Excess of reagent was removed by passage of the reaction mixture through a pad of silica gel which converted it to isobutene and dicyclohexylurea. Extraction with citric acid solution then allowed efficient removal of copper ions.

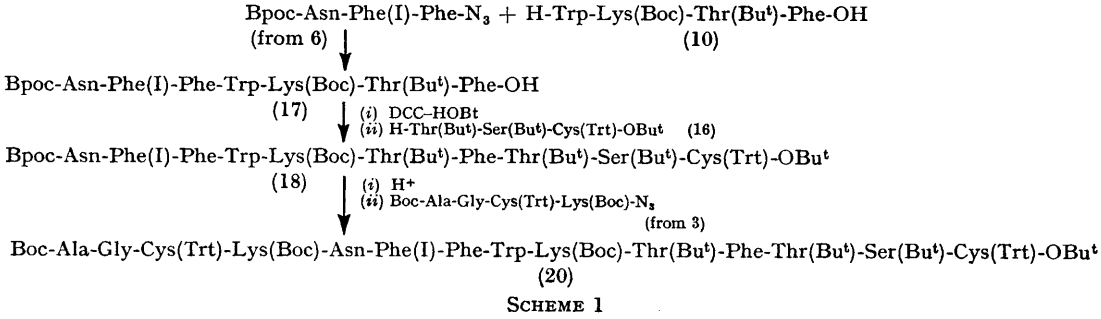
The dipeptide Bpoc-Ser(But)-Cys(Trt)-OBut (13) and

† Somatostatin,

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

In this paper, all amino-acid residues are L. Abbreviations for amino-acids and their use in the formulation of derivatives follow the revised recommendations of the I.U.P.A.C.–I.U.B. Commission on Biochemical nomenclature entitled 'Symbols for Amino-Acid Derivatives and Peptides. Recommendations (1971).'

the tripeptide Bpoc-Thr(But)-Ser(But)-Cys(Trt)-OBut (15) were exceptionally lipophilic molecules which could not be crystallised. After removal of the biphenylylisopropoxy-groups, the hydrochlorides could be crystallised and characterised. These hydrochlorides [(14) and (16)] were so lipophilic that they could be handled by techniques usually employed for fully-protected molecules.



SCHEME 1

For example, they were crystallised from solvent mixtures such as cyclohexane–hexane or ether–light petroleum and could be chromatographed on columns of silica gel using chloroform–methanol for elution.

Exposure of the fully-protected tripeptide (15) to the conditions ultimately used for reductive deiodination showed that the *S*-tritylcysteine residue slowly gave rise to alanine. A similar generation of alanine from cystine under harsh reduction conditions has been reported in an attempted synthesis of tritiated oxytocin.[11]

Examination of the reduction product of the protected tetradecapeptide (20) after 15 min reaction showed that 28% of the 4-iodophenylalanine had not been reduced. After 30 min reaction, all the 4-iodophenylalanine had been converted to phenylalanine and decomposition of *S*-tritylcysteine to alanine was not significant (<2%). After reduction for 1 h, nearly 10% excess of alanine was present in the reaction mixture.

The above results confirmed that some poisoning of the catalyst mixture, presumably by triphenylmethanethiol, was occurring because the reduction of 4-iodophenylalanine was much slower than in the corticotrophin series[12] where this residue was completely dehalogenated in <5 min (unpublished result) and a high efficiency (93%) of isotopic incorporation was observed. The much lower level (53%) observed in this instance indicates that the labelling medium is diluted by exchangeable protons in the protected peptide at a comparable rate to that of the deiodination. Calculation shows that, if all tritons and potentially-exchangeable protons equilibriate before de-iodination commences, an incorporation efficiency of 79% would be achieved. Probably, this result is due to a combination of the effects of incorporation of traces of water in the system and a slow rate of dehalogenation brought about by poisoning.

As reported in our studies on labelled calcitonin,[3] we observed that the t-butyl ether protecting group on serine was only slowly removed by 90% trifluoroacetic acid. After 30 min at room temperature, about 5% of the protecting group remained. This was completely removed by extending the treatment with acid to a period of 1 h. The product was purified by ion-exchange chromatography followed by high-pressure liquid chromatography, as described for tritiated calcitonin.[3]

To enable analysis of the product by enzymic digestion,[13] it was necessary to open the disulphide bridge by reduction with β-mercaptoethanol and to block the thiol functions generated by reaction with ethyleneimine. The product recovered by gel-filtration contained 0.94 residues of alanine and 1.81 residues of lysine (Table 1).

TABLE 1

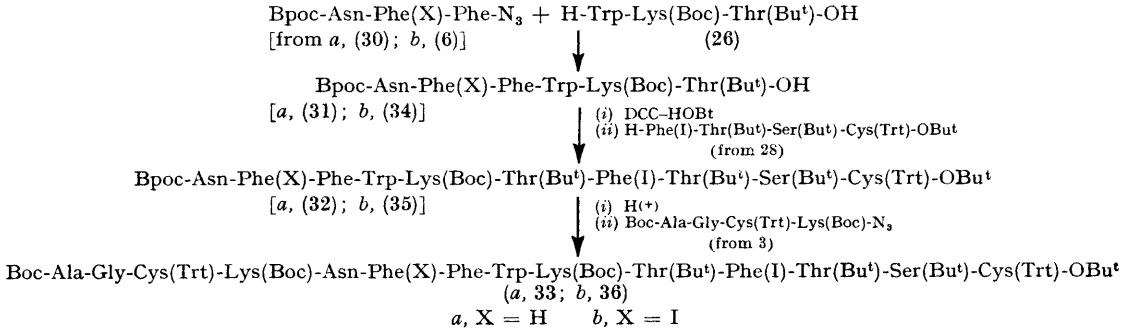
Amino-acid analysis after acidic and enzymic [a] hydrolysis of tritiated somatostatins

	Somatostatin analogue					
	[4-^{3}H-Phe^6]		[4-^{3}H-Phe^{11}]		[4-^{3}H-Phe^{6,11}]	
	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme
Ala	0.99	0.94	1.00	0.92	1.00	0.93
Asn		0.97		0.96		0.98
Asp	1.00		0.99		1.01	
Cys [b]	1.88		1.88		1.89	
Cys (AE)		1.51		1.69		1.71
Gly	1.00	0.91	1.00	0.90	1.00	0.93
Lys	2.05	1.81	2.05	1.98	2.01	1.90
Phe	3.00	3.00	3.00	3.00	3.00	2.91
Ser	2.66 [c]	0.97	2.65 [c]	0.98	2.68 [c]	0.98
Thr		2.01		1.94		1.91
Trp	0.44	0.94	0.52	0.97	0.49	0.92
Specific activity (Ci mmol^{-1})	15.5		13.8		14.15	

[a] After reduction and aminoethylation. [b] Sum of all Cys species. [c] Value for Ser + Thr.

This is due to a small degree of N-aminoethylation by ethyleneimine. The radioactivity was associated exclusively with phenylalanine. This was observed for all three radioactive peptides described in this paper.

Following the successful synthesis of compound (40), we embarked on the preparation of a precursor containing a 4-iodophenylalanine residue at position 11. The synthesis (Scheme 1) was modified so that most efficient use of the scarce 4-iodophenylalanine could be achieved. This entailed constructing fragments corresponding to the 8—10 and 11—14 sequences (instead of 8—11 and 12—14), otherwise the approach was identical (Scheme 2*a*). We found that, whereas the



SCHEME 2

phenylalanine methyl ester function in the 8—11 compound (8) could be saponified by sodium hydroxide solution quickly and without racemisation, the methyl ester of threonine in the 8—10 fragment could not be cleaved without partial racemisation. We therefore used the trimethylsilylethyl ester protecting group,[14] which was successfully removed by fluoride ion without racemisation. In all other respects, the synthesis of the precursor (33) and its labelling proceeded exactly as for the previous synthesis.

We next wished to prepare a precursor containing 4-iodophenylalanine residues at both positions 6 and 11. From this we hoped to be able to obtain somatostatin labelled with tritium to *ca.* 30 Ci mmol^{-1}, as the two singly-labelled derivatives already described could be consistently prepared at specific radioactivities averaging *ca.* 15 Ci mmol^{-1} over many preparations.

Using intermediates prepared for the two syntheses already described and the pathway used for the synthesis of the analogue containing 4-iodophenylalanine at position 11, we prepared the bis-iodophenylalanine precursor without difficulty (Scheme 2*b*). The labelling procedure gave a product which had a specific radioactivity of only 14.1 Ci mmol^{-1}, no higher than could have been achieved by simply mixing the two singly-labelled molecules already described. The failure to generate a product of substantially higher isotopic content was shown to be due to slower exchange of the increased amount of iodine for tritium, presumably inhibited by triphenylmethanethiol release as already explained. After 15 min reduction, 35% of the two iodine atoms per molecule had been replaced. This compared with 72% removal of iodine from the precursor molecule containing only one iodine atom per molecule, using the same conditions.

These results indicate that, with the catalysts presently used, products of very high specific radioactivity are not obtained merely by increasing the halogen content of the precursor molecule when poisoning of the catalysts by slow degradation of sulphur-containing amino-acids is an accompanying reaction.

The analytical data for all three radioactive products are summarised in Table 1.

It has been reported that [de-Ala^1]-somatostatin is a metabolite of somatostatin in rats.[15] We report the synthesis of this compound, which was constructed from appropriate precursors by the fragment condensation route given in Scheme 1. We were unable to distinguish this compound from somatostatin by high-pressure liquid chromatography using the conditions given in this report for compound (40). This finding agreed with that suggested by the work of McMartin and Purdon.[15]

We also describe the preparation of the somatostatin analogues containing 4-iodophenylalanine singly at positions 6 and 11 and the bis-Phe(I)^{6,11} compound. It was not possible to analyse these peptides by enzymic hydrolysis because the products obtained after reduction and S-aminoethylation were insoluble in water.

EXPERIMENTAL

Characteristics of compounds not described individually are given in Table 2.

NS-Ditritylcysteinyl-N$^{\epsilon}$-t-butoxycarbonyl-lysine Methyl Ester (1).—A solution of *NS*-ditritylcysteine *N*-hydroxysuccinimide ester (16.4 g) and *N$^{\epsilon}$*-t-butoxycarbonyl-lysine methyl ester hydrochloride (6.4 g) and NEt_3 (3.3 ml) in DMF (125 ml) was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in EtOAc (75 ml), washed with water (75 ml), and the solution was evaporated to dryness. The residue was dissolved in benzene (10 ml) and applied to a column (40 × 4.5 cm) of silica gel G. Elution with benzene–ether (9 : 1, v/v) gave the *product* which was crystallised from ether–light petroleum, (b.p. 60—80 °C) to afford 11.3 g (62%) of material which, even after drying *in vacuo* at 65 °C, still contained solvent of crystallisation. It had m.p. 83—86 °C, $[\alpha]_D^{25}$ +54.9 ± 0.6° (*c* 0.9, MeOH) (Found: C, 75.55; H, 6.75; N, 4.7; S, 3.8. $C_{53}H_{57}N_3O_5S\cdot0.5C_6H_{14}$ requires C, 75.47; H, 7.23; N, 4.71; S, 3.59%).

TABLE 2

Compound	Prepared from	Method as for compound	M.p. (°C)	$[\alpha]_D^{25}$ (°)	Yield (%)	Found (%) C	H	N	I	S	Formula	Required (%) C	H	N	I	S
(29) Bpoc-Asn-Phe-Phe-OMe	Bpoc-Asn-OH + Boc-Phe-Phe-OMe	(5)	149—152	−27.5 ± 0.2 *m*	63	68.72	6.20	8.25			$C_{39}H_{42}N_4O_7$	69.0	6.23	8.25		
(30) Bpoc-Asn-Phe-Phe-$NHNH_2$	(29) + $N_2H_4{\cdot}H_2O$	(6)	184—186	−42.5 ± 0.4 *m*	81	66.46	6.27	12.27			$C_{38}H_{42}N_6O_6{\cdot}0.5H_2O$	66.35	6.30	12.21		
(31) Bpoc-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-OH	(30) + (26)	(17)	196—197 (decomp.)	−34.0 ± 0.6 *p*	66 *a*	64.73	6.95	10.04			$C_{68}H_{85}N_9O_{15}{\cdot}1.5H_2O$	64.64	7.02	9.97		
(32) Bpoc-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-Phe(I)-Thr(But)-Ser(But)-Cys(Trt)-OBut	(31) + (28)	(18)	196—198	−1.8 ± 0.6 *p*	56 *b*	63.80	6.77	8.19	5.85	1.53	$C_{118}H_{148}IN_{13}O_{19}S$	64.08	6.74	8.23	5.73	1.44
(33) Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-Phe(I)-Thr(But)-Ser(But)-Cys(Trt)-OBut	(3) + (32)	(20)	233—235	−1.9 ± 0.3 *p*	68	62.46	6.85	9.14	4.70	2.25	$C_{146}H_{189}IN_{16}O_{25}S$	62.75	6.86	9.08	4.57	2.31
(34) Bpoc-Asn-Phe(I)-Phe-Trp-Lys(Boc)-Thr(But)-OH	(6) + (26)	(31) *c*	200 (decomp.)	−16.4 ± 0.3 *p*	58 *d*	59.52	6.20	9.25	9.27		$C_{68}H_{84}IN_9O_{13}{\cdot}0.5H_2O$	59.55	6.24	9.19	9.25	
(35) Bpoc-Asn-Phe(I)-Phe-Trp-Lys(Boc)-Thr(But)-Phe(I)-Thr(But)-Ser(But)-Cys(Trt)-OBut	(34) + (28)	(32) *e*	176	−0.7 ± 0.3 *p*	72 *f*	60.25	6.30	7.77	10.85	1.41	$C_{118}H_{147}I_2N_{13}O_{19}S{\cdot}H_2O$	60.17	6.37	7.73	10.77	1.36
(36) Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe(I)-Phe-Trp-Lys-(Boc)-Thr(But)-Phe(I)-Thr(But)-Ser(But)-Cys(Trt)-OBut	(3) + (35)	(33) *g*	240 (decomp.)	+1.2 ± 0.3 *p*	76 *h*	60.25	6.53	8.81	8.91	2.20	$C_{145}H_{188}I_2N_{16}O_{25}S_2$	60.03	6.53	8.69	8.74	2.21
(38) [4-Iodo-Phe[11]]-Somatostatin	(33)	(37)			42	49.36	6.00	12.76	6.17	2.95	$C_{76}H_{103}IN_{18}O_{19}{\cdot}2MeCO_2H{\cdot}3H_2O$	49.58	6.08	13.01	6.54	3.30
(39) [4-Iodo-Phe[6-11]]-Somatostatin	(36)	(37)			48	44.69	5.62	11.55	11.95	2.62	$C_{75}H_{102}I_2N_{18}O_{19}{\cdot}2MeCO_2H{\cdot}8H_2O$	44.60	5.89	11.70	11.78	2.97
(41) [4-^{3}H-Phe[11]]-Somatostatin	(33)	(40)			21											
(42) [4-^{3}H-Phe[6-11]]-Somatostatin	(36)	(40)			17											
(44) Boc-Gly-Cys(Trt)-Lys(Boc)-$NHNH_2$	(43)	(3)	142—145	+9.7 ± 0.3 *k*	82 *i*	61.87	6.93	10.81		4.28	$C_{40}H_{54}N_6O_7S{\cdot}0.5H_2O$	62.23	7.18	10.88		4.15
(45) Bpoc-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-Phe-OH	(30) + (10)	(17)	209 (decomp.)	−8.8 ± 0.3 *m*	54 *j*	65.77	6.72	9.98			$C_{77}H_{94}N_{10}O_4{\cdot}H_2O$	65.98	6.90	9.99		
(46) Bpoc-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-Phe-Thr(But)-Ser(But)-Cys(Trt)-OBut	(45) + (16)	(18)	192—194	−9.7 ± 0.8 *k*	77	67.51	7.20	8.63		1.65	$C_{118}H_{149}N_{15}O_{19}S{\cdot}H_2O$	67.37	7.23	8.65		1.52
(47) Boc-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe-Phe-Trp-Lys(Boc)-Thr(But)-Phe-Thr(But)-Ser(But)-Cys(Trt)-OBut	(44) + (46)	(20)	221 (decomp.)	−1.7 ± 0.6 *m*	78	65.88	7.29	9.15		2.54	$C_{143}H_{185}N_{17}O_{24}S_2{\cdot}0.5H_2O$	65.92	7.24	9.20		2.47
(48) [des-Ala[1]]-Somatostatin	(47)	(37)		−41.8 ± 0.4 *n*	36	53.00	6.36	13.77		3.60	$C_{73}H_{99}N_{17}O_{18}S_2{\cdot}2MeCO_2H{\cdot}3H_2O$	53.12	6.54	13.67		3.68

a From MeOH–H_2O (3 : 1 v/v), three crystallisations. *b* From MeOH–DMF–H_2O (6 : 3 : 1 v/v). *c* Work-up by trituration (ice–H_2O), dried, dissolved in $CHCl_3$–MeOH (9 : 1 v/v), filtered (silica pad), evaporated, and residue crystallised. *d* From MeOH–DMF–H_2O (5 : 1 : 2 v/v). *e* Purified on silica pad, elution with $CHCl_3$–MeOH (97 : 3 v/v). *f* From MeOH–DMF–H_2O (12 : 5 : 1 v/v). *g* Trituration with ice–water, then dried. *h* From trifluoroethanol–H_2O (8 : 1 v/v). *i* From methanol. *j* From aqueous methanol. *k* *c* 1.0, $CHCl_3$. *l* From MeOH–DMF (5 : 1 v/v). *m* *c* 1.0, DMF. *n* *c* 0.5, AcOH–H_2O (1 : 99 v/v). *p* *c* 1.0, MeOH.

N-t-Butoxycarbonylalanyl-glycyl-S-tritylcysteinyl-N$^{\epsilon}$-t-butoxycarbonyl-lysine Methyl Ester (2).—Compound (1) (37.5 g) was dissolved in warm glacial acetic acid (330 ml), cooled to 18 °C, and 2M-HCl (22.1 ml) added dropwise to the stirred solution at a rate at which the solution remained clear. Water (44.3 ml) was added dropwise, the mixture was stirred for 1 h, and the precipitated triphenylmethanol was filtered off. The filtrate was evaporated to dryness below 25 °C, water (50 ml) was added, and the solution again evaporated to dryness. The residue was crystallised from ethyl acetate to give 21.3 g (75%) of hydrochloride, m.p. 189—191 °C.

The hydrochloride was suspended in MeCN (150 ml), cooled to 5 °C, and NEt_3 (4.71 ml) added dropwise to the stirred solution. The mixture was stirred for 20 min at 5 °C and triethylamine hydrochloride was removed by filtration. The fitrate was added to a solution of Boc-Ala-Gly-OH [9] (8.95 g), *N*-hydroxybenzotriazole hydrate (HOBt) (5.1 g), and dicyclohexylcarbodi-imide (DCC) (7.7 g) in a mixture of DMF (18 ml) and MeCN (180 ml) which had been pre-incubated for 1 h at 0 °C and then 1 h at 10 °C, and from which dicyclohexylurea (DCU) had been removed by filtration. The mixture was then stirred at 4 °C for 16 h. The precipitated solid was recovered by filtration and crystallised from benzene–cyclohexane to give the solvated product (27.1 g, *ca.* 90%), m.p. 135 °C (softens), 166—170 °C. A sample for analysis was dried *in vacuo* at 140 °C for 1.5 h. The *protected tetrapeptide* had m.p. 175—178 °C, $[\alpha]_D^{20}$ −12.7 ± 0.2° (*c* 0.9, MeOH) (Found: C, 63.2; H, 7.1; N, 8.4; S, 4.0. $C_{44}H_{59}N_5O_9S$ requires C, 63.4; H, 7.1; N, 8.4; S, 3.8%).

N-t-Butoxycarbonylalanyl-glycyl-S-tritylcysteinyl-N$^{\epsilon}$-t-butoxycarbonyl-lysine Hydrazide (3).—Compound (2) (5.0 g) was dissolved in methanol (50 ml), $N_2H_4{\cdot}H_2O$ (3.0 ml) was added, and the mixture was stirred at room temperature under N_2 for 24 h. The precipitate was recovered by filtration, washed with cold methanol (20 ml) and water (100 ml), and dried *in vacuo* over concentrated H_2SO_4. The crude material was crystallised twice from methanol to give the *hydrazide* (2.6 g, 52%), m.p. 234—236 °C (decomp.), $[\alpha]_D^{25}$ −16.0 ± 0.3° (*c* 1.0, MeOH) (Found: C, 61.9; H, 7.1; N, 11.5; S, 3.9. $C_{43}G_{59}N_7O_8S$ requires C, 61.9; H, 7.1; N, 11.75; S, 3.8%).

N-t-Butoxycarbonyl-4-iodophenylalanyl-phenylalanine Methyl Ester (4).—A solution of Boc-Phe(I)-OH [12] (10.1 g), H-Phe-OMe·HCl (5.6 g) and NEt_3 (3.6 ml) in DMF (40 ml) was cooled to 0 °C and a solution of DCC (6.4 g) in DMF (20 ml) was added. The mixture was stirred at 4 °C for 16 h, some product (7.7 g) was recovered by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (100 ml), cooled, and DCU removed by filtration. The filtrate was extracted with cold aqueous saturated citric acid (2 × 100 ml), water (100 ml), and saturated aqueous $NaHCO_3$ (100 ml), dried (Na_2SO_4), and evaporated to dryness. The residue and the solid recovered above were combined and crystallised from ethyl acetate to give the *product* (10.55 g, 74%), m.p. 184 °C, $[\alpha]_D^{26}$ −0.6 ± 1.0° (*c* 0.5, MeOH) (Found: C, 52.3; H, 5.3; I, 22.9; N, 5.2. $C_{24}H_{29}IN_2O_5$ requires C, 52.2; H, 5.25; I, 23.0; N, 5.1%).

N-2-(4-Biphenylyl)isopropoxycarbonylasparaginyl-4-iodophenylalanyl-phenylalanine Methyl Ester (5).—Compound (4) (5.15 g) was dissolved in acetic acid (100 ml), 1.7M-HCl in MeOH (180 ml) was added, and the mixture was stirred for 3 h. The solution was evaporated to dryness and the residue obtained was kept *in vacuo* over NaOH pellets for 16 h. The solid ester salt was dissolved in DMF (25 ml) with addition of NEt_3 (1.2 ml), cooled to 0 °C, and added to a solution of Bpoc-Asn-OH (3.2 g), HOBt (1.32 g), and DCC (1.8 g) in DMF (25 ml) which had been pre-incubated for 1 h at 0 °C and then for 1 h at 10 °C. The mixture was stirred at 4 °C for 16 h, DCU was removed by filtration, and the filtrate was evaporated to dryness. The residue was crystallised three times from DMF–MeOH–H_2O (1 : 4 : 2 v/v) to give the *protected tripeptide* (5.1 g, 75%) which had m.p. 197—199 °C, $[\alpha]_D^{25}$ −26.2 ± 0.6° (*c* 1.0, DMF) (Found: C, 57.9; H, 5.2; I, 15.9; N, 7.2. $C_{39}H_{41}IN_4O_7$ requires C, 58.2; H, 5.1; I, 15.8; N, 7.0%).

N-2-(p-Biphenylyl)isopropoxycarbonylasparaginyl-4-iodophenylalanyl-phenylalanine Hydrazide (6).—Compound (5) (1.81 g) was dissolved in DMF (22 ml), $N_2H_4{\cdot}H_2O$ (1.08 ml) was added, and the mixture was stirred under N_2 for 18 h. The solution was evaporated to dryness and the residue was triturated with ice–water (50 ml) to yield a solid which was crystallised twice from methanol to give the *hydrazide* (1.55 g, 86%), m.p. 210—215 °C (decomp.), $[\alpha]_D^{25}$ −30.8 ± 0.6° (*c* 1.0, DMF) (Found: C, 55.8; H, 5.1; I, 15.55; N, 10.5, $C_{38}H_{41}IN_6O_6{\cdot}0.5H_2O$ requires C, 56.1; H, 5.2; I, 15.6; N, 10.3%).

N$^{\alpha}$-Benzyloxycarbonyl-N$^{\epsilon}$-butoxycarbonyl-lysyl-O-t-butylthreonyl-phenylalanine Methyl Ester (7).—(a) Z-Thr-(But^t)-Phe-OMe. Triethylamine (13.9 ml) was added dropwise with stirring to a solution of phenylalanine methyl ester hydrochloride (21.6 g) in DMF (160 ml) at −5 °C. Z-Thr(But^t)-OSu (40.6 g) was added in portions, and the mixture was stirred for 1 h at −5 to 0 °C, then at room temperature overnight. After filtration and evaporation of the solvent the residue was dissolved in ethyl acetate (200 ml), washed successively with water, aqueous citric acid, saturated $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Evaporation of the solvent left a viscous oil (46.3 g; 99%).

(b) H-Thr(But^t)-Phe-OMe·HCl. A solution of dipeptide ester (13 g) in methanol (130 ml) containing acetic acid (6.5 ml) was hydrogenated at room temperature over palladium–charcoal (5%: 0.9 g). When hydrogen uptake had ceased the catalyst was filtered off, the filtrate concentrated, and the residue dissolved in aqueous methanol (1 : 1, 50 ml). The solution was cooled to 3 °C and 1M HCl (27.5 ml) was added below 5 °C. Concentration on the oil pump was followed by addition of water (30 ml) and concentration once more. The white solid dipeptide hydrochloride (10.3 g) was dried over KOH.

(c) *Compound* (7). A solution of Z-Lys(Boc)-OH (18.6 g) and HOBt (7.4 g) in DMF (75 ml) was cooled to 0 °C and DCC (10.8 g) in DMF (25 ml) was added. The mixture was stirred for 1 h at 0 °C and 1 h at 10 °C then treated with a cold solution of dipeptide hydrochloride (17.1 g) and NEt_3 (7.3 ml) in DMF (95 ml). Next day the solid was filtered off and the filtrate concentrated. The residue was treated with water, and the solid filtered off and recrystallised several times from propan-2-ol. The product (19.4 g; 61%) had m.p. 150—152 °C, $[\alpha]_D^{26}$ +9.5 ± 0.3° (*c* 2.0, MeOH) (Found: C, 63.6; H, 7.7; N, 8.05. $C_{37}H_{54}N_4O_9$ requires C, 63.58; H, 7.78; N, 8.01%).

N-Benzyloxycarbonyltryptophyl-N$^{\epsilon}$-butoxycarbonyl-lysyl-O-t-butylthreonyl-phenylalanine Methyl Ester (8).—A solution of (7) (16.3 g) in methanol (400 ml) was hydrogenated over 10% palladium–charcoal (2 g). The recovered free ester was dissolved in DMF (28 ml). A solution of Z-Trp-OH (7.9 g)

and HOBt (3.73 g) in DMF (20 ml) was cooled to 0 °C, a solution of DCC (5.13 g) in DMF (10 ml) was added and the mixture stirred at 0 °C for 1 h, then at 10 °C for 1 h. The ester solution was added and stirring continued overnight. The DCU was filtered off and the filtrate concentrated. The residue was treated with ethyl acetate (200 ml) and the first crop of product filtered off. The filtrate was diluted with an equal volume of ethyl acetate, then washed successively with water, aqueous citric acid, saturated $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated. The residue was combined with the first crop which had been filtered from ethyl acetate, and the whole recrystallised several times from methanol. The *protected tetrapeptide* (14.1 g: 73%) had m.p. 190—192 °C, $[\alpha]_D^{26}$ +2.1 ± 0.5° (*c* 1.9, MeOH) (Found: C, 65.2; H, 7.25; N, 9.5. $C_{48}H_{64}N_6O_{10}$ requires C, 65.13; H, 7.28; N, 9.49%).

N-*Benzyloxycarbonyltryptophyl*-N$^{\epsilon}$-*butoxycarbonyl-lysyl*-O-*t-butylthreonyl-phenylalanine* (9).—Protected tetrapeptide methyl ester (8) (9.2 g) was dissolved in dioxan (40 ml) with warming, the solution cooled, and 4M-NaOH solution (5.5 ml) added. After 1 h the solution was cooled to 4 °C, 1M-HCl (22 ml) added, and the solution concentrated. The residue was treated with ethyl acetate (700 ml) and water (200 ml). The water layer was separated and the organic layer shaken with saturated $NaHCO_3$ solution (200 ml) and water (500 ml). This formed a gel which was filtered off, washed with ethyl acetate, and sucked dry. The gel was added to the separated aqueous layer, cooled to 4 °C, and the suspension acidified with citric acid. The solution was extracted with ethyl acetate (3 × 400 ml) and the combined organic layer was washed with brine to neutrality, dried (Na_2SO_4), and concentrated. The residue was crystallised from aqueous methanol (1 : 4) and then methanol to give the *tetrapeptide acid* (5.9 g; 65%), m.p. 164—167 °C, $[\alpha]_D^{26}$ +11.7 ± 0.3° (*c* 1.9, MeOH) (Found: C, 64.6; H, 7.15; N, 9.65. $C_{47}H_{62}N_6O_{10}$ requires C, 64.8; H, 7.17; N, 9.64%).

Tryptophyl-N$^{\epsilon}$-*butoxycarbonyl-lysyl*-O-*t-butylthreonyl-phenylalanine* (10).—Compound (9) (7.1 g) was dissolved in methanol (200 ml) and water (50 ml) and hydrogenated over 5% palladium–charcoal (0.6 g). The catalyst was filtered off, the filtrate concentrated, and the residue recrystallised from methanol–ether. The *hydrated product* (3.8 g; 61%) had m.p. 164—167 °C, $[\alpha]_D^{26}$ +42.8 ± 0.3° (*c* 1.5, MeOH) (Found: C, 61.35; H, 7.45; N, 10.9. $C_{39}H_{56}N_6O_8 \cdot 1.5H_2O$ requires C, 61.31; H, 7.78; N, 11.0%).

NS-*Ditritylcysteine t-Butyl Ester* (11).—Copper(I) chloride (0.39 g) was added to a solution of DCC (39.8 g) in t-BuOH (17.0 g) and the mixture was stirred for 18 h with exclusion of moisture. Ethyl acetate (135 ml) was added and the mixture was cooled to 5 °C. A cooled solution of Trt-Cys-(Trt)-OH (19.4 g) in EtOAc (167 ml) was added and the mixture was stirred at 5 °C for 1 h and then at 20 °C for 18 h. The mixture was cooled to 0 °C and filtered through a pad (2 × 12 cm) of silica gel (70—230 mesh) which was washed with EtOAc until the filtrate was colourless. The filtrate was extracted at 0 °C with 7% (w/v) aqueous citric acid solution (2 × 200 ml), washed to neutrality with 10% aqueous NaCl solution, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in light petroleum (b.p. 60—80 °C) (350 ml), insoluble matter removed by filtration, and the filtrate passed through a pad (3 × 15 cm) of silica gel (70—230 mesh) in a Buchner funnel. Product was recovered by washing the pad with light petroleum–ether (96 : 4 v/v). Crystallisation from propan-2-ol gave the *ester* (15.4 g, 75%) which had m.p. 134—136 °C, $[\alpha]_D^{28}$ +60.2 ± 1.0° (*c* 1.0, $CHCl_3$) (Found: C, 81.7; H, 6.6; N, 2.1; S, 4.8. $C_{45}H_{43}NO_2S$ requires C, 81.65; H, 6.5; N, 2.1; S, 4.8%).

O-*t-Butylseryl*-S-*tritylcysteine t-Butyl Ester Hydrochloride* (14).—Compound (11) (9.4 g) was dissolved in acetic acid (100 ml) and water (25 ml) was added dropwise during 10 min with vigorous stirring. The suspension was cooled to 0 °C and set aside for 2 h, the triphenylmethanol was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (125 ml), extracted at 0 °C with saturated $NaHCO_3$ (3 × 100 ml), washed with aqueous 10% NaCl, dried (Na_2SO_4), and evaporated to dryness to give the base (12) (5.9 g, 100%).

Bpoc-Ser(Bu^t)-OH (26.4 g) was prepared by regeneration from its dicyclohexylamine salt (33 g) with cold citric acid solution, dissolved in dry DMF (200 ml) and the solution was cooled to −15 °C. *N*-Methylmorpholine (7.25 ml) was added followed by isobutyl chloroformate (8.65 ml) and the mixture was stirred at −10 °C for 10 min with the exclusion of moisture. A solution of the base (12) (31.3 g) in dry DMF (400 ml) at −10 °C was added and the mixture was stirred for 1 h at −10 °C and then for 18 h at 4 °C. A small amount of insoluble material was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (500 ml), washed with 10% aqueous NaCl (2 × 500 ml), dried (Na_2SO_4), and evaporated to dryness. The product (13) (55 g) could not be crystallised; it was dissolved in acetic acid (320 ml), water (80 ml) was added, and the mixture was stirred at 50 °C for 1 h. Water (400 ml) was then added and the suspension was stirred at 0 °C for 2 h. Biphenylylpropan-2-ol was removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in EtOAc (450 ml), extracted at 0 °C with saturated aqueous $NaHCO_3$ (3 × 300 ml), washed with 10% aqueous NaCl, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in EtOAc (30 ml), cooled to −20 °C, 2M-HCl in EtOAc (33 ml) added, and the mixture stirred for 15 min at −20 °C. Light petroleum (b.p. 60—80 °C) was added to precipitate the hydrochloride (38.4 g, 98%) which was obtained by filtration as a hygroscopic solid. A sample (5.2 g) of material was purified for analysis by applying a solution in chloroform (15 ml) to a column (15 × 3 cm) of silica gel and eluting the product with chloroform–methanol (98 : 2 v/v). Crystallisation from cyclohexane–hexane gave the *hydrochloride* (4.4 g), m.p. 85—95 °C, $[\alpha]_D^{25}$ +30.8 ± 0.6° (*c* 1.0, $CHCl_3$) (Found: C, 66.4; H, 7.5; Cl, 5.9; N, 4.7; S, 5.1. $C_{33}H_{43}ClN_2O_4S$ requires C, 66.1; H, 7.2; Cl, 5.9; N, 4.7; S, 5.35%).

O-*t-Butylthreonyl*-O-*t-butylseryl*-S-*tritylcysteine t-Butyl Ester Hydrochloride* (16).—Compound (14) (11.2 g) was dissolved in EtOAc (150 ml), the solution was washed with saturated aqueous $NaHCO_3$ (3 × 150 ml), 10% aqueous NaCl solution (3 × 150 ml), dried (Na_2SO_4), and evaporated to dryness to give the base (8.86 g, 84%). This was coupled with Bpoc-Thr(Bu^t)-OH by the mixed anhydride procedure and worked up as described above for compound (13). The neutral product (15.2 g) was dissolved in benzene (20 ml) and applied to a pad (1.5 × 10 cm) of alumina (neutral, grade 1) which was washed with benzene (750 ml), benzene–ether (1 : 1 v/v) (1 l), and ether (1 l). Fractions of eluate which contained the product were combined and evaporated to give the non-crystalline tripeptide (15) (10.2 g, 68%) which was dissolved in acetic acid (120 ml), deprotected, worked up, and converted to the hydrochloride as described above for compound (14). The white, hygro-

scopic solid obtained (5.7 g, 60%) was applied in chloroform (25 ml) to a column (22 × 3 cm) of silica gel and eluted with increasing concentrations (1, 2, and 4% v/v) of methanol in chloroform. Fractions containing the product were pooled and evaporated to dryness to give the hydrochloride (5.0 g, 53%). A sample crystallised from ether–light petroleum (b.p. 40—60 °C) gave the *hemihydrate* which had m.p. 85—90 °C, $[\alpha]_D^{28}$ +28.9 ± 0.6° (*c* 1.0, $CHCl_3$) (Found: C, 64.4; H, 7.8; Cl, 4.7; N, 5.3; S, 4.2. $C_{41}H_{58}ClN_3O_6S\cdot 0.5H_2O$ requires C, 64.3; H, 7.8; Cl, 4.6; N, 5.5; S, 4.2%).

N-2-(p-*Biphenylyl*)*isopropoxycarbonylasparaginyl*-4-*iodophenylalanyl*-*phenylalanyl*-*trytophyl*-N$^{\epsilon}$-*t*-*butoxycarbonyllysyl*-O-*t*-*butylthreonyl*-*phenylalanine Monohydrate* (17).—Compound (6) (1.55 g) was dissolved in DMF (10 ml) and cooled to −10 °C, and 4M-HCl in EtOAc (0.98 ml) was added with stirring at this temperature. t-Butyl nitrite (0.23 ml) was added, the mixture was stirred for 10 min at −10 °C, and NEt_3 (0.55 ml) was added. A solution of compound (10) (1.71 g) and NEt_3 (0.32 ml) in DMF (10 ml) at −10 °C was added and the mixture was stirred at −10 °C for 1 h and then at 4 °C for 72 h. Glacial acetic acid (0.14 ml) was added, insoluble matter was removed by filtration, and the filtrate was evaporated to dryness below 30 °C. The residue was dried *in vacuo* over concentrated H_2SO_4 for 16 h and then triturated with ice–water to yield a white solid (3.01 g) which was crystallised from DMF–MeOH–H_2O (2 : 3 : 1 v/v) (60 ml) to give material (2.09 g) which, based on amino-acid analysis, was *ca.* 90% pure. The material was purified in two batches by counter-current distribution using the solvent system toluene–chloroform–methanol–water (5 : 5 : 8 : 2 v/v). The recovered material was crystallised from DMF–MeOH–H_2O (2 : 3 : 1 v/v) to give the *product* (1.11 g, 38%) which had m.p. 223° (decomp.), $[\alpha]_D^{25}$ −9.6 ± 1.6° (*c* 0.8, DMF) (Found: C, 60.6; H, 6.2; N, 9.3; I, 8.3. $C_{77}H_{91}IN_{10}O_{14}\cdot H_2O$ requires C, 60.5; H, 6.3; N, 9.2; I, 8.3%).

N-2-(p-*Biphenylyl*)*isopropoxycarbonylasparaginyl*-4-*iodophenylalanyl*-*phenylalanyl*-*tryptophyl*-N$^{\epsilon}$-*t*-*butoxycarbonyllysyl*-O-*t*-*butylthreonyl*-*phenylalanyl*-O-*t*-*butylthreonyl*-O-*t*-*butylseryl*-S-*tritylcysteine t-Butyl Ester* (18).—A solution of compound (17) (1.03 g) and HOBt (104 mg) in DMF (2.25 ml) was cooled to −10 °C and a solution of DCC (155 mg) in DMF (0.25 ml) was added. The mixture was stirred at −10 °C for 1 h and then at 0 °C for 1 h and a solution of compound (16) (600 mg) and NEt_3 (0.11 ml) in DMF (1.75 ml) at 0 °C was added. The mixture was stirred at 0 °C for 1 h, insoluble matter was removed by filtration, and the filtrate was evaporated to dryness below 30 °C. The residue was dried *in vacuo* over concentrated H_2SO_4 for 4 h. The material was purified by counter-current distribution in solvent system A, methanol–buffer–chloroform–carbon tetrachloride (11 : 3 : 6 : 7 v/v).[16] The recovered material was triturated with light petroleum (b.p. 60—80 °C) and dried to constant weight. The *product* (1.36 g, 90%) had m.p. 216—218 °C (decomp.), $[\alpha]_D^{25}$ +2.3 ± 0.6° (*c* 1.0, DMF) (Found: C, 63.9; H, 6.75; I, 5.5; N, 8.35; S, 1.4. $C_{118}H_{148}IN_{13}O_{19}S$ requires C, 64.1; H, 6.7; I, 5.7; N, 8.2; S, 1.4%).

N-*Butoxycarbonylalanyl*-*glycyl*-S-*tritylcysteinyl*-N$^{\epsilon}$-*t*-*butoxycarbonyl*-*lysyl*-*asparaginyl*-4-*iodophenylalanyl*-*phenylalanyl*-*tryptophyl*-N$^{\epsilon}$-*t*-*butoxycarbonyl*-*lysyl*-O-*t*-*butylthreonyl*-*phenylalanyl*-O-*t*-*butylthreonyl*-O-*t*-*butylseryl*-S-*tritylcysteine t-Butyl Ester* (20).—Compound (18) (1.27 g) was dissolved in a mixture of glacial acetic acid (40 ml) and water (10 ml). The mixture was kept at 50 °C for 1 h and then evaporated to dryness. The residue was dissolved in DMF–MeOH (1 : 1 v/v) (10 ml) and EtOAc (25 ml) and light petroleum (b.p. 60—80 °C) 25 ml) were added. The mixture was kept at 4 °C for 1.5 h and the gelatinous material was recovered by filtration, suspended in light petroleum (100 ml) and stirred vigorously; the granular solid was filtered off and dried to constant weight. The base acetate salt (19) (1.09 g) had a content of 87.4% by amino-acid analysis, corresponding to a yield of 955 mg (0.47 mmol, 82%).

Compound (3) (624 mg) was dissolved in DMF (3.75 ml), the solution was cooled to −10 °C and 3.9M-HCl in EtOAc (0.48 ml) was added below −5 °C. t-Butyl nitrite (90 μl) was added, the mixture was stirred at −10 °C for 10 min and di-isopropylethylamine (DIEA) (0.35 ml) was added. A solution of (19) (0.47 mmol) and DIEA (80 μl) in DMF (2.25 ml) at −10 °C was added and the mixture was stirred at −10 °C for 1 h and then at 4 °C for 20 h. The mixture was evaporated to dryness and the residue was triturated with water. The solid obtained was crystallised twice from DMF–MeOH (1 : 5 v/v) to give the *product* (1.04 g, 81%), m.p. 226 °C (decomp.), $[\alpha]_D$ −0.1 ± 0.3° (*c* 1.1, DMF) (Found: C, 62.6; H, 7.0; I, 4.5; N, 8.9; S, 2.2. $C_{145}H_{189}IN_{18}O_{25}S_2$ requires C, 62.75; H, 6.9; I, 4.6; N, 9.1; S, 2.3%).

N$^{\alpha}$-*Benzyloxycarbonyltryptophyl*-N$^{\epsilon}$-*butoxycarbonyl*-*lysyl*-O-*t*-*butylthreonine* 2-*Trimethylsilylethyl Ester* (24).—(a) N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\epsilon}$-*t*-*butoxycarbonyl*-*lysyl*-O-*t*-*butylthreonine* 2-*trimethylsilylethyl ester* (22). Z-Thr(But^t)-OTmse [14] (16.4 g) was dissolved in propan-2-ol (200 ml), acetic acid (2.5 ml) was added, and the mixture was hydrogenated for 3 h at room temperature in the presence of 10% palladium–charcoal (2 g). The recovered acetate salt was dissolved in ethyl acetate (20 ml), the solution was cooled to −20 °C and 3.2M-HCl in ethyl acetate (12.5 ml) was added. The hydrochloride (21) could not be crystallised by addition of light petroleum and the solution was evaporated to dryness below 25 °C.

Z-Lys(Boc)-OH (15.2 g) and HOBt (6.1 g) were dissolved in DMF (100 ml), the solution was cooled to 0 °C, and DCC (8.25 g) in DMF (15 ml) was added. The solution was stirred for 1 h at 0 °C, then at 10 °C for 1 h, and then cooled again to 0 °C. The hydrochloride (21) (13.1 g) was dissolved in DMF (100 ml) at 0 °C, NEt_3 (5.6 ml) was added, and the solution was added to the active ester solution. The mixture was stirred at 0 °C for 1 h and then at 8 °C for 18 h, insoluble matter was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (200 ml) and extracted at 0 °C with 7% citric acid solution (2 × 100 ml), brine (100 ml), saturated $NaHCO_3$ solution (2 × 100 ml), brine (100 ml), and the solution was dried (Na_2SO_4) and evaporated. The oil (31.6 g) was dissolved in benzene (50 ml), and applied to a column (100 × 4 cm) of silica gel. Elution with benzene–ether (4 : 1 v/v) afforded the pure dipeptide (16.2 g; 65%), which was not crystallised.

(b) Compound (22) (16.2 g) was dissolved in propan-2-ol (130 ml), acetic acid (1.6 ml) was added, and the mixture was hydrogenated for 16 h at room temperature in the presence of 10% palladium–charcoal (1.5 g). The hydrochloride (23) (13.1 g) was obtained as described above for compound (21) and coupled with Z-Trp-OH (7.4 g) as described above for compound (22). The reaction mixture was purified by extraction as described for compound (22). Crystallisation from ethyl acetate–light petroleum (b.p. 60—80 °C) gave the *product* (12.5 g, 65%), m.p. 122—124 °C,

$[\alpha]_D^{25}$ $-16.1 \pm 0.6°$ (*c* 1.0, MeOH) (Found: C, 62.7; H, 7.85; N, 8.55. $C_{43}H_{65}N_5O_9Si$ requires C, 62.66; H, 7.95; N, 8.49%).

N$^{\alpha}$-*Benzyloxycarbonyltryptophyl*-*N*$^{\epsilon}$-*butoxycarbonyllysyl*-O-*t-butylthreonine* (25).—Compound (24) (4.8 g) was dissolved in DMF (16 ml) and added to a solution of Et_4NF (2.58 g) in DMF (100 ml). The solution was stirred at 30 °C for 0.5 h and then poured onto a mixture of crushed ice (800 ml) and 1M-HCl (17.5 ml). The precipitate was collected, washed with water, and dried over KOH pellets. The dry material (3.9 g, 99%) was dissolved in DMF (95 ml) and added dropwise with vigorous stirring to ice–water (950 ml). The collected, dried *acid* (3.1 g, 80%) had m.p. 149—153 °C, $[\alpha]_D^{25}$ $-9.9 \pm 0.6°$ (*c* 1.1, MeOH) (Found: C, 62.85; H, 7.45; N, 9.6. $C_{38}H_{53}N_5O_9$ requires C, 63.05; H, 7.38; N, 9.67%).

Tryptophyl-*N*$^{\epsilon}$-*butoxycarbonyl-lysyl*-O-*t-butylthreonine* (26).—Compound (25) (3.65 g) was dissolved in methanol–H_2O (9 : 1 v/v) (100 ml) and hydrogenated in the presence of 10% palladium–charcoal (0.6 g) for 2 h at room temperature. Catalyst was removed by filtration, the filtrate was evaporated to dryness, and the residue was crystallised twice from methanol–ether. The *sesquihydrate* (2.1 g, 71%) had m.p. 134—136 °C, $[\alpha]_D^{25}$ $+13.9 \pm 0.6°$ (*c* 1.0, MeOH) (Found: C, 58.25; H, 8.15; N, 11.35. $C_{30}H_{47}N_5O_7 \cdot 1.5H_2O$ requires C, 58.42; H, 8.17; N, 11.35%).

N-2-(p-*Biphenylyl*)*isopropoxycarbonyl*-4-*iodophenylalanine Cyclohexylamine salt* (27).—This was obtained from 4-iodophenylalanine and 2-(p-biphenylyl)isopropyl phenyl carbonate in 21% yield by the method of Sieber and Iselin [17] and repeatedly crystallised from acetone. The *salt* had m.p. 159—161 °C, $[\alpha]_D^{25}$ $+56.0 \pm 0.6°$ (*c* 1.0, DMF) (Found: C, 59.1; H, 5.95; I, 20.25; N, 4.5. $C_{31}H_{37}IN_2O_4$ requires C, 59.23; H, 5.93; I, 20.19; N, 4.45%).

N-2-(p-*Biphenylyl*)*isopropoxycarbonyl*-4-*iodophenylalanyl*-O-*t-butylthreonyl*-O-*t-butylseryl*-S-*tritylcysteine t-Butyl Ester* (28).—Bpoc-Phe(I)-OH (4.2 g) was regenerated from compound (27), dissolved in DMF (15 ml), cooled to −15 °C, and *N*-methylmorpholine (0.88 ml) and isobutyl chloroformate (1.04 ml) were added. The solution was stirred with exclusion of moisture for 10 min at −10 °C and then added to a solution in DMF (25 ml) at −10 °C of the free base (5.1 g) derived from compound (16) (6.05 g) by treatment with cold saturated aqueous $NaHCO_3$. The mixture was stirred for 1 h at −10 °C and then evaporated to dryness after removal of insoluble material by filtration. The residue was dissolved in ethyl acetate, purified by extraction, and crystallised from propan-2-ol. The *product* (6.2 g, 74%) had m.p. 130—132 °C, $[\alpha]_D^{25}$ $+22.8 \pm 0.6°$ (*c* 1.0, MeOH) (Found: C, 64.25; H, 6.5; I, 10.1; N, 4.65; S, 2.65. $C_{66}H_{79}IN_4O_9S$ requires C, 64.39; H, 6.46; I, 10.30; N, 4.55; S, 2.60%).

[4-*Iodo-Phe*^{6}]-*Somatostatin* (37).—Compound (20) (350 mg) was dissolved in DMF (21.3 ml) and added dropwise over 20 min to a well-stirred solution of I_2 (314 mg) in methanol (57 ml). The solution was stirred for a further 20 min and then 10% aqueous ascorbic acid was added dropwise until all the colour had disappeared. The solution was brought to pH 7.0 with 1M-NaOH, evaporated to dryness below 30 °C, the residue was triturated with ice–water, and the product was collected by filtration and dried. The material was dissolved in 90% aqueous trifluoroacetic acid (280 ml) and the solution was kept for 1 h at room temperature. The solution was evaporated to dryness below 30 °C and the residue was kept *in vacuo* over KOH pellets for 16 h. The material was dissolved in DMF (17.5 ml) and water (15 ml) and passed through a column (15 × 2 cm) of Dowex 1 (acetate form) resin which was eluted with 55% aqueous DMF (250 ml). The eluate was evaporated to dryness below 30 °C and the residue was dissolved in a mixture of 10 ml of each of the phases of the solvent system butan-1-ol–acetic acid–water (4 : 1 : 5 v/v), put in tube 4 of a 123-tube counter-current machine (10 ml each phase) and subjected to 118 transfers of upper phase. The *product* (101 mg, 52%) was recovered from tubes 29—53 by evaporation, solution of the residue in water, and freeze-drying. The amino-acid analysis is given in Table 1 (Found: C, 48.6; H, 6.1; I, 6.15; N, 12.3; S, 3.05. $C_{76}H_{103}IN_{18}O_{19}S_2 \cdot 2MeCO_2H \cdot 6H_2O$ requires C, 48.23; H, 6.22; I, 6.37; N, 12.65; S, 3.21%).

[4-^{3}H-*Phe*^{6}]-*Somatostatin* (40).—Compound (20) (10.8 mg) was dissolved in freshly distilled DMF (0.5 ml) and reduced using 98% tritium gas (3.2 ml, 8 Ci) in the presence of 10% palladium–charcoal (11.4 mg) and 5% rhodium–calcium carbonate (7.7 mg) for 30 min at room temperature. Catalysts were removed by filtration through a pad of cellulose and the filtrate was evaporated to dryness. The residue was dissolved in DMF (0.75 ml) and added over 20 min in a thin stream to a well-stirred solution of iodine (11.0 mg) in methanol (2 ml) at room temperature. The mixture was stirred for a further 20 min and aqueous ascorbic acid (10% w/v) was added dropwise until the colour of iodine was discharged. The solution was partitioned between the upper (10 ml) and lower (15 ml) phases of the solvent system A [see under compound (18)] and the lower phase and one further washing of the upper phase with fresh lower phase (5 ml) were combined and washed with fresh upper phase (2 × 15 ml). The solution was evaporated to dryness and the residue was treated with 90% trifluoroacetic acid (10 ml) for 1 h at room temperature. The solution was evaporated to dryness and the residue was dissolved in water (10 ml) and washed with chloroform (2 × 10 ml). The aqueous solution was evaporated to dryness and the residue was dissolved in water (1 ml) and passed through a column (1 ml) of Dowex 1 (acetate form) resin. The eluate was evaporated to dryness and the residue was dissolved in water (1 ml) and applied to a column (9 × 0.7 cm) of carboxymethylcellulose (trimethylammonium form). The column was eluted with a linear gradient (0—0.5M, 40 ml) of trimethylammonium acetate, pH 5.0, and fractions (1 ml) were collected automatically and monitored for radioactivity. Fractions 22—25 were combined and evaporated. Portions (1 ml) of water were evaporated from the residue until all the buffer had been removed. The residue was dissolved in water (0.53 ml) and the bulk (0.5 ml) of the solution was applied to a column (50 × 0.7 cm) of Nucleosil $10C_{18}$ ODS-silica using a Rheodyne six-port injection valve. The column was eluted at 6 ml min^{-1} with a constant volume (100 ml) gradient starting with methanol–water–H_3PO_4 (400 : 600 : 1 v/v) and ending with a composition of 800 : 200 : 1 (v/v). The eluate was monitored at 210 nm and fractions (0.5 min) were collected automatically. Fractions 21—25 were combined and evaporated to a volume of *ca.* 3 ml. The solution was passed through a column (1 ml) of Dowex 1 (acetate form) resin and the eluate was evaporated to dryness. The residue (1.03 μmol, 26%, 15.5 Ci $mmol^{-1}$) was dissolved in water (3.0 ml) and stored in liquid nitrogen. Analytical data are given in Table 3.

A portion of the peptide solution was reduced with β-mercaptoethanol and the thiol groups generated were reacted with ethyleneimine as previously detailed.[13] The

TABLE 3

Amino-acid analyses after acidic hydrolysis of iodine-containing somatostatins and of the metabolite [de-Ala^1]-somatostatin

	Somatostatin analogue			[de-Ala^1]	
	[4-Phe(I)^6]	[4-Phe(I)^{11}]	[4-Phe(I)^{6,11}]	Acidic digest	Enzymic digest [a]
Ala	1.00	1.02	1.01		
Asn					0.92
Asp	1.00	1.01	1.01	1.00	
Cys [b]	1.89	1.90	1.96	1.80	
Cys (AE)					1.72
Gly	1.00	1.02	1.01	1.00	0.91
Lys	2.03	1.99	2.02	2.02	1.87
Phe	2.00	2.00	1.00	3.00	3.00
Ser + Thr	2.76	2.72	2.72	2.71	3.04
Trp	0.26	0.27	0.23	0.34	0.94
Phe(I)	1.01	1.03	2.01		

[a] After reduction and aminoethylation. [b] Sum of all Cys species.

recovered derivative was digested with a mixture of carrier-bound enzymes. The results are given in Table 1.

N-t-Butoxycarbonyl-glycyl-S-tritylcysteinyl-N$^\epsilon$-t-butoxycarbonyl-lysine Methyl Ester (43).—H-Cys(Trt)-Lys(Boc)-OMe·HCl (6.5 g) was converted to the free base by the method described in the preparation of compound (2). The solution was added to a solution of Boc-Gly-OH (1.78 g), *N*-hydroxybenzotriazole hydrate (1.51 g), and dicyclohexylcarbodiimide (2.27 g) in a mixture of MeCN (53 ml) and DMF (5.3 ml) which had been pre-incubated for 1 h at 0 °C and then 1 h at 10 °C, and from which dicyclohexylurea had been removed by filtration. After 16 h at 4 °C the solid was filtered off and recrystallised first from benzene–cyclohexane, then from benzene, to give the required *product* (4.40 g; 61%), m.p. 135—138 °C, $[\alpha]_D^{25}$ −2.1 ± 0.3° (*c* 1.0, MeOH) and +3.5 ± 0.3° (*c* 1.0, $CHCl_3$) (Found: C, 64.8; H, 7.2; N, 7.25; S, 4.2. $C_{41}H_{54}N_4O_8S$ requires C, 64.54; H, 7.13; N, 7.34; S, 4.20).

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