Tritiated Peptides. Part 15.[1] Synthesis of Tritium Labelled Biologically Active Analogues of Somatostatin

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The syntheses are described of *cyclo*-{[D-Trp^{8},Gaba^{12}]somatostatin-(5—12)-peptide}† (**77**), and (**79**)—(**82**) labelled singly at positions 6, 7, 8, and 11 and doubly at residues 6 and 11 to specific radioactivities of between 4.8 and 22.4 Ci mmol^{-1}. The linear sequence (5—12) and the related *cyclo*-{[D-Trp^{8},Nag^{12}]somatostatin-(5—12)-peptide} (**78**) and (**83**) were also prepared to specific radioactivities of 19.2 and 19.6 Ci mmol^{-1} respectively. The syntheses of the labelled hexapeptide *cyclo*-{[4-^{3}H-Phe^{7},D-Trp^{8},Pro^{12}]somatostatin-(7—12)-peptide} (**84**) and the full sequences [4-^{3}H-Phe^{6},D-Trp^{8},D-Cys^{14}]somatostatin (**94**) and [D-Trp^{8},4-^{3}H-Phe^{11},D-Cys^{14}]somatostatin (**95**) labelled at *ca.* 13.0 Ci mmol^{-1} are described. Labelling was effected by reductive dehalogenation in the presence of tritium of the fully protected precursors and the purity of the final products was assessed by amino acid analysis after acidic hydrolysis following purification by ion-exchange and h.p.l.c. as appropriate.

Attempts to modify the activity profile of somatostatin by structural alterations has shown the octapeptide *cyclo*-{[D-Trp^{8},Gaba^{12}]somatostatin-(5—12)-peptide} to be of interest in terms of both enhanced biological activity and duration of action.[2] Specifically labelled highly radioactive forms of the cyclic analogue were required to enable studies of its handling *in vivo*.[3] Analogues possessing tritium located specifically in positions 6 and 11, compounds (**77**), (**79**), and (**80**) were prepared and metabolic studies[4] with these compounds though generating much information showed that analogues with tritium in positions 7 and 8 (**81**) and (**82**) were necessary to provide unequivocal data regarding initial proteolysis of the tritiated peptide. These compounds and a linear octapeptide sequence (**78**) were subsequently prepared.

Substitution in the cyclic octapeptide of the residue of 4-aminobutanoic acid by 4-amino-3-(1-naphthyl)butanoic acid has been shown to increase markedly certain of the molecule's activities.[5] In order to synthesize this analogue in a labelled form (**83**) it was first necessary to develop a resolution of 4-amino-3-(1-naphthyl)butanoic acid.

The cyclic tetradecapeptide [D-Trp^{5},D-Cys^{14}]somatostatin had been claimed in early experiments[6] to differentiate selectively between some of the activities of the parent compound in such a way as to be of potential value in the treatment of diabetes mellitus.[7] The synthesis of two tritiated analogues (**94**) and (**95**) of this peptide are described. The hexapeptide, *cyclo*-{[D-Trp^{8},Pro^{12}]somatostatin-(7—12)-peptide} has also been shown to be of potential clinical value in the treatment of diabetic conditions[8] and its preparation in a specifically tritiated form (**84**) is also described.

The labelled analogues were prepared by catalytic dehalogenation of suitably protected precursors in the presence of tritium gas by a method first described by us for β-corticotrophin-(1—24)-tetracosapeptide.[9]

† Somatostatin =

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys. In this paper, all amino-acid residues are L unless specifically stated otherwise. 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. Committee on Biochemical Nomenclature entitled 'Nomenclature and Symbolism for Amino Acids and Peptides' (Recommendations 1983). In addition Gaba = 4-aminobutanoic acid, Nag = 4-amino-3-(1-naphthyl)butanoic acid, and TFE = 2,2,2-trifluoroethanol. To avoid ambiguity the numbering of the parent peptide is retained.

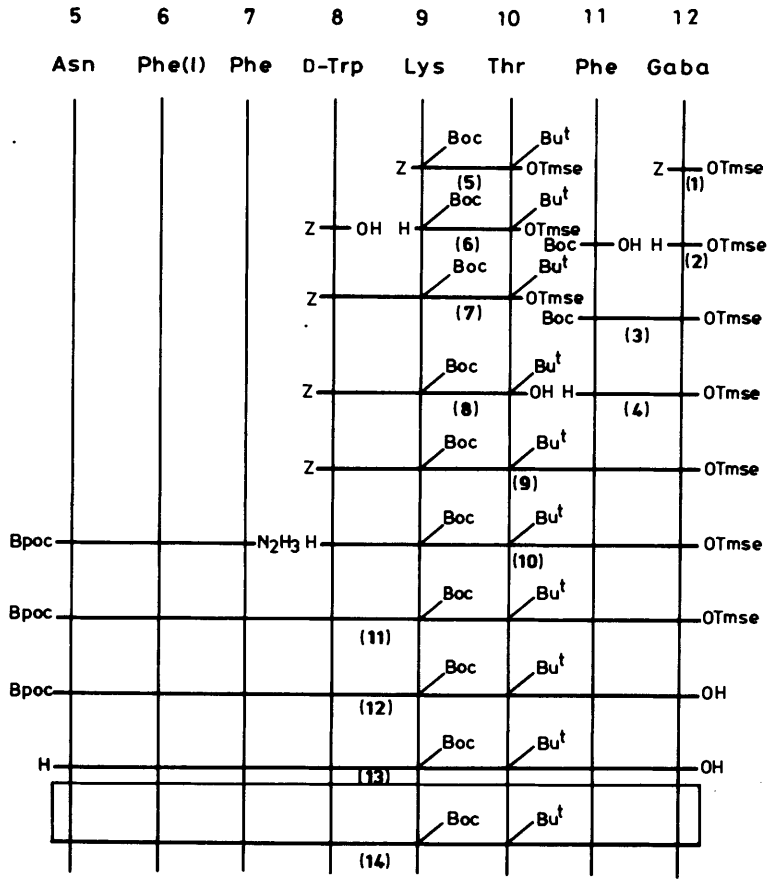
Results and Discussion

All the peptides described were synthesized by solution-phase procedures. The presence of individual residues in the sequences that are resistant to racemisation under coupling conditions, *i.e.* 4-aminobutanoic acid, proline, and 4-amino-3-(1-naphthyl)-butanoic acid, strongly recommended their use as the carboxy terminal residues for cyclisation. The efficient cyclisation at 4-aminobutanoic acid of the equivalent unhalogenated linear octapeptide has already been reported[10] using a large excess of activating agent (dicyclohexylcarbodi-imide–hydroxybenzotriazole, DCC–HOBt).

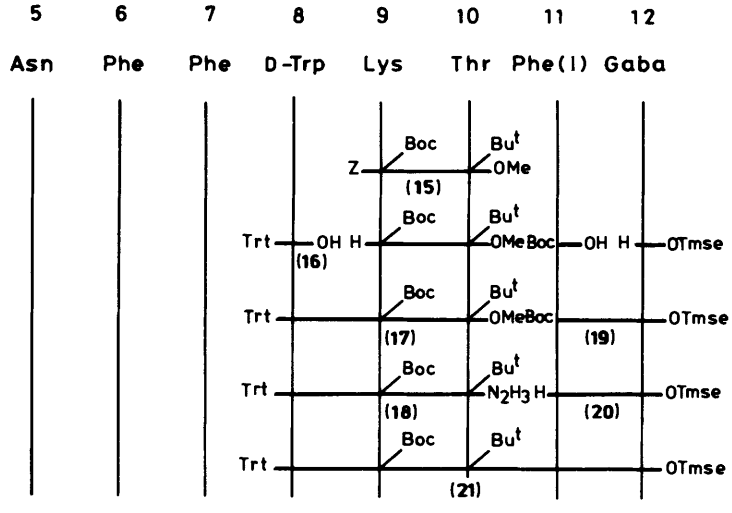
't-Butyl Tactics' as described by Kamber[11] were employed for functional group protection with the additional use of 2-trimethylsilylethyl ester to permit selective de-esterification in the presence of acid- and reductively-labile groups, and the trityl group for thiol- and occasionally amino-protection. The routes adopted were dictated both by the scarcity of the unusual or halogenated amino acid and the availability of fragments described here and previously.[3] Those fragments required for the synthesis of the octapeptide (**14**) containing 4-iodophenylalanine at residue 6 that had not been previously prepared were synthesised by stepwise addition to the carboxy terminal amino acid. The two fragments (**4**) and (**8**) (Scheme 1) were coupled by the DCC–HOBt procedure.[12] Addition of the suitably protected 5—7 fragment[3] was accomplished by the azide method[13] and selective removal of both α-carboxy and α-amino protection was performed by established methods to provide the side-chain protected octapeptide (**13**). The cyclic product (**14**) was obtained in 31% yield from (**13**) after treatment with a 10-fold molar excess of both DCC and HOBt for 16 h at 50 °C in dilute dimethylformamide solution, followed by purification by counter-current distribution, adsorption chromatography, and crystallisation.

Synthesis of an octapeptide analogue containing 4-iodophenylalanine in the 11-position by the route outlined previously (Scheme 1) restricted the choice of amino-protection of the 8—10 fragment to either the 2-(biphenyl-4-yl)isopropoxycarbonyl (Bpoc)‡ or trityl groups. Neither group proved to very satisfactory though trityl protection allowed the synthesis of the protected *C*-terminal pentapeptide (**21**) (Scheme 2) albeit in very low yield. The difficulties encountered during this synthesis probably resulted from steric interference—a problem often

‡ Bpoc = 2-biphenyl-4-yl-1-methylethoxycarbonyl



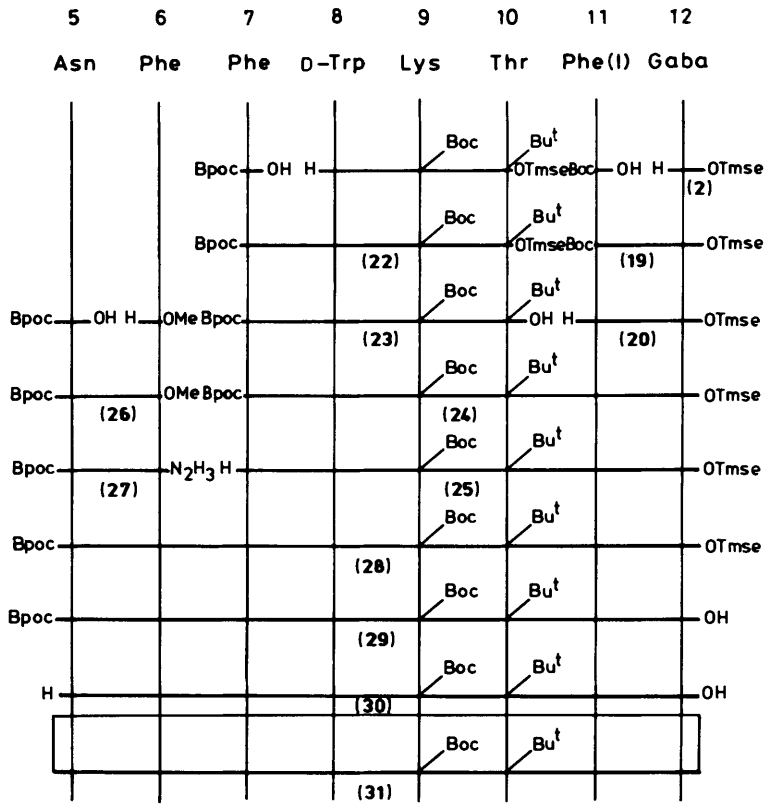
Scheme 1.



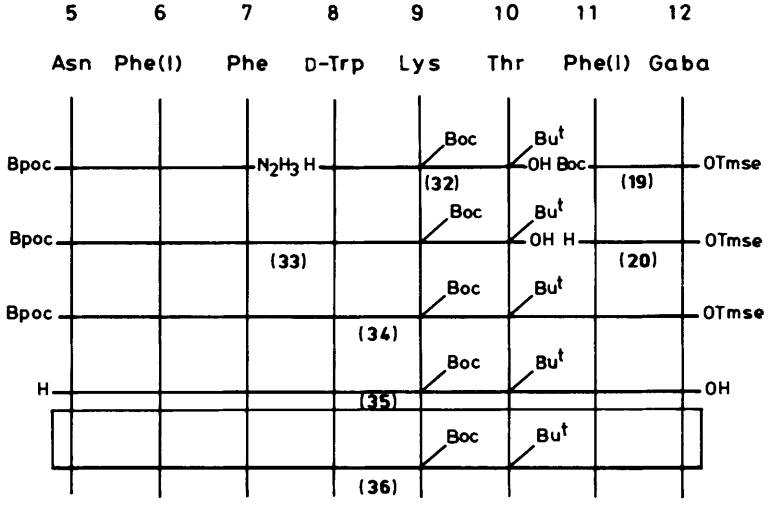
Scheme 2.

associated with this protecting group. Alteration of the route to incorporate a final 2 + 6 coupling with initial 2-(biphenyl-4-yl)isopropoxycarbonyl amino-protection of the 7—10 fragment (Scheme 3) however, was found to be satisfactory, and provided the required product (**31**) in good overall yield.

A slight alteration in the order of fragment coupling from that described for the previous analogues allowed the use of already available fragments for the synthesis of the analogue containing 4-iodophenylalanine in both the 6- and 11-positions. The fragments corresponded to the 5—7, 8—10, and 11—12 sequences (Scheme 4). Formation of the 5—10 followed by the 5—12 sequence then avoided the earlier problem of *N*-terminal protection for the 8—10 fragment. Fragment couplings, terminal deprotection and cyclisation were carried out in a



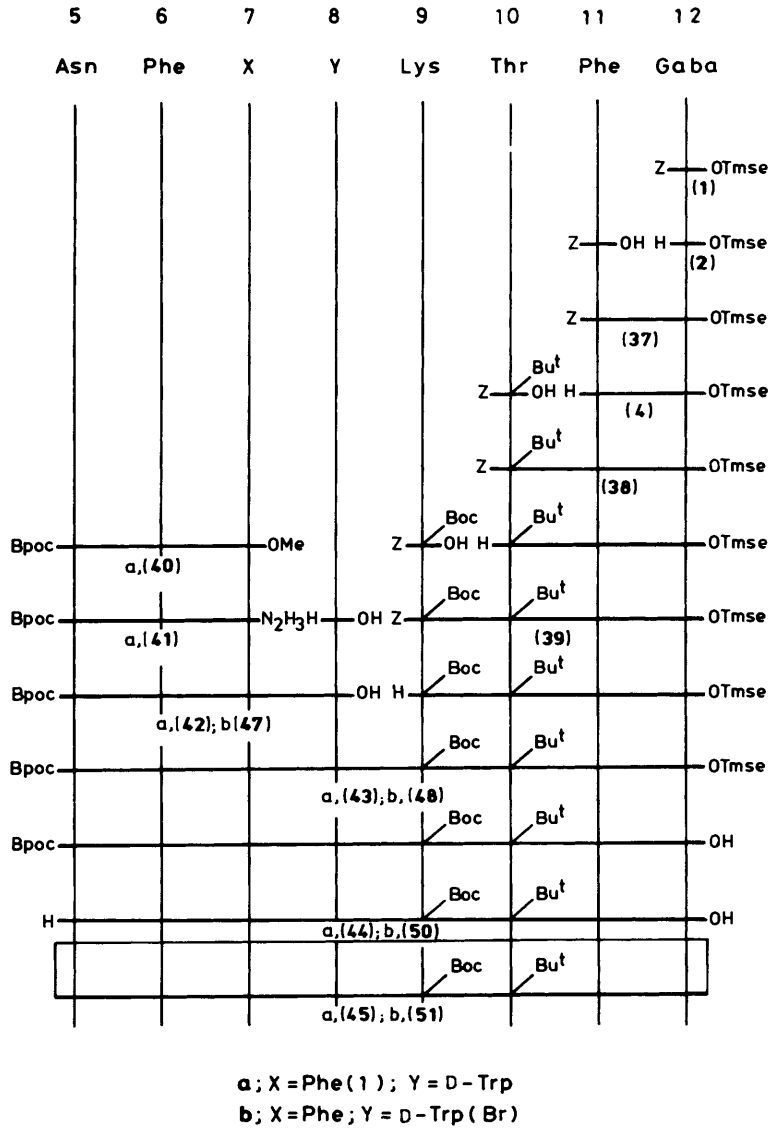
Scheme 3.



Scheme 4.

manner exactly analogous to the previous examples and gave the pure protected cyclic octapeptide (**36**) in 32% yield from compound (**34**).

Earlier results had shown a need for analogues with tritium in positions 7 and 8. It was expected that the synthesis of the [4-I-Phe^7] precursor would be achieved more easily than the synthesis of the brominated D-tryptophan precursor, though it was probable that unequivocal metabolic data could only be obtained from the last named analogue. Consequently, both analogues were prepared and similar routes were followed enabling the use of common intermediates. The approach adopted made use of a final 4 + 4 coupling with the *C*-terminal tetrapeptide built up by DCC–HOBt-mediated stepwise couplings and the *N*-terminal tetrapeptide constructed by a 3 + 1 azide coupling. In this way, the synthesis of the [4-I-Phe^7] analogue became a model for the synthesis of the labelled [D-Trp^8] analogue, involving the residue in position 8 in as few operations as possible and without the need for either its N^{α} or



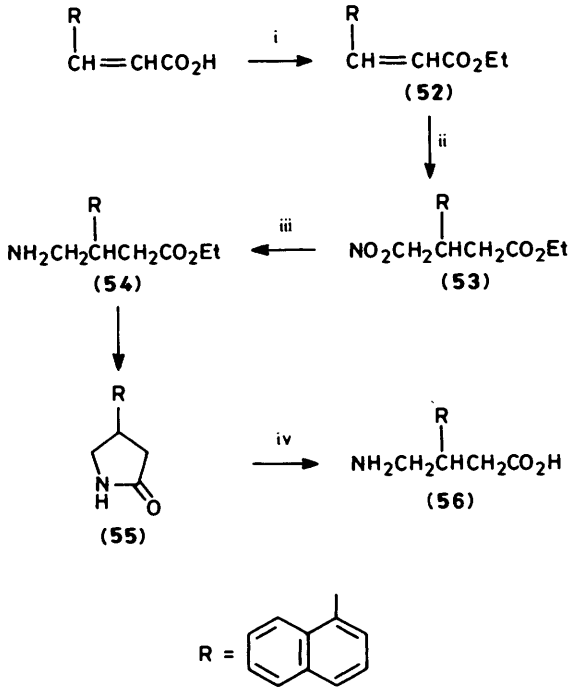
Scheme 5.

C^{α} protection. This last consideration was crucial considering the lengthy synthesis of the halogenated amino acid precursor of tritiated tryptophan.

A novel synthesis of 4-amino-3-(1-naphthyl)butanoic acid based on a Michael addition of nitromethane to ethyl 3-(1-naphthyl)prop-2-enoate (**52**) followed by catalytic hydrogenation, lactam formation, and hydrolysis (Scheme 6) provided the racemic amino acid (**56**) in 46% yield from compound (**52**). The Michael addition reaction was monitored by u.v. spectroscopy, observing the disappearance of the acrylate ester absorption at 321 nm and was complete in 6 h—considerably shorter than the corresponding reaction time (68 h) for the synthesis of ethyl 4-nitro-3-phenylbutanoate.[14] Complete resolution was accomplished by the fractional crystallisation of the strychnine salt of the phthaloyl derivative and was confirmed after reaction of both the resolved and racemic amino acid with D-glutamic acid n-carboxyanhydride[15] by h.p.l.c. analysis of the diastereoisomers.

Efficient use of the appropriate isomer of 4-amino-3-(1-naphthyl)butanoic acid was effected by its incorporation in the required sequence during the final coupling by an unconventional 7 + 1 condensation (Scheme 7). Synthesis of the *N*-terminal heptapeptide (**65**) proceeded along conventional lines, with an azide coupling between fragments 5—7 and 8—11 (**64**). The final 7 + 1 coupling was DCC–HOBt-mediated using 1,1,3,3-tetramethylguanidine as the only base tried that enabled dissolution of the free *C*-terminal amino acid (**60**) in dimethylformamide at the temperature (−5 °C) and concentration (0.6M) required. Removal of dicyclohexylurea from the solution of the activated carboxyl compound showed that precipitation of the starting materials, already at the limits of their solubility, had not occurred. Trituration gave a crude product which on crystallisation afforded the protected octapeptide acid (**66**) in 65% yield from compound (**65**). *N*-Terminal deprotection, cyclisation, and purification were performed by the methods described previously to provide the iodinated precursor in good yield.

The synthesis of the iodinated, protected cyclic hexapeptide (**76**) was achieved in a stepwise fashion by DCC–HOBt mediated couplings starting from proline 2-trimethylsilylethyl ester with benzyloxycarbonyl α-amino protection for all except the final step where 4-iodophenylalanine was added as its 2-



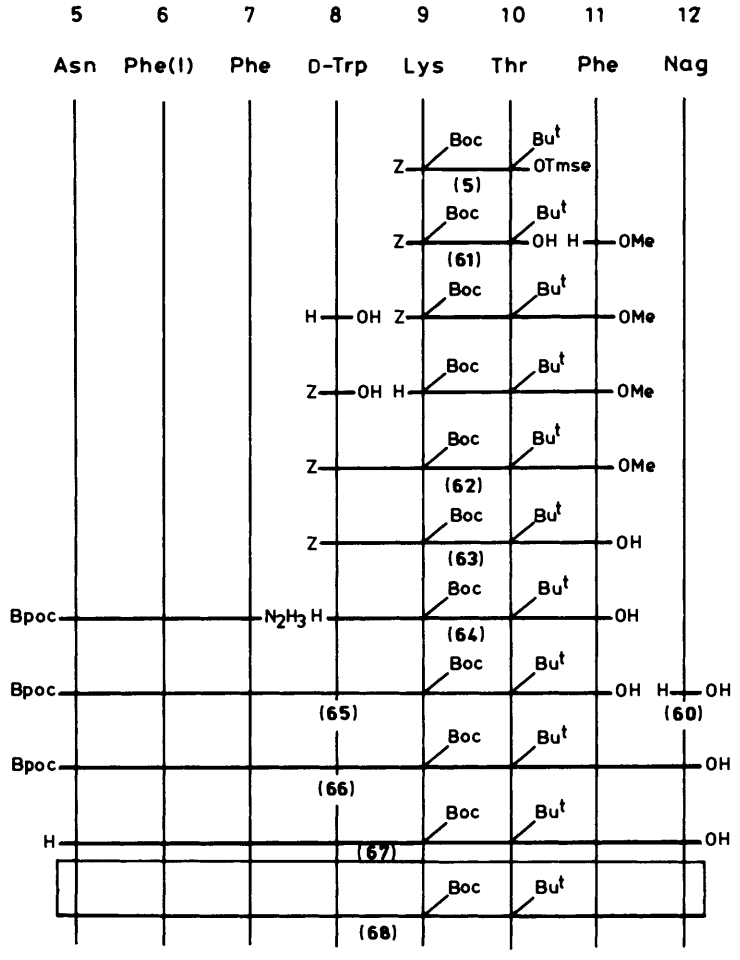
Scheme 6. *Reagents:* i, EtOH–HCl–reflux, 1.5 h; ii, $MeNO_2$–Triton B–68 °C, 6 h; iii, H_2–10% Pd–C; iv, HCl–HOAc–reflux, 24 h

(biphenyl-4-yl)isopropoxycarbonyl derivative. Deprotection at the amino and carboxy termini and cyclisation of the linear peptide was performed as described previously [for compound (**14**)] and gave the cyclic peptide (**76**) in 38% yield overall from the protected linear hexapeptide (**73**) following purification by counter-current distribution and crystallisation from benzene–light petroleum (b.p. 60—80 °C).

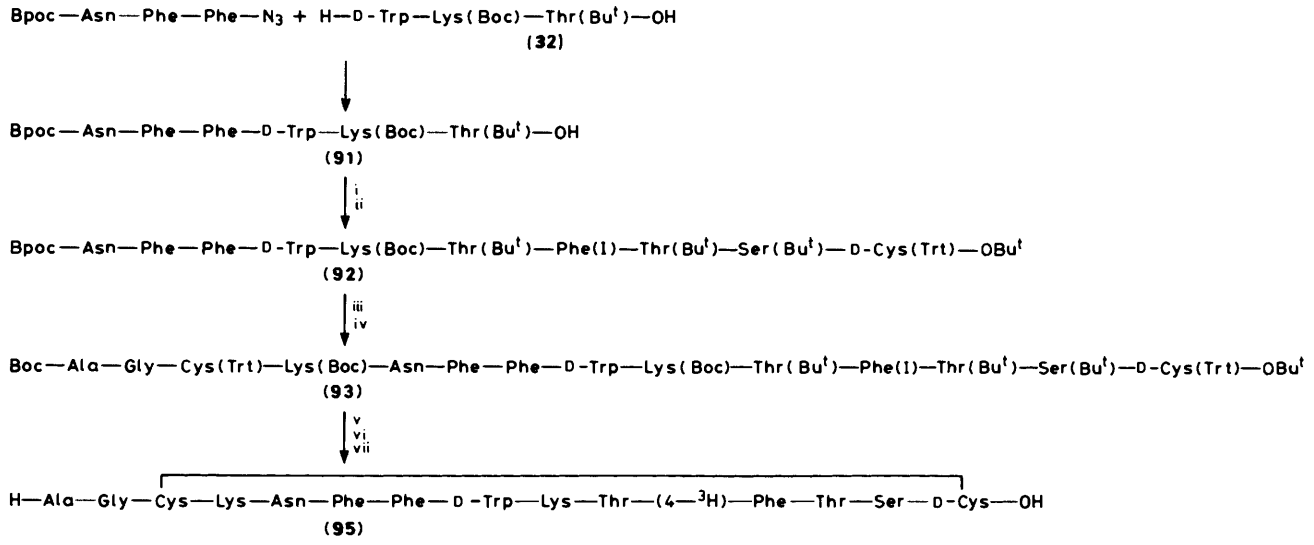
The two tritiated tetradecapeptide analogues, compounds (**94**) and (**95**), were synthesized by routes (Schemes 8 and 9) analogous to those for the all L-peptides.[3] The low solubility of both products required that they were dissolved in aqueous acetic acid and not water alone for application to the column for h.p.l.c. purification. The specific radioactivities obtained upon catalytic tritiation and the analytical data are shown in Table 1 and are of the same order as those obtained for the native molecule.[3]

The optical integrity of all the final products was assessed by amino acid analysis following enzymic hydrolysis.[3] Enzymic digestion of the cyclic octapeptides established that the 7—9 and 12—5 sequences were resistant to enzymolysis, revealing only the phenylalanine residues at positions 6, 11, and threonine—all in good agreement with theory. Tryptophan and lysine were detected at levels no greater than 0.03 residues (*i.e.* within experimental error). Similar treatment of the aminoethylated tetradecapeptides provided results entirely consistent with those expected for a racemisation-free product.

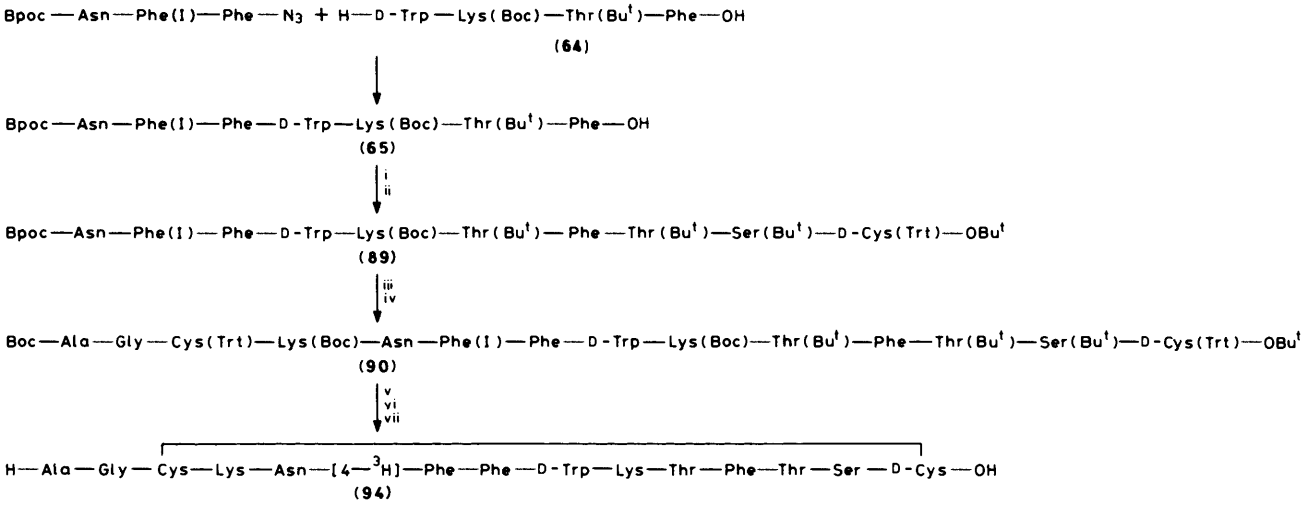
Labelling of the shorter sequences [Compounds (**77**)—(**84**)] was effected in a similar manner to that described above except that the purification procedure was simplified by omitting both



Scheme 7.



Scheme 8. *Reagents:* i, DCC–HOBt; ii, H-Phe(I)-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t; iii, H^+; iv, Boc-Ala-Gly-Cys-(Trt)-Lys(Boc)-N_3; v, $^3H_2$–Pd–C, Rh–$CaCO_3$; vi, I_2–MeOH; vii, 90% aqueous TFA.



Scheme 9. *Reagents:* i, DCC–HOBt; ii, H-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t; iii, H^+; iv, Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-N_3; v, $^3H_2$–Pd–C, Rh–$CaCO_3$; vi, I_2–MeOH; vii, 90% aqueous TFA.

the first Dowex and subsequent carboxymethylcellulose ion-exchange treatments which had been found to be essential in removing products arising from desulphurisation in those cases. The specific radioactivities and analytical details of these eight compounds are given in Table 1.

The labelling results revealed several interesting features we feel worthy of further mention. An earlier attempt in these laboratories[3] to increase the specific radioactivity of a tritiated somatostatin analogue by including two residues of 4-iodophenylalanine in its precursor had resulted in the achievement of no greater specific activity than was possible through the incorporation of a single 4-iodophenylalanine residue. It was suggested this was a result of catalyst poisoning by sulphur-containing amino acids. Therefore the absence of sulphur in the octapeptide sequence increased the likelihood of achieving an enhancement of specific radioactivity by incorporation of 4-iodophenylalanine in both the 6 and 11-position. This was substantiated by the result obtained. Although the extent of tritium incorporation was virtually doubled, the activity obtained was less than half that theoretically possible. The possibility that the conformational rigidity of the molecule imposed by its cyclic structure may have resulted in allowing only a reduced accessability of the iodinated residues to the catalyst during reduction was examined by labelling the linear protected octapeptide (**11**). Incorporation of tritium as a percentage of that theoretically achievable increased from 43% for the cyclic (**77**) to 66% for the linear peptide (**78**). The small increase indicated that restraint of conformation was probably a factor affecting efficient labelling.

The successful tritiation of a tryptophan residue in compound (**82**) has been achieved through the corresponding 5-bromo derivative. This was in contrast to earlier work where the synthesis and inclusion of the 5-bromo derivative into an analogue of ACTH had been achieved,[16] but experimental

Table 1. Amino acid analysis of tritiated somatostatin analogues

Compound	Prepared from	Method as for	Yield (%)	Amino acid analysis after acidic hydrolysis: Als	Asp	Cys[a]	Gaba	Gly	Lys	Nag	Phe	Pro	Ser	Thr	Trp	Specific activity Ci mmol^{-1}
-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba- (5 6 7 8 9 10 11 12)																
(77) *cyclo*[4-^{3}H-Phe^6]-	(14)		69	—	0.98	—	0.98	—	1.02	—	3.00	—	—	0.93	0.83	12.6
(78) linear[4-^{3}H-Phe^6]-	(11)	(77)	24	—	1.03	—	0.98	—	1.05	—	3.00	—	—	1.06	0.77	19.2
(79) *cyclo*[4-^{3}H-Phe^{11}]-	(31)	(77)	46	—	1.00	—	1.01	—	1.04	—	3.00	—	—	0.96	0.67	12.4
(80) *cyclo*[4-^{3}H-Phe^{6,11}]-	(36)	(77)	59	—	0.98	—	0.99	—	1.01	—	3.00	—	—	0.95	0.77	22.4
(81) *cyclo*[4-^{3}H-Phe^7]-	(45)	(77)	63	—	0.99	—	1.00	—	0.99	—	3.00	—	—	0.94	0.84	14.7
(82) *cyclo*[5-^{3}H-D-Trp^8]-	(51)	(77)	40	—	1.00	—	1.00	—	1.00	—	3.00	—	—	0.95	0.79	4.8
(83) *cyclo*[4-^{3}H-Phe^6-Nag^{12}]-	(68)	(77)[b]	36	—	0.99	—	—	—	1.01	1.00	3.00	—	—	0.94	0.70	19.6
(84) *cyclo*-des-Asn^5,Phe^6-[4-^{3}H-Phe^7,Pro^{12}]-	(76)	(77)	49	—	—	—	—	—	1.02	—	2.00	1.00	—	1.00	0	13.0
(94) [4-^{3}H-Phe^6,D-Trp^8,D-Cys^{14}]somatostatin	(90)		20	1.00	1.01	1.88	—	1.02	2.01	—	3.00	—	—	2.73[c]	0.43	12.5
(95) [D-Trp^8,4-^{3}H-Phe^{11},D-Cys^{14}] somatostatin	(93)	(94)	18	0.97	1.02	1.76	—	1.01	1.98	—	3.00	—	—	2.64[c]	0.49	12.7

[a] Sum of all Cys species. [b] Eluting gradient from Nucleosil 10C$_{18}$ column: acetonitrile–water–phosphoric acid (300:700:1 to 600:400:1). [c] Ser + Thr

conditions for its complete reduction could not be found. We felt that this was due to catalyst poisoning by sulphur and therefore that the sulphur-free octapeptide analogue of somatostatin should be more amenable to reductive dehalogenation than the ACTH analogue. The level of tritiation obtained (Table 1) validated this view.

Experimental

Experimental details of compounds not described individually are given in Tables 2 and 3.

4-(*t-Butoxycarbonylphenylalanylamino*)*butanoic Acid* 2-*Trimethylsilylethyl Ester* (**3**).—(*a*) 4-*Benzyloxycarbonylaminobutanoic acid* 2-*trimethylsilylethyl ester* (**1**). This was prepared from Z-Gaba-OH and 2-trimethylsilylethyl alcohol by the method of Sieber[17] using pyridine and dicyclohexylcarbodiimide (DCC) and purification on a column of silica by elution with ethyl acetate yielded a chromatographically homogeneous viscous oil (74%).

(*b*) 4-*Aminobutanoic acid* 2-*trimethylsilylethyl ester hydrochloride* (**2**). Hydrogenolysis of Z-Gaba-OTmse (**1**) (14.3 g) in propan-2-ol (210 ml) containing glacial acetic acid (2.7 ml) over 10% palladium–charcoal (2.5 g) provided the base acetate salt which was converted to the hydrochloride salt (**2**) (10.3 g, 100%) by dissolution in ethyl acetate (10 ml) and treatment with 3.1M-HCl in ethyl acetate (13.7 ml) at −20 °C. After evaporation under reduced pressure the solid residue was pumped over KOH pellets.

A solution of Boc-Phe-OH (6.06 g) and *N*-hydroxybenzotriazole hydrate (HOBt) (3.5 g) in DMF (57 ml) was cooled to 0 °C and treated with a solution of DCC (5.2 g) in DMF (7 ml). The mixture was stirred for 1 h at 0 °C and 1 h at 10 °C. The reaction mixture was cooled to 0 °C and a solution of H-Gaba-OTmse·HCl (**2**) (5.5 g) and di-isopropylethylamine (DIEA) (3.94 ml) in DMF (57 ml) was added. The mixture was stirred for 1 h at 0 °C and for 16 h at 4 °C. The mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (150 ml), cooled to 0 °C and extracted with 7% (w/v) aqueous citric acid solution (2 × 100 ml), saturated aqueous $NaHCO_3$ solution (2 × 100 ml), and aqueous 10% NaCl solution (2 × 100 ml). After drying (Na_2SO_4) the solution was evaporated to give a brown oil (9.2 g, 89%), which was dissolved in chloroform (25 ml) and applied to a silica pad (9 × 17 cm) which was eluted with chloroform and evaporated to yield a chromatographically homogeneous product (7.6 g, 72%) as a viscous oil.

4-*Phenylalanylaminobutanoic Acid* 2-*Trimethylsilylethyl Ester Hydrochloride* (**4**).—Boc-Phe-Gaba-OTmse (**3**) (5.0 g) was dissolved in 3M-HCl in ethyl acetate (220 ml), the solution was stored for 30 min at room temperature then evaporated to dryness below 30 °C, and the residue (4.6 g) was stored overnight *in vacuo* (NaOH pellets). The product was dissolved in chloroform (25 ml) and applied to a pad (5 × 11 cm) of silica which was eluted with chloroform–methanol (95:5 v/v). Fractions containing purified product were combined, evaporated to dryness and pumped to give the hydrochloride (3.25 g, 76%).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonine* 2-*Trimethylsilylethyl Ester* (**5**).—A solution containing *N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\varepsilon}$-t-butoxycarbonyl-lysine *N*-hydroxysuccinimide ester (7.7 g) and *O*-t-butylthreonine methyl ester hydrochloride (4.4 g) in acetonitrile (43 ml) was treated with NEt_3 (2.6 ml) and stirred for 6 h at 20 °C before pouring onto cold water (175 ml). The oily precipitate was extracted into ethyl acetate (2 × 150 ml), and the extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in chloroform and filtered through a silica pad (10 × 20 cm), eluting with chloroform. Fractions containing homogeneous product were combined, evaporated to dryness and dried *in vacuo* to provide the dipeptide ester (8.5 g, 95%) as a crisp foam.

N$^{\alpha}$-*Benzyloxycarbonyl*-D-*tryptophyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonine* 2-*Trimethylsilylethyl Ester* (**7**).—Z-Lys(Boc)-Thr(Bu^t)-OTmse (**5**) (17 g) was debenzyloxycarbonylated by the method used to prepare H-Gaba-OTmse·HCl (**2**). Z-D-Trp-OH (7.8 g) and H-Lys(Boc)-Thr(Bu^t)-OTmse·HCl (**6**) (13.9 g) were coupled by the DCC–HOBt procedure (NEt_3 as the base) and washed as described for compound (**3**).

Table 2.

	Compound	Prepared from	Method as for	M.p. (°C)	$[\alpha]_D^{25}$ (°)	Yield (%)	Found (%) C	H	N	Br	I	Formula	Requires (%) C	H	N	Br	I
(15)	Z-Lys(Boc)-Thr(Bu^t)-OMe	Z-Lys(Boc)-OH + H-Thr(Bu^t)-OMe	**(5)**	oil		95											
(19)	Boc-Phe(I)-Gaba-OTmse	Boc-Phe(I)-OH + H-Gaba-OTmse	**(3)**	99—101[a]	+6.9 ± 0.6[b]	50	48.05	6.45	4.9		21.95	$C_{23}H_{37}IN_2O_5Si$	47.91	6.46	4.85		22.01
(22)	Bpoc-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-OTmse	Bpoc-Phe-OH + **(7)**	**(3)**	123—124[a]	−15.0 + 0.6[b]	46	66.9	7.65	7.75			$C_{60}H_{82}N_6O_{10}Si$	67.01	7.68	7.81		
(23)	Bpoc-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-OH	**(22)**	**(12)**[c]	118[a] (decomp)	−11.0 ± 0.6[b]	94	67.55	7.3	8.6			$C_{55}H_{70}N_6O_{10}$	67.74	7.23	8.62		
(24)	Bpoc-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-OTmse	**(23)** + **(3)**	**(3)**	174—175[d]		76	61.0	6.7	7.75		8.85	$C_{73}H_{97}IN_8O_{12}Si$	61.15	6.82	7.81		8.85
(26)	Bpoc-Asn-Phe-OMe	Bpoc-Asn-OH + H-Phe-OMe	**(3)**	107—109[a]	−10.8 ± 0.6[b]	64	67.7	6.45	7.6			$C_{30}H_{33}N_3O_6$	67.77	6.25	7.90		
(27)	Bpoc-Asn-Phe-$NHNH_2$	**(26)**	**(18)**	148—150[e]	−27.4 ± 0.6[f]	66	65.0	6.25	13.15			$C_{29}H_{33}N_5O_5 \cdot 0{\cdot}25H_2O$	64.96	6.29	13.06		
(28)	Bpoc-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-OTmse	**(27)** + **(24)**	**(11)**	195[d] (decomp)	−8.1 ± 0.6[f]	70	60.89	6.6	9.15		7.4	$C_{86}H_{112}IN_{11}O_{15}Si$	60.94	6.66	9.09		7.48
(30)	H-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-OH	**(28)**	**(12)**, **(13)**	320[g] (decomp)	−3.7 ± 0.6[f]	75	57.15	6.35	11.35		9.15	$C_{65}H_{86}IN_{11}O_{13} \cdot 0.5H_2O$	57.17	6.42	11.28		9.29
(31)	*cyclo*[-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-]	**(30)**	**(14)**	176 (decomp)	+17.2 ± 0.6[b]	49	57.45	6.25	11.3		9.2	$C_{65}H_{84}IN_{11}O_{12} \cdot H_2O$	57.55	6.39	11.36		9.35
(32)	H-D-Trp-Lys(Boc)-Thr(Bu^t)-OH	**(8)**	**(17)**	132—135[h]	−33.8 ± 0.5[b]	93	58.8	8.1	11.45			$C_{30}H_{47}N_5O_7 \cdot 1.5H_2O$	58.42	8.17	11.35		
(33)	Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-OH	Bpoc-Asn-Phe(I)-Phe-$NHNH_2$ + **(32)**	**(11)**[i]	137[j] (decomp)	−14.6 ± 0.6[b]	25	59.6	6.25	9.15		9.6	$C_{68}H_{84}IN_9O_{13} \cdot 0.5H_2O$	59.55	6.24	9.19		9.25

(34)	Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-OTmse	**(33)** + H-Phe(I)-Gaba-OTmse	**(3)**	240[k] (decomp)	−7.9 ± 1.2[f]	68	56.5	6.15	8.5		13.8	$C_{86}H_{111}I_2N_{11}O_{15}Si$	56.72	6.14	8.46		13.93
(35)	H-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-OH	**(34)**	**(13)**	320[i] (decomp)	−1.6 ± 1.8[f]	80	52.55	5.85	10.35		16.95	$C_{65}H_{85}I_2N_{11}O_{13}$	52.66	5.78	10.39		17.12
(36)	*cyclo*[-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Gaba-]	**(35)**	**(14)**	163 (decomp)	+1.5 ± 1.0[f]	40	52.65	5.75	10.35		16.95	$C_{65}H_{83}I_2N_{11}O_{12}\cdot H_2O$	52.66	5.78	10.39		17.12
(37)	Z-Phe-Gaba-OTmse	Z-Phe-OH + H-Gaba-OTmse	**(3)**	91—92[d]	−0.5 ± 0.6[b]	61	64.55	7.45	5.75			$C_{26}H_{36}N_2O_5Si$	64.63	7.48	5.78		
(38)	Z-Thr(Bu^t)-Phe-Gaba-OTmse	Z-Thr(Bu^t)-OH + **(37)**	**(3)**	107—109[d]	−3.1 ± 0.6[b]	55	63.65	8.15	6.5			$C_{34}H_{51}N_3O_7Si$	63.61	8.00	6.54		
(39)	Z-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse	Z-Lys(Boc)-OH + **(38)**	**(3)**	142—144[d]	−12.3 ± 0.6[b]	58	62.4	8.4	8.0			$C_{45}H_{71}N_5O_{10}Si$	62.11	8.22	8.04		
(40)	Bpoc-Asn-Phe-Phe(I)-OMe	Bpoc-Asn-OH + Boc-Phe-Phe(I)-OMe[l]	**(3)**	164—166[k]	−31.7 ± 0.6[f]	21	57.75	5.1	6.9		16.05	$C_{39}H_{41}IN_4O_7\cdot 0.5H_2O$	57.56	5.20	6.88		15.59
(41)	Bpoc-Asn-Phe-Phe(I)-NHNH$_2$	**(40)**	**(18)**	148—150[m]	−27.4 ± 0.6[f]	80	55.8	5.05	10.4		15.75	$C_{38}H_{41}IN_6O_6\cdot 0.5H_2O$	56.09	5.20	10.32		15.59
(45)	*cyclo*[Asn-Phe-Phe(I)-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-]	**(44)**	**(14)**	166—168[n]	+5.3 ± 1.0[f]	27	58.4	6.15	11.25		9.35	$C_{65}H_{84}IN_{11}O_{12}$	58.33	6.33	11.51		9.48
(47)	Bpoc-Asn-Phe-Phe-D-Trp-(Br)-OH	Bpoc-Asn-Phe-Phe-NHNH$_2$ + **(46)**	**(42)**	154[p] (decomp)	−7.5 ± 0.5[f]	58	60.45	5.3	8.8	8.32		$C_{49}H_{49}BrN_6O_8\cdot 2.5H_2O$	60.36	5.58	8.62	8.19	
(48)	Bpoc-Asn-Phe-Phe-D-Trp-(Br)-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse	**(47)** + **(39)**	**(43)**	209[q] (decomp)		36	62.15	6.85	9.05	4.65		$C_{86}H_{112}BrN_{11}O_{15}Si\cdot H_2O$	62.00	6.89	9.24	4.79	
(50)	H-Asn-Phe-Phe-D-Trp(Br)-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OH	**(48)**	**(12)**, **(13)**	290[r] (decomp)	+5.7 ± 0.4[f]	51	58.9	6.65	11.5	5.79		$C_{65}H_{86}BrN_{11}O_{13}\cdot H_2O$	58.81	6.68	11.60	6.02	
(51)	*cyclo*[-Asn-Phe-Phe-D-Trp(Br)-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-]	**(50)**	**(14)**	160—163[s]	+9.2 ± 1.5[f]	25	58.9	6.45	11.55	5.97		$C_{65}H_{84}BrN_{11}O_{12}\cdot 2H_2O$	58.81	6.68	11.60	6.02	

Table 2 (*continued*)

	Compound	Prepared from	Method as for	M.p. (°C)	$[\alpha]_D^{25}$ (°)	Yield (%)	Found (%) C	H	N	Br	I	Formula	Requires (%) C	H	N	Br	I
(61)	Z-Lys(Boc)-Thr(Bu^t)-OH	**(5)**	**(8)**	oil													
(62)	Z-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OMe	Z-D-Trp-OH + H-Lys(Boc)-Thr(Bu^t)-Phe-OMe[1]	**(3)**	170—172[s]	+2.8 ± 0.3[b]	75	65.0	7.25	9.5			$C_{42}H_{64}N_6O_{10}$	65.13	7.28	9.49		
(64)	H-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OH	**(63)**	**(17)**	140—143[h]	−7.9 ± 0.4[b]	60	62.6	7.8	11.15			$C_{39}H_{56}N_6O_8 \cdot 0.5H_2O$	62.79	7.70	11.26		
(65)	Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OH	Bpoc-Asn-Phe(I)-Phe-OH[1] + **(64)**	**(11)**	195[s] (decomp)	+4.5 ± 0.3[f]	58	60.65	6.3	9.15		8.5	$C_{77}H_{93}IN_{10}O_{14} \cdot H_2O$	60.54	6.26	9.16		8.30
(67)	H-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Nag-OH	**(66)**	**(13)**	216[g] (decomp)		77											
(68)	*cyclo*[-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Nag-]	**(67)**	**(14)**	185 (decomp)	+2.2 ± 0.9[b]	31	60.45	6.05	10.4		8.55	$C_{75}H_{90}IN_{11}O_{12} \cdot H_2O$	60.76	6.25	10.39		8.56
(69)	Z-Phe-Pro-OTmse	Z-Phe-OH + H-Pro-OTmse	**(3)**[t]	oil		60											
(70)	Z-Thr(Bu^t)-Phe-Pro-OTmse	Z-Thr(Bu^t)-OH + H-Phe-Pro-OTmse	**(3)**[u]	oil		50											
(71)	Z-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OTmse	Z-Lys(Boc)-OH + H-Thr(Bu^t)-Phe-Pro-OTmse	**(3)**[v]	oil		43											
(72)	Z-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OTmse	Z-D-Trp-OH + H-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OTmse	**(3)**[w]	114—115[w]	−36.4 ± 0.6[b]	54	63.75	7.6	9.06			$C_{57}H_{81}N_7O_{11}Si$	64.07	7.64	9.17		
(73)	Bpoc-Phe(I)-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OTmse	Bpoc-Phe(I)-OH + H-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OTmse	**(3)**[w]	163—164[a]	−15.9 ± 0.5[b]	40	60.85	6.65	7.63			$C_{74}H_{97}IN_8O_{12}Si \cdot H_2O$	60.72	6.81	7.65		
(74)	Bpoc-Phe(I)-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OH	**(73)**	**(12)**	144—145		97	60.15	6.35	8.02			$C_{69}H_{85}IN_8O_{12} \cdot 1.5H_2O$	60.38	6.46	8.16		

(75)	H-Phe(I)-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Pro-OH	(74)	(13)[u]	144—145[d]	+12.4 ± 0.5[b]	54	55.9	6.35	9.51	11.05	$C_{53}H_{71}IN_8O_{10}\cdot 2H_2O$	55.68	6.61	9.80	11.10
(76)	*cyclo*[-Phe(I)-D-Trp-Lys-(Boc)Thr(Bu^t)-Phe-Pro-]	(75)	(14)[x]	182—185[y]	−25.6 ± 0.7[b]	72	57.1	6.4	10.05	11.0	$C_{53}H_{69}IN_8O_9\cdot 1.5H_2O$	57.03	6.50	10.04	11.37

[a] From ethyl acetate–light petroleum (b.p. 60—80 °C). [b] *c* 1, MeOH. [c] Et_4NF (5 equiv.)–20 min–30 °C. [d] From aqueous methanol. [e] From MeOH–DMF–water (30:7:4 v/v). [f] *c* 1, DMF. [g] From MeOH–DMF–water (10:4:3 v/v). [h] From MeOH–Et_2O. [i] Work-up by CCD, chloroform–toluene–methanol–water (5:5:8:2 v/v). [j] From ethanol. [k] From MeOH–DMF–water (5:2:1 v/v). [l] From MeOH–DMF–H_2O (3:2:2 v/v). [m] From MeOH–DMF–H_2O (40:9:5 v/v). [n] From benzene–cyclohexane. [p] From aqueous acetonitrile following CCD, chloroform–toluene–methanol–water (5:5:8:2 v/v). [q] From aqueous methanol following CCD, chloroform–carbon tetrachloride–methanol–0.5M-aq. NH_4OAc (4:7:12:3 v/v). [r] From MeOH–DMF–water (6:1:4 v/v) following CCD: chloroform–toluene–methanol–water (5:5:8.2 v/v). [s] From ethanol. [t] Solvent for chromatographic clean up: ether–benzene (10:90 v/v). [u] Work-up by silica pad, elution with ether–benzene (20:80 v/v) [w] From ether–light petroleum (b.p. 40—60 °C). [x] Work-up by CCD, chloroform–carbon tetrachloride–methanol–0.5M-aq. NH_4OAc (4:7:12:3 v/v). [y] From benzene–light petroleum (b.p. 60—80 °C).

Table 3.

					Found (%)							Requires (%)					
	Compound	M.p. (°C)	$[\alpha]_D^{25}$ (°)	Yield (%)	C	H	N	Cl	I	S	Formula	C	H	N	Cl	I	S
(85)	Trt-D-Cys(Trt)-OBu^t	134—136	−59.9 ± 0.6[a]	62	81.7	6.55	2.15			4.85	$C_{45}H_{43}NO_2S$	81.66	6.54	2.11			4.84
(86)	H-Ser(Bu^t)-D-Cys(Trt)-OBu^t	85—95	−43.7 ± 0.5[a]	46	65.0	7.45	4.65			5.37	$C_{33}H_{43}ClN_2O_4S\cdot 0.5H_2O$	65.16	7.29	4.65			5.27
(87)	H-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t·HCl	110—120	+7.4 ± 0.1[a]	84	64.8	7.75	5.4	4.7		4.13	$C_{41}H_{58}ClN_3O_6S$	65.09	7.72	5.55	4.68		4.23
(88)	Bpoc-Phe(I)-Thr(Bu^t)-Ser(Bu^t)-D-Cys-(Trt)-OBu^t	105—108	+18.4 ± 0.3[a]	46	64.1	6.3	4.5		10.25	2.46	$C_{66}H_{79}IN_4O_9S$	64.37	6.46	4.55		10.30	2.60
(89)	Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t	185—187[b]	+4.6 ± 0.3[c]	72	63.5	6.7	8.25		5.73	1.51	$C_{118}H_{148}IN_{13}O_{19}S$	63.56	6.78	8.16		5.69	1.43
(90)	Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t	214—216[d]	+5.8 ± 0.3[c]	71	62.65	6.9	8.95		4.58	2.33	$C_{145}H_{189}IN_{18}O_{25}S$	62.75	6.86	9.08		4.57	2.31
(91)	Bpoc-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-OH	163—164[e]	−22.7 ± 0.3[f]	43	64.5	7.0	9.9				$C_{68}H_{148}IN_{13}O_{19}S$	64.64	7.02	9.97			
(92)	Bpoc-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Thr(Bu^t)-Ser(Bu^t)-D-Cys(Trt)-OBu^t	164—166[g]	+6.2 ± 0.2[c]	74	63.55	6.75	8.2		6.11	1.49	$C_{118}H_{148}IN_{13}O_{19}S$	63.56	6.78	8.16		5.69	1.43
(93)	Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe(I)-Thr(Bu^t)-Ser(Bu^t)-D-Cys-(Trt)-OBu^t	234	+5.1 ± 0.3[c]	62	62.5	6.85	8.9		4.70	2.33	$C_{145}H_{189}IN_{18}O_{25}S$	62.75	6.86	9.08		4.57	2.31

[a] *c* 1, $CHCl_3$. [b] Eluted from a silica column with $CHCl_3$–MeOH (97:3 v/v). Crystallised from aqueous MeOH. [c] *c* 1, DMF. [d] Purified by crystallisation from DMF–MeOH–water, then by elution from a silica column with $CHCl_3$–MeOH (96:4 v/v), followed by crystallisation from aqueous TFE. [e] Purified by elution from a silica column by $CHCl_3$–MeOH (93:7 v/v). [f] *c* 1, MeOH. [g] Purified by elution from a silica column by $CHCl_3$–MeOH (97:3 v/v) and then crystallised from aqueous MeOH.

Application of the crude product, dissolved in chloroform, to a pad of silica (5 × 11 cm) eluting first with chloroform (16 × 200 ml) and then with chloroform–methanol (98:2 v/v; 5 × 200 ml) provided a chromatographically homogenous product (11.7 g, 61%). A sample of this material was crystallised from ethyl acetate–light petroleum to give the *protected tripeptide*, m.p. 62—65 °C, $[\alpha]_D^{25}$ −14.3 ± 0.6° (*c* 1, MeOH) (Found: C, 63.2; H, 8.05; N, 8.1. $C_{43}H_{65}N_5O_9Si$ requires C, 62.66; H, 7.95; N, 8.49%).

N$^{\alpha}$-Benzyloxycarbonyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonine (**8**).—A solution of Et_4NF (5.9 g) in DMF (229 ml) was added to a solution of Z-D-Trp-Lys(Boc)-Thr(Bu^t)-OTmse (**7**) (11 g) in DMF (36 ml) and the mixture was stirred for 30 min at 30 °C. It was poured onto 1 840 ml of ice–water containing M-HCl (40 ml). The suspended solid was collected by filtration, washed thoroughly with water and dried *in vacuo* (9.0 g, 93%). Three reprecipitations from aqueous DMF gave a sample of the *tripeptide acid monohydrate* with m.p. 75—80 °C, $[\alpha]_D^{25}$ −3.7° (*c* 1, MeOH) (Found: C, 61.95; H, 7.4; N, 9.55. $C_{38}H_{53}N_5O_9 \cdot H_2O$ requires C, 61.52; H, 7.47; N, 9.44%).

4-(N$^{\alpha}$-Benzyloxycarbonyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanyl)aminobutanoic Acid 2-Trimethylsilylethyl Ester (**9**).—Z-D-Trp-Lys(Boc)-Thr(Bu^t)-OH (**8**) (4.2 g) and the dipeptide hydrochloride (**4**) (2.24 g) were coupled as described for (**3**). The reaction mixture was filtered, the filtrate evaporated and the residue triturated with ice-cold water. The solid obtained was crystallised twice from aqueous methanol to give the *protected pentapeptide* (3.9 g, 65%), m.p. 176—177 °C $[\alpha]_D^{25}$ −10.1 ± 0.6° (*c* 1.0, MeOH) (Found: C, 63.7; H, 7.75; N, 9.4. $C_{56}H_{81}N_7O_{11}Si$ requires C, 63.66; H, 7.72; N, 9.28%).

4-(N-2-Biphenyl-4-ylisopropoxycarbonylasparaginyl-4-iodophenylalanylphenylalanyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanylamino)butanoic Acid 2-Trimethylsilyethyl Ester (**11**).—(*a*) *4-(D-Tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanylamino)butanoic Acid 2-Trimethylsilylethyl Ester Hydrochloride* (**10**). A solution of Z-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse (**9**) (4.22 g) in 90% aqueous trifluoroethanol (33 ml) containing acetic acid (0.24 ml) was hydrogenated at room temperature over palladium–charcoal (10%, 0.4 g). When hydrogen uptake had ceased the catalyst was removed by filtration, the filtrate concentrated and the residue dissolved in ethyl acetate (10 ml). The solution was cooled to −20 °C and treated with 3M-HCl in ethyl acetate (1.33 ml). Addition of light petroleum (b.p. 60—80 °C) caused the precipitation of a solid (3.3 g, 86%), which was filtered off under nitrogen and dried *in vacuo* (KOH pellets). A solution of this material in chloroform (10 ml) was applied to a column (45 × 3 cm) of silica which was eluted with a mixture of chloroform–methanol (96:4 v/v) to provide chromatographically pure hydrochloride (2.75 g, 72%).

(*b*) Bpoc-Asn-Phe(I)-Phe-$NHNH_2$[3] (2.32 g) in DMF (13 ml), was cooled to −10 °C and treated with 3.0M-HCl in ethyl acetate (2.39 ml) followed by t-butyl nitrite (0.34 ml). The solution was stirred for 10 min at −10 °C and DIEA (1.24 ml) was added. A solution of the pentapeptide hydrochloride salt (**10**) (2.75 g) in DMF (11 ml) containing DIEA (0.49 ml) was added and the mixture was stirred for 1 h at −10 °C and then for 84 h at 8 °C. Evaporation of the reaction mixture to dryness, followed by trituration of the residue under cold water provided the crude product which was crystallised twice from DMF–methanol (50:3 v/v) to give the *protected octapeptide* (3.27 g, 68%), m.p. 230 °C (decomp.), $[\alpha]_D^{25}$ −4.8 ± 0.6° (*c* 1, DMF) (Found: C, 60.85; H, 6.7: I, 7.6; N, 8.95. $C_{86}H_{112}IN_{11}O_{15}Si$ requires C, 60.94; H, 6.66; I, 7.48; N, 9.09%).

4-(Asparaginyl-4-iodophenylalanylphenylalanyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanylamino)butanoic Acid (**13**).—(*a*) *4-[N-(2-Biphenyl-4-ylisopropoxycarbonylasparaginyl)-4-iodophenylanalylphenylalanyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanylamino]butanoic Acid* (**12**). Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse (**11**) (2.67 g) was added to a solution of Et_4NF (2.36 g) in DMF (104 ml) which was stirred for 10 min at 30 °C then poured into ice–water (900 ml) containing M-HCl (15.7 ml). The precipitate was collected by filtration, washed thoroughly with water and dried. Crystallisation from DMF–methanol gave the protected acid (**12**) (2.24 g, 90%).

(*b*) A solution of Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OH (**12**) (2.24 g) in acetic acid (14.4 ml) and water (3.6 ml) was stirred for 1 h at 50 °C. The solution was evaporated under reduced pressure and the residue triturated under light petroleum (b.p. 60—80 °C) (2 × 100 ml). Crystallisation of the crude product from DMF–methanol–water (5:10:3 v/v) gave the *octapeptide hemihydrate* (1.55 g, 82%), m.p. 320 °C (decomp.), $[\alpha]_D^{26}$ +1.9 ± 0.6° (*c* 1, DMF) (Found: C, 57.1; H, 6.25; I, 9.3; N, 11.45. $C_{65}H_{86}IN_{11}O_{13} \cdot 0.5H_2O$ requires C, 57.18; H, 6.42; I, 9.29; N, 11.28%).

cyclo-[4-(*Asparaginyl-4-iodophenylalanylphenylalanyl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonylphenylalanylamino)butanoic Acid*] (**14**)—HOBt (0.79 g) and DCC (1.07 g) were added to a solution of H-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OH (**13**) (0.7 g) in DMF (525 ml) which was stirred for 20 h at 50 °C. The solvent was removed by evaporation and the residue was dissolved in 20 ml of the lower phase of the solvent system, chloroform–carbon tetrachloride–methanol–water (4:7:12:3 v/v). Dicyclohexylurea (DCU) was filtered off and the filtrate was shaken with 20 ml of the upper phase of the same solvent system. The layers were separated and loaded into tubes 3 and 4 of a 123-tube counter-current distribution (CCD) machine and subjected to a total of 739 upper phase transfers in the recycling mode. The contents of tubes containing product and DCU (95—28) were combined and concentrated to 100 ml. The precipitate (400 mg) was collected by filtration and washed thoroughly with water and dried. The solid was dissolved in chloroform (20 ml) and applied to a silica pad (3 × 11 cm) which was eluted with chloroform (3 × 100 ml), and 2%, 4%, and 8% methanol in chloroform (8 × 100 ml, 5 × 50 ml, and 12 × 50 ml respectively). Fractions containing homogeneous product (19—24) were combined and evaporated to dryness. Precipitation of the product from aqueous methanol gave the *cyclic octapeptide sesquihydrate* (0.21 g, 31%). A sample for analysis was prepared by crystallisation from benzene–cyclohexane and had m.p. 153—155 °C (decomp.), $[\alpha]_D^{22}$ +9.4 ± 0.6° (*c* 1, MeOH) and amino acid analysis after acid hydrolysis: Asp, 0.99; Gaba, 0.98; Lys, 1.01; Thr, 0.94; Trp, 0.73; Phe, 2.00; Phe(I), 1.06 (Found: C, 57.2; H, 6.45; I, 9.15; N, 11.4. $C_{65}H_{84}IN_{11}O_{12} \cdot 1.5H_2O$ requires C, 57.17; H, 6.42; I, 9.29; N, 11.28%).

N$^{\alpha}$-Trityl-D-tryptophan Diethylammonium Salt (**16**).—This was prepared in 37% yield by the method of Stelakatos, *et al.*,[18] m.p. 114 °C (decomp.), $[\alpha]_D^{25}$ −3.5 ± 0.1° (*c* 5, MeOH) {for L compound lit.,[18] m.p. 150—151 °C, $[\alpha]_D$ +4.5° (*c* 5, MeOH)} (Found: C, 78.6; H, 6.95; N, 7.3. $C_{34}H_{37}N_3O_2$ requires C, 78.57; H, 7.17; N, 8.08%).

N$^{\alpha}$-Trityl-D-tryptophyl-N$^{\varepsilon}$-t-butoxycarbonyl-lysyl-O-t-butylthreonine Methyl Ester (**17**).—*N$^{\alpha}$*-Trityl-D-tryptophan (3.5 g) was generated from Trt-D-Trp-OH·DEA (**16**) (4.1 g) in ethyl acetate by shaking with ice-cold 10% aqueous citric acid. The organic layer was separated, dried (Na_2SO_4) and then

evaporated. The free acid was coupled to H-Lys(Boc)-Thr(Bu^t)-OMe·HCl (3.4 g) [prepared from Z-Lys(Boc)-Thr(Bu^t)-OMe (**15**) by hydrogenolysis in aqueous methanol, by a method analagous to that described for H-Lys(Boc)-Thr(Bu^t)-OTmse (**6**)] and washed by the method used to prepare Boc-Phe-Gaba-OTmse (**3**). The evaporated product was applied to a column of silica in chloroform, eluting with chloroform, chloroform–methanol (99:1 v/v), and chloroform–methanol (98:2 v/v). Fractions containing homogeneous product were combined and evaporated, and the residue was crystallised from ether–light petroleum (b.p. 40—60 °C) to provide the *tripeptide* (1.85 g, 30%), m.p. 112—115 °C (Found: C, 71.25; H, 7.7; N, 7.85. $C_{50}H_{63}N_5O_7$ requires C, 70.97; H, 7.50; N, 8.27%).

N$^{\alpha}$-*Trityl*-D-*tryptophyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonine Hydrazide* (**18**).—A solution of Trt-D-Trp-Lys(Boc)-Thr(Bu^t)-OMe (**17**) (1.7 g) in DMF and methanol (5.1 ml) was cooled to 0 °C and treated with hydrazine hydrate (1.02 ml). The solution was stirred for 16 h at room temperature then added dropwise to vigorously stirred water (50 ml) and the precipitated product collected by centrifugation. The dried product (1.1 g) was redissolved in methanol (1.86 ml) and DMF (0.55 ml) cooled to 0 °C and treated with further hydrazine hydrate (1.32 ml) for 16 h at room temperature, the solution was poured into water and the precipitate was collected by centrifugation. Crystallisation of the dried product from acetonitrile gave the *tripeptide hydrazide* (0.60 g, 35%), m.p. 203—205 °C (Found: C, 69.3; H, 7.35; N, 11.8. $C_{49}H_{63}N_7O_6$ requires C, 69.55; H, 7.50; N, 11.58%).

4-(N$^{\alpha}$-*Trityl*-D-*trytophyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonyl*-4-*iodophenylalanylamino*)*butanoic Acid* 2-*Trimethylsilylethyl Ester* (**21**).—Trt-D-Trp-Lys(Boc)-Thr(Bu^t)-$NHNH_2$ (**18**) was converted to the azide and coupled to H-Phe(I)-Gaba-OTmse·HCl (**20**) (0.30 g) [prepared from Boc-Phe(I)-Gaba-OTmse (**19**) as for H-Phe-Gaba-OTmse·HCl (**4**)] by the method used to prepare compound (**11**). The solution was stirred for 1 h at −10 °C and for 88 h at 8 °C then evaporated to dryness and the residue triturated with cold water. Purification by chromatography on a column of silica eluting with chloroform–methanol (99:1 v/v) followed by crystallisation from ethyl acetate–light petroleum (b.p. 60—80 °C) gave the *protected pentapeptide* (0.2 g, 26%), m.p. 113—115 °C (Found: C, 62.4; H, 6.85; I, 9.45; N, 7.5. $C_{67}H_{88}IN_7O_9Si$ requires C, 62.36; H, 6.87; I, 9.83; N, 7.60%).

N-(2-*Biphenyl*-4-*ylisopropoxycarbonylasparaginylphenylalanyl*-4-*iodophenylalanyl*)-D-*tryptophan* (**42**).—Bpoc-Asn-Phe-Phe(I)-$NHNH_2$ (**41**) was coupled to H-D-Trp-OH by the azide method as described for compound (**11**). The reaction mixture was stirred for 1 h at −10 °C and for 16 h at 8 °C. Evaporation of the clear solution followed by trituration of the residue with ice–water gave a solid product which when crystallised twice from DMF–methanol–water (3:15:2 v/v) provided the *tetrapeptide acid* (1.8 g, 62%), m.p. 214—216 °C (decomp.), $[\alpha]_D^{25}$ −12.0 ± 0.6° (*c* 1, DMF) (Found: C, 59.4; H, 5.1; I, 12.75; N, 8.65. $C_{49}H_{49}IN_6·H_2O$ requires C, 59.15; H, 5.16; I, 12.75; N, 8.44%).

4-[N-(2-*Biphenyl*-4-*ylisopropoxycarbonylasparaginyl*)-*phenylalanyl*-4-*iodophenylalanyl*-D-*tryptophyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonylphenylalanylamino*]*butanoic Acid* 2-*Trimethylsilylethyl Ester* (**43**).—Z-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse (**39**) was hydrogenated and converted to the hydrochloride salt in 77% yield by the methods employed for H-Gaba-OTmse·HCl (**2**).

Bpoc-Asn-Phe-Phe(I)-D-Trp-OH (**42**) and H-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse·HCl were coupled by the DCC-HOBt procedure as previously described for Bpoc-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse (**24**). Crystallisation of the crude reaction product from DMF–methanol–water (3:16:4 v/v) gave a material which was dissolved in the solvent system chloroform–carbon tetrachloride–methanol–0.05M aqueous ammonium acetate (4:7:12:3 v/v) and was subjected to 370 transfers of the upper phase in this system. Crystallisation from DMF–methanol–water (1:3:1 v/v) gave material (1.73 g, 47%) with amino acid analysis after acid hydrolysis: Asp, 1.00; Gaba, 0.93; Lys, 0.94; Phe, 2.00; Phe(I), 0.97; Thr, 0.91; Trp, 0.75.

4-(*Asparaginylphenylalanyl*-4-*iodophenylalanyl*-D-*tryptophyl*-N$^{\varepsilon}$-*t-butoxycarbonyl-lysyl*-O-*t-butylthreonylphenylalanylamino*)*butanoic Acid* (**44**).—Bpoc-Asn-Phe-Phe(I)-D-Trp-Lys-(Boc)-Thr(Bu^t)-Phe-Gaba-OTmse (**43**) was treated first with Et_4NF then with aqueous acetic acid by the procedure described for the [Phe(I)^6] analogue (**13**). Crystallisation of the crude product from DMF–methanol–water (5:7:8 v/v) gave a mixture of major product (0.5 g, 78%) with traces of three other materials. Repeated recrystallisation from DMF–methanol–water failed to remove the impurities.

N-*Acetyl*-5-*bromo*-D-*trpytophan*.—N-Acetyl-5-bromo-D-tryptophan derived from the fungal α-amylase resolution of *N*-acetyl-5-bromo-DL-tryptophan[14] was recrystallised from aqueous methanol and had m.p. 120—122 °C (decomp.), $[\alpha]_D^{26}$ −15.1° (*c* 2, MeOH) (Found: C, 46.6; H, 4.5; Br, 23.65; N, 7.95. $C_{13}H_{13}BrN_2O_3·0.5H_2O$ requires C, 46.72; H, 4.22; Br, 23.91; N, 8.38%).

5-*Bromo*-D-*tryptophan* (**46**).—*N*-Acetyl-5-bromo-D-tryptophan (15.7 g) was refluxed for 1 h in 6M-H_2SO_4 (500 ml), filtered hot and the filtrate neutralised by the addition of solid lithium hydroxide. The precipitate that formed was collected by filtration, washed with water and dried to give 13.0 g (95%) of the crude amino acid. Suspension of the solid in warm water (1 800 ml) followed by the addition of concentrated HCl to complete solution, hot filtration and neutralisation of the filtrate with ammonia (*d* 0.88), gave a precipitate which was collected by filtration and washed thoroughly with water. The filtrate was concentrated by evaporation to yield a further crop of solid. This process was repeated several times to provide a total of 6.7 g (51%) of product which after crystallisation from aqueous methanol gave the *amino acid*, m.p. 289—290 °C, $[\alpha]_D$ +27.1 ± 0.1° (*c* 1.1, AcOH) (Found: C, 46.4; H, 3.95; Br, 28.2; N, 9.75. $C_{11}H_{11}BrN_2O_2$ requires C, 46.67; H, 3.92; Br, 28.20; N, 9.89%).

Ethyl 3-(1-*Naphthyl*)*prop*-2-*enoate* (**52**).—Dry hydrogen chloride gas was passed through a refluxing solution of 3-(1-naphthyl)prop-2-enoic acid (34.8 g) in absolute ethanol (175 ml) for 1.5 h. The solution was cooled to room temperature and poured into water (1 500 ml) and carbon tetrachloride (100 ml). The organic phase was separated, washed with saturated aqueous $NaHCO_3$, followed by 10% aqueous NaCl, dried (Na_2SO_4) and evaporated to dryness. The resulting oil was crystallised from ethanol (30 ml) to provide the ester (38.4 g, 97%) in two crops, m.p. 36—38 °C (lit.,[19] m.p. 37—38.5 °C), $\lambda_{max.}$ 321 nm (ε 11 450); $\nu_{max.}$(KBr) 1 715 (C=O, α,β-unsaturated ester) and 1 635 cm^{-1} (C=C).

Ethyl 3-(1-*Naphthyl*)-4-*nitrobutanoate* (**53**).—Ethyl 3-(1-naphthyl)prop-2-enoate (**52**) (22.6 g) was dissolved in nitromethane (18.2 ml). To this solution was added a solution of Triton B in butan-1-ol [generated immediately before use from a 40% (w/w) solution of Triton B in methanol (8.4 ml) by evaporation followed by dissolution in butan-1-ol (10 ml), re-

evaporation and making up to the required concentration with butan-1-ol (5.2 ml)] and the solution was stirred for 6 h at 68 °C. The mixture was acidified with M-HCl (40 ml), dichloromethane (60 ml) was added and the organic layer separated after vigorous shaking, washed thoroughly with water and dried (Na_2SO_4). Evaporation of the solution under reduced pressure gave a mobile red oil (35.2 g), distillation of which gave a viscous yellow oil (22.9 g, 80%), b.p. 185—195 °C (0.3—1.0 mmHg). Repeated fractional distillation of a portion of this material failed to provide an analytically pure sample. However, chromatographic treatment on a silica column, eluting with redistilled benzene gave the pure *nitro ester*, $\lambda_{max.}$ 281 nm (ε 6 200), 272 nm (ε 5 150), and 292 nm (ε 4 300); $\nu_{max.}$(film) 1 745 (C=O, saturated ester), 1 565, 1 385 (NO_2), and 1 190 cm^{-1} (C–O, ester) (Found: C, 66.8; H, 5.9; N, 4.9. $C_{16}H_{17}NO_4$ requires C, 66.88; H, 5.96; N, 4.87%).

4-(1-*Naphthyl*)-2-*pyrrolidone* (**55**).—(*a*) *Ethyl* 4-*amino*-3-(1-*naphthyl*)*butanoate* (**54**). A solution of ethyl 3-(1-naphthyl)-4-nitrobutanoate (**53**) (5.0 g) in ethanol (20 ml) was hydrogenated over 10% palladium–carbon catalyst (1.0 g). Rapid hydrogen uptake ceased after 5 h, when the catalyst was filtered off and the filtrate was evaporated under reduced pressure to yield the amino ester (**54**) as a mobile oil (3.8 g, 85%), $\nu_{max.}$(film) 1 740 (C=O ester), and 1 190 cm^{-1} (C–O, ester).

(*b*) Compound (**54**) (1.5 g) was stirred for 10 min in a pre-heated oil bath (150 °C). Crystallisation of the viscous residue from benzene–hexane (3:2 v/v) provided the pyrrolidone (0.6 g, 49%) as colourless crystals, m.p. 131—133 °C (reference material[20] m.p. 132—134 °C), $\nu_{max.}$ (KBr disc) 1 715 (C=O, γ-lactam) and 1 290 cm^{-1} (Amide III).

4-*Amino*-3-(1-*naphthyl*)*butanoic Acid* (**56**).—A solution of 4-(1-naphthyl)-2-pyrrolidone (**55**) (2.5 g) in concentrated hydrochloric acid (25 ml) and glacial acetic acid (25 ml) was refluxed for 24 h. Removal of the solvent by evaporation under reduced pressure followed by evaporation of an added portion of benzene and pumping over KOH pellets gave the amino acid hydrochloride as a white powder (3.1 g, 100%). Suspension of the powder (1.4 g) in chloroform (30 ml), cooling to 0 °C and shaking with a cold aqueous M-$NaHCO_3$ solution gave after filtration and drying the *product* (1.1 g, 91%), m.p. 182—183 °C (decomp.) (Found: C, 67.65; H, 6.85; N, 5.8. $C_{14}H_{15}NO_2 \cdot H_2O$ requires C, 67.99; H, 6.92; N, 5.66%).

3-(1-*Naphthyl*)-4-*phthalimidobutanoic Acid* (**57**).—An intimate mixture of 4-amino-3-(1-naphthyl)butanoic acid (**56**) (1.0 g) and phthalic anhydride (0.715 g) in a round-bottomed flask was immersed in a pre-heated oil-bath (145—150 °C) for 30 min, stirring continuously with a glass rod. Trituration of the cooled glass with hot methanol (5 ml), followed by cooling and filtration gave an impure product (1.0 g, 64%). A single crystallisation from propan-2-ol (20 ml) provided the N-*protected amino acid* (0.96 g, 91%) as colourless plates, m.p. 186—187 °C, $[\alpha]_D^{30}$ +0.2 ± 1.2° (*c* 0.5, $CHCl_3$) (Found: C, 73.45; H, 4.95; N, 3.85. $C_{22}H_{17}NO_4$ requires C, 73.52; H, 4.76; N, 3.89%).

(+)-3-(1-*Naphthyl*)-4-*phthalimidobutanoic Acid Strychnine Salt* (**58**).—3-(1-Naphthyl)-4-phthalimidobutanoic acid (**57**) (3.0 g) and (−)-strychnine (2.8 g) were dissolved in the minimum quantity of boiling methanol (350 ml). Water (100 ml) was added and the solution stored at 5 °C for 65 h. The crystals of the *strychnine salt* that formed were collected by filtration, dried (1.8 g) and had m.p. 143 °C (decomp.), $[\alpha]_D^{25}$ +4.8 ± 0.3° (*c* 2.2, $CHCl_3$) (Found: C, 72.4; H, 5.9; N, 5.9. $C_{43}H_{39}N_3O_6 \cdot H_2O$ requires C, 72.55; H, 5.80; N, 5.90%).

(+)-3-(1-*Naphthyl*)-4-*phthalimidobutanoic Acid* (**59**).—The strychnine salt (**58**) (1.64 g) was suspended in ethyl acetate (60 ml) and shaken with M-HCl (40 ml) until all solid had dissolved. The organic phase was separated, washed with water, dried (Na_2SO_4) and evaporated to dryness to yield the *free acid* (0.85 g, 100%). A sample crystallised from propan-2-ol had m.p. 164—165 °C, $[\alpha]_D^{26}$ +48.2 ± 0.3° (*c* 1, $CHCl_3$) (Found: C, 73.7; H, 4.8; N, 3.9. $C_{22}H_{17}NO_4$ requires C, 73.52; H, 4.76; N, 3.89%).

(−)-4-*Amino*-3-(1-*naphthyl*)*butanoic Acid* (**60**).—A solution of (+)-3-(1-naphthyl)-4-phthalimidobutanoic acid (**59**) (0.85 g) in ethanol (8 ml) containing hydrazine hydrate (0.135 ml) was refluxed for 1 h, and evaporated to dryness. T.l.c. showed the presence of unchanged starting material and the residue was redissolved in ethanol (5 ml), a further portion of hydrazine hydrate (0.135 ml) was added and the solution was refluxed for another 1 h, after which time all starting material had been consumed. The mixture was evaporated and the residue dissolved in cold aqueous 5M-acetic acid. Neutralisation of the solution with cold M-NaOH followed by collection of the precipitate by filtration provided a crude product (0.3 g). Crystallisation of this from ethanol (60 ml) gave the resolved *amino acid* (0.20 g, 37%) in two crops, m.p. 165—166 °C, $[\alpha]_D^{25}$ −0.7 ± 0.4° (*c* 1, HOAc) (Found: C, 65.9; H, 6.85; N, 5.7. $C_{14}H_{15}NO_2 \cdot 1.5H_2O$ requires C, 65.60; H, 7.07; N, 5.46%).

The resolved amino acid (**60**) and the racemic mixture (**56**) (about 2.0 mg) were derivatised with D-glutamic acid *N*-carboxy anhydride (1.9 mg) by the method of Manning and Moore.[16] Samples of the derivatised amino acid (20 μl) were applied to a column (30 × 0.46 cm) of Nucleosil $10C_{18}$ using a Rheodyne six-port injection valve. The column was eluted with MeCN–0.083*N* triethylammonium phosphate (pH 3.0) (15:85 v/v) at a flow rate of 2.4 ml min^{-1} and the eluate was monitored at 210 nm. The pair of diastereomeric products from the unresolved sample eluted at 17 min 6 sec and 15 min 10 sec. The resolved derivative eluted at 17 min 16 sec.

N^{α}-*Benzyloxycarbonyl*-D-*tryptophyl*-N^{ε}-*t*-*butoxycarbonyl*-*lysyl*-O-*t*-*butylthreonylphenylalanine* (**63**).—4M-NaOH (6.3 ml) was added to a solution of Z-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OMe (**62**) (7.5 g) in dioxane (30.6 ml) and stirred for 1 h at room temperature. The solution was cooled to 5 °C, adjusted to pH 7 with M-HCl and evaporated to dryness. The residue was dissolved in ethyl acetate–butan-1-ol (180 ml; 1:1), cooled to 5 °C and shaken with 7% aqueous citric acid. The organic layer was separated, washed to neutrality with 10% NaCl solution and dried (Na_2SO_4). Evaporation to dryness gave an oil which crystallised on storage at 5 °C (7.3 g, 99%). A sample was crystallised from aqueous methanol to give the *acid* which had m.p. 116—120 °C (decomp.), $[\alpha]_D^{25}$ +12.9 (*c* 2, MeOH) (Found: C, 64.5; H, 7.15; N, 9.4. $C_{47}H_{62}N_6O_{10}$ requires C, 64.80; H, 7.17; N, 9.64%).

(−)-4-[N-(2-*Biphenyl*-4-*ylisopropoxycarbonylasparaginyl*)-4-*iodophenylalanylphenylalanyl*-D-*tryptophyl*-N^{ε}-*t*-*butoxycarbonyl*-*lysyl*-O-*t*-*butylthreonylphenylalanylamino*]-3-(1-*naphthyl*)*butanoic Acid* (**66**).—A solution of Bpoc-Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OH (**65**) (2.0 g) and HOBt (0.203 g) in DMF (2.7 ml) was cooled to −5 °C and a solution of DCC (0.27 g) in DMF (0.5 ml) was added and the mixture was stirred for 1 h at −5 °C and for 1 h at 5 °C then cooled again to −5 °C. The suspension was rapidly centrifuged and the supernatant was added to a cold (−5 °C) solution of (−)-4-amino-3-(1-naphthyl)butanoic acid (**60**) (0.28 g) and 1,1,3,3-tetramethylguanidine (0.15 ml) in DMF (2 ml). A cold DMF wash (1 ml) of the centrifuged material was also added. The mixture was stirred for 30 min at −5 °C and for 48 h at 8 °C,

evaporated to dryness and the residue triturated with cold water. The solid was collected by filtration and dried (2.1 g). Two crystallisations from DMF–methanol–water (5:40:7 v/v) gave the *octapeptide acid* (1.2 g, 60%), m.p. 207 °C (decomp.), $[\alpha]_D^{25}$ +0.7 ± 1.4° (*c* 0.3, DMF), amino acid analysis after acid hydrolysis: Asp, 1.01; Lys, 0.99; Nag, 1.08; Phe, 2.00; Phe(I), 1.01; Thr, 0.92; Trp, 0.71 (Found: C, 63.6; H, 6.3; I, 7.05; N, 8.65. $C_{91}H_{106}IN_{11}O_{15}$ requires C, 63.51; H, 6.20; I, 7.37; N, 8.95%).

cyclo-{4-(*Asparaginyl*-[4-^{3}H]*phenylalanyl*-D-*tryptophyl-lysylthreonylphenylalanylamino*)*butanoic Acid*}. (**77**).—*cyclo*-[Asn-Phe(I)-Phe-D-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Gaba] (**14**) (9.6 mg) was dissolved in DMF (0.5 ml) and stirred in an atmosphere of tritium gas (3.2 ml; 8 Ci) for 40 min in the presence of the catalysts 10% palladium–charcoal (11.4 mg) and 5% rhodium–calcium carbonate (9.6 mg) at room temperature. The catalysts were removed by filtration through a pad of cellulose and the filtrate was evaporated to dryness. The residue was kept at room temperature for 1 h with 90% aqueous trifluoroacetic acid (5 ml) and the solution was then evaporated to dryness. The residue was dissolved in water (2 ml) and the solution, after ultrafiltration (0.45 μ Millex filter), was applied, using a Rheodyne six-port injection valve, onto a column (50 × 0.7 cm) of Nucleosil $10C_{18}$ which was eluted at a flow rate of 5 ml min^{-1} with a constant volume (100 ml) gradient of acetonitrile–water–phosphoric acid (from 250:750:1 to 500:500:1). The eluate was monitored at 210 nm and fractions (30 s) were collected automatically. Fractions 19—23 were combined, reduced to *ca.* 1 ml and the solution passed through a column (0.5 ml) of Dowex 1 (acetate form) resin which was eluted with water. The eluate was evaporated to dryness and the residue (4.48 μmol, 69%; 12.6 Ci mmol^{-1}) was dissolved in water to give a solution of concentration 5 mCi ml^{-1}, which was stored in liquid nitrogen (− 196 °C). Analytical data are given in Table 1.

[4-^{3}H-Phe^6,D-Trp^8,D-Cys^{14}]*Somatostatin*. (**94**).—Compound (**90**) was reduced with tritium, cyclised with I_2 and deprotected with trifluoroacetic acid as described for the corresponding somatostatin analogue.[3] After evaporation of the trifluoroacetic acid, the residue was shaken with chloroform and water (10 ml each) and the aqueous fraction was passed through a column (0.5 ml) of Dowex 1 (acetate form) resin. The eluate (12 ml) was applied directly to a column (3 × 0.7 cm) of carboxymethyl cellulose (trimethylammonium form) and eluted as described previously.[3] Fractions (1 ml) were collected in tubes containing glacial acetic acid (1 ml) to avoid precipitation of the product. Evaporation of fractions 13—18 gave a residue which was dissolved in HOAc–water (2:3, 1.05 ml) and purified by h.p.l.c. as described previously.[3] Fractions 17—20 of the eluate were combined and concentrated to 1.5 ml, passed through Dowex 1 (acetate form) resin and the eluate (3 ml) was stored at − 196 °C. The product (0.91 μmoles, 20%) had specific activity 12.5 Ci mmol^{-1}. Radioactivity was associated exclusively with phenylalanine.[3] The purity of the product was assessed using a Panax E.0111/XPD-05 scanner system after t.l.c. in the solvent systems [propan-2-ol–acetic acid–water (30:1:15 v/v] and [butan-1-ol–pyridine–acetic acid–water (30:6:20:24 v/v)] giving values of 95.8 and 99.7% respectively.

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