[Chem. Pharm. Bull.
32(2) 510—519 (1984)]

Studies on Peptides. CXVI.[1,2] Synthesis of the Protected Docosapeptide Corresponding to the C-Terminal Portion of Human Growth Hormone Releasing Factor (GRF, Somatocrinin)

NOBUTAKA FUJII, MASANORI SHIMOKURA, KENICHI AKAJI,
SHINYA KIYAMA, and HARUAKI YAJIMA*

Faculty of Pharmaceutical Sciences, Kyoto University,
Yoshida, Sakyo-ku, Kyoto 606, Japan

(Received June 13, 1983)

As a starting material for the solution synthesis of a tetratetracontapeptide amide corresponding to the entire sequence of growth hormone releasing factor derived from human pancreatic tumor, a C-terminal-protected docosapeptide amide (positions 23—44) was synthesized by successive azide condensation of three peptide fragments (positions 33—44, 28—32, and 23—27). Amino acid derivatives bearing protecting groups removable by trifluoromethanesulfonic acid were employed; *i.e.*, benzyloxycarbonyl (Z), benzyl (Bzl), and mesitylene-2-sulfonyl (Mts).

Keywords——growth hormone releasing peptide; somatocrinin; GRF-44-amide synthesis; β,β,β-trichloroethyloxycarbonylhydrazine; N^G-mesitylene-2-sulfonylarginine; thioanisole-mediated deprotection; hard-soft concept; push-pull mechanism; methionine sulfoxide; acid-catalyzed succinimide formation of Asp

Since Green and Harris[3] postulated in 1949 that the secretion of the anterior pituitary hormones is under the control of hypothalamic factors, several hypothalamic peptides responsible for these secretions have been chemically characterized, *i.e.*, thyrotropin releasing hormone (TRH),[4,5] luteinizing hormone releasing hormone (LH-RH),[6,7] growth hormone release inhibiting factor (somatostatin)[8,9] and recently corticotropin releasing factor (CRF).[10,11] However, even after the isolation of somatostatin, structural elucidation of the hypothalamic growth hormone (GH) releasing factor continued to elude investigators, because of the minute quantities of this substance in hypothalamic organs. Quite recently, observations that acromegalic patients with pancreatic islet tumor show elevated GH plasma level[12] have shed light on the nature of the long-sought hypothalamic releasing factor for GH.

In 1982, two groups of investigators reported independently the isolation of peptides with GH releasing activity from human tumor of the pancreas, and these peptides were simultaneously sequenced. Guillemin's group elucidated the structures of three active peptides;[13-15] the tetratetracontapeptide amide [H-(hpGRF 1—44)-NH_2], the tetracontapeptide [H-(hpGRF 1—40)-OH][14] and the heptatriacontapeptide [H-(hpGRF 1—37)-OH]. The latter two peptides are shorter chain peptides lacking the tetra- or the heptapeptide from the C-terminus of the former larger form of GRF, respectively, as shown in Fig. 1. They

H-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-
Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2
37 40 44

Fig. 1. The Primary Structure of Human Pancreatic Tumor-Derived Growth Hormone Releasing Factor

	5 10 15 20 25 30 35 40
GRF(1—44)	YADA I FTNSYRKVLGQL SARKL LQD I MSRQQGE SNQERGARARL[a]
PHI	HADGVFT SDF SRL LGQL SAKKYLE SL I[a]
VIP	HSDAVFTDNYTRLRKQMAVKKYLNS I LN[a]
Glucagon	HSQGTFT SDY SKYLD SRRAQDFVQWLMNT
Secretin	HSDGTFT SEL SRLRD SARLQRL LQGLV[a]
GIP	YAEGTF I SDYS I AMDK I RQQDFVNWL LAQKGKK SDWKHN I TQ

a) Amidated C-terminus.

Fig. 2. Sequence Homologies between hpGRF and Various Gut-Pancreatic Peptides

reported that the tumor-derived peptide, H–(hpGRF 1—44)–NH_2, is identical in biological activity and similar in physicochemical properties to the still uncharacterized GRF present in extracts of hypothalamic tissues in all studies conducted so far[16,17] and proposed the name somatocrinin[16] for GRF. The other group[18,19] at the Salk Institute simultaneously reported the sequence of 40 residues of human pancreatic tumor GH-releasing peptide, H–(hpGRF 1—40)–OH. From these sequence studies, it has become evident that there are sequence homologies between hpGRF and several peptides of gut-pancreas origin (*i.e.*, peptides of the secretin–glucagon family[20]), especially between hpGRF and PHI,[21] as shown in Fig. 2.

In connection with their structural studies, two groups of investigators[13-15,18,19] synthesized a number of related peptides by the solid phase method to confirm the structures of the natural peptides and to obtain information about chainlength and activity relationship of GRF.

We wish to report the first solution synthesis of a tetratetracontapeptide amide corresponding to the entire amino acid sequence of a larger form of hpGRF in two consecutive papers. In this paper, synthesis of the protected docosapeptide amide corresponding to positions 23—44 of hpGRF is reported.

In the present synthesis, the thioanisole-mediated TFMSA deprotecting procedure[22] was applied in the final step of the synthesis. The effectiveness of thioanisole as a cation scavenger was previously pointed out.[23,24] To explain this phenomenon, the hard-soft concept[25] or the push-pull mechanism[23,26] was proposed. Thus, the thioanisole-mediated cleavage of protecting groups seems to proceed through an S_N2 type substitution reaction,[27] instead of simple S_N1 type acidolytic cleavage. Recently, dimethylsulfide-mediated HF deprotection based on the S_N2 mechanism was discussed by Tam *et al.*[28] In addition, we have concluded on the basis of several model experiments that the acid-catalyzed succinimide formation of the Asp residue[29,30] can be minimized by the use of 1 M TFMSA–thioanisole/TFA deprotection.[27] The present target peptide contains one residue of Met at position 27. We decided to carry out the synthesis using the corresponding sulfoxide[31] in order to avoid partial *S*-alkylation[32] during the N^{α}-deprotection with TFA. As pointed out by us[33] and others,[34] the Met(O) can be reduced back to Met, when the deprotection with TFMSA/TFA or HF is performed in the presence of thioanisole or other sulfur compounds. Thus, the deprotecting procedure that we chose for the present synthesis is expected to have many advantageous features. Accordingly, amino acid derivatives bearing protecting groups removable by TFMSA–thioanisole/TFA were employed, *i.e.*, Z, Z(OMe), Mts[35] and Bzl.

In order to construct the tetratetracontapeptide backbone of hpGRF, we selected eight fragments (Fig. 3). The azide procedure[36] was employed as a sole tool to condense these fragments, since much less risk of racemization is involved in this procedure, compared to other amide-forming reactions. Of these fragments, fragment [3] containing the Asp(OBzl)-group was prepared with the aid of a substituted hydrazine, Troc–$NHNH_2$,[37] the protecting group of which is known to be cleaved by Zn in AcOH[38] without affecting the side-chain protecting Bzl group.

Based on the strategies described above, we undertook the synthesis of the tetratetracontapeptide (hpGRF). The synthesis of the half molecule, the protected docosapeptide amide derived by condensation of three fragments from [1] to [3], is reported herein.

First, the C-terminal-protected dodecapeptide amide, Z(OMe)–Glu(OBzl)–Ser–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2, Z(OMe)–(hpGRF 33—44)–NH_2 [1], was prepared according to the scheme illustrated in Fig. 4. For this synthesis, two fragments, Z(OMe)–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2 (1-a position 40—44) and Z(OMe–Glu(OBzl)–Arg(Mts)–Gly–$NHNH_2$ (1-b, position 37—39), served as the starting materials. In order to prepare the former pentapeptide (1-a), two dipeptide units, Z(OMe)–Arg(Mts)–Leu–NH_2 and Z(OMe)–Arg(Mts)–Ala–OMe, were prepared by the DCC+HOBT procedure[39] and the latter, after conversion to the corresponding hydrazide, was condensed with the TFA-treated sample of the former dipeptide amide, *via* the azide, to give the protected tetrapeptide amide, Z(OMe)–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2. Next, Z(OMe)–Ala–OH was added to the TFA-treated sample of this tetrapeptide, *via* the active Np ester,[40] to give the protected C-terminal pentapeptide (1-a). The other protected tripeptide hydrazide (1-b) was prepared in a stepwise manner starting with H–Gly–NHNH–Troc[41] by successive addition of Z(OMe)–Arg(Mts)–OH and Z(OMe)–Glu(OBzl)–OH, where the DCC+HOBT and the Np ester procedures were employed, respectively. From the resulting tripeptide derivative, Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–NHNH–Troc, the Troc group was removed smoothly by treatment with Zn powder in the presence of AcOH to give the protected tripeptide hydrazide (1-b). The azide condensation of (1-b) and the TFA-treated sample of (1-a) went smoothly to give the protected octapeptide amide, Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2. Chain elongation of the octapeptide to the dodecapeptide was carried out by stepwise addition of the corresponding Z(OMe)–amino acids, *via* the Np ester or the azide. The desired protected dodecapeptide amide [1] was purified by precipitation from DMF with MeOH.

Fragment [2], Z(OMe)–Ser–Arg(Mts)–Gln–Gln–Gly–$NHNH_2$, Z(OMe)–(hpGRF 28—32)–$NHNH_2$, was prepared in a stepwise manner starting with the known dipeptide, Z(OMe)–

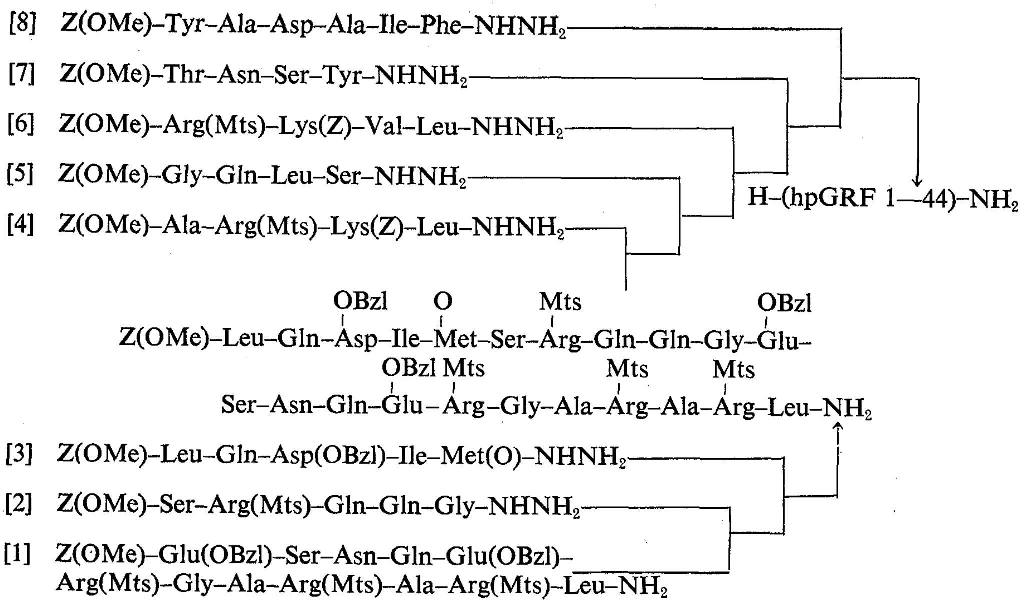

Fig. 3. Synthetic Route to hpGRF

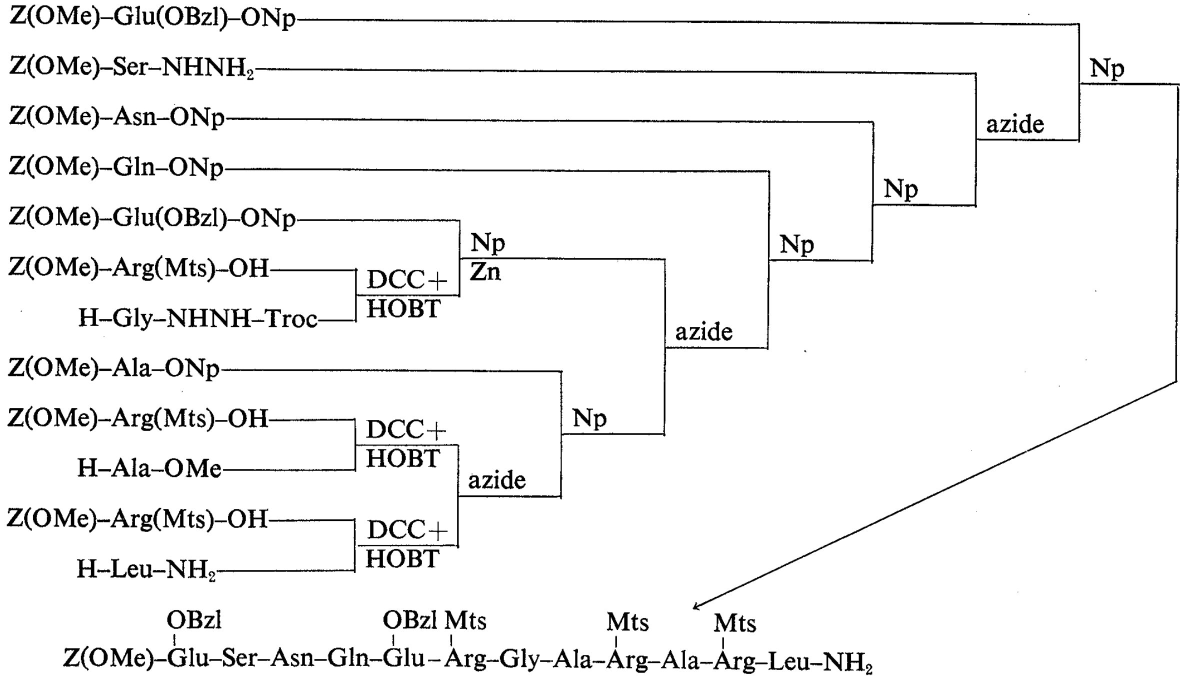

Fig. 4. Synthetic Scheme for the Protected Dodecapeptide Amide, Z(OMe)–(hpGRF 33—44)–NH_2 [1]

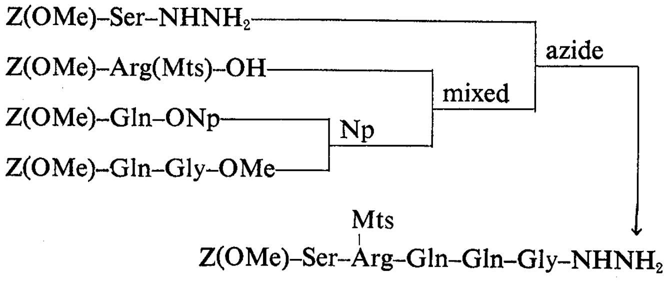

Fig. 5. Synthetic Scheme for the Protected Pentapeptide Hydrazide, Z(OMe)–(hpGRF 28—32)–$NHNH_2$ [2]

Gln–Gly–OMe,[42] as shown in Fig. 5. Onto the TFA-treated sample of this dipeptide, the Gln residue was introduced by the Np method, the Arg(Mts) residue by the mixed anhydride method[43] and the Ser residue by the azide procedure. The resulting pentapeptide ester, Z(OMe)–Ser–Arg(Mts)–Gln–Gln–Gly–OMe, was converted smoothly to the corresponding hydrazide [2] by the usual hydrazine treatment.

Fragment [3], Z(OMe)–Leu–Gln–Asp(OBzl)–Ile–Met(O)–$NHNH_2$, was also prepared in a stepwise manner starting with H–Met(O)–NHNH–Troc.[44] The base-catalyzed succinimide formation of the Asp(OBzl) residue seems to be sequence-dependent[45] and much less risk of this side reaction seems to be involved in the Asp(OBzl)–Ile linkage. Thus, we decided to keep the Bzl protecting group at this Asp residue. The substituted hydrazine was used to obtain the desired hydrazide [3]. Onto the Met(O) residue, the Ile residue was introduced by the DCC method, the Asp(OBzl) residue by the mixed anhydride procedure, the Gln residue by the Np method and the Leu residue by the mixed anhydride procedure, successively. The resulting pentapeptide, Z(OMe)–Leu–Gln–Asp(OBzl)–Ile–Met(O)–NHNH–Troc, was treated with Zn–AcOH to give the desired hydrazide [3].

The three fragments thus prepared were condensed successively by the azide procedure as

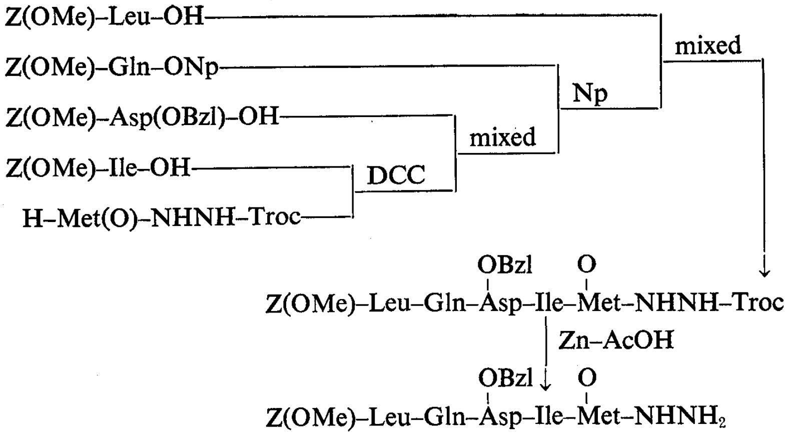

Fig. 6. Synthetic Scheme for the Protected Pentapeptide Hydrazide, Z(OMe)-(hpGRF 23—27)-$NHNH_2$ [3]

Table I. Amino Acid Ratios in 6 N HCl Hydrolysates of Z(OMe)-(hpGRF 23—44)-NH_2 and Intermediates

	40—44	37—44	36—44	35—44	34—44	33—44	28—44	23—44	Residue
Asp				1.01	1.03	0.93	0.93	1.81	2
Ser					0.96	0.79	1.65	1.75	2
Glu		1.16	1.98	1.89	1.95	2.89	4.78	5.88	6
Gly		1.01	1.01	0.99	1.02	0.95	1.92	2.01	2
Ala	2.12	2.09	2.16	2.16	2.17	2.12	2.08	2.13	2
Met[a]								0.79	1
Ile								0.89	1
Leu	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	2
Arg	1.89	2.82	2.86	2.91	2.94	2.81	3.75	3.81	4
Rec.	88.6%	86.7%	78.0%	93.0%	88.1%	87.2%	91.1%	94.6%	

a) Met(O) was not calculated (110 °C, for 24 h).

shown in Fig. 3. Every reaction proceeded smoothly, though a slight excess of the acyl component was required to bring each reaction to completion. Each product was easily purified by washing with 5% citric acid and 5% $NaHCO_3$, followed by precipitation from DMF with MeOH, without encountering any particular solubility problem. Throughout this synthesis, Leu was selected as the diagnostic amino acid in acid hydrolysis. By comparison of the recovery of Leu with those of newly incorporated amino acids, satisfactory condensation was ascertained, as shown in Table I.

Further elongation of this intermediate to the tetratetracontapeptide will be described in the following paper.

Experimental

General experimental procedures used were those described in Part LXXXVIII of this series.[46] An azide was prepared according to Honzl and Rudinger[36] with isoamyl nitrite and a mixed anhydride was prepared according to Vaughan and Osato[43] with isobutyl chloroformate.

Unless otherwise mentioned, products were purified by one of the following two procedures. Procedure A: For purification of protected peptides soluble in AcOEt, the extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, then dried over Na_2SO_4 and concentrated. The residue was recrystallized or precipitated from appropriate solvents. Procedure B: For purification of protected peptides less soluble in AcOEt, the crude product was triturated with ether and 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O and recrystallized or precipitated from appropriate solvents.

Thin layer chromatography of products obtained in this series was performed on silica gel (Kieselgel G, Merk). *Rf* values refer to the following v/v solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_2 $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5), Rf_3 $CHCl_3$–MeOH–AcOH (9 : 3 : 0.5).

Z(OMe)–Arg(Mts)–Leu–NH_2——Z(OMe)–Leu–NH_2 (3.50 g, 11.9 mmol) was treated with TFA–anisole (10 ml–3 ml) as usual and the TFA salt of the deprotected peptide precipitated by isopropyl ether was next treated with 4.28 N HCl–dioxane (5 ml). The resulting HCl salt, isolated as a powder by addition of ether, was dried over KOH pellets *in vacuo* for 3 h then dissolved in DMF (30 ml), together with Et_3N (1.65 ml, 11.9 mmol), Z(OMe)–Arg(Mts)–OH [prepared from 7.23 g (11.9 mmol) of the CHA salt] and HOBt (1.61 g, 11.9 mmol). After addition of DCC (2.94 g, 14.3 mmol), the solution was stirred at room temperature overnight, and filtered. The filtrate was concentrated and the product was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 6.88 g (91%), mp 107—109 °C, $[\alpha]_D^{20}$ $-10.0°$ ($c=2.0$, MeOH), Rf_1 0.68. *Anal.* Calcd for $C_{30}H_{44}N_6O_7S$: C, 56.94; H, 7.01; N, 13.28. Found: C, 56.74; H, 7.15; N, 13.05.

Z(OMe)–Arg(Mts)–Ala–$NHNH_2$——DCC (5.71 g, 27.7 mmol) was added to a mixture of Z(OMe)–Arg(Mts)–OH [prepared from 14.05 g (23.1 mmol) of the CHA salt], H–Ala–OMe [prepared from 3.87 g (27.7 mmol) of the HCl salt] and HOBt (3.12 g, 23.1 mmol) in DMF (100 ml). After being stirred overnight, the solution was filtered, then the filtrate was concentrated, and the product was purified by procedure A (yield 10.77 g, 77%). The oily dipeptide ester thus obtained was dissolved in MeOH (100 ml) and treated with 80% hydrazine hydrate (5.56 ml, 5 eq) for 24 h. The solvent was removed by evaporation and the residue was dissolved in AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from AcOEt and ether; yield 7.92 g (73%), mp 124—125 °C, $[\alpha]_D^{20}$ $+6.89°$ ($c=1.2$, DMF), Rf_1 0.64. *Anal.* Calcd for $C_{27}H_{39}N_7O_7S$: C, 53.54; H, 6.49. N, 16.19. Found: C, 53.57; H, 6.47; N, 16.16.

Z(OMe)–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2——Z(OMe)–Arg(Mts)–Leu–NH_2 (6.86 g, 10.8 mmol) was treated with TFA–anisole (14 ml–4 ml) as usual, then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (50 ml) containing Et_3N. The azide [prepared from 6.54 g (10.8 mmol) of Z(OMe)–Arg(Mts)–Ala–$NHNH_2$] in DMF (50 ml) and Et_3N (1.5 ml, 10.8 mmol) were added to the above ice-chilled solution and the mixture was stirred at 5 °C overnight. After evaporation of the solvent, the residue was treated with H_2O and the resulting powder was purified by procedure B followed by recrystallization from MeOH and ether; yield 9.77 g (87%), mp 197—198 °C, $[\alpha]_D^{20}$ $-3.2°$ ($c=1.2$, DMF), Rf_1 0.66. *Anal.* Calcd for $C_{48}H_{71}N_{11}O_{11}S_2$: C, 55.31; H, 6.87; N, 14.78. Found: C, 55.13; H, 6.81; N, 14.85.

Z(OMe)–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH_2——The above protected tetrapeptide amide (5.22 g, 5.0 mmol) was treated with TFA–anisole (10 ml–3 ml) and the N^α-deprotected peptide isolated as described above was dissolved in DMF (50 ml), together with Et_3N (1.39 ml, 10 mmol) and Z(OMe)–Ala–ONp (2.25 g, 6.0 mmol). After being stirred for 40 h, the solution was concentrated and the residue was treated with 5% Na_2CO_3. The resulting powder was purified by procedure B followed by recrystallization from MeOH and AcOEt; yield 4.36 g (78%), mp 142—145 °C, $[\alpha]_D^{20}$ $-7.6°$ ($c=0.9$, DMF), Rf_1 0.72. *Anal.* Calcd for $C_{51}H_{76}N_{12}O_{12}S_2 \cdot H_2O$: C, 54.14; H, 6.95; N, 14.85. Found: C, 54.18; H, 7.04; N, 14.81.

Z(OMe)–Arg(Mts)–Gly–NHNH–Troc——Z(OMe)–Gly–NHNH–Troc (5.01 g, 11.7 mmol) was treated with TFA–anisole (12 ml–3 ml) as usual, then dry *n*-hexane was added and the oily precipitate was dissolved in 4.2 N HCl–dioxane (5 ml). The hydrochloride was precipitated with *n*-hexane, washed 3 times with *n*-hexane and dissolved in DMF (50 ml), together with Et_3N (1.8 ml, 12.9 mmol), Z(OMe)–Arg(Mts)–OH [prepared from 7.11 g (11.7 mmol) of the CHA salt] and HOBt (1.58 g, 11.7 mmol). After addition of DCC (2.66 g, 12.9 mmol), the solution was stirred overnight and filtered. The filtrate was concentrated and the residue was purified by procedure A, followed by recrystallization from AcOEt and ether; yield 7.68 g (86%), mp 114—116 °C, $[\alpha]_D^{20}$ $+0.8°$ ($c=2.6$, MeOH), Rf_1 0.68. *Anal.* Calcd for $C_{29}H_{38}Cl_3N_7O_9S$: C, 45.40; H, 4.99; N, 12.78. Found: C, 45.69; H, 5.05; N, 12.57.

Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–NHNH–Troc——Z(OMe)–Arg(Mts)–Gly–NHNH–Troc (7.66 g, 10 mmol) was treated with TFA–anisole (15 ml–6 ml) and the N^α-deprotected peptide isolated as usual was dissolved in DMF (60 ml), together with Et_3N (2.1 ml, 15 mmol) and Z(OMe)–Glu(OBzl)–ONp (6.27 g, 12.0 mmol). After being stirred overnight, the solution was concentrated and the product was purified by procedure A, followed by recrystallization from AcOEt and isopropyl ether; yield 7.52 g (76%), mp 108—110 °C, $[\alpha]_D^{20}$ $-5.4°$ ($c=0.7$, MeOH), Rf_1 0.72. *Anal.* Calcd for $C_{41}H_{51}Cl_3N_8O_{12}S$: C, 49.92; H, 5.21; N, 11.36. Found: C, 50.29; H, 5.13; N, 11.58.

Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–$NHNH_2$——The above protected tripeptide (7.52 g, 7.6 mmol) dissolved in MeOH–AcOH (70 ml–3 ml) was treated with Zn powder (5.0 g) at room temperature overnight. The solution was filtered, the filtrate was concentrated and the residue, after being treated with 2% EDTA for 30 min, was extracted with AcOEt. The organic phase was washed with 2% EDTA, 5% $NaHCO_3$, and H_2O–NaCl, dried over Na_2SO_4 and concentrated. Trituration of the residue afforded a powder, which was recrystallized from AcOEt and 2-propanol; yield 4.22 g (68%), mp 83—87 °C, $[\alpha]_D^{20}$ $-7.6°$ ($c=0.4$, MeOH), Rf_1 0.68. Amino acid ratios in 6 N HCl hydrolysate: Glu 1.12, Arg 0.85, Gly 1.00 (recovery of Gly 71%). *Anal.* Calcd for $C_{38}H_{50}N_8O_{10}S$: C, 56.28; H, 6.22; N, 13.82.

Found: C, 55.96; H, 6.14; N, 13.30.

Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$——Z(OMe)–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$ (4.35 g, 3.9 mmol) was treated with TFA–anisole (10 ml–3 ml) and the N^{α}-deprotected peptide isolated as usual was dissolved in DMF–DMSO (3 : 1, 40 ml) containing Et$_3$N (0.54 ml, 3.9 mmol). The azide [prepared from 4.75 g (5.9 mmol) of Z(OMe)–Glu(OBzl)–Arg(Mts)–Gly–NHNH$_2$] in DMF (20 ml) and Et$_3$N (0.81 ml, 5.9 mmol) were added to the above ice-chilled solution and the mixture was stirred overnight. The solution (negative to the ninhydrin test) was then concentrated and the residue was treated with 5% citric acid. The resulting powder was purified by procedure B followed by precipitation from DMF with MeOH; yield 4.90 g (73%), mp 189—190 °C, $[\alpha]_D^{20}$ −4.2° (c=0.7, DMSO). *Rf*$_1$ 0.51. *Anal.* Calcd for $C_{80}H_{114}N_{18}O_{19}S \cdot H_2O$: C, 55.03; H, 6.70; N, 14.44. Found: C, 55.21; H, 6.78; N, 14.04.

Z(OMe)–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$——The above protected octapeptide (4.90 g, 2.8 mmol) was treated with TFA–anisole (12 ml–4 ml) and the N^{α}-deprotected peptide isolated as described above was dissolved in DMF–DMSO (3 : 1, 40 ml), together with Et$_3$N (0.39 ml, 2.8 mmol), Z(OMe)–Gln–ONp (1.83 g, 4.3 mmol) and NMM (0.29 ml, 2.8 mmol). After being stirred overnight, the solution was concentrated and the residue was treated with 5% citric acid. The resulting powder was purified by procedure B followed by precipitation from DMF with MeOH; yield 5.0 g (95%), mp 197—199 °C, $[\alpha]_D^{20}$ −6.9° (c=0.4, DMSO), *Rf*$_1$ 0.56. *Anal.* Calcd for $C_{85}H_{122}N_{20}O_{21}S_3 \cdot H_2O$; C, 54.47; H, 6.67; N, 14.95. Found: C, 54.28; H, 6.66; N, 15.02.

Z(OMe)–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$——The above protected nonapeptide (4:98 g, 2.7 mmol) was treated with TFA–anisole (10 ml–4 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMSO–DMF (1 : 3, 50 ml), together with Et$_3$N (0.37 ml, 2.7 mmol), Z(OMe)–Asn–ONp (1.68 g, 4.0 mmol) and NMM (0.27 ml, 2.7 mmol). After being stirred at 4 °C overnight, the solution was concentrated and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid and H$_2$O and precipitated twice from DMF with MeOH; yield 4.12 g (78%), mp 199—201 °C, $[\alpha]_D^{20}$ +12.3° (c=0.5, DMSO), *Rf*$_1$ 0.65. *Anal.* Calcd for $C_{89}H_{128}N_{22}O_{23}S_3 \cdot H_2O$: C, 53.76; H, 6.59; N, 15.50. Found: C, 53.99; H, 6.75; N, 15.17.

Z(OMe)–Ser–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$——The above protected decapeptide (4.11 g, 2.1 mmol) was treated with TFA–anisole (10 ml–3 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF–DMSO (1 : 1, 30 ml) containing Et$_3$N (0.29 ml, 2.1 mmol). The azide [prepared from 0.89 g (3.1 mmol) of Z(OMe)–Ser–NHNH$_2$] in DMF (5 ml) and Et$_3$N (0.44 ml, 3.1 mmol) were added. After being stirred at 4 °C overnight, the solution was concentrated and the residue was treated with 5% citric acid. The resulting powder was purified as stated above; yield 3.97 g (92%), mp 196—198 °C, $[\alpha]_D^{20}$ +6.1° (c=0.7, DMSO), *Rf*$_1$ 0.57. *Anal.* Calcd for $C_{92}H_{133}N_{23}O_{25}S_3 \cdot H_2O$: C, 53.24; H, 6.56; N, 15.46. Found: C, 53.33; H, 6.69; N, 15.09.

Z(OMe)–Glu(OBzl)–Ser–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$ [1]——The above protected undecapeptide (3.96 g 1.9 mmol) was treated with TFA–anisole (10 ml–3 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF–DMSO (1 : 1, 40 ml) together with Et$_3$N (0.53 ml 3.8 mmol) and Z(OMe)–Glu(OBzl)–ONp (1.20 g, 2.3 mmol). Stirring was continued for an additional 24 h, then H$_2$O (200 ml) was added and the resulting powder was purified by washing with 5% citric acid and H$_2$O, followed by precipitation from DMF with MeOH; yield 3.98 g (91%), mp 209—210 °C, $[\alpha]_D^{20}$ −8.9° (c=0.6, DMSO), *Rf*$_1$ 0.62. *Anal.* Calcd for $C_{104}H_{149}N_{24}O_{28}S_3 \cdot 3H_2O$; C, 53.59; H, 6.57; N, 14.42. Found: C, 53.62; H, 6.50; N, 14.41.

Z(OMe)–Gln–Gln–Gly–OMe——Z(OMe)–Gln–Gly–OMe (9.30 g, 24.4 mmol) was treated with TFA–anisole (21 ml–5.3 ml) and the N^{α}-deprotected peptide isolated as usual was dissolved in DMF–DMSO (1 : 1, 150 ml), together with Et$_3$N (7.2 ml, 51.2 mmol) and Z(OMe)–Gln–ONp (11.57 g, 26.8 mmol). After being stirred overnight, the solution was concentrated and the residue was purified by procedure B followed by precipitation from a mixture of DMF–DMSO (1 : 1) with MeOH; yield 12.10 g (97%), mp 242—243 °C, $[\alpha]_D^{20}$ +1.8° (c=1.1, DMSO), *Rf*$_1$ 0.49. *Anal.* Calcd for $C_{22}H_{31}N_5O_9$: C, 51.86; H, 6.13; N, 13.75. Found: C, 51.85; H, 5.91; N, 13.77.

Z(OMe)–Arg(Mts)–Gln–Gln–Gly–OMe——The above protected tripeptide (11.0 g, 21.6 mmol) was treated with TFA–anisole (23 ml—4.7 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (100 ml) containing Et$_3$N (3.0 ml, 21.6 mmol). A mixed anhydride [prepared from 15.40 g (24.8 mmol) of Z(OMe)–Arg(Mts)–OH, CHA] in THF (100 ml) was added to the above ice-chilled solution and the mixture was stirred in an ice-bath for 5 h. The solvent was removed by evaporation and the residual solid was purified by procedure B followed by precipitation from DMF with EtOH; yield 16.0 g (87%), mp 198—199 °C, $[\alpha]_D^{20}$ −1.1° (c=0.9, DMSO). *Rf*$_1$ 0.48. *Anal.* Calcd for $C_{37}H_{53}N_9O_{12}S$: C, 52.41; H, 6.30; N, 14.87. Found: C, 52.25; H, 6.16; N, 14.63.

Z(OMe)–Ser–Arg(Mts)–Gln–Gln–Gly–OMe——The above protected tetrapeptide (15.0 g, 17.7 mmol) was treated with TFA–anisole (31 ml–7.7 ml) and the N^{α}-deprotected peptide isolated as stated above was dissolved in DMF (150 ml) containing Et$_3$N (2.5 ml, 17.7 mmol). The azide [prepared from 5.51 g (19.5 mmol) of Z(OMe)–Ser–NHNH$_2$] in DMF (50 ml) and Et$_3$N (3.0 ml, 21.4 mmol) were added to the above ice-chilled solution and the mixture, after being stirred overnight, was concentrated. The residual solid was purified by procedure B, followed by precipitation from DMF with EtOH; yield 14.95 g (90%), mp 199—200 °C, $[\alpha]_D^{20}$ −3.6° (c=1.1, DMSO), *Rf*$_1$ 0.35. *Anal.* Calcd for $C_{40}H_{58}N_{10}O_{14}S$: C, 51.38; H, 6.25; N, 14.98. Found: C, 51.29; H, 6.53; N, 15.13.

Z(OMe)–Ser–Arg(Mts)–Gln–Gln–Gly–NHNH$_2$ [2]——The above protected pentapeptide ester (7.0 g, 7.5 mmol) in DMF–MeOH (1 : 1, 100 ml) was treated with 80% hydrazine hydrate (4.7 ml, 10 eq) overnight and the solvent was removed by evaporation. Treatment of the residue with EtOH afforded a powder, which was precipitated form DMF with EtOH; yield 5.50 g (79%), mp 207—208 °C, $[\alpha]_D^{20}$ $-3.3°$ ($c=1.2$, DMSO), Rf_1 0.13. Amino acid ratios in 6 N HCl hydrolysate: Ser 0.92, Arg 0.95, Glu 1.99, Gly 1.00 (recovery of Gly 89%). *Anal.* Calcd for $C_{39}H_{58}N_{12}O_{13}S$: C, 50.09; H, 6.25; N, 17.98. Found: C, 49.80; H, 6.37; N, 17.82.

Z(OMe)–Ile–Met(O)–NHNH–Troc——Z(OMe)–Met(O)–NHNH–Troc (17.82 g, 34.3 mmol) was treated with TFA–anisole (20 ml–11.1 ml) in an ice-bath for 30 min, then most of the solvent was removed by evaporation. The residue was dissolved in 5% citric acid and washed with ether. The aqueous phase was made alkaline with 0.1 N NaOH and extracted with *n*-BuOH–AcOEt (1 : 1). The organic phase was dried over $MgSO_4$ and concentrated. The residue was dissolved in DMF (170 ml) together with Z(OMe)–Ile–OH (11.14 g, 37.7 mmol) and then DCC (8.48 g, 41.2 mmol) was added. After being stirred overnight, the solution was filtered, the filtrate was concentrated and the residue was treated with H_2O and ether. The resulting solid was recrystallized from MeOH and ether; yield 17.42 g (80%), mp 171—173 °C, $[\alpha]_D^{20}$ $-31.9°$ ($c=1.3$, MeOH), Rf_2 0.48. *Anal.* Calcd for $C_{23}H_{33}Cl_3N_4O_8S$: C, 43.71; H, 5.26; N, 8.87. Found: C, 43.57; H, 5.47; N, 8.82.

Z(OMe)–Asp(OBzl)–Ile–Met(O)–NHNH–Troc——Z(OMe)–Ile–Met(O)–NHNH–Troc (17.21 g, 27.2 mmol) was treated with TFA–anisole (25 ml–8.8 ml) and the N^{α}-deprotected peptide, precipitated by *n*-hexane as an oil, was dried over KOH pellets *in vacuo* for 3 h then dissolved in DMF (70 ml) containing Et_3N(3.8 ml, 27.2 mmol). A mixed anhydride [prepared from 11.59 g (29.9 mmol) of Z(OMe)–Asp(OBzl)–OH] in DMF (100 ml) was added to the above ice-chilled solution. After being stirred in an ice-bath for 3 h, the solution was concentrated and the residue was treated with H_2O and ether. The resulting powder was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 19.11 g (84%), mp 187—189 °C, $[\alpha]_D^{20}$ $-8.6°$ ($c=1.0$, DMF), Rf_1 0.66, Rf_2 0.44. *Anal.* Calcd for $C_{34}H_{44}Cl_3N_5O_{11}S$: C, 48.78; H, 5.30; N, 8.37. Found: C, 48.56; H, 5.37; N, 8.39.

Z(OMe)–Gln–Asp(OBzl)–Ile–Met(O)–NHNH–Troc——The above protected tripeptide (18.56 g, 22.2 mmol) was treated with TFA–anisole (23 ml–7.2 ml) and the N^{α}-deprotected peptide, isolated in the usual manner, was dissolved in DMF (140 ml), together with Et_3N (3.1 ml, 22.2 mmol), Z(OMe)–Gln–ONp (10.53 g, 24.4 mmol) and NMM (2.7 ml, 24.4 mmol). After being stirred for 6 h, the solution was diluted with H_2O–NaCl (200 ml). The resulting powder was purified by procedure B followed by precipitation from DMF with EtOH; yield 16.44 g (77%), mp 203—204 °C, $[\alpha]_D^{20}$ $-16.5°$ ($c=1.1$, DMF), Rf_1 0.69, Rf_2 0.41. *Anal.* Calcd for $C_{39}H_{52}Cl_3N_7O_{13}S$: C, 48.52; H, 5.43; N, 10.16. Found: C, 48.49; H, 5.44; N, 10.21.

Z(OMe)–Leu–Gln–Asp(OBzl)–Ile–Met(O)–NHNH–Troc——The above protected tetrapeptide (15.01 g, 15.5 mmol) was treated with TFA–anisole (25 ml–5.0 ml), and the N^{α}-deprotected peptide isolated in the usual manner was dissolved in DMF (70 ml) containing NMM (1.7 ml, 15.5 mmol). A mixed anhydride [prepared from 5.04 g (17.1 mmol) of Z(OMe)–Leu–OH] in DMF (80 ml) was added to the above ice-chilled solution and the mixture, after being stirred in an ice-bath for 3 h, was diluted with H_2O–NaCl. The resulting powder was purified by procedure B followed by precipitation from DMF with MeOH; yield 12.81 g (77%), mp 213—215 °C, $[\alpha]_D^{20}$ $-22.1°$ ($c=1.2$, DMF), Rf_1 0.72, Rf_2 0.23, Rf_3 0.75. *Anal.* Calcd for $C_{45}H_{63}Cl_3N_8O_{14}S$: C, 50.11; H, 5.89; N, 10.39. Found: C, 50.03; H, 6.03; N, 10.43.

Z(OMe)–Leu–Gln–Asp(OBzl)–Ile–Met(O)–NHNH$_2$ [3]——AcOH (5 ml) and then Zn powder (3.09 g, 10 eq) were added to a solution of the above protected pentapeptide (5.09 g, 4.7 mmol) in DMF–HMPA (1 : 1, 50 ml). A gelatinous mass, formed during stirring at 40 °C for 3 h, was dissolved by addition of DMSO (50 ml) and the solution was filtered. The filtrate was mixed with 2% EDTA (400 ml) and the resulting powder was washed with 2% EDTA, 5% $NaHCO_3$ and H_2O, then precipitated three times from DMSO with MeOH; yield 2.41 g (57%), mp 238—240 °C, $[\alpha]_D^{20}$ $-4.8°$ ($c=0.4$, DMSO), Rf_1 0.49. Amino acid ratios in 6 N HCl hydrolysate: Asp 1.02, Glu 1.04, Met 0.79, Ile 1.00, Leu 1.01 (recovery of Ile 88%). *Anal.* Calcd for $C_{42}H_{62}N_8O_{12}S \cdot H_2O$: C, 54.77; H, 7.00; N, 12.17. Found: C, 54.55; H, 6.85; N, 11.98.

Z(OMe)–Ser–Arg(Mts)–Gln–Gln–Gly–Glu(OBzl)–Ser–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$, Z(OMe)–(hpGRF 28—44)–NH$_2$——Z(OMe)–(hpGRF 33—44)–NH$_2$ (3.98 g, 1.75 mmol) was treated with TFA–anisole (10 ml–4 ml) in an ice-bath for 60 min as usual, then dry ether was added. The N^{α}-deprotected peptide, precipitated by dry ether, was dried over KOH pellets *in vacuo* for 3 h, then dissolved in DMSO–DMF (1 : 1, 40 ml) containing Et_3N (0.25 ml, 1.75 mmol). The azide [prepared from 2.45 g (2.62 mmol) of Z(OMe)–(hpGRF 28—32)–NHNH$_2$] in DMF (10 ml) and NMM (0.26 ml, 2.62 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C overnight. The ninhydrin-negative solution was diluted with H_2O (200 ml) and the resulting powder was purified by procedure B, followed by precipitation twice from DMF with MeOH; yield 4.78 g (91%); mp 229—230 °C, $[\alpha]_D^{20}$ $+3.1°$ ($c=0.3$, DMSO), Rf_1 0.46. *Anal.* Calcd for $C_{134}H_{192}N_{34}O_{38}S_4 \cdot 4H_2O$: C, 52.12; H, 6.58; N, 15.43. Found: C, 52.45; H, 6.49; N, 15.03.

Z(OMe)–Leu–Gln–Asp(OBzl)–Ile–Met(O)–Ser–Arg(Mts)–Gln–Gln–Gly–Glu(OBzl)–Ser–Asn–Gln–Glu(OBzl)–Arg(Mts)–Gly–Ala–Arg(Mts)–Ala–Arg(Mts)–Leu–NH$_2$, Z(OMe)–(hpGRF 23—44)–NH$_2$——The above protected hexadecapeptide amide, Z(OMe)–(hpGRF 28—44)–NH$_2$ (4.76 g, 1.58 mmol) was treated with TFA–anisole (10 ml–4 ml) and the N^{α}-deprotected peptide obtained as described above was dissolved in DMSO–DMF (1 : 1, 40 ml) containing Et_3N (0.22 ml, 1.58 mmol). The azide [prepared from 2.14 g (2.37 mmol) of Z(OMe)–(hpGRF 23—27)–NHNH$_2$] in DMF–DMSO (2 : 1, 10 ml) and Et_3N (0.33 ml, 2.37 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C overnight. The ninhydrin-negative solution was diluted with H_2O (180 ml) and the resulting powder was purified by procedure B, followed by precipitation twice from DMSO–MeOH; yield 5.40 g (92%), mp 265—267 °C, $[\alpha]_D^{20}$ $-8.6°$ (c = 0.6, DMSO), Rf_1 0.41. *Anal.* Calcd for $C_{167}H_{242}N_{40}O_{47}S_5$: C, 53.88; H, 6.55; N, 15.05. Found: C, 53.59; H, 6.65; N, 14.80.

References and Notes

1) Part CXV: K. Okamoto, K. Yasuharu, K. Fujitani, S. Katakura, K. Akaji, H. Yajima, Y. Nakata, A. Inoue, and T. Segawa, *Chem. Pharm. Bull.*, **32**, 430 (1984).
2) Amino acids and peptide derivatives mentioned in this investigation are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Bzl = benzyl, Mts = mesitylene-2-sulfonyl, Troc = *β*,*β*,*β*-trichloroethyloxycarbonyl, Np = *p*-nitrophenyl, Su = *N*-hydroxysuccinimidyl, DCC = dicyclohexylcarbodiimide, HOBT = *N*-hydroxybenzotriazole, CHA = cyclohexylamine, NMM = *N*-methylmorpholine, EDTA = ethylenediaminetetraacetic acid disodium salt, TFA = trifluoroacetic acid, DMF = dimethylformamide, DMSO = dimethylsulfoxide, TFMSA = trifluoromethanesulfonic acid, VIP = vasoactive intestinal polypeptide, GIP = gastric inhibitory polypeptide.
3) J. D. Green and G. W. Harris, *J. Physiol.* (London), **108**, 359 (1949).
4) R. Burgus, T. F. Dunn, D. Desiderio, D. N. Ward, W. Vale, and R. Guillemin, *Nature* (London), **226**, 321 (1970).
5) R. M. G. Nair, J. F. Barrett, C. Y. Bowers, and A. V. Schally, *Biochemistry*, **9**, 1103 (1970).
6) H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **43**, 1334 (1971).
7) R. Burgus, M. Butcher, M. Amoss, N. Ling, M. Monahan, J. Rivier, R. Fellows, R. Blackwell, W. Vale, and R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 278 (1972).
8) P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin, *Science*, **179**, 77 (1973).
9) R. Burgus, N. Ling, M. Butcher, and R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 684 (1973).
10) W. Vale, J. Spiess, C. Rivier, and J. Rivier, *Science*, **213**, 1394 (1981).
11) J. Spiess, J. Rivier, C. Rivier, and W. Vale, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6517 (1981).
12) C. Beck, R. G. Larkins, T. J. Martin, and H. G. Burger, *J. Endocrinol.*, **59**, 325 (1973); R. H. Caplan, L. Koob, R. M. Abellera, A. S. Pagliara, K. Kovacs, and R. V. Randall, *Am. J. Med.*, **64**, 874 (1978); M. O. Thorner, R. L. Perryman, M. J. Cronin, M. Draznin, A. Johanson, A. D. Rogol, J. Jane, L. Rudolf, E. Horvath, K. Kovacs, and W. Vale, *Clin. Res.*, **30**, 555A (1982).
13) R. Guillemin, P. Brazeau, P. Böhlen, F. Esch, N. Ling, and W. B. Wehrenberg, *Science*, **218**, 585 (1982).
14) F. S. Esch, P. Böhlen, N. C. Ling, P. E. Brazeau, W. B. Wehrenberg, M. O. Thorner, M. J. Cronin, and R. Guillemin, *Biochem. Biophys. Res. Commun.*, **109**, 152 (1982).
15) F. S. Esch, P. Böhlen, N. C. Ling, P. E. Brazeau, W. B. Wehrenberg, and R. Guillemin, *J. Biol. Chem.*, **258**, 1806 (1983).
16) P. Brazeau, N. Ling, P. Böhlen, F. Esch, S.-Y. Ying, and R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 7909 (1982).
17) W. B. Wehrenberg, N. Ling, P. Brazeau, F. Esch, P. Böhlen, A. Baird, S. Ying, and R. Guillemin, *Biochem. Biophys. Res. Commun.*, **109**, 382 (1982).
18) J. Rivier, J. Spiess, M. Thorner, and W. Vale, *Nature* (London), **300**, 276 (1982).
19) J. Spiess, J. Rivier, M. Thorner, and W. Vale, *Biochemistry*, **21**, 6037 (1982).
20) S. Sundler, R. Hakanson, and S. Leander, *Clinics in Gastroenterology*, **9**, 517 (1980); C. B. Irvine and R. F. Murphy, *Gut.*, **22**, 1048 (1981); S. R. Bloom and J. M. Polak, "Gut Hormones," Churchill Livingstone, London, 1981 and other references cited therein.
21) K. Tatemoto and V. Mutt, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6603 (1981).
22) K. Akaji, N. Fujii, H. Yajima, M. Moriga, M. Aono, and A. Takagi, *Int. J. Peptide Protein Res.*, **18**, 180 (1981).
23) Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, *J. Chem. Soc., Chem. Commun.*, **1979**, 971.
24) N. Fujii, S. Funakoshi, T. Sasaki, and H. Yajima, *Chem. Pharm. Bull.*, **25**, 3096 (1977).
25) H. Irie, N. Fujii, H. Ogawa, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, **1976**, 922.
26) Y. Kiso, K. Ukawa, S. Nakamura, K. Ito, and T. Akita, *Chem. Pharm. Bull.*, **28**, 673 (1980); Y. Kiso, K. Ukawa, and T. Akita, *J. Chem. Soc., Chem. Commun.*, **1980**, 101.
27) H. Yajima and N. Fujii, *J. Am. Chem. Soc.*, **103**, 5867 (1981).
28) J. P. Tam, W. F. Heath, and R. B. Merrifield, *Int. J. Peptide Protein Res.*, **21**, 57 (1983); W. F. Heath, J. P. Tam, and R. B. Merrifield, *J. Chem. Soc., Chem. Commun.*, **1982**, 896.
29) J. Martinez and M. Bodanszky, *Int. J. Peptide Protein Res.*, **12**, 277 (1978); M. Bodanszky, J. C. Tolle, S. S. Deshmane, and A. Bodanszky, *ibid.*, **12**, 57 (1978) and other references cited therein.
30) I. Schön and L. Kisfaludy, *Int. J. Peptide Protein Res.*, **14**, 485 (1979).

31) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961); N. Fujii, T. Sasaki, S. Funakoshi, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 650 (1978).

32) R. L. Noble, D. Yamashiro, and C. H. Li, *J. Am. Chem. Soc.*, **98**, 2324 (1976); B. F. Lundt, N. L. Johansen, A. Vølund, and J. Markussen, *Int. J. Peptide Protein Res.*, **12**, 258 (1978).

33) S. Funakoshi, N. Fujii, H. Yajima, C. Shigeno, I. Yamamoto, R. Morita, and K. Torizuka, *Chem. Pharm. Bull.*, **30**, 1706 (1982).

34) J. P. Tam, W. F. Heath, and R. B. Merrifield, *Tetrahedron Lett.*, **23**, 2939 (1982); D. Yamashiro, *Int. J. Peptide Protein Res.*, **20**, 63 (1982).

35) H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *J. Chem. Soc., Chem. Commun.*, **1978**, 482.

36) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).

37) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.*, **19**, 420 (1971).

38) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).

39) W. König and R. Geiger, *Chem. Ber.*, **103**, 788, 2024, 2034 (1970).

40) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

41) F. Tamura, H. Ogawa, N. Fujii, H. Yajima, K. Miyata, M. Nakamura, and A. Tanaka, *Chem. Pharm. Bull.*, **25**, 767 (1977).

42) H. Ogawa, M. Sugiura, H. Yajima, H. Sakurai, and K. Tsuda, *Chem. Pharm. Bull.*, **26**, 1549 (1978).

43) Th. Wieland and H. Bernhard, *Justus Liebigs Ann. Chem.*, **572**, 190 (1951); R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

44) N. Fujii and H. Yajima, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 819.

45) M. Bodanszky and J. Z. Kwei, *Int. J. Peptide Protein Res.*, **12**, 69 (1978).

46) N. Fujii and H. Yajima, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 789.