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4-Methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr): a New Amino and Imidazole Protecting Group in Peptide Synthesis[1)]

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The 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group was introduced as a protecting group for the amino function, especially the ε-amino function of lysine, and the imidazole ring of histidine. The N^{ε}-Mtr group was removable by dilute methanesulfonic acid-containing trifluoroacetic acid–thioanisole, but was stable to trifluoroacetic acid or catalytic hydrogenation. The N^{im}-Mtr group was removable by trifluoroacetic acid–dimethylsulfide or 1-hydroxybenzotriazole, but was more stable than the N^{im}-Tos or the N^{im}-Mbs group with triethylamine. To examine the usefulness of the Mtr group as a protecting group for use in peptide synthesis, mastoparan X and chicken gastrin releasing peptide (c-GRP) were synthesized, and this group was found to be very convenient for general solution synthesis. In particular, the introduction of Lys(Mtr) made possible a new strategy in peptide synthesis.

Keywords——4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr); N^{ε}-4-methoxy-2,3,6-trimethylbenzenesulfonyllysine; N^{im}-4-methoxy-2,3,6-trimethylbenzenesulfonylhistidine; methanesulfonic acid–trifluoroacetic acid–thioanisole deprotection; trifluoroacetic acid–dimethylsulfide deprotection; mastoparan X; chicken gastrin releasing peptide

Among the various kinds of amino-protecting groups that have been reported to date, the Z[2)] and Boc[3)] groups are especially useful. However, these groups are not sufficient for synthesizing complicated, sulfur-containing peptides. Also, protecting groups which resist either TFA treatment or catalytic hydrogenation but can be removed under mild acidic conditions are desired. The N^{ε}-Tos group[4)] is known to be resistant to both TFA and catalytic hydrogenation but can be removed with Na in liquid ammonia and is very stable to all acidic conditions used for deprotection in peptide chemistry.

In our comparative studies on multi-substituted benzenesulfonyl-protecting groups for the guanidino function of arginine[5)] and indole nitrogen of tryptophan,[6)] we observed that the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group is the most acid-labile protecting group among the arylsulfonyl groups tested. Therefore, we introduced this group for protection of the amino function (*e.g.*, the α-amino group of Gly or Ile, and the ε-amino group of Lys) and examined its acid lability (shown in Table I).

Introduction of the Mtr group at the α-amino function of Gly or Ile was easily achieved by treating the amino acid with Mtr-Cl in the presence of $NaHCO_3$ in aqueous THF. In the case of Lys, this group was introduced at the ε-amino function according to the method of

Table I. Deproection of the Mtr Group attached to the Amino Group

Compound	0.075 M MSA in TFA–thioanisole (9:1) at 20°C	0.15 M MSA in TFA–thioanisole (9:1) at 20°C	0.3 M MSA in TFA–thioanisole (9:1) at 20°C	TFA–thioanisole[a)] (9:1) at 50°C	HF–thioanisole at 0°C	MSA–thioanisole at 20°C	TFA (neat) at 20°C
Lys (Mtr) 1h	70.6	80.7	95.1[b)]	15.1	3.6	2.3	0.0
2h	—	91.9[b)]	99.3[b)]	33.6	—	—	0.0

The reaction products were subjected to quantitative amino acid analysis to estimate the content of regenerated Lys (%).
a) Mtr-Gly-OH and Mtr-Ile-OH were treated for 1 h, and 101.4 % of Gly and 95.6 % of Ile were recovered.
b) The starting material did not appear on TLC.

Neuberger *et al.*[7] by treating the Lys. Cu(II) complex with Mtr-Cl in the presence of $NaHCO_3$ in aqueous acetone. After removal of Cu ion by H_2S, Lys(Mtr) could be obtained as crystals.

The Mtr group attached to the α-amino function of Gly or Ile could be removed by TFA–thioanisole at 50°C within 1 h, but the same group attached to the ε-amino function of Lys was somewhat more resistant to these conditions, and could be removed only partially. However, addition of a small amount of methanesulfonic acid (MSA) to TFA–thioanisole considerably accelerated the cleavage of the N^{ε}-Mtr group, and 0.15—0.3 M MSA-containing TFA–thioanisole could be used to remove this group. These conditions limited aspartimide formation of the model peptide, H–Trp–Asp(OBu^t)–Asn–Gln–OBu^t, to within 20% which is comparable to that seen with the HF treatment reported previously.[8] On the other hand, N^{ε}-Mtr was very stable to TFA treatment at room temperature. Surprisingly, it was also very stable to both anhydrous HF–thioanisole and MSA–thioanisole. These results strongly suggested that the Mtr group would be very useful as an N^{ε}-amino protecting group as well as an α-amino protecting one.

Next, we used this group to protect the imidazole ring of histidine. As N^{im}-benzenesulfonyl-type protecting groups, the N^{im}-Tos[9] and the N^{im}-Mbs[10] groups are known. These groups can be removed by treatment under mild acidic conditions such as with TFA–dimethylsulfide, but they are somewhat unstable under basic conditions. Since the N^{G}–Mtr group is more acid-labile than the other multi-substituted benzenesulfonyl-type protecting groups, we considered that the N^{im}-Mtr group might be more resistant than others to basic conditions and we therefore examined its stability to base.

Boc–His(Mtr)–OH was prepared from Boc–His–OH and Mtr-Cl by the procedure used for the preparation of Boc–His(Tos)–OH,[9] and obtained as a crystalline DCHA salt. Z–His(Mtr)–OH was prepared similarly and characterized as a crystalline DCHA compound. H–His(Mtr)–OH was also obtained as a crystalline compound after catalytic hydrogenation of Z–His(Mtr)–OH.

The stability of Boc–His(Mtr)–OH was examined with 0.5 M triethylamine in 50% aqueous MeOH and compared with those of Boc–His(Tos)–OH and Boc–His(Mbs)–OH by high performance liquid chromatography (HPLC)[11] analysis (shown in Table II).

Table II. Stability of Boc-His (X)-OH in 0.5 M TEA

Protecting group (X)	Recovery (%) of the starting material 1.0 h	2.0 h	4.0 h
Mtr	98.4	92.4	86.4
Mbs	81.4	67.5	43.3
Tos	55.5	15.3	5.1

Boc–His(X)–OH·DCHA was treated with 0.5 M TEA in 50 % aq. MeOH at 26 °C, and the amount of the starting material which remained was estimated by HPLC.[11]

The N^{im}-Tos and the N^{im}-Mbs groups were removed with 0.5 M TEA (at room temperature for 4 h) to the extents of 94.9% and 56.7%, respectively. Unlike these groups, the N^{im}-Mtr group was considerably stable and only 13.6% split off under the same conditions. Like the N^{im}-Tos or the N^{im}-Mbs group, the N^{im}-Mtr group could be removed by either TFA–dimethylsulfide or HOBt within 1 h. However, this group was stable to catalytic hydrogenation and also relatively stable to TFA treatment (checked by thin layer chromatography (TLC)). These results suggested that the Mtr group could be used not only for protection of the ε-amino function of Lys and the indole ring of Trp, but also for temporary protection of the imidazole ring of His.

In order to demonstrate the usefulness of this group in practical peptide synthesis, we synthesized two biologically active peptides, mastoparan X and chicken gastrin releasing peptide (c-GRP).

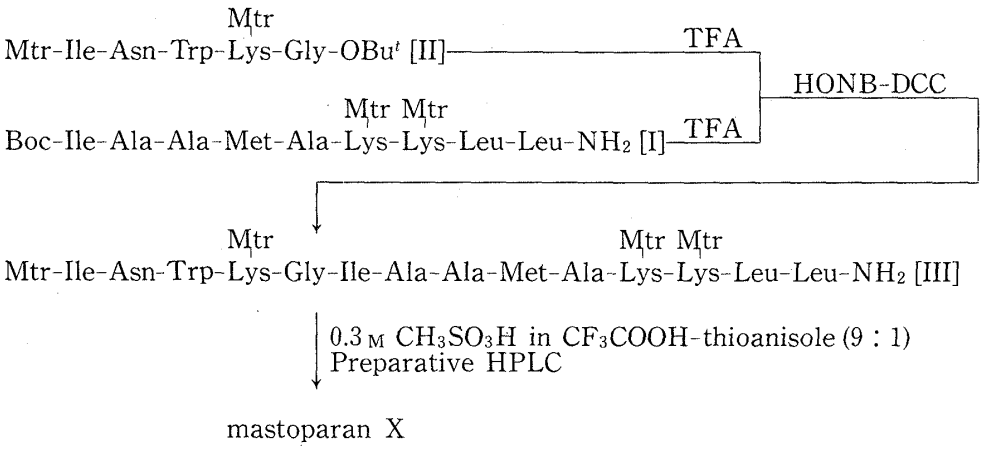

Fig. 1. Synthetic Scheme for Mastoparan X

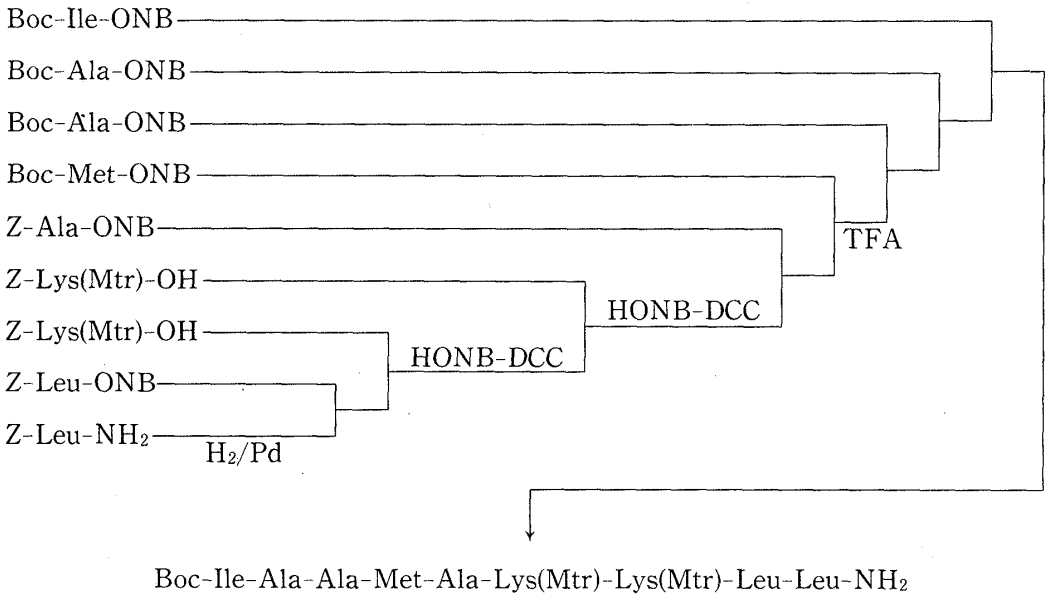

Fig. 2. Preparation of the Protected Nonapeptide I

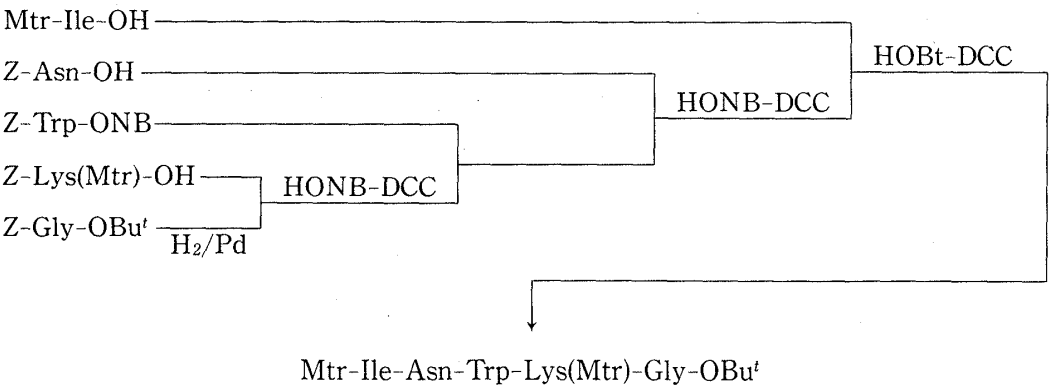

Fig. 3. Preparation of the Protected Pentapeptide II

Mastoparan X is a tetradecapeptide amide having mast cell degranulating activity and was isolated from the Japanese hornet, *Vespa xanthoptera*. Its structure was elucidated as H–Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–Ala–Lys–Lys–Leu–Leu–NH_2 by Nakajima *et al.*,[12] and its synthesis was first reported by Yajima *et al.*[13] This peptide contains four free amino groups; one α-amino group of Ile and three ε-amino groups of Lys. Our synthetic scheme for mastoparan X is outlined in Fig. 1.

Both the N-terminal and the side chain amino functions of Lys were protected by the Mtr group, and the α-amino function of the intermediates was temporarily protected by either the Z or the Boc group.

As shown in Fig. 1, two peptide fragments, Boc–Ile–Ala–Ala–Met–Ala–Lys(Mtr)–Lys(Mtr)–Leu–Leu–NH_2 [I] and Mtr–Ile–Asn–Trp–Lys(Mtr)–Gly–OBu^t [II] were chosen for the construction of the total sequence. Both peptides were prepared by stepwise chain elongation starting from the carboxy-end amino acid amide, H–Leu–NH_2, or ester, H–Gly–OBu^t, using the HONB–DCC or HOBt–DCC method. The synthetic routes to these fragments are shown in Figs. 2 and 3.

Fragment I contains the Met residue at the 4th position. Since the ε-amino function of Lys was protected by the Mtr group, which is resistant to both catalytic hydrogenation and TFA, we chose the Z and the Boc groups for protection of the α-amino function of the intermediates before and after introduction of the Met residue, respectively.

In synthesizing fragment II, we could use the *tert*-butyl ester to protect the carboxyl function of Gly in combination with the Z group which protected the α-amino function of the intermediates, because the amino function of the N-terminal Ile and the side chain of Lys were protected by the Mtr group. Treatment of Mtr–Ile–Asn–Trp–Lys(Mtr)–Gly–OBu^t withTFA gave the free acid.

For synthesis of the entire amino acid sequence of the tetradecapeptide, the protected nonapeptide I was treated with TFA and the resulting amine of I was condensed with the free acid of fragment II by the HONB–DCC procedure, giving Mtr–Ile–Asn–Trp–Lys(Mtr)–Gly–Ile–Ala–Ala–Met–Ala–Lys(Mtr)–Lys(Mtr)–Leu–Leu–NH_2 [III] in good yield. Most of the intermediates were obtained in crystalline form. To remove all the protecting groups, protected tetradecapeptide III was treated with 0.3 M MSA–containing TFA–thioanisole (9: 1) at room temperature for 1 h. After addition of ammonium acetate, concentration of the mixture yielded a residue, which was triturated with ether. After desalting on a Sephadex G-25 column (1 N AcOH), the product was converted into the corresponding acetate with Amberlite IRA-410 (acetate form). The acetate was purified on a carboxymethylcellulose column by gradient elution using pH 6.8 ammonium acetate buffer (0.005—0.6 M), and then further purified by preparative HPLC.[14] The peptide thus obtained gave a single spot on TLC and a single peak on HPLC[15] which were identical with those for natural purified mastoparan X[16] (shown in Fig. 4). Amino acid analysis of the acid hydrolysate gave results which agreed well with the theoretical values.

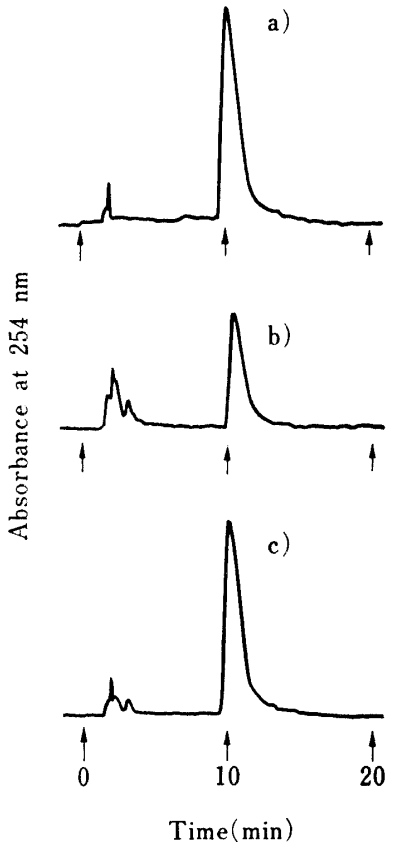

Fig. 4. Reverse-Phase HPLC of Mastoparan X

a) synthetic mastoparan X, b) natural mastoparan X, c) a mixture of synthetic and natural mastoparan X.

Another example of the use of the Mtr group for protection of the ε-amino function of Lys and the imidazole ring of His is the synthesis of chicken gastrin releasing peptide (c-GRP), which is a heptacosapeptide amide having gastrin releasing activity isolated from chicken proventricular tissue,[17] H–Ala–Pro–Leu–Gln–Pro–Gly–Gly–Ser–Pro–Ala–Leu–Thr–Lys–Ile–Tyr–Pro–Arg–Gly–Ser–His–Trp–Ala–Val–Gly–His–Leu–Met–NH_2. Very recently, the synthesis of this peptide in solution was reported by Akaji *et al.*[18] Our synthetic scheme for c-GRP is outlined in Fig. 5.

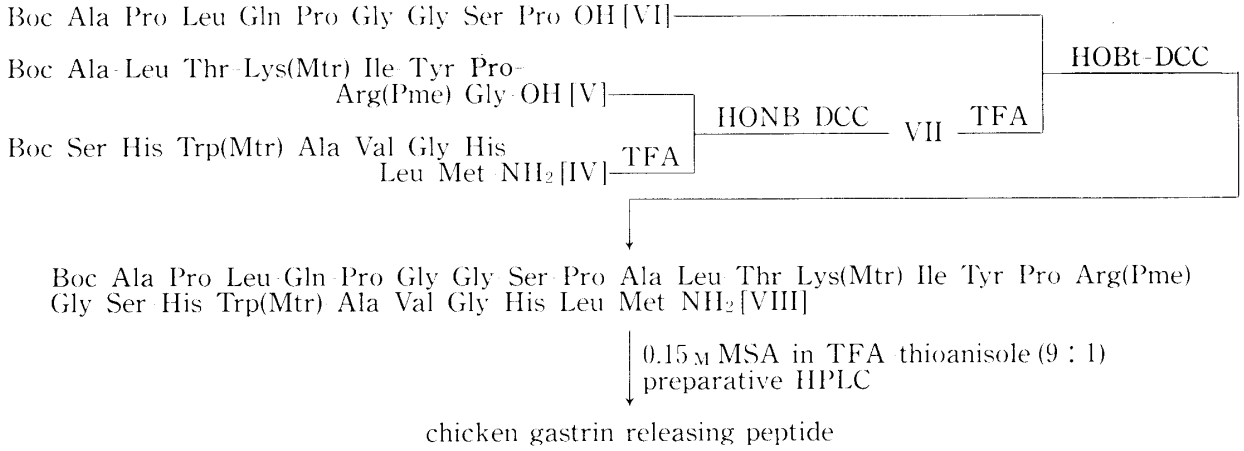

Fig. 5. Synthetic Scheme for Chicken Gastrin Releasing Peptide

This peptide contains a variety of amino acids, *e.g.*, Lys, Trp, His, and Arg. The Mtr group was used for semipermanent protection of the ε-amino function of Lys, the indole ring of Trp,[6] and for temporary protection of the imidazole function, in addition to the Pme group for semipermanent protection of the guanidino function of Arg. The α-amino function was protected by the Z or the Boc group.

As shown in Fig. 5, three peptide fragments, Boc–Ser–His–Trp(Mtr)–Ala–Val–Gly–His–Leu–Met–NH_2 [IV], Boc–Ala–Leu–Thr–Lys(Mtr)–Ile–Tyr–Pro–Arg(Pme)–Gly–OH [V], and

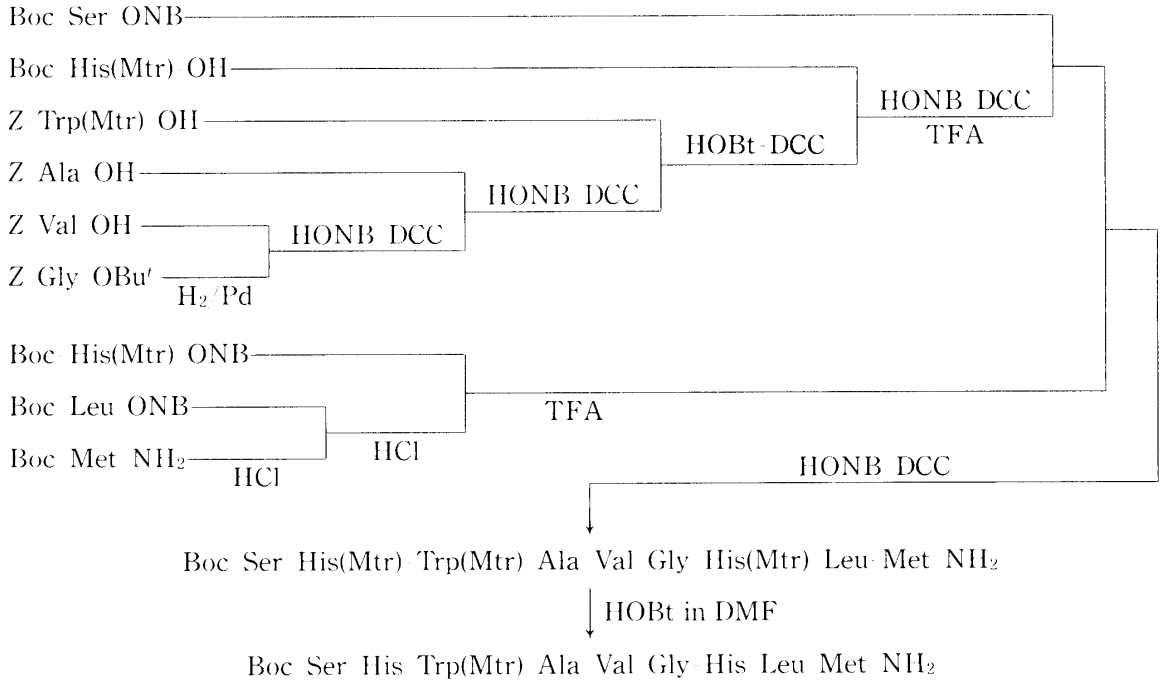

Fig. 6. Preparation of the Protected Nonapeptide IV

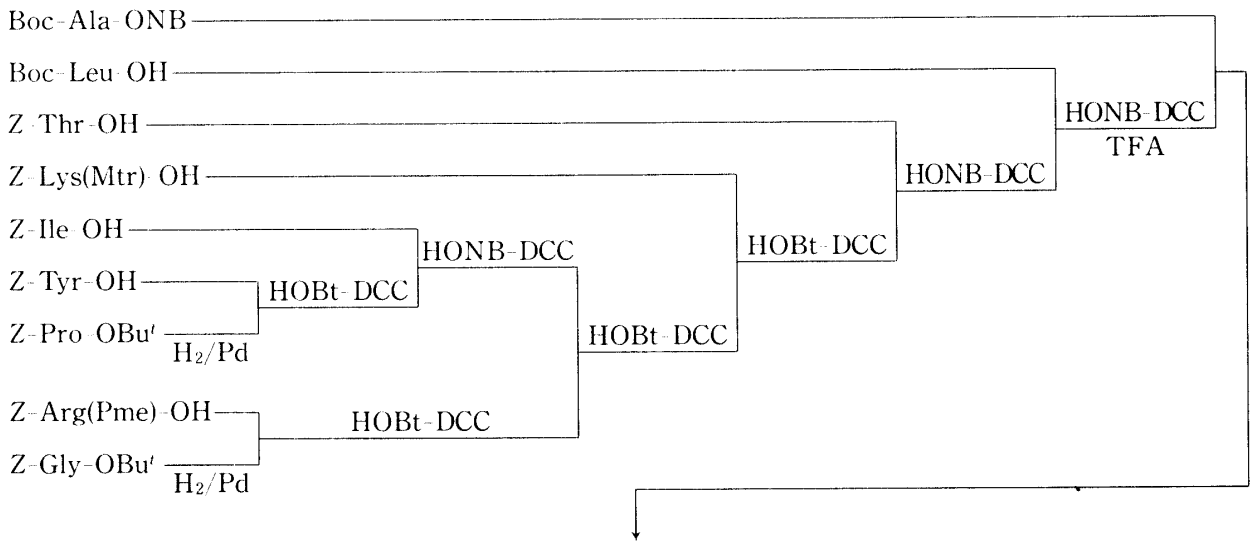

Fig. 7. Preparation of the Protected Nonapeptide V

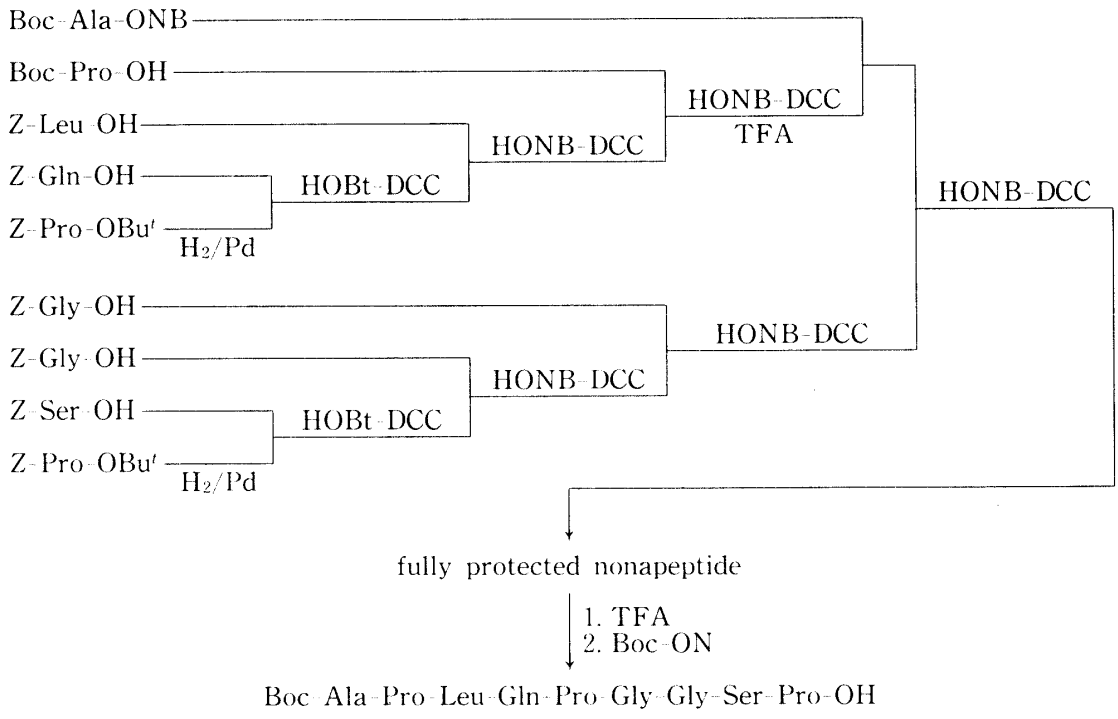

Fig. 8. Preparation of the Protected Nonapeptide VI

Boc–Ala–Pro–Leu–Gln–Pro–Gly–Gly–Ser–Pro–OH [VI], were chosen for construction of the total sequence. The synthetic routes to these fragments are shown in Figs. 6—8.

To synthesize fragment IV, the N-terminal hexapeptide, Boc–Ser–His(Mtr)–Trp(Mtr)–Ala–Val–Gly–OH, and the C-terminal tripeptide, Boc–His(Mtr)–Leu–Met–NH_2, were synthesized by stepwise chain elongation starting from the carboxy-end amino acid ester or amide, respectively. After treatment of Boc–His(Mtr)–Leu–Met–NH_2 with TFA, the resulting free base was coupled to the N-terminal hexapeptide by the HONB–DCC procedure to give the fully protected nonapeptide, Boc–Ser–His(Mtr)–Trp(Mtr)–Ala–Val–Gly–His(Mtr)–Leu–Met–NH_2, which was easily purified from aq. EtOH and obtained in 91.7% yield. However, when this nonapeptide was treated with TFA for fragment condensation with compound V, some

cleavage of the N^{im}-Mtr group was observed. Therefore, we decided to remove the N^{im}-Mtr group by treating the protected nonapeptide with HOBt in DMF and obtained compound IV in a pure form.

In synthesizing fragment V, the carboxyl function of Gly was protected with *tert*-butyl ester and the α-amino function of the intermediates was protected with the Z group. Two peptide fragments, Z–Ile–Tyr–Pro–OH and H–Arg(Pme)–Gly–OBu^t, were synthesized and coupled by the HOBt–DCC procedure, and then each amino acid was introduced stepwise to obtain the protected nonapeptide V in 83.9% yield.

To synthesize fragment VI, the N-terminal pentapeptide, Boc–Ala–Pro–Leu–Gln–Pro–OH, and the C-terminal tetrapeptide, Z–Gly–Gly–Ser–Pro–OBu^t, were synthesized by stepwise chain elongation starting from the carboxy-end amino acid ester, H–Pro–OBu^t. After hydrogenation of Z–Gly–Gly–Ser–Pro–OBu^t over Pd-black, the resulting free base was condensed with the N-terminal pentapetide, giving the protected nonapeptide, Boc–Ala–Pro–Leu–Gln–Pro–Gly–Gly–Ser–Pro–OBu^t in 99.2% yield. After treatment of this peptide with TFA, acylation of the resulting free nonapeptide with Boc–ON gave compound VI.

To synthesize the entire amino acid sequence of the heptacosapeptide, the protected nonapeptide IV was treated with TFA, and the resulting free base of IV was condensed with fragment V by the HONB–DCC procedure, giving Boc–Ala–Leu–Thr–Lys(Mtr)–Ile–Tyr–Pro–Arg(Pme)–Gly–Ser–His–Trp(Mtr)–Ala–Val–Gly–His–Leu–Met–NH_2 [VII]. VII was obtained in 94.2% yield. The Boc group of the octadecapeptide VII was removed by TFA treatment, and the free base was coupled with fragment VI by the HOBt–DCC procedure, giving the heptacosapeptide [VIII]. VIII was obtained in 76.0% yield.

To remove all the protecting groups, the protected heptacosapeptide VIII was treated with 0.15 M MSA–containing TFA–thioanisole (9 : 1) at room temperature for 2 h. After addition of ammonium acetate, the solution was concentrated and the resulting residue was triturated with ether, giving a precipitate. This was passed through a column of Sephadex G-25 (1 N AcOH) and the product was converted into the corresponding acetate with Amberlite IRA-410 (acetate form) and then purified on a carboxymethyl-cellulose column by gradient elution using pH 6.8 ammonium acetate buffer (0.005—0.4 M), giving a white powder in 37% yield. Final purification by preparative HPLC[19] gave c-GRP in 32% yield. The peptide showed a single spot on TLC and a single peak on HPLC[20] (shown in Fig. 9). This substance was identical to a reference sample of c-GRP supplied by Dr. Yajima on the basis of TLC in several solvent systems and reverse-phase HPLC. Amino acid analysis of the acid hydrolysate gave results which agreed well with the theoretical values.

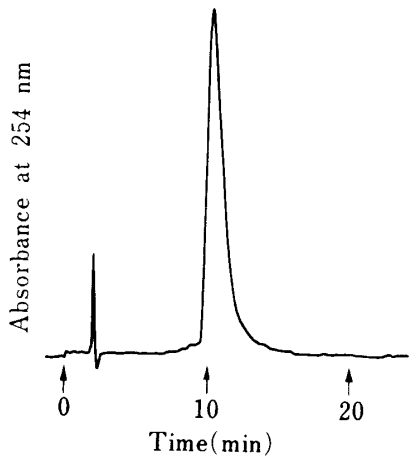

Fig. 9. Reverse-Phase HPLC of c-GRP

From these results, we concluded that the Mtr group is a useful protecting group for the amino function, the imidazole ring of His and the indole ring of Trp; in particular, the introduction of Lys(Mtr) should make possible new strategies in solution peptide synthesis.

Experimental

All melting points were taken by the capillary method and are uncorrected. Rotations were determined with a Perkin-Elmer model 141 polarimeter. Acid hydrolysis was carried out in 4% thioglycolic acid-containing 6 N HCl at 110°C for 24 h. Amino acid analysis was performed on a Hitachi 835 amino acid analyzer. Solutions were concentrated in a rotary evaporator under reduced pressure at a temperature

of 20—40°C. Catalytic hydrogenations were performed at room temperature with palladium (Pd) black as a catalyst. The purity of the product was tested by thin-layer chromatography on silica gel (precoated silica gel $60F_{254}$, Merck) or cellulose (Avicel, Funakoshi Yakuhin Co. Ltd.) plates. Solvent systems used were $CHCl_3$–MeOH (19: 1, *Rf*[1]); $CHCl_3$–MeOH–AcOH (9: 1: 0.5, *Rf*[2]); AcOEt–pyridine–AcOH–H_2O (30: 10: 3: 5, *Rf*[3]); $CHCl_3$–MeOH–H_2O (7: 3: 0.5, *Rf*[4]); *n*-BuOH–pyridine–AcOH–H_2O (15: 10: 3: 12, *Rf*[5]); AcOEt–*n*-BuOH–AcOH–H_2O (1: 1: 1: 1, *Rf*[6]). *Rf* values are given for silica gel unless otherwise mentioned.

H-Lys(Mtr)-OH——Lys·HCl (54.0 g) was dissolved in hot water (1000 ml) and to this was added $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (116 g). The whole was refluxed for 5 h. The insoluble material was filtered off and the filtrate was concentrated to 500 ml. $NaHCO_3$ (96.0 g) and acetone (300 ml) were added to the solution, then Mtr-Cl (82.0 g) in acetone (300 ml) was added dropwise and the mixture was stirred for 15 h. Acetone was evaporated off and the crystals formed were collected by filtration. The product was dissolved in 50% aq. AcOH and exposed to H_2S gas. The precipitate was filtered off and the solution was concentrated to give white crystals, which were collected and recrystallized from AcOH–H_2O: yield 43.5 g (40.5%), mp 228—230°C, $[\alpha]_D^{23}$ +1.1° (*c*=0.9 in MeOH), *Rf*[3] 0.18. *Anal.* Calcd for $C_{16}H_{26}N_2O_5S$: C, 53.61; H, 7.31; N, 7.82; S, 8.95. Found: C, 53.78; H, 7.36; N, 7.89; S, 8.79.

Z-Lys(Mtr)-OH·DCHA——H-Lys(Mtr)-OH (40.0 g) was dissolved in 1 N NaOH (130 ml) and the solution was cooled with ice. To this, Z-Cl (20.6 g) and 1 N NaOH (110 ml) were added and the mixture was stirred for 4 h. After addition of citric acid (36 g), the oily product was extracted with AcOEt. The extract was washed with water and dried over anhydr. Na_2SO_4. After removal of Na_2SO_4 by filtration, DCHA (20 ml) was added and the mixture was concentrated. The residue was crystallized from ether and recrystallized from MeOH–ether: yield 53.4 g (72.0%), mp 164—165°C, $[\alpha]_D^{23}$ +5.8° (*c*=0.9 in MeOH), *Rf*[2] 0.72. *Anal.* Calcd for $C_{36}H_{55}N_3O_7S$: C, 64.16; H, 8.23; N, 6.24; S, 4.76. Found: C, 63.80; H, 8.28; N, 6.13; S, 4.70.

Boc-Lys(Mtr)-OH·DCHA——H-Lys(Mtr)-OH (1.10 g) was suspended in a mixture of dioxane (10 ml) and water (10 ml) then TEA (0.63 ml) and Boc-ON (0.81 g) were added. The whole was stirred for 10 h, and after the usual work-up, DCHA (0.60 ml) was added. The crystals that formed were obtained from ether: yield 1.26 g (65.6%), mp 169—170°C, $[\alpha]_D^{22}$ +8.6° (*c*=0.9 in MeOH), *Rf*[2] 0.71. *Anal.* Calcd for $C_{33}H_{57}N_3O_7S$: C, 61.94; H, 8.98; N, 6.57; S, 5.01. Found: C, 62.18; H, 9.22; N, 6.50; S, 5.07.

Mtr-Gly-OH——Gly (0.75 g) was dissolved in H_2O (10 ml) together with $NaHCO_3$ (1.75 g), and the solution was cooled with ice. To this was added Mtr-Cl (2.48 g) in THF (10 ml), and the mixture was stirred for 10 h at room temperature, then worked up as usual. The crystals that formed were filtered off and recrystallized from AcOEt: yield 1.60 g (55.6%), mp 149—152°C, *Rf*[2] 0.49. *Anal.* Calcd for $C_{12}H_{17}NO_5S$: C, 50.16; H, 5.97; N, 4.88; S, 11.16. Found: C, 50.21; H, 6.17; N, 4.78; S, 11.14.

Mtr-Ile-OH·CHA——Ile (2.16 g) was dissolved in H_2O (30 ml) together with $NaHCO_3$ (2.80 g) and the solution was cooled with ice. To this was added Mtr-Cl (3.73 g) in THF (30 ml) and the mixture was stirred for 10 h, then worked up as usual. CHA (1.0 ml) was added to the residue, and the crystals that formed were filtered off and washed well with ether: yield 3.50 g (52.7%), mp 189—190°C, $[\alpha]_D^{23}$ +13.6° (*c*=1.1 in MeOH), *Rf*[2] 0.64. *Anal.* Calcd for $C_{22}H_{38}N_2O_5S$: C, 59.70; H, 8.65; N, 6.33; S, 7.25. Found: C, 59.99; H, 8.41; N, 6.28; S, 7.09.

Bos-His(Mtr)-OH·DCHA——Boc-His-OH (5.11 g) was dissolved in H_2O–acetone (30 ml–30 ml) together with TEA (5.6 ml) under ice-cooling. To this was added Mtr-Cl (4.97 g) in acetone (30 ml). The whole was stirred for 2 h and worked up as usual. The oily material thus obtained was dissolved in ether together with DCHA (3.6 ml). The crystals that formed were collected and washed with ether: yield 7.80 g (60.1%), mp 136—137°C, $[\alpha]_D^{23}$ +18.8° (*c*=1.0 in MeOH), *Rf*[2] 0.63. *Anal.* Calcd for $C_{33}H_{52}N_4O_7S$: C, 61.08; H, 8.08; N, 8.64; S, 4.94. Found: C, 61.19; H, 8.05; N, 8.89; S, 4.73.

Z-His(Mtr)-OH·DCHA——Z-His-OH (2.90 g) was dissolved in H_2O–acetone (15 ml–15 ml) together with TEA (2.8 ml) under ice-cooling. To this was added Mtr-Cl (2.49 g) in acetone (15 ml). The whole was stirred for 1 h and worked up as usual. The oily material obtained was dissolved in ether together with DCHA (2.0 ml). The crystals that formed were collected and washed with ether: yield 4.10 g (60.0%), mp 149—150°C, $[\alpha]_D^{24}$ +14.7° (*c*=0.9 in MeOH), *Rf*[2] 0.62. *Anal.* Calcd for $C_{36}H_{50}N_4O_7S$: C, 63.32; H, 7.38; N, 8.21; S, 4.70. Found: C, 62.94; H, 7.11; N, 8.11; S, 4.80.

H-His(Mtr)-OH——Z-His(Mtr)-OH·DCHA (3.4 g) was suspended in AcOEt (100 ml) and the mixture was washed with 0.2 N H_2SO_4 (30 ml) then concentrated. The residue was hydrogenated in MeOH in the usual manner, and the crystals that formed were collected and washed with ether: yield 1.80 g (98.3%), mp 162—164°C, $[\alpha]_D^{23}$ −27.1° (*c*=1.0 in AcOH), *Rf*[3] 0.35. *Anal.* Calcd for $C_{16}H_{20}N_3O_5S \cdot 1/3H_2O$: C, 51.60; H, 5.63; N, 11.28; S, 8.61. Found: C, 51.76; H, 5.63; N, 11.08; S, 8.63.

Z-Leu-Leu-NH$_2$ [Ia]——Z-Leu-NH_2 (5.29 g) was hydrogenated in MeOH (200 ml) and the free base obtained was coupled with Z-Leu-ONB (8.53 g) in DMF (80 ml). After the usual work-up, the product was crystallized from AcOEt–pet. ether: yield 6.82 g (90.3%), mp 201—202°C, $[\alpha]_D^{23}$ −17.3° (*c*=0.3 in DMF), *Rf*[1] 0.19. *Anal.* Calcd for $C_{20}H_{31}N_3O_4$: C, 63.63; H, 8.28; N, 11.13. Found: C, 63.90; H, 8.68; N, 11.29.

Z-Lys(Mtr)-Leu-Leu-NH$_2$ [Ib]——Compound Ia (3.78 g) was hydrogenated in MeOH (200 ml) and the free base was dissolved in DMF (60 ml). To this, Z-Lys(Mtr)-OH (prepared from the DCHA salt (6.6 g)), HONB (2.0 g) and DCC (2.2 g) were added under ice-cooling. The mixture was stirred for 10 h and worked up as usual. The product was crystallized from AcOEt to give needles: yield 5.4 g (75.2%), mp 158—159°C,

$[\alpha]_D^{23}$ −28.4° (c=0.3 in MeOH), Rf^1 0.23. *Anal.* Calcd for $C_{36}H_{55}N_5O_8S$: C, 60.23; H, 7.72; N, 9.96; S, 4.97. Found: C, 60.33; H, 7.57; N, 9.82; S, 4.28.

Z-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [Ic]——Compound Ib (3.59 g) was hydrogenated in MeOH (100 ml) and the free base was dissolved in DMF (50 ml). To this, Z-Lys(Mtr)-OH (prepared from the DCHA salt (3.3 g)), HONB (1.0 g) and DCC (1.2 g) were added under ice-cooling. The mixture was stirred for 10 h and worked up as usual. The product was crystallized from MeOH–AcOEt to give needles: yield 4.7 g (88.8 %), mp 222°C, $[\alpha]_D^{23}$ −25.6° (c=0.4 in MeOH), Rf^1 0.08. *Anal.* Calcd for $C_{52}H_{79}N_7O_{12}S_2$: C, 59.01; H, 7.52; N, 9.27; S, 6.06. Found: C, 59.29; H, 7.72; N, 9.28; S, 6.08.

Z-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [Id]——Compound Ic (4.23 g) was hydrogenated in MeOH (100 ml) and the free base was coupled with Z-Ala-ONB (1.54 g) in DMF (50 ml) for 10 h. The solution was concentrated and the resulting residue was triturated with AcOEt to give a precipitate, which was reprecipitated from MeOH–AcOEt: yield 4.2 g (92.9%), mp 206—209°C, $[\alpha]_D^{23}$ −28.7° (c=0.6 in MeOH), Rf^1 0.11. *Anal.* Calcd for $C_{55}H_{84}N_8O_{13}S_2$: C, 58.49; H, 7.50; N, 9.92; S, 5.68. Found: C, 58.59; H, 7.56; N, 9.83; S, 5.80.

Boc-Met-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [Ie]——Compound Id (2.83 g) was hydrogenated in MeOH (100 ml) and the free base was coupled with Boc-Met-ONB (1.03 g) in DMF (20 ml) for 6 h. The solution was concentrated and the residue was triturated with MeOH–ether to give a precipitate, which was reprecipitated from aq. MeOH: yield 3.08 g (97.0%), mp 217—219°C, $[\alpha]_D^{24}$ −17.6° (c=0.4 in DMF), Rf^1 0.10. *Anal.* Calcd for $C_{57}H_{95}N_9O_{14}S_3$: C, 55.81; H, 7.81; N, 10.28; S, 7.84. Found: C, 55.54; H, 7.82; N, 10.20; S, 7.82.

Boc-Ala-Met-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [If]——Compound Ie (2.94 g) was treated with TFA (20 ml) for 30 min. The mixture was concentrated and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in DMF (20 ml) together with TEA (0.36 ml). Boc-Ala-ONB (0.84 g) was added and the whole was stirred for 6 h. The solution was concentrated and the residue was triturated with ether to give a precipitate, which was crystallized from MeOH–AcOEt: yield 2.90 g (96.0 %), mp 227—228°C, $[\alpha]_D^{24}$ −19.4° (c=0.3 in DMF), Rf^1 0.10. *Anal.* Calcd for $C_{60}H_{100}N_{10}O_{15}S_3$: C, 55.53; H, 7.77; N, 10.79; S, 7.41. Found: C, 55.55; H, 7.80; N, 10.70; S, 7.59.

Boc-Ala-Ala-Met-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [Ig]——Compound If (2.6 g) was treated with TFA (20 ml) for 30 min and the resulting free base was coupled with Boc-Ala-ONB (0.77 g) in DMF (10 ml) for 6 h. The solution was concentrated, and the product was crystallized from hot MeOH: yield 2.45 g (89.4%), mp 243—245°C, $[\alpha]_D^{24}$ −17.8° (c=0.4 in DMF), Rf^2 0.50. *Anal.* Calcd for $C_{63}H_{105}N_{11}O_{16}S_3$: C, 55.28; H, 7.73; N, 11.25; S, 7.03. Found: C, 55.28; H, 7.71; N, 11.20; S, 6.98.

Boc-Ile-Ala-Ala-Met-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [I]——Compound Ig (2.0 g) was treated with TFA–anisole (20: 1, 12 ml) for 30 min and the resulting product was dissolved in DMF (10 ml) together with TEA (0.23 ml) and then coupled with Boc-Ile-ONB (prepared from Boc-Ile-OH (0.46 g)) for 10 h. The solution was concentrated and the residue was triturated with AcOEt to give a precipitate, which was washed with hot MeOH: yield 1.82 g (82.0%), mp 273—274°C, $[\alpha]_D^{24}$ −15.6° (c=0.3 in DMF), Rf^1 0.04, Rf^2 0.73. *Anal.* Calcd for $C_{69}H_{116}N_{12}O_{17}S_3$: C, 55.92; H, 7.89; N, 11.34; S, 6.49. Found: C, 55.61; H, 8.17; N, 11.33; S, 6.71.

Z-Lys(Mtr)-Gly-OBu^t [IIa]——Z-Gly-OBu^t (2.7 g) was hydrogenated in MeOH (100 ml) and the free base was coupled with Z-Lys(Mtr)-OH (prepared from the DCHA salt (6.6 g)) in the presence of HONB (2.0 g) and DCC (2.3 g) in acetonitrile (100 ml) for 6 h. After the usual work-up, the product was crystallized from pet. ether: yield 4.60 g (76.7%), mp 53—56°C, $[\alpha]_D^{23}$ −8.4° (c=1.0 in MeOH), Rf^1 0.42. *Anal.* Calcd for $C_{30}H_{43}N_3O_8S$: C, 59.48; H, 7.16; N, 6.94; S, 5.29. Found: C, 60.27; H, 7.39; N, 6.80; S, 5.04.

Z-Trp-Lys(Mtr)-Gly-OBu^t [IIb]——Compound IIa (3.63 g) was hydrogenated in MeOH (50 ml) and the free base was coupled with Z-Trp-ONB (3.0 g) in acetonitrile (50 ml). After the usual work-up, the product was crystallized from AcOEt–pet. ether: yield 4.30 g (90.5%), mp 120—124°C, $[\alpha]_D^{23}$ −8.4° (c=0.3 in MeOH), Rf^1 0.39. *Anal.* Calcd for $C_{41}H_{53}N_5O_9S$: C, 62.18; H, 6.75; N, 8.84; S, 4.05. Found: C, 62.47; H, 6.98; N, 9.04; S, 4.09.

Z-Asn-Trp-Lys(Mtr)-Gly-OBu^t [IIc]——Compound IIb (3.96 g) was hydrogenated in MeOH (50 ml) and the resulting free base was coupled with Z-Asn-OH (1.33 g) in the presence of HONB (1.0 g) and DCC (1.2 g) in DMF (40 ml). The mixture was stirred for 10 h and concentrated to dryness. The residue was triturated with AcOEt–MeOH (5: 2) to give a precipitate, which was washed with MeOH–acetonitrile (1: 1): yield 3.96 g (87.4%), mp 200—203°C, $[\alpha]_D^{24}$ −14.8° (c=0.5 in DMF), Rf^2 0.44. *Anal.* Calcd for $C_{45}H_{59}N_7O_{11}S$: C, 59.65; H, 6.56; N, 10.82; S, 3.54. Found: C, 59.60; H, 6.48; N, 10.83; S, 3.80.

Mtr-Ile-Asn-Trp-Lys(Mtr)-Gly-OBu^t [II]——Compound IIc (2.70 g) was hydrogenated in DMF (60 ml) and the resulting free base was coupled with Mtr-Ile-OH prepared from the CHA salt (1.35 g) in the presence of HOBt (0.40 g) and DCC (0.68 g) in DMF. The mixture was stirred for 10 h, filtered and concentrated. The product was triturated with ether to give a precipitate, which was crystallized from hot MeOH to give needles: yield 2.93 g (95.8%), mp 207—208°C, $[\alpha]_D^{24}$ −21.7° (c=0.3 in DMF), Rf^2 0.61. *Anal.* Calcd for $C_{53}H_{76}N_8O_{13}S_2$: C, 58.00; H, 6.98; N, 10.21; S, 5.84. Found: C, 58.16; H, 7.14; N, 10.03; S, 5.93.

Mtr-Ile-Asn-Trp-Lys(Mtr)-Gly-Ile-Ala-Ala-Met-Ala-Lys(Mtr)-Lys(Mtr)-Leu-Leu-NH$_2$ [III]——Compound II (1.02 g) was treated with TFA–anisole (10 ml–1 ml) at 20°C for 30 min. After removal of the TFA by

evaporation, the residue was triturated with ether to give a precipitate. Compound I (1.50 g) was also treated with TFA–anisole (10 ml–1 ml) at 20°C for 30 min, and the resulting free base was coupled with the free acid obtained above in the presence of HONB (0.20 g) and DCC (0.25 g) in DMF (20 ml). The mixture was stirred for 10 h and concentrated to dryness. The residue was triturated with water to give a precipitate, which was further purified by washing with hot MeOH–AcOEt (1: 1): yield 1.89 g (77.0%), mp 256—259°C, $[\alpha]_D^{23}$ −8.6° (c=0.8 in DMF), Rf^2 0.22, Rf^4 0.89. *Anal.* Calcd for $C_{113}H_{174}N_{20}O_{27}S_5 \cdot 2H_2O$: C, 55.60; H, 7.27; N, 11.48; S, 6.57. Found: C, 55.33; H, 7.30; N, 11.23; S, 6.65.

H-Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2 (Mastoparan X)——Compound III (200 mg) was treated with 0.3 M MSA in TFA–thioanisole (9: 1) (20 ml) at room temperature for 1 h. After addition of $AcONH_4$ (240 mg), the solution was concentrated and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in 1 N AcOH and passed through a column (2.2×120 cm) of Sephadex G-25 (1 N AcOH). The fractions (180—290 ml) were pooled and lyophilized. The product was dissolved in water and then passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form). The eluates were applied to a column (2.2×17 cm) of carboxymethyl-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005 M/0.6 M=400 ml/400 ml). The fractions (375—505 ml) containing the desired product were pooled and lyophilized: yield 60 mg (40%). A part of the powder (45 mg) was purified by preparative HPLC.[14] After the removal of acetonitrile by evaporation, the solution was passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form), and lyophilized. Finally, the product was passed through a column (2.2×120 cm) of Sephadex G-25 (1 N AcOH) and mastoparan X was obtained as a white powder after lyophilization: yield 38 mg (34%), $[\alpha]_D^{22}$ −63.5° (c=0.3 in 3% AcOH), Rf^5 (cellulose) 0.66, Rf^6 (cellulose) 0.64. Amino acid ratios in acid hydrolysate (4% thioglycolic acid in 6 N HCl): Lys 3.02(3); Trp 1.05(1); Asp 0.68(1); Gly 1.09(1); Val 3.26(3); Met 1.00 (1); Ile 1.97(2); Leu 2.08(2) (average recovery 77%).

Boc-Leu-Met-NH_2 [IVa]——Boc–Met–NH_2 (10.3 g) was treated with 4 N HCl/AcOH (40 ml) for 20 min, and the crystals that formed were collected and washed with ether. The material obtained was dissolved in DMF (200 ml) together with TEA (7.0 ml) under ice-cooling, and then Boc–Leu–ONB [prepared from Boc–Leu–OH·H_2O (8.0 g), HONB (6.85 g), and DCC (7.83 g)] was added. The mixture was stirred for 15 h, then worked up as usual. The product was crystallized from ether and recrystallized from MeOH–ether: yield 10.8 g (93.1%), mp 153—154°C, $[\alpha]_D^{23}$ −34.2° (c=1.0 in DMF), Rf^2 0.65. *Anal.* Calcd for $C_{16}H_{31}N_3O_4S$: C, 53.15; H, 8.64; N, 11.63; S, 8.87. Found: C, 53.56; H, 8.72; N, 11.47; S, 8.91.

Boc-His(Mtr)-Leu-Met-NH_2 [IVb]——Compound IVa (10.0 g) was treated with 4 N HCl/AcOH (30 ml) for 20 min, and the mixture was triturated with ether to give a precipitate. The powder was dissolved in DMF (100 ml) together with TEA (4.6 ml) under ice-cooling, and then Boc–His(Mtr)–ONB (prepared from Boc–His(Mtr)–OH (prepared from the DCHA salt (16.0 g)), HONB (5.0 g), and DCC (5.8 g)) was added. The mixture was stirred at room temperature for 6 h, then worked up as usual. The oily product was triturated with ether to give a precipitate: yield 15.1 g (76.8%), mp 129—131°C, $[\alpha]_D^{23}$ −13.8° (c=0.9 in DMF), Rf^2 0.64. *Anal.* Calcd for $C_{32}H_{50}N_6O_8S_2$: C, 54.06; H, 7.09; N, 11.82; S, 9.02. Found: C, 54.02; H, 7.25; N, 11.61; S, 9.04.

Z-Val-Gly-OBu^t [IVc]——Z–Gly–OBu^t (12.0 g) was hydrogenated in MeOH and the free base was coupled with Z–Val–OH (8.80 g) in the presence of HONB (7.20 g) and DCC (8.24 g) in DMF (200 ml). After 10 h, the mixture was worked up as usual and the product was crystallized from pet. ether, and recrystallized from AcOEt–pet. ether: yield 11.1 g (87.0%), mp 141°C, $[\alpha]_D^{23}$ −2.1° (c=1.2 in DMF), Rf^2 0.80. *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.49; H, 7.60; N, 7.72.

Z-Ala-Val-Gly-OBu^t [IVd]——Compound IVc (10.0 g) was hydrogenated in MeOH and the free amine was coupled with Z–Ala–OH (5.7 g) in the presence of HONB (4.1 g) and DCC (6.2 g) in DMF (150 ml). After 20 h, the mixture was worked up as usual and the product was crystallized from AcOEt: yield 10.5 g (94.4%), mp 184—185°C, $[\alpha]_D^{23}$ −7.8° (c=1.1 in DMF), Rf^2 0.67. *Anal.* Calcd for $C_{22}H_{33}N_3O_6$: C, 60.67; H, 7.64; N, 9.65. Found: C, 60.93; H, 7.86; N, 9.77.

Z-Trp(Mtr)-Ala-Val-Gly-OBu^t [IVe]——Compound IVd (5.0 g) was hydrogenated in MeOH and the free amine was coupled with Z–Trp(Mtr)–OH (6.34 g) in the presence of HOBt (1.86 g) and DCC (2.85 g) in DMF (70 ml). After 10 h, the mixture was worked up as usual and the oily product obtained was triturated with ether to give a precipitate, which was crystallized from MeOH–AcOEt–ether: yield 8.80 g (96.5%), mp 154—155°C, $[\alpha]_D^{23}$ −22.7° (c=0.9 in DMF), Rf^2 0.68. *Anal.* Calcd for $C_{43}H_{55}N_5O_{10}S$: C, 61.92; H, 6.65; N, 8.40; S, 3.85. Found: C, 62.06; H, 7.01; N, 8.58; S, 3.58.

Boc-His(Mtr)-Trp(Mtr)-Ala-Val-Gly-OBu^t [IVf]——Compound IVe (8.0 g) was hydrogenated in DMF (300 ml) and the resulting free base was condensed with Boc–His(Mtr)–OH prepared from the DCHA salt (6.22 g) in the presence of HONB (1.90 g) and DCC (2.18 g) in DMF (150 ml). After 15 h, the mixture was worked up as usual and the material obtained was triturated with ether to give a precipitate, which was washed with MeOH–AcOEt–ether: yield 10.2 g (92.5%), mp 202—204°C, $[\alpha]_D^{23}$ −16.1° (c=1.2 in DMF), Rf^2 0.68. *Anal.* Calcd for $C_{56}H_{76}N_8O_{14}S_2$: C, 58.52; H, 6.67; N, 9.74; S, 5.58. Found: C, 58.32; H, 6.54; N, 9.58; S, 5.51.

Boc-Ser-His(Mtr)-Trp(Mtr)-Ala-Val-Gly-OH [IVg]——Compound IVf (4.0 g) was treated with TFA (30 ml) at room temperature for 50 min. The TFA was evaporated off, and the residue was triturated with

ether to give a precipitate. The powder obtained was dissolved in DMF (60 ml) together with TEA (1.0 ml) under ice-cooling. To this was added Boc-Ser-ONB prepared from Boc-Ser-OH (0.76 g), HONB (0.72 g) and DCC (0.83 g), and the mixture was stirred for 10 h. The usual work-up gave an oily product; this was triturated with aq. AcOH to give a precipitate, which was reprecipitated from DMF–H_2O: yield 3.60 g (85.0 %), mp 148—152°C, $[\alpha]_D^{23}$ −16.4° (c=1.1 in DMF), Rf^2 0.40. *Anal.* Calcd for $C_{55}H_{73}N_9O_{16}S_2 \cdot 2H_2O$: C, 54.31; H, 6.38; N, 10.36; S, 5.27. Found: C, 54.58; H, 6.33; N, 10.82; S, 5.51.

Boc-Ser-His(Mtr)-Trp(Mtr)-Ala-Val-Gly-His(Mtr)-Leu-Met-NH_2 [IVh]——Compound IVb (3.97 g) was treated with TFA (40 ml) at room temperature for 10 min. TFA was evaporated off, then 1.3 N HCl/AcOH (4.1 ml) was added and the mixture was triturated with ether to give a precipitate. The powder obtained was dissolved in DMF (40 ml) together with TEA (0.86 ml) under ice-cooling. To this, compound IVg (5.50 g), HONB (1.0 g) and DCC (1.15 g) were added, and the mixture was stirred for 20 h. After the usual work-up, the oily product was triturated with H_2O to give a precipitate, which was washed with aq. EtOH: yield 7.35 g (91.7%), mp 192—193°C, $[\alpha]_D^{23}$ −12.5° (c=1.1 in DMF), Rf^2 0.50. *Anal.* Calcd for $C_{82}H_{113}N_{15}O_{21}S_4$: C, 55.54; H, 6.42; N, 11.85; S, 7.23. Found: C, 55.56; H, 6.74; N, 11.83; S, 6.55.

Boc-Ser-His-Trp(Mtr)-Ala-Val-Gly-His-Leu-Met-NH_2 [IV]——Compound IVh (3.0 g) was dissolved in DMF (15 ml). HOBt (2.30 g) was added, and the mixture was stirred for 30 min. After removal of the solvent by evaporation, the residue was triturated with ether to give a precipitate: yield 2.15 g (93.0%), mp 191—193°C, $[\alpha]_D^{23}$ −18.8° (c=0.9 in DMF), Rf^4 0.64. *Anal.* Calcd for $C_{62}H_{89}N_{15}O_{15}S_2 \cdot H_2O$: C, 54.49; H, 6.71; N, 15.38; S, 4.69. Found: C, 53.92; H, 6.43; N, 15.80; S, 4.34.

Z-Arg(Pme)-Gly-OBu^t [Va]——Z-Gly-OBu^t (13.0 g) was hydrogenated in MeOH (400 ml) and the resulting amine was condensed with Z-Arg(Pme)-OH prepared from the CHA salt (20.0 g) in the presence of HOBt (5.4 g) and DCC (8.2 g) in DMF (200 ml). After 15 h, the mixture was worked up as usual and the oily product was triturated with pet. ether to give a precipitate: yield 19.8 g (96.8%), mp 71—73°C, $[\alpha]_D^{23}$ +0.2° (c=0.9 in DMF), Rf^2 0.62. *Anal.* Calcd for $C_{31}H_{45}N_5O_7S$: C, 58.93; H, 7.18; N, 11.09; S, 5.08. Found: C, 59.42; H, 7.58; N, 10.95; S, 4.84.

Z-Tyr-Pro-OBu^t [Vb]——Z-Pro-OBu^t (15.0 g) was hydrogenated in MeOH (500 ml) and the resulting free amine was condensed with Z-Tyr-OH prepared from the DCHA salt (20.0 g) in the presence of HOBt (6.75 g) and DCC (10.4 g) in DMF (400 ml). After 15 h, the mixture was worked up as usual and the material obtained was purified by column chromatography on silica gel (7.5×9 cm, 1% MeOH/$CHCl_3$). The desired fractions were pooled and concentrated. The resulting residue was triturated with pet. ether to give a precipitate: yield 15.4 g (80.1%), mp 59—62°C, $[\alpha]_D^{23}$ −39.9° (c=0.8 in DMF), Rf^2 0.62. *Anal.* Calcd for $C_{26}H_{32}N_2O_6 \cdot 1/2H_2O$: C, 65.39; H, 6.97; N, 5.87. Found: C, 65.70; H, 6.93; N, 5.66.

Z-Ile-Tyr-Pro-OBu^t [Vc]——Compound Vb (15.2 g) was hydrogenated in MeOH (500 ml) and the free amine was condensed with Z-Ile-OH (8.0 g) in the presence of HONB (6.5 g) and DCC (7.4 g) in DMF (200 ml). After 10 h, the mixture was worked up as usual and the product was crystallized from pet. ether then recrystallized from MeOH–ether–pet. ether: yield 10.9 g (62.0%), mp 177—178°C, $[\alpha]_D^{23}$ −38.3° (c=1.1 in DMF), Rf^2 0.62. *Anal.* Calcd for $C_{32}H_{43}N_3O_7$: C, 66.07; H, 7.45; N, 7.22. Found: C, 66.07; H, 7.74; N, 7.19.

Z-Ile-Tyr-Pro-OH [Vd]——Compound Vc (6.0 g) was treated with TFA (60 ml) at room temperature for 1 h. TFA was evaporated off, and the residue was dissolved in AcOEt (300 ml). The solution was washed with water and then concentrated. The residue was triturated with ether to give a precipitate: yield 5.10 g (92.6%), mp 72—74°C, $[\alpha]_D^{23}$ −25.2° (c=1.0 in DMF), Rf^2 0.44. *Anal.* Calcd for $C_{28}H_{35}N_3O_7 \cdot 1/2H_2O$: C, 62.88; H, 6.79; N, 7.86. Found: C, 63.00; H, 6.53; N, 7.66.

Z-Ile-Tyr-Pro-Arg(Pme)-Gly-OBu^t [Ve]——Compound Va (7.58 g) was hydrogenated in MeOH (400 ml) in the presence of 1 N HCl (12 ml) and the resulting product was dissolved in DMF (150 ml) together with TEA (2.0 ml) under ice-cooling. To this solution, compound Vd (5.78 g), HOBt (2.23 g) and DCC (3.44 g) were added, and the mixture was stirred for 20 h. After the usual work-up, the oily material obtained was purified by column chromatography on a silica gel column (5.5×10 cm, 2% MeOH/$CHCl_3$). The resulting residue was triturated with ether to give a precipitate: yield 7.20 g (65.1%), mp 110—112°C, $[\alpha]_D^{23}$ −28.3° (c=1.2 in DMF), Rf^2 0.59. *Anal.* Calcd for $C_{51}H_{72}N_8O_{11}S \cdot H_2O$: C, 59.85; H, 7.29; N, 10.93; S, 3.13. Found: C, 60.16; H, 7.56; N, 10.95; S, 2.98.

Z-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-OBu^t [Vf]——Compound Ve (7.0 g) was hydrogenated in MeOH (500 ml) in the presence of 1 N HCl (7 ml), and the resulting product was dissolved in DMF (300 ml) together with TEA (1.0 ml) under ice-cooling. To this, Z-Lys(Mtr)-OH prepared from the DCHA salt (4.74 g), HOBt (1.20 g) and DCC (1.80 g) were added, and the mixture was stirred for 15 h. After the usual work-up, the oily material was triturated with ether to give a precipitate, which was reprecipitated from AcOEt–ether: yield 8.40 g (90.0%), mp 116—118°C, $[\alpha]_D^{23}$ −19.9° (c=1.1 in DMF), Rf^2 0.59. *Anal.* Calcd for $C_{67}H_{96}N_{10}O_{15}S_2 \cdot H_2O$: C, 59.01; H, 7.24; N, 10.27; S, 4.70. Found: C, 59.10; H, 7.44; N, 10.46; S, 4.98.

Z-Thr-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-OBu^t [Vg]——Compound Vf (7.50 g) was hydrogenated in MeOH (400 ml) and the resulting free base was condensed with Z-Thr-OH (1.49 g) in the presence of HONB (1.51 g) and DCC (1.73 g) in DMF (100 ml). After 15 h, the mixture was worked up as usual and the residue was triturated with ether to give a precipitate: yield 7.7 g (96.8%), mp 122—124°C, $[\alpha]_D^{23}$ −25.5° (c=0.8 in DMF), Rf^2 0.59. *Anal.* Calcd for $C_{71}H_{103}N_{11}O_{17}S_2$: C, 58.94; H, 7.18; N, 10.65; S, 4.43. Found: C, 58.61; H, 7.29; N, 10.47; S, 4.02.

Boc-Leu-Thr-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-OBu^t [Vh]——Compound Vg (7.5 g) was hydrogenated in MeOH (400 ml) in the presence of *p*-Tos-OH·H_2O (0.99 g) and the resulting product was dissolved in DMF (150 ml) together with TEA (0.74 ml) under ice-cooling. To this, Boc–Leu–OH·H_2O (1.37 g), HONB (1.41 g) and DCC (1.62 g) were added, and the mixture was stirred for 10 h. After the usual work-up, the oily material was triturated with ether to give a precipitate: yield 7.55 g (94.3%), mp 136—138°C, $[\alpha]_D^{23}$ −26.6° (c=1.0 in DMF), Rf^2 0.59. *Anal.* Calcd for $C_{74}H_{116}N_{12}O_{18}S_2 \cdot H_2O$: C, 57.56; H, 7.70; N, 10.89; S, 4.15. Found: C, 57.53; H, 8.12; N, 10.65; S, 3.90.

Boc-Ala-Leu-Thr-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-OH [V]——Compound Vh (4.20 g) was treated with TFA (40 ml) at room temperature for 1 h. TFA was evaporated off, and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in DMF (80 ml) together with TEA (0.80 ml) under ice-cooling, and to this was added Boc-Ala-ONB prepared from Boc-Ala-OH (0.52 g), HONB (0.54 g) and DCC (0.62 g). After 15 h, the mixture was concentrated and the residue was triturated with aq. AcOH to give a precipitate, which was reprecipitated from MeOH–H_2O: yield 3.60 g (83.9%), mp 169—171°C, $[\alpha]_D^{23}$ −32.9° (c=1.0 in DMF), Rf^2 0.40. *Anal.* Calcd for $C_{73}H_{113}N_{13}O_{19}S_2 \cdot 2H_2O$: C, 55.60; H, 7.48; N, 11.55; S, 4.07. Found: C, 55.79; H, 7.56; N, 11.75; S, 3.91.

Z-Ser-Pro-OBu^t [VIa]——Z–Pro–OBu^t (11.0 g) was hydrogenated in MeOH (500 ml) and the resulting free amine was coupled with Z–Ser–OH (7.2 g) in the presence of HOBt (4.90 g) and DCC (7.50 g) in DMF (300 ml). After 10 h, the mixture was worked up as usual. The product was crystallized from pet. ether, and recrystallized from AcOEt–ether: yield 9.50 g (67.2%), mp 126—127°C, $[\alpha]_D^{23}$ −50.9° (c=1.0 in DMF), Rf^2 0.65. *Anal.* Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.45; H, 7.16; N, 7.31.

Z-Gly-Ser-Pro-OBu^t [VIb]——Compound VIa (10.0 g) was hydrogenated in MeOH (400 ml) and the resulting free base was coupled with Z–Gly–OH (5.06 g) in the presence of HONB (5.13 g) and DCC (5.89 g) in DMF (300 ml). After 8 h, the mixture was worked up as usual. The product was crystallized from pet. ether and washed with ether: yield 7.70 g (72.1%), mp 96—98°C, $[\alpha]_D^{23}$ −53.4° (c=1.1 in DMF), Rf^2 0.61. *Anal.* Calcd for $C_{22}H_{31}N_3O_7$: C, 58.78; H, 6.95; N, 9.35. Found: C, 58.86; H, 7.04; N, 9.46.

Z-Gly-Gly-Ser-Pro-OBu^t [VIc]——Compound VIb (7.0 g) was hydrogenated in MeOH (300 ml) and the resulting free amine was condensed with Z–Gly–OH (3.0 g) in the presence of HONB (3.10 g) and DCC (3.60 g) in DMF (150 ml). After 8 h, the mixture was worked up as usual and the product was crystallized from ether, then recrystallized from AcOEt: yield 5.70 g (78.5%), mp 129—130°C, $[\alpha]_D^{23}$ −47.0° (c=0.8 in DMF), Rf^2 0.47. *Anal.* Calcd for $C_{24}H_{34}N_4O_8$: C, 56.90; H, 6.77; N, 11.06. Found: C, 56.75; H, 6.68; N, 10.90.

Z-Gln-Pro-OBu^t [VId]——Z–Pro–OBu^t (16.2 g) was hydrogenated in MeOH (600 ml) and the resulting amine was coupled with Z–Gln–OH (12.4 g) in the presence of HOBt (7.16 g) and DCC (10.9 g) in DMF (400 ml). After 15 h, the mixture was worked up as usual and the product was crystallized from ether then recrystallized from AcOEt–pet. ether: yield 15.8 g (82.5%), mp 106—107°C, $[\alpha]_D^{23}$ −51.2° (c=1.1 in DMF), Rf^2 0.62. *Anal.* Calcd for $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.21; N, 9.69. Found: C, 60.95; H, 7.36; N, 9.41.

Z-Leu-Gln-Pro-OBu^t [VIe]——Compound VId (8.0 g) was hydrogenated in MeOH (400 ml) in the presence of *p*-Tos–OH·H_2O (3.5 g), and the resulting product was dissolved in DMF (300 ml) together with TEA (2.6 ml) under ice-cooling. To this, Z–Leu–OH prepared from the DCHA salt (8.24 g), HONB (4.0 g) and DCC (4.6 g) were added, and the mixture was stirred for 15 h. After the usual work-up, the product was crystallized from pet. ether, and recrystallized from AcOEt-pet. ether: yield 7.70 g (76.3%), mp 62—64°C, $[\alpha]_D^{23}$ −51.7° (c=1.1 in DMF), Rf^2 0.61. *Anal.* Calcd for $C_{28}H_{42}N_4O_7$: C, 61.52; H, 7.75; N, 10.25. Found: C, 61.19; H, 7.75; N, 10.11.

Boc-Pro-Leu-Gln-Pro-OBu^t [VIf]——Compound VIe (7.0 g) was hydrogenated in MeOH (300 ml) and the resulting free base was condensed with Boc–Pro–OH (2.42 g) in the presence of HONB (2.76 g) and DCC (3.17 g) in DMF (100 ml). After 10 h, the mixture was worked up as usual and the product was triturated with ether–pet. ether to give a precipitate: yield 6.50 g (83.3%), mp 74—76°C, $[\alpha]_D^{23}$ −76.3° (c=1.2 in DMF), Rf^2 0.60. *Anal.* Calcd for $C_{30}H_{51}N_5O_8$: C, 59.05; H, 8.43; N, 11.49. Found: C, 58.89; H, 8.12; N, 11.08.

Boc-Ala-Pro-Leu-Gln-Pro-OH [VIg]——Compound VIf (6.0 g) was treated with TFA (60 ml) at room temperature for 1 h. TFA was evaporated off, and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in DMF (100 ml) together with TEA (2.80 ml) under ice-cooling, and to this was added Boc–Ala–ONB prepared from Boc–Ala–OH (1.95 g), HONB (2.07 g) and DCC (2.38 g). The mixture was stirred for 15 h. The solvent was evaporated off, then AcOH (3 ml) was added and the mixture was triturated with ether to give a precipitate, which was purified by column chromatography on a silica gel column (5.5×8 cm, 5% MeOH/$CHCl_3$). The desired fractions were pooled and concentrated, and the residue was triturated with ether to give a precipitate: yield 4.50 g (71.2%), mp 129—133°C, $[\alpha]_D^{23}$ −82.1° (c=1.0 in DMF), Rf^2 0.18. *Anal.* Calcd for $C_{29}H_{48}N_6O_9 \cdot H_2O$: C, 54.19; H, 7.84; N, 13.08. Found: C, 54.60; H, 7.88; N, 13.06.

Boc-Ala-Pro-Leu-Gln-Pro-Gly-Gly-Ser-Pro-OBu^t [VIh]——Compound VIc (2.23 g) was hydrogenated in MeOH (100 ml) and the resulting free base was coupled with compound VIg (2.50 g) in the presence of HONB (0.90 g) and DCC (1.03 g) in DMF (50 ml). After 15 h, the mixture was worked up as usual and the product obtained was triturated with ether to give a precipitate: yield 3.85 g (99.2%), mp 123—126°C, $[\alpha]_D^{23}$ −74.0° (c=0.9 in DMF), Rf^2 0.19. *Anal.* Calcd for $C_{45}H_{74}N_{10}O_{14} \cdot H_2O$: C, 54.20; H, 7.68; N, 14.05. Found: C, 54.01; H, 7.45; N, 13.44.

Boc-Ala-Pro-Leu-Gln-Pro-Gly-Gly-Ser-Pro-OH [VI]——Compound VIh (1.0 g) was treated with TFA (10 ml) at room temperature for 1 h. TFA was evaporated off and the residue was triturated with ether to give a precipitate. The powder was dissolved in DMF (10 ml) together with TEA (0.46 ml) under ice-cooling. Boc–ON (0.27 g) was added and the mixture was stirred for 4 h. After removal of the solvent by evaporation, AcOH (1.5 ml) was added and the mixture was triturated with ether to give a precipitate, which was reprecipitated from MeOH–ether: yield 0.87 g (91.3%), mp 156—158°C, $[\alpha]_D^{23}$ −72.9° (c=1.0 in DMF), Rf^4 0.29. *Anal.* Calcd for $C_{41}H_{66}N_{10}O_{14}\cdot 3/2H_2O$: C, 51.83; H, 7.32; N, 14.74. Found: C, 51.77; H, 7.33; N, 14.86.

Boc-Ala-Leu-Thr-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-Ser-His-Trp(Mtr)-Ala-Val-Gly-His-Leu-Met-NH_2 [VII]——Compound IV (2.0 g) was treated with TFA (20 ml) at room temperature for 15 min. TFA was evaporated off and the residue was triturated with ether to give a precipitate. The powder was dissolved in DMF (15 ml) together with TEA (1.24 ml), followed by precipitation with ether. The free base thus obtained was dissolved in DMF (20 ml), and compound V (1.78 g), HONB (0.41 g) and DCC (0.47 g) were added under ice-cooling. After 20 h, the mixture was worked up as usual and the residue was triturated with EtOH–AcOEt to give a precipitate, which was washed well with hot EtOH: yield 3.10 g (94.2%), mp 222—223°C (dec.), $[\alpha]_D^{23}$ −22.8° (c=1.0 in DMF), Rf^4 0.67. *Anal.* Calcd for $C_{130}H_{192}N_{28}O_{31}S_4\cdot 8H_2O$: C, 53.55; H, 7.19; N, 13.45; S, 4.40. Found: C, 53.35; H, 6.98; N, 12.91; S, 4.58.

Boc-Ala-Pro-Leu-Gln-Pro-Gly-Gly-Ser-Pro-Ala-Leu-Thr-Lys(Mtr)-Ile-Tyr-Pro-Arg(Pme)-Gly-Ser-His-Trp(Mtr)-Ala-Val-Gly-His-Leu-Met-NH_2 [VIII]——Compound VII (500 mg) was treated with TFA (5 ml) at room temperature for 15 min. TFA was evaporated off, and the residue was triturated with ether to give a precipitate. The powder was dissolved in DMF (5 ml) together with TEA (0.2 ml), followed by precipitation with ether. The free base thus obtained was dissolved in DMF (5 ml), and to this compound VI (217 mg), HOBt (50 mg) and DCC (160 mg) were added under ice-cooling. After 25 h, the mixture was worked up as usual and the residue was triturated with AcOEt to give a precipitate. The powder obtained was dissolved in DMF–MeOH (10 ml–10 ml) and heated for 30 min. After removal of the solvent by evaporation, the residue was triturated with AcOEt to give a precipitate, which was washed well with aq. MeOH: yield 480 mg (76.0%), mp 203—208°C (dec.), $[\alpha]_D^{23}$ −35.2° (c=0.9 in DMF), Rf^4 0.66. *Anal.* Calcd for $C_{166}H_{248}N_{38}O_{42}S_4\cdot 6H_2O$: C, 54.11; H, 7.11; N, 14.45; S, 3.48. Found: C, 54.08; H, 6.87; N, 14.19; S, 3.43.

H-Ala-Pro-Leu-Gln-Pro-Gly-Gly-Ser-Pro-Ala-Leu-Thr-Lys-Ile-Tyr-Pro-Arg-Gly-Ser-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (c-GRP)——Compound VIII (200 mg) was treated with 0.15 M MSA/TFA–thioanisole (9: 1) (30 ml) at room temperature for 2 h. After addition of $AcONH_4$ (200 mg), the solution was concentrated and the residue was triturated with ether to give a precipitate. The powder was dissolved in 1 N AcOH and passed through a column (2.2×120 cm) of Sephadex G-25 (1 N AcOH). The desired fractions (170—270 ml) were pooled and lyophilized. The powder was dissolved in water and the solution was passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form). The eluates were then applied to a column (2.2×17 cm) of carboxymethyl-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005 M/0.4 M=400 ml/400 ml). The desired fractions (325—375 ml) were pooled and lyophilized: yield 61 mg (37%). A part of the product (44 mg) was purified by preparative HPLC.[19] Acetonitrile was evaporated off and the solution was passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form), then lyophilized. Finally, the product was passed through a column (2.2×120 cm) of Sephadex G-25 (1 N AcOH), and c-GRP was obtained as a white powder after lyophilization: yield 38 mg (32%), $[\alpha]_D^{22}$ −102.2° (c=0.3 in 1% AcOH), Rf^5 (cellulose) 0.69; Rf^6 (cellulose) 0.64. Amino acid ratios in acid hydrolysate (4% thioglycolic acid in 6 N HCl): Lys 1.00(1); His 1.85(2); Arg 0.98(1); Trp 1.03(1); Thr 0.99(1); Ser 1.76(2); Glu 1.08(1); Pro 4.37(4); Gly 4.06(4); Ala 2.95(3); Val 0.97(1); Met 0.97(1); Ile 0.93(1); Leu 3.00(3); Tyr 1.00(1) (average recovery 79%).

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References and Notes

1) Amino acids, peptides and their derivatives in this paper are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mbs=*p*-methoxybenzenesulfonyl, Tos=*p*-toluenesulfonyl, Pme=2,3,4,5,6-pentamethylbenzenesulfonyl, Mtr=4-methoxy-2,3,6-trimethylbenzenesulfonyl, OBu^t=*tert*-butyl ester, HONB=*N*-hydroxy-5-norbornene-2,3-dicarboximide, HOBt=1-hydroxybenzotriazole, DCC=*N*,*N*′-dicyclohexylcarbodiimide, DCU=*N*,*N*′-dicyclohexylurea, Boc-ON=2-*tert*-butoxycarbonyloxyimino-2-phenyl-acetonitrile, THF=tetrahydrofuran, DMF=dimethylformamide, TFA=trifluoroacetic acid, TEA=triethylamine, CHA=cyclohexylamine, DCHA=dicyclohexylamine. Preliminary communication, M. Fujino, M. Wakimasu, and C. Kitada, *J. Chem. Soc., Chem. Commun.*, **1982**, 445.

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11) Column: Toyo Soda LS-410 (0.4×20 cm); solvent, CH_3CN–0.1 M $AcONH_4$ (1: 2); flow rate, 1.0 ml/min; elution times, Boc-His(Mtr)–OH=12.0 min, Boc–His(Tos)–OH=5.0 min, Boc–His(Mbs)–OH=4.4 min.
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14) Column: Toyo Soda LS-410 (2.14×7.5 cm+2.14×30 cm); solvent, 0.1% TFA/CH_3CN–H_2O (35: 65); flow rate, 13 ml/min; elution time, mastoparan X=67.2 min.
15) Column: Toyo Soda LS-410 (0.4×20 cm); solvent, 0.1% TFA/CH_3CN–H_2O (37: 63); flow rate, 1.0 ml/min; elution time, mastoparan X=10.2 min.
16) Natural mastoparan X was a gift from Professor T. Nakajima (Tokyo Medical and Dental Univ.).
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19) Column: Toyo Soda LS-410 (2.14×7.5 cm+2.14×30 cm); solvent, 0.1% TFA/CH_3CN–H_2O (1: 3); flow rate, 11 ml/min; elution time, c-GRP=40 min.
20) Column: Toyo Soda LS-410 (0.4×20 cm); solvent, 0.1% TFA/CH_3CN–H_2O (1: 3); flow rate, 1 ml/min; elution time, c-GRP=10.1 min.