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Synthesis of a Wasp Venom Tetradecapeptide, Mastoparan, with a New Cleaving System for 4-Methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) Amino-Protecting Group[1)]

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The 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group was introduced for protection of the ε-amino function of lysine and its acid lability was examined. The cleavage of Lys(Mtr) was accelerated by addition of methyl sulfide, as a second scavenger, to methanesulfonic acid-containing trifluoroacetic acid–thioanisole. In order to examine the usefulness of the new cleaving system with methyl sulfide, a wasp venom peptide, mastoparan, was synthesized. This system was found to give a highly pure product in good yield.

Keywords——4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr); N^{ε}-4-methoxy-2,3,6-trimethylbenzenesulfonyllysine; methanesulfonic acid–trifluoroacetic acid–thioanisole–methyl sulfide deprotection; mastoparan; wasp venom peptide

Since du Vigneaud *et al.*[2)] synthesized oxytocin in 1953, a number of biologically active peptides have been chemically synthesized. In these studies of peptide syntheses, the Z group[3)] and Boc group[4)] were used as important protecting groups, although these groups are not satisfactory for synthesizing complicated and/or sulfur-containing peptides. For syntheses of such peptides, protecting groups are required that are resistant to either TFA treatment or catalytic hydrogenation and then are removable under mild acidic conditions.

In our previous studies on protecting groups for the guanidino function of arginine[5)] and the indole imino function of tryptophan,[6)] we have observed that the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group is the most acid-labile protecting group among the arylsulfonyl groups so far examined. Further, we have introduced this group for protection of the ε-amino group of lysine and examined its acid lability.[7)] The Mtr group attached to the ε-amino function of lysine may be removed by 0.15—0.30 M methanesulfonic acid (MSA)-containing TFA–thioanisole system.

In recent years, for the deprotection of Lys(Z),[8)] Tyr(Bzl),[9)] Ser(Bzl) and Thr(Bzl),[10)] acid treatments with various scavengers have been examined. In the case of cleaving systems containing MSA or trifluoromethanesulfonic acid (TFMSA), thioethers have been found to be stable and potent cation acceptors.[11)] In particular, thioanisole has been found to be effective because the sulfonium ion is then stabilized by π-resonance.[8-11)] The TFA–thioanisole system has also been used for the deprotection of Arg(Mtr).[5)]

In the present study on the Mtr group, we have observed that the addition of methyl sulfide, as a second scavenger, to MSA-containing TFA–thioanisole considerably accelerated the cleavage of the N^{ε}-Mtr group. In order to demonstrate the usefulness of this cleaving system with methyl sulfide, we synthesized a wasp venom peptide, mastoparan. Mastoparan is

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a tetradecapeptide amide with mast cell degranulating activity and was isolated from the vespid wasp, *Vespula lewisii*.[12] Its primary structure was determined as H–Ile–Asn–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–Lys–Ile–Leu–NH_2 by Nakajima *et al.*,[12] and chemical syntheses have been reported by Fujino *et al.*,[13] Yanaihara *et al.*,[14] Yajima *et al.*[15] and Colombo.[16] By the use of the new milder cleaving system with methyl sulfide, in the present study, we have synthesized highly pure mastoparan in good yield.

Results and Discussion

The acid lability of Lys(Mtr) was examined in the presence or absence of methyl sulfide in the cleaving system of TFA–thioanisole, in the concentration range of 0—0.3 M MSA. As shown in Table I, the addition of methyl sulfide accelerated the cleavage of Lys(Mtr) at any concentration of MSA tested. More than 98% of N^{ε}-Mtr groups were removed within 2 h by 0.075 M MSA in the presence of methyl sulfide, whereas only 95% of the Mtr groups were removed by such a high concentration as 0.3 M MSA in the absence of methyl sulfide. Thus, by the addition of methyl sulfide, the Mtr group may be removed efficiently at a lower concentration of MSA. Yajima *et al.*[15] used *m*-cresol, as an additional scavenger, to trap all alkylating carbonium ions in the TFA–thioanisole system when removing the Z group attached to the ε-amino function of lysine. In the case of the N^{ε}-Mtr group, the combination of thioanisole and methyl sulfide is quite effective as scavenger. This new cleaving system with methyl sulfide has been employed for the synthesis of mastoparan.

The synthetic scheme for mastoparan (Fig. 1) was designed according to the procedure for the synthesis of mastoparan-X.[7] Two heptapeptide fragments, Z–Ala–Leu–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [I] and Mtr–Ile–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–OBu^t [II] were chosen for the construction of the total sequence. The heptapeptides I and II were both prepared by stepwise chain elongation, starting from H–Leu–NH_2 and H–Ala–OBu^t, respectively, where the HONB–DCC or HOBt–DCC method was used. The Z group was chosen for protection of the α-amino function of the intermediates. Treatment of Mtr–Ile–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–OBu^t with TFA gave the free acid without any effect on Mtr groups. The protected heptapeptide I was hydrogenated and then the free base of I was condensed with the free acid of fragment II by the HONB–DCC method, giving Mtr–Ile–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–Ala–Leu–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [III] in good yield. In this procedure, intermediate peptides were soluble in various organic solvents and were generally obtained in crystalline form. By contrast, in the syntheses of mastoparan reported previously,[13,15] the intermediate peptides were not readily soluble in organic solvents such as DMF.

TABLE I. Deprotection of Lys (Mtr) at 20 °C

Concn. of MSA (M)	TFA–thioanisole (9 : 1)		TFA–thioanisole–methyl sulfide (8 : 1 : 1)	
	1 h	2 h	1 h	2 h
0	1.5%	2.2%	6.8%	12.2%
0.075	52.1	80.2	82.7	98.7[a]
0.15	78.6	89.8[a]	93.4[a]	97.8[a]
0.3	82.6	95.2[a]	98.9[a]	101.4[a]

a) The starting material was not detectable on TLC.

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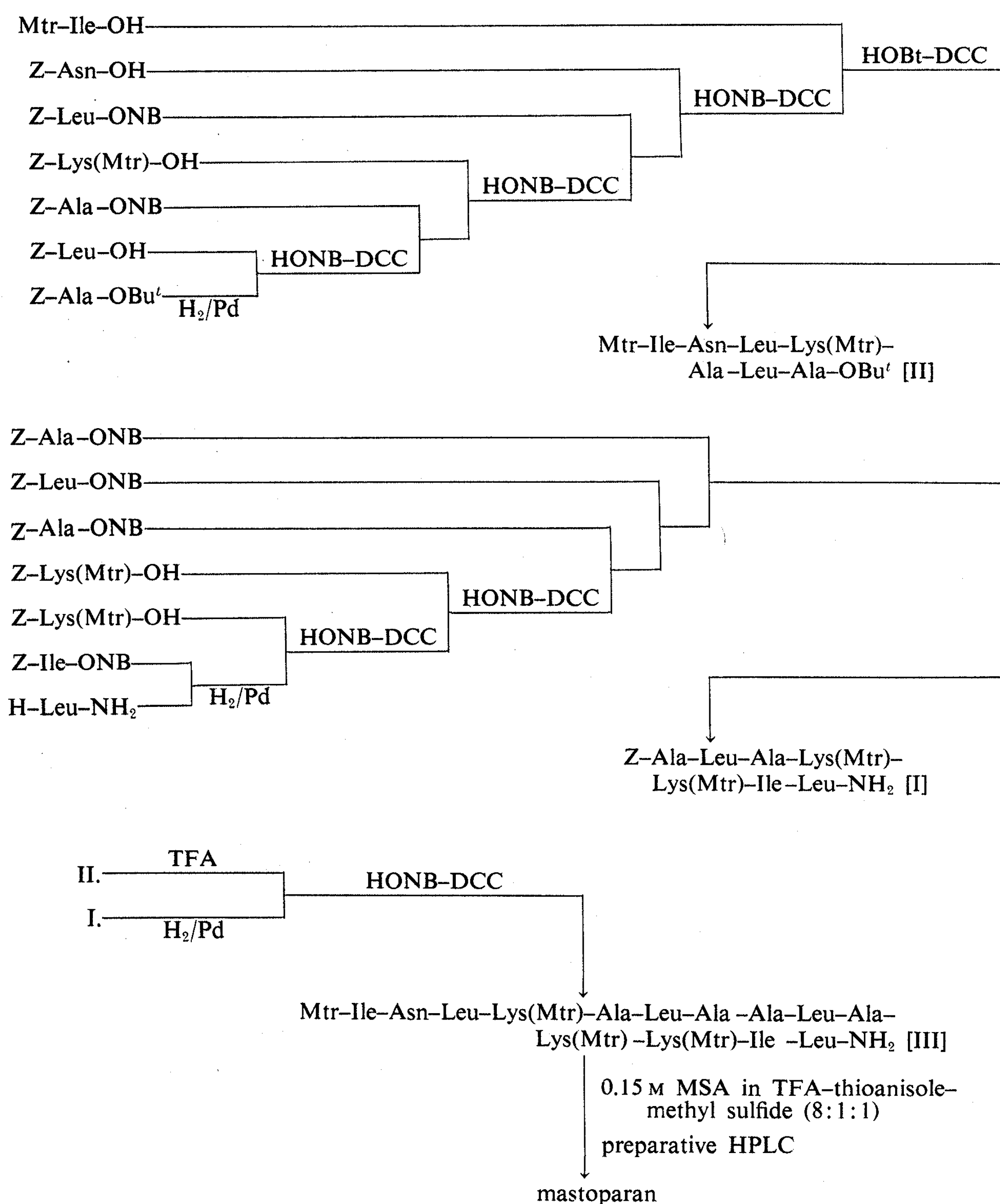

Fig. 1. Synthetic Scheme for Mastoparan

In order to clarify the effect of the addition of methyl sulfide on the removal of the Mtr group in peptide synthesis, deprotecting reactions of III were performed under several conditions. The protected tetradecapeptide III was treated for 2 h at room temperature with TFA–thioanisole (9:1) or TFA–thioanisole–methyl sulfide (8:1:1) containing 0.075 or 0.15 M MSA. After the addition of ammonium acetate, the mixture was concentrated. The resulting residue was triturated with ether and desalted on a Sephadex G-25 column (30% AcOH). The ninhydrin-positive fractions were pooled and converted into the corresponding acetate with Amberlite IRA-410 (acetate form). The acetates were examined by reversed phase HPLC.[17] As shown in Fig. 2, methyl sulfide remarkably accelerated the deprotecting reaction at either concentration of MSA. In the presence of 0.15 M MSA with methyl sulfide, partially protected peptides disappeared almost completely.

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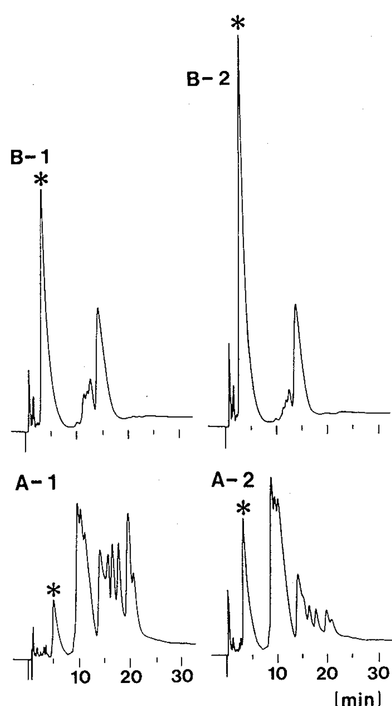

Fig. 2. HPLC Elution Patterns (Absorbance at 220 nm)[17] of the Cleaving Reaction Mixtures of Protected Mastoparan with TFA–Scavenger System

The reaction conditions were as follows: the protected mastoparan [III] was treated with 0.075 M (A-1) or 0.15 M (A-2) MSA in TFA–thioanisole (9 : 1), or 0.075 M (B-1) or 0.15 M (B-2) MSA in TFA–thioanisole–methyl sulfide (8 : 1 : 1), for 2 h at room temperature.
Asterisks (*) show the peak of mastoparan.

Accordingly, the protected peptide III was treated with 0.15 M MSA-containing TFA–thioanisole–methyl sulfide (8 : 1 : 1) at room temperature for 2 h. The deprotected peptide acetate obtained as described above was purified on a carboxymethyl-cellulose column by gradient elution using pH 6.8 ammonium acetate buffer (0.005—0.6 M), and was further purified by preparative HPLC.[18] The peptide thus obtained showed a single spot on TLC and a single peak on HPLC[19] which were identical with those of an authentic sample. The amino acid analysis of the acid hydrolysate gave results in agreement with the theoretical values.

In conclusion, the Mtr group is a useful protecting group for the amino function. Most of the Mtr-peptide intermediates are obtained in crystalline form, and are readily soluble in various organic solvents. Furthermore, the new cleaving system with methyl sulfide does not require a high concentration of MSA, and the Mtr group may be removed under milder acidic conditions.

Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were observed with a Union automatic polarimeter PM-201. Acid hydrolysis was carried out in 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 (TLC) on silica gel (precoated Silica gel $60F_{254}$, Merck) or cellulose (Avicel, Funakoshi Yakuhin Co., Ltd.) plates. Solvent systems used were $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5, Rf^1); $CHCl_3$–MeOH (5 : 1, Rf^2); $CHCl_3$–MeOH–AcOH (95 : 5 : 1, Rf^3); *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2, Rf^4). *Rf* values are given for silica gel unless otherwise mentioned.

Deprotection of Lys(Mtr)——(A) Reaction with MSA-containing TFA–Thioanisole: Lys(Mtr) (10.7 mg, 30 μmol) was treated with 10 eq of MSA in TFA–thioanisole (9 : 1) at 20 °C; the MSA concentration was adjusted to 0.075, 0.15 or 0.3 M by altering solution volume appropriately. As a standard, the same amount of Lys(Mtr) was treated with TFA–thioanisole (9 : 1) at 20 °C in the absence of MSA. After 1 or 2 h, the solution was evaporated *in vacuo*. The resulting residue was dissolved in 0.2 M sodium citrate buffer (pH 2.2, 30 ml), and after being washed with ether, was applied to an amino acid analyzer to estimate the content of regenerated lysine. The results are shown in Table I.

(B) Reaction with MSA-containing TFA–Thioanisole–Methyl Sulfide: The procedure described in (A) was

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repeated with TFA–thioanisole–methyl sulfide (8 : 1 : 1) in place of TFA–thioanisole (9 : 1).

Deprotection of Protected Mastoparan——(A) Reaction with MSA-containing TFA–Thioanisole: Protected mastoparan [III] (50 mg, 21.5 μmol, synthesized as described below) was treated with 40 eq of MSA in TFA–thioanisole (9 : 1) at 20 °C for 2 h; the MSA concentration was adjusted to 0.075 or 0.15 M by altering the solution volume appropriately. After the addition of $AcONH_4$ (155 mg), the solution was evaporated *in vacuo* and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in 30% AcOH and passed through a column (2.2 × 120 cm) of Sephadex G-25 (30% AcOH). The ninhydrin-positive fractions (180—250 ml) were pooled and evaporated. The residue was dissolved in water and then passed through a column (1.5 × 15 cm) of Amberlite IRA-410 (acetate form). 0.1 ml of TFA was added to the eluates, which were subsequently lyophilized. The purity of the powder was checked by HPLC.[17] The results are shown in Fig. 2.

(B) Reaction with MSA-containing TFA–Thioanisole–Methyl Sulfide: The procedure described in (A) was repeated with TFA–thioanisole–methyl sulfide (8 : 1 : 1) in place of TFA–thioanisole (9 : 1).

Z–Ile–Leu–NH_2 [Ia]——Z–Ile–ONB (42.65 g) was added to a chilled solution of H–Leu–NH_2·HCl (18.33 g) and Et_3N (15.4 ml) in DMF (500 ml). The mixture was stirred overnight and concentrated. The resulting residue was triturated with AcOEt to give a precipitate, which was reprecipitated from MeOH: yield 23.32 g (61.8%), mp 223—225 °C, $[\alpha]_D^{22}$ −17.4 ° (*c* = 1.0 in DMF), *Rf*[1] 0.63. *Anal.* Calcd for $C_{20}H_{31}N_3O_4$: C, 63.64; H, 8.28; N, 11.13. Found: C, 63.56; H, 7.97; N, 11.16.

Z–Lys(Mtr)–Ile–Leu–NH_2 [Ib]——Compound Ia (8.30 g) was hydrogenated in MeOH (250 ml) in the presence of TsOH·H_2O (4.2 g), and the resulting product was dissolved in DMF (130 ml) together with Et_3N (3.08 ml) under ice-cooling. To this solution, Z–Lys(Mtr)–OH (prepared from the DCHA salt (14.83 g)), HONB (4.34 g) and DCC (4.99 g) were added, and the mixture was stirred overnight. The solution was filtered and concentrated, and the product was crystallized from AcOEt: yield 9.34 g (59.1%), mp 214—216 °C, $[\alpha]_D^{22}$ −37.4 ° (*c* = 1.0 in MeOH), *Rf*[1] 0.58. *Anal.* Calcd for $C_{36}H_{55}N_5O_8S$: C, 60.23; H, 7.72; N, 9.75; S, 4.47. Found: C, 60.33; H, 7.67; N, 9.89; S, 5.07.

Z–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [Ic]——Compound Ib (9.00 g) was hydrogenated in MeOH (220 ml)–DMF (110 ml) in the presence of TsOH·H_2O (2.4 g), and MeOH was removed by evaporation. To this DMF solution, Et_3N (1.75 ml), Z–Lys(Mtr)–OH (prepared from the DCHA salt (8.87 g)), HONB (2.46 g) and DCC (2.84 g) were added under ice-cooling. The mixture was stirred overnight, filtered and concentrated. The residue was triturated with AcOEt to give a precipitate, which was washed with hot MeOH: yield 11.80 g (89.2%), mp 232—235 °C, $[\alpha]_D^{22}$ −16.0 ° (*c* = 1.0 in DMF), *Rf*[1] 0.50. *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, 59.20; H, 7.43; N, 9.28; S, 6.23.

Z–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [Id]——Compound Ic (11.64 g) was hydrogenated in MeOH (200 ml)–DMF (100 ml) in the presence of TsOH·H_2O (2.1 g), and MeOH was removed by evaporation. To this DMF solution, Et_3N (1.7 ml) and Z–Ala–ONB (prepared from Z–Ala–OH (2.58 g)) were added under ice-cooling. The mixture was stirred overnight, filtered and concentrated. The product was triturated with AcOEt to give a precipitate, which was washed with hot MeOH: yield 11.09 g (89.3%), mp 246—247 °C, $[\alpha]_D^{22}$ −18.5 ° (*c* = 1.0 in DMF), *Rf*[1] 0.49, *Rf*[2] 0.58. *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, 58.65; H, 7.33; N, 9.95; S, 5.66.

Z–Leu–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [Ie]——Compound Id (5.08 g) was hydrogenated in DMF (200 ml) in the presence of TsOH·H_2O (0.86 g) and concentrated to 150 ml, and then Z–Leu–ONB (prepared from Z–Leu–OH (1.31 g)) and Et_3N (0.63 ml) were added under ice-cooling. The mixture was stirred overnight, filtered and concentrated. The product was triturated with AcOEt to give a precipitate, which was washed with hot MeOH: yield 4.03 g (72.1%), mp 236—238 °C, $[\alpha]_D^{22}$ −19.3 ° (*c* = 1.0 in DMF), *Rf*[1] 0.50, *Rf*[2] 0.63. *Anal.* Calcd for $C_{61}H_{95}N_9O_{14}S_2$: C, 58.96; H, 7.71; N, 10.14; S, 5.16. Found: C, 58.69; H, 7.57; N, 10.16; S, 5.28.

Z–Ala–Leu–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH_2 [I]——Compound Ie (3.73 g) was hydrogenated in DMF (150 ml) in the presence of TsOH·H_2O (0.57 g) and concentrated to 80 ml, and then Et_3N (0.42 ml) and Z–Ala–ONB (prepared from Z–Ala–OH (0.74 g)) were added under ice-cooling. The mixture was stirred overnight, filtered and concentrated. The product was triturated with AcOEt to give a precipitate, which was washed with MeOH–acetonitrile (1 : 1): yield 2.28 g (57.8 %), mp 246—248 °C, $[\alpha]_D^{22}$ −21.1 ° (*c* = 1.0 in DMF), *Rf*[1] 0.44, *Rf*[2] 0.53. *Anal.* Calcd for $C_{64}H_{100}N_{10}O_{15}S_2$: C, 58.52; H, 7.67; N, 10.66; S, 4.88. Found: C, 58.42; H, 7.45; N, 10.82; S, 4.66.

Z–Leu–Ala–OBu^t [IIa]——Z–Ala–OBu^t (15.36 g) was hydrogenated in MeOH (350 ml) and the resulting free amine was condensed with Z–Leu–OH (11.22 g) in the presence of HONB (8.34 g) and DCC (9.60 g) in THF (300 ml). After 15 h, the mixture was worked up as usual and the material obtained was purified by column chromatography on silica gel (5.5 × 18.5 cm, $CHCl_3$). The product was obtained as an oily substance: yield 15.3 g (92%), *Rf*[3] 0.80.

Z–Ala–Leu–Ala–OBu^t [IIb]——Compound IIa (15.0 g) was hydrogenated in MeOH (300 ml) and the free base was coupled with Z–Ala–ONB (prepared from Z–Ala–OH (7.69 g)) in DMF (200 ml). After 15 h, the mixture was worked up as usual and the product was crystallized from ether: yield 13.98 g (87.4%), mp 135—136 °C, $[\alpha]_D^{22}$ −70.6 ° (*c* = 1.0 in MeOH), *Rf*[1] 0.74, *Rf*[3] 0.57. *Anal.* Calcd for $C_{24}H_{37}N_3O_6$: C, 62.18; H, 8.05; N, 9.06. Found: C, 62.27; H, 7.81; N, 9.01.

Z–Lys(Mtr)–Ala–Leu–Ala–OBu^t [IIc]——Compound IIb (4.64 g) was hydrogenated in MeOH (100 ml) and the free amine was coupled with Z–Lys(Mtr)–OH prepared from the DCHA salt (6.74 g) in the presence of HONB

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(1.97 g) and DCC (2.27 g) in DMF (80 ml). After 15 h, the mixture was worked up as usual and the product was crystallized from ether: yield 7.16 g (89.1%), mp 168—170 °C, $[\alpha]_D^{22}$ $-46.8°$ (c=1.0 in MeOH), Rf^1 0.71, Rf^3 0.31. *Anal.* Calcd for $C_{40}H_{61}N_5O_{10}S$: C, 59.75; H, 7.65; N, 8.71; S, 3.99. Found: C, 60.01; H, 7.76; N, 8.88; S, 3.81.

Z–Leu–Lys(Mtr)–Ala–Leu–Ala–OBu^t [IId]——Compound IIc (68.3 g) was hydrogenated in MeOH (150 ml) in the presence of TsOH·H_2O (1.62 g), and the resulting product was dissolved in DMF (100 ml) together with Et_3N (1.19 ml) under ice-cooling, and coupled with Z–Leu–ONB prepared from Z–Leu–OH (2.48 g). After 15 h, the mixture was worked up as usual and the product was triturated with ether to give a precipitate, which was washed with hot MeOH: yield 5.39 g (69.1%), mp 158—161 °C, $[\alpha]_D^{22}$ $-31.8°$ (c=1.0 in DMF), Rf^1 0.60, Rf^3 0.75. *Anal.* Calcd for $C_{46}H_{72}N_6O_{11}S$: C, 60.24; H, 7.91; N, 9.16; S, 3.50. Found: C, 60.37; H, 7.98; N, 9.03; S, 3.47.

Z–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–OBu^t [IIe]——Compound IId (5.00 g) was hydrogenated in MeOH (150 ml) and the free base was coupled with Z–Asn–OH (1.74 g) in the presence of HONB (1.29 g) and DCC (1.49 g) in DMF (80 ml). After 15 h, the mixture was filtered and concentrated, and the product was precipitated from AcOEt and washed with MeOH–acetonitrile (1 : 1): yield 3.46 g (61.6%), mp 217 °C, $[\alpha]_D^{22}$ $-30.7°$ (c=1.0 in DMF), Rf^1 0.42, Rf^2 0.53. *Anal.* Calcd for $C_{50}H_{78}N_8O_{13}S$: C, 58.23; H, 7.62; N, 10.87; S, 3.11. Found: C, 57.98; H, 7.50; N, 11.01; S, 3.25.

Mtr–Ile–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–OBu^t [II]——Compound IIe (3.00 g) was hydrogenated in DMF (200 ml) and the resulting free base was coupled with Mtr–Ile–OH (prepared from the CHA salt (1.55 g)) in the presence of HOBt (0.94 g) and DCC (1.08 g) in DMF (50 ml). The mixture was stirred for 15 h, filtered and concentrated. The product was triturated with AcOEt–ether (1 : 1) to give a precipitate, which was crystallized from hot MeOH: yield 2.72 g (76.5%), mp 231—233 °C, $[\alpha]_D^{22}$ $-43.0°$ (c=1.0 in DMF), Rf^1 0.44, Rf^2 0.54. *Anal.* Calcd for $C_{58}H_{95}N_9O_{15}S_2$: C, 56.98; H, 7.83; N, 10.31; S, 5.25. Found: C, 57.17; H, 7.83; N, 10.39; S, 5.23.

Mtr–Ile–Asn–Leu–Lys(Mtr)–Ala–Leu–Ala–Ala–Leu–Ala–Lys(Mtr)–Lys(Mtr)–Ile–Leu–NH$_2$ [III]——Compound II (0.93 g) was treated with TFA (10 ml) at 20 °C for 50 min. After removal of the TFA by evaporation, the residue was triturated with ether to give a precipitate. Compound I (1.00 g) was hydrogenated in DMF (100 ml) and the resulting free base was coupled with the free acid obtained above in the presence of HONB (0.55 g) and DCC (0.31 g) in DMF (50 ml). The mixture was stirred for 15 h and concentrated to dryness. The residue was triturated with ether–AcOEt (8 : 2) to give a precipitate, which was further purified by washing with water: yield 1.71 g (96.7%), mp >300 °C, $[\alpha]_D^{22}$ $-16.6°$ (c=1.0 in DMF), Rf^1 0.13, Rf^2 0.43. *Anal.* Calcd for $C_{110}H_{179}N_{19}O_{27}S_4$: C, 56.75; H, 7.75; N, 11.43; S, 5.51. Found: C, 56.53; H, 7.72; N, 11.69; S, 5.53.

H–Ile–Asn–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–Lys–Ile–Leu–NH$_2$ (Mastoparan)——Compound III (500 mg) was treated with 0.15 M MSA in TFA–thioanisole–methyl sulfide (8 : 1 : 1) (57.2 ml) at room temperature for 2 h. After addition of $AcONH_4$ (1.5 g), the solution was concentrated and the residue was triturated with ether to give a precipitate. The powder obtained was dissolved in 30% AcOH and passed through a column (2.2 × 120 cm) of Sephadex G-25 (30% AcOH). The fractions (185—315 ml) were pooled and lyophilized. The product was dissolved in water and then passed through a column (1.6 × 17 cm) of Amberlite IRA-410 (acetate form). The eluates were applied to a column of carboxymethyl-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005 M/0.6 M=800 ml/800 ml). The fractions (800—950 ml) containing the desired product were pooled and lyophilized. The powder obtained was purified by preparative HPLC.[18] After the removal of acetonitrile by evaporation, the solution was passed through a column (1.5 × 20 cm) of Amberlite IRA-410 (acetate form), and mastoparan was obtained as a white powder after lyophilization: yield 278 mg (75.4%), $[\alpha]_D^{24.5}$ $-76.0°$ (c=0.50 in 3% AcOH) (lit.[15] $-71.4°$), Rf^4 0.39 (lit.[15] 0.38), Rf^4 (cellulose) 0.59. Amino acid ratio in acid hydrolysate: Lys 3.00 (3); Asp 1.10 (1); Ala 3.67 (4); Ile 2.05 (2); Leu 4.24 (4) (average recovery 87%).

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References and Notes

1) Amino acids, peptides and their derivatives in the present study are of the L-configuration. The abbreviations used are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mtr=4-methoxy-2,3,6-trimethylbenzenesulfonyl, OBu^t=*tert*-butyl ester, Bzl=benzyl, HONB=*N*-hydroxy-5-norbornene-2,3-dicarboximide HOBt=1-hydroxybenzotriazole, DCC=dicyclohexylcarbodiimide, THF=tetrahydrofuran, DMF=*N*, *N*-dimethylformamide, MSA=methanesulfonic acid, TFMSA=trifluoromethanesulfonic acid, TFA=trifluoroacetic acid, CHA=cyclohexylamine, DCHA=dicyclohexylamine, TLC=thin-layer chromatography, HPLC=high performance liquid chromatography.

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17) Column: Shimadzu Shim-Pack PCN-03 (0.46 × 5 cm); solvent, 0.1% TFA/CH_3CN–H_2O (gradient from 15:85 to 25:75 over a period of 20 min); flow rate, 1.0 ml/min; temperature, 50 °C, elution time, mastoparan = *ca.* 3.1 min.
18) Column: Toyo Soda LS-410 (2.14 × 7.5 + 2.14 × 30 cm); solvent, 0.1% TFA/CH_3CN–H_2O (39:61); flow rate, 8.1 ml/min; elution time, mastoparan = 31.5 min.
19) Column: DuPont Zorbax-ODS (0.46 × 25 cm); solvent, 0.1% TFA/CH_3CN–H_2O (39:61); flow rate 1.0 ml/min; elution time, mastoparan = 9.3 min.

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