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Studies on Peptides. LXXI.[1,2] Synthesis of the Octadecapeptide corresponding to the Entire Amino Acid Sequence of β-Melanocyte-stimulating Hormone from the Dogfish (*Scyliorhinus canicula*)

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H–Asp–Gly–Ile–Asp–Tyr–Lys–Met–Gly–His–Phe–Arg–Trp–Gly–Ala–Pro–Met–Asp–Lys–OH, dogfish β-MSH (*Scyliorhinus canicula*), was synthesized in a conventional manner by applying the hydrogen fluoride procedure.

Keywords——dogfish α-MSH; dogfish β-MSH (*Scyliorhinus canicula*); MSH common tetrapeptide core; hydrogen fluoride procedure; transformation of Asp-Gly; 5-chloro-8-quinolyl ester procedure; DCC plus HOBT procedure; histidine DCC adduct; TFA-anisole-2% ethandithiol system; AP–M digestion of Asp-Gly-peptides

Following to the structural confirmation of the α-type melanocyte-stimulating hormone (MSH) in the neurointermediate lobe of dogfish pituitary glands,[4] Bennett, *et al.*[5] has succeeded in identifying the presence of the 2nd principle, β-MSH, in the dogfish (*Squalus acanthias*). Independently, Love and Pickering[6] isolated β-MSH from another dogfish (*Scyliorhinus canicula*) in 1974. These two β-melanotropic principles from non-vertebrates were thus clari fied at the first time in structural bases. It was found that these structures are greatly different from those of the hitherto known mammarian species. Mammarian α and β-MSHs so far known possess the common heptapeptide core, Met-Glu-His-Phe-Arg-Trp-Gly.[7] This is true in the dogfish α-MSH,[4] but not in these β-MSHs. Their findings claimed that the core has to be reduced to the tetrapeptide, His-Phe-Arg-Trp and offered thus valuable evolutionary informations about pituitary intermediate lobe peptides.

Squalus acanthias
H–Asp–Gly–Asp–Asp–Tyr–Lys–Phe–Gly–His–Phe–Arg–Trp–Ser–Val–Pro–Leu–OH
Scyliorhinus canicula
H–Asp–Gly–Ile–Asp–Tyr–Lys–Met–Gly–His–Phe–Arg–Trp–Gly–Ala–Pro–Met–Asp–Lys–OH

Amino Acid Sequence of Dogfish β-MSH[5]

1) Part LXX: F. Tamura, H. Ogawa, N. Fujii, H. Yajima, M. Nakamura, K. Miyata, and A. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 767 (1977).
2) Amino acids, peptides and their derivatives mentioned here are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=tosyl, Bzl=benzyl, NP=*p*-nitrophenyl, QCl=5-chloro-8-quinolyl, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, TFA=trifluoroacetic acid, DMF=dimethylformamide.
3) Location: *a) 606, Sakyo-ku, Kyoto; b) 553, Fukushima, Osaka.*
4) P.J. Lowry and A. Candwick, *Nature*, **226**, 219 (1970); *idem*, *Biochem. J.*, **118**, 713 (1970).
5) H.P.J. Bennett, P.J. Lowry, C. McMartin, and A.P. Scott, *Biochem. J.*, **141**, 439 (1974).
6) R.M. Love and B.T. Pickering, *General. Comp. Endocrinol.*, **24**, 398 (1974).
7) See review article, H. Yajima, and Y. Kiso, *Pharm. Therap. B.*, **1**, 529 (1975) and other references therein.

Because of these structural differences in MSHs between mammarian species and non-vertebrates, we have been interested in synthesizing these two hormones in a conventional manner. In this paper, we wish to record the synthesis of the octadecapeptide corresponding to the entire amino acid sequence of *caniculus* β-MSH.[5,6] Main strategy we employed in this synthesis (Fig. 1) is essentially the same as described in our previous synthesis of porcine β-MSH[8] in a respect that amino acid derivatives bearing protecting groups removable by hydrogen fluoride[9] were employed, *i. e.*, Asp(OBzl), Arg(Tos) and Lys(Z). By taking advantage of the suitable location of the Gly residue at positions 8 and 13, the following three peptide fragments, A (position 14—18), B (9—13) and C (2—8) were selected as building blocks. Because of the absence of an asymmetrical center in Gly, racemization free condensation of these fragments is thus feasible. Introduction of the Asp-Gly unit to the peptide chain is known to suffer serious disadvantages, because of easy transformation from the α to β peptide bond through the succinimide type intermediate[10] as we also discussed in the synthesis of camel β-MSH,[1] in which we choosed the azide prodedure.[11] In the present synthesis, we decided to try to introduce a single Asp residue at the final step of condensations reaction.

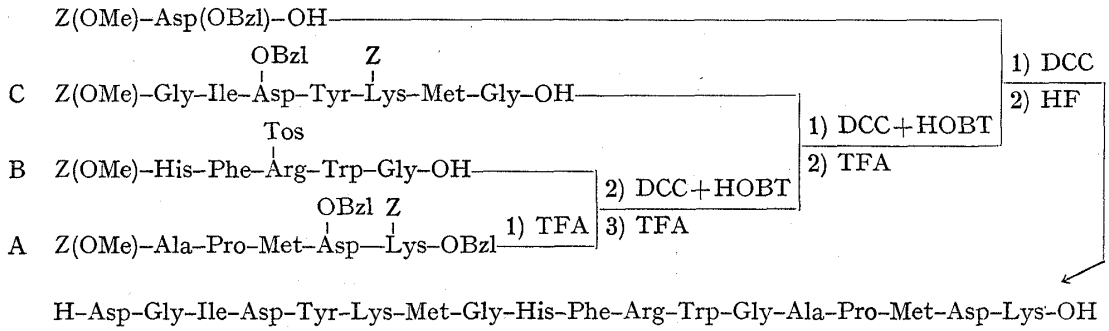

Fig. 1. Synthetic Route to β-MSH (*Scyliorhinus canicula*)

Synthetic scheme of the protected C-terminal pentapeptide ester, Z(OMe)-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl (A), is illustrated in Fig. 2. First, H-Lys(Z)-OBzl prepared according to Abe *et al.*[12] was condensed with Z(OMe)-Asp(OBzl)-OH by the DCC procedure.[13] When the resulting protected dipeptide ester was treated with TFA,[14] the deprotected dipeptide ester, H-Asp(OBzl)-Lys(Z)-OBzl, could not be precipitated with ether or *n*-hexane as powders. Therefore, this, after extraction with AcOEt followed by neutralization with $NaHCO_3$, was next submitted to the DCC condensation with Z(OMe)-Met-OH. Location of the Met residue near the C-terminal portion of the molecule is one of the structural features of *canicula* β-MSH, when compared with mammarian β-MSHs. In order to prevent

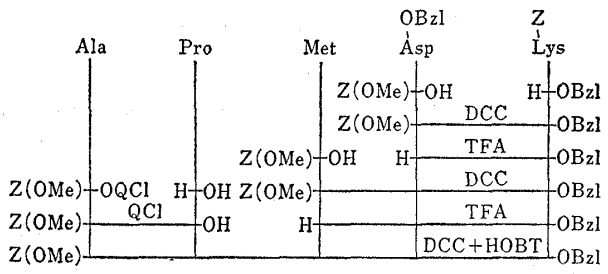

Fig. 2. Synthetic Scheme of the Protected C-Terminal Pentapeptide Ester

8) H. Watanabe, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 375 (1975).
9) S. Sakakibara, Y. Shimonishi, Y. Kishida, H. Okada, and H. Suguhara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
10) M. Bodanszky and S. Natarajan, *J. Org. Chem.*, **40**, 2495 (1975) and see other references therein.
11) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
12) O. Abe, H. Takiguchi, M. Ohno, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Japan*, **40**, 1945 (1967).
13) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
14) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

the partial oxidation of Met, each reaction was performed under the nitrogen atmosphere and *n*-hexane, instead of ether, was preferably employed to precipitate deblocked peptides throughout this synthesis, though it gave oily precipitates in most cases. The protected tripeptide ester, Z(OMe)-Met-Asp(OBzl)-Lys(Z)-OBzl thus obtained, was condensed, after the similar deprotection and the subsequent neutralization, with Z(OMe)-Ala-Pro-OH perpared by the 5-chloro-8-quinolyl ester procedures.[15] This 2 plus 3 condensation reaction proceeded smoothly by means of the DCC plus HOBT procedure[16] and the purity of the resulting protected pentapeptide ester (A) was confirmed by three criteria, thin-layer chromatography, acid hydrolysis and elemental analysis.

The protected pentapeptide, Z(OMe)-His-Phe-Arg(Tos)-Trp-Gly-OH (B), was prepared, like the corresponding Z-derivative,[8] by the azide condensation of Z(OMe)-His-$NHNH_2$[17] with H-Phe-Arg(Tos)-Trp-Gly-OH.[8]

The protected heptapeptide, Z(OMe)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-OH (C), was synthesized in a stepwise manner starting with the known dipeptide, Z(OMe)-Met-Gly-OH[18] as illustrated in Fig. 3, in which various condensation procedures were applied, *i. e.*, the *p*-nitrophenyl ester procedure[19] was employed to introduce Asp(OBzl), Ile and Gly, the 5-chloro-8-quinolyl ester procedure[15] for Lys(Z) and the azide procedure for Tyr respectively. Again *n*-hexane, instead of ether, was applied for precipitation of these Met-containing peptides, after each deprotecting step.

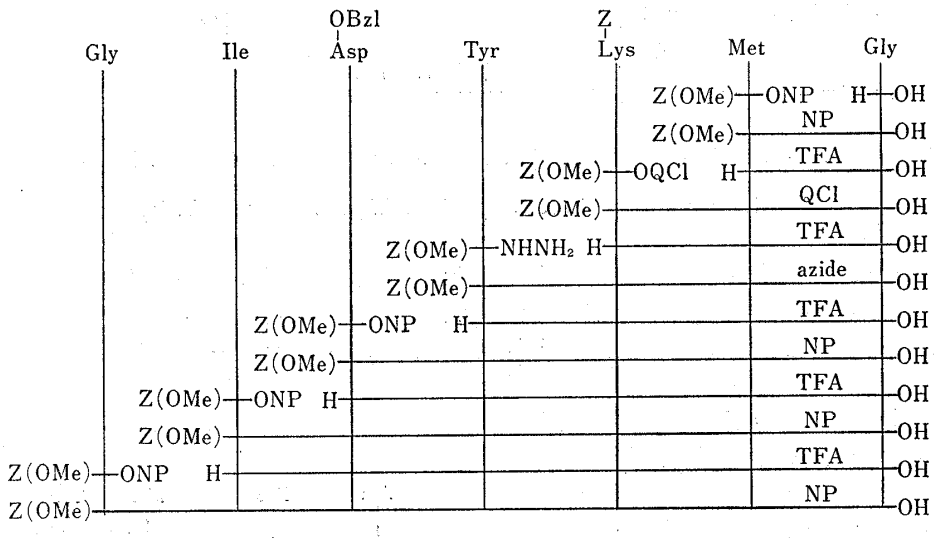

Fig. 3. Synthetic Scheme of the Protected Heptapeptide

Three building blocks, A,B and C, were then assembled by the DCC plus HOBT procedure[16] according to the scheme illustrated in Fig. 1. Since a possibility exists that the DCC adduct at the His residue might formed under this condensation reaction, each crude product was treated with methanol according to Rink and Riniker.[20] Column chromatography on silica was next employed for purification of the respective products, Z(OMe)-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl and Z(OMe)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl. For elution, the solvent

15) H.D. Jakubke and A. Voigt, *Chem. Ber.*, **99**, 2944 (1966); H. Yajima, H. Ogawa, H. Watanabe, N. Fujii, M. Kurobe, and S. Miyamoto, *Chem. Pharm. Bull.* (Tokyo), **23**, 371 (1975).
16) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
17) H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *Chem. Pharm. Bull.* (Tokyo), **21**, 380 (1973).
18) Y. Mori and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2781 (1976).
19) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
20) H. Rink and B. Riniker, *Helv. Chim. Acta*, **57**, 831 (1974).

system of chloroform, methanol and water was found efficient to isolate the desired products in an analytically pure form. In the latter case, DMF was added in the above solvent system to establish the adequate solubility. Prior to performing the column chromatographic purification, each sample was examined by thin-layer chromatography in various solvent systems to find an efficient resolution conditions of impurities, since elution pattern from the column matched well with their *Rf* values in the thin-layer chromatography.

We wish to mention here also, as described in the preceding paper,[1] that during the TFA treatment of Trp-containing peptides, anisole containing 2% ethanedithiol was employed as a cation scavenger to minimize its destruction. This deblocking condition was extended to the final step of the synthesis for the condensation of the N-terminal Z(OMe)-Asp(OBzl)-OH residue. The deprotected heptadecapeptide ester, after neutralization with triethylamine, was isolated in a free form in order to perform the DCC condensation reaction in a neutral condition as possible by ther eason stated earlier. The protected octadecapeptide ester, Z-(OMe)-Asp(OBzl)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl, was purified by precipitation from DMF with methanol and its purity was assessed by three criteria: thin-layer chromatography, elemental analysis and hydrolysis with 3 N Tos-OH.[21]

Removal of all protecting groups from the above protected octadecapeptide was performed in an ice-bath by hydrogen fluoride in the presence of anisole, skatole and dithiothreitol to minimize possible alkylation reactions.[22] Subsequent isolation of the desired product was performed in essentially the same manner as described in the purification of camel β-MSH;[1] *i.e.*, conversion to the corresponding acetate by Amberlite CG-4B, column chromatographies on Sephadex G-15 and CM-cellulose and the final desalting on Sephadex G-25. Purity of synthetic canicula β-MSH thus obtained was established by thin-layer chromatography, hydrolysis with 3 N Tos-OH and elemental analysis. Since the Asp-Gly bond locates at the N-terminal portion of the molecule as like as camel β-MSH, recovery of amino acids in aminopeptidase (AP-M)[23] digestion is of our particular attention. After 40 hours' incubation, average recovery of amino acids was 79%. Previously, Beacham *et al.*[24] mentioned that hydrolysis of the Asp-Gly bond with AP-M was extremely slow and 88% hydrolysis was obtained after 40 hour' incubation. Refering to this result, it seems difficult to determine the correct content of the β-Asp-Gly bond, if any, in the molecule, beyond the limitation of the experimental tolerance with this enzymatic technique. When the above thin-layer chromatographically homogeneous peptide was further examined by the disc electrophoresis at pH 2.3, a single band with a slight tailing was observed. This tailing portion might be attributed to a little amount of the contamination with the β-Asp-peptide.

The *in vitro* MSH activity was determined by using frog skins (*Rana pipiens*, six points design) according to Nakamura *et al.*[25] When synthetic α-MSH (2×10^{10} MSH U/g) was taken as a standard, the relative potency of synthetic dogfish MSH was 0.092 (1.8×10^{9} MSH U/g). Thus, a somewhat higher activity was recorded than that expected from the literature.[5]

Experimental

General experimental methods employed here are essentially the same as described in the Part 62[26] of this series. Thin-layer chromatography (TLC) was performed on silica gel (Kieselgel G, Merck). *Rf* values

21) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **246**, 2842 (1971).
22) S. Sano and S. Kawanishi, *J. Am. Chem. Soc.*, **97**, 3480 (1975); R.S. Feinberg and R.B. Merrifield, *ibid.*, **97**, 3485 (1975).
23) G. Pfleiderer and G.P. Celliers, *Biochem. Z.*, **339**, 186 (1963); K. Hofmann, F.M. Finn, M. Limetti, J. Montbeller, and G. Zanetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966).
24) J. Beacham, G. Dappis, F.M. Finn, H.T. Story, C. Yanaihara, N. Yanaihara, and K. Hofmann, *J. Am. Chem. Soc.*, **93**, 5526 (1971).
25) M. Nakamura, A. Tanaka, M. Hirata, and S. Inoue, *Endocrinol. Japonica*, **19**, 383 (1972).
26) H. Ogawa, M. Kubota, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24**, 2428 (1976).

refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (90: 10: 1), Rf_2 $CHCl_3$–MeOH–H_2O (45: 10: 1), Rf_3 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_4 $CHCl_3$–MeOH (97: 3), Rf_5 *n*-BuOH–AcOH–pyridine–H_2O (30: 6: 20: 24).

Z(OMe)-Asp(OBzl)-Lys(Z)-OBzl——Et_3N (5.21 ml) was added to a solution of H-Lys(Z)-OBzl tosylate (20.20 g) in $CHCl_3$ (100 ml) and the organic phase was washed with 10% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then combined with a solution of Z(OMe)–Asp(OBzl)–OH (15.50 g) in DMF (100 ml). DCC (8.24 g) was added and the mixture, after stirring at room temperature for 16 hr was filtered. The filtrate was condensed *in vacuo*. The residue was extracted with AcOEt, which was washed with 10% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The resulting mass was recrystallized from AcOEt; yield 23.57 g (86%), mp 115—117°, $[\alpha]_D^{23}$ −12.5° (c=0.4, DMF), Rf_1 0.88. *Anal.* Calcd. for $C_{41}H_{45}N_3O_{10}$: C, 66.43; H, 6.13; N, 5.68. Found: C, 66.54; H, 6.23; N, 5.73.

Z(OMe)-Met-Asp(OBzl)-Lys(Z)-OBzl——Z(OMe)–Asp(OBzl)–Lys(Z)–OBzl (14.80 g) was treated with TFA (30 ml) in the presence of anisole (6.5 ml) in an ice-bath for 30 min and *n*-hexane was added. The oily residue, after washing with *n*-hexane, was dissolved in AcOEt. This ice-chilled solution was basified with $NaHCO_3$, washed with H_2O–NaCl, dried over Na_2SO_4 and then filtered. To this filtrate, Z(OMe)–Met–OH (6.70 g) in DMF (100 ml) and DCC (4.33 g) were successively added. The mixture was stirred at room temperature overnight, filtered and the filtrate was evaporated. The oily residue was extracted with AcOEt, which was washed with 10% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The resulting mass was recrystallized from MeOH; yield 12.0 g (69%), mp 117—119°, $[\alpha]_D^{33}$ −22.6° (c=0.5, DMF), Rf_4 0.52. *Anal.* Calcd. for $C_{46}H_{54}N_4O_{11}S$: C, 63.43; H, 6.25; N, 6.43. Found: C, 63.59; H, 6.00; N, 6.38.

Z(OMe)-Ala-OQCl——The title compound was prepared according to Jakubke and Voigt[15] from Z(OMe)–Ala–OH (21.72 g), HOQCl (15.34 g) and DCC (17.59 g) and purified by recrystallization from AcOEt; yield 30.04 g (85%), mp 132—133°, $[\alpha]_D^{27}$ −24.4° (c=0.5, DMF), Rf_3 0.87. *Anal.* Calcd. for $C_{21}H_{19}N_2O_5Cl$: C, 60.80; H, 4.62; N, 6.75. Found: C, 61.02; H, 4.67; N, 6.87.

Z(OMe)-Ala-Pro-OH——H–Pro–OH (6.33 g) was dissolved in H_2O (50 ml) with an aid of Et_3N (14.7 ml). To this solution, Z(OMe)–Ala–OQCl (20.74 g) in THF (150 ml) was added and the mixture, after stirring at room temperature for 18 hr, was condensed *in vacuo*. The oily residue was dissolved in H_2O, which was washed with ether and then acidified with 10% citric acid. The resulting oily precipitate was extracted with AcOEt, which was washed with 10% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and recrystallized from AcOEt; yield 14.80 g (84%), mp 131—132°, $[\alpha]_D^{22}$ −54.7° (c=0.8, DMF), Rf_1 0.16, Rf_3 0.70. *Anal.* Calcd. for $C_{17}H_{22}N_2O_6$: C, 58.27; H, 6.33; N, 8.00. Found: C, 58.22; H, 6.33; N, 8.00.

Z(OMe)-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl (A)——Z(OMe)–Met–Asp(OBzl)–Lys(Z)–OBzl (8.0 g) was treated with TFA (17 ml) in the presence of anisole (4 ml) containing 2% ethanedithiol (We applied this cation scavenger to Met–peptides as well as Trp–peptides.) in an ice-bath for 90 min and dry *n*-hexane was added. The resulting oily precipitate was washed three times with *n*-hexane and then dissolved in AcOEt. The ice-chilled organic phase was washed with 10% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then filtered. The filtrate was combined with a solution of Z(OMe)–Ala–Pro–OH (3.54 g) in DMF (50 ml). DCC (2.09 g) and HOBT (1.62 g) were then added and the mixture was stirred at room temperature for 16 hr. The solution was filtered, the filtrate was condensed *in vacuo*. The oily residue was dissolved in AcOEt, which was washed with 10% $NaHCO_3$, 10% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The resulting mass was recrystallized with MeOH; yield 5.96 g (62%); mp 135—137°, $[\alpha]_D^{23}$ −32.2° (c=0.7, DMF), Rf_1 0.65. Amino acid ratios in an acid hydrolysate: Ala 1.06, Pro 1.00, Met 0.81, Asp 1.15, Lys 0.94 (average recovery 99%). *Anal.* Calcd. for $C_{54}H_{66}N_6O_{13}S$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.63; H, 6.42; N, 7.96.

Z(OMe)-His-Phe-Arg(Tos)-Trp-Gly-OH (B)——The title compound was prepared according to the procedure desribed in the preparation of the corresponding Z-derivative[8] from H–Phe–Arg(Tos)–Trp–Gly–OH[8] (7.50 g) and Z(OMe)–His–$NHNH_2$[17] (3.37 g) by means of the azide procedure[11] and purified by precipitation from DMF with AcOEt; yield 8.24 g (84%), mp 161—162°, $[\alpha]_D$ −14.7° (c=0.6, DMF). Rf_1 0.63. Amino acid ratios in 3 N Tos–OH hydrolysate: His 0.93, Phe 1.06, Arg (Tos) 0.80, Arg 0.28, Trp 0.85, Gly 1.00 (average recovery 93%). *Anal.* Calcd. for $C_{50}H_{57}N_{11}O_{11}S \cdot 2H_2O$: C, 56.86; H, 5.82; N, 14.59. Found: C, 56.59; H, 5.83; N, 14.62.

Z(OMe)-Lys(Z)-Met-Gly-OH——Z(OMe)–Met–Gly–OH[18] (8.15 g) was treated with TFA (18 ml) in the presence of anisole (16 ml) containing 2% ethanedithiol in an ice-bath for 50 min and dry *n*-hexane was added. The resulting oily precipitate was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (60 ml), to which Et_3N (2.8 ml) and Z(OMe)–Lys(Z)–OQCl[15] (12.12 g) were added. The mixture was stirred at room temperature for 18 hr, the solvent was evaporated and the residue was dissolved in H_2O, which after washing with AcOEt, was acidified with citric acid. The resulting oily precipitate was extracted with AcOEt. The organic phase was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with *n*-hexane and recrystallized from AcOEt and *n*-hexane; yield 8.52 g (67%), mp 138—139°, $[\alpha]_D^{22}$ −21.4° (c=0.4, DMF), Rf_1 0.29. *Anal.* Calcd. for $C_{30}H_{40}N_4O_9S$: C, 56.94; H, 6.37; N, 8.96. Found: C, 56.69; H, 6.30; N, 8.71.

Z(OMe)-Tyr-Lys(Z)-Met-Gly-OH——In the usual manner, Z(OMe)–Lys(Z)–Met–Gly–OH (8.24 g) was treated with TFA (19 ml) in the presence of anisole (4 ml) containing 2% ethanedithiol in an ice-bath for 50

min and *n*-hexane was added to form an oily precipitate, which after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF (6 ml). To this ice-cold solution, Et_3N (3.6 ml) and the azide (prepared from 4.67 g of Z(OMe)–Tyr–$NHNH_2$[27] with 8.7 ml of 2.98 N HCl–DMF, 1.74 ml of isoamylnitrite and 3.64 ml of Et_3N) in DMF (35 ml) were combined and the mixture was stirred at 4° for 18 hr. The solvent was evaporated and the residue was dissolved in H_2O with an aid of Et_3N (4 ml). The aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt, which was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The resulting solid mass was recrystallized from MeOH and AcOEt; yield 7.53 g (73%), mp 158—160°, $[\alpha]_D^{23}$ −26.8° (c=0.8, DMF), Rf_1 0.17, Rf_2 0.37. *Anal.* Calcd. for $C_{39}H_{49}N_5O_{11}S$: C, 58.85; H, 6.21; N, 8.80. Found: C, 58.82; H, 6.35; N, 8.94.

Z(OMe)-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-OH——In the usual manner, Z(OMe)–Tyr–Lys(Z)–Met–Gly–OH (3.10 g) was treated with TFA (6 ml) in the presence of anisole (3.4 ml) containing 2% ethanedithiol in an ice-bath for 30 min and *n*-hexane was added. The resulting oily precipitate was dried over KOH pellets *in vacuo* and then dissolved in DMF (10 ml), to which Et_3N (1.1 ml) and Z(OMe)–Asp(OBzl)–ONP (2.0 g) were combined. The mixture was stirred at room temperature for 16 hr, condensed *in vacuo* and the residue was treated with AcOEt and H_2O. The resulting powder was washed with 5% citric acid and H_2O and recrystallized from MeOH and AcOEt; yield 2.52 g (65%), mp 182—183°, $[\alpha]_D^{20}$ −18.7° (c=0.5, DMF). Rf_2 0.40. *Anal.* Calcd. for $C_{50}H_{60}N_6O_{14}S\cdot H_2O$: C, 58.92; H, 6.13; N, 8.25. Found: C, 59.02; H, 5.90; N, 8.24.

Z(OMe)-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-OH——The above protected pentapeptide (2.47 g) was treated as usual with TFA (4 ml) in the presence of anisole (2.7 ml) containing 2% ethanedithiol (0.2 ml) in an ice-bath for 50 min and dry *n*-hexane was added to form an oily precipitate, which after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF (5 ml). To this solution, Et_3N (0.7 ml) and Z(OMe)–Ile–ONP (1.08 g) were added and the mixture was stirred at room temperature for 16 hr. The solvent was evaporated and AcOEt was added. The resulting powder was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 1.18 g (42%), mp 199—202°, $[\alpha]_D^{23}$ −24.0° (c=0.3, DMF), Rf_2 0.48. Amino acid ratios in 3 N Tos–OH hydrolysate: Ile 1.22, Asp 1.00, Tyr 0.72, Lys 0.85, Met 0.83, Gly 1.04 (average recovery 97%). *Anal.* Calcd. for $C_{56}H_{71}N_7O_{15}S\cdot H_2O$: C, 59.40; H, 6.50; N, 8.66. Found: C, 59.56; H, 6.30; N, 9.26.

Z(OMe)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-OH (C)——The above protected hexapeptide (1.15 g) was treated with TFA (2 ml) in the presence of anisole (1 ml) containing 2% ethanedithiol as stated above and *n*-hexane was added. The resulting oil was washed with *n*-hexane, dried over KOH pellets and then dissolved in DMF (10 ml). To this solution, Et_3N (0.4 ml), Z(OMe)–Gly–ONP (0.39 g) were added and the mixture, after stirring at room temperature for 18 hr, was condensed *in vacuo*. The residue was purified by batchwise washing followed by precipitation from DMF with MeOH; yield 1.09 g (92%), mp 224—226°, $[\alpha]_D^{23}$ −21.4° (c=0.5, DMF). Rf_2 0.35. Amino acid ratios in 3 N Tos–OH hydrolysate: Gly 2.00, Ile 1.04, Asp 1.01, Tyr 0.97, Lys 0.80, Met 0.95 (average recovery 95%). *Anal.* Calcd. for $C_{58}H_{74}N_8O_{16}S$: C, 59.47; H, 6.37; N, 9.57. Found: C, 59.17; H, 6.40; N, 9.36.

Z(OMe)-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp(OBzl)-Lys(Z)-OBzl——Z(OMe)–Ala–Pro–Met–Asp(OBzl)–Lys(Z)–OBzl (4.16 g) was treated with TFA (10 ml) in the presence of anisole (9 ml) containing 2% ethanedithiol in an ice-bath for 60 min and *n*-hexane was added. The resulting oily precipitate was washed with *n*-hexane and dissolved in AcOEt, which was washed with 10% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then filtered. The filtrate was combined with a DMF solution (10 ml) containing Z(OMe)–His–Phe–Arg(Tos)–Trp–Gly–OH (4.08 g) and HOBT (0.55 g). DCC (0.85 g) was then added to the mixture, which after stirring at room temperature for 18 hr, was filtered and the filtrate was condensed *in vacuo*. The residue was treated with 5% citric acid and the resulting powder was dissolved in DMF–MeOH–3% AcOH (20 ml—10 ml—5 ml). After heating at 60° for 3 hr, the solution was filtered, condensed *in vacuo* and the residue was treated with H_2O. The resulting powder was dissolved in a small amount of the solvent consisting of $CHCl_3$–MeOH–H_2O (200: 10: 1) and the solution was applied to a column of silica (5.5×7 cm), which was eluted with the same solvent. Fractions containing the substance of Rf_2 0.65 were combined and the solvent was evaporated. The residue was treated with H_2O and the resulting powder was precipitated from DMF with AcOEt; yield 4.46 g (59%), mp 105—109°, $[\alpha]_D^{20}$ −30.6° (c=0.6, DMF). Rf_1 0.35, Rf_2 0.65. Amino acid ratios in 3 N Tos–OH hydrolysate: His 0.90, Phe 1.15, Arg not det. Trp 1.22, Gly 1.00, Ala 1.00, Pro 0.98, Met 0.95, Asp 1.01, Lys 1.03 (average recovery 86%). *Anal.* Calcd. for $C_{95}H_{113}N_{17}O_{20}S\cdot H_2O$: C, 60.20; H, 6.12; N, 12.57. Found: C, 60.58; H, 6.00; N, 11.91.

Z(OMe)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp(OBzl)-Lys-(Z)-OBzl——The above protected decapeptide ester (1.50 g) was treated with as stated above with TFA (6 ml) in the presence of anisole (2.2 ml) containing 2% ethanedithiol and *n*-hexane was added to form an oily precipitate, which after addition of 2 N HCl (0.8 ml), was lyophilized with an aid of benzene. The resulting powder was dissolved in DMF (3.5 ml), to which Et_3N (0.11 ml), Z(OMe)–Gly–Ile–Asp(OBzl)–Tyr–Lys(Z)–Met–Gly–OH (0.95 g), HOBT (0.11 g) and DCC (0.17 g) were added successively and the mixture was stirred

27) N. Fujii and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 2446 (1975).

at room temperature for 18 hr. The solvent was evaporated and the residue was treated with H_2O. The resulting powder was treated with MeOH–3% AcOH as stated above and dissolved in a small amount of the solvent consisting of $CHCl_3$–MeOH–H_2O–DMF (20: 4: 1: 5) and the solution was applied to a column of silica (5.6×16 cm), which was eluted with the same solvent. Fractions containing a substance of Rf_2 0.60 were combined and the solvent was removed by evaporation. The residue was treated with H_2O and the resulting powder was precipitated from DMF with MeOH; yield 1.93 g (84%); mp 216—218°, $[\alpha]_D^{22}$ −28.7°, (c=0.4, DMF), Rf_1 0.15, Rf_2 0.60. Amino acid ratios in 3 N Tos–OH hydrolysate: Gly 3.03, Ile 0.92, Asp 1.98, Tyr 0.85, Lys 1.90, Met 1.96, His 0.84, Phe 0.90, Arg(Tos) 1.08, Arg 0.10, Trp 1.08, Ala 1.08, Pro 1.00 (average recovery 97%). *Anal.* Calcd. for $C_{144}H_{177}N_{25}O_{32}S_3$: C, 60.34; H, 6.22; N, 12.22. Found: C, 60.23; H, 6.26; N, 12.11.

Z(OMe)-Asp(OBzl)-Gly-Ile-Asp(OBzl)-Tyr-Lys(Z)-Met-Gly-His-Phe-Arg(Tos)-Trp-Gly-Ala-Pro-Met-Asp-(OBzl)-Lys(Z)-OBzl——The above protected heptadecapeptide ester (1.49 g) was treated with TFA (4 ml) in the presence of anisole (2 ml) containing 2% ethanedithiol as stated above. The deprotected peptide, precipitated with *n*-hexane, was dissolved in a small amount of DMF and the solution was neutralized with Et_3N. The fine powder formed by addition of H_2O, was collected by filtration, washed with H_2O, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (3 ml). Z(OMe)-Asp(OBzl)-OH (0.21 g) and DCC (0.11 g) were added and the mixture was stirred at room temperature for 16 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was treated with MeOH. The resulting powder was precipitated twice from DMF with MeOH; yield 1.31 g (82%), mp 225—229°, $[\alpha]_D^{22}$ −28.7° (c=0.4, DMF), Rf_1 0.20, Rf_2 0.65. Amino acid ratios in 3 N Tos–OH hydrolysate: Asp 3.14, Gly 3.28, Ile 1.02, Tyr 1.00, Lys 2.06, Met 2.02, His 0.96, Phe 0.90, Trp 0.84, Arg+Arg(Tos) 1.17, Ala 1.12, Pro 1.03 (average recovery 89%). *Anal.* Calcd. for $C_{155}H_{188}N_{26}O_{35}S_3 \cdot 4H_2O$: C, 59.22; H, 6.28; N, 11.59. Found: C, 59.10; H, 6.06; N, 12.04.

H-Asp-Gly-Ile-Asp-Tyr-Lys-Met-Gly-His-Phe-Arg-Trp-Gly-Ala-Pro-Met-Asp-Lys-OH (Scyliorhinus canicu-lus β-MSH)——The above protected octadecapeptide ester (967 mg) was treated with HF (approximately 10 ml) in the presence of anisole (3.4 ml), skatole (830 mg) and dithiothreitol (490 mg) in an ice-bath for 60 min. The excess HF was evaporated at 0° *in vacuo* and the residue was dissolved in a small amount of H_2O, which was washed with AcOEt and then treated with Amberlite CG-4B (type 1, acetate form, 3 g) for 30 min. The resin was removed by filtration, the filtrate was lyophilized and the residue was dissolved in a small amount of 3% AcOH. The solution was applied to a column of Sephadex G-15 (3.2×142 cm), which was eluted with the same solvent. Individual fractions (5 ml each) were collected and absorbancy at 280 mμ was determined. Fractions corresponding to the front main peak (tube No. 72—123) were combined and the solvent was removed by lyophilization to give a fluffy powder; yield 691 mg. The product was then dissolved in a small amount of H_2O and the solution was applied to a column of CM-Sephadex C-25 (3.0×6.0 cm), which was eluted first with 0.005 M $AcONH_4$ buffer (pH 6.9, 150 ml) and then with 0.1 M buffer (pH 6.9, 1500 ml) through a mixing flask containing 0.005 M buffer (800 ml). Individual fractions (15 ml each) were collected and absorbancy at 280 mμ was determined. Fractions corresponding to the main peak in the gradient eluates (tube No. 40—74) were combined and the solvent was condensed *in vacuo*. The residue was applied to a column of Sephadex G-25 (2.6×142 cm) for desalting. The desired fractions were collected as described above and the product was finally lyophilized as a fluffy white powder; yield 316 mg (43%), $[\alpha]_D^{24}$ −56.1° (c= 0.4, 3% AcOH). Rf_5 0.48. Amino acid ratios in 3 N Tos–OH hydrolysate: Asp 3.15, Gly 3.05, Ile 1.09; Tyr 1.03, Lys 1.91, Met 1.75, His 0.85, Phe 0.98, Arg 0.93, Trp 0.65, Ala 1.00, Pro 1.08 (average recovery 89%). Amino acid ratios in AP-M digest: Asp 2.92, Gly 2.77, Ile 1.02, Tyr 1.33, Lys 2.25, Met 1.50, His 0.95, Phe 0.91, Arg 0.95, Trp 0.81, Ala 1.00, Pro 1.07 (average recovery 79%). Disc electrophoretic mobility on 15% polyacrylamide gel (0.5×6.0 cm, 5 mA/tube) at pH 2.3 (0.37 M glycine buffer) was 2.3 cm (with a slight tailing) to the cathod after 70 min. *Anal.* Calcd. for $C_{95}H_{138}N_{26}O_{26}S_2 \cdot 3AcOH \cdot 6H_2O$: C, 50.28; H, 6.77; N, 15.10. Found: C, 50.48; H, 6.27; N, 14.75.