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Studies on Peptides. XLVII.[1,2] Synthesis of Dogfish Melanocyte-Stimulating Hormone (MSH)

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Synthesis of dogfish MSH P_{II}, H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH_2, was described, in which N^{ε}-β,β,β-trichloroethyloxycarbonyllysine and β,β,β-trichloroethyloxycarbonylhydrazine, bearing the protecting group removable by Zn, were applied.

The amino acid sequence of melanocyte-stimulating hormone (MSH) from dogfish (*Squalus acanthias*) was elucidated by Lowry and Chadwick[4] in 1970. It was found that the sequence of the hormone, Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met, is resemble to mammalian α-MSH.[5] About half of its molecules have the carboxyl group at the C-terminus free (P_I) and about half are amidated (P_{II}). They also mentioned that about a fifth have an extra tyrosine residue on the N-terminus. This hormone, as claimed by these authors, is the first peptide hormone from non-mammalian vertebrates as far as MSH is concerned. Among these hetrogeneous melanotropic principles of dogfish, we wish to record the synthesis

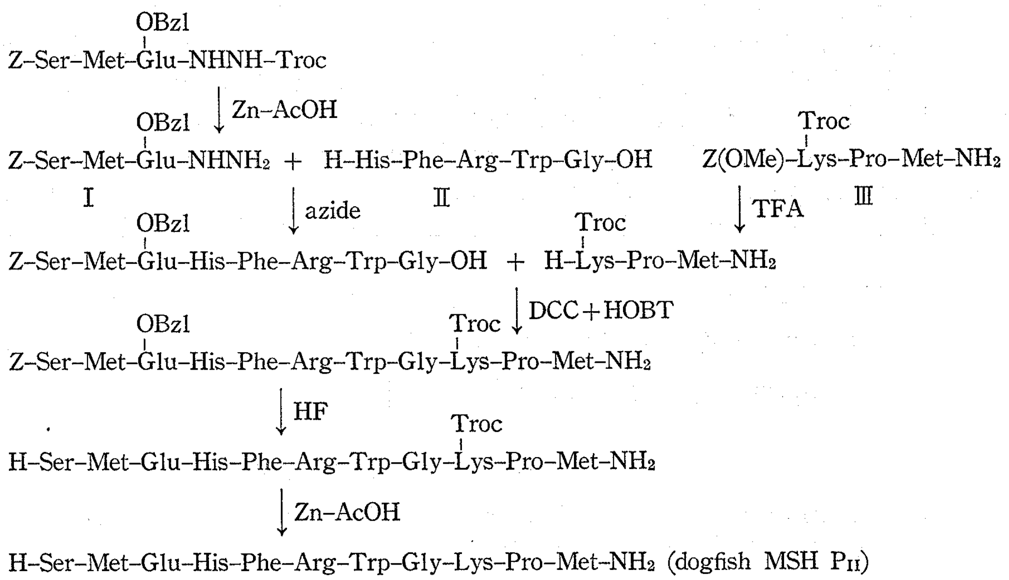

Fig. 1. Synthetic Route to Dogfish MSH P_{II}

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[1] Part XLVI: H. Kawatani, F. Tamura and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **22**, 1879 (1974).

[2] Amino acid, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC–IUB Commission of Biochemistry Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=p-methoxybenzyloxycarbonyl, OBzl=benzyl ester, Troc=trichloroethyloxycarbonyl, ONP=p-nitrophenyl ester, OTCP=2,4,5-trichlorophenyl ester.

[3] Location: *a) Sakyo-ku, Kyoto; b) 2-Sagisu, Fukushima, Osaka.*

[4] P.J. Lowry and A. Chadwick, *Nature*, **226**, 219 (1970); *idem, Biochem. J.*, **118**, 713 (1970).

[5] J.L. Harris and A.B. Lerner, *Nature*, **179**, 1346 (1957).

of P_{II}, in which our newly introduced H–Lys(Troc)–OH⁶⁾ and NH_2NH–Troc⁷⁾ were applied as illustrated in Fig. 1.

Three subunits, Z–Ser–Met–Glu(OBzl)–$NHNH_2$ (I, positions 1—3), H–His–Phe–Arg–Trp–Gly–OH (II, positions 4—8) and Z(OMe)–Lys(Troc)–Pro–Met–NH_2 (III, positions 9—11), served to construct the entire amino acid sequence of this hormone.

First, Z–Ser–Met–Glu(OBzl)–$NHNH_2$ (I) was synthesized, as illustrated in Fig. 2, in a stepwise manner starting from Z(OMe)–Glu(OBzl)–NHNH–Troc.⁷⁾ This starting protected hydrazide derivative, reported previously as oil, could be obtained as a crystalline compound by column chromatographic purification on silica. This, after treatment with trifluoroacetic acid (TFA)⁸⁾ followed by neutralization with triethylamine, was condensed with Z(OMe)–Met–OH by the mixed anhydride procedure.⁹⁾ The resulting oily product, Z(OMe)–Met–Glu(OBzl)–NHNH–Troc, after treatment with TFA and subsequent neutralization, was allowed to react with Z(OMe)–Ser–OPCP to give Z(OMe)–Ser–Met–Glu(OBzl)–NHNH–Troc. Attempt to crystallize this compound has been unsuccessful. However upon treatment with zinc in acetic acid,¹⁰⁾ the Troc group was cleaved and Z–Ser–Met–Glu(OBzl)–$NHNH_2$ was isolated as a crystalline compound in analytically pure form, though the yield was somewhat moderate. Its homogenity was assessted by the positive hydrazine test¹¹⁾ on thin–layer chromatography. Next, as illustrated in Fig. 1, the modified azide procedure¹²⁾ was applied to unit Z–Ser–Met–Glu(OBzl)–$NHNH_2$ with H–His–Phe–Arg–Trp–Gly–OH prepared according to Hofmann and Lande¹³⁾ and the resulting protected octapeptide, Z–Ser–Met–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH, was obtained in analytically pure form after recrystallization from dimethylformamide (DMF) and ethyl acetate.

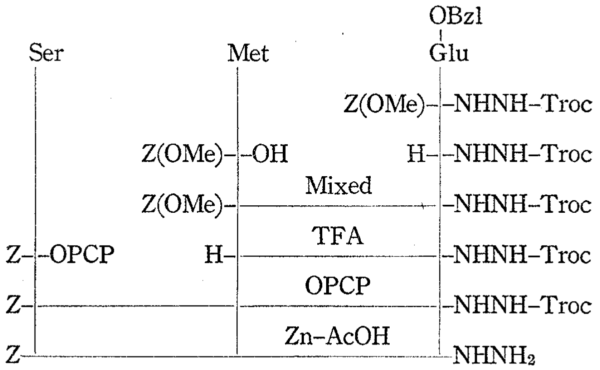

Fig. 2. Synthetic Scheme of Z–Ser–Met–Glu(OBzl)–$NHNH_2$

In order to confirm the rationality of the azide procedure of such a peptide containing the benzyl ester, an unequivocal synthesis of the above protected octapeptide was undertaken according to the scheme illustarated in Fig. 3. Z(OMe)–Glu(OBzl)–ONP was allowed to

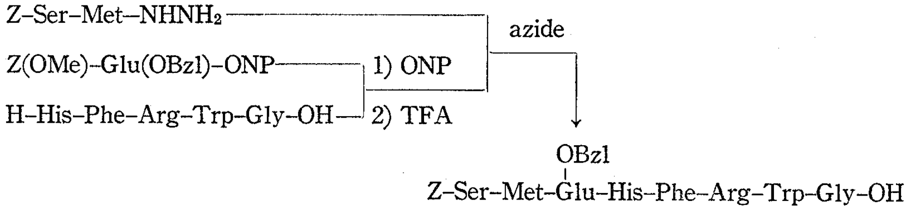

Fig. 3. Alternate Synthesis of the Protected Octapeptide, Z–Ser–Met–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH

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6) H. Yajima, H. Watanabe and M. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2185 (1971).
7) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 420 (1971).
8) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
9) T. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); R.A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J.R. Vaughan, Jr., *J. Am. Chem. Soc.*, 73, 3547 (1951).
10) R.B. Woodward, K. Heusler, J. Gosteli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966); T.B. Windholz and D.B.R. Johnston, *Tetrahedron Letters*, **1967** 2555.
11) K. Hofmann, R. Schmiechen, R.D. Wells, Y. Wolman and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
12) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
13) K. Hofmann and S. Lande, *J. Am. Chem. Soc.*, **83**, 2286 (1961).

react with H–His–Phe–Arg–Trp–Gly–OH (II) to give Z(OMe)–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH, which after treatment with TFA followed by neutralization, was coupled with Z–Ser–Met–$NHNH_2$[14] by the modified azide procedure. Identity of the protected octapeptides obtained by the two alternate routes was established by comparison of their melting points, *Rf* values, specific rotation values and infrared (IR) spectra.

To prepare the protected tripeptide amide, Z(OMe)–Lys(Troc)–Pro–Met–NH_2 (III), Z(OMe)–Lys(Troc)–OH[6] was first converted to the corresponding 2,4,5-trichlorophenyl ester[15] in the usual manner. This crystalline active ester was allowed to react with H–Pro–OH in the presence of triethylamine to afford Z(OMe)–Lys(Troc)–Pro–OH, which was then coupled with H–Met–NH_2 by the dicyclohexylcarbodiimide (DCC) plus N-hydroxybenztriazole (HOBT) procedure[16] as illustrated in Fig. 4. The resulting protected tripeptide amide (III) was isolated in pure form after recrystallization from ethanol. For the final coupling reaction, the above protected tripeptide amide (III) was treated with TFA as usual. As established previously, under this condition, the Troc group remained intact.[6] The product, after conversion to the corresponding hydrochloride followed by neutralization with triethylamine, was condensed with the protected octapeptide obtained above by the DCC plus HOBT procedure. The resulting protected undecapeptide amide, Z–Ser–Met–Glu(OBzl)–His–Phe–Arg–Trp–Gly–Lys(Troc)–Pro–Met–NH_2, was treated, without further purification, with hydrogen fluoride according to Sakakibara, *et al.*[17] As scavengers, H–Met–OH and H–Trp–OH were added during this treatment, under which the Z and benzyl ester groups could be selectively removed. The resulting partially protected undecapeptide amide, H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys(Troc)–Pro–Met–NH_2, was purified by column chromatography on carboxymethyl (CM)-cellulose using pyridine acetate buffers.

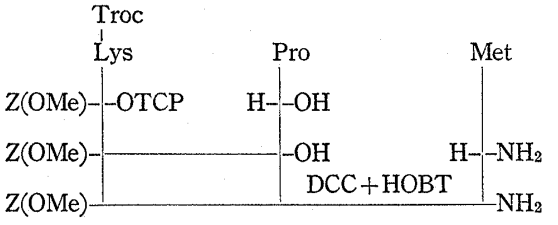

Fig. 4. Synthetic Scheme of the Protected Tripeptide Amide Z(OMe)–Lys(Troc)–Pro–Met–NH_2

In order to remove the Troc group from the above partially protected undecapeptide amide, this was then treated with zinc in acetic acid at 50° for 30 minutes and the product was isolated after purification by column chromatography on CM-cellulose. The desired undecapeptide amide was emerged from the column by pH 6.9, 0.05 M ammonium acetate buffer. The synthetic dogfish MSH P_{II} thus isolated was homogeneous on thin-layer chromatography. An acid hydrolysate contained constituent amino acids in ratios predicted by theory except for Trp which was destroyed during the hydrolysis. However the average recovery of each amino acid was somewhat low. When tested by dithizone,[18] the synthetic peptide gave deep green color, indicating the presence of zinc ion in the molecule. This zinc seems to be not a contaminant, but rather bind with the peptide, since rechromatography of this sample on CM-cellulose or Sephadex G-10 was not effective to diminish this typical green color by this reagent. From the carbon content of the elemental analysis, presence of four zinc atoms could tentatively be assumed. By analogy of the zinc preparation of the synthetic ACTH tetracosapeptide,[19] it can be concluded that this particular amino acid sequence possesses an ability to form a very tight complex with zinc, though the actual mode of binding is not known.

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[14] K. Hofmann, A. Johl, A.E. Furlenmeier and H. Kappeler, *J. Am. Chem. Soc.*, **79**, 1636 (1957).
[15] R.L. Huguenin, *Helv. Chim. Acta*, **47**, 1934 (1964).
[16] W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
[17] S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
[18] V.I. Kuznetov, Y.A. Bamkovsky and A.F. Iyevinshi, *Zh. Anal. Khim.*, **13**, 267 (1958).
[19] CIBA Ltd., Fr. Patent M. 480813 (1967).

Synthetic peptides were submitted to the *in vivo* bioassay according to Nakamura, *et al.*,[20] using african frog, *xenopus laevis* D. The relative potency of synthetic dogfish MSH P_{II} was 1/25 of α-MSH (1×10^{10} *in vitro* MSH U/g).[21] This value is closely in agreement with the reported value of the natural sourse[4] and its Troc derivative was 1/14 of the above synthetic dogfish MSH. It seems noteworthy that the presence of zinc does not interfere the MSH activity of synthetic peptides.

Experimental

General experimental methods employed are essentially the same as described in the Part XXII[22] of this series. Thin-layer chromatography was performed on silica gel (Kieselgel G. Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_2 $CHCl_3$–MeOH–AcOH (9: 1: 0.5), Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2), Rf_4 $CHCl_3$–MeOH–pyridine–H_2O (8: 3: 2: 1).

Z(OMe)-Glu(OBzl)-NHNH-Troc——Z(OMe)–Glu(OBzl)–OH (10.0 g) was condensed with NH_2NH–Troc (5.2 g) by the mixed anhydride procedure as described previously.[7] The crude product was applied to a column of silica (3×40 cm), which was eluted with $CHCl_3$ and the eluates were examined by thin-layer chromatography. The fractions which contained the desired compound (Rf_1 0.78) were combined. Condensation of the solvent afforded the solid which was recrystallized from $CHCl_3$; yield 6.52 g (44%). mp 85—88°. $[\alpha]_D^{22}$ $-11.2°$ (c=0.8, DMF). *Anal.* Calcd. for $C_{24}H_{26}O_8N_3Cl_3$: C, 48.78; H, 4.44; N, 7.11. Found: C, 48.63; H, 4.52; N, 7.00.

Z(OMe)-Met-Glu(OBzl)-NHNH-Troc——Z(OMe)–Glu(OBzl)–NHNH–Troc (8.86 g) was treated with TFA (11 ml) in the presence of anisole (3.2 ml) at 0° for 45 min. Dry ether was added and the resulting oily precipitate, after drying over KOH pellets *in vacuo* overnight, was dissolved in tetrahydrofuran (THF) (20 ml). To this ice-cold solution, Et_3N (2.8 ml) and a mixed anhydride (prepared from 4.7 g of Z(OMe)–Met–OH with 2.1 ml of Et_3N and 1.4 ml of ethyl chloroformate) in dry THF (50 ml), were added and the mixture was stirred at room temperature for 2 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which was washed with 10% citric acid, 5% $NaHCO_3$ and NaCl–H_2O, dried over Na_2SO_4 and then evaporated to give an oily product. Rf_1 0.82. Attempt to solidify this compound has been unsuccessful, yield 7.2 g (67%).

Z-Ser-Met-Glu(OBzl)-NHNH-Troc——Z(OMe)–Met–Glu(OBzl)–NHNH–Troc (7.20 g) was treated with TFA (7.4 ml) in the presence of anisole (3.2 ml) as stated above. The oily precipitate formed by addition of dry ether was dried over KOH pellets *in vacuo* overnight and then dissolved in DMF (30 ml). To this solution, Et_3N (2.8 ml) and Z–Ser–OPCP (4.90 g) were added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was dissolved in AcOEt, which was washed with 10% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated to give an oily residue, Rf_1 0.86; yield 4.31 g (55%).

Z-Ser-Met-Glu(OBzl)-NHNH$_2$——Zinc dust (1 g) was added to a solution of Z–Ser–Met–Glu(OBzl)–NHNH–Troc (1.0 g) in AcOH (10 ml) and the solution was stirred at room temperature for 12 hr. After filtration, the filtrate was condensed and the residue wsa basified with Na_2CO_3. The resulting precipitate was extracted with AcOEt, which after filtration and washing with H_2O–NaCl, was dried over Na_2SO_4 and then evaporated. The solid residue was recrystallized from AcOEt; yield 0.42 g (54%). mp 153—155°. Rf_1 0.65. *Anal.* Calcd. for $C_{28}H_{37}O_8N_5S\cdot1/2H_2O$: C, 54.89; H, 6.25; N, 11.43. Found: C, 54.96; H, 6.20; N, 11.28.

Z(OMe)-Glu(OBzl)-His-Phe-Arg-Trp-Gly-OH——Z(OMe)–Glu(OBzl)–ONP (2.20 g) was added to a solution of H–His–Phe–Arg–Trp–Gly–OH (1.67 g) in DMF (10 ml) containing Et_3N (1.5 ml) and the mixture was stirred at room temperature for 18 hr. The solution, after neutralization with AcOH, was condensed *in vacuo* and the residue was tritulated with AcOEt and H_2O. The resulting powder was recrystallized from DMF and MeOH; yield 2.34 g (94%). mp 179—181°. $[\alpha]_D^{22}$ $-14.8°$ (c=0.6, DMF), Rf_3 0.79. *Anal.* Calcd. for $C_{55}H_{64}O_{12}N_{12}\cdot CH_3COOH\cdot 2H_2O$: C, 57.95; H, 6.14; N, 14.23. Found: C, 58.00; H, 6.11; N, 14.43.

Z-Ser-Met-NHNH$_2$——The title compound was prepared according to Hofmann, *et al.*[14] mp 214—216°. $[\alpha]_D^{22}$ $-22.5°$ (c=0.5, DMF). (lit.[14] mp 215—216°). Rf_1 0.50. *Anal.* Calcd. for $C_{16}H_{24}O_5N_4S\cdot 1/2H_2O$: C, 48.84; H, 6.40; N, 14.24. Found: C, 48.48; H, 6.27; N, 14.29.

Z-Ser-Met-Glu(OBzl)-His-Phe-Arg-Trp-Gly-OH——a) To a solution of Z–Ser–Met–Glu(OBzl)–$NHNH_2$ (0.33 g) in DMF (1 ml), 2.3N HCl–DMF (0.48 ml) and isoamylnitrite (0.07 ml) were added under cooling with ice-NaCl. After 5 min, when the hydrazine test[11] of the reaction mixture became negative, the solution was neutralized with Et_3N (0.21 ml). To this solution, H–His–Phe–Arg–Trp–Gly–OH[13] in DMF

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20) M. Nakamura, A. Tanaka, M. Hirata and S. Inoue, *Endocrinol. Japon.*, **19**, 383 (1972).

21) H. Yajima, K. Kawasaki, Y. Okada, H. Minami, K. Kubo and I. Yamashita, *Chem. Pharm. Bull.* (Tokyo), **16**, 919 (1968).

22) H. Yajima, Y. Okada, H. Kawatani and N. Mizokami, *Chem. Pharm. Bull.* (Tokyo), **17**, 1229 (1969).

(1.5 ml) containing Et_3N (0.14 ml) was combined and the mixture was stirred at 4° for 24 hr. After evaporation of the solvent, AcOEt was added to the residue. The resulting solid was washed with H_2O and then recrystallized from DMF and MeOH; yield 0.46 g (70%). mp 223—224°. $[\alpha]_D^{26}$ $-24.4°$ (c=0.1, DMF). Rf_2 0.46, Rf_3 0.72. Amino acid ratios in an acid hydrolysate $\mathrm{Ser_{0.95}Met_{1.04}Glu_{1.05}His_{0.91}Phe_{0.96}Arg_{0.91}Gly_{1.00}}$ (average recovery 97%). *Anal.* Calcd. for $C_{62}H_{76}O_{14}N_{14}S\text{–}CH_3COOH$: C, 57.64; H, 6.05; N, 14.71. Found: C, 57.87; H, 5.96; N, 14.66.

b) Z(OMe)–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH (0.83 g) was treated with TFA (2 ml) in the presence of anisole (0.8 ml) at 0° for 60 min, when dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and then dissolved in DMF (3 ml). To this solution, Et_3N (0.2 ml) and the azide (prepared as stated above from 0.55 g of Z–Ser–Met–$NHNH_2$ with 0.7 ml of 4N HCl–DMF, 0.2 ml of isoamylnitrite and 0.7 ml of Et_3N) in DMF (2.5 ml) and the mixture was stirred at 4° for 18 hr. Purification and recrystallization of the product were performed as stated in (a); yield 0.65 g (69%). mp 224—225°. $[\alpha]_D^{26}$ $-22.2°$ (c=0.2, DMF). Rf_3 0.73. IR spectra of the compounds obtained in (a) and (b) were identical. Amino acid ratios in an acid hydrolysate: $\mathrm{Ser_{0.81}Met_{0.95}Glu_{1.13}His_{1.08}Phe_{1.00}Arg_{1.05}Gly_{1.05}}$ (average recovery 94%).

Z(OMe)-Lys(Troc)-OTCP——DCC (20.8 g) was added to a solution of Z(OMe)–Lys(Troc)–OH (47.0 g) and 2,4,5-trichlorophenol (18.2 g) in AcOEt (200 ml) and the mixture was stirred at room temperature for 18 hr. The solution was filtered and the filtrate was condensed *in vacuo*. The residue was triturated with petrolleum ether and recrystallized from AcOEt and petroleum ether; yield 54.0 g (88%). mp 60—63°. $[\alpha]_D^{22}$ $-4.0°$ (c=0.9, DMF). Rf_1 0.95. *Anal.* Calcd. for $C_{24}H_{24}O_7N_2Cl_6$: C, 43.33; H, 3.64; N, 4.21. Found: C, 43.59; H, 3.63; N, 4.25.

Z(OMe)-Lys(Troc)-Pro-OH——Z(OMe)–Lys(Troc)–OTCP (33.26 g) in THF (125 ml) was added to a solution of H–Pro–OH (6.91 g) in H_2O (100 ml) containing Et_3N (15.4 ml) and the mixture was stirred at room temperature for 18 hr. After evaporation of the solvent, the residue was dissolved in 5% NH_4OH (300 ml), which was washed with ether and then acidified with citric acid. The resulting precipitate was extracted with AcOEt, which was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was tritulated with petroleum ether and recrystallized from AcOEt and petroleum ether; yield 25.04 g (86%). mp 37—40°. $[\alpha]_D^{22}$ $-21.9°$ (c=0.6, DMF). Rf_1 0.50. *Anal.* Calcd. for $C_{23}H_{30}O_8N_3Cl_3$: C, 47.39; H, 5.19; N, 7.21. Found: C, 47.54; H, 5.25; N, 6.93.

Z(OMe)-Lys(Troc)-Pro-Met-NH$_2$——Z(OMe)–Met–NH_2 (0.68 g) was treated with TFA (3.7 ml) in the presence of anisole (0.5 ml) at 0° for 30 min, when dry ether was added. An oily precipitate was washed with ether, dried over KOH pellects *in vacuo* and then dissolved in 3.15N HCl-dioxane (2 ml). This solution was lyophilized and the residue, after drying over KOH pellets *in vacuo*, was dissolved in DMF (5 ml). To this solution, Et_3N (0.9 ml), Z(OMe)–Lys(Troc)–Pro–OH (1.46 g), HOBT (0.52 g) and DCC (0.62 g) were added and the mixture was stirred at room temperature overnight. The solution was filtered and the solvent was evaporated *in vacuo*. The residue was dissolved in AcOEt, which was washed with 10% citric acid, 3% NH_4OH, H_2O–NaCl, dried over Na_2SO_4 and then evaporated and the resulting solid was recrystallized from EtOH; yield 1.35 g (87%). mp 143—144°. $[\alpha]_D^{22}$ $-18.4°$ (c=0.4, DMF). Rf_1 0.80, Rf_2 0.50. *Anal.* Calcd. for $C_{28}H_{40}O_8N_5SCl_3$: C, 47.16; H, 5.65; N, 9.82. Found: C, 47.45; H, 5.85; N, 9.88.

H-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(Troc)-Pro-Met-NH$_2$——In the usual manner, Z(OMe)–Lys(Troc)–Pro–Met–NH_2 (0.11 g) was treated with TFA (0.5 ml) in the presence of anisole (0.1 ml) at room temperature for 30 min. The precipitate formed by addition of petroleum ether, was dissolved in 1N HCl (0.15 ml) and the solution was lyophilized. The residue was dissolved in DMF (2 ml), to which, Et_3N (0.02 ml), Z–Ser–Met–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH (0.14 g), DCC (31 mg) and HOBT (20 mg) were added and the mixture was stirred at room temperature for 18 hr. The solution, after filtration, was condensed *in vacuo* and the residue was treated with AcOEt to give the solid; yield 0.18 g, Rf_3 0.84, Rf_4 0.37 (main spot). This was then treated with HF (approximately 2 ml) in the presence of anisole (0.1 ml), H–Met–OH (15 mg) and H–Trp–OH (20 mg) in an ice-bath for 30 min. The excess HF was evaporated *in vacuo* and the residue, after drying over KOH pellets *in vacuo* overnight, was dissolved in H_2O. The aqueous solution was washed with ether and then treated with Amberlite CG-4B (acetate cycle, approximately 0.5 g). The resin was removed by filtration and the filtrate was applied to a column of CM-cellulose (1.5×16 cm), which was eluted with following pH 5.9 pyridine acetate buffers: 0.01M (250 ml), 0.025M (250 ml), 0.05M (250 ml) and 0.1M (1400 ml). Individual fractions, 6 ml each, were collected and absorbancy at 280 mμ was determined. The fractions corresponding to a single peak present in 0.1M buffer (tube No. 190—240), were collected and the solvent was evaporated. Lyophilization of the residue gave fluffy white powder; yield 80 mg (45%). $[\alpha]_D^{26}$ $-31.2°$ (c=0.1, DMF). Rf_3 0.60, Rf_4 0.33. Amino acid ratios in an acid hydrolysate: $\mathrm{Ser_{0.94}Met_{1.78}Glu_{1.07}His_{0.85}Phe_{1.00}Arg_{0.94}Gly_{1.07}Lys_{0.83}Pro_{1.03}}$ (average recovery 80%). *Anal.* Calcd. for $C_{66}H_{94}O_{16}N_{19}S_2Cl_3\cdot 2CH_3COOH$: (dried at 100° for 4 hr): C, 49.45; H, 6.05; N, 15.65. Found: C, 49.20; H, 6.30; N, 15.50.

H-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met-NH$_2$ (Dogfish MSH P$_{\text{II}}$)——The above partially protected undecapeptide amide (100 mg) was dissolved in AcOH (2 ml), to which Zn dust (60 mg) was added and the solution, under N_2, was stirred at 50° for 30 min. The solution was filtered, the filtrate was condensed and the residue was lyophilized. The crude product was then applied to a column of Sephadex G-10

(2.6 × 50 cm) for desalting. The column was eluted with 10% AcOH and the absorbancy at 280 mμ was determined in each fraction (3 ml). Fractions corresponding to the front peak (tube No. 34—41) were combined and the solvent was removed by lyophilization. This product was then applied to a column of CM-cellulose (2.6 × 5 cm), which was eluted with the following ammonium acetate buffers (pH 6.9): 0.005 M (500 ml), 0.01 M (500 ml), 0.02 M (500 ml) and 0.04 M (2000 ml). Individual fractions (10 ml each) were collected and the absorbancy at 280 mμ was determined. Fractions of the main peak present in 0.04 M eluates (tube No. 175—205) were pooled and the solvent was removed by lyophilization to give fluffy white powder; yield 39 mg (42%). $[\alpha]_D^{22}$ −22.5° (c=0.1, 3% AcOH). Rf_3 0.40. The dithizone test gave green color. Rechromatography on Sephadex G-10 or CM-cellulose was not effective to remove the zinc complex. Amino acid ratios in an acid hydrolysate: $Ser_{0.95}Met_{1.97}Glu_{1.09}His_{0.97}Phe_{1.00}Arg_{0.91}Gly_{1.03}Lys_{1.05}$-$Pro_{0.92}$ (average recovery 63%). *Anal.* Calcd. for $C_{63}H_{93}O_{14}N_{19}S_2 \cdot 4Zn(CH_3COO)_2$ (dried at 100° for 5 hr): C, 44.36; H, 5.52; N, 12.45. Found: C, 44.64; H, 5.73; N, 12.48.

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