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Studies on Peptides. XLIX.[1,2] Synthesis of the Octadecapeptide corresponding to the Entire Amino Acid Sequence of Porcine β-Melanocyte-stimulating Hormone

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The octadecapeptide corresponding to the entire amino acid sequence of porcine β-MSH was synthesized by assembling four peptide subunits; Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–OH (I), Z(OMe)–Pro–Tyr–Lys(Z)–Met–$NHNH_2$ (II), Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–OH (III) and Z(OMe)–Ser–Pro–Pro–Lys(Z)–$Asp(OBzl)_2$, followed by deblocking of all protecting groups with hydrogen fluoride. The synthetic porcine β-MSH was 3.16 times active than our synthetic human β-MSH (6.2×10^9 MSH unit/g).

The structure of porcine β-melanocyte-stimulating hormone (β-MSH) was determined independently in 1956 by two research groups, *i.e.*, the one by Harris and Ross[4] and the other by Geschwind and Li.[5] It consists of the octadecapeptide sequence, H–Asp–Glu–Gly–Pro–Tyr–Lys–Met–Glu–His–Phe–Arg–Trp–Gly–Ser–Pro–Pro–Lys–Asp–OH. However, its total synthesis has remained to be accomplished up to date. Among various mammalian β-MSH, bovine β-MSH was synthesized by Schwyzer, *et al.*[6] in 1963. Total syntheses of monkey and human β-MSHs were achieved in our laboratory.[7,8] Later, solid phase synthesis of latter two β-MSHs were reported by Wang, *et al.*[9]

Our synthetic outline of porcine β-MSH is illustrated in Fig. 1. This scheme is different from those employed in the syntheses of the above analogous β-MSH molecules in the respect that protecting groups removable by hydrogen fluoride[10] were employed. For protection of the side functional groups of basic amino acid residues, Lys and Arg, the Z and Tos groups were applied respectively. The ω-carboxyl function of Glu and Asp were masked by the Bzl ester. The Z(OMe) group removable by trifluoroacetic acid (TFA),[11] was employed for

1) Part XLVIII: H. Yajima, H. Ogawa, H. Watanabe, N. Fujii, M. Krobe and S. Miyamoto, *Chem. Pharm. Bull.* (Tokyo), **23**, 371 (1975).
2) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC–IUB Commission on Biochemistry Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z(OMe)=*p*-methoxybenzyloxycarbonyl, Z=benzyloxycarbonyl, Tos=tosyl, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, OQCl=5-chloro-8-quinolyl ester.
3) Location: *Sakyo-ku, Kyoto.*
4) J.I. Harris and P. Ross, *Nature*, **179**, 90 (1956); *idem*, *Biochem. J.*, **71**, 434 (1959).
5) I.I. Geschwind, C.H. Li and L. Bernafi, *J. Am. Chem. Soc.*, **78**, 4494 (1956); I.I. Geschwind and C.H. Li, *ibid.*, **79**, 615 (1957).
6) R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel and H. Zuber, *Helv. Chim. Acta*, **46**, 1975 (1963).
7) H. Yajima, Y. Okada, Y. Kinomura and H. Minami, *J. Am. Chem. Soc.*, **90**, 527 (1968); H. Yajima, Y. Okada, Y. Kinomura, N. Mizokami and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **17**, 1237 (1969).
8) H. Yajima, K. Kawasaki, H. Minami, H. Kawatani, N. Mizokami and Y. Okada, *Biochim. Biophys. Acta*, **175**, 228 (1969); H. Yajima, K. Kawasaki, H. Minami, H. Kawatani, N. Mizokami, Y. Kiso and F. Tamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 1394 (1970).
9) K.T. Wang, J. Blake and C.H. Li, *Int. J. Peptides Protein Res.*, **5**, 33 (1973).
10) S. Sakakibara, Y. Shimonishi, Y. Kishida, H. Okada and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
11) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962); H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **17**, 1962 (1969); H. Yajima, F. Tamura and Y. Kiso, *ibid.*, **18**, 2574 (1970).

the temporary protection of the α-amino function of peptides containing Lys(Z) as well as Asp (OBzl) and Glu(OBzl). Four subunits prepared by using these amino acid derivatives in suit served as stems to construct the entire amino acid sequence of this melanotropic principle.

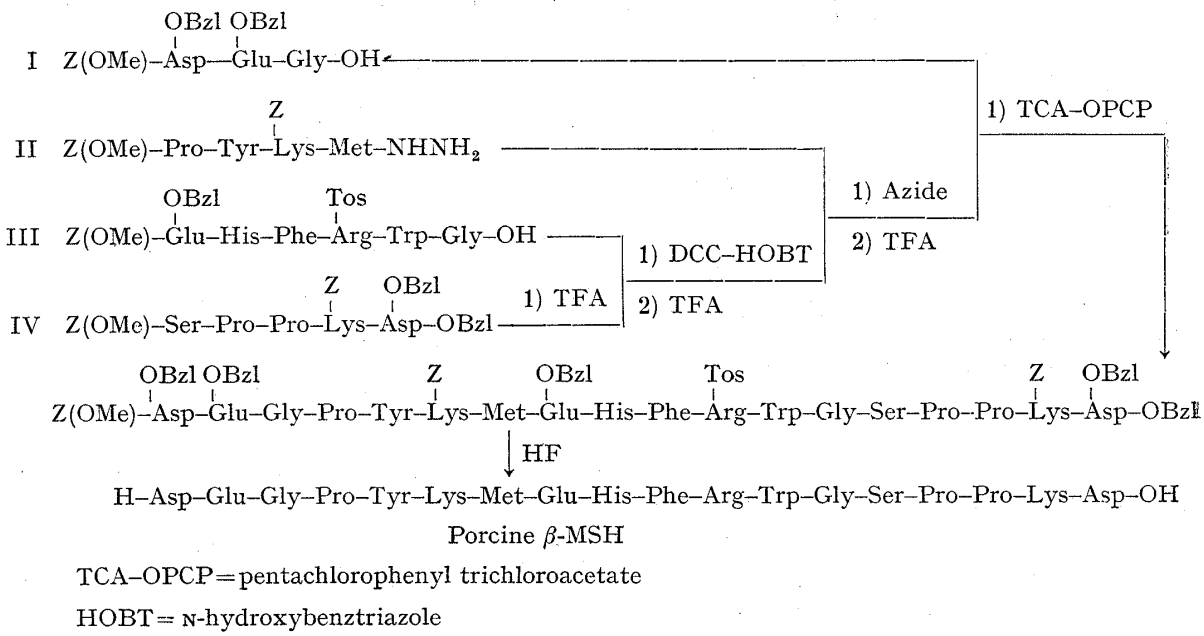


Fig. 1. Synthetic Route to Porcine β-MSH

The N-terminal protected tripeptide subunit, Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–OH (I), was prepared stepwisely by the active ester procedure starting from H–Gly–OH as shown in Fig. 2. The condensation of Z(OMe)–Glu(OBzl)–ONP[12] with the triethylammonium salt of H–Gly–OH gave Z(OMe)–Glu(OBzl)–Gly–OH as a fine crystalline compound. This protected dipeptide was also prepared by the 5-chloro-8-quinolyl ester procedure (OQCl)[1,13] in comparable high yield. This, after treatment with TFA, was allowed to react with Z(OMe)–Asp(OBzl)–ONP[12] to give (I) as also a fine crystalline derivative.

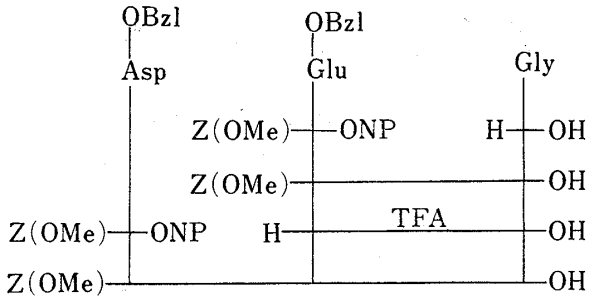
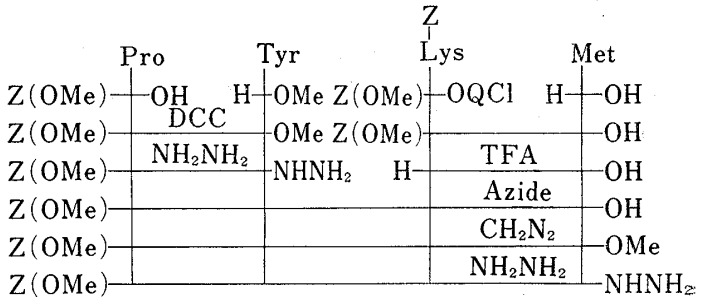


Fig. 2. Synthetic Scheme of Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–OH (I)



OQCl=5-chloro-8-quinolyl ester

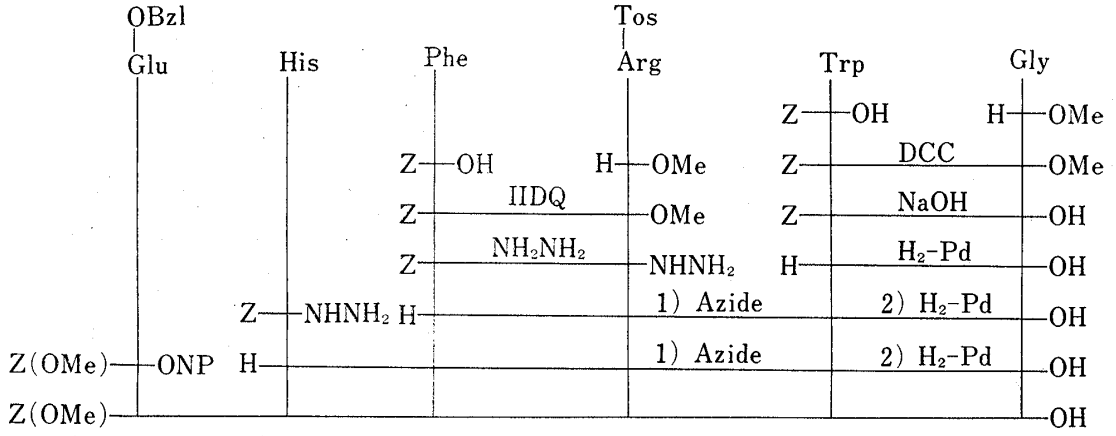
Fig. 3. Synthetic Scheme of Z(OMe)–Pro–Tyr–Lys(Z)–Met–$NHNH_2$ (II)

Synthetic route to the subunit (II), Z(OMe)–Pro–Tyr–Lys(Z)–Met–$NHNH_2$, is illustrated in Fig. 3. The newly prepared active ester, Z(OMe)–Lys(Z)–OQCl[1] was allowed to react

12) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1061 (1974).
13) H.D. Jakubke and A. Voigt, *Chem. Ber.*, **97**, 2944 (1966).

with the triethylammonium salt of H–Met–OH to give the protected dipeptide, Z(OMe)–Lys(Z)–Met–OH in 94% yield. This, after treatment with TFA, was condensed with Z(OMe)–Pro–Tyr–$NHNH_2$ by the modified azide procedure.[14] The latter hydrazide was prepared by the dicyclohexylcarbodiimide (DCC) condensation of Z(OMe)–Pro–OH with H–Tyr–OMe, followed by treatment of the resulting Z(OMe)–Pro–Tyr–OMe with hydrazine. The protected tetrapeptide, Z(OMe)–Pro–Tyr–Lys(Z)–Met–OH, prepared by the above coupling reaction of two dipeptide units, was converted in the usual manner to the corresponding hydrazide through its methyl ester. Homogeneity of the subunit (II) was assessed by thin-layer chromatography and elemental analysis.

Next, synthetic route to the protected hexapeptide (subunit III), Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–OH, is illustrated in Fig. 4. This unit is known as a key intermediate for the synthesis of various ACTH active peptides and analogous.[15,16] In the present synthesis, H–Arg(Tos)–OH was adopted as did by Li, *et al*,[15,17] since the azide procedure of Arg(Tos)-containing peptides was successfully demonstrated by Otsuka and Inouye.[18] Z–Phe–Arg(Tos)–OMe was prepared by the condensation reaction of Z–Phe–OH and H–Arg(Tos)–OMe with N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ).[19] This was converted to the corresponding hydrazide, Z–Phe–Arg(Tos)–$NHNH_2$ in the usual manner. This hydrazide was then coupled with H–Trp–Gly–OH by means of the modified azide procedure to give the protected tetrapeptide, Z–Phe–Arg(Tos)–Trp–Gly–OH, which after hydrogenation, was condensed with Z–His–$NHNH_2$ by the similar azide procedure. The resulting protected pentapeptide, Z–His–Phe–Arg(Tos)–Trp–Gly–OH, after the similar hydrogenation, was condensed with Z(OMe)–Glu(OBzl)–ONP to give the subunit (III). Through this synthetic route, the key intermediate of the synthesis of porcine β-MSH, bearing the protecting groups different from others,[15,16] was easily prepared and its homogeneity was confirmed by thin-layer chromatography and amino acid and elemental analyses.



IIDQ=N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline

Fig. 4. Synthetic Scheme of Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp-Gly–OH (III)

14) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
15) C.H. Li, J. Meienhofer, E. Schnabel, D. Chung, T.B. Lo and J. Ramachandran, *J. Am. Chem. Soc.*, **82**, 5760 (1960); *ibid.*, **83**, 4449 (1961).
16) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, **44**, 1136 (1961); R. Schwyzer and H. Kappeler, *ibid.*, **46**, 1550 (1963); R. Geiger, K. Strum and W. Siedel, *Chem. Ber.*, **97**, 1207 (1964); H. Otsuka, K. Watanabe and K. Inouye, *Bull. Chem. Soc. Japan*, **43**, 2278 (1970).
17) J. Ramachandran and C.H. Li, *J. Org. Chem.*, **27**, 4506 (1962).
18) H. Otsuka and K. Inouye, *Bull. Chem. Soc. Japan*, **37**, 1465 (1964).
19) Y. Kiso and H. Yajima, *Chem. Commun.*, **1972**, 942; Y. Kiso, Y. Kai and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **21**, 2507 (1973).

The C-terminal pentapeptide unit (IV), Ser–Pro–Pro–Lys–Asp, is a common amino acid sequence of various mammalian β-MSHs except for horse β-MSH.[20] Schwyzer, *et al.*[6] applied the protecting groups derived from *tert*-butanol to Lys and Asp in their early synthesis of bovine β-MSH. In our former syntheses of monkey and human β-MSHs,[7,8] N^{ε}-formyl-lysine was employed and the C-terminal Asp was not protected. As noticed at that time, with such a C-terminal free subunit, low yield of the subsequent fragment condensation reaction could not be avoided. Therefore, in this synthesis, the C-terminal carboxyl function was masked by the Bzl ester. Thus H–Asp(OBzl)$_2$ was condensed with Z(OMe)–Lys(Z)–OH by DCC to give Z(OMe)–Lys(Z)–Asp(OBzl)$_2$. The protecting groups employed here, the Bzl and Z, are known to be cleaved by hydrogen fluoride[10] as mentioned above. This crystalline protected dipeptide ester was treated with TFA and the resulting deprotected dipeptide ester, precipitated by addition of dry ether as fine powder, was converted to the corresponding hydrochloride. This, after neutralization with triethylamine, was condensed with Z(OMe)–Pro–OH by DCC to give Z(OMe)–Pro–Lys(Z)–Asp(OBzl)$_2$ in crystalline form. The similar TFA treatment of this protected tripeptide ester and the subsequent DCC condensation of Z(OMe)–Pro–OH were repeated in essentially the same manner as described above to give Z(OMe)–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ in satisfactory yield. Next, this protected tetrapeptide ester was similarly treated with TFA. However addition of dry ether did not afford the fine powder. The deprotected product was then precipitated as an oil by petroleum ether, neutralized with $NaHCO_3$ and then extracted with ethyl acetate. This solution containing H–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ was submitted to the coupling reaction with Z(OMe)–Ser–$NHNH_2$ by the modified azide procedure. The resulting protected pentapeptide ester, Z(OMe)–Ser–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ (IV) was obtained, after precipitation from ethyl acetate and petroleum ether, as fine powder with satisfactory elemental and amino acid analyses.

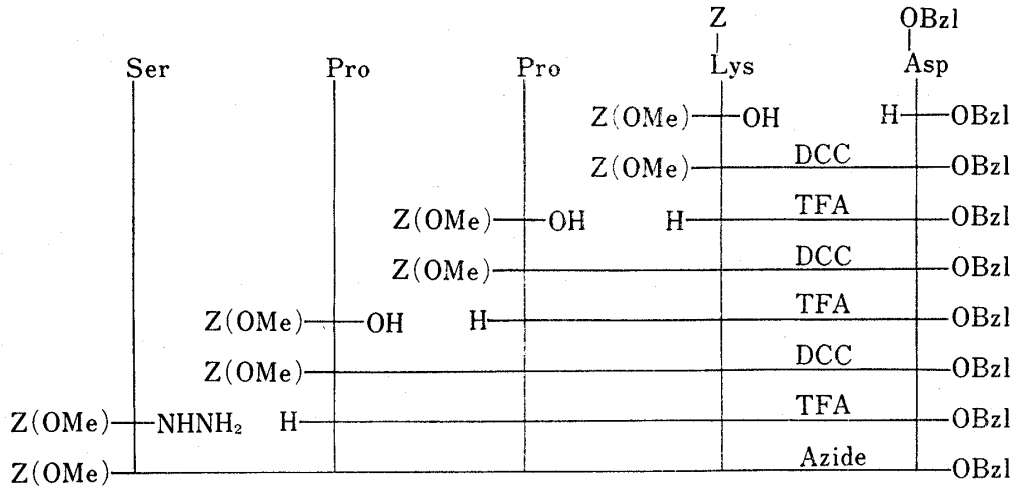


Fig. 5. Synthetic Scheme of Z(OMe)–Ser–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ (IV)

Four peptide subunits synthesized as outlined above were then assembled according to the scheme illustrated in Fig. 1. After deprotection of the Z(OMe) group from IV by TFA, the resulting product was converted to the corresponding hydrochloride, which after neutralization with triethylamine, was condensed with Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–OH (III) by DCC in the presence of N-Hydroxybenztriazole (HOBT)[21] and the resulting protected undecapeptide ester, Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–Ser–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$, was purified by column chromatography on silica, which was eluted

20) J.S. Dixon and C.H. Li, *General Comparative Endocrinol.*, **1**, 161 (1961).
21) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

with chloroform–methanol–water (90: 10: 1). This solvent system was quite effective to isolate the desired compound from other impurities. Such purification procedure was applied also in the latter steps of this synthesis. Deprotection of the protected undecapeptide ester gave rise some problem, since it contains the acid sensitive Trp residue. Addition of a small amount of mercaptoethanol[22] and large excess of anisole druing the TFA treatment prevented this destruction to some extent. The deprotected undecapeptide ester, which was slightly brownish, was then condensed with Z(OMe)–Pro–Tyr–Lys(Z)–Met–$NHNH_2$ (II) by the modified azide procedure. The reaction proceeded smoothly to give the protected pentadecapeptide ester, Z(OMe)–Pro–Tyr–Lys(Z)–Met–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–Ser–Pro–Pro–Lys(Z)–$Asp(Bzl)_2$. The homogeneous sample was obtained in satisfactory yield after column chromatographic purification as stated above.

For the final coupling reaction, the above protected pentadecapeptide ester was treated with TFA as mentioned above. The resulting α-amino deblocked product, after neutralization with triethylamine, was allowed to react with the pentachlorophenyl ester of Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–OH. Pentachlorophenyl trichloroacetate (TCA–OPCP) introduced by Fujino and Hatanaka[23] served to prepare this active ester. After this coupling reaction, the similar chromatographic purification was also found effective to purify the fully protected octadecapeptide ester, Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–Pro–Tyr–Lys(Z)–Met–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–Ser–Pro–Pro–Lys(Z)–$Asp(OBzl)_2$. Thin-layer chromatography and amino acid and elemental analyses supported the criteria of its homogeneity. In addition, hydrolysis of a sample by 3N p-toluenesulphonic acid was performed according to Liu and Chang.[24] As an additive, skatole, instead of tryptamine, was used. It seems noteworthy that the recovery of Tyr was quantitative. It is known that its recovery in a hydrochloric acid hydrolysate of protected peptides is generally poor.[25] Arg(Tos) emmerged after Phe in the long column or prior to Lys in the short column of an analyser. In the latter case, its interfered the Trp peak.

Finally, in the presence of scavengers, anisole and dithiothreitol, the above protected porcine β-MSH was treated with hydrogen fluoride at 0° for 60 minutes to remove all protecting groups. The deblocked peptide was converted to the corresponding acetate by treatment with Amberlite CG-4B. The most of scavengers was next removed from the product by Sephadex G-10. The column chromatography on CM-Sephadex C-25 was then performed and the desired compound was eluted from the column by gradient elution with ammonium acetate buffers. Besides one minor component, a large main peak was detected by measurement of absorbancy in each eluate at 277 mμ. Fractions of the main peak were combined and this solution was applied to a column of Amberlite XAD-II for desalting. The product was eluted with aqueous methanol and then lyophilized. The compound thus purified exhibited a sharp single spot on thin-layer chromatography and its homogeneity was assessed by acid hydrolysis and elemental analysis.

Considering the method of choice we employed here, *i.e.*, the azide procedure and the activation of the Gly terminal peptide subunits, it can be realized that the synthesis of porcine β-MSH carried out under conditions which risks of racemization were minimized. Complete digestion of synthetic β-MSH was confirmatively achieved by exposure to the action of aminopeptidase (AP-M) we obtained, though this peptidase was reported not to cleave the Pro bond.[26] Enzymatic assessment of the L-configuration of our synthetic peptide was thus

22) G.R. Marshall, *Advan. Exp. Med.*, **2**, 48 (1969); H. Yajima, H. Kawatani and H. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **18**, 1333 (1970).
23) M. Fujino and C. Hatanaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 929 (1968).
24) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **216**, 2842 (1971).
25) B. Iselin, *Helv. Chim. Acta*, **45**, 1510 (1962).
26) E.C. Jorgensen, G.C. Windridge and W. Patton, *J. Med. Chem.*, **12**, 733 (1969). AP-M (Rohm and Haas Co. Lot No. 48112) was purchased from Protein Research Fundation (Osaka).

achieved. In addition, the content of Trp was estimated by the *p*-toluenesulphonic acid hydrolysis. The recovery obtained here was somewhat low but close to the value which could be judged as one mole.

The *in vivo* bioassay of the synthetic octadecapeptide corresponding to the entire amino acid sequence of porcine β-MSH thus obtained was conducted according to Nakamura, *et al.*,[27] using african flogs, *xenopus laevis* D. The synthetic peptide was 3.16 times active than our synthetic human β-MSH (6.2×10^9 MSH unit/g).[8] The result confirmed the view derived from the natural sourses that, as far as the MSH activity is concerned, the octadecapeptide (natural porcine β-MSH, 5×10^9 unit/g)[28] exhibited a little higher activity than the docosapeptide (natural human β-MSH, 3.3×10^9 unit/g).

Experimental

Thin-layer chromatography was performed on silica (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–H_2O (45:10:1), Rf_3 $CHCl_3$–MeOH–AcOH (9:1:0.5), Rf_4 $CHCl_3$–MeOH–H_2O (100:10:1), Rf_5 *n*-BuOH–AcOH–pyridine–H_2O (15:3:10:12).

Z(OMe)-Glu(OBzl)-Gly-OH——Z(OMe)–Glu(OBzl)–ONP (10.45 g) in dioxane (50 ml) was added to a solution of H–Gly–OH (2.25 g) and Et_3N (5.6 ml) in H_2O (50 ml) and the mixture was stirred at room temperature for 18 hr. The solvent was evaporated and the residue was dissolved in 10% $NaHCO_3$, which was washed with ether. The aqueous phase was acidified with citric acid and the resulting precipitate was extracted with AcOEt, which after washing with 10% citric acid and H_2O–NaCl, was dried over Na_2SO_4 and then evaporated. The resulting solid was recrystallized from AcOEt; yield 7.85 g (86%), mp 125—128°, $[\alpha]_D^{22}$ −9.0° (c=0.5, DMF). Rf_1 0.56, Rf_2 0.42. *Anal.* Calcd. for $C_{23}H_{26}O_8N_2$: C, 60.25; H, 5.72; N, 6.11. Found: C, 60.24; H, 5.66; N, 6.00. This compound was also prepared using Z(OMe)–Glu(OBzl)–OQCl[1] in essentially the same manner as described above; yield 81%, mp 118—120°, $[\alpha]_D^{22}$ −9.4° (c=1.0, DMF). *Anal.* Found: C, 60.01; H, 5.77; N, 5.86.

Z(OMe)-Asp(OBzl)-Glu(OBzl)-Gly-OH (I)——In the usual manner, Z(OMe)–Glu(OBzl)–Gly–OH (5.91 g) was treated with TFA (15 ml) in the presence of anisole (3.2 ml) at 0° for 30 min, when petroleum ether was added to form an oily precipitate. The supernatant was removed by decantation. The deblocked peptide, after similar washing with petroleum ether, was dissolved in H_2O (10 ml) and Et_3N (9.6 ml) was added. To this solution, Z(OMe)–Asp(OBzl)–ONP (6.56 g) in DMF (50 ml) was combined and the mixture was stirred at room temperature for 18 hr. The solvent was evaporated and the residue was dissolved in 10% $NaHCO_3$, which was washed with ether. The aqueous phase was acidified with citric acid and the resulting 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 solid residue was recrystallized from EtOH; yield 6.24 g (73%), mp 94—95°, $[\alpha]_D^{22}$ −5.6° (c=0.8, DMF). Rf_1 0.59, Rf_2 0.42, Rf_3 0.53. *Anal.* Calcd. for $C_{34}H_{37}O_{11}N_3$: C, 61.53; H, 5.62; N, 6.33. Found: C, 61.59; H, 5.44; N, 6.24.

Z(OMe)-Pro-Tyr-OMe——DCC (5.67 g) was added to a solution of Z(OMe)–Pro–OH (8.38 g) and H–Tyr–OMe (prepared from 5.79 g of the hydrochloride with 3.5 ml of Et_3N) in DMF (20 ml) and the mixture was stirred at room temperature for 18 hr. The solution was filtered, the filtrate was condensed and the residue was dissolved in AcOEt. The solution was washed with 10% citric acid, 10% Na_2CO_3 and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 8.98 g (79%), mp 98—100°, $[\alpha]_D^{21}$ −13.3° (c=1.0, DMF). Rf_1 0.81. *Anal.* Calcd. for $C_{24}H_{28}O_7N_2$: C, 63.14; H, 6.18; N, 6.14. Found: C, 63.24; H, 6.42; N, 6.43.

Z(OMe)-Pro-Tyr-NHNH$_2$——In the usual manner, Z(OMe)–Pro–Tyr–OMe (18.24 g) in MeOH (200 ml) was converted to the corresponding hydrazide with 80% hydrazine (25 ml). The crude product was recrystallized from DMF and MeOH; yield 17.73 g (97%), mp 202—204°, $[\alpha]_D^{22}$ −48.0° (c=0.9, DMF). Rf_1 0.66. *Anal.* Calcd. for $C_{23}H_{28}O_6N_4\cdot1/2H_2O$: C, 59.34; H, 6.28; N, 12.04. Found: C, 59.59; H, 6.42; N, 12.07.

Z(OMe)-Lys(Z)-Met-OH——Z(OMe)–Lys(Z)–OQCl[1] (10.0 g) in THF (100 ml) was added to a solution of H–Met–OH (3.94 g) and Et_3N (6.5 ml) in H_2O (50 ml) and the mixture was stirred at room temperature under the nitrogen atmosphere for 18 hr. The solvent was evaporated and the residue was dissolved in H_2O, which, after washing with ether was acidified with 0.1N HCl. The resulting precipitate was extracted with AcOEt, which was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated; yield 8.90 g (94%), mp 134—135°, $[\alpha]_D^{21}$ −1.0° (c=1.0, DMF). Rf_1 0.57. *Anal.* Calcd. for $C_{28}H_{37}O_8N_3S$: C, 58.42; H, 6.48; N, 7.30. Found: C, 58.12; H, 6.43; N, 7.27.

27) M. Nakamura, A. Tanaka, M. Hirata and S. Inouye, *Endocrinol. Japon*, **19**, 383 (1972).
28) B.T. Pickering and C.H. Li, *Biochim. Biophys. Acta*, **74**, 156 (1963).

Z(OMe)-Pro-Tyr-Lys(Z)-Met-OH——Z(OMe)–Lys(Z)–Met–OH (9.21 g) was treated with TFA (12 ml) in the presence of anisole (3.5 ml) at 0° for 30 min, when dry ether (peroxide free) was added. The resulting oily precipitate was washed with ether, dried over KOH pellets *in vacuo* overnight and then dissolved in DMF (60 ml). To this solution, Et_3N (5 ml) and Z(OMe)–Pro–Tyr–azide (prepared from 8.75 g of the corresponding hydrazide with 13.1 ml of 2.9N HCl–DMF, 2.6 ml of isoamylnitrite and 5.4 ml of Et_3N) in DMF (20 ml) were added and the mixture was stirred at 4° for 18 hr. After evaporation of the solvent, the residue was dissolved in 10% Na_2CO_3, which was washed with AcOEt. The aqueous phase was then acidified with citric acid and 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 residue was treated with ether and then recrystallized from AcOEt and ether; yield 11.58 g (87%), mp 102—106°, $[\alpha]_D^{21}$ −20.4° (c=0.3, DMF). Rf_1 0.57, Rf_3 0.39. *Anal.* Calcd. for $C_{42}H_{53}O_{11}N_5S$: C, 60.34; H, 6.39; N, 8.38. Found: C, 60.43; H, 6.44; N, 8.17.

Z(OMe)-Pro-Tyr-Lys(Z)-Met-OMe——An ethereal solution of diazomethane was added to a solution of Z(OMe)–Pro–Tyr–Lys(Z)–Met–OH (11.0 g) in MeOH (100 ml) as usual. The yellow color persisted for 30 min, when a few drop of AcOH was added. The solvent was evaporated and the residue was triturated with ether. The product was recrystallized from AcOEt; yield 10.83 g (97%), mp 114—115°, $[\alpha]_D^{22}$ −37.7° (c=1.0, DMF). Rf_1 0.83, Rf_3 0.57. *Anal.* Calcd. for $C_{43}H_{55}O_{11}N_5S$: C, 60.76; H, 6.52; N, 8.24. Found: C, 60.58; H, 6.41; N, 7.97.

Z(OMe)-Pro-Tyr-Lys(Z)-Met-$NHNH_2$ (II)——Hydrazine hydrate (80%, 7.6 ml) was added to a solution of Z(OMe)–Pro–Tyr–Lys(Z)–Met–OMe (10.20 g) in MeOH (50 ml) and the solution was kept on standing at room temperature for 36 hr. The resulting crystalline mass was collected by filtration and recrystallized from DMF and MeOH; yield 6.57 g (64%), mp 170—171°, $[\alpha]_D^{23}$ −34.2° (c=1.2, DMF). Rf_1 0.67, Rf_2 0.56, Rf_3 0.31. *Anal.* Calcd. for $C_{42}H_{55}O_{10}N_7S$: C, 59.34; H, 6.52; N, 11.54. Found: C, 59.28; H, 6.54; N, 11.65.

H-Trp-Gly-OH——According to Hofmann, *et al.*[29] Z–Trp–Gly–OMe was saponified and then hydrogenated; mp 155—158°, $[\alpha]_D^{25}$ +81.9° (c=0.8, H_2O). (lit.[29] mp 176—178°, $[\alpha]_D^{26}$ +81.7° in H_2O). *Anal.* Calcd. for $C_{13}H_{15}O_3N_3 \cdot H_2O$: C, 58.74; H, 5.87; N, 15.81. Found: C, 58.50; H, 6.13; N, 15.69.

Z-Phe-Arg(Tos)-OMe——IIDQ (1.80 g) was added to a mixture of Z–Phe–OH (1.50 g) and H–Arg-(Tos)–OMe (1.70 g) in THF (15 ml) and the solution was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which was washed with 0.1N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The oily residue was triturated with petroleum ether; yield 2.68 g (86%), mp 78—80°, $[\alpha]_D^{25}$ −24.2° (c=0.8, MeOH). Rf_1 0.64. *Anal.* Calcd. for $C_{31}H_{37}O_7N_5S$: C, 59.79; H, 5.98; N, 11.23. Found: C, 59.49; H, 5.83; N, 10.96.

Z-Phe-Arg(Tos)-$NHNH_2$——To a solution of Z–Phe–Arg(Tos)–OMe (20.0 g) in MeOH (150 ml), 80% hydrazine hydrate (15 ml) was added and the solution was kept on standing in a refrigerator overnight. After evaporation of the solvent, the residue was extracted with AcOEt, which was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and petroleum ether and recrystallized from EtOH and AcOEt; yield 18.0 g (90%), mp 105—108°, $[\alpha]_D^{25}$ −10.9° (c=0.6, DMF). Rf_1 0.53. *Anal.* Calcd. for $C_{31}H_{37}O_7N_5S$: C, 59.79; H, 5.98; N, 11.23. Found: C, 59.49; H, 5.83; N, 10.96.

Z-Phe-Arg(Tos)-Trp-Gly-OH——To a solution of Z–Phe–Arg(Tos)–$NHNH_2$ (12.48 g) in 1N HCl–DMF (40 ml), isoamylnitrite (2.9 ml) was added at −15°. After stirring for 5 min, when the hydrazine test became negative, the solution was neutralized with Et_3N (5.6 ml). This solution was then combined with a solution of H–Trp–Gly–OH (7.30 g) and Et_3N (7 ml) in 90% DMF (60 ml). The mixture was stirred at 4° for 24 hr and the solvent was evaporated. The residue was dissolved in 10% Na_2CO_3. The aqueous phase, after washing with AcOEt, was acidified with citric acid and 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 treated with ether and the resulting powder was precipitated from THF and ether; yield 14.4 g (84%), mp 123—125°, $[\alpha]_D^{25}$ −14.2° (c=0.5, DMF). Rf_1 0.50. *Anal.* Calcd. for $C_{43}H_{48}O_9N_8S$: C, 60.55; H, 5.67; N, 13.14. Found: C, 60.26; H, 5.44; N, 12.97.

Z-His-Phe-Arg(Tos)-Trp-Gly-OH——Z–Phe–Arg(Tos)–Trp–Gly–OH (25.59 g) in MeOH (200 ml) was hydrogenated over a Pd catalyst in the usual manner. After evaporation of the solvent, the hygroscopic powder was obtained when the residue was precipitated from MeOH with ether; yield 20.44 g (95%). This free tetrapeptide (13.25 g) was dissolved in DMF (100 ml) containing Et_3N (8.4 ml). To this solution, the azide (prepared from 10.92 g of Z–His–$NHNH_2$ with 14.8 ml of 5.4N HCl–DMF, 5.3 ml of isoamylnitrite and 11.2 ml of Et_3N) in DMF (100 ml) was added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the powder formed by addition of AcOEt and H_2O, was washed with 2% AcOH and H_2O and then recrystallized from THF and AcOEt; yield 15.48 g (87%), mp 170—173°, $[\alpha]_D^{25}$ −27.9° (c=0.7, DMF). Rf_1 0.34. *Anal.* Calcd. for $C_{49}H_{55}O_{10}N_{11}S \cdot 2H_2O$: C, 57.35; H, 5.79; N, 15.00. Found: C, 57.03; H, 5.70; N, 14.67.

H-His-Phe-Arg(Tos)-Trp-Gly-OH——Z–His–Phe–Arg(Tos)–Trp–Gly–OH (15.48g) was hydrogenated over a Pd catalyst in the usual manner and the product was precipitated from THF with ether; yield 12.01 g

29) K. Hofmann, M.E. Woolner, G. Spuhler and E.T. Schwartz, *J. Am. Chem. Soc.*, **80**, 1486 (1958).

(84%), mp 161—165°, $[\alpha]_D^{25}$ +25.4° (c=0.5, 10% AcOH), Rf_2 0.82. *Anal.* Calcd. for $C_{43}H_{53}O_{10}N_{11}S \cdot 2H_2O$: C, 54.24; H, 6.03; N, 16.18. Found: C, 54.02; H, 6.44; N, 15.70.

Z(OMe)-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-OH (III)——H–His–Phe–Arg(Tos)–Trp–Gly–OH (2.26 g) was dissolved in DMF (25 ml). To this solution, Et_3N (0.7 ml) and Z(OMe)–Glu(OBzl)–ONP (2.61 g) were combined and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was triturated with ether. The resulting powder was washed with 2% AcOH and precipitated from THF and ether; 2.50 g (77%), mp 157—161°, $[\alpha]_D^{26}$ −14.8° (c=1.3, DMF). Rf_1 0.44. Amino acid ratios in an acid hydrolysate: $Glu_{0.82}His_{0.98}Phe_{1.00}Arg_{0.93}Gly_{1.02}$ (average recovery 96%), in p-toluenesulphonic acid hydrolysate $Glu_{0.79}His_{0.79}Phe_{1.00}Arg_{1.04}Trp_{0.75}Gly_{1.01}$. *Anal.* Calcd. for $C_{62}H_{70}O_{14}N_{12}S \cdot 2H_2O$: C, 58.38; H, 5.84; N, 13.17. Found: C, 58.07; H, 5.74; N, 13.61.

Z(OMe)-Lys(Z)-Asp(OBzl)$_2$——To a suspension of HCl·H–Asp(OBzl)$_2$ (3.50 g), Et_3N (1.4 ml), Z(OMe)–Lys(Z)–OH (5.34 g) and DCC (2.47 g) were added and the mixture was stirred at room temperature for 18 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was dissolved in AcOEt, which was washed with 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The resulting crystalline solid was recrystallized from hot AcOEt; yield 7.10 g (96%), mp 133—134°, $[\alpha]_D^{23}$ −14.2° (c=1.0, DMF). Rf_3 0.80. *Anal.* Calcd. for $C_{41}H_{45}O_{10}N_3$: C, 66.43; H, 6.13; N, 5.68. Found: C, 66.35; H, 5.96; N, 5.82.

Z(OMe)-Pro-Lys(Z)-Asp(OBzl)$_2$——Z(OMe)–Lys(Z)–Asp(OBzl)$_2$ (3.70 g) was treated with TFA (3.7 ml) in the presence of anisole (2 ml) in an ice-bath for 50 min, when dry ether was added. The resulting powder, after drying over KOH pellets *in vacuo*, was dissolved in dioxane (10 ml), to which 3.15N HCl–DMF (1.4 ml) was added. The solution was evaporated *in vacuo* and evaporation was repeated after addition of dioxane (10 ml). The residue was suspended in THF (20 ml), to which Et_3N (0.6 ml), Z(OMe)–Pro–OH (1.16 g) and DCC (0.85 g) were added. The mixture was stirred at room temperature for 18 hr and then filtered. The filtrate was evaporated and the residue was dissolved in AcOEt. The organic phase was washed with 5% $NaHCO_3$. 5% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then evaporated to dryness. The resulting solid was recrystallized from AcOEt; yield 2.67 g (78%), mp 129—130°, $[\alpha]_D^{23}$ −29.2° (c=0.9, DMF). Rf_3 0.87. *Anal.* Calcd. for $C_{46}H_{52}O_{11}N_4$: C, 66.01; H, 6.26; N, 6.69. Found: C, 66.17; H, 6.31; N, 6.79.

Z(OMe)-Pro-Pro-Lys(Z)-Asp(OBzl)$_2$——In the usual manner, Z(OMe)–Pro–Lys(Z)–Asp(OBzl)$_2$ (2.43 g) was treated with TFA (2.2 ml) in the presence of anisole (1.6 ml) in an ice-bath for 50 min. Addition of dry ether did not afford the precipitate. The solvent was then evaporated *in vacuo* and the residue was washed with petroleum ether. The oily residue was dissolved in AcOEt, which was washed with 5% $NaHCO_3$ This solution was then combined with a solution of Z(OMe)–Pro–OH (0.90 g) in THF (20 ml) and DCC (0.60 g) was added. The mixture, after stirring at room temperature for 12 hr, was filtered, the filtrate was condensed and the residue was dissolved in AcOEt, which was washed with base and acid as stated above, dried over Na_2SO_4 and then evaporated. Trituration of the residue with ether gave the solid which was recrystallized from AcOEt and ether; yield 1.96 g (72%), mp 55—58°, $[\alpha]_D^{23}$ −38.5° (c=1.0, DMF). Rf_3 0.81. *Anal.* Calcd. for $C_{51}H_{59}O_{12}N_5$: C, 65.58; N, 6.37; N, 7.50. Found: C, 65.28; H, 6.44; N, 7.31.

Z(OMe)-Ser-Pro-Pro-Lys(Z)-Asp(OBzl)$_2$ (IV)——In the usual manner, Z(OMe)–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ (2.81 g) was treated with TFA (2.2 ml) in the presence of anisole (2 ml) at 0° for 50 min, when the solvent was evaporated. The residue, after washing with petroleum ether was dried over KOH pellets *in vacuo* overnight and then dissolved in DMF (5 ml). To this solution, Et_3N (0.5 ml) and the azide (prepared from 0.93 g of Z(OMe)–Ser–$NHNH_2$ with 1.2 ml of 5.4N HCl–DMF, 0.48 ml of isoamylnitrite and 0.9 ml of Et_3N) in DMF (5 ml) were combined and the mixture was stirred at 4° for 18 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which after washing with 5% citric acid and H_2O–NaCl, was dried over Na_2SO_4 and then evaporated. The residue was triturated with petroleum ether and recrystallized from AcOEt and petroleum ether; yield 2.25 g (73%), mp 40—43°, $[\alpha]_D^{23}$ −43.4° (c=0.7, DMF). Rf_3 0.68. Amino acid ratios in an acid hydrolysate: $Ser_{0.94}Pro_{1.92}Lys_{1.13}Asp_{1.00}$ (average recovery 94%). *Anal.* Calcd. for $C_{54}H_{64}O_{14}N_6$: C, 63.51; H, 6.32; N, 8.23. Found: C, 63.80; H, 6.53; N, 7.93.

Z(OMe)-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-Ser-Pro-Pro-Lys(Z)-Asp(OBzl)$_2$——Z(OMe)–Ser–Pro–Pro–Lys(Z)–Asp(OBzl)$_2$ (2.09 g) was treated with TFA (4.5 ml) in the presence of anisole (1.1 ml) at 0° for 60 min, when petroleum ether was added. The resulting oily precipitate was washed with the same solvent and then dissolved in 3.15N HCl–DMF (0.65 ml). The solution, after addition of benzene (30 ml), was lyophilized and the resulting hydrochloride was dissolved in DMF (5 ml), to which Et_3N (0.3 ml), Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–OH (2.48 g), DCC (0.43 g) and HOBT (0.3 g) were combined. After the mixture was stirred at room temperature for 40 hr, the solvent was evaporated *in vacuo*. Addition of H_2O afforded the solid, which was dissolved in a small amount of $CHCl_3$–MeOH–H_2O (90 : 10 : 1). This solution was applied to a column of silica (4.6 × 6.5 cm), which was eluted with the same solvent and the eluates was examined by thin-layer chromatography. The fractions containing the substance of Rf_1 0.56 were combined. After evaporation of the solvent, the resulting powder was washed with H_2O and recrystallized from AcOEt and ether; yield 2.45 g (59%), mp 112—114°, $[\alpha]_D^{25}$ −35.9° (c=0.5, DMF). Rf_1 0.56, Rf_2 0.60. Amino acid ratios in an acid hydrolysate: $Glu_{1.15}His_{1.00}Phe_{0.91}Arg_{0.89}Gly_{0.86}Ser_{0.98}Pro_{1.95}Lys_{1.09}Asp_{1.08}$

(average recovery 98%). *Anal.* Calcd. for $C_{107}H_{124}O_{24}N_{18}S \cdot H_2O$: C, 61.30; H, 6.06; N, 12.03. Found: C, 61.29; H, 5.91; N, 11.93.

Z(OMe)-Pro-Tyr-Lys(Z)-Met-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-Ser-Pro-Pro-Lys(Z)-Asp(OBzl)$_2$——The above protected undecapeptide ester (2.04 g) immersed well with anisole (5.4 ml) was treated with TFA (7.4 ml) containing mercaptoethanol (0.01 ml) at 0° for 60 min. The product precipitated by addition of petroleum ether was triturated with ether, dried over KOH pellets *in vacuo* (yield 1.95 g, 98%) and dissolved in DMF (2 ml). To this solution, Et_3N (0.14 ml) and the azide (prepared from 1.28 g of Z(OMe)–Pro–Tyr–Lys(Z)–Met–$NHNH_2$ with 0.84 ml of 3.58N HCl–DMF, 0.2 ml of isoamylnitrite and 0.63 ml of Et_3N) in DMF (3 ml) were combined and the mixture was stirred at 4° for 12 hr. The solvent was evaporated and the residue was dissolved in $CHCl_3$–MeOH–H_2O (120: 10: 1) and this solution was applied to a column of silica (5.6×5.0 cm), which was eluted with the same solvent. The fractions containing the substance of Rf_2 0.70 were combined and the solvent was evaporated. The residue was triturated with H_2O and then recrystallized from $CHCl_3$ and MeOH; yield 1.73 g (66%), mp 196—198°, $[\alpha]_D^{23}$ −34.2° (c=0.7, DMF). Rf_1 0.65, Rf_2 0.70. Amino acid ratios in an acid hydrolysate: $Pro_{3.47}Tyr_{0.63}Lys_{2.19}Met_{0.68}Glu_{1.14}His_{1.00}Phe_{1.00}Arg_{0.88}Gly_{0.99}Ser_{0.96}Asp_{1.05}$ (average recovery 93%). *Anal.* Calcd. for $C_{140}H_{167}O_{31}N_{23}S_2 \cdot 2H_2O$: C, 60.74; H, 6.23; N, 11.64. Found: C, 60.76; H, 6.09; N, 11.53.

Z(OMe)-Asp(OBzl)-Glu(OBzl)-Gly-Pro-Tyr-Lys(Z)-Met-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-Ser-Pro-Pro-Lys(Z)-Asp(OBzl)$_2$——The above protected pentadecapeptide ester (1.15 g), immersed well with anisole (2.3 ml) was treated with TFA (1.6 ml) containing mercaptoethanol (0.01 ml) at 0° for 60 min. The product was precipitated as fine powder by addition of dry ether, dried over KOH pellets *in vacuo* and then dissolved in DMF (1.5 ml). To this solution, Et_3N (0.12 ml), Z(OMe)–Asp(OBzl)–Glu(OBzl)–Gly–OPCP (prepared from 0.33 g of Z(OMe)-tripeptide with 0.07 ml of Et_3N and 0.21 g of TCA–OPCP) in DMF (1.5 ml) were combined and the mixture was stirred at room temperature for 12 hr. The solvent was evaporated *in vacuo* and the residue was applied to a column of silica (5.6×2.5 cm), which was eluted with $CHCl_3$–MeOH–H_2O (120: 10: 1). The fractions containing the substance of Rf_1 0.70 were combined and the solvent was evaporated. The residue was triturated with H_2O and then recrystallized from $CHCl_3$ and MeOH; yield 0.75 g (55%), mp 207—208°, $[\alpha]_D^{23}$ −38.6° (c=0.4, DMF). Rf_1 0.70, Rf_2 0.63, Rf_4 0.17. Amino acid ratios in HCl and *p*-toluenesulphonic acid (number in blacket) hydrolysates: $Asp_{2.16(2.15)}Glu_{2.33(2.20)}Gly_{2.00)2.00)}Pro_{3.44(3.20)}Tyr_{0.49(1.02)}Lys_{2.26(2.22)}Met_{0.60(0.83)}His_{1.00(0.91)}Phe_{1.00(1.00)}Arg_{0.93(trace)}Arg(Tos)_{trace(1.00)}Trp_{trace(not\ det.)}Ser_{0.97(0.99)}$ [average recovery 93% (89%)]. *Anal.* Calcd. for $C_{165}H_{194}O_{38}N_{26}S_2 \cdot 4H_2O$: C, 60.31; H, 6.20; N, 11.09. Found: C, 60.33; H, 6.09; N, 11.18.

H-Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH——The above protected octadecapeptide ester (0.64 g) was immersed well with anisole (2.2 ml) and then treated with HF (approximately 6 ml) in the presence of skatole (0.52 g) and dithiothreitol (0.62 g) at 0° for 60 min. The excess HF was evaporated *in vacuo* and the residue was dissolved in H_2O (5 ml), which after washing with ether, was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min. The resin was removed by filtration and the filtrate was lyophilized to give fine powder (0.77 g). This was then applied to a column of Sephadex G-10 (2.7×69 cm), which was eluted with 3% AcOH and the absorbancy at 277 mμ was determined in each fraction (3 ml each). The fractions of the front peak (tube No. 46—66) were combined and the solvent was removed by lyophilization to give fluffy powder (0.47 g). For further purification, this (in 5 ml of H_2O) was then applied to a column of CM-Sephadex C-25 (H form) (3.4×3.0 cm), which was eluted with H_2O (160 ml) and 0.02M, pH 6.9 ammonium acetate buffer (900 ml). Gradient elution was then established with 0.06M, pH 6.9 ammonium acetate buffer (1400 ml) through a mixing flask containing the above 0.02M buffer (150 ml). Individual fractions (20 ml each) were collected and absorbancy at 277 mμ was determined. Two peaks were detected: the minor one in the 0.02M eluates (tube No. 31—36) and the other large peak (tube No. 87—115) in the gradient eluates. The fractions corresponding to the main large peak were combined and this solution was applied to a column of Amberlite XAD-II (2.4×6.5 cm), which after washing with H_2O (300 ml), was eluted with 70% MeOH. Each fraction was examined in the same manner as described above. The eluates containing a single component (Rf_5 0.32, tube No. 19—70) were combined, the solvent was evaporated and the residue was lyophilized to give white fluffy powder; yield 0.22 g (44%), $[\alpha]_D^{23}$ −74.1° (c=0.5, 10% AcOH). Amino acid ratios in HCl and *p*-toluenesulphonic acid (number in blacket) hydrolysates: $Asp_{2.05(2.25)}Glu_{2.14(2.30)}Gly_{1.93(2.14)}Pro_{3.07(3.29)}Tyr_{0.99(1.03)}Lys_{2.10(2.15)}Met_{0.94(1.00)}His_{0.94(0.91)}Phe_{1.00(1.00)}Arg_{1.00(0.97)}Trp_{trace(0.74)}Ser_{0.91(1.03)}$ [average recovery 95% (99%)]. Amino acid ratios in AP–M digest: $Asp_{1.93}Glu_{2.22}Gly_{1.81}Pro_{3.16}Tyr_{1.10}Lys_{2.16}Met_{0.72}His_{1.13}Phe_{1.06}Arg_{1.00}Trp_{0.76}Ser_{0.80}$ (average recovery 95%). *Anal.* Calcd. for $C_{106}H_{154}O_{37}N_{26}S \cdot 3CH_3COOH \cdot 4H_2O$: C, 51.43; H, 6.56; N, 15.00. Found: C, 51.70; H, 6.51; N, 14.65.

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