Synthesis of the Nonatriacontapeptide corresponding to the Entire Amino Acid Sequence of Dogfish Adrenocorticotropic Hormone (*Squalus acantias*)[1]

Kenji Okamoto, Koichi Yasumura, Shinichi Shimamura,[2a]
Masuhisa Nakamura, Akira Tanaka,[2b]
and Haruaki Yajima[2c]

*Kyoto College of Pharmacy,[2a] Shionogi Research Laboratory, Shionogi Co. Ltd.[2b]
and Faculty of Pharmaceutical Sciences, Kyoto University[2c]*

The nonatriacontapeptide corresponding to the entire amino acid sequence of dogfish adrenocorticotropic hormone was synthesized by successive condensations of three peptide fragments; Z(OMe)-(15—19)-OH, Z(OMe)-(11—14)-NHNH$_2$ and Z-(1—10)-OH, with H-(20—39)-OBzl, a synthetic intermediate of dogfish corticotropin-like intermediate lobe peptide. The synthetic peptide exhibited the *in vivo* steroidogenetic activity of 21.3 IU/mg.

Keywords——dogfish adrenocorticotropic hormone; deprotection by hydrogen fluoride; 4 N methanesulfonic acid hydrolysis of Trp-peptides; column chromatographic purification of protected peptides; 4-morpholinyl active ester; *in vivo* steroidogenetic activity

In 1974, Lowry *et al.*[3] isolated a new adrenocorticotropic hormone (ACTH) from the pars distalis of pituitary of dogfish, *squalus acantias*, and determined its primary structure (I). The number of amino acid residue of this hormone is identical with that of the known mammalian ACTHs.[4] However, on comparison with the amino acid composition of human ACTH, eleven amino acid differences are seen, nine of which are in the 20—39 region of the dogfish ACTH molecule. Thus, the amino acid sequence of N-terminal nonadecapeptide (1—19 region) of this hormone is identical with that of mammalian ACTHs, except at two positions; 13 and 15.

In this paper, we wish to describe the synthesis of the nonatriacontapeptide corresponding to the entire amino acid sequence of the dogfish ACTH (I). For this synthesis, eicosapeptide ester (II), a synthetic intermediate of the dogfish corticotropic-like intermediate lobe peptide (CLIP),[5] served as an amino component, with which three fragments, III (position 15—19), IV (position 11—14) and V (position 1—10) were successively condensed as shown in Fig. 1.

1) The amino acids (except glycine) employed in this paper are of the L-configuration. Abbreviation used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochemistry*, 5, 2485 (1966); *ibid.*, 6, 362 (1967); *ibid.*, 11, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, MBS=*p*-methoxybenzenesulfonyl, Tos=*p*-toluenesulfonyl, OBzl=benzyl ester, OMor=4-morpholinyl ester, ONP=*p*-nitrophenyl ester, DCC=dicyclohexylcarbodiimide, DMF=dimethylformamide, THF=tetrahydrofuran, TFA=trifluoroacetic acid, OQCl=5-chloro-8-quinolyl ester.
2) Location: *a*) *Yamashina-ku, Kyoto, 607, Japan*; *b*) *Fukushima-ku, Osaka, 553, Japan*; *c*) *Sakyo-ku, Kyoto, 606, Japan.*
3) P.J. Lowry, H.P.J. Bennett, C. McMartin, and A.P. Scott, *Biochem. J.*, 141, 427 (1974).
4) L. Graf, S. Bajusz, A. Patthy, E. Barat, and G. Cseh, *Acta Biochim. Biophys.*, 6, 415 (1971); B. Riniker, P. Sieber, W. Rittel, and H. Zuber, *Nature New Biol.*, 235, 114 (1972); C.H. Li, *Biochem. Biophys. Res. Commun.*, 49, 835 (1972).
5) K. Okamoto, S. Shimamura, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), 26, 1698 (1978).

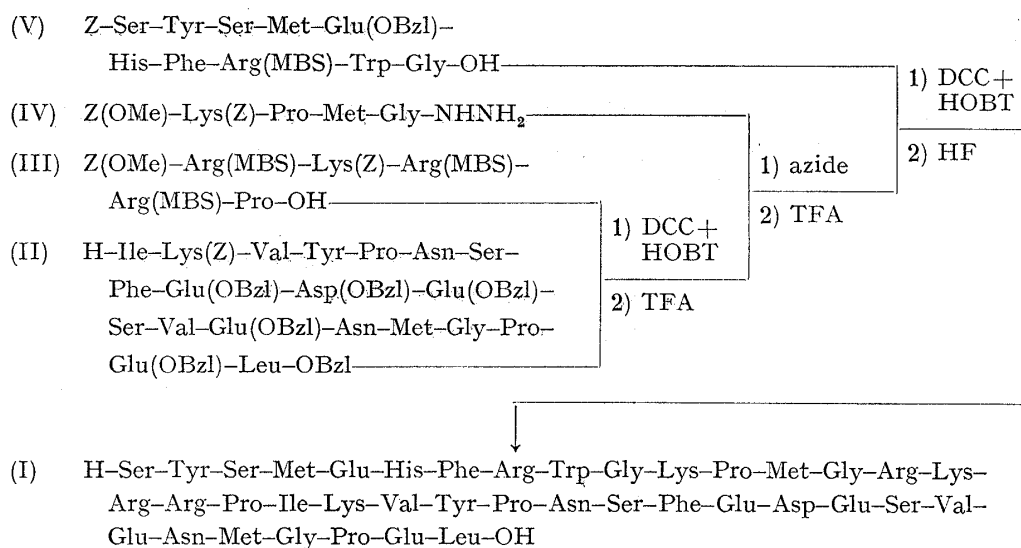(V)   Z–Ser–Tyr–Ser–Met–Glu(OBzl)–
      His–Phe–Arg(MBS)–Trp–Gly–OH

(IV) Z(OMe)–Lys(Z)–Pro–Met–Gly–NHNH₂

(III) Z(OMe)–Arg(MBS)–Lys(Z)–Arg(MBS)–
      Arg(MBS)–Pro–OH

(II) H–Ile–Lys(Z)–Val–Tyr–Pro–Asn–Ser–
      Phe–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–
      Ser–Val–Glu(OBzl)–Asn–Met–Gly–Pro–
      Glu(OBzl)–Leu–OBzl

1) DCC+ HOBT   2) TFA

1) azide   2) TFA

1) DCC+ HOBT   2) HF

(I)   H–Ser–Tyr–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–Gly–Arg–Lys–
      Arg–Arg–Pro–Ile–Lys–Val–Tyr–Pro–Asn–Ser–Phe–Glu–Asp–Glu–Ser–Val–
      Glu–Asn–Met–Gly–Pro–Glu–Leu–OH

Fig. 1.   Synthetic Route to the Dogfish ACTH (I)

The α-amino function of intermediates was protected by the TFA-labile Z(OMe) group[6] and the side chain protecting groups adopted to the synthesis of dogfish CLIP[5] were also employed in our present synthesis; i.e., Arg(MBS),[7] Asp(OBzl), Glu(OBzl), and Lys(Z).

First, N-terminal protected decapeptide (V) was prepared by azide coupling[8] of Z–Ser–Tyr–Ser–Met–NHNH₂ (VI)[9] with the TFA treated hexapeptide of Z(OMe)–Glu(OBzl)–His–Phe–Arg(MBS)–Trp–Gly–OH (VII) as shown in Fig. 2.   In order to prepare the necessary
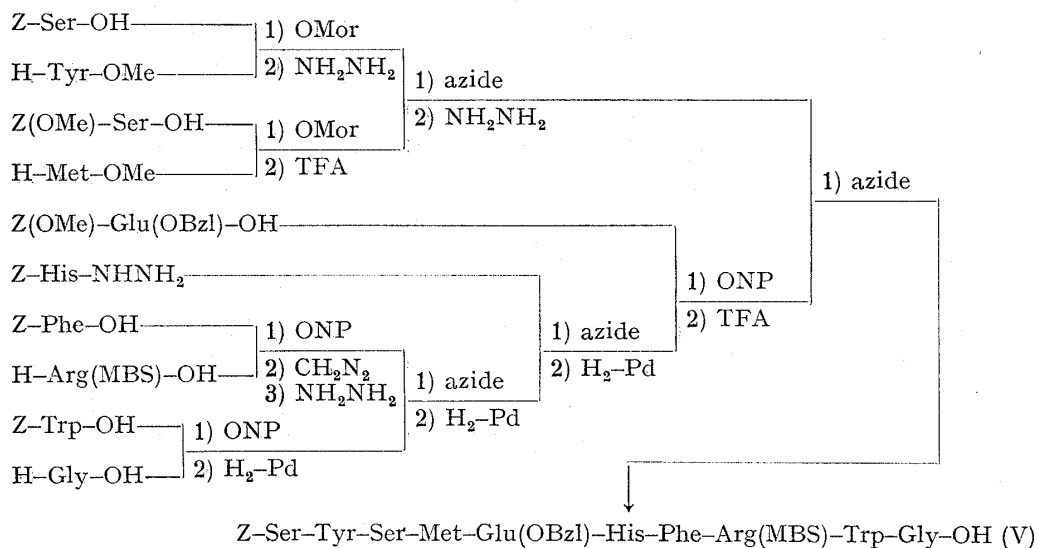
Z–Ser–OH   1) OMor
H–Tyr–OMe   2) NH₂NH₂   1) azide
                                2) NH₂NH₂
Z(OMe)–Ser–OH   1) OMor
H–Met–OMe   2) TFA   1) azide

Z(OMe)–Glu(OBzl)–OH

Z–His–NHNH₂

Z–Phe–OH   1) ONP
H–Arg(MBS)–OH   2) CH₂N₂
                  3) NH₂NH₂   1) azide   1) ONP
                               2) H₂–Pd   2) TFA
                                             1) azide
                                             2) H₂–Pd
Z–Trp–OH   1) ONP
H–Gly–OH   2) H₂–Pd

Z–Ser–Tyr–Ser–Met–Glu(OBzl)–His–Phe–Arg(MBS)–Trp–Gly–OH (V)

Fig. 2.   Synthetic Route to the Z–(1–10)–OH (V)

fragment VI, Z–Ser–Tyr–NHNH₂[10] was condensed with H–Ser–Met–OMe by the azide procedure[8] and the resulting protected tetrapeptide was converted to the corresponding hydrazide (VI) in the usual manner.   In this synthesis, the two intermediates, Z–Ser–

6) F. Weygand and K. Hunger, Chem. Ber., 95, 1 (1962).
7) O. Nishimura and M. Fujino, Chem. Pharm. Bull. (Tokyo), 24, 1568 (1976).
8) J. Honzl and J. Rudinger, Coll. Czech. Chem, Commun., 26, 2333 (1961).
9) C.H. Li, J. Meienhofer, E. Schnabel, D. Chung, T.B. Lo, and J. Ramachandran, J. Am. Chem. Soc., 83, 4449 (1961).
10) a) M. Fujino, O. Nishimura, and C. Hatanaka, Chem. Pharm. Bull. (Tokyo), 17, 2135 (1969);   b) K. Hofmann, A. Jöhl, A.E. Furlenmeier, and H. Kappeler, J. Am. Chem. Soc., 79, 1636 (1957).

Tyr–OMe[11] and Z(OMe)–Ser–Met–OMe, were prepared by our newly introduced 4-morpholinyl ester method[12] in good yields. Since, without protection of the hydroxyl function, Ser can be incorporated into the peptide chain by this method. VII was synthesized in essentially the same manner as those described in the synthesis of the fragment of porcine β-melanocyte-stimulating hormone by Yajima *et al.*,[13] except that Arg(MBS),[7] instead of Arg(Tos), was employed. For the α-deprotection, the TFA treatment of VII was performed in the presence of anisole containing 2% ethanedithiol, in order to suppress the side reactions on Trp residue.[14] The purity of N-terminal protected decapeptide was assessed by thin layer chromatography (TLC), elemental analysis and acid hydrolysis with 4N methanesulfonic acid (MSA).[15] As shown in Fig. 3, protected tetrapeptide hydrazide, Z(OMe)–Lys(Z)–Pro–Met–Gly–NHNH$_2$ (IV), was prepared by DCC plus N-hydroxybenzotriazole (HOBT) coupling[16] of Z(OMe)–Lys(Z)–Pro–OH[17] with the TFA treated sample of Z(OMe)–Met–Gly–OMe followed by treatment of the resulting protected tetrapeptide ester with hydrazine hydrate. The above dipeptide ester was prepared by the 4-morpholinyl ester method in excellent yield. Homogeneity of IV was assessed by elemental analysis and TLC.
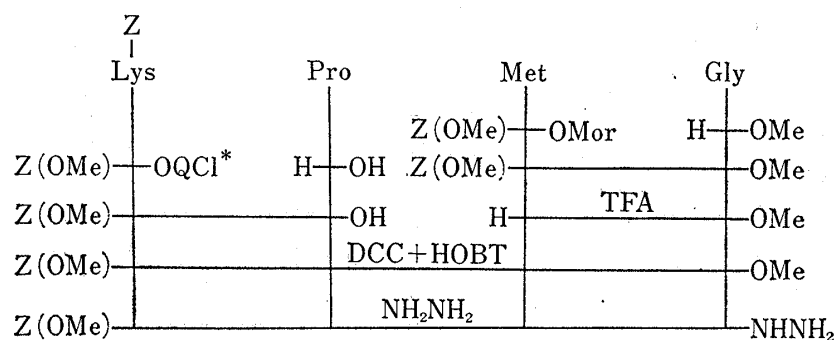

Fig. 3.   Synthetic Route to the Z(OMe)–(11–14)–NHNH$_2$ (IV)

*H. Yajima, H. Ogawa, H. Watanabe, N. Fujii, M. Kurobe, and S. Miyamoto,
Chem. Pharm. Bull. (Tokyo), **23**, 371 (1975).

The protected pentapeptide, Z(OMe)–Arg(MBS)–Lys(Z)–Arg(MBS)–Arg(MBS)–Pro–OH (III) was prepared by azide coupling of Z(OMe)–Arg(MBS)–Lys(Z)–NHNH$_2$ with the hydrogenated sample of Z–Arg(MBS)–Arg(MBS)–Pro–OBzl (VIII) as shown in Fig. 4. The former
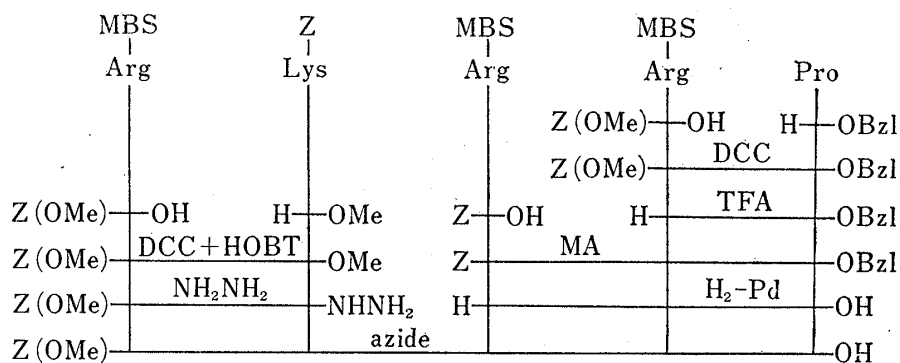

Fig. 4.   Synthetic Route to the Z(OMe)–(15–19)–OH (III)

11) *a*) R.F. Fisher and R.R. Whetstone, *J. Am. Chem. Soc.*, **76**, 5076 (1954);   *b*) S. Guttmann and R.A. Boissonnas, *Helv. Chim. Acta*, **41**, 1852 (1958).
12) K. Okamoto and S. Shimamura, *Yakugaku Zasshi*, **93**, 333 (1973).
13) H. Watanabe, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 375 (1975).
14) J.J. Sharp, A.B. Robinson, and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973).
15) R.J. Simpson, M.R. Neuberger, and T.Y. Liu, *J. Biol. Chem.*, **251**, 1936 (1976).
16) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
17) H. Yajima, K. Kitagawa, and T. Segawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 2500 (1973).

hydrazide was prepared by DCC plus HOBT coupling of Z(OMe)–Arg(MBS)–OH and H–Lys-(Z)–OMe followed by treatment of the resulting protected dipeptide ester with hydrazine hydrate in the usual manner. Protected tripeptide ester (VIII) was prepared as follows. Z(OMe)–Arg(MBS)–OH was condensed with H–Pro–OBzl by the DCC procedure[18] to give the corresponding Z(OMe)–Arg(MBS)–Pro–OBzl (IX), which after treatment with TFA, was further condensed with Z–Arg(MBS)–OH by mixed anhydride method[19] to give VIII. Homogeneity of the protected pentapeptide (III) was confirmed by elemental analysis, TLC and amino acid analysis.

Assembling of four subunits obtained as mentioned above was then performed according to the scheme illustrated in Fig. 1. The protected eicosapeptide ester, Z(OMe)–Ile–Lys(Z)–Val–Tyr–Pro–Asn–Ser–Phe–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Val–Glu(OBzl)–Asn–Met–Gly–Pro–Glu(OBzl)–Leu–OBzl,[5] was treated with TFA in the presence of anisole and the resulting trifluoroacetate was converted to the corresponding hydrochloride, which after neutralization with Et_3N, was condensed with III by the DCC plus HOBT procedure. The protected pentacosapeptide ester thus obtained, Z(OMe)–Arg(MBS)–Lys(Z)–Arg(MBS)–Arg-(MBS)–Pro–Ile–Lys(Z)–Val–Tyr–Pro–Asn–Ser–Phe–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Val–Glu(OBzl)–Asn–Met–Gly–Pro–Glu(OBzl)–Leu–OBzl (X), was purified by column chromatography on silica using solvent system of $CHCl_3$–MeOH–H_2O (8:3:1). This solvent system was effectively employed also for purifications of other protected intermediates. X was treated with TFA as mentioned above and the resulting pentacosapeptide ester, after neutralization with Et_3N, was condensed with IV by the azide procedure to give the corresponding Z(OMe)–Lys(Z)–Pro–Met–Gly–Arg(MBS)–Lys(Z)–Arg(MBS)–Arg(MBS)–Pro–Ile–Lys(Z)–Val–Tyr–Pro–Asn–Ser–Phe–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Val–Glu(OBzl)–Asn–Met–Gly–Pro–Glu(OBzl)–Leu–OBzl(XI). Its purification was achieved by column chromatography on silica and the homogeneity was also examined by three criteria as mentioned above.

Finally, XI, after treatment with TFA, conversion to the corresponding hydrochloride and subsequent neutralization with Et_3N, was condensed with V by the DCC plus HOBT procedure to give the corresponding Z–Ser–Tyr–Ser–Met–Glu(OBzl)–His–Phe–Arg(MBS)–Trp–Gly–Lys(Z)–Pro–Met–Gly–Arg(MBS)–Lys(Z)–Arg(MBS)–Arg(MBS)–Pro–Ile–Lys(Z)–Val–Tyr–Pro–Asn–Ser–Phe–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Val–Glu(OBzl)–Asn–Met–Gly–Pro–Glu(OBzl)–Leu–OBzl (XII). Rink and Riniker[20] pointed out that DCC has a tendency to mask the N^{im}-function of His residue during the DCC plus HOBT condensation reaction, the crude product (XII) was therefore heated in a mixture of DMF and methanol containing acetic acid at 65° for 8 hr to remove the masking group of the His residue and then purified by column chromatography on silica as stated above. Homogeneity of XII thus isolated was examined by three criteria; TLC, elemental analysis and acid hydrolysis with 4 N MSA.

According to the method developed by Sakakibara et al.,[21] all of the protecting groups, i.e. Z, MBS, and Bzl were deblocked from XII by hydrogen fluoride in the presence of anisole containing 2% ethanedithiol. The product, after conversion to the corresponding acetate by Amberlite IR-45 (acetate form), was incubated in methanol containing 2 N acetic acid at 60° for 14 hr to secure the removal of the dicyclohexylamidino moiety from the N^{im} of His residue. Subsequently, the product was incubated with dithiothreitol at 50° for 24 hr to reduce Met-sulfoxide formed possibly during the above deblocking process.[22] The crude product thus obtained was passed through a column on Sephadex G-25 and then purified by column chromatography on CM-Sephadex. After the column was eluted with water, gradient elution

18) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
19) J.R. Vaughan, Jr. and R.L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
20) H. Rink and B. Riniker, *Helv. Chim. Acta*, **57**, 831 (1974).
21) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
22) D. Yamashiro and C.H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).

was established using 0.3 M ammonium acetate at pH 6.9. The gradient elutes were examined by measurement of the absorbancy at 280 nm. Ammonium acetate was mostly removed from desired fractions by Sephadex G-25 and finally by repeated lyophilization. The fluffy powder thus obtained exhibited a single spot on TLC in two different solvent systems. The hydrolysate with 4 N MSA contained the constituent amino acids in ratios predicted by theory. Also this Pro-containing nonatriacontapeptide was completely digested by aminopeptidase M (AP-M).[23] In amino acid analysis of the enzymatic hydrolysate, the Asn peak overlapped with that of Ser. From difference of the Asp recovery between acid and enzymatic hydrolysates and that of Ser recovery, the presence of the Asn residue could be confirmed.

The *in vivo* steroidogenetic activity of our synthetic dogfish ACTH was judged as 21.3 IU/mg, when synthetic human ACTH (98.6 IU/mg)[24] was taken as a standard. It has been reported by Lowry *et al.*[3] that natural dogfish ACTH has about 15% of the activity of synthetic human ACTH.

Experimental

The reactions of Met-containing peptides were performed in N_2 atmosphere. The melting points are uncorrected. Rotations are determined in a Union Automatic Polarimeter Model P-101 (cell length: 1 cm). The amino acid composition of the acid and enzymatic hydrolysates were determined with a Hitachi Amino Acid Analyzer, Model KLA-5 according to the method of Moor *et al.*[25] Solvents were evaporated *in vacuo* at a bath temperature of 40 to 50° in a rotary evaporator. Thin-layer chromatography was performed on silica gel (kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 CHCl₃–MeOH (29: 1), Rf_2 CHCl₃–MeOH (9: 1), Rf_3 CHCl₃–MeOH–H₂O (90: 15: 5), Rf_4 CHCl₃–MeOH–H₂O (8: 3: 1), Rf_5 CHCl₃–MeOH–AcOH (9: 1: 0.5), Rf_6 CHCl₃–MeOH–AcOH (9: 1: 1), Rf_7 CHCl₃–MeOH–pyridine–H₂O (8: 3: 0.05: 1), Rf_8 *n*-BuOH–AcOH–pyridine–H₂O (15: 3: 10: 12) and Rf_9 *n*-BuOH–AcOH–pyridine–H₂O (4: 1: 1: 2).

1) Z(OMe)-Amino Acid 4-Morpholinyl Esters——The title compounds were prepared by DCC coupling of Z(OMe)-amino acid with 4-hydroxymorpholine according to the previous paper.[12]

i) Z(OMe)–Met–OMor: Yield 68.0%, mp 69—71°. $[\alpha]_D^{15}$ −2.0° (*c*=3.9, CHCl₃). Rf_1 0.51. *Anal.* Calcd. for C₁₈H₂₆N₂O₆S: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.24; H, 6.48; N, 7.08.

ii) Z(OMe)–Ser–OMor: Yield 65.0%, mp 113—115°. $[\alpha]_D^{22}$ −5.3° (*c*=1.5, CHCl₃). Rf_2 0.36. *Anal.* Calcd. for C₁₆H₂₂N₂O₇: C, 54.23; H, 6.26; N, 7.91. Found: C, 53.99; H, 6.23; N, 7.87.

2) Z-Ser-Tyr-OMe——Z-Ser-OMor (1.30 g) was added to a suspension of H–Tyr–OMe·HCl (1.40 g) and sodium acetate (0.5 g) in DMF (10 ml). The reaction mixture was stirred at room temperature for 72 hr. After filtration, the filtrate was evaporated *in vacuo* to dryness. The residue was extracted with AcOEt. The AcOEt layer was washed successively with 10% citric acid, 5% NaHCO₃ and H₂O–NaCl, dried over Na₂SO₄ and then evaporated *in vacuo*. The residue was recrystallized from AcOEt and petroleum ether; yield 1.05 g (62.9%), mp 111.5—113°, $[\alpha]_D^{15}$ +15.1° (*c*=1.9, DMF). (Lit.[11]) mp 112—113°, $[\alpha]_D^{21}$ +13.5° (*c*= 2.0, DMF). Rf_2 0.38. *Anal.* Calcd. for C₂₁H₂₄N₂O₇: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.32; H, 5.88; N, 6.93.

3) Z(OMe)-Ser-Met-OMe——Z(OMe)–Ser–OMor (1.42 g) was added to a suspension of H–Met–OMe·HCl (1.20 g) and sodium acetate (0.50 g) in DMF (10 ml). The mixture was stirred at room temperature for 72 hr. The title compound was isolated from the reaction mixture as stated above (Exp. 2); yield 1.20 g (72.3%). mp 93.5—95°. $[\alpha]_D^{25}$ −18.1° (*c*=1.6, MeOH), Rf_2 0.28. *Anal.* Calcd. for C₁₈H₂₆N₂O₇S: C, 52.16; H, 6.32; N, 6.76. Found: C, 52.32; H, 6.54; N, 6.87.

4) Z-Ser-Tyr-Ser-Met-NHNH₂——Z-Ser-Tyr-Ser-Met-OMe was prepared as described by Hofmann *et al.*[10b] using Z(OMe)–Ser–Met–OMe prepared above; yield 56.3%, mp 189.5—192°, $[\alpha]_D^{25}$ −30.0° (*c*=1.0, MeOH). Rf_5 0.45. (Lit.[10b] mp 190° (dec.). $[\alpha]_D^{27}$ −39.0° (*c*=0.7, MeOH)). *Anal.* Calcd. for C₂₉H₃₈N₄O₁₀S: C, 54.88; H, 6.03; N, 8.83. Found: C, 55.13; H, 6.26; N, 8.70. The tetrapeptide ester was converted to the title compound according to Li *et al.*,[9] yield 55.0%. mp 244—245°. $[\alpha]_D^{25}$ −12.4° (*c*=1.1, DMF). (Lit.[9] mp 245—246° (dec.), $[\alpha]_D^{24}$ −12.0° (*c*=0.5, DMF). Rf_4 0.40. *Anal.* Calcd. for C₂₈H₃₈N₆O₉S: C, 52.99; H, 6.03; N, 13.24. Found: C, 53.27; H, 6.24; N, 13.48.

5) Z-Phe-Arg(MBS)-OH——To a solution of H–Arg(MBS)–OH (6.80 g) in DMF (40 ml) containing Et₃N (2.80 ml), Z–Phe–ONP (8.40 g) and HOBT (5 mg) were added. After the mixture was stirred at room temperature for 72 hr, the solvent was evaporated *in vacuo*. The residue was dissolved in 5% NaHCO₃ and washed with AcOEt. The aqueous phase was acidified with 1 N HCl and the resulting precipitate was ex-

23) G. Pfleiderer and P.G. Celliers, *Biochem. Z.*, **339**, 186 (1963).
24) K. Inouye, K. Watanabe, and H. Otsuka, *Bull. Chem. Soc. Japan*, **50**, 211 (1977).
25) S. Moor, D.H. Spackman, and W.H. Stain, *Anal. Chem.*, **30**, 1185 (1958).

tracted with AcOEt. The AcOEt layer was washed with 1 N HCl and H_2O–NaCl, dried over Na_2SO_4 and then evaporated *in vacuo*. The residue was purified by column chromatography on silica using solvent system of $CHCl_3$–MeOH–H_2O (8 : 3 : 1); yield 10.1 g (80.8%), amorphous powder, $[\alpha]_D^{29}$ $-6.5°$ ($c=4.6$, MeOH). Rf_5 0.47. *Anal*. Calcd. for $C_{30}H_{35}N_5O_8S \cdot 3/2H_2O$: C, 55.20; H, 5.87; N, 10.73. Found: C, 55.54; H, 5.52; N, 10.62.

6) **Z-Phe-Arg(MBS)-OMe**——An ethereal solution of diazomethane was excessively added to a solution of Z–Phe–Arg(MBS)–OH (8.0 g) in MeOH (150 ml). The yellow color persisted for 60 min and a few drops of AcOH were added. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica using $CHCl_3$; yield 6.0 g (73.3%), amorphous powder, $[\alpha]_D^{29}$ $-6.5°$ ($c=2.8$, MeOH). Rf_2 0.25. *Anal*. Calcd. for $C_{31}H_{37}N_5O_8S$: C, 58.20; H, 5.83; N, 10.95. Found: C, 58.36; H, 6.03; N, 10.67.

7) **Z-Phe-Arg(MBS)-NHNH$_2$**——To a solution of Z–Phe–Arg(MBS)–OMe (6.0 g) in MeOH (20 ml), 80% hydrazine hydrate (2.2 ml) was added. The mixture was kept on standing at room temperature for 48 hr. H_2O was added to the reaction mixture and the resulting precipitate was collected by filtration and then reprecipitated from MeOH with H_2O; yield 5.0 g (83.3%), amorphous powder, $[\alpha]_D^{31}$ $-17.1°$ ($c=1.9$, DMF), Rf_5 0.41. *Anal*. Calcd. for $C_{30}H_{37}N_7O_7S$: C, 56.33; H, 5.83; N, 15.33. Found: C, 56.47; H, 5.95; N, 15.19.

8) **Z-Phe-Arg(MBS)-Trp-Gly-OH**——To a solution of Z–Phe–Arg(MBS)–NHNH$_2$ (5.10 g) in DMF (10 ml), 1.25 N HCl/DMF (13 ml) and isoamyl nitrite (1.1 ml) were added under cooling with ice-NaCl and the mixture was stirred until the hydrazine test became negative. The solution, after neutralization with Et_3N (2.21 ml), was combined with a solution of H–Trp–Gly–OH[26] (1.72 g) in 90% aqueous DMF (20 ml) containing Et_3N (1.83 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was washed batchwisely with 10% citric acid and H_2O–NaCl. The crude product was purified by column chromatography on silica using solvent system of $CHCl_3$–MeOH–H_2O (8 : 3 : 1) and further reprecipitated from THF–MeOH (1 : 3 by vol.) with ether; yield 3.30 g (61.9%), amorphous powder, $[\alpha]_D^{33}$ $-11.4°$ ($c=2.9$, DMF). Rf_5 0.12. *Anal*. Calcd. for $C_{43}H_{48}N_8O_{10}S \cdot 3H_2O$: C, 55.96; H, 5.90; N, 12.14. Found: C, 56.19; H, 5.71; N, 12.19.

9) **Z-His-Phe-Arg(MBS)-Trp-Gly-OH**——In the usual manner, Z–Phe–Arg(MBS)–Trp–Gly–OH (1.70 g) in MeOH (30 ml)–THF (20 ml) containing AcOH (1.0 ml) was hydrogenated over a Pd catalyst. After filtration, the filtrate was evaporated *in vacuo*. The residue was dissolved in DMF (12 ml) and Et_3N (0.44 ml) was added. To this ice-chilled solution, Et_3N (0.42 ml) and the azide solution (prepared from Z–His–NHNH$_2$ (0.92 g)/DMF (6 ml) with 0.5 N HCl/DMF (12 ml), isoamyl nitrite (0.4 ml) and Et_3N (0.83 ml)) were added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was washed batchwisely with 2% AcOH and H_2O. The crude product was reprecipitated from MeOH–THF (3 : 1 by vol.) with AcOEt; yield 0.8 g (49.7%), mp 155—157°, $[\alpha]_D^{33}$ $-28.9°$ ($c=1.14$, DMF), Rf_4 0.21. *Anal*. Calcd. for $C_{49}H_{55}N_{11}O_{11}S \cdot H_2O$: C, 57.47; H, 5.61; N, 15.04. Found: C, 57.62; H, 5.69; N, 14.89.

10) **Z(OMe)-Glu(OBzl)-His-Phe-Arg(MBS)-Trp-Gly-OH**——Z–His–Phe–Arg(MBS)–Trp–Gly–OH (0.97 g) in MeOH (40 ml)–THF (20 ml) containing AcOH (0.5 ml) was hydrogenated over a Pd catalyst in the usual manner. After filtration, the filtrate was evaporated *in vacuo*. The residue was dissolved in DMF (20 ml) and Et_3N (0.13 ml) was added. To this solution, Z(OMe)–Glu(OBzl)–ONP (0.60 g), Et_3N (0.26 ml) and HOBT (20 mg) were added and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated *in vacuo*. The residue was washed batchwisely with 2% AcOH and H_2O. The crude product thus obtained was purified by column chromatography on silica using solvent system of $CHCl_3$–MeOH–H_2O (8 : 3 : 1) and further precipitated from MeOH–THF (3 : 1 by vol.) with ether; yield 0.47 g (41.6%), amorphous powder, $[\alpha]_D^{29}$ $-11.8°$ ($c=0.7$, DMF). Rf_4 0.24. Amino acid ratios in an acid (4 N MSA) hydrolysate: $Glu_{1.12}His_{1.03}Phe_{1.01}Arg_{0.94}Trp_{0.64}Gly_{1.00}$ (average recovery 94.4%). *Anal*. Calcd. for $C_{62}H_{70}N_{12}O_{15}S \cdot 3H_2O$: C, 56.87; H, 5.85; N, 12.84. Found: C, 57.01; H, 5.57; N, 13.22.

11) **Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg(MBS)-Trp-Gly-OH, III, Z-(1—10)-OH**——Z(OMe)–Glu-(OBzl)–His–Phe–Arg(MBS)–Trp–Gly–OH (0.35 g) was treated with TFA (1.5 ml) in the presence of anisole (0.2 ml) containing 2% ethanedithiol at 0° for 60 min and dry ether was added. The resulting precipitate was collected by filtration and dried over KOH pellets *in vacuo*. The powder was dissolved in DMF (5 ml) and Et_3N (0.078 ml) was added. To this ice-chilled solution, Et_3N (0.039 ml) and the azide solution (prepared from Z–Ser–Tyr–Ser–Met–NHNH$_2$ (0.26 g)/DMF (5 ml) with 0.5 N HCl/DMF (1.67 ml), isoamyl nitrite (0.056 ml) and Et_3N (0.116 ml)) were added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was washed batchwisely with 2% AcOH and H_2O. The crude product thus obtained was purified by column chromatography on silica using solvent system of $CHCl_3$–MeOH–H_2O (8 : 3 : 1) and further reprecipitated from DMF with AcOEt; yield 291 mg (61.8%), mp 186—190°, $[\alpha]_D^{21}$ $-21.3°$ ($c=0.8$, DMF), Rf_7 0.20. Amino acid ratios in an acid (4 N MSA) hydrolysate: $Ser_{1.70}Tyr_{0.90}Met_{0.81}Glu_{1.13}His_{1.04}Phe_{0.99}Arg_{0.91}Trp_{0.76}Gly_{1.00}$ (average recovery 89.5%). *Anal*. Calcd. for $C_{81}H_{96}N_{16}O_{21}S_2 \cdot 2H_2O$: C, 56.24; H, 5.83; N, 12.96. Found: C, 55.93; H, 5.98; N, 12.79.

12) **Z(OMe)-Met-Gly-OMe[5]**——Z(OMe)–Met–OMor (1.40 g) was added to a suspension of H–Gly–OMe·HCl (0.94 g) and sodium acetate (1.02 g) in dioxane (10 ml). The mixture was stirred at room temperature

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

for 72 hr. The title compound was isolated from the reaction mixture as stated above (Exp. 2); yield 1.14 g (84.3%), mp 94—95.5°, $[\alpha]_D^{15}$ −5.0° (c=1.4, CHCl₃). This compound was identical with authentic sample[5] by mixed melting point determination and TLC.

13) **Z(OMe)-Lys(Z)-Pro-Met-Gly-OMe**——Z(OMe)–Met–Gly–OMe (3.85 g) was treated with TFA (7 ml) in the presence of anisole (1.9 ml) at 0° for 40 min. The excess TFA was evaporated at 0°. The resulting oily product was washed with n-hexane and then dried over KOH pellets *in vacuo*. The oily product was dissolved in THF (30 ml) and the solution was neutralized with Et₃N (1.4 ml). To this ice-chilled solution, a solution of Z(OMe)–Lys(Z)–Pro–OH (5.40 g) in THF (20 ml), HOBT (1.45 g) and DCC (2.06 g) were added and the mixture was stirred at room temperature for 18 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue was recrystallized from AcOEt and petroleum ether; yield 3.0 g (40.5%), mp 116.5—121°, $[\alpha]_D^{20}$ −55.9° (c=1.0, CHCl₃). Rf_3 0.63. *Anal.* Calcd. for C₃₆H₄₉N₅O₁₀S: C, 58.13; H, 6.68; N, 9.41. Found: C, 58.37; H, 6.87; N, 9.52.

14) **Z(OMe)-Lys(Z)-Pro-Met-Gly-NHNH₂**——To a solution of Z(OMe)–Lys(Z)–Pro–Met–Gly–OMe (4.0 g) in MeOH (40 ml), 80% hydrazine hydrate (3.2 ml) was added. The mixture was kept on standing at room temperature for 48 hr and H₂O was added. The resulting precipitate was collected by filtration. The crude product was reprecipitated from dioxane with H₂O; yield 3.0 g (75.0%). mp 90.5—94°, $[\alpha]_D^{33}$ −39.3° (c=1.6, DMF), Rf_4 0.56. *Anal.* Calcd. for C₃₅H₄₉N₇O₉S: C, 56.51; H, 6.64; N, 13.18. Found: C, 56.43; H, 6.88; N, 13.32.

15) **Z(OMe)-Arg(MBS)-Pro-OBzl**——A solution of Z(OMe)–Arg(MBS)–OH (4.80 g) in THF (40 ml) and DCC (1.94 g) were added to a solution of H–Pro–OBzl (prepared from the hydrochloride (2.30 g) and Et₃N (1.3 ml) in DMF (5 ml)) under cooling with ice. The mixture was stirred at room temperature for 18 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue was dissolved in AcOEt and the AcOEt layer was washed successively with 10% citric acid, 5% NaHCO₃ and H₂O–NaCl, dried over Na₂SO₄ and evaporated *in vacuo*. The residue was purified by column chromatography on silica using solvent system of CHCl₃–MeOH (100: 1); yield 4.10 g (63.0%), amorphous powder, $[\alpha]_D^{13}$ −34.2° (c=1.6, CHCl₃). Rf_2 0.44. *Anal.* Clacd. for C₃₄H₄₁N₅O₉S: C, 58.69; H, 5.94; N, 10.07. Found: C, 58.49; H, 5.70; N, 10.04.

16) **Z-Arg(MBS)-Arg(MBS)-Pro-OBzl**——Z(OMe)–Arg(MBS)–Pro–OBzl (3.50 g) was treated with TFA (7.0 ml) in the presence of anisole (1.0 ml) at 0° for 60 min and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and then dissolved in DMF (20 ml). The solution was neutralized with Et₃N (0.7 ml). Separately, Et₃N (0.83 ml) and isobutyl chloroformate (0.79 ml) were added to a solution of Z–Arg(MBS)–OH (2.90 g) in DMF (20 ml) at −10°. After stirring for 15 min, the above amino component was added to this solution and further stirred at room temperature for 18 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue was extracted with AcOEt and the AcOEt layer was washed successively with 10% citric acid, 5% NaHCO₃ and H₂O–NaCl, dried over Na₂SO₄ and then evaporated *in vacuo*. The residue was purified by column chromatography on silica using solvent system of CHCl₃–MeOH (50: 1); yield 3.8 g (76.5%), amorphous powder, $[\alpha]_D^{19}$ −44.2° (c=1.9, CHCl₃), Rf_3 0.48. *Anal.* Calcd. for C₄₆H₅₇N₉O₁₂S₂: C, 55.69; H, 5.79; N, 12.71. Found: C, 55.74; H, 5.50; N, 12.87.

17) **Z(OMe)-Arg(MBS)-Lys(Z)-OMe**——Z(OMe)–Arg(MBS)–OH (5.10 g), DCC (2.10 g) and HOBT (1.40 g) were added to a solution of H–Lys(Z)–OMe (prepared from the hydrochloride (3.30 g) and Et₃N (1.39 ml) in THF (50 ml)–DMF (4 ml)) under cooling with ice. The mixture was stirred at room temperature for 18 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue was extracted with AcOEt and the AcOEt layer was washed successively with 10% citric acid, 5% NaHCO₃ and H₂O–NaCl, dried over Na₂SO₄ and then evaporated *in vacuo*. The residue was purified by column chromatography on silica using solvent system of CHCl₃–MeOH (70: 1); yield 5.80 g (74.4%), amorphous powder, $[\alpha]_D^{30}$ −16.8° (c=1.3, CHCl₃), Rf_3 0.55. *Anal.* Calcd. for C₃₇H₄₈N₆O₁₁S: C, 56.62; H, 6.16; N, 10.71. Found: C, 56.53; H, 6.28; N, 10.73.

18) **Z(OMe)-Arg(MBS)-Lys(Z)-NHNH₂**——To a solution of Z(OMe)–Arg(MBS)–Lys(Z)–OMe (3.20 g) in MeOH (20 ml), 80% hydrazine hydrate (1.0 ml) was added. After standing at room temperature for 48 hr, the solvent was evaporated *in vacuo*. The residue was reprecipitated from MeOH with H₂O; yield 1.90 g (59.3%), mp 108—115°. $[\alpha]_D^{29}$ −13.9° (c=1.0, DMF). Rf_4 0.66. *Anal.* Calcd. for C₃₆H₄₈N₈O₁₀S: C, 55.09; H, 6.16; N, 14.28. Found: C, 54.94; H, 6.37; N, 14.41.

19) **Z(OMe)-Arg(MBS)-Lys(Z)-Arg(MBS)-Arg(MBS)-Pro-OH, III**——In the usual manner, Z–Arg(MBS)–Arg(MBS)–Pro–OBzl (1.0 g) in MeOH (20 ml) containing AcOH (5 ml) was hydrogenated over a Pd catalyst. The catalyst was removed by filtration and the filtrate was condensed. The residue was dried over KOH pellets *in vacuo* and dissolved in DMF (5 ml). To this ice-chilled solution, Et₃N (0.42 ml) and the azide solution (prepared from Z(OMe)–Arg(MBS)–Lys(Z)–NHNH₂ (0.87 g)/DMF (5 ml) with 1.25 N HCl/DMF (1.8 ml), isoamyl nitrite (0.16 ml) and Et₃N (0.31 ml)) were added. The mixture was stirred at 4° for 48 hr and the solvent was evaporated *in vacuo*. The residue was extracted with CHCl₃ and the CHCl₃ layer was washed with 10% citric acid and H₂O–NaCl, dried over Na₂SO₄ and then evaporated *in vacuo*. The residue was further purified by column chromatography on silica using solvent system of CHCl₃–MeOH–H₂O (8: 3: 1); yield 0.8 g (52.6%), mp 168—170°. $[\alpha]_D^{29}$ −9.8° (c=2.2, DMF). Rf_6 0.17. Amino acid ratios in an acid hydrolysate; Arg₃.₀₀Lys₀.₉₂Pro₁.₀₉ (average recovery 98.8%). *Anal.* Calcd. for C₆₇H₈₉N₁₅O₂₀S₃·3H₂O; C, 51.10; H, 6.08; N, 13.34. Found: C, 51.00; H, 5.95; N, 13.41.

20) Z(OMe)-Arg(MBS)-Lys(Z)-Arg(MBS)-Arg(MBS)-Pro-Ile-Lys(Z)-Val-Tyr-Pro-Asn-Ser-Phe-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Val-Glu(OBzl)-Asn-Met-Gly-Pro-Glu(OBzl)-Leu-OBzl, Z(OMe)-(d-ACTH 15—39)-OBzl——Z(OMe)-(d-ACTH 20—39)-OBzl[5]) (0.78 g) was treated with TFA (1.5 ml) in the presence of anisole containing 2% ethanedithiol at 0° for 60 min and dry ether was added. The resulting precipitate was collected by filtration and then dissolved in 0.5 N HCl/DMF (1 ml). To this solution, dry ether was added and the resulting eicosapeptide ester hydrochloride was collected by filtration and then dried over KOH pellets *in vacuo*. The powder was dissolved in DMF (5 ml) and Et_3N (0.032 ml) was added. To this ice-chilled solution, Z(OMe)–Arg(MBS)–Lys(Z)–Arg(MBS)–Arg(MBS)–Pro–OH (0.53 g), HOBT (47 mg) and DCC (72 mg) were added and the mixture was stirred at room temperature for 72 hr. The solvent was evaporated *in vacuo* and the residue was washed batchwisely with 10% citric acid, 5% $NaHCO_3$, H_2O and MeOH. The crude product thus obtained was purified by column chromatography on silica using solvent system of $CHCl_3$-MeOH-H_2O (8:3:1); yield 0.77 g (68.6%), mp 223—230°, $[\alpha]_D^{27}$ −30.4° ($c=0.6$, DMF), Rf_6 0.31. Amino acid ratios in an acid hydrolysate: $Asp_{3.06}Ser_{1.89}Glu_{4.16}Pro_{2.77}Gly_{1.00}Val_{1.57}Met_{0.97}Ile_{0.81}Leu_{1.07}Tyr_{0.79}Lys_{2.21}$-$Arg_{3.11}$ (average recovery 87.8%). *Anal.* Calcd. for $C_{218}H_{282}N_{38}O_{57}S_4\cdot2H_2O$: C, 58.04; H, 6.39; N, 11.80. Found: C, 57.95; H, 6.47; N, 11.75.

21) Z(OMe)-Lys(Z)-Pro-Met-Gly-Arg(MBS)-Lys(Z)-Arg(MBS)-Arg(MBS)-Pro-Ile-Lys(Z)-Val-Tyr-Pro-Asn-Ser-Phe-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Val-Glu(OBzl)-Asn-Met-Gly-Pro-Glu(OBzl)-Leu-OBzl, Z-(OMe)-(d-ACTH 10—39)-OBzl——The above Z(OMe)-(d-ACTH 15—39)-OBzl (0.46 g) was treated with TFA (2 ml) in the presence of anisole containing 2% ethanedithiol at 0° for 60 min and dry ether was added. The resulting powder was dried over KOH pellets *in vacuo*. The powder was dissolved in DMF (4 ml) and Et_3N (0.014 ml) was added. To this ice-chilled solution, Et_3N (0.014 ml) and the azide solution (prepared from Z(OMe)–Lys(Z)–Pro–Met–Gly–NHNH$_2$ (0.12 g)/DMF (3 ml) with 0.5 N HCl/DMF (0.61 ml), isoamyl nitrite (0.02 ml) and Et_3N (0.042 ml)) were added and the mixture was stirred at 4° for 72 hr. The solvent was evaporated *in vacuo* and the residue was washed batchwisely with 10% citric acid, 5% $NaHCO_3$, H_2O and MeOH. The crude product thus obtained was purified by column chromatography on silica using $CHCl_3$-MeOH-H_2O (8:3:1); yield 0.41 g (75.9%), mp 222—226°, $[\alpha]_D^{23}$ −33.6° ($c=0.5$, DMF), Rf_7 0.62. Amino acid ratios in an acid hydrolysate: $Asp_{3.28}Ser_{1.99}Glu_{5.06}Pro_{3.69}Gly_{2.00}Val_{2.02}Met_{1.84}Ile_{0.96}Leu_{1.08}Tyr_{0.96}$-$Phe_{1.11}Lys_{2.72}Arg_{2.90}$ (average recovery 97.0%). *Anal.* Calcd. for $C_{244}H_{319}N_{43}O_{63}S_5\cdot5H_2O$: C, 57.32; H, 6.47; N, 11.78. Found: C, 57.07; H, 6.37; N, 11.53.

22) Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg(MBS)-Trp-Gly-Lys(Z)-Pro-Met-Gly-Arg(MBS)-Lys(Z)-Arg(MBS)-Arg(MBS)-Pro-Ile-Lys(Z)-Val-Tyr-Pro-Asn-Ser-Phe-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Val-Glu(OBzl)-Asn-Met-Gly-Pro-Glu(OBzl)-Leu-OBzl, Protected Dogfish ACTH——The above Z(OMe)-(d-ACTH 11—39)-OBzl (179 mg) was treated with TFA (1 ml) in the presence of anisole (0.18 ml) at 0° for 60 min and dry ether was added. The resulting powder was converted to the corresponding hydrochloride as stated above. The resulting nonacosapeptide ester hydrochloride was dissolved in DMF (5 ml) and Et_3N (0.049 ml of 10% DMF solution) was added. To this ice-chilled solution, Z–Ser–Tyr–Ser–Met–Glu(OBzl)–His–Phe–Arg(MBS)–Trp–Gly–OH (87.4 mg), HOBT (7.0 mg) and DCC (11 mg) were added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated *in vacuo*. The residue was washed batchwisely with acid and base as stated above and then dissolved in a mixture of DMF–MeOH–2 N AcOH (5:4:1 by vol., 10 ml). The solution, after heating at 65° for 8 hr, was condensed *in vacuo* and the residue was purified by column chromatography on silica as stated above. The desired product thus isolated was reprecipitated from DMF with MeOH; yield 116 mg (48.7%), mp 220—228°. $[\alpha]_D^{23}$ −19.0° ($c=0.6$, DMF). Rf_7 0.67. Amino acid ratios in an acid (4 N MSA) hydrolysate: $Ser_{3.92}Tyr_{1.81}Met_{2.69}Glu_{5.25}His_{0.70}Phe_{2.12}Arg_{3.51}$-$Trp_{0.45}Gly_{3.00}Lys_{2.60}Pro_{3.43}Ile_{0.88}Val_{2.07}Asp_{3.20}Leu_{1.16}$ (average recovery 89.5%). *Anal.* Calcd. for $C_{316}H_{405}$-$N_{59}O_{80}S_7\cdot8H_2O$: C, 56.83; H, 6.35; N, 12.37. Found: C, 57.09; H, 6.21; N, 12.05.

23) H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met-Gly-Arg-Lys-Arg-Arg-Pro-Ile-Lys-Val-Tyr-Pro-Asn-Ser-Phe-Glu-Asp-Glu-Ser-Val-Glu-Asn-Met-Gly-Pro-Glu-Leu-OH (Dogfish ACTH)——The above protected nonatriacontapeptide ester (85 mg) was treated with HF (approximately 3 ml) in the presence of anisole containing 2% ethanedithiol (0.8 ml) at −5° for 60 min. The excess HF was removed by evaporation and the residue dissolved in water (10 ml), then was treated with Amberlite IR-45 (acetate form, approximately 4 g) for 30 min. The resin was removed by filtration, the filtrate was lyophilized. The residue was dissolved in MeOH–2 N AcOH (4:1 by vol., 20 ml) and heated at 60° for 14 hr. The solvent was removed by evaporation and the residue was dissolved in water (20 ml). The solution, after addition of dithiothreitol (30 mg), was incubated at 50° for 24 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in a small amount of 0.2 N AcOH and then applied to a column of Sephadex G-25 (2.6 × 76 cm), which was eluted with 0.2 N AcOH. Individual fractions (5 ml each) were collected and absorbancy at 280 nm was determined for each fraction. The fractions corresponding to the front peak (tube No. 23—37) were combined and the solvent was removed by lyophilization; yield 41 mg (deblocking step: 68.9%). The product was dissolved in water (30 ml) and the solution was applied to a column of CM-Sephadex (3.0 × 1.5 cm), which was eluted first with H_2O (100 ml) and then with 0.3 M ammonium acetate buffer (pH 6.9), through a mixing flask containing water (150 ml). Individual fractions (4 ml each) were collected and absorbancy at 280 nm was determined. A main peak present in the gradient elutes (tube No. 71—79) were combined and the solvent was condensed to approximately 5 ml. This solution was then applied to a column of Sephadex G-25 (2.8 ×

120 cm), which was eluted with 0.2 N AcOH.   The desired fractions (5 ml each, tube No. 69—80) were collected and the solvent was removed by lyophilization to give a fluffy powder; yield 18.4 mg (overall yield: 27.4%), $[\alpha]_D^{25}$ —88.0° (c=0.13, 0.2 N AcOH), Rf_9 0.17, Rf_8 0.28, Rf (cellulose) 0.42.[27]   Amino acid ratios in an acid (4 N MSA) hydrolysate: $Asp_{3.09}Ser_{3.42}Glu_{5.16}Pro_{3.95}Gly_{3.00}Val_{2.00}Met_{2.67}Ile_{0.92}Leu_{1.00}Tyr_{1.76}Phe_{1.82}Trp_{0.66}Lys_{2.91}$ $His_{0.84}Arg_{4.22}$ (average recovery 77.8%).   Amino acid ratios in AP–M digest (peptide 0.2 μmol/AP–M 2U): $Asp_{1.05(1)}$ $Ser+Asn_{5.09(4+2,\ Calcd.\ as\ Ser)}Glu_{4.79(5)}Pro_{3.55(4)}Gly_{2.79(3)}Val_{1.92(2)}Met_{2.47(3)}Ile_{0.99(1)}Leu_{1.00(1)}Tyr_{1.69(2)}$ $Phe_{1.83(2)}Trp_{0.82(1)}His_{0.71(1)}Lys_{2.99(3)}Arg_{3.66(4)}$ (average recovery 82.9%).   Number in parentheses are theoretical.   *Anal.* Calcd. for $C_{207}H_{315}N_{59}O_{60}S_3 \cdot 7CH_3COOH \cdot 20H_2O$: C, 48.55; H, 7.06; N, 15.12.   Found: C, 48.40; H, 7.06; N, 15.47.

27) TLC was performed on cellulose (solvent system: *n*-BuOH–AcOH–pyridine–H_2O=15: 3: 10: 12).