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Studies on Peptides. LXVII.[1,2] Synthesis of the Hexadecapeptide corresponding to Position 1 through 16 of Porcine Cholecystokinin-Pancreozymin (CCK-PZ)

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The hexadecapeptide corresponding to the N-terminal portion of porcine cholecystokinin-pancreozymin (CCK-PZ) was synthesized by the successive azide condensation of four peptide fragments.

Previously, Ondetti, *et al.*[4~6] demonstrated through their synthetic studies, that the C-terminal heptapeptide amide of cholecystokinin-pancreozymin (CCK–PZ) is the smallest fragment which elicits the CCK-like pattern of biological activity and the C-terminal octapeptide amide is more potent on a molar basis than CCK–PZ itself, as far as the gallbladder contracting activity is concerned. In both cases, the Tyr residue located at the 7th position from the C-terminus must be sulfated. Otherwise, their potencies decrease to approximately three hundredth or even more. These results imply that as expressed by Grossman[7] that elongation of the peptide chain from the octa peptide amide to the full chain length of CCK–PZ with 33 amino acid residues may bring about certain decrease of CCK activity, but increase its specificity (ratios of gallbladder contracting activity and gastric acid secreting activity). In 1972, Bodanszky, *et al.*[8] reported the synthesis of the protected N-terminal octapeptide hydrazide, Z–Lys(Z)–Ala–Pro–Ser–Gly–Arg–Val–Ser–$NHNH_2$. However, any free peptides related to the N-terminal portion of CCK–PZ have never been tested biologically.

In order to cast further light on the structure-activity correlationship of this upper intestinal polypeptide, we have synthesized the protected hexadecapeptide corresponding to positions 1 through 16 of CCK–PZ and a part of the sample was deprotected for bioassay. Prior to the chain elongation of the heptadecapeptide amide (position 17—33) prepared previously, detailed account of the synthesis of the hexadecapeptide, the N-terminal portion of CCK–PZ, is presented in this paper.

The hexadecapeptide was synthesized by uniting four peptide fragments as shown in Fig. 1. Two residues of Lys locate within this N–terminal portion of CCK–PZ. It seems interest to note that this basic residue does not distribute in the last half of the molecule.

1) Part LXVI: Y. Mori and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24** 2781 (1976).
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 Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=tosyl, ONP=*p*-nitrophenyl ester, Boc=*tert*-butoxycarbonyl, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
4) M.A. Ondetti, J. Pluscec, E.F. Sabo, J.T. Sheehan, and N. Williams, *J. Am. Chem. Soc.*, **92**, 195 (1970).
5) J. Pluscec, J.T. Sheehan, E.F. Sabo, N. Williams, O. Kocy, and M.A. Ondetti, *J. Med. Chem.*, **13**, 349 (1970).
6) M.A. Ondetti, B. Rubin, S.L. Engel, J. Pluscec, and J.T. Sheehan, *Am. J. Digestive Diseases*, **15**, 149 (1970).
7) M.I. Grossman, Gastrointestinal Hormones. A Panoramic View, a State of the Arg Lecture delivered to the Endocrine Society in New York City on June 20, 1975.
8) M. Bodanszky, N. Chaturvedi, D. Hudson, and M. Itoh, *J. Org. Chem.* **37**, 2303 (1972).

Lys(Z) was applied by the reason mentioned in the previous paper.[1] This side chain protecting group, like the Tos group of Arg, survives mostly intact under limited TFA treatment, which requires for the selective removal of the α-amino protecting Z(OMe) group.[9]

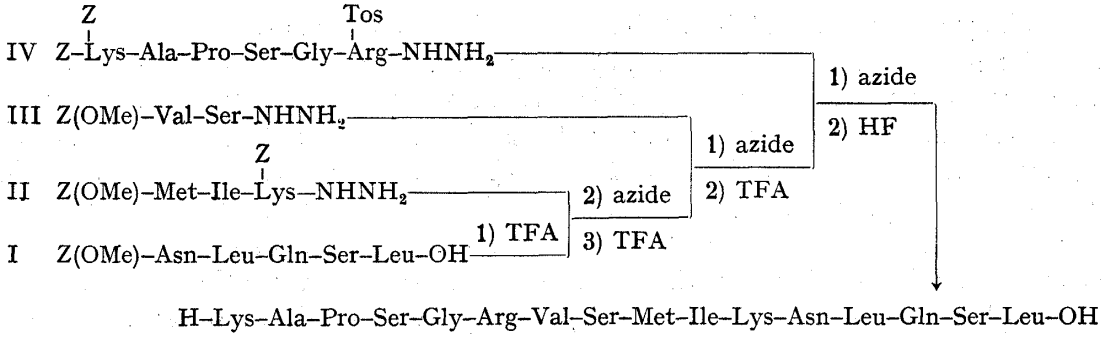

Fig. 1. Synthetic Route to the Hexadecapeptide, H-(CCK-PZ 1—16)-OH

The protected pentapeptide, Z(OMe)-Asn-Leu-Gln-Ser-Leu-OH (I) was synthesized as illustrated in Fig. 2. First, the known dipeptide ester, Z-Gln-Ser-OMe,[10] was converted in the usual manner to the corresponding hydrazide, which was then condensed with the triethylammonium salt of H-Leu-OH by the Honzl and Rudinger's azide procedure.[11] The azide procedure was further extended to condense the hydrogenated sample of Z-Gln-Ser-Leu-OH obtained above and Z(OMe)-Asn-Leu-$NHNH_2$, which was prepared by condensation of Z(OMe)-Asn-OH and H-Leu-OMe with our newly introduced reagent, N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ)[12] followed by exposure the resulting Z(OMe)-Asn-Leu-OMe to hydrazine hydrate. Since the azide reaction was both carried out in a mixture of DMF and water, a certain amount of the amino component came out from the solution and this sacrificed the yield in some extent in both cases.

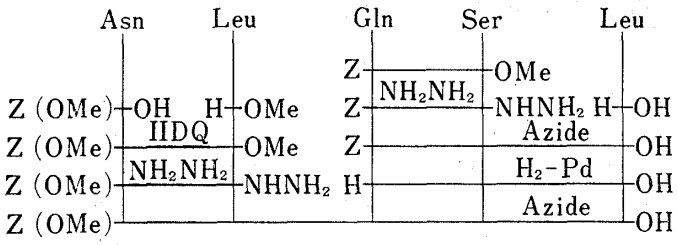

Fig. 2. Synthetic Scheme of the Protected Pentapeptide Z(OMe)-(CCK-PZ 12—16)-OH

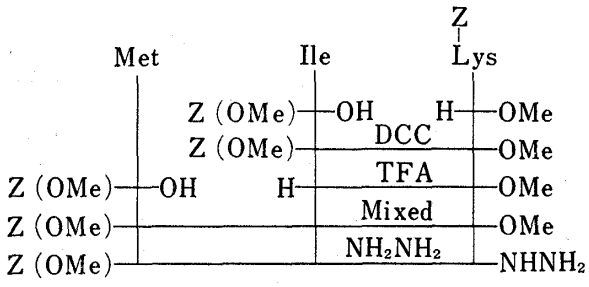

Fig. 3. Synthetic Scheme of the Protected Tripeptide Hydrazide, Z(OMe)-(CCK-PZ 9—11)-$NHNH_2$

The protected tripeptide hydrazide, Z(OMe)-Met-Ile-Lys(Z)-$NHNH_2$, was synthesized according to the scheme illustrated in Fig. 3. The DCC condensation[13] of Z(OMe)-Ile-OH and H-Lys(Z)-OMe afforded Z(OMe)-Ile-Lys(Z)-OMe, which after treatment with TFA, was condensed with Z(OMe)-Met-OH by the mixed anhydride procedure.[14] The resul ing

9) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962); H. Yajima, F. Tamura, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **18**, 2574 (1970).
10) E. Sondheimer and R.W. Holley, *J. Am. Chem. Soc.*, **76**, 2816 (1954).
11) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
12) Y. Kiso and H. Yajima, *J.C.S. Chem. Commun.*, **1972**, 942; Y. Kiso, Y. Kai, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **21**, 2507 (1973).
13) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
14) Th. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); R.A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J.R. Vaughan, Jr. and R.L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

protected tripeptide ester, Z(OMe)-Met-Ile-Lys(Z)-OMe, was converted to the corresponding hydrazide, Z(OMe)-(CCK-PZ 9—11)-$NHNH_2$ (II), in the usual manner. Z(OMe)-Val-Ser-$NHNH_2$, Z(OMe)-(CCK-PZ 7—8)-$NHNH_2$ (III), was also prepared in the usual manner by the DCC condensation of Z(OMe)-Val-OH and H-Ser-OMe followed by exposure the resulting protected dipeptide ester, Z(OMe)-Val-Ser-OMe, to hydrazine hydrate.

In order to avoid racemization, usually the peptide fragment condensation is performed by either activating the C-terminal Gly which has no asymmetrical center or the azide procedure of suitable subunits. It is reasonable that when Bodanszky, *et al.*[8] prepared the protected octapeptide hydrazide mentioned above, Boc-Lys(Boc)-Ala-Pro-Ser-Gly-OH was chosen as one fragment. The pentachlorophenyl ester procedure[15] or the azide procedure was applied to unit this pentapeptide unit to H-Arg-Val-Ser-OMe.[8] Despite of the location of the Gly residue at position 5, we selected in this instance the protected hexapeptide hydrazide, Z-Lys-(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-$NHNH_2$ (IV), as one building block to persuade the azide fragment condensation procedure exclusively. This hexapeptide unit was also prepared by the above authors in a different manner from that of our present synthesis, but their peptide was not used for the coupling reaction.

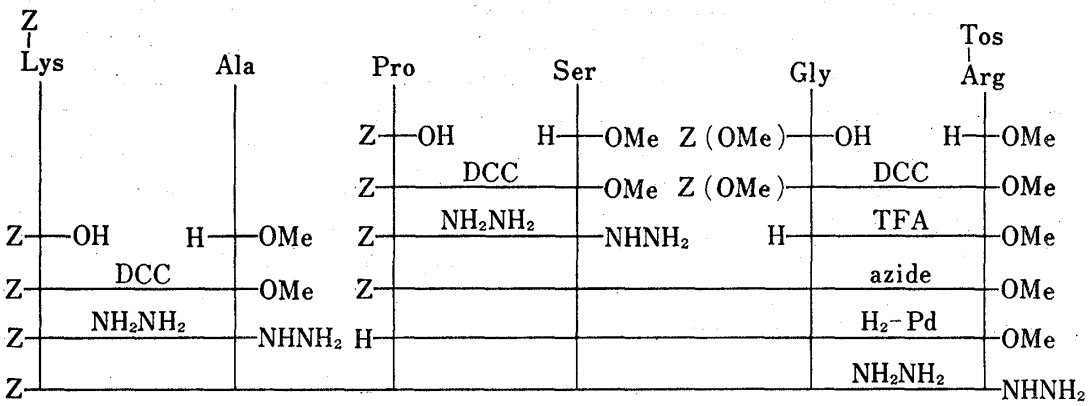

Fig. 4. Synthetic Scheme of the Protected Hexapeptide Hydrazide, Z(OMe)-(CCK-PZ 1—6)-$NHNH_2$

The N-terminal hexapeptide unit was synthesized by uniting three dipeptide units, Z-Lys(Z)-Ala-$NHNH_2$, Z-Pro-Ser-$NHNH_2$ and the TFA treated sample of Z(OMe)-Gly-Arg(Tos)-OMe as shown in Fig. 4, of these Z-Pro-Ser-$NHNH_2$ is the known compound.[16] Two dipeptide ester, Z-Lys(Z)-Ala-OMe and Z(OMe)-Gly-Arg(Tos)-OMe, were prepared by the DCC condensation of respective amino acid derivatives easily available. The former was treated with hydrazine hydrate to give the corresponding hydrazide. The protected hexapeptide ester, Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-OMe thus synthesized after two consecutive azide condensation, was converted to the corresponding hydrazide, Z-(CCK-PZ 1—6)-$NHNH_2$ (IV) in the usual manner.

The azide assembling of four fragments prepared as outlined above was then performed according to the scheme illustrated in Fig. 1. Batchwise washing and precipitation procedures were quite efficient to isolate the chromatographically and analytically pure samples of Z(OMe)-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH and Z(OMe)-Val-Ser-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH. Next, column chromatography on Sephadex LH-20 was employed to purify the protected hexadecapeptide, Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-Val-Ser-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH. The solvent system of DMSO-DMF (5:1) was employed to isolate the desired product, from which the rearrangement product of the hexapeptide azide was well separated.

15) J. Kovacs, L. Kisfaludy, and M.Q. Ceprini, *J. Am. Chem. Soc.*, **89**, 183 (1967).
16) K. Lubke, E. Schroder, R. Schmiechen and H. Gibian, *Ann. Chem.*, **679**, 195 (1964).

In order to prepare the sample for bioassay, the protected hexadecapeptide obtained above was exposed to hydrogen fluoride[17] to remove all protecting groups. The deblocked peptide was purified by column chromatography on Sephadex G-25. Assay results obtained with this partially purified sample will be reported in the latter paper. The peptide fragments prepared in the present synthesis were used, with slight modification, for the chain elongation from the previously synthesized heptadecapeptide amide to the tritriacontapeptide amide which covers the entire amino acid sequence of the CCK-PZ molecule as we will report also in the latter paper.

Experimental

General experimental methods employed here are essentially the same as those described in the Part LXII[18] 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 *n*-BuOH–AcOH–pyridine–H_2O (30: 6: 20: 24).

Z-Gln-Ser-NHNH$_2$——To a solution of Z-Gln-Ser-OMe[10] (19.05 g) in DMF–MeOH (20—250 ml), 80% hydrazine hydrate (18 ml) was added. The gelatinous mass formed on standing at room temperature overnight, was collected by filtration and washed with MeOH; yield 16.55 g (87%), mp 223—225°, $[\alpha]_D^{18}$ +19.3° (c=0.7, DMSO), Rf_1 0.19. *Anal.* Calcd. for $C_{16}H_{23}O_6N_5$: C, 50.39; H, 6.08; N, 18.36. Found: C, 50.20; H, 5.94; N, 18.20.

Z-Gln-Ser-Leu-OH——Under cooling with ice-NaCl, 3.13N HCl–DMF (10.5 ml) and isoamylnitrite (2.28 ml) were added consecutively to a solution of Z-Gln-Ser-NHNH$_2$ (5.75 g) in DMF (80 ml). After stirring for 5 min, the solution, negative of the hydrazine test, was neutralized with Et_3N (4.62 ml) and combined with a solution of H-Leu-OH (3.90 g) and Et_3N (6.3 ml) in H_2O–pyridine (40—20 ml). The mixture was stirred at 4° for 48 hr, the solvent was evaporated *in vacuo* and the residue was dissolved in 5% NH_4OH, which was washed with AcOEt and then acidified with 5N HCl. The resulting gelatinous mass was collected by filtration, washed with H_2O and then recrystallized from THF and ether; yield 3.05 g (42%), mp 133—135°, $[\alpha]_D^{18}$ +39.9° (c=0.3, DMSO), Rf_1 0.26. *Anal.* Calcd. for $C_{22}H_{32}O_8N_4 \cdot 1/2H_2O$: C, 53.97; H, 6.79; N, 11.44. Found: C, 53.99; H, 6.86; N, 11.29.

Z(OMe)-Asn-Leu-OMe——IIDQ (18.18 g) was added to a mixture of Z(OMe)-Asn-OH (13.95 g) and H-Leu-OMe (prepared from 9.08 g of the hydrochloride with 7 ml of Et_3N) in DMF (250 ml) and the solution, after stirring at room temperature for 48 hr, was condensed. Treatment of the residue with ether and H_2O afforded a fine powder, which was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and then recrystallized from THF and *n*-hexane; yield 14.90 g (70%), mp 165—168°, $[\alpha]_D^{18}$ +19.5° (c=0.5, DMSO), Rf_1 0.40. *Anal.* Calcd. for $C_{20}H_{29}O_7N_3$: C, 56.72; H, 6.90; N, 9.92. Found: C, 56.49; H, 6.83; N, 9.47.

Z(OMe)-Asn-Leu-NHNH$_2$——To a solution of Z(OMe)-Asn-Leu-OMe (13.40 g) in DMF–MeOH (20—120 ml), 80% hydrazine hydrate (9 ml) was added. The gelatinous mass formed on standing overnight, was collected by filtration and washed with MeOH; yield 11.20 g (84%), mp 228—230°, $[\alpha]_D^{18}$ +30.0° (c=0.3, DMSO), Rf_1 0.39. *Anal.* Calcd. for $C_{19}H_{29}O_6N_5 \cdot H_2O$: C, 51.69; H, 7.07; N, 15.86. Found: C, 51.63; H, 6.94; N, 15.87.

Z(OMe)-Asn-Leu-Gln-Ser-Leu-OH——Z-Gln-Ser-Leu-OH (4.80 g) dissolved in THF–H_2O (70—20 ml), was hydrogenated over a Pd catalyst in the usual manner. After addition of H_2O (100 ml), the catalyst was removed by filtration, the filtrate was condensed *in vacuo* and the residue was treated with MeOH. The resulting powder was collected by filtration; yield 2.49 g (72%). This free tripeptide was dissolved in H_2O (30 ml) containing Et_3N (2.02 ml). To this ice-cold solution, the azide (prepared from 3.10 g of Z(OMe)-Asn-Leu-NHNH$_2$, 7.8 ml of 2.03N HCl–DMF, 1.07 ml of isoamylnitrite and 2.24 ml of Et_3N) in DMF (60 ml) was combined. The mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 5% citric acid and H_2O and precipitated from DMF with MeOH; yield 2.60 g (49%), mp 215—217°, $[\alpha]_D^{18}$ −14.3° (c=0.5, DMSO), Rf_1 0.28. Anal. Calcd. for $C_{33}H_{51}O_{12}N_7$: C, 53.72; H, 6.96; N, 13.28. Found: C, 53.76; H, 7.14; N, 13.27.

Z(OMe)-Ile-Lys(Z)-OMe——DCC (21.30 g) was added to a mixture of Z(OMe)-Ile-OH (30.53 g) and H-Lys(Z)-OMe (prepared from 28.50 g of the hydrochloride with 12 ml of Et_3N) in DMF (300 ml) and the mixture was stirred at room temperature for 48 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, 5% sodium bicarbonate and H_2O-NaCl, dried over sodium sulfate and then evaporated. Treatment of the residue with ether afforded a powder, which was recrystallized from AcOEt; yield 28.26 g (57%), mp 121—123°, $[\alpha]_D^{18}$ −5.4° (c=0.6, DMF), Rf_1 0.92.

17) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).

18) H. Ogawa, M. Kubota, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **24** 2428 (1976).

Anal. Calcd. for $C_{35}H_{50}O_9N_4S$: C, 63.02; H, 7.22; N, 7.35. Found: C, 62.97; H, 7.20; N, 7.42.

Z(OMe)-Met-Ile-Lys(Z)-OMe——Z(OMe)-Ile-Lys(Z)-OMe (5.71 g) was treated with TFA (10 ml) in the presence of anisole (5 ml) in an ice-bath for 45 min and the excess TFA was removed by evaporation. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (40 ml) containing Et_3N (1.4 ml). To this solution, a mixed anhydride (prepared in the usual manner from 3.76 g of Z(OMe)-Met-OH, 1.92 ml of Et_3N, 1.56 ml of isobutyl chloroformate) in AcOEt (50 ml) was combined. The mixture was stirred in an ice-bath for 2 hr, the solvent was evaporated and the residue was treated with *n*-hexane. The resulting powder was washed batchwisely with 5% citric acid, 5% sodium bicarbonate and H_2O and then recrystallized from MeOH and *n*-hexane; yield 5.50 g (78%), mp 169—170°, $[\alpha]_D^{18}$ −32.0° (c=0.4, DMSO), Rf_2 0.73. *Anal.* Calcd. for $C_{35}H_{50}O_9N_4S$: C, 59.81; H, 7.17; N, 7.97. Found: C, 59.72; H, 7.33; N, 8.09.

Z(OMe)-Met-Ile-Lys(Z)-NHNH$_2$——To a solution of Z(OMe)-Met-Ile-Lys(Z)-$NHNH_2$ (4.90 g) in DMF–MeOH (40—30 ml), 90% hydrazine hydrate (8 ml) was added. The gelatinous mass formed on standing overnight was collected by filtration and washed with MeOH; yield 3.97 g (81%), mp 227—229°, $[\alpha]_D^{18}$ −2.4° (c=0.6, DMSO), Rf_1 0.54. *Anal.* Calcd. for $C_{34}H_{50}O_8N_6S$: C, 58.10; H, 7.17; N, 11.96. Found: C, 57.85; H, 7.16; N, 11.78.

Z(OMe)-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH——Z(OMe)-Asn-Leu-Gln-Ser-Leu-OH (1.44 g) was treated with TFA (6 ml) in the presence of anisole (2 ml) in an ice–bath for 60 min and the excess TFA was removed by evaporation *in vacuo* and the residue was treated with ether. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (5 ml) containing Et_3N (0.84 ml). To this ice-cold solution, the azide (prepared from 1.41 g of Z(OMe)-Met-Ile-Lys(Z)-$NHNH_2$, with 1.28 ml of 3.13N HCl–DMF, 0.29 ml of isoamylnitrite and 0.56 ml of Et_3N) in DMF (40 ml) was combined and the mixture was stirred at 4° for 72 hr. The solvent was evaporated *in vacuo*, the residue was treated with 5% citric acid and AcOEt. The resulting powder was washed batchwisely with 5% citric acid and H_2O and precipitated from DMF with MeOH; yield 1.30 g (52%), mp 260° decomp., $[\alpha]_D^{18}$ −34.6° (c=0.7, DMSO), Rf_2 0.87. *Anal.* Calcd. for $C_{58}H_{81}O_{17}N_{11}S \cdot H_2O$: C, 55.53; H, 6.67; N, 12.28. Found: C, 55.56; H, 7.08; N, 12.16.

Z(OMe)-Val-Ser-OMe——DCC (22.70 g) was added to a mixture of Z(OMe)-Val-OH (30 94 g) and H-Ser-OMe (prepared from 15.59 g of the hydrochloride with 14 ml of Et_3N) in DMF (300 ml) and the mixture was stirred at room temperature for 48 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, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was treated with ether to afford a fine powder, which was recrystallized from AcOEt and ether; yield 21.88 g (57%), mp 155—157°, $[\alpha]_D^{18}$ −13.7° (c= 0.2, DMF), Rf_1 0.80. *Anal.* Calcd. for $C_{18}H_{26}O_7N_2$: C, 56.53; H, 6.85; N, 7.33. Found: C, 56.50; H, 6.95; N, 7.38.

Z(OMe)-Val-Ser-NHNH$_2$——To a solution of Z(OMe)-Val-Ser-OMe (19.07 g) in MeOH (200 ml), 90% hydrazine hydrate (17 ml) was added. The gelatinous mass formed on standing overnight was collected by filtration and washed with MeOH; yield 17.02 g (89%); mp 243—245°, $[\alpha]_D^{18}$ −2.4° (c=0.6, DMSO), Rf_1 0.69. *Anal.* Calcd. for $C_{17}H_{26}O_6N_4$: C, 53.39; H, 6.85; N, 14.65. Found: C, 53.49; H, 6.64; N, 14.69.

Z(OMe)-Val-Ser-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH——Z(OMe)-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH (1.00 g) was treated with TFA (6 ml) in the presence of anisole (2 ml) as stated above. The TFA salt isolated as a fine powder, was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMSO (35 ml) containing Et_3N (0.34 ml). To this ice-cold solution the azide (prepared from 0.61 g of Z(OMe)-Val-Ser-$NHNH_2$, with 1.58 ml of 2.03N HCl–DMF, 0.23 ml of isoamylnitrite and 0.45 ml of Et_3N) in DMF (30 ml) was combined. The mixture was stirred at 4° for 72 hr and the solvent was evaporated and the residue was treated with AcOEt. The resulting powder was purified as stated above by batchwise washing followed by precipitation from DMF with MeOH; yield 0.75 g (66%), mp 263° decomp., $[\alpha]_D^{18}$ −32.5° (c=1.0, DMSO), Rf_2 0.85. Amino acid ratios in an acid hydrolysate: Val 0.95, Ser 1.73, Met 1.01, Ile 1.11, Lys 0.93, Asp 1.06, Leu 2.00 Glu 1.07 (average recovery 81%). *Anal.* Calcd. for $C_{64}H_{95}O_{20}N_{13}S$: C, 54.96; H, 6.85; N, 13.02. Found: C, 55.21; H, 6.94; N, 12.84.

Z(OMe)-Gly-Arg(Tos)-OMe——DCC (3.09 g) was added to a solxtion of Z(OMe)-Gly-OH (3.59 g) and H-Arg(Tos)-OMe (prepared from 5.10 g of the hydrochloride with 1.96 ml of Et_3N) in THF–DMF (40—30 ml) and the mixture was stirred at room temperature for 48 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, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. Treatment of the residue with *n*-hexane afforded a powder, which was recrystallized from ether and *n*-hexane; yield 5.97 g (79%), mp 77—80° $[\alpha]_D^{18}$ −18.4° (c=0.3, DMF), Rf_1 0.89. *Anal.* Calcd. for $C_{25}H_{33}O_8N_5S$: C, 53.28; H, 5.90; N, 12.43. Found: C, 53.57; H, 6.12; N, 12.11.

Z-Pro-Ser-Gly-Arg(Tos)-OMe——Z(OMe)-Gly-Arg(Tos)-OMe (7.60 g) was treated with TFA (15 ml) in the presence of anisole (4 ml) in an ice-bath for 60 min and excess TFA was removed by evaporation *in vacuo*. The oily residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* and then dissolved in DMF (70 ml) containing Et_3N (3.8 ml). To this ice-cold solution, the azide (prepared from 4.73 g of Z-Pro-Ser-$NHNH_2$ with 13.3 ml of 2.03N HCl–DMF, 1.8 ml of isoamylnitrite and 3.8 ml of Et_3N) in DMF (70 ml) and the mixture was stirred at 4° for 72 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which was

washed with 1N HCl, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. Treatment of the residue with ether gave a fine powder, which was recrystallized from AcOEt and ether; yield 5.62 g (58%), mp 90—93°, $[\alpha]_D^{18}$ −28.6° (c=0.9, DMF), Rf_1 0.69. *Anal.* Calcd. for $C_{32}H_{43}O_{10}N_7S \cdot H_2O$: C, 52.24; H, 6.16; N, 13.33. Found: C, 52.51; H, 6.21; N, 12.99.

Z-Lys(Z)-Ala-OMe——The corresponding ethyl ester was prepared by the azide procedure previously. DCC (17.06 g) was added to a mixture of Z-Lys(Z)-OH (34.30 g) and H-Ala-OMe (prepared from 9.61 g of the hydrochloride with 9.6 ml of Et_3N) in DMF (100 ml) and the mixture was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was dissolved in AcOEt, which after washing with 5% citric acid, 5% sodium bicarbonate and H_2O–NaCl, was dried over sodium sulfate and then evaporated. The residue was treated with ether to afford a powder, which was recrystallized from ethanol and ether; yield 16.88 g (76%), mp 143—145°, $[\alpha]_D^{19}$ −9.9° (c=0.9, DMF), Rf_1 0.89. *Anal.* Calcd. for $C_{26}H_{33}O_7N_3$: C, 62.50; H, 6.65; N, 8.41. Found: C, 62.62; H, 6.69; N, 8.53.

Z-Lys(Z)-Ala-NHNH$_2$——To a solution of Z-Lys(Z)-Ala-OMe (24.98 g) in MeOH (250 ml), 90% hydrazine hydrate (20 ml) was added. The solid mass formed on standing overnight, was collected by filtration and washed with MeOH; yield 21.20 g (85%), mp 153—155°, $[\alpha]_D^{18}$ −5.1°, (c=0.9, DMSO), Rf_1 0.62. *Anal.* Calcd. for $C_{25}H_{33}O_6N_5 \cdot H_2O$ C, 58.02; H, 6.81; N, 13.53. Found: C, 58.08; H, 6.37; N, 13.31.

Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-OMe——Z-Pro-Ser-Gly-Arg(Tos)-OMe (5.02 g) dissolved in MeOH (100 ml) containing 1N HCl (7 ml) was hydrogenated over a Pd catalyst in the usual manner. The catalyst was removed by filtration, the filtrate was condensed and the residue was dissolved in DMF (40 ml) containing Et_3N (0.98 ml). To this ice-cold solution, the azide (prepared from 3.50 g of Z-Lys(Z)-Ala-NHNH$_2$ with 6.9 ml of 2.03N HCl–DMF, 0.94 ml of isoamylnitrite and 1.96 ml of Et_3N) in DMF (40 ml) was combined. The mixture was stirred at 4° for 72 hr, the solvent was evaporated and the residue was dissolved in AcOEt, which was washed with 1N HCl, 5% sodium bicarbonate and H_2O–NaCl, dried over sodium sulfate and then evaporated. The residue was triturated with ether and recrystallized from MeOH and ether; yield 5.20 g (71%), mp 98—101° $[\alpha]_D^{18}$ −40.5° (c=0.8, DMSO), Rf_1 0.77. Amino acid ratios in acid hydrolysate: Lys 1.08, Ala 0.95, Pro 1.06, Ser 0.95, Gly 1.00, Arg 0.98 (average recovery 84%). *Anal.* Calcd. for $C_{49}H_{66}O_{14}N_{10}S \cdot H_2O$: C, 55.05; H, 6.41; N, 13.10. Found: C, 55.32; H, 6.44; N, 12.87.

Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-NHNH$_2$——To a solution of Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-OMe (3.0 g) in MeOH (30 ml), 90% hydrazine hydrate (3 ml) was added. After standing at room temperature overnight, the solution was condensed *in vacuo*. The residue was triturated with ether and recrystallized from MeOH and ether; yield 2.50 g (83%), mp 125—128°, $[\alpha]_D^{18}$ −31.1° (c=0.6, DMSO), Rf_1 0.47. *Anal.* Calcd. for $C_{48}H_{66}O_{13}N_{12}S \cdot H_2O$: C, 53.92; H, 6.41; N, 15.72. Found: C, 54.15; H, 6.51; N, 15.43.

Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-Val-Ser-Met-Ile-Lys(Z)-Asn-Leu-Gln-Ser-Leu-OH——The protected decapeptide (0.35 g) prepared above was treated with TFA (1 ml) in the presence of anisole (0.7 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in ice-cold DMSO (2.5 ml) containing Et_3N (0.7 ml). The azide (prepared from 0.53 g of Z-Lys(Z)-Ala-Pro-Ser-Gly-Arg(Tos)-NHNH$_2$ with 0.4 ml of 2.98N HCl–DMF, 0.97 ml of isoamylnitrite and 1.68 ml of Et_3N) in DMF (4 ml) was combined with the above solution containing the decapeptide and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder, after washing with H_2O, was dissolved in a small amount of the solvent consisting of DMSO and DMF (5: 1) and the solution was applied to a column of Sephadex LH-20 (3 × 126 cm), which was eluted with the same solvent. Individual fractions (4 ml each) were collected and absorbancy at 272 mμ was determined. Fractions corresponding to the front main peak (tube No. 70—92) were combined, the solvent was evaporated *in vacuo* and the residue was treated with H_2O. The resulting powder was precipitated from DMSO with AcOEt; yield 0.40 g (70%), mp 255—258°, $[\alpha]_D^{18}$ −39.5° (c=0.3, DMSO), Rf_1 0.18. Amino acid ratios in an acid hydrolysate: Lys 1.76, Ala 1.01, Pro 1.01, Ser 3.07, Gly 1.22, Arg 0.91, Val 1.00, Met 0.96, Ile 1.01, Asp 1.00, Leu 2.21, Glu 1.02 (average recovery 94%). *Anal.* Calcd. for $C_{105}H_{157}O_{30}N_{23}S_2 \cdot 3H_2O$: C, 53.90; H, 7.02; N, 13.77. Found: C, 53.77; H, 7.16; N, 13.85.

H-Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-OH——The above protected hexadecapeptide (200 mg) was treated with HF (approximately 5 ml) in the presence of anisole (2 ml) in an ice-bath for 60 min and dry ether was added. The resulting powder was collected by filtration and then dissolved in H_2O (10 ml), which was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min. The resin was removed by filtration, the filtrate was lyophilized. The resulting powder was dissolved in a small amount of 5% AcOH and the solution was applied to a column of Sephadex G-25 (3 × 130 cm), which was eluted with the same solvent. Individual fractions (5 ml each) were collected and tested by the ninhydrin test on paper. The fractions corresponding to the front main peak (tube No. 54—87) were collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 129 mg (85%), Rf_3 0.22, Rf_4 0.53. Amino acid ratios in an acid hydrolysate: Lys 2.18, Ala 0.90, Pro 1.10, Ser 3.29, Gly 1.00, Arg 0.90 Val 1.02, Met 1.01, Ile 1.03, Asp 1.24, Leu 2.25, Glu 1.14 (average recovery 91%).

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