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Studies on Peptides. XLIII.[1,2] Synthesis of Two Protected Peptides Related to the C-Terminal Portion of the Basic Trypsin Inhibitor from Bovine Pancreas (Kunitz and Northrop)

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The protected nonadecapeptide and the C-terminal dipeptide corresponding to positions 38 through 56 and 57—58 of the basic trypsin inhibitor from bovine pancreas were prepared. Synthesis of the protected nonadecapeptide, Z(OMe)–Cys(Bzl)–Arg(Tos)–Ala–Lys(Z)–Arg(Tos)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH, was achieved by condensation of three subunits: Z(OMe)–Cys(Bzl)–Arg(Tos)–Ala–Lys(Z)–Arg(Tos)–$NHNH_2$ (positions 38—42), Z(OMe)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–$NHNH_2$ (positions 43—48) and Z(OMe)–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH (positions 49—56). The C-terminal dipeptide was prepared in a form of Z(OMe)–Gly–Ala–OH.

In the preceding papers, we reported the syntheses of three protected peptide fragments corresponding to positions 1—12 (I),[4] 13—28 (II)[5] and 29—37 (III)[1] of bovine pancreatic basic trypsin inhibitor (BTI). In this paper, we wish to report the syntheses of the protected nonadecapeptide and the C-terminal dipeptide corresponding to positions 38 through 56 and 57 to 58 of BTI.

Synthesis of the protected nonadecapeptide (IV) was achieved by condensation of three subunits: penta (IV-a, 38—42), hexa (IV-b, 43—48) and octa (IV-c, 49—56)-peptide units as shown in Chart 1. Protecting groups removable by hydrogen fluoride[6] were also adopted to this synthesis, in which 9 out of 19 amino acid residues required the protecting groups; the benzyl group for Cys, Asp and Glu, the tosyl group for Arg and the Z group for Lys.

Synthetic scheme of the protected octapeptide (IV-c) is illustrated in Chart 2. Z–Cys(Bzl)–Gly–OH[7] was treated with hydrogen bromide in acetic acid and the resulting crystalline hydrobromide of H–Cys(Bzl)–Gly–OH[8] was allowed to react with Z(OMe)–Thr–OPCP in the presence of triethylamine to give Z(OMe)–Thr–Cys(Bzl)–Gly–OH. The Z(OMe) group of this tripeptide was removed by trifluoroacetic acid (TFA)[9] and the resulting tripeptide, H–Thr–Cys(Bzl)–Gly–OH, was precipitated as white powder by the addition of dry ether. Attempt

1) Part XLII: H. Yajima, N. Mizokami, M. Kiso, T. Jinnouchi, Y. Kai, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1075 (1974).
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: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, ONP=*p*-nitrophenyl ester. Tos=*p*-toluenesulfonyl, OPCP=pentachlorophenyl ester, DCC=dicyclohexylcarbodiimide.
3) Location: *Sakyo-ku, Kyoto.*
4) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1061 (1974).
5) H. Yajima, Y. Okada, H. Watanabe, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **22**, 1067 (1974).
6) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965).
7) B. Hagedus, *Helv. Chim. Acta*, **31**, 739 (1948); R. Consden and A.H. Gordon, *Biochem. J.*, **46**, 8 (1950); T. Wieland and H.L. Weidenmüller, *Ann. Chem.*, **597**, 111 (1955); S. Gold Schmidt and G. Rosculet, *Chem. Ber.*, 93, 2387 (1960).
8) G. Losse, H. Jeschkeit, and R. Hohn, *Ann. Chem.*, **676**, 222 (1964).
9) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

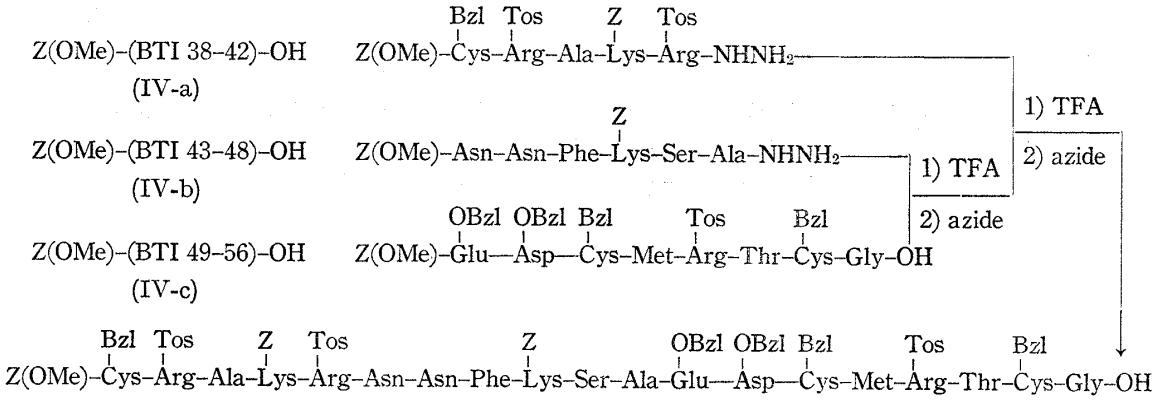

Chart 1. Synthetic Route to the Protected Nonadecapeptide (IV) Z(OMe)-(BTI 38-56)-OH

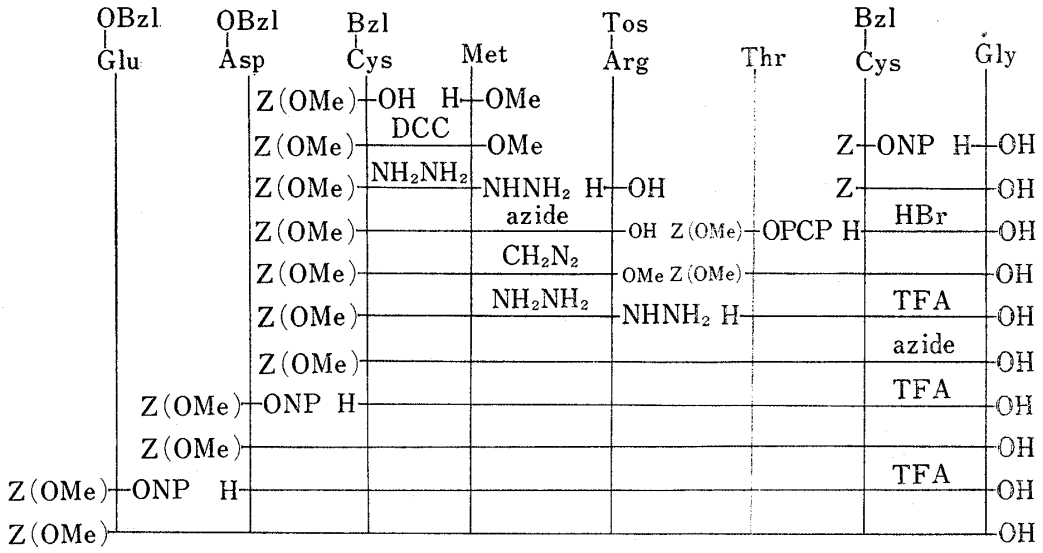

Chart 2. Synthetic Route to the Protected Octapeptide Z(OMe)-(BTI 49—56)-OH (IV-c)

to condense Z(OMe)-Arg(NO_2)-OH to this tripeptide by either the mixed anhydride or the active ester procedure gave impure product unable to purify by single recrystallization. Since the azide procedure of Arg(Tos)-peptides has been shown to work satisfactory in the synthesis of II, Z(OMe)-(BTI 13—28)-OH,[5] Arg(Tos)-terminal peptide hydrazide was undertaken to elongate the peptide chain. Z(OMe)-Cys(Bzl)-OH was condensed with H-Met-OMe by DCC to give Z(OMe)-Cys(Bzl)-Met-OMe, which was converted to the corresponding hydrazide in the usual manner. According to the azide procedure of Honzl and Rudinger,[10] Z(OMe)-Cys(Bzl)-Met-$NHNH_2$ thus obtained was condensed with the triethylammonium salt of H-Arg(Tos)-OH[11] to give Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-OH. Methylation of this protected tripeptide with diazomethane followed by hydrazinolysis gave the protected tripeptide hydrazide, Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-$NHNH_2$, which was condensed with H-Thr-Cys(Bzl)-Gly-OH by the modified azide procedure stated above. Batchwise washing with citric acid and organic solvents was effective in purifying the desired protected hexapeptide, Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH.

This hexapeptide was treated with TFA and the resulting partially protected hexapeptide, H-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH, was then allowed to react with Z(OMe)-

10) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, **26**, 2333 (1961).
11) J. Ramachandran and C.H. Li, *J. Org. Chem.*, **27**, 4006 (1962).

Asp(OBzl)–ONP.[4] The batchwise washing procedure was adopted to purify the resulting protected heptapeptide, Z(OMe)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH. Addition of Z(OMe)–Glu(OBzl)–OH to this heptapeptide was performed in essentially the same manner as described above. Homogeneity of the resulting protected octapeptide, Z(OMe)–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH (IV-c) abbreviated as Z(OMe)–(BTI 49—56)–OH, was confirmed by thin-layer chromatography and elemental analysis.

As has been mentioned previously,[4] there is one discrepancy between the formula presented by Kassell, *et al.*[12] and Anderer, *et al.*[13] with regards to the amino acid residue at position 50 of the trypsin inhibitor isolated from different bovine organs. The former assigned Asp and the latter Asn. Our present synthesis followed the former formula of Kassell, *et al.*[12] as outlined above.

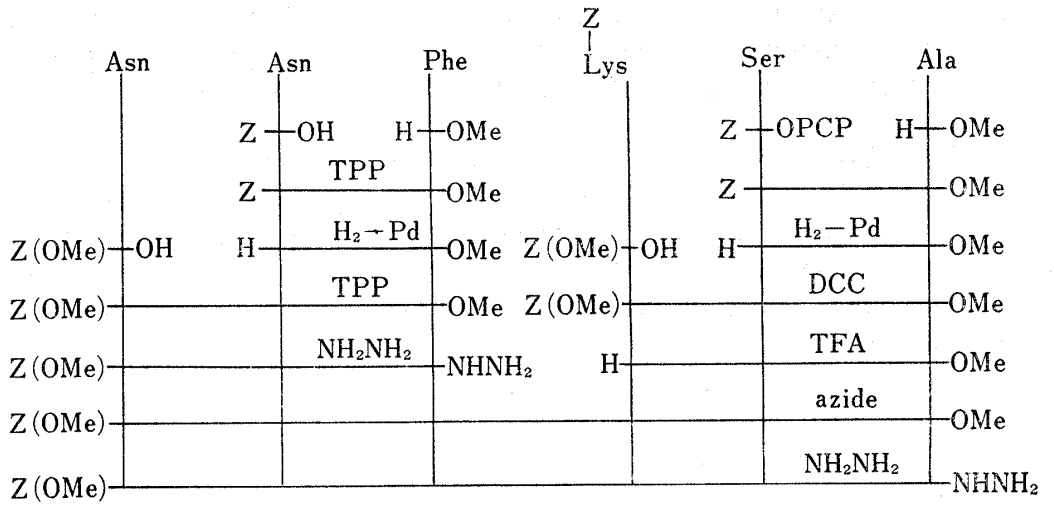

Chart 3. Synthetic Route to the Protected Hexapeptide Z(OMe)–(BTI 43—48)–OH (IV-b)
TPP=triphenylphosphite

The middle hexapeptide unit, Z(OMe)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–$NHNH_2$ (IV-b) abbreviated as Z(OMe)–(BTI 43—48)–OH, was prepared, as illustrated in Chart 3, by coupling two tripeptide subunits, Z(OMe)–Asn–Asn–Phe–$NHNH_2$ and Z(OMe)–Lys(Z)–Ser–Ala–OMe.

The dipeptide methyl ester, Z–Ser–Ala–OMe,[14] was hydrogenated and the resulting H–Ser–Ala–OMe was condensed with Z(OMe)–Lys(Z)–OH[9,15] by DCC to give Z(OMe)–Lys(Z)–Ser–Ala–OMe. In order to prepare the other tripeptide subunit, Z–Asn–OH was coupled with H–Phe–OMe by triphenylphosphite in the presence of imidazole according to Mitin and Glinskaya.[16] Z–Asn–Phe–OMe[17] was obtained in somewhat moderate yield, but the product did not exhibit any CN absorption in infrared (IR) spectrum indicating that the dehydration of the amide group[18] of Asn did not take place during this coupling reaction. As has been mentioned previously in the synthesis of II, the Asn–Ala bond was also established by

12) B. Kassell and M. Laskowski, Sr., *Biochem. Biophys. Res. Commun.*, **20**, 463 (1965); R. Acher and J. Chauvet, *Bull. Soc. Chim. France*, **1967**, 3954; V. Dlouha, D. Pospisilova, B. Meloun, and F. Sorm, *Coll. Czech. Chem. Commun.*, 33, 1363 (1968).
13) F.A. Anderer and S. Hornle, *J. Biol. Chem.*, **241**, 1568 (1966).
14) J.S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942); H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971), Y. Kiso, Y. Kai, and H. Yajima, *ibid.*, **21**, 2507 (1973).
15) H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *Chem. Pharm. Bull.* (Tokyo), **21**, 1380 (1973).
16) Y. V.Mitin and O.V. Glinskaya, *Tetrahedron Letters*, **1965**, 5267.;
17) *a*) W.L. Haas, E.V. Krumkalns, and K. Gerzon, *J. Am. Chem. Soc.*, **88**, 1988 (1966); *b*) G. Gawne, G.W. Kenner, and R.C. Sheppard, *ibid.*, **91**, 5669 (1969).
18) D.T. Gish, P.G. Katsoyannis, G.P. Hess, and R.J. Stedman, *J. Am. Chem. Soc.*, **78**, 5954 (1956).

the same method. It appears that this coupling reagent is particularly useful for the synthesis of Asn peptides. The Z group of the above protected dipeptide ester was removed by hydrogenation and the resulting dipeptide methyl ester, H–Asn–Phe–OMe[19] was condensed with Z(OMe)–Asn–OH by the same coupling reagent to give Z(OMe)–Asn–Asn–Phe–OMe. This protected tripeptide ester is less soluble in methanol and therefore it was converted to the corresponding hydrazide in a dimethylformamide (DMF) solution. Since the hydrazide dissolved in DMF containing hydrochloride, the modified azide procedure worked out without difficulty in the coupling reaction with H–Lys(Z)–Ser–Ala–OMe derived from the above protected tripeptide ester by the TFA treatment. The resulting protected hexapeptide ester, Z(OMe)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–OMe, was converted to the corresponding hydrazide (IV-b) in the usual manner.

The synthetic outline of the N-terminal pentapeptide unit, Z(OMe)–Cys(Bzl)–Arg(Tos)–Ala–Lys(Z)–Arg(Tos)–$NHNH_2$ (IV-a) abbreviated as Z(OMe)–(BTI 38—42)–OH, is illustrated in Chart 4. Since the application of the azide procedure of the Arg-terminal peptide fragment was successfully demonstrated as mentioned above, this pentapeptide was prepared by the combination of di and tripeptide units, Z(OMe)–Cys(Bzl)–Arg(Tos)–$NHNH_2$ and Z(OMe)–Ala–Lys(Z)–Arg(Tos)–OH.

Z(OMe)–Cys(Bzl)–ONP[20] was allowed to react with H–Arg(Tos)–OH to give Z(OMe)–Cys(Bzl)–Arg(Tos)–OH. Attempt to crystallize this dipeptide has been unsuccessful. It was then methylated by diazomethane and the methyl ester was converted to the corresponding hydrazide in the usual manner. The dipeptide hydrazide, Z(OMe)–Cys(Bzl)–Arg(Tos)–$NHNH_2$ dissolved fairly well in methanol and was less soluble in ethanol, from the latter it could be recrystallized.

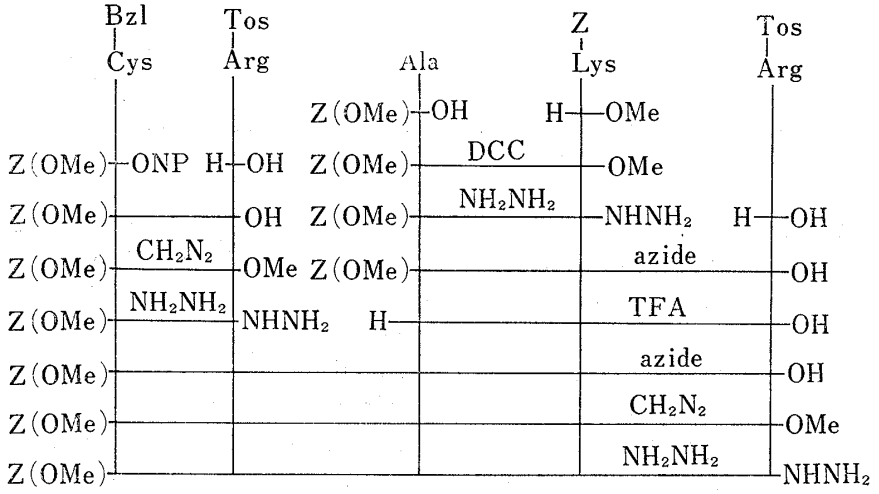

Chart 4. Synthetic Route to the Protected Pentapeptide Z(OMe)–(BTI 38—42)–OH (IV-a)

Z(OMe)–Ala–Lys(Z)–Arg(Tos)–OH was synthesized by coupling Z(OMe)–Ala–Lys(Z)–$NHNH_2$ with H–Arg(Tos)–OH by the modified azide procedure. The former protected dipeptide hydrazide was obtained through Z(OMe)–Ala–Lys(Z)–OMe, resulted from the DCC coupling of Z(OMe)–Ala–OH with H–Lys(Z)–OMe. The protected tripeptide, after treatment with TFA, was condensed with Z(OMe)–Cys(Bzl)–Arg(Tos)–$NHNH_2$ by the azide procedure to give the protected pentapeptide, Z(OMe)–Cys(Bzl)–Arg(Tos)–Ala–Lys(Z)–Arg(Tos)–OH, in satisfactory yield. Conversion of this pentapeptide to the corresponding hydrazide (IV-a) was carried out in the usual manner.

19) E. Schröder, *Ann. Chem.*, **688**, 250 (1965).
20) H. Yajima, N. Shirai, and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 1900 (1971).

Assembling of three peptide fragments thus obtained was performed as illustrated in Chart 1. The hexapeptide hydrazide (IV-b) is also less soluble in DMF, however it could be smoothly converted to the corresponding azide by isoamylnitrite. The hydrazine test[21] is useful in following the progress of this reaction. The azide was then allowed to react with the octapeptide, H–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH, derived from the Z(OMe)-derivative (IV-c) by the TFA treatment. The protected tetradecapeptide, Z(OMe)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg-(Tos)–Thr–Cys(Bzl)–Gly–OH, was obtained in analytically pure form after purification by batchwise washing and recrystallization.

For the final coupling reaction, the α-amino protecting group of the protected tetradecapeptide was cleaved by TFA and the resulting deblocked peptide was coupled with the protected pentapeptide hydrazide (IV-a) again by the modified azide procedure. Purification of the product was performed in essentially the same manner as described in the purification of the protected tetradecapeptide. The desired protected nonadecapeptide (IV) exhibited a single spot on thin-layer chromatography and acid hydrolysate contained every constituent amino acid in ratios predicted by theory. Elemental analysis supported further the homogeneity of this final product.

The C-terminal dipeptide, Z(OMe)–Gly–Ala–OH (V) assigned as Z(OMe)–(BTI 57—58)–OH, was prepared by the reaction of *p*-methoxybenzyl azidoformate[9,22] and H–Gly–Ala–OH.[23] First attachment of the peptide on the resin was established with this small peptide fragment as will be mentioned in the subsequent paper.

Experimental

General experimental methods employed are essentially the same as described in the Part XXII[24] of this series. Unless otherwise mentioning, products described herein were purified by either one of the following procedures: Procedure A. For the purification of neutral compounds soluble in AcOEt, the extract was washed successively with 10% citric acid, 5% Na_2CO_3 and H_2O–NaCl, dried over Na_2SO_4 and then condensed *in vacuo*. The residue was recrystallized from appropriate solvents. Procedure B. For the purification of acidic compounds soluble in AcOEt, the extract was washed with 10% citric acid and H_2O–NaCl, dried over Na_2SO_4 and then condensed *in vacuo*. The residue was recrystallized from appropriate solvents. Procedure C. For the purification of fully protected peptides less soluble in AcOEt, the crude product was washed batchwisely with 10% citric acid, 0.1 N Na_2CO_3 and AcOEt and recrystallized from appropriate solvents. 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 (40:15:5), Rf_2 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2).

Z(OMe)-Thr-OPCP——A mixture of Z(OMe)–Thr–OH (6.0 g), PCP–OH (6.20 g) and DCC (4.80 g) in AcOEt (50 ml) was stirred at room temperature overnight. After filtration, the filtrate was condensed and the residue was recrystallized from EtOH; yield 10.33 g (91%), mp 142—145°. *Anal.* Calcd. for $C_{19}H_{16}O_6N$-Cl_5: C, 42.93; H, 3.03; N, 2.63. Found: C, 43.23; H, 3.08; N, 2.72.

Z(OMe)-Thr-Cys(Bzl)-Gly-OH——The HBr salt of H–Cys(Bzl)–Gly–OH[8] (17.50 g), prepared from Z–Cys(Bzl)–Gly–OH[7] with 2.7 N HBr in AcOH, was dissolved in 60% aqueous DMF (240 ml). To this solution, triethylamine (7.0 ml) and Z(OMe)–Thr–OPCP (27.0 g) were added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was dissolved in H_2O, which was washed with ether and then acidified with 10% citric acid. The resulting crystalline solid was collected by filtration, washed with H_2O, dried over P_2O_5 and then recrystallized from MeOH and AcOEt; yield 24.70 g (93%), mp 179—182°, $[\alpha]_D^{26}$ −16.5° (c=1.0, DMF). Rf_1 0.25. *Anal.* Calcd. for $C_{26}H_{33}O_8N_8S$: C, 56.27; H, 5.85; N, 7.87. Found: C, 56.00; H, 5.97; N, 7.91.

21) H.E. Ertel and L. Horner, *J. Chromatog.*, **7**, 268 (1962); K. Hofmann, R. Schmiechen, R.D. Wells, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
22) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **17**, 1962 (1969); H. Yajima, H. Kawatani, and Y. Kiso, *ibid.*, **18**, 850 (1970).
23) B.F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508 (1951); F. Weygand and W. Steglich, *Chem. Ber.*, **93**, 2983 (1960); F.H.C. Stewart, *Aust. J. Chem.*, **23**, 1073 (1970).
24) H. Yajima, Y. Okada, H. Kawatani, and N. Mizokami, *Chem. Pharm. Bull.* (Tokyo), **17**, 1229 (1969).

Z(OMe)-Cys(Bzl)-Met-OMe——A mixture of Z(OMe)-Cys(Bzl)-OH (18.30 g), H-Met-OMe (prepared from 8.20 g of the hydrochloride with 6.9 ml of triethylamine) and DCC (10.3 g) in DMF (180 ml) was stirred at room temperature overnight. The solution was filtered and the filtrate was condensed *in vacuo*. The residue was purified according to the procedure A. The product was recrystallized from MeOH and AcOEt; yield 21.20 g (81%), mp 82—85°, $[\alpha]_D^{26}$ −37.5° (c=1.2, DMF). *Anal.* Calcd. for $C_{25}H_{32}O_6N_2S_2$: C, 57.67; H, 6.19; N, 5.38. Found: C, 57.79; H, 6.26; N, 5.67.

Z(OMe)-Cys(Bzl)-Met-NHNH$_2$——To a solution of the above dipeptide methyl ester (20.0 g) in MeOH (200 ml), 80% hydrazine hydrate (10 ml) was added and the solution was kept on standing overnight. The gelatinous mass formed was collected by filtration and recrystallized from MeOH; yield 18.04 g (90%), mp 168—172°. *Anal.* Calcd. for $C_{24}H_{32}O_5N_4S_2$: C, 55.33; H, 6.19; N, 10.76. Found: C, 55.45; H, 6.34; N, 10.61.

Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-OH——Z(OMe)-Cys(Bzl)-Met-NHNH$_2$ (10.40 g) was dissolved in DMF (100 ml). Under cooling with ice-NaCl, 1.8 N HCl-DMF (22 ml) and isoamylnitrite (3.0 ml) were added. Stirring was continued for 10 min, when the hydrazine test[21] became negative. The solution, after neutralization with triethylamine (5.6 ml), was combined with a solution of H-Arg(Tos)-OH[11] (6.0 g) and triethylamine (5.6 ml) in H_2O (40 ml). After the mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was dissolved in 3% NH_4OH, which after washing with AcOEt, was acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt, which was purified as described in the procedure B. The product was recrystallized from MeOH and AcOEt; yield 9.88 g (61%), mp 129—133°, $[\alpha]_D^{30}$ −15.9° (c=0.9, DMF). Rf_1 0.50, Rf_2 0.80. *Anal.* Calcd. for $C_{37}H_{48}O_9N_6S_3$: C, 54.39; H, 5.93; N, 10.28. Found: C, 54.25; H, 5.94; N, 10.00.

Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-OMe——To a solution of Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-OH (9.80 g) in MeOH (200 ml), diazomethane in ether (stored over $FeSO_4$) was added. After the solution was stirred for 1 hr, the excess diazomethane was destroyed with AcOH and the solvent was evaporated. The residue turned to the solid on standing; yield 9.20 g (92%), mp 120—123°, $[\alpha]_D^{25}$ −13.6° (c=1.5, DMF). Rf_1 0.72. *Anal.* Calcd. for $C_{38}H_{50}O_9N_6S_3$: C, 54.92; H, 6.06; N, 10.11. Found: C, 54.68; H, 6.27; N, 9.83.

Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-NHNH$_2$——To a solution of the above protected tripeptide methyl ester (9.0 g) in MeOH (30 ml), 80% hydrazine hydrate (20 ml) was added. The crystalline mass formed on standing at room temperature for 48 hr, was recrystallized from MeOH; yield 7.97 g (89%), mp 173—176°. *Anal.* Calcd. for $C_{37}H_{50}O_8N_8S_3$: C, 53.47; H, 6.06; N, 13.48. Found: C, 53.20; H, 6.16; N, 13.21.

Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH——Z(OMe)-Thr-Cys(Bzl)-Gly-OH (2.70 g) was treated with TFA (5 ml) in the presence of anisole (1 ml) at room temperature for 40 min. The product was precipitated by addition of dry ether and dried over KOH pellets *in vacuo* overnight. The TFA salt thus obtained was dissolved in DMF (15 ml) and triethylamine (2.1 ml) was added. To this solution, the azide, prepared from Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-NHNH$_2$ (4.20 g), 1 N HCl-DMF (10 ml), isoamylnitrite (0.7 ml) and triethylamine (1.4 ml) in DMF (20 ml), was combined and the mixture was stirred at 4° for 48 hr. A few drop of AcOH was added and the solvent was evaporated *in vacuo*. AcOEt and 10% citric acid were added to the residue and the resulting solid was collected by filtration, washed with 10% citric acid, H_2O and AcOEt, dried over P_2O_5 and then recrystallized from MeOH and AcOEt; yield 3.90 g (66%), mp 127—132°, $[\alpha]_D^{28}$ −15.9° (c=0.2, DMF). *Anal.* Calcd. for $C_{53}H_{69}O_{13}N_9S_4 \cdot H_2O$: C, 53.65; H, 6.03; N, 10.63. Found: C, 53.60; H, 6.25; N, 10.69.

Z(OMe)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH——Z(OMe)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly OH (2.37 g) was treated with TFA (2.5 ml) in the presence of anisole (1.5 ml) at room temperature for 40 min. The product was precipitated by addition of dry ether, collected by filtration and dried over KOH pellets and P_2O_5 *in vacuo* overnight. To a solution of the TFA salt thus obtained in DMF (12 ml), triethylamine (0.8 ml) and Z(OMe)-Asp(OBzl)-ONP[4] (1.32 g) were added and the mixture was stirred at room temperature for 5 hr. A few drop of AcOH was added to the solution and the solvent was evaporated *in vacuo*. AcOEt and 10% citric acid were added to the residue and the resulting solid powder was collected by filtration, washed batchwisely with AcOEt, H_2O and recrystallized from DMF and AcOEt; yield 2.43 g (87%), mp 126—129°, $[\alpha]_D^{28}$ −27.9° (c=0.2, DMF). Rf_1 0.43. *Anal.* Calcd. for $C_{64}H_{80}O_{16}N_{10}S_4 \cdot H_2O$: C, 55.23; H, 5.93; N, 10.07. Found: C, 55.28; H, 6.03; N, 10.24.

Z(OMe)-Glu(OBzl)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH (IV-c)——Z(OMe)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH (2.23 g) was treated with TFA (1.5 ml) in the presence of anisole (1 ml) at room temperature for 40 min. The precipitate formed by addition of dry ether was collected by filtration, dried over KOH pellets and P_2O_5 *in vacuo* overnight. To a solution of the TFA salt in DMF (14 ml), triethylamine (0.7 ml) and Z(OMe)-Glu(OBzl)-ONP[4] (1.25 g) was added and the mixture was stirred at room temperature for 72 hr. After evaporation of the solvent, AcOEt and 10% citric acid were added to the residue and the resulting solid was collected by filtration, washed batchwisely with AcOEt and H_2O and recrystallized from DMF and AcOEt; yield 1.85 g (73%), mp 185—188°, $[\alpha]_D^{28}$ −21.6° (c=0.3, DMF). Rf_3 0.36. *Anal.* Calcd. for $C_{76}H_{93}O_{19}N_{11}S_4$: C, 57.30; H, 5.88; N, 9.76. Found: C, 57.08; H, 6.13; N, 9.61.

Z(OMe)-Lys(Z)-Ser-Ala-OMe——Z-Ser-Ala-OMe[14] (15.0 g) in MeOH (250 ml) containing 1 N HCl (43 ml) was hydrogenated over a Pd catalyst in the usual manner. After filtration, the filtrate was condensed

and the residue was dried over KOH pellets and P_2O_5 *in vacuo* over night. The residue was then dissolved in DMF (300 ml). To this solution, triethylamine (6.0 ml), Z(OMe)–Lys(Z)–OH[9,15] (19.09 g) and DCC (8.85 g) were added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was purified according to the procedure A. The resulting solid was recrystallized from MeOH and AcOEt; yield 24.77 g (89%), mp 176—178°, $[\alpha]_D^{23}$ −7.4° (c=2.0, DMF). Rf_1 0.65. *Anal.* Calcd. for $C_{30}H_{40}O_{10}N_4$: C, 58.42; H, 6.53; N, 9.08. Found: C, 58.44; H, 6.67; N, 9.12.

Z-Asn-Phe-OMe——A mixture of Z–Asn–OH (60.0 g), H–Phe–OMe (prepared from 43.0 g of the hydrochloride with 27 ml of triethylamine), triphenylphosphite (108 g) and imidazole (24.0 g) in DMF (400 ml) was stirred at 40° for 48 hr. After filtration, the filtrate was condensed *in vacuo* and the residue was treated with AcOEt to give the solid, which was purified according to the procedure C. The product was recrystallized from MeOH; yield 53.70 g (65%), mp 193—196°, $[\alpha]_D^{22}$ −1.6° (c=1.2, DMF). (lit.[17a] mp 195—197°, $[\alpha]_D$ +16.5° in AcOH). *Anal.* Calcd. for $C_{22}H_{25}O_6N_3$: C, 61.12; H, 5.90; N, 9.83. Found: C, 61.56; H, 6.07; N, 9.93.

Z(OMe)-Asn-Asn-Phe-OMe——Over a Pd catalyst, H_2 gas was bubbled through a suspension of Z–Asn–Phe–OMe (34.26 g) in MeOH (350 ml) containing 1 N HCl (60 ml). The clear solution was obtained when the hydrogenolysis proceeded. The catalyst was removed by filtration and the filtrate was condensed. The residue (Rf_1 0.47), after drying over KOH pellets and P_2O_5 *in vacuo*, was dissolved in DMF (150 ml) and triethylamine (8.3 ml) was added. To this solution, Z(OMe)–Asn–OH (16.80 g), triphenylphosphite (37.2 g) and imidazole (8.20 g) were combined and the mixture was stirred at 40° for 48 hr. The solvent was evaporated and the resulting solid was purified as described in the procedure C. The product was recrystallized from DMF and ether; yield 24.0 g (51%), mp 196—200°, $[\alpha]_D^{23}$ −9.5° (c=1.6, DMF). Rf_1 0.62. *Anal.* Calcd. for $C_{27}H_{33}O_9N_5$: C, 56.73; H, 5.82; N, 12.25. Found: C, 57.00; H, 5.57; N, 12.08.

Z(OMe)-Asn-Asn-Phe-NHNH$_2$——To a solution of Z(OMe)–Asn–Asn–Phe–OMe (28.90 g) in DMF (300 ml), 80% hydrazine hydrate (12 ml) was added and the solution was kept on standing overnight. The resulting gelatinous precipitate was collected by filtration and recrystallized from DMF and ether; yield 25.0 g (86%), mp 260—261°. *Anal.* Calcd. for $C_{26}H_{33}O_8N_7 \cdot 1/2H_2O$: C, 53.80; H, 5.90; N, 17.00. Found: C, 53.72; H, 5.86; N, 17.34.

Z(OMe)-Asn-Asn-Phe-Lys(Z)-Ser-Ala-OMe——Z(OMe)–Lys(Z)–Ser–Ala–OMe (4.35 g) was treated with TFA (7 ml) in the presence of anisole (2.5 ml). The TFA salt, precipitated by ether and dried over KOH pellets, was dissolved in DMF (30 ml) and triethylamine (2.9 ml) was added. This solution was combined with a solution of the azide (prepared from 4.06 g of Z(OMe)–Asn–Asn–Phe–NHNH$_2$ with 14 ml of 1 N HCl–DMF, 0.94 ml of isoamylnitrite and 1.9 ml of triethylamine) in DMF (20 ml). The mixture was stirred at 4° for 48 hr and then the solvent was evaporated. The residue was triturated with AcOEt and the resulting powder was purified according to the procedure C. The product was further washed with MeOH and recrystallized from DMF and AcOEt; yield 4.96 g (70%), mp 212—215°, $[\alpha]_D^{26}$ −45.7° (c=0.2, DMF). Rf_1 0.44. *Anal.* Calcd. for $C_{47}H_{61}O_{15}N_9 \cdot H_2O$: C, 55.89; H, 6.29; N, 12.48. Found: C, 55.88; H, 6.42; N, 12.91.

Z(OMe)-Asn-Asn-Phe-Lys(Z)-Ser-Ala-NHNH$_2$ (IV-b)——To a solution of the above protected hexapeptide methyl ester (10.26 g), 80% hydrazine hydrate (2 ml) was added. The gelatinous mass formed on standing overnight was collected by filtration and washed with MeOH; yield 10.20 g (99%), mp 218—224°. *Anal.* Calcd. for $C_{46}H_{61}O_{14}N_{11} \cdot H_2O$: C, 54.69; H, 6.28; N, 15.25. Found: C, 54.65; H, 6.14; N, 15.72.

Z(OMe)-Ala-Lys(Z)-OMe——A mixture of Z(OMe)–Ala–OH (5.06 g), H–Lys(Z)–OMe (prepared from 6.60 g of the hydrochloride and 2.8 ml of triethylamine) and DCC (4.1 ml) in DMF (60 ml) was stirred at room temperature for 48 hr. After filtration, the filtrate was condensed *in vacuo* and the residue was purified according to the procedure A. The product was recrystallized from MeOH and AcOEt; yield 5.96 g (56%), mp 110—112°, $[\alpha]_D^{29}$ +8.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{27}H_{35}O_8N_3$: C, 61.23; H, 6.66; N, 7.94. Found: C, 61.46; H, 6.64; N, 8.09.

Z(OMe)-Ala-Lys(Z)-NHNH$_2$——Z(OMe)–Ala–Lys(Z)–OMe (5.90 g) was dissolved in MeOH (70 ml) and 80% hydrazine hydrate (3.2 ml) was added. The gelatinous mass formed on standing overnight was collected by filtration and recrystallized from MeOH; yield 5.24 g (88%), mp 138—140°. *Anal.* Calcd. for $C_{26}H_{35}O_7N_5$: C, 58.96; H, 6.66; N, 13.23. Found: C, 58.92; H, 6.83; N, 13.46.

Z(OMe)-Ala-Lys(Z)-Arg(Tos)-OH——To a solution of Z(OMe)–Ala–Lys(Z)–NHNH$_2$ (10.90 g) in DMF (30 ml) and 1.2 N HCl–DMF (33 ml), isoamylnitrite (3.2 ml) was added at −15°. After stirring was continued for 5 min, triethylamine (5.5 ml) was added. This solution was then combined with a solution of H–Arg(Tos)–OH (6.0 g) in 75% aqueous DMF (60 ml) containing triethylamine (5.5 ml). The mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in 3% NH_4OH, which after washing with AcOEt, was acidified with 10% citric acid. The resulting precipitate was treated according to the procedure B. The residue solidified with AcOEt, was recrystallized from MeOH and AcOEt; yield 6.90 g (42%), mp 87—91°, $[\alpha]_D^{26}$ +0.2° (c=1.4, DMF). *Anal.* Calcd. for $C_{39}H_{51}O_{11}N_7S$: C, 56.71; H, 6.22; N, 11.87. Found: C, 56.54; H, 6.50; N, 11.58.

Z(OMe)-Cys(Bzl)-Arg(Tos)-NHNH$_2$——Z(OMe)–Cys(Bzl)–ONP[20] (4.96 g) in DMF (40 ml) was added to a solution of H–Arg(Tos)–OH (2.96 g) and triethylamine (2.7 ml) in H_2O (10 ml) and the solution was stirred at room temperature for 48 hr. The solvent was evaporated and the residue was dissolved in H_2O, which

after washing with ether, was acidified with 10% citric acid and the resulting precipitate was purified according to the procedure B to give an oily residue; yield 5.90 g. Z(OMe)–Cys(Bzl)–Arg(Tos)–OH thus obtained was dissolved in MeOH (60 ml) and treated with diazomethane in the usual manner to give an oily residue (Rf_1 0.76). The methyl ester, Z(OMe)–Cys(Bzl)–Arg(Tos)–OMe, was then dissolved in MeOH (50 ml) and 80 hydrazine hydrate (2.5 ml) was added. After standing overnight, the solution was condensed *in vacuo*. The residue was treated with EtOH and the resulting solid was recrystallized from EtOH; yield 4.97 g (77%), mp 86—89°. *Anal.* Calcd. for $C_{32}H_{41}O_7N_7S \cdot 2H_2O$: C, 54.61; H, 6.44; N, 13.93. Found: C, 54.49; H, 5.93; N, 13.82.

Z(OMe)-Cys(Bzl)-Arg(Tos)-Ala-Lys(Z)-Arg(Tos)-OH——Z(OMe)–Ala–Lys(Z)–Arg(Tos)–OH (1.65 g) was treated with TFA (1.5 ml) in the presence of anisole (1.0 ml) at room temperature for 40 min. The TFA salt, precipitated by addition of dry ether and dried over KOH pellets *in vacuo* overnight, was dissolved in DMF (10 ml) and triethylamine (1.2 ml) was added. This solution was combined with a solution of the azide (prepared from 2.10 g of Z(OMe)–Cys(Bzl)–Arg(Tos)–$NHNH_2$ with 5.0 ml of 1.2 N HCl–DMF, 0.48 ml of isoamylnitrite and 0.83 ml of triethylamine) in DMF (12 ml). After the solution was stirred at 4° for 48 hr, the solvent was evaporated and the residue was dissolved in 3% NH_4OH, which after washing with AcOEt, was acidified with 10% citric acid and the product was treated according to the procedure B. Trituration of the residue with ether gave amorphous powder; yield 2.25 g (84%), mp 108—112°, $[\alpha]_D^{26}$ −15.8° (c=0.2, DMF). *Anal.* Cacld. for $C_{62}H_{80}O_{15}N_{12}S_3$: C, 56.00; H, 6.06; N, 12.64. Found: C, 55.77; H, 6.32; N, 12.39.

Z(OMe)-Cys(Bzl)-Arg(Tos)-Ala-Lys(Z)-Arg(Tos)-$NHNH_2$ (IV-a)——The above pentapeptide (2.20 g) was dissolved in MeOH (30 ml) and etheral diazomethane was added. After 1 hr, the excess diazomethane was discharged by addition of AcOH. The solvent was evaporated and the residue was dissolved in EtOH (10 ml). After addition of 80% hydrazine hydrate (0.3 ml), the product formed on standing overnight was collected and washed MeOH; yield 1.89 g (86%), mp 119—124°. *Anal.* Calcd. for $C_{62}H_{82}O_{14}N_{14}S_3$: C, 55.42; H, 6.15; N, 14.59. Found: C, 55.18; H, 5.88; N, 14.67.

Z(OMe)-Asn-Asn-Phe-Lys(Z)-Ser-Ala-Glu(OBzl)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH——Z(OMe)–Asn–Asn–Phe–Lys(Z)–Ser–Ala–$NHNH_2$ (2.26 g) was suspended in DMF (20 ml) and under cooling with ice–NaCl, 1 N HCl–DMF (4.0 ml) was added. To a clear solution thus obtained, isoamylnitrite (0.3 ml) was added and the solution was stirred for 20 min. When the hydrazine test became negative, the solution was neutralized with triethylamine (0.46 ml) and then combined with a solution of the TFA salt of H–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH (prepared from 2.74 g of the Z(OMe) derivative by treatment with 3.0 ml of TFA in the presence of 1.5 ml of anisole for 40 min followed by addition of dry ether) and triethylamine (0.8 ml) in DMF (30 ml). After the mixture was stirred at 4° for 48 hr, a few drop of AcOH was added and the solvent was evaporated *in vacuo*. The residue was treated with AcOEt. The resulting powder was collected, washed batchwisely with 10% citric acid, H_2O, AcOEt and then hot MeOH and recrystallized twice from DMF and AcOEt; yield 3.64 g (88%), mp 231—235°, $[\alpha]_D^{28}$ −26.6° (c=0.4, DMF). Rf_2 0.82. *Anal.* Calcd. for $C_{113}H_{142}O_{30}N_{20}S_3$: C, 56.81; H, 5.99; N, 11.73. Found: C, 56.64; H, 6.27; N, 11.93.

Z(OMe)-Cys(Bzl)-Arg(Tos)-Ala-Lys(Z)-Arg(Tos)-Asn-Aan-Phe-Lys(Z)-Ser-Ala-Glu(OBzl)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH (IV)——Z(OMe)–Cys(Bzl)–Arg(Tos)–Ala–Lys(Z)–Arg(Tos)–$NHNH_2$ (1.41 g) was dissolved in DMF (15 ml). Under cooling with ice–NaCl, 1 N HCl in DMF (1.4 ml) and isoamylnitrite (0.14 ml) were added. After 5 min, when the hydrazine test became negative, the solution was neutralized with triethylamine (0.29 ml) and then combined with a solution containing the TFA salt of H–Asn–Asn–Phe–Lys(Z)–Ser–Ala–Glu(OBzl)–Asp(OBzl)–Cys(Bzl)–Met–Arg(Tos)–Thr–Cys(Bzl)–Gly–OH (prepared from 1.52 g of the protected tetradecapeptide by treatment with 1.5 ml of TFA in the presence of 0.7 ml of anisole for 40 min followed by addition of dry ether) and triethylamine (0.3 ml) in DMF (15 ml). The mixture was stirred at 4° for 72 hr. After addition of a few drop of AcOH, the solvent was evaporated and the residue was treated with 10% citric acid and AcOEt. The resulting solid was washed with 10% citric acid, H_2O, AcOEt and MeOH and recrystallized twice from DMF and AcOEt; yield 1.68 g (71%), mp 229—233°, $[\alpha]_D^{26}$ −29.1° (c=0.2, DMF). Rf_2 0.89. Amino acid ratios in an acid hydrolysate $Arg_{2.50}$ $Ala_{2.01}$ $Lys_{2.00}$ $Asp_{3.00}$ $Phe_{0.90}$ $Ser_{1.07}$ $Glu_{1.03}$ $Met_{0.50}$ $Thr_{0.92}$ $Gly_{1.09}$ (average recovery 89%). *Anal.* Calcd. for $C_{166}H_{212}O_{41}N_{32}S_7 \cdot 3H_2O$: C, 55.53; H, 6.12; N, 12.49. Found: C, 55.51; H, 6.26; N, 12.63.

Z(OMe)-Gly-Ala-OH (V)——To a solution of H–Gly–Ala–OH[23] (9.60 g) and MgO (5.2 g) in 50% aqueous dioxane (250 ml), *p*-methoxybenzyl azidoformate[9,22] (17.7 g) was added and the mixture was stirred at room temperature for 50 hr. After filtration, the filtrate was condensed and the residue was dissolved in H_2O, which was washed with ether and then acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt and then purified according to the procedure B. The product was recrystallized from MeOH and AcOEt; yield 13.80 g (66%), mp 136—138°, $[\alpha]_D^{29}$ −18.4° (c=1.8, DMF). *Anal.* Calcd. for $C_{14}H_{18}O_6N_4 \cdot 1/2H_2O$: C, 52.62; H, 6.00; N, 8.77. Found: C, 52.82; H, 5.74; N, 8.78.

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