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Synthesis of Hexapeptides Having Cysteines at N- and C-Terminals

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In an attempt to evaluate a propensity of turn or β-structure formation of an amino acid residue, several hexapeptides having a sequence of R_1-CysXABYCys-R_2 (R_1=H or Ac, R_2=OH or $NHCH_3$) were synthesized with a solution method. Residues A and B were selected to form a turn (Gly or Pro), X and Y to form a β-structure (Gly, Val, Lys, Glu).

KEY WORDS: Peptide synthesis/ Solution method/ Hexapeptides/ Cysteine-cystine/ Disulfide/

β-Structure is one of the two ordered secondary structures essential in proteins. The other structure, α-helix, has been studied exhaustively, both experimentally and theoretically. The Zimm-Bragg's parameters, s and σ, which determine the stability of an α-helix, have been determined for almost all twenty amino acids found in protein (for the most recent result, see Ref. 1). On the contrary, β-structure has been studied only scarcely. The reason was, in most cases experiments carried out, an inevitable presence of intermolecular interactions in β-structure, which was due to characteristic long-range interaction in β-structure and not appeared in the structure of α-helix. Only few examples of synthetic polypeptides assuming exclusively intramolecular β-structures have been reported.[2,3]

Very recently, Scheraga, Konishi and coworkers proposed a new model system which was consisted of a small peptide and would enable a quantitative evaluation of a tendency of an amino acid residue to form β-structure. The method is based on disulfide bond formation between two cysteine residues located at the N- and C-terminal of a β-forming peptide. Coupled with a new theory of a β-coil transition,[4] which is somewhat similar to Zimm-Bragg's treatment of α-helix, it will be possible to analyze the thermodynamic stabilities of β-structures as in the same level as for α-helices. In this report, we will describe the synthesis of several hexapeptides containing cysteines at the N- and C-terminal, which are necessary for a study in these lines.

RESULTS AND DISCUSSION

Seven hexapeptides *8*, *14*, *21*, *22*, *31*, *38*, and *45* were synthesized by conventional procedures as outlined in Fig. 1. The design of these compounds was as follows: (1) In peptides *21*, *22*, *31*, *38*, and *45*, proline and glycine were selected

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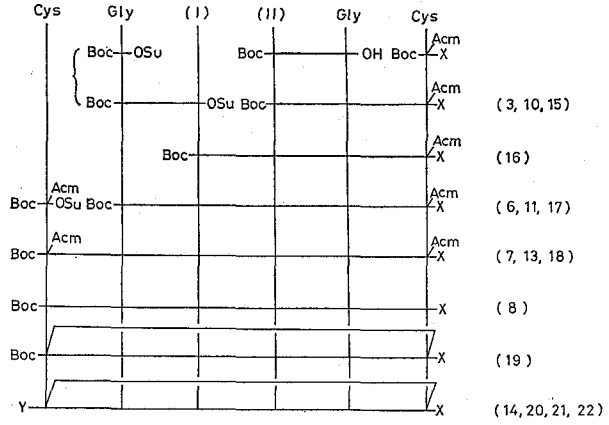


Fig. 1 A

Fig. 1A. Synthesis of *14*, *20*, *21*, and *22*.
X=OH (*14*, *21*, *22*) ; OBzl (others)
Y=H (*14*, *22*); Ac (*20*, *21*)
(I)=Gly (*3—14*) ; Pro (*16–22*)
(II)=Gly (*3—8*, *15–22*) ; Pro (*10–14*)

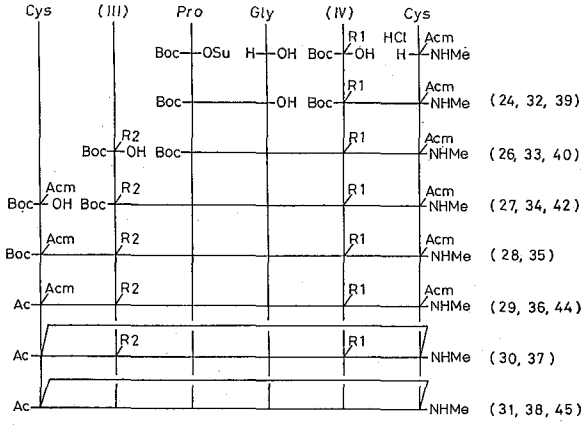


Fig. 1 B

Fig. 1B. Synthesis of *31*, *38*, and *45*.
R_1=Z (*24–30* ; OBzl (*32–37*)
R_2=OBzl (*27–30*; Z (*34–37*)
(III))=Glu (*27–31*) ; Lys (*34–38*) ; Val (*42–45*)
(IV)=Lys (*24–31*) ; Glu (*32–38*); (*39–45*)

as the third and fourth residues, respectively, which would give the most probable sequence for a β-turn. (2) Peptide *14* was designed to see the effect of the sequence reversed as Gly^3-Pro^4. (3) In peptide *8*, a neutral sequence Gly^3-Gly^4 was introduced in the position of peptide turn. (4) Four peptides were synthesized to see the effect of electric charges at terminals (peptides *21* and *22*) or at the penultimate positions (peptides *31* and *38*). (5) In peptide *45*, valine was selected for the positions 2 and 5, the amino acid had been assumed the highest propensity for β-structure.

Peptide coupling was carried out in most cases with dicyclohexylcarbodiimide in the presence of 1.5 eq. of 1-hydroxybenzotriazole. Disulfide bonds formation in hexapeptide was achieved by an oxidation with iodine. The products were characterized by elemental analysis, amino acid analysis and ^{1}H-NMR. The determination of thermodynamic constants of disulfide-thiols equilibrium for each hexapeptide will be published elsewhere.[13]

EXPERIMENTAL

All amino acids are of L-configuration except glycine. Melting points were measured on a micro hotplate and described without correction. Proton magnetic resonance spectra were taken with a JEOL FX100 (100 MHz) or a JEOL GX400 (400 MHz) spectometer as solutions in dimethyl-d_6 sulfoxide, and the chemical shifts are reported in ppm from internal tetramethylsilane. Chemical shifts without any notation mean the center of multiplets. Amino acid analysis of acid hydrolyzates (6N HCl, 110°C, 24 h, unless otherwise noted) were carried out on a JASCO amino acid analyzer with $NaBH_4$-ninhydrin system[5]. Solvents for TLC had the following ratio of chloroform: methanol:acetic acid, (I) 95:5:1, (II) 90:10:1, (III) 80:20:1, (IV) 100:14:1, (V) 60:40:1. Merck HPTLC plates were used with these solvents. Diisopropylethylamine (Sigma) was distilled over ninhydrin after treatment with KOH pellets.

Elemental analyses were carried at the Facility of Elemental Analysis, Institute for Chemical Research, Kyoto University.

BOC-Cys(Acm)-OCH$_2$Ph(p-NO$_2$-), (*1*). The ester was synthesized from the cesium salt of BOC-Cys(Acm)-OH, which was prepared from H-Cys(Acm)-OH[6] by the method of Itoh *et al.*[7], and p-nitrobenzyl bromide according to the method of Wang *et al.*[8] Yield, 68%. After recrystallization from ethanolether, the compound had m.p. 110–112°C. Anal. Found: C, 50.58; H, 5.80; N, 9.94%. Calcd for $C_{18}H_{25}O_7N_3S$: C, 50.58; H, 5.90; N, 9.63%. TLC: Rf(I)=0.66.

HCl·H-Cys(Acm)-OCH$_2$Ph(p-NO$_2$-), (*2*). Removal of Boc group of compound *1* was carried out by the addition of 5 ml of HCl-saturated ethyl acetate to the solution of 2.14 g of *1* in 5 ml of ethyl acetate. The mixture was kept at room temperature for 1 h. The hydrochloride was filtered and washed with ether. Yield, quantitative. m.p. 157–159°C (decomposition). Anal. Found: C, 42.84; H, 4.93; N, 11.63%. Calcd for $C_{13}H_{18}O_5N_3ClS$: C, 42.91; H, 4.99; N, 11.55%. TLC: Rf(I)=0.17.

Boc-GlyGlyCys(Acm)-OCH$_2$Ph(p-NO$_2$-), (*3*). p-Nitrophenylester of Boc-GlyGly-OH (1.59 g, 4.5 mmol) and *2* (1.64 g, 4.5 mmol) were treated with DIEA (1.74 ml, 9.9 mmol) in 10 ml of dry DMF at room temperature overnight. The reaction mixture was evaporated in *vacuo* to leave the residue, which was dissolved in ethyl acetate. The solution was washed successively with 10% aq. citric acid, 3% aq. sodium hydrogen carbonate, water, and finally with saturated aq. sodium chloride, dried over sodium sulfate, then evaporated to afford *3*, which was crystallized from ethanol-ether or ethyl acetate. Yield, 82%. m.p. 130–133°C. Anal. Found: C, 48.82; H, 5.71; N, 13.00%. Calcd for $C_{22}H_{31}O_9N_5S$: C, 48.79; H, 5.77; N, 12.93%. TLC: Rf(II)=0.47. NMR: 1.36(s, 9H: Boc), 1.82(s, 3H: Ac), 2.94(2H: (Cys)Cβ-2H), 3.56(2H: (Gly^1)Cα−2H), 3.76(2H: (Gly^2)Cα−2H), 4.2(2H: (Acm)CH_2), 4.6(1H: (Cys)Cα−H), 5.26(s, 2H: CH_2 of p-nitrobenzyl), 6.92(t, 1H: (Gly^1)NH), 7.6–8.2(4H: p-nitrophenyl), 8.00(t, 1H: (Gly^2)NH), 8.42(d, 1H: (Cys)NH), 8.48(t, 1H: (Acm)NH).

HCl·H-GlyGlyCys(Acm)-CH$_2$Ph(p-NO$_2$), (*4*). Boc-tripeptide ester *3*, 3.72 g, was dissolved in 10 ml of dichloromethane and treated with 5 ml of TFA at room temperature for 1.5 h. The solution was evaporated and deblocking was completed with HCl/ethyl acetate. Yield, quantitative. Found: C, 39.36; H, 4.68; N, 12.46%. Calcd for $C_{17}H_{24}O_7N_5SCl\cdot 1/2\ CF_3CO_2H\cdot H_2O$: C, 39.06; H, 4.83; N, 12.66%.

Boc-GlyGly-OSu, (*5*). The compound was synthesized from Boc-GlyGly-OH and HOSu with DCC in DMF. The DCC reaction was carried out at −12°C for first two hours, then at 0°C overnight. Yield

Abbreviations used: Acm, S-acetamidomethyl; DCC, dicyclohexylcarbodiimide; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; HOBT, hydroxybenzotriazole; TFA, trifluoroacetic acid; TLC, thin-layer chromatography.

of *5*, 79%; m.p. 163–166°C (recrystallized from ethyl acetate). Found: C, 47.41; H, 5.58; N, 12.81%. Calcd for $C_{13}H_{19}O_7N_3$: C, 47.41; H, 5.82; N, 12.76%.

Boc-GlyGlyGlyGlyCys(Acm)-CH_2Ph(p-NO_2-), (*6*). Hydrochloride *4*, 3.4 g (6.15 mmol), was reacted with 2.02 g (6.15 mmol) of *5* and DIEA (1.18 ml, 6.77 mmol) in 20 ml of DMF at room temperature for 15 h. Additional 0.2 ml of DIEA and 0.16 g of glycine were added to the reaction mixture, and the whole mixture was kept at room temperature for 24 h, then evaporated. The residue eas dissolved in 1-butanol and the solution was washed with acid and alkali. Evaporation of the solvent gave an oil, which was extracted with ethanol to remove some solids. Ethanol solution afforded *6* upon the addition of ether. Yield, 3.6 g (89%). TLC: Rf(III) 0.66. NMR: 1.38(s,9H: Boc), 1.84(s, 3H: Ac), 2.8–3.1(2H: (Cys)Cβ−2H), 3.58(d, 2H: (Gly[1])Cα−2H), 3.7–3.8(6H: 3(Gly)Cα−2H), 4.2–4.3(2H: (Acm) CH_2), 4.62(1H: (Cys)Cα−H), 5.29(s, 2H: CH_2 of p-nitrophenyl), 7.01(t, 1H: (Gly[1])NH), 7.7−8.2(4H: p-nitrophenyl), four triplets for (Gly) NH at 8.04, 8.16, 8.18, and 8.55; 8.48(d, 1H: (Cys)NH).

Boc-Cys(Acm)GlyGlyGlyGlyCys(Acm)-CH_2Ph(p-NO_2-), (*7*). Boc-pentapeptide *6*, 3 g, was treated first with 50% TFA in dichloromethane then with 3M HCl in ethyl acetate. Yield of HCl·H-GlyGlyGlyGly-Cys(Acm)-CH_2Ph(p-NO_2-) was 2.8 g (quantitative). This hydrochloride was reacted with 1.8 g (4.6 mmol) of Boc-Cys(Acm)-OSu[6] and 0.87 ml (5 mmol) of DIEA in 20 ml of dry DMF at room temperature for 15 h. Additional 0.3 ml of DIEA was added to the reaction mixture, the whole mixture was stood for 6 h, and evaporated. The residue was dissolved in 1-butanol and the solution was washed successively with KCl-saturated aq. K_2CO_3, saturated aq. NaCl. The product was crystallized from 1-butanol-saturated water. Yield, 2.74 g (69%); mp. 194–195°C (dec.). Found: C, 44.52; H, 5.65; N, 14.47%. Calcd for $C_{32}H_{47}O_{13}N_9S_2\cdot 2H_2O$: C, 44.38; H, 5.94; N, 14.56%. NMR: 1.38 (s, 9H: Boc), 1.836 (s, 3H: (Acm)CH_3), 1.844 (s, 3H: (Acm)CH_3), 2.88 and 3.04 (a pair of quartet, 2H: (Cys[6])Cβ-2H), 2.67 and 2.91 (a pair of quartet, 2H: (Cys[1])Cβ-2H), 3.7–3.8 (8H: 4(Gly)Cα-2H), 4.18 (q, 1H: (Cys)Cα-H), 4.25 (4H: 2(Acm)CH_2), 4.62 (1H: (Cys)Cα-H), 5.30 (s, 2H: CH_2 of p-nitrobenzyl), 7.02 (d, 1H: (Cys[1])NH), 7.66–8.24 (4H: p-nitrophenyl), 8.1–8.2 and 8.4–8.6 (seven NH protons).

H-Cys(Acm)GlyGlyGlyGlyCys(Acm)-OH, (*8*). Compound *7*, 0.83 g (1 mmol), was dissolved in 12 ml of methanol and saponified with 1.1 ml of 1 M NaOH. After 1 h at room temperature, the solution was neutralized with dil. HCl and evaporated to dryness. The residue was redissolved in water and p-nitrobenzyl alcohol was removed by extraction with ether. The aqueous layer was lyophilized to give 0.7 g of crude free acid. This material was dissolved in chloroform-methanol (80:20) and chromatographed on SiO_2. Elution with chloroform-methanol (60:40) afforded Boc-Cys(Acm)Gly_4-Cys(Acm)-OH which gave a single spot on TLC, Rf(V), 0.45. Yield, 0.6 g. This Boc-hexapeptide acid was treated with 5 ml of 50% TFA in dichloromethane at room temperature for 3 h. The residue which was obtained by evaporation of the mixture was dissolved in ethyl acetate and treated with 3 M HCl in ethyl acetate. Hydrochloride of 8 was filtered and washed with ether. Yield, 0.56 g. The compound was purified by chromatography on a BioRad AG50W-X2 column under the conditions of Schroeder[9]. Amino acid analysis of the air-oxidized hydrolyzate as described[6] gave the value of Gly:Cys_2=1:0.126. Under the same conditions, both of Boc-GlyGlyCys(Acm)-OBzl and Boc-GlyGly-Cys(Acm)-OCH/Ph(p-nitro-) gave the amount of Cys_2 between 0.116 and 0.118. Therefore, when we adopted the value 0.117 as the reference, the amino acid composition of 8 was obtained as Gly:Cys= 4:2.14. NMR: 1.83 (s, 3H: (Acm)CH_3), 1.85 (s, 3H: (Acm)CH_3), 2.7–3.0 (4H: 2(Cys)Cβ-2H), 3.6–3.9 (9H: 4(Gly)Cα-2H), (Cys)Cα-H), 4.2–4.3 (5H: 2(Acm)CH_2, (Cys)Cα-H), 7.82 (d, 1H: (Cys[6])NH), six triplets (1H, each) for four (Gly)NH and two (Acm)NH at 8.18, 8.23, 8.32, 8.49, 8.72, and 8.89.

Boc-GlyCys(Acm)-OBzl, (*9*). Boc-Cys(Acm)-OBzl[10], 3.82 g (10 mmol), was converted to HCl·H-Cys(Acm)-OBzl with the same procedure as for the preparation of *2*. The hydrochloride was neutralized with 2.1 ml of DIEA in 50 ml of DMF and reacted with 1.74 g (10 mmol) of Boc-Gly-OH, 2.1 g of DCC (10 mmol), and 1.62 g (12 mol) of HOBT at 0°C for 15 h. Product *9* was recrystallized from ethyl acetate-ether. Yield, 3.7 g (84%); m.p. 92–93°C. Found: C, 54.54; H, 6.48; N, 9.52%. Calcd for $C_{20}H_{29}O_6N_3S$: C, 54.66; H, 6.65, N, 9.56%. NMR: 1.36 (s, 9H: Boc), 1.81 (s, 3H: (Acm) CH_3), 2.92 (2H: (Cys)Cβ-2H), 3.56 (2H: (Gly)Cα-2H), 4.2 (2H: (Acm) CH_2), 4.56 (1H: (Cys)Cα-H), 5.10 (s, 2H: (Bzl)CH_2), 6.92 (t, 1H: (Gly)NH), 7.3 (5H: Ph), 8.28 (d, 1H: (Cys)NH), 8.46 (t, 1H: (Acm)NH).

Boc-Pro-GlyCys(Acm)-OBzl, (10). To a solution of *9* (4.66 g, 10.6 mmol) in 40 ml of ethyl acetate was added 10 ml of HCl-saturated ethyl acetate. The mixture was stood at room temperature for 2 h, then evaporated. The solid was washed thoroughly with ether to afford 4.0 g of HCl·H-Gly-Cys(Acm)-OBzl. Yield, quantitative. To a suspension of this hydrochloride (4.0 g), in 20 ml of DMF, 2.1 ml of DIEA (1.2 eq)., 2.25 g of Boc-Pro-OH (1.05 eq.), 2.05 g (1.5 eq.) of HOBT, and 2.17 g (1.05 eq.) of DCC in 2 ml of ethyl acetate were added in this order at 0°C. The reaction mixture was kept at 2–3°C overnight, filtered, and evaporated. The residue was dissolved in ethyl acetate and washed with acid and alkali. Compound *10* was recrystallized from ethyl acetate-ether; m.p. 123°C. Yield, 4.5 g (84%). Found: C, 55.94; H, 6.63; N, 10.44%. Calcd for $C_{25}H_{36}O_7N_4S$: C, 55.96; H, 6.76; N, 10.44%. NMR: The spectrum was somewhat complex due to the presence of a cis-trans isomerism about the prolyl peptide bond. 1.35 and 1.39 (two singlets with intensity ratio=57:43, respectively, total 9H: Boc), 1.82 (s, 3H: (Acm)CH_3), 1.7–2.1 (4H: (Pro)Cβ, γ-4H), 2.8–3.0 (2H: (Cys)Cβ-2H), 3.2–3.4 (2H: (Pro)Cδ-2H), 3.65–3.9 (2H: (Gly) Cα-2H), 4.1 (2H: (Acm)CH_2), 4.2 (1H: (Pro)Cα-H), 4.5 (1H: (Cys)Cα-H), 5.14 (s, 2H: (Bzl)CH_2), 7.37 (5H: Ph), 8.06 and 8.16 (two triplets with intensity ratio about 6:4, total 1H: (Gly)NH), 8.28 and 8.43 (two doublets with intensity ratio about 6:4, total 1H: (Cys)NH), 8.52 (t, 1H: (Acm)NH).

Boc-GlyGlyProGlyCys(Acm)-OBzl, (11). Compound *10* (4 g, 7.5 mmol) was dissolved in 50 ml of hot ethyl acetate. The solution was cooled with an ice-bath and 10 ml of HCl-saturated ethyl acetate was added. The hydrochloride separated as an oil solidified on standing at room temperature for 3 h. This hydrochloride was collected and dissolved in 5 ml of DMF and neutralized with 1.55 ml of DIEA at 0°C. To the solution were added 1.52 g (1.5 eq.) of HOBT, 1.74 g (7.5 mmol) of Boc-Gly-Gly-OH, and 1.55 g of DCC in 4 ml of DMF in this sequence at 0°C. The reaction mixture was kept at 2–3°C for 24 h, filtered and evaporated. The residue dissolved in 1-butanol was worked up as described above. The crude products were chromatographed on SiO_2 with chloroform-methanol (95:5) and pentapeptide *11* was recrystallized from methanol-ether; m.p. 105–107°C (dec.). Found: C, 52.88; H, 6.46; N, 12.58%. Calcd for: $C_{29}H_{42}O_9N_6S\cdot 1/2\ H_2O$: C, 52.80; H, 6.57; N, 12.74%. TLC: Rf(I) 0.31.

HCl·H-GlyGlyProGlyCys(Acm)-OBzl, (12). TFA (5 ml) was added to a suspension of 3.5 g (5.4 mmol) of compound *11* in 8 ml of dichloromethane and 1.25 ml of anisole at 0°C. The mixture was kept at room temperature for 2 h. After concentration to a smaller volume, the residue was dissolved in small amount of ethyl acetate and treated with 3M HCl-ethyl acetate at room temperature for 0.5 h. Hydrochloride *12* was filtered and washed with ether. Yield, quantitative. NMR: 1.6–2.2 (4H except Ac: (Pro)Cβ, γ-4H), 1.82 (s, 3H: Ac), 2.8–3.0 (2H: (Cys)Cβ-2H), 3.3–3.9 (6H), 3.9–4.1 (2H), 4.19 (d, 2H: (Acm)CH_2), 4.4–4.6 (2H), 5.08 (s, 2H: (Bzl)CH_2), 7.33 (5H: Ph), 8.0–8.6 (4H: NH); ammonium protons appeared as a very broad signal around 5.4 ppm.

Boc-Cys(Acm)GlyGlyProGlyCys(Acm)-OBzl, (13). Compound *12*, obtained from 3.5 g (5.4 mmol) of *11*, was dissolved in 15 ml of DMF and neutralized with 1.13 ml (1.2 eq.) of DIEA with cooling in an ice-bath. The coupling with Boc-Cys(Acm)-OH (1.73 g, 1.1 eq.) was carried out with 1.16 g (1.05 (eq.) of DCC and 1.1 g (1.5 eq.) of HOBT at −13°C for initial 1 h, then at 0°C overnight. Because the presence of the starting pentapeptide was shown on a TLC plate, additional amounts of reagents (0.7 ml of DIEA, 0.25 g of Boc-Cys(Acm)-OH, and 0.25 g of DCC) were added and the mixture was kept at 2–3°C for 2 days, and finally at room temperature for 24 h. Although the TLC spot, Rf of which was same as that for *12*, still persisted at this stage, the reaction mixture was filtered, evaporated to give the residue which was dissolved in 1-butanol, and worked up as previously described. The product was chromatographed on SiO_2; the desired product was eluted at the concentration of methanol 5 to 7% in chloroform. Yield of the powder from methanol-chloroform having m.p. 83–85°C (dec. at about 100°C) was 2.67 g (60%). Found: C, 50.63; H, 6.35; N, 13.20%. Calcd for $C_{35}H_{52}O_{11}N_8S_2\cdot 1/2\ H_2O$: C, 50.40; H, 6.41; N, 13.44%. NMR: 1.38 (s, 9H: Boc), 1.82 (s, 3H: Ac), 1.85 (s, 3H: Ac), 1.7–2.0 (4H: (Pro)Cβ, γ-4H), 2.6–3.0 (4H: 2(Cys) Cβ-2H), 3.4–3.6 (2H: (Pro)Cδ-2H), 3.6–4.3 (6H: 3(Gly)Cα-2H), 4.2 (t, 1H: (Pro)Cα-H), 4.6 (2H: 2(Cys) Cα-H), 5.14 (s, 2H: (Bzl)-CH_2), 7.01 (d, 1H: (Cys^1)NH), 7.49 (5H: Ph), 7.94, 8.11, and 8.21 (three triplets, each 1H: 3(Gly)-NH), 8.26 (d, 1H: (Cys^6)NH), 8.50 (t, 2H: 2(Acm)NH).

H-CysGlyGlyProGlyCys-OH, (14). Compound *13*, 0.8 g (0.96 mmol), was treated with 10 ml of HF

in the presence of 1 ml of anisole at 0°C for 1 h. The residue obtained after evaporation of HF was dissolved in 50 ml of water (pH was adjusted to ca. 4 by the addition of solid $NaHCO_3$) and washed with ether. To the aqueous layer was added 0.64 g (2 mmol) of mercury (II) acetate and the solution was kept at room temperature for 1 h. The solution was bubbled with H_2S and filtered. The filtrate was diluted to 500 ml, bubbled with oxygen for 18 h in the presence of 10^{-6}M copper (II) sulfate. The solution, after concentration to ca. 50 ml and ajdsted to pH 4 with formic acid, was applied on a column of BioRad AG50W X-2 column and chromatography was carried out under the conditions of Schroeder[9]. Yield, 0.15 g (32%). Amino acid analysis: (1) Direct hydrolysis of *14* gave the value, Gly:Pro-Cys_2=3.0:1.15:0.9. (2) Hydrolysis after reduction of the disulfide bond and pyridylethylation of the resulting thiols with 4-vinylpyridine gave Gly:Pro-Cys=3.0:1.1:1.6. NMR: 1.7–2.2 (4H: (Pro)Cβ, γ-4H), 2.9–3.5 (4H: 2(Cys)Cβ-2H), 3.5–4.1 (8H: 3(Gly)Cα-2H, (Pro)Cδ-2H), 4.03 (t, 1H: (Pro)Cα-H), 4.2 (1H: (Cys)Cα-H), 4.3 (1H: (Cys)Cα-H), 7.66 (d, 1H: (Cys^6)NH), 7.92 (t, 1H: (Gly)NH), 8.54 (t, 1H: (Gly)NH), 9.12 (1H: (Gly)NH); ammonium protons appeared as a very broad signal ranging from 3 to 4.5 ppm.

Boc-GlyGlyCys(Acm)-OBzl, (15). HCl·H-Cys(Acm)-OBzl, 6.2 g (0.0195 mol), which was neutralized with 3.83 ml of DIEA, and Boc-GlyGly-OH (4.64 g, 0.02 mol) in 40 ml of DMF were coupled with DCC (4.33 g, 1.05 eq.)—HOBT (3.25 g, 1.2 eq.). Temperature of the reaction was kept at about −13°C for initial 2 h, then at 2–3°C overnight, and finally at room temperature for 1 day. Compound *15* was recrystallized from ethyl acetate-ether. Yield, 7.7 g (80%); m.p. 110°C. Found: C, 53.14; H, 6.39; N, 11.20%. Calcd for $C_{22}H_{32}O_7N_4S$: C, 53.22; H, 6.50; N, 11.29%. TLC: Rf(I), 0.49.

Boc-ProGlyGlyCys(Acm)-OBzl, (16). Compound *15*, 7.0 g, in a mixture of 50 ml of dichloromethane and 3 ml of anisole was treated with 6 ml of TFA at room temperature for 30 min. The mixture was concentrated and redissolved in 20 ml of ethyl acetate. To the solution was added 20 ml of HCl-saturated ethyl acetate and the mixture was kept at room temperature for 30 min. Evaporation of the solvent and addition of ether afforded 5.6 g (91%) of HCl·H-GlyGlyCys(Acm)-OBzl. A mixture of this hydrochloride (4.35 g, 0.01 mol), Boc-Pro-OSu (3.11 g, 0.01 mol), and DIEA (2.0 ml, 1.15 eq.) was stirred in 30 ml of DMF at room temperature overnight. Since TLC showed the presence of the starting materials, 0.5 ml of DIEA was added to the reaction mixture and the reaction was continued for 24 h. After evaporation of the solvent, the residue taken in ethyl acetate was washed with acid and alkali, then chromatographed on SiO_2. Elution with chloroform-methanol (95:5) afforded 2.8 g of *16* (47%). TLC: Rf(II) 0.63.

Boc-GlyProGlyGlyCys(Acm)-OBzl, (17). Compound *16*, 2.8 g, was dissolved in 40 ml of ethyl acetate and mixed with 20 ml of HCl-saturated ethyl acetate. The solution was kept at room temperature for 1 h and concentrated to a small volume. Addition of dry ether afforded HCl·H-ProGlyGly-Cys(Acm)-OBzl with quantitative yield (2.5 g). This hydrochloride (2.5 g, 4.69 mmol) was mixed with Boc-Gly-OSu (1.275 g, 4.69 mmol) and DIEA (1.0 ml, 1.2 eq.) in DMF (15 ml) under ice-cooling. The mixture was stirred at room temperature for 24 h and worked up as described above. The crude material was chromatographed on SiO_2, elution with 4% methanol in chloroform afforded *17*, which was recrystallized from ethyl acetate, m.p. 115–117°C. Yield, 1.51 g (50%). Found: C, 53.46; H, 6.41; N, 13.01%. Calcd for $C_{29}H_{42}O_9N_6S$: C, 53.52; H, 6.51; N, 12.92%. TLC: Rf(I), 0.23. NMR: 1.37 (s, 9H: Boc), 1.83 (s, 3H: Ac), 1.85–2.0 (4H: (Pro)Cβ, γ-4H), 2.87 (1H: (Cys)Cβ-H), 3.00 (1H: (Cys)Cβ-H), 3.42–3.53 (2H: (Pro)Cδ-2H), 3.70 (d, 2H: (Gly) Cα-2H), 3.72–3.85 (4H: 2(Gly) Cα-2H), 4.22 (t, 2H: (Acm)CH_2), 4.28 (1H: (Pro)Cα-H), 4.58 (1H: (Cys)Cα-H), 5.14 (s, 2H: (Bzl) CH_2), 6.74 (t, 1H: (Gly^1)NH), 7.37 (5H: Ph), 7.98 (t, 1H: (Gly)NH), 8.25 (t, 1H: (Gly)NH), 8.44 (d, 1H: (Cys)NH), 8.53 (t, 1H: (Acm)NH).

Boc-Cys(Acm)GlyProGlyGlyCys(Acm)-OBzl, (18). Compound *17*, 1.35 g, was dissolved in 20 ml of hot ethyl acetate. The solution was cooled to room temperature and was added 20 ml of HCl-saturated ethyl acetate. After 2 h at room temperature, the precipitate was washed out with dry ether. Yield of HCl·H-GlyProGlyGlyCys(Acm)-OBzl was 1.22 g (quantitative). This hydrochloride (1.22 g, 2.08 mmol) dissolved in 10 ml of DMF was neutralized with 0.45 ml (1.2 eq.) of DIEA and reacted with 0.81 g of Boc-Cys(Acm)-OSu at room temperature for 24 h. After working up the reaction mixture, the crude material was dissolved in 3 ml of chloroform and chromatographed on SiO_2. Elution was carried out with $CHCl_3$-MeOH (92.5:7.5, v/v). Yield, 1.1 g (64%). TLC: Rf(II), 0.32; (III),

0.54. Found: C, 50.43; H, 6.37; N, 13.41%. Calcd for $C_{35}H_{52}O_{11}N_8S_2 \cdot 1/2\ H_2O$: C, 50.40; H, 6.41; N, 13.44%. NMR: 1.37 (s, 9H: Boc), 1.84 (s, 3H: Ac), 1.85 (s, 3H: Ac), 1.75–2.1 (4H: (Pro)-Cβ, γ-H), 2.6–2.7 (1H: (Cys)Cβ-H), 2.83–3.03 (3H: (Cys)Cβ-3H), 3.4–4.05 (8H), 4.123–4.33 (6H), 4.55–4.62 (1H: (Cys)Cα-H), 5.14 (s, 2H: (Bzl)CH_2), 7.01 (d, 1H: (Cys^1)NH), 7.83, 8.01, and 8.19 (three t, 1H each: 3(Gly)NH), 8.41 (d, 1H: (Cys^6)NH), 8.50 (t, 1H: (Acm)NH), 8.51 (t, 1H: (Acm)-NH).

S——S

Boc-CysGlyProGlyGlyCys-OBzl, (19). The method of Ref. 10–12 was slightly modified. A solution of S-protected hexapeptide *18* (1.0 g, 1.2 mmol) in a mixture of methanol (200 ml) and water (50 ml) was added dropwise to a stirred iodine solution (1.22 g, 4.8 mmol, in 200 ml of methanol and 50 ml of water). After the addition of the peptide solution was finished, the stirring was continued for 1 h. Excess iodine was reduced by the addition of 350 ml of 0.02 M sodium thiosulfate. The resultant solution was passed through a column of Amaberlite MB-1 (250 ml, washed before use successively with 400 ml of water and 500 ml of methanol-water (4:1, v/v)) and then evaporated to give crystals of *19*. Yield, 0.59 g (89%). The compound was recrystallized from DMF-ethanol; m.p. 240–241°C (dec.). Found: C, 49.64; H, 6.01; N, 11.72%. Calcd for $C_{29}H_{40}O_9N_6S_2 \cdot H_2O$: C, 49.84; H, 6.06; N, 12.03%. NMR: 1.36 (s, 9H: Boc, a satellite peak appeared at 1.38 ppm with an intensity 15.4% of 1.36 ppm peak), 1.7–1.8 (1H: (Pro) Cγ-H), 1.83–1.97 (2H: (Pro) Cβ, γ-2H), 2.13–2.22 (1H: (Pro)Cβ-H), 2.77–2.82 (1H: (Cys)Cβ-H), 2.92–3.02 (2H: (Cys)Cβ-2H), 3.13–3.18 (1H: (Cys)Cβ-H), 3.46–3.67 (4H: (Gly) Cα-2H),; (Pro)Cδ-2H), 3.83–3.96 (2H: (Gly) Cα-2H), 4.1–4.14 (1H: (Pro)Cα-H), 4.21–4.29 (1H: (Gly)Cα-H), 4.33–4.41 ((Gly)Cα-H), 4.37–4.46 (1H: (Cys)Cα-H), 4.98–5.06 (1H: (Cys)Cα-H), 5.16 (q, 2H: (Bzl)CH_2), 7.08 (d, 1H: (Cys^1)NH), 7.36 (5H: Ph), 7.62 (q, 1H: NH), 7.92 (q, 1H: NH), 8.49 (m, 1H: NH), 8.79 (d, 1H: NH).

S——S

Ac-CysGlyProGlyGlyCys-OBzl, (20). Boc-hexapeptide *19*, 1.1 g (1.62 mmol) was converted to HCl·H-CysGlyProGlyGlyCys-OBzl(-SS-) in a similar way as described for the preparation of *16*. The hydrochloride was dissolved in 3 ml of dry DMF and treated with 0.65 ml of triethylamine (the amount was determined to make the solution basic to a wet pH-test paper) and 0.5 g (3.23 mmol) of AcOSu at room temperature overnight. The crystals separated were collected by filtration, the filtrate gave another crop of *20* upon concentration. Yield, 0.81 g (80%); m.p. 249–255°C. Analytical samples which were recrystallized from DMF-H_2O were found to contain 0.44 mol DMF/mol of *20* from NMR analysis (the value was estimated from intensities of 2.72 and 2.88 ppm peaks of DMF). Found: C, 49.29; H, 5.58; N, 13.88%. Calcd for $C_{26}H_{24}O_8N_6S_2 \cdot 1/2\ C_3H_7ON \cdot 1/2\ H_2O$: C, 49.42; H, 5.81; N, 13.62%. NMR: Pro-β-, γ-protons appeared at 1.7–1.8 (1H), 1.85–2.0 (2H), and 2.1–2.21 (1H). 1.85 (s, 3H: Ac), 2.84–3.0 (3H: (Cys)Cβ-H), 3.16–3.23 (1H: (Cys)Cβ-H), 3.48–3.66 (4H: (Gly)Cα-2H, (Pro)Cδ-2H), 3.82–3.93 (2H: (Gly)Cα-2H), 4.13 (1H: (Pro) Cα-H), 4.22–4.36 (2H: (Gly) Cα-2H), 4.73–4.79 (1H: (Cys)Cα-H), 4.98–5.04 (1H: (Cys)Cα-H), 5.16 (s, 2H: (Bzl)CH_2), 7.3–7.4 (5H: Ph), 7.64 (q, 1H: (Gly)NH), 8.04 (q, 1H: (Gly)NH), 8.20 (d, 1H: (Cys^1)NH), 8.51 (t, 1H: (Gly)-NH), 8.79 (d, 1H: (Cys^6)NH).

S——S

Ac-CysGlyProGlyGlyCys-OH, (21). Compound *20*, 0.5 g, was treated with HF (5 ml)-anisole (1 ml) at 0°C for 30 min. After evaporation of HF, the residue was washed with ether, dissolevd in water, and lyophilized. Yield, 0.436 g. Amino acid composition: Pro:Gly:Cys_2=1.00:2.95:1.00. NMR: 1.7–1.8 (1H: (Pro) Cγ-H), 1.85 (s, 3H: Ac), 1.85–1.96 (2H: (Pro) Cβ, γ-2H), 2.13–2.21 (1H: (Pro)-Cβ-H), 2.83–3.0 and 3.14–3.2 (4H: (Cys)Cβ-H), 3.46–3.63 (4H: (Gly) Cα-2H, (Pro) Cδ-2H), 3.82–3.93 (2H: (Gly)Cα-2H), 4.12 (1H: (ProCα-H), 4.24–4.36 (2H: (Gly)Cα-2H), 4.73–4.79 (1H: (Cys)Cα-H), 4.81–4.87 (1H: (Cys)Cα-H), 7.62 (q, 1H: (Gly)NH), 8.06 (q, 1H: (Gly)NH), 8.21 (d, 1H: (Cys^1)-NH), 8.51 (t, 1H: (Gly)NH) 8.67 (d, 1H: (Cys^6)NH).

S——S

H-CysGlyProGlyGlyCys-OH, (22). Peptide benzyl ester *19*, 0.28 g, was treated with 1 ml of anisole and 5 ml of HF and worked up as similarly as in the preparation of *21*. Yield of *22*, 0.21 g. Amino acid composition: Pro:Gly:Cys_2=1.00:2.91:1.03. NMR: 1.72–1.8 (1H: (Pro)Cδ-H), 1.85–2.0 (2H: (Pro)Cβ,δ-2H), 2.13–2.21 (1H: (Pro)-Cβ-H), 2.83–3.0 (3H: (Cys) Cβ-H), 3.19–3.24 (1H: (Cys)Cβ-

H), 3.48–3.65 (4H: (Gly)Cα-2H, (Pro) Cδ-2H), 3.84–3.97 (3H: Cα-H), 4.14 (t, 1H: (Pro) Cα-H), 4.18–4.26 (2H: Cα-H), 4.47–4.54 (1H: (Cys)Cα-H), 7.59 (q, 1H: (Gly)NH), 8.20 (1H: (Gly)NH), 8.55 (d, 1H: (Cys^6)NH), 8.58 (t, 1H: (Gly)NH).

HCl·H-Cys(Acm)-NHMe, (23). Boc-Cys(acm)-OH, 11.7 g, was reacted with 5.4 ml of isobutyl chloroformate and 4.4 ml of N-methylmorpholine in 200 ml of tetrahydrofuran at −18°C for 5 min under stirring. To the solution was added 10 g of solution of methylamine in DMF (4 mmol methylamine/g of the solution), and after 5 min, additional 1 g of the methylamine solution. The temperature of the reaction mixture gradually increased to nearly 0°C during 2 hours due to the consumption of cooling material (ice-salt). The mixture was kept at room temperature overnight and filtered. The filtrate was evaporated *in vacuo* to afford a solid mass of BocCys(acm)NHMe which was washed with diethyl ether and recrystallized from ca. 30 ml of water. M.p. 129–130°C, yield, 10.7 g (88%). This Boc-compound was dissolved in the mixture of 10 ml of dichloromethane, 3 ml of anisole, and 10 ml of TFA, and kept at room temperature for 1 hr. The solution was evaporated and to the residue taken in 50 ml of ethyl acetate was added ca. 20 ml of 3M HCl in ethyl acetate to afford *23*, which was washed with ether. Yield, quantitative.

Boc-Lys(ε-Z)Cys(Acm)-NHMe, (24). The compound *23*, 2.75 g, was neutralized with 2.05 ml of DIEA in 175 ml of DMF, and reacted with 4.35 g of Boc-Lys(Z)-OH, 2.3 g of HOBT, and 2.35 g of DCC in 5 ml of ethyl acetate at −12°C for first two hours, then at 2–5°C overnight. Compound *24* was recrystallized from ehtanol-ethyl acetate. M.p. 158–160°C. Anal. Found: C, 54.90; H, 7.30; N, 12.23 %. Calcd for $C_{26}H_{41}O_7N_5S$: C, 55.01; H, 7.28; N, 12.34%. NMR: 1.36(Boc); 1.83(Acm); 2.55 and 2.58 (amide Me); 1.5–2.95 (Lys protons); ca. 2.8(Cys, Cβ-H); 3.8 (Lys, Cα-H); ca. 4.2 (Acm, CH_2); ca. 4.3 (Cys, Cα-H); 4.96 (Z, benzyl protons); 6.9 (Boc-NH); ca. 7.1 (Lys, ε-NH), 7.27 (Z, aromatic protons); 7.8 (methylamide, NH); 6.9–8.4 (Acm, Cys, NH). TLC: 0.6 (I).

Boc-ProGly-OH, (25). N-Hydroxysuccinimido ester of Boc-proline, 6.24 g, in 70 ml of ethanol was mixed with 1.5 g of glycine and 3.36 g of $NaHCO_3$ in 50 ml water under vigorous stirring. TLC-analysis showed that the reaction completed within 30 min. After overnight stirring, the reaction mixture was evaporated to remove ethanol, and redissolved in saturated aq. NaCl, acidified with 10% aq. citric acid, and extracted with ethylacetate. The ethyl acetate layer was washed five times with saturated aq. NaCl, dried over sodium sulfate, then evaporated. The residue was recrystallized from ethanol-ethyl acetate-cyclohexane to give *25*, m.p. 169–170° (dec.) Yield, 4.5 g (83%). Anal. Found: C, 53.03; H, 7.29; N, 10.23%. Calcd for $C_{12}H_{20}O_5N_2$: C, 52.93; H, 7.40; N, 10.29%. TLC: 0.47 (II).

Boc-ProGlyGlyLys(ε-Z)Cys(Acm)-NHMe, (26). Compound *24* was converted to HCl·H-Lys(Z)-Cys(Acm)-NHMe in the similar way as described for *23* (yield, quantitative). This hydrochloride, 3.8 g, was neutralized with 1.3 ml of DIEA in ca. 40 ml of DMF and reacted with 2.2 g of *25* and 1.65 g of DCC, in the presence of 2.05 g of HOBT, at −10°C. After about 2 hr at −10°C, the mixture was stood in a cold room (2–3°C) for two days with stirring. Since TLC showed the presence of the starting materials, 0.25 ml of DIEA and 0.2 g or DCC were added to the mixture and the reaction was continued at 0°C overnight. The reaction mixture wsa worked up as described above to give *26*, which was precipitated from ethanol-ethyl acetate-ether. Yield, 3.3 g (62%). Anal. Found: C, 55.15; H, 7.08; N, 13.48%. Calcd for $C_{33}H_{51}O_9N_7S$: C, 54.91; H, 7.12; N, 13.59%. NMR: 1.32 and 1.38 (Boc, intensity ratio=9:7. A presence of these two peaks suggests the existence of cis-trans isomerism of prolyl peptide bond.); 1.83 (Ac); Z (4.99 for CH_2; 7.31 for phenyl); 2.55 and 2.59 ((methylamide)CH_3); 4.24 (d, (Acm)CH_2). Other protons at 1.0–2.2 (10H), 2.7–3.1 (3H), 3.1–3.6 (3H), 3.6–3.8 (2H), 4.0–4.5 (5H), 7.2 (t, Z-NH), 7.7–7.9 (1H), 7.9–8.3 (2H), and 8.5 (t, (Acm)NH). TLC: 0.50 (II), the value is close to that of compound *25*, but the spot of *26* develops brown color quickly in iodine fumes, on the other hand, *25* responds only slowly.

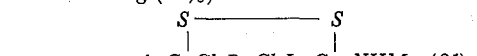
Boc-Glu(γ-Bzl)ProGlyLys(ε-Z)Cys(Acm)-NHMe, (27). To the susepnsion of *26* (2.5 g) in dichloromethane (25 ml), ethyl acetate (5 ml), anisole (2.5 ml), and TFA (2 ml) were added. The clear solution was evaporated immediately and the residue was dissolved in 3 M HCl in ethyl acetate. After 30 min standing at room temperature, precipitates were filtered and washed thoroughly with ether. Yield, 2.4 g (quantitative). The hydrochloride was suspended in 25 ml of DMF, neutralized with 0.45 g of DIEA, and reacted with Boc-Glu (γ-Bzl)-OH (2.22 g, 1.05 eq.) and DCC (0.75 g, 1.05 eq.)

in the presence of HOBT (0.7 g, 1.4 eq.) at ca. −10°C for 1 hr. The reaction was continued overnight at 2–3°C and the mixture was worked up as usual. The compound *27* was precipitated from ethanol-ether. Yield, 2.1 g (65%). Anal. Found: C, 56.74; H, 6.71; N, 11.51%. Calcd for $C_{45}H_{64}O_{12}N_8S\cdot 1/2H_2O$: C, 56.88; H, 6.90; N, 11.80%.

Boc-Cys(Acm)Glu(γ-Bzl)ProGlyLys(ε-Z)Cys(Acm)-NHMe, (28). The Boc group of the compound *27* was removed by the treatment with a combination of TFA and HCl/ ethyl acetate as in the preparation of *23*. The compound, HCl·H-Glu(Bzl)ProGlyLys(Z)Cys(Acm)-NHMe (1.44 g, 1.64 mmol) was neutralized with DIEA (0.60 ml, 1.05 eq.) and coupled to Boc-Cys(Acm)-OH (1.01 g, 1.05 eq.) with DCC (0.71 g, 1.05 eq.)-HOBT (0.67 g, 1.5 eq.) in 25 ml of DMF. The coupling reaction was carried out at first at −15°C for about two hours, then at about 2°C for 20 hr, and for further 12 hr after additional introduction of 0.05 ml of DIEA. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate—n-BuOH (1:1, v/v) and washed with acid and alkali as described previously. As the product showed a minor ninhydrin-positive spot on TLC at Rf=0.33 in addition to a main spot at Rf=0.41 (solvent IV), silica gel chromatography (Merck, Lobar column) with chloroform-methanol (100:7, v/v) was applied to obtain a pure *28*. Yield, 1.4 g (77%). Anal. Found: C, 54.34; H, 6.54; N, 12.38%. Calcd for $C_{51}H_{74}O_{14}N_{10}S_2\cdot 1/2H_2O$: C, 54.48; H, 6.72; N, 12.46%. NMR: 1.1–2.2 (12H, except for Boc and Ac: (Pro) Cβ, γ-4H), (Glu) Cβ-2H, (Lys) Cβ, γ, δ-6H), 1.34 (Boc); 1.83 (6H: 2Ac); 2.55 and 2.60 (3H: (methylamide)CH_3); 4.98 (2H: (Z)CH_2); 5.07 (2H: (Bzl)CH_2); 6.99 (d, 1H: (Boc)-NH); 7.31 (phenyl protons of Z); 7.32 (phenyl protons of Bzl); 8.48 (t, 2H: 2(Acm)-NH).

Ac-Cys(Acm)Glu(γ-Bzl)ProGlyLys(ε-Z)Cys(Acm)-NHMe, (29). The compound *28*, 1.0 g, was dissolved in a mixture of dichloromethane (5 ml), anisole (3 ml), and TFA (4 ml), and kept at room temperature for 1 hr. The residue obtained after evaporation of volatile materials was redissolved in 50 ml of ethyl acetate and was added 20 ml of HCl-saturated ethyl acetate. The hydrochloride (0.91 g) was dissolved in 4 ml of DMF and neutralized with triethylamine until pH 8 on a wet indicator paper. The solution was mixed with 0.25 g of AcOSu and 0.25 ml of triethylamine and stood at room temperature for 1 day. Evaporation of DMF gave the residue which was dissolved in 1-butanol and washed with water, the organic phase was concentrated *in vacuo*. The residue was redissolved in 1-butanol and the insoluble inorganic substances were removed by filtration. Addition of ether to the residue obtained by evaporation of the solvent gave a crude product which was dissolved in a mixture of chroloform (8 ml), AcOH (1 ml), and methanol (0.5 ml) and chromatographed on SiO_2 (Merck, Lichroprep, 25–40 μm, 30×400 mm) with chloroform—methanol (95:5, 1.5 l, then 90:10, 1.5 l). The fraction containing *29* were collected and evaporated. Yield, 0.563 g (58%). Anal. Found: C, 53.52; H, 6.34; N, 12.94%. Calcd for $C_{48}H_{68}O_{13}N_{10}S_2\cdot H_2O$: C, 53.61; H, 6.56; N, 13.03%. TLC: Rf(II), 0.26; (IV), 0.35. NMR: 1.2–1.3 (2H), 1.35–1.4 (2H), 1.5–1.55 (1H), 1.6–1.7 (1H), 1.75–1.9 (4H, except for Ac), 1.84 (s, 9H: 3Ac), 1.95–2.05 (2H), 2.4–2.5 (2H), 2.58 (d, 3H: (methylamide)CH_3), 2.6–2.67 (q, 1H), 2.7–2.77 (q, 1H), 2.82–2.87 (1q, 1H), 2.87–2.93 (q, 1H), 2.93–3.1 (4H), 3.5–3.6 (2H), 3.71 (t, 1H), 4.13–4.2 (1H), 4.21 (d, 4H: 2(Acm)CH_2), 4.23–4.3 (2H), 4.31–4.38 (1H), 4.4–4.52 (1H: (Cys)Cα-H), 4.52–4.58 (1H: (Cys)Cα-H), 5.00 (s, 2H: (Z)CH_2), 5.099 and 5.091 (two singlets, nearly equal intensities, total 2H: (Bzl)CH_2. These two peaks may reflect the presence of a locked benzyl group or an isomerism about a prolyl peptide bond), 7.20 (t, 1H: (Lys) Cε-NH), 7.32 (10H: 2Ph), 7.76 (q, 1H: (methylamide)NH), 7.84 (d, 1H), 8.06 (d, 1H), 8.12 (t, 2H: 2(Acm) NH), 8.19 (t, 1H: (Gly)NH), 8.52 (two doublets, 2H).

Ac-CysGlu(γ-Bzl)ProGlyLys(ε-Z)Cys-NMHe, (30). The *hexapeptide 29* (0.4 g, 0.379 mmol) was dissolved in 125 ml of MeOH-H_2O (4:1, v/v) and the oxidative deprotection of S-Acm groups were achieved with 0.385 g of iodine in a similar way as described for the preparation of *19*. Yield, 0.29 g (85%).



Ac-CysGluProGlyLysCys-NHMe, (31). Protected hexapeptide disulfide *30*, 0.25 g, was treated with 5 ml of HF in the presence of 1 ml of anisole at 0°C for 1 h. The yield of *31* was 0.188 g (97%). Amino acid composition: Glu:Pro:Gly:Cys_2:Lys=1.14:1.06:1.00:1.00:0.99. NMR: 1.15–1.35 (2H), 1.45–1.7 (4H), 1.75–1.95 (4H, except for Ac), 1.85 (s, 3H: Ac), 2.0–2.1 (1H), 2.1–2.2 (3H), 2.60 (d,

3H: (methylamide)CH_3), 2.62–2.8 (2H), 2.82–3.13 (four quasiquartets, 4H: 2(Cys)Cβ-2H), 3.4–3.5 (1H), 3.57–3.62 (1H), 3.78–3.83 (1H), 3.9–3.98 (1H), 4.12 (t, 1H), 4.29–4.35 (1H), 4.38–4.43 (1H), 4.5–4.56 (1H), 4.64–4.7 (1H), 7.72 (d, 1H), 7.93 (q, 1H: (methylamide)NH), 7.97 (d, 1H), 8.19 (d, 1H: (Cys^1)NH), 8.43–8.46 (1H), 8.89 (t, 1H: (Gly)NH). Protons of ε-ammonio group of Lys were observed as a very broad signal ranging from 3.5 to 4.8 ppm.

Boc-Glu(γ-Bzl)Cys(Acm)-NHMe, (32). *23*, 3.02 g (0.013 mol), was dissolved in 150 ml of DMF and neutralized with 2.35 ml (0.0135 mol) of DIEA at 0°C. Boc-Glu (γ-Bzl)-OH (4.55 g, 0.0135 mol), and HOBT (2.8 g, 0.021 mol) were added and 2.78 g (0.135 mol) of DCC in 10 ml of DMF was introduced into the solution at −10°C. The reaction mixture was kept at about −10°C for 1 h, at 2–3°C overnight, and worked up as usual. Compound *32* was recrystallized from ethanol-ether; m.p. 138–140°C. Yield, 4.1 g (62%). TLC: Rf(I), 0.54. Found: C, 55.24; H, 6.85; N, 10.73%. Calcd for $C_{24}H_{36}O_7SN_4$: C, 54.95; H, 6.92; N, 10.68%. NMR: 1.39 (s, 9H: Boc), 1.85 (s, 3H: Ac), 1.9 (2H: (Glu)Cβ-2H), 2.4 (2H: (Glu) Cγ-2H), 2.58 (d, 3H: (methylamide) CH_3), 2.8 (2H: (Cys) Cβ-2H), 4.0 (1H: (Glu)Cα-H), 4.21 (d, 2H: (Acm)CH_2), 4.4 (1H: (Cys)Cα-H), 5.11 (s, 2H: (Bzl)CH_2), 7.0 (d, 1H: (Glu)NH), 7.38 (5H: Ph), 7.8 (1H: (methylamide)NH), 8.0 (d, 1H: (Cys)NH), 8.4 (t, 1H: (Acm)NH).

Boc-ProGlyGlu(γ-Bzl)Cys(Acm)-NHMe, (33). Compound *32* was treated with HCl/ethyl acetate in a similar way as descrived for *2*. Yield of HCl·Glu (Bzl)Cys(Acm)-NHMe was 3.5 g (quantitative). This hydrochloride (3.5 g, 7.6 mmol) dissolved in DMF (30 ml) was neutralized with 1.35 ml of DIEA and coupled to 2.1 g (7.7 mmol) of *25* with DCC (1.6 g, 7.6 mmol) and HOBT (1.6 g, 11.8 mmol) at −10°C for first 1 h, then at 2–3°C for 36 h. The product obtained after a usual workup procedure was recrystallized from ethanol-ether; m.p. 187–191°C. TLC: Rf(I), 0.32. Found: C, 54.66; H, 6.75; N, 12.42%. Calcd for $C_{31}H_{46}O_9N_6S$: C, 54.85; H, 6.83; N, 12.38%. NMR (distinct signals): 1.35 (s, 9H: Boc), 1.85 (s, 3H: Ac), 2.4 (2H: (Glu) Cγ-2H), 2.59 (d, 3H: (methylamide)CH_3), 4.2 (d, 2H: (Acm)CH_2), 5.10 (s, 2H: (Bzl)CH_2), 7.38 (5H: PH), 7.78 (q, 1H: (methylamide)NH), 8.1 (3H), 8.48 (t, 1H: (Acm)NH).

Boc-Lys (ε-Z) ProGlyGlu (γ-Bzl)Cys (Acm)-NHMe, (34). Compound *33*, 3.0 g (4.42 mmol), was converted to HCl·H-ProGlyGlu(Bzl)Cys(Acm)-NHMe as described in the preaparation of *23*. Yield, quantitative. This hydrochloride, 2.75 g (4.42 mmol), was neutralized with 0.72 ml of DIEA in 30 ml DMF at 0°C and mixed with 1.28 g (4.42 mmol) of Boc-Lys (ε-Z)-OH and 0.9 g (6.63 mmol) of HOBT. The reaction with 0.92 g of DCC in 5 ml of DMF was carried out at −10°C for 1 h, and at 2–3°C afterwards. After 12 h from the start of the reaction, 0.5 ml of DIEA was added to the reaction mixture and the reaction was continued for 24 h. The filtrate was worked up in the usual manner to afford 2.8 g (66%) of *34* (the product was precipitated from ethyl acetate solution by the addition of ether); m.p. 98–100°C. TLC: Rf (II), 0.58. Found: C, 57.45; H, 6.79; N, 11.81%. Calcd for $C_{45}H_{64}O_{12}N_8S$: C, 57.43; H, 6.86; N, 11.91%. NMR (distinct peaks): 1.34 (s, 9H: Boc), 1.83 (s, 3H: Ac), 2.32–2.39 (2H: (Glu)Cγ-2H), 2.47 (d, 3H: (methylamide)CH_3), 4.21 (d, 2H: (Acm) CH_2), 4.95 (s, 2H: (Z)CH_2), 5.02 (s, 2H: (Bzl)CH_2), 6.81 (d, 1H: (Lys)NH), 7.20 (1H: (Lys)Cα-NH), 7.3 (5H: PH), 7.32 (5H: Ph), 7.7–8.0 (2H), 8.05–8.3 (2H), 8.48 (t, 1H: (Acm)NH).

Boc-Cys (Acm)Lys (ε-Z)ProGlyGlu (γ-Bzl)Cys (Acm)-NHMe, (35). To a solution of compound *34*, 2.42 g (2.57 mmol) in 50 ml of ethylacetate, was added 25 ml of HCl-saturated ethyl acetate. Solid mass of HCl·H-Lys(Z)ProGlyGlu(Bzl)Cys(Acm)-NHMe was washed out with ether, dried, dissolved in 25 ml of DMF, and neutralized with 0.45 ml (2.58 mmol) of DIEA. DCC reaction (Boc-Cys(Acm)-OH, 0.79 g, 1.05 eq.; HOBT, 0.52 g, 1.5 eq.; DCC, 0.56 g, 1.05 eq.) was carried out at −13°C for first 1 h, then at 2–3°C afterwards. After 24 h, 0.15 g of Boc-Cys(Acm)-OH, 0.15 ml of DIEA, and 0.11 g of DCC were added to the reaction mixture and the reaction was continued for further 48 h. The residue obtained after evaporation of the solvent was taken in 1-butanol and washed as described above. The crude hexapeptide was chromatographed on SiO_2, which was eluted with $CHCl_3$-MeOH (10: 1, v/v). Yield, 1.28 g (43%); m.p. 148–150°C. TLC: Rf (IV), 0.60. Found: C, 54.51; H, 6.68; N, 12.38%. Calcd for $C_{51}H_{74}O_{14}N_{10}S_2 \cdot 1/2H_2O$: C, 54.48; H, 6. 72; N, 12.46. NMR: 1.38 (s, 9H: Boc), 1.2–1.55 (6H), 1.6–1.7 (1H), 1.8–1.86 (2H), 1.9–2.05 (3H), 2.41 (t, 2H: (Glu)Cγ-2H), 2.58 (d, 3H: (methylamide)CH_3), 2.6–2.95 (4H: 2(Cys)Cβ-H), 2.97 (2H: (Lys)Cε-2H), 3.5–3.57 (1H), 3.6–3.66 (1H), 3.69–3.75 (2H), 4.1–4.2 (2H), 4.22 (d, 2H: (Gly)Cα-H), 4.25–4.4 (4H), 4.44–4.45 (1H), 5.00 (s, 2H: (Bzl)CH_2), 5.09 (s, 2H: (Bzl)CH_2), 6.96 (d, 1H: (Cys^1)NH), 7.21 (t, 1H), 7.77 (q, 1H), 7.83 (d, 1H),

7.92 (d, 1H), 8.17 (d and t, 2H), 8.51 (two t, 2H: (Acm)NH).

Ac-Cys(Acm)Lys(ε-Z)ProGlyGlu(γ-Bzl)Cys(Acm)-NHMe, (*36*). HCl·H-Cys (Acm) Lys (Z) ProGly-Glu(Bzl)Cys(Acm)-NHMe, 0.79 g (0.756 mmol), which was derived from 0.96 g of *35*, was dissolved in 5 ml of DMF-0.21 ml triethylamine (2 eq.) and reacted with 0.18 g (1.5 eq.) of AcOSu at 0°C for 2 days. The mixture was concentrated to a smaller volume and water was added to separate 0.83 g (91%) of *36*; m.p. 183–185°C. Found: C, 54.04; H, 6.43; N, 13.01%. Calcd for $C_{48}H_{68}O_{13}N_{10}S_2 \cdot 1/2\ H_2O$: C, 54.07; H, 6.52; N, 13.14%. NMR (distinct peaks): 1.84 (s, 9H: 3Ac), 2.58 (d, 3H: (methylamide)-CH_3), 4.98 (s, 2H: (Z)CH_2), 5.06 (s, 2H: (Bzl)CH_2), 7.2 (2H: 2NH), 7.3 (5H: PH), 7.32 (5H: Ph), 7.78 (q, 1H: (methylamide)NH), 7.95 (1H), 8.0–8.3 (3H), 8.48 (t, 2H: 2(Acm)NH).

Ac-CysLys(ε-Z)ProGlyGlu(γ-Bzl)Cys-NHMe, (*37*). A solution of 0.5 g (0.48 mmol) of *36* in 150 ml of MeOH-H_2O (4: 1) was treated with 0.48 g of iodine in a similar way as described in the preparation of *19*. Yield of *37*, 0.4 g (93%). TLC: Rf (III), 0.31. Found: C, 54.25; H, 5.99; N, 12.12%. Calcd for $C_{42}H_{56}O_{11}N_8S_2 \cdot H_2O$: C, 54.18; H, 6.28; N, 12.04%. NMR: 1.28–1.40 (4H), 1.43–1.52 (1H), 1.57–1.67 (1H), 1.74–1.91 (3H, except for Ac), 1.85 (3H: Ac), 2.0–2.14 (3H), 2.22–2.42 (2H: (Glu)-Cγ-2H), 2.61 (d, 3H: (methylamide)CH_3), 2.82–2.92 (2H: (Cys) Cβ-2H), 2.93–2.98 (2H: (Lys) Cε-2H), 3.02–3.08 (1H: (Cys)Cβ-H), 3.10–3.16 (1H: (Cys)Cβ-H), 3.39–3.51 (2H: (Pro)Cδ-2H), 3.75–3.80 (1H: (Glu) or (Lys)Cα-H, coupled to 7.81 ppm signal), 3.97–4.03 (1H: (Glu) or (Lys)Cα-H, coupled to 8.09 ppm signal), 4.11 (t, 1H: (Pro)Cα-H), 4.4–4.49 (2H: (Gly)Cα-2H), 4.63–4.68 (1H: (Cys)Cα-H), 4.72–4.77 (1H: (Cys)Cα-H), 4.99 (s, 2H: (Z)CH_2), 5.06 (d, 2H: (Bzl)CH_2, see a comment for *29*), 7.19 (t, 1H: (Lys)ε-NH), 7.27–7.37 (10H: 2Ph), 7.81 (d, 1H: (Glu) or (Lys) NH), 8.03 (q, 1H: (methylamide)NH), 8.09 (d, 1H: (Glu) or (Lys)NH), 8.21 (d, 1H: (Cys) NH), 8.26 (d, 1H: (Cys)NH), 8.70 (q, 1H: (Gly)NH).

Ac-CysLysProGlyGluCys-NHMe, (*38*). Compound *37*, 0.4 g, was treated with 5 ml HF and 1 ml of anisole at 0°C for 30 min and the product *38* was lyophilized from water. Yield, 0.32 g. Amino acid analysis: Pro: Glu: Gly: Lys: Cys_2=1.00: 1.08: 0.98: 1.00: 0.91. NMR: 1.3–1.7 (6H), 1.75–1.97 (4H, except Ac), 1.85 (s, 3H: Ac), 2.0–2.2 (4H), 2.59 (d,3H: (methylamide)CH_3), 2.74 (2H: (Lys)-Cε-2H), 2.88–2.93 (1H: (Cys)Cβ-H), 3.01–3.08 (1H: (Cys)Cβ-H), 3.10–3.21 (2H: (Cys)Cβ-2H), 3.38–3.44 (1H: (Pro)Cδ-H), 3.46–3.52 (1H: (Pro)Cδ-H), 3.74–3.80 (1H: (Lys)Cα-H), 4.00–4.08 (1H: (Glu)Cα-H), 4.12 (t, 1H: (Pro)Cα-H), 4.31–4.37 (1H: (Cys)Cα-H), 4.38–4.44 (2H: (Gly)Cα-2H), 4.56–4.61 (1H: (Cys)Cα-H), 7.87 (d, 1H: (Glu) or (Lys)NH), 7.89 (d, 1H: (Glu) or (Lys)NH), 8.02 (q, 1H: (methylamide)NH), 8.10 (d, 1H: (Cys^1)NH), 8.75 (d, 1H: (Cys^6)NH), 8.78 (q, 1H: (Gly)NH). Lys-ε-ammonium protons appeared as a broad signal ranging from 4.0 to 4.7 ppm.

Boc-ValCys(Acm)-NHMe, (*39*). Compound *23*, 5 g (0.0205 mol), was suspended in 200 ml of DMF and warmed. When most of the hydrochloride was dissolved, the mixture was cooled, neutralized with 3.65 ml (0.021 mol) of DIEA, and added 4.56 g (0.021 mol) of Boc-Val-OH and 4.3 g (1.5 eq.) of HOBT. Addition of DCC (4.33 g, 0.021 mol) was carried out at −10°C, the mixture was stood at 2–3°C for 2 days, filtered, and the solvent was evaporated. The residue taken in wet 1-butanol was washed with aq. citric acid and aq. hydrogen carbonate several times. These aqueous washes were extracted with ethyl acetate which was combined with the 1-butanol layer. The combined organic phase was dried and evaporated to afford 5.5 g (66%) of *39*; m.p. 159–160°C. Found: C, 50.33; H, 7.98; N, 13.91%. Calcd for $C_{17}H_{32}O_5N_4S$: C, 50.47; H, 7.97; N, 13.85%.

Boc-ProGlyValCys(Acm)-NHMe, (*40*). HCl·H-ValCys(Acm)-NHMe, 3.8 g (0.011 mol), which was obtained from 4.5 g (0.011 mol) of *39* by the method described for *23* was dissolved in 150 ml of DMF, neutralized with 1.96 ml (0.011 mol) of DIEA, and reacted with 3.03 g (0.011 mol) of *25*, 2,28 g of DCC, and 2.26 g (1.5 eq.) of HOBT. DCC reaction was carried out at first at −10°C for 2 h, then at 2–3°C for 24 h, and the mixture was filtered. The filtrate was concentrated, the residue taken in 1-butanol was worked up as described above. Yield, 5.2 g (84%); m.p. 222–223°C (EtOH-Et_2O). Found: C, 51.03; H, 7.52; N, 14.88%. Calcd for $C_{24}H_{42}O_7N_6S \cdot 1/4\ H_2O$: C, 51.18; H, 7.61; N, 14.92%. NMR: 0.9 (6H: (Val)Cγ-6H), 1.34 (9H: Boc), 1.83 (s, 3H: Ac), 1.8 (4H: (Pro)Cβ, γ-4H), 2.0 (1H: (Val)Cβ-H), 2.57 (d, 3H: (methylamide)CH_3), 2.8 (2H: (Cys)Cβ-2H), 3.3 (2H: (Pro)Cδ-2H), 3.7 (2H: (Gly)Cα-

2H), 3.9–4.5 (3H except (Acm)CH_2), 4.19 (d, 2H: (Acm)CH_2), 7.7 (2H), 8.2 (2H: (Gly)NH, (methylamide) NH), 8.49 (t, 1H: (Acm)NH).

HCl·H-ProGlyValCys(Acm)-NHMe, (41). Compound *40*, 4 g (7.2 mmol), was deblocked by a similar method described for *23*. Yield of *41*, 3.2 g; m.p. 225-226°C (recrystallized from DMF). Found: C, 45.71; H, 7.07; N, 17.00%. Calcd for $C_{19}H_{35}O_5N_6SCl$: C, 46.09; H, 7.13; N, 16.98%.

Boc-ValProGlyValCys(Acm)-NHMe, (42). To a suspension of 3.0 g (6.1 mmol) of *41* in 100 ml of DMF was added 1.14 ml (6.5 mmol) of DIEA, Boc-Val-OH (1.4 g, 6.4 mmol), HOBT (1.3 g, 9.6 mmol), and DCC (1.33 g, 6.4 mmol, addition at −10°C). After the mixture was stood at 2–3°C for 48 h, 0.28 g of Boc-Val-OH and 0.27 g of DCC were added, and the whole mixture was worked up after 24 h at 0°C as described in the preparation of *15*. Yield of powders of *41* from ethanol-ethyl acetate was 3.29 g (82%). TLC: Rf (I), 0.34; (II), 0.56. Found: C, 52.50; H, 7.58; N, 14.45%. Calcd for $C_{29}H_{51}O_8N_7S\cdot 1/4\ H_2O$: C, 52.59; H, 7.84; N, 14.81%. NMR; 0.8–0.9 (12H: 2(Val)Cγ-6H), 1.55 (s, 9H: Boc), 1.6–2.2 (6H except for Ac: (Pro)Cβ, γ-4H, 2(Val)Cβ-H), 1.82 (3H: Ac), 2.57 (d, 3H: (methylamide) CH_3), 2.7–2.9 (2H: (Cys)Cβ-2H), 3.2–3.4 (2H: (Pro)Cδ-2H), 3.6–4.5 (4H, except for (Acm)CH_2: Cα-protons other than Gly), 4.19 (d, 2H: (Acm)CH_2), 6.79 (d, 1H: (Val^1)NH), 7.6–7.9 (2H), 8.0–8.3 (2H), 8.49 (t, 1H: (Acm)NH).

Boc-Cys(Acm)ValProGlyValCys(Acm)-NHMe, (43). HCl·H-ValProGlyValCys(Acm)-NHMe, which was prepared from 2.9 g (4.4 mmol) of *42* in a similar manner as for *23*, was dissolved in 20 ml of DMF and neutralized with 0.80 ml (1.05 eq.) of DIEA. Boc-Cys(Acm)-OH (1.35 g, 1.05 eq.) was coupled to this peptide with DCC (0.95 g, 1.05 eq.)—HOBT (0.89 g, 1.5 eq.) at first at about −10°C for 2 h then at 0°C for 48 h with intermittent addition of DCC and Boc-Cys(Acm)-OH (total 95 mg and 135 mg, respectively). The residue after concentration of the filtrate was dissolved in 1-butanol and washed successively with aq. citric acid and 3% hydrogen carbonate–0.01 M NaOH. The crude product was chromatographed on SiO_2 with $CHCl_3$–MeOH (100:7). Yield, 1.5 g (41%). Found: C, 49.56; H, 7.15; N, 14.84%. Calcd for $C_{35}H_{61}O_{10}N_9S_2\cdot H_2O$: C, 49.45; H, 7.47; N, 14.83%. NMR: 0.8–0.92 (12H: 2(Val)Cγ-H), 1.35 (9H: Boc), 1.77–1.88 (2H: (Pro) Cβ-2H), 1.9–2.1 (4H: (Pro)-Cγ-2H, 2(Val)Cβ-H), 2.59 (d, 3H: (methylamide)CH_3), 2.6–2.91 (4H: 2(Cys)Cβ-2H), 3.55–3.61 (1H), 3.65–3.81 (3H), 4.10–4.23 (5H), 4.26–4.40 (4H), 7.01 (S, 1H: (Cys^1)NH), 7.64 (d, 1H: (Val)-NH), 7.69 (d, 1H: (Val)NH), 7.77 (q, 1H: (methylamide)NH), 8.15 (d, 1H: (Cys^6)NH), 8.18 (t, 1H: (Gly)NH), 8.50 (t, 1H: (Acm)NH), 8.51 (t, 1H: (Acm)NH).

Ac-Cys(Acm)ValProGlyValCys(Acm)-NHMe, (44). A mixture of HCl·H-Cys(Acm)ValProGlyVal-Cys(Acm)-NHMe, which was prepared from 1.25 g (1.1 mmol) of compound *43* in the similar way as described for *23*, triethylamine (0.32 ml) and AcOSu (0.36 g, 2.3 mmol) in 20 ml of DMF was stirred at room temperature for 48 h. Evaporation of DMF afforded a residue, which was dissolved in ethanol. Precipitates obtained by an addition of ethyl acetate were dissolved in water (a small volume of MeOH was added to make the solution homogeneous) and the solution was passed through a column of Amberlite MB-1. Evaporation of the solvent afforded 0.82 g (71%) of *44*, which was recrystallized from EtOH-Et_2O; m.p. 197–198°C. TLC: Rf(III), 0.54. Found: C, 48.63; H, 6.98; N, 15.81%. Calcd for $C_{32}H_{55}O_9N_9S_2\cdot H_2O$: C, 48.53; H, 7.26; N, 15.92%. NMR: 0.82–0.91 (12H: 2(Val)Cγ-H), 1.846 (s, 3H: Ac), 1.854 (s, 3H: Ac), 1.860 (s, 3H: Ac), 1.77–2.1 (6H, except three Ac: (Pro)Cβ,γ-4H, 2(Val)Cβ-H), 2.59 (d, 3H: (methylamide)CH_3), 2.6–2.9 (4H: 2(Cys)Cβ-2H), 3.54–3.61 (1H), 3.68–3.81 (3H), 4.10–4.23 (4H), 4.28–4.34 (3H), 4.35–4.40 (1H: (Cys)Cα-H), 4.51–4.58 (1H: (Cys)Cα-H), 7.65 (d, 1H: (Val)NH), 7.77 (q, 1H: (methylamide) NH), 7.83 (d, 1H: (Val)-NH), 8.13 (d, 1H: (Cys)NH), 8.16 (d, 1H: (Cys)NH), 8.19 (t, 1H: (Gly)NH), 8.59 (t, 1H: (Acm)-NH), 8.52 (t, 1H: (Acm)NH).

Ac-CysValProGlyValCys-NHMe, (45). A solution of Acm-peptide *44*, 0.5 g (0.646 mmol), in a mixture of 200 ml MeOH and 50 ml H_2O was treated with iodine (0.66 g, 2.6 mmol) and worked up in a similar way as described for *19*. After treatment with Amberlite MB-1, concentration of the solution afforded 0.33 g (80%) of *45*, which was recrystallized from DMF-H_2O; m.p. 262–264°C. Found: C, 48.91; H, 6.67; N, 15.57%. Calcd for $C_{26}H_{43}O_7N_7S_2\cdot 1/2\ H_2O$: C, 48.88; H, 6.94; N, 15.35%. Amino acid composition: Pro:Gly:Val:Cys_2=1:0.99:1.78:0.93. NMR: 0.76–0.95 (a pair of quartet, 12H: 2(Val)Cγ-H), 1.76–1.91 (2H, except Ac: (ProCγ-2H), 1.87 (s, 3H: Ac), 2.0–2.2 (4H: (Pro)-Cβ-2H, 2(Val)Cβ-H), 2.62 (d, 3H: (methylamide) CH_3), 2.83–3.03 (4H: 2(Cys)Cβ-2H), 3.3–3.56

(2H: (Pro)Cδ-2H), 3.85–3.91 (1H), 4.08–4.26 (3H), 4.37–4.41 (1H), 4.63–4.69 (1H), 4.81–4.87 (1H), 7.65 (d, 1H), 8.02 (q, 1H: (methylamide)NH), 8.05 (d, 1H), 8.07 (d, 1H), 8.29 (d, 1H: (Cys^1)-NH), 8.67 (broad, 1H).

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