Synthetic Study on Peptide Antibiotic Nisin. IV. Synthesis of Ring D-E[1]

Koichi FUKASE, Yoshiaki ODA, Akira KUBO, Tateaki WAKAMIYA,* and Tetsuo SHIBA†
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560
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In the series of synthetic study on peptide antibiotic nisin, a bicyclic sulfide part ring D-E in this molecule was successfully synthesized. Conjunctive sulfide rings were constructed either by one step or stepwise desulfurization reaction from corresponding disulfides by use of hexaethylphosphorus triamide. Thus, all of the ring moieties in nisin were prepared for total synthesis of this antibiotic.

An antibacterial activity of nisin was found in culture broth of *Streptococcus lactis* before the discovery of penicillin,[2] although the isolation was delayed until the work by Mattick in 1947.[3] Nisin is widely used as a food preservative in Europe because of its antibacterial activity particularly against *Clostridium botulinum*.[4] Its unique structure composed of 34 amino acid residues was proposed by Gross in 1971 as shown in Fig. 1.[5] It contains three dehydro amino acids and five cyclic sulfide parts which are composed of *meso*-lanthionine residue in ring A and *threo*-methyllanthionine residues in rings B to E, respectively.

From standpoint of synthetic interest for such a unique structure as well as need of confirmation of the proposed structure, we started the synthetic study of nisin, and already finished syntheses of the rings A, B, and C.[6-8] In these previous studies, we developed a versatile method for the synthesis of lanthionine peptide by desulfurization[9] from the corresponding disulfide peptide by use of hexaethylphosphorus triamide [$P(NEt_2)_3$]. Particularly, in the synthetic studies of rings B and C containing *threo*-methyllanthionine residue, we revealed that these peptides could be prepared from disulfide peptides involving L-cysteine and *threo*-3-methyl-D-cysteine, where the configuration on β-carbon atom of *threo*-3-methyl-D-cysteine was retained in the course of desulfurization reaction.[7,8]

Among the five ring moieties in nisin, ring D-E part seems to be most difficult to be synthesized because of its unique bicyclic structure composed of two sulfide bridges (Fig. 2). On the basis of the above desulfurization reaction, we considered two possible synthetic routes as shown in Fig. 3. The first approach is a simultaneous construction of both sulfide rings from bis-disulfide peptide by one step desulfurization [method (A)]. On the other hand, second method is based on a stepwise construction of these sulfide rings [method (B)]. Method (A) seems to be advantageous in view of less reaction steps although the rearrangement of the rings would occur more possibly during the desulfurization reaction. On the other hand, a more secure formation of bicyclic sulfide structure could be expected in method (B). In fact, according to each strategy, we attempted to synthesize conjunctive ring D-E moiety as shown in Figs. 4 and 5, respectively.

For protection of thiol groups of four mercapto-amino acids, we chose both trityl (Trt) and acetamidomethyl (Acm) groups in view of differentiation of their removals.[10] The amino group and the *C*-terminal carboxyl group were protected with *t*-butoxycarbonyl (Boc) group and methyl (Me) ester respectively. The imidazole ring in histidine residue

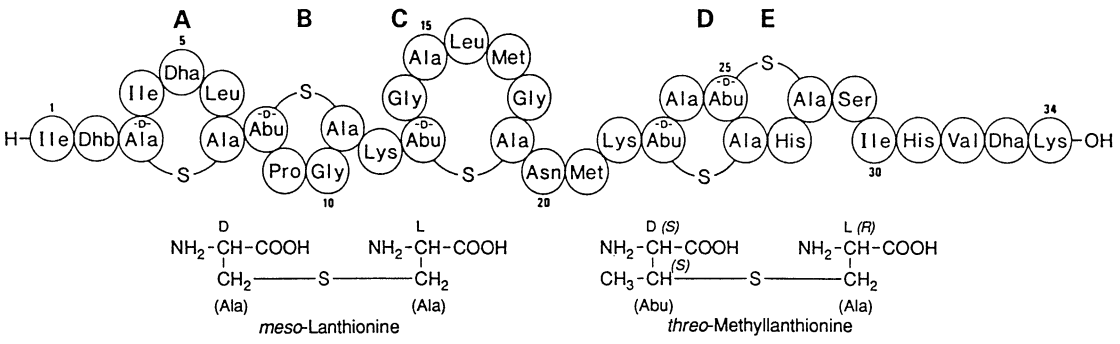

Fig. 1. Structure of nisin. Abu: 2-aminobutyric acid; Dha: dehydroalanine, Dhb: dehydrobutyrine (=3-methyldehydroalanine).

† Present address: Peptide Institute, Protein Research Foundation, Minoh, Osaka 562.

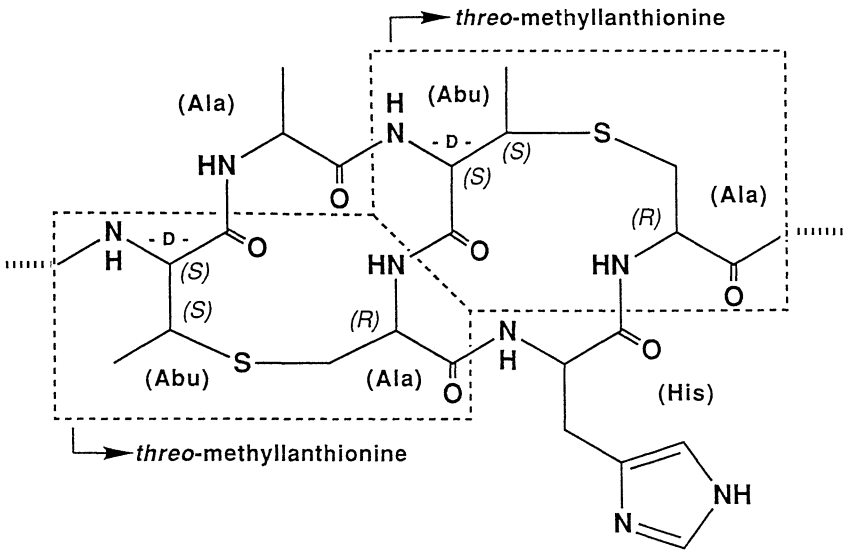

Fig. 2. Structure of ring D-E part of nisin.

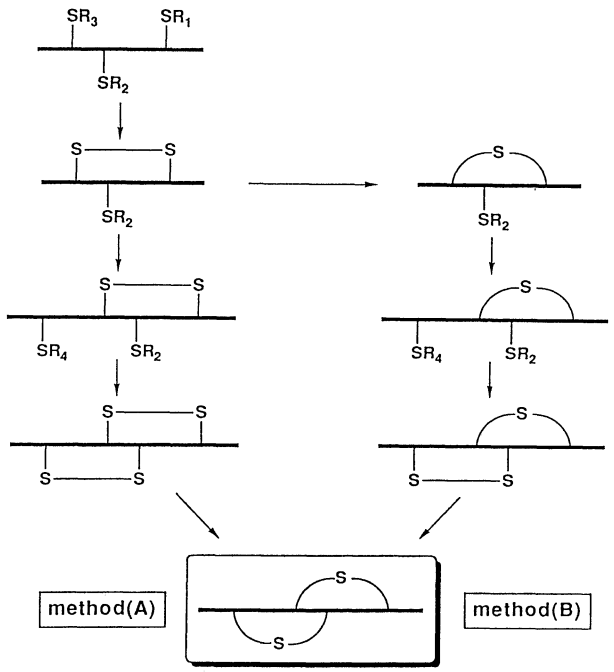

Fig. 3. Synthetic strategies of ring D-E.

was protected with tosyl (Ts) group. The peptide elongation was carried out successively from *C*-terminal by either active ester of *N*-hydroxysuccinimide (HOSu) (peptide **1**, **2**), dicyclohexylcarbodiimide (DCC) (peptide **3**), or symmetrical anhydride method (peptides **5**). In the case of very slow rate of coupling reaction owing to steric influence in cyclic sulfide or disulfide peptide, benzotriazol-1-yl diethylphosphate (BDP),[11] known as a particularly active reagent, was used for the coupling (peptide **6**, **10**, **11**). In this case, N^{im}-Ts group removed by resulting 1-hydroxybenzotriazole should be reintroduced with tosyl chloride (TsCl) and triethylamine (TEA) after the coupling reaction.

In the synthesis of ring D-E according to method (A) (Fig. 4), we first prepared disulfide peptide **4** by iodine oxidation of peptide **3**. Previously, Kamber reported that *S*-Trt group could be selectively removed in the presence of *S*-Acm group by iodine oxidation in $CHCl_3$ or CH_2Cl_2 with or without either CF_3CH_2OH or $(CF_3)_2CHOH$.[10] However, undesirable oligomerization products were observed predominantly in $CHCl_3$ or CH_2Cl_2 if we performed the reaction without any fluoro alcohols in the solvent. On the other hand, when fluoro alcohol such as CF_3CH_2OH, $(CF_3)_2CHOH$ or $CF_3CF_2CH_2OH$, was added to CH_2Cl_2, oligomerizations were depressed effectively. Among these fluoro alcohols, we chose CF_3CH_2OH as the best additive in view of effectiveness and availability. Iodine oxidation of **3** was carried out in CH_2Cl_2-CF_3CH_2OH=10 : 1 to obtain cyclic disulfide **4**, whose molecular weight was confirmed by field-desorption mass spectrometry (FD-MS) (*m*/*z* 815, M^+). Disulfide peptide **4** was then elongated to hexapeptide **6**. Mono-disulfide peptide **6** thus obtained was subjected to second disulfide linkage formation by oxidative removal of *S*-Trt and *S*-Acm groups with iodine in MeOH. Although desired bis-disulfide peptide **7** was secured, we only obtained unsatisfactory yield (50%) which may be caused by possible disulfide exchange reaction during iodine oxidation. In order to avoid this disadvantage, we then used MeOH-H_2O=9 : 1 as solvent, expecting an acceleration of rate in the cleavage reaction of *S*-Trt and *S*-Acm groups by addition of water.[10] Desired bis-disulfide peptide **7** was thus obtained in 78% yield. The molecular weight of **7** was confirmed by fast atom bombardment mass spectrometry (FAB-MS) [*m*/*z* 931, $(M+H)^+$].

Finally, the formation of bicyclic sulfide rings was attempted by one step desulfurization reaction of **7**

with $P(NEt_2)_3$. In the syntheses of rings A, B, and C, the most suitable solvent to desulfurization varied depending on the case i.e., *N,N*-dimethylformamide (DMF) for ring A,[6] benzene for ring B,[7] tetrahydrofuran (THF) for ring C.[8] Whereas, in the present study, the desulfurization reaction was carried out most smoothly in anhydrous benzene[12] to give desired ring D-E **8** in 40% yield, whose molecular weight was confirmed by FAB-MS [*m/z* 867, $(M+H)^+$].

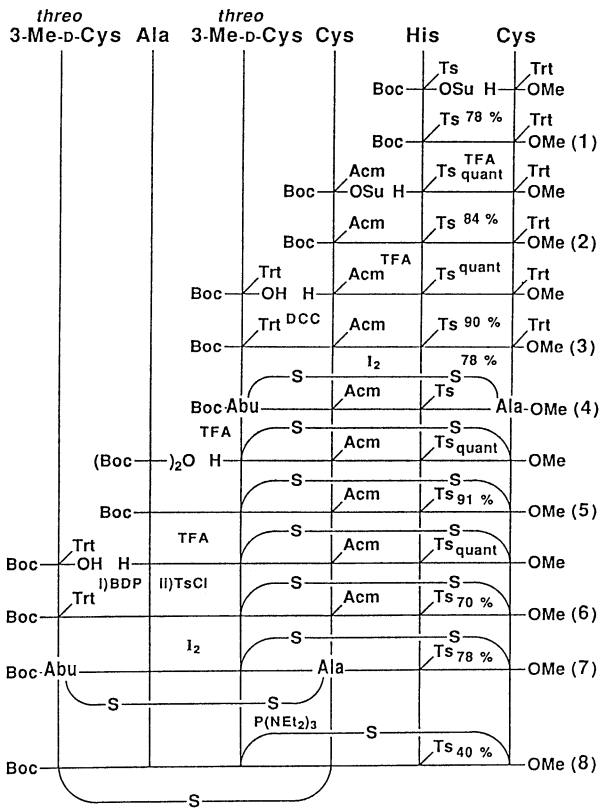

Fig. 4. Synthetic scheme according to method (A).

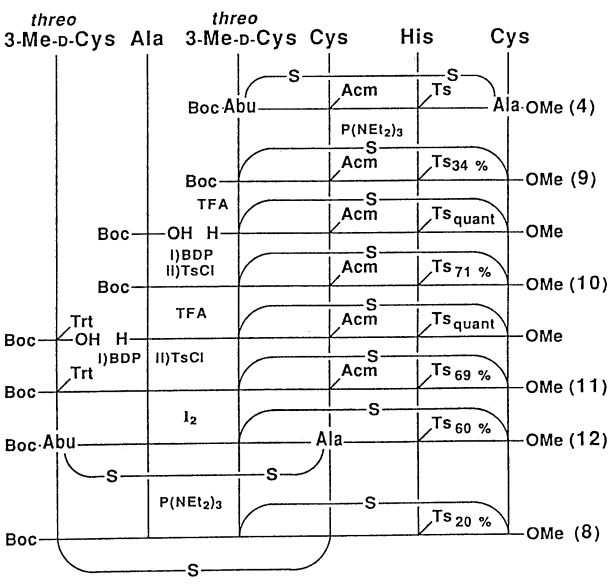

Fig. 5. Synthetic scheme according to method (B).

To discuss an advantage of simultaneous or stepwise method for desulfurization in this case, we then attempted a synthesis of the same compound of ring D-E according to method (B) (Fig. 5). The first desulfurization step from disulfide peptide **4** in anhydrous benzene gave the cyclic sulfide peptide **9** corresponding to ring E. However, the yield (34%) was rather low in comparison with that in method (A), since a considerable amount of oligomers were formed even under high dilution conditions [0.31 mM (1 M=1 mol dm^{-1})]. After the cyclic peptide **9** was elongated to hexapeptide **11**, both *S*-Trt and *S*-Acm groups in **11** were removed with iodine in MeOH to give the bicyclic peptide **12**. The second desulfurization step produced the cyclic sulfide part corresponding to ring D consequently affording the desired ring D-E **8**, but again in unsatisfactory yield.

The products **8** of the ring D-E part thus prepared by the different methods were completely identical with each other in high performance liquid chromatography (HPLC), ^1H NMR,[13] and FAB-MS. Amino acid analyses of their hydrolyzates not only showed an identity of both compounds, but also certified a retention of threo configuration of methyllanthionine residue as natural one in both products.

Although we succeeded in the synthesis of ring D-E part of nisin by two methods, the method (A) gave a better result than the method (B) in view of the less reaction steps, better yield of desulfurization step, and easiness of peptide elongation. Consequently by this study in addition to the previous investigations, we have now been able to accomplish the syntheses of all ring parts in nisin. Furthermore, we have recently succeeded in the total synthesis of nisin by couplings of these lanthionine peptide fragments,[14,15] details of which will soon be reported elsewhere.

Experimental

All melting points are uncorrected. The FD-MS and FAB-MS spectra were obtained with a JEOL JMS-DX 300 mass spectrometer. ^1H NMR spectra were obtained with an FX-90Q NMR spectrometer. Specific rotations were obtained with a Perkin-Elmer 141 polarimeter. HPLC was carried out with Shimadzu LC-5A liquid chromatograph [column: Cosmosil $5C_{18}$ column, 4×125 mm; solvent: CH_3CN-0.1% aq TFA, gradient: 40—70% (2% min^{-1}); flow rate: 1.0 ml min^{-1}; detection: UV at 220 nm]. Amino acid analysis was carried out with a Hitachi KLA-5 analyzer [column: Hitachi custom #2618 resin, 9×250 mm, 55 °C; buffer: 0.20 M sodium citrate pH 3.00 (0—80 min), pH 4.25 (80—120 min), 0.80 M sodium citrate pH 6.60 (120—230 min); flow rate 1.0 ml min^{-1}]. Samples for amino acid analysis were hydrolyzed with constant boiling 6 M HCl in sealed tubes at 110 °C for 48 h. Silica-gel column chromatography was carried out using Merck silica gel 60 (70—230 mesh) for the preparation of **1** and **3** or Merck silica gel 60 (230—400 mesh) for the preparation of **4**, **6**, **7**, **8**, **9**, **10**, and **11**.

Boc-His(Ts)-Cys(Trt)-OMe (1). To a solution of Boc-His(Ts)-OH (27.6 g, 67.3 mmol) in 200 ml of THF were

added HOSu (7.75 g, 67.3 mmol) and DCC (13.9 g, 67.3 mmol) under ice cooling. The mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. To this mixture was added H-Cys(Trt)-OMe (24.6 g, 67.3 mmol) in 200 ml of THF under ice cooling. The mixture was stirred at room temperature overnight and then filtered. The residue obtained by vacuum concentration was dissolved in EtOAc and the solution was washed successively with 10% aq citric acid, saturated $NaHCO_3$, and saturated NaCl solutions. The organic layer was dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by silica-gel column chromatography (500 g, 7×23 cm, benzene-EtOAc-MeOH=40:1:1) to give oily product: yield 40.3 g (77.9%); $[\alpha]_D^{23}$ +3.7° (*c* 0.55, MeOH). Found: C, 63.69; H, 5.71; N, 7.20; S, 8.30%. Calcd for $C_{41}H_{44}N_4O_7S_2 \cdot 0.25H_2O$: C, 63.67; H, 5.80; N, 7.24; S, 8.29%.

Boc-Cys(Acm)-His(Ts)-Cys(Trt)-OMe (2). Boc group of dipeptide **1** (7.00 g, 9.10 mmol) was removed with 28 ml of trifluoroacetic acid (TFA). The solution was allowed to stand at room temperature for 30 min, and then concentrated in vacuo. The residue was dissolved in 15 ml of EtOAc. To this solution were added TEA (2.34 g, 23.1 mmol) and Boc-Cys(Acm)-OSu (5.45 g, 14.0 mmol) under ice cooling. The mixture was stirred at 0 °C for 1.5 h and then washed with 10% aq citric acid, saturated $NaHCO_3$, and saturated NaCl solutions. The organic layer dried over $MgSO_4$ was concentrated in vacuo. The residue was precipitated from EtOAc and hexane: yield 7.18 g (83.7%); mp 92—105 °C; $[\alpha]_D^{23}$ −4.8° (*c* 0.52, MeOH). Found: C, 59.13; H, 5.78; N, 8.81; S, 9.93%. Calcd for $C_{47}H_{54}N_6O_9S_3 \cdot 0.5H_2O$: C, 59.29; H, 5.82; N, 8.83; S, 10.10%.

Boc-3-Me-D-Cys(Trt)-Cys(Acm)-His(Ts)-Cys(Trt)-OMe (3). Boc group of **2** (28.7 g, 30.4 mmol) was removed with 30 ml of TFA as described above. The residue obtained by vacuum concentration was dissolved in EtOAc and then washed with saturated $NaHCO_3$ solution. The organic layer dried over $MgSO_4$ was concentrated in vacuo. The residue and Boc-3-Me-D-Cys(Trt)-OH (14.5 g, 30.4 mmol) were dissolved in 120 ml of THF. To this solution was added DCC (6.28 g, 30.4 mmol) under ice cooling. The mixture was stirred at 0 °C for 1 h and at room temperature overnight, and then filtered. The residue obtained by vacuum concentration was purified by silica-gel column chromatography (500 g, 7×23 cm, $CHCl_3$-MeOH=40:1) to give oily product: yield 35.6 g (89.9%). The product was precipitated from EtOAc and hexane: mp 116—120 °C; $[\alpha]_D^{23}$ −42.6° (*c* 0.560, MeOH). Found: C, 63.60; H, 5.82; N, 7.39; S, 9.67%. Calcd for $C_{70}H_{75}N_7O_{10}S_4 \cdot H_2O$: C, 63.66; H, 5.88; N, 7.42; S, 9.71%.

Boc-3-Me-D-Cys-Cys(Acm)-His(Ts)-Cys-OMe (4). To a solution of tetrapeptide **3** (11.6 g, 8.90 mmol) in 3.2 l of CH_2Cl_2 and 350 ml of CF_3CH_2OH was added a solution of I_2 (6.78 g, 26.7 mmol) in 270 ml of CH_2Cl_2. After the mixture was stirred at room temperature for 20 min, 0.2 M $Na_2S_2O_3$ solution was added until the color of I_2 disappeared. The organic layer was separated, dried over $MgSO_4$, and then concentrated in vacuo. The oily residue was purified by silica-gel column chromatography (200 g, 3×60 cm, $CHCl_3$-acetone=1:1). The product was precipitated from EtOAc and hexane: yield 5.68 g (78.2%); mp 117—118 °C; $[\alpha]_D^{23}$ +57.3° (*c* 0.450, MeOH); FD-MS, *m/z* 815 (M^+). Found: C, 45.91; H, 5.61 ; N, 11.56; S, 15.11%. Calcd for $C_{32}H_{45}N_7O_{10}S_4 \cdot H_2O$: C, 46.08; H, 5.68; N, 11.76; S, 15.38%.

TFA · H-3-Me-D-Cys-Cys(Acm)-His(Ts)-Cys-OMe. Boc group of **4** (4.55 g, 5.58 mmol) was removed with 17 ml of TFA as described above to give oily residue which was subjected to the following reaction without purification.

Boc-Ala-3-Me-D-Cys-Cys(Acm)-His(Ts)-Cys-OMe (5). To a solution of Boc-Ala-OH (3.16 g, 16.7 mmol) in 13 ml of CH_2Cl_2 was added DCC (1.72 g, 8.34 mmol) under ice cooling and the mixture was stirred at 0 °C for 3 h. To the anhydride solution were added the TFA salt described above in 10 ml of CH_2Cl_2 and TEA (1.69 g, 16.7 mmol) under ice cooling. The mixture was stirred at room temperature overnight and then filtered. The filtrate was washed, dried, and concentrated as described for the preparation of **2**. Addition of EtOAc and hexane to the residue gave crystalline product: yield 4.50 g (90.9%); mp 114—116 °C; $[\alpha]_D^{23}$ +44.0° (*c* 0.430, MeOH). Found: C, 45.58; H, 5.73; N, 12.03; S, 14.08%. Calcd for $C_{35}H_{50}N_8O_{11}S_4 \cdot 2H_2O$: C, 45.54; H, 5.90; N, 12.14; S, 13.89%.

HCl · H-Ala-3-Me-D-Cys-Cys(Acm)-His(Ts)-Cys-OMe. Boc group of **5** (2.00 g, 2.25 mmol) was removed with 10 ml of TFA as described above. The residue obtained by vacuum concentration was dissolved in a small amount of MeOH. To the solution were added successively 12.9 M HCl/MeOH (0.70 ml, 9 mmol) and ether. The precipitate was filtered and washed with ether. This peptide was subjected to the following reaction without purification.

Boc-3-Me-D-Cys(Trt)-Ala-3-Me-D-Cys-Cys(Acm)-His-(Ts)-Cys-OMe (6). Boc-3-Me-D-Cys(Trt)-OH (1.13 g, 2.36 mmol) and the hydrochloride described above were dissolved in 15 ml of DMF. TEA (784 mg, 7.75 mmol) and BDP (753 mg, 2.75 mmol) were added to the solution under ice cooling. The mixture was stirred at room temperature overnight and then concentrated in vacuo. EtOAc and 10% aq citric acid were added to the residue. Organic layer was washed with saturated $NaHCO_3$ and saturated NaCl solutions, and dried over $MgSO_4$. EtOAc was evaporated in vacuo until the volume of the solution became 10 ml. In order to reintroduce N^{im}-Ts group, TsCl (430 mg, 2.25 mmol) and TEA (227 mg, 2.25 mmol) were added to the solution. The mixture was stirred at room temperature for 1 h, washed with water, and then dried over $MgSO_4$. The oily residue obtained by vacuum concentration was purified by silica-gel column chromatography (100 g, 3×30 cm, $CHCl_3$-MeOH=50:1) to give **6** which was precipitated from EtOAc and hexane: yield 2.06 g (70.1%); mp 146—153 °C; $[\alpha]_D^{23}$ +9.6° (*c* 1.0, MeOH). Found: C, 54.75; H, 5.77; N, 9.67; S, 12.55%. Calcd for $C_{58}H_{71}N_9O_{12}S_5 \cdot 1.5H_2O$: C, 54.70; H, 5.86; N, 9.90; S, 12.59%.

Boc-3-Me-D-Cys-Ala-3-Me-D-Cys-Cys-His(Ts)-Cys-OMe (7). To a solution of peptide **6** (3.44 g, 2.76 mmol) in the mixture of 980 ml of MeOH and 120 ml of H_2O was added I_2 (2.10 g, 8.28 mmol) in 83 ml of MeOH at room temperature under vigorous stirring. After the solution was stirred for 1 min, a solution of L-ascorbic acid (3.5 g), citric acid monohydrate (6.3 g), and NaOH (2.4 g) in 300 ml of water was added until the color of I_2 disappeared. The mixture was concentrated in vacuo and the residue was extracted with 150 ml of $CHCl_3$. The extract was washed with saturated NaCl solu-

tion and then dried over $MgSO_4$. The residue obtained by vacuum concentration was purified by silica-gel column chromatography (70 g, 2×45 cm, $CHCl_3$-MeOH=70:1) to give **7** which was precipitated from EtOAc and hexane: yield 2.00 g (77.8%); mp 189—193 °C; $[\alpha]_D^{23}$ +139° (*c* 0.659, MeOH); FAB-MS, *m/z* 931 $[(M+H)^+]$. Found: C, 45.59; H, 5.43; N, 11.73; S, 16.94%. Calcd for $C_{36}H_{50}N_8O_{11}S_5 \cdot H_2O$: C, 45.55; H, 5.52; N, 11.81; S, 16.89%.

Boc-D-Abu-Ala-D-Abu-Ala-His(Ts)-Ala-OMe (8). Method (A). To a solution of **7** (350 mg, 0.376 mmol) in 610 ml of anhydrous benzene was added $P(NEt_2)_3$ (3.70 g, 15.0 mmol). The mixture was stirred at room temperature for 3 d and then concentrated in vacuo. The residue was dissolved in EtOAc and washed with 10% aq citric acid and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica-gel column chromatography (20 g, 1.5×25 cm, $CHCl_3$-MeOH=30:1) to give **8** which was precipitated from EtOAc and hexane: yield 131 mg (40.0%); mp 154—158 °C; $[\alpha]_D^{23}$ −40.5° (*c* 0.306, MeOH); FAB-MS, *m/z* 867 $[(M+H)^+]$; ^{1}H NMR ($CDCl_3$) δ=1.26—1.44 (9H, m, Ala C(3)-CH_3, 2×Abu C(4)-CH_3), 1.49 (9H, s, $(CH_3)_3C$-), 2.45 (3H, s, $C_6H_4CH_3$), 2.8—3.7 (8H, m), 3.74 (3H, s, $COOCH_3$), 4.0 (1H, m), 4.3—4.7 (3H, m), 4.8—5.2 (3H, m), 6.8—7.2 (4H, m), 7.36 (2H, d, *J*=8.2 Hz), 7.5 (2H, m), 7.83 (2H, d, *J*=8.2 Hz), 8.02 (1H, d, *J*=1.3 Hz); retention time in HPLC: 8.1 min. Amino acid analysis: Melan (2.44), Ala (1.00), His (0.96). Found: C, 48.40; H, 5.88; N, 12.41; S, 10.98%. Calcd for $C_{36}H_{50}N_8O_{11}S_3 \cdot 1.5H_2O$: C, 48.36; H, 5.97; N, 12.53; S, 10.76%.

Boc-D-Abu-Cys(Acm)-His(Ts)-Ala-OMe (9). To a solution of **4** (800 mg, 0.980 mmol) in 3.0 l of anhydrous benzene was added $P(NEt_2)_3$ (9.70 g, 39.3 mmol). The mixture was stirred at room temperature for 4 d and then treated as described for the preparation of **8** [method (A)]. The product purified by silica-gel column chromatography (25 g, 1.5×30 cm, $CHCl_3$-acetone=1:1) was precipitated from EtOAc and hexane: yield 261 mg (34.0%); mp 115—117 °C; $[\alpha]_D^{23}$ −58.0° (*c* 0.188, MeOH); FD-MS, *m/z* 784 $[(M+H)^+]$. Found: C, 48.19; H, 5.76; N, 12.11; S, 11.90%. Calcd for $C_{32}H_{45}N_7O_{10}S_3 \cdot H_2O$: C, 47.93; H, 5.91; N, 12.33; S, 11.99%.

HCl · H-D-Abu-Cys(Acm)-His(Ts)-Ala-OMe. Boc group of **9** (380 mg, 0.485 mmol) was removed with 1.5 ml of TFA. The residue obtained by vacuum concentration was dissolved in 1.4 ml of THF. To the solution were added 5.26 M HCl-THF (0.40 ml, 2.1 mmol) and ether. The precipitate obtained was subjected to the following reaction without purification.

Boc-Ala-D-Abu-Cys(Acm)-His(Ts)-Ala-OMe (10). The condensation of Boc-Ala-OH (106 mg, 0.561 mmol) and the hydrochloride described above (0.485 mmol) was carried out using BDP (166 mg, 0.612 mmol) and TEA (181 mg, 1.79 mmol) in 3 ml of DMF as described for the preparation of **6**. TsCl (265 mg, 1.39 mmol) and TEA (140 mg, 1.39 mmol) were added to DMF solution to reintroduce N^{im}-Ts group. After the mixture was stirred at room temperature for 1 h, EtOAc and 10% aq citric acid were added to the mixture. The EtOAc layer was washed with saturated $NaHCO_3$ and saturated NaCl solutions, and then dried over $MgSO_4$. The oily residue obtained by vacuum concentration was purified by silica-gel column chromatography (8 g, 1×18 cm, $CHCl_3$-acetone=2:1) to give **10** which was precipitated from EtOAc and hexane: yield 293 mg (70.6%); mp 136—142 °C; $[\alpha]_D^{23}$ −20.9° (*c* 0.297, MeOH). Found: C, 47.74; H, 6.05; N, 12.63; S, 11.14%. Calcd for $C_{35}H_{50}N_8O_{11}S_3 \cdot 1.5H_2O$: C, 47.66; H, 6.06; N, 12.70; S, 10.91%.

HCl · H-Ala-D-Abu-Cys(Acm)-His(Ts)-Ala-OMe. Boc group of **10** (259 mg, 0.303 mmol) was removed with 1.0 ml of TFA. The residue obtained by vacuum concentration was dissolved in CH_2Cl_2. To the solution were added 5.26 M HCl-THF (0.40 ml, 2.1 mmol) and ether successively. The hydrochloride precipitated was filtered and then subjected to the following reaction without purification.

Boc-3-Me-D-Cys(Trt)-Ala-D-Abu-Cys(Acm)-His(Ts)-Ala-OMe (11). The condensation of Boc-3-Me-D-Cys(Trt)-OH (159 mg, 0.333 mmol) and the hydrochloride described above (0.303 mmol) was carried out using BDP (99 mg, 0.364 mmol) and TEA (107 mg, 1.06 mmol) in 4 ml of DMF as described for the preparation of **6**. To the solution were added $CHCl_3$ and saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo until the volume of the solution became 4 ml. To this solution were added TsCl (116 mg, 0.606 mmol) and TEA (97 mg, 0.91 mmol). The mixture was stirred at room temperature for 1 h, and then concentrated in vacuo. The residue was purified by silica-gel column chromatography (13 g, 1×30 cm, $CHCl_3$-acetone=3:2) to give **11** which was precipitated from EtOAc and hexane: yield 255 mg (69.3%); mp 158—162 °C; $[\alpha]_D^{23}$ −40.3° (*c* 0.176, MeOH). Found: C, 56.03; H, 5.99; N, 9.92; S, 10.25%. Calcd for $C_{58}H_{71}N_9O_{12}S_4 \cdot 2H_2O$: C, 55.71; H, 6.04; N, 10.08; S, 10.26%.

Boc-3-Me-D-Cys-Ala-D-Abu-Cys-His(Ts)-Ala-OMe (12). To a solution of **11** (95 mg, 0.078 mmol) in 28 ml of MeOH was added I_2 (58 mg, 0.23 mmol) in 2.3 ml of MeOH at room temperature under vigorous stirring. The mixture was stirred for 50 min, and then treated as described for the preparation of **7**. The crude product was reprecipitated from $CHCl_3$ and hexane: yield 42 mg (60%); mp 155—162 °C; $[\alpha]_D^{23}$ +22.4° (*c* 0.541, MeOH); FD-MS, *m/z* 899 $[(M+H)^+]$. Found: C, 46.29; H, 5.70; N, 11.83; S, 13.95%. Calcd for $C_{36}H_{50}N_8O_{11}S_4 \cdot 2H_2O$: C, 46.24; H, 5.82; N, 11.98; S, 13.72%.

Compound 8. Method (B). To a solution of **12** (19.0 mg, 0.0211 mmol) in 30 ml of anhydrous benzene and 2 ml of anhydrous 1,2-dimethoxyethane was added $P(NEt_2)_3$ (137 mg, 0.554 mmol). The mixture was stirred at room temperature overnight and then treated as described for the preparation of **8** [method (A)]. An oily residue obtained was purified by thin-layer silica-gel chromatography (Merck silica gel F_{254}, 0.5 mm, 20×20 cm, $CHCl_3$-MeOH=15:1): yield 3.7 mg (20%); FAB-MS, *m/z* 867 $[(M+H)^+]$; ^{1}H NMR ($CDCl_3$) δ=1.26—1.44 (9H, m, Ala C(3)-CH_3, 2×Abu C(4)-CH_3), 1.49 (9H, s, $(CH_3)_3C$-), 2.45 (3H, s, $C_6H_4CH_3$), 2.8—3.7 (8H, m), 3.74 (3H, s, $COOCH_3$), 4.0 (1H, m), 4.3—4.7 (3H, m), 4.8—5.2 (3H, m), 6.8—7.2 (4H, m), 7.36 (2H, d, *J*=8.2 Hz), 7.5 (2H, m), 7.83 (2H, d *J*=8.2 Hz), 8.02 (1H, d, *J*=1.3 Hz); retention time in HPLC: 8.1 min. Amino acid analysis: Melan (2.39), Ala (1.00), His (1.01).

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