Pergamon

Tetrahedron, Vol. 53, No. 2, pp. 567-584, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)00982-9

Efficient Synthesis of Peptaibol using a Chloroimidazolidium Coupling Reagent, CIP

Kenichi Akaji, Yasunori Tamai, and Yoshiaki Kiso*

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

Abstract: Two peptaibols, alamethicin F-30 and trichovirin I 4A, have been synthesized in solution by using a 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate in the presence of an additive (CIP-additive) as a coupling agent and TFA as a final deprotecting reagent. Alamethicin F-30 is one of the most common peptaibols and consists of 19 amino acids including 8 α,α–dimethyl amino acid (aminoisobutylic acid, Aib) and phenylalaninol residues. Trichovirin I 4A consists of 13 amino acids including 5 Aib residues and leucinol. In the synthesis of both, all couplings including those between sterically hindered Aib residues were successfully achieved within 60 min using a newly developed coupling agent, the CIP-additive. In the synthesis of trichovirin I 4A, no racemization was detected during the CIP-mediated coupling of peptide fragments having an optically active α–amino acid at its C-terminus. The synthesis of 2 peptaibols shows that the CIP-additive method is efficient not only for the coupling of sterically hindered amino acids but also for general fragment coupling. Copyright © 1996 Elsevier Science Ltd

Peptaibols are a class of linear antibiotic peptides biosynthesized by widespread soil fungi, such as *Trichoderma, Hypocrea, or Stilbella* [1]. Structurally, peptides of this class are characterized by several α,α–dialkyl amino acid residues such as α,α–dimethylamino acid (α–aminoisobutyric acid, Aib), an N-terminal acetyl group, and the presence of a C-terminal amino alcohol[2]. Because of the relatively high content of Aib, the strongest known helix-forming amino acid[3], peptaibols have a strong tendency to form amphipathic helices resulting in voltage-dependent transmembrane channels in lipid bilayer membrane[4]. These properties are closely related to the biological activities displayed by peptaibols, particularly their antibiotic activity[5].

Despite their interesting membrane-modifying properties, relatively few works regarding peptaibols have been described because of synthetic difficulties[6]. The reactivity of sterically hindered α,α–dialkylamino acids is much lower than that of typical α–amino acids[7]. Consequently, a solid phase approach employing standard stepwise protocols is not suitable for the synthesis of peptaibols rich in α,α–dialkyl amino acids. The increased risk of racemization due to low reactivities is another concern. Difficulties also arise because of inherent degradation in the presence of acid labile Aib-Pro linkage[8]. Thus, solution synthesis is still the preferred route for the synthesis of peptaibols because coupling at each step can be optimized, although an activated form such as fluoride makes the solid-phase incorporation of Aib into peptides feasible[9].

Recently, we reported that CIP (2-chloro-1,3-dimethylimidazolidium hexafluorophosphate)[10] in the presence of an additive (HOAt[11] or HODhbt[12]) is a suitable coupling agent for N^{α}-protected Aib (Figure 1).

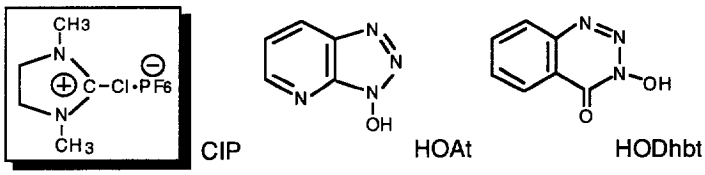


Figure 1. Structure of CIP, HOAt, and HODhbt

In the coupling reaction using the CIP-additive, the first intermediate, 2-[(9-fluorenylmethyl)oxy]-4,4-dimethyl-5-oxazolone, is transformed to a highly reactive active ester by the catalytic additive to give the desired peptide without detectable racemization. These model studies suggest that efficient synthesis of peptaibol containing a high proportion of Aib would be feasible using the CIP-additive as a coupling agent. Herein, we report the efficient synthesis of alamethicin F-30, one of the most common peptaibols, and trichovirin I 4A using a combination of the CIP-additive method and the TFA deprotection procedure.

RESULTS AND DISCUSSION

Synthesis of alamethicin F-30

Alamethicins are peptide antibiotics produced by the fungus *Trichoderma viride*. [13] The variant F-30 is a major component of natural alamethicin which is a mixture of closely related compounds. Alamethicin F-30 is a linear peptide consisting of 19 α–amino acids including 8 Aib residues and a C-terminal α–amino alcohol, phenylalaninol (Pheol) (Figure 2).

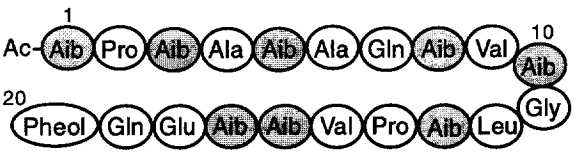
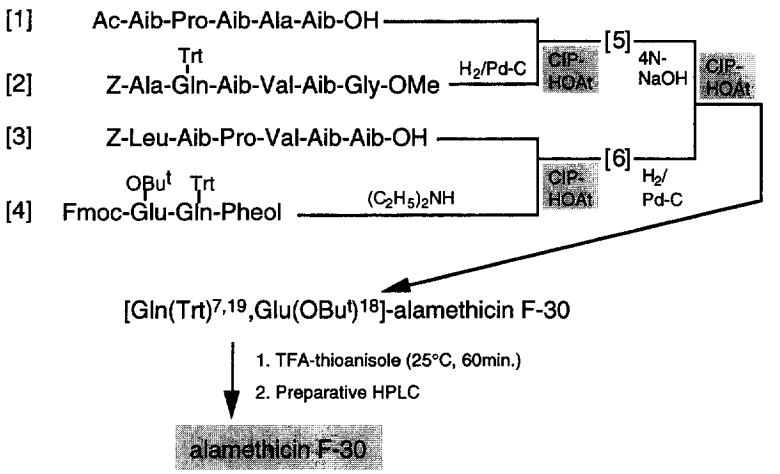


Figure 2. Alamethicin F-30

In previous synthetic work on alamethicins, a combination of DCC-mediated coupling and N^{α}-Boc protection was mainly employed[14]. However, DCC-mediated coupling has been reported to give moderate yields even after activation at an elevated temperature (40°C) and need a long reaction time (12 h or more). In addition, care should be taken to suppress possible peptide bond cleavages during repetitive acidolytic Boc removal. To overcome these difficulties, we adopted a synthetic strategy employing the newly developed CIP-mediated coupling technique combined with N^{α}-Z or -Fmoc protection.

Scheme 1 shows the synthetic route for alamethicin F-30. Four fragments were selected to construct the peptaibol backbone. The fragments [1] to [3] have Aib or Gly residues at their C-terminal positions, this makes the fragment condensation free of racemization. We selected a Z group cleavable by hydrogenolysis as the temporary N^{α}-protecting group for aliphatic amino acids to avoid repetitive acid cleavage treatment. As the side chain groups protecting Glu and Gln, Bu^{t} and Trt groups were used, respectively, since these groups are

easily cleaved by treatment with weak acid such as TFA during the final stage of synthesis. Thus, the commercially available Fmoc derivatives of these 2 amino acids were used for the preparation of fragments [2] and [4]. The N^{α}-Fmoc group can be cleaved by treatment with a weak base, such as diethylamine, without affecting the peptide chain.



Scheme 1. Synthesis of alamethicin F-30

Each fragment was synthesized by a stepwise procedure in which every amino acid was coupled by a 60 min reaction at 25°C with the CIP-additive. As the additive, HOAt which has the highest catalytic enhancing effect was used for the coupling of Aib whereas HODhbt, the second one, was used for typical α–amino acids[10]. Fragments [1] to [4] and the intermediates were obtained with similar quantitative yields and were well characterized by TLC and elemental analysis.

The peptaibol backbone was constructed according to the route shown in Scheme 1. The N^{α}-Fmoc group of fragment [4] was removed by diethylamine treatment and the product was coupled with fragment [3] by a reaction with CIP-HOAt for 60 min at 25°C to give a 9-residue C-terminal fragment [6] with a 75% isolation yield. The N-terminal 11-residue fragment [5] was similarly prepared by the condensation of fragment [1] and the N^{α}-deprotected product of fragment [2] with a 52 % yield using CIP-HOAt (60 min at 25°C). Finally, the N-terminal fragment obtained by saponification of fragment [5] and the C-terminal fragment obtained by hydrogenolysis of fragment [6] were coupled by a reaction with CIP-HOAt (60 min at 25°C). The product was then purified by column chromatography using LH-20 to give [$Glu(Trt)^{7,19}$, $Glu(OBu^t)^{18}$]-alamethicin F-30 with a 66% isolation yield. Thus, all fragment couplings including those between large fragments (ca 10 residues) were conducted within 60 min by CIP-mediated reactions. Each condensation product was well characterized by TLC, elemental analysis, and amino acid analysis after acid hydrolysis. The integrity of the protected alamethicin F-30 was further confirmed by fast atom bombardment-mass spectrometry (FAB-MS).

To remove the side chain protecting groups, the protected alamethicin F-30 was treated with TFA-thioanisole (10 : 1) for 60 min at 25°C and the deprotected peptide was isolated by column chromatography

using LH-20. The crude product gave a single main peak on the HPLC as shown in Figure 3-1; this indicates that no serious peptide bond cleavage at Aib-Pro had occurred during the final deprotection step. The product was purified by preparative HPLC, and a homogeneous peptaibol was obtained with a 30 % yield calculated from the protected alamethicin F-30.

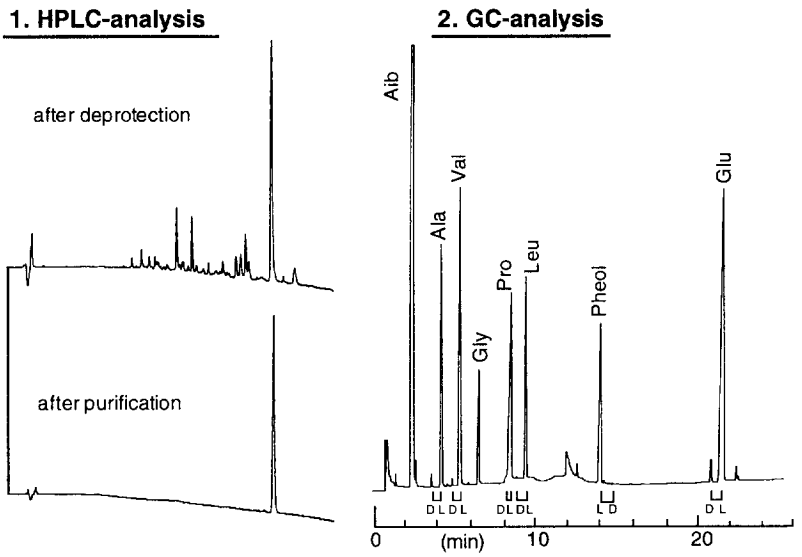


Figure 3. HPLC and GC spectrum of the synthetic alamethicin F-30

The integrity of the purified product was determined by analytical HPLC (Figure 3-1), amino acid analysis after acid hydrolysis, FAB-MS, and by ^{1}H- and ^{13}C-NMR[4]. Efficient structure determination as well as conformation analysis of the synthetic alamethicin F-30 and its analogues using 2 dimensional NMR techniques will be reported separately. The optical purity of the constituent amino acids in the synthetic alamethicin F-30 was confirmed by gas chromatography (GC) using a chiral column. The (pentafluoropropionyl) amino acid *n*-butyl esters derived from a total hydrolysate of synthetic alamethicin F-30 were analyzed in a Chirasil Val-L capillary column (Figure 3-2). The content of each D-amino acid was less than 0.5 %, the value due to hydrolysis. In addition, the circular dichroism (CD) spectra of synthetic alamethicin F-30 concurred with that reported for natural alamethicin F-30[15].

Synthesis of trichovirin I 4A

In the coupling of fragment having an optically active α–amino acid at its C-terminus, racemization at the activated carboxyl function is still a serious problem. The low reaction rate for fragment condensation is expected to be one of the major reasons for racemization[16]. Thus, we examined the application of CIP-mediated coupling for general fragment condensation yielding peptaibols.

For this purpose, we selected trichovirin I 4A isolated from *Trichoderma viride* (NRLL5234)[17] as a model peptaibol. Trichovirin I 4A consists of 13 α–amino acids including 5 Aib residues and a C-terminal leucinol (Figure 4). This particular variant of tricovirin I contains 3 consecutive amino acids having increasing

steric hindrance (Ala, Val, and Aib) in the middle part of the 14-residue molecule, which would be at a suitable position to allow fragment condensation to be examined. Thus, we synthesized trichovirin I 4A according to 2 different schemes; one by CIP-mediated amide bond formation between Val and Aib residues (path A) and the other by that between Ala and Val residues (path B)(Figure 4). In both synthetic schemes, the N-terminal fragment had an optically active α–amino acid at its C-terminus (Val or Ala), the former would be at a more hindered coupling position than the later.

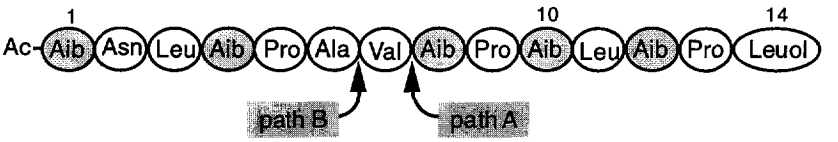
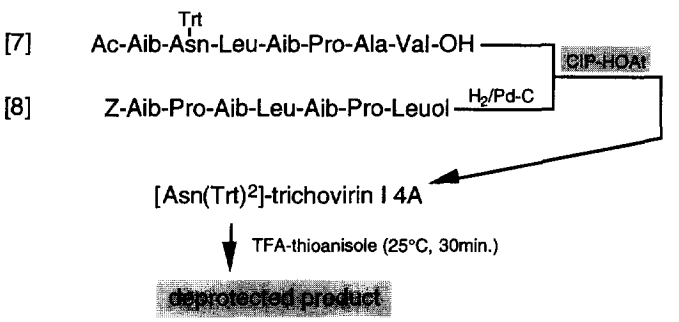


Figure 4. Trichovirin I 4A

Each fragment necessary for the above synthetic schemes was prepared by the same stepwise procedure as described in the synthesis of alametnicin F-30. As the temporary N^{α}-protecting group, Z was used except for Fmoc-Asn(Trt)-OH. All coupling reaction were conducted by 60 min reactions at 25°C with CIP-HOAt or HODhbt and gave similar quantitative yields. Each product was characterized by TLC and elemental analysis.

To construct the peptaibol backbone, we first conducted fragment coupling according to pathway A (Scheme 2). The 7-residue N-terminal fragment [7] and the 7-residue C-terminal fragment obtained by hydrogenolysis of [8] were coupled by a reaction with CIP-HOAt (60 min, 25°C) to give [Asn(Trt)^2]-trichovirin I 4A with a 60 % isolation yield. The amino acid composition and mass value of the protected trichovirin I 4A concurred with the theoretical values, respectively.



Scheme 2. Synthesis (path A) of trichovirin I 4A

The Trt side chain protecting group of the product was removed by brief treatment with TFA-anisole (25°C, 30 min) and the deprotected peptaibol was isolated by LH-20 column chromatography. The product, however, was found to be an equal mixture of 2 compounds, as determined by HPLC analysis (Figures 5-1, compound 1 and 2). In addition, each purified compound had an amino acid composition and mass value consistent with the theoretical values, and there was no significant difference between the values of the 2 compounds. These results strongly suggest that the difference in the 2 products would be their optical purity.

Thus, the optical purity of each compound was examined by GC analysis using a chiral capillary column, as described in alamethicin synthesis. The analysis indicated that compound 2 contained all the expected L-amino acids and was identified as tricovirin I 4A, while compound 1 contained a D-Val instead of a L-Val residue (Figure 5-2); this clearly shows that racemization occurred during fragment coupling conducted between the Val and Aib residues (path A) to give a mixture of an equal amount of [D-Val^7]- and the expected trichovirin I 4A.

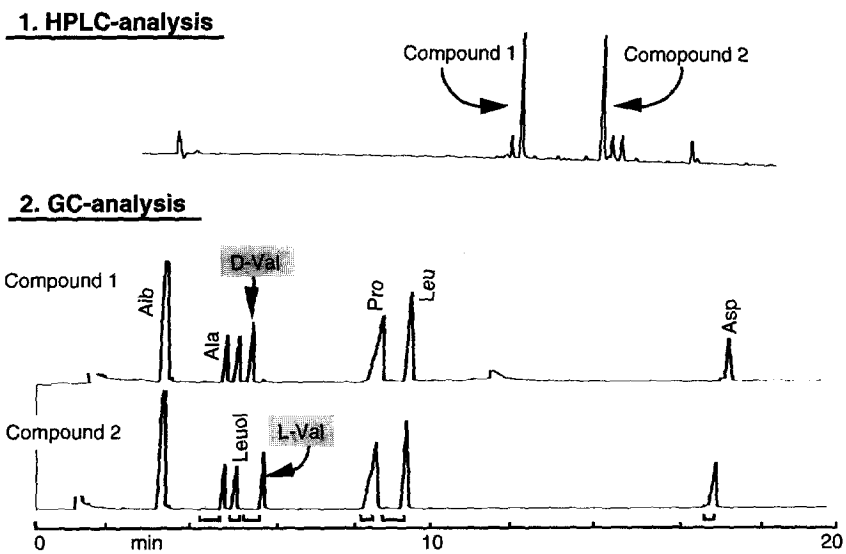
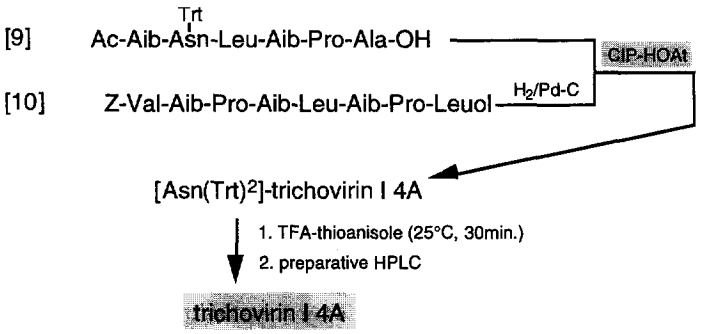


Figure 5. HPLC and GC spectrum of the products obtained by the path A synthesis

We then synthesized trichovirin I 4A according to pathway B; fragment coupling between the less hindered Ala and Val residues (Scheme 3). The coupling reaction was conducted with CIP-HOAt (60 min, 25°C) as described in pathway A synthesis to give [Asn(Trt)^7]-trichovirin I 4A with a 81 % isolation yield. The amino acid composition and mass value of the product concurred with the theoretical values.

Removal of a Trt group from the protected trichovirin I 4A with TFA-anisole (25°C, 30min) and the isolation of the deprotected product with LH-20 was conducted as described for pathway A synthesis. The crude product obtained in pathway B synthesis gave a single main HPLC peak having the same retention time as that of compound 2 obtained in pathway A synthesis (Figure 6-1); this indicates no serious racemization had occurred during fragment coupling in pathway B synthesis. Thus, CIP-mediated fragment coupling between the general α-amino acid proceeded so quickly that no serious racemization had occurred, whereas the fragment having an Aib residue at its N-terminus reacted more slowly, even with CIP-HOAt, to give a racemized product with a moderate yield (pathway A synthesis).



Scheme 3. Synthesis (path B) of trichovirin I 4A

The crude product obtained by pathway B synthesis was purified by preparative HPLC, and homogeneous peptaibol was obtained with a 43 % yield calculated from the protected trichovirin I 4A. The integrity of the purified product was determined by analytical HPLC, amino acid analysis after acid hydrolysis, FAB-MS, and by ^{1}H- and ^{13}C-NMR. The optical purity of the constituent amino acids in the synthetic trichovirin I 4A was confirmed by GC-analysis using a chiral column; the content of each D-amino acid was less than 0.5 % (Figure 6-2).

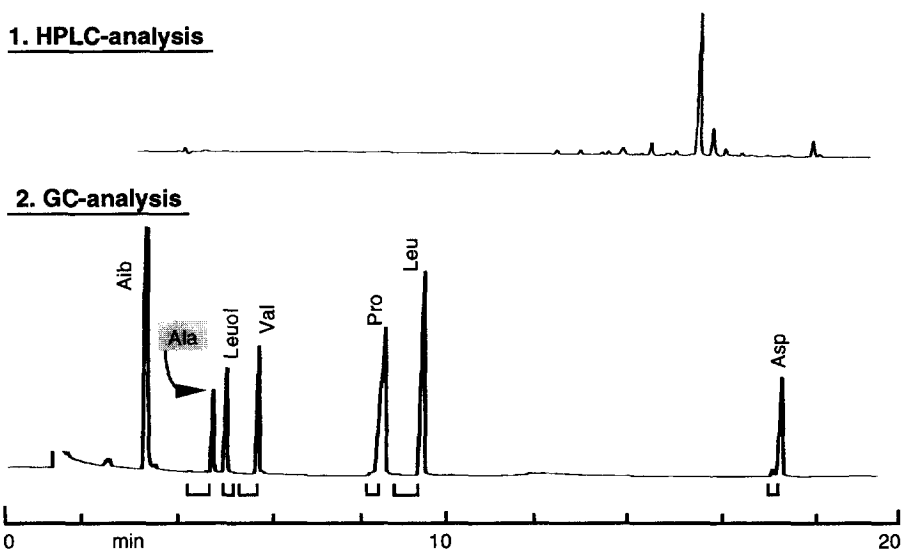


Figure 6. HPLC and GC spectrum of the products obtained by the path B synthesis

Conclusion

We have synthesized 2 peptaibols, alamethicin F-30 and trichovirin I 4A using a CIP-additive as the coupling agent. In the synthesis of alamethicin F-30, every coupling including that between the large fragments was successfully completed by a 60 min reaction; this clearly shows that CIP-mediated coupling is a more suitable reaction for peptaibol synthesis than conventional carbodiimide-mediated coupling. In the synthesis of trichovirin I 4A, CIP-mediated fragment coupling between general α–amino acids proceeded so quickly that practically no racemization occurred at the activated carboxyl function. Thus, we have shown that a newly developed coupling reaction using the CIP-additive is efficient not only for sterically hindered α,α-dimethylamino acids but also for peptide fragments having an optically active α-amino acid at their C-terminus. We think that the CIP-additive method should be generally applicable for the efficient synthesis of peptaibols.

EXPERIMENTAL

General. Melting points were uncorrected. TLC was performed on silica-gels (Kiesel-gel $60F_{254}$, Merck). Rf values refer to the following v/v solvent systems: Rf_1 $CHCl_3$-MeOH-AcOH (9:1:0.5), Rf_2 $CHCl_3$-MeOH (9:1), Rf_3 $CHCl_3$-MeOH (10:0.5), Rf_4 $CHCl_3$-MeOH (20:0.5). Silica-gel column chromatography was carried out using Kiesel-gel 60 (70-230 mesh, Merck). Fmoc-Glu(OBu^t)-OH, Fmoc-Gln(Trt)-OH, and Fmoc-Asn(Trt)-OH were obtained from CALBIOCHEM NOVABIOCHEM and were used without further purification. Pheol and Leuol were prepared according to the published procedure[18].

Analytical HPLC was conducted with a Hitach L-6200, and preparative HPLC was conducted with a Shimadzu LC-6A. NMR was performed on a Bruker AM600 or AC300 spectrometer. FAB-MS was obtained on a JEOL JMS-SX102A spectrometer equipped with the JMA-DA7000 data system. Optical rotation was determined with a Horiba SEPA-200 polarimeter using a 1 ml cell. Gas chromatography was conducted with a Shimadzu GC-14A chromatograph. Acid hydrolysis and GC analysis using Chirasil Val-L capillary columns was conducted according to the published procedure[10].

General method for the removal of the Z group. To the MeOH solution containing N^{α}-Z derivative a few drops of AcOH and catalytic amount of 10% Pd-C was added under an Ar atmosphere. The mixture was stirred in an H_2 atmosphere for 60 min at 25°C. The Pd-C was removed by filtration and the solvent was removed by evaporation. The N^{α}-deprotected product was obtained as oil and used directly in subsequent reactions without further purification. The pH of the subsequent reaction mixture was adjusted to 7-8 with DIEA after addition of the N^{α}-deprotected product.

General procedure for the isolation of the condensation product. The condensation product was dissolved in AcOEt and the extract was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O-NaCl. The organic layer was dried over Na_2SO_4 and the solvent was removed by evaporation. The residue was purified by recrystallization in an appropriate solvent, or by silica-gel column chromatography.

Z-Ala-Aib-OMe. To the CH_2Cl_2 (100 ml) solution of Z-Ala-OH (5.0 g, 22.4 mmol) 1.83 g (11.2 mmol) of HODhbt, 16.6 ml (95.4 mmol) of DIEA, 6.2 g (22.4 mmol) of CIP, and 3.8 g (24.6 mmol) of HCl•H-Aib-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated using general methods and was purified by silica-gel column chromatography (2/3, AcOEt:hexane) to yield 6.2 g (85 %) of Z-Ala-Aib-OMe as an oil. Rf_3 0.48.

Z-Aib-Ala-Aib-OMe. Z-Ala-Aib-OMe (5.2 g, 16.2 mmol) was treated with H_2/Pd-C to remove the Z-group according to general methods. To the DMF (40 ml) solution of Z-Aib-OH (4.2 g, 17.8 mmol) the following were added: 1.2 g (8.9 mmol) of HOAt, 11.9 ml (68.4 mmol) of DIEA, 5.0 g (17.8 mmol) of CIP, and H-Ala-Aib-OMe (in 40 ml of DMF) obtained above. The mixture was stirred for 60 min at 25°C. The

product was isolated using general methods and was purified by silica-gel column chromatography (3/1, AcOEt:hexane) to yield 3.88 g (59 %) of Z-Aib-Ala-Aib-OMe as an oil. Rf_3 0.37.

Z-Pro-Aib-Ala-Aib-OMe. Z-Aib-Ala-Aib-OMe (4.4 g, 10.9 mmol) was treated with H_2/Pd by general methods to give H-Aib-Ala-Aib-OMe as an oil. To the CH_2Cl_2 (30 ml) solution of Z-Pro-OH (3.0 g, 12.0 mmol), 0.98 g (6.0 mmol) of HODhbt, 8.0 ml (45.7 mmol) of DIEA, 3.3 g (12.0 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by general methods and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 4.4 g (80 %) of a white powder, mp 70-71°C. $[\alpha]_D^{25}$ -45.04 (c=0.9, MeOH), Rf_3 0.38. Anal. Calcd. for $C_{25}H_{36}N_4O_7$: C, 59.51; H, 7.19; N, 11.10. Found: C, 59.32; H, 7.49; N, 11.70.

Z-Aib-Pro-Aib-Ala-Aib-OMe. Z-Pro-Aib-Ala-Aib-OMe (2.0 g, 3.9 mmol) was treated with H_2/Pd by the general method to give H-Pro-Aib-Ala-Aib-OMe as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Aib-OH (1.1 g, 4.6 mmol), 0.32 g (2.4 mmol) of HOAt, 3.0 ml (17.4 mmol) of DIEA, 1.1 g (3.9 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by general methods and was purified by recrystallized from AcOEt with ether to yield 1.8 g (79 %) of a white powder, mp 215-216°C. $[\alpha]_D^{25}$ +55.80 (c=0.9, MeOH), Rf_2 0.65. Anal. Calcd. for $C_{29}H_{43}N_5O_8$: C, 59.06; H, 7.35; N, 11.88. Found: C, 58.76; H, 7.38; N, 11.82.

Ac-Aib-Pro-Aib-Ala-Aib-OMe. Z-Aib-Pro-Aib-Ala-Aib-OMe (2.4 g, 4.0 mmol) was treated with H_2/Pd by the general method and the resulting H-Aib-Pro-Aib-Ala-Aib-OMe was dissolved in 18 ml of CH_2Cl_2. To this solution 0.76 ml (8.1 mmol) of acetic anhydride and 1.1 ml (8.3 mmol) of Et_3N were added. The mixture was stirred for 60 min at 25°C. The solvent was removed by evaporation and the residue was purified by silica-gel column chromatography (9/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with ether to yield 1.8 g (92 %) of a white powder, mp 225-226°C. $[\alpha]_D^{25}$ +19.76 (c=0.9, MeOH), Rf_2 0.43. Anal. Calcd. for $C_{23}H_{39}N_5O_7$: C, 55.51; H, 7.90; N, 14.08. Found: C, 55.45; H, 7.96; N, 13.78.

Ac-Aib-Pro-Aib-Ala-Aib-OH [1]. To the MeOH (10 ml) solution of Ac-Aib-Pro-Aib-Ala-Aib-OMe (1.1 g, 2.2 mmol), 4N NaOH (2.7 ml, 10.8 mmol) was added at an ice-bath temperature and the mixture was stirred for 60 min at 25°C. The pH of the solution was adjusted to ca 7 with conc. HCl and the solvent was removed by evaporation. The residue was dissolved in CH_3CN (ca 20 ml) at 40 °C and the solution was filtered. CH_3CN of the filtrate was evaporated and the residue was recrystallized from AcOEt with hexane to yield 1.0 g (96 %) of a white powder, mp 117-120 °C. $[\alpha]_D^{25}$ +16.46 (c=0.8, MeOH), Rf_1 0.43. Anal. Calcd. for $C_{22}H_{37}N_5O_7$: C, 52.68; H, 7.84; N, 13.96. Found: C, 52.07; H, 7.55; N, 13.87.

Z-Val-Aib-OMe. To the CH_2Cl_2 (80 ml) solution of Z-Val-OH [prepared from 8.0 g (18.5 mmol) of the corresponding DCHA salt], 1.5 g (9.2 mmol) of HODhbt, 12.9 ml (74.1 mmol) of DIEA, 5.2 g (18.7 mmol) of CIP, and 3.1 g (20.2 mmol) of HCl•H-Aib-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (1/2, AcOEt:hexane) followed by recrystallization from AcOEt with hexane to yield 5.6 g (87 %) of a white powder, mp 79-80°C. $[\alpha]_D^{25}$ -24.28 (c=0.9, MeOH), Rf_4 0.54. Anal. Calcd. for $C_{18}H_{26}N_2O_5$: C, 61.69; H, 7.48; N, 8.00. Found: C, 61.94; H, 7.60; N, 8.18.

Z-Val-Aib-Gly-OMe. To the MeOH (20 ml) solution of Z-Val-Aib-OMe (1.9 g, 5.3 mmol), 4N NaOH (13.2 ml, 52.8 mmol) was added at an ice-bath temperature and the mixture was stirred for 60 min at 25°C. The solvent was removed by evaporation and the residue was dissolved in H_2O. The aqueous phase was washed with ether. The pH of the aqueous phase was adjusted to ca 2 with conc. HCl and the resulting oily product was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and then the solvent was removed by evaporation. The resulting powder was dissolved in CH_2Cl_2 (20 ml). To the solution, 0.36 g (2.6 mmol) of HOAt, 4.1 ml (23.8 mmol) of DIEA, 1.5 g (5.3 mmol) of CIP, and 1.0 g (2.6 mmol) of HCl•H-Gly-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method to yield 2.0 g (91 %) of Z-Val-Aib-Gly-OMe as an oil. Rf_3 0.36.

Z-Aib-Val-Aib-Gly-OMe. Z-Val-Aib-Gly-OMe (2.0 g, 4.8 mmol) was treated with H_2/Pd by the general method to give H-Val-Aib-Gly-OMe as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Aib-OH (1.3 g,

5.3 mmol), 0.36 g (2.6 mmol) of HOAt, 3.4 ml (19.3 mmol) of DIEA, 1.5 g (5.3 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by a general method to yield 2.1 g (92 %) of Z-Aib-Val-Aib-Gly-OMe as an oil. Rf_2 0.55.

Fmoc-Gln(Trt)-Aib-Val-Aib-Gly-OMe. Z-Aib-Val-Aib-Gly-OMe (5.8 g, 11.7 mmol) was treated with H_2/Pd by the general method to give H-Aib-Val-Aib-Gly-OMe as an oil. To the CH_2Cl_2 (65 ml) solution of Fmoc-Gln(Trt)-OH (7.8 g, 12.8 mmol), 1.1 g (7.0 mmol) of HODhbt, 8.5 ml (49.0 mmol) of DIEA, 3.6 g (12.9 mmol) of CIP, and the deprotected tetrapeptide ester obtained above in CH_2Cl_2 (40 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 8.0 g (72 %) of a white powder, mp 115-119°C. $[\alpha]_D^{25}$ -0.31 (c=1.0, MeOH), Rf_3 0.37. Anal. Calcd. for $C_{55}H_{62}N_6O_9 \cdot 0.5H_2O$: C, 68.80; H, 6.61; N, 8.75. Found: C, 68.81; H, 7.07; N, 8.35.

Z-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OMe [2]. To the CH_3CN (50 ml) solution of Fmoc-Gln(Trt)-Aib-Val-Aib-Gly-OMe (8.0 g, 8.4 mmol), 4.2 ml (43.3 mmol) of diethylamine was added. The mixture was stirred for 20 min at 25°C. The solution was removed by evaporation to give H-Gln(Trt)-Aib-Val-Aib-Gly-OMe as an oil. To the CH_2Cl_2 (20 ml) solution of Z-Ala-OH (2.1 g, 9.2 mmol), 0.82 g (5.0 mmol) of HODhbt, 6.1 ml (35.2 mmol) of DIEA, 2.6 g (9.2 mmol) of CIP, and the deprotected pentapeptide ester obtained above in CH_2Cl_2 (40 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 6.6 g (84 %) of a white powder, mp 112-115°C. $[\alpha]_D^{25}$ -9.02 (c=1.1, MeOH), Rf_2 0.53. Anal. Calcd. for $C_{51}H_{63}N_7O_{10} \cdot 0.5H_2O$: C, 65.02; H, 6.85; N, 10.41. Found: C, 65.01; H, 7.29; N, 10.42.

Z-Aib-Aib-OMe. To the CH_2Cl_2 (35 ml) solution of Z-Aib-OH (2.0 g, 8.4 mmol), 0.57 g (4.2 mmol) of HOAt, 5.9 ml (33.7 mmol) of DIEA, 2.4 g (8.4 mmol) of CIP, and 1.4 g (9.2 mmol) of HCl•H-Aib-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 2.7 g (93 %) of a white powder, mp 107-108°C. Rf_3 0.56. Anal. Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.61; H, 7.40; N, 8.14.

Z-Val-Aib-Aib-OMe. Z-Aib-Aib-OMe (7.1 g, 21.1 mmol) was treated with H_2/Pd by the general method to give H-Aib-Aib-OMe as an oil. To the CH_2Cl_2 (40 ml) solution of Z-Val-OH [prepared from 9.1 g (21.1 mmol) of the corresponding DCHA salt], 1.7 g (10.5 mmol) of HODhbt, 14.7 ml (84.3 mmol) of DIEA, 5.9 g (21.1 mmol) of CIP, and the deprotected dipeptide ester obtained above in CH_2Cl_2 (50 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (6/4, AcOEt:hexane) followed by recrystallization from AcOEt with hexane to yield 8.2 g (90 %) of a white powder, mp 82-85°C. $[\alpha]_D^{25}$ -2.16 (c=0.8, MeOH), Rf_3 0.32. Anal. Calcd. for $C_{22}H_{33}N_3O_6 \cdot 2H_2O$: C, 56.03; H, 7.05; N, 8.91. Found: C, 56.08; H, 7.27; N, 8.93.

Z-Pro-Val-Aib-Aib-OMe. Z-Val-Aib-Aib-OMe (8.2 g, 18.9 mmol) was treated with H_2/Pd by the general method to give H-Val-Aib-Aib-OMe as an oil. To the CH_2Cl_2 (50 ml) solution of Z-Pro-OH (5.2 g, 20.8 mmol), 1.5 g (9.4 mmol) of HODhbt, 13.1 ml (75.3 mmol) of DIEA, 5.8 g (20.8 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (50 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) to yield 8.9 g (88 %) of Z-Pro-Val-Aib-Aib-OMe as an oil. Rf_2 0.57.

Z-Aib-Pro-Val-Aib-Aib-OMe. Z-Pro-Val-Aib-Aib-OMe (3.7 g, 7.0 mmol) was treated with H_2/Pd by the general method to give H-Pro-Val-Aib-Aib-OMe as an oil. To the CH_2Cl_2 (20 ml) solution of Z-Aib-OH (2.5 g, 10.5 mmol), 0.71 g (5.2 mmol) of HOAt, 6.3 ml (36.3 mmol) of DIEA, 2.9 g (10.5 mmol) of CIP, and the deprotected tetrapeptide ester obtained above in CH_2Cl_2 (25 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with ether to yield 4.0 g (92 %) of a white powder, mp 170-173°C. $[\alpha]_D^{25}$

+9.37 (c=0.9, MeOH), Rf_2 0.43. Anal. Calcd. for $C_{31}H_{47}N_5O_8$: C, 60.27; H, 7.67; N, 11.34. Found: C, 60.43; H, 7.96; N, 11.12.

Z-Leu-Aib-Pro-Val-Aib-Aib-OMe. Z-Aib-Pro-Val-Aib-Aib-OMe (1.5 g, 2.5 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Val-Aib-Aib-OMe as an oil. To the CH_2Cl_2 (6 ml) solution of Z-Leu-OH [prepared from 1.3 g (3.0 mmol) of the corresponding DCHA salt], 0.24 g (1.5 mmol) of HODhbt, 2.0 ml (11.5 mmol) of DIEA, 0.84 g (3.0 mmol) of CIP, and the deprotected pentapeptide ester obtained above in CH_2Cl_2 (10 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 1.3 g (73 %) of a white powder, mp 100-103°C. $[\alpha]_D^{25}$ -23.83 (c=0.9, MeOH), Rf_2 0.49. Anal. Calcd. for $C_{37}H_{58}N_6O_9$: C, 60.80; H, 8.00; N, 11.50. Found: C, 61.06; H, 8.22; N, 11.27.

Z-Leu-Aib-Pro-Val-Aib-Aib-OH [3]. To the MeOH (2 ml) solution of Z-Leu-Aib-Pro-Val-Aib-Aib-OMe (0.14 g, 0.19 mmol), 4N NaOH (0.96 ml, 3.8 mmol) was added at an ice-bath temperature and the mixture was stirred for 30 min at 40°C. The pH of the mixture was adjusted to ca 7 with conc. HCl and the solvent was removed by evaporation. The residue was dissolved in H_2O and the pH of the solution was adjusted to ca 2 with conc. HCl. The resulting oily product was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and then the solvent was removed by evaporation. The oily residue was purified by silica-gel column chromatography (9/1/0.5, $CHCl_3$:MeOH:AcOH) followed by recrystallization from AcOEt with hexane to yield 0.12 g (88 %) of a white powder, mp 118-123°C. $[\alpha]_D^{25}$ -21.94 (c=1.0, MeOH), Rf_1 0.50. Anal. Calcd. for $C_{36}H_{56}N_6O_9 \cdot 0.5H_2O$: C, 59.56; H, 7.92; N, 11.58. Found: C, 59.22; H, 7.90; N, 11.30.

Fmoc-Gln(Trt)-Pheol. To the CH_2Cl_2 (60 ml) solution of Fmoc-Gln(Trt)-OH (5.0 g, 8.2 mmol) , 0.67 g (4.1 mmol) of HODhbt, 5.7 ml (32.8 mmol) of DIEA, 2.3 g (8.2 mmol) of CIP, and 1.4 g (9.0 mmol) of Pheol were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 5.1 g (85 %) of a white powder, mp 175-178°C. $[\alpha]_D^{25}$ -14.37 (c=0.7, MeOH), Rf_3 0.39. Anal. Calcd. for $C_{48}H_{45}N_3O_5$: C, 77.50; H, 6.10; N, 5.65. Found: C, 77.25; H, 6.25; N, 5.60.

Fmoc-Glu(OBu^t)-Gln(Trt)-Pheol [4] .To the CH_3CN (25 ml) solution of Fmoc-Gln(Trt)-Pheol (5.1 g, 6.9 mmol), 35.5 ml (342 mmol) of diethylamine was added. The mixture was stirred for 20 min at 25°C. The solution was removed by evaporation to give H-Gln(Trt)-Pheol as an oil. To the CH_2Cl_2 (30 ml) solution of Fmoc-Glu(OBu^t)-OH (3.2 g, 7.5 mmol), 0.62 g (3.8 mmol) of HODhbt, 4.8 ml (27.5 mmol) of DIEA, 2.1 g (7.5 mmol) of CIP, and the deprotected dipeptide obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 5.4 g (85 %) of a white powder, mp 97-99°C. $[\alpha]_D^{25}$ -32.35 (c=0.7, MeOH), Rf_2 0.56. Anal. Calcd. for $C_{57}H_{60}N_4O_8 \cdot 0.5H_2O$: C, 72.92; H, 6.55; N, 5.97. Found: C, 72.91; H, 6.52; N, 6.09.

Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OMe [5]. Z-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OMe (230 mg, 0.25 mmol) was treated with H_2/Pd by the general method to give H-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OMe as an oil. To the CH_2Cl_2 (2 ml) solution of Ac-Aib-Pro-Aib-Ala-Aib-OH (180 mg, 0.37 mmol), 34 mg (0.25 mmol) of HOAt, 240 μl (1.4 mmol) of DIEA, 110 mg (0.39 mmol) of CIP, and the deprotected hexapeptide ester obtained above in CH_2Cl_2 (1 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 160 mg (52 %) of a white powder, mp 156-159°C. $[\alpha]_D^{25}$ +2.72 (c=0.7, MeOH), Rf_2 0.39. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Glu 1.05 (1), Gly 1.00 (1), Ala 2.00 (2), Aib 4.64 (5), Val 1.00 (1), Pro 1.02 (1). Anal. Calcd. for $C_{65}H_{92}N_{12}O_{14} \cdot 3H_2O$: C, 59.16; H, 7.49; N, 12.74. Found: C, 59.16; H, 7.09; N, 12.33.

Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OH. To the MeOH (7 ml) solution of Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OMe (0.88 g, 0.70 mmol), 4N NaOH (3.5 ml, 13.9 mmol) was added at an ice-bath temperature and the mixture was stirred for 30 min at 25°C. The pH of the mixture was adjusted to ca 7 with conc. HCl and the solvent was removed by evaporation. The residue was dissolved in H_2O and the pH of the solution was adjusted to ca 2 with conc. HCl. The resulting oily product was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and then the solvent was removed by evaporation. The oily residue was purified by recrystallization from AcOEt with ether to yield 0.80 g (91 %) of a white powder, mp 165-168°C. $[\alpha]_D^{25}$ +2.30 (c=0.4, MeOH), Rf_1 0.18. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Glu 1.04 (1), Gly 1.00 (1), Ala 1.90 (2), Aib 4.84 (5), Val 1.00 (1), Pro 1.38 (1). Anal. Calcd. for $C_{64}H_{90}N_{12}O_{14}\cdot 4H_2O$: C, 58.08; H, 7.46; N, 12.70. Found: C, 57.87; H, 6.94; N, 12.77.

Z-Leu-Aib-Pro-Val-Aib-Aib-Glu(OBu^t)-Gln(Trt)-Pheol [6]. To the CH_3CN (8 ml) solution of Fmoc-Glu(OBu^t)-Gln(Trt)-Pheol (1.9 g, 2.0 mmol), 10.5 ml (101 mmol) of diethylamine was added. The mixture was stirred for 20 min at 25°C. The solvent was removed by evaporation to give H-Glu(OBu^t)-Gln(Trt)-Pheol as an oil. To the CH_2Cl_2 (15 ml) solution of Z-Leu-Aib-Pro-Val-Aib-Aib-OH (2.2 g, 3.0 mmol), 0.41 g (3.0 mmol) of HOAt, 2.1 ml (12.1 mmol) of DIEA, 0.90 g (3.2 mmol) of CIP, and the deprotected tripeptide obtained above in CH_2Cl_2 (10 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with ether to yield 2.1 g (75 %) of a white powder, mp 122-124°C. $[\alpha]_D^{25}$ -22.23 (c=0.9, MeOH), Rf_2 0.55. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Glu 2.00 (2), Aib 2.99 (3), Val 1.00 (1), Leu 1.04 (1), Pro 1.32 (1). Anal. Calcd. for $C_{78}H_{104}N_{10}O_{14}$: C, 66.64; H, 7.46; N, 9.97. Found: C,66.61; H, 7.48; N, 9.96.

[Gln(Trt)^{7,19}, Glu(OBu^t)^{18}]-alamethicin F-30. Z-Leu-Aib-Pro-Val-Aib-Aib-Glu(OBu^t)-Gln(Trt)-Pheol [6] (120 mg, 85 μmol) was treated with H_2/Pd by the general method to give H-Leu-Aib-Pro-Val-Aib-Aib-Glu(OBu^t)-Gln(Trt)-Pheol as an oil. To the CH_2Cl_2 (2 ml) solution of Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln(Trt)-Aib-Val-Aib-Gly-OH (160 mg, 130 μmol), 17 mg (120 μmol) of HOAt, 89 μl (0.51 mmol) of DIEA, 40 mg (140 μmol) of CIP, and the deprotected nonapeptide obtained above in CH_2Cl_2 (2 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by gel-filtration using the Sephadex LH-20 column (20 x 280 mm, MeOH) followed by reprecipitation from AcOEt with hexane to yield 110 mg (66 %) of a white powder, mp 172-175°C. $[\alpha]_D^{25}$ -6.70 (c=0.2, MeOH), Rf_2 0.56. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Glu 3.06 (3), Gly 1.01 (1), Ala 2.00 (2), Aib 5.86 (8), Val 2.05 (2), Leu 0.90 (1), Pro 2.41 (2). FAB-MS: m/z 2504.3899 for $[M+H]^+$ (Calcd. 2504.4038 for $C_{134}H_{187}N_{22}O_{25}$). Anal. Calcd. for $C_{134}H_{186}N_{22}O_{25}$: C, 64.25; H, 7.48; N, 12.30. Found: C,64.27; H, 7.61; N, 12.31.

Alamethicin F-30. [Gln(Trt)^{7,19}, Glu(OBu^t)^{18}]-alamethicin F-30 (110 mg, 43.9 μmol) was treated with TFA (3 ml) in the presence of thioanisole (300 μl) for 60 min at 25°C. The TFA of the reaction mixture was removed by evaporation at 4°C. Ether (50 ml) was added to the residue and the resulting precipitate was dissolved in MeOH (5 ml). The solution was applied to a Sephadex LH-20 column (20 x 280 mm, MeOH). The solvent of the fractions corresponding to the main peak was removed by evaporation to give a powder (63 mg). The crude product was further purified by preparative HPLC [YMC SH-343-5AM column (20 x 250 mm), CH_3CN /0.1 % TFA; CH_3CN 60 % to 100 % (60 min)] and yielded 26 mg (30 %) of a white powder. HPLC on a YMC AM302 (4.6 x 150 mm) [retention time; 28.47 min, CH_3CN /0.1 % TFA; CH_3CN 20 % to 100 % (30 min)]. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Glu 3.02 (3), Gly 1.00 (1), Ala 1.96 (2), Aib 6.48 (8), Val 1.99 (2), Leu 1.00 (1), Pro 2.39 (2). ^{1}H-NMR (600 MHz, MeOH-D4): δ, 0.914 (d, J=6.5Hz, 3H), 0.938 (d, J=6.5Hz, 3H), 0.978 (d, J=6.8Hz, 3H), 1.005 (d, J=6.8Hz, 3H), 1.068 (d, J=6.6Hz, 3H), 1.135 (d, J=6.6Hz, 3H), 1.464 (s, 3H), 1.489 (s, 3H), 1.502 (s, 3H), [1.529(s)1.531(s)1.537(s)1.544(s)1.548(s)1.558(s)1.563(s), total 13H], 1.589 (s, 3H), 1.612 (s, 3H), 1.610 (m, 1H), 1.800 (m, 2H), 2.056 (s, 3H), 1.890~2.397 (m, 19H), 2.533 (m, 2H),

2.707 (dd, J=5.3Hz, 10.1Hz, 1H), 2.744 (dd, J=7.3Hz, 13.8Hz, 1H), 2.937 (dd, J=5.5Hz, 13.7Hz, 1H), 3.494 (dt, J=6.1Hz, 10.3Hz, 1H), 3.594 (dd, J=5.6Hz, 8.4Hz, 1H), 3.626 (d, J=5.0Hz, 1H), 3.662 (dd, J=6.4Hz, 16.5Hz, 1H), 3.728 (m, 2H), 3.879 (ddt, J=6.1Hz, 11.7Hz, 11.1Hz, 1H), 3.941 (m, 3H), 4.024 (dd, J=4.7Hz, 7.3Hz, 1H), 4.034 (m, 1H), 4.096 (dd, J=6.0Hz, 7.3Hz, 1H), 4.162 (m, 2H), 4.250 (t, J=8.4Hz, 1H), 4.374 (dd, J=6.8Hz, 8.7Hz, 1H), 4.460 (ddd, J=4.0Hz, 8.0Hz, 11.3Hz, 1H), 6.599 (br s, 1H), 6.744 (br s, 1H), 7.142 (t, J=7.3Hz, 1H), 7.225 (t, J=7.8Hz, 2H), 7.291 (d, J=7.0Hz, 1H), 7.285 (br s, 1H), 7.340 (d, J=9.1Hz, 1H), 7.417 (br s, 1H), 7.481 (d, J=5.5Hz, 1H), 7.562 (d, J=5.5Hz, 1H), 7.567 (s, 1H), 7.589 (d, J=7.5Hz, 1H), 7.623 (s, 1H), 7.680~7.905 (m, 3H), 7.925 (s, 1H), 7.979 (d, J=5.1Hz, 1H), 8.067 (s, 1H), 8.092 (d, J=8.0Hz, 1H), 8.203 (s, 1H), 8.327 (t, J=5.9Hz, 1H), 8.346 (s, 1H), 8.604 (s, 1H). ^{13}C-NMR (75 MHz, MeOH-D4): δ, 16.93, 17.00, 19.46, 19.57, 20.23, 20.77, 21.29, 22.38, 23.01, 23.07, 23.19, 23.32, 23.38, 23.41, 23.73, 23.80, 25.57, 26.60, 26.76, 26.91, 27.03, 27.08, 27.21, 27.31, 27.38, 28.05, 29.68, 30.03, 30.28, 30.48, 31.51, 31.75, 32.55, 32.90, 39.98, 41.53, 42.30, 45.02, 49.94, 50.54, 53.87, 54.00, 54.04, 54.45, 55.83, 56.83, 57.27, 57.43, 57.55, 57.61, 57.67, 58.03, 58.08, 64.17, 64.66, 64.88, 65.67, 127.09, 129.09, 130.40, 139.77, 172.50, 172.96, 174.05, 174.87, 174.93, 175.25, 175.33, 175.43, 175.59, 175.72, 175.74, 175.79, 176.27, 176.29, 176.43, 177.18, 177.27, 177.58, 177.83, 178.02, 178.20, 178.47, 178.84, 178.98. FAB-MS: m/z 1986.1140 for $[M+Na]^+$ (Calcd. 1986.1040 for $C_{92}H_{150}N_{22}O_{25}Na$). CD spectrum: $[\theta]_{222}= -1.298 \times 10^{-4}$ deg•cm^2•dmol^{-1} (MeOH) [lit[15]; $[\theta]_{221}= -1.169 \times 10^{-4}$ deg•cm^2•dmol^{-1} (MeOH)]. The GC-spectrum of the acid hydrolysate obtained [95°C for 2min, then 95°C to 200°C (4°C/min)] is shown in Figure 3-2.

Z-Ala-Val-OMe. To the CH_2Cl_2 (40 ml) solution of Z-Ala-OH (2.0 g, 9.0 mmol), 0.73 g (4.5 mmol) of HODhbt, 6.2 ml (35.9 mmol) of DIEA, 2.5 g (9.0 mmol) of CIP, and 1.7 g (9.8 mmol) of HCl•H-Val-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (2/3, AcOEt:hexane) followed by recrystallization from AcOEt with hexane to yield 2.2 g (72 %) of a white powder, mp 73-76°C. $[\alpha]_D^{25}$ -30.70 (c=1.2, MeOH), Rf_3 0.45. Anal. Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.32. Found: C, 60.32; H, 7.13; N, 8.42.

Z-Pro-Ala-Val-OMe. Z-Ala-Val-OMe (4.5 g, 13.2 mmol) was treated with H_2/Pd by the general method to give H-Ala-Val-OMe as an oil. To the CH_2Cl_2 (30 ml) solution of Z-Pro-OH (3.6 g, 14.6 mmol), 1.3 g (7.9 mmol) of HODhbt, 9.7 ml (55.6 mmol) of DIEA, 4.1 g (14.5 mmol) of CIP, and the deprotected dipeptide ester obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (3/1, AcOEt:hexane) followed by recrystallization from AcOEt with hexane to yield 4.7 g (83 %) of a white powder, mp 126-127°C. $[\alpha]_D^{25}$ -98.04 (c=0.5, MeOH), Rf_3 0.40. Anal. Calcd. for $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.21; N, 9.69. Found: C, 61.43; H, 7.32; N, 9.70.

Z-Aib-Pro-Ala-Val-OMe. Z-Pro-Ala-Val-OMe (2.2 g, 5.1 mmol) was treated with H_2/Pd by the general method to give H-Pro-Ala-Val-OMe as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Aib-OH (1.3 g, 5.6 mmol), 0.42 g (3.1 mmol) of HOAt, 3.7 ml (21.4 mmol) of DIEA, 1.6 g (5.6 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 2.0 g (76 %) of a white powder, mp 151-156°C. $[\alpha]_D^{25}$ +3.05 (c=0.8, MeOH), Rf_2 0.54. Anal. Calcd. for $C_{26}H_{38}N_4O_7$: C, 60.21; H, 7.39; N, 10.80. Found: C, 60.18; H, 7.52; N, 10.83.

Z-Leu-Aib-Pro-Ala-Val-OMe. Z-Aib-Pro-Ala-Val-OMe (2.7 g, 5.2 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Ala-Val-OMe as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Leu-OH [prepared from 2.6 g (5.8 mmol) of the corresponding DCHA salt], 0.51 g (3.1 mmol) of HODhbt, 3.8 ml (22.0 mmol) of DIEA, 1.6 g (5.8 mmol) of CIP, and the deprotected tetrapeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 2.3 g (71 %) of a white powder, mp 145-148°C. $[\alpha]_D^{25}$ -

42.46 (c=0.6, MeOH), Rf_2 0.46. Anal. Calcd. for $C_{32}H_{49}N_5O_8$: C, 60.83; H, 7.82; N, 11.09. Found: C, 60.81; H, 7.98; N, 11.10.

Fmoc-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe. Z-Leu-Aib-Pro-Ala-Val-OMe (1.5 g, 2.4 mmol) was treated with H_2/Pd by the general method to give H-Leu-Aib-Pro-Ala-Val-OMe as an oil. To the CH_2Cl_2 (13 ml) solution of Fmoc-Asn(Trt)-OH (1.6 g, 2.6 mmol), 0.23 g (1.4 mmol) of HODhbt, 1.8 ml (10.1 mmol) of DIEA, 0.73 g (2.6 mmol) of CIP, and the deprotected pentapeptide ester obtained above in CH_2Cl_2 (10 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 2.3 g (89 %) of a white powder, mp 149-151°C. $[\alpha]_D^{25}$ -6.09 (c=1.2, MeOH), Rf_2 0.57. Anal. Calcd. for $C_{62}H_{73}N_7O_{10}$: C, 69.19; H, 6.84; N, 9.11. Found: C, 69.22; H, 7.19; N, 8.99.

Z-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe. To the CH_3CN (15 ml) solution of Fmoc-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe (2.3 g, 2.1 mmol), 11.0 ml (106 mmol) of diethylamine was added. The mixture was stirred for 20 min at 25°C. The solution was removed by evaporation to give H-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe as an oil. To the CH_2Cl_2 (7 ml) solution of Z-Aib-OH (0.56 g, 2.4 mmol), 0.16 g (1.2 mmol) of HOAt, 1.5 ml (8.5 mmol) of DIEA, 0.65 g (2.3 mmol) of CIP, and the deprotected hexapeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 1.8 g (79 %) of a white powder, mp 127-130°C. $[\alpha]_D^{25}$ -15.33 (c=0.6, MeOH), Rf_2 0.50. Anal. Calcd. for $C_{59}H_{76}N_8O_{11}$: C, 66.02; H, 7.14; N, 10.44. Found: C, 65.95; H, 7.29; N, 10.25.

Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe. Z-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe (0.50 g, 0.47 mmol) was treated with H_2/Pd by the general method to give H-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe as an oil. The product was dissolved in CH_2Cl_2 (5 ml). To the solution 88 µl (0.93 mmol) of acetic anhydride and 162 µl (0.93 mmol) of DIEA were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 0.43 g (94 %) of a white powder, mp 158-163°C. $[\alpha]_D^{25}$ -13.27 (c=0.5, MeOH), Rf_2 0.49. Anal. Calcd. for $C_{53}H_{72}N_8O_{10}\cdot 0.5H_2O$: C, 64.28; H, 7.43; N, 11.32. Found: C, 64.39; H, 7.57; N, 11.04.

Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OH [7]. To the MeOH (4 ml) solution of Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OMe (0.43 g, 0.44 mmol), 4N NaOH (1.7 ml, 6.6 mmol) was added at an ice-bath temperature and the mixture was stirred for 60 min at 25°C. The pH of the mixture was adjusted to ca 7 with conc. HCl and the solvent was removed by evaporation. The residue was dissolved in H_2O and the pH of the solution was adjusted to ca 2 with conc. HCl. The resulting oily product was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and then the solvent was removed by evaporation. The oily residue was purified by recrystallization from AcOEt with ether to yield 0.40 g (94 %) of a white powder, mp 171-175°C. $[\alpha]_D^{25}$ -3.37 (c=0.7, MeOH), Rf_1 0.29. Anal. Calcd. for $C_{52}H_{70}N_8O_{10}\cdot H_2O$: C, 63.39; H, 7.37; N, 11.38. Found: C, 63.59; H, 7.41; N, 11.24.

Z-Pro-Leuol. To the CH_2Cl_2 (25 ml) solution of Z-Pro-OH (2.0 g, 8.0 mmol), 0.65 g (4.0 mmol) of HODhbt, 5.6 ml (32.1 mmol) of DIEA, 2.2 g (8.0 mmol) of CIP, and 1.1 g (8.8 mmol) of Leuol were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 2.3 g (83 %) of a white powder, mp 83-85°C. $[\alpha]_D^{25}$ -65.80 (c=1.0, MeOH), Rf_3 0.48. Anal. Calcd. for $C_{19}H_{28}N_2O_4$: C, 65.49; H, 8.10; N, 8.04. Found: C, 65.32; H, 8.18; N, 8.24.

Z-Aib-Pro-Leuol. Z-Pro-Leuol (5.1 g, 14.7 mmol) was treated with H_2/Pd by the general method to give H-Pro-Leuol as an oil. To the CH_2Cl_2 (30 ml) solution of Z-Aib-OH (3.8 g, 16.2 mmol), 1.2 g (8.8 mmol) of HOAt, 10.8 ml (61.8 mmol) of DIEA, 4.5 g (16.2 mmol) of CIP, and the deprotected dipeptide obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 4.6 g

(71 %) of a white powder, mp 151-153°C. $[\alpha]_D^{25}$ +21.16 (c=0.9, MeOH), Rf_3 0.28. Anal. Calcd. for $C_{23}H_{35}N_3O_5$: C, 63.72; H, 8.14; N, 9.69. Found: C, 63.87; H, 8.23; N, 9.51.

Z-Leu-Aib-Pro-Leuol. Z-Aib-Pro-Leuol (2.0 g, 4.6 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Leu-OH [prepared from 2.2 g (5.0 mmol) of the corresponding DCHA salt], 0.45 g (2.8 mmol) of HODhbt, 3.3 ml (19.2 mmol) of DIEA, 1.4 g (5.0 mmol) of CIP, and the deprotected tripeptide obtained above in CH_2Cl_2 (10 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 2.0 g (79 %) of a white powder, mp 129-131°C. $[\alpha]_D^{25}$ -29.51 (c=1.0, MeOH), Rf_3 0.30. Anal. Calcd. for $C_{29}H_{46}N_4O_6$: C, 63.71; H, 8.48; N, 10.25. Found: C, 63.86; H, 8.68; N, 10.35.

Z-Aib-Leu-Aib-Pro-Leuol. Z-Leu-Aib-Pro-Leuol (2.0 g, 3.6 mmol) was treated with H_2/Pd by the general method to give H-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (8 ml) solution of Z-Aib-OH (0.95 g 4.0 mmol), 0.27 g (2.0 mmol) of HOAt, 2.7 ml (15.2 mmol) of DIEA, 1.1 g (4.0 mmol) of CIP, and the deprotected tetrapeptide obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 2.2 g (97 %) of a white powder, mp 130-131°C. $[\alpha]_D^{25}$ -33.26 (c=0.9, MeOH), Rf_2 0.44. Anal. Calcd. for $C_{33}H_{53}N_5O_7$: C, 62.73; H, 8.32; N, 11.09. Found: C, 62.60; H, 8.54; N, 11.18.

Z-Pro-Aib-Leu-Aib-Pro-Leuol. Z-Aib-Leu-Aib-Pro-Leuol (3.8 g, 6.0 mmol) was treated with H_2/Pd by the general method to give H-Aib-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Pro-OH (1.7 g, 6.7 mmol), 0.59 g (3.6 mmol) of HODhbt, 4.4 ml (25.3 mmol) of DIEA, 1.9 g (6.6 mmol) of CIP, and the deprotected pentapeptide obtained above in CH_2Cl_2 (25 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 4.2 g (96 %) of a white powder, mp 214-216°C. $[\alpha]_D^{25}$ -34.48 (c=1.0, MeOH), Rf_2 0.58. Anal. Calcd. for $C_{38}H_{60}N_6O_8$: C, 62.61; H, 8.30; N, 11.53. Found: C, 62.42; H, 8.36; N, 11.79.

Z-Aib-Pro-Aib-Leu-Aib-Pro-Leuol [8]. Z-Pro-Aib-Leu-Aib-Pro-Leuol (4.0 g, 5.5 mmol) was treated with H_2/Pd by the general method to give H-Pro-Aib-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (15 ml) solution of Z-Aib-OH (2.0 g, 8.2 mmol), 0.56 g (4.1 mmol) of HOAt, 4.8 ml (27.5 mmol) of DIEA, 2.3 g (8.2 mmol) of CIP, and the deprotected hexapeptide obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 3.1 g (69 %) of a white powder, mp 231-233°C. $[\alpha]_D^{25}$ +30.93 (c=1.1, MeOH), Rf_2 0.48. Anal. Calcd. for $C_{42}H_{67}N_7O_9$: C, 61.97; H, 8.30; N, 12.05. Found: C, 61.87; H, 8.52; N, 11.65.

Z-Pro-Ala-OMe. To the CH_2Cl_2 (25 ml) solution of Z-Pro-OH (2.0 g, 8.0 mmol), 0.65 g (4.0 mmol) of HODhbt, 5.6 ml (32.1 mmol) of DIEA, 2.2 g (8.0 mmol) of CIP, and 1.2 g (8.8 mmol) of HCl•H-Ala-OMe were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (1/1, AcOEt:hexane) to yield 2.4 g (90 %) of Z-Pro-Ala-OMe as an oil. Rf_3 0.56.

Z-Aib-Pro-Ala-OMe. Z-Pro-Ala-OMe (2.4 g, 7.2 mmol) was treated with H_2/Pd by the general method to give H-Pro-Ala-OMe as an oil. To the CH_2Cl_2 (15 ml) solution of Z-Aib-OH (1.9 g, 7.9 mmol), 0.54 g (4.0 mmol) of HOAt, 5.3 ml (30.3 mmol) of DIEA, 2.2 g (7.9 mmol) of CIP, and the deprotected dipeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method to yield 1.9 g (64 %) of Z-Aib-Pro-Ala-OMe as an oil. Rf_3 0.42. The product was used in subsequent reactions without further purification.

Z-Leu-Aib-Pro-Ala-OMe. Z-Aib-Pro-Ala-OMe (1.9 g, 4.6 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Ala-OMe as an oil. To the CH_2Cl_2 (10 ml) solution of Z-Leu-OH [prepared from 2.3 g (5.0 mmol) of the corresponding DCHA salt], 0.45 g (2.8 mmol) of HODhbt, 3.4 ml (19.3 mmol) of DIEA, 1.4 g (5.0 mmol) of CIP, and the deprotected tripeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and

was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 1.9 g (80 %) of a white powder, mp 67-68°C. $[\alpha]_D^{25}$ -75.28 (c=1.0, MeOH), Rf_2 0.63. Anal. Calcd. for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.52. Found: C, 60.64; H, 7.72; N, 10.44.

Fmoc-Asn(Trt)-Leu-Aib-Pro-Ala-OMe. Z-Leu-Aib-Pro-Ala-OMe (1.9 g, 3.6 mmol) was treated with H_2/Pd by the general method to give H-Leu-Aib-Pro-Ala-OMe as an oil. To the CH_2Cl_2 (18 ml) solution of Fmoc-Asn(Trt)-OH (2.4 g, 4.0 mmol), 0.36 g (2.2 mmol) of HODhbt, 2.7 ml (15.3 mmol) of DIEA, 1.1 g (4.0 mmol) of CIP, and the deprotected tetrapeptide ester obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 2.8 g (80 %) of a white powder, mp 140-142°C. $[\alpha]_D^{25}$ -28.38 (c=0.9, MeOH), Rf_2 0.66. Anal. Calcd. for $C_{57}H_{64}N_6O_9 \cdot 0.5H_2O$: C, 69.42; H, 6.64; N, 8.52. Found: C, 69.31; H, 6.78; N, 8.39.

Z-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OMe. To the CH_3CN (40 ml) solution of Fmoc-Asn(Trt)-Leu-Aib-Pro-Ala-OMe (5.5 g, 5.6 mmol), 29.2 ml (282 mmol) of diethylamine was added. The mixture was stirred for 20 min at 25°C. The solution was removed by evaporation to give H-Asn(Trt)-Leu-Aib-Pro-Ala-OMe as an oil. To the CH_2Cl_2 (14 ml) solution of Z-Aib-OH (1.5 g, 6.2 mmol), 0.46 g (3.4 mmol) of HOAt, 4.1 ml (23.7 mmol) of DIEA, 1.7 g (6.2 mmol) of CIP, and the deprotected pentapeptide ester obtained above in CH_2Cl_2 (30 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (40/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 4.8 g (87 %) of a white powder, mp 184-185°C. $[\alpha]_D^{25}$ -32.52 (c=1.1, MeOH), Rf_2 0.70. Anal. Calcd. for $C_{54}H_{67}N_7O_{10}$: C, 66.58; H, 6.93; N, 10.07. Found: C, 66.42; H, 6.96; N, 9.96.

Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OMe. Z-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OMe (4.8 g, 4.9 mmol) was treated with H_2/Pd by the general method to give H-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OMe as an oil. The product was dissolved in CH_2Cl_2 (40 ml). To the solution 0.93 ml (9.9 mmol) of acetic anhydride and 1.7 ml (9.8 mmol) of DIEA were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by recrystallization from AcOEt with hexane to yield 2.9 g (67 %) of a white powder, mp 143-144°C. $[\alpha]_D^{25}$ -30.04 (c=0.8, MeOH), Rf_2 0.56. Anal. Calcd. for $C_{48}H_{63}N_7O_9 \cdot 0.5H_2O$: C, 64.70; H, 7.24; N, 11.00. Found: C, 64.54; H, 7.29; N, 10.85.

Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OH [9]. To the MeOH (4 ml) solution of Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OMe (0.33 g, 0.37 mmol), 4N NaOH (0.46 ml, 1.8 mmol) was added at an ice-bath temperature and the mixture was stirred for 60 min at 25°C. The pH of the mixture was adjusted to ca 7 with conc. HCl and the solvent was removed by evaporation. The residue was dissolved in H_2O and the pH of the solution was adjusted to ca 2 with conc. HCl. The resulting oily product was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and then the solvent was removed by evaporation. The oily residue was purified by recrystallization from AcOEt with ether to yield 0.32 g (98 %) of a white powder, mp 165-166°C. $[\alpha]_D^{25}$ -26.02 (c=0.7, MeOH), Rf_1 0.24. Anal. Calcd. for $C_{47}H_{61}N_7O_9 \cdot 1.5H_2O$: C, 63.07; H, 6.87; N, 10.96. Found: C, 63.19; H, 7.08; N, 10.77.

Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol [10]. Z-Aib-Pro-Aib-Leu-Aib-Pro-Leuol [8] (1.9 g, 2.3 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Aib-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (8 ml) solution of Z-Val-OH [prepared from 1.2 g (2.8 mmol) of the corresponding DCHA salt], 0.23 g (1.41 mmol) of HODhbt, 1.82 ml (10.46 mmol) of DIEA, 0.78 g (2.80 mmol) of CIP, and the deprotected heptapeptide obtained above in CH_2Cl_2 (15 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (20/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 1.4 g (64 %) of a white powder, mp 227-229°C. $[\alpha]_D^{25}$ -7.73 (c=0.9, MeOH), Rf_2 0.50. Anal. Calcd. for $C_{47}H_{76}N_8O_{10}$: C, 61.82; H, 8.39; N, 12.27. Found: C, 61.86; H, 8.49; N, 12.30.

[Asn(Trt)^2]-trichovirin I 4A by the path A. Z-Aib-Pro-Aib-Leu-Aib-Pro-Leuol [8] (100 mg, 0.12 mmol) was treated with H_2/Pd by the general method to give H-Aib-Pro-Aib-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (3 ml) solution of Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-Val-OH [7] (140 mg, 0.14 mmol),

20 mg (0.15 mmol) of HOAt, 110 µl (0.63 mmol) of DIEA, 40 mg (0.14 mmol) of CIP, and the deprotected heptapeptide obtained above in CH_2Cl_2 (1 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (30/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 120 mg (60 %) of a white powder. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Asp 1.00 (1), Ala 0.95 (1), Aib 3.60 (5), Val 0.93 (1), Leu 1.95 (2), Pro 3.74 (3). FAB-MS: m/z 1650.9679 for $[M+Na]^+$ (Calcd. 1650.9639 for $C_{86}H_{129}N_{15}O_{16}Na$).

Trichovirin I 4A by the path A (Compound 1 and 2). [Asn(Trt)^2]-trichovirin I 4A (120 mg, 74 µmol) obtained above was treated with TFA (3 ml) in the presence of anisole (300 µl) for 30 min at 25°C. The TFA in the reaction mixture was removed by evaporation at 4°C. $CHCl_3$ (50 ml) was added to the residue and the solution was washed with H_2O, 5% $NaHCO_3$, H_2O, and then dried over Na_2SO_4. The solvent was removed by evaporation and the residue was dissolved in MeOH (5 ml). The solution was applied to a Sephadex LH-20 column (20 x 280 mm, MeOH). The solvent of the fractions corresponding to the main peak was removed by evaporation to give a powder (49 mg). The crude product was further purified by preparative HPLC [YMC SH-343-5AM column (20 x 250 mm), CH_3CN /0.1 % TFA; CH_3CN 60 % to 100 % (60 min)] and yielded 16 mg (10 %) of compound 1 and 12 mg (7 %) of compound 2, respectively. HPLC on a YMC AM302 (4.6 x 150 mm) [retention time; 20.38 min for compound 1 and 24.74 min for compound 2, CH_3CN /0.1 % TFA; CH_3CN 20 % to 100 % (30 min)]. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Compound 1; Asp 1.00 (1), Ala 0.97 (1), Aib 4.03 (5), Val 1.00 (1), Leu 2.04 (2), Pro 3.78 (3); Compound 2; Asp 0.99 (1), Ala 0.96 (1), Aib 4.19 (5), Val 1.00 (1), Leu 2.05 (2), Pro 3.84 (3). FAB-MS: Compound 1; m/z 1408.8599 for $[M+Na]^+$ (Calcd. 1408.8544 for $C_{67}H_{115}N_{15}O_{16}Na$); Compound 2; m/z 1408.8684 for $[M+Na]^+$ (Calcd. 1408.8544 for $C_{67}H_{115}N_{15}O_{16}Na$); GC-spectra of the acid hydrolysate derived from compound 1 and 2 [95°C for 2 min, then 95°C to 200°C (4°C/min)] are shown in Figure 5.

[Asn(Trt)^2]-trichovirin I 4A by the path B. Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol [10] (200 mg, 0.22 mmol) was treated with H_2/Pd by the general method to give H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol as an oil. To the CH_2Cl_2 (3 ml) solution of Ac-Aib-Asn(Trt)-Leu-Aib-Pro-Ala-OH [9] (270 mg, 0.31 mmol), 42 mg (0.31 mmol) of HOAt, 210 µl (1.2 mmol) of DIEA, 85 mg (305 mmol) of CIP, and the deprotected octapeptide obtained above in CH_2Cl_2 (2 ml) were added. The mixture was stirred for 60 min at 25°C. The product was isolated by the general method and was purified by silica-gel column chromatography (30/1, $CHCl_3$:MeOH) followed by recrystallization from AcOEt with hexane to yield 290 mg (81 %) of a white powder, mp 184-185°C. $[\alpha]_D^{25}$ +1.73 (c=0.8, MeOH), Rf_2 0.50. Anal. Calcd. for $C_{86}H_{129}N_{15}O_{16}\cdot H_2O$: C, 62.67; H, 8.01; N, 12.75. Found: C, 62.88; H, 8.15; N, 12.41. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Asp 0.95 (1), Ala 0.93 (1), Aib 3.60 (5), Val 0.97 (1), Leu 2.00 (2), Pro 3.96 (3). FAB-MS: m/z 1650.9675 for $[M+Na]^+$ (Calcd. 1650.9639 for $C_{86}H_{129}N_{15}O_{16}Na$).

Trichovirin I 4A by the path B. [Asn(Trt)^2]-trichovirin I 4A (100 mg, 61 µmol) obtained above was treated with TFA (3 ml) in the presence of anisole (300 µl) for 30 min at 25°C. The same isolation procedure followed by LH-20 column chromatography, as described for pathway A synthesis, was performed to yield a powder (70 mg). The crude product was further purified by preparative HPLC [YMC SH-343-5AM column (20 x 250 mm), CH_3CN /0.1 % TFA; CH_3CN 60 % to 100 % (60 min)] to yield 36 mg (43 %) of a white powder. HPLC on a YMC AM302 (4.6 x 150 mm) [retention time; 24.70 min, CH_3CN /0.1 % TFA; CH_3CN 20 % to 100 % (30 min)]. Amino acid analysis after 6 N HCl hydrolysis (numbers in parentheses are theoretical values): Asp 0.99 (1), Ala 1.00 (1), Aib 3.92 (5), Val 1.00 (1), Leu 2.01 (2), Pro 3.97 (3). ^{1}H-NMR (600 MHz, MeOH-D4): δ, 0.842 (d, J=6.2Hz, 3H), 0.859 (d, J=6.5Hz, 3H), 0.896 (d, J=6.1Hz, 3H), 0.901 (d, J=6.5Hz, 3H), 0.949 (d, J=6.5Hz, 3H), 0.957 (d, J=6.6Hz, 3H), 0.977 (d, J=6.8Hz, 3H), 0.998 (d, J=6.8Hz, 3H), 1.356 (ddd, J=4.2Hz, 9.3Hz, 13.5Hz, 1H), 1.450 (s, 3H), 1.456 (s, 3H), 1.476 (s, 3H), 1.488 (s, 3H), 1.495 (s, 3H), 1.509 (s, 3H), 1.521 (s, 6H), 1.523 (d, J=7.3Hz, 3H), 1.543 (s, 3H), 1.594 (s, 3H), 1.564~1.977 (m, 18H), 2.027 (s, 3H), 2.020~2.095 (m, 3H), 2.140 (ddd, J=6.7Hz,

13.5Hz, 13.5Hz, 1H), 2.257~2.371 (m, 3H), 2.731 (dd, J=5.0Hz, 15.5Hz, 1H), 2.818 (dd, J=5.0Hz, 15.5Hz, 1H), 3.356 (m, 1H), 3.466 (ddd, J=6.6Hz, 8.3Hz, 11.5Hz, 1H), 3.512 (dd, J=5.5Hz, 11.3Hz, 1H), 3.558 (dd, J=5.5Hz, 11.3Hz, 1H), 3.592 (ddd, J=8.5Hz, 6.5Hz, 11.5Hz, 1H), 3.838~3.915 (m, 3H), 3.951~3.991 (m, 1H), 4.1502 (t, J=8.5Hz, 1H), 4.201 (t, J=8.3Hz, 1H), 4.224 (t, J=7.3Hz, 1H), 4.335~4.392 (m, 3H), 4.417 (t, J=5.9Hz, 1H), 4.436 (t, J=8.0Hz, 1H), 6.954 (br s, 1H), 7.450 (d, J=9.1Hz, 1H), 7.526 (d, J=8.9Hz, 2H), 7.613 (d, J=8.5Hz, 1H), 7.706 (br s, 1H), 7.769 (s, 1H), 7.802 (d, J=7.0Hz, 1H), 7.826 (s, 1H), 7.849 (s, 2H), 8.567 (t, J=6.1Hz, 1H), 8.635 (s, 1H). ^{13}C-NMR (75 MHz, MeOH-D4): δ, 17.098, 19.541, 19.645, 20.758, 21.247, 22.010, 23.029, 23.358, 23.415, 23.541, 23.774, 23.961, 24.209, 25.611, 25.838, 25.929, 26.061, 26.584, 26.783, 27.082, 27.521, 29.720, 30.131, 31.767, 35.573, 40.283, 40.892, 41.170, 50.092, 50.250, 50.521, 51.126, 52.333, 53.298, 53.628, 57.638, 57.734, 57.847, 58.125, 61.020, 64.164, 64.917, 65.790, 65.976, 173.030, 173.673, 173.924, 174.369, 174.783, 174.909, 174.985, 175.078, 175.245, 175.493, 176.344, 177.765, 178.013. FAB-MS: m/z 1408.8517 for $[M+Na]^+$ (Calcd. 1408.8544 for $C_{67}H_{115}N_{15}O_{16}Na$). GC-spectrum of the derived acid hydrolysate [95°C for 2 min, then 95°C to 200°C (4°C/min)] is shown in Figure 6.

Abbreviations. Bu^t=*tert*-butyl, DIEA=N,N-diisopropylethylamine, Fmoc=fluoren-9-ylmethyloxycarbonyl, HOAt=1-hydroxy-7-azabenzortiazole, HODhbt=3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine, TFA= trifuloroacetic acid, TLC=thin layer chromatography, Trt=trityl, Z=benzyloxycarbonyl.

Acknowledgment. The authors are grateful to Dr. Shiroh Futaki (Institute for Medicinal Resources, The University of Tokushima) for measuring the CD spectra and Mrs. Masako Akaji (Faculty of Pharamaceutical Sciences, Kyoto University) for measuring the ^{1}H- and ^{13}C-NMR spectra.

REFERENCES

1. Sansom, M.S.P. *Prog. Biophys. Mol. Biol*, **1991**, 55, 139-235.
2. Nagaraj, R.; Balaram, P. *Acc. Chem. Res.* **1981**, 14, 356-362.
3. Karle, I.L.; Balaram, P. *Biochemistry*, **1990**, 29, 6747-6756.
4. Brachais, L.; Davoust, D.; Molle, G. *Int. J. Peptide Protein Res.* **1995**, 45, 164-172; and references cited therein.
5. Cafiso, D.S. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, 23, 141-165.
6. Slomczynska, U.; Beusen, D.D.; Zabrocki, J.; Kociolek, K.; Redlinski, A.; Reusser, F.; Hutton, W.C.; Leplawy, M.T.; Marshall, G.R. *J. Am. Chem. Soc.* **1992**, 114, 4095-4106.
7. Spencer, J.R.; Antonenko, V.V.; Delaet, N.G.J.; Goodman, M. *Int. J. Peptide Protein Res.* **1992**, 40, 282-293.
8. Brückner, H.; König, W.A.; Greiner, M.; Jung, G. *Angew. Chem. Int. Ed. Engl.* **1979**, 18, 476-477.
9. Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L.A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, 60, 405-410.
10. Akaji, K.; Kuriyama, N.; Kiso, Y. *J. Org. Chem.* **1996**, 61, 3350-3357; *idem,. Tertrahedron Lett.* **1994**, 35, 3315-3318.
11. Carpino, L.A. *J. Am. Chem. Soc.* **1993**, 115, 4397-4398.
12. König, W.; Geiger, R. *Chem. Ber.*, **1970**, 103, 2034-2040.; Aterton, E.; Cameron, L.; Meldal, M.;Sheppard, R. *J. Chem. Soc., Chem. Commun.* **1986**, 1763-1765.
13. Meyer, C.E.; Reusser, F. *Experientia*, **1967**, 23, 85-86.
14. Schmitt, H.; Jung, G. *Liebigs Ann. Chem.* **1985**, 321-344; and references cited therein.
15. Jung, G.; Dubischar, N.;Leibfritz, D. *Eur. J. Biochem.* **1975**, 54, 395-409.
16. Carpino, L.A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, 61, 2460-2465.
17. Brückner, H.; Kripp, T.; Kieβ, M. *Peptides, 1990*, Proceedings of the 21st European Peptide Symposium, eds. Giralt, E. and Andreu, D., Escom Science Publishers, Leiden, **1991**, pp347-349.
18. Seki, H.; Koga, K.; Matsuo, H.; Ohki, S.; Matsuo, I.; Yamada, S. *Chem. Pharm. Bull.* **1965**, 13, 995-1000.

(*Received in Japan* 30 *September* 1996; *accepted* 23 *October* 1996)