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Rhodopeptins, Novel Cyclic Tetrapeptides with Antifungal Activities from *Rhodococcus* sp.

III. Synthetic Study of Rhodopeptins

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Total syntheses of *cyclo* (-Gly-L-Lys-L-Val-(*R*)-3-aminododecanoyl-); LV9nA and its diastereomer *cyclo* (-Gly-L-Lys-L-Val-(*S*)-3-aminododecanoyl-); LV9nB, congeners of rhodopeptin B5 on β-amino acid moiety, were achieved. The β-amino acid moiety was prepared as a racemate by the thermal Michael addition of an amine to α,β-unsaturated ester. The racemic β-amino acids were converted to their L-Valylamide derivatives and the obtained diastereomers were separated. Coupling of both diastereomers, L-Val-β-amino acids with Gly-L-Lys gave linear tetrapeptides, and tetrapeptides were cyclized by diphenylphosphoryl azide (DPPA) method between *C*-terminus of β-amino acid and *N*-terminus of Gly to give cyclic tetrapeptides.

The deprotected cyclic tetrapeptides, LV9nA and LV9nB, both exhibited almost the same antifungal activity as the naturally obtained rhodopeptins. Furthermore, comparison of the ^{1}H NMR spectra of two congeners and rhodopeptin B5 suggested that the stereochemistry of β-amino acid moiety in natural rhodopeptin B5 has (*R*)-configuration.

During our screening search for antifungal antibiotics, Rhodopeptins were isolated from the bacterial pellet of *Rhodococcus* sp. Mer-N1033.[1] Their structures were determined as novel cyclic tetrapeptides composed of a lipophilic β-amino acid (abbreviated as βAA) and three usual α-amino acids.[2]

Because they have interesting biological activity and unique structures, we attempted to establish the synthetic method which could be applied for studies on the structure-activity relationship of rhodopeptin derivatives. In this paper, we report the syntheses of rhodopeptin analogues.

Chemistry

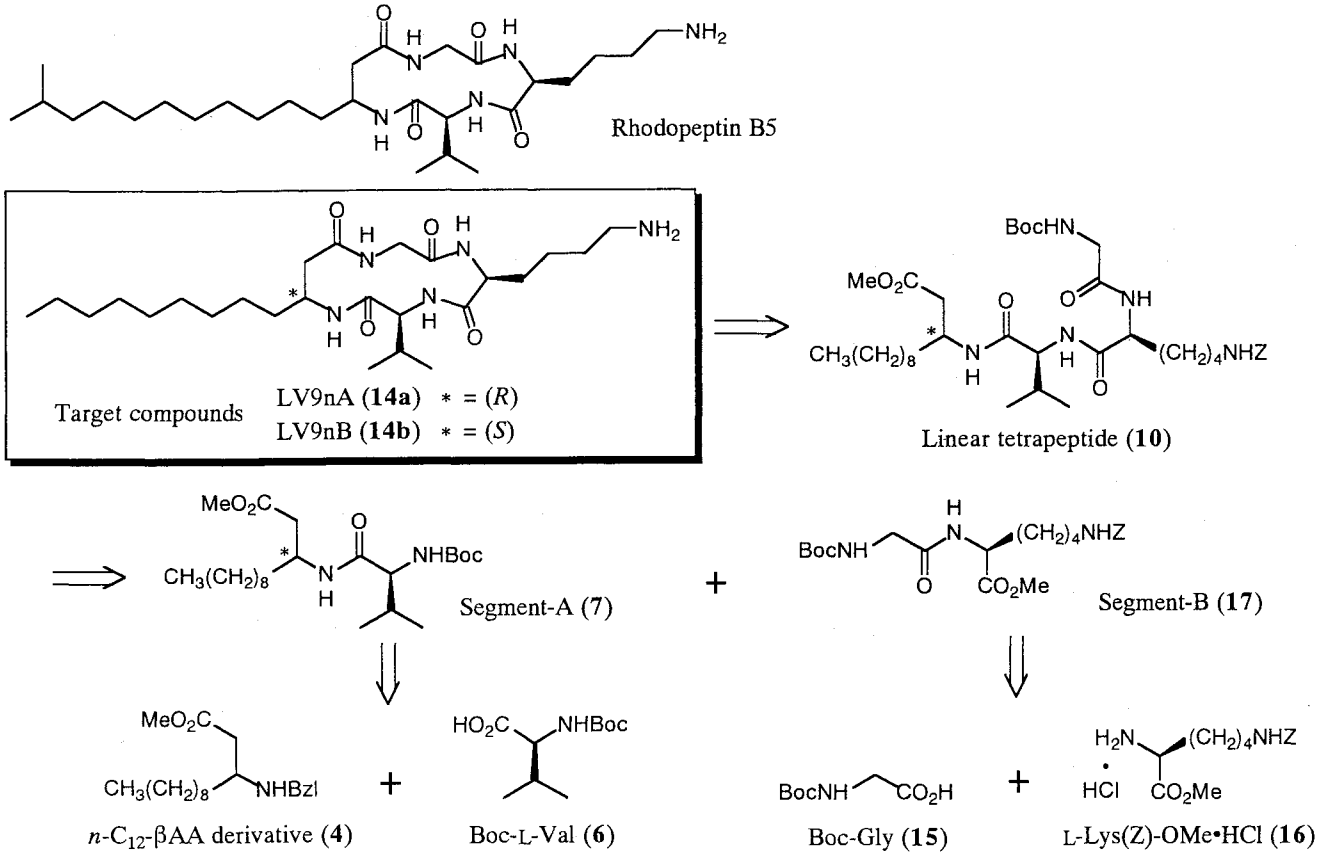
We chose the rhodopeptin B5 analogue that was composed of Gly, Lys, Val and 3-aminododecanoic acid (abbreviated as *n*-C_{12}-βAA) as the synthetic target shown in Fig. 1. *n*-C_{12}-βAA was selected as the βAA moiety in consideration of the following two points: (i) it will be convenient to synthesize βAA with no second chiral center in the side chain; (ii) the target analogues will be interested in the biological activities, because the side chain of the βAAs composing naturally occurring rhodopeptins was isolated as an *iso*- or *anteiso*-type and not isolated as a *normal*-type.[1,2]

The synthetic strategy is shown in Fig. 1. Also as to determine the stereochemistry of the natural products, two epimers in the β-position of the βAA moiety should be synthesized. The synthetic procedure was planned to obtain racemic βAA.

The racemic βAA and L-Val will be coupled and the resulting dipeptides (abbreviated as segment-A) will be obtained as a mixture of two diastereomers. The diastereomers should be separated to obtain two optically pure segment-As. The another dipeptide (abbreviated as segment-B) will be obtained with Gly and L-Lys.

One of the diastereomers of segment-A will be coupled

Fig. 1. Retro synthesis of LV9nA (**14a**), LV9nB (**14b**).



with segment-B to give a linear tetrapeptide, Gly-Lys-Val-βAA.

In the final step *C*-terminus of βAA and *N*-terminus of Gly will be coupled to cyclize the linear tetrapeptide. The reasons of the ring closure with that position were considered with the next two points: (i) minimum steric hindrance will cause high flexibility of the molecule; (ii) racemization at the coupling site will be out of consideration.

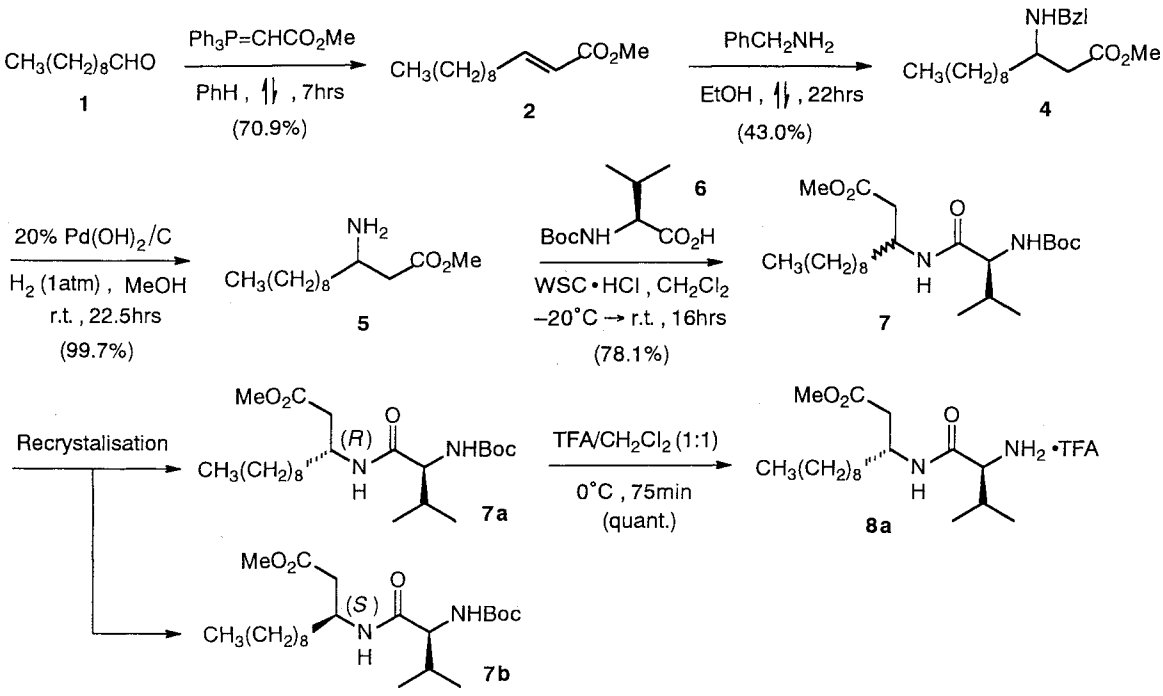
Synthesis of *n*-C_{12}-βAA was planned as follows. Wittig reaction to an aldehyde, *n*-decanal (**1**) will give α,β-unsaturated ester (**2**)[3,4] and thermal Michael addition of an amine to this will be performed.[5,6]

βAAs are of considerable interest as constituents of natural peptides, as precursors for β-lactam antibiotics and taxane diterpenes, and as versatile building block for compounds with biological interest. With this growing interest over the past few years, several methods have been reported for the racemic and asymmetric syntheses of βAAs.[7] Conceptually, one of the simplest methods for the construction of βAAs is through the thermal Michael addition of amines to α,β-unsaturated esters. And for this type of conjugate addition, enantioselective reaction with optically active lithium amides to α,β-unsaturated esters and many other modified asymmetric Michael additions have been reported recently.[7] The approach with this type of addition has the future expansion. So we selected the thermal Michael addition for the procedure that the preparation of *n*-C_{12}-βAA was carried out surely by the simple operations.

The synthesis of rhodopeptin analogue, LV9nA (**14a**) was achieved by following the procedure shown in Scheme 1. Wittig reaction of *n*-decanal (**1**) with (carbomethoxymethylene)triphenylphosphorane gave *trans*-α,β-unsaturated ester (**2**).[3,4] The *cis*-compound (**3**) was gave as a byproduct and the ratio of **2** to **3** were 16 to 1.

Thermal Michael addition to *trans*-ester (**2**) was carried out by refluxing in EtOH for 22 hours with benzylamine to give *N*-Bzl-β-amino ester (**4**)[5,6] in 43.0% yield. The starting material (**2**) was recovered in 45.4% yield. Debenzylation of **4** by using Pearlman's catalyst gave *N*-free β-amino ester (**5**)[9,10] almost quantitatively. Coupling of **5** with Boc-L-Val (**6**) by using 1-(3-dimethylaminopropyl)-3-ethylcar-

Scheme 1. Synthetic route of (*R*)-segment-A, TFA salt (**8a**).



bodiimide hydrochloride (abbreviated as WSC·HCl) as a coupling reagent gave the crude dipeptide, segment-A (**7**) in 90.5% yield. Crystallization differentiation (*n*-hexane-EtOAc) of the mixture of the diastereomers (**7**) gave the one diastereomer (called as (*R*)-segment-A) (**7a**) as colorless crystals in 26.5% yield. The residue containing the other (called as (*S*)-segment-A) (**7b**) as a main product was obtained in 51.6% yield (Scheme 1). **7a** and **7b** were indistinguishable on TLC analysis, but could be distinguished by means of ^{1}H NMR spectrometry.

The stereochemistry of β-position of *n*-C_{12}-βAA in **7a** was determined (*R*) by comparison of the values of optical rotation, which was measured with *n*-C_{12}-βAA obtained from **7a** by acid hydrolysis (6 N HCl, 150°C, 8 hours), and with those of (*R*)-3-aminooctanoic acid and (*R*)-3-aminodecanoic acid described in the literatures[11,12]: $[\alpha]_D^{22}$ −25.14° (*c* 0.44, H_2O-MeOH (1 : 1)) [ref, (*R*)-3-aminooctanoic acid, $[\alpha]_D^{23}$ −22° (*c* 0.5, H_2O-MeOH (1 : 1))],[11] $[\alpha]_D^{22}$ −13.84° (*c* 0.44, 1 N HCl) [ref, (*R*)-3-aminodecanoic acid, $[\alpha]_D^{24}$ −13.1° (*c* 0.41, 1 N HCl)].[12]

Segment-B, Boc-Gly-L-Lys(*Z*)-OMe (**17**) was prepared by coupling reaction of Boc-Gly (**15**) to L-Lys(*Z*)-OMe·HCl (**16**) by using triethylamine and WSC·HCl.

The prepared two dipeptide segments were coupled to provide the tetrapeptide. First, alkaline hydrolysis of ester of **17** gave the free acid (**9**) in 93.3% yield. On the other hand, deprotection of (*R*)-segment-A (**7a**) by using TFA gave the TFA salt (**8a**)[13] almost quantitatively.

Coupling reaction of **9** to **8a** by using WSC·HCl, 1-hydroxybenzotriazole (abbreviated as HOBt) and triethylamine gave the protected tetrapeptide (**10a**)[13] in 93.6% yield. Alkaline hydrolysis of ester of **10a** in THF/MeOH/H_2O (3 : 1 : 1) by using 0.1 M LiOH·H_2O gave the free acid (**11a**)[14] in 86.6% yield. Then deprotection of **11a** in TFA/CH_2Cl_2 (1 : 1) stirring in an ice bath gave the tetrapeptide TFA salt (**12a**) almost quantitatively. Cyclization of **12a** in DMF by using diphenylphosphoryl azide (abbreviated as DPPA) and $NaHCO_3$ gave the crude product (**13a**).[14,15] Sequential debenzyloxycarbonylation of **13a** in TFA by catalytic reduction over 10% Pd/C gave the crude desired product quantitatively though the reaction was slow. After purification of this crude product by preparative HPLC, *cyclo* (-Gly-L-Lys-L-Val-(*R*)-3-aminododecanoyl-) (named as LV9nA) (**14a**) was obtained (Scheme 2).

In the same way the synthesis using the protected (*S*)-segment-A (**8b**) that was the diastereomer of **8a** gave *cyclo* (-Gly-L-Lys-L-Val-(*S*)-3-aminododecanoyl-) (named as LV9nB) (**14b**).

Scheme 2. Synthetic route of LV9nA (**14a**).

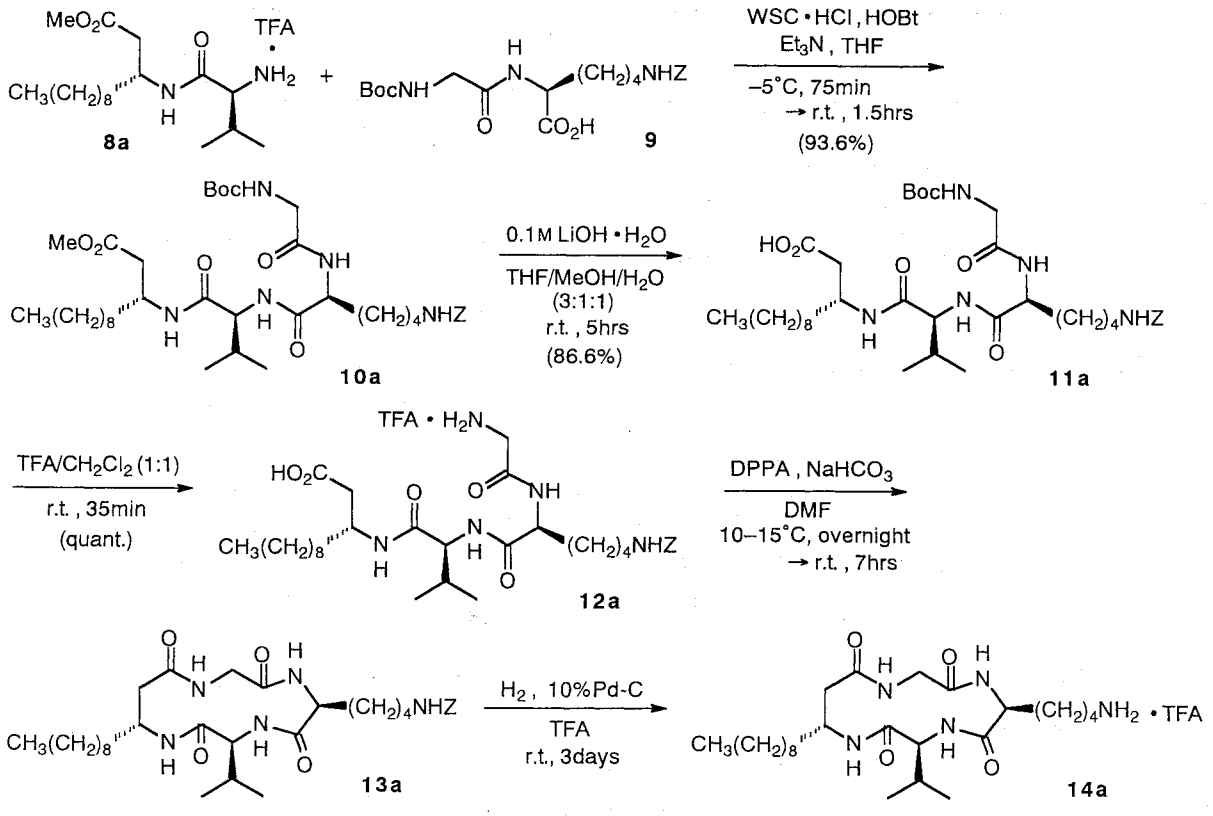


Table 1. Antifungal activity of rhodopeptin analogues.

Microorganism	MIC (μg/ml)[a]		
	LV9nA(**14a**)	LV9nB(**14b**)	rhodopeptin B5 [1]
Candida albicans ATCC10231	3.2	1.6	2.5
Candida albicans ATCC24433	3.2	3.2	—

[a] Broth dilution method using Sabouraud-Dextrose broth as a medium.

Biological Evaluation and Stereochemistry

Antifungal activities of LV9nA (**14a**) and LV9nB (**14b**) were measured. The results were shown in Table 1. They indicated that both compounds exhibited almost the same activity and there was little difference between the activities of **14a** and **14b** and those of the naturally obtained rhodopeptins.[1]

With those results, the stereochemistry of β-position of β-AA moiety in naturally occurring rhodopeptins remained unknown. By comparison with **14a** and **14b** on the basis of ^{1}H NMR spectrum data and physicochemical properties shown in Table 2 and 3, LV9nA (**14a**) was assumed to be the natural type. In the ^{1}H NMR spectrum data C2 (δ 2.24, 2.55) and C3 (δ 4.36) of βAA in **14a** were different from C2 (δ 2.53 (2H)) and C3 (δ 4.26) of βAA in **14b**, but similar to those of rhodopeptin B5 (C2 (δ 2.26, 2.56) and C3 (δ 4.35)). Also Cα (δ 3.33, 4.19) of Gly, Cβ (δ 2.24) and Cγ (δ 0.91 (6H)) of Val, and Cα (δ 4.19) of Lys of **14a** were different from those of **14b** (δ 3.63, 3.84; 2.13; 0.94 (3H),

Table 2. ^{1}H NMR data for rhodopeptin analogues (400 MHz, CD_3OD).

Assignment	δ_H (mult, J (Hz))			
	rhodopeptin C1	rhodopeptin B5	LV9nA(**14a**)	LV9nB(**14b**)
Val				
Cα	3.97 (d, 8.1)	3.97 (d, 7.7)	4.00 (d, 8.4)	3.98 (d, 9.5)
Cβ	2.24 (m)	2.23 (m)	2.24 (m)	2.13 (m)
Cγ	0.92 (6H, d, 6.6)	0.92 (6H, d, 7.0)	0.91 (6H, d, 6.6)	0.94 (3H, d, 7.0) 0.96 (3H, d, 7.0)
Gly				
Cα	3.33 (d, 13.9) 4.18 (d, 13.9)	ca.3.3 (obscured) 4.18 (d, 13.6)	3.33 (d, 13.9) 4.19 (d, 13.9)	3.63 (d, 14.7) 3.84 (d, 14.7)
Lys (Orn)				
Cα	4.19 (t, 7.3)	4.19 (m)	4.19 (m)	3.94 (m)
Cβ	1.84 (2H, m)	1.80 (2H, m)	1.80 (2H, m)	1.85 (2H, m)
Cγ	1.80 (2H, m)	1.29 (2H, m)	1.50 (2H, m)	1.52 (2H, m)
Cδ	2.99 (2H, br t, 7.3)	1.29 (m) 1.71 (m)	1.71 (2H, m)	1.70 (2H, m)
Cε	———	2.98 (2H, m)	2.93 (2H, br t, 7.0)	2.92 (2H, br t, 7.3)
β-Amino acid				
C2	2.27 (dd, 13.9, 8.4) 2.56 (dd, 13.9, 4.8)	2.26 (dd, 14.3, 8.4) 2.56 (dd, 13.9, 5.1)	2.24 (m) 2.55 (dd, 14.3, 5.1)	2.53 (2H, m)
C3	4.35 (m)	4.35 (m)	4.36 (m)	4.26 (br t, 7.3)
C4	1.57 (2H, m)	1.55 (2H, m)	1.56 (2H, m)	1.52 (2H, m)
C5 C6 C7 C8 C9	1.30 (10H, m)	1.29 (14H, m)	1.29 (14H, m)	1.29 (14H, m)
C10	1.12 (m)			
C11	1.30 (2H, m)			
C12	0.87 (3H, t, 7.0)	1.16 (2H, m)	0.89 (3H, t, 7.0)	0.89 (3H, t, 6.6)
C13	0.85 (3H, d, 6.2)	1.50 (m)	———	———
C14	———	0.88 (3H, d, 6.6)	———	———
C15	———	0.88 (3H, d, 6.6)	———	———

0.96 (3H); 3.94), but similar to those of rhodopeptin B5 (δ *ca.* 3.3, 4.18; 2.23; 0.92 (6H); 4.19). These data suggested that the stereochemistry of β-position of βAA moiety in natural rhodopeptins was (*R*).

We will describe the structure-activity relationship of rhodopeptin derivatives on the basis of the obtained synthetic procedure mentioned above elsewhere.

Experimental

General

The mp was measured with a Yanagimoto micro-mp apparatus and is uncorrected. FAB-MS was obtained on a Jeol JMS-SX102A spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-GSX400 spectrometer at 400 MHz

Table 3. Physico-chemical properties of rhodopeptin analogues.

	rhodopeptin C1	rhodopeptin B5	LV9nA(**14a**)	LV9nB(**14b**)
Appearance	White powder	Colorless solid	White powder	White powder
mp	> 240 ℃		> 260℃	250~260℃(dec)
Molecular Formula	$C_{25}H_{47}N_5O_4$	$C_{28}H_{53}N_5O_4$	$C_{25}H_{47}N_5O_4$	$C_{25}H_{47}N_5O_4$
Rf value of TLC (*n* -BuOH-AcOH-H_2O, 4:1:2)	0.5	0.5	0.5	0.45
FAB-MS (*m/z*) positive	482 $(M+H)^+$	524 $(M+H)^+$	482 $(M+H)^+$	482 $(M+H)^+$
Optical Rotation	$[\alpha]_D^{26}$ –15.6° (*c* 0.14, MeOH)		$[\alpha]_D^{26}$ –17.6° (c0.14, MeOH)	$[\alpha]_D^{25}$ –94.0° (*c* 0.02, MeOH) $[\alpha]_D^{25}$ –83.5° (*c* 0.11, TFA)
IR (KBr) ν cm^{-1}	3434, 3277, 3083, 2963, 2926, 2855, 1680, 1649, 1553, 1437, 1400, 1381, 1209, 1140, 841, 802, 725	3432, 3283, 3117, 2963, 2930, 2857, 1680, 1653, 1559, 1437, 1399, 1209, 1140, 839, 804, 725	3476, 3281, 3081, 2959, 2924, 2855, 1678, 1647, 1553, 1468, 1437, 1383, 1206, 1138, 839, 804, 721	3430, 3308, 3057, 2961, 2926, 2855, 1658 (br), 1545, 1468, 1425, 1335, 1289, 1246, 1208, 1182, 1132, 837, 801, 723, 683

and 100 MHz, respectively. Chemical shifts are given on the δ scale (ppm). TMS was used as an internal standard at δ 0.0. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Coupling constants (*J* values) are given in Hz. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a Jasco FT/IR-5300 spectrometer, respectively. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. TLC was carried out on silica gel 60 F_{254} (Merck). The products were detected by ninhydrin reagent at 120°C. Preparative HPLC was performed by using TSKgel ODS-120T column (Tosoh, 55×300 mm) with UV detection at 210 nm and a flow rate of 50 ml/minute. Solvent systems are indicated in each case.

Methyl (*E*)-Dodec-2-enoate (**2**)

A suspension of (carbomethoxymethylene)triphenylphosphorane (7.36 g, 1.1 equiv) in benzene (60 ml) was stirred at room temperature under a nitrogen atmosphere. A solution of *n*-decanal (**1**) (3.13 g, 20 mmol) in benzene (10 ml) was added dropwise to this suspension. The mixture was refluxed for 7 hours, cooled to room temperature, and evaporated. The solid residue was suspended with *n*-hexane and allowed to stand at room temperature overnight. After filtration of the precipitate, the filtrate was evaporated to give the residue (4.92 g). This was chromatographed on silica gel (150 g) using *n*-hexane - toluene (4 : 1~2 : 1) to give *cis*-ester (**3**) (188.4 mg, yield 4.4%) as an oil, and using *n*-hexane - toluene (2 : 1~1 : 1) to give *trans*-ester (**2**) (3.01 g, yield 70.9%) as an oil, respectively.

2: IR (neat) cm^{-1} 2928, 2857, 1728, 1659, 1460, 1435, 1314, 1269, 1198, 1175, 1128, 1042, 981, 851, 720; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=7.3 Hz, C(12)), 1.26 (12H, br s, C(5)~(10)), 1.45 (2H, m, C(11)), 2.19 (2H, ddd, *J*=14.7, 7.0, 1.5 Hz, C(4)), 3.73 (3H, s, CO_2Me), 5.82 (1H, dt, *J*=15.8, 1.5 Hz, C(2)), 6.97 (1H, dt, *J*=15.4, 7.0 Hz, C(3)); Rf 0.5 (*n*-hexane - toluene, 1 : 1).

3: IR (neat) cm^{-1} 2926, 2855, 1728, 1645, 1439, 1408, 1314, 1198, 1175, 1019, 818, 723; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=7.0 Hz, C(12)), 1.26 (12H, br s, C(5)~(10)), 1.45 (2H, m, C(11)), 2.64 (2H, ddd, *J*=15.0, 7.7, 1.8 Hz, C(4)), 3.71 (3H, s, CO_2Me), 5.76 (1H, dt, *J*=11.4, 1.8 Hz, C(2)), 6.23 (1H, dt, *J*=11.4, 7.7Hz, C(3)); Rf 0.4 (*n*-hexane - toluene, 1 : 1).

Methyl 3-*N*-Benzylaminododecanoate (**4**)

Benzylamine (76.3 ml, 1.0 equiv) was added to a solution of *trans*-ester (**2**) (148.1 mg, 0.70 mmol) in EtOH (5 ml). The mixture was refluxed for 5 hours under a nitrogen atmosphere. After addition of benzylamine (1.0 equiv), this solution was refluxed again for 17 hours under a nitrogen atmosphere, cooled to room temperature, and evaporated to give the residue (266.8 mg). The residue was chromatographed on silica gel (13.5 g) using *n*-hexane - toluene (2 : 1) to recover **2** (67.2 mg, yield 45.4%) as an oil, and using toluene - EtOAc (5 : 1) to give **4** (95.9 mg, yield

43.0%) as an oil, respectively: FAB-MS (Matrix; 3-nitrobenzyl alcohol (abbreviated as NBA)) positive *m/z* 320 $(M+H)^+$; IR (neat) cm^{-1} 2926, 2855, 1738, 1456, 1437, 1194, 1173, 735, 698; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz, C(12)), 1.26 (14H, br s, C(5)~(11)), 1.35~1.60 (2H, m, C(4)), 1.57 (1H, br s, NH), 2.46 (2H, d, J=5.9 Hz, C(2)), 3.01 (1H, quintet, J=6.2 Hz, C(3)), 3.67 (3H, s, CO_2Me), 3.78 (2H, s, PhCH_2NH), 7.25 (1H, m, Ph(*p*)), 7.31 (4H, m, Ph(*o*,*m*)); Rf 0.4 (toluene - EtOAc, 5 : 1).

Methyl 3-Aminododecanoate (**5**)

A solution of **4** (886.4 mg, 2.77 mmol) in MeOH (30 ml) was hydrogenated in the presence of 20% $Pd(OH)_2/C$ (*ca.* 40 mg) at an atmospheric pressure for 19 hours at room temperature. After addition of 20% $Pd(OH)_2/C$ (*ca.* 40 mg), the mixture was further hydrogenated for 3.5 hours. After removal of the catalyst by filtration through a Celite pad, the filtrate was evaporated to give **5** (633.1 mg, yield 99.7%) as an oil: FAB-MS (Matrix; NBA) positive *m/z* 230 $(M+H)^+$; IR (neat) cm^{-1} 3382, 2926, 2855, 1738, 1458, 1437, 1167, 1017, 839, 721; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz, C(12)), 1.26 (14H, br s, C(5)~(11)), 1.35 (2H, m, C(4)), 1.52 (2H, br s, NH_2), 2.25 (1H, dd, J=15.4, 8.8 Hz, C(2)Ha), 2.47 (1H, dd, J=15.4, 3.7 Hz, C(2)Hb), 3.16 (1H, m, C(3)), 3.69 (3H, s, CO_2Me); Rf 0.7 (*n*-BuOH - AcOH - H_2O, 4 : 1 : 2).

Boc-L-Val-3-aminododecanoic Acid Methyl Ester (**7**)

A solution of **5** (633.1 mg, 2.77 mmol) and **6** (696.4 mg, 1.1 equiv) in CH_2Cl_2 (15 ml) was stirred at −20°C under a nitrogen atmosphere. A solution of WSC·HCl (583.0 mg, 1.1 equiv) in CH_2Cl_2 (10 ml) was added dropwise to this solution. The mixture was stirred at −20°C for 80 minutes and at room temperature for 3 hours. The mixture was cooled to −20°C again, and a solution of WSC·HCl (212.0 mg, 0.4 equiv) in CH_2Cl_2 (5 ml) was added dropwise to the mixture. The mixture was stirred at −20°C for 1 hour, and was further stirred for 15 hours allowing to warm to room temperature. The solution was diluted with CH_2Cl_2, washed sequentially with cold 0.1 N HCl, cold satd aq $NaHCO_3$, water (twice), and satd aq NaCl, dried over Na_2SO_4, and evaporated to give crude **7** (1.07 g, yield 90.5%). This was recrystallized from *n*-hexane - EtOAc by standing overnight at 5°C. The crystals were collected, washed with *n*-hexane, and dried. The pure (*R*)-segment-A (**7a**) 257.5 mg (21.8%) was obtained as colorless crystals. The filtrate was evaporated to give the residue (933.9 mg). This was chromatographed on silica gel (45 g) using toluene - EtOAc (5 : 1) to give the mixture of diastereomers (**7**) (738.9 mg, yield 62.4%). This was recrystallized from *n*-hexane - EtOAc to give the crystals (**7a**) (56.4 mg, yield 4.7%) again. The filtrate was evaporated to give the residue containing the diastereomer (**7b**) mainly (610.2 mg, yield 51.6%).

7a: mp 113~115°C; FAB-MS (Matrix; NBA) positive *m/z* 429 $(M+H)^+$; IR (KBr) cm^{-1} 3329, 3293, 3083, 2957, 2926, 1732, 1684, 1651, 1524, 1468, 1437, 1373, 1310, 1248, 1206, 1171, 1022, 720, 664; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz, βAA-C(12)), 0.90 (3H, d, J=7.0 Hz, Val-Me), 0.96 (3H, d, J=7.0 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.45 (9H, s, Boc), 1.53 (2H, m, β-AA-C(4)), 2.13 (1H, m, Val-βH), 2.54 (2H, d, J=5.9 Hz, β-AA-C(2)), 3.68 (3H, s, CO_2Me), 3.87 (1H, dd, J=8.4, 5.5 Hz, βAA-C(3)), 4.22 (1H, m, Val-αH), 5.04 (1H, m), 6.33 (1H, m); Rf 0.4 (toluene - EtOAc, 3 : 1); $[\alpha]_D^{28}$ +12.04° (*c* 0.525, $CHCl_3$).

7b: FAB-MS (Matrix; NBA) positive *m/z* 429 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=7.0 Hz, βAA-C(12)), 0.90 (3H, d, J=7.0Hz, Val-Me), 0.95 (3H, d, J=7.0 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.44 (9H, s, Boc), 1.52 (2H, br s, βAA-C(4)), 2.13 (1H, m, Val-βH), 2.53 (2H, t, J=5.5 Hz, βAA-C(2)), 3.67 (3H, s, CO_2Me), 3.84 (1H, dd, J=8.8, 5.9 Hz, βAA-C(3)), 4.23 (1H, m, Val-αH), 5.05 (1H, br s), 6.37 (1H, br s); Rf 0.4 (toluene - EtOAc, 3 : 1).

L-Val-3(*R*)-aminododecanoic Acid Methyl Ester, TFA Salt (**8a**)

TFA (3 ml) was added dropwise to a stirred ice-cooled solution of **7a** (257.5 mg, 0.602 mmol) in CH_2Cl_2 (3 ml) and the mixture was stirred for 75 minutes in an ice bath. After removal of the solvent, the solid residue was coevaporated with toluene (twice) and $CHCl_3$. The TFA salt (**8a**) (275.0 mg) was obtained quantitatively. This was used in the next reaction without further purification: mp 128~134°C; FAB-MS (Matrix; NBA) positive *m/z* 329 $(M+H)^+$; IR (KBr) cm^{-1} 3351, 3042, 2961, 2928, 2857, 1713, 1686, 1671, 1549, 1514, 1466, 1443, 1375, 1314, 1206, 1181, 1144, 839, 801, 723; ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, J=7.0 Hz, βAA-C(12)), 1.03 (3H, d, J=6.6 Hz, Val-Me), 1.06 (3H, d, J=6.6 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.51 (2H, m, βAA-C(4)), 2.19 (1H, m, Val-βH), 2.50 (2H, t, J=5.9 Hz, βAA-C(2)), 3.64 (3H, s, CO_2Me), 3.91 (1H, d, J=5.9 Hz, βAA-C(3)), 4.20 (1H, m, Val-αH), 7.70 (1H, d, J=8.8 Hz, CO-*NH*), 8.09 (2H, br s, NH_2); Rf 0.34 ($CHCl_3$ - MeOH, 10 : 1); $[\alpha]_D^{28}$ +17.88° (*c* 2.08, $CHCl_3$), $[\alpha]_D^{27}$ +11.30° (*c* 2.10, MeOH).

Boc-Gly-L-Lys(*Z*)-OMe (**17**)

A solution of **16** (1.99 g, 6.0 mmol) in CH_2Cl_2 (20 ml)

was stirred at −20°C under a nitrogen atmosphere. Triethylamine (0.92 ml, 1.1 equiv), CH_2Cl_2 (20 ml), and Boc-Gly (**15**) (1.10 g, 1.05 equiv) were added to this solution sequentially. A solution of WSC·HCl (1.27 g, 1.1 equiv) in CH_2Cl_2 (20 ml) was added dropwise to the mixture. The mixture was stirred at −20°C for 1 hour and at room temperature for 3 hours. The mixture was diluted with CH_2Cl_2, washed sequentially with cooled 0.1 N HCl, water, and satd aq NaCl, dried over Na_2SO_4, and evaporated to give an oil (**17**) (2.80 g, yield 103.5%). This was used in the next reaction without further purification: FAB-MS (Matrix; NBA) positive *m/z* 452 $(M+H)^+$, 352 $(M-Boc+2H)^+$; IR (neat) cm^{-1} 3326, 2936, 1703 (br), 1530, 1454, 1368, 1250, 1171, 1051, 1026, 864, 739, 698; ^1H NMR ($CDCl_3$) δ 1.34 (2H, m), 1.45 (9H, s, Boc), 1.51 (2H, m), 1.68 (1H, m), 1.87 (1H, m), 3.18 (2H, br dd, J=12.8, 6.6 Hz, Lys-εH_2), 3.74 (3H, s, CO_2Me), 3.80 (2H, br d, J=5.9 Hz, Gly), 4.60 (1H, td, J=8.1, 5.1 Hz, Lys-αH), 4.92 (1H, br s), 5.10 (2H, s, PhCH_2), 5.16 (1H, br s), 6.66 (1H, br d, J=6.6 Hz), 7.35 (5H, m, *Ph*CH_2); Rf 0.3 (toluene - EtOAc, 1 : 1); $[\alpha]_D^{28}$ +9.00° (*c* 2.72, $CHCl_3$).

Boc-Gly-L-Lys(*Z*) (**9**)

One N NaOH (3.38 ml, 1.1 equiv) was added dropwise to a stirred ice-cooled solution of **17** (1.39 g, 3.08 mmol) in MeOH (34 ml). The mixture was stirred for 6.5 hours at room temperature and evaporated. After addition of water and ether to the residue, this was suspended. The aqueous layer was separated from this suspension, cooled in an ice bath, acidified to pH 4 with 0.1 N HCl, and extracted three times with EtOAc. The EtOAc extracts were washed sequentially with water and satd aq NaCl, dried over Na_2SO_4, and evaporated to give crude **9** (1.25 g, yield 93.3%). This was used in the next reaction without further purification: FAB-MS (Matrix; NBA) positive *m/z* 438 $(M+H)^+$, 338 $(M-Boc+2H)^+$; IR (neat) cm^{-1} 3326, 3065, 2936, 2868, 2614 (br), 1699 (br), 1532, 1454, 1368, 1252 (br), 1167, 1051, 1028, 943, 862, 737, 698; ^1H NMR ($CDCl_3$) δ 1.38 (2H, m), 1.43 (9H, s, Boc), 1.50 (2H, m), 1.76 (1H, m), 1.89 (1H, m), 3.17 (2H, br d, J=6.2 Hz, Lys-εH_2), 3.77, 3.89 (2H, ABq, J=15.4 Hz, Gly), 4.59 (1H, m, Lys-αH), 4.92 (1H, br s), 5.08, 5.14 (2H, ABq, PhCH_2), 5.59 (1H, br d), 6.33 (1H, br d), 7.18 (1H, br d), 7.33 (5H, m, *Ph*CH_2) Rf 0.35 ($CHCl_3$ - MeOH, 3 : 1); $[\alpha]_D^{27}$ +15.53° (*c* 3.57, $CHCl_3$).

Boc-Gly-L-Lys(*Z*)-L-Val-3(*R*)-aminododecanoic Acid Methyl Ester (**10a**)

Triethylamine (249 ml, 3.0 equiv) was added to a stirred solution of **8a** (263.0 mg, 0.595 mmol) in THF (20 ml) at −5°C under a nitrogen atmosphere. After the dropwise addition of a solution of **9** (312.0 mg, 1.2 equiv) in THF (10 ml) to the mixture, HOBt (120.6 mg, 1.5 equiv) and WSC·HCl (171.1 mg, 1.5 equiv) were also added to the mixture successively at −5°C under a nitrogen atmosphere. The mixture was stirred at −5°C for 75 minutes and at room temperature for 1.5 hours. After removal of the solvent, the residue was dissolved in water and EtOAc. The EtOAc layer was separated from this suspension, washed sequentially with 1 N HCl, satd aq $NaHCO_3$, water (twice), and satd aq NaCl, dried over Na_2SO_4, and evaporated to give **10a** (416.1 mg, yield 93.6%) as a white powder. This was used in the next reaction without further purification: mp 150~152°C; FAB-MS (Matrix; NBA) positive *m/z* 748 $(M+H)^+$,770 $(M+Na)^+$, 648 $(M-Boc+2H)^+$; IR (KBr) cm^{-1} 3285, 3073, 2959, 2928, 2857, 1732, 1692, 1636, 1545, 1456, 1368, 1271, 1256, 1175, 1026, 698; ^1H NMR ($CDCl_3$) δ 0.875 (3H, t, J=7.0 Hz, βAA-C(12)), 0.884 (3H, d, J=7.0 Hz, Val-Me), 0.92 (3H, d, J=7.0 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.37 (2H, m, Lys-γH_2), 1.44 (9H, s, Boc), 1.50 (4H, m, Lys-βH_2, βAA-C(4)), 1.75 (2H, m, Lys-δH_2), 2.16 (1H, m, Val-βH), 2.46 (1H, br s, βAA-C(2)Ha), 2.49 (1H, m, βAA-C(2)Hb), 3.18 (2H, br d, J=5.9 Hz, Lys-εH_2), 3.62 (3H, s, CO_2Me), 3.86 (2H, m, Gly), 4.23 (1H, m, βAA-C(3)), 4.29 (1H, m), 4.47 (1H, m), 5.09 (2H, br t, J=12.8 Hz, PhCH_2), 5.31 (1H, br s, NH), 5.45 (1H, br s, NH), 6.71 (1H, br s, NH), 6.85 (1H, br s, NH), 7.33 (5H, m, *Ph*CH_2); Rf 0.4 ($CHCl_3$ - MeOH, 10 : 1); $[\alpha]_D^{27}$ −2.87° (*c* 1.03, $CHCl_3$).

Boc-Gly-L-Lys(*Z*)-L-Val-3(*R*)-aminododecanoic Acid (**11a**)

The protected tetrapeptide (**10a**) (415.3 mg, 0.556 mmol) was dissolved in 0.1 M LiOH THF - MeOH - H_2O (3 : 1 : 1) soln (15 ml). This solution was stirred for 5 hours at room temperature. After addition of 0.1 N HCl (15 ml) to this solution in an ice bath, water and EtOAc were added to the mixture. The mixture was shaken and then the EtOAc layer was separated from that suspension. The aqueous layer was further extracted with EtOAc and $CHCl_3$ successively. The combined EtOAc extracts and the $CHCl_3$ extracts were respectively washed with satd aq NaCl, dried over Na_2SO_4, and evaporated together to give the crude product (391.2 mg). This was chromatographed on silica gel (20 g) with $CHCl_3$ - MeOH (10 : 1~5 : 1) to give **11a** (352.7 mg, yield 86.6%) as a white powder: mp 148~160°C; FAB-MS (Matrix; NBA) positive *m/z* 734 $(M+H)^+$, 756 $(M+Na)^+$, 772 $(M+K)^+$, 634 $(M-Boc+2H)^+$, 672 $(M-Boc+K+H)^+$; IR (KBr) cm^{-1} 3293 (br), 3069, 2959, 2928, 2857, 1698, 1642, 1547, 1454, 1393, 1368, 1252, 1171, 735, 698; ^1H NMR ($CDCl_3$ - CD_3OD, 5:1) δ 0.87 (3H, t, J=7.0 Hz, βAA-

C(12)), 0.92 (3H, d, J=6.6 Hz, Val-Me), 0.93 (3H, d, J=6.6 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.35 (2H, m, Lys-γH_2), 1.44 (9H, s, Boc), 1.50 (4H, m, Lys-βH_2, β-AA-C(4)), 1.67 (1H, m, Lys-δHa), 1.79 (1H, m, Lys-δHb), 2.08 (1H, m, Val-βH), 2.33 (1H, m, βAA-C(2)Ha), 2.42 (1H, br d, J=10.3 Hz, βAA-C(2)Hb), 3.14 (2H, t, J=6.6 Hz, Lys-εH_2), 3.78 (2H, br t like(AB), Gly), 4.01 (1H, m, βAA-C(3)H), 4.17 (1H, m, Val-αH), 4.33 (1H, m, Lys-αH), 5.10 (2H, br d, J=17.6 Hz, PhCH_2), 7.36 (5H, m, PhCH$_2$); Rf 0.2 ($CHCl_3$ - MeOH, 10 : 1); $[\alpha]_D^{28}$ −14.87° (c 1.03, $CHCl_3$ - MeOH (10 : 1)).

Gly-L-Lys(Z)-L-Val-3(R)-aminododecanoic Acid, TFA Salt (**12a**)

After the dropwise addition of TFA (15 ml) to a stirred ice-cooled suspension of **11a** (341.7 mg, 0.466 mmol) in CH_2Cl_2 (15 ml), the mixture was stirred at room temperature for 35 minutes, and evaporated. The residue was co-evaporated with toluene and MeOH successively to give **12a** (376.8 mg) almost quantitatively as a white powder. This was used in the next reaction without further purification: mp 170~177°C; FAB-MS (Matrix; Glycerin) positive m/z 634 $(M+H)^+$, 500, 485, 315, 216; IR (KBr) cm^{-1} 3439 (br), 3291, 3092, 2959, 2928, 2857, 1692 (br), 1638, 1553, 1437, 1385, 1267, 1208, 1140, 841, 802, 723, 696; ^{1}H NMR (CD_3OD) δ 0.89 (3H, t, J=7.0 Hz, βAA-C(12)), 0.94 (3H, d, J=6.6 Hz, Val-Me), 0.95 (3H, d, J=6.6 Hz, Val-Me), 1.28 (14H, br s, βAA-C(5)~(11)), 1.39 (2H, m, Lys-γH_2), 1.52 (4H, m, Lys-βH_2, βAA-C(4)), 1.64 (1H, m, Lys-δHa), 1.81 (1H, m, Lys-δHb), 2.04 (1H, dq, J=13.9, 7.0 Hz, Val-βH), 2.42, 2.50 (2H, ABX, J=15.8, 6.6 Hz, βAA-C(2)), 3.12 (2H, br t, J=6.6 Hz, Lys-εH_2), 3.72 (2H, br t like(AB), Gly), 4.15 (2H, m, βAA-C(3)H, Val-αH), 4.37 (1H, dd, J=8.8, 5.5 Hz, Lys-αH), 5.06 (2H, s, PhCH_2), 7.30 (1H, m, Ph(p)), 7.34 (4H, m, Ph(o,m)), 7.90 (1H, d, J=8.4 Hz, NH), 7.96 (1H, d, J=8.8 Hz, NH); Rf 0.2 ($CHCl_3$ - MeOH - AcOH, 40 : 10 : 1); $[\alpha]_D^{27}$ −17.63° (c 1.02, MeOH).

cyclo (-Gly-L-Lys(Z)-L-Val-3(R)-aminododecanoyl-) (**13a**)

A degassed solution of **12a** (336.7 mg, 0.451 mmol) in DMF (50 ml) was stirred in an ice bath. $NaHCO_3$ (189.3 mg, 5.0 equiv) was added to this solution and DPPA (146 ml, 1.5 equiv) was successively added dropwise to the mixture in an ice bath. This was stirred for 1 hour in an ice bath, 18.5 hours at 10~15°C, and then 7 hours at room temperature under a nitrogen atmosphere. This was cooled in an ice bath again, and the precipitate was obtained by addition of water to this mixture. The precipitate was collected and dried to give crude **13a** (313.3 mg, yield 112.2%) as a colorless solid. This was used in the next reaction without further purification: mp 260°C~ ; FAB-MS (Matrix; Glycerin) positive m/z 616$(M+H)^+$, 638 $(M+Na)^+$; IR (KBr) cm^{-1} 3432 (br), 3289, 3071, 2926, 2857, 1690, 1647, 1545, 1456, 1379, 1267, 1142, 698; Rf 0.8 ($CHCl_3$ - MeOH, 4 : 1).

cyclo (-Gly-L-Lys-L-Val-3(R)-aminododecanoyl-) (**14a**)

A catalytic amount of 10% Pd-C (2 spatulas) was added to a solution of **13a** (290.8 mg, 0.473 mmol) in TFA (6 ml). The mixture was vigorously stirred under a hydrogen atmosphere for 3 hours at room temperature. After addition of 10% Pd-C (1 spatula) to the mixture, this was hydrogenated again for additional 1 hour. After filtration of the catalyst, the filtrate was evaporated. The above hydrogenation and workup were repeated twice with the obtained residue for 7 hours and 3.3 hours to give crude **14a** as TFA salt (277.2 mg, yield 98.5%). Crude **14a** (250 mg) was purified by preparative HPLC (eluent, 50% CH_3CN - 0.1% TFA). The fractions containing the desired product (Rt 23 minutes) were combined, concentrated *in vacuo*, and lyophilized to give a white powder **14a** (100 mg) as TFA salt: mp 260°C~ (dec); FAB-MS (Matrix; Glycerin) positive m/z 482 $(M+H)^+$, 574 $(M+H+Glycerin)^+$; IR (KBr) cm^{-1} 3476 (br), 3281, 3081, 2959, 2924, 2855, 1678, 1647, 1553, 1468, 1437, 1383, 1206, 1138, 839, 804, 721; ^{1}H NMR (CD_3OD) δ 0.89 (3H, t, J=7.0 Hz, βAA-C(12)), 0.91 (6H, d, J=6.6 Hz, Val-Me_2), 1.29 (14H, br s, βAA-C(5)~(11)), 1.50(1H, m, Lys-γH_2), 1.56 (2H, m, βAA-C(4)), 1.71 (2H, m, Lys-δH_2), 1.80 (2H, m, Lys-βH_2), 2.24 (2H, m, βAA-C(2)Ha, Val-βH), 2.55 (2H, dd, J=14.3, 5.1 Hz, βAA-C(2)Hb), 2.93 (2H, br t, J=7.0 Hz, Lys-εH_2), 3.33 (2H, d, J=13.9 Hz, Gly-Ha), 4.00 (1H, t, J=8.4 Hz, Val-αH), 4.19 (2H, m, Gly-Hb, Lys-αH), 4.36 (1H, m, βAA-C(3)H); Rf 0.5 (n-BuOH - AcOH - H_2O, 4 : 1 : 2); $[\alpha]_D^{26}$ −17.6° (c 0.14, MeOH).

L-Val-3(S)-aminododecanoic Acid Methyl Ester, TFA Salt (**8b**)

TFA (4 ml) was added dropwise to a stirred ice-cold solution of the solid residue containing **7b** mainly (598.9 mg, 1.40 mmol) in CH_2Cl_2 (4 ml). The mixture was stirred in an ice bath for 40 minutes and at room temperature for additional 40 minutes. After removal of the solvent, the solid residue was coevaporated with toluene (twice) and $CHCl_3$ to give crude **8** (775 mg). This was chromatographed on silica gel (40 g) using $CHCl_3$ - MeOH (50 : 1~10 : 1) to give TFA salt (**8b**) (443.1 mg, yield 71.6%) as an oil, and using $CHCl_3$ - MeOH (20 : 1~5 : 1) to give diastereomer (**8a**)

(235.0 mg, yield 38.0%) as a white powder, respectively.

8b: FAB-MS (Matrix; NBA) positive *m/z* 329 $(M+H)^+$; IR (neat) cm^{-1} 3291, 3090, 2959, 2928, 2857, 1734, 1684, 1644, 1564 (br), 1464, 1441, 1375, 1304, 1206, 1138, 1011, 837, 801, 721; ^1H NMR ($CDCl_3$) δ 0.84 (3H, d, J=7.0 Hz, Val-Me), 0.87 (3H, t, J=6.6 Hz, βAA-C(12)), 0.99 (3H, d, J=7.0 Hz, Val-Me), 1.24 (14H, br s, βAA-C(5)~(11)), 1.51 (2H, m, βAA-C(4)), 2.24 (1H, m, Val-βH), *ca.* 2.5 (2H, br s, NH_2), 2.54 (2H, ABX, J=15.8, 5.5 Hz, βAA-C(2)), 3.35 (1H, br s, βAA-C(3)), 3.68 (3H, s, CO_2Me), 4.25 (1H, m, Val-αH), 7.52 (1H, br s, CONH); Rf 0.52 ($CHCl_3$-MeOH, 10:1); $[\alpha]_D^{27}$ +1.48° (*c* 2.11, MeOH).

Boc-Gly-L-Lys(*Z*)-L-Val-3(*S*)-aminododecanoic Acid Methyl Ester (**10b**)

By a procedure similar to that used for the preparation of **10a** from **8a**, **10b** was obtained as a white powder in 80.6% yield from **8b**. This was used in the next reaction without further purification: mp 180~182°C; FAB-MS (Matrix; NBA) positive *m/z* 748 $(M+H)^+$, 770 $(M+Na)^+$, 648 $(M-Boc+2H)^+$, 614 $(M-Z+2H)^+$; IR (KBr) cm^{-1} 3425 (br), 3291, 3079, 2959, 2930, 2857, 1734, 1692, 1638, 1545, 1456, 1368, 1271, 1252, 1175, 696; ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, J=7.0 Hz, βAA-C(12)), 0.89 (3H, d, J=6.6 Hz, Val-Me), 0.92 (3H, d, J=6.6 Hz, Val-Me), 1.23 (14H, br s, βAA-C(5)~(11)), 1.38 (2H, m, Lys-γH_2), 1.44 (9H, s, Boc), 1.50 (4H, m, Lys-βH_2, βAA-C(4)), 1.70, 1.83 (1H×2, m, Lys-δH_2), 2.14 (1H, m, Val-βH), 2.48 (2H, m, βAA-C(2)), 3.18 (2H, m, Lys-εH_2), 3.65 (3H, s, CO_2Me), 3.82 (2H, m, Gly), 4.22 (2H, m), 4.46 (1H, m), 5.10 (1H, br t, PhCH_2), 5.26 (2H, m, NH), 6.71 (2H, m, NH), 7.11 (1H, m), 7.34 (4H, m); Rf 0.44 ($CHCl_3$-MeOH, 10:1); $[\alpha]_D^{26}$ −27.90° (*c* 1.04, $CHCl_3$).

Boc-Gly-L-Lys(*Z*)-L-Val-3(*S*)-aminododecanoic Acid (**11b**)

By a procedure similar to that used for the preparation of **11a** from **10a**, **11b** was obtained as a white powder in 99.1% yield from **10b**. This was used in the next reaction without further purification: mp 158~160°C; FAB-MS (Matrix; Glycerin) positive *m/z* 734 $(M+H)^+$, 600 $(M-Z+2H)^+$; IR (KBr) cm^{-1} 3296 (br), 3071, 2959, 2930, 2857, 1699, 1640, 1541, 1456, 1393, 1368, 1252, 1171, 1051, 1028, 698; ^1H NMR (CD_3OD) δ 0.89 (3H, t, J=7.0 Hz, βAA-C(12)), 0.93 (3H, d, J=7.0 Hz, Val-Me), 0.94 (3H, d, J=6.6 Hz, Val-Me), 1.27 (14H, br s, βAA-C(5)~(11)), 1.36 (2H, m, Lys-γH_2), 1.44 (9H, s, Boc), 1.50 (4H, m, Lys-βH_2, βAA-C(4)), 1.67(1H, m, Lys-δHa), 1.77 (1H, m, Lys-δHb), 2.03 (1H, m, Val-βH), 2.43 (2H, m, βAA-C(2)), 3.11 (2H, t, J=6.6 Hz, Lys-εH_2), 3.71 (2H, br s, Gly), 4.06 (1H, td, J=8.4, 2.6 Hz), 4.24 (1H, m), 4.39 (1H, m), 5.06 (2H, s, PhCH_2), 7.30 (1H, m), 7.34 (4H, d, J=2.9 Hz), 7.90 (1H, d, J=7.3 Hz, NH), 7.97 (1H, d, J=8.0 Hz, NH); Rf 0.20 ($CHCl_3$-MeOH, 10:1); $[\alpha]_D^{26}$ −23.74° (*c* 1.06, MeOH).

Gly-L-Lys(*Z*)-L-Val-3(*S*)-aminododecanoic Acid, TFA Salt (**12b**)

By a procedure similar to that used for the preparation of **12a** from **11a**, **12b** was almost quantitatively obtained as a white powder from **11b**. This was used in the next reaction without further purification: mp 204~208°C; FAB-MS (Matrix; Glycerin) positive *m/z* 634 $(M+H)^+$, 500, 485, 315, 216, 590, 419, 320; ^1H NMR (CD_3OD) δ 0.88 (3H, t, J=7.0 Hz, βAA-C(12)), 0.931 (3H, d, J=6.6 Hz, Val-Me), 0.934 (3H, d, J=6.6 Hz, Val-Me), 1.27 (14H, br s, βAA-C(5)~(11)), 1.40 (2H, m, Lys-γH_2), 1.52 (4H, m, Lys-βH_2, βAA-C(4)), 1.67(1H, m, Lys-δHa), 1.77 (1H, m, Lys-δHb), 2.01 (1H, sextet, J=7.0 Hz, Val-βH), 2.44 (2H, ABX, βAA-C(2)), 3.11 (2H, t, J=7.0 Hz, Lys-εH_2), 3.71 (2H, br s, Gly), 4.09 (1H, td, J=8.1, 2.9 Hz), 4.21 (1H, m), 4.40 (1H, dd, J=8.1, 2.9 Hz), 5.06 (2H, s, PhCH_2), 7.30 (1H, m), 7.34 (4H, d, J=4.0 Hz), 8.02 (1H, d, J=10.6 Hz, NH); Rf 0.26 ($CHCl_3$-MeOH-AcOH, 40:10:1); $[\alpha]_D^{26}$ −20.85° (*c* 1.06, MeOH).

cyclo (-Gly-L-Lys(*Z*)-L-Val-3(*S*)-aminododecanoyl-) (**13b**)

By a procedure similar to that used for the preparation of **13a** from **12a**, **13b** was obtained as a colorless solid in 85.2% yield from **12b**. This was used in the next reaction without further purification: mp 260°C~; FAB-MS (Matrix; Glycerin) positive *m/z* 616 $(M+H)^+$, 482 $(M-Z+2H)^+$; ^1H NMR (CF_3CO_2D) δ 0.91 (3H, t, J=6.3 Hz), 1.05 (3H, t, J=6.3 Hz), 1.14 (3H, t, J=9.3 Hz), 1.33 (12H, br s), 1.42 (2H, br s), 1.55 (1H, m), 1.69 (4H, m), 1.87 (1H, m), 1.97 (2H, m), 2.19 (1H, m), 2.86 (1H, dd, J=16.1, 10.3 Hz), 2.98 (1H, br d, J=16.1 Hz), 3.36 (2H, m), 4.04 (1H, d, J=13.7 Hz), 4.19 (1H, d, J=14.7 Hz), 4.25 (1H, m), 4.31 (1H, d, J=11.2 Hz), 4.54 (1H, t, J=7.8 Hz), 5.28 (2H, s, PhCH_2), 7.38 (4H, s), 7.42 (1H, m); IR (KBr) cm^{-1} 3308 (br), 3067, 2959, 2926, 2854, 1659 (br), 1543, 1466, 1254 (br), 696; Rf 0.61 ($CHCl_3$-MeOH-AcOH, 40:10:1).

cyclo (-Gly-L-Lys-L-Val-3(*S*)-aminododecanoyl-) (**14b**)

A catalytic amount (2 spatulas) of 10% Pd-C was added to a solution of **13b** (357.2 mg, 0.58 mmol) in TFA (9 ml). The mixture was vigorously stirred under a hydrogen atmosphere for 6 hours at room temperature. After filtration of the catalyst, filtrate was evaporated. This hydrogenation was repeated with the obtained residue for 7 hours to give

crude **14b** as TFA salt (456.1 mg). Crude **14b** (440 mg) was purified by preparative HPLC (eluent, 45% CH_3CN - 0.01 N HCl). The fraction containing the desired product (Rt 20 minutes) was combined, concentrated *in vacuo*, and lyophilized to give a white powder **14b** (180 mg, converted yield 62.1% from **13b**) as HCl salt: mp 250~255°C (dec); FAB-MS (Matrix; Glycerin) positive *m/z* 482 $(M+H)^+$, 574 $(M+H+Glycerin)^+$; IR (KBr) cm^{-1} 3430, 3308, 3057, 2961, 2926, 2855, 1658 (br), 1545, 1468, 1425, 1335, 1289, 1246, 1208, 1182, 1132, 837, 801, 723, 683; ^1H NMR (CD_3OD) δ 0.89 (3H, t, J=6.6 Hz, βAA-C(12)), 0.94 (3H, d, J=7.0 Hz, Val-Me), 0.96 (3H, d, J=7.0 Hz, Val-Me), 1.29 (14H, br s, βAA-C(5)~(11)), 1.52 (4H, m, Lys-γH_2, βAA-C(4)), 1.70 (2H, m, Lys-δH_2), 1.85 (2H, m, Lys-βH_2), 2.13 (1H, m, Val-βH), 2.53 (2H, m, βAA-C(2)), 2.92 (2H, br t, J=7.3 Hz, Lys-εH_2), 3.63 (2H, d, J=14.7 Hz, Gly-Ha), 3.84 (2H, d, J=14.7 Hz, Gly-Hb), 3.94 (1H, m, Lys-αH), 3.98 (1H, d, J=9.5 Hz, Val-αH), 4.26 (1H, br t, J=7.3 Hz, βAA-C(3)H); Rf 0.45 (*n*-BuOH - AcOH - H_2O, 4 : 1 : 2); $[\alpha]_D^{25}$ −94.0° (*c* 0.02, MeOH), $[\alpha]_D^{25}$ −83.5° (*c* 0.11, TFA).

Stereochemistry of β-Position of *n*-C_{12}-βAA in **7a**

(*R*)-segment-A (**7a**) (17.5 mg) was hydrolyzed with 6 N HCl (1.0 ml) at 150°C for 8 hours in a sealed tube. The reaction mixture was diluted with methanolic water and evaporated. The residue was dissolved with water, washed with AcOEt, and extracted with *n*-BuOH. Alcoholic extract was washed with water and evaporated to give a residue (*n*-C_{12}-βAA) (8.8 mg): $[\alpha]_D^{22}$ −25.14° (*c* 0.44, H_2O - MeOH (1 : 1)), $[\alpha]_D^{22}$ −13.84° (*c* 0.44, 1 N HCl).

Biological Assay

The antifungal activities of **14a** and **14b** were determined by a two-fold micro-dilution method with Sabouraud-Dextrose broth after incubation 35°C for 48 hours.

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