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Synthesis of stable analogs in blood and conformational analysis of arenastatin A, a potent cytotoxic spongean depsipeptide

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Abstract—In order to produce stable analogs in blood of arenastatin A, a potent cytotoxic depsipeptide from the marine sponge *Dysidea arenaria*, we synthesized four analogs in which the 15-20 ester linkage was modified. Among them, the carba analog and 20-deoxo analog showed stability in serum. The conformation of arenastatin A and its three analogs were analyzed by distance-restrained molecular dynamic calculation to elucidate a three-dimensional stereostructure contributing to the extremely potent cytotoxicity of arenastatin A.

Bioassay-guided separation disclosed a cyclic depsipeptide, arenastatin A (**1**), with extremely potent cytotoxicity against tumor cell lines (IC_{50}: 5 pg/ml against KB cells) as a minute amount of principle from the Okinawan marine sponge, *Dysidea arenaria*.[1,2] Thereafter, we achieved the total synthesis[3,4] of **1** by using four segments and found that arenastatin A (**1**) displayed cytotoxicity by inhibiting microtubule assembly through binding to the rhizoxin/maytansine site on tubulin.[5,6] Although **1** was found to exhibit in vivo anti-tumor activity by ip–ip administration (71% ILS at 5 mg/kg dose against P388 mice), ip–iv administration reduced its potency significantly because of instability in mouse serum. Under the assumption that this regrettable biological behavior is ascribable to metabolic degradation of the ester linkages in **1**, we synthesized three amide analogs, in which one or two ester moieties were replaced by an amide group. Assessment of their stability elucidated that the 15,20-ester linkage in arenastatin A (**1**) must be subject to degradation in blood.[7] On the other hand, despite the nearly complete stability and potent cytotoxicity of triamide analog (**2**), poor solubility in polar solvents (such as MeOH, DMSO, and water) made **2** inapplicable to in vivo anti-tumor test.[7]

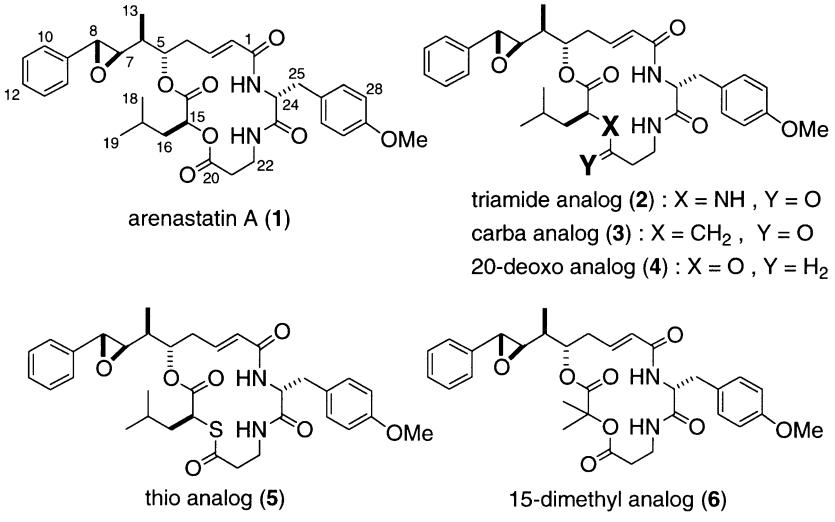

Chart 1.

Keywords: arenastatin A; depsipeptide; marine metabolite; restrained molecular dynamics.
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In this context, we synthesized four analogs [carba analog (**3**), 20-deoxo analog (**4**), thio analog (**5**), and 15-dimethyl analog (**6**)], which were expected to exhibit good stability in serum, and evaluated their biological property (Chart 1). In addition, conformational analyses of **1**–**4** were performed by molecular dynamic calculation with the distance restraints obtained from their NOESY spectra in order to search for a contributive three-dimensional stereostructure to the potent cytotoxic activity of **1**. This paper deals with the synthesis and biological property of the four analogs (**3**–**6**) of arenastatin A (**1**)[8] and the conformational comparison of **1**–**4**.

1. Synthesis and biological property of carba (3) and 20-deoxo analogs (4)[8]

In comparison with arenastatin A (**1**), the triamide analog (**2**) exhibited about 1000-fold weaker cytotoxicity against KB cells. Taking into consideration the contribution of the respective 20-carbonyl residue and 15,20-ether oxygen, we undertook the synthesis of carba (**3**) and 20-deoxo analogs (**4**). After reporting the total synthesis of **1**, we further modified the synthetic protocol to establish a more facile route. Chart 2 illustrates our present outline for the synthesis of arenastatin A (**1**) and its analogs. Namely, introduction of the 7,8-epoxy portion was carried out in the final stage and the precursory cyclic depsipeptide **i** was disconnected into four segments A–D. After construction of segments A-B and C-D, both segments were connected through 5,14-ester linkage. Removal of the protecting group of **ii** followed by macrolactamization at the C-22,23 position provided the desired cyclic depsipeptide **i**. The modified part of the synthesis of arenastatin A (**1**) is as follows.

After protecting the hydroxyl group in segment A (**7**) as a methoxymethyl (MOM) ether, removal of the *t*-butyldiphenylsilyl (TBDPS) group with tetra-*n*-butylammonium fluoride (TBAF) afforded a primary alcohol **8** in 92% yield for two steps. Dess–Martin oxidation of **8** followed by Horner–Emmons reaction with segment B in the presence of NaH provided an α,β-unsaturated amide, which was treated with pyridinium *p*-toluenesulfonate (PPTS) in *t*-BuOH to give segment A-B (**9**) in 82% yield for three steps. On the other hand, condensation of segments C (**10**) and D (**11**) with 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDCI·HCl) in the presence of 4-dimethylaminopyridine (DMAP) followed by deprotection of the benzyl ester using Pd-C under hydrogen atmosphere gave segment C-D (**12**). Subsequently, segments A-B (**9**) and C-D (**12**) were connected with EDCI·HCl and DMAP to afford **13**. We executed the macrolactamization of **13** followed by epoxidation as reported before.[4] Namely, simultaneous cleavage of the two protecting groups in **13** followed by HCl treatment afforded a *seco* amino acid as a hydrochloride salt, which was further subjected to intramolecular macrolactamization by use of diphenylphosphorus azide (DPPA) and $NaHCO_3$ to give a cyclic depsipeptide **14** in 92% yield from **13**. Finally, epoxidation of **14** was successfully treated with dimethyldioxirane to furnish the desired arenastatin A (**1**) predominantly in a ratio of ca. 2:1 (Scheme 1).

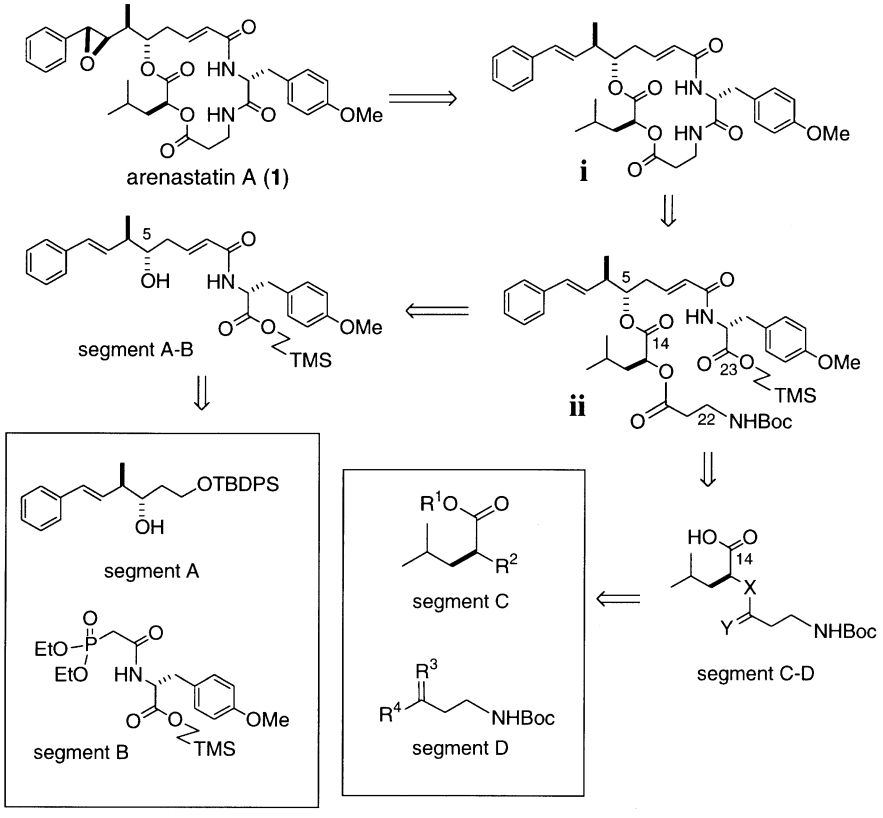
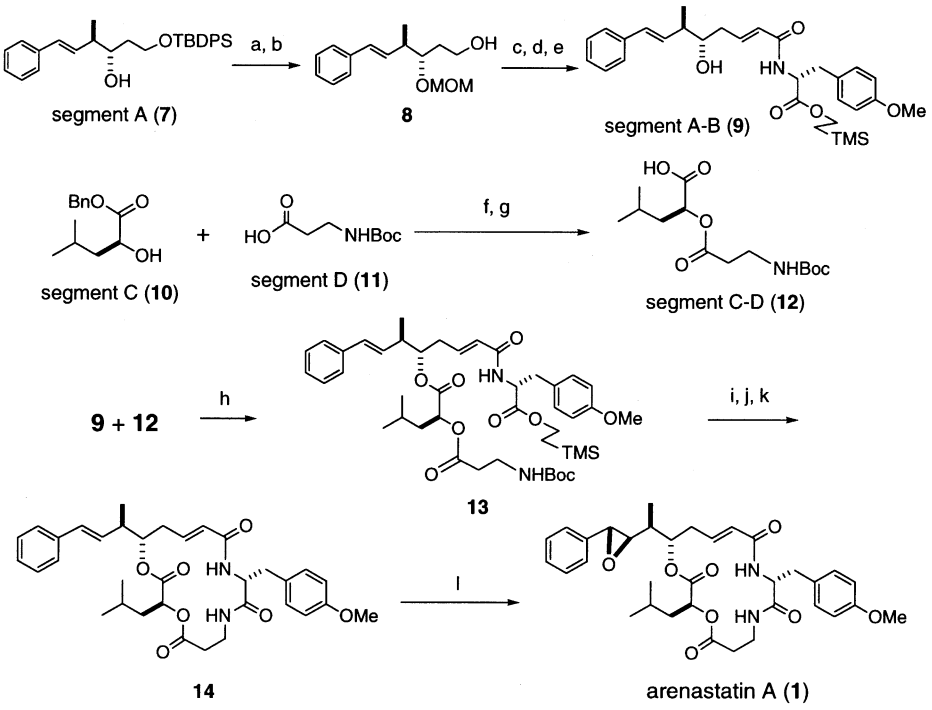
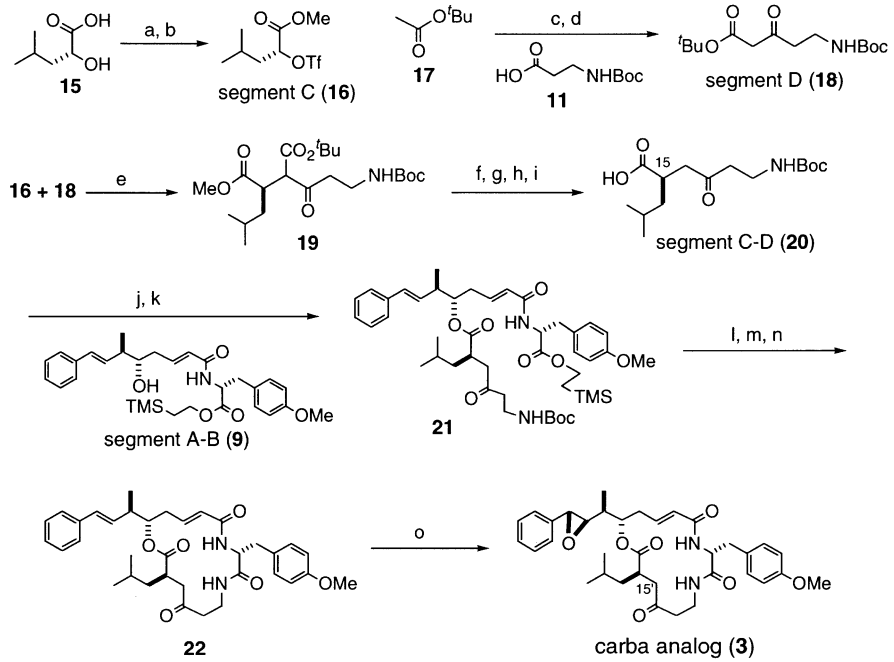


Chart 2.

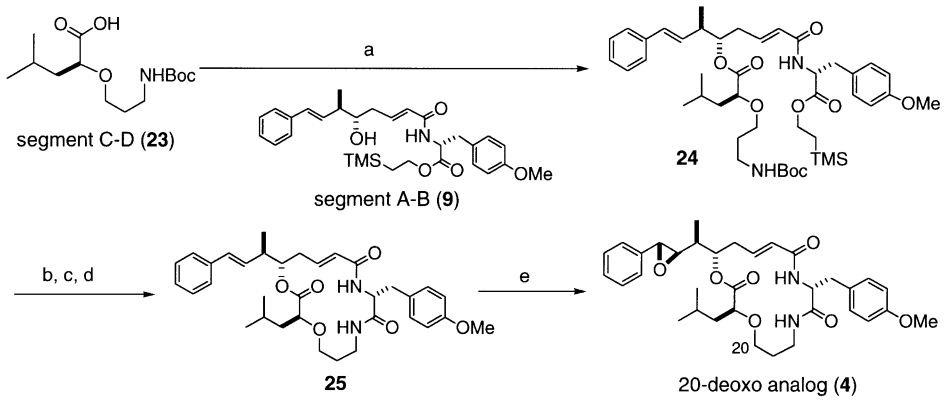


Scheme 1. Synthesis of arenastatin A (**1**). Reagents and conditions: (a) MOMCl, *i*Pr_2NEt, CH_2Cl_2; (b) TBAF, THF, 92% 2 steps; (c) Dess–Martin periodinane, CH_2Cl_2, 0°C; (d) segment B, NaH, THF, 0°C; (e) PPTS, *t*-BuOH, reflux, 82% 3 steps; (f) EDCI·HCl, DMAP, CH_2Cl_2, 97%; (g) H_2, Pd-C, MeOH, 89%; (h) EDCI·HCl, DMAP, CH_2Cl_2, quant.; (i) TFA, CH_2Cl_2; (j) HCl–Et_2O; (k) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps, 92%; and (l) dimethyldioxirane, CH_2Cl_2, 0°C, 61%.

Carba analog (**3**), in which a methylene group is substituted for the oxygen atom linked to C-15 of **1**, was synthesized as illustrated in Scheme 2. The key segment C-D (**20**) was prepared by SN2 nucleophilic substitution of the carbanion of segment D (**18**) for segment C (**16**) and the following decarboxylation reaction. Thus, treatment of (*R*)-leucinic acid (**15**) with trimethylsilyldiazomethane followed by trifluoromethanesulfonylation using Tf_2O and 2,6-lutidine gave segment C (**16**). Carbonyldiimidazole induced the coupling of *N*-Boc-β-alanine (**11**) and lithium enolate



Scheme 2. Synthesis of carba analog (**3**). Reagents and conditions: (a) CHN_2TMS, Et_2O–MeOH (5:1); (b) Tf_2O, 2,6-lutidine, −20°C, 2 steps 95%; (c) LDA, THF, −78°C; (d) **11**, carbonyldiimidazole, THF, 2 steps 83%; (e) NaH, THF, −78°C, 71%; (f) TFA; (g) $(Boc)_2O$, Et_3N, CH_2Cl_2; (h) benzene, reflux; (i) LiOH, THF–H_2O (3:1), 4 steps 84%; (j) 2,4,6-trichlorbenzoyl chloride, Et_3N, THF; (k) **9**, Et_3N DMAP toluene, 2 steps 97%; (l) TFA, CH_2Cl_2; (m) HCl–Et_2O; (n) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 66%; and (o) dimethyldioxirane, CH_2Cl_2, −10°C, 66%.



Scheme 3. Synthesis of 20-deoxo analog (**4**). Reagents and conditions: (a) **9**, EDCI·HCl, DMAP, CH_2Cl_2, quant.; (b) TFA, CH_2Cl_2; (c) HCl–Et_2O; (d) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 74%; and (e) dimethyldioxirane, CH_2Cl_2, 65%.

generated from *t*-butyl acetate (**17**) to provide segment D (**18**) in 83% yield. Condensation of **16** and **18** in the presence of NaH in THF afforded a diastereomeric mixture **19** in 71% yield in a ratio of ca. 2:1. Removal of the *t*-butyl group in **19** and subsequent re-introduction of a Boc group gave a half-ester, which was subjected to decarboxylation under reflux in benzene to yield a ketoester. Saponification of the resulting ketoester by LiOH furnished the desired segment C-D (**20**) with high enantioselectivity in a ratio of 30:1 in 84% overall yield through four steps. The C-15*R* configuration in segment C-D (**20**) was confirmed by Kusumi's method,[9] for determining the configuration at a chiral center adjacent to a carboxyl group. Thus, **20** was converted to (*S*)- and (*R*)-phenylglycine methyl ester (PGME) amides (**20a**, **20b**) and the respective ^1H NMR data were compared.[8]

Condensation of segment A-B (**9**) and segment C-D (**20**) with EDCI·HCl, which afforded the coupled product in good yield in the syntheses of arenastatin A (**1**) and the other analogs (**4**–**6**), proceeded in poor yield in the synthesis of **3**. Activation of the carboxyl group using isopropenyl chloroformate (IPCF), diphenylphosphoryl azide (DPPA), and PyBOP[10] also gave unsatisfactory results. Examination of various activating reagents for the condensation of **9** and **20** led to the finding that usage of 2,4,6-trichlorobenzoyl chloride[11] and Et_3N furnished the desired diester **21** in 97% yield. Conversion from **21** to a hydrochloride salt followed by macrolactamization afforded a cyclic depsipeptide **22** in 66% overall yield through three steps along with a stereo isomer (33%). The chemical structure of this isomer was deduced to be the epimer at C-24 by comparison of the proton signals due to 2-H, 3-H, 25-H_2 (δ: 5.84, 6.51, 3.20, 2.97) from those (δ: 5.72, 6.44, 3.24, 2.91) of the congener obtained during the synthesis of 24-*epi*-arenastatin A. Although the epimerization might have occurred in the stage of condensation of **9** and **20**, the presence of C-24 epimer could not be detected from the NMR spectrum of **21**. Epoxidation of **22** with dimethyldioxirane afforded the desired carba analog (**3**) as a major product together with an α-epoxide in a ratio of ca. 2:1.[12]

Scheme 3 shows the synthesis of 20-deoxo analog (**4**) lacking the 20-carbonyl group of **1**. An alkoxy carboxylic acid **23** was prepared from D-leucine according to TenBrink's procedure.[13] The resulting carboxylic acid **23** was coupled with **9** using EDCI·HCl in the presence of DMAP in CH_2Cl_2 to give a conjugated product **24** quantitatively. After removal of the two protecting groups followed by conversion to a hydrochloride salt, the resulting *seco* acid was also cyclized intramolecularly to give a cyclic depsipeptide **25** in moderate overall yield (74%). Treatment of **25** with dimethyldioxirane furnished 20-deoxo analog (**4**) as a major oxygenated product (α-epoxide: β-epoxide=1:2).

The synthesized two analogs were evaluated for stability in mouse serum, and the results are shown in Fig. 1. Carba (**3**) and 20-deoxo analog (**4**) displayed nearly complete stability in mouse serum as well as moderate cytotoxic efficacy against KB cells (IC_{50}, **3**: 70 ng/ml, **4**: 40 ng/ml), respectively. It should be noted that the two analogs had higher solubility applicable to in vivo anti-tumor test than that of the triamide analog (**2**).[8]

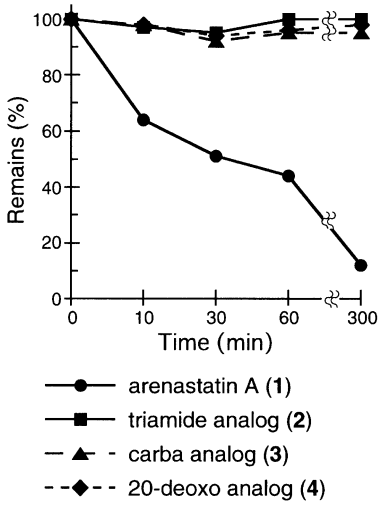
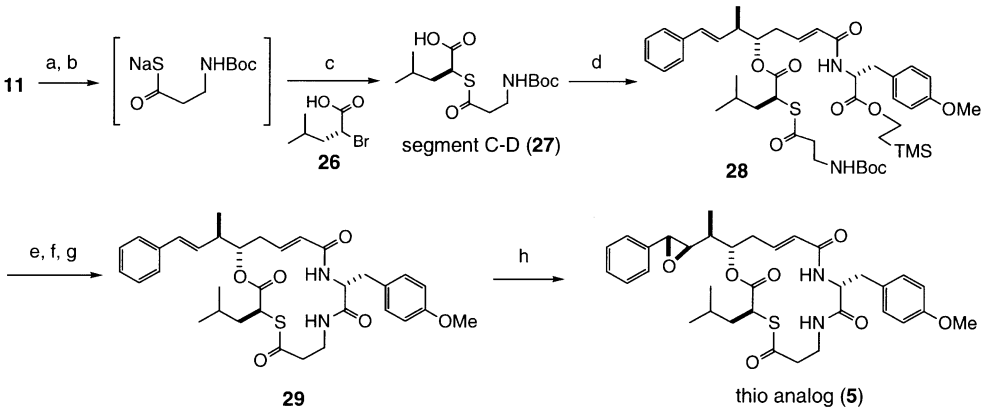


Figure 1. Stability of arenastatin A (**1**) and its analogs (**2**–**4**) in mouse serum. Each sample (10 μl of 0.1 mg/ml solution) was treated with fresh mouse serum (100 μl) and incubated at 37°C for 0, 10, 30, 60, 300 min, respectively. After extraction of the reaction mixture with EtOAc, each extract was analyzed by reversed-phase HPLC to determine the remaining amounts of **1**, **2**, **3**, and **4**.



Scheme 4. Synthesis of thio analog (**5**). Reagents and conditions: (a) Piv–Cl, Et_3N, THF, 0°C; (b) NaSH, 15-crown-5, 0°C; (c) **26**, 3 steps 51%; (d) **9**, EDCI·HCl, DMAP, CH_2Cl_2, 82%; (e) TFA, CH_2Cl_2; (f) HCl–Et_2O; (g) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 58%; and (h) dimethyldioxirane, CH_2Cl_2, −20°C, 31%.

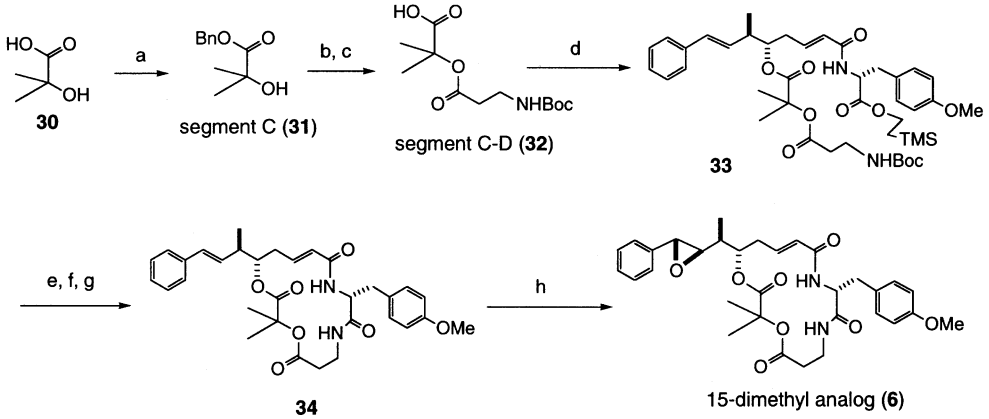
2. Synthesis and biological property of thio (5) and 15-dimethyl analogs (6)

Judging from the reduction of cytotoxicity observed in **3** and **4** relative to arenastatin A (**1**), it was presumed that the 15,20-ester function must be required to exert potent cytotoxic activity. On the other hand, cryptophycin 1, the congener of **1**, bearing the methyl residue on C-21 was reported by Moore's group[14] to display excellent in vivo anti-tumor effect. We assumed that the different biological behavior between arenastatin A (**1**) and cryptophycin 1 was derived from steric hindrance around the 15,20-ester linkage. Therefore, we further designed thio analog (**5**) and 15-dimethyl analog (**6**), assuming that replacement of the ester function by a thioester group or steric hindrance due to two methyl residues on C-15 may prevent metabolic degradation in blood in mice.

The synthetic protocol of thio analog (**5**), in which the C-15 ester oxygen was replaced by a sulfur atom, is summarized in Scheme 4. The key intermediate, segment C-D (**27**), was synthesized by SN2 nucleophilic substitution of thiocarboxylic acid generated in situ from *N*-Boc-β-alanine (**11**) and (*R*)-2-bromo-4-methylpentanoic acid (**26**).[15] Thus, treatment of **11** with pivaloyl chloride and Et_3N afforded a mixed anhydride, which was subjected to substitution reaction with NaSH in the presence of 15-crown-5 to generate a sodium salt of thiocarboxylic acid. Without purification, the sodium salt was directly coupled with **26** to give segment C-D (**27**) in 51% yield from **11**. The condensation between segments A-B (**9**) and C-D (**27**) afforded **28** in 82% yield. Subsequent removal of the protecting groups and macrolactamization under the same conditions in the synthesis of **1** provided **29**, which was submitted to epoxidation using dimethyldioxirane to furnish thio analog (**5**).

The synthesis of 15-dimethyl analog (**6**) was performed using a benzyl ester of 2-hydroxyisobutyric acid (**31**) in the same manner as that of arenastatin A (**1**) as shown in Scheme 5.

The analog (**5**) showed potent cytotoxicity against KB cells (IC_{50}:0.9 ng/ml); nevertheless, the degradation rate in mouse serum was higher than that of **1**. On the contrary, 15-dimethyl analog (**6**) was somewhat more stable than **1** in mouse serum; however, the biological potency of **6** was reduced (IC_{50}: 200 ng/ml against KB cells) (Fig. 2).



Scheme 5. Synthesis of 15-dimethyl analog (**6**). Reagents and conditions: (a) BnBr, $NaHCO_3$, TBAI, CH_2Cl_2, 92%; (b) **11**, EDCI·HCl, DMAP, $(CH_2Cl)_2$, 95%; (c) H_2, Pd-C, MeOH, 88%; (d) **9**, EDCI·HCl, DMAP, CH_2Cl_2, 96%; (e) TFA, CH_2Cl_2; (f) HCl–Et_2O; (g) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 90%; and (h) dimethyldioxirane, CH_2Cl_2, 0°C, 60%.

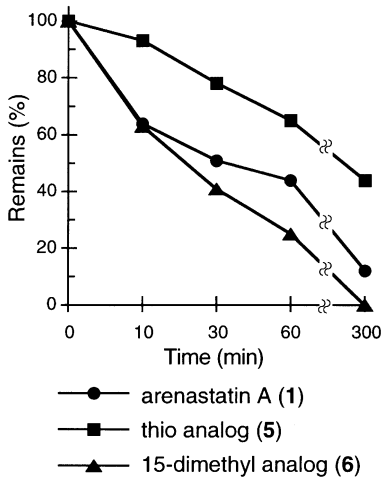


Figure 2. Stability of arenastatin A (**1**), thio analog (**5**), and 15-dimethyl analog (**6**) in mouse serum.

3. Conformational analysis for arenastatin A (1) and three analogs (2–4)

The foregoing large difference in cytotoxic outcome among arenastatin A (**1**) and the analogs of **1** led us to presume that a conclusive conformation of the 16-membered ring portion in **1** is contributive to biological potency. We, therefore, undertook analysis of the conformational features with respect to arenastatin A (**1**) and the three analogs (**2**–**4**) by molecular dynamic calculation with distance restraints using SYBYL 6.5 (Tripos Ass., St Louis, MO).

In the first instance, the NOESY spectra of **1**–**4** in d_6-DMSO were measured to obtain distance restraints.[16] The observed NOE cross peaks were classified into three classes depending on their intensities as strong, medium, or weak and then translated into distance restraints (upper bound of distance: 2.8, 3.5, and 5.0 Å), respectively. Based on the NOE data, an initial structure was built with a complete random array of atoms and refined by energy-minimization using the BFGS method.[17] Then, the refined structure was subjected to 50 cycles of molecular dynamic calculation. The 50 structures obtained were refined by energy-minimization once again. Among the resulting conformers, acceptable conformers were selected on the basis of the criterion that torsion angles of the amide linkages and enone moieties were within ±15°.

The average value of pairwise root-mean-square distance deviation (RMSD) for the backbone (16-membered ring) heavy atoms and the restraint violations of arenastatin A (**1**) and the three analogs (**2**–**4**) are summarized in Table 1. Each compound exhibited not only good convergence on the 16-membered ring portions, but also good compatibility with NOE information. Since the two ester linkages in arenastatin A (**1**) were clarified to play an important role for potent cytotoxicity from our structure-activity relationship study, we compared the 16-membered ring structure of each compound focusing on the two torsion angles [C4–C5–O–C14 and C14–C15–O–C20].

In each compound, a characteristic cluster consisting of predominant conformers appeared as depicted in Fig. 3. Furthermore, the % abundance of conformers belonging to

Table 1. Structural statistics and distance restraint violations

	Arenastatin A (**1**)	Triamide (**2**)	Carba (**3**)	20-Deoxo (**4**)
Number of accepted conformers	43	31	50	47
RMSD (Å) for backbone heavy atom[a]	0.62±0.22	0.42±0.17	0.44±0.21	0.62±0.22
Restraint violation (kcal/mol)[a,b]	0.18±0.27	0.56±0.45	1.20±1.94	0.67±0.41

[a] The given numbers are the mean±standard deviation. Backbone means 16-membered ring.
[b] When the calculated distance is greater than the distance restraint, the energy incurred is $200(d_{measure}-d_{restrain})^2$.

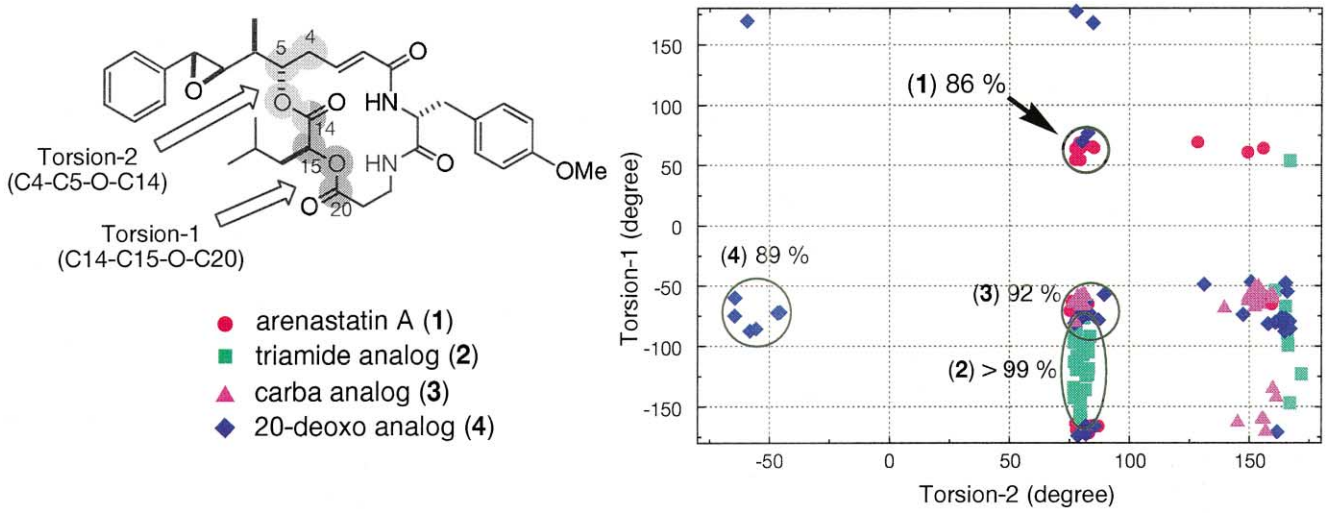


Figure 3. Distribution of conformers obtained by molecular dynamic calculation.

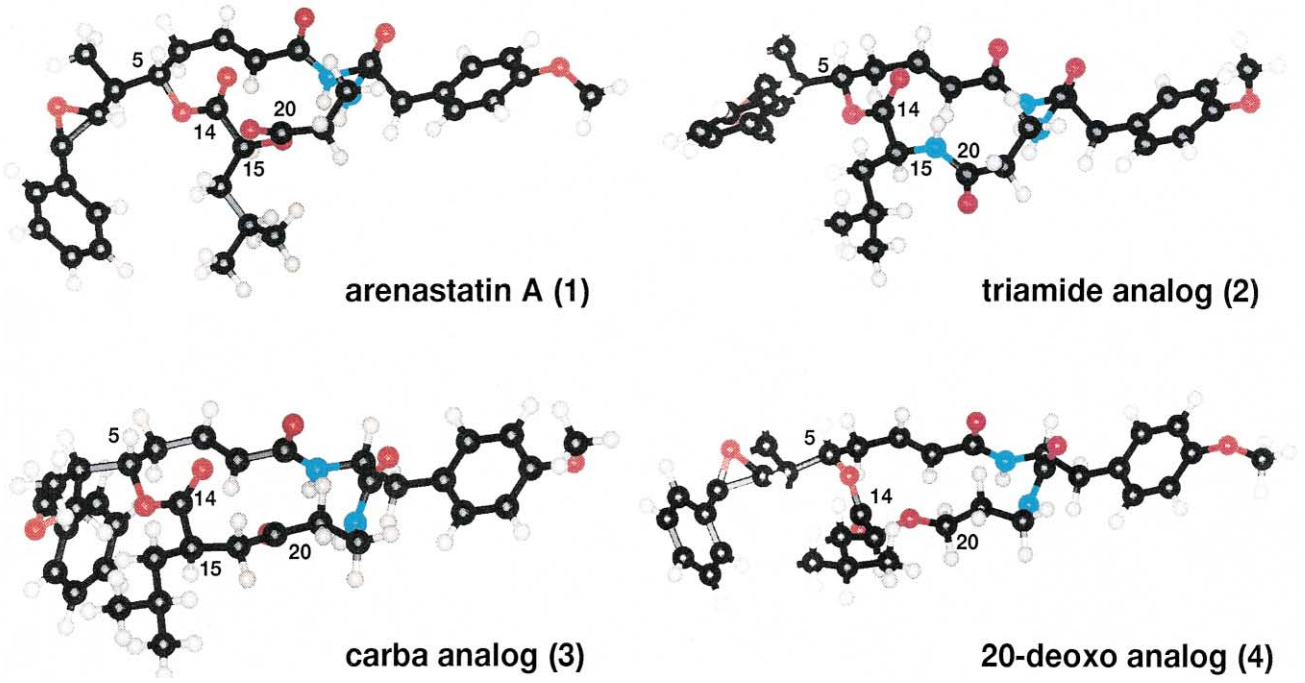


Figure 4. The lowest energy structures of arenastatin A (**1**) and the three analogs (**2**–**4**).

each cluster was estimated by Boltzmann distribution. Apart from the three analogs (**2**–**4**), arenastatin A (**1**) adopted the torsion-1 angle in the vicinity of +60°, leading to a specific conformation.

Fig. 4 illustrates the lowest energy structure of each compound, which was contained in the respective predominant cluster. With respect to arenastatin A (**1**), the carbonyl residue at C-20 was pointed toward the outside region of the 16-membered ring. In contrast, the directions of the C-20 carbonyl groups of **2** and **3** were deviated from that of **1**. Namely, the C-20 carbonyl residue of triamide analog (**2**) was oriented to the downward region of the macrocyclic ring. In the case of carba analog (**3**), the C-20 carbonyl function was turned toward the inside of the 16-membered ring moiety. As for the C-14 carbonyl groups, that of 20-deoxo analog (**4**) pointed toward a different spatial region.

The present conformational analysis led us to presume that the characteristic spatial orientation of the C-20 carbonyl residue and the 16-membered ring structure in **1** would be associated with rigid binding to the active site in tubulin to exert the extremely potent cytotoxicity of **1**. A program to develop new analogs utilizing this template structure of arenastatin A (**1**), obtained by the present molecular dynamic calculation, is proceeding in our laboratory.

4. Experimental

The following instruments were used to obtain physical data: a Jasco DIP-181 digital polarimeter for specific rotations; a Hitachi 260-30 infrared spectrometer for IR spectra; a JEOL JNM Lambda-500 (500 MHz) NMR spectrometer for ^{1}H NMR spectra [$CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard unless otherwise specified]. Silica gel (Merck 60–230 mesh) and pre-coated thin layer chromatography (TLC) plates (Merck, Kiesel gel, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying vanillin/H_2SO_4 (vanillin 5 g, H_2SO_4 95 ml) or acidic *p*-anisaldehyde solution (*p*-anisaldehyde 25 ml, H_2SO_4 25 ml, AcOH 5 ml, EtOH 425 ml) with subsequent heating.

4.1. Conversion from 7 to 8

A solution of segment A (**7**, 250 mg, 0.56 mmol) in dry CH_2Cl_2 (10 ml) was treated with methoxymethylchloride (130 μl) in the presence of iPr$_2$NEt (400 μl) for 10 h. The reaction mixture was poured into aqueous saturated NaCl, and then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a MOM ether. A solution of the MOM ether in dry THF (5.6 ml) was treated with tetra-*n*-butylammonium fluoride (TBAF, 1.0 M in THF, 0.8 ml) for 3 h. The reaction mixture was poured into water, then the whole was extracted with EtOAc. The EtOAc extract was washed with water, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 8 g, hexane:EtOAc=2:1) to furnish **8** (130 mg, 92% 2 steps).

8: Colorless oil, $[\alpha]^{23}_D$ −32.7° (c=0.40 in $CHCl_3$). IR (KBr): 3422, 2953, 2885, 1599 cm^{-1}. ^{1}H NMR δ: 7.40–7.17 (5H, m, 10, 11, 12-H), 6.40 (1H, d, J=15.8 Hz, 8-H), 6.15 (1H, dd, J=7.6, 15.8 Hz, 7-H), 4.70 (2H, m, CH_2OCH_3), 3.83–3.70 (3H, m, 3, 5-H), 3.42 (3H, s, CH_2OCH_3), 2.60 (1H, m, 6-H), 2.40 (1H, m, OH), 1.75 (2H, m, 4-H), 1.13 (3H, d,

J=6.9 Hz, 13-H). FABMS *m/z*: 273 $[M+Na]^+$. FABHRMS *m/z*: Calcd for $C_{15}H_{22}O_3$+Na: 273.1467. Found: 273.1442.

4.2. Conversion from 8 to 9

A solution of **8** (75 mg, 0.30 mmol) in dry CH_2Cl_2 (5 ml) was treated with Dess–Martin periodinane (500 mg) for 1 h at 0°C. After adding aqueous saturated $NaHCO_3$ (4 ml) and $Na_2S_2O_3$ (4 ml), the reaction mixture was further stirred for 1 h at room temperature. The whole was extracted with EtOAc, and then the EtOAc extract was dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave an aldehyde. A solution of segment B (260 mg, 0.56 mmol) in dry THF (5.5 ml) was treated with NaH (67 mg, ca. 60% in paraffin liquid) for 1 h at 0°C. To this reaction mixture was added a solution of the aldehyde in dry THF (1.5 ml), then the whole was stirred for 20 min at 0°C. The reaction mixture was quenched with aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc. The EtOAc extract was dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2: 8 g, hexane:EtOAc=5:1) to furnish an amide (136 mg). A solution of the amide in dry *t*-BuOH (5 ml) was treated with PPTS (600 mg) for 6 h under reflux. The whole was extracted with EtOAc, and then the EtOAc extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 8 g, hexane:EtOAc=3:1) to furnish segment A-B (**9**, 120 mg, 82% 3 step).

9: Colorless oil, $[\alpha]^{20}_D$−21.2° (c=0.33 in $CHCl_3$). IR (KBr): 3366, 2955, 1738, 1668, 1631, 1514 cm^{-1}. ^1H NMR δ: 7.39–7.20 (5H, m, 10, 11, 12-H), 7.01 (2H, d, J=8.6 Hz, 27-H), 6.91 (1H, m, 3-H), 6.80 (2H, d, J=8.6 Hz, 28-H), 6.46 (1H, d, J=15.8 Hz, 8-H), 6.12 (1H, dd, J=8.6, 15.8 Hz, 7-H), 5.91 (1H, d, J=7.6 Hz, 24-NH), 5.88 (1H, d, J=15.5 Hz, 2-H), 4.86 (1H, ddd, J=5.3, 5.3, 7.6 Hz, 24-H), 4.22 (2H, m, CH_2CH$_2$TMS), 3.77 (3H, s, 29-OMe), 3.65 (1H, m, 5-H), 3.09 (2H, d-like, J=5.3 Hz, 25-H), 2.45–2.33 (3H, m, 4, 6-H), 1.14 (3H, d, J=6.8 Hz, 13-H), 0.94 (2H, t, J=8.6 Hz, CH$_2$CH_2TMS), 0.04 (9H, s, TMS). FABMS *m/z*: 524 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{30}H_{41}NO_2Si$+H: 524.2832: Found: 524.2857.

4.3. Preparation for segment C-D (12)

A solution of segment C (**10**, 220 mg, 1.0 mmol) and segment D (**11**, 490 mg, 2.5 mmol) in dry CH_2Cl_2 (10 ml) were treated with EDCI·HCl (1.2 g) in the presence of DMAP (320 mg) at room temperature for 3 h. The reaction mixture was poured into aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 25 g, hexane:EtOAc=12:1) to furnish a diester (380 mg, 97%). To a solution of this diester (380 mg) in MeOH (20 ml) was added 10% Pd-C (100 mg), then the reaction mixture was stirred under H_2 atmosphere at room temperature for 2 h. The reaction mixture was filtered through Celite, then removal of solvent from the filtrate gave a product, which was purified by column chromatography (SiO_2: 8 g, $CHCl_3$:MeOH=7:1) to furnish segment C-D (**12**, 260 mg, 89%).

12: A white powder, $[\alpha]^{22}_D$−24.0° (c=1.1 in $CHCl_3$). IR (KBr): 3371, 2959, 1734, 1523 cm^{-1}. ^1H NMR δ: 4.98 (1H, brs, 22-NH), 4.16 (1H, t, J=7.3 Hz, 15-H), 3.43 (2H, m, 22-H), 2.53 (2H, t, J=6.3 Hz, 21-H), 1.80–1.60 (3H, m, 16, 17-H), 1.41 (9H, s, *t*Bu), 0.98, 0.96 (both 3H, d, J=6.7 Hz, 18, 19-H), FABMS *m/z*: 326 $[M+Na]^+$. FABHRMS *m/z*: Calcd for $C_{14}H_{25}NO_6$+Na: 326.1580. Found: 326.1583.

4.4. Condensation of segment A-B (9) and C-D (12) giving 13

A solution of **9** (140 mg, 0.26 mmol) and **12** (160 mg, 0.54 mmol) in dry CH_2Cl_2 (4 ml) was treated with EDCI·HCl (320 mg) in the presence of DMAP (34 mg) at room temperature for 1 h. The reaction mixture was poured into aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated NaCl, and then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 7 g, hexane:EtOAc=3:1) to furnish **13** (190 mg, quant.).

13: Colorless oil, $[\alpha]^{22}_D$−3.3° (c=1.2 in $CHCl_3$). IR (KBr): 2969, 1711, 1670, 1610, 1512 cm^{-1}. ^1H NMR δ: 7.38–7.30 (5H, m, 10, 11, 12-H), 7.02 (2H, d, J=8.5 Hz, 27-H), 6.79 (2H, d, J=8.5 Hz, 28-H), 6.76 (1H, m, 3-H), 6.40 (1H, d, J=15.8 Hz, 8-H), 6.01 (1H, dd, J=8.5, 15.8 Hz, 7-H), 5.88 (1H, d, J=15.9 Hz, 2-H), 5.02 (1H, q-like, J=ca. 6 Hz, 5-H), 4.92 (1H, dd, J=3.7, 10.4 Hz, 15-H), 4.87 (1H, dt, J=7.5, 6.1 Hz, 24-H), 4.18 (2H, m, CH_2CH$_2$TMS), 3.76 (3H, s, 29-OMe), 3.35 (2H, brd, J=6.1 Hz, 22-H), 3.08 (2H, m, 25-H), 2.56 (5H, m, 4, 6, 21-H), 1.64 (3H, m, 16, 17-H), 1.42 (9H, s, *t*Bu), 1.10 (3H, d, J=7.4 Hz, 13-H), 0.96 (2H, m, CH$_2$CH_2TMS), 0.85 (3H, d, J=6.1 Hz), 0.81 (3H, d, J=6.1 Hz, 18, 19-H), 0.03 (9H, s, TMS). FABMS *m/z*: 809 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{44}H_{64}N_2O_{10}Si$+H: 809.4409. Found: 809.4429.

4.5. Deprotection of 13 followed by macrolactamization giving 14

A solution of **13** (88 mg, 0.11 mmol) in dry CH_2Cl_2 (2 ml) was treated with TFA (2 ml) at room temperature for 5 h to give a *seco* amino acid as a TFA salt, which was treated with saturated HCl gas–Et_2O three times to furnish an HCl salt. A solution of HCl salt in dry DMF (20 ml) was treated with DPPA (45 μl) and $NaHCO_3$ (45 mg) at 0°C for 48 h. The reaction mixture was poured into aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 7 g, hexane:EtOAc=1:3) to furnish **14** (59 mg, 92%).

14: A white powder, $[\alpha]^{25}_{D}$+17.5° (c=0.14 in $CHCl_3$). IR (KBr): 2920, 1741, 1728, 1651, 1602, 1516 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.11 (2H, d, J=8.5 Hz, 27-H), 6.81 (2H, d, J=8.5 Hz, 28-H), 6.71 (1H, ddd, J=4.5, 10.1, 15.1 Hz, 3-H), 6.40 (1H, d, J=16.0 Hz, 8-H), 6.01 (1H, dd, J=9.3, 16.0 Hz, 7-H), 5.73 (1H, d, J=15.1 Hz, 2-H), 5.57 (1H, d, J=7.9 Hz, 24-NH), 5.05 (1H, m, 5-H), 4.90 (1H, dd, J=3.5, 9.6 Hz, 15-H), 4.63 (1H, ddd, J=5.3, 7.2, 7.9 Hz, 24-H), 3.78 (3H, s, 29-OMe), 3.53 (1H, m, 22-Ha), 3.44 (1H, m, 22-Hb), 3.16 (1H, dd, J=5.3, 14.3 Hz, 25-Ha), 3.05 (1H, dd, J=7.2, 14.3 Hz, 25-Hb), 2.54, 2.37 (total 5H, m, 4, 6, 21-H), 1.63, 1.33 (total 3H, m, 16, 17-H), 1.13 (3H, d, J=6.9 Hz, 13-H), 0.75 (3H, d, J=6.6 Hz), 0.72 (3H, d, J=6.4 Hz, 18, 19-H). FABMS *m/z*: 591 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{34}H_{42}N_2O_7$+H: 591.3070. Found: 591.3072.

4.6. Epoxidation of 14 giving 1

A solution of **14** (27 mg, 0.046 mmol) in CH_2Cl_2 (20 ml) was treated with dimethyldioxirane (0.074 M in acetone, 10 ml) for 3 h at 0°C. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column: YMC-Pack ODS (20 mm i.d.×250 mm), mobile phase; MeOH:H_2O=75:25, detection] to furnish **1** (17 mg, 61%).

1: A white powder, $[\alpha]^{18}_{D}$+35.5° (c=0.21 in $CHCl_3$). IR (KBr): 2928, 1742, 1680, 1660, 1614, 1535, 1514 cm^{-1}. ^1H NMR δ: 7.42–7.23 (5H, m, 10, 11, 12-H), 7.10 (2H, d, J=8.5 Hz, 27-H), 6.80 (2H, d, J=8.5 Hz, 28-H), 6.70 (1H, ddd, J=4.9, 10.4, 15.3 Hz, 3-H), 5.70 (1H, d, J=15.3 Hz, 2-H), 6.62 (1H, brd, J=7.3 Hz, 24-NH), 5.19 (1H, ddd, J=3.6, 5.5, 9.7 Hz, 5-H), 4.89 (1H, dd, J=3.7, 10.4 Hz, 15-H), 4.73 (1H, q-like, J=ca. 7 Hz, 24-H), 3.77 (3H, s, 29-OMe), 3.68 (1H, d, J=1.8 Hz, 8-H), 3.48 (1H, m, 22-Ha), 3.44 (1H, m, 22-Hb), 3.13 (1H, dd, J=5.9, 14.0 Hz, 25-Ha), 3.02 (1H, dd, J=7.3, 14.0 Hz, 25-Hb), 2.92 (1H, dd, J=1.8, 7.9 Hz, 7-H), 2.53 (total 4H, m, 4-H, 21-H), 1.80 (1H, m, 6-H), 1.67, 1.29 (total 3H, m, 16-H, 17-H), 1.29 (1H, m, 16-Hb), 1.14 (3H, d, J=7.3 Hz, 13-H), 0.84, 0.82 (both 3H, d, J=6.1 Hz, 18, 19-H). ^1H NMR (d_6-DMSO) δ: 8.20 (1H, d, J=8.5 Hz, 24-NH), 7.38–7.30 (5H, m, 10, 11, 12-H), 7.21 (1H, m, 22-NH), 7.12 (2H, d, J=8.5 Hz, 27-H), 6.82 (2H, d, J=8.5 Hz, 28-H), 6.42 (1H, ddd, J=3.5, 10.2, 15.1 Hz, 3-H), 5.77 (1H, d, J=15.1 Hz, 2-H), 5.12 (1H, ddd, J=3.6, 5.2, 10.6 Hz, 5-H), 4.97 (1H, dd, J=3.9, 9.4 Hz, 15-H), 4.27 (1H, m, 24-H), 3.88 (1H, d, J=1.8 Hz, 8-H), 3.70 (3H, s, 29-OMe), 3.37 (1H, m, 22-Ha), 3.24 (1H, m, 22-Hb), 2.99 (1H, dd, J=1.8, 7.4 Hz, 7-H), 2.97 (1H, dd, J=4.5, 12.3 Hz, 25-Ha), 2.68 (1H, m, 4-Ha), 2.65 (1H, m, 25-Hb), 2.61 (1H, m, 21-Ha), 2.27 (2H, m, 21-Hb, 4-Hb), 1.83 (1H, m, 6-H), 1.56 (1H, m, 17-H), 1.50 (1H, m, 16-Ha), 1.24 (1H, m, 16-Hb), 1.05 (3H, d, J=7.3 Hz, 13-H), 0.78 (3H, d, J=6.8 Hz), 0.76 (3H, d, J=6.4 Hz, 18, 19-H). FABMS *m/z*: 607 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{34}H_{42}N_2O_8$+H: 607.3019. Found: 607.3015.

4.7. Coupling between 11 and *t*-butyl acetate (17) giving segment D (18)

A solution of **11** (2.5 g, 13 mmol) in dry THF (15 ml) was treated with carbonyldiimidazole (2.4 g) for 30 min at 0°C, and then the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was added to a solution of lithium enolate of **17**, which was prepared by treatment of *t*-butyl acetate (5.4 ml) with a solution of LDA (2.0 M, 20 ml) for 1 h at −78°C, and then the whole was stirred for 30 min. The reaction mixture was poured into aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NH_4Cl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 150 g, hexane:EtOAc=5:1) to furnish **18** (2.9 g, 83%).

18: Colorless oil, IR (KBr): 2920, 1728, 1708, 1518 cm^{-1}. ^1H NMR δ: 4.95 (1H, brs, 22-NH), 3.38 (2H, q-like, J=ca. 5 Hz, 22-H), 3.36 (2H, s, 15′-H), 2.76 (2H, t, J=5.5 Hz, 21-H), 1.49, 1.42 (both 9H, s, *t*Bu×2). FABMS *m/z*: 288 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{14}H_{25}NO_5$+H: 288.1811. Found: 288.1814.

4.8. Preparation for diester 19

A solution of (*R*)-leucinic acid (**15**, 1.3 g, 9.7 mmol) in Et_2O (5 ml)-MeOH (1 ml) was treated with CHN_2TMS (2.0 M in hexane, 5 ml) for 1 h. Removal of solvent from the reaction mixture under reduced pressure gave a methyl ester. A solution of 2,6-lutidine (1.0 ml) in dry CH_2Cl_2 (10 ml) was treated with trifluoromethanesulfonic anhydirde (1.4 ml) for 10 min at −20°C. A solution of the methyl ester in CH_2Cl_2 (3 ml) was added to the reaction mixture, and then the whole was stirred at −20°C for 15 min and at room temperature for 1 h. The reaction mixture was filtered, and then the filtrate was dried over $MgSO_4$. Removal of solvent from the filtrate under reduced pressure gave a crude triflate **16**, which was not further purified because of its instability. A solution of **18** (1.1 g, 3.8 mmol) in dry THF (20 ml) was treated with NaH (130 mg) for 40 min at 0°C. After the reaction mixture was cooled to −78°C, a solution of **16** in THF (3 ml) was added to the reaction mixture and the whole was stirred for 48 h at −78°C. The reaction mixture was poured into aqueous saturated NH_4Cl, and then the whole was extracted with EtOAc and dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2:40 g, hexane:EtOAc=10:1) to furnish **19** (1.1 g, 71%) as a mixture of two diastereomers.

19: Colorless oil, IR (KBr): 2925, 1728, 1712, 1698, 1510 cm^{-1}. ^1H NMR δ (major diastereomer): 4.95 (1H, brs, 22-NH), 3.72 (1H, d, J=4.5 Hz, 15′-H), 3.67 (3H, s, COOMe), 3.36 (2H, m, 22-H), 3.19 (1H, dt, J=4.5, 10.0 Hz, 15-H), 2.71 (2H, m, 21-H), 1.60–1.51 (3H, m, 16, 17-H), 1.46, 1.42 (both 9H, s, *t*Bu×2), 0.94, 0.88 (both 3H, d, J=6.5 Hz, 18, 19-H). ^1H NMR δ (minor diastereomer): 4.95 (1H, brs, 22-NH), 3.74 (1H, d, J=3.8 Hz, 15′-H), 3.69 (3H, s, COOMe), 3.36 (2H, m, 22-H), 2.80–2.75 (3H, m, 15, 21-H), 1.60–1.51 (3H, m, 16, 17-H), 1.48, 1.43 (both 9H, s, *t*Bu×2), 0.93, 0.86 (both 3H, d, J=6.5 Hz, 18, 19-H). FABMS *m/z*: 416 $[M+H]^+$.

FABHRMS *m/z*: Calcd for $C_{21}H_{37}NO_7$+H: 416.2726. Found: 416.2632.

4.9. Preparation for segment C-D (20)

A solution of **19** (390 mg, 0.94 mmol) in TFA (4 ml) was stirred for 2 h at room temperature. Removal of TFA from the reaction mixture gave an aminocarboxylic acid. A solution of the aminocarboxylic acid in dry CH_2Cl_2 (5 ml) was treated with Et_3N (530 μl) and $(Boc)_2O$ (330 mg) for 3 h at room temperature. The reaction mixture was poured into aqueous NaH_2PO_4, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a half-ester. A solution of the half-ester in benzene (10 ml) was heated under reflux for 5 h. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 20 g, hexane:EtOAc=5:1) to furnish a ketoester (250 mg, 84%). A solution of the ketoester in THF–H_2O (3:1, 9 ml) was treated with 1N LiOH (2.4 ml) for 1 h at room temperature. The reaction mixture was acidified with 5% HCl to pH 3, and then the whole was extracted with EtOAc. The EtOAc extract was dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 10 g, benzene: acetone=5:1) to furnish **20** (240 mg, quant.).

20: Colorless oil, $[\alpha]^{22}_D$+3.8° (*c*=2.5 in $CHCl_3$). IR (KBr): 3362, 2959, 1714, 1683, 1521 cm^{-1}. ^1H NMR δ: 4.95 (1H, brs, 22-NH), 3.28 (2H, m, 22-H), 2.87 (1H, m, 15-H), 2.74 (1H, dd, *J*=9.9, 18.0 Hz, 15′-Ha), 2.59 (2H, m, 21-H), 2.43 (1H, brd, *J*=18.0 Hz, 15′-Hb), 1.59–1.49 (3H, m, 16, 17-H), 1.40 (9H, s, *t*Bu), 0.87, 0.83 (both 3H, d, *J*=6.5 Hz, 18, 19-H). FABMS *m/z*: 324 $[M+Na]^+$. FABHRMS *m/z*: Calcd for $C_{15}H_{27}NO_5$+Na: 324.1787. Found: 324.1798.

4.10. Preparation for (*S*)-PGME amide of 20

To a solution of segment C-D (**20**, 5.5 mg, 0.018 mmol)and (*S*)-PGME (4 mg, 0.023 mmol) in dry DMF (0.2 ml) was added Py-BOP (11 mg), 1-hydroxybenzotriazole (3 mg) and *N*-methylmorpholine (6 μl) at 0°C, and then the whole was stirred for 2 h at room temperature. The reaction mixture was diluted with benzene (1 ml) and EtOAc (1 ml), and then the whole was washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated NaCl, then dried over Na_2SO_4. Removal of solvent from the organic layer gave a product, which was purified by column chromatography (SiO_2: 600 mg, hexane:EtOAc=2:1) to furnish **20a** (6.0 mg, 79%).

20a: Colorless oil, $[\alpha]^{22}_D$−3.9° (*c*=0.55 in $CHCl_3$). IR (KBr): 2948, 2851, 1740, 1639, 1560, 1515 cm^{-1}. ^1H NMR δ: 7.36–7.20 (5H, m, Ph), 6.67 (1H, d, *J*=6.7 Hz, amide-NH), 5.50 (1H, d, *J*=6.7 Hz, PGME-α-H), 4.88 (1H, brs, 22-NH), 3.73 (3H, s, COOMe), 3.25 (2H, m, 22-H), 2.82 (1H, m, 15-H), 2.80 (1H, m, 15′-Ha), 2.56 (1H, dt, *J*=17.5, 5.5 Hz, 21-Ha), 2.46 (1H, dt, *J*=17.5, 5.5 Hz, 21-Hb), 2.39 (1H, dd, *J*=8.0, 14.0 Hz, 15′-Hb), 1.61 (2H, m, 16-Ha, 17-H), 1.41 (9H, s, *t*Bu), 1.20 (1H, m, 16-Hb), 0.95 (3H, d, *J*=6.7 Hz), 0.89 (3H, d, *J*=6.1 Hz, 18, 19-H). FABMS *m/z*: 449 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{24}H_{37}N_2O_6$+H: 449.2651. Found: 449.2672.

4.11. Preparation for (*R*)-PGME amide of 20

Segment C-D (**20**, 6.5 mg, 0.021 mmol) was converted to **20b** (6.5 mg, 66%) in the same manner as preparation for **20a**.

20b: Colorless oil, $[\alpha]^{22}_D$+15.0° (*c*=0.58 in $CHCl_3$). IR (KBr): 2953, 2851, 1740, 1642, 1561, 1515 cm^{-1}. ^1H NMR δ: 7.36–7.20 (5H, m, Ph), 6.75 (1H, d, *J*=6.7 Hz, amide-NH), 5.45 (1H, d, *J*=6.7 Hz, PGME-α-H), 5.16 (1H, brs, 22-NH), 3.71 (3H, s, COOMe), 3.40 (2H, brd, *J*=5.5 Hz, 22-H), 2.88 (1H, m, 15′-Ha), 2.87 (1H, m, 15-H), 2.65 (2H, m, 21-H), 2.43 (1H, m, 15′-Hb),1.60 (1H, m, 16-Ha), 1.50 (1H, m, 17-H), 1.42 (9H, s, *t*Bu), 1.12 (1H, m, 16-Hb), 0.87 (3H, d, *J*=6.7 Hz), 0.83 (3H, d, *J*=6.1 Hz, 18, 19-H). FABMS *m/z*: 449 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{24}H_{37}N_2O_6$+H: 449.2651. Found: 449.2686.

4.12. Condensation of segment A-B (9) with segment C-D (20)

A solution of **20** (75 mg, 0.25 mmol) and Et_3N (70 μl) in dry THF (2.5 ml) was treated with 2,4,6-trichlorobenzoyl-chloride (44 μl) for 3 h at room temperature. The solvent of the reaction mixture was removed, then to the residue was added the solution of **9** (47 mg, 0.090 mmol) in dry toluene (5 ml). After addition of Et_3N (70 μl) and DMAP (2.0 mg), the reaction mixture was stirred for 2 h at room temperature. Work-up in the same manner as preparation for **13** gave a product, which was purified by column chromatography (SiO_2: 4 g, benzene: acetone=50:1) to furnish **21** (70 mg, 97%).

21: Colorless oil, $[\alpha]^{22}_D$−0.83° (*c*=5.2 in $CHCl_3$). IR (KBr): 3360, 2959, 1731, 1716, 1672, 1608, 1512 cm^{-1}. ^1H NMR δ: 7.38–7.30 (5H, m, 10, 11, 12-H), 7.20 (1H, m, 22-NH), 7.02 (2H, d, *J*=8.6 Hz, 27-H), 6.80 (1H, m, 3-H), 6.79 (2H, d, *J*=8.6 Hz, 28-H), 6.39 (1H, d, *J*=16.0 Hz, 8-H), 6.05 (1H, dd, *J*=8.6, 16.0 Hz, 7-H), 5.92 (1H, d, *J*=15.0 Hz, 2-H), 4.98 (1H, m, 5-H), 4.88 (1H, m, 24-H), 4.20 (2H, m, CH_2CH_2TMS), 3.76 (3H, s, 29-OMe), 3.25 (2H, m, 22-H), 3.08 (2H, m, 25-H), 2.88, 2.59, 2.50 (total 6H, m, 4, 6, 15, 21-H), 2.77 (1H, dd, *J*=10.4, 17.7 Hz, 15′-Ha), 2.42 (1H, dd, *J*=4.2, 17.7 Hz, 15′-Hb), 1.77–1.47 (3H, m, 16, 17-H), 1.41 (9H, s, *t*Bu), 1.09 (3H, d, *J*=6.7 Hz, 13-H), 0.96 (2H, m, CH_2CH_2TMS), 0.82 (3H, d, *J*=6.7 Hz), 0.79 (3H, d, *J*=6.1 Hz, 18 or 19-H), 0.03 (9H, s, TMS). FABMS *m/z*: 807 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{45}H_{66}N_2O_9Si$+H: 807.4616. Found: 807.4630.

4.13. Deprotection of 21 followed by macrolactamization giving 22

A solution of **21** (40 mg, 0.050 mmol) in dry CH_2Cl_2 (1.5 ml) was treated with TFA (3 ml) at room temperature for 8 h to give a *seco* amino acid as a TFA salt, which was treated with saturated HCl gas–Et_2O three times to furnish

an HCl salt. A solution of the HCl salt in dry DMF (10 ml) was treated with DPPA (14 μl) and $NaHCO_3$ (13 mg) at 0°C for 48 h. Work-up in the same manner as preparation for **14** gave a product, which was purified by column chromatography (SiO_2: 4 g, benzene: acetone=6:1) to furnish **22** (20 mg, 66%).

22: A white powder, $[\alpha]^{22}_D$+28.0° (c=0.20 in $CHCl_3$). IR (KBr): 2920, 1735, 1714, 1660, 1602, 1530 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.10 (2H, d, J=8.6 Hz, 27-H), 6.82 (2H, d, J=8.6 Hz, 28-H), 6.71 (1H, ddd, J=4.8, 10.4, 15.5 Hz, 3-H), 6.39 (1H, d, J=16.0 Hz, 8-H), 6.06 (1H, dd, J=8.6, 16.0 Hz, 7-H), 5.75 (1H, d, J=15.5 Hz, 2-H), 5.52 (1H, d, J=7.4 Hz, 24-NH), 5.11 (1H, m, 5-H), 4.63 (1H, ddd, J=5.5, 7.3, 7.4 Hz, 24-H), 3.79 (3H, s, 29-OMe), 3.67 (1H, m, 22-Ha), 3.20 (1H, m, 22-Hb), 3.11 (1H, dd, J=5.5, 14.7 Hz, 25-Ha), 3.05 (1H, dd, J=7.3, 14.7 Hz, 25-Hb), 2.88 (2H, m, 15-H, 15′-Ha), 2.59 (1H, m, 21-Ha), 2.54 (1H, m, 21-Hb), 2.50–2.30 (3H, m, 4, 6-H), 2.35 (1H, m, 15′-Hb), 1.65–1.40 (3H, m, 16, 17-H), 1.13 (3H, d, J=7.3 Hz, 13-H), 0.73 (6H, d, J=6.7 Hz, 18, 19-H). FABMS *m/z*: 589 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{35}H_{44}N_2O_6$+H: 589.3277. Found: 589.3285.

4.14. Epoxidation of 22 giving 3

A solution of **22** (5.5 mg, 0.0090 mmol) in CH_2Cl_2 (3 ml) was treated with dimethyldioxirane (0.074 M in acetone, 3.0 ml) for 3 h at −10°C. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; COSMOSIL 5C18AR (10 mm i.d.×250 mm), mobile phase; CH_3CN:H_2O:CH_2Cl_2=60:55:0.1] to furnish **3** (3.6 mg, 66%).

3: A white powder, $[\alpha]^{22}_D$+59.0° (c=0.15 in $CHCl_3$). IR (KBr): 2930, 2860, 1728, 1720, 1696, 1668, 1514 cm^{-1}. ^1H NMR δ: 7.40–7.25 (5H, m, 10, 11, 12-H), 7.18 (1H, m, 22-NH), 7.09 (2H, d, J=8.5 Hz, 27-H), 6.82 (2H, d, J=8.5 Hz, 28-H), 6.71 (1H, ddd, J=8.0, 14.7, 15.3 Hz, 3-H), 5.73 (1H, d, J=15.3 Hz, 2-H), 5.50 (1H, d, J=7.3 Hz, 24-NH), 5.25 (1H, m, 5-H), 4.65 (1H, ddd, J=5.5, 7.3, 7.4 Hz, 24-H), 3.79 (3H, s, 29-OMe), 3.69 (1H, d, J=1.9 Hz, 8-H), 3.63 (1H, m, 22-Ha), 3.14 (1H, m, 22-Hb), 3.10 (1H, dd, J=5.5, 14.0 Hz, 25-Ha), 3.05 (1H, dd, J=7.4, 14.0 Hz, 25-Hb), 2.94 (1H, dd, J=1.9, 7.3 Hz, 7-H), 2.90 (1H, dd, J=8.5, 16.2 Hz, 15′-Ha), 2.87 (1H, m, 15-H), 2.61 (1H, m, 21-Ha), 2.54–2.47 (3H, m, 4-H, 21-Hb), 2.33 (1H, brd, J=16.2 Hz, 15′-Hb), 1.65–1.40 (3H, m, 16, 17-H), 1.40 (1H, m, 6-H), 1.13 (3H, d, J=7.4 Hz, 13-H), 0.86, 0.83 (both 3H, d, J=6.8 Hz, 18, 19-H). ^1H NMR (d_6-DMSO) δ: 8.10 (1H, d, J=8.0 Hz, 24-NH), 7.34–7.23 (5H, m, 10, 11, 12-H), 7.33 (1H, m, 22-NH), 7.05 (2H, d, J=8.5 Hz, 27-H), 6.76 (2H, d, J=8.5 Hz, 28-H), 6.30 (1H, ddd, J=3.2, 11.0, 15.2 Hz, 3-H), 5.73 (1H, d, J=15.2 Hz, 2-H), 5.05 (1H, ddd, J=3.8, 5.5, 10.6 Hz, 5-H), 4.15 (1H, m, 24-H), 3.84 (1H, d, J=1.8 Hz, 8-H), 3.64 (3H, s, 29-OMe), 3.21 (1H, m, 22-Ha), 3.09 (1H, m, 22-Hb), 2.86 (2H, m, 7-H, 25-Ha), 2.70 (1H, m, 15′-Ha), 2.57 (2H, m, 4-Ha, 25-Hb), 2.47 (1H, m, 15′-Hb), 2.42 (1H, m, 21-Ha), 2.28 (2H, m, 4-Hb, 21-Hb), 1.75 (1H, m, 6-H), 1.37 (1H, m, 17-H), 1.21 (1H, m, 16-Ha), 1.05 (1H, m, 15-H), 0.95 (3H, d, J=7.3 Hz, 13-H), 0.78 (1H, m, 16-Hb), 0.74 (3H, d, J=6.7 Hz), 0.70 (3H, d, J=6.4 Hz, 18, 19-H). FABMS *m/z*: 605 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{35}H_{44}N_2O_7$+H: 605.3227. Found: 605. 3224.

4.15. Condensation of segment A-B (9) with segment C-D (23)

A solution of **9** (71 mg, 0.13 mmol) and **23** (79 mg, 0.27 mmol) in dry CH_2Cl_2 (2 ml) was treated with EDCI·HCl (160 mg) in the presence of DMAP (17 mg) at room temperature for 1 h. Work-up in the same manner as preparation for **13** gave a product, which was purified by column chromatography (SiO_2: 5 g, hexane:EtOAc=3:1) to furnish **24** (110 mg, quant.).

24: Colorless oil, $[\alpha]^{21}_D$+5.5° (c=0.99 in $CHCl_3$). IR (KBr): 2935, 1734, 1637, 1560, 1514 cm^{-1}. ^1H NMR δ: 7.32–7.27 (5H, m, 10, 11, 12-H), 7.21 (1H, brt, J=ca. 6 Hz, 22-NH), 7.01 (2H, d, J=8.5 Hz, 27-H), 6.81 (2H, d, J=8.5 Hz, 28-H), 6.78 (1H, m, 3-H), 6.40 (1H, d, J=15.8 Hz, 8-H), 6.04 (1H, dd, J=8.5, 15.8 Hz, 7-H), 5.85 (1H, d, J=15.3 Hz, 2-H), 5.13 (1H, brs, 24-NH), 5.05 (1H, m, 5-H), 4.84 (1H, q-like, J=ca. 6 Hz, 24-H), 4.17 (2H, m, CH_2CH_2TMS), 3.80 (1H, dd, J=3.7, 9.8 Hz, 15-H), 3.77 (3H, s, 29-OMe), 3.54 (1H, m, 22-Ha), 3.30 (1H, m, 22-Hb), 3.18 (2H, m, 20-H), 3.08 (2H, m, 25-H), 2.60 (1H, m, 6-H), 2.50 (2H, m, 4-H), 1.71 (3H, m, 17, 21-H), 1.56 (1H, m, 16-Ha), 1.43 (9H, s, *t*Bu), 1.38 (1H, m, 16-Hb), 1.11 (3H, d, J=6.7 Hz, 13-H), 0.97 (2H, m, CH_2CH_2TMS), 0.84, 0.79 (both 3H, d, J=6.7 Hz, 18, 19-H), 0.04 (9H, s, TMS). FABMS *m/z*: 796 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{44}H_{66}N_2O_9Si$+H: 796.1118. Found: 796.1129.

4.16. Deprotection of 24 followed by macrolactamization giving 25

Deprotection of **24** (110 mg, 0.13 mmol) and subsequent conversion to an HCl salt and macrolactamization was carried out in the same manner as preparation for **14** to give a product, which was purified by column chromatography (SiO_2: 7 g, benzene:acetone=6:1) to furnish **25** (56 mg, 74%).

25: A white powder, $[\alpha]^{23}_D$+77.2° (c=0.27 in $CHCl_3$). IR (KBr): 2934, 1743, 1657, 1626, 1538, 1514 cm^{-1}. ^1H NMR δ: 7.32–7.19 (5H, m, 10, 11, 12-H), 7.13 (2H, d, J=8.5 Hz, 27-H), 6.85 (1H, ddd, J=4.3, 11.6, 15.0 Hz, 3-H), 6.80 (2H, d, J=8.5 Hz, 28-H), 6.56 (1H, brs, 22-NH), 6.39 (1H, d, J=15.9 Hz, 8-H), 6.00 (1H, dd, J=9.2, 15.9 Hz, 7-H), 5.74 (1H, d, J=8.0 Hz, 24-NH), 5.70 (1H, d, J=15.0 Hz, 2-H), 5.18 (1H, ddd, J=2.4, 6.1, 11.6 Hz, 5-H), 4.66 (1H, q-like, J=ca. 8Hz, 24-H), 3.77 (3H, s, 29-OMe), 3.76 (1H, m, 22-Ha), 3.63 (1H, dd, J=3.4, 10.1 Hz, 15-H), 3.25 (1H, m, 22-Hb), 3.19 (1H, dd, J=7.0, 14.6 Hz, 25-Ha), 3.10 (1H, m, 20-Ha), 2.90 (1H, dd, J=7.9, 14.6 Hz, 25-Hb), 2.86 (1H, m, 20-Hb), 2.55 (2H, m, 4-Ha, 6-H), 2.29 (1H, m, 4-Hb), 1.81 (2H, m, 21-H), 1.73, 1.46, 1.23 (total 3H, m, 16, 17-H), 1.13 (3H, d, J=6.7 Hz, 13-H), 0.79, 0.70 (both 3H, d, J=6.7 Hz, 18, 19-H). FABMS *m/z*: 577 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{34}H_{44}N_2O_6$+H: 577.3278. found: 577.3264.

4.17. Epoxidation of 25 giving 4

A solution of **25** (12 mg, 0.020 mmol) in CH_2Cl_2 (7 ml) was treated with dimethyldioxirane (0.074 M in acetone, 4.0 ml) for 4 h at room temperature. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; YMC-Pack ODS (20 mm i.d.×250 mm), mobile phase; MeOH:H_2O=75:25] to furnish **4** (7.6 mg, 65%).

4: A white powder, $[\alpha]^{18}_D$+44.1° (c=0.11 in $CHCl_3$). IR (KBr): 2928, 1745, 1660, 1614, 1535, 1514 cm^{-1}. ^1H NMR δ: 7.36–7.23 (5H, m, 10, 11, 12-H), 7.12 (2H, d, J=8.5 Hz, 27-H), 6.84 (1H, ddd, J=3.7, 11.0, 14.6 Hz, 3-H), 6.81 (2H, d, J=8.5 Hz, 28-H), 6.52 (1H, brd, J=6.7 Hz, 22-NH), 5.67 (1H, d, J=14.6 Hz, 2-H), 5.66 (1H, d, J=8.5 Hz, 24-NH), 5.33 (1H, ddd, J=2.4, 5.5, 9.2 Hz, 5-H), 4.66 (1H, q-like, J=ca. 8 Hz, 24-H), 3.77 (3H, s, 29-OMe), 3.76 (1H, m, 22-Ha), 3.67 (1H, d, J=1.8 Hz, 8-H), 3.60 (1H, dd, J=3.1, 10.4 Hz, 15-H), 3.26 (1H, m, 22-Hb), 3.18 (1H, dd, J=7.3, 14.6 Hz, 25-Ha), 3.08 (1H, m, 20-Ha), 2.92 (1H, dd, J=1.8, 7.9 Hz, 7-H), 2.89 (1H, m, 25-Hb), 2.84 (1H, m, 20-Hb), 2.59 (1H, m, 4-Ha), 2.37 (1H, m, 4-Hb), 1.78 (3H, m, 6, 21-H), 1.64, 1.49, 1.23 (total 3H, m, 16, 17-H), 1.14 (3H, d, J=73 Hz, 13-H), 0.84, 0.83 (both 3H, d, J=6.7 Hz, 18, 19-H). ^1H NMR (d_6-DMSO) δ: 8.47 (1H, m, 24-NH), 7.40–7.25 (5H, m, 10, 11, 12-H), 7.38 (1H, m, 22-NH), 7.10 (2H, d, J=8.5 Hz, 27-H), 6.80 (2H, d, J=8.5 Hz, 28-H), 6.48 (1H, ddd, J=3.8, 9.5, 14.8 Hz, 3-H), 5.84 (1H, d, J=14.8 Hz, 2-H), 5.14 (1H, m, 5-H), 4.38 (1H, q-like, J=ca. 7 Hz, 24-H), 3.86 (1H, d, J=1.8 Hz, 8-H), 3.70 (3H, s, 29-OMe), 3.58 (1H, m, 15-H), 3.38 (1H, m, 22-Ha), 3.24 (1H, m, 20-Ha), 2.91 (3H, m, 7-H, 20-Hb, 25-Ha), 2.77 (1H, m, 22-Hb), 2.67 (2H, m, 4-Ha, 25-Hb), 2.23 (1H, m, 4-Hb), 1.78 (1H, m, 6-H), 1.67 (1H, m, 17-H), 1.58 (2H, m, 21-H), 1.25 (2H, m, 16-H), 1.03 (3H, d, J=6.7 Hz, 13-H), 0.82 (3H, d, J=6.8 Hz), 0.78 (3H, d, J=6.3 Hz, 18, 19-H). FABMS m/z: 593 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{34}H_{44}N_2O_7$+H: 593.3277. Found: 593.3217.

4.18. Preparation for segment C-D (27)

A solution of **11** (50 mg, 0.27 mmol) in dry THF (2 ml) was treated with Et_3N (38 μl) and pivaloyl chloride (34 μl) at 0°C for 1 h, and then to the reaction mixture was added NaSH (28 mg) and 15-crown-5 (68 μl) at 0°C. The whole mixture was stirred for 1 h, and then a solution of **26** (80 mg, 0.41 mmol) in dry THF (0.5 ml) was added to the reaction mixture. After stirring at room temperature overnight, the reaction mixture was quenched with aqueous saturated $NaHCO_3$, and then the whole was washed with Et_2O. The water phase was acidified with 1N $NaHSO_4$, and then the whole was extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by HPLC [column; YMC-Pack ODS-A (10 mm i.d.×250 mm), mobile phase; MeOH:H_2O=75:25] to furnish **27** (40 mg, 51%).

27: A pale yellow powder, $[\alpha]^{30}_D$−51.0° (c=3.5 in $CHCl_3$). IR (KBr): 3377, 2959, 2928, 1695, 1530, 1510 cm^{-1}. ^1H NMR δ: 4.95 (1H, brs, 22-NH), 4.22 (1H, t, J=7.3 Hz, 15-H), 3.42 (2H, m, 22-H), 2.88 (2H, t, J=6.1 Hz, 21-H), 1.86 (1H, ddd, J=7.3, 7.3, 14.0 Hz, 16-Ha), 1.71 (1H, m, 17-H), 1.59 (1H, m, 16-Hb), 1.44 (9H, s, tBu), 0.96, 0.92 (both 3H, d, J=6.4 Hz, 18, 19-H). FABMS m/z: 342 $[M+Na]^+$. FABHRMS m/z: Calcd for $C_{14}H_{25}NO_5S$+Na: 342.1351. Found: 342.1361.

4.19. Condensation of segment A-B (9) with segment C-D (27)

Condensation of **9** (10 mg, 0.019 mmol) and **27** (12 mg, 0.038 mmol) was carried out in the same manner as preparation for **13** to give a product, which was purified by column chromatography (SiO_2: 1.5 g, hexane:EtOAc=3:1) to furnish **28** (13 mg, 82%).

28: Colorless oil, $[\alpha]^{30}_D$−2.8° (c=0.85 in $CHCl_3$). IR (KBr): 2965, 2928, 1714, 1695, 1534, 1516 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.01 (2H, d, J=8.7 Hz, 27-H), 6.80 (2H, d. J=8.7 Hz, 28-H), 6.75 (1H, m, 3-H), 6.40 (1H, d, J=15.7 Hz, 8-H), 6.13 (1H, d, J=7.4 Hz, 24-NH), 6.04 (1H, dd, J=8.4, 15.7 Hz, 7-H), 5.87 (1H, d, J=15.4 Hz, 2-H), 4.98 (1H, m, 5-H), 4.86 (1H, m, 24-H), 4.18 (3H, m, 15-H, CH_2CH_2TMS), 3.77 (3H, s, 29-OMe), 3.32 (2H, m, 22-H), 3.09 (2H, m, 25-H), 2.71, 2.61 (total 3H, m, 4, 6-H), 2.49 (2H, m, 21-H), 1.77–1.47 (3H, m, 16, 17-H), 1.41 (9H, s, tBu), 1.10 (3H, d, J=7.0 Hz, 13-H), 0.97 (2H, m, CH$_2$CH_2TMS), 0.81, 0.78 (both 3H, d, J=6.4 Hz, 18, 19-H), 0.03 (9H, s, TMS). FABMS m/z: 825 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{44}H_{64}N_2O_9SSi$+H: 825.4180. Found: 825.4176.

4.20. Deprotection of 28 followed by macrolactamization giving 29

Deprotection of **28** (21 mg, 0.026 mmol) and subsequent conversion to an HCl salt and macrolactamization was carried out in the same manner as preparation for **14** to give a product, which was purified by column chromatography (SiO_2: 800 mg, hexane:EtOAc=1:1) to furnish **29** (9.5 mg, 58%).

29: A white powder, $[\alpha]^{30}_D$+32.0° (c=0.30 in $CHCl_3$). IR (KBr): 2925, 1730, 1718, 1685, 1533, 1516 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.09 (2H, d, J=8.5 Hz, 27-H), 6.82 (2H, d, J=8.5 Hz, 28-H), 6.69 (1H, ddd, J=3.8, 10.6, 14.7 Hz, 3-H), 6.38 (1H, d, J=15.8 Hz, 8-H), 6.02 (1H, dd, J=8.5, 15.8 Hz, 7-H), 5.78 (1H, dd, J=1.2, 14.7 Hz, 2-H), 5.54 (1H, d, J=7.2 Hz, 24-NH), 5.14 (1H, ddd, J=3.0, 6.5, 10.9 Hz, 5-H), 4.66 (1H, ddd, J=5.3, 7.2, 7.4 Hz, 24-H), 4.09 (1H, dd, J=6.9, 8.9 Hz, 15-H), 3.79 (3H, s, 29-OMe), 3.65 (1H, ddd, J=4.6, 8.4, 17.4 Hz, 22-Ha), 3.34 (1H, ddd, J=3.6, 7.9, 17.4 Hz, 22-Hb), 3.11 (1H, dd, J=5.3, 14.3 Hz, 25-Ha), 3.02 (1H, dd, J=7.4, 14.3 Hz, 25-Hb), 2.74, 2.56, 2.45 (total 5H, m, 4, 6, 21-H), 1.65–1.40 (3H, m, 16, 17-H), 1.13 (3H, d, J=6.8 Hz, 13-H), 0.71, 0.70 (both 3H, d, J=6.1 Hz, 18, 19-H). FABMS m/z: 607 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{34}H_{42}N_2O_6S$+H: 607.2842. Found: 607.2845.

4.21. Epoxidation of 29 giving 5

A solution of **29** (3.5 mg, 0.0058 mmol) in CH_2Cl_2 (1 ml)

was treated with dimethyldioxirane (0.074 M in acetone, 2.0 ml) for 3 h at −20°C. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; YMC-Pack ODS-A (10 mm i.d.×250 mm), mobile phase; MeOH:H_2O=75:25] to furnish **5** (1.2 mg, 31%).

5: A white powder, $[\alpha]^{30}_D$+48.0° (c=0.10 in $CHCl_3$). IR (KBr): 2925, 1732, 1716, 1685, 1535, 1516 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.00 (2H, d, J=8.0 Hz, 27-H), 6.83 (2H, d, J=8.0 Hz, 28-H), 6.67 (1H, ddd, J=6.0, 8.0, 14.9 Hz, 3-H), 5.76 (1H, d, J=14.9 Hz, 2-H), 5.52 (1H, d, J=7.4 Hz, 24-NH), 5.11 (1H, m, 5-H), 4.65 (1H, q-like, J=ca. 8 Hz, 24-H), 4.21 (1H, dd, J=6.2, 8.5 Hz, 15-H), 3.79 (3H, s, 29-OMe), 3.77 (1H, d, J=2.4 Hz, 8-H), 3.65 (1H, m, 22-Ha), 3.33 (1H, m, 22-Hb), 3.09 (1H, dd, J=5.5, 14.5 Hz, 25-Ha), 3.01 (1H, dd, J=8.6, 14.5 Hz, 25-Hb), 2.93 (1H, dd, J=2.4, 6.9 Hz, 7-H), 2.75 (1H, m, 21-Ha), 2.53 (1H, m, 21-Hb), 2.30 (2H, m, 4-H), 1.65–1.40 (3H, m, 16, 17-H), 1.40 (1H, m, 6-H), 1.11 (3H, d, J=7.3 Hz, 13-H), 0.88, 0.86 (both 3H, d, J=7.6 Hz, 18, 19-H). FABMS m/z: 623 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{34}H_{42}N_2O_7S$+H: 623.2791. Found: 623.2790.

4.22. Preparation for segment C-D (32)

A solution of 2-hydroxyisobutyric acid (**30**, 280 mg, 2.7 mmol) and $NaHCO_3$ (230 mg) in CH_2Cl_2 (5 ml) and water (5 ml) was treated with benzyl bromide (BnBr, 0.35 ml) and tetra-*n*-butylammonium iodide (TBAI, 980 mg) at room temperature for 2 days. After removal of about half volume of solvent from reaction mixture, the whole was extracted with Et_2O. The Et_2O extract was dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2: 3 g, hexane:EtOAc=4:1) to furnish a benzyl ester (**31**, 480 mg, 92%). A solution of **31** (200 mg, 1.0 mmol) and **11** (490 mg, 2.5 mmol) in dry $(CH_2Cl)_2$ (10 ml) was treated with EDCI·HCl (1.2 g) in the presence of DMAP (320 mg) at room temperature for 6 h. Work-up in the same manner as preparation for **13** gave a product, which was purified by column chromatography (SiO_2: 25 g, hexane:EtOAc=12:1) to furnish a diester (360 mg, 95%). To a solution of the diester (350 mg, 0.92 mmol) in MeOH (20 ml) was added 10% Pd-C (100 mg), and then the reaction mixture was stirred under H_2 atmosphere at room temperature for 2 h. The reaction mixture was filtered through Celite, and then removal of solvent from the filtrate gave a product, which was purified by column chromatography (SiO_2: 1 g, $CHCl_3$:MeOH=7:1) to furnish **32** (230 mg, 88%).

32: A white powder, IR (KBr): 3362, 2980, 1720, 1520 cm^{-1}. ^1H NMR δ: 5.07 (1H, brs, 22-NH), 3.40 (2H, m, 22-H), 2.54 (2H, t, J=6.0 Hz, 21-H), 1.60 (6H, s, 16, 17-H), 1.44 (9H, s, *t*Bu). FABMS m/z: 298$[M+Na]^+$. FABHRMS m/z: Calcd for $C_{12}H_{21}NO_6$+Na: 298.1267. Found: 298.1281.

4.23. Condensation of segment A-B (9) with segment C-D (32)

Condensation of **9** (85 mg, 0.16 mmol) and **32** (110 mg, 0.4 mmol) was carried out in the same manner as preparation for **13** to give a product, which was purified by column chromatography (SiO_2: 7 g, hexane:EtOAc=2:1) to furnish **33** (120 mg, 96%).

33: Colorless oil, $[\alpha]^{21}_D$−6.1° (c=0.30 in $CHCl_3$). IR (KBr): 2972, 1740, 1669, 1639, 1530, 1512 cm^{-1}. ^1H NMR δ: 7.38–7.30 (5H, m, 10, 11, 12-H), 7.01 (2H, d, J=8.3 Hz, 27-H), 6.79 (2H, d, J=8.3 Hz, 28-H), 6.75 (1H, m, 3-H), 6.41 (1H, d, J=15.8 Hz, 8-H), 6.17 (1H, brs, 24-NH), 6.04 (1H, dd, J=8.5, 15.8 Hz, 7-H), 5.86 (1H, d, J=15.6 Hz, 2-H), 5.20 (1H, brs, 22-NH), 5.04 (1H, m, 5-H), 4.86 (1H, m, 24-H), 4.23–4.15 (2H, m, CH_2CH_2TMS), 3.77 (3H, s, 29-OMe), 3.30 (2H, m, 22-H), 3.09 (2H, m, 25-H), 2.64 (1H, m, 6-H), 2.49 (4H, m, 4, 21-H), 1.50 (6H, s, 16, 17-H), 1.41 (9H, s, *t*Bu), 1.11 (3H, d, J=6.7 Hz, 13-H), 0.98 (2H, m, CH_2CH_2TMS), 0.04 (9H, s, TMS). FABMS m/z: 781 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{42}H_{60}N_2O_{10}Si$+H: 781.4096. Found: 781.4040.

4.24. Deprotection of 33 followed by macrolactamization giving 34

Deprotection of **33** (70 mg, 0.09 mmol) and subsequent conversion to an HCl salt and macrolactamization was carried out in the same manner as preparation for **14** to give a product, which was purified by column chromatography (SiO_2: 6 g, hexane:EtOAc=1:3) to furnish **34** (46 mg, 90%).

34: A white powder, $[\alpha]^{21}_D$+39.0° (c=0.75 in $CHCl_3$). IR (KBr): 2930, 1738, 1676, 1640, 1528, 1514 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.12 (2H, d, J=8.5 Hz, 27-H), 6.80 (2H, d, J=8.5 Hz, 28-H), 6.69 (1H, ddd, J=6.1, 8.5, 15.3 Hz, 3-H), 6.41 (1H, d, J=15.9 Hz, 8-H), 6.03 (1H, dd, J=8.6, 15.9 Hz, 7-H), 5.79 (1H, d, J=15.3 Hz, 2-H), 5.75 (1H, d, J=9.2 Hz, 24-NH), 4.98 (1H, ddd, J=2.4, 5.5, 11.0 Hz, 5-H), 4.82 (1H, dt, J=9.2, 6.7 Hz, 24-H), 3.76 (3H, s, 29-OMe), 3.51 (1H, ddd, J=5.5, 6.1, 14.1 Hz, 22-Ha), 3.36 (1H, dt, J=14.1, 5.5 Hz, 22-Hb), 3.11 (2H, m, 25-H), 2.56 (1H, m, 6-H), 2.50–2.38 (4H, m, 4, 21-H), 1.56, 1.36 (both 3H, s, 16, 17-H), 1.12 (3H, d, J=6.7 Hz, 13-H). FABMS m/z: 563 $[M+H]^+$. FABHRMS m/z: Calcd for $C_{32}H_{38}N_2O_7$+H: 563.2757. Found: 563.2778.

4.25. Epoxidation of 34 giving 6

A solution of **34** (15 mg, 0.027 mmol) in CH_2Cl_2 (15 ml) was treated with dimethyldioxirane (0.074 M in acetone, 7 ml) for 8 h. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; COSMOSIL 5C18AR (10 mm i.d.×250 mm), mobile phase; MeOH:H_2O=65:35] to furnish **6** (9.3 mg, 60%).

6: A white powder, $[\alpha]^{21}_D$+40.0° (c=0.80 in $CHCl_3$). IR (KBr): 2932, 1739, 1676, 1646, 1533, 1514 cm^{-1}. ^1H NMR δ: 7.34–7.20 (5H, m, 10, 11, 12-H), 7.11 (2H, d, J=8.6 Hz, 27-H), 6.80 (2H, d, J=8.6 Hz, 28-H), 6.68 (1H, ddd, J=6.1, 8.5, 15.3 Hz, 3-H), 5.77 (1H, d, J=15.3 Hz, 2-H), 5.62 (1H, d, J=8.8 Hz, 24-NH), 5.08 (1H, ddd, J=3.0, 5.6, 11.3 Hz, 5-H), 4.83 (1H, dt, J=8.8, 6.8 Hz, 24-H), 3.77 (3H, s, 29-OMe), 3.68 (2H, d, J=1.9 Hz, 8-H), 3.46 (1H, m, 22-Ha),

3.41 (1H, m, 22-Hb), 3.11 (2H, d, J=6.8 Hz, 25-H), 2.88 (1H, dd, J=1.9, 7.6 Hz, 7-H), 2.75 (1H, m, 4-Ha), 2.43 (3H, m, 4-Hb, 21-H), 1.80 (1H, sextet, J=ca. 6.5 Hz, 6-H), 1.50, 1.24 (both 3H, s, 16, 17-H), 1.13 (3H, d, J=6.9 Hz, 13-H). FABMS *m/z*: 579 $[M+H]^+$. FABHRMS *m/z*: Calcd for $C_{32}H_{38}N_2O_8$+H: 579.2706. Found: 579.2722.

4.26. Conformational analysis

The NOESY spectra were measured by Varian Inova 600 (600 MHz for ^{1}H) at 24°C in d_6-DMSO, and a total of 48 (for **1**), 47 (for **2**), 33 (for **3**), and 43 (for **4**) NOEs were used as distance restraints for molecular dynamic calculations, respectively. The pro-chiral methylene proton and methyl proton were treated as pseudo-atoms[18] and additional distance terms were imposed to the upper bound of distance in the following manner. As the NOEs observed between the protons less than five covalent bonds, the methyl and methylene groups were allowed to have 0.9 and 0.6 Å of distance terms, respectively. In the case of NOE via more than four covalent bonds, 1.3 and 1.0 Å of distance terms were added to the methyl and methylene groups, respectively. The calculation was performed using SYBYL 6.5 (Tripos) on SGI R10000-O2 (Silicon Graphics) with Tripos force field.[19] The initial structure built up with reference to the NOE data was energy-minimized without restraints using the BFGS method until the derivative became less than 0.005 kcal/mol Å or the iteration steps reached 20,000. This refined structure was subjected to 50 cycles of simulated annealing in vacuo with distance restraints. Simulated annealing was executed for 500 fs at 700 K at an initial equilibrium step, and then the temperature was exponentially cooled down to 100 K over 1500 fs. The resulting 50 structures, which were extracted at each cycle end, were subjected to 20,000 steps of BFGS energy minimization with the restraints until the derivative became less than 0.005 kcal/mol Å. During both the simulated annealing and the secondary energy minimization, 200 kcal/Å^2 of force constant was employed as the violation of the restraint.

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