Pergamon

Tetrahedron 56 (2000) 9121–9128

TETRAHEDRON

Synthesis of Amide Analogs of Arenastatin A

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This paper is dedicated to Professor P. J. Scheuer, Hawaii University, on the occasion of his 85th birthday

Received 20 April 2000; accepted 27 July 2000

Abstract—In order to probe the metabolism of arenastatin A, a potent cytotoxic depsipeptide from the marine sponge *Dysidea arenaria*, we synthesized three analogs in which the ester linkages were replaced by amide bonds. Triamide analog-II and tetraamide analog, both of which contained a 15,20-amide linkage, showed stability in serum. However, arenastatin A and triamide analog-I, which both contained a 15,20-ester moiety, were readily metabolized in serum. Among the three amide analogs, only triamide analog-II exhibited potent cytotoxic activity against KB cells.

Introduction

During the course of our investigation in a search for new biologically active substances from marine organisms, we isolated and characterized an extremely potent cytotoxic (IC_{50} 5 pg/ml against KB cells) depsipeptide named arenastatin A (**1**) from the Okinawan marine sponge *Dysidea arenaria*.[1,2] In addition, we established the total synthesis of **1** and elucidated its structure-activity relationship among several synthesized stereoisomers.[3,4] Arenastatin A (**1**) was also shown to inhibit microtubule assembly and bind to the rhizoxin/maytansine site on tubulin specifically.[5,6]

Arenastatin A (**1**) bears a close structural similarity to the potent anti-tumor depsipeptide cryptophycin 1 (**3**), which was isolated from a cyanobacterium of *Nostoc* sp.[7] The ring portions and overall frameworks of the two compounds are identical, except that arenastatin A (**1**) lacks an aryl chlorine and a methyl on C-21 which are found in cryptophycin 1 (**3**). Cryptophycin 1 (**3**) exhibits potent in vivo anti-tumor activity.[8] Arenastatin A (**1**) was found to exhibit in vivo anti-tumor activity (71% ILS at 5 mg/kg dose against P388) by ip–ip administration, but little activity by ip–iv administration. In addition, when placed in fresh mouse serum, arenastatin A (**1**) was found to be readily metabolized. These observations led us to presume that one of the ester linkages in **1** is hydrolyzed (presumably enzymatically) in the case of intravenous administration, leading to loss of anti-tumor activity. In order to elucidate the specific portions of arenastatin A (**1**) which were susceptible to metabolic breakdown, we synthesized three amide analogs (**2**, **4**, **5**) in which one or two ester moieties were replaced by an amide group. This paper describes the synthesis and biological activities of these amide analogs (**2**, **4**, **5**) (Chart 1).

Syntheses of amide analogs

According to our synthetic protocol of arenastatin A (**1**),[4] we synthesized three amide analogs (**2**, **4**, **5**). The strategic disconnections of the three analogs (**2**, **4**, **5**) are summarized as depicted in Chart 2. Since arenastatin A (**1**) having an epoxy moiety adjacent to a phenyl group as well as a cyclic diester structure is fairly unstable under both acidic and alkaline conditions,[4] the epoxy function was introduced at the final stage of the synthesis.

Scheme 1 illustrates the synthetic route of triamide analog-I (**2**) having a 5,14-amide function in place of the ester linkage in **1**. Introduction of an amino group at C-5 in segment A (**6**)[4] with *S*-configuration was carried out by Mitsunobu reaction,[9] followed by S_N2-type nucleophilic substitution reaction of an azide group. The treatment of **6** with benzoic acid in the presence of tributylphosphine and diethylazodicarboxylate (DEAD) afforded a 5*R*-benzoate, which was then saponified by NaOMe in MeOH to give an alcohol **13** in 68% yield. After mesylation of **13**, the resulting mesylate was treated with NaN_3 in DMF to provide a 5*S*-azide, which was then converted to the desired segment A′ (**7**) by $LiAlH_4$ reduction in 87% yield from **13**. Segments A′ (**7**) and C–D (**14**)[10] were conjugated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) and 4-dimethylaminopyridine (DMAP) to give an amide, the methoxymethyl (MOM) group of which was removed by Me_2BBr[11] treatment to provide **15** in 72% yield. The

Keywords: depsipeptide; cytotoxin; marine metabolite; marine sponge.
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PII: S0040-4020(00)00766-3

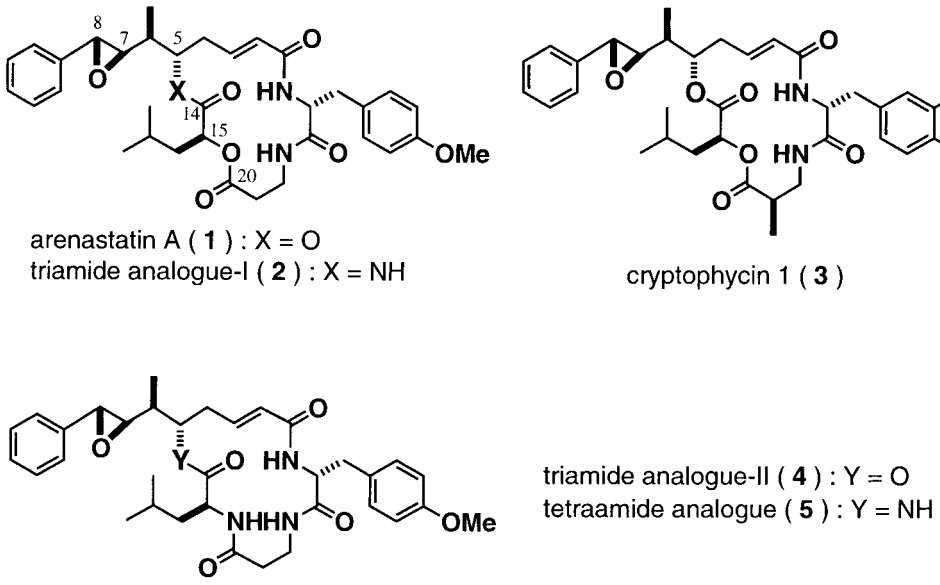


Chart 1.

primary alcohol in **15** was converted by Dess–Martin oxidation[12] to the corresponding aldehyde, which was further subjected to Horner–Emmons reaction with segment B (**8**)[4] to afford the diamide **16** in 96% yield. Removal of both protecting groups in **16** by trifluoroacetic acid (TFA) and subsequent HCl treatment gave a *seco* aminocarboxylic acid as an HCl salt. The *seco* aminocarboxylic acid was subjected to intramolecular macrolactamization by use of diphenylphosphorus azide (DPPA)[13,14] in the presence of $NaHCO_3$ in DMF to afford a cyclic depsipeptide **17** in 93% yield from **16**. Epoxidation of **17** using dimethyldioxirane proceeded stereoselectively to give triamide analog-I (**2**). The stereochemistry of the 7*R*,8*R*-epoxy function in **2** was confirmed by comparison of the chemical shifts of the C6-methyl proton (δ 1.06) and C8-proton (δ 3.62) of **2** in $CDCl_3$–CD_3OD (5:1) with those of **1** and its epoxy isomer. Thus, the C6-methyl proton and C8-proton in **1** were observed at δ 1.07 and δ 3.65 in $CDCl_3$–CD_3OD (5:1), while those of the epoxy isomer of **1** were observed at δ 1.00 and δ 3.56.

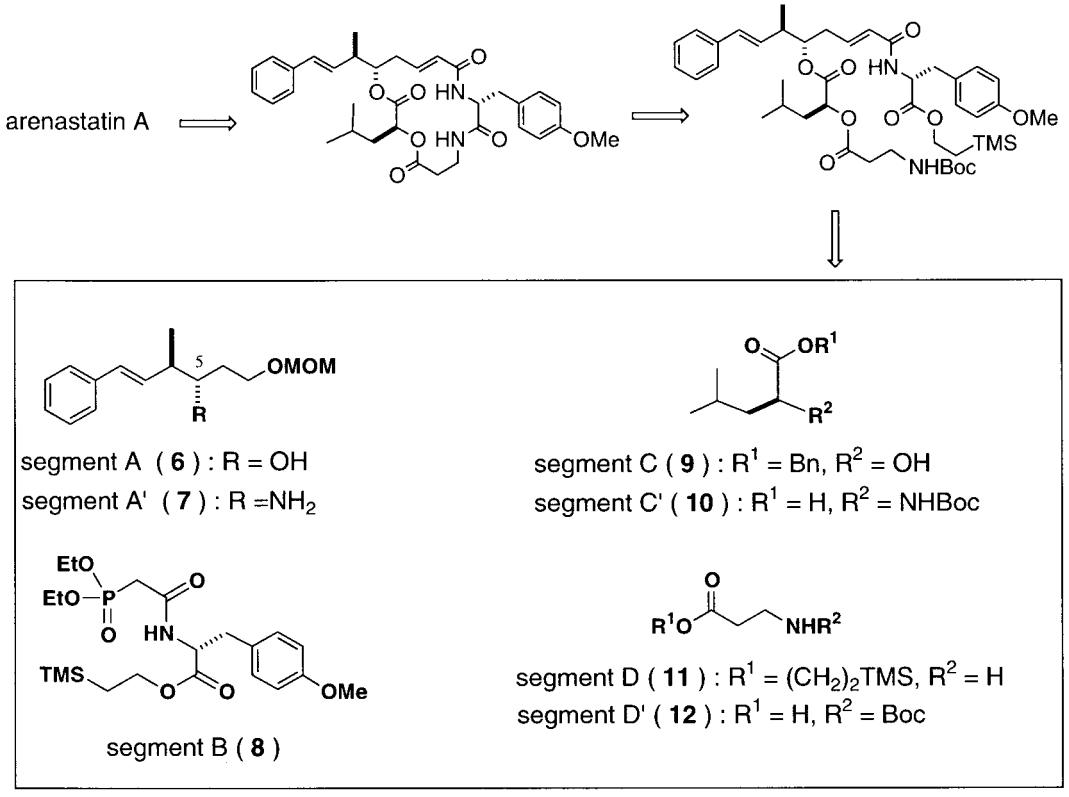
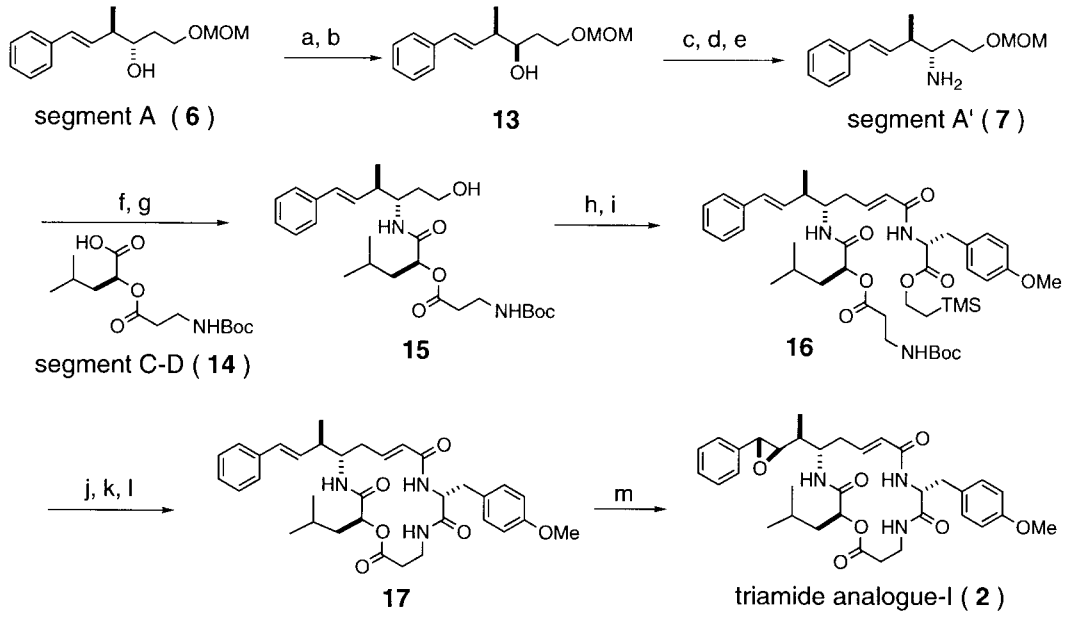


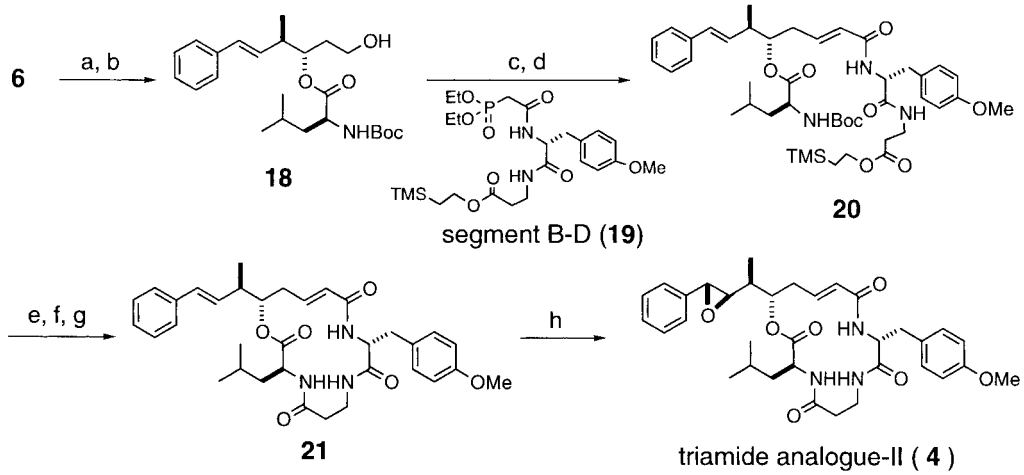
Chart 2.



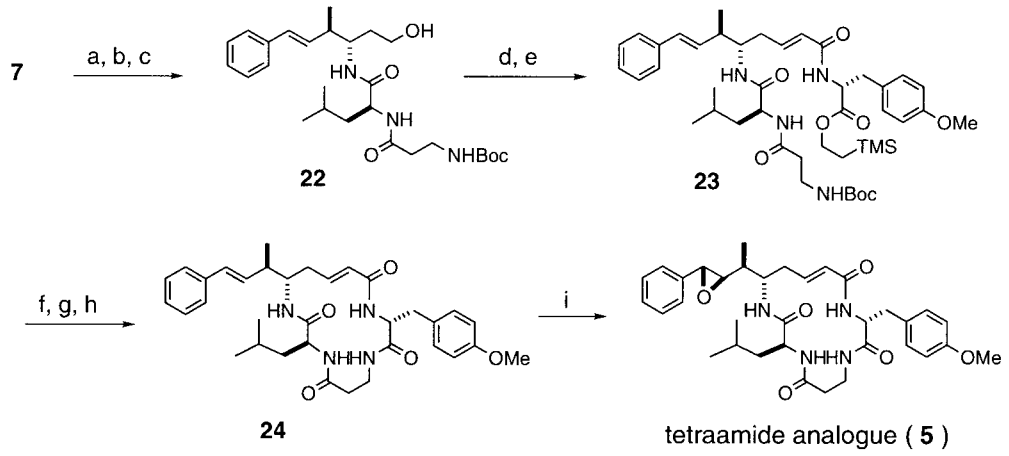
Scheme 1. Synthesis of triamide analog-I (**2**). *Reagents and conditions*: (a) PBu_3, DEAD, benzoic acid, THF; (b) NaOMe–MeOH, 2 steps 68%; (c) MsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF; (e) $LiAlH_4$, Et_2O, 0°C, 3 steps 91%; (f) **14**, EDCI·HCl, DMAP, CH_2Cl_2; (g) Me_2BBr, CH_2Cl_2, −78°C, 2 steps 72%; (h) Dess–Martin periodinane, CH_2Cl_2; (i) **8**, DBU, LiCl, CH_3CN, 2 steps 96%; (j) TFA, CH_2Cl_2; (k) HCl–ether; (l) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 93%; (m) dimethyldioxirane, CH_2Cl_2–MeOH (3:1), quant.

Triamide analog-II (**4**), in which an amide function was substituted for the 15,20-ester linkage in **1**, was synthesized as shown in Scheme 2. Condensation of segment A (**6**) and segment C′ (**10**) in the presence of dicyclohexylcarbodiimide (DCC) and DMAP afforded an ester, the MOM group of which was removed by Me_2BBr treatment to give **18**. Dess–Martin oxidation of the primary alcohol in **18** provided an aldehyde, which was subjected to Horner–Emmons reaction with the phosphonate **19**[3] to give an unsaturated amide **20**. Simultaneous deprotection of the *t*-butoxycarbonyl and the 2-trimethylsilylethyl groups using TFA and subsequent HCl treatment gave an HCl salt of a *seco* aminocarboxylic acid, which was then cyclized intramolecularly by use of DPPA and $NaHCO_3$ to give a cyclic depsipeptide **21**. Finally, dimethyldioxirane oxidation of **21** furnished the desired triamide analog-II (**4**)[6,15] and its epoxy isomer in a ratio of 2:1. The stereochemistry of the 7,8-epoxy function in **4** and the epoxy isomer of **4** were also confirmed by comparison of the chemical shifts of the C6-methyl proton and C8-proton in $CDCl_3$. Thus, the C6-methyl proton and C8-proton of **4** and its epoxy isomer were observed at δ 1.14, 1.04 and δ 3.67, 3.59 in $CDCl_3$, respectively. On the other hand, the C6-methyl proton and C8-proton of **1** and its epoxy isomer were observed at δ 1.14, 1.05 and δ 3.68, 3.59 in $CDCl_3$, respectively.

Additionally, tetraamide analog (**5**), in which both ester linkages in **1** were replaced by amide bonds, was prepared in a similar manner as for the synthesis of **2** (Scheme 3). The



Scheme 2. Synthesis of triamide analog-II (**4**). *Reagents and conditions:* (a) **10**, DCC, DMAP, CH_2Cl_2; (b) Me_2BBr, CH_2Cl_2, −78°C, 2 steps 92%; (c) Dess–Martin periodinane, CH_2Cl_2; (d) NaH, THF, 2 steps 76%; (e) TFA, CH_2Cl_2; (f) HCl–ether; (g) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 68%; (h) dimethyldioxirane, CH_2Cl_2–MeOH (2:1), 62%.



Scheme 3. Synthesis of tetraamide analog (**5**). *Reagents and conditions:* (a) **10**, DCC, DMAP, CH_2Cl_2, 82%; (b) 10% HCl–MeOH; (c) **12**, EDCI·HCl, DMAP, CH_2Cl_2, 2 steps 77%; (d) Dess–Martin periodinane, CH_2Cl_2; (e) **8**, DBU, LiCl, CH_3CN, 2 steps 93%; (f) TFA, CH_2Cl_2; (g) HCl–ether; (h) DPPA, $NaHCO_3$, DMF, 0°C, 3 steps 87%; (i) dimethyldioxirane, $CHCl_3$–MeOH (3:1), −30°C, 61%.

stereochemistry of the 7*R*,8*R*-epoxy function in **5** was also confirmed by comparison of the chemical shifts of the C6-methyl proton (δ 1.01) and C8-proton (δ 3.82). Thus, the C6-methyl proton and C8-proton in **1** were observed at δ 1.05 and δ 3.82 in d_6-DMSO, respectively.

Discussion

When tested for cytotoxicity against KB cells, triamide analog-II (**4**) exhibited potent cytotoxicity (IC_{50} 6 ng/ml), although this was 10^3 weaker than the activity of **1**. Triamide analog-I (**2**) and tetraamide analog (**5**) showed only weak cytotoxic activity of 4 and 6 μg/ml, respectively. As mentioned earlier, it was suspected that the hydrolysis of one of the ester linkages contributed to the loss of activity during intravenous administration. In order to test this presumption, the stability of arenastatin A (**1**) was compared with each of the three analogs in mouse serum. The compounds were allowed to incubate in mouse serum, then extracted and the remaining compound was evaluated by HPLC. Arenastatin A (**1**) and triamide analog-I (**2**), both of which contain the 15,20-ester linkage, were rapidly metabolized in mouse serum. In contrast, triamide analog-II (**4**) and tetraamide analog (**5**), in which the 15,20-ester is replaced by an amide functional group, were recovered unchanged from the mouse serum (Fig. 1).

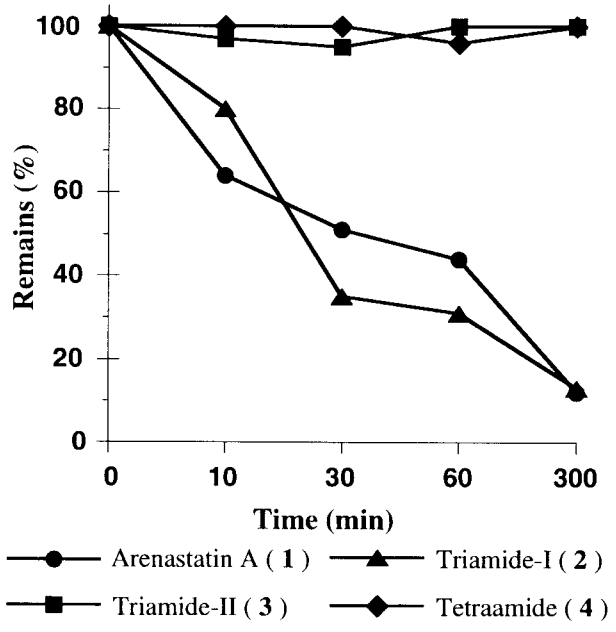


Figure 1. Stability of arenastatin A (**1**) and amide analogs (**2**, **4**, **5**) in mouse serum. Each sample (10 μl of 0.1 mg/ml solution in DMSO) was treated with fresh mouse serum (100 μl) and incubated at 37°C for 0, 10, 30, 60, 300 min, respectively. After extraction of the reaction mixture with EtOAc, each extract was analyzed by reversed phase HPLC to determine the remain of **1**, **2**, **4**, and **5**.

The results found with arenastatin A (**1**) and these three analogs suggest the following observations regarding cytotoxicity: (1) substitution of the 5,14-ester linkage by an amide results in a loss of cytotoxicity, (2) the 15,20-ester linkage is readily metabolized in serum, and (3) replacement of the 15,20-ester by an amide function results in reduced metabolism of the compound. Recently, the Lilly research group[16] synthesized a 15,20-amide analog of cryptophycin 1 (**3**) and reported that this analog exhibited a similar cytotoxicity as that of **3**.

In spite of exhibiting potent cytotoxicity, triamide analog-II (**4**) was poorly soluble in polar solvents such as H_2O, MeOH and DMSO to be applied to in vivo anti-tumor tests.[17] A program aiming at producing analogs of arenastatin A (**1**) for further anti-tumor and cytotoxic evaluation, and which will overcome these solubility limitations, is currently in progress in our laboratory.

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 ^1H 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, *c*-H_2SO_4 95 ml) or acidic *p*-anisaldehyde solution (*p*-anisaldehyde 25 ml, *c*-H_2SO_4 25 ml, AcOH 5 ml, EtOH 425 ml) with subsequent heating.

Inversion of hydroxy group in 6. DEAD (480 μl, 3.0 mmol) was added to a THF solution (2.0 ml) of **6** (250 mg, 1.0 mmol), PBu_3 (760 μl, 3.0 mmol) and benzoic acid (360 mg, 3.0 mmol) at 0°C, then the whole was stirred at 40°C for 8 h. The reaction mixture was evaporated in vacuo to afford a product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane–Et_2O=10:1) to give a benzoate (320 mg). A solution of the benzoate in MeOH (5 ml) was treated with 28% NaOMe–MeOH (2.5 ml) at room temperature for 2 h. The reaction mixture was poured into saturated aqueous NH_4Cl, 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 crude product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane–EtOAc=3:1) to furnish **13** (170 mg, 68%). **13:** Colorless oil, $[\alpha]_D^{26}$=+39.9° (*c*=2.0 in $CHCl_3$). IR (KBr): 3441, 2928, 1450, 1151, 1107 cm^{-1}. ^1H NMR δ: 7.33–7.16 (5H, m, Ph), 6.36 (1H, d, *J*=15.8 Hz, 8-H), 6.11 (1H, dd, *J*=7.9, 15.8 Hz, 7-H), 4.56 (2H, s, OCH_2OCH_3), 3.74–3.56 (3H, m, 3, 5-H), 3.30 (3H, s, OCH_2OCH_3), 2.38 (1H, m, 6-H), 1.76 (1H, m, 4-Ha), 1.67 (1H, m, 4-Hb), 1.09 (3H, d, *J*=6.6 Hz, 13-H). FAB-MS *m/z*: Calcd for $C_{15}H_{22}LiO_3^+$=257.1729. Found: 257.1754 $(M+Li)^+$.

Preparation for segment A′ (7). A solution of **13** (145 mg, 0.58 mmol) in dry CH_2Cl_2 (5.8 ml) was treated with Et_3N (160 μl, 1.2 mmol) and methansulfonyl chloride (54 μl, 0.7 mmol) for 10 min under cooling in an ice-water bath. The reaction mixture was poured into saturated aqueous NH_4Cl, 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 crude mesylate. A solution of this mesylate in dry DMF (5.8 ml) was treated with NaN_3 (110 mg, 1.7 mmol) for 3 h at 90°C. After removing the residue by filtration, the filtrate was poured into the water, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 10 g, *n*-hexane–Et_2O=15:1) to furnish an azide (145 mg, 91%). A solution of azide compound (82 mg, 0.30 mmol) in dry Et_2O (2.9 ml) was stirred in the presence of $LiAlH_4$ (34 mg, 0.90 mmol) at 0°C for 1 h. The reaction mixture was successively treated with water saturated Et_2O, 3 drops of 1N NaOH, and then the whole was filtered. Removal of solvent from the filtrate under reduced pressure gave segment A′ (**7**, 71 mg, quant.). **7:** Colorless oil, $[\alpha]_D^{23}$=+17.7° (*c*=2.39 in $CHCl_3$). IR (KBr): 3306, 1736, 1718, 1523 cm^{-1}. ^1H NMR δ:7.37–7.18 (5H, m, Ph), 6.44 (1H, d, *J*=15.9 Hz, 8-H), 6.14 (1H, dd, *J*=8.4, 15.9 Hz, 7-H), 4.63 (2H, s, OCH_2OCH_3), 3.69 (2H, m, 3-H), 3.37 (3H, s, OCH_2OCH_3), 2.88 (1H, ddd, *J*=3.7, 9.0, 12.6 Hz, 5-H), 2.33 (1H, m, 6-H), 1.89 (1H, m, 4-Ha), 1.54 (1H, ddt, *J*=9.0, 14.1, 5.9 Hz, 4-Hb), 1.14 (3H, d, *J*=6.6 Hz, 13-H). FAB-MS *m/z*: Calcd for $C_{15}H_{24}NO_2^+$=250.1807. Found: 250.1742 $(M+H)^+$.

Amidation of segment C–D (14) with segment A′ (7). A solution of segment A′ (**7**, 71 mg, 0.30 mmol) and segment C–D (**14**, 130 mg, 0.43 mmol) in dry CH_2Cl_2 (2.0 ml) was treated with EDCI·HCl (170 mg, 0.89 mmol) in the presence of DMAP (27 mg, 0.22 mmol) at room temperature for 1 h. The reaction mixture was poured into water, then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, then 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 8 g, *n*-hexane–EtOAc=1:1) to furnish an amide. (123 mg, 78%). A solution of the amide (40 mg, 0.075 mmol) in dry CH_2Cl_2 (0.75 ml) was treated with Me_2BBr (1.67 M in CH_2Cl_2, 130 μl) at −78°C for 1 h. After a mixture of THF and aqueous saturated $NaHCO_3$ (2:1) was dropped into the reaction mixture, 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 2 g, *n*-hexane–EtOAc=1:1) to furnish **15** (33.6 mg, 92%). **15:** Colorless oil, $[\alpha]_D^{23}$=−44.9° (*c*=0.51 in $CHCl_3$). IR (KBr): 3306, 2962, 1736, 1718, 1695, 1664, 1523 cm^{-1}. ^1H NMR δ: 7.42–7.22 (5H, m, Ph), 6.41 (1H, d, *J*=15.9 Hz, 8-H), 6.14 (1H, dd, *J*=7.9, 15.9 Hz, 7-H), 6.02 (1H, brd, *J*=6.1 Hz, 5-NH), 5.08 (1H, dd, *J*=3.1, 12.2 Hz, 15-H), 4.93 (1H, brs, 22-NH), 4.13 (1H, m, 5-H), 3.67 (1H, dt, *J*=12.2, 4.3 Hz, 3-Ha), 3.53 (1H, dt, *J*=12.2, 3.1 Hz, 3-Hb), 3.22 (2H, m, 22-H), 2.54 (1H, m, 6-H), 2.44 (1H, m, 21-Ha), 2.34 (1H, m, 21-Hb), 2.01 (1H, m, 4-Ha), 1.69–1.48 (4H, m, 4-Hb, 16, 17-H), 1.43 (9H, s, ^{t}Bu), 1.15 (3H, d, *J*=6.7 Hz, 13-H), 0.85, 0.84 (both 3H, d-like, *J*=ca. 6.0 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{27}H_{43}N_2O_6^+$=491.6495. Found: 491.6493 $(M+H)^+$.

Conversion from 15 to 16. A solution of **15** (10.5 mg, 0.021 mmol) in dry CH_2Cl_2 (0.4 ml) was treated with Dess–Martin periodinane (45.6 mg, 0.11 mmol) for 30 min at room temperature. Saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ were successively dropped into the reaction mixture, then the whole was stirred for 30 min. After extracting with EtOAc, the EtOAc extract was dried over Na_2SO_4. Removal of solvent from the EtOAc extract under reduced pressure gave a crude aldehyde. A solution of segment B (**8**, 19 mg, 0.04 mmol) in dry CH_3CN (1.9 ml) was treated with LiCl (8.8 mg, 0.2 mmol) and DBU (6 μl, 0.04 mmol) at room temperature for 10 min, the crude aldehyde was added to the solution with CH_2Cl_2 (0.5 ml). After further 10 min stirring, the reaction mixture was poured into saturated aqueous NaCl, 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 2 g, *n*-hexane–EtOAc=1:1) to furnish **16** (16.6 mg, 96%). **16:** Colorless oil, $[\alpha]_D^{23}$=+10.6° (*c*=0.04 in $CHCl_3$). IR (KBr): 2959, 1739, 1718, 1655, 1637 cm^{-1}. ^1H NMR δ: 7.38–7.22 (5H, m, Ph), 7.03 (2H, d, *J*=8.6 Hz, 27-H), 6.81 (2H, d, *J*=8.6 Hz, 28-H), 6.76 (1H, m, 3-H), 6.39 (1H, d, *J*=15.8 Hz, 8-H), 6.15 (1H, brs, 5-NH), 6.06 (1H, dd, *J*=8.3, 15.8 Hz, 7-H), 5.90 (1H, d, *J*=8.3 Hz, 24-NH), 5.89 (1H, d, *J*=15.5 Hz, 2-H), 5.04 (1H, m, 22-NH), 5.04

(1H, dd, *J*=4.6, 12.9 Hz, 15-H), 4.85 (1H, q-like, *J*=ca. 6.0 Hz, 24-H), 4.19 (2H, m, OCH_2CH_2TMS), 3.99 (1H, m, 5-H), 3.77 (3H, s, 30-H), 3.19 (2H, m, 22-H), 3.08 (2H, d, *J*=5.9 Hz, 25-H), 2.56 (1H, m, 6-H), 2.54–2.23 (4H, m, 4, 21-H), 1.68–1.40 (3H, m, 16, 17-H), 1.42 (9H, s, ^{t}Bu), 1.12 (3H, d, *J*=6.6 Hz, 13-H), 0.97 (2H, t-like, *J*=ca. 8.5 Hz, OCH_2CH_2TMS), 0.82 (6H, d, *J*=5.3 Hz, 18, 19-H), 0.04 (9H, s, TMS). FAB-MS *m/z*: Calcd for $C_{44}H_{66}N_3O_9Si^+$=808.4568. Found: 808.4576 $(M+H)^+$.

Deprotection of 16 followed by macrolactamization giving 17. A solution of **16** (16.6 mg, 0.02 mmol) in dry CH_2Cl_2 (0.4 ml) was treated with TFA (0.8 ml) under cooling in an ice-water bath for 4 h to give a *seco* amino acid as TFA salt. A solution of the TFA salt was treated with saturated HCl gas–Et_2O three times to furnish an HCl salt. A solution of the HCl salt in dry DMF (2.6 ml) was treated with DPPA (5 μl, 0.024 mmol) and $NaHCO_3$ (9 mg, 0.1 mmol) at 0°C for 44 h. The reaction mixture was poured into aqueous saturated NH_4Cl, then the whole was extracted with EtOAc, and 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 1 g, $CHCl_3$–MeOH=30:1) to furnish **17** (11.3 mg, 93%). **17:** A white powder, $[\alpha]_D^{19}$=+39.7° (*c*=0.07 in $CHCl_3$–MeOH=5:1). IR (KBr): 2922, 1797, 1743, 1651, 1514 cm^{-1}. ^{1}H NMR ($CDCl_3$–CD_3OD=5:1) δ: 7.31–7.04 (5H, m, Ph), 7.00 (2H, d, *J*=8.5 Hz, 27-H), 6.67 (2H, d, *J*=8.5 Hz, 28-H), 6.47 (1H, ddd, *J*=4.9, 10.4, 15.3 Hz, 3-H), 6.23 (1H, d, *J*=15.9 Hz, 8-H), 5.90 (1H, dd, *J*=9.2, 15.9 Hz, 7-H), 5.65 (1H, d, *J*=15.3 Hz, 2-H), 4.73 (1H, dd, *J*=5.5, 9.8 Hz, 15-H), 4.39 (1H, dd, *J*=5.5, 9.2 Hz, 24-H), 3.93 (1H, m, 5-H), 3.63 (3H, s, 30-H), 3.31 (1H, m, 22-Ha), 3.21 (1H, m, 22-Hb), 3.03 (1H, dd, *J*=5.5, 14.0 Hz, 25-Ha), 2.73 (1H, dd, *J*=9.2, 14.0 Hz, 25-Hb), 2.37 (4H, m, 4-Ha, 6, 21-H), 2.04 (1H, m, 4-Hb), 1.41–1.11 (3H, m, 16,17-H), 0.99 (3H, d, *J*=6.7 Hz, 13-H), 0.54, 0.53 (both 3H, d, *J*=6.7 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{34}H_{44}N_3O_6^+$=590.3228. Found: 590.3229 $(M+H)^+$.

Epoxidation of 17 giving 2. A solution of **17** (2.5 mg, 0.004 mmol) in CH_2Cl_2–MeOH (3:1, 0.1 ml) was treated with dimethyldioxirane (0.074 M in acetone, 0.1 ml) for 6 h. Removal of solvent of the reaction mixture under reduced pressure gave **2** (2.6 mg, quant.). **2:** A white powder, $[\alpha]_D^{20}$=+13.5° (*c*=0.06 in $CHCl_3$–MeOH=5:1). IR (KBr): 2932, 1747, 1657, 1635, 1537 cm^{-1}. ^{1}H NMR ($CDCl_3$–CD_3OD=5:1) δ: 7.34–7.08 (5H, m, Ph), 7.07 (2H, d, *J*=8.5 Hz, 27-H), 6.73 (2H, d, *J*=8.5 Hz, 28-H), 6.54 (1H, ddd, *J*=4.9, 11.6, 15.9 Hz, 3-H), 5.76 (1H, d, *J*=15.9 Hz, 2-H), 4.83 (1H, dd, *J*=7.3, 10.4 Hz, 15-H), 4.42 (1H, dd, *J*=4.9, 9.8 Hz, 24-H), 4.03 (1H, m, 5-H), 3.66 (3H, s, 30-H), 3.62 (1H, d, *J*=1.8 Hz, 8-H), 3.53 (1H, m, 22-Ha), 3.22 (1H, m, 22-Hb), 3.03 (1H, dd, *J*=4.9, 14.6 Hz, 25-Ha), 2.89 (1H, dd, *J*=1.8, 7.9 Hz, 7-H), 2.70 (1H, dd, *J*=9.8, 14.6 Hz, 25-Hb), 2.52 (1H, m, 4-Ha), 2.43 (2H, m, H-21), 2.14 (1H, m, 4-Hb), 1.58 (1H, m, 6-H), 1.47 (2H, m, 16-Ha, 17-H), 1.27 (1H, m, 16-Hb), 1.06 (3H, d, *J*=6.7 Hz, 13-H), 0.68, 0.64 (both 3H, d, *J*=6.1 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{34}H_{44}N_3O_7^+$=606.7410. Found: 606.7431 $(M+H)^+$.

Conversion from 6 to 18. A solution of segment A (**6**, 50 mg, 0.2 mmol) and segment C′ (**10**, 92 mg, 0.4 mmol) in CH_2Cl_2 (4 ml) was treated with DCC (206 mg, 1.0 mmol) and DMAP (73 mg, 0.6 mmol) at room temperature for 2 h. To the reaction mixture 10% HCl was added until the whole was neutralized. Then, the whole was extracted with Et_2O. The Et_2O extract was dried over Na_2SO_4. Removal of solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2 15 g, *n*-hexane–EtOAc=6:1) to furnish an ester (89 mg, 96%). A solution of the ester (46 mg, 0.099 mmol) in dry CH_2Cl_2 (1.0 ml) was treated with Me_2BBr (1.67 M in CH_2Cl_2, 190 μl) at −78°C for 20 min. The mixture of THF and aqueous saturated $NaHCO_3$ (2:1) was dropped into the reaction mixture. 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 crude product, which was purified by column chromatography (SiO_2 4 g, *n*-hexane–EtOAc=3:1) to furnish **18** (40 mg, 96%). **18:** Colorless oil, $[\alpha]_D^{25}$=−18.5° (*c*=1.1 in $CHCl_3$). IR (KBr): 2962, 1712, 1367, 1168 cm^{-1}. ^{1}H NMR δ: 7.31–7.07 (5H, m, Ph), 6.31 (1H, d, *J*=15.8 Hz, 8-H), 5.99 (1H, dd, *J*=8.6, 15.8 Hz, 7-H), 5.00 (1H, ddd, *J*=3.0, 5.9, 10.5 Hz, 5-H), 4.73 (1H, d, *J*=7.6 Hz, 15-NH), 4.11 (1H, m, 15-H), 3.55 (2H, m, 3-H), 2.49 (1H, m, 6-H), 1.83 (1H, m, 4-Ha), 1.68-1.23 (4H, m, 4-Hb, 16, 17-H), 1.33 (9H, s, ^{t}Bu), 1.03 (3H, d, *J*=6.9 Hz, 13-H), 0.76, 0.70 (both 3H, d, *J*=6.6 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{24}H_{37}NNaO_5^+$=442.2569. Found: 442.2546 $(M+Na)^+$.

Dess–Martin oxidation of 18 followed by Horner–Emmons reaction giving 20. A solution of **18** (36 mg, 0.086 mmol) in dry CH_2Cl_2 (1.7 ml) was treated with Dess–Martin periodinane (110 mg, 0.26 mmol) at room temperature for 1.5 h. The reaction mixture was worked up in the same manner as preparation for **16** to give a crude aldehyde. After a solution of segment B–D (**19**, 70 mg, 0.13 mmol) in dry THF (1.3 ml) was treated with NaH (20 mg, 60% in oil, 0.5 mmol) at −10°C for 1 h, the crude aldehyde was added to the solution with CH_2Cl_2 (0.4 ml). And the whole was stirred for 30 min. The reaction mixture was poured into saturated aqueous NH_4Cl, 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 5 g, *n*-hexane–EtOAc=1:1) to furnish **20** (52.5 mg, 76%). **20:** Colorless oil, $[\alpha]_D^{24}$=+31.9° (*c*=0.79 in $CHCl_3$). IR (KBr): 3285, 2957, 1730, 1658, 1631, 1512 cm^{-1}. ^{1}H NMR δ: 7.33–7.19 (5H, m, Ph), 7.12 (2H, d, *J*=8.6 Hz, 27-H), 6.80 (2H, d, *J*=8.6 Hz, 28-H), 6.77 (1H, m, 3-H), 6.56 (1H, d, *J*=7.7 Hz, 24-NH), 6.40 (1H, d, *J*=15.8 Hz, 8-H), 6.26 (1H, brs, 22-NH), 6.05 (1H, dd, *J*=8.6, 15.8 Hz, 7-H), 5.86 (1H, d, *J*=15.8 Hz, 2-H), 4.98 (1H, m, 5-H), 4.87 (1H, d, *J*=8.6 Hz, 15-NH), 4.57 (1H, q-like, *J*=ca. 7 Hz, 24-H), 4.21 (1H, m, 15-H), 4.13 (2H, m, OCH_2CH_2TMS), 3.77 (3H, s, 30-H), 3.47 (1H, m, 22-Ha), 3.34 (1H, m, 22-Hb), 3.01 (2H, d, *J*=6.0 Hz, 25-H), 2.61 (1H, m, 6-H), 2.50 (2H, m, 21-H), 2.38 (2H, m, 4-H), 1.52-1.22 (3H, m, 16, 17-H), 1.44 (9H, s, ^{t}Bu), 1.11 (3H, d, *J*=6.8 Hz, 13-H), 0.96 (2H, t, *J*=8.6 Hz, OCH_2CH_2TMS), 0.87, 0.81 (both 3H, d, *J*=6.4 Hz, 18, 19-H), 0.04 (9H, s, TMS). FAB-MS

m/z: Calcd for $C_{44}H_{66}N_3O_9Si^+$=808.4568. Found: 808.4592 $(M+H)^+$.

Deprotection of 20 followed by macrolactamization giving 21. Deprotection of **20** (50 mg, 0.062 mmol) and subsequent conversion to HCl salt and macrolactamization was carried out in the same manner as preparation for **17** to give a crude product, which was purified by HPLC [column; COSMOSIL $5C_{18}$ AR (10 mm i.d.×250 mm), mobile phase; $CH_3CN–H_2O–CH_2Cl_2$=60:40:1, detection; UV (λ= 220 nm), flow rate; 2.0 ml/min] to furnish **21** (25 mg, 68%). **21:** A white powder, $[\alpha]_D^{24}$=+40.8° (*c*=0.35 in $CHCl_3$). IR (KBr): 3294, 2957, 1736, 1662, 1541, 1514 cm^{-1}. ^1H NMR δ: 7.36–7.19 (5H, m, Ph), 7.07 (2H, d, *J*=8.6 Hz, 27-H), 6.96 (1H, m, 22-NH), 6.80 (2H, d, *J*=8.6 Hz, 28-H), 6.71 (1H, ddd, *J*=3.6, 10.5, 14.8 Hz, 3-H), 6.39 (1H, d, *J*=15.8 Hz, 8-H), 6.01 (1H, dd, *J*=8.5, 15.8 Hz, 7-H), 5.90 (1H, d, *J*=8.5 Hz, 15-NH), 5.70 (1H, d, *J*=14.8 Hz, 2-H), 5.64 (1H, d, *J*=6.9 Hz, 24-NH), 5.12 (1H, ddd, *J*=2.6, 6.6, 11.2 Hz, 5-H), 4.61 (1H, m, 15-H), 4.51 (1H, m, 24-H), 3.79 (1H, m, 22-Ha), 3.78 (3H, s, 30-H), 3.30 (1H, m, 22-Hb), 3.12 (1H, dd, *J*=4.9, 14.2 Hz, 25-Ha), 2.96 (1H, dd, *J*=8.3, 14.2 Hz, 25-Hb), 2.51 (1H, m, 4-Ha), 2.37 (4H, m, 4-Hb, 6, 21-H), 1.75–1.22 (3H, m, 16, 17-H), 1.13 (3H, d, *J*=6.9 Hz, 13-H), 0.74 (6H, d, *J*=6.6 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{34}H_{44}N_3O_6^+$=590.3228. Found: 590.3229 $(M+H)^+$.

Epoxidation of 21 giving 4. A solution of **21** (4.7 mg, 0.008 mmol) in CH_2Cl_2–MeOH (2:1, 0.3 ml) was treated with dimethyldioxirane (0.074 M in acetone, 0.8 ml) for 1 h. Removal of solvent of the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; COSMOSIL $5C_{18}$ AR (20 mm i.d.×250 mm), mobile phase; $CH_3CN–H_2O–CH_2Cl_2$= 50:50:1, detection; UV (λ=240 nm), flow rate; 3.0 ml/min] to furnish **4** (3.0 mg, 62%) and its epoxy isomer in 2:1 ratio. **4:** A white powder, $[\alpha]_D^{17}$=+18.6° (*c*=0.14 in $CHCl_3$–MeOH=1:1). IR (KBr): 3287, 2928, 1660, 1635, 1539, 1514 cm^{-1}. ^1H NMR δ:7.29–7.15 (5H, m, Ph), 7.07 (2H, d, *J*=8.6 Hz, 27-H), 6.94 (1H, brd, *J*=6.5 Hz, 22-NH), 6.82 (2H, d, *J*=8.6 Hz, 28-H), 6.70 (1H, ddd, *J*=3.9, 7.3, 15.0 Hz, 3-H), 5.82 (1H, d, *J*=5.0 Hz, 15-NH), 5.68 (1H, d, *J*=15.0 Hz, 2-H), 5.54 (1H, d, *J*=6.4 Hz, 24-NH), 5.28 (1H, m, 5-H), 4.60 (1H, q-like, *J*=ca. 7 Hz, 24-H), 4.50 (1H, m, 15-H), 3.78 (3H, s, 30-H), 3.77 (1H, m, 22-Ha), 3.67 (1H, d, *J*=1.7 Hz, 8-H), 3.23 (1H, m, 22-Hb), 3.12 (1H, dd, *J*=5.1, 14.5 Hz, 25-Ha), 2.96 (1H, dd, *J*=8.0, 14.5 Hz, 25-Hb), 2.92 (1H, dd, *J*=1.7, 7.7 Hz, 7-H), 2.55 (1H, m, 4-Ha), 2.42 (1H, m, 4-Hb), 2.35 (2H, m, 21-H), 1.80 (1H, m, 6-H), 1.52 (1H, m, 17-H), 1.37 (1H, m, 16-Ha), 1.29 (1H, m, 16-Hb), 1.14 (3H, d, *J*=6.8 Hz, 13-H), 0.85, 0.83 (both 3H, d, *J*=6.6 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{34}H_{43}LiN_3O_7^+$= 612.3261. Found: 612.3250 $(M+Li)^+$. *Epoxy isomer of **4**:* A white powder, $[\alpha]_D^{17}$=+19.5° (*c*=0.11 in $CHCl_3$–MeOH=1:1). IR (KBr): 3279, 2910, 1667, 1633, 1534, 1514 cm^{-1}. ^1H NMR δ: 7.29–7.15 (5H, m, Ph), 7.07 (2H, d, *J*=8.6 Hz, 27-H), 6.96 (1H, brd, *J*=6.5 Hz, 22-NH), 6.83 (2H, d, *J*=8.6 Hz, 28-H), 6.71 (1H, ddd, *J*=3.9, 7.3, 15.0 Hz, 3-H), 5.84 (1H, d, *J*=5.0 Hz, 15-NH), 5.68 (1H, d, *J*=15.0 Hz, 2-H), 5.54 (1H, d, *J*=6.4 Hz, 24-NH), 5.27 (1H, m, 5-H), 4.60 (1H, q-like, *J*=ca. 7.0 Hz, 24-H), 4.50 (1H, m, 15-H), 3.78 (3H, s, 30-H), 3.77 (1H, m, 22-Ha), 3.59 (1H, d, *J*=1.7 Hz, 8-H), 3.23 (1H, m, 22-Hb), 3.14 (1H, dd, *J*=5.1, 14.5 Hz, 25-Ha), 2.98 (1H, dd, *J*=7.9, 14.5 Hz, 25-Hb), 2.90 (1H, dd, *J*=1.7, 7.7 Hz, 7-H), 2.54 (1H, m, 4-Ha), 2.43 (1H, m, 4-Hb), 2.35 (2H, m, 21-H), 1.81 (1H, m, 6-H), 1.53 (1H, m, 17-H), 1.35 (1H, m, 16-Ha), 1.30 (1H, m, 16-Hb), 1.04 (3H, d, *J*=6.8 Hz, 13-H), 0.90, 0.87 (both 3H, d, *J*=6.6 Hz, 18,19-H). FAB-MS *m/z*: Calcd for $C_{34}H_{43}LiN_3O_7^+$=612.3261. Found: 612.3255 $(M+Li)^+$.

Conversion from 7 to 22. A solution of segment A′ (**7**, 60 mg, 0.24 mmol) and segment C′ (**10**, 86 mg, 0.36 mmol) in dry CH_2Cl_2 (2.0 ml) was treated with DCC (190 mg, 0.96 mmol) in the presence of DMAP (28 mg, 0.24 mmol) at 0°C for 2 h. The reaction mixture was worked up in the same manner as preparation for **18** to give a crude product, which was purified by column chromatography (SiO_2 7 g, *n*-hexane–EtOAc=5:1) to furnish an amide (92 mg, 82%). A solution of the amide (77 mg, 0.18 mmol) was treated with 10% dry HCl–MeOH for 2 h. Removal of solvent from the reaction mixture under reduced pressure gave a crude amide. A solution of the crude amide and segment D′ (**12**, 34 mg, 0.18 mmol) in dry CH_2Cl_2 (5.0 ml) was treated with EDCI·HCl (51.4 mg, 0.24 mmol) and DMAP (26.4 mg, 0.22 mmol) at 0°C for 2 h. Usual work-up was carried out in the same manner as preparation for **15**. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 3 g, $CHCl_3$–MeOH=50:1) to furnish **22** (63 mg, 77%). **22:** Colorless oil, $[\alpha]_D^{24}$= −32.6° (*c*=0.33 in $CHCl_3$). IR (KBr): 2955, 1691, 1637, 1545 cm^{-1}. ^1H NMR δ: 7.33–7.19 (5H, m, Ph), 6.42 (1H, d, *J*=15.8 Hz, 8-H), 6.12 (1H, dd, *J*=7.9, 15.8 Hz, 7-H), 6.09 (2H, m, 5, 15-NH), 5.04 (1H, m, 22-NH), 4.26 (1H, q-like, *J*=ca. 7 Hz, 15-H), 4.04 (1H, m, 5-H), 3.49 (1H, m, 3-Ha), 3.38 (1H, m, 3-Hb), 3.35 (2H, m, 22-H), 2.50 (1H, m, 6-H), 2.37 (2H, t-like, *J*=ca. 6 Hz, 21-H), 1.98 (1H, m, 4-Ha), 1.63-1.36 (4H, m, 4-Hb, 16, 17-H), 1.43 (9H, s, ^{t}Bu), 1.16 (3H, d, *J*=6.6 Hz, 13-H), 0.79, 0.77 (both 3H, d, *J*=6.3 Hz, 18, 19-H). FAB-MS *m/z*: Calcd for $C_{27}H_{44}N_3O_5^+$=490.3262. Found: 490.3260. $(M+H)^+$.

Dess–Martin oxidation of 22 followed by Horner–Emmons reaction. A solution of **22** (59 mg, 0.12 mmol) in dry CH_2Cl_2 (2 ml) was treated with Dess–Martin periodinane (102 mg, 0.24 mmol) for 30 min at room temperature. The reaction mixture was worked up in the same manner as preparation for **16** to give a crude aldehyde. After a solution of segment B (**8**, 91 mg, 0.19 mmol) in dry CH_3CN (3 ml) was treated with LiCl (16.3 mg, 0.38 mmol) and DBU (32 μl, 0.21 mmol) for 10 min at room temperature, the crude aldehyde was added to the solution with CH_2Cl_2 (0.5 ml). Then the reaction mixture was stirred for 10 min. The reaction mixture was worked up in the same manner as preparation for **16**. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 7 g, *n*-hexane–EtOAc=6:1) to furnish **23** (89 mg, 92%). **23:** Colorless oil, $[\alpha]_D^{24}$=−21.9° (*c*=2.9 in $CHCl_3$). IR (KBr): 3312, 2957, 1734, 1691, 1643 cm^{-1}. ^1H NMR δ: 7.37–7.18 (5H, m, Ph), 7.08 (2H, d, *J*=8.6 Hz, 27-H), 6.81 (2H, d, *J*=8.6 Hz, 28-H), 6.69 (2H, m, 3-H,15-NH), 6.41 (1H, d, *J*=15.8 Hz, 8-H), 6.40 (1H, m, 5-NH), 6.23 (1H, d, *J*=7.7 Hz, 24-NH), 6.08 (1H, dd, *J*=8.6, 15.8 Hz, 7-H), 5.78 (1H, d, *J*=15.2 Hz,

2-H), 5.16 (1H, brs, 22-NH), 4.79 (1H, q-like, J=ca. 7 Hz, 24-H), 4.31 (1H, m, 15-H), 4.20 (2H, m, OCH_2CH_2TMS), 4.05 (1H, m, 5-H), 3.77 (3H, s, 30-H), 3.30 (2H, m, 22-H), 3.11 (1H, dd, J=5.9, 14.1 Hz, 25-Ha), 3.02 (1H, dd, J=6.1, 14.1 Hz, 25-Hb), 2.45 (1H, m, 6-H), 2.37–2.24 (4H, m, 4, 21-H), 1.68–1.40 (3H, m, 16, 17-H), 1.39 (9H, s, ^{t}Bu), 1.14 (3H, d, J=6.6 Hz, 13-H), 0.88 (2H, m, OCH_2CH_2TMS), 0.79, 0.78 (both 3H, d, J=6.3 Hz, 18, 19-H), 0.04 (9H, s, TMS). FAB-MS m/z: Calcd for $C_{44}H_{66}LiN_4O_8Si^+$= 813.4810. Found: 813.4811 $(M+Li)^+$.

Deprotection of 23 followed by macrolactamization. Compound **23** (26 mg, 0.033 mmol) was converted to a macrolactam **24** in the same manner as preparation for **21**. The crude product was purified by HPLC [column; COSMOSIL $5C_{18}$ AR (10 mm i.d.×250 mm), mobile phase; CH_3CN–EtOH–H_2O–CH_2Cl_2=40:10:50:0.5, detection; UV (λ=220 nm), flow rate; 3.0 ml/min] to furnish **24** (16.5 mg, 87%). **24:** A white powder, $[\alpha]_D^{18}$=+28.9° (c=0.09 in $CHCl_3$–MeOH=5:1). IR (KBr): 2962, 1651, 1639, 1633, 1556 cm^{-1}. FAB-MS m/z: Calcd for $C_{34}H_{45}N_4O_5^+$=589.3390. Found: 589.3361 $(M+H)^+$. This compound was characterized by the data of IR and FAB-MS spectrum because of its extremely low solubility in various solvent for NMR measurement.

Epoxidation of 24 giving 5. A solution of **24** (4.3 mg 0.007 mmol) in $CHCl_3$–MeOH (3:1, 1.0 ml) was treated with dimethyldioxirane (0.074 M in acetone, 0.2 ml) for 1 h at −30°C. Removal of solvent from the reaction mixture under reduced pressure gave a product, which was purified by HPLC [column; COSMOSIL $5C_{18}$ AR (10 mm i.d.×250 mm), mobile phase; CH_3CN–H_2O=50:50, detection; UV (λ=220 nm), flow rate; 2.5 ml/min] to furnish **5** (2.7 mg, 61%). **5:** A white powder, $[\alpha]_D^{19}$=+19.6° (c=0.06 in $CHCl_3$–MeOH=5:1). IR (KBr): 1637, 1554, 1516, 1381 cm^{-1}. ^{1}H NMR (d_6-DMSO) δ: 8.27 (1H, d, J=7.9 Hz, 24-NH), 7.97 (1H, d, J=9.8 Hz, 5-NH), 7.90 (1H, d, J=7.9 Hz, 15-NH), 7.37–7.16 (5H, m, Ph), 7.13 (2H, d, J=8.5 Hz, 27-H), 7.07 (1H, brd, J=8.5 Hz, 22-NH), 6.82 (2H, d, J=8.5 Hz, 28-H), 6.39 (1H, ddd, J=3.1, 11.0, 14.6 Hz, 3-H), 5.73 (1H, d, J=14.6 Hz, 2-H), 4.28 (1H, m, 15-H), 4.22 (1H, m, 24-H), 4.03 (1H, m, 5-H), 3.82 (1H, d, J=2.0 Hz, 8-H), 3.70 (3H, s, 30-H), 3.66 (1H, m, 22-Ha), 3.47 (1H, m, 22-Hb), 2.99 (1H, dd, J=2.0, 7.0 Hz, 7-H), 2.96 (1H, dd, J=4.0, 12.0 Hz, 25-Ha), 2.69 (1H, m, 25-Hb), 2.52 (2H, m, 4-Ha, 21-Ha), 2.16 (1H, m, 21-Hb), 2.09 (1H, m, 4-Hb), 1.50 (2H, m, 6-H, 16-Ha), 1.38 (1H, m, 17-H), 1.17 (1H, m, 16-Hb), 1.01 (3H, d, J=6.8 Hz, 13-H), 0.74, 0.69 (both 3H, d, J=6.7 Hz, 18, 19-H). FAB-MS m/z: Calcd for $C_{34}H_{44}N_4NaO_6^+$=627.3158. Found: 627.3151 $(M+Na)^+$.

Evaluation for stability of 1, 2, 4, and 5 in mouse serum. Each sample (10 μl of 0.1 mg/ml solution in DMSO) 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 (500 μl), the solvent from each EtOAc (400 μl) extract was removed by nitrogen stream. Each residue was dissolved with CH_3CN–H_2O (1:1, 100 μl), then an aliquot (45 μl) was analyzed by reversed phase HPLC [column; mightysil RP-18 GP (4.6 mm i.d.×150 mm), mobile phase; CH_3CN–H_2O=50:50, detection; UV (λ=220 nm), flow rate; 1.0 ml/min] to determine the remain of **1**, **2**, **4**, and **5**.

Acknowledgements

The authors are grateful to Dr K. Sugita, the Research Laboratory of Shionogi Pharmaceutical Co., Ltd., for in vivo anti-tumor test. The authors are also grateful to the Naito Foundation, the Houansha Foundation, and the Ministry of Education, Sciences, Sports, and Culture of Japan for financial support.

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17. For example, the solubility of arenastatin A (**1**) and its amide analogs (**2**, **4**, **5**) in MeOH were as follows; **1**: 2.8 mg/ml, **2**: 1.1 mg/ml, **4**: 0.20 mg/ml, **5**: 0.028 mg/ml.