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Synthesis of 15,20-triamide analogue with polar substituent on the phenyl ring of arenastatin A, an extremely potent cytotoxic spongean depsipeptide

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Abstract—In order to increase metabolic stability and water solubility of arenastatin A, an extremely potent cytotoxic depsipeptide from the Okinawan marine sponge of *Dysidea arenaria*, several 15,20-triamide analogues with a polar substituent on the phenyl ring were synthesized. The 15,20-triamide analogues with a polar substituent (**24**, **30**, and **31**) showed increased solubility to MeOH and stronger cytotoxicity against KB cells in comparison with the parental 15,20-triamide analogue (**2**). Furthermore, the diethylamine analogue (**30**) exhibited in vivo anti-tumor activity against subcutaneously implanted murine sarcoma.
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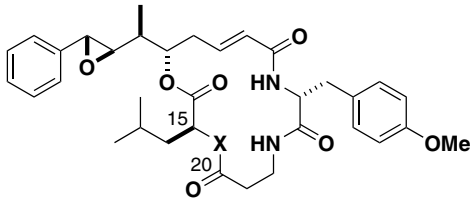
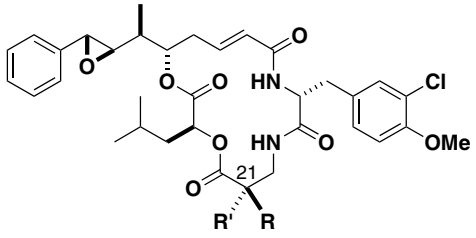
1. Introduction

In the course of our search for bioactive substances from marine organisms, we have isolated and characterized arenastatin A (**1**), a cyclic depsipeptide having extremely potent cytotoxic activity (IC_{50} = 5 pg/mL) against KB cells, from the Okinawan marine sponge of *Dysidea arenaria*.[1] Thereafter, we achieved the total synthesis of **1**[2] and elucidated the activity of **1** to be ascribable to the inhibition of microtubule assembly by binding to the rhizoxin/maytansine site on tubulin.[3] On the other hand, **1** was found to exhibit only marginal in vivo anti-tumor activity in the case of intravenous administration, because of a rapid metabolism of the 15,20-ester linkage in **1** in mouse serum. In order to overcome this biological lability of **1**, we have synthesized 15,20-triamide analogue (**2**), in which the labile ester function of **1** was replaced by an amide moiety, and found that analogue **2** showed sufficient stability in serum and moderate cytotoxicity (IC_{50} = 6 ng/mL). However, **2** was almost insoluble in polar solvents such as MeOH, DMSO, and water, so it could not be applied to in vivo biological evaluation (see Fig. 1).[4]



arenastatin A (**1**): X = O
15,20-triamide analogue (**2**): X = NH



cryptophycin 1 (**3**): R = Me, R' = H
cryptophycin 52 (**4**): R = R' = Me

Figure 1.

Keywords: Arenastatin A; Depsipeptide; Marine sponge; Structure–activity relationship.
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Cryptophycins, a family of closely related depsipeptides, have been found from terrestrial cyanobacterium of *Nostoc* sp.[5] Some of them, such as cryptophycin 1 (**3**) having a C_{21}-methyl group which prevents hydrolysis of the 15,20-ester function, exhibited not only potent cytotoxicity but also excellent in vivo anti-tumor activity. Due to their potent bioactivity and synthetically attractive structure, many synthetic studies have been reported, including the total synthesis of these natural products and structure–activity relationship (SAR) studies through the synthesis of several analogues.[6] Among them, cryptophycin 52 (**4**), a synthetic analogue with a *gem*-dimethyl group on the C-21 position, has been selected for clinical development.[7] Recently, the SAR study of cryptophycin 52 (**4**) by Al-awar and co-workers revealed that substitution on the phenyl ring of the left side of the molecule was well tolerated and some analogues with improved aqueous solubility showed potent anti-tumor activity.[8] This information led us to synthesize the 15,20-triamide analogues with a polar substituent on the phenyl ring, in order to improve their solubility and to evaluate their biological activity. Here we report the full details of our synthetic study and biological evaluation.

2. Chemistry

2.1. Synthetic strategy

Figure 2 shows our strategy toward synthesis of 15,20-triamide analogues with a polar substituent. Relatively unstable β-7,8-epoxide should be introduced in the final step by the oxidation of olefin. In order to prepare various analogues with a polar substituent on the phenyl ring efficiently, the aryl moiety was introduced through Heck reaction between aryl halide and a cyclic depsipeptide **5** having a terminal olefin. This approach made the synthesis flexible for incorporation of various aryl substituents. The cyclic peptide **5** would be synthesized in a similar manner with our synthesis of 15,20-triamide analogue (**2**),[4] namely, sequential condensation of four segments A–D (**8–11**) and macrocyclization at the 15,20-amide bond.

2.2. Preparation of segment A

For the stereoselective synthesis of segment A, we decided to use the same strategy with our total synthesis of arenastatin A (**1**) as shown in Scheme 1.[2b] Evans' *syn*-selective asymmetric aldol reaction of chiral *N*-crotonyl oxazolidinone (**12**)[9] and an aldehyde **13** proceeded stereoselectively to afford the desired (2*R*,3*S*)-adduct **14** in good yield. Removal of the chiral auxiliary with $LiBH_4$ and reductive deoxygenation of the resulting primary hydroxyl group of **15** gave segment A (**8**).

2.3. Synthesis of cyclic depsipeptide

Segment A (**8**) and commercially available *N*-Boc-L-leucine (**9**) were coupled by 1,3-dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP) to give an ester **17**. After deprotection of the TBS group of **17** by tetra-*n*-butylammonium fluoride (TBAF) and following Dess–Martin oxidation of the alcohol **18**, the resulting aldehyde **6** was subjected to Horner–Emmons reaction with segment BD (**7**)[4] to give an α,β-unsaturated amide (**19**). After deprotection of the *N*-Boc group and *O*-(2-trimethylsilyl)ethyl group of **19** by trifluoroacetic acid (TFA), HCl/Et_2O treatment of the resulting amine **20** and intramolecular macrocyclization by using diphenylphosphoryl azide (DPPA) afforded the objective cyclic depsipeptide **5** in good yield (Scheme 2).

2.4. Heck reaction—preliminary study

With the required cyclic peptide **5** in hand, the introduction of an aryl moiety with a polar substituent was examined. As a similar synthetic strategy using the Heck reaction has been reported by Georg's group,[10] we first tried a coupling reaction between aryl iodide **21** and the cyclic peptide **5** according to their method. Thus, **5** and **21** were treated with $Pd(OAc)_2$ and Et_3N in hot CH_3CN, but the reaction hardly proceeded and resulted in recovery of the starting material. In order to find better reaction conditions, we examined the model reaction using compound **17**, as shown in Scheme 3 and Table 1. It was revealed that the condition using an inorganic base and a phosphine ligand was effective for this reaction (entry 2), and the reaction was further activated by the addition of tetra-*n*-butylammonium chloride to give desired coupling product **22** in good yield (entry 3).[11] Either DMF and CH_3CN can be used for the reaction solvent (entry 4).

2.5. Synthesis of ether and ester analogues

With the use of the conditions developed above, Heck coupling between cyclic peptide **5** and aryl iodide **21** proceeded to give **23**, but the yield of the coupling product **23** was not satisfactory. After further investigation, we found that the addition of water accelerated the reaction and gave the coupling product **23** in improved yield (54%). Finally, treatment of **23** with dimethyldioxirane at −20 °C gave the desired 15,20-triamide analogue **24** having β-epoxide, after separation of the α/β mixture (α/β = 1:2.6) by reversed-phase HPLC.[12] Analogue **27** having an ester moiety was synthesized from **23** in three steps, for example, deprotection of the MOM group of **23**, condensation of the resulting alcohol **25** with *N*-Boc-glycine, and epoxidation of **26** by dimethyldioxirane (Scheme 4).

2.6. Synthesis of amine and ammonium analogues

Next, we examined synthesis of the analogues having an amine moiety on the phenyl ring. We planned that such analogues would be efficiently prepared from benzyl chloride **28**, by treatment with the corresponding amines. First, we executed a direct approach to synthesize the key compound **28** through Heck reaction between cyclic peptide **5** and 4-iodobenzyl chloride. However, the objective product could not be obtained. So, the analogues having amine moieties

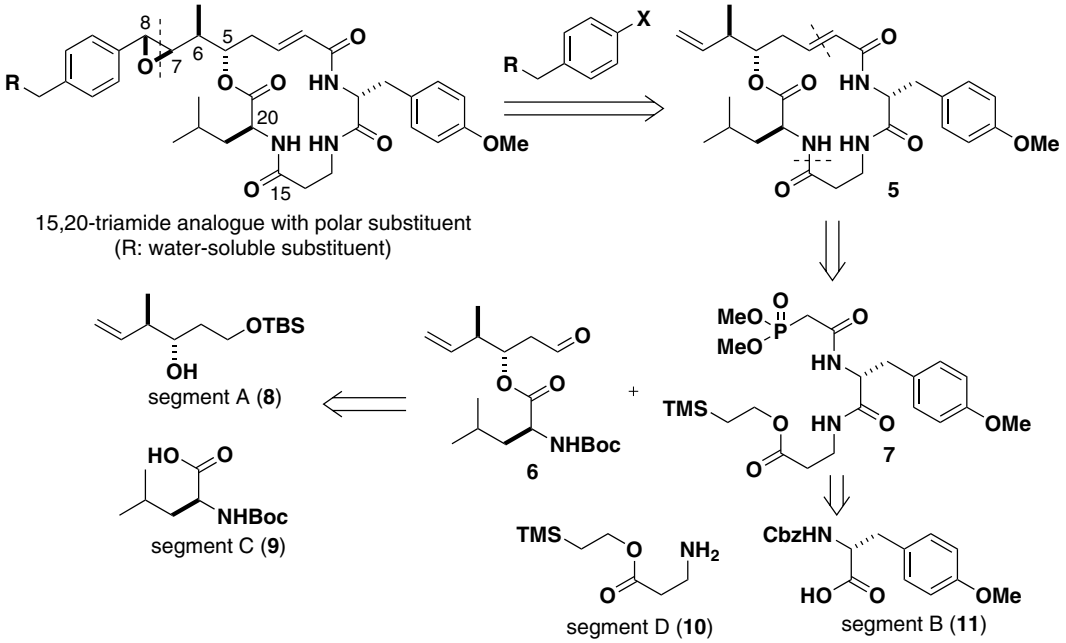
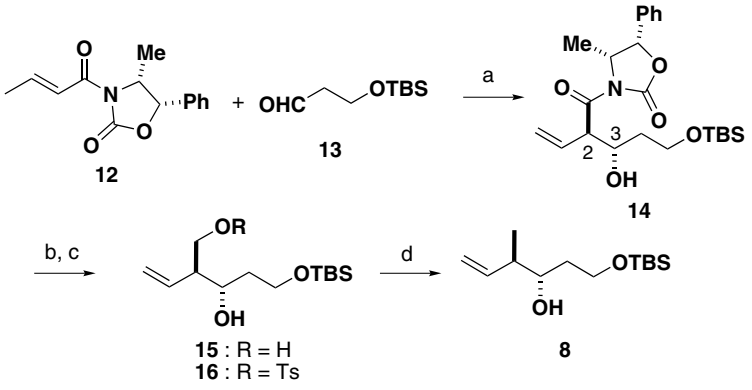
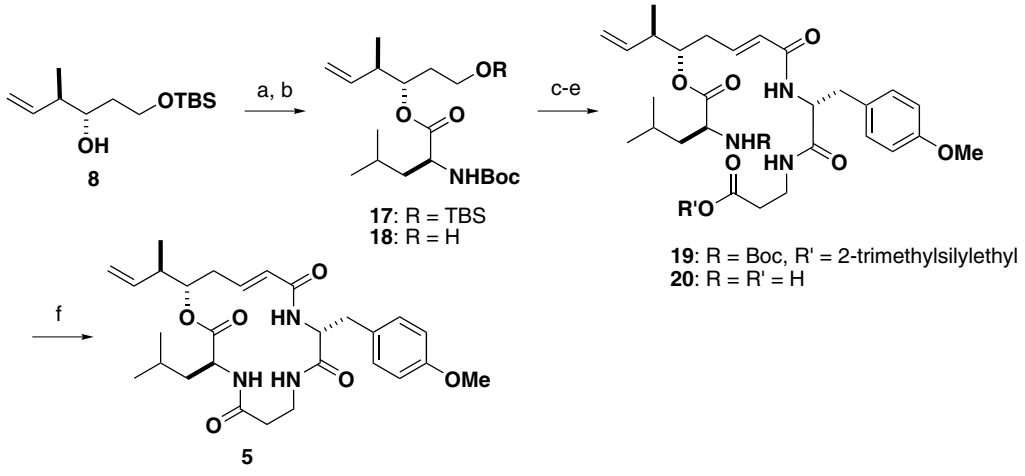


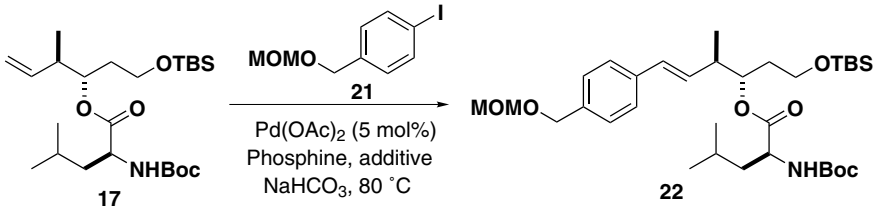
Figure 2. Retrosynthetic analysis.



Scheme 1. Reagents and conditions: (a) Bu_2BOTf, Et_3N, CH_2Cl_2, −78 °C, 91%; (b) Bu_3B, AcOH, $LiBH_4$, THF, 88%; (c) TsCl, pyridine, 90%; (d) $NaBH_4$, DMSO, 73%.



Scheme 2. Reagents and conditions: (a) **9**, DCC, DMAP, CH_2Cl_2, 100%; (b) TBAF, AcOH, THF, 100%; (c) Dess–Martin periodinane, CH_2Cl_2; (d) **7**, NaH, THF, 75% (two steps); (e) TFA, CH_2Cl_2; HCl/Et_2O; (f) DPPA, $NaHCO_3$, DMF, 89% (two steps).



Scheme 3.

Table 1. Heck reaction between **17** and **21**[a]

Entry	PPh_3 (10 mol%)	n-Bu_4NCl (100 mol%)	Solvent	Yield (%)
1	–	–	DMF	n.d.
2	+	–	DMF	45
3	+	+	DMF	77
4	+	+	CH_3CN	79

[a] All reaction was performed in the presence of $Pd(OAc)_2$ (5 mol%) and $NaHCO_3$ (250 mol%) at 80 °C.

were synthesized from benzyl alcohol **25**, as shown in Scheme 5. Benzyl alcohol **25** was converted to the corresponding chloride **28** in good yield, by treatment with MsCl and LiCl.[13] Oxidation of **28** with dimethyldioxirane and the following HPLC separation gave β-epoxide **29**,[12] and subsequent treatment with diethylamine or piperazine afforded the corresponding tertiary amine analogues (**30**, **31**) without affecting the labile epoxide moiety. Quaternary ammonium analogues (**32** and **33**), aiming at improved water solubility by their constantly charged structure, were also prepared by the treatment of **29** with triethylamine and 1,4-diazabicyclo[2.2.2]octane (DABCO), respectively.

3. Biological evaluation

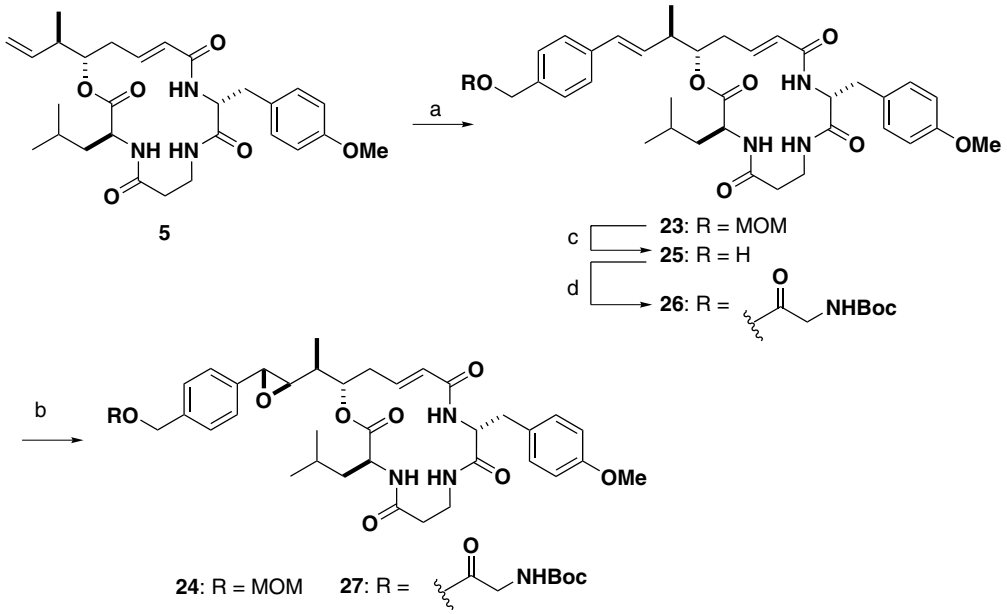
3.1. In vitro cell growth inhibition

The growth inhibitory effect of the synthesized 15-triamide analogues with a polar substituent on the phenyl ring against KB 3-1 cells was evaluated as summarized in Table 2, together with their solubility in MeOH. All analogues showed better solubility than the parental 15,20-triamide analogue (**2**). In the case of **31**, having a piperazine moiety at the phenyl ring, 85-fold solubility was observed.

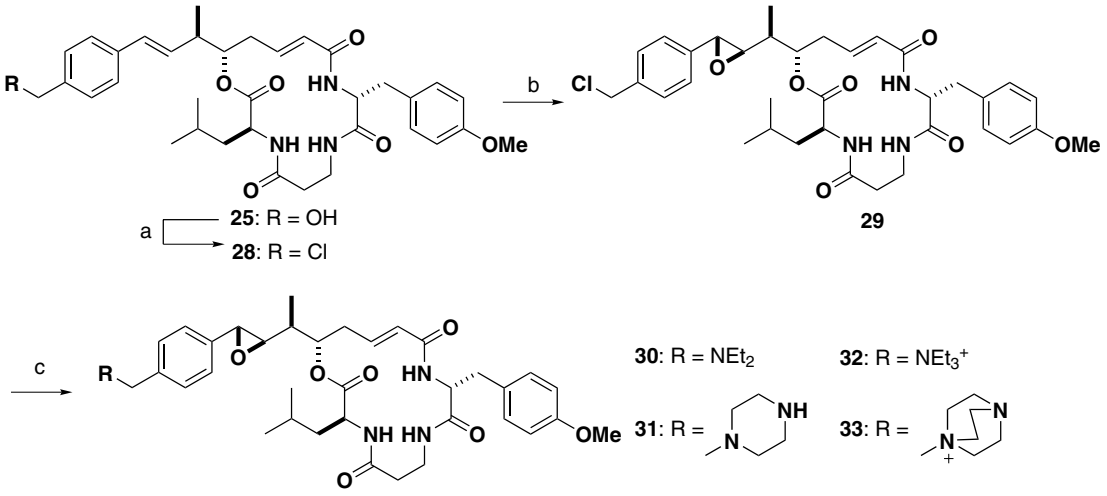
Among them, diethylamine analogue (**30**) exhibited the most potent cytotoxic activity (IC_{50} = 0.18 ng/mL), which was 30-fold greater than that of **2**. MOM-ether derivative (**24**) also showed increasing activity, 10 times as potent as **2**. On the other hand, quaternary ammonium analogues (**32** or **33**) showed disappointing results in terms of both cytotoxicity and solubility in MeOH.

3.2. In vivo test for anti-tumor activity

The in vivo anti-tumor testing of diethylamine analogue (**30**), possessing both potent cytotoxicity and good solu-



Scheme 4. Reagents and conditions: (a) **21**, $Pd(OAc)_2$, Ph_3P, $NaHCO_3$, Bu_4NCl, DMF/H_2O, 80 °C, 54%; (b) dimethyldioxirane, CH_2Cl_2/MeOH, 53% for **24**; 48% for **27**; (c) 6 M HCl, MeOH, 72%; (d) *N*-Boc-Gly, DCC, DMAP, CH_2Cl_2, 100%.



Scheme 5. Reagents and conditions: (a) MsCl, LiCl, γ-collidine, DMF, 93%; (b) dimethyldioxirane, CH_2Cl_2/MeOH, 60%; (c) amine, DMF, 85% for **30**; 80% for **31**; 86% for **32**; 85% for **33**.

Table 2. In vitro growth inhibitory activity of arenastatin A analogues and their solubility in MeOH

Compound	IC_{50} (ng/mL)	Solubility (mg/mL)
2	6	0.2
24	0.61	2
27	2.16	2.6
30	0.18	13
31	1.5	17
32	19	7.2
33	44	4.9

bility in MeOH, was carried out. Murine sarcoma S180 cells were implanted subcutaneously, and the testing compound was administered intraperitoneally every other day for 2 weeks. The effectiveness of the testing compound was determined by measuring the median tumor diameter. It was found that diethylamine analogue (**30**) inhibited the growth of tumor at the dose of 1 mg/kg, exhibiting comparable efficacy as that of doxorubicin (positive control) (Table 3). Even though diethylamine analogue (**30**) showed toxicity at the 5 mg/kg dose, 1 mg/kg administration of **30** showed no significant acute toxicity such as body weight loss. As expected, arenastatin A analogue with improved stability and water solubility could be a promising candidate for anti-tumor agent.

Table 3. In vivo anti-tumor effect of analogue **30**

Compound	Dose (mg/kg)[a]	Tumor size (mm)[b]
30	1	11.7 ± 1.7
	0.5	12.7 ± 2.3
Doxorubicin	5	11.4 ± 2.4
	3	11.5 ± 1.6
Control		13.1 ± 2.0

[a] Ip administration (days 3, 5, 7, 9, 11, and 13).
[b] Mean ± SD (n = 8).

4. Summary

From the SAR study of 15,20-triamide analogues with a polar substituent on the phenyl ring, the analogues with diethylamine or MOM-ether substituent (**30** or **24**, respectively) were found to exhibit more than 10-fold potent cytotoxic activity against KB cells in comparison with that of the parental 15,20-triamide analogue (**2**). Furthermore, diethylamine analogue (**30**) showed to exhibit in vivo anti-tumor activity against subcutaneously implanted murine sarcoma.

5. Experimental

5.1. General

All the reaction solvents were distilled prior to use. The following instruments were used to obtain physical data: a JASCO DIP-370 digital polarimeter for specific rotations; a JASCO FT/IR-5300 infrared spectrometer for IR spectra; a Waters Q-Tof Ultima API mass spectrometer for ESI-TOF MS; a JEOL JNM AL-500 NMR spectrometer for ^1H and ^{13}C NMR using tetramethylsilane as an internal standard. HPLC was performed using a Hitachi L-6000 pump equipped with Hitachi L-4000H UV detector. Silica gel (Fuji Silysia BW-200 or Chromatorex®-NH_2) and pre-coated thin-layer chromatography (TLC) plates (Merck, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying acidic *p*-anisaldehyde solution (*p*-anisaldehyde: 25 mL, *c*-H_2SO_4: 25 mL, AcOH: 5 mL, and EtOH: 425 mL) with subsequent heating.

5.1.1. (4*R*,5*S*)-3-{(2*R*)-2-[(1*S*)-3-(*tert*-Butyldimethylsilanyloxy)-1-hydroxypropyl]-but-3-enoyl}-4-methyl-5-phenyloxazolidin-2-one (14). Under argon atmosphere, a THF solution of Bu_2BOTf (1.0 M, 7.3 mL, 7.3 mmol) was added dropwise to a solution of **12** (1.5 g, 6.1 mmol) in CH_2Cl_2 (23 mL) at −78 °C. After being stirred for 10 min, Et_3N (1.3 mL, 9.2 mmol) was added to the reac-

tion mixture, and the whole mixture was stirred at this temperature for 20 min, then warmed to 0 °C and stirred for 30 min. After the reaction mixture was cooled again to −78 °C, **13** (1.4 g, 7.3 mmol) was added dropwise to the reaction mixture, and the whole mixture was stirred at this temperature for 2 h, then warmed to 0 °C and stirred for 1 h. The reaction was quenched with water, and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$) to give **14** (2.41 g, 91%) as colorless oil.

Compound **14**: $[\alpha]_D^{20}$ +45 (*c* 1.46, $CHCl_3$). IR (KBr): 3491, 2930, 2858, 1782, 1699 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.37–7.44 (m, 3H), 7.30 (d, J = 6.7 Hz, 2H), 6.06 (ddd, J = 8.5, 10.4, 17.1 Hz, 1H), 5.68 (d, J = 7.3 Hz, 1H), 5.33 (d, J = 10.4 Hz, 1H), 5.32 (d, J = 17.1 Hz, 1H), 4.81 (dq, J = 6.7, 7.3 Hz, 1H), 4.54 (dd, J = 4.3, 8.5 Hz, 1H), 4.24–4.27 (m, 1H), 3.80–3.90 (m, 2H), 3.67 (d, J = 1.8 Hz, 1H), 1.75–1.82 (m, 1H), 1.65–1.70 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.90 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.2, 152.6, 133.3, 131.9, 128.9 (2C), 128.8 (2C), 125.7, 120.4, 78.9, 71.3, 61.7, 55.0, 53.0, 36.3, 25.9 (3C), 18.2, 14.3, −5.5 (2C). ESI-MS: *m*/*z* 456 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 456.2182, calcd for $C_{23}H_{35}NO_5SiNa$. Found: 456.2180. Anal. Calcd for $C_{23}H_{35}NO_5Si$: C, 63.71; H, 8.14; N, 3.23. Found: C, 63.62; H, 8.11; N, 3.21.

5.1.2. (2*R*,3*S*)-5-(*tert*-Butyldimethylsilanyloxy)-2-vinylpentane-1,3-diol (15). Bu_3B solution (1.0 M in THF, 13.8 mL, 13.8 mmol) was added to a solution of **14** (5.0 g, 11.5 mmol) and AcOH (2.0 mL, 34.5 mmol) in THF (30 mL) at 0 °C under argon atmosphere, and the whole mixture was stirred for 30 min, then cooled to −78 °C. $LiBH_4$ (2.0 M in THF, 11.5 mL, 23.0 mmol) was added dropwise to the reaction mixture and stirred at this temperature for 4 h, then warmed to 0 °C and stirred for 30 min. The reaction mixture was successively treated with H_2O (10 mL), 3N NaOH (10 mL), and 30% H_2O_2 (10 mL) at 0 °C. After being stirred for 15 min, the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 3:1) to give **15** (2.55 g, 88%) as colorless oil.

Compound **15**: $[\alpha]_D^{20}$ +1.3 (*c* 2.30, $CHCl_3$). IR (KBr): 3391, 2930, 2858, 1639, 1471 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 5.87 (ddd, J = 8.5, 10.4, 17.1 Hz, 1H), 5.22 (dd, J = 1.2, 10.4 Hz, 1H), 5.15 (dd, J = 1.2, 17.1 Hz, 1H), 4.10 (dt, J = 2.4, 9.8 Hz, 1H) 3.92 (dt, J = 4.3, 14.0 Hz, 1H), 3.83 (dt, J = 3.7, 10.4 Hz, 1H), 3.73–3.79 (m, 3H), 2.65 (br s, 1H), 2.34 (m, 1H), 1.80 (ddt, J = 4.3, 9.8, 14.6 Hz, 1H), 1.52–1.57 (m, 1H), 0.90 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 135.3, 118.3, 73.8, 64.5, 63.1, 51.2, 35.7, 25.9 (3C), 18.1, −5.5 (2C). ESI-MS: *m*/*z* 283 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 283.1705, calcd for $C_{13}H_{28}O_3SiNa$. Found: 283.1709. Anal. Calcd for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84. Found: C, 59.76; H, 10.52.

5.1.3. (2*R*)-2-[(1*S*)-3-(*tert*-Butyldimethylsilanyloxy)-1-hydroxypropyl]-but-3-enyl toluene-4-sulfonate (16). *p*-TsCl (1.37 g, 7.2 mmol) was added to a solution of **15** (1.80 g, 6.9 mmol) in pyridine (10 mL) at 0 °C. The whole mixture was stirred at this temperature for 30 min, then warmed to rt and stirred for 5 h. The reaction was quenched with saturated aqueous $NaHCO_3$, and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 10:1) to give **16** (2.57 g, 90%) as colorless oil.

Compound **16**: $[\alpha]_D^{20}$ +20 (*c* 1.70, $CHCl_3$). IR (KBr): 3495, 2955, 2858, 1599, 1469 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.79 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.5 Hz, 2H), 5.70 (ddd, J = 8.5, 10.4, 17.7 Hz, 1H), 5.19 (dd, J = 1.2, 10.4 Hz, 1H), 5.12 (dd, J = 1.2, 17.7 Hz, 1H), 4.30 (dd, J = 7.3, 9.8 Hz, 1H), 4.02 (dd, J = 6.7, 9.8 Hz, 1H), 3.99–4.01 (m, 1H), 3.86 (dt, J = 4.3, 9.8 Hz, 1H), 3.78 (dd, J = 3.1, 9.8 Hz, 1H), 3.33 (br s, 1H), 2.44 (s, 3H), 2.40–2.45 (m, 1H), 1.69 (ddt, J = 4.3, 9.8, 14.0 Hz, 1H), 1.43–1.48 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 144.7, 133.1, 133.0, 129.8 (2C), 128.0 (2C), 119.7, 70.4, 70.4, 62.8, 49.3, 36.1, 25.9 (3C), 21.6, 18.1, −5.6 (2C). ESI-MS: *m*/*z* 437 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 437.1794, calcd for $C_{20}H_{34}O_5SiSNa$. Found: 437.1810. Anal. Calcd for $C_{20}H_{34}O_5SiS$: C, 57.93; H, 8.27; S, 7.73. Found: C, 57.68; H, 8.10; S, 7.74.

5.1.4. (3*S*,4*R*)-1-(*tert*-Butyldimethylsilanyloxy)-4-methylhex-5-en-3-ol (8). A solution of **16** (346 mg, 0.83 mmol) in DMSO (1.6 mL) was treated with $NaBH_4$ (63 mg, 1.7 mmol) at rt for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 10:1) to give **8** (148 mg, 73%) as colorless oil.

Compound **8**: $[\alpha]_D^{20}$ +9 (*c* 0.67, $CHCl_3$). IR (KBr): 3506, 2957, 2858, 1464, 1255 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 5.80–5.87 (m, 1H), 5.05–5.08 (m, 2H), 3.89 (dt, J = 4.9, 10.4 Hz, 1H), 3.79–3.85 (m, 1H), 3.66–3.71 (m, 1H), 3.22 (d, J = 1.8 Hz, 1H), 2.21–2.28 (m, 1H), 1.62–1.66 (m, 2H), 1.04 (d, J = 6.7 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 140.8, 115.1, 75.0, 62.8, 44.0, 35.6, 25.9 (3C), 18.2, 15.8, −5.5 (2C). ESI-MS: *m*/*z* 267 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 267.1756, calcd for $C_{13}H_{28}O_2SiNa$. Found: 267.1758. Anal. Calcd for $C_{13}H_{28}O_2Si$: C, 63.87; H, 11.55. Found: C, 63.65; H, 11.29.

5.1.5. (1*S*,2*R*)-1-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-2-methylbut-3-enyl (2*S*)-2-*tert*-butoxycarbonylamino-4-methylpentanoate (17). *N*-Boc-L-leucine (**9**, 1.87 g, 7.5 mmol), DMAP (910 mg, 7.5 mmol), and DCC (2.58 g, 12.5 mmol) were successively added to a solution of **8** (630 mg, 2.5 mmol) in CH_2Cl_2 (10 mL), then the whole mixture was stirred at rt for 2 h. 5% HCl was added to the reaction mixture, then diluted with Et_2O. The whole mixture was filtered to remove the precipitate, then the filtrate was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 10:1) to give **17** (1.15 g, 100%) as colorless oil.

Compound **17**: $[\alpha]_D^{20}$ −26 (*c* 1.49, $CHCl_3$). IR (KBr): 3373, 2959, 2860, 1716, 1501 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 5.69–5.76 (m, 1H), 5.00–5.07 (m, 3H), 4.86 (d, J = 8.5 Hz, 1H), 4.25–4.35 (m, 1H), 3.54–3.65 (m, 2H), 2.42–2.49 (m, 1H), 1.69–1.79 (m, 3H), 1.56–1.62 (m, 1H), 1.42–1.48 (m, 1H), 1.44 (s, 9H), 1.01 (d, J = 7.3 Hz, 3H), 0.95 (d, J = 6.1 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.88 (s, 9H), 0.03 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.0, 155.6, 139.2, 115.8, 79.6, 75.0, 59.5, 52.4, 42.1, 41.5, 34.4, 28.4 (3C), 25.9 (3C), 24.9, 23.0, 21.9, 18.3, 15.8, −5.4 (2C). ESI-MS: *m*/*z* 480 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 480.3121, calcd for $C_{24}H_{47}NO_5SiNa$. Found: 480.3113. Anal. Calcd for $C_{24}H_{47}NO_5Si$: C, 62.98; H, 10.35; N, 3.06. Found: C, 62.93; H, 10.24; N, 3.09.

5.1.6. (1*S*,2*R*)-1-(2-Hydroxyethyl)-2-methylbut-3-enyl (2*S*)-2-*tert*-butoxycarbonylamino-4-methylpentanoate (18). A solution of **17** (500 mg, 1.1 mmol) and AcOH (0.37 mL, 6.6 mmol) in THF (10 mL) was treated with TBAF (1.0 M in THF, 3.3 mL, 3.3 mmol) at 0 °C for 4 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 3:1) to give **18** (374 mg, 100%) as colorless oil.

Compound **18**: $[\alpha]_D^{20}$ −47 (*c* 1.01, $CHCl_3$). IR (KBr): 3368, 2962, 2874, 1711, 1518 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 5.69–5.77 (m, 1H), 5.04–5.09 (m, 3H), 4.86 (d, J = 7.9 Hz, 1H), 4.21–4.26 (m, 1H), 3.60–3.64 (m, 2H), 2.58 (br s, 1H), 2.38-2.45 (m, 1H), 1.80–1.86 (m, 1H), 1.65–1.79 (m, 2H), 1.57–1.62 (m, 1H), 1.47–1.49 (m, 1H), 1.44 (s, 9H), 1.04 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.6, 155.7, 139.1, 116.0, 80.1, 75.0, 58.5, 52.3, 42.1, 41.4, 34.6, 28.3 (3C), 24.9, 22.9, 21.8, 16.3. ESI-MS: *m*/*z* 366 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 366.2256, calcd for $C_{18}H_{33}NO_5SiNa$. Found: 366.2255.

5.1.7. (1*S*,2*R*)-1-(3-{(1*R*)-2-(4-Methoxyphenyl)-1-[2-(2-trimethylsilanylethoxycarbonyl)ethylcarbamoyl]ethylcarbamoyl}allyl)-2-methylbut-3-enyl (2*S*)-2-*tert*-butoxycarbonylamino-4-methylpentanoate (19). A solution of **18** (7.3 mg, 0.021 mmol) in CH_2Cl_2 (1 mL) was treated with Dess–Martin periodinane (14 mg, 0.042 mmol) at rt for 2 h. Then saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ were added to the reaction mixture and stirred for 30 min. The whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude aldehyde **6**.

NaH (2.5 mg, 0.105 mmol) was added to a solution of segment BD (**7**) (22 mg, 0.042 mmol) in THF (1 mL) at −10 °C, then the whole mixture was stirred for 30 min. A solution of the above aldehyde **6** in THF (0.3 mL) was added dropwise to the reaction mixture, then the whole mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; *n*-hexane/EtOAc 2:1) to give **19** (12 mg, 75% in two steps) as yellow oil.

Compound **19**: $[\alpha]_D^{20}$ +3.3 (*c* 0.79, $CHCl_3$). IR (KBr): 3279, 3076, 2957, 1716, 1512 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.11 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 6.72 (dt, J = 6.7, 15.8 Hz, 1H), 6.55 (d, J = 7.3 Hz, 1H), 6.25 (brs, 1H), 5.84 (d, J = 15.8 Hz, 1H), 5.70 (dt, J = 9.2, 17.7 Hz, 1H), 5.04–5.08 (m, 2H), 4.88–4.93 (m, 2H), 4.54–4.59 (m, 1H), 4.19–4.24 (m, 1H), 4.08–4.16 (m, 2H), 3.77 (s, 3H), 3.44–3.49 (m, 1H), 3.32–3.36 (m, 1H), 2.92–3.08 (m, 2H), 2.29–2.46 (m, 5H), 1.69–1.75 (m, 2H), 1.55–1.61 (m, 1H), 1.45 (s, 9H), 1.02 (d, J = 6.7 Hz, 3H), 0.96 (t, J = 7.9 Hz, 2H), 0.93 (m, 6H), 0.02 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.3, 172.1, 170.8, 165.4, 158.6, 155.6, 139.3, 138.7, 130.3 (2C), 128.8, 125.9, 116.4, 114.1 (2C), 80.0, 76.1, 63.0, 55.2, 55.0, 54.8, 41.4, 41.0, 37.6, 34.8, 34.0, 33.5, 28.4 (3C), 24.9, 23.0, 21.8, 17.3, 16.3, −1.5 (3C). ESI-MS: *m*/*z* 754 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 754.4075, calcd for $C_{38}H_{61}N_3O_9SiNa$. Found: 754.4075. Anal. Calcd for $C_{38}H_{61}N_3O_9Si$: C, 62.35; H, 8.40; N, 5.74. Found: C, 62.06; H, 8.35; N, 5.68.

5.1.8. (3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-16-[(1*R*)-1-methylallyl]-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (5). A solution of **19** (16 mg, 0.022 mmol) in CH_2Cl_2 (0.5 mL) was treated with TFA (1 mL) at rt for 3 h. Removal of the solvent from the reaction mixture under reduced pressure gave a TFA salt of **20**. Then the TFA salt was treated with HCl in Et_2O (1 mL, 4×) to furnish a HCl salt. DPPA (7 μL, 0.033 mmol) and $NaHCO_3$ (9.2 mg, 0.11 mmol) were added to a solution of the HCl salt in DMF (0.5 mL) at 0 °C, and the whole mixture was stirred at this temperature for 10 h. The reaction mixture was poured into water, and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by

column chromatography (SiO_2; $CHCl_3$/MeOH 30:1) to give **5** (10 mg, 89%) as a white powder.

Compound **5**: $[\alpha]_D^{20}$ +33 (*c* 0.81, $CHCl_3$). IR (KBr): 3294, 3074, 2957, 1736, 1658 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.08 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 6.7 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.72 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 6.15 (d, J = 8.5 Hz, 1H), 5.64–5.72 (m, 2H), 5.71 (d, J = 14.6 Hz, 1H), 5.05–5.23 (m, 3H), 4.59–4.63 (m, 1H), 4.54 (dd, J = 6.7, 8.5 Hz, 1H), 3.80–3.82 (m, 1H), 3.78 (s, 3H), 3.25–3.30 (m, 1H), 3.12 (dd, J = 4.9, 14.6 Hz, 1H), 2.97 (dd, J = 7.9, 14.6 Hz, 1H), 2.28–2.42 (m, 5H), 1.56–1.65 (m, 1H), 1.47 (t, J = 6.7 Hz, 2H), 1.04 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.6, 170.1, 165.0, 158.8, 142.1, 138.7, 130.1 (2C), 128.2, 124.9, 116.5, 114.3 (2C), 75.9, 55.3, 55.0, 50.6, 42.3, 41.8, 36.0, 35.8, 34.5, 34.2, 24.8, 22.6, 22.0, 16.5. ESI-MS: *m*/*z* 536 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 536.2737, calcd for $C_{28}H_{39}N_3O_6Na$. Found: 536.2741.

5.1.9. (3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-16-{(1*R*)-3-[4-(methoxymethoxymethyl)phenyl]-1-methylallyl}-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (23). Compound **21** (140 mg, 0.50 mmol), $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), PPh_3 (5.2 mg, 0.02 mmol), *n*-Bu_4NCl (83 mg, 0.3 mmol), and $NaHCO_3$ (42 mg, 0.50 mmol) were successively added to a solution of **5** (100 mg, 0.20 mmol) in DMF-H_2O (9:1, 4 mL), then the whole mixture was stirred at 80 °C for 40 h. Water was added to the reaction mixture, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$/MeOH 30:1) and further purified by HPLC (COSMOSIL 5C18-AR-II, MeOH/H_2O 4:1) to give **23** (71 mg, 54%) as a white powder.

Compound **23**: $[\alpha]_D^{20}$ +62 (*c* 0.20, $CHCl_3$). IR (KBr): 3288, 2951, 1736, 1658, 1541 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.27–7.32 (m, 4H), 7.08 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 7.9 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.73 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 6.39 (d, J = 15.9 Hz, 1H), 6.02 (dd, J = 7.3, 15.9 Hz, 1H), 5.99–6.02 (m, 1H), 5.70 (d, J = 14.6 Hz, 1H), 5.59 (d, J = 4.9 Hz, 1H), 5.11–5.14 (m, 1H), 4.68 (s, 2H), 4.59–4.62 (m, 1H), 4.57 (s, 2H), 4.52 (dd, J = 7.3, 8.5 Hz, 1H), 3.78 (s, 3H), 3.75–3.82 (m, 1H), 3.40 (s, 3H), 3.23–3.28 (m, 1H), 3.12 (dd, J = 4.9, 14.0 Hz, 1H), 2.97 (dd, J = 7.9, 14.0 Hz, 1H), 2.49–2.56 (m, 2H), 2.30–2.41 (m, 3H), 1.47–1.55 (m, 1H), 1.36 (t, J = 7.3 Hz, 2H), 1.12 (d, J = 7.3 Hz, 3H), 0.75 (d, J = 6.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.2, 171.6, 170.6, 165.0, 158.8, 141.9, 137.3, 136.3, 131.4, 130.3, 130.1 (2C), 128.2 (3C), 126.2 (2C), 125.0, 114.3 (2C), 95.6, 76.1, 68.8, 55.4, 55.3, 55.0, 50.7, 42.2, 41.7, 36.4, 35.8, 34.5, 34.1, 24.7, 22.3, 21.8, 17.1. ESI-MS: *m*/*z* 686 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 686.3417, calcd for $C_{37}H_{49}N_3O_8Na$. Found: 686.3424.

5.1.10. (3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-16-(1*S*)-1-[(2*R*,3*R*)-{3-[4-(methoxymethoxymethyl)phenyl]oxiranyl}ethyl]-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (24). A solution of **23** (5.0 mg, 7.5 μmol) in CH_2Cl_2–MeOH (3:1, 0.3 mL) was treated with dimethyldioxirane (0.074 M in acetone, 0.3 mL) at −20 °C for 2 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by HPLC (COSMOSIL 5C18-AR-II, MeOH/H_2O 65:35) to give **24** (2.7 mg, 53%) as a white powder.

Compound **24**: $[\alpha]_D^{20}$ +47 (*c* 0.23, $CHCl_3$). IR (KBr): 3288, 2928, 1738, 1660, 1543 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (d, J = 7.9 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.71 (ddd, J = 4.0, 11.0, 14.6 Hz, 1H), 5.91 (d, J = 9.2 Hz, 1H), 5.67 (d, J = 14.6 Hz, 1H), 5.52 (d, J = 6.1 Hz, 1H), 5.26–5.29 (m, 1H), 4.71 (s, 2H), 4.60 (s, 2H), 4.57–4.59 (m, 1H), 4.50 (dd, J = 5.5, 9.8 Hz, 1H), 3.79 (s, 3H), 3.75–3.82 (m, 1H), 3.69 (d, J = 1.2 Hz, 1H), 3.42 (s, 3H), 3.21–3.26 (m, 1H), 3.13 (dd, J = 4.9, 14.6 Hz, 1H), 2.95 (dd, J = 7.9, 14.6 Hz, 1H), 2.91 (dd, J = 1.2, 7.3 Hz, 1H), 2.52–2.55 (m, 1H), 2.30–2.43 (m, 3H), 1.77–1.83 (m, 1H), 1.53–1.61 (m, 1H), 1.37–1.42 (m, 1H), 1.28–1.32 (m, 1H), 1.14 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.1 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.7, 170.6, 164.8, 158.8, 141.4, 138.4, 136.7, 130.4 (2C), 128.2 (2C), 128.0, 125.8 (2C), 125.1, 114.4 (2C), 95.7, 75.0, 68.7, 63.1, 58.8, 55.4, 55.3, 55.1, 50.5, 41.4, 40.5, 36.6, 35.8, 34.4, 34.0, 24.7, 22.6, 21.6, 13.3. ESI-MS: *m*/*z* 702 $(M + Na)^+$. HR-ESI-MS: *m*/*z* 702.3367, calcd for $C_{37}H_{49}N_3O_9Na$. Found: 702.3342.

5.1.11. (3*S*,10*R*,16*S*)-16-{(1*R*)-3-[4-(Hydroxymethyl)-phenyl]-1-methylallyl}-3-isobutyl-10-(4-methoxybenzyl)-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (25). A solution of **23** (10 mg, 15 μmol) in MeOH (0.5 mL) was treated with 6 N HCl (0.3 mL) at 0 °C for 3 h. Saturated aqueous $NaHCO_3$ was added to the reaction mixture, then the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$/MeOH 20:1) to give **25** (6.6 mg, 72%) as a white powder.

Compound **25**: $[\alpha]_D^{20}$ +87 (*c* 0.20, $CHCl_3$/MeOH = 10:1). IR (KBr): 3452, 3285, 2959, 1732, 1660, 1628, 1539, 1512 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.29–7.34 (m, 4H), 7.08 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 6.7 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.72 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 6.40 (d, J = 15.9 Hz, 1H), 6.01 (dd, J = 8.5, 15.9 Hz, 1H), 5.83 (d, J = 8.5 Hz, 1H), 5.70 (d, J = 14.6 Hz, 1H), 5.49 (d, J = 6.7 Hz, 1H), 5.13–5.16 (m, 1H), 4.68 (s, 2H), 4.59–4.62 (m, 1H), 4.52 (dd, J = 7.3, 8.5 Hz, 1H), 3.78 (s, 3H), 3.79–3.83 (m, 1H), 3.49 (s, 1H), 3.22–3.27 (m, 1H), 3.12 (dd, J = 4.9, 14.6 Hz, 1H), 2.97 (dd, J = 7.9, 14.6 Hz, 1H), 2.50–2.56 (m, 2H), 2.31–2.38 (m, 3H), 1.48–1.55 (m, 1H),

1.36 (t, J = 7.3 Hz, 2H), 1.13 (d, J = 7.3 Hz, 3H), 0.76 (d, J = 6.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.6, 170.6, 164.9, 158.8, 141.9, 140.2, 136.3, 131.4, 130.3, 130.1 (2C), 128.1, 127.3 (2C), 126.4 (2C), 125.0, 114.3 (2C), 76.1, 65.1, 55.3, 55.0, 50.6, 42.2, 41.7, 36.4, 35.8, 34.5, 34.1, 24.7, 22.3, 21.8, 17.2. ESI-MS: m/z 642 $(M + Na)^+$. HR-ESI-MS: m/z 642.3155, calcd for $C_{35}H_{45}N_3O_7Na$. Found: 642.3146.

5.1.12. 4-((2*R*,3*R*)-3-{(1*S*)-1-[(3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-2,5,9,12-tetraoxo-1-oxa-4,8,11-triazacyclohexadec-13-en-16-yl]ethyl}oxiranyl)benzyl *tert*-butoxycarbonylaminoacetate (27). *N*-Boc-glycine (6.0 mg, 0.03 mmol), DMAP (0.9 mg, 8 μmol), and DCC (6.6 mg, 0.03 mmol) were successively added to a solution of **25** (9.0 mg, 14 μmol) in CH_2Cl_2 (0.14 mL), and the whole mixture was stirred at rt for 3 h. After filtration through a Celite pad with Et_2O as eluent, removal of the solvent from the filtrate under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$/MeOH 20:1) to give glycine ester **26** (11 mg, 100%) as a white powder.

Compound **26**: $[\alpha]_D^{20}$ +42 (c 0.64, $CHCl_3$). IR (KBr): 3290, 2957, 1736, 1660, 1628, 1541, 1514 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28–7.31 (m, 5H), 7.07 (d, J = 8.5 Hz, 2H), 7.05–7.07 (m, 1H), 6.81 (d, J = 8.5 Hz, 2H), 6.73 (ddd, J = 3.7, 11.6, 14.6 Hz, 1H), 6.38 (d, J = 15.9 Hz, 1H), 6.34–6.37 (m, 1H), 6.03 (dd, J = 8.5, 15.9 Hz, 1H), 5.80 (br s, 1H), 5.72 (d, J = 14.6 Hz, 1H), 5.13 (s, 2H), 5.11–5.29 (m, 1H), 5.00–5.05 (m, 1H), 4.58–4.62 (m, 1H), 4.48–4.53 (m, 1H), 3.93 (d, J = 4.9 Hz, 2H), 3.78 (s, 3H), 3.75–3.83 (m, 1H), 3.22–3.27 (m, 1H), 3.11 (dd, J = 4.9, 14.6 Hz, 1H), 2.97 (dd, J = 7.9, 14.6 Hz, 1H), 2.49–2.56 (m, 2H), 2.29–2.41 (m, 3H), 1.48–1.53 (m, 1H), 1.43 (s, 9H), 1.29–1.39 (m, 1H), 1.12 (d, J = 6.7 Hz, 3H), 0.73 (d, J = 5.5 Hz, 3H), 0.72 (d, J = 4.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.2, 171.8, 170.8, 170.3, 165.1, 158.7, 155.7, 142.0, 137.1, 134.4, 131.1, 131.0, 130.1 (2C), 128.8 (2C), 128.1, 126.4 (2C), 124.9, 114.2 (2C), 80.1, 76.0, 66.8, 55.2, 55.1, 50.6, 42.5, 42.2, 41.4, 36.3, 35.8, 34.3, 34.2, 28.3 (3C), 24.6, 22.3, 21.7, 17.1. ESI-MS: m/z 799 $(M + Na)^+$. HR-ESI-MS: m/z 799.3894, calcd for $C_{42}H_{56}N_4O_{10}Na$. Found: 799.3909.

A solution of the compound **26** (11 mg, 16 μmol) in CH_2Cl_2 (0.5 mL) was treated with dimethyldioxirane (0.074 M in acetone, 0.5 mL) at 0 °C for 3 h. Removal of the solvent from the reaction mixture gave a crude product, which was purified by HPLC (COSMOSIL 5C18-MS-II, CH_3CN/H_2O 3:2) to give **27** (6.1 mg, 48%) as a white powder.

Compound **27**: $[\alpha]_D^{20}$ +54 (c 0.51, $CHCl_3$). IR (KBr): 3294, 2961, 2932, 1738, 1664, 1514 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35 (d, J = 7.9 Hz, 2H), 7.23 (d, J = 7.9 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.70 (ddd, J = 4.0, 11.6, 14.6 Hz, 1H), 5.88 (d, J = 9.2 Hz, 1H), 5.67 (d, J = 14.6 Hz, 1H), 5.55 (d, J = 6.1 Hz, 1H), 5.26–5.29 (m, 1H), 5.18 (s, 2H), 5.02 (br s, 1H), 4.57–4.61 (m, 1H), 4.47–4.51 (dt, J = 5.5, 9.2 Hz, 1H), 3.96 (d, J = 5.5 Hz, 2H), 3.79 (s, 3H), 3.75–3.83 (m, 1H), 3.66 (d, J = 1.8 Hz, 1H), 3.21–3.26 (m, 1H), 3.13 (dd, J = 4.9, 14.6 Hz, 1H), 2.95 (dd, J = 7.9, 14.6 Hz, 1H), 2.94 (dd, J = 1.8, 7.3 Hz, 1H), 2.52–2.55 (m, 1H), 2.31–2.43 (m, 3H), 1.77–1.81 (m, 1H), 1.48–1.53 (m, 1H), 1.43 (s, 9H), 1.36–1.39 (m, 1H), 1.28–1.31 (m, 1H), 1.14 (d, J = 7.3 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.7, 170.6, 170.2, 164.9, 158.8, 156.1, 141.3, 137.2, 135.7, 130.1 (2C), 128.8 (2C), 128.1, 126.0 (2C), 125.2, 114.4 (2C), 81.0, 75.0, 66.6, 63.2, 58.7, 55.3, 55.1, 50.5, 42.5, 41.4, 40.5, 36.6, 35.8, 34.4, 34.1, 28.3 (3C), 24.7, 22.6, 21.6, 13.3. ESI-MS: m/z 815 $(M + Na)^+$. HR-ESI MS: m/z 815.3843, calcd for $C_{42}H_{56}N_4O_{11}Na$. Found: 815.3859.

5.1.13. (3*S*,10*R*,16*S*)-16-{(1*R*)-3-[4-(Chloromethyl)phenyl]-1-methylallyl}-3-isobutyl-10-(4-methoxybenzyl)-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (28). 2,4,6-Collidine (3 μL, 0.024 mmol) and MsCl (1 μL, 0.015 mmol) were added to a solution of **25** (6.0 mg, 9.6 μmol) in DMF (0.5 mL), then the whole mixture was stirred for 15 min under argon atmosphere. LiCl (4.9 mg, 0.115 mmol) was added to the reaction mixture, and the whole mixture was further stirred for 6 h. The reaction was quenched with 5% HCl, and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$/MeOH 20:1) to give **28** (5.7 mg, 93%) as a white powder.

Compound **28**: $[\alpha]_D^{20}$ +62 (c 0.22, $CHCl_3$/MeOH 1:1). IR (KBr): 3290, 2961, 2928, 1736, 1658, 1637, 1545, 1514 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.29–7.35 (m, 4H), 7.08 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 6.7 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.72 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 6.39 (d, J = 15.9 Hz, 1H), 6.03 (dd, J = 8.5, 15.9 Hz, 1H), 5.90 (m, 1H), 5.70 (d, J = 14.6 Hz, 1H), 5.54 (m, 1H), 5.11–5.16 (m, 1H), 4.59–4.61 (m, 1H), 4.56 (s, 2H), 4.50–4.53 (m, 1H), 3.78 (s, 3H), 3.77–3.83 (m, 1H), 3.22–3.27 (m, 1H), 3.13 (dd, J = 4.9, 14.6 Hz, 1H), 2.98 (dd, J = 7.9, 14.6 Hz, 1H), 2.50–2.57 (m, 2H), 2.33–2.41 (m, 3H), 1.47–1.52 (m, 1H), 1.32–1.36 (m, 2H), 1.13 (d, J = 6.7 Hz, 3H), 0.75 (d, J = 6.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.2, 171.6, 170.7, 165.0, 158.8, 141.9, 137.0, 136.7, 131.1, 131.1, 130.1 (2C), 128.8 (2C), 128.0, 126.5 (2C), 125.0, 114.4 (2C), 76.1, 55.3, 55.0, 50.7, 46.0, 42.2, 41.6, 36.4, 35.8, 34.5, 34.2, 24.7, 22.3, 21.8, 17.2. ESI-MS: m/z 660 $(M + Na)^+$. HR-ESI-MS: m/z 660.2816, calcd for $C_{35}H_{44}ClN_3O_6Na$. Found: 660.2813.

5.1.14. (3*S*,10*R*,16*S*)-16-((1*S*)-1-{(2*R*,3*R*)-3-[4-(Chloromethyl)phenyl]oxiranyl}ethyl)-3-isobutyl-10-(4-methoxybenzyl)-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (29). A solution of **28** (80 mg, 0.12 mmol) in CH_2Cl_2–MeOH (1:1, 2 mL) was treated with dimethyldioxirane (0.074 M in acetone, 1 mL) at −30 °C for 5 h. Removal of the solvent from the reaction mix-

ture under reduced pressure gave a crude product, which was purified by HPLC (COSMOSIL 5C18-MS-II, MeOH/H_2O 7:3) to give **29** (41 mg, 50%) as a white powder.

Compound **29**: $[\alpha]_D^{20}$ +51 (*c* 0.17, $CHCl_3$/MeOH 1:1). IR (KBr): 3290, 2955, 1738, 1660, 1637, 1541, 1514 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.39 (d, J = 7.9 Hz, 2H), 7.23 (d, J = 7.9 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 6.7 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.70 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 5.80 (d, J = 9.2 Hz, 1H), 5.67 (d, J = 14.6 Hz, 1H), 5.49 (d, J = 6.7 Hz, 1H), 5.26–5.29 (m, 1H), 4.59 (s, 2H), 4.57–4.61 (m, 1H), 4.50 (dt, J = 5.4, 9.2 Hz, 1H), 3.79 (s, 3H), 3.77–3.83 (m, 1H), 3.68 (d, J = 1.8 Hz, 1H), 3.21–3.26 (m, 1H), 3.13 (dd, J = 4.9, 14.6 Hz, 1H), 2.95 (dd, J = 7.9, 14.6 Hz, 1H), 2.90 (dd, J = 1.8, 7.3 Hz, 1H), 2.53–2.55 (m, 1H), 2.31–2.43 (m, 3H), 1.77–1.81 (m, 1H), 1.47–1.52 (m, 1H), 1.36–1.42 (m, 1H), 1.27–1.32 (m, 1H), 1.14 (d, J = 7.3 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.7, 170.5, 164.8, 157.3, 141.3, 137.9, 137.1, 130.0 (2C), 129.0 (2C), 128.0, 126.1 (2C), 125.2, 114.4 (2C), 74.9, 63.2, 58.6, 55.3, 55.0, 50.4, 45.8, 41.4, 40.4, 36.6, 35.8, 34.4, 34.0, 24.7, 22.6, 21.6, 13.3. ESI-MS: *m/z* 676 $(M + Na)^+$. HR-ESI-MS: *m/z* 676.2765, calcd for $C_{35}H_{44}ClN_3O_7Na$. Found: 676.2766.

5.1.15. (3*S*,10*R*,16*S*)-16-((1*S*)-1-{(2*R*,3*R*)-3-[4-(Diethylaminomethyl)phenyl]oxiranyl}ethyl)-3-isobutyl-10-(4-methoxybenzyl)-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (30). A solution of **29** (3.2 mg, 4.8 μmol) in DMF (1 mL) was treated with diethylamine (25 μL, excess) at rt for 3 h. Removal of the solvent from the reaction mixture gave a crude product, which was purified by column chromatography (SiO_2; $CHCl_3$/MeOH 10:1) to give **30** (2.8 mg, 85%) as a white powder.

Compound **30**: $[\alpha]_D^{20}$ +33 (*c* 0.24, $CHCl_3$). IR (KBr): 3292, 2966, 2928, 1738, 1660, 1631, 1539 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35 (d, J = 7.9 Hz, 2H), 7.18 (d, J = 7.9 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 7.9 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.71 (ddd, J = 3.6, 11.0, 14.6 Hz, 1H), 5.94 (d, J = 9.2 Hz, 1H), 5.68 (d, J = 14.6 Hz, 1H), 5.56 (d, J = 6.7 Hz, 1H), 5.26–5.30 (m, 1H), 4.59 (m, 1H), 4.49 (dd, J = 5.5, 9.2 Hz, 1H), 3.78 (s, 3H), 3.75–3.83 (m, 1H), 3.66 (d, J = 1.8 Hz, 1H), 3.60 (s, 3H), 3.19-3.25 (m, 1H), 3.13 (dd, J = 4.9, 14.6 Hz, 1H), 2.96 (dd, J = 7.9, 14.6 Hz, 1H), 2.92 (dd, J = 1.8, 6.7 Hz, 1H), 2.52–2.57 (m, 5H), 2.31–2.45 (m, 3H), 1.74–1.81 (m, 1H), 1.52–1.61 (m, 1H), 1.37–1.43 (m, 1H), 1.29–1.34 (m, 1H), 1.13 (d, J = 6.7 Hz, 3H), 1.08 (t, J = 6.7 Hz, 6H), 0.86 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 172.0, 171.0, 165.1, 158.6, 141.0, 139.0, 131.6 (2C), 130.1 (2C), 128.8 (2C), 128.4, 128.2, 126.5 (2C), 125.3, 114.2 (2C), 77.2 (2C), 74.9, 63.5, 58.3, 55.5, 55.3, 55.2, 50.6, 45.9 (2C), 41.3, 40.3, 36.6, 35.7, 34.3, 34.1, 24.8, 22.7, 21.8, 13.5, 8.6 (2C). ESI-MS: *m/z* 691 $(M + H)^+$. HR-ESI-MS: *m/z* 691.4071, calcd for $C_{39}H_{55}N_4O_7$. Found: 691.4099.

5.1.16. (3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-16-((1*S*)-1-{(2*R*,3*R*)-3-[4-(piperazin-1-ylmethyl)phenyl]oxiranyl}ethyl)-1-oxa-4,8,11-triazacyclohexadec-13-ene-2,5,9,12-tetraone (31). A solution of **29** (4.3 mg, 7.9 μmol) in DMF (0.5 mL) was treated with piperadine (50 μL, excess) at rt for 12 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (Chromatorex®-NH_2; $CH_3CN \rightarrow$ MeOH) to give **31** (3.7 mg, 80%) as a white powder.

Compound **31**: $[\alpha]_D^{20}$ +43 (*c* 0.22, $CHCl_3$). IR (KBr): 3287, 2949, 1738, 1660, 1537, 1514 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.32 (d, J = 7.9 Hz, 2H), 7.17 (d, J = 7.9 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 6.7 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 6.71 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 5.88 (d, J = 9.2 Hz, 1H), 5.67 (d, J = 14.6 Hz, 1H), 5.49 (d, J = 6.7 Hz, 1H), 5.26–5.30 (m, 1H), 4.57–4.61 (m, 1H), 4.50 (dt, J = 5.4, 9.2 Hz, 1H), 3.79 (s, 3H), 3.77–3.83 (m, 1H), 3.66 (d, J = 1.8 Hz, 1H), 3.49 (s, 2H), 3.20–3.25 (m, 1H), 3.12 (dd, J = 4.9, 14.6 Hz, 1H), 2.96 (dd, J = 8.5, 14.6 Hz, 1H), 2.92 (dd, J = 1.8, 6.7 Hz, 1H), 2.90 (t, J = 4.9 Hz, 1H), 2.52–2.55 (m, 1H), 2.30–2.45 (m, 7H), 1.75–1.79 (m, 1H), 1.53–1.61 (m, 1H), 1.29–1.43 (m, 2H), 1.14 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.8, 170.5, 164.8, 158.8, 141.5, 138.8, 135.4, 130.1 (2C), 129.5 (2C), 128.0, 125.6 (2C), 125.1, 114.4 (2C), 75.0, 63.4, 63.1, 59.0, 55.3, 55.0, 54.6 (2C), 50.5, 46.2 (2C), 41.4, 40.6, 36.6, 35.8, 34.4, 34.0, 24.7, 22.6, 21.6, 13.4. ESI-MS: *m/z* 704 $(M)^+$. HR-ESI-MS: *m/z* 704.4023, calcd for $C_{39}H_{54}N_5O_7$. Found: 704.4015.

5.1.17. Triethyl-[4-((2*R*,3*R*)-3-{(1*S*)-1-[(3*S*,10*R*,16*S*)-3-isobutyl-10-(4-methoxybenzyl)-2,5,9,12-tetraoxo-1-oxa-4,8,11-triazacyclohexadec-13-en-16-yl]ethyl}oxiranyl) benzyl]ammonium chloride (32). A solution of **29** (5.2 mg, 7.9 μmol) in DMF (1 mL) was treated with triethylamine (50 μL, excess) at rt for 24 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (Chromatorex®-NH_2; $CH_3CN \rightarrow$ MeOH) to give **32** (4.8 mg, 86%) as a white powder.

Compound **32**: $[\alpha]_D^{20}$ +32 (*c* 0.34, $CHCl_3$). IR (KBr): 3391, 3172, 2957, 2916, 1738, 1657, 1514, 1467 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.61 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 7.21 (br s, 1H), 7.14 (d, J = 8.5 Hz, 2H), 7.03–7.12 (m, 1H), 6.78 (d, J = 8.5 Hz, 2H), 6.63 (ddd, J = 3.7, 10.4, 14.6 Hz, 1H), 6.55–6.66 (m, 1H), 5.85 (d, J = 14.6 Hz, 1H), 5.26 (br d, J = 11.0 Hz, 1H), 4.78 (ABq, J = 14.0 Hz, 2H), 4.56 (m, 1H), 4.46 (m, 1H), 3.79 (s, 3H), 3.75–3.82 (m, 1H), 3.73 (d, J = 1.8 Hz, 1H), 3.42 (d-like, J = 6.7 Hz, 6H), 3.18–3.26 (m, 1H), 3.11 (dd, J = 4.9, 14.6 Hz, 1H), 2.97 (dd, J = 8.5, 14.6 Hz, 1H), 2.87 (dd, J = 1.8, 7.9 Hz, 1H), 2.29–2.52 (m, 4H), 1.74–1.78 (m, 1H), 1.55–1.63 (m, 1H), 1.50 (t, J = 6.7 Hz, 9H), 1.40–1.45 (m, 1H), 1.28–1.33 (m, 1H), 1.15 (d, J = 7.3 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.3, 172.0, 170.7, 165.6,

158.7, 140.3, 140.2, 133.3 (2C), 131.0, 130.4 (2C), 127.4, 126.8 (2C), 126.3, 114.3 (2C), 75.1, 64.0, 61.2, 58.6, 56.0, 55.5, 53.3 (3C), 51.1, 41.8, 40.6, 37.0, 35.9, 35.0, 34.7, 25.1, 22.9, 22.3, 14.0, 8.6 (3C). ESI-MS: *m/z* 719 $(M)^+$. HR-ESI-MS: *m/z* 719.4384, calcd for $C_{41}H_{59}N_4O_7$. Found: 719.4404.

5.1.18. 1-[4-(2*R*,3*R*)-3-{(1*S*)-1-[(3*S*,10*R*,16*S*)-3-Isobutyl-10-(4-methoxybenzyl)-2,5,9,12-tetraoxo-1-oxa-4,8,11-triazacyclohexadec-13-en-16-yl]ethyl}oxiranyl]benzyl]-4-aza-1-azoniabicyclo[2.2.2]octane (33). A solution of **29** (5.0 mg, 7.9 μmol) in DMF (0.5 mL) was treated with DABCO (50 μL, excess) at rt for 24 h. Removal of the solvent from the reaction mixture under reduced pressure gave a crude product, which was purified by column chromatography (Chromatorex®-NH_2; $CH_3CN \rightarrow$ MeOH) to give **33** (4.9 mg, 85%) as a white powder.

Compound **33**: $[\alpha]_D^{20}$ +26 (*c* 0.35, $CHCl_3$). IR (KBr): 3402, 3285, 3252, 2959, 2928, 1738, 1668, 1537, 1514 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.62 (d, J = 7.9 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.17 (br s, 1H), 6.79 (d, J = 8.5 Hz, 2H), 6.67 (ddd, J = 3.7, 11.0, 14.6 Hz, 1H), 6.62–6.79 (m, 1H), 6.56 (m, 1H), 5.80 (d, J = 14.6 Hz, 1H), 5.27 (br d, J = 11.0 Hz, 1H), 4.99 (ABq, J = 12.8 Hz, 2H), 4.56–4.60 (m, 1H), 4.45 (dt, J = 5.4, 9.2 Hz, 1H), 3.76 (s, 3H), 3.73–3.78 (m, 1H), 3.70 (s, 1H), 3.18-3.25 (m, 7H), 3.10 (dd, J = 4.9, 14.6 Hz, 1H), 3.01 (dd, J = 8.5, 14.6 Hz, 1H), 2.86 (d, J = 6.7 Hz, 1H), 2.50–2.55 (m, 1H), 2.33–2.46 (m, 9H), 1.75–1.81 (m, 1H), 1.58–1.62 (m, 1H), 1.49–1.55 (m, 1H), 1.38–1.44 (m, 1H), 1.14 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 171.9, 171.0, 165.2, 158.6, 140.9, 139.9, 133.9 (2C), 130.2 (2C), 128.0, 126.6, 126.4 (2C), 125.4, 114.2 (2C), 74.9, 67.2, 63.6, 58.1, 55.5, 55.3, 52.2 (3C), 50.7, 45.5 (3C), 41.4, 40.3, 36.7, 35.7, 34.5, 34.2, 24.8, 22.7, 21.9, 13.5. ESI-MS: *m/z* 730 $(M)^+$. HR-ESI-MS: *m/z* 730.4180, calcd for $C_{41}H_{56}N_5O_7$. Found: 730.4206.

5.2. Solubility test in MeOH

A saturated solution of the test compounds in MeOH was sonicated at 25 °C for 10 min, then filtered through the filter (pore size = 0.45 μm, Millex-FH, Millipore). The solubility was determined through analyzing UV (230 nm) absorption of the filtrate, by calculating from the value of ε = 12,500, a molar absorbance coefficient of arenastatin A (**1**) at 230 nm.

5.3. In vitro biological evaluation

KB 3-1 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, kanamycin (50 μg/mL), and L-glutamine (4 μM). Cells were plated into 96-well microplates at 5×10^3 cells/100 μL assay medium/well, and various concentration of test compounds were added to each well as 1 μL DMSO solution. The plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 72 h, and cell proliferation was determined by MTT colorimetric assay.

5.4. In vivo anti-tumor experiment

Murine sarcoma cells, S180 (2×10^5 cells/body), were implanted subcutaneously into the right ventral flank of ddY mice (5 weeks old). Three days after implantation, analogue **30** or doxorubicin was administered at various doses on every other day (total 6 times), by ip injection as a suspension in 1% CMC. Tumor diameter was measured with calipers. Tumor size was evaluated by the larger diameter.

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References and notes

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12. The stereochemistry of the epoxy moieties of **24**, **27**, and **29** was established by comparison of the proton chemical shifts due to the 8-H and 6-Me groups from the same behavior observed between arenastatin A (**1**, 8-H: δ 3.68, 6-Me: δ 1.14) and the α-epoxy isomer of **1** (δ 3.59, 1.05). Namely, an obvious difference in chemical shifts of the 8-H and 6-Me groups was observed; the signals of **24**, **27**, and **29** appeared in lower field (**24**: δ 3.69, 1.14; **27**: δ 3.66, 1.14; **29**: δ 3.68, 1.14) than those of the α-isomers (**24′**: δ 3.59, 1.04; **27′**: δ 3.59, 1.04; **29′**: δ 3.59, 1.04).
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