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DIHYDROAZAMACROSPHELIDES: SYNTHESIS AND APOPTOSIS INDUCING ACTIVITIES

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Abstract – Dihydroazamacrosphelides, which were aza-analogues of 3-epi macrosphelide L, were prepared according to ring-closing metathesis strategy. Their biological assay revealed that the oxidized diketo-analogues showed stronger apoptosis inducing activity than their dihydroxy precusors.

INTRODUCTION

Macrosphelides are well known 16-membered macrolides having potent biological activities such as a dose-dependent inhibition against the adhesion of HL-60 to human-umbilical-vein endothelial cells (HUVECs) without an affection on various mammalian cell lines nor microorgasms in vitro, and an immunosuppressant activity equal to that of rapamycin.[1] Considering those novel biological profiles as a new lead for pharmaceuticals, macrosphelides have kindled much interest of organic chemists as attractive synthetic targets and numerous creative total syntheses of natural congeners have been established[2–9] since the pioneering work by Omura and Smith.[2] We are also interested in their potency as an antitumor agent and developed total syntheses of macrosphelides A (**1**), B (**2**), E (**7**), I (**4**), and L (**5**) utilizing ring-closing metathesis (RCM) strategy.[7] Furthermore, based on the RCM strategy, we furnished natural-type and 'non-natural' macrosphelides to reveal that the diketomacrosphelide **3**, oxidized derivative of macrosphelides A (**1**) and B (**2**), could induce apoptotic cell death more effectively than their parent compounds. Notably, among the macrosphelide analogues we reported, 12,13-dihydro-diketomacrosphelide **6** derived from macrosphelides I (**4**) and L (**5**) exhibited comparable potency with **3**.[7c,10] On the other hand, novel aza-macrosphelides (azaMSs), which were designed by the

replacement of the lactone oxygen with nitrogen, were found to be a potential apoptosis-inducer and *N*-phenylethyl 10-aza congener (**8**; R = phenylethyl, X = Y = O) demonstrated the best biological property.[10g] With these findings, next we focused our attention on the aza-analogues of dihydromacrosphelides, 12,13-dihydro-10-azaMSs **9** and herein we describe the syntheses of such derivatives and their biological activities toward human lymphoma cell (U937) by the measurement of early apoptosis and secondary necrosis.

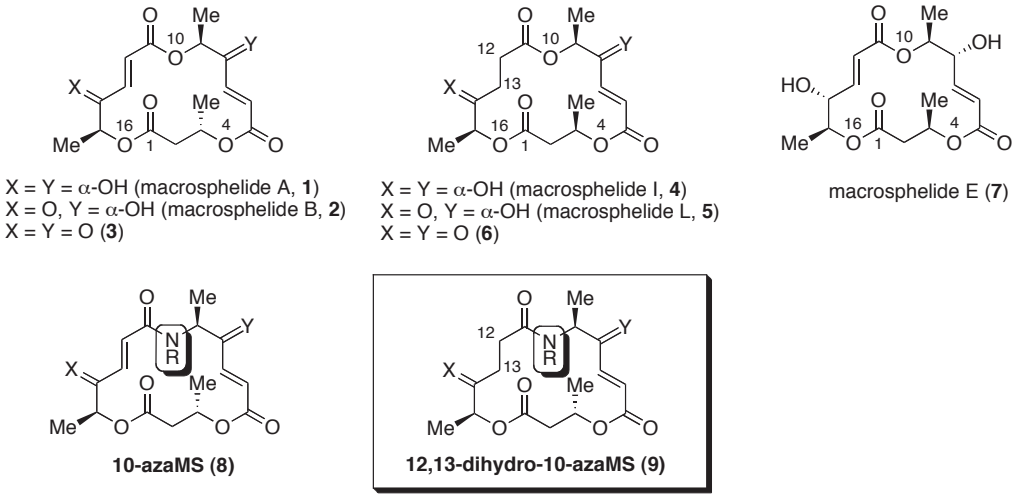
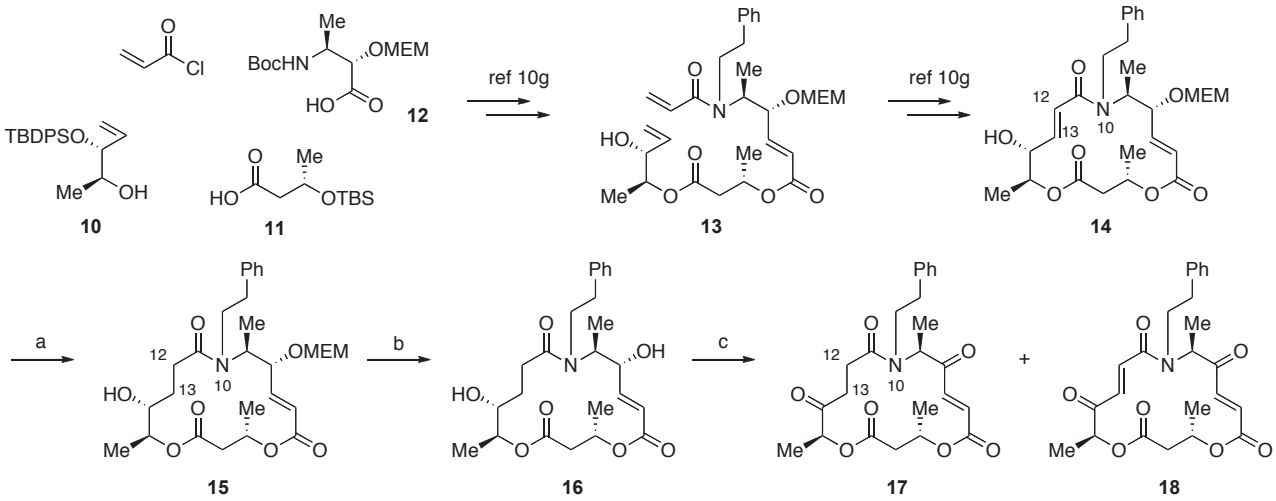


Figure 1. Natural and 'non-natural' macrosphelides

RESULTS AND DISCUSSION

Considering our previous result, we started the syntheses of *N*-phenylethyl-aza-analogues of dihydromacrosphelides as shown in Scheme 1.



Scheme 1. Preparation of *N*-phenylethyl-12,13-dihydro-10-azaMSs. Reagents and conditions: a) H_2 (1 atm), Rh/Al_2O_3, EtOH, rt, 1 h, quant.; b) TFA, CH_2Cl_2, rt, 4 h, 29%; c) Dess-Martin periodinane, CH_2Cl_2, rt, 1 h, 56% (**17**) and 31% (**18**)

According to our preceding synthesis of aza-MSs,[10g] RCM precursor **13** was assembled from the corresponding homoallyl alcohol **10**, β-hydroxycarboxylic acid **11**, β-amino acid derivative **12**, and acryloyl chloride, and then RCM with Grubbs' catalyst second generation successfully afforded the macrolactam **14**. Catalytic hydrogenation with Rh/Al_2O_3 selectively reduced the olefin adjacent to the free hydroxyl group to furnish the desired MEM-protected 12,13-dihydro-10-azaMS **15** quantitatively. The conformational analysis of **14** with Monte Carlo simulation well rationalized the observed chemoselectivity as that the outer periferal face of the olefin adjacent to the MEM ether was hindered with the MEM group to be prevented from the approach of the reagents (Figure 2). Acidic deacetalization[11] followed by Dess-Martin oxidation established the corresponding diketo-10-azaMS **17** with further oxidized **18**.[10g]

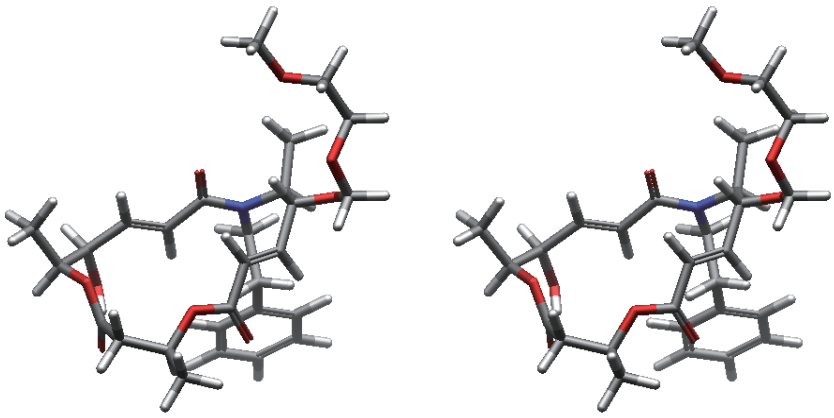
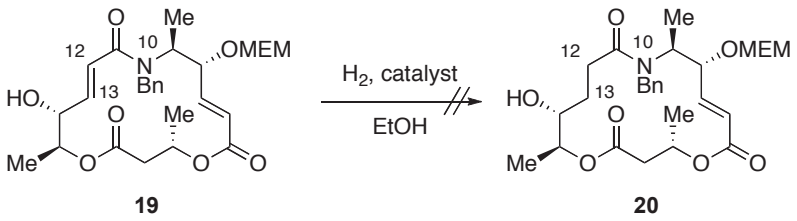


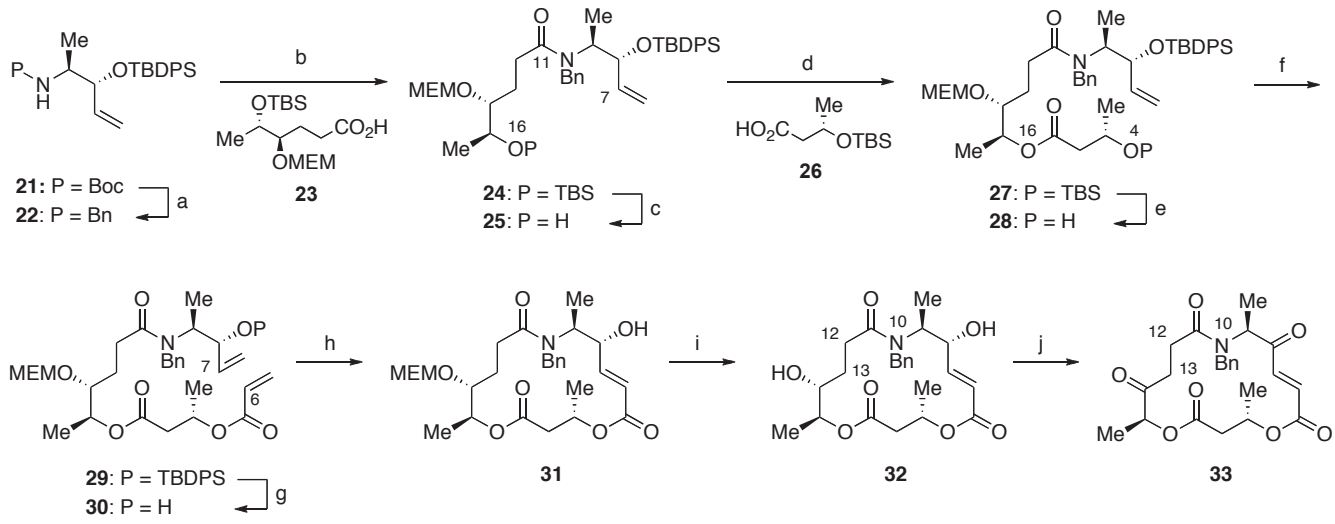
Figure 2. Stereoview of a stable conformer of **14** obtained by Monte Carlo simulation. Condition: OPLS2005 in octanol

Next, we made an attempt on the preparation of the *N*-benzyl-12,13-dihydro-10-azaMSs under similar way. The MEM ether **19**[10g] was exposed to several typical hydrogenation conditions, however, desired dihydro-derivative was not observed at all (Scheme 2).



Scheme 2. Attempt on the hydrogenation of the N-benzyl-azaMS **19**

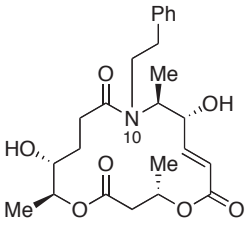
Because the ^{1}H NMR analysis on the resultant crude mixture indicated that debenzylation of the substrate was inevitable under the conditions for hydrogenation of this olefin, we gave up this late stage hydrogenation and turned our attention on the alternative approach using the carboxylic acid **23**[7c] for the saturated C11–O16 fragment as depicted in Scheme 3. The synthesis commenced with the protected allyl alcohol **21**,[10g] an intermediate of formerly reported our macrosphelides syntheses. The protecting group on the nitrogen was switched to benzyl group then dehydrative condensation of resulting amine **22** with the carboxylic acid **23**[7c] furnished the C7–O16 segment **24**. After twice of the selective desilylation and esterification sequence, the corresponding RCM precursor **30** was uneventfully prepared. In the presence of Grubbs' catalyst second generation, the requisite macrolactam **31** was smoothly constructed with satisfactory yield. Acidic deacetalization afforded *N*-benzyl-12,13-dihydro-azaMS **32** then finally Dess-Martin oxidation established desired diketone **33**.



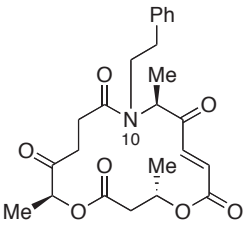
Scheme 3. Preparation of *N*-benzyl-10-aza-12,13-dihydromacrosphelides. Reagents and conditions: a) TFA, CH_2Cl_2, 0 °C, 15 min then benzaldehyde, 2-picoline·BH_3, MeOH/AcOH (10:1), rt, 1 h, 78%; b) **23**, EDC·HCl, DMAP, CH_2Cl_2, rt, 1 day, 99%; c) AcOH/H_2O/THF (3:1:1), 60 °C, 12 h, 71%; d) **26**, 2,4,6-trichlorobenzoyl chloride, NEt_3, toluene, 0 °C, 0.5 h then **25**, DMAP, rt, 1 h, 90%; e) AcOH/H_2O/THF (3:1:1), 50 °C, 12 h, 82%; f) acryloyl chloride, diisopropylethylamine, CH_2Cl_2, rt, 1 h, 83%; g) AcOH, TBAF, THF, rt, 2.5 days, 82%; h) Grubbs' cat. second generation, CH_2Cl_2, rt, 3 h, 63%; i) TFA, CH_2Cl_2, rt, 1 h, 88%; j) Dess-Martin periodinane, CH_2Cl_2, rt, 1 h, 85%

Having prepared novel four analogues of dihydro-azaMSs, their apoptosis-inducing activities were evaluated by the assay on human lymphoma cell line (U937) (Table 1).[10]

Table 1. Evaluation of Apoptosis-inducing Activity of Synthesized Dihydro-AzaMS Compounds



16



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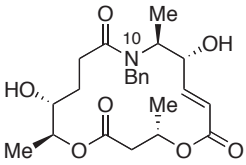
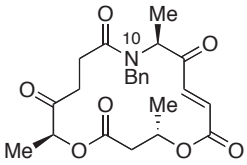


32



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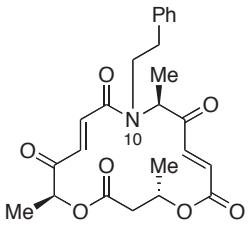

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Compound	Early apoptosis[a] (Secondary necrosis)[a]	Compound	Early apoptosis[a] (Secondary necrosis)[a]
16	0.53 (0.39)	**17**	4.36 (7.26)
32	0.74 (0.47)	**33**	3.98 (6.01)
control	0.46 (0.42)	**8**	30.7 (2.67)

[a] Represented as Fraction of Cells (%). Human lymphoma cells (U937) were treated with 10 μM concentrations of each azaMS compound for 12 h. Percentage of early apoptotic and secondary necrotic cells weremeasured by means of flow cytometry. Results are presented as an average of three experiments.

The cells were exposed to 10 μM of each candidate and incubated for 12 h, then the percentages of the early apoptotic and the secondary necrotic cell death were measured with flow cytometry. Disappointingly, the dihydroderivatives **16** and **32** never induced the desired apoptotic cell death. However, despite the activities were lower than that of previously reported aza-MS **8**, diketo-derivatives **17** and **33** were found to be significant apoptosis-inducers on lymphoma cells with relatively higher secondary necrosis inducing activity.

In conclusion, we proved that RCM approach could be applicable for the frequent preparation of various types of macrosphelide analogues. Additionally, the chemoselective reduction of the functionally resembled two olefins in our azamacrocycle was found to be possible because of its structural rigidity. Furthermore, it was revealed that the structurally more flexible dihydro-analogue of the aza-MSs induced not only the desired apoptosis but also the undesired necrosis. Now the relevance between the biological activities and the three dimensional structures is under investigation through conformational analyses on natural and designed macrosphelides.

EXPERIMENTAL

Materials were obtained from commercial suppliers and used without further purification unless otherwise noted. Anhydrous THF was purchased from Kanto Chemical Co., Inc. Anhydrous Et_2O, CH_2Cl_2, dioxane, DMF, DMSO, toluene, and MeCN were purchased from Wako Pure Chemical Industries. Anhydrous MeOH, EtOH, *i*PrOH, NEt_3 were dried and distilled according to the standard protocols. Otherwise noted, all reactions were performed using oven-dried glassware, sealed with a

rubber septum under a slight positive pressure of argon. Flash column chromatography was carried out using Kanto silica gel 60N (spherical, neutral, 40–50 μm). Analytical TLC was performed on Merck 60 F_{254} glass plates precoated with a 0.25 mm thickness of silica gel. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. All melting points were determined on Yanagimoto micro melting point apparatus and uncorrected. IR spectra were measured on a JNM FT/IR-660 spectrometer. NMR spectra were measured on a VARIAN Gemini 300 spectrometer. For ^{1}H spectra, chemical shifts are expressed in parts par million (ppm) downfield from internal tetramethylsilane (δ 0) or relative internal $CHCl_3$ (δ 7.26). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. *J* values are given in hertz. For ^{13}C spectra, chemical shifts are expressed in ppm, relative to the central line of a triplet at 77.0 ppm for internal $CDCl_3$. Mass spectra were recorded on a JEOL D-200, JEOL JMS-GCmate II, SHIMADZU GCMS-QP 500, or JEOL AX 505 spectrometer.

(3*S*,6*E*,8*R*,9*S*,14*R*,15*S*)-14-Hydroxy-8-(2-methoxyethoxy)methoxy-3,9,15-trimethyl-10-phenethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,11-trione (15). Under an H_2 atmosphere, to a solution of cyclic compound **14** (26.7 mg, 0.0500 mmol) in EtOH (2.0 mL) was added Rh/Al_2O_3 (20.0 mg), and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with CH_2Cl_2, and filtered through Celite. The filtrate was concentrated in vacuo, and the residue was identified as the **15** (26.8 mg, 0.0500 mmol, quant.) by ^{1}H-NMR, and used for next reaction without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.32–7.17 (5H, m), 6.67 (1H, dd, *J* = 15.7 Hz, 9.1 Hz), 6.01 (1H, d, *J* = 15.7 Hz), 5.39–5.11 (1H, m), 5.00–4.80 (2H, m), 4.79–4.60 (2H, m), 3.80–3.51 (5H, m), 3.43–3.23 (5H, m), 3.09–3.01 (1H, m), 2.99–2.76 (2H, m), 2.63–2.24 (5H, m), 2.19–2.04 (1H, m), 1.55–1.20 (9H, m); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.95, 171.21, 164.66, 145.36, 137.59, 128.65, 128.56, 126.64, 124.50, 94.12, 78.17, 74.44, 73.62, 71.58, 68.60, 67.29, 61.55, 59.04, 53.65, 41.53, 35.89, 30.92, 28.61, 20.13, 15.93, 15.68; IR ($CHCl_3$): 3432 cm^{-1} (O–H), 1732 cm^{-1} (C=O), 1627 cm^{-1} (C=O); MS (EI) *m/z* 535 (M^+); HRMS (EI) Calcd for $C_{28}H_{41}NO_9$: 535.2781 (M^+), found: 535.2768; $[\alpha]_D^{24}$ –12.2 (*c* 1.375, $CHCl_3$).

(3*S*,6*E*,8*R*,9*S*,14*R*,15*S*)-8,14-Dihydroxy-3,9,15-trimethyl-10-phenethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,11-trione (16). Under an Ar atmosphere, to a solution of the MEM ether **15** (26.8 mg, 0.0500 mmol) in CH_2Cl_2 (0.50 mL) was added TFA (0.50 mL) at 0 °C, and the mixture was stirred for 4 h at rt. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 3:2) to afford the diol **16** (6.4 mg, 0.0143 mmol, 29%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34–7.08 (5H, br), 6.91–6.59 (3H, br), 6.11–5.94 (1H, br), 5.44–5.28 (1H, br), 5.16–4.68 (1H, br), 4.36–4.08 (1H, br), 3.82–3.10 (4H, br), 2.86–2.18 (8H, br), 1.59–1.25 (9H, br); IR ($CHCl_3$): 3418 cm^{-1} (O–H), 1731 cm^{-1} (C=O), 1715 cm^{-1} (C=O), 1647 cm^{-1} (C=O); MS (EI) *m/z*

447 (M^+); HRMS (EI) Calcd for $C_{24}H_{33}NO_7$: 447.2257 (M^+), found: 447.2241; $[\alpha]_D^{24}$ +33.3 (*c* 0.270, $CHCl_3$).

(3*S*,6*E*,9*S*,15*S*)-3,9,15-Trimethyl-10-phenethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,8,11,14-pentaone (17). Under an Ar atmosphere, to a solution of the diol **16** (7.2 mg, 0.0160 mmol) in CH_2Cl_2 (1.0 mL) was added Dess-Martin periodinane (33.9 mg, 0.080 mmol) at rt and the mixture was stirred for 1 h. The reaction mixture was directly purified by column chromatography on silica gel (hexane:AcOEt = 7:1) to afford the diketone **17** (4.0 mg, 0.0090 mmol, 56%, as a colorless oil) and diketone **18**[10g] (2.2 mg, 0.0050 mmol, 31%, as a colorless oil).

17: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34–7.18 (5H, m), 7.03 (1H, d, *J* = 15.8 Hz), 6.54 (1H, d, *J* = 15.8 Hz), 5.41–5.35 (1H, m), 5.22 (1H, q, *J* = 7.1 Hz), 4.67–4.65 (1H, m), 3.82–3.72 (1H, m), 3.52–3.34 (1H, m), 3.03–2.87 (3H, m), 2.69–2.35 (5H, m), 1.50 (3H, d, *J* = 7.1 Hz), 1.45 (3H, d, *J* = 6.3 Hz), 1.42 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 204.79, 197.25, 171.96, 169.52, 164.77, 137.33, 137.12, 130.36, 128.79, 128.50, 126.91, 74.93, 68.44, 60.24, 49.17, 40.69, 36.86, 29.79, 26.54, 20.02, 16.34, 13.29; IR ($CHCl_3$): 1742 cm^{-1} (C=O), 1719 cm^{-1} (C=O), 1636 cm^{-1} (C=O); MS (EI) *m/z* 443 (M^+); HRMS (EI) Calcd for $C_{24}H_{29}NO_7$: 443.1944 (M^+), found: 443.1970; $[\alpha]_D^{24}$ –129.4 (*c* 0.185, $CHCl_3$).

***N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]amine (22).** Under an Ar atmosphere, to a solution of the TBDPS ether **21**[10g] (439.7 mg, 1.00 mmol) in CH_2Cl_2 (10.0 mL) was dropped TFA (5.0 mL), and the mixture was stirred for 15 min at 0 °C. The reaction was quenched with sat. $NaHCO_3$ aq., and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo.

To a solution of the residue in MeOH/AcOH (10:1, 10.0 mL) were added benzaldehyde (0.10 mL, 1.50 mmol) and 2-picoline·BH_3 (106.9 mg, 1.00 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred for 30 min at rt. The reaction was quenched with 10% HCl aq., and the mixture was alkalinized with sat. $NaHCO_3$ aq., extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting oil was purified by column chromatography on silica gel (hexane:AcOEt = 5:2) to afford the amine **22** (335.2 mg, 0.78 mmol, 78% in 2 steps) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.64–7.61 (4H, m), 7.45–7.21 (11H, m), 5.89 (1H, ddd, *J* = 17.3 Hz, 10.4 Hz, 6.9 Hz), 5.03 (1H, ddd, *J* = 10.4 Hz, 1.1 Hz, 0.8 Hz), 4.92 (1H, ddd, *J* = 17.3 Hz, 1.1 Hz, 1.1 Hz), 4.13–4.09 (1H, m), 3.80 (1H, d, *J* = 13.4 Hz), 3.62 (1H, d, *J* = 13.4 Hz), 2.75–2.67 (1H, m), 1.90 (1H, br), 1.07 (9H, s), 0.97 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 140.41, 137.16, 135.93, 135.76, 134.68, 133.83, 133.68, 129.50, 129.42, 128.21, 127.98, 127.58, 127.40, 127.22, 126.54, 116.54, 78.15, 56.76, 51.16, 27.15, 19.47, 15.94; IR ($CHCl_3$): 3421 cm^{-1} (N–H), 1112 cm^{-1} (C–N); MS (EI) *m/z* 372 (M^+–57); HRMS (EI) Calcd for $C_{24}H_{26}NOSi$: 372.1784 (M^+–57), found: 372.1791; $[\alpha]_D^{24}$ +49.1 (*c* 0.865, $CHCl_3$).

(4*R*,5*S*)-*N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]-5-*tert*-butyldimethylsilyloxy-4-(2-methoxyethoxy)methoxyhexanamide (24). Under an Ar atmosphere, to a solution of the amine **22** (344.6 mg, 0.80 mmol) and carboxylic acid **23**[7c] (396.1 mg, 1.13 mmol) in CH_2Cl_2 (7.5 mL) were added EDC·HCl (216.6 mg, 1.13 mmol) and DMAP (18.3 mg, 0.15 mmol) at 0 °C, and the mixture was stirred for 24 h at rt. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 15:1) to afford the amide **24** (588.6 mg, 0.77 mmol, 99%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.70–7.60 (4H, m), 7.48–7.14 (9H, m), 7.11–7.03 (2H, m), 5.82–5.59 (1H, m), 4.91–4.39 (6H, m), 4.24–3.97 (2H, m), 3.87–3.67 (2H, m), 3.57–3.45 (1H, m), 3.43–3.23 (6H, m), 2.59–2.16 (1H, m), 2.00–1.45 (3H, m), 1.15–1.01 (15H, m), 0.89–0.82 (9H, m), 0.06–0.03 (6H, m); IR ($CHCl_3$): 1648 cm^{-1} (C=O); MS (EI) *m/z* 761 (M^+); HRMS (EI) Calcd for $C_{44}H_{67}NO_6Si_2$: 761.4507 (M^+), found: 761.4475; $[\alpha]_D^{25}$ +1.3 (*c* 0.595, $CHCl_3$).

(4*R*,5*S*)-*N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]-5-hydroxy-4-(2-methoxyethoxy)methoxyhexanamide (25). A solution of the TBS ether **24** (571.6 mg, 0.75 mmol) in AcOH/THF/H_2O (3:1:1, 3.0 mL) was stirred for 12 h at 60 °C. The reaction mixture was diluted with Et_2O, washed with sat. $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 10:1) to give the alcohol **25** (346.2 mg, 0.53 mmol, 71%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.71–7.61 (4H, m), 7.48–7.15 (9H, m), 7.06–7.04 (2H, m), 5.83–5.60 (1H, m), 4.93–4.86 (2H, m), 4.81–4.39 (4H, m), 4.17–3.99 (2H, m), 3.86–3.33 (9H, m), 2.19–2.09 (1H, m), 1.96–1.65 (4H, m), 1.16–1.06 (15H, m); IR ($CHCl_3$): 3445 cm^{-1} (O–H), 1634 cm^{-1} (C=O); MS (EI) *m/z* 647 (M^+); HRMS (EI) Calcd for $C_{38}H_{53}NO_6Si$: 647.3642 (M^+), found: 647.3607; $[\alpha]_D^{25}$ –60.2 (*c* 0.740, $CHCl_3$).

(1*S*,2*R*)-4-{*N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]carbamoyl}-2-(2-methoxyethoxy)methoxy-1-methylbutyl (*S*)-3-*tert*-Butyldimethylsilyloxybutyrate (27). Under an Ar atmosphere, to a solution of the carboxylic acid **26**[10g] (147.4 mg, 0.675 mmol) in toluene (4.5 mL) were added NEt_3 (0.11 mL, 0.81 mmol) and 2,4,6-trichlorobenzoyl chloride (0.11 mL, 0.675 mmol) at 0 °C, and the mixture was stirred for 30 min at the same temperature. To this solution were added a solution of the alcohol **25** (324.0 mg, 0.50 mmol) in toluene (4.5 mL) and DMAP (82.5 mg, 0.675 mmol), and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with Et_2O, washed with 10% HCl aq. and sat. $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 10:1) to give the ester **27** (381.0 mg, 0.45 mmol, 90%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.71–7.60 (4H, m), 7.47–7.11 (9H, m), 7.04–7.01 (2H, m), 5.85–5.61

(1H, m), 4.94–4.89 (2H, m), 4.84–4.39 (4H, m), 4.31–3.97 (3H, m), 3.76–3.69 (1H, m), 3.56–3.42 (2H, m), 3.40–3.21 (6H, m), 2.54–2.08 (4H, m), 1.99–1.64 (2H, m), 1.27–1.12 (9H, m), 1.06 (9H, s), 0.89–0.80 (9H, m), 0.09–0.03 (6H, m); IR ($CHCl_3$): 1734 cm^{-1} (C=O), 1648 cm^{-1} (C=O); MS (EI) *m/z* 790 (M^+–57); HRMS (EI) Calcd for $C_{44}H_{64}NO_8Si_2$: 790.4170 (M^+–57), found: 790.4208; $[\alpha]_D^{24}$ –0.09 (*c* 0.705, $CHCl_3$).

(1*S*,2*R*)-4-{*N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]carbamoyl}-2-(2-methoxyethoxy)methoxy-1-methylbutyl (*S*)-3-Hydroxybutyrate (28). A solution of the TBS ether **27** (373.2 mg, 0.44 mmol) in AcOH/THF/H_2O (3:1:1, 1.75 mL) was stirred for 12 h at 50 °C. The reaction mixture was diluted with Et_2O, washed with sat. $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Et_2O only) to give the alcohol **28** (264.3 mg, 0.36 mmol, 82%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.71–7.60 (4H, m), 7.46–7.14 (9H, m), 7.04–7.02 (2H, m), 5.84–5.61 (1H, m), 5.14–4.71 (3H, m), 4.65–4.38 (4H, m), 4.19–3.97 (2H, m), 3.74–3.71 (1H, m), 3.56–3.25 (6H, m), 2.73 (1H, br), 2.57–2.29 (3H, m), 2.20–1.67 (5H, m), 1.25–1.10 (9H, m), 1.06 (9H, s); IR ($CHCl_3$): 3446 cm^{-1} (O–H), 1732 cm^{-1} (C=O), 1646 cm^{-1} (C=O); MS (EI) *m/z* 733 (M^+); HRMS (EI) Calcd for $C_{42}H_{59}NO_8Si$: 733.4010 (M^+), found: 733.3970; $[\alpha]_D^{24}$ –2.4 (*c* 0.540, $CHCl_3$).

(1*S*,2*R*)-4-{*N*-Benzyl-*N*-[(1*S*,2*R*)-2-*tert*-butyldiphenylsilyloxy-1-methyl-3-butenyl]carbamoyl}-2-(2-methoxyethoxy)methoxy-1-methylbutyl (*S*)-3-Acryloyloxybutyrate (29). Under an Ar atmosphere, to a solution of the alcohol **28** (256.9 mg, 0.35 mmol) in CH_2Cl_2 (3.5 mL) were added diisopropylethylamine (0.15 mL, 0.84 mmol) and acryloyl chloride (56.9 μL, 0.700 mmol) at 0 °C, and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with CH_2Cl_2, successively washed with 10% HCl aq. and sat. $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 6:1) to give the ester **29** (229.6 mg, 0.29 mmol, 83%, mixture of rotamers) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.71–7.60 (4H, m), 7.46–7.14 (9H, m), 7.04–7.01 (2H, m), 6.40–6.32 (1H, m), 6.12–6.00 (1H, m), 5.84–5.58 (2H, m), 5.36–5.27 (1H, m), 5.10–4.70 (3H, m), 4.64–4.38 (4H, m), 4.19–3.97 (2H, m), 3.74–3.67 (1H, m), 3.55–3.47 (1H, m), 3.40–3.24 (6H, m), 2.71–2.42 (2H, m), 2.19–2.08 (1H, m), 1.91–1.62 (3H, m), 1.36–1.28 (3H, m), 1.21–1.14 (6H, m), 1.05 (9H, s); IR ($CHCl_3$): 1728 cm^{-1} (C=O), 1645 cm^{-1} (C=O); MS (EI) *m/z* 787 (M^+); HRMS (EI) Calcd for $C_{45}H_{61}NO_9Si$: 787.4115 (M^+), found: 787.4145; $[\alpha]_D^{24}$ –6.1 (*c* 0.720, $CHCl_3$).

(1*S*,2*R*)-4-{*N*-Benzyl-*N*-[(1*S*,2*R*)-2-hydroxy-1-methyl-3-butenyl]carbamoyl}-2-(2-methoxyethoxy)-methoxy-1-methylbutyl (*S*)-3-Acryloyloxybutyrate (30). Under an Ar atmosphere, to a solution of the TBDPS ether **29** (220.6 mg, 0.280 mmol) in THF (2.2 mL) were added AcOH (0.38 mL, 6.7 mmol) and TBAF (1.0 M solution in THF, 5.6 mL, 5.6 mmol), and the mixture was stirred for 2.5 days at rt. The

reaction mixture was diluted with Et_2O, washed with sat. $NaHCO_3$ aq., dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2: AcOEt = 5:1) to give the alcohol **30** (126.3 mg, 0.23 mmol, 82%) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.38–7.18 (5H, m), 6.37 (1H, dd, *J* = 17.3 Hz, 1.4 Hz), 6.06 (1H, dd, *J* = 17.3 Hz, 10.2 Hz), 5.78 (1H, dd, *J* = 10.2 Hz, 1.6 Hz), 5.74 (1H, ddd, *J* = 17.3 Hz, 10.4 Hz, 6.9 Hz), 5.35–5.28 (1H, m), 5.21 (1H, dd, *J* = 17.3 Hz, 1.6 Hz), 5.08 (1H, dd, *J* = 10.4 Hz, 1.4 Hz), 5.07–5.00 (1H, m), 4.74 (1H, d, *J* = 7.0 Hz), 4.64 (1H, d, *J* = 17.6 Hz), 4.62 (1H, d, *J* = 7.0 Hz), 4.50 (1H, d, *J* = 17.6 Hz), 4.35–4.33 (1H, m), 3.87–3.80 (1H, m), 3.66–3.61 (1H, m), 3.57–3.52 (2H, m), 3.50–3.35 (2H, m), 3.32 (3H, s), 2.63 (1H, dd, *J* = 15.4 Hz, 7.7 Hz), 2.51 (1H, dd, *J* = 15.4 Hz, 5.5 Hz), 2.49–2.40 (2H, m), 1.99–1.84 (1H, m), 1.81–1.74 (1H, m), 1.31 (3H, d, *J* = 6.3 Hz), 1.18 (3H, d, *J* = 6.9 Hz), 1.16 (3H, d, *J* = 5.5 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.66, 169.34, 165.07, 138.26, 137.04, 130.70, 128.79, 128.40, 127.48, 126.19, 115.46, 94.99, 78.10, 75.53, 71.71, 71.66, 67.50, 67.41, 59.54, 59.04, 51.74, 41.11, 30.39, 25.58, 19.90, 15.21, 11.09; IR ($CHCl_3$): 3432 cm^{-1} (O–H), 1727 cm^{-1} (C=O), 1632 cm^{-1} (C=O); MS (EI) *m*/*z* 549 (M^+); HRMS (EI) Calcd for $C_{29}H_{43}NO_9$: 549.2938 (M^+), found: 549.2894; $[\alpha]_D^{25}$ +5.4 (*c* 0.765, $CHCl_3$).

(3*S*,6*E*,8*R*,9*S*,14*R*,15*S*)-10-Benzyl-8-hydroxy-14-(2-methoxyethoxy)methoxy-3,9,15-trimethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,11-trione (31). Under an Ar atmosphere, to a solution of the cyclization precursor **30** (82.4 mg, 0.150 mmol) in CH_2Cl_2 (150 mL) was added Grubbs' catalyst second generation (25.5 mg, 0.0300 mmol), and the mixture was stirred for 3 h at rt. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (CH_2Cl_2: AcOEt = 3:1) to give the macrocyclic compound **31** (49.5 mg, 0.095 mmol, 63%) as a colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.37–7.21 (3H, m), 7.18–7.15 (2H, m), 6.50 (1H, dd, *J* = 15.7 Hz, 8.2 Hz), 5.90 (1H, d, *J* = 15.7 Hz), 5.41–5.28 (1H, m), 5.02–4.90 (2H, m), 4.80 (1H, d, *J* = 6.9 Hz), 4.68 (1H, d, *J* = 6.9 Hz), 4.32 (2H, s), 3.77–3.46 (5H, m) 3.39–3.28 (4H, m), 2.75 (1H, br), 2.63–2.25 (4H, m), 2.00–1.88 (1H, m), 1.75–1.66 (1H, m), 1.39 (3H, d, *J* = 6.6 Hz), 1.31 (3H, d, *J* = 6.3 Hz), 1.22 (3H, d, *J* = 6.3 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.78, 169.77, 165.18, 147.87, 135.99, 128.66, 127.72, 127.59, 122.03, 94.36, 77.62, 72.96, 71.58, 69.87, 67.88, 67.37, 61.53, 59.01, 53.94, 41.29, 29.01, 25.23, 19.87, 16.65, 14.85; IR ($CHCl_3$): 3433 cm^{-1} (O–H), 1732 cm^{-1} (C=O), 1641 cm^{-1} (C=O); MS (EI) *m*/*z* 521 (M^+); HRMS (EI) Calcd for $C_{27}H_{39}NO_9$: 521.2625 (M^+), found: 521.2624.

(3*S*,6*E*,8*R*,9*S*,14*R*,15*S*)-10-Benzyl-8,14-dihydroxy-3,9,15-trimethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,11-trione (32). Under an Ar atmosphere, to the solution of the MEM ether **31** (14.1 mg, 0.0270 mmol) in CH_2Cl_2 (0.27 mL) was added TFA (0.27 mL) at 0 °C, and the mixture was stirred for 1 h at rt. The reaction mixture was concentrated in vacuo, and the residue was purified by column

chromatography on silica gel (CH_2Cl_2:AcOEt = 3:2) to give the diol **32** (10.3 mg, 0.0238 mmol, 88%) as a colorless solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.38–7.26 (3H, br), 7.18–7.16 (2H, br), 6.76–6.63 (1H, br), 6.00–5.95 (1H, br), 5.45–5.38 (1H, br), 4.99–4.62 (2H, br), 4.42–4.35 (2H, br), 3.49–3.31 (2H, br), 3.22–2.91 (3H, br), 2.66–2.38 (5H, br), 1.34–1.25 (9H, br); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.76, 169.77, 165.02, 147.75, 128.78, 128.37, 127.42, 122.66, 74.31, 73.42, 73.07, 68.64, 53.75, 41.75, 31.10, 29.74, 29.29, 28.83, 20.31, 15.24, 15.02; IR ($CHCl_3$): 3409 cm^{-1} (O–H), 1730 cm^{-1} (C=O), 1623 cm^{-1} (C=O); MS (EI) *m/z* 433 (M^+); HRMS (EI) Calcd for $C_{23}H_{31}NO_7$: 433.2101 (M^+), found: 433.2082; $[\alpha]_D^{25}$ +12.6 (*c* 0.515, $CHCl_3$); mp. 58–61 °C.

(3*S*,6*E*,9*S*,15*S*)-10-Benzyl-3,9,15-trimethyl-10-aza-4,16-dioxa-6-cyclohexadecene-1,5,8,11,14-pentaone (33). Under an Ar atmosphere, to a solution of the diol **32** (13.0 mg, 0.0300 mmol) in CH_2Cl_2 (1.0 mL) was added Dess-Martin periodinane (30.5 mg, 0.0720 mmol) at rt, and the mixture was stirred for 1 h. The reaction mixture was directly purified by column chromatography on silica gel (CH_2Cl_2:AcOEt = 2:1) to give the diketone **33** (10.9 mg, 0.0254 mmol, 85%) as a colorless solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42–7.26 (5H, m), 6.98 (1H, d, *J* = 15.9 Hz), 6.47 (1H, d, *J* = 15.9 Hz), 5.43–5.38 (1H, m), 5.22 (1H, q, *J* = 7.1 Hz), 4.91 (1H, d, *J* = 17.0 Hz), 4.70–4.68 (1H, br), 4.33 (1H, d, *J* = 17.0 Hz), 3.02–2.93 (1H, m), 2.78–2.43 (5H, m), 1.50 (3H, d, *J* = 7.1 Hz), 1.47 (3H, d, *J* = 6.3 Hz), 1.29 (3H, d, *J* = 6.6 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 204.79, 197.18, 172.48, 169.56, 164.74, 137.37, 136.48, 130.30, 128.94, 127.92, 126.87, 74.78, 68.41, 59.72, 50.60, 40.73, 33.95, 27.12, 20.08, 16.36, 12.62; IR ($CHCl_3$): 1722 cm^{-1} (C=O), 1641 cm^{-1} (C=O), 1632 cm^{-1} (C=O); MS (EI) *m/z* 429 (M^+); HRMS (EI) Calcd for $C_{23}H_{27}NO_7$: 429.1788 (M^+), found: 429.1809; $[\alpha]_D^{26}$ –179.7 (*c* 0.545, $CHCl_3$); mp. 37–40 °C.

Biological evaluation procedures

Cell line and culture: A human lymphoma cell line, U937, was obtained from the Human Sciences Research Resource Bank (Japan Human Sciences Foundation, Tokyo, Japan) and was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in humidified air with 5% CO_2.

Apoptosis assay by flow cytometry

Flow cytometry was performed with PI and FITC-labeled annexin V (Immunotech, Marselille, France) to detect phosphatidylserine externalization (on the surface of the cell membrane) as an endopoint indicator of early apoptosis. After treatment of the cells with test compounds, the remaining intact cells were incubated at 37 °C for 12 h, collected, washed with cold phosphate-buffered saline (PBS) at 4 °C, and centrifuged at 500 *g* for 3 min. FTIC-labeled annexin V (5 μL) and PI (5 μL) were added to the cell suspension (490 μL) and mixed in gently. After incubation at 4 °C for 10 min in the dark, the cells were

analyzed by flow cytometry (Epics XL, Beckman-Coulter, Miami, FL).

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11. The reaction conditions were not optimized. This acidic deacetalization afforded the desired diol **16** with unidentifiable, highly polar material. Since the elongation of the reaction time (9 h) gave the similar result with 24% yield and analogous N-Bn 14-MEM ether **31** was uneventfully deprotected by TFA without the decomposition of diol **32**, this unsuccessful result might to be caused by the instability of the substrate **15** under the acidic condition, not by the further undesired reaction of the diol **16**.