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Synthesis of penaresidin derivatives and its biological activity

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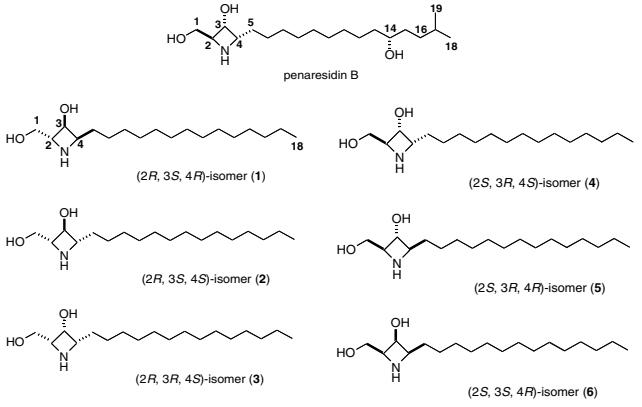
Abstract—A series of stereoisomers for the azetidine ring of penaresidin B was synthesized and their cytotoxic and antimicrobial activities were evaluated. Among six synthetic isomers **1**–**6**, isomers **4** and **5** showed relatively potent cytotoxic activity against A549 (lung) and HT29 (colon) tumor cells as well as antibacterial activity.

1. Introduction

Marine sponges have frequently afforded a variety of sphingosine-related compounds,[1] in which penaresidins A and B isolated from an Okinawan marine sponge *Penares* sp. are the first sphingosine-derived alkaloids possessing an azetidine ring,[2] although some azetidine alkaloids from marine sources have been reported so far.[3] Since a modest cytotoxic activity against L1210 (murine lymphoma) cells was recently found for penaresidin B, in order to investigate the structure–activity relationships of stereoisomers for the azetidine ring of penaresidin B for cytotoxicity and antimicrobial activity, we synthesized six stereoisomers **1**–**6** with a C_{14} alkyl side chain at C-3 without any substituents. Among six synthetic isomers **1**–**6**, isomers **4** and **5** showed relatively potent cytotoxic activity against A549 (lung) and HT29 (colon) tumor cells as well as antibacterial activity. In this paper, we describe synthesis of penaresidin derivatives and its biological activity.

2. Results and discussion

In Scheme 1 is shown our synthetic plan for isomers **1**–**3** according to a synthetic strategy by Mori et al.[3d] Isomers **1** and **2** can be prepared from sphingosine derivatives **7a** and **7b**, which are to be obtained from **9** via **8a** and **8b**, respectively. The protected sphingosine **9** can be prepared from **10** and **11** employing Garner's general method of sphingosine synthesis.[4] Isomer **3** can be prepared from **12**, which is to be obtained from **10** and **11** via *threo* predominant coupling.[5]



The synthesis of isomers **1** and **2** is summarized in Scheme 2. Garner's aldehyde **10**[4] was coupled with lithium alkynide from **11**. This reaction is known to proceed stereoselectively yielding the *anti*-product **13** (>95% de from ^{1}H NMR).[3d] Reduction of the triple bond of **13** with lithium and ethylamine afforded the protected sphingosine with concomitant deprotection of both oxazolidine and *tert*-butoxycarbonyl (Boc) protecting group. The two hydroxy groups were then protected

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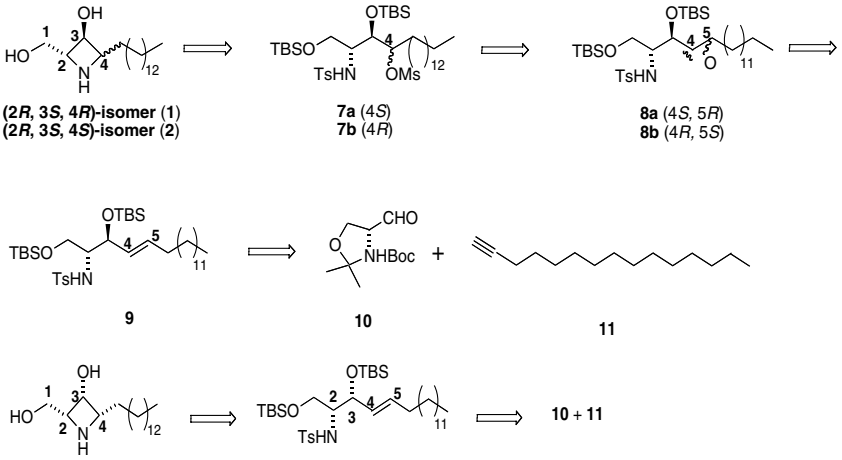
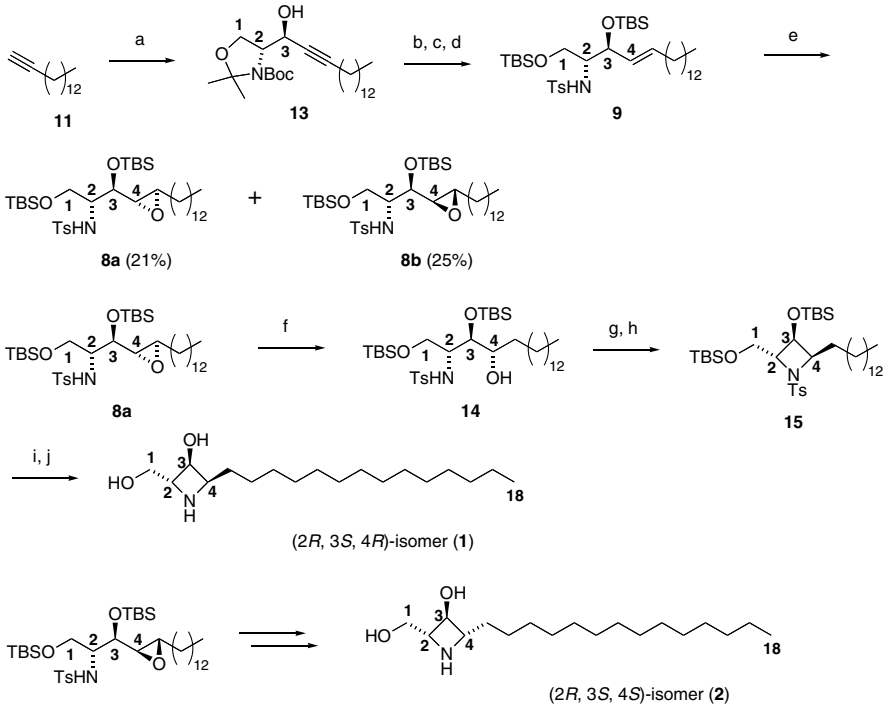


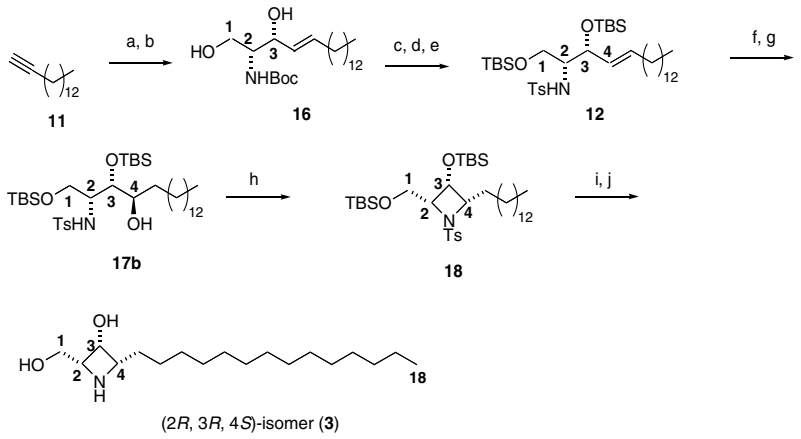
Scheme 1. Synthetic plan for stereoisomers **1**–**3** of penaresidin B derivative.



Scheme 2. Synthesis of (2*R*, 3*S*, 4*R*)-isomer (**1**) and (2*R*, 3*S*, 4*S*)-isomer (**2**). Reagents: (a) *n*-BuLi/THF, then Garner's aldehyde (**10**) (73%); (b) Li/EtNH$_2$; (c) TBSOTf, 2,6-lutidine/CH$_2$Cl$_2$ (27% for 2 steps); (d) TsC/pyridine (98%); (e) *m*CPBA, NaHCO$_3$/hexane; (f) DIBAL/toluene (86%); (g) MsCl/pyridnie; (h) NaH/THF (60% for 2 steps); (i) Na, naphthalene/DME (91%); (j) HF/MeCN (77%).

as the corresponding *tert*-butyldimethylsilyl (TBS) ethers and then an amino group was protected to provide **9**. Epoxidation of **9** with mCPBA (*m*-chloroperoxybenzoic acid) in hexane afforded **8a** and **8b** as a diastereomeric mixture, which were separated by silica gel column chromatography. The stereochemistry of **8a** and **8b** was elucidated by ^{1}H NMR spectra compared with those of authentic known epoxides.[3d] Reduction of **8a** with DIBAL (diisobutyoaluminum hydride) gave **14**. Alcohol **14** was treated with MsCl (methanesulfonyl chloride) to give mesylate and the mesylate was treated with sodium hydride to effect the ring closure, affording the azetidine **15** in 60% yield by the S$_N$2-type intramolecular N-alkylation. The tosyl group of **15** was then removed reductively by treatment with sodium naphthalenide and then removal of the TBS protecting groups furnished (2*R*, 3*S*, 4*R*)-isomer (**1**). Similarly, by starting from epoxide **8b**, (2*R*, 3*S*, 4*S*)-isomer (**2**) was synthesized.

Scheme 3 is shown synthesis of isomer **3**. *threo*-*N*-Boc-sphingosine **16**[5] was prepared from Garner's aldehyde (**10**) with 1-alkenyl nucleophiles via hydrozirconation of terminal alkyne and purified by recrystalization to give single isomer. Removal of Boc group, protection of two hydroxy groups with TBS, and tosylation of amino group afforded **12**. Epoxidation of **12** with mCPBA in hexane yielded epoxides as a diastereomeric mixture. One epoxide was reduced with DIBAL to give **17b**, while another epoxide was decomposed by DIBAL



Scheme 3. Synthesis of (2*R*, 3*R*, 4*S*)-isomer (**3**). Reagents: (a) $Cp_2Zr(H)Cl$, Et_2Zn/THF, then Garner's aldehyde (**10**) (90%); (b) AcOH/H_2O, then recrystalized from hexane (77%); (c) TFA/H_2O (10:3); (d) TBSOTf, 2,6-lutidine/CH_2Cl_2 (74% for 2 steps); (e) TsC/pyridine (quant.); (f) *m*CPBA, $NaHCO_3$/hexane (98%); (g) DIBAL/toluene (16%); (h) PPh_3, DIAD/THF (20%); (i) Na, naphthalene/DME; (j) HF/MeCN (89% for 2 steps).

reduction. The alcohol **17b** was treated with DIAD (diisopropyl azodicarboxylate) and PPh_3 to effect the ring closure, affording the azetidine **18** by the S_N2-type intramolecular Mitsunobu reaction. The tosyl group of **18** was then removed reductively by treatment with sodium naphthalenide and then removal of the TBS protecting groups furnished (2*R*, 3*R*, 4*S*)-isomer (**3**). The relative stereochemistry of **3** was elucidated by NOESY spectral data. Enantiomers **4**–**6** of **1**–**3** were also synthesized as described above.

The cytotoxicity of the synthetic isomers **1**–**6** was evaluated using human solid tumor cell lines, KB (carcinoma), A549 (lung), and HT29 (colon), in addition to murine lymphoma L1210 (lymphoma) (Table 1). Among them, isomers **4** and **5** showed relatively potent cytotoxicity against A549 and HT29 cells. Furthermore, antimicrobial activities of **1**–**5** were examined against bacteria and fungi as shown in Table 2. Isomers **1**, **2**, **4**, and **5** showed antibacterial activity against Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*), while only an isomer **5** exhibited antibacterial activity against Gram-negative bacterium (*Escherichia coli*). All the compounds **1**–**5** did not show antifungal activity.

Table 1. Cytotoxicity of isomers **1**–**6** and penaresidin B against tumor cells

Compound	IC_{50}[e] (μg/mL)			
	L1210[a]	KB[b]	A549[c]	HT529[d]
1	4.5	6	>2	1.23
2	4.5	>10	1.97	0.79
3	7.5	>10	>2	>2
4	4.5	3.4	0.34	0.56
5	1.6	3.2	0.22	0.17
6	>10	>10	1.38	1.65
Penaresidin B	2.9	>10	>2	>2

[a] Murine lymphoma cells.
[b] Human epidermoid carcinoma cells.
[c] Human lung epithelial cells.
[d] Human colon cancer cells.
[e] 50% inhibition concentration.

In this study, it was found that the stereochemistry of the azetidine ring in penaresidine derivatives (**1**–**6**) affected significantly its cytotoxicity and antibacterial activity.

3. Experimental

3.1. (2*R*, 3*S*, 4*E*)-2-Amino-1,3-bis(*tert*-butyldimethylsilyoxy)-2-(*p*-tolylsulfonylamino)-4-octade-cene (9)

To a cooled (−20 °C) solution of **11** (1.15 g, 5.53 mmol) in THF (20 mL), *n*-BuLi (1.6 M in hexane; 2.0 mL, 3.2 mmol) was added and stirred for 1 h at −20 °C. To the reaction mixture was added a solution of Garner's aldehyde (633.4 mg, 2.76 mmol) in THF (2 mL) at −20 °C and the reaction mixture was stirred at 0 °C for 15 h. The reaction mixture was quenched with satd aq NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Silica gel column chromatography (hexane/EtOAc 10:1) provided **13** (883.5 mg, 2.02 mmol) in 73% yield. **13**: $[\alpha]_D^{22}$25.1 (*c* 0.55, $CHCl_3$); IR (KBr) 3340, 2923, 1670, 1396, 1092 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 4.58 (1H, br s), 4.12 (1H, br s), 3.61 (1H, br s), 3.30–3.12 (2H, m), 1.59 (2H, t, J = 6.7 Hz), 1.26–0.71 (37H, m), 0.44 (3H, t, J = 6.7 Hz). ^{13}C NMR (100 MHz, C_6D_6) δ 32.43, 30.22, 30.05, 29.92, 29.66, 29.33, 29.14, 28.43, 26.20, 23.22, 19.20, 14.48. ESIMS *m*/*z* 460 $(M+Na)^+$. Calcd for $C_{26}H_{47}O_4NNa$ $(M+Na)^+$ *m*/*z* 460.3403. Found: *m*/*z* 460.3409.

To a cooled solution of ethylamine (18 mL) and THF (8 mL) was added Li wire (203 mg, 29 mmol) and stirred for 2 h. To the reaction mixture was added a solution of **13** (883 mg, 3.85 mmol) in THF (6 mL) and the reaction mixture was allowed to warm to ambient temperature. After 15 h, the reaction mixture was quenched with satd aq NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give crude alcohol (183.9 mg). Without further purification, the alcohol

Table 2. Antimicrobial activities of isomers **1**–**5** and penaresidin B

Microorganisms	MIC[a] values (μg/mL)					
	1	**2**	**3**	**4**	**5**	Penaresidin B
Bacillus subtilis PC 1219	8.35	16.7	>16.7	4.18	4.18	>16.7
Escherichia coli ATCC25922	>16.7	>16.7	>16.7	>16.7	8.35	>16.7
Micrococcus luteus IFM 2066	4.18	8.35	>16.7	1.84	4.18	>16.7
Staphylococcus aureus 209P	16.7	>16.7	>16.7	8.35	8.35	>16.7
Cryptococcus neoformans IFM 46914	>16.7	>16.7	>16.7	>16.7	>16.7	>16.7
Candida albicans ATCC 90028	>16.7	>16.7	>16.7	>16.7	>16.7	>16.7
Aspergillus niger IFM 5368	>16.7	>16.7	>16.7	>16.7	>16.7	>16.7

[a] Minimum inhibitory concentration.

was used for the next step. To a solution of crude alcohol (183.9 mg) and 2,6-lutidine (0.26 mL, 2.2 mmol) in CH_2Cl_2 (2.1 mL) was added TBSOTf (0.4 mL, 1.7 mmol) at 0 °C and the reaction mixture was allowed to warm to ambient temperature. After 1.5 h, the reaction mixture was quenched with MeOH (0.1 mL) and partitioned between H_2O and EtOAc. The organic extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Silica gel column chromatography (hexane/EtOAc 20:1) provided TBS ether (280.8 mg, 0.532 mmol) in 28% yield for 2 steps. $[\alpha]_D^{22} - 13.2$ (*c* 0.10, $CHCl_3$); IR (KBr) 2925, 2855, 1458, 1255, 837, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.67 (1H, dt, *J* = 15.2, 6.8 Hz), 5.39 (1H, dd, *J* = 15.4, 7.5 Hz), 4.17–4.11 (1H, m), 3.78–3.72 (1H, m), 3.67–3.59 (1H, m), 2.89 (1H, q, *J* = 5.6 Hz), 2.09–2.01 (2H, m), 1.46–1.19 (24H, m), 1.02–0.79 (21H, m), 0.12–0.00 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.55, 129.19, 74.23, 63.18, 57.71, 56.48, 32.36, 31.98, 29.86, 29.75, 29.57, 29.43, 29.32, 29.24, 25.99, 25.94, 22.76, 18.32, 14.20, −3.88, −4.74, −5.25, −5.30. ESIMS *m*/*z* 528 $(M+H)^+$. Calcd for $C_{30}H_{66}O_2NSi_2$ $(M+H)^+$ *m*/*z* 528.4632. Found: *m*/*z* 528.4635.

To a solution of amine (273.6 mg, 0.518 mmol) in pyridine (3 mL) was added TsCl (195.7 mg, 1.03 mmol) at 0 °C. After being stirred for 15 h at ambient temperature, the reaction mixture was quenched with MeOH, and partitioned between H_2O and EtOAc. The organic extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Silica gel column chromatography (hexane/EtOAc 20:1) provided **9** (219.7 mg, 0.322 mmol) in 56% yield. **9**: $[\alpha]_D^{22} - 1.50$ (*c* 0.36, $CHCl_3$); IR (KBr) 2926, 1471, 1337, 1254, 1165, 1093, 1004, 837, 777 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* = 8.5 Hz), 7.27 (2H, d, *J* = 8.5 Hz), 5.57 (1H, dt, *J* = 15.0, 7.1 Hz), 5.23 (1H, dd, *J* = 15.4, 7.3 Hz), 4.63 (1H, d, *J* = 7.0 Hz), 4.24 (1H, t, *J* = 6.2 Hz), 3.80 (1H, dd, *J* = 10.2, 4.1 Hz), 3.46 (1H, dd, *J* = 9.9, 6.5 Hz), 3.16–3.09 (1H, m), 2.41 (1H, s), 1.99–1.92 (1H, m), 1.37–1.21 (25H, m), 0.91–0.79 (21H, m), 0.06–0.01 (12H, m). ^{13}C NMR ($CDCl_3$) δ 142.99, 137.73, 134.09, 129.50, 128.83, 127.20, 72.53, 71.74, 61.42, 60.86, 59.82, 58.24, 31.98, 29.75, 29.57, 29.42, 29.16, 25.96, 25.94, 24.70, 22.75, 21.55, 21.48, 18.25, 14.17, −5.29, −5.33. ESIMS *m*/*z* 704 $(M+Na)^+$. Calcd for $C_{37}H_{71}O_4NSi_2SNa$ $(M+Na)^+$ *m*/*z* 704.4540. Found: *m*/*z* 704.536.

3.2. 1,3-Bis(*tert*-butyldimethylsiloxy)-4,5-epoxy-2-(*p*-tolylsulfonylamino)-4-octadecene (8a and 8b)

To a suspension of **9** (2.27 g, 3.32 mmol) and $NaHCO_3$ (1.43 g, 17.0 mmol) in hexane was added mCPBA (1.65 g, 9.57 mmol) and the reaction mixture was stirred at ambient temperature for 3 day. The reaction mixture was quenched with satd aq $N_2S_2O_3$ (5.5 mL), diluted with water, and extracted with EtOAc. The organic extract was washed H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/Et_2O, 1:0 → 80:1 → 60:1 → 40:1) afforded epoxides **8a** (492.2 mg, 0.706 mmol) and **8b** (577.8 mg, 0.829 mmol) in 21 and 25% yield, respectively.

Compound **8a**: $[\alpha]_D^{22} + 14.6°$ (*c* 1.00, $CHCl_3$); IR (KBr) 2927, 1338, 1254, 1165, 1094, 837, 814, 779 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.75 (2H, d, *J* = 8.3 Hz), 7.28 (2H, d, *J* = 8.5 Hz), 4.78 (1H, d, *J* = 6.8 Hz), 3.77 (1H, t, *J* = 5.2 Hz), 3.71 (1H, dd, *J* = 10.3, 4.9 Hz), 3.55 (1H, dd, *J* = 10.4, 5.0 Hz), 3.48 (1H, q, *J* = 7.0 Hz), 3.27 (1H, dd, *J* = 6.8, 4.9 Hz), 2.79 (1H, dd, *J* = 6.2, 1.9 Hz), 2.67 (1H, dd, *J* = 5.2, 2.2 Hz), 2.41 (3H, s), 1.44–1.18 (23H, m), 0.88 (3H, t, *J* = 6.9 Hz), 0.85 (9H, s), 0.82 (9H, s), 0.05 (3H, s), 0.04 (3H, s), −0.02 (3H, s), −0.05 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.24, 137.27, 129.55, 129.51, 127.26, 127.20, 70.80, 65.85, 60.72, 58.37, 58.23, 58.17, 56.57, 56.52, 31.97, 31.55, 29.71, 29.60, 29.56, 29.52, 29.41, 26.02, 25.89, 22.75, 21.56, 21.51, 18.23, 14.19, −4.30, −4.83, −5.36, −5.49. ESIMS *m*/*z* 720 $(M+Na)^+$. Calcd for $C_{37}H_{71}O_5NSi_2SNa$ $(M+Na)^+$ *m*/*z* 720.4489. Found: *m*/*z* 720.4486.

Compound **8b**: ^1H NMR (400 MHz,$CDCl_3$) δ 7.73 (2H, d, *J* = 8.1 Hz), 7.29 (2H, d, *J* = 8.1 Hz), 4.85 (1H, d, *J* = 7.5 Hz), 3.75 (1H, dd, *J* = 9.5, 2.7 Hz), 3.32–3.20 (2H, m), 2.84–2.77 (2H, m), 2.42 (3H, s), 1.38–1.18 (28H, m), 0.90–0.80 (21H, m), 0.12 (3H, s), 0.00 (3H, s), −0.04 (3H, s), −0.09 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.39, 137.84, 129.72, 129.67, 126.91, 126.83, 73.63, 60.38, 60.09, 57.84, 57.76, 57.70, 31.97, 31.69, 29.71, 29.62, 29.42, 25.94, 25.87, 22.75, 21.58, 18.15, 14.19, −4.13, −5.32, −5.35, −5.59.

3.3. (2*R*, 3*R*, 4*S*)-1,3-Bis(*tert*-butyldimethylsiloxy)-2-*p*-tolylsulfonylaminooctadeca-4-ol (14)

To a cooled (−78 °C) solution of epoxide **8a** (436.2 mg, 0.625 mmol) in toluene (2.5 mL) was added a solution of DIBAL (1.04 M, in toluene; 2 mL, 2.08 mmol). After being stirred for 4 h at −78 °C, the reaction mixture was quenched with $Na_2SO_4{\cdot}10H_2O$ (173 mg) and allowed to warm to ambient temperature. To the mixture was added Celite (500 mg), and the reaction mixture was stirred for 1 h and then filtered through Celite plug and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ether 40:1 → 30:1 → 20:1) to afford alcohol (242.8 mg, 0.346 mmol) in 55% yield.

$[\alpha]_D^{22}$ + 4.27 (*c* 1.00, $CHCl_3$); IR (KBr) 3524, 2926, 1254, 1163, 1093, 837, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* = 8.3 Hz), 7.29 (2H, d, *J* = 7.9 Hz), 4.82 (1H, d, *J* = 6.8 Hz), 3.81 (1H, dd, *J* = 4.8, 3.3 Hz), 3.69 (1H, dd, *J* = 10.3, 6.5 Hz), 3.58 (2H, dd, *J* = 10.2, 5.6 Hz), 3.41 (1H, dd, *J* = 6.0, 3.2 Hz), 2.42 (3H, s), 1.44 (2H, d, *J* = 8.8 Hz), 1.26 (26H, s), 0.88 (9H, s), 0.82 (9H, s), 0.12 (3H, s), 0.09 (3H, s), −0.02 (3H, s), −0.05 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.34, 137.20, 129.63, 129.59, 127.19, 127.13, 75.72, 73.63, 61.10, 56.35, 33.02, 31.97, 29.74, 29.42, 26.10, 25.99, 25.89, 22.75, 21.57, 21.52, 18.26, 18.22, 14.19, −4.09, −4.31, −5.45, −5.56. ESIMS *m*/*z* 722 $(M+Na)^+$. Calcd for $C_{37}H_{73}O_5NSi_2SNa$ $(M+Na)^+$ *m*/*z* 722.4646. Found: *m*/*z* 722.4656.

3.4. (2*R*, 3*R*, 4*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-(*tert*-butyldimethylsilanyloxymethyl)-4-tetradecyl-1-(toluene-4-sulfonyl)-azetidine (15)

To a cooled (0 °C) solution of alcohol **8a** (234.5 mg, 0.335 mmol) in pyridine (2.5 mL) was added MsCl (80 μL, 0.751 mmol). After being stirred for 12 h at ambient temperature, the reaction mixture was diluted with H_2O and extracted with ether. The extract was washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford crude mesylate. The crude mesylate was used for the next step without further purification. To a cooled (0 °C) solution of crude mesylate in THF (3.7 mL) was added NaH (45 mg, 1.1 mmol) and the reaction mixture was allowed to warm to ambient temperature. After 15 h, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic extract was washed with satd aq NH_4Cl and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Silica gel column chromatography (hexane/EtOAc 50:1) provided azetidine **15** (137.6 mg, 1.96 mmol) in 59% yield for 2 steps.

Compound **15**: $[\alpha]_D^{22}$ − 59.1 (*c* 1.09, $CHCl_3$); IR (KBr) 2926, 1471, 1345, 1254, 1159, 838, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (2H, d, *J* = 8.3 Hz), 7.26 (2H, d, *J* = 8.1 Hz), 4.41 (1H, dd, *J* = 6.4, 3.2 Hz), 4.23 (1H, dt, *J* = 10.3, 4.5 Hz), 3.97 (1H, dd, *J* = 7.6, 3.3 Hz), 3.83 (2H, ddd, *J* = 22.7, 11.2, 4.0 Hz), 2.41 (3H, s), 1.84–1.70 (2H, m), 1.33–1.15 (24H, m), 0.93–0.81 (21H, m), 0.05 (3H, s), 0.03 (3H, s), 0.03 (3H, s), 0.01 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.73, 138.43, 129.32, 129.28, 127.18, 127.12, 73.64, 69.13, 65.76, 61.49, 31.97, 29.74, 29.64, 29.42, 26.98, 25.97, 25.66, 22.75, 21.56, 21.50, 18.40, 17.90, 14.19, −4.48, −5.17, −5.31, −5.49. ESIMS *m*/*z* 704 $(M+Na)^+$. Calcd for $C_{37}H_{71}O_4NSi_2SNa$ $(M+Na)^+$ *m*/*z* 704.4540. Found: *m*/*z* 704.4546.

3.5. (2*R*, 3*R*, 4*S*)-2-Hydroxymethyl-4-tetradecylazetidin-3-ol (isomer 1)

Sodium naphthalenide was prepared from naphthalene (628.6 mg, 2.4 mmol) and Na (102.5 mg, 4.46 mmol) in DME (6.6 mL) in the usual manner. To a cooled solution of tosylate **15** (111.7 mg, 0.164 mmol) in DME (1.0 mL), the prepared sodium naphthalenide solution (2.2 mL) was added at −78 °C. After being stirred for 40 min at −78 °C, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 15:1) to amine (79 mg, 0.149 mmol) in 91% yield. $[\alpha]_D^{22}$ + 11.3 (*c* 1.02, $CHCl_3$); IR (KBr) 3447, 2926, 1464, 1254, 837, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (1H, dd, *J* = 7.0, 5.5 Hz), 3.66 (2H, d, *J* = 3.8 Hz), 3.62 (1H, t, *J* = 4.4 Hz), 3.56 (1H, dd, *J* = 14.2, 7.2 Hz), 2.19 (1H, br s), 1.72–1.58 (2H, m), 1.26–1.24 (24H, m), 0.92–0.85 (21H, m), 0.07 (3H, s), 0.07 (3H, s), 0.02 (3H, s), 0.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.03, 67.67, 63.40, 61.54, 31.98, 30.59, 29.90, 29.73, 29.42, 25.99, 25.84, 22.76, 18.41, 18.16, 14.20, −4.65, −4.91, −5.32. ESIMS *m*/*z* 528.4 (M^+). Calcd for $C_{30}H_{66}O_2NSi_2$ (M^+) *m*/*z* 528.4632. Found: *m*/*z* 528.4640.

To a solution of crude amine (68.4 mg, 0.13 mmol) in CH_3CN (2 mL), aq HF (46%, 50 μL, 1.15 mmol) was added. After being stirred for 12 h at ambient temperature, the reaction mixture was neutralized with $NaHCO_3$ (345 mg), filtered through Celite plug, and concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_3Cl/MeOH 10:1 → 0:1) to afford isomer **1** (30.0 mg, 0.10 mmol) in 77% yield. $[\alpha]_D^{23}$ − 9.0° (*c* 0.10, MeOH); IR (KBr) 3442, 2920, 2850, 1558, 1458, 1287, 1124 cm^{-1}; ^1H NMR (600 MHz, CD_3OD, 323 K) δ 4.36 (1H, dd, *J* = 6.9, 5.5 Hz), 3.71–3.64 (4H, m), 1.83–1.80 (1H, m), 1.67–1.64 (1H, m), 1.43–1.22 (27H, m), 0.90 (3H, t, *J* = 3.4 Hz). ^{13}C NMR (150 MHz, CD_3OD, 323 K) δ 69.38, 69.17, 64.17, 63.75, 33.78, 31.53, 31.48, 31.44, 31.41, 31.15, 27.65, 24.42, 15.09. ESIMS *m*/*z* 300 $(M+H)^+$. Calcd for $C_{18}H_{38}O_2N$ $(M+H)^+$ *m*/*z* 300.2903. Found: *m*/*z* 300.2908.

3.6. (2*R*, 3*S*, 4*E*)-2-Amino-1,3-bis(*tert*-butyldimethylsiloxy)-2-(*p*-tolylsulfonylamino)-4-octadecene (12)

To a *threo*-N-Boc-sphingosine **16**[5] (1.21 g, 3.03 mmol) was added 77% TFA aqueous solution and the mixture was stirred for 1 h at ambient temperature. The reaction mixture was concentrated in vacuo, partitioned between aq satd $NaHCO_3$ and EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give crude alcohol. The alcohol

was used for next step without further purification. To a solution of crude alcohol and 2,6-lutidine (1.8 mL, 15.5 mmol) in CH_2Cl_2 (15 mL) was added TBSOTf (2 mL, 8.7 mmol) at 0 °C and the reaction mixture was allowed to warm to ambient temperature. After 1.5 h, the reaction mixture was quenched with MeOH (0.3 mL) and partitioned between H_2O and EtOAc. The organic extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc 40:1 → 30:1 → 20:1) to afford TBS ether (1.18 g, 2.24 mmol) in 74% yield for 2 steps. $[\alpha]_D^{22}$ − 3.86 (*c* 1.00, $CHCl_3$); IR (KBr) 2926, 2856, 1459, 1252, 1119, 838, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.58 (1H, dt, *J* = 15.1, 6.8 Hz), 5.40 (1H, dd, *J* = 15.6, 7.3 Hz), 4.07 (1H, t, *J* = 6.3 Hz), 3.60 (1H, dd, *J* = 9.8, 5.1 Hz), 3.47 (1H, dd, *J* = 10.3, 5.8 Hz), 2.63 (1H, dd, *J* = 11.2, 5.2 Hz), 2.01 (2H, dd, *J* = 13.7, 7.1 Hz), 1.70 (3H, s), 1.35 (2H, s), 1.25 (19H, br s), 0.90–0.87 (21H, m), 0.05 (3H, s), 0.04 (3H, s), 0.04 (3H, s), 0.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.77, 130.79, 74.42, 64.29, 58.08, 58.03, 32.32, 31.97, 29.73, 29.56, 29.42, 29.29, 25.98, 25.95, 22.75, 18.28, 18.24, 14.19, −3.77, −3.84, −4.74, −5.29. ESIMS *m*/*z* 528 $(M+H)^+$. Calcd for $C_{30}H_{66}O_2NSi_2$ $(M+H)^+$ *m*/*z* 528.4632. Found: *m*/*z* 528.4639.

To a solution of amine (1.16 g, 2.2 mmol) in pyridine (14 mL) was added TsCl (840.2 mg, 4.41 mmol) at 0 °C. After being stirred for 15 h at ambient temperature, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated under reduce pressure. Silica gel column chromatography (hexane/EtOAc 10:1) provided **12** (1.49 g, 2.18 mmol) in 99% yield.

Compound **12**: $[\alpha]_D^{22}$ − 9.59 (*c* 1.00, $CHCl_3$); IR (KBr) 3279, 2927, 1470, 1338, 1254, 1164, 1094, 1006, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.61–5.54 (1H, m), 5.40 (1H, dd, *J* = 15.6, 7.3 Hz), 4.07 (1H, t, *J* = 6.3 Hz), 3.60 (1H, dd, *J* = 9.8, 5.1 Hz), 3.47 (1H, dd, *J* = 10.3, 5.8 Hz), 2.63 (1H, dd, *J* = 11.2, 5.2 Hz), 2.01 (2H, dd, *J* = 13.7, 7.1 Hz), 1.70 (3H, s), 1.35 (2H, s), 1.25 (19H, br s), 0.89 (21H, dd, *J* = 4.4, 3.3 Hz), 0.05 (3H, s), 0.04 (3H, s), 0.04 (3H, s), 0.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.77, 130.79, 74.42, 64.29, 58.08, 58.03, 32.32, 31.97, 29.73, 29.56, 29.42, 29.29, 25.98, 25.95, 22.75, 18.28, 18.24, 14.19, −3.82, −4.75, −5.27, −5.31. ESIMS *m*/*z* 704 $(M+Na^+)$. Calcd for $C_{37}H_{71}O_4NSi_2SNa$ $(M+Na^+)$ *m*/*z* 704.4540. Found: *m*/*z* 704.4536.

3.7. (2*R*, 3*S*, 4*R*)-1,3-Bis(*tert*-butyldimethylsiloxy)-2-*p*-tolylsulfonylaminooctadeca-4-ol (17b)

To a suspension of **12** (727.1 mg, 1.07 mmol) and $NaHCO_3$ (454 mg, 5.41 mmol) in hexane was added mCPBA (524 mg, 3.04 mmol) and the reaction mixture was stirred at ambient temperature for 3 day. The reaction mixture was quenched with satd aq $N_2S_2O_3$ (2 mL), diluted with water, and extracted with EtOAc. The organic extract was washed H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/Et_2O, 1:0 → 80:1 → 60:1 → 40:1) afforded epoxides (747.2 mg; 8:5) as a diastereomeric mixture in 98% yield. To a cooled (−78 °C) solution of epoxides (531.5 mg, 0.761 mmol) in toluene (3 mL) was added a solution of DIBAL (1.0 M, in toluene; 2.1 mL, 2.02 mmol). After being stirred for 4 h at −78 °C, the reaction mixture was quenched with $Na_2SO_4{\cdot}10H_2O$ (205 mg) and allowed to warm to ambient temperature. To the mixture was added Celite (600 mg), and the reaction mixture was stirred for 1 h and then filtered through Celite plug and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ether 40:1 → 30:1 → 20:1) to afford alcohol **17b** (85.2 mg, 0.118 mmol) in 16% yield.

Compound **17b**: $[\alpha]_D^{22}$ − 26.1 (*c* 1.09, $CHCl_3$); IR (KBr) 3735, 3288, 2926, 1257, 1162, 1088, 839, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* = 8.3 Hz), 7.30 (2H, d, *J* = 8.1 Hz), 5.30 (1H, d, *J* = 7.7 Hz), 3.61 (1H, dd, *J* = 7.1, 1.6 Hz), 3.57 (1H, dd, *J* = 10.0, 4.1 Hz), 3.51–3.44 (2H, m), 3.35 (1H, tdd, *J* = 7.9, 4.3, 1.9 Hz), 2.68 (1H, br s), 2.42 (3H, s), 1.39–1.20 (26H, m), 0.90–0.83 (21H, m), 0.01 (6H, s), −0.01 (3H, s), −0.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.33, 137.66, 129.55, 129.48, 127.36, 127.27, 73.40, 72.50, 61.14, 60.39, 55.59, 33.11, 31.97, 29.72, 29.57, 29.41, 25.96, 25.83, 25.74, 22.74, 21.57, 21.50, 18.15, −4.41, −4.49, −5.41, −5.46. ESIMS *m*/*z* 722 $(M+Na)^+$. Calcd for $C_{37}H_{73}O_5NSi_2SNa$ $(M+Na)^+$ *m*/*z* 722.4646. Found: *m*/*z* 722.4645.

3.8. (2*R*, 3*R*, 4*R*)-3-(*tert*-Butyldimethylsiloxy)-2-(*tert*-butyldimethylsilanyloxymethyl)-4-tetrad-ecyl-*N*-(*p*-toluenesulfonyl)azetidine (18)

To a cooled (0 °C) solution of PPh_3 (43.5 mg, 0.166 mmol) in THF (0.22 mL) was added DIAD (25 μL, 0.127 mmol). After being stirred for 30 min at 0 °C, to the reaction mixture was added a solution of alcohol **17b** (42.1 mg, 0.061 mmol) in THF (0.25 mL). The reaction mixture was stirred for 12 h at 0 °C and then partitioned with H_2O and EtOAc. The organic extract was washed brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by silica gel column chromatography (hexane/EtOAc, 40:1 → 30:1 → 20:1) afforded azetidine **18** (8.2 mg 0.012 mmol) in 20% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (2H, d, *J* = 8.1 Hz), 7.34–7.28 (2H, m), 4.23 (1H, dd, *J* = 6.6, 3.3 Hz), 4.07 (1H, td, *J* = 9.6, 1.6 Hz), 3.73–3.66 (3H, m), 2.46 (3H, s), 1.88–1.78 (1H, m), 1.71–1.60 (1H, m), 1.35–1.23 (24H, m), 0.89 (9H, s), 0.88 (9H, s), 0.84 (3H, t, *J* = 6.0 Hz), 0.07 (3H, s), 0.05 (3H, s), 0.02 (3H, s), −0.02 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.70, 133.74, 133.55, 129.52, 129.46, 128.63, 128.41, 67.46, 67.39, 64.43, 60.15, 31.98, 29.72, 29.43, 26.03, 25.72, 25.64, 22.76, 18.24, 14.21, −4.83, −4.89, −5.12, −5.18.

3.9. (2*R*, 3*R*, 4*R*)-2-Hydroxymethyl-4-tetradecylazetidin-3-ol (isomer 3)

Sodium naphthalenide was prepared from naphthalene (628.6 mg, 2.4 mmol) and Na (102.5 mg, 4.46 mmol) in

DME (6.6 mL) in the usual manner. To a cooled solution of tosylate **18** (8.2 mg, 12 μmol) in DME (0.1 mL), the prepared sodium naphthalenide solution (0.6 mL) at was added −78 °C. After being stirred for 40 min at −78 °C, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 15:1) to amine. To a solution of amine in CH_3CN (0.8 mL), aq HF (46%, 20 μL, 0.46 mmol) was added. After being stirred for 12 h at ambient temperature, the reaction mixture was neutralized with $NaHCO_3$ (140 mg), filtered through Celite plug, and concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_3Cl/MeOH 10:1 → 0:1) to afford isomer **3** (3.2 mg, 10.7 μmol) in 89% yield for 2 steps.

$[\alpha]_D^{23}$ − 1.2 (*c* 0.50, MeOH); IR (KBr) 3430, 2920, 2850, 1646, 1385, 1051, 783 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 4.60 (1H, dd, J = 6.0, 6.0 Hz), 4.45–4.42 (1H, m), 4.40 (1H, dt, J = 5.9, 8.0 Hz), 3.97 (1H, dd, J = 12.3, 8.1 Hz), 3.85 (1H, dd, J = 12.3, 4.8 Hz), 1.90–1.80 (2H, m), 1.43–1.29 (27H, m), 0.90 (3H, t, J = 6.9 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 68.44, 66.07, 65.67, 59.34, 33.85, 31.56, 31.53, 31.42, 31.31, 31.24, 31.13, 28.00, 26.72, 24.51, 15.21. ESIMS *m*/*z* 300 ($M+H^+$). Calcd for $C_{18}H_{38}O_2N$ ($M+H^+$) *m*/*z* 300.2903. Found: *m*/*z* 300.2902.

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