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Diastereoselective Epoxidation of the Double Bond at C-4 of Sphingosines to Provide Phytosphingosine Relatives such as α-Galactosylceramide KRN7000†

Hirosato Takikawa, Shin-etsu Muto and Kenji Mori*

Department of Chemistry, Science University of Tokyo, Kagurazaka 1-3, Shinjuku-ku, Tokyo 162-0825. Japan

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Abstract: Diastereoselective epoxidation of the double bond at C-4 of two sphingosine derivatives [**3**, R = $(CH_2)_7Me$ or $(CH_2)_{12}Me$] was studied. Dimethyldioxirane was found to be the best oxidant for that purpose. Application of this epoxidation resulted in a new synthesis of the α-galactosylceramide KRN7000 (**2**), which has an enhancing effect on the activity of natural killer cells.

Studies on sphingosine and its relatives are now flourishing due to their important role in biological processes especially on cell surfaces. Since 1982 we have worked on the synthesis of sphingosine relatives,[1] and recently reported the synthesis of penaresidin A (**1a**), B (**1b**)[2] and penazetidine A (**1c**).[3] They are unusual sphingosines with an azetidine ring. As shown in Figure 1, one of the key steps for the synthesis of penaresidins A, B and penazetidine A was the epoxidation of the double bond at C-4 of the sphingosine derivatives **3** to give **4** followed by its conversion to the target molecules via phytosphingosines **5**.

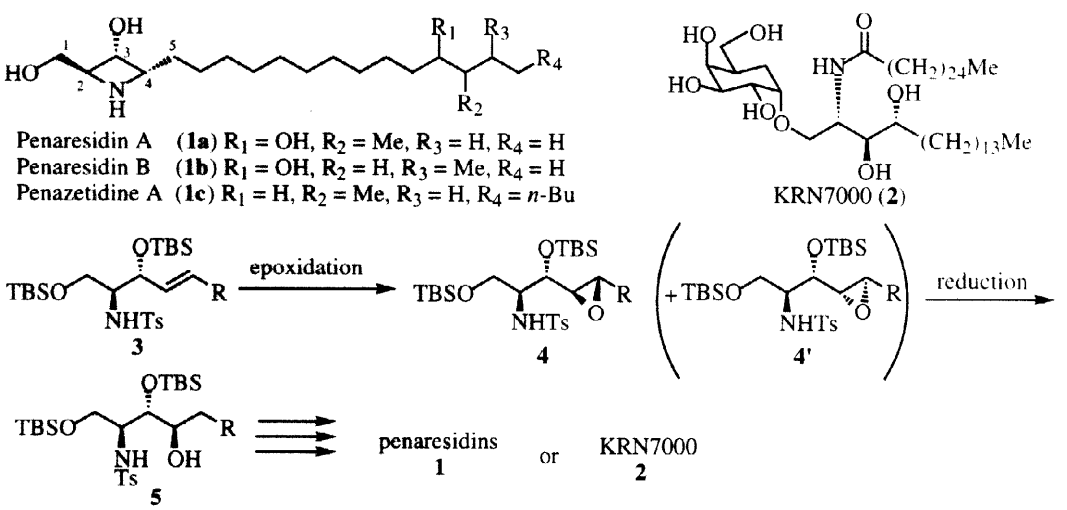

Figure 1. Structures of Penaresidins A and B, Penazetidine A, and KRN7000.

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Unfortunately, however, the diastereoselectivity of this epoxidation was unsatisfactory (**4** : **4'** = ca. 2 : 3).[2c,2d,3b] To circumvent this drawback, we examined various reagents for epoxidation under different conditions as listed in Table 1. After all, our attempts failed to find out appropriate conditions for selective epoxidation.[4] Our effort to convert **4'** back to **3** was also in vain.[5]

Our further study on this epoxidation to improve the diastereoselectivity revealed dimethyldioxirane (DMD) to be the reagent of choice for the synthesis of **4**. Epoxidation of **3** with DMD to give **4** as the major product facilitates the synthesis of phytosphingosines **5**. Indeed, the use of this reagent enabled us to achieve a new synthesis of the α-galactosylceramide KRN7000 (**2**). This new glycosphingolipid **2** markedly activates natural killer cells which are antitumor effector cells both in vitro and in vivo.[6]

Table 1. Epoxidation of **3** under various conditions.[a]

entry	reagents	temp.	time	ratio (**4**:**4'**)	isolated yield of **4**
1	*m*-CPBA, $NaHCO_3/CH_2Cl_2$	r.t.	12 h	33:67	21%
2[b]	*m*-CPBA, $NaHCO_3$/hexane	r.t.	48 h	40:60	40%
3	MMPP[f]/MeOH[7]	r.t.	72 h	42:58	9% (36%[c])
4	$AcOOH/CH_2Cl_2$	r.t.	20 h	34:66	20%[d]
5	TBHP[f], $VO(acac^f)_2/CH_2Cl_2$	40°C	24 h	—	N.R.
6	PhCN, $KHCO_3$, H_2O_2, MeOH[8]	r.t.	2 days	—	N.R.
7	TBHP, $Ti(O^iPr)_4$, DET[e,f]/CH_2Cl_2	-25°C-r.t.	5 days	—	N.R.

a) A model compound **3** (R = *n*-octyl) was employed as the substrate. b) These reaction conditions were applied to our synthesis of **1a**, **1b** and **1c**. c) This was the corrected yield based on the consumed **3**. d) This was the calculated yield based on HPLC analysis. e) Both the enantiomers were examined. f) MMPP (magnesium monoperoxyphthalate), TBHP (*t*-butyl hydroperoxide), acac (acetylacetonato), DET (diethyl tartrate)

Table 1 summarizes the results of our preliminary attempts for the diastereoselective epoxidation of **3**. Since all of the results were unsatisfactory, we reluctantly adopted the conditions of the entry 2 for our synthesis of **1a**, **1b** and **1c**. The low diastereoselectivity in the epoxidation and the poor reactivity of **3** were presumably due to the presence of the hydrophobic and bulky side-chain. In a polar solvent the hydrophobic and long side-chain of **3** might behave as a bulky shield to prevent the attack of the oxidant. We therefore reasoned that the key to solve this problem might depend on the reduction in the size of the oxidant and choice of a less polar solvent.

Accordingly, we selected a smaller oxidant, dimethyldioxirane (DMD), as the candidate and carried out a series of experiments as shown in Table 2. The conventional methods of employing DMD generated in situ did not work (entries 1 and 2). The failure was thought to be due to the presence of water in the solvent system. Thus in the subsequent attempt DMD was used as an acetone solution (entry 3) according to Murray and

Jeyaraman.[9] This resulted in the surprising reversal in the ratio of **4** : **4'** to give the desired **4**. A similar reversal of diastereoselectivity was also observed by Kocienski et al. in the case of the epoxidation of an allylic alcohol with dioxirane.[10] Our experiments were continued to find out the optimum conditions. At a low temperature (-23°C) the reaction became very sluggish, although the diastereoselectivity could be improved (see entries 3-5). Use of a co-solvent was then attempted, because Murray et al. reported an interesting co-solvent effect.[11] In our case, however, no distinct effect caused by co-solvents could be observed (entries 6-10). Further modification using ethylmethyldioxirane (EMD) in 2-butanone[9] did not cause any remarkable improvement (entries 11 and 12). Consequently the conditions listed in the entry 4 was selected as the best one for preparative works to obtain the desired stereoisomer **4** as the major product.

Table 2. Epoxidation of **3** using dioxiranes.[a]

entry	reagents	temp.	time	ratio (**4**:**4'**)	isolated yield of **4**
1	Oxone®[d]/acetone-H_2O	r.t	48 h	—	N.R.
2	Oxone®, acetone, 18-crown-6	6-8°C	48 h	—	trace
3	DMD/acetone	r.t.	24 h	80:20	56% (62%[b])
4	DMD/acetone	4°C	72 h	84:16	57% (67%[b])
5	DMD/acetone	-23°C	96 h	87:13	trace
6	DMD/acetone+hexane	r.t.	24 h	58:42	(16%[c], 73%[b,c])
7	DMD/acetone+MeOH	r.t.	24 h	—	trace
8	DMD/acetone+CH_2Cl_2	r.t.	24 h	71:29	(30%[c], 38%[b,c])
9	DMD/acetone+$CHCl_3$	r.t.	24 h	—	trace
10	DMD/acetone+CCl_4	r.t.	24 h	80:20	(52%[c], 73%[b,c])
11	EMD/2-butanone	r.t.	96 h	82:18	47% (58%[b])
12	EMD/2-butanone	4°C	96 h	72:18	trace

a) A model compound **3** (R = *n*-tridecyl) was employed as the substrate. b) This was the corrected yield based on the consumed **3**. c) This was the calculated yield based on HPLC analysis. d) Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$)

This improvement in the diastereoselectivity of epoxidation had two implications. Firstly, the drawback of our previous synthesis of penaresidins A, B[2d] and penazetidine A[3b] was overcome. Secondly, the selective epoxidation enabled a new and synthesis of phytosphingosines and its relative KRN7000.

Figure 2 shows our synthetic route leading to KRN7000 (**2**). The C_{18}-sphingosine **7** was prepared from (*S*)-serine and 1-pentadecyne by means of Garner's general sphingosine synthesis.[12] It was then converted into the protected sphingosine **8b** in two steps according to our procedure.[2d] This was employed for the key epoxidation step using DMD, and the desired β-epoxide **9** was obtained in 69% yield (α:β = 17:83). The reductive opening of the epoxide **9** with diisobutylaluminum hydride (DIBAL) in toluene took place

regioselectively to give the alcohol **10a** in 88% yield. After the removal of the *N*-Ts group of **10a** by treatment with sodium naphthalenide (83%), the amino group of **10b** was acylated selectively with *p*-nitrophenyl hexacosanoate to furnish the amide **11a** in 70% yield. The 1-*O*-TBS group was then selectively cleaved, and the resulting 1,4-diol was employed for the glycosylation. Glycosylation, however, did not take place with this substrate. We therefore protected the 4-OH of **11a** as the TBS ether to give **11b** in 96% yeild. The selective cleavage of the 1-*O*-TBS group was achieved by treatment of **11b** with diluted trifluoroacetic acid to give **11c** in 70% yield.[13] Glycosylation of **11c** with tetra-*O*-benzyl α-D-galactopyranosyl fluoride

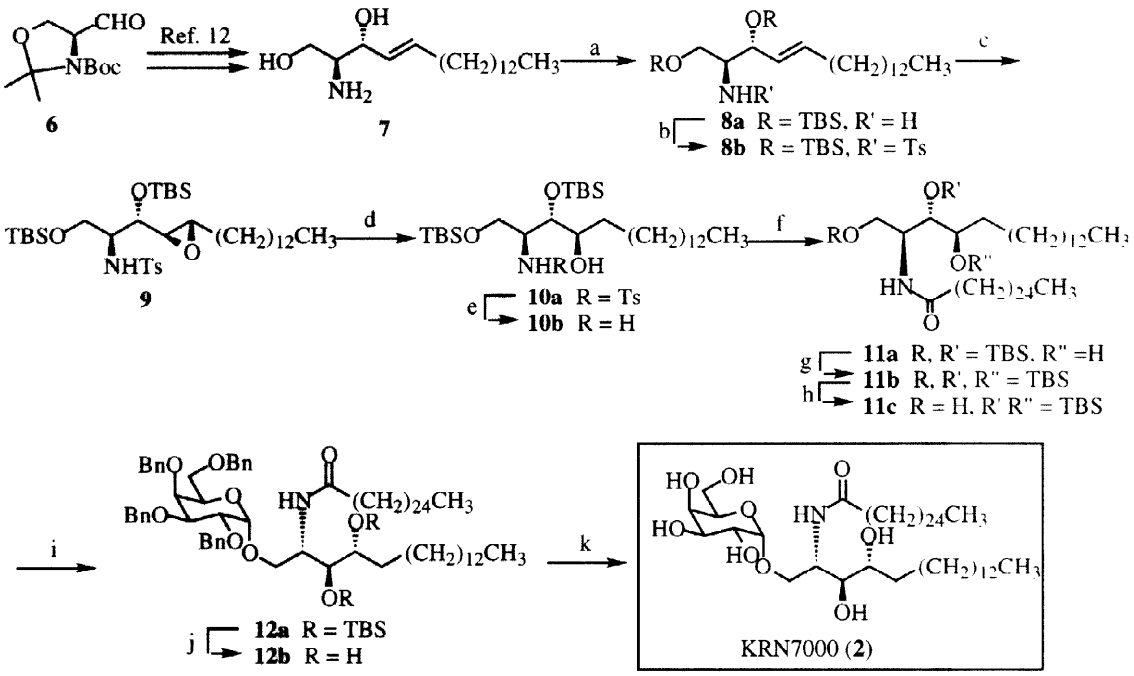

Figure 2. Synthesis of KRN7000 (**2**).

Reagents: a) TBSOTf, 2,6-lutidine, CH_2Cl_2 (80%). – b) TsCl, pyridine (97%). – c) DMD, acetone, 4°C (69%). – d) DIBAL, toluene (88%). – e) Na, naphthalene, DME (83%). – f) *p*-nitrophenyl hexacosanoate, pyridine (70%). – g) TBSOTf, 2,6-lutidine, CH_2Cl_2 (96%). – h) CF_3CO_2H, H_2O, THF (70%). – i) *O*-Bn-α-fluorogalactose, Sn_2Cl_2, $AgClO_4$, MS 4A, THF (53%). – j) TBAF, THF (100%). – k) 20% $Pd(OH)_2$-C, H_2, $CHCl_3$, EtOH (71%).

under Mukaiyama's conditions[14] yielded α-galactopyranoside **12a** in 53% yield. After removal of the TBS group of **12a** (quantitative), the cleavage of benzyl groups was executed by hydrogenolysis to give KRN7000 (**2**) in 71% yield, mp 189.5-190.0°C (lit.[6a] mp 189.5-190.5°C) and $[\alpha]_D^{23}$ = +42.2 (pyridine) [lit.[6a] $[\alpha]_D^{23}$ = +43.6 (pyridine)]. The overall yield of **2** was 8.7% in 9 steps based on **8b**. The IR, ^1H and ^{13}C NMR spectral data of our KRN7000 are in good accord with those reported.[6]

In summary, diastereoselective epoxidation of protected sphingosines **3** to yield **4** was achieved with dimethyldioxirane in acetone at 4°C. This selective epoxidation was employed in the new synthesis of KRN7000, a phytosphingosine relative.

Experimental

All mps were uncorrected. Mps were measured with Yanaco micro-melting point apparatus. IR spectra were measured as films for oils or as KBr disks for solids on a Jasco IRA-102 spectrometer. ^{1}H NMR spectra were recorded at 90 MHz on a Jeol JNM-EX 90A spectrometer, at 300 MHz on a Bruker DPX 300 spectrometer, at 500 MHz on a Jeol GSX 500 spectrometer. The peak for TMS or solvent ($CHCl_3$: δ 7.26, C_5D_4HN: δ 7.55) was used as an internal standard. ^{13}C NMR spectra were recorded at 125 MHz on a Jeol GSX 500 spectrometer. Solvent peak (C_5D_5N: δ 135.5) was used as an internal standard. Optical rotations were measured on a Jasco DIP-1000 polarimeter. Mass spectra were recorded on a Jeol JMS-SX102A mass spectrometer. Refractive indices were measured on an ATAGO Abbe refractometer 1T.

(2*S*,3*R*,4*E*)-2-Amino-1,3-bis-*t*-butyldimethylsilyloxy-4-octadecene (8a)

To a solution of **7** (6.91 g, 23.1 mmol) and 2,6-lutidine (13.4 ml, 115 mmol) in CH_2Cl_2 (70 ml), TBSOTf (21.2 ml, 92.3 mmol) was added at 0 °C under Ar. The reaction mixture was stirred for 1 h at room temperature and then quenched with MeOH. It was then poured into water and extracted with diethyl ether. The extract was washed with water, saturated aq. $NaHCO_3$ and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *amine* **8a** (9.72 g, 80%) as an oil, n_D^{26} 1.4510; Anal. Calcd. for $C_{30}H_{65}NO_2Si_2$: C, 68.24; H, 12.41; N, 2.65, Found. C, 67.95; H, 12.64; N, 2.77; $[\alpha]_D^{23}$ = -1.2 (*c* 1.0 in $CHCl_3$); IR (film)/cm^{-1} 3400 (w, N-H), 1255 (m, Si-Me); δ_H(90 MHz; $CDCl_3$) 0.01 and 0.04 (total 12H, each s, Si-Me), 0.70-0.96 (21H, m, *t*-Bu, 18-H), 1.26 (24H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H and NH_2), 2.00 (2H, m, 6-H), 2.75 (1H, m, 2-H), 3.60 (2H, m, 1-H), 4.00 (1H, t, *J* = 6.0 Hz, 3-H), 5.22-5.80 (2H, m, 4, 5-H).

(2*S*,3*R*,4*E*)-1,3-bis-*t*-Butyldimethylsilyloxy-2-*p*-toluenesulfonylamino-4-octadecene (8b)

To an ice-cooled solution of **8a** (2.16 g, 3.71 mmol) in dry pyridine (20 ml), TsCl (1.57 g, 8.23 mmol) was added, and the stirring was continued for 24 h at room temperature. The reaction mixture was then poured into dil. HCl and extracted with diethyl ether. The extract was washed with saturated aq. $CuSO_4$, water, saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *amide* **8b** (2.46 g, 97%) as a colorless oil, n_D^{24} 1.4837; Anal. Calcd. for $C_{37}H_{71}NO_4SSi_2$: C, 65.14; H, 10.49; N, 2.05, Found. C, 65.11; H, 10.90; N, 2.02; $[\alpha]_D^{24}$ = -1.9 (*c* 1.0 in $CHCl_3$); IR (film)/cm^{-1} 3290 (m, N-H), 1600 (w, aromatic), 1335 (m, SO_2), 1255 (m, Si-Me), 1170 (s, SO_2), 1080 (s, Si-O); δ_H(90 MHz; $CDCl_3$) -0.05 (6H, s, Si-Me), -0.01 (6H, s, Si-Me), 0.77-0.95 (21H, m, *t*-Bu, 18-H), 1.26 (22H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H), 1.92 (2H, m, 6-H), 2.41 (3H, s, Ar-Me), 3.11 (1H, m, 2-H), 3.45 (1H, dd, *J* = 10.2, 6.0 Hz, 1-Ha), 3.81 (1H, dd, *J* = 10.2, 4.2 Hz, 1-Hb), 4.24 (1H, t, *J* = 6.2 Hz, 3-H), 4.63 (1H, d, *J* = 6.7 Hz, N-H), 5.21 (1H, dd, *J* = 15.5, 6.8 Hz, 4-H), 5.60 (1H, dt, *J* = 15.5, 6.6 Hz, 5-H), 7.27 (2H, d, *J* = 8.4 Hz, *m*-Ar), 7.74 (2H, d, *J* = 8.4 Hz, *o*-Ar).

(2*S*,3*S*,4*R*,5*S*)-1,3-bis-*t*-Butyldimethylsilyloxy-4,5-epoxy-2-*p*-toluenesulfonylamino-4-octadecane (9)

To **8b** (579 mg, 0.849 mmol), a solution of DMD in acetone (0.03-0.05 M, 100 ml) was added at 0 °C, and the stirring was continued for 84 h at 4°C. The reaction mixture was concentrated under reduced pressure.

The residue was chromatographed over SiO_2 to give the undesired α-epoxide (84.2 mg, 14%) and the β-*epoxide* **9** (406 mg, 69%) as a colorless oil, n_D^{24} 1.4840; Anal. Calcd. for $C_{37}H_{71}NO_5SSi_2$: C, 63.65; H, 10.25; N, 2.01, Found. C, 63.34; H, 9.86; N, 1.86; $[\alpha]_D^{24}$ = -18.8 (*c* 1.00 in $CHCl_3$); IR (film)/cm^{-1} 3300 (m, N-H), 1600 (w, aromatic), 1335 (m, SO_2), 1255 (m, Si-Me), 1165 (s, SO_2); δ_H(300 MHz; $CDCl_3$) -0.05, 0.02, 0.04 and 0.05 (total 12H, each s, Si-Me), 0.82 (9H, s, *t*-Bu), 0.84 (9H, s, *t*-Bu), 0.85 (3H, t, *J* = 7.2 Hz, 18-H), 1.26 (24H, br. s, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H), 2.41 (3H, s, Ar-Me), 2.66 (1H, dd, *J* = 5.1, 2.1 Hz, 4-H), 2.78 (1H, m, 5-H), 3.27 (1H, m, 2-H), 3.55 (1H, dd, *J* = 10.4, 4.9 Hz, 1-Ha), 3.71 (1H, dd, *J* = 10.4, 4.9 Hz, 1-Hb), 3.77 (1H, t, *J* = 5.1 Hz, 3-H), 4.76 (1H, d, *J* = 6.8 Hz, N-H), 7.27 (2H, d, *J* = 8.3 Hz, *m*-Ar), 7.75 (2H, d, *J* = 8.3 Hz, *o*-Ar).

(2*S*,3*S*,4*R*)-1,3,-bis-*t*-Butyldimethylsilyloxy-2-*p*-toluenesulfonylamino-4-octadecanol (10a)

To a stirred and cooled solution of **9** (1.79 g, 2.56 mmol) in dry toluene (10 ml), DIBAL (1.01 M in toluene; 12.7 ml, 12.6 mmol) was added dropwise at -78 °C under Ar. This mixture was then warmed gradually to -10 °C with stirring during 4 h and quenched with saturated aq. Rochelle's salt and then diethyl ether was added. The resulting slurry was stirred at room temperature until two clear layers separated. The layers were separated, and the aqueous layer was extracted with diethyl ether. The extract was washed with water and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *alcohol* **10a** (1.58 g, 88%) as a colorless oil, n_D^{24} 1.4860; Anal. Calcd. for $C_{37}H_{73}NO_5SSi_2$: C, 63.47; H, 10.51; N, 2.00, Found. C, 63.25; H, 10.36; N, 2.11; $[\alpha]_D^{23}$ = -0.4 (*c* 0.5 in $CHCl_3$); IR (film)/cm^{-1} 3550 (m, O-H), 3300 (m, N-H), 1600 (w, aromatic), 1335 (m, SO_2), 1255 (m, Si-Me), 1170 (s, SO_2); δ_H(300 MHz; $CDCl_3$) -0.05, -0.02, 0.09 and 0.12 (total 12H, each s, Si-Me), 0.82 (9H, s, *t*-Bu), 0.85 (3H, t, *J* = 7.2 Hz, 18-H), 0.88 (9H, s, *t*-Bu), 1.20-1.55 (26H, m, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H), 2.42 (3H, s, Ar-Me), 2.57 (1H, d, *J* = 5.3 Hz, O-H), 3.42 (1H, m, 4-H), 3.55-3.60 (2H, m, 1-Ha, 2-H), 3.69 (1H, dd, *J* = 10.2, 6.5 Hz, 1-Hb), 3.81 (1H, dd, *J* = 4.7, 3.2 Hz, 3-H), 4.82 (1H, d, *J* = 6.7 Hz, N-H), 7.29 (2H, d, *J* = 8.3 Hz, *m*-Ar), 7.73 (2H, d, *J* = 8.3 Hz, *o*-Ar).

(2*S*,3*S*,4*R*)-2-Amino-1,3,-bis-*t*-butyldimethylsilyloxy-4-octadecanol (10b)

Preparation of sodium naphthalenide: To a stirred solution of naphthalene (1.90 g, 14.8 mmol) in dry DME (15 ml), Na (270 mg, 11.7 mmol) was added under Ar. The mixture was stirred for 2 h at room temperature. —Detosylation of **10a**: To a solution of **10a** (1.09 g, 1.56 mmol) in DME (20 ml), the prepared sodium naphthalenide was added dropwise at -78 °C under Ar. The reaction mixture was then warmed gradually to -10 °C with stirring in the course of 3 h and diluted with water. The reaction mixture was extracted with diethyl ether. The extract was washed with water and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give recovered **10a** (377 mg, 35%) and the *amine* **10b** (462 mg, 83% based on consumed **10a**) as an oil, n_D^{24} 1.4612; Anal. Calcd. for $C_{30}H_{67}NO_3Si_2$: C, 65.99; H, 12.37; N, 2.57, Found. C, 66.32; H, 12.53; N, 2.52; $[\alpha]_D^{26}$ = +2.7 (*c* 1.0 in $CHCl_3$); IR (film)/cm^{-1} 3360 (m, O-H), 3150 (m, N-H), 1255 (m, Si-Me); δ_H(300 MHz; $CDCl_3$) 0.07 and 0.08 (total 12H, each s, Si-Me), 0.88 (3H, t, *J* = 7.0 Hz, 18-H), 0.89 (9H, s, *t*-Bu), 0.90 (9H, s, *t*-Bu), 1.20-1.35 (22H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-H), 1.42-1.65 (6H, m, 5, 6-H, NH_2), 2.60-2.90 (1H, br.

OH), 2.86 (1H, m, 2-H), 3.47 (1H, dd, J = 7.2, 4.5 Hz, 3-H), 3.66 (1H, dd, J = 9.9, 6.0 Hz, 1-Ha), 3.80 (1H, dd, J = 9.9, 3.3 Hz, 1-Hb), 3.86 (1H, m, 4-H).

(2*S*,3*S*,4*R*)-1,3,-bis-*t*-Butyldimethylsilyloxy-2-hexacosanoylamino-4-octadecanol (11a)

To a solution of **10b** (185 mg, 0.339 mmol) in dry pyridine (5 ml), *p*-nitrophenyl hexacosanoate (190 mg, 0.367 mmol) was added, and the stirring was continued for 24 h at room temperature. The reaction mixture was then poured into water and extracted with diethyl ether. The extract was washed with saturated aq. $CuSO_4$, water, saturated aq. $NaHCO_3$ and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *amide* **11a** (216 mg, 69%) as a colorless oil, n_D^{25} 1.4674; Anal. Calcd. for $C_{56}H_{117}NO_4Si_2$: C, 72.74; H, 12.75; N, 1.52, Found. C, 73.17; H, 12.94; N, 1.59; $[\alpha]_D^{26}$ = +15.4 (*c* 1.00 in $CHCl_3$); IR (film)/cm^{-1} 3450-3350 (m, O-H and N-H), 1640 (m, C=O), 1255 (m, Si-Me); δ_H(300 MHz; $CDCl_3$) 0.057, 0,064 and 0.08 (total 12H, each s, Si-Me), 0.84-0.90 (24H, m, 18-, 26'-H and *t*-Bu), 1.20-1.35 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.35-1.70 (6H, m, 5, 6, 3'-H), 2.19 (2H, br. t, J = 7.3 Hz, 2'-H), 3.19 (1H, d, J = 6.6 Hz, OH), 3.60 (1H, dt, J = 5.9, 5.9 Hz, 4-H), 3.71-3.75 and 4.06-4.08 (total 4H, each m, 1-H, 2-H, 3-H), 6.07 (1H, d, J = 8.8 Hz, N-H).

(2*S*,3*S*,4*R*)-1,3,4-tris-*t*-Butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecane (11b)

To a solution of **11a** (121 mg, 0.131 mmol) and 2,6-lutidine (60 µl, 0.51 mmol) in CH_2Cl_2 (1.5 ml), TBSOTf (80 µl, 0.35 mmol) was added at 0 °C under Ar. The reaction mixture was stirred for 1 h at room temperature and then quenched with MeOH. It was then poured into water and extracted with diethyl ether. The extract was washed with water, saturated aq. $NaHCO_3$ and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *bis-TBS ether* **11b** (131 mg, 96%) as an oil, n_D^{24} 1.4636; Anal. Calcd. for $C_{62}H_{131}NO_4Si_3$: C, 71.67; H, 12.71; N, 1.35, Found. C, 72.09; H, 12.96; N, 1.35; $[\alpha]_D^{25}$ = +3.0 (*c* 1.0 in $CHCl_3$); IR (film)/cm^{-1} 3450 (w, N-H), 1685 (m, C=O), 1250 (m, Si-Me); δ_H(300 MHz; $CDCl_3$) 0.03, 0.046, 0.054, 0.10 and 0.12 (total 18H, each s, Si-Me), 0.84-0.91 (33H, m, 18-, 26'-H and *t*-Bu), 1.20-1.40 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.40-1.65 (6H, m, 5, 6, 3'-H), 2.14 (2H, br. t, J = 7.3 Hz, 2'-H), 3.62 (1H, dd, J = 10.0, 4.4 Hz, 1-Ha), 3.62 (1H, m, 4-H), 3.82 (1H, dd, J = 7.3, 1.0 Hz, 3-H), 3.87 (1H, dd, J = 10.0, 4.1 Hz, 1-Hb), 3.94 (1H, m, 2-H), 5.82 (1H, d, J = 8.5 Hz, N-H).

(2*S*,3*S*,4*R*)-3,4,-bis-*t*-Butyldimethylsilyloxy-2-hexacosanoylamino-4-octadecanol (11c)

To a solution of **11b** (346 mg, 0.333 mmol) in THF (4 ml), CF_3CO_2H (~10% in water, 1.5 ml) was added dropwise at -10 °C. The reaction mixture was then warmed gradually to 10 °C with stirring during 2.5 h and neutralized with dil. NaOH. The reaction mixture was extracted with diethyl ether. The extract was washed with water, saturated aq. $NaHCO_3$ and brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *alcohol* **11c** (216 mg, 70%) as a colorless oil, n_D^{24} 1.4712; Anal. Calcd. for $C_{56}H_{117}NO_4Si_2$: C, 72.74; H, 12.75; N, 1.52, Found. C, 72.53; H, 12.74;

N, 1.53; $[\alpha]_D^{26}$ = -10.9 (*c* 1.00 in $CHCl_3$); IR (film)/cm^{-1} 3400-3280 (m, O-H and N-H), 1640 (m, C=O), 1250 (m, Si-Me); δ_H(500 MHz; $CDCl_3$) 0.08 and 0.11 (total 12H, s, Si-Me), 0.88 (6H, t, *J* = 7.4 Hz, 18-, 26'-H), 0.91 (9H, s, *t*-Bu), 0.92 (9H, s, *t*-Bu), 1.20-1.36 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.48-1.66 (6H, m, 5, 6, 3'-H), 2.18 (2H, br. t, *J* = 6.7 Hz, 2'-H), 3.16 (1H, dd, *J* = 9.1, 3.4 Hz, OH), 3.59 (1H, ddd, *J* = 11.3, 9.1, 3.6 Hz, 1-Ha), 3.76 (1H, dt, *J* = 2.8, 6.4 Hz, 4-H), 3.90 (1H, t, *J* = 2.8 Hz, 3-H), 4.06 (1H, m, 2-H), 4.21 (1H, dt, *J* = 11.3, 3.0 Hz, 1-Hb), 6.24 (1H, d, *J* = 7.9 Hz, N-H).

(2*S*,3*S*,4*R*)-3,4,-bis-*t*-Butyldimethylsilyloxy-2-hexacosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane (12a)

To a solution of **11c** (100 mg, 0.108 mmol) in THF (2 ml), stannous chloride (61.5 mg, 0.324 mmol), silver perchlorate (68.2 mg, 0.329 mmol) and molecular sieves 4A powder (500 mg) were added, and the stirring was continued for 30 min at room temperature. A solution of benzylgalactosyl fluoride (91.2 mg, 0.168 mmol) in THF (2 ml) was added at -10 °C. The reaction mixture was then warmed gradually to room temperature with stirring in the course of 2 h, and filtered through Celite. The filter cake was washed with diethyl ether. The combined filtrate and washings were washed with brine, dried (K_2CO_3), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *compound* **12a** (82 mg, 53%) as a colorless oil, n_D^{25} 1.5021; $[\alpha]_D^{24}$ = +15.4 (*c* 1.00 in $CHCl_3$); positive HR FABMS *m/z* 1447.1011 (M+H) (Calcd. for $C_{90}H_{152}NO_9Si_2$ 1447.1006); IR (film)/cm^{-1} 3360 (w, N-H), 1680 (m, C=O), 1250 (m, Si-Me); δ_H(300 MHz; $CDCl_3$) 0.030, 0.034, 0.06 and 0.08 (total 12H, each s, Si-Me), 0.86 (6H, m, 18-, 26'-H), 0.88 (9H, s, *t*-Bu), 0.90 (9H, s, *t*-Bu), 1.15-1.38 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.48-1.68 (6H, m, 5, 6, 3'-H), 1.99 (2H, br. t, *J* = 7.3 Hz, 2'-H), 3.50 (2H, m, 6"-H), 3.65 (1H, m, 4-H), 3.75-3.97 (6H, m, 1-Ha, 3, 2", 3", 4", 5"-H), 4.04 (1H, dd, *J* = 10.0, 3.6 Hz, 1-Hb), 4.09 (1H, m, 2-H), 4.38-4.81 (7H, m, ph-C*H*), 4.84 (1H, d, *J* = 3.6 Hz, 1"-H), 4.92 (1H, d, *J* = 11.4 Hz, ph-C*H*), 6.04 (1H, d, *J* = 7.1 Hz, N-H), 7.26-7.34 (20H, m, Ar-H).

(2*S*,3*S*,4*R*)-2-Hexacosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl) octadecane-3,4-diol (12b)

To a solution of **12a** (50 mg, 0.035 mmol) in THF (2 ml), TBAF (1.0 M in THF; 0.14 ml, 0.14 mmol) was added dropwise at room temperature. After stirring for 1.5 h, the reaction mixture was diluted with water and extracted with diethyl ether. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *diol* **12b** (42 mg, 100%) as a solid, $[\alpha]_D^{25}$ = +33.3 (*c* 1.00 in $CHCl_3$); mp 70.5-71.5°C; positive HR FABMS *m/z* 1218.9269 (M+H) (Calcd. for $C_{78}H_{124}NO_9$ 1218.9276); IR (KBr)/cm^{-1} 3320 (O-H), 1640 (C=O), 1620, 1550; δ_H(300 MHz; $CDCl_3$) 0.88 (6H, m, 18-, 26'-H), 1.15-1.35 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.36-1.83 (6H, m, 5, 6, 3'-H), 2.12 (2H, t, *J* = 8.1 Hz, 2'-H), 2.15-2.30 (1H, br, OH), 3.46-3.52 (4H, m, 4, 6"-H, OH), 3.83-3.96 (6H, m, 1-Ha, 3, 2", 3", 4", 5"-H), 4.04 (1H, dd, *J* = 10.0, 3.7 Hz, 1-Hb), 4.20

(1H, m, 2-H), 4.36-4.93 (9H, m, 1"-H, ph-C*H*), 6.42 (1H, d, *J* = 8.2 Hz, N-H), 7.24-7.37 (20H, m, Ar-H).

KRN7000, (2*S*,3*S*,4*R*)-1-(α-D-Galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol (2)

To a solution of **12b** (36 mg, 0.030 mmol) in EtOH (2 ml) and $CHCl_3$ (0.5 ml), $Pd(OH)_2$ (20% on carbon, 150 mg) was added, and the stirring was continued vigorously at room temperature for 24 h under H_2. It was filtered through Celite, and the filter cake was washed with $CHCl_3$ and MeOH. The combined filtrate and washings were concentrated under reduced pressure. The residue was chromatographed over SiO_2 to give the *compound* **2** (18 mg, 85%) as a colorless solid, $[\alpha]_D^{23}$ = +42.2 (*c* 0.54 in pyridine) [lit.[6a] $[\alpha]_D^{23}$ = +43.6 (*c* 1.0 in pyridine)]; mp 189.5-190.0°C (lit.[6a] mp 189.5-190.5°C); positive HR FABMS *m/z* 858.7380 (M+H) (Calcd. for $C_{56}H_{117}NO_4Si_2$ 858.7398); ν_{max}(KBr)/cm^{-1} 3300, 2930, 2850, 1640, 1540, 1470, 1070; δ_H(500 MHz; C_5D_5N) 0.85 (6H, m, 18, 26'-H), 1.17-1.45 (66H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13', 14', 15', 16', 17', 18', 19', 20', 21', 22', 23', 24', 25'-H), 1.60-1.68 (1H, m, 6-Ha), 1.76-1.82 (2H, m, 3'-H), 1.83-1.92 (2H, m, 5-Ha, 6-Hb), 2.21-2.28 (1H, m, 5-Hb), 2.49 (2H, t, *J* = 7.3 Hz, 2'-H), 4.25-4.35 (2H, m, 3, 4-H), 4.38 (3H, m, 1-Ha, 6"-H), 4.47-4.52 (2H, m, 4", 5"-H), 4.55 (1H, m, 3"-H), 4.62-4.67 (2H, m, 1-Hb, 2"-H), 5.26 (1H, m, 2-H), 5.55 (1H, d, *J* = 3.6 Hz, 1"-H), 8.79 (1H, d, *J* = 8.9 Hz, N-H); δ_C(125 MHz; C_5D_5N) 14.3, 22.9, 26.4, 26.5, 29.6, 29.7, 29.82, 29.87, 29.92, 29.99, 30.02, 30.12, 30.4, 32.1, 34.4, 36.8, 51.4, 62.6, 68.6, 70.3, 70.9, 71.6, 72.6, 72.9, 76.6, 101.3, 173.5.

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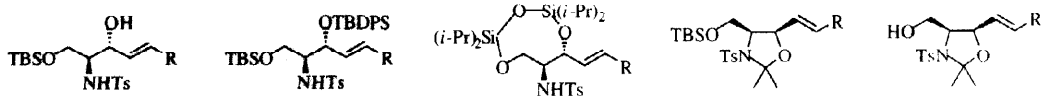
4. We prepared modified substrates as shown below and examined epoxidation of these compounds under various conditions. However, we could not obtain any satisfactory result.



a b c d e

5. We made efforts to convert the undesired epoxide **4'** back to the olefin **3** under various conditions, but in vain. Conditions: (a) WCl_6, *n*-BuLi/THF[15] (b) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$[16] (c) 3-Methylbenzothiazole -2-selone[17] (d) TMSCl, NaI,[18] etc.
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