

Tetrahedron 54 (1998) 3141-3150

TETRAHEDRON

# Diastereoselective Epoxidation of the Double Bond at C-4 of Sphingosines to Provide Phytosphingosine Relatives such as $\alpha$ -Galactosylceramide KRN7000<sup>+</sup>

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Received 19 December 1997; accepted 19 January 1998

**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  $\alpha$ -galactosylceramide KRN7000 (2), which has an enhancing effect on the activity of natural killer cells.  $\bigcirc$  1998 Elsevier Science Ltd. All rights reserved.

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.<sup>1</sup> and recently reported the synthesis of penaresidin A (1a), B (1b)<sup>2</sup> and penazetidine A (1c).<sup>3</sup> 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.

<sup>†</sup>Synthesis of Sphingosine Relatives, Part 20. For Part 19, see ref. 2d.

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.<sup>4</sup> Our effort to convert 4' back to 3 was also in vain.<sup>5</sup>

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  $\alpha$ -galactosylceramide KRN7000 (2). This new glycosphingolipid 2 markedly activates natural killer cells which are antitumor effector cells both in vitro and in vivo.<sup>6</sup>

entry	reagents	temp.	time	ratio (4:4')	isolated yield of 4
1	m-CPBA, NaHCO <sub>3</sub> /CH <sub>2</sub> Cl <sub>2</sub>	r.t.	12 h	33:67	21%
2 <sup>b</sup>	m-CPBA, NaHCO <sub>3</sub> /hexane	r.t.	48 h	40:60	40%
3	MMPPf/MeOH7	r.t.	72 h	42:58	9% (36%°)
4	AcOOH/CH <sub>2</sub> Cl <sub>2</sub>	r.t.	20 h	34:66	20% <sup>d</sup>
5	TBHPf, VO(acacf)2/CH2Cl2	40°C	24 h	_	N.R.
6	PhCN, KHCO <sub>3</sub> , H <sub>2</sub> O <sub>2</sub> , MeOH <sup>8</sup>	r.t.	2 days		N.R.
7	TBHP, Ti(OiPr)4, DETe,f/CH2Cl2	-25°C-r.t.	5 days	-	N.R.

Table 1. Epoxidation of 3 under various conditions.<sup>a</sup>

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

entries 3-5). Use of a co-solvent was then attempted, because Murray et al. reported an interesting co-solvent effect.<sup>11</sup> 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<sup>9</sup> 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.

entry	reagents	temp.	time	ratio (4:4')	isolated yield of 4
1	Oxone®d/acetone-H <sub>2</sub> O	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% <sup>h</sup> )
4	DMD/acetone	4°C	72 h	84:16	57% (67% <sup>h</sup> )
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 <sub>2</sub> Cl <sub>2</sub>	r.t.	24 h	71:29	(30%°, 38% <sup>h.c</sup> )
9	DMD/acetone+CHCl <sub>3</sub>	r.t.	24 h	_	trace
10	DMD/acetone+CCl <sub>4</sub>	r.t.	24 h	80:20	(52%c, 73%b.c)
11	EMD/2-butanone	r.t.	96 h	82:18	47% (58% <sup>h</sup> )
12	EMD/2-butanone	4°C	96 h	72:18	trace

Table 2. Epoxidation of 3 using dioxiranes.<sup>a</sup>

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<sub>5</sub>•KHSO<sub>4</sub>•K<sub>2</sub>SO<sub>4</sub>)

This improvement in the diastereoselectivity of epoxidation had two implications. Firstly, the drawback of our previous synthesis of penaresidins A, B<sup>2d</sup> and penazetidine A<sup>3b</sup> 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.<sup>12</sup> It was then converted into the protected sphingosine **8b** in two steps according to our procedure.<sup>2d</sup> This was employed for the key epoxidation step using DMD, and the desired  $\beta$ -epoxide 9 was obtained in 69% yield ( $\alpha:\beta = 17:83$ ). 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.<sup>13</sup> Glycosylation of 11c with tetra-O-benzyl  $\alpha$ -D-galactopyranosyl fluoride



Figure 2. Synthesis of KRN7000 (2).

Reagents: a) TBSOTf, 2,6-lutidine, CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>Cl<sub>2</sub> (96%). - h) CF<sub>3</sub>CO<sub>2</sub>H, H<sub>2</sub>O, THF (70%). - i) *O*-Bn- $\alpha$ -fluorogalactose. Sn<sub>2</sub>Cl<sub>2</sub>. AgClO<sub>4</sub>, MS 4A, THF (53%). - j) TBAF, THF (100%). - k) 20% Pd(OH)<sub>2</sub>-C, H<sub>2</sub>, CHCl<sub>3</sub>, EtOH (71%).

under Mukaiyama's conditions<sup>14</sup> yielded  $\alpha$ -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.<sup>6a</sup> mp 189.5-190.5°C) and  $[\alpha]_D^{23} = +42.2$  (pyridine) [lit.<sup>6a</sup>  $[\alpha]_D^{23} = +43.6$  (pyridine)]. The overall yield of 2 was 8.7% in 9 steps based on 8b. The IR, <sup>1</sup>H and <sup>13</sup>C NMR spectral data of our KRN7000 are in good accord with those reported.<sup>6</sup>

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. <sup>1</sup>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<sub>3</sub>:  $\delta$  7.26, C<sub>5</sub>D<sub>4</sub>HN:  $\delta$  7.55) was used as an internal standard. <sup>13</sup>C NMR spectra were recorded at 125 MHz on a Jeol GSX 500 spectrometer. Solvent peak (C<sub>5</sub>D<sub>5</sub>N:  $\delta$  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.

#### (2S,3R,4E)-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<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub> and brine, dried (K<sub>2</sub>CO<sub>3</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *amine* **8a** (9.72 g. 80%) as an oil. n<sub>D</sub><sup>26</sup> 1.4510; Anal. Calcd. for C<sub>30</sub>H<sub>65</sub>NO<sub>2</sub>Si<sub>2</sub>: C, 68.24; H, 12.41; N, 2.65, Found. C, 67.95; H. 12.64; N. 2.77; [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -1.2 (*c* 1.0 in CHCl<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3400 (w, N-H), 1255 (m, Si-Me);  $\delta_{\rm H}$ (90 MHz; CDCl<sub>3</sub>) 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<sub>2</sub>), 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).

#### (2S,3R,4E)-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<sub>4</sub>, water. saturated aq. NaHCO<sub>3</sub> and brine, dried (MgSO<sub>4</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *amide* **8b** (2.46 g, 97%) as a colorless oil,  $n_D^{24}$  1.4837; Anal. Calcd. for C<sub>37</sub>H<sub>71</sub>NO<sub>4</sub>SSi<sub>2</sub>: 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<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3290 (m, N-H), 1600 (w, aromatic), 1335 (m, SO<sub>2</sub>), 1255 (m, Si-Me). 1170 (s. SO<sub>2</sub>), 1080 (s, Si-O);  $\delta_H$ (90 MHz; CDCl<sub>3</sub>) -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).

## (2S,3S,4R,5S)-1,3-bis-t-Butyldimethylsilyloxy-4,5-epoxy-2-p-toluenesulfonylamino-4octadecane (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<sub>2</sub> to give the undesired  $\alpha$ -epoxide (84.2 mg, 14%) and the  $\beta$ epoxide 9 (406 mg, 69%) as a colorless oil,  $n_D^{24}$  1.4840; Anal. Calcd. for C<sub>37</sub>H<sub>71</sub>NO<sub>5</sub>SSi<sub>2</sub>: 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<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3300 (m, N-H), 1600 (w, aromatic), 1335 (m, SO<sub>2</sub>), 1255 (m, Si-Me), 1165 (s, SO<sub>2</sub>);  $\delta_H(300 \text{ MHz}; \text{ CDCl}_3) - 0.05$ . 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).

#### (2S,3S,4R)-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<sub>2</sub> to give the *alcohol* **10a** (1.58 g, 88%) as a colorless oil,  $n_D^{24}$  1.4860; Anal. Calcd. for C<sub>37</sub>H<sub>73</sub>NO<sub>5</sub>SSi<sub>2</sub>: C, 63.47; H, 10.51; N, 2.00, Found. C, 63.25; H, 10.36; N, 2.11; [ $\alpha$ ]<sub>D</sub><sup>23</sup> = -0.4 (*c* 0.5 in CHCl<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3550 (m, O-H), 3300 (m, N-H), 1600 (w, aromatic), 1335 (m, SO<sub>2</sub>). 1255 (m. Si-Me), 1170 (s, SO<sub>2</sub>);  $\delta_{H}$ (300 MHz; CDCl<sub>3</sub>) -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).

### (2S,3S,4R)-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<sub>2</sub>CO<sub>3</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give recovered **10a** (377 mg, 35%) and the *amine* **10b** (462 mg, 83% based on consumed **10a**) as an oil, n<sub>D</sub><sup>24</sup> 1.4612; Anal. Calcd. for C<sub>30</sub>H<sub>67</sub>NO<sub>3</sub>Si<sub>2</sub>: 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<sub>3</sub>): IR (film)/cm<sup>-1</sup> 3360 (m, O-H), 3150 (m, N-H), 1255 (m, Si-Me);  $\delta_H(300 \text{ MHz}; \text{CDCl}_3)$  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<sub>2</sub>), 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).

#### (25,35,4R)-1,3,-bis-t-Butyldimethylsilyloxy-2-hexacosanoylamino-4-octadecanol (11a)

To a solution of 10b (185 mg, 0.339 mmol) in dry pyridine (5 mł), *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<sub>4</sub>, water, saturated aq. NaHCO<sub>3</sub> and brine, dried (K<sub>2</sub>CO<sub>3</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *amide* 11a (216 mg, 69%) as a colorless oil, n<sub>D</sub><sup>25</sup> 1.4674; Anal. Calcd. for C<sub>56</sub>H<sub>117</sub>NO<sub>4</sub>Si<sub>2</sub>: 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<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3450-3350 (m, O-H and N-H), 1640 (m, C=O), 1255 (m, Si-Me);  $\delta_H$ (300 MHz; CDCl<sub>3</sub>) 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

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

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).

To a solution of **11a** (121 mg, 0.131 mmol) and 2,6-lutidine (60 µl, 0.51 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub> and brine, dried (K<sub>2</sub>CO<sub>3</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *bis-TBS ether* **11b** (131 mg, 96%) as an oil,  $n_D^{24}$  1.4636; Anal. Calcd. for C<sub>62</sub>H<sub>131</sub>NO<sub>4</sub>Si<sub>3</sub>: C, 71.67; H, 12.71; N, 1.35, Found. C. 72.09; H. 12.96; N, 1.35; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +3.0 (*c* 1.0 in CHCl<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3450 (w, N-H), 1685 (m. C=O). 1250 (m. Si-Me);  $\delta_{H}(300 \text{ MHz}; \text{CDCl}_3)$  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).

#### (25,35,4R)-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<sub>3</sub> and brine, dried (K<sub>2</sub>CO<sub>3</sub>), and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *alcohol* **11c** (216 mg, 70%) as a colorless oil,  $n_D^{24}$  1.4712; Anal. Calcd. for C<sub>56</sub>H<sub>117</sub>NO<sub>4</sub>Si<sub>2</sub>: 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<sub>3</sub>); IR (film)/cm<sup>-1</sup> 3400-3280 (m, O-H and N-H), 1640 (m, C=O). 1250 (m, Si-Me);  $\delta_H(500 \text{ MHz}; \text{CDCl}_3)$  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).

# (2S, 3S, 4R)-3, 4, -bis-t-Butyldimethylsilyloxy-2-hexacosanoylamino-1-(2, 3, 4, 6-tetra-O-benzyl- $\alpha$ -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<sub>2</sub>CO<sub>3</sub>), 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<sub>3</sub>); positive HR FABMS m/z 1447.1011 (M+H) (Calcd. for C<sub>90</sub>H<sub>152</sub>NO<sub>9</sub>Si<sub>2</sub> 1447.1006); IR (film)/cm<sup>-1</sup> 3360 (w, N-H), 1680 (m, C=O), 1250 (m. Si-Me);  $\delta_{\rm H}(300 \text{ MHz}; \text{CDCl}_3) 0.030, 0.034, 0.06 and 0.08 (total 12H, each s, Si-Me), 0.86 (6H, m, 18-, 26-$ 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-CH), 4.84 (1H, d, J = 3.6 Hz, 1"-H), 4.92 (1H, d, J = 11.4 Hz, ph-CH), 6.04 (1H, d, J = 7.1Hz, N-H), 7.26-7.34 (20H, m, Ar-H).

# (2S, 3S, 4R)-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<sub>4</sub>). and concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *diol* **12b** (42 mg, 100%) as a solid,  $[\alpha]_D^{25} = +33.3$  (*c* 1.00 in CHCl<sub>3</sub>); mp 70.5-71.5°C; positive HR FABMS *m/z* 1218.9269 (M+H) (Calcd. for C<sub>78</sub>H<sub>124</sub>NO<sub>9</sub> 1218.9276); IR (KBr)/cm<sup>-1</sup> 3320 (O-H), 1640 (C=O), 1620. 1550;  $\delta_H(300 \text{ MHz; CDCl}_3) 0.88$  (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-CH), 6.42 (1H, d, J = 8.2 Hz, N-H), 7.24-7.37 (20H, m, Ar-H).

# KRN7000, (2S,3S,4R)-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<sub>3</sub> (0.5 ml), Pd(OH)<sub>2</sub> (20% on carbon, 150 mg) was added, and the stirring was continued vigorously at room temperature for 24 h under H<sub>2</sub>. It was filtered through Celite, and the filter cake was washed with CHCl<sub>3</sub> and MeOH. The combined filtrate and washings were concentrated under reduced pressure. The residue was chromatographed over SiO<sub>2</sub> to give the *compound* **2** (18 mg, 85%) as a colorless solid,  $[\alpha]_D^{23} = +42.2$  (*c* 0.54 in pyridine) [lit.<sup>6a</sup>  $[\alpha]_D^{23} = +43.6$  (*c* 1.0 in pyridine)]; mp 189.5-190.0°C (lit.<sup>6a</sup> mp 189.5-190.5°C); positive HR FABMS *m/z* 858.7380 (M+H) (Calcd. for C<sub>56</sub>H<sub>117</sub>NO<sub>4</sub>Si<sub>2</sub> 858.7398); v<sub>max</sub>(KBr)/cm<sup>-1</sup> 3300, 2930, 2850, 1640, 1540, 147C, 1070;  $\delta_H(500 \text{ MHz}; \text{C}_5\text{D}_5\text{N})$  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);  $\delta_C(125 \text{ MHz}; \text{C}_5\text{D}_5\text{N})$  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.

#### Acknowledgement

We thank Messrs A. Yajima and T. Maeda for their contribution in the early phase of this work. This work was supported by the Grant-in-Aid for Scientific Research on Priority Area No. 08245103 from the Ministry of Education, Science, Sports and Culture, of Japanese Government.

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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.



- We made efforts to convert the undesired epoxide 4' back to the olefin 3 under various conditions, but in vain. Conditions: (a) WCl<sub>6</sub>, *n*-BuLi/THF<sup>15</sup> (b) N<sub>2</sub>C(CO<sub>2</sub>Me)<sub>2</sub>, Rh<sub>2</sub>(OAc)<sub>4</sub><sup>16</sup> (c) 3-Methylbenzothiazole -2-selone<sup>17</sup> (d) TMSCl, NaI,<sup>18</sup> etc.
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