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## Supplementary Materials and Methods

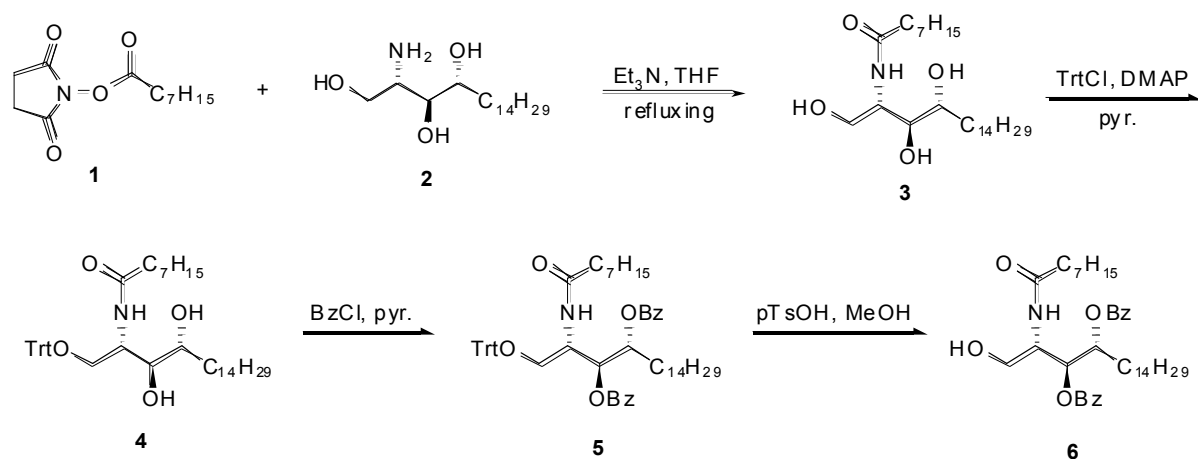
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## 1. GENERAL INFORMATION

All solvents were dried with solvent-purification system from Innovative Technology, Inc. All reagents were obtained from commercial sources and used without further purification. Analytical TLC was carried out on silica gel 60 F254 aluminum-backed plates (E. Merck). The 230 - 400 mesh size of the same absorbent was utilized for all chromatographic purifications.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at the indicated field strengths. The high-resolution mass spectra were collected at The Ohio State University Campus Chemical Instrumentation Center.

## 2. PREPARATION OF THE CERAMIDE ACCEPTOR



**Scheme 1.** Synthetic scheme for ceramide acceptor

**(2S,3S,4R)-2-Octanoylamino-octadecan-1,3,4-triol (3)**<sup>1</sup>. A suspension of ester **1** (1.38 g, 5.71 mmol), sphingosine **2** (1.51 g, 4.76 mmol) and  $\text{Et}_3\text{N}$  (2.4 mL, 17.1 mmol) in THF (50 mL) was stirred at 50 °C for 14 h. After cooled to room temperature, the solvent was removed *in vacuo*. The residue was dissolved in hot ethyl acetate (10 mL). The precipitate was collected by centrifuge (3000 rpm, 30 min) to give 1.84 g of product in 89% yield.  $^1\text{H}$  NMR

(400 MHz, Py-d5)  $\delta$  8.39 (d,  $J$  = 10.6 Hz, 1H), 5.01-5.07 (m, 2H), 4.41-4.49 (m, 2H), 4.34 (d,  $J$  = 7.6 Hz, 1H), 4.22-4.27 (m, 1H), 2.38-2.43 (m, 2H), 2.17-2.24 (m, 1H), 1.86-1.97 (m, 2H), 1.74-1.81 (m, 2H), 1.63-1.69 (m, 1H), 1.09-1.36 (m, 28H), 0.94 (t,  $J$  = 8.9 Hz, 1H), 0.85 (t,  $J$  = 8.1 Hz, 3H), 0.79 (t,  $J$  = 6.6 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz, Py-d5)  $\delta$  174.2, 77.6, 73.9, 63.0, 54.6, 47.5, 37.7, 34.9, 33.0, 32.8, 31.2, 31.0, 30.9, 30.87, 30.81, 30.52, 30.50, 30.3, 27.5, 27.3, 26.9, 23.8, 23.7, 15.2, 15.1, 13.1.

**(2*S*,3*S*,4*R*)-1-Triphenylmethyl-2-octanoylamino-octadecan-1,3,4-triol (4)**. To a solution of triol **3** (1.84 g, 4.15 mmol) in Pyridine (40 mL) were added TrtCl (5.78 g, 20.7 mmol) and DMAP (100 mg, 0.82 mmol) and stirred at 50 °C overnight. The solution was worked up by adding H<sub>2</sub>O (1 ml) and stirred for 1 h. The solution was concentrated under vacuum. The residue was dissolved by EtOAc and washed by water. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The concentrated residue was purified by column chromatography with hexane/ethyl acetate (4:1) to give 2.62 g of product in 92% yield as colorless oil.  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.42-7.44 (m, 6H), 7.31-7.35 (m, 6H), 7.25-7.28 (m, 3H), 6.06 (d,  $J$  = 8.4 Hz, 1H), 4.25-4.30 (m, 1H), 3.58-3.60 (m, 1H), 3.51-3.54 (m, 2H), 3.35-3.42 (m, 2H), 3.15 (d,  $J$  = 8.4 Hz, 1H), 2.28 (d,  $J$  = 7.6 Hz, 1H), 2.17 (t,  $J$  = 7.6 Hz, 2H), 1.71 (b, 2H), 1.60-1.66 (m, 2H), 1.44-1.46 (m, 2H), 1.25-1.32 (m, 28H), 0.90 (t,  $J$  = 6.4 Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.2, 143.2, 128.5, 128.1, 127.4, 87.7, 75.6, 73.2, 65.9, 63.0, 50.4, 36.9, 33.3, 31.9, 31.7, 29.7, 29.7, 29.6, 29.4, 29.3, 29.0, 25.8, 25.7, 22.7, 22.6, 14.1, 14.1; HRMS calcd for C<sub>45</sub>H<sub>67</sub>NO<sub>4</sub>Na ([M + Na]<sup>+</sup>) 708.4962, found 708.4969.

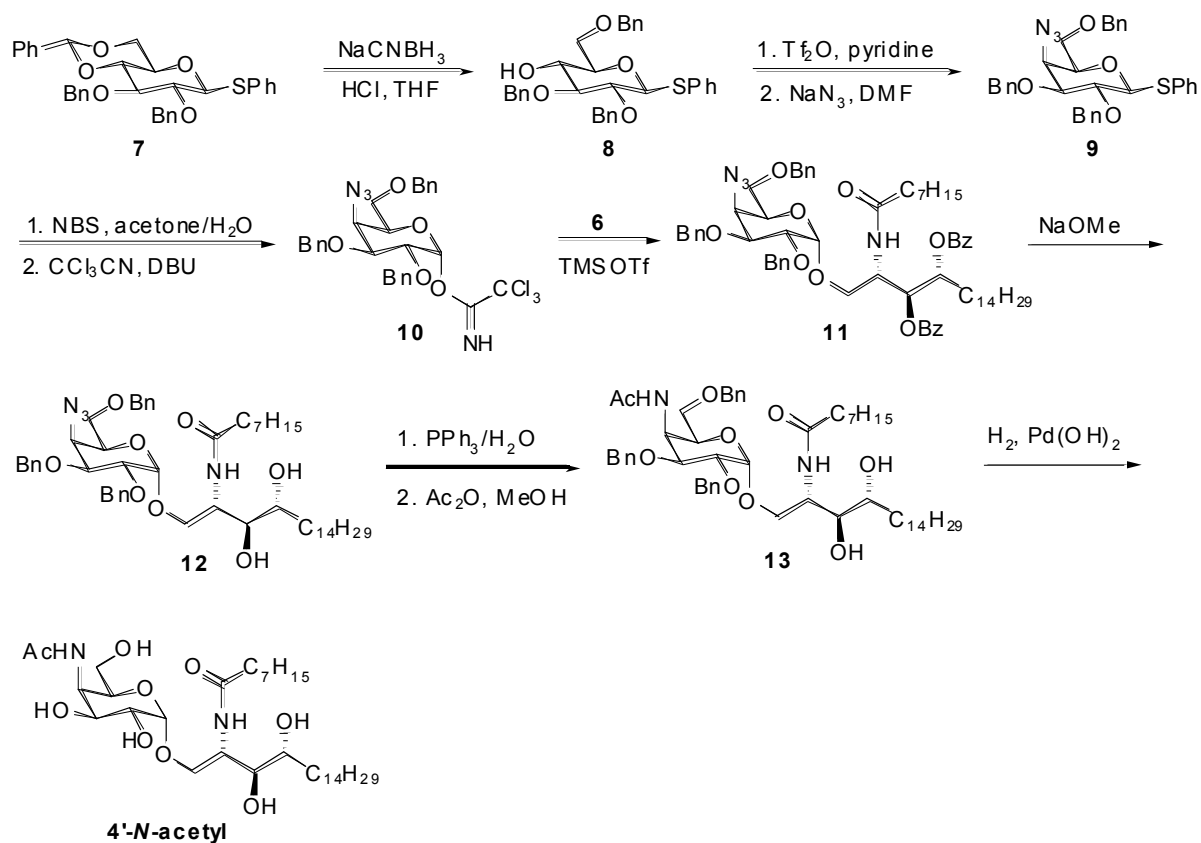
**(2S,3S,4R)-1-Triphenylmethyl-3,4-di-O-benzoyl-2-octanoylamino-octadecan-1,3,4-triol**

**(5)**. To a solution of compound **4** (2.8 g, 4.08 mmol) in Pyridine (40 mL) were added BzCl (2.8 ml, 24.5 mmol) and DMAP (49 mg, 0.4 mmol) and stirred at 50 °C for 8 h. The solution was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic layer was separated and washed with cooled 1N HCl, saturated aqueous NaHCO<sub>3</sub> and brine. After dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, it was purified by column chromatography with hexane/ethyl acetate (15:1) to give 3.14 g of product in 86% yield as colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.98 (d, *J* = 8.0 Hz, 2H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.55-7.62 (m, 2H), 7.42 (dd, *J* = 14.8, 7.2 Hz, 4H), 7.31-7.33 (m, 6H), 7.19-7.7.17 (m, 9H), 6.02 (d, *J* = 9.6 Hz, 1H), 5.81 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.36-5.38 (m, 1H), 4.57-4.64 (m, 1H), 3.32-3.35 (m, 2H), 2.16-2.23 (m, 2H), 1.86-1.91 (m, 2H), 1.62-1.68 (m, 2H), 1.23-1.40 (m, 32H), 0.88-0.911 (m, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.8, 166.4, 165.1, 143.3, 133.07, 132.9, 130.2, 129.9, 129.79, 129.76, 128.6, 128.41, 128.36, 127.8, 127.0, 86.8, 74.2, 72.8, 61.7, 48.6, 36.9, 31.9, 31.7, 29.68, 29.66, 29.62, 29.57, 29.52, 29.36, 29.34, 291, 25.80, 25.71, 25.69, 22.70, 22.66, 14.13, 14.09; HRMS calcd for C<sub>59</sub>H<sub>75</sub>NO<sub>6</sub>Na ([M + Na]<sup>+</sup>) 916.5487, found 916.5466.

**(2S,3S,4R)-3,4-Di-O-benzoyl-2-octanoylamino-octadecan-1,3,4-triol (6)**. To a solution of **5** (1.8 g, 2.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> and MeOH (2:1, 20 mL) were added *p*-toluenesulfonic acid monohydrate (400 mg, 2.1 mmol) and stirred for 3 h. The solution was quenched by addition Et<sub>3</sub>N (0.2 ml). The solution was concentrated under vacuum. The concentrated residue was purified by column chromatography with hexane/ethyl acetate (5:1) to give 1.2 g of product

in 92% yield as colorless oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.04 (d,  $J = 1.0$  Hz, 2H), 7.95 (d,  $J = 1.0$  Hz, 2H), 7.63 (t,  $J = 7.5$  Hz, 1H), 7.49-7.54 (m, 3H), 7.38 (d,  $J = 7.5$  Hz, 2H), 6.50 (d,  $J = 9.5$  Hz, 1H), 5.46 (dd,  $J = 9.5, 2.5$  Hz, 1H), 5.38-5.40 (m, 1H), 4.38-4.43 (m, 1H), 3.66 (dd,  $J = 12.0, 2.0$  Hz, 2H), 2.95 (br, 1H), 2.29 (d,  $J = 7.5$  Hz, 2H), 2.02-2.04 (m, 2H), 1.68-1.72 (m, 2H), 1.24-1.46 (m, 32H), 0.88 ( $J = 7.0$  Hz, 6H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  173.3, 167.0, 166.4, 133.8, 133.1, 130.0, 129.9, 129.7, 129.2, 128.7, 128.4, 86.8, 74.0, 73.7, 61.6, 50.0, 36.9, 31.9, 31.7, 29.70, 29.68, 29.65, 29.59, 29.57, 29.40, 29.36, 29.3, 29.1, 28.5, 25.84, 25.75, 22.69, 22.63, 14.11, 14.07; HRMS calcd for  $\text{C}_{40}\text{H}_{61}\text{NO}_6\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 674.4391, found 674.4407.

### 3. PREPARATION OF THE 4'-N-ACETYL- $\alpha$ -GALCER



**Scheme 2.** Synthetic scheme for 4'-N-acetyl- $\alpha$ -GalCer.

**Phenyl 1-thio-2,3,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranoside (8)**<sup>2</sup>. To a mixture of Phenyl 1-thio-2,3-di-*O*-benzyl-4,6-*O*-benzyliden- $\alpha$ -D-glucopyranoside **7**<sup>3</sup> (2.53 g, 4.6 mmol), NaCNBH<sub>3</sub> (3.63 g, 57.8 mmol) and 4 Å molecular sieves (3 g) in dry THF (60 mL) was added dropwise a solution of HCl-Et<sub>2</sub>O under nitrogen atmosphere at room temperature. The reaction mixture was stirred for 10 min, and then filtered off through Celite. The filtrate was washed sequentially with saturated NaHCO<sub>3</sub> aqueous and brine. After dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, it was purified by flash chromatography (hexane/ethyl acetate 3:1) to give 2.1 g of product in 86% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.43-7.26 (m, 20 H), 4.93 (d, *J* = 11.0 Hz, 1H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.76 (d, *J* = 9.8 Hz, 1H), 4.71 (d, *J* = 9.4 Hz, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.58 (d, *J* = 11.8 Hz, 1H), 3.83-3.78 (m, 2H), 3.68 (t, *J* = 8.8 Hz, 1H), 3.56-3.49 (m, 3H).

**Phenyl 1-thio-4-deoxy-4-azido-2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranoside (9)**. To a solution of glucoside **8** (1.83 g, 3.37 mmol) and anhydrous pyridine (0.44 mL, 5.39 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was added Tf<sub>2</sub>O (0.85 mL, 5.06 mmol) slowly. After being stirred for 30 min, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, washed with water, saturated NaHCO<sub>3</sub> aqueous and brine. Dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solution was concentrated and dissolved with dry DMF (30 mL). NaN<sub>3</sub> (0.88 g, 13.5 mmol) was added and the resulting suspension was stirred for 20 h at room temperature. The solvent was removed in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The residue was purified by flash chromatography (hexane/ethyl acetate 6:1) to give 1.41 g of product in 74% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

$\delta$  7.57 (m, 2H), 7.42-7.28 (m, 18H), 4.83 (d,  $J = 10.2$  Hz, 1H), 4.79 (d,  $J = 11.5$  Hz, 1H), 4.77 (d,  $J = 10.2$  Hz, 1H), 4.74 (d,  $J = 11.5$  Hz, 1H), 4.61 (d,  $J = 8.6$  Hz, 1H), 4.58 (d,  $J = 12.4$  Hz, 1H), 4.55 (d,  $J = 12.4$  Hz, 1H), 4.07 (d,  $J = 1.8$  Hz, 1H), 3.79-3.73 (m, 2H), 3.71-3.63 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  138.1, 137.7, 137.5, 133.6, 132.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.0, 127.96, 127.9, 127.88, 127.5, 88.1, 82.8, 77.2, 75.9, 75.6, 73.7, 72.8, 68.7, 60.0; HRMS calcd for  $\text{C}_{33}\text{H}_{33}\text{N}_3\text{O}_4\text{SNa}$  ( $[\text{M} + \text{Na}]^+$ ) 590.2084, found 590.2076.

**4-Deoxy-4-azido-2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranosyl-trichloroacetimide (10).** To a solution of thio-compound (1.30 g, 2.3 mmol) in acetone and water (9:1, 20 mL) cooled at 0 °C was added NBS (0.90 g, 5.0 mmol). The resulting mixture was stirred for 30 min and the reaction was quenched by addition of saturated  $\text{NaHCO}_3$  aqueous. The organic solvent was removed under reduced pressure. The aqueous was extracted with ethyl acetate. After dried over anhydrous  $\text{Na}_2\text{SO}_4$ , it was purified by flash chromatography.

The above galactose derivative was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (10 mL),  $\text{CCl}_3\text{CN}$  (2.3 mL, 23 mmol) and DBU (0.18 mL, 1.2 mmol) were added. The resulting mixture was stirred for 2h. The solvent was removed in vacuo. The residue was purified by chromatography (hexane/ethyl acetate, 5:1) to give 1.12 g of product in 79% yield for two steps.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.59 (s, 1H), 7.42-7.29 (m, 15H), 6.46 (d,  $J = 3.3$  Hz, 1H), 4.85 (d,  $J = 11.7$  Hz, 1H), 4.81 (d,  $J = 11.7$  Hz, 1H), 4.78 (d,  $J = 11.7$  Hz, 1H), 4.75 (d,  $J = 11.7$  Hz, 1H), 4.58 (d,  $J = 11.7$  Hz, 1H), 4.54 (d,  $J = 11.7$  Hz, 1H), 4.22 (t,  $J = 7.4$  Hz, 1H), 4.16 (m, 1H), 4.13 (dd,  $J = 9.6, 3.3$  Hz, 1H), 4.07 (dd,  $J = 9.6, 3.4$  Hz, 1H), 3.67-3.59 (m, 2H); HRMS calcd for  $\text{C}_{29}\text{H}_{29}\text{Cl}_3\text{N}_4\text{O}_5\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 641.1096, found 641.1105.

**4-Deoxy-4-azido-2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-3,4-di-*O*-benzoyl-octadecan-1,3,4-triol (11).** A suspension of trichloroacetimide donor **10** (0.87 g, 1.40 mmol), ceramide acceptor **6** (0.61 g, 0.94 mmol) and molecular sieves (1.0 g) in Et<sub>2</sub>O and THF (5:1, 12 mL) was stirred at room temperature for 30 min. Then the mixture was cooled at -25 °C and TMSOTf (20  $\mu$ L, 0.09 mmol) was added by syringe. The resulting mixture was continued stirring for 2h. The molecular sieves were filtered through Celite pad. The filtrate was diluted with ethyl acetate and washed with saturated aqueous NaHCO<sub>3</sub> and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The product was purified by flash chromatography (hexane/ethyl acetate/ dichloromethane 12:1:1) to give 0.70 g of product in 67% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.06 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.97 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.63 (tt, *J* = 7.5, 1.2 Hz, 1H), 7.55 (tt, *J* = 7.4, 1.1 Hz, 1H), 7.50-7.20 (m, 19H), 6.77 (d, *J* = 9.4 Hz, 1H), 5.70 (dd, *J* = 9.4, 2.8 Hz, 1H), 5.42 (m, 1H), 4.73 (d, *J* = 11.5 Hz, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.69 (d, *J* = 3.7 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.64 (m, 2H), 4.59 (m, 1H), 4.53 (d, *J* = 12.1 Hz, 1H), 4.13 (t, *J* = 6.5 Hz, 1H), 3.98 (m, 1H), 3.96 (dd, *J* = 9.3, 3.6 Hz, 1H), 3.91 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.81 (dd, *J* = 9.3, 3.7 Hz, 1H), 3.66 (dd, *J* = 11.6, 2.7 Hz, 1H), 3.57 (dd, *J* = 9.4, 6.6 Hz, 1H), 3.51 (dd, *J* = 9.3, 6.5 Hz, 1H), 2.19 (d, *J* = 7.4 Hz, 2H), 1.92 (m, 2H), 1.66 (m, 2H), 1.36-1.22 (m, 32H), 0.91 (t, *J* = 6.5 Hz, 3H), 0.90 (t, *J* = 6.7 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.1, 166.1, 165.3, 138.1, 138.0, 137.6, 133.3, 133.0, 130.1, 129.8, 129.78, 128.6, 128.5, 128.4, 128.35, 128.0, 127.9, 127.85, 127.8, 127.7, 99.8, 77.4, 77.3, 76.4, 73.8, 73.6, 73.5, 73.1, 72.4, 69.8, 69.0, 68.1, 61.3, 48.6, 36.7, 31.9, 31.8, 29.7, 29.68, 29.6, 29.59, 29.55, 29.4, 29.37, 29.3, 29.1, 28.5, 25.7, 22.7, 22.67, 14.1, 14.11; HRMS calcd for C<sub>67</sub>H<sub>88</sub>N<sub>4</sub>O<sub>10</sub>Na ([M + Na]<sup>+</sup>) 1131.6393, found 1131.6385.



**4-Deoxy-4-azido-2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (12).** To a solution of protected glycosceramide **11** (400 mg, 0.36 mmol) in dry MeOH (4 mL) was added a freshly prepared NaOMe (19 mg, 0.36 mmol). The resulting mixture was stirred at 50 °C for 10 h. The reaction was neutralized by addition of Dowex ion-exchange resin and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography (hexane/ethyl acetate, 2:1) to give 0.31 g of product in % yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.35-7.28 (m, 15H), 6.40 (d, *J* = 8.4 Hz, 1H), 4.89 (d, *J* = 11.5 Hz, 1H), 4.81 (d, *J* = 3.6 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.76 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.5 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1), 4.23 (m, 1H), 4.08 (dd, *J* = 3.4, 1.1 Hz, 1H), 4.00-3.93 (m, 2H), 3.89-3.84 (m, 2H), 3.75 (m, 1H), 3.61-3.54 (m, 2H), 3.48 (m, 1H), 2.28 (m, 1H), 2.16 (m, 2H), 1.63 (m, 2H), 1.61 (m, 1H), 1.32-1.24 (m, 32H), 0.92 (t, *J* = 6.5 Hz, 3H), 0.91 (t, *J* = 6.6 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.2, 137.6, 137.58, 137.4, 128.6, 128.2, 128.1, 128.0, 127.98, 127.8, 99.0, 78.2, 77.3, 76.2, 75.6, 74.5, 73.8, 73.3, 72.7, 70.0, 68.7, 67.7, 60.7, 49.4, 36.8, 33.4, 32.0, 31.7, 29.7, 29.4, 29.3, 29.1, 25.9, 25.7, 22.7, 22.66, 14.2, 14.1; HRMS calcd for C<sub>53</sub>H<sub>80</sub>N<sub>4</sub>O<sub>8</sub>Na ([M + Na]<sup>+</sup>) 923.5868, found 923.5852.

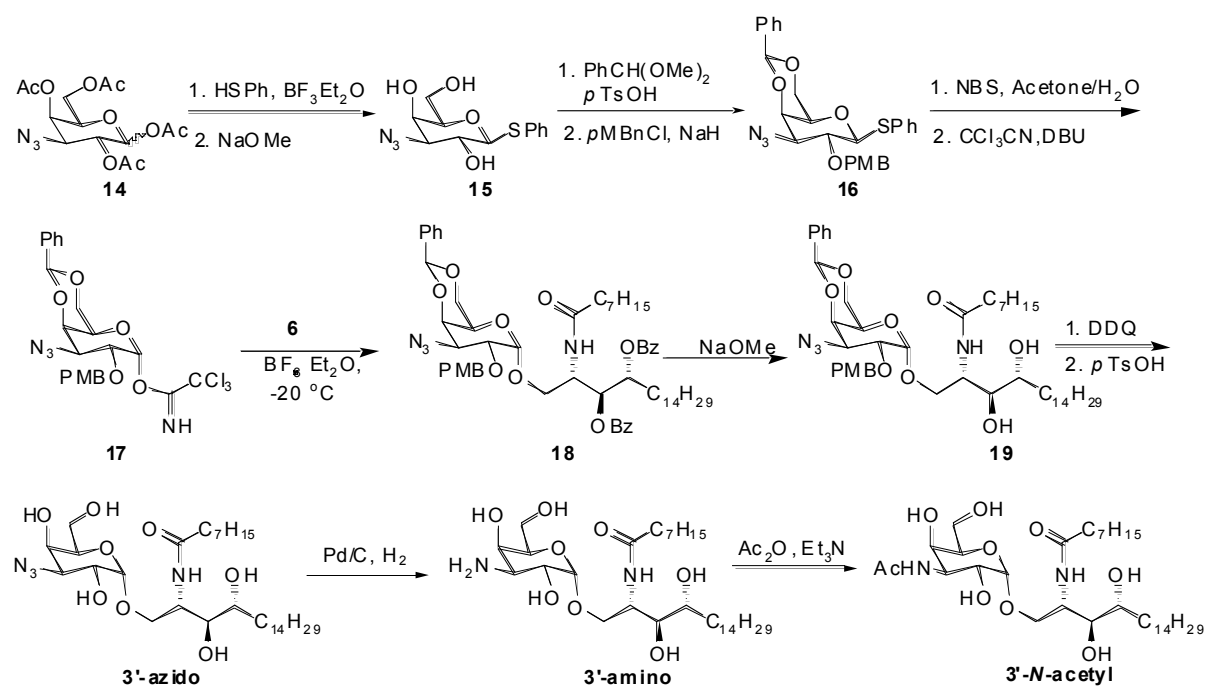
**4-Deoxy-4-acetamido-2,3,6-tri-*O*-benzyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (13).** A solution of azidogalactosylceramide **12** (82 mg, 0.091 mmol) and PPh<sub>3</sub> (48 mg, 0.18 mmol) in benzene (2 mL) containing trace amount of water was heated to 60 °C for 5 h. After cooling to room temperature, the solvent was removed in vacuo, and the residue was directly used for next steps without further purification.

The above residue was dissolved in anhydrous MeOH (2 mL) and Ac<sub>2</sub>O (12 μL, 0.13 mmol) was added. The resulting mixture was stirred for 8 h at room temperature and then the solvent was concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 1:2) to give 70 mg of product in 84% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.48-7.29 (m, 15H), 6.45 (d, *J* = 8.3 Hz, 1H), 5.56 (d, *J* = 9.9 Hz, 1H), 4.92 (d, *J* = 11.6 Hz, 1H), 4.87 (d, *J* = 4.0 Hz, 1H), 4.86 (d, *J* = 10.7 Hz, 1H), 4.83 (m, 1H), 4.65 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 11.9 Hz, 1H), 4.51 (d, *J* = 10.9 Hz, 1H), 4.28 (d, *J* = 11.8 Hz, 1H), 4.22 (m, 1H), 4.13 (t, *J* = 5.1 Hz, 1H), 3.95-3.92 (m, 3H), 3.56-3.47 (m, 5H), 2.14 (m, 2H), 2.01 (s, 3H), 1.60 (m, 3H), 1.48 (m, 1H), 1.33-1.22 (m, 32H), 0.91 (t, *J* = 5.7 Hz, 3H), 0.90 (t, *J* = 6.2 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.3, 170.3, 137.9, 137.8, 137.5, 128.5, 128.49, 128.4, 128.2, 128.1, 127.9, 127.7, 98.9, 83.7, 77.2, 76.2, 75.4, 74.4, 73.7, 73.3, 71.4, 70.1, 69.3, 68.5, 49.7, 47.7, 36.7, 33.3, 31.9, 31.7, 29.7, 29.67, 29.4, 29.3, 29.0, 25.9, 25.7, 23.5, 22.7, 22.6, 14.1, 14.08; HRMS calcd for C<sub>55</sub>H<sub>84</sub>N<sub>2</sub>O<sub>9</sub>Na ([M + Na]<sup>+</sup>) 939.6069, found 939.6081.

**4-Deoxy-4-acetamido-α-D-galactopyranosyl-(1,1)-(2S,3S,4R)-2-octanoylamino-octadecan-1,3,4-triol (4'-N-acetyl).** A suspension of tribenzylGalCer **13** (20 mg, 0.022 mmol) and 20 % Pd(OH)<sub>2</sub> on carbon (10 mg) in EtOH (1 mL) and CHCl<sub>3</sub> (0.25 mL) was stirred overnight under 1atm H<sub>2</sub> overnight. The catalyst was filtered through a Celite pad and the filtrate was concentrated. The residue was purified by silica gel chromatography (CHCl<sub>3</sub>/MeOH, 5:1) to afford 9 mg of product in 63% yield. <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>) δ 8.71 (d, *J* = 9.5 Hz, 1H), 8.47 (d, *J* = 8.6 Hz, 1H), 5.39 (d, *J* = 3.9 Hz, 1H), 5.17 (m, 1H), 5.08 (m, 1H), 4.61-4.53

(m, 3H), 4.42 (dd,  $J = 10.2, 4.0$  Hz, 1H), 4.32-4.25 (m, 3H), 4.09 (dd,  $J = 11.5, 6.6$  Hz, 1H), 4.03 (dd,  $J = 11.2, 6.6$  Hz, 1H), 2.39 (td,  $J = 7.5, 2.5$  Hz, 2H), 2.23 (m, 1H), 2.06 (s, 3H), 1.86 (m, 2H), 1.75 (m, 2H), 1.63 (m, 1H), 1.38-1.10 (m, 32H), 0.84 (t,  $J = 6.5$  Hz, 3H), 0.78 (t,  $J = 6.6$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ ; HRMS calcd for  $\text{C}_{34}\text{H}_{66}\text{N}_2\text{O}_9\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 669.4661, found 699.4685.

#### 4. PREPARATION OF C3'-SUBSTITUTED $\alpha$ -GALCER ANALOGUES



**Scheme 3.** Synthetic scheme for C3'-substituted  $\alpha$ -GalCer analogues

**Phenyl 3-deoxy-3-azido-1-thio- $\beta$ -D-galactopyranoside (**15**)**<sup>4</sup>. The peracetyl protected 3-azido galactose **14** (4.0 g, 10.7 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (80 mL) and 4 Å MS (1 g) and thiophenol (1.65 mL, 16.1 mmol) was added. The mixture was stirred at r.t. for 0.5 h and then cooled to 0 °C. After that, boron trifluoride ether solution (2.0 mL, 16.1 mmol) was injected into the solution. The solution was slowly warmed to room temperature and stirred for 48h. The reaction was neutralized by  $\text{NaHCO}_3$  and washed with water and brine. The

organic layer was dried by  $\text{Na}_2\text{SO}_4$  and the residue was chromatographed by hexane/EtOAc (5/1, v/v) to give compound (3.5 g, 77 %) as yellowish solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.05 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.63-3.66 (m, 1H), 3.90 (t,  $J = 6.4$ , 1H), 4.12 (d,  $J = 6.4$  Hz, 2H), 4.70 (dd,  $J = 9.9, 1.3$  Hz, 1H), 5.18-5.24 (m, 1H), 5.18-5.44-5.45 (m, 1H), 7.31-7.33 (m, 3H), 7.50-7.53 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  20.59, 20.66, 20.86, 61.85, 62.89, 67.81, 68.34, 86.91, 128.17, 128.91, 132.52, 169.37, 169.95, 170.37.

To a solution of of the above compound (2.70 g, 6.38 mmol) in MeOH (20 mL) were added NaOMe (10.3 mg, 0.19 mmol). The reaction mixture was stirred at room temperature for 5 h. Neutralize the solution by Amberlyst 15 cation exchange resin and filter the resin. The solution was concentrated under reduced pressure and then purified by column chromatography (EtOAc/Hexane 3:2) to give white solid **15** (82.3 %).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.52 (d,  $J = 7.5$  Hz, 2H), 7.28-7.20 (m, 3H), 3.93 (d,  $J = 2.7$  Hz, 1H), 3.79 (t, 1H), 3.72-3.63 (m, 2H), 3.55 (t,  $J = 6.0$  Hz, 1H), 3.35 (dd,  $J = 9.9, 3.0$  Hz, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  174.2, 134.4, 130.9, 128.5, 126.8, 89.5, 79.5, 68.1, 67.7, 61.1.

**Phenyl 1-thio-2-O-p-methoxybenzyl-3-deoxy-3-azido-4,6-O-benzylidene- $\beta$ -D-galactopyranoside (16)**<sup>5</sup>. To a solution of azido compound **15** (1.13 g, 3.80 mmol) in DMF (20 mL) were added benzaldehyde dimethylacetal (0.87 mL, 5.70 mmol) and *p*-toluenesulfonic acid (36 mg, 0.19 mmol) and stirred at 60 °C for 2h. The solution was worked up by adding  $\text{Et}_3\text{N}$  (0.25 mL) and stirred for 10 min. The solution was concentrated under vacuum. The residue was dissolved by DMF (20 mL) and added NaH (228 mg, 5.70 mmol). The solution was stirred at room temperature for 0.5 h and then added *p*-methoxybenzyl chloride. The reaction

is continued overnight. The concentrated residue was purified by column chromatography with hexane/dichloromethane (1:3) to give **16** in 83.3% yields as white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.75 (d,  $J = 7.5$  Hz, 2H), 7.55 (m, 2H), 7.45-7.40 (m, 5H), 7.29-7.23 (m, 3H), 6.92 (d,  $J = 8.5$  Hz, 2H), 5.59 (s, 1H), 4.82 (d,  $J = 9.6$  Hz, 1H), 4.67 (d,  $J = 9.4$  Hz, 1H), 4.59 (d,  $J = 9.6$  Hz, 1H), 4.39 (d,  $J = 12.3$  Hz, 1H), 4.23 (d,  $J = 3.2$  Hz, 1H), 4.04 (d,  $J = 12.3$  Hz, 1H), 3.96 (t,  $J = 9.6$  Hz, 1H), 3.83 (s, 3H), 3.57 (dd,  $J = 9.7, 3.2$  Hz, 1H), 3.45 (s, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  159.6, 137.5, 132.8, 132.3, 130.4, 129.7, 129.2, 129.1, 128.3, 127.6, 126.4, 113.9, 101.2, 86.9, 75.0, 74.7, 74.4, 70.1, 69.3, 65.0, 55.3.

**2-*O*-*p*-methoxybenzyl-3-deoxyl-3-azido-4,6-*O*-benzylidene-1-*O*- $\alpha$ -D-galactopyranosyl-**

**trichloroacetimidate (17).** To a solution of thiophenyl **16** (0.20 g, 0.48 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) were added DBU (36  $\mu\text{L}$ , 0.24 mmol) and trichloroacetonitrile (0.39 mL, 3.87 mmol) and stirred at room temperature for 2 h. The solution was concentrated under vacuum. The concentrated residue was purified by column chromatography with hexane/ethyl acetate (4:1) to give **17** in 89% yields as white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.67 (s, 1H), 7.54 (dd,  $J = 7.9, 1.7$  Hz, 2H), 7.43-7.39 (m, 3H), 7.29 (d,  $J = 8.4$  Hz, 2H), 6.88 (d,  $J = 8.6$  Hz, 2H), 6.68 (d,  $J = 3.1$  Hz, 1H), 5.62 (s, 1H), 4.68 (dd,  $J = 20.0, 11.0$  Hz, 2H), 4.37 (d,  $J = 3.0$  Hz, 1H), 4.34 (dd,  $J = 12.6, 0.8$  Hz, 1H), 4.29 (dd,  $J = 10.6, 3.2$  Hz, 1H), 4.09 (dd,  $J = 12.6, 1.3$  Hz, 1H), 3.97 (dd,  $J = 10.7, 3.2$  Hz, 1H), 3.83 (s, 1H), 3.82 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  161.0, 159.5, 137.2, 129.4, 129.1, 128.3, 126.1, 113.9, 101.0, 94.3, 91.2, 73.1, 72.5, 69.0, 64.9, 58.9, 55.3; HRMS calculated for  $\text{C}_{23}\text{H}_{23}\text{Cl}_3\text{N}_4\text{O}_6\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 579.0575, found 579.0612.

**2-*O*-*p*-methoxybenzyl-3-deoxy-3-azido-4,6-*O*-benzylidene-1-*O*- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-3,4-di-*O*-benzoyl-2-octanoylamino-octadecan-1,3,4-triol (18)**. To a solution of trichloroacetimide donor **17** (0.24 g, 0.43 mmol) in Ether/THF (2:1, 6 mL) were added ceramide acceptor **6** (0.19 g, 0.29 mmol) and 4 Å molecular sieves (2g) and stirred for 0.5h. The solution was cooled to -20 °C and stirred for 15 min. Then, TMSOTf (15  $\mu$ L, 0.086 mmol) was injected and the solution was stirred at -20 °C for 2 h. The solution was concentrated under vacuum. The concentrated residue was purified by column chromatography with hexane/ethyl acetate (4:1) to give **18** in 88% yields as white solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.05 (m, 2H), 7.96 (m, 2H), 7.63 (m, 1H), 7.56 (m, 1H), 7.52-7.48 (m, 4H), 7.43-7.40 (m, 2H), 7.39-7.35 (m, 3H), 7.24 (d,  $J$  = 8.7 Hz, 2H), 6.79 (d,  $J$  = 9.6 Hz, 1H), 6.73 (d,  $J$  = 8.7 Hz, 2H), 5.67 (dd,  $J$  = 9.4, 2.9 Hz, 1H), 5.56 (s, 1H), 5.29 (m, 1H), 4.90 (d,  $J$  = 3.3 Hz, 1H), 4.63 (d,  $J$  = 11.0 Hz, 1H), 4.60-4.55 (m, 2H), 4.27 (dd,  $J$  = 12.8, 1.3 Hz, 1H), 4.23 (d,  $J$  = 3.2 Hz, 1H), 4.07-4.02 (m, 2H), 3.80-3.75 (m, 6H), 3.68 (dd,  $J$  = 11.6, 3.1 Hz, 1H), 2.23 (t,  $J$  = 7.6 Hz, 2H), 1.90 (dd,  $J$  = 13.5, 6.6 Hz, 2H), 1.68-1.64 (m, 2H), 1.38-1.40 (m, 1H), 1.33-1.21 (m, 34H), 0.90-0.87 (m, 6H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  173.2, 166.4, 165.5, 159.5, 137.3, 133.6, 133.1, 130.0, 129.8, 129.7, 129.5, 129.4, 129.0, 128.7, 128.4, 128.2, 126.1, 113.9, 100.9, 98.9, 75.5, 74.2, 74.0, 72.7, 72.3, 69.3, 68.9, 63.3, 58.7, 55.2, 48.6, 36.9, 31.9, 31.8, 29.70, 29.69, 29.66, 29.63, 29.58, 29.55, 29.4, 29.30, 29.29, 29.1, 28.2, 25.8, 25.7, 22.70, 22.66, 14.14, 14.11; ESI-MS calculated for C<sub>61</sub>H<sub>82</sub>N<sub>4</sub>O<sub>11</sub>Na (M+Na<sup>+</sup>) 1069.6, found 1069.5.

**2-*O-p*-Methoxybenzyl-3-deoxy-3-azido-4,6-*O*-benzylidene-1-*O*- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (19).** To a solution of compound **18** (0.27 g, 0.248 mmol) in MeOH (20 mL) were added sodium methoxide (0.67 mg, 0.0124 mmol) and stirred at 60 °C overnight. The solution was cooled to room temperature and concentrated under vacuum. The concentrated residue was purified by column chromatography with isopropanol/dichloromethane (1:50 V/V) to give product in 61% yields as white solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.50 (m, 2H), 7.38-7.34 (m, 3H), 7.31(m, 2H), 6.89 (m, 2H), 6.29 (d, *J* = 8.1 Hz, 1H), 5.57 (s, 1H), 5.00 (d, *J* = 3.5 Hz, 1H), 4.75 (d, *J* = 11.0 Hz, 1H), 4.59 (d, *J* = 11.1 Hz, 1H), 4.28 (d, *J* = 2.9 Hz, 1H), 4.25-4.22 (m, 2H), 4.07-4.03 (m, 2H), 3.98 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.83-3.75 (m, 4H), 3.62 (br, 1H), 3.49-3.46 (m, 3H), 2.15 (t, *J* = 7.4 Hz, 2H), 2.09 (d, *J* = 5.4 Hz, 1H), 1.61-1.58 (m, 3H), 1.49-1.43 (m, 2H), 1.47-1.26 (m, 35H), 0.90-0.87 (m, 6H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  173.2, 159.7, 137.2, 129.9, 129.1, 129.0, 128.2, 126.1, 114.1, 101.0, 98.3, 75.8, 75.5, 73.8, 73.4, 73.36, 69.5, 69.2, 62.9, 59.5, 55.3, 49.8, 36.8, 33.4, 31.9, 31.7, 29.7, 29.67, 29.4, 29.3, 29.1, 25.8, 25.79, 25.7, 22.7, 22.6, 14.11, 14.08; HRMS calculated for C<sub>47</sub>H<sub>74</sub>N<sub>4</sub>O<sub>9</sub>Na ([M + Na]<sup>+</sup>) 861.5348, found 861.5359.

**3-Deoxy-3-azido-1-*O*- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (3'-azido)** To a solution of **19** (80 mg, 0.095 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) with trace amount of water was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (40 mg, 0.143 mmol) and stirred at room temperature overnight. The solution was concentrated in vacuo. Flash chromatographic purification (isopropanol/chloroform, 3:100) give a compound in 76% yield

as white solid. This compound (60 mg, 0.084 mmol) in MeOH (8 mL) was added *p*-toluene sulfonic acid (16 mg, 0.084 mmol) and stirred overnight. The solution was concentrated and purified by flash chromatography (MeOH/CHCl<sub>3</sub>, 6%) to give colorless oil (58 % yield for two steps). <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>) δ 8.66 (d, *J* = 8.6 Hz, 1H), 7.43 (br, 1H), 7.27 (d, *J* = 5.7 Hz, 1H), 6.69 (br, 1H), 6.52 (d, *J* = 6.5 Hz, 1H), 6.16 (br, 1H), 5.52 (d, *J* = 3.6 Hz, 1H), 5.26 (m, 1H), 4.81 (m, 1H), 4.68 (dd, *J* = 10.5, 4.4 Hz, 1H), 4.51 (br, 1H), 4.46-4.41 (m, 2H), 4.33 (m, 3H), 4.28 (m, 1H), 3.95 (dd, *J* = 10.6, 2.7 Hz, 1H), 2.43 (t, *J* = 7.7 Hz, 2H), 2.28 (m, 1H), 1.92-1.86 (m, 2H), 1.81-1.75 (m, 2H), 1.69 (m, 1H), 1.43-1.10 (m, 35H), 0.84 (t, *J* = 5.9 Hz, 3H), 0.78 (t, *J* = 5.9 Hz, 3H); <sup>13</sup>C NMR (125 MHz, pyridine-d<sub>5</sub>) δ 173.2, 100.8, 76.4, 72.6, 72.4, 69.4, 68.6, 67.5, 63.8, 61.9, 51.5, 36.5, 34.0, 31.9, 31.7, 30.1, 29.9, 29.8, 29.7, 29.41, 29.39, 29.2, 26.3, 26.1, 22.7, 22.6, 14.1, 14.0; HRMS calculated for C<sub>32</sub>H<sub>62</sub>N<sub>4</sub>O<sub>8</sub>Na ([M + Na]<sup>+</sup>) 653.4460, found 653.4465.

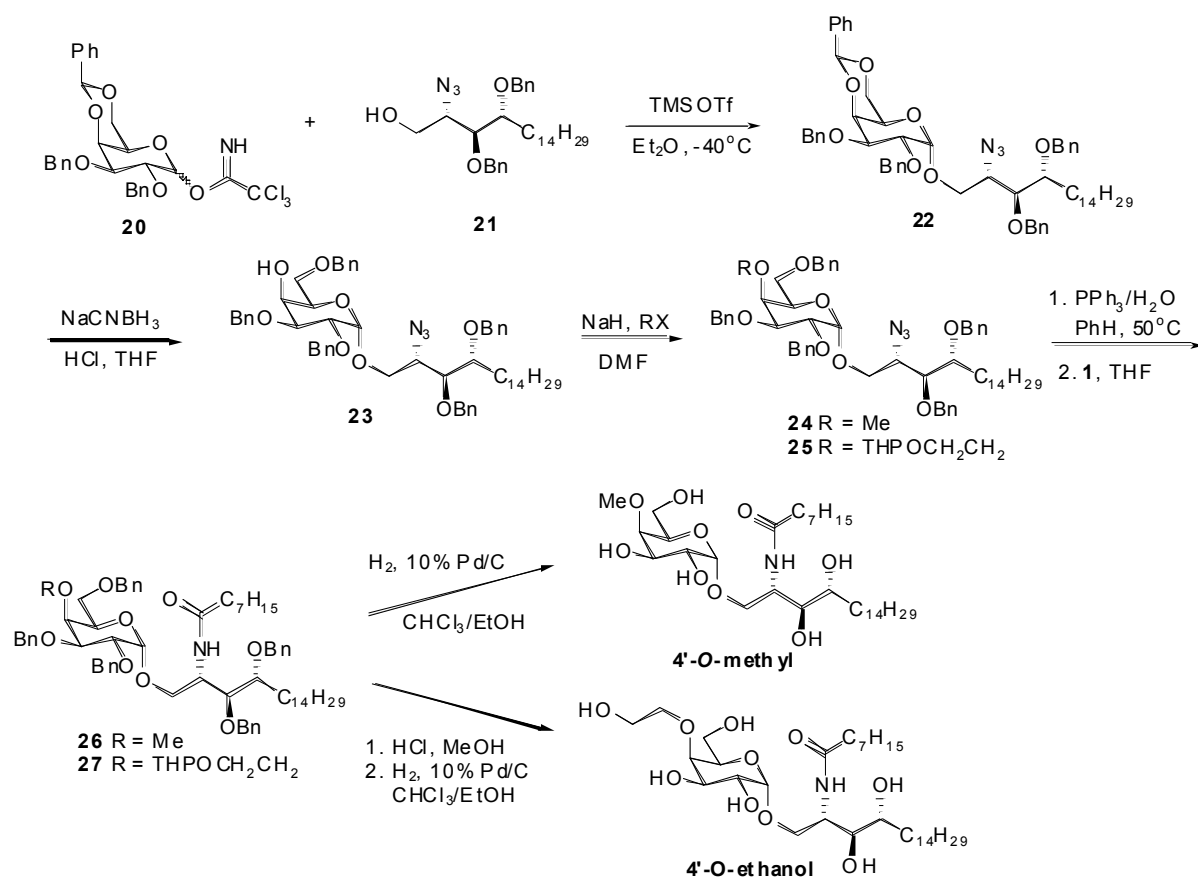
**3-Deoxy-3-amino-*O*-α-D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (3'-amino)** To a solution of **3'-azido** (20 mg, 0.032 mmol) in EtOH/CHCl<sub>3</sub> (2:1, 6 mL) was added Pd/C (6 mg) and stirred under H<sub>2</sub> (atmospher) at room temperature for 8h. The solution was concentrated in vacuo to give compound **9** as colorless solid (quantitative). <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>) δ 8.90 (d, *J* = 8.6 Hz, 1H), 5.52 (br, 1H), 5.16 (m, 1H), 5.10 (br, 1H), 4.73 (d, *J* = 10.0 Hz, 1H), 4.58 (dd, *J* = 10.4, 3.7 Hz, 1H), 4.46 (t, *J* = 5.9 Hz, 1H), 4.43-4.33 (m, 4H), 4.28-4.22 (m, 2H), 2.51 (t, *J* = 7.2 Hz, 2H), 2.24 (m, 1H), 1.92-1.86 (m, 2H), 1.82-1.75 (m, 2H), 1.60 (m, 1H), 1.43-1.09 (m, 34H), 0.83 (t, *J* = 5.5 Hz, 3H), 0.76 (t, *J* = 5.6 Hz, 3H); <sup>13</sup>C NMR (125 MHz, pyridine-d<sub>5</sub>) δ 174.9, 101.6, 77.4, 73.8, 73.4, 70.1,



68.5, 68.0, 62.8, 55.5, 53.0, 38.1, 35.1, 33.4, 33.2, 31.7, 31.5, 31.34, 31.32, 31.28, 31.2, 30.93, 30.89, 30.7, 27.8, 27.7, 24.2, 24.1, 15.6, 15.5; HRMS calculated for  $C_{32}H_{64}N_2O_8Na$  ( $[M + Na]^+$ ) 627.4560, found 627.4553.

**3-Deoxy-3-acetamido-*O*- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octade**

**can-1,3,4-triol (10)** To a solution of amino **9** (10 mg, 0.017 mmol) in EtOH/CHCl<sub>3</sub> (2:1, 2 ml) was added acetyl anhydride (7  $\mu$ l, 0.066 mmol) and Et<sub>3</sub>N (10  $\mu$ l). The solution was stirred at room temperature for 1h then worked up by adding one drop of concentrated ammonium hydroxide. The concentrated residue (22 mg) was purified by column chromatography with CHCl<sub>3</sub>/MeOH (8:1) to give **19** in 49.3% yields as a white solid. <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>)  $\delta$  8.78 (d,  $J = 3.0$  Hz, 1H), 8.56 (d,  $J = 8.5$  Hz, 1H), 5.49 (d,  $J = 3.5$  Hz, 1H), 4.60-4.54 (m, 3H), 4.47-4.41 (m, 2H), 4.37-4.34 (m, 1H), 4.22-4.32 (m, 3H), 2.45-2.40 (m, 2H), 2.21 (m, 1H), 2.03 (s, 3H), 1.91-1.85 (m, 2H), 1.79-1.73 (m, 2H), 1.65 (m, 1H), 1.27-1.10 (m, 36H), 0.84 (t,  $J = 7.0$  Hz, 3H), 0.78 (t,  $J = 7.0$  Hz, 3H); <sup>13</sup>C NMR (125 MHz, pyridine-d<sub>5</sub>)  $\delta$  173.4, 170.7, 100.9, 76.0, 72.6, 72.3, 69.1, 68.4, 67.9, 62.3, 52.9, 51.7, 36.6, 33.7, 31.9, 31.7, 30.1, 29.9, 29.80, 29.76, 29.7, 29.4, 29.37, 29.1, 26.3, 26.1, 23.0, 22.7, 22.6, 14.0, 13.1; HRMS calculated for  $C_{34}H_{66}N_2O_9Na$  ( $[M + Na]^+$ ) 669.4666, found 669.4670.

5. PREPARATION OF 4'-*O*-SUBSTITUTED  $\alpha$ -GALCER ANALOGUESScheme 4. Synthetic scheme for 4'-*O*-substituted  $\alpha$ -GalCer analogues

**2,3-Di-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (22)**. Powered 4Å molecule sieves (1.0 g) were added to a stirred solution of benzylidene protected trichloroacetimidate donor **20** (192 mg, 0.32 mmol) and azido lipid **21** (170 mg, 0.32 mmol) in fresh dried CH<sub>2</sub>Cl<sub>2</sub> (4 mL). After 30 min, the mixture was cooled to -40 °C, TMSOTf (6  $\mu$ L, 0.03 mmol) was added by syringe and the resulting mixture was stirred for 2h. The reaction was quenched by addition of Et<sub>3</sub>N (0.1 mL), and the mixture was filtered through Celite pad. The filtered was concentrated and purified by column chromatography with hexane/ethyl acetate (8:1) to give 205 mg of product in 67%

yield.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.56-7.24 (m, 25H), 5.48 (s, 1H), 5.00 (d,  $J = 3.4$  Hz, 1H), 4.89 (d,  $J = 11.8$  Hz, 1H), 4.84 (d,  $J = 12.4$  Hz, 1H), 4.77 (d,  $J = 12.4$  Hz, 1H), 4.70 (d,  $J = 12.1$  Hz, 1H), 4.69 (d,  $J = 11.3$  Hz, 1H), 4.64 (d,  $J = 11.6$  Hz, 1H), 4.61 (d,  $J = 11.8$  Hz, 1H), 4.53 (d,  $J = 11.6$  Hz, 1H), 4.19 (d,  $J = 3.2$  Hz, 1H), 4.13 (dd,  $J = 10.3, 3.4$  Hz, 1H), 4.10 (d,  $J = 11.5$  Hz, 1H), 4.06 (dd,  $J = 10.4, 3.3$  Hz, 1H), 4.03 (d,  $J = 7.9$  Hz, 1H), 3.91 (d,  $J = 12.0$  Hz, 1H), 3.78-3.72 (m, 3H), 3.66 (m, 1H), 3.60 (br, 1H), 1.70 (m, 1H), 1.57 (m, 1H), 1.44 (m, 1H), 1.34-1.25 (m, 23H), 0.92 (t,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  138.8, 138.4, 138.1, 137.9, 128.9, 128.4, 128.38, 128.3, 128.2, 128.1, 127.9, 127.8, 127.78, 127.73, 127.70, 127.6, 127.5, 127.48, 126.4, 101.1, 99.2, 79.5, 79.0, 75.9, 75.5, 74.7, 73.8, 73.5, 72.1, 72.0, 69.4, 68.5, 63.0, 61.7, 31.9, 30.1, 29.8, 29.7, 29.68, 29.66, 29.6, 29.4, 25.5, 22.7, 14.1; HRMS calcd for  $\text{C}_{59}\text{H}_{75}\text{N}_3\text{O}_8\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 976.5446, found 976.5467.

**2,3,6-Tri-*O*-benzyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (23).** The compound **22** (190 mg, 0.20 mmol) was concentrated twice from toluene and then dissolved in fresh dried THF (5 mL) containing 4Å molecule sieves (0.50 g). After stirring for 20 min,  $\text{NaCNBH}_3$  (125 mg, 2.0 mmol) was added, followed by a crystal of methyl orange. A solution of hydrochloride in ethyl ether (2 M) was added dropwise until a pink color persists. After 90 min, an additional portion of the hydrochloride in ethyl ether solution (0.5 mL) was added and the reaction mixture was continued stirring for 2.5 h. The reaction mixture was filtered into a separatory funnel containing a 1:1 mixture of  $\text{CH}_2\text{Cl}_2$  and water. The organic layer was separated and the aqueous was extracted with additional  $\text{CH}_2\text{Cl}_2$  ( $2 \times 5$  mL). The combined organic layers were washed successively with saturated aqueous

NaHCO<sub>3</sub> and brine. After dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated, the residue was purified by column chromatography with hexane/ethyl acetate (5:1) to give 174 mg of product as colorless oil in 91% yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.42-7.25 (m, 25H), 4.94 (d, *J* = 2.8 Hz, 1H), 4.82 (d, *J* = 11.7 Hz, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 4.75 (d, *J* = 11.7 Hz, 1H), 4.71 (d, *J* = 12.4 Hz, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.10 (m, 1H), 4.08 (dd, *J* = 10.3, 1.9 Hz, 1H), 3.97 (t, *J* = 5.9 Hz, 1H), 3.95 (m, 2H), 3.80-3.75 (m, 3H), 3.72 (m, 1H), 3.68-3.64 (m, 2H), 2.67 (br, 1H), 1.70 (m, 1H), 1.58 (m, 1H), 1.44 (m, 1H), 1.36-1.25 (m, 23H), 0.93 (t, *J* = 6.7 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 138.6, 138.5, 138.2, 138.16, 138.0, 128.5, 128.4, 128.37, 128.35, 128.3, 127.9, 127.8, 127.7, 127.68, 127.6, 98.4, 79.4, 79.1, 77.5, 75.7, 73.8, 73.6, 73.2, 72.6, 72.0, 69.6, 68.9, 68.1, 68.1, 62.0, 32.0, 30.0, 29.8, 29.74, 29.72, 29.70, 29.68, 29.65, 29.4, 25.4, 22.7, 14.2; HRMS calcd for C<sub>59</sub>H<sub>77</sub>N<sub>3</sub>O<sub>8</sub>Na ([M + Na]<sup>+</sup>) 978.5603, found 978.5589.

**2,3,6-Tri-*O*-benzyl-4-*O*-methyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (24).** To a solution of the hydroxyl compound **23** (85 mg, 0.089 mmol) in dry DMF (2 mL) was added 60% NaH (5 mg, 0.12 mmol). The resulting suspension was stirred for 30 min. MeI (8  $\mu$ L, 0.12 mmol) was added by syringe, and the reaction mixture was continued stirring for 8 h. DMF was removed in vacuo. The residue was dissolved in water (5 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  5 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography with hexane/ethyl acetate (9:1) to give 81 mg of product as

colorless oil in 94% yield.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43-7.24 (m, 25H, 5 $\times$ Ph), 4.91 (d,  $J = 2.9$  Hz, 1H, H-1'), 4.84 (d,  $J = 12.3$ , 2H, PhCH<sub>2</sub>), 4.77 (d,  $J = 11.8$  Hz, 1H, PhCH<sub>2</sub>), 4.70 (d,  $J = 11.6$  Hz, 2H, PhCH<sub>2</sub>), 4.65 (d,  $J = 11.4$  Hz, 1H, PhCH<sub>2</sub>), 4.61 (d,  $J = 11.6$  Hz, 1H, PhCH<sub>2</sub>), 4.57 (d,  $J = 11.8$  Hz, 1H, PhCH<sub>2</sub>), 4.51 (d,  $J = 11.5$  Hz, 1H, PhCH<sub>2</sub>), 4.50 (d,  $J = 11.8$  Hz, 1H, PhCH<sub>2</sub>), 4.04 (d,  $J = 8.6$  Hz, 1H), 3.99 (m, 3H), 3.77-3.72 (m, 4H), 3.66 (dd,  $J = 9.2, 7.5$ , 1H), 3.64 (m, 1H), 3.59 (s, 3H), 3.54 (dd,  $J = 9.2, 5.9$  Hz, 1H), 1.70 (m, 1H), 1.58 (m, 1H), 1.44 (m, 1H), 1.36-1.25 (m, 23H), 0.93 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  138.8, 138.77, 138.5, 138.2, 138.0, 128.4, 128.35, 128.3, 127.9, 127.8, 127.7, 127.69, 127.67, 127.64, 127.6, 127.5, 127.4, 98.7, 79.4, 79.1, 78.6, 76.6, 73.7, 73.6, 73.4, 73.0, 72.1, 69.5, 68.6, 68.5, 62.0, 61.4, 32.0, 30.0, 29.8, 29.74, 29.71, 29.70, 29.67, 29.6, 29.4, 25.4, 22.7, 14.1; HRMS calcd for  $\text{C}_{60}\text{H}_{79}\text{N}_3\text{O}_8\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 992.5759, found 992.5782.

**2,3,6-Tri-*O*-benzyl-4-*O*-methyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (26)** A solution of the azido compound **24** (70 mg, 0.072 mmol) and  $\text{PPh}_3$  (37 mg, 0.14 mmol) in benzene (5 ml) and stoichiometric amount of water was stirred at 50 °C for 12 h. The solvent was removed in vacuo, and the residue was co-evaporated twice with benzene to remove the remaining trace amount of water. Then the residue was re-dissolved in anhydrous THF (4 mL) and the activated ester **1** (26 mg, 0.11 mmol) was added. The reaction was stirred at room temperature overnight. The solvent was removed in vacuo and the residue was purified by column chromatography with hexane/ethyl acetate (5:1) to give 72 mg of product in 91% yield.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.40-7.23 (m, 25H, 5 $\times$ Ph), 6.06 (d,  $J = 8.5$  Hz, 1H, CONH), 4.85 (d,  $J = 3.7$  Hz, 1H, H-1'), 4.82 (d,  $J =$

11.7 Hz, 1H, PhCH<sub>2</sub>), 4.80 (d,  $J = 11.7$  Hz, 1H, PhCH<sub>2</sub>), 4.78 (d,  $J = 11.1$  Hz, 1H, PhCH<sub>2</sub>), 4.76 (d,  $J = 11.4$  Hz, 1H, PhCH<sub>2</sub>), 4.64 (d,  $J = 11.7$  Hz, 1H, PhCH<sub>2</sub>), 4.61 (d,  $J = 11.4$  Hz, 1H, PhCH<sub>2</sub>), 4.58 (d,  $J = 10.9$  Hz, 1H, PhCH<sub>2</sub>), 4.54 (d,  $J = 11.5$  Hz, 1H, PhCH<sub>2</sub>), 4.49 (d,  $J = 11.9$  Hz, 1H, PhCH<sub>2</sub>), 4.46 (d,  $J = 11.6$  Hz, 1H, PhCH<sub>2</sub>), 4.21 (m, 1H), 3.99 (dd,  $J = 10.9, 5.6$  Hz, 1H), 3.95 (m, 2H), 3.88 (t,  $J = 2.4$  Hz, 1H), 3.86 (dd,  $J = 5.4, 2.9$  Hz, 1H), 3.78 (dd,  $J = 10.9, 4.1$  Hz, 1H), 3.71 (d,  $J = 2.0$  Hz, 1H), 3.63 (dd,  $J = 9.2, 7.0$  Hz, 1H), 3.57 (s, 3H), 3.55 (dd,  $J = 9.3, 3.0$  Hz, 1H), 3.52 (m, 1H), 1.96 (m, 2H), 1.66 (m, 3H), 1.49 (m, 3H), 1.35-1.21 (m, 30H), 0.91 (t,  $J = 6.9$  Hz, 3H), 0.89 (t,  $J = 7.2$  Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  172.9, 138.7, 138.68, 138.6, 138.5, 137.7, 128.4, 128.38, 128.36, 128.34, 128.3, 127.9, 127.84, 127.83, 127.8, 127.7, 127.6, 127.54, 127.52, 99.5, 80.1, 78.8, 78.7, 77.2, 73.64, 73.60, 73.5, 72.8, 71.8, 69.8, 69.1, 68.8, 61.4, 50.4, 36.7, 31.9, 31.8, 29.9, 29.86, 29.73, 29.68, 29.4, 29.3, 29.1, 26.1, 25.7, 22.7, 22.6, 14.11, 14.08; HRMS calcd for C<sub>68</sub>H<sub>95</sub>NO<sub>9</sub>Na ([M + Na]<sup>+</sup>) 1092.6899, found 1092.6911.

**4-*O*-Methyl- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (4'-*O*-methyl).** A suspension of compound **26** (32 mg, 0.030 mmol) and Pd(OH)<sub>2</sub> (10 mg) in a mixture of EtOH and CHCl<sub>3</sub> (4:1, 2.5 mL) was stirred under 1 atm H<sub>2</sub> for 2 h. The catalyst was filtered through Celite pad, and the filtrate was concentrated and purified by column chromatography (CHCl<sub>3</sub>/MeOH, 6:1) to give 14 mg product in 74% yield. <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>)  $\delta$  8.40 (d,  $J = 8.7$  Hz, 1H), 6.42 (br, 2H), 6.04 (br, 1H), 5.49 (d,  $J = 3.8$  Hz, 1H), 5.20 (m, 1H), 4.96 (br, 1H), 4.60 (dd,  $J = 10.9, 5.3$  Hz, 1H), 4.52 (dd,  $J = 10.0, 3.8$  Hz, 1H), 4.44 (t,  $J = 6.5$  Hz, 1H), 4.41 (dd,  $J = 10.1, 3.1$  Hz, 1H), 4.31 (dd,  $J = 10.9, 5.0$

Hz, 1H), 4.26 (m, 2H), 4.23 (dd,  $J = 10.5, 6.5$  Hz, 1H), 4.18 (dd,  $J = 10.5, 6.6$  Hz, 1H), 4.00 (d,  $J = 3.0$  Hz, 1H), 3.08 (s, 3H), 2.38 (t,  $J = 7.2$  Hz, 2H), 2.24 (m, 1H), 1.86 (m, 2H), 1.73 (m, 2H), 1.64 (m, 1H), 1.38 (m, 2H), 1.30-1.08 (m, 28H), 0.85 (t,  $J = 6.7$  Hz, 3H), 0.78 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (125 MHz, pyridine- $d_5$ )  $\delta$  173.0, 101.2, 80.6, 76.4, 72.9, 72.2, 71.8, 70.3, 68.4, 61.4, 51.1, 36.5, 34.1, 31.9, 31.7, 30.1, 29.9, 29.78, 29.75, 29.67, 29.4, 29.1, 26.2, 26.1, 22.7, 22.6, 14.04, 13.98; HRMS calcd for  $\text{C}_{33}\text{H}_{65}\text{NO}_9\text{Na}$  ( $[\text{M} + \text{Na}]^+$ ) 642.4552, found 642.4546.

**2,3,6-Tri-*O*-benzyl-4-*O*-(tetrahydro-2*H*-pyran-2-yloxyethyl)- $\alpha$ -D-galactopyranosyl-(1,1)**

**-(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (25).** To a solution of the hydroxyl compound **23** (50 mg, 0.052 mmol) in dry DMF (2 mL) was added 60% NaH (3 mg, 0.073 mmol). The resulting suspension was stirred for 30 min. THPOCH<sub>2</sub>CH<sub>2</sub>Cl (11  $\mu\text{L}$ , 0.073 mmol) was added by syringe, and the reaction mixture was continued stirring for 8 h. DMF was removed in vacuo. The residue was dissolved in water (5 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  5 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography with hexane/ethyl acetate (9:1) to give 48 mg of product as colorless oil in 85% yield.  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.39-7.22 (m, 25H), 4.92 (d,  $J = 3.5$  Hz, 0.5H), 4.91 (d,  $J = 3.5$  Hz, 0.5H), 4.81 (d,  $J = 11.9$  Hz, 2H), 4.75 (d,  $J = 11.8$  Hz, 1H), 4.69-4.48 (m, 8H), 4.12 (m, 1H), 4.04-3.94 (m, 4H), 3.90-3.79 (m, 3H), 3.76-3.70 (m, 5H), 3.63-3.54 (m, 3H), 3.47 (m, 1H), 1.82 (m, 1H), 1.68 (m, 2H), 1.61-1.53 (m, 5H), 1.50 (m, 2H), 1.42 (m, 1H), 1.34-1.23 (m, 22H), 0.91 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  138.84, 138.78, 138.5, 138.3, 138.2, 128.5,

128.44, 128.39, 128.34, 128.30, 128.24, 128.19, 127.93, 127.87, 127.73, 127.70, 127.66, 127.61, 127.57, 127.53, 127.46, 127.4, 98.9, 98.74, 98.70, 98.5, 79.4, 79.0, 78.69, 78.67, 76.6, 76.5, 76.48, 76.1, 73.7, 73.5, 73.3, 73.25, 73.0, 72.9, 72.6, 72.3, 72.0, 69.9, 69.8, 69.1, 69.0, 68.5, 67.2, 66.6, 62.3, 62.0, 61.9, 32.0, 30.7, 30.6, 30.0, 29.8, 29.73, 29.69, 29.67, 29.65, 29.4, 25.51, 25.49, 25.4, 22.7, 19.6, 19.4, 14.1; HRMS calcd for C<sub>66</sub>H<sub>89</sub>N<sub>3</sub>O<sub>10</sub>Na ([M + Na]<sup>+</sup>) 1106.6440, found 1106.6429.

**2,3,6-Tri-*O*-benzyl-4-*O*-(tetrahydro-2*H*-pyran-2-yloxyethyl)- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-3,4-di-*O*-benzyl-octadecan-1,3,4-triol (27).** A solution of the azido compound **25** (48 mg, 0.044 mmol) and PPh<sub>3</sub> (23 mg, 0.088 mmol) in benzene (5 ml) and stoichiometric amount of water was stirred at 50 °C for 12 h. The solvent was removed in vacuo, and the residue was co-evaporated twice with benzene to remove the remaining trace amount of water. Then the residue was re-dissolved in anhydrous THF (4 mL) and the activated ester **1** (16 mg, 0.066 mmol) was added. The reaction was stirred at room temperature overnight. The solvent was removed in vacuo and the residue was purified by column chromatography with hexane/ethyl acetate (5:1) to give 46 mg of product in 87% yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.39-7.22 (m, 25H), 6.19 (d, *J* = 8.6 Hz, 0.5H), 6.15 (d, *J* = 8.6 Hz, 0.5H), 4.88 (d, *J* = 3.7 Hz, 0.5 H), 4.87 (d, *J* = 3.7 Hz, 0.5H), 4.82-4.74 (m, 4H), 4.66-4.50 (m, 7H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.19 (m, 1H), 4.14-3.94 (m, 4H), 3.90-3.69 (m, 9H), 3.64-3.50 (m, 3H), 3.46 (m, 1H), 2.36 (t, *J* = 7.5 Hz, 2H), 1.97 (m, 2H), 1.80 (m, 1H), 1.70-1.61 (m, 5H), 1.58-1.45 (m, 7H), 1.38-1.21 (m, 25H), 0.91 (t, *J* = 6.7 Hz, 3H), 0.89 (t, *J* = 7.1 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ; HRMS calcd for C<sub>74</sub>H<sub>105</sub>NO<sub>11</sub>Na ([M + Na]<sup>+</sup>)



1206.7580, found 1206.7601.

**4-*O*-(2-Hydroxyethyl)- $\alpha$ -D-galactopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-octanoylamino-octadecan-1,3,4-triol (4'-*O*-ethanol).** To a solution of the compound **27** (40 mg, 0.034 mmol) in a mixture of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (2:1, 3 mL) was added p-TsOH (10 mg, 0.053 mmol). The resulting mixture was stirred for 2h. The reaction was quenched by addition of Et<sub>3</sub>N (100  $\mu$ L). The solvent was removed in vacuo, and the residue was purified by column chromatography (hexane/ethyl acetate, 3;1) to give 32 mg of product.

A suspension of the above product (32 mg, 0.030 mmol) and Pd(OH)<sub>2</sub> (10 mg) in a mixture of EtOH and CHCl<sub>3</sub> (4:1, 2.5 mL) was stirred under 1 atm H<sub>2</sub> for 3 h. When TLC showed that reaction completed, the catalyst was filtered through Celite pad, and the filtrate was concentrated and purified by column chromatography (CHCl<sub>3</sub>/MeOH, 6:1) to give 14 mg of product in 61% yield for two steps. <sup>1</sup>H NMR (500 MHz, pyridine-d<sub>5</sub>)  $\delta$  8.41 (d, *J* = 8.7 Hz, 1H), 7.96 (br, 1H), 6.48 (br, 2H), 6.05 (br, 1H), 5.50 (d, *J* = 3.7 Hz, 1H), 5.21 (m, 1H), 4.95 (m, 1H), 4.60 (dd, *J* = 10.8, 5.3 Hz, 1H), 4.55 (dd, *J* = 10.0, 3.8 Hz, 1H), 4.44 (t, *J* = 6.7 Hz, 1H), 4.42 (dd, *J* = 9.6, 3.2 Hz, 1H), 4.32-4.17 (m, 8H), 3.95 (t, *J* = 4.6 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.24 (m, 1H), 1.86 (m, 2H), 1.74 (m, 2H), 1.63 (m, 1H), 1.37 (m, 2H), 1.29-1.08 (m, 28H), 0.85 (t, *J* = 6.9 Hz, 3H), 0.78 (t, *J* = 6.9 Hz, 3H); <sup>13</sup>C NMR (125 MHz, pyridine-d<sub>5</sub>)  $\delta$  173.0, 101.2, 79.6, 76.4, 75.7, 72.6, 72.2, 71.6, 70.3, 68.3, 62.2, 61.2, 51.1, 36.5, 34.1, 31.9, 31.7, 30.1, 29.9, 29.8, 29.76, 29.69, 29.4, 29.1, 26.2, 26.1, 22.7, 22.6, 14.1, 14.0; HRMS calcd for C<sub>34</sub>H<sub>67</sub>NO<sub>10</sub>Na ([M + Na]<sup>+</sup>) 672.4657, found 672.4682.