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Synthesis of a GM3 ganglioside analogue carrying a phytoceramide moiety by intramolecular glycosylation as a key step ☆

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ABSTRACT

A novel analogue of ganglioside GM3, in which sphingosine was replaced with a phytosphingosine moiety, was synthesized by intramolecular glycosylation as a key step. Glucose, a reducing terminal of the saccharide, and phytoceramide were first tethered by succinic acid and the derivative used for the subsequent glycosidic bond formation. The obtained glycosyl phytoceramide was further glycosylated with the sialyl galactose residue to afford a fully protected GM3 derivative, which was converted into the desired, final compound by using conventional deprotection procedures.

1. Introduction

Gangliosides, sialic acid-containing glycosphingolipids, are commonly present in cell-surface membranes. These molecules are considered to be involved in many biological processes such as cell growth, cell differentiation, cell adhesion, microbial and viral infections, immune response, oncogenesis, and many other receptor-mediated reactions.[1–3] Since gangliosides have been found to play important functional roles, synthetic gangliosides are of critical importance to investigate structure–activity relationships. Therefore, numerous reports on the synthesis of gangliosides have been published, most of them employ a typical synthetic strategy to introduce the ceramide moiety[4] or an azido derivative of sphingosine[5] as an equivalent of sphingosine, after having completed the construction of the oligosaccharides. However, reduction in yield during the introduction of the ceramide moiety or multiple steps required for the conversion of the azido derivative to a ceramide moiety having an oligosaccharide unit makes the synthesis of glycolipids in large quantities a formidable task. Intramolecular glycosylation can be a promising approaches to overcome this problem because of enhanced reactivity and stereo-/regiospecificity[6] as already demonstrated in α-glycosylation and β-mannosylation.[7] As a part of our continuous interest in the synthesis of glycolipids, we describe herein the efficient synthesis of a GM3 analogue by employing intramolecular glycosylation as a key step.

2. Results and discussion

The retro-synthetic scheme of the target compound is shown in Chart 1, where a sialyl galactosyl donor **11**, which has already been employed successfully in the synthesis of a series of gangliosides,[8–12] and glycosyl phytoceramide acceptor **10**, which was synthesized by intramolecular glycosylation and also serves as a glycosyl acceptor for the first time in this study, have been employed as the key building blocks. The glucose derivative **3** was synthesized from the known 4,6-*O*-benzylidene protected glucose derivative **1**.[13] First, **1** was benzolyated at O-2 to give **2**, then removal of the benzylidene acetal from **2** gave the desired glucose derivative **3** (Scheme 1).

The phytoceramide derivative **6** was synthesized from phytoceramide **4**,[14] which was previously prepared from the commercially available phytosphingosine. Regioselective silylation of the primary hydroxyl with *tert*-butyldiphenylsilyl chloride gave **5**, then subsequent treatment with succinic anhydride and acetic anhydride gave **6** in 40% yield accompanied by **7** (40%) as by-product.

Linking of glucose derivative **3** with phytoceramide **6** was accomplished in the presence of 2,4,6-trichlorobenzoyl chloride, triethylamine and 4-(dimethylamino)pyridine[15] to give the desired compound **8** in 76% yield (Scheme 2). In intramolecular glycosylation, succinyl and malonyl spacers are well investigated and are known to provide good results in terms of yield and/or stereocontrol.[16] The *tert*-butyldiphenylsilyl group was removed by treatment with acetic acid and tetrabutylammonium fluoride to yield **9** in 77% yield.

☆ Part 145 in the series: Synthetic studies on sialoglycoconjugates.

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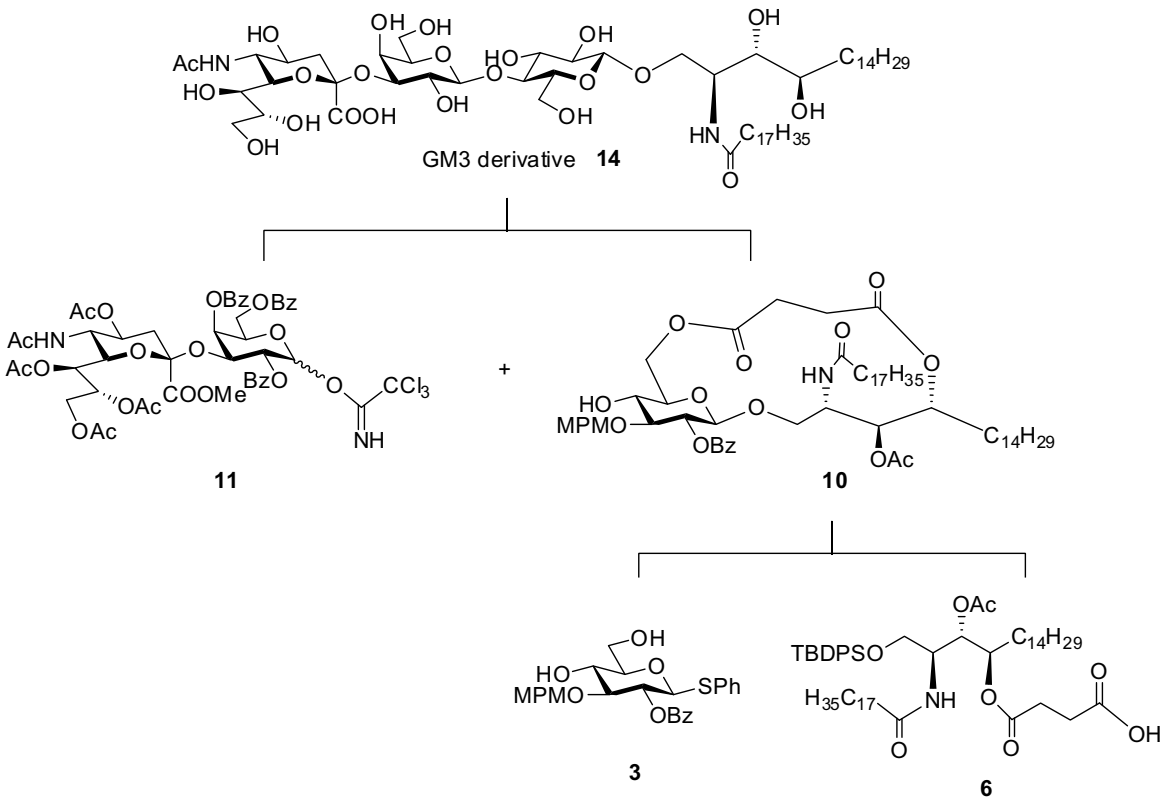
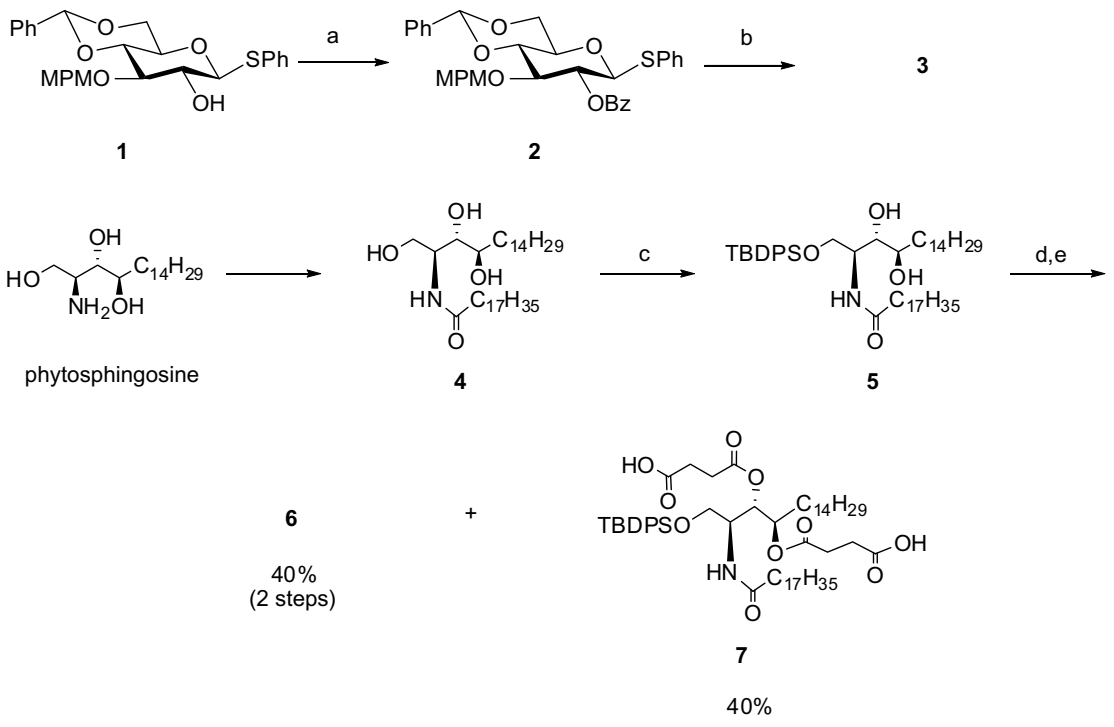
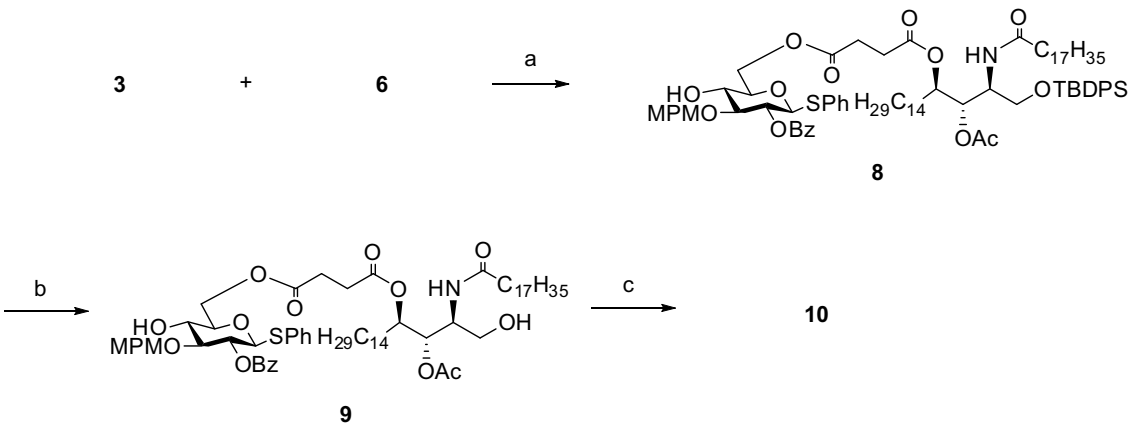


Chart 1. Retro-synthetic scheme for ganglioside GM3 derivative.



Scheme 1. Reagents and conditions: (a) BzCl, py, rt, 80%; (b) 85% aq AcOH, 40 °C, 86%; (c) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, rt, 76%; (d) succinic anhydride, DMAP, py, rt to 40 °C; (e) Ac_2O, DMAP, py, rt, 40% in two steps.

The pre-arranged glycolipid **9** was then treated with *N*-iodosuccinimide and trifluoromethanesulfonic acid (NIS–TfOH)[17] in dichloromethane to give the desired glucosyl ceramide **10** in 85% yield as an intramolecular glycosylation product. When the 2-hydroxy group was protected with a non-participating MPM group, this glycosylation reaction gave an anomeric mixture of the



Scheme 2. Reagents and conditions: (a) 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 76%; (b) TBAF, AcOH, THF, 0 °C, 77%; (c) NIS, TfOH, CH_2Cl_2, 0 °C, 85%.

glycoside unless nitriles were used as a solvent (data not shown). The anomeric signal of **10** at δ 4.47 (d, $J_{1,2}$ 7.5 Hz, H-1 of Glc) confirmed the β-glycosidic linkage.

The obtained glycosyl ceramide was then further glycosylated to give the fully protected GM3 derivative **12** (Scheme 3). Glycosylation of **10** with the trichloroacetimidate[18] derivative of sialyl galactose (**11**) was carried out by use of trimethylsilyl trifluoromethanesulfonic acid (TMSOTf) to give the desired GM3 derivative **12** in 70% yield. A proton doublet in the ^{1}H NMR spectrum of **12** at δ 5.10 (d, $J_{1,2}$ 8.2 Hz, H-1 of Gal) showed that the sialyl galactose unit was incorporated into the glycolipid as a β-glycoside. These results suggest that the glycosyl acceptor **10** could be applied as versatile building unit for the synthesis of more complicated gangliosides such as GM1 and GM2.

Finally, all the protecting groups were removed by conventional deprotection procedures, such as treatment with trifluoroacetic acid to remove the MPM group (→**13**) sodium methoxide deacetylation to remove acetyl and benzoyl groups, and a final saponification of the methyl ester to afford the target compound **14** in quantitative yield.

In conclusion, the present study demonstrates the usefulness of the intramolecular glycosylation of newly developed glucose-ceramide derivatives and its application in the synthesis of a ganglioside GM3 analogue carrying a phytoceramide moiety in place of mammalian ceramide. The key intermediate glucosylceramide could be used as an acceptor in glycosylation reactions for the efficient synthesis of higher gangliosides and their analogues. Further study on this subject is currently underway.

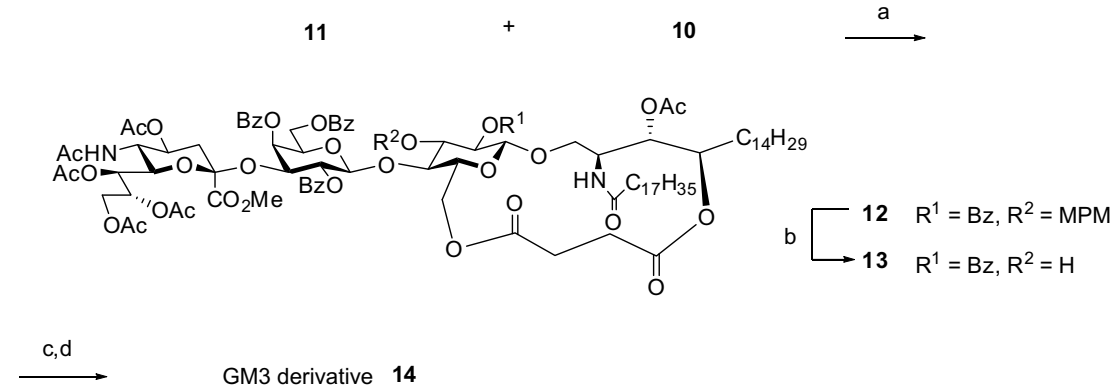
3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were measured using Varian INOVA 400 and 500 equipments. Chemical shifts are expressed in ppm (δ) relative to the signal of Me_4Si adjusted to δ 0 ppm. High-resolution mass spectra were recorded in positive ion mode on a Bruker micro TOF (ESI-TOFMS). Molecular sieves were purchased from Wako Chemicals Inc. and dried at 300 °C for 2 h in muffle furnace prior to use. Solvents as reaction media were dried over molecular sieves and used without purification. TLC analysis was performed on Merck TLC (Silica Gel $60F_{254}$ on glass plate). Silica gel (80 mesh and 300 mesh) manufactured by Fuji Silysia Co. was used for flash column chromatography. Amount of silica gel was usually estimated as 200–400-fold weight of sample to be charged. Solvent systems for chromatography are specified in v/v. Evaporation and concentration were carried out under diminished pressure. Specific rotations were determined with a Horiba SEPA-300 high sensitive polarimeter.

3.2. Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-4-methoxybenzyl-1-thio-β-D-glucopyranoside (2)

To a soln of **1** (171 mg, 0.356 mmol) in pyridine (3.5 mL) were added benzoyl chloride (62.0 μL, 0.534 mmol) and DMAP (4.35 mg, 0.036 mmol), and the mixture was stirred for 4 h at room temperature. After complete consumption of the starting material



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 70%; (b) TFA, CH_2Cl_2, rt; (c) NaOMe, MeOH, rt; (d) NaOMe, MeOH and water, rt, quantitative in three steps.

(TLC 50:1 toluene–MeOH), MeOH was added to the reaction mixture at 0 °C and the soln was co-evaporated with toluene. The mixture was diluted with $CHCl_3$ and washed with 2 M HCl, water, satd $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on a column of silica gel ($CHCl_3$) to give **2** (166 mg, 80%) as an amorphous product: $[\alpha]_D$ +47.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–8.01 (m, 15H, 3Ph), 6.55–7.10 (2d, 4H, MeO*Ph*), 5.60 (s, 1H, PhC*H*), 5.26 (t, 1H, $J_{1,2} = J_{2,3}$ 9.9 Hz, H-2), 4.84 (d, 1H, H-1), 4.73 (d, 1H, J_{gem} 11.7 Hz, PhCH_2), 4.59 (d, 1H, PhCH_2), 4.41 (t, 1H, H-4), 3.68–3.89 (m, 3H, H-6, H-6′, H-3), 3.68 (s, 3H, O*Me*), 3.56 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 159.1, 137.2, 133.1, 132.9, 132.2, 129.9, 129.8, 129.7, 129.0, 128.9, 128.3, 128.2, 128.1, 126.0, 113.5, 101.2, 87.0, 81.4, 78.7, 73.8, 72.0, 70.6, 68.6, 55.0; HRESIMS: calcd for $C_{34}H_{32}O_7S$: 607.1761 $[M+Na]^+$, found: *m*/*z* 607.1773.

3.3. Phenyl 2-*O*-benzoyl-3-*O*-4-methoxybenzyl-1-thio-β-D-glucopyranoside (3)

To a soln of **2** (200 mg, 0.330 mmol) in AcOH (15 mL) was added water (3.0 mL). After stirring at 40 °C for 12 h (TLC monitoring 1:1 toluene–EtOAc), the mixture was diluted with $CHCl_3$, and washed with water, satd $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (3:1 toluene–EtOAc) to give **3** (145 mg, 86%) as an amorphous mass: $[\alpha]_D$ +0.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–8.10 (m, 10H, 2Ph), 6.60–7.15 (2d, 4H, MeO*Ph*), 5.23 (t, 1H, $J_{1,2} = J_{2,3}$ 9.9 Hz, H-2), 4.83 (d, 1H, H-1), 4.63 (d, 1H, J_{gem} 11.7 Hz, PhCH_2), 4.59 (d, 1H, PhCH_2), 3.90 (m, 1H, H-6′), 3.83 (m, 1H, H-6), 3.74 (m, 1H, H-4), 3.70 (m, 1H, H-3), 3.65 (s, 3H, O*Me*), 3.44 (m, 1H, H-5), 3.42 (d, 1H, $J_{4,4\text{-OH}}$ 3.6 Hz, 4-OH), 2.77 (m, 1H, 6-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 159.1, 133.2, 132.8, 132.0, 129.8, 129.6, 129.6, 128.8, 128.3, 127.8, 113.7, 86.3, 83.1, 79.5, 74.3, 72.2, 70.2, 62.3, 55.0; HRESIMS: calcd for $C_{27}H_{28}O_7S$: 519.1448 $[M+Na]^+$, found: *m*/*z* 519.1454.

3.4. (2*S*,3*S*,4*R*)-1-*O*-*tert*-butyldiphenylsilyl-2-octadecanoylamino-octadecane (5)

To a soln of **4** (500 mg, 0.857 mmol) and Et_3N (10 mL) in CH_2Cl_2 (8.6 mL) were added TBDPSCl (260 μL, 1.03 mmol) and DMAP (209 mg, 1.71 mmol). After stirring at room temperature for 20 h (TLC monitoring 50:1 $CHCl_3$–MeOH), MeOH was added to the reaction mixture at 0 °C. After concentration, the residue was chromatographed on a column of silica gel (100:1 $CHCl_3$–MeOH) to give **5** (533 mg, 76%) as an amorphous product: $[\alpha]_D$ +1.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30–7.80 (m, 10H, 2Ph), 6.15 (d, 1H, $J_{2,NH}$ 8.4 Hz, NH), 4.17 (m, 1H, $J_{1,2}$ 3.6 Hz, $J_{1',2}$ 4.4 Hz, H-2), 4.05 (dd, 1H, J_{gem} 10.6 Hz, H-1), 3.79 (dd, 1H, H-1′), 3.58 (m, 2H, $J_{3,3\text{-OH}}$ 6.8 Hz, $J_{4,4\text{-OH}}$ 7.6 Hz, H-3, H-4), 3.46 (d, 1H, 4-OH), 2.80 (d, 1H, 3-OH), 2.08 (m, 2H, HNC(=O)CH_2–), 1.70 (m, 2H, H-6, H-6′), 1.50 (m, 2H, HNC(=O)CH$_2$CH_2–), 1.45 (m, 2H, H-5, H-5′), 1.00–1.40 (m, 50H, –CH_2–), 0.85 (t, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 135.5, 135.4, 134.8, 132.5, 132.2, 130.0, 127.9, 127.5, 75.6, 73.3, 63.8, 51.3, 36.7, 33.3, 31.9, 29.7, 29.6, 29.5, 29.34, 29.33, 29.28, 26.9, 26.5, 25.9, 25.6, 22.7, 19.1, 14.1; HRESIMS: calcd for $C_{52}H_{91}NO_4Si$: 844.6615 $[M+Na]^+$, found: *m*/*z* 844.6623.

3.5. (2*S*,3*S*,4*R*)-3-*O*-acetyl-1-*O*-*tert*-butyldiphenylsilyl-4-*O*-succinoyl-2-octadecanoylamino-octadecane (6)

To a soln of **5** (300 mg, 0.365 mmol) in pyridine (1.8 mL) was added succinic anhydride (40.2 mg, 0.402 mmol) at 0 °C. The mixture was stirred for 24 h at 40 °C. After complete consumption of the starting material (TLC: 40:1:1 $CHCl_3$–MeOH–5% aq $CaCl_2$), Ac_2O (2.5 mL, 26.5 mmol) was added at 0 °C. After stirring at 40 °C for 12 h (TLC monitoring: 40:1:1 $CHCl_3$–MeOH–5% aq $CaCl_2$), MeOH was added to the mixture at 0 °C. The reaction mixture was co-evaporated with toluene and extracted with EtOAc. The organic layer was washed with 2 M HCl, water, satd $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (1:6 EtOAc–hexane) to give **6** (138 mg, 40%) as an amorphous solid: $[\alpha]_D$ +60.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.65 (m, 10H, 2Ph), 5.97 (d, 1H, $J_{2,NH}$ 9.5 Hz, NH), 5.33 (dd, 1H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 2.4 Hz, H-3), 4.95 (m, 1H, H-4), 4.25 (m, 1H, H-2), 3.66 (m, 2H, H-1,1′), 2.54–2.67 (m, 4H, –OCOCH_2CH_2COO–), 2.15 (m, 2H, H-6, H-6′), 1.92 (s, 3H, OAc), 1.60 (m, 2H, H-5,5′), 1.00–1.70 (m, 54H, –CH_2–), 0.90 (t, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.0, 172.8, 172.0, 169.8, 135.6, 135.5, 132.9, 132.6, 129.9, 127.8, 127.8, 73.9, 71.4, 62.3, 49.2, 36.8, 31.9, 29.7, 29.6, 29.52, 29.47, 29.4, 29.34, 29.30, 29.28, 29.1, 28.9, 27.8, 26.8, 25.6, 25.5, 22.7, 20.7, 19.2, 14.1; HRESIMS: calcd for $C_{58}H_{97}NO_8Si$: 986.6876 $[M+Na]^+$, found: *m*/*z* = 986.6843.

3.6. Phenyl 6-*O*-[{(2*S*,3*S*,4*R*)-3-*O*-acetyl-1-*O*-*tert*-butyldiphenylsilyl-2-octadecanoylamino-octadecane-4-yloxy}carbonylpropanoyl]-2-*O*-benzoyl-3-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (8)

To a soln of **6** (204 mg, 0.212 mmol) in CH_2Cl_2 (2.1 mL) were added 2,4,6-trichlorobenzoyl chloride (50.0 μL, 0.318 mmol), DMAP (39.0 mg, 0.318 mmol), Et_3N (44.4 μL, 0.318 mmol) and **3** (105 mg, 0.212 mmol). The mixture was stirred for 2 h at room temperature. After complete consumption of the starting material (TLC: 2:1 toluene–EtOAc), the mixture was diluted with $CHCl_3$ and washed with satd $NaHCO_3$, water and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (1:6 EtOAc–hexane) to give **8** (229 mg, 76%) as an amorphous solid: $[\alpha]_D$ +0.4 (*c* 1.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20–8.10 (m, 20H, 4Ph), 6.62–7.15 (2d, 4H, MeO*Ph*), 5.95 (d, 1H, $J_{2,NH}$ 9.5 Hz, NH), 5.35 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 2.4 Hz, H-3^{Cer}), 5.19 (t, 1H, $J_{1,2} = J_{2,3}$ 9.9 Hz, H-2^{Glc}), 4.95 (m, 1H, H-4^{Cer}), 4.76 (d, 1H, H-1^{Glc}), 4.66 (d, 1H, J_{gem} 11.0 Hz, PhCH_2), 4.63 (d, 1H, PhCH_2), 4.46 (dd, 1H, $J_{5,6}$ 2.4 Hz, J_{gem} 12.0 Hz, H-6′^{Glc}), 4.39 (dd, 1H, $J_{5,6'}$ 4.0 Hz, H-6′^{Glc}), 4.22 (m, 1H, H-2^{Cer}), 3.72 (m, 1H, $J_{4,4\text{-OH}}$ 2.8 Hz, H-4^{Glc}), 3.71 (s, 3H, O*Me*), 3.63 (m, 1H, H-3^{Glc}), 3.53 (m, 1H, H-5^{Glc}), 3.10 (d, 1H, 4-OH^{Glc}), 2.60–2.66 (m, 4H, –OCOCH_2CH_2COO–), 2.10 (m, 2H, H-6^{Cer}, H-6′^{Cer}), 1.95 (s, 3H, OAc), 1.52 (m, 2H, H-5^{Cer}, H-5′^{Cer}), 1.00–1.60 (m, 54H, –CH_2–), 0.90 (t, 6H, 2Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 172.2, 171.9, 170.5, 165.1, 159.3, 135.7, 135.5, 133.2, 132.8, 132.6, 129.9, 129.8, 129.7, 128.7, 128.4, 127.9, 127.84, 127.79, 113.8, 86.3, 82.9, 73.9, 72.1, 71.6, 69.8, 63.0, 62.3, 55.1, 49.2, 36.8, 31.9, 29.70, 29.66, 29.6, 29.5, 29.39, 29.35, 29.1, 27.9, 26.8, 25.7, 22.7, 20.7, 19.2, 14.1; HRESIMS: calcd for $C_{85}H_{123}NO_{14}SSi$: 1464.8289 $[M+Na]^+$, found: *m*/*z* 1464.8357.

3.7. Phenyl 6-*O*-[{(2*S*,3*S*,4*R*)-3-*O*-acetyl-2-octadecanolyamino-octadecane-4-yloxy}carbonylpropanoyl]-2-*O*-benzoyl-3-*O*-4-methoxybenzyl-1-thio-β-D-glucopyranoside (9)

To a soln of **8** (48.8 mg, 0.034 mmol) in THF (338 μL) were added AcOH (6.0 μL, 0.101 mmol) and tetrabutylammonium fluoride (102 μL, 0.102 mmol). After stirring at 0 °C for 12 h (TLC monitoring: 1:2 EtOAc–hexane), the mixture was diluted with $CHCl_3$, and washed with satd $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (1:5 toluene–EtOAc) to give **9** (30 mg, 77%) as an amorphous solid: $[\alpha]_D$ +7.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20–8.05 (m, 10H, 2Ph), 6.65–7.19 (2d, 4H, MeO*Ph*), 6.23 (d, 1H,

$J_{2,NH}$ = 9.0 Hz, NH), 5.21 (t, 1H, $J_{1,2} = J_{2,3}$ 10.0 Hz, H-2^{Glc}), 5.01–5.06 (m, 2H, H-3^{Cer}, H-4^{Cer}), 4.78 (d, 1H, H-1^{Glc}), 4.63 (2d, 2H, J_{gem} 11.5 Hz, PhCH_2), 4.48 (dd, 1H, $J_{5,6'}$ 4.0 Hz, J_{gem} 12.0 Hz, H-6$'^{Glc}$), 4.39 (dd, 1H, $J_{5,6}$ 2.0 Hz, H-6^{Glc}), 4.19 (m, 1H, $J_{2,NH}$ 9.0 Hz, H-2^{Cer}), 3.65–3.74 (m, 6H, H-1$'^{Cer}$, H-3^{Glc}, H-4^{Glc}, O*Me*), 3.54–3.59 (m, 2H, H-1^{Cer}, H-5^{Glc}), 2.60–2.75 (m, 4H, –OCOCH_2CH_2COO–), 2.20 (m, 2H, H-6^{Cer}, H-6$'^{Cer}$), 2.15 (s, 3H, OAc), 1.60 (m, 2H, H-5^{Cer}, H-5$'^{Cer}$), 1.20–1.79 (m, 54H, –CH_2–), 0.89 (t, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 172.6, 171.9, 171.5, 165.2, 159.3, 133.2, 132.8, 132.5, 129.83, 129.79, 129.7, 129.0, 128.7, 128.4, 128.2, 127.8, 125.3, 113.7, 86.4, 82.9, 77.8, 74.5, 73.4, 73.2, 72.0, 69.7, 63.3, 61.4, 55.1, 49.6, 36.7, 31.9, 29.7, 29.6, 29.5, 29.34, 29.28, 29.1, 28.6, 25.6, 25.5, 22.7, 20.9, 14.1; HRESIMS: calcd for $C_{69}H_{105}NO_{14}S$: 1226.7148 [M+Na]$^+$, found: *m*/*z* 1226.7180.

3.8. 2-*O*-Benzoyl-3-*O*-4-methoxybenzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*)-3-*O*-acetyl-2-octadecanoylamino-octadecane-4,6-succinate (10)

To a soln of **9** (43 mg, 0.036 mmol) in CH_2Cl_2 (1.2 mL) was added MS4Å (40 mg). After stirring for 1 h, NIS (16.0 mg, 0.071 mmol) and TfOH (0.6 μL, 0.0079 mmol) were added to the suspension at 0 °C. The mixture was stirred for 5 h at 0 °C. After completion of the reaction (TLC 1:1 toluene–EtOAc), the mixture was filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$, washed with satd $NaHCO_3$, satd $Na_2S_2O_3$ and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (3:1 toluene–EtOAc) to give **10** (33 mg, 85%) as an amorphous solid: $[\alpha]_D$ +3.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–8.05 (m, 5H, Ph), 6.75–7.12 (2d, 4H, MeO*Ph*), 6.02 (d, 1H, $J_{2,NH}$ 9.0 Hz, NH), 5.18 (m, 2H, $J_{1,2}$ 7.5 Hz, H-4^{Cer}, H-2^{Glc}), 5.10 (t, 1H, H-3^{Cer}), 4.66 (d, 1H, J_{gem} 11.5 Hz, PhCH_2), 4.55 (d, 1H, PhCH_2), 4.47 (d, 1H, H-1^{Glc}), 4.39 (d, 1H, $J_{1,2}$ 5.5 Hz, H-2^{Cer}), 4.37–4.41 (m, 2H, H-6^{Glc}, H-6$'^{Glc}$), 3.81 (dd, 1H, J_{gem} 11.5 Hz, H-1^{Cer}), 3.73 (s, 3H, O*Me*), 3.61–3.69 (m, 3H, H-1$'^{Cer}$, H-3^{Glc}, H-5^{Glc}), 3.49 (dt, 1H, $J_{4,4\text{-OH}}$ 2.0 Hz, H-4^{Glc}), 2.50–2.80 (m, 4H, –OCOCH_2CH_2COO–), 2.50 (d, 1H, 4-OH^{Glc}), 2.10 (s, 3H, OAc), 2.00 (m, 2H, H-6^{Cer}, H-6$'^{Cer}$), 1.50 (m, 2H, H-5^{Cer}, H-5$'^{Cer}$), 1.10–1.60 (m, 54H, –CH_2–), 0.84 (t, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 171.4, 171.2, 170.8, 165.0, 159.3, 133.3, 129.8, 129.7, 129.6, 129.5, 128.5, 114.0, 113.9, 100.1, 81.5, 74.7, 74.3, 73.8, 73.7, 72.7, 70.4, 63.8, 55.1, 47.4, 37.4, 37.1, 36.5, 33.5, 32.7, 31.9, 30.2, 30.0, 29.7, 29.64, 29.55, 29.52, 29.48, 29.4, 29.34, 29.32, 29.2, 27.4, 27.1, 25.4, 25.0, 24.4, 22.7, 21.0, 19.7, 14.1; HRESIMS: calcd for $C_{63}H_{99}NO_{14}$: 1116.6963 [M+Na]$^+$, found: *m*/*z* 1116.6923.

3.9. (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-4-methoxybenzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*)-3-*O*-acetyl-2-octadecanoylamino-octadecane-4,6-succinate (12)

To a soln of **10** (63 mg, 0.0578 mmol) and **11** (152 mg, 0.137 mmol) in CH_2Cl_2 (1.1 mL) were added molecular sieves (AW-300) (200 mg). After stirring for 1 h, TMSOTf (1.0 μL, 0.00548 mmol) was added to the suspension at 0 °C. The progress of the reaction was monitored by TLC (1:3 toluene–EtOAc). After stirring for 4 h at 0 °C, the reaction mixture was filtered through a pad of Celite. The combined filtrate and washings were extracted with $CHCl_3$, and washed with satd $NaHCO_3$, and brine, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel (1:2 toluene–EtOAc) to give **12** (83 mg, 70%) as an amorphous solid: $[\alpha]_D$ +24.4 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–8.21 (m, 20H, 4Ph), 6.43–7.01 (2d, 4H, MeO*Ph*), 5.94 (d, 1H, $J_{2,NH}$ 8.2 Hz, NH^{Cer}), 5.68 (m, 1H, $J_{7,8}$ 9.2 Hz, H-8^{Neu}), 5.55 (t, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 8.2 Hz, H-2^{Gal}), 5.36 (d, 1H, H-4^{Gal}), 5.24 (dd, 1H, $J_{6,7}$ 2.8 Hz, $J_{7,8}$ 9.2 Hz, H-7^{Neu}), 5.10 (d, 1H, H-1^{Gal}), 5.08 (m, 1H, H-4^{Cer}), 5.02 (d, 1H, $J_{1,2}$ 7.8 Hz, H-2^{Glc}), 4.91–4.98 (m, 3H, H-3^{Cer}, NH^{Neu}, H-3^{Gal}), 4.81 (m, 1H, H-4^{Neu}), 4.66–4.87 (2d, 2H, J_{gem} 11.0 Hz, PhCH_2), 4.42 (dd, 1H, J_{gem} 12.4 Hz, H-9$'^{Neu}$), 4.35 (d, 1H, H-1^{Glc}), 4.14–4.37 (m, 6H, H-5^{Gal}, H-6^{Gal}, H-6$'^{Gal}$, H-6^{Glc}, H-6$'^{Glc}$, H-2^{Cer}), 4.05 (dd, 1H, $J_{8,9}$ 6.0 Hz, J_{gem} 12.4 Hz, H-9^{Neu}), 3.71–3.82 (m, 8H, H-1^{Cer}, H-1$'^{Cer}$, H-3^{Glc}, H-4^{Glc}, H-5^{Neu}, O*Me*), 3.61 (dd, 1H, H-6^{Neu}), 3.57 (s, 3H, O*Me*), 3.50 (m, 1H, H-5^{Glc}), 2.36–2.67 (m, 4H, –OCOCH_2CH_2COO–), 2.48 (dd, 1H, $J_{3eq.,4}$ 4.6 Hz, H-3eq.^{Neu}), 1.64 (t, 1H, J_{gem} 12.4 Hz, H-3ax.^{Neu}), 1.52–2.18 (6s, 18H, OAc), 1.80 (m, 2H, H-6^{Cer}, H-6$'^{Cer}$), 1.40 (m, 2H, H-5^{Cer}, H-5$'^{Cer}$), 1.10–1.50 (m, 54H, –CH_2–), 0.87 (t, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 171.0, 170.9, 170.8, 170.7, 170.6, 170.3, 170.1, 168.2, 165.7, 165.6, 165.1, 158.8, 133.3, 133.1, 130.4, 130.2, 129.9, 129.8, 129.7, 129.6, 129.4, 128.6, 128.5, 128.3, 113.4, 101.3, 98.8, 96.9, 79.5, 78.7, 77.7, 74.5, 74.4, 73.4, 73.1, 73.1, 72.0, 71.6, 71.4, 70.9, 69.4, 68.2, 67.6, 66.6, 63.3, 62.4, 61.7, 55.0, 53.2, 48.8, 46.6, 37.4, 36.3, 31.9, 30.8, 30.0, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 25.3, 25.2, 23.2, 22.7, 21.5, 20.9, 20.8, 20.7, 20.4, 14.1; HRESIMS: calcd for $C_{110}H_{148}N_2O_{34}$: 2063.9811 [M+Na]$^+$, found: *m*/*z* 2063.9823.

3.10. *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(*O*-β-D-galactopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*)-2-octadecanoylamino-octadecane-1,3,4-triol (14)

To a soln of **12** (38 mg, 0.0184 mmol) in CH_2Cl_2 (800 μL) was added trifluoroacetic acid (370 μL). The mixture was stirred for 7 h at room temperature. After complete consumption of the starting material (TLC 6:1 EtOAc–hexane), Et_3N was added at 0 °C. The reaction mixture was co-evaporated with toluene. After concentration, the residue was dissolved in MeOH (1.0 mL) and NaOMe (7.2 mM soln in MeOH, 100 μL, 5.2 μmol) was added at 0 °C. After stirring at room temperature for 26 h (TLC monitoring: 10:1:1 BuOH–MeOH–water), water was added to the mixture at 0 °C. The mixture was stirred at room temperature for 10 h (TLC monitoring: 10:1:1 BuOH–MeOH–water). The reaction mixture was neutralized with Dowex (H^+) and filtered. The combined filtrate and washings were concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (200 g) gave **14** (22.1 mg, quantitative) as an amorphous solid: $[\alpha]_D$ +8.0 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 4.42 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1^{Gal}), 4.31 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1^{Glc}), 2.85 (dd, 1H, J_{gem} 11.7 Hz, H-3eq.^{Neu}), 2.20 (m, 2H, H-6^{Cer}, H-6$'^{Cer}$), 2.00 (s, 3H, NAc), 1.73 (t, 1H, H-3ax.^{Neu}), 1.50 (m, 2H, H-5^{Cer}, H-5$'^{Cer}$), 1.10–1.70 (m, 54H, –CH_2–), 0.90 (t, 6H, 2Me); ^{13}C NMR (150 MHz, CD_3OD): δ 176.0, 175.5, 175.0, 105.1, 104.5, 101.1, 80.9, 77.7, 77.1, 76.5, 76.2, 75.1, 75.0, 74.8, 73.1, 73.0, 70.9, 70.2, 70.1, 69.4, 69.0, 64.6, 62.7, 61.8, 54.0, 51.9, 49.9, 49.6, 42.1, 37.3, 33.1, 32.3, 30.9, 30.9, 30.8, 30.8, 30.7, 30.6, 30.5, 30.5, 30.4, 27.1, 23.7, 22.6, 14.4; HRESIMS: calcd for $C_{59}H_{110}N_2O_{22}$: 1221.7442 [M+Na]$^+$, found: *m*/*z* 1221.7450.

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