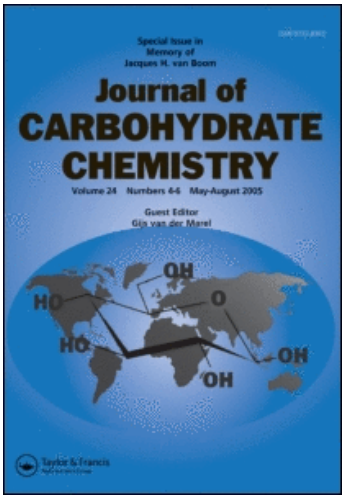

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Ganglioside GM_3 Derivatives with Truncated Ceramide Moiety: Facial Synthesis and Inhibitory Activity against KB Cell Growth

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Ganglioside GM_3 Derivatives with Truncated Ceramide Moiety: Facial Synthesis and Inhibitory Activity against KB Cell Growth

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An expeditious sialylation reaction with phenylthioglycoside **4** as a sialyl donor and MeSOTf as a promoter was developed. These conditions are very useful for synthesizing ganglioside GM_3 (**1**), its C8-ceramide analog **2**, and 3-deoxy analog **3** of **2** in an efficient manner. The GM_3 analog **2**, whose hydrophilicity is increased by shortening the ceramide moiety, exhibits increased growth inhibiton of KB cells. The 3-hydoxy group of ceramide does not influence its activity against KB cells.

Keywords Ganglioside GM_3, truncated ceramide, inhibitory activity against KB cell growth

INTRODUCTION

Gangliosides are sialic acid-containing glycosphingolipids that are present on all mammalian plasma membranes, where they participate in recognition and signaling activity.[1] Ganglioside GM_3 (**1**, herein referred to as GM_3), a simple

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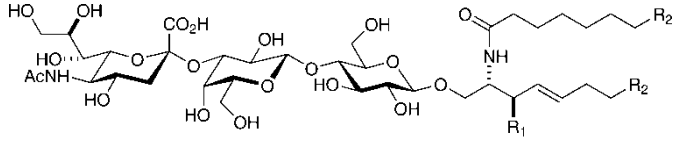
and widely distributed glycosphingolipid, is known as (1) a negative regulator of insulin signaling, which makes it a potential therapeutic target in type II diabetes,[2] (2) a modulator of cell growth through inhibition of EGF receptor-associated tyrosine kinase,[3] (3) a tumor-associated antigen in humans,[4] (4) a modulator of EGF receptor phosphorylation, (5) a regulator of protein kinase A (PKA) activity, and (6) an inducer of CD4 internalization in human lymphocytes.[5] GM_3 also interacts with HIV surface envelope glycoproteins and modulates Ca^{2+} flux in triad membranes.[6] Although large-scale isolation of GM_3 from hybridoma cells has been developed, the purification typically produces as a mixture of the ceramide moiety (*N*-acylsphingosine) with C16 : 0-, C18 : 0-, C20 : 0-, C22 : 0-, and C24 : 1-fatty acids.[7] Interestingly, the ceramide itself plays an important role in the regulation of several biological processes of the cell. Some diseases are related to ceramide accumulation and subsequent apoptosis induction.[8] Because glycolipids isolated from eukaryotes and prokaryotes generally exhibit both amphiphilic and amphiphatic nature,[9] it is important to (1) improve the physiochemical properties of glycolipids that facilitate the penetration of a biological membrane and (2) design the mimic of lipid moieties that can be recognized by glycosylases. As a part of our studies on the synthesis of bioactive glycolipids and on the development of inhibitors of glycosyltransferases and fatty acid biosyntheses, we wish to address the function of the ceramide of glycolipids.[10] For these investigations we first selected GM_3 because of its significant biological activity (*vide infra*) and because of its structural simplicity compared to the other higher gangliosides.

The ceramides are biosynthesized from L-serine and the C3-OH group, and the configuration is important for their function as an acceptor for glycosyltransferases.[11] However, C3-OH group may not be an essential functional group for gangliosides to exhibit their biological activities.[12] It is also important to understand the minimum chain-length of the lipids required for enzymatic recognitions and biological activities. In order to gain information about these questions, we need to synthesize GM_3 analogs containing shorter carbon-chain ceramides. We now wish to report an expeditious sialylation of the lactose derivative **5** and syntheses of GM_3 (**1**, as a control), C8-GM_3 **2**,[13] and its 3-deoxy analog **3** (Fig. 1), and a preliminary biological evaluation of these analogs against KB cells.



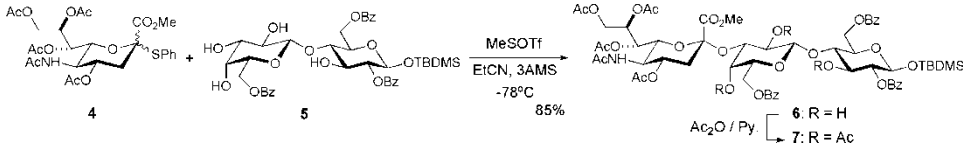
Ganglioside GM_3 (1): R_1, R_2 = OH, $C_{11}H_{23}$
C8-GM_3 (2): R_1, R_2 = OH, CH_3
3-Deoxy-C8-GM_3 (3): R_1, R_2 = H, CH_3

Figure 1: Structures of ganglioside GM_3 and its analogues.

Syntheses of GM_3 Analogs

A number of elegant syntheses of gangliosides have been reported to date.[14] One of challenges in the syntheses of gangliosides is how efficiently one can achieve the sialylation selectively at the desired position of the acceptor. In order to achieve efficient α-selective sialylation reactions, it is very important to find an appropriate combination between leaving group of the sialyl donor and its promoter.[14c,d] Some sialylation conditions require long reaction times at controlled temperatures and do not afford exclusively the desired α-glycoside in good yield. On the contrary, enzymatic sialylations have an advantage over chemical methods because of exclusive regio- and stereoselectivity with unprotected acceptors.[15] However, chemical synthesis of structurally less complicated GM_3 derivatives is more feasible. We selected phenythio glycoside **4** (a 1 : 6.6 mixture of α- and β-anomers) as a silayl donor,[16] which could be synthesized in 85% overall yield in three steps from sialic acid. We observed that MeSOTf[17] generated from AgOTf and MeSBr was very effective to activate **4** in propionitrile at $-78^{\circ}C$ or in CH_3CN. (Importance of using a nitrile solvent in α-selective sialylation was described in a number of literatures (see ref. 12)) at $-45^{\circ}C$ in which the sialylations with benzyl alcohol and 2-trimethylsilylethanol were completed within 1 min to afford the corresponding sialyl glycoside as a $6 \sim 7 : 1$ mixture of α- and β-glycosides in quantitative yields. Gratifyingly, when these conditions were applied to partially protected TBDMS lactoside **5**, which was synthesized by Hasegawa's procedures,[14d,18] the α-sialyl lactoside **6** was obtained within 5 min in 85% yield without a detectable amount of undesired β-glycoside. (The structure of **6** was confirmed after conversion of **6** into the imidate **16** whose physical data was identical to that reported in the literature. Hasegawa also reported better α-selectivity of sialylations with the partially protected lactoside than those with the primary alcohols (see ref. 14d)) The same reaction was demonstrated on a gram-scale and we confirmed reproducibility of selectivity and reaction yield. α-Sialyl lactoside **6** was protected with Ac_2O and pyridine to give the per-protected trisaccharide **7** in quantitative yield. We stored **7** as a building block for the systematic syntheses of GM_3 analogs (Sch. 1).[19]

The synthesis of ceramide analogs began with the corresponding sphingosine analogs. By using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline

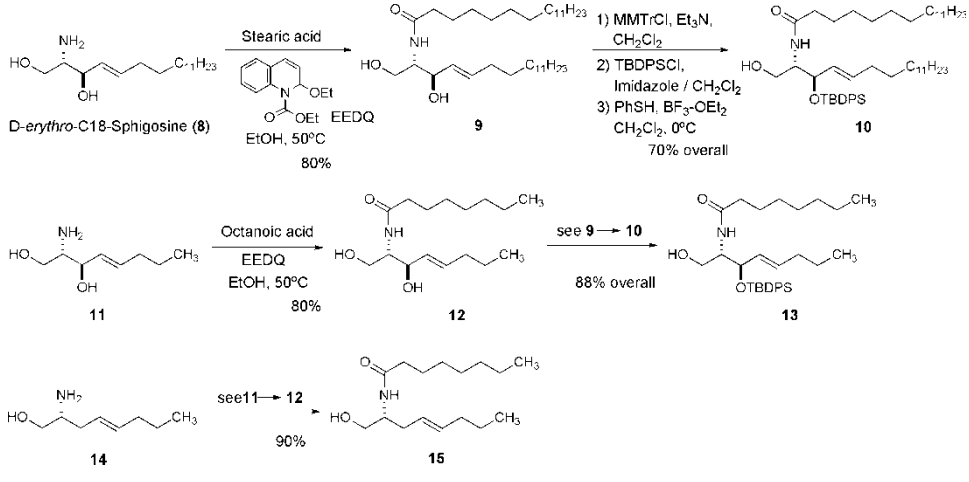


4 5

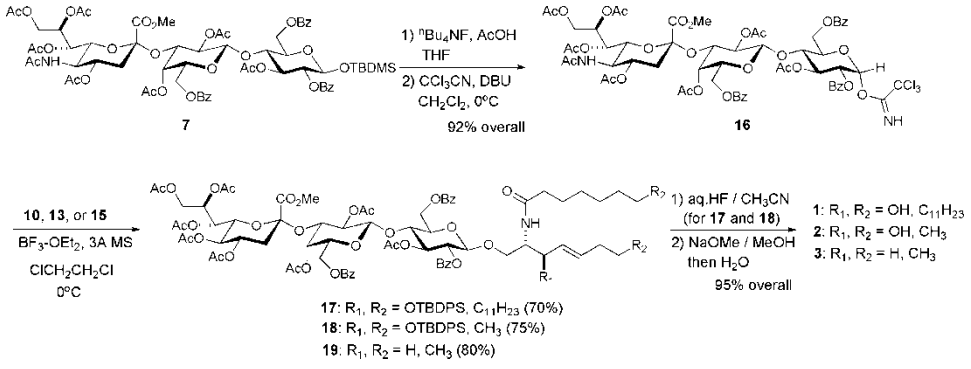
Scheme 1:

(EEDQ), D-*erythro*-C18-sphingosine (**8**) was condensed with stearic acid to provide the ceramide **9** in 80% yield. The C3-hydroxyl group of the ceramide **9** was protected as its TBDPS ether in three steps (1, tritylation of primary alcohol; 2, TBDPS silylation; and 3, deprotection of trityl group using PhSH in the presence of $BF_3 \cdot OEt_2$) to afford **10** in 70% overall yield. (The corresponding C3-benzoyl and acetyl-protected ceramide were not useful in the glycosylations (**16** → **17** and **18**); the coupling between **16** and the acylated ceramides gave the desired products in less than 30% yields. It was speculated that C3-acyl carbonyl deactivates the reactivity of the C1-alcohol by the formation of an eight-membered Lewis acid complex) In a similar manner C8-sphingosine **11**[20] was converted to its TBDPS ether **13**. (*R,E*)-2-aminooct-4-en-1-ol (**14**) was synthesized from D-glyceraldehyde derivative in eight steps in 40% overall yield and coupled with octanoic acid using EEDQ (Sch. 2).[20]

With the protected ceramide **10** (a natural form) and its analogs **13** and **15** in our hands, the fully protected trisaccharide **7** was then converted to the imidate **16** in two steps: selective desialylation of the anomeric position, and imidate formation.[21] The coupling reactions between the sialyl-lactosylimidate **16** and the ceramide analogs **10**, **13**, and **15** smoothly underwent at 0°C under Schmidt's conditions to give exclusively β-glycosides **17**, **18**, and **19** in good yields of 70%, 75%, and 80%, respectively. Global deprotections of these coupling products were achieved by the treatment of aq. HF (for **17** and **18**) followed by saponifications to provide **1**, **2**, and **3** in excellent yields. Thus, we could synthesize the GM_3 ceramide analog in a convergent manner as illustrated in Scheme 3.



Scheme 2:



Scheme 3:

Activity of GM_3 Ceramide Analogs on KB Cell Proliferation

As a preliminary study on the biological activity of GM_3 analogs, we first examined the growth-inhibitory property of the synthesized GM_3 ceramide analogs **2** and **3** against KB cells in vitro. We observed a pronounced growth inhibitory effect of **2** and **3** against KB cells compared to the natural form **1**; the analogs **2** and **3** inhibited the growth of KB cells at 0.5 nmol/mL; however, **1** did not inhibit growth at the same concentrations. The analogs **2** and **3** are about 100-fold more potent than **1** in inhibiting the growth of KB cells (Figure 2).

DISCUSSION AND CONCLUSION

We have demonstrated expeditious α-sialylation with phenylthioglycoside **4** as a sialyl donor and methylthiotrilate as a promoter. Introduction of the ceramide

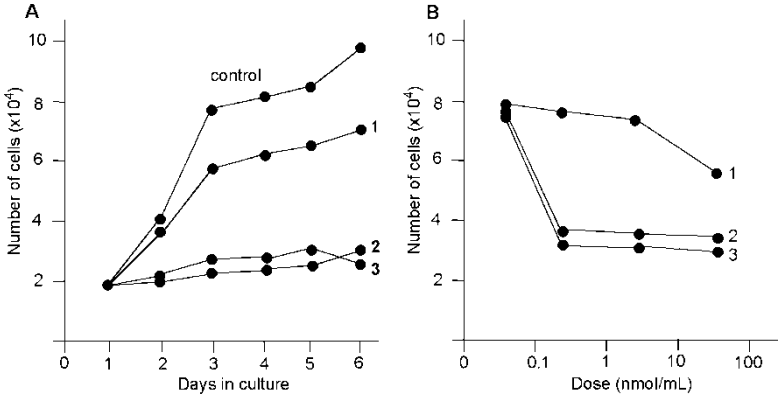


Figure 2: A. Effect of GM_3 derivatives **2** and **3** on growth of KB cells. Cells were treated at 50 nmol/mL. **B**. Dose response of **2** and **3** on growth of KB cells at various concentrations.

analogs to the trisaccharide donor was also achieved in very good yield by using ceramide **13** possessing a TBDPS-protecting group at C3 position. The shorter chain length of ceramide portion of GM_3 significantly improved its biological activity as demonstrated by the inhibition of KB cell proliferation. The C3-hydroxy group of the ceramide moiety did not contribute to the inhibitory activity against KB cells.

In studies of the immunosuppressive activity of gangliosides it is reported that ceramide containing a shorter fatty acyl chain influences its immunosuppressive activity. Ceramides with chain lengths of 16 and 18 carbons were 6- to 10-fold more active than those with 22 and 24 carbons.[22] Thus, the subtle difference in the chain length of fatty acid of ganglioside makes a dramatic difference in immunosuppressive activity. The structures of the carbohydrate moiety in certain glycolipids are identical between normal and tumor cells. However, structural diversity in the ceramide moiety has been observed. Therefore, synthesis of diverse ceramide analogs and their biological evaluations at the molecular level have been of great interest.[23] As a result, it was realized that certain ceramide analogs have important functional roles such as cell adhesion and apoptosis.[24] However, there is lack of data of biological function for ceramide structures conjugated with oligosaccharides. No rationale has been found for the optimization of ceramide structure of each biologically significant glycolipid. Our study described here and others[13,22,24a] strongly suggest that biological properties of gangliosides would be altered by the change in length of ceramide chains. We are currently validating the effects of ceramide-chain truncated GM_3 on other cancer cell lines as well as enzymatic recognition of the truncated ceramides and their glycoconjugates.

EXPERIMENTAL

Methyl (phenyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-dideoxy-2-thio-D-*galacto*-2-nonulopyranoside)onate (4). To a stirred solution of 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid methyl ester[25] (5.0 g, 9.57 mmol) and PhSH 3.93 mL, 38.3 mmol) in CH_2Cl_2 (95 mL) at 0°C was added $BF_3 \cdot OEt_2$ (10.9 g, 76.6 mmol). The reaction mixture was warmed to r.t. After being stirred for 12 h, the reaction mixture was poured into cold aq. $NaHCO_3$ solution. The water phase was extracted with CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification by silica gel chromatography (toluene:acetone = 15 : 1) gave **4** (5.1 g, 92%) as a white solid: $[\alpha]_D = -94.0$ (*c* 1.2, $CHCl_3$); β-glycoside; ^{1}H NMR (500 MHz, $CDCl_3$) 3.26 ppm (3H, s), 2.26 (1H, dd, $J = 12.5$, 4.8 Hz), 2.08, 2.04, 1.96, 1.90, 1.85 (3H each, s), 2.00 (1H, dd, $J = 13.9$, 11.4 Hz); α-glycoside; 3.57 (3H, s), 2.83 (1H, dd, $J = 12.5$, 4.5 Hz), 2.15, 2.14, 2.05, 2.02, 1.87 (3H each, s), 2.00 (1H, dd, $J = 12.5$,

10.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) 86.0 ppm, 85.9 (anomeric carbons); IR ($CHCl_3$) 3065 cm^{-1}, 2957, 2936, 1745, 1602. FAB-HRMS calcd for $C_{26}H_{33}NO_{12}S$ $[M + H^+]$: 454.17.2244. Found: 454.2246. The anomeric ratio was determined to be 1 : 6.6 (α/β) from the integration value of C3-equatorial protons in ^{1}H NMR.

General procedure for sialylation with 4. MeSBr was prepared from Me_2S_2 (200 μL, 2.72 mmol) and Br_2 (133 μL, 2.59 mmol) in $ClCH_2CH_2Cl$ (3 mL) at r.t. This solution could be stored in the dark for over 1 month without loss of activity. Thiophenyl glycoside **4** (2.5 eq), the glycosyl acceptor, AgOTf (3 eq), and MS 3Å in EtCN (0.1 ∼ 0.3 M) were stirred at r.t. for 1 h. The reaction mixture was cooled to −78°C and MeSBr (ca. 1.55 M, 2.8 eq) was added. After 1 ∼ 15 min, the reaction mixture was diluted with CH_2Cl_2 and filtered through celite. The organic phase was washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel chromatography (toluene:acetone = 10 : 1).

***tert*-Butyldimetylsilyl *O*-(methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-dideoxy-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (6):** 85% yield as a white solid: $[\alpha]_D = +45.3$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 8.1–7.2 ppm (15H, m), 3.79 (3H, s), 2.57 (1H, dd, J = 12.5, 4.6 Hz), 2.14, 2.06, 2.04, 1.95 (3H each s), 1.90 (3H, s), 0.89 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) 104.3 ppm, 97.5, 90.9 (anomeric carbons); IR (KBr) 3470 cm^{-1}, 3065, 2957, 2932, 1745, 1602. FAB-HRMS calcd for $C_{59}H_{76}NO_{26}Si$ $[M + Na^+]$: 1264.4244. Found: 1264.4237.

***tert*-Butyldimetylsilyl *O*-(methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucopyranoside (7).** To a stirred solution of **6** (2.0 g, 1.61 mmol) in pyridine (5 mL) was added Ac_2O (5 mL). After 12 h, all volatiles were evaporated under high vacuum to give the crude product. Purification by silica gel chromatography (toluene: acetone = 15 : 1) gave **7** (2.2 g, 100%) as a white solid: $[\alpha]_D = +42.8$ (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 8.1–7.4 ppm (15H, m), 5.78 (1H, t, J = 9.9 Hz), 4.58 (1H, dd, J = 9.9, 3.3 Hz), 4.19 (1H, dd, J = 11.6, 7.6 Hz), 4.30 (1H, m), 3.68 (3H, s), 2.58 (1H, dd, J = 11.9, 4.28 Hz), 2.21, 2.12, 2.04, 2.01, 1.98, 1.95 (3H each s), 1.82 (3H, s), 1.69 (1H, t, J = 11.9), 0.89 (9H, s), 0.01 (3H, s), 0.00 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) 101.4 ppm, 96.7, 90.5 (anomeric carbons); IR (KBr) 3470 cm^{-1}, 3378, 3067, 2859, 1748, 1603. FAB-HRMS calcd for $C_{65}H_{81}NO_{29}Si$ $[M + Na^+]$: 1390.4561. Found: 1390.4618.

***O*-(Methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucopyranosyl)trichloroacertymidate (16).** To a stirred solution of **7** (113.9 mg, 0.0833 mmol) in THF (2.8 mL) was added AcOH (194 mL, 0.28 mmol, 3.4 eq.) and TBAF (1.0 M, 183 μL, 2.2 eq). After 48 h, the reaction was quenched with MeOH (1 mL). All volatiles were evaporated in vacuo. Purification by silica gel chromatography (toluene:acetone = 5 : 1) gave *O*-(methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucopyranose (101.3 mg, 97%) as a white solid. This was dissolved in CH_2Cl_2 (1 mL). Into the solution Cl_3CCN (156 μL, 1.3 eq) and DBU (38 μL, 0.026 mmol, 0.5 eq) were added at 0°C. After 1 h, all volatiles were evaporated in vacuo to provide the crude imidate. Purification by silica gel chromatography (toluene: acetone = 6 : 1) gave **16**[18] (68.3 mg, 95%) as a white solid: $[\alpha]_D = +33.0$ (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 8.55 ppm (1H, s), 8.1–7.3 (15H, m), 6.66 (1H, d, $J = 9.9$ Hz), 5.86 (1H, t, $J = 9.9$ Hz), 5.6–4.9 (m), 4.60 (1H, dd, $J = 9.9$, 3.3 Hz), 4.4–3.9 (m), 3.71 (3H, s), 3.58 (1H, dd, $J = 10.6$, 2.3 Hz), 2.59 (1H, dd, $J = 13.6$, 4.28 Hz), 2.21, 2.13, 2.10, 2.00, 1.97, 1.83 (3H each s); IR (KBr) 3327 cm^{-1}, 2961, 1746, 1678, 1602. Anal. Calcd for $C_{61}H_{67}N_2O_{29}Cl_3$: C, 52.39; H, 4.83; N, 2.00. Found: C, 52.42; H, 4.85; N, 1.90.

(2*S*,3*R*,4*E*)-3-*O*-*tert*-Butyldiphenylsilyl-2-octadecanamide-octadec-4-ene-1,3-diol (10): $[\alpha]_D = -11.6$ (*c* 2.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 7.7–7.3 ppm (10H, m), 5.94 (1H, br.d), 5.42–5.32 (1H, m), 4.35–4.32 (1H, m), 3.91–3.81 (2H, m), 3.64–3.57 (1H, m), 2.00–1.93 (2H, m), 1.90–1.86 (2H, m), 1.53–1.48 (2H, m), 1.45–1.20 (50H, m), 1.07 (9H, s), 0.88 (6H, t-like); IR (KBr) 3300 cm^{-1}, 2924, 2855, 1645. FAB-HRMS calcd for $C_{52}H_{89}NO_3Si$ $[M + Na^+]$: 826.6500. Found: 826.6499.

(2*S*,3*R*,4*E*)-3-*O*-*tert*-Butyldiphenylsilyl-2-octanamide-oct-4-ene-1,3-diol (13): $[\alpha]_D = -30.6$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 7.7–7.3 ppm (10H, m), 5.94 (1H, br.d), 5.67 (1H, m), 4.53 (1H, m), 3.91–3.81 (2H, m), 3.60–3.57 (1H, m), 2.00–1.93 (2H, m), 1.90–1.86 (2H, m), 1.53–1.20 (12H, m), 1.07 (9H, s), 0.96 (6H, t-like); IR (KBr) 3305 cm^{-1}, 2924, 2855, 1648. FAB-HRMS calcd for $C_{32}H_{49}NO_3Si$ $[M + Na^+]$: 548.3392. Found: 548.3389.

(2*R*,4*E*)-2-Octamido-oct-4-en-1-ol (15): $[\alpha]_D = -3.5$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) 8.19 ppm (1H, br.d), 5.4–5.7 (2H, m), 4.5 (1H, m), 3.9 (2H, m), 2.7–2.5 (2H, m), 2.37 (2H, t, $J = 7.25$ Hz), 1.4–1.1 (12H, m), 1.53–1.48 (2H, m), 1.4–1.1 (12H, m), 0.82 (6H, m); IR (KBr) 3300 cm^{-1}, 2924, 2854, 1644. FAB-HRMS calcd for $C_{16}H_{31}NO_2$ $[M + Na^+]$: 292.4236. Found: 292.4231.

***O*-(Methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-di-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 1)-(2*S*,3*R*,4*E*)-3-*O*-*tert*-butyldiphenylsilyl-2-octadecanamide-octadec-4-ene-1,3-diol (17).** To a stirred suspension of **16** (20.9 mg, 0.026 mmol, 1.3 eq), **10** (28 mg, 0.020 mmol), and MS 3A (100 mg), $ClCH_2CH_2Cl$ (1 mL) was stirred for 1 h at r.t. and cooled to $-20 \sim -25^\circ C$. Into the reaction mixture $BF_3 \cdot OEt_2$ (11.3 mg, 0.080 mmol, 4 eq) was added. The reaction mixture was stirred for 12 h at 0°C and diluted with CH_2Cl_2 and filtered through celite. The organic phase was washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel chromatography (toluene:acetone = 5 : 1) to give **17** (28.5 mg, 70%) as a white solid: $[\alpha]_D = -2.0$ (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 8.2–7.3 ppm (25H, m), 5.6–3.6 (21H, m), 3.71 (3H, s), 2.58 (1H, dd, $J = 13.4$, 4.8 Hz), 2.20, 2.12, 2.04, 2.02, 2.01, 2.00, 1.92, 1.84 (3H each s), 1.2 (52H, m), 0.87 (6H, s), 0.86 (9H, s); IR (KBr) 3312 cm^{-1}, 3070, 2926, 1748, 1685, 1602. FAB-HRMS calcd for $C_{111}H_{154}NO_{31}Si$ $[M + Na^+]$: 2062.0202. Found: 2062.0327.

***O*-(Methyl 5-acetoamido-4,7,8,9-*tetra*-*O*-acetyl-3,5-di-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-*O*-(2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 1)-(2*S*, 3*R*, 4*E*)-3-*O*-*tert*-butyldiphenylsilyl-2-octanamide-oct-4-ene-1,3-diol (18).** To a stirred suspension of **16** (21.0 mg, 0.026 mmol, 1.3 eq), **13** (10.5 mg, 0.020 mmol), and MS 3A (100 mg), $ClCH_2CH_2Cl$ (1 mL) was stirred for 1 h at r.t. and cooled to $-20 \sim -25^\circ C$. Into the reaction mixture $BF_3 \cdot OEt_2$ (11.3 mg, 0.080 mmol, 4 eq) was added. The reaction mixture was stirred for 12 h at 0°C and diluted with CH_2Cl_2 and filtered through celite. The organic phase was washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel chromatography (toluene:acetone = 5 : 1) to give **18** (26.4 mg, 75%) as a white solid: $[\alpha]_D = -10$ (*c* 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 8.1–7.2 ppm (25H, m), 5.5–3.5 (21H, m), 3.71 (3H, s), 2.58 (1H, dd, $J = 13.4$, 4.8 Hz), 2.21, 2.13, 2.05, 2.02, 2.01, 2.00, 1.92, 1.84 (3H each s), 1.9–1.3 (12H, m), 0.90 (6H, s), 0.86 (9H, s); IR (KBr) 3313 cm^{-1}, 3077, 2928, 1749, 1680, 1602. FAB-HRMS calcd for $C_{91}H_{113}NO_{32}Si$ $[M + Na^+]$: 1783.1192. Found: 1783.1203.

***O*-(Sodium 5-acetoamido-3,5-di-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-β-D-galactopyranosyl)-(1 → 4)-3-*O*-β-D-glucopyranosyl)-(1 → 1)-(2*S*,3*R*,4*E*)-2-octadecanamide-octadec-4-ene-1,3-diol (ganglioside GM$_3$, 1).** To a stirred solution of **17** (18 mg, 0.0088 mmol) in THF (1 mL) was added an HF solution (46% $HF/CH_3CN = 3/7$, 0.5 mL). After

16 h at r.t. the reaction mixture was poured into aq. $NaHCO_3$. The water phase was extracted with $CHCl_3$. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. This was dissolved in absolute MeOH (0.5 mL) and NaOMe (3.8 mg, 0.0352 mmol, 8eq) was added. After 12 h at r.t., water (0.3 mL) was added. The reaction mixture was stirred for an additional 4 h and all volatile were evaporated under high vaccum to give the crude product. This was purified by Sephadex LH-20 ($CHCl_3$:MeOH:H_2O = 24 : 24 : 1) to provide **1**[26] (10.2 mg, 95%) as a white solid: $[\alpha]_D = +4.0$ (*c* 0.5, $CHCl_3$:MeOH=1 : 1); ^{1}H NMR (500 MHz, d_6-DMSO:D_2O = 1 : 1) 5.55 (1H, dt, $J = 15.3$, 6.8 Hz), 5.37 (1H, dd, $J = 15.3$, 6.8 Hz), 4.19 (1H, d, $J = 7.3$ Hz), 4.14 (1H, d, $J = 7.9$ Hz), 2.73 (1H, dd, $J = 11.6$, 4.3 Hz), 2.04 (2H, t, $J = 7.9$ Hz), 1.9 (2H, m), 1.5–1.3 (13H, m), 0.84 (6H, t, $J = 7.3$ Hz); ^{13}C NMR (125 MHz, d_6-DMSO:D_2O = 1 : 1) 173.7 ppm, 173.5, 172.0, 132.7, 131.5, 104.2, 100.0, 93.7; IR (KBr) 3312 cm^{-1}, 3070, 2926, 1748, 1685, 1602. FAB-HRMS calcd for $C_{39}H_{67}N_2O_{21}Na$ $[M + Na_2^+]$: 1225.7161. Found: 1225.7203.

***O*-(Sodium 5-acetoamido-3,5-di-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-β-D-galacto-pyranosyl)-(1 → 4)-3-*O*-β-D-glucopyranosyl)-(1 → 1)-(2*S*,3*R*,4*E*)-2-octanamide-oct-4-ene-1,3-diol (2):** $[\alpha]_D = +19.6$ (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, d_6-DMSO:D_2O = 1 : 1) 5.55 (1H, dt, $J = 15.3$, 6.8 Hz), 5.37 (1H, dd, $J = 15.3$, 6.8 Hz), 4.19 (1H, d, $J = 7.3$ Hz), 4.14 (1H, d, $J = 7.9$ Hz), 2.73 (1H, dd, $J = 11.6$, 4.3 Hz), 2.04 (2H, t, $J = 7.9$ Hz), 1.9 (2H, m), 1.5–1.3 (13H, m), 0.84 (6H, t, $J = 7.3$ Hz); ^{13}C NMR (125 MHz, d_6-DMSO:D_2O = 1 : 1) 173.6 ppm, 173.4, 172.1, 132.7, 131.5, 104.2, 100.7, 93.6; IR (KBr) 3327 cm^{-1}, 2926, 1749, 1624. FAB-HRMS calcd for $C_{39}H_{67}N_2O_{21}Na$ $[M + Na_2^+]$: 945.4032. Found: 945.4048.

***O*-(Sodium 5-acetoamido-3,5-di-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-onate)-(2 → 3)-β-D-galacto-pyranosyl)-(1 → 4)-3-*O*-β-D-glucopyranosyl)-(1 → 1)-(2*R*,4*E*)-2-octanamide-oct-4-ene-1-ol (3):** $[\alpha]_D = +5.1$ (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, d_6-DMSO:D_2O = 50 : 1) 5.5–5.3 (2H, m), 4.19 (1H, d, $J = 7.9$ Hz), 4.15 (1H, d, $J = 7.9$ Hz), 2.74 (1H, dd, $J = 11.6$, 4.3 Hz), 2.04 (2H, t, $J = 7.9$ Hz), 1.9 (2H, m), 1.5–1.1 (13H, m), 0.84 (6H, t-like); IR (KBr) 3320 cm^{-1}, 2926, 1734, 1626. FAB-HRMS calcd for $C_{39}H_{67}N_2O_{20}Na$ $[M + Na_2^+]$: 929.4032. Found: 929.4048.

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