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Efficient diversification of GM3 ganglioside *via* late-stage sialylation and dynamic glycan structural studies with ^{19}F solid-state NMR

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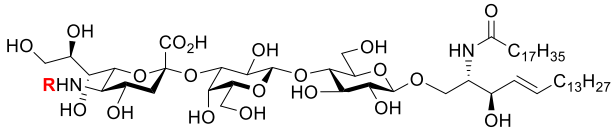
Sialic acid-containing glycoconjugates are involved in important biological processes such as immune response, cancer metastasis, and viral infection. However, their chemical syntheses have been challenging, mainly due to the difficulties in the α-sialylation of oligosaccharides. Very recently, we established a completely stereoselective sialidation method using a macrobicyclic sialyl donor. Herein, we describe a rational and efficient synthesis of sialoglycolipids *via* direct sialylation of a glycolipid at a late-stage, based on our novel sialidation method. The synthetic method enabled the development of GM3 ganglioside analogs with various C5-modifications of the sialosyl moiety. Furthermore, the synthesized analog was subjected to solid-state ^{19}F NMR analysis on the model membranes and revealed the influence of cholesterol on glycan dynamics.

Introduction

Sialic acid is a 3-deoxy-2-ketoaldonic acid comprising a nine carbon backbone and has diverse functions in various biological processes such as immune response, cancer metastasis, and viral infections.[1,2] The chemical synthesis of sialic acid-containing glycoconjugates,[3] such as glycolipids and glycoproteins, remains a challenge mainly due to the difficulty in the formation of the α-sialoside linkage. The formation of the α-sialoside linkage is unfavourable due to anomeric effect.[4-6] Further, the formation of α-sialosides is very difficult to control due to the absence of neighboring group participation from the deoxy C3 position of the sialic acid framework. In addition, the presence of the electron-withdrawing carboxyl group on the anomeric center destabilizes the oxocarbenium intermediate of sialic acid and promotes 1,2-elimination. These drawbacks hamper the efficient and rational chemical synthesis of sialoglycans. For example, the direct sialylation of oligosaccharyl acceptors typically results in stereoisomeric mixtures of the sialosides. The chromatographic purification of such mixtures is highly challenging, due to which oligosaccharyl acceptors are not widely used in chemical sialylation. Thus, the synthesis of the α-sialoside linkage has been confined to the use of monosaccharide building blocks in the earlier stages of the synthesis, despite its positioning on the outermost ends of naturally-occurring oligosaccharides.

Very recently, we reported a fully α-selective sialidation method[7] employing macrobicyclic sialic acid donors, wherein the C1 and C5 positions are tethered using an alkyl chain. In the glycosidation reactions of these donors, the macrocyclic tether moiety sterically blocks the β-face, thereby ensuring completely α-selective sialoside formation. Importantly, the bicyclic sialosyl moiety can be converted into the C5-amino derivative *via* chemoselective cleavage of the 2,2-dichololoethoxycarbamoyl moiety of the tether, which enabled various C5-modifications after sialidations. We expected that our method, which confers exclusive α-sialidation, would streamline the synthesis of various sialo-glycoconjugates. In this study, we focused on the synthesis of GM3 gangliosides with various C5-modified sialic acids. Herein, we describe the synthesis of naturally-occurring GM3s (**1** and **2**), C5-modified GM3 analogs designed for NMR study (**3** and **4**) *via* direct sialylation of a glycolipid acceptor and subsequent C5-modifications at a late-stage of the synthesis



1 (C5-NHAc-GM3): **R** = $C(=O)CH_3$
2 (C5-NHGc-GM3): **R** = $C(=O)CH_2OH$
3 (C5-^{13}C-NHAc-GM3): **R** = $^{13}C(=O)CH_3$
4 (C5-NHTFAc-GM3): **R** = $C(=O)CF_3$

Fig. 1 Structure of GM3 analogs.

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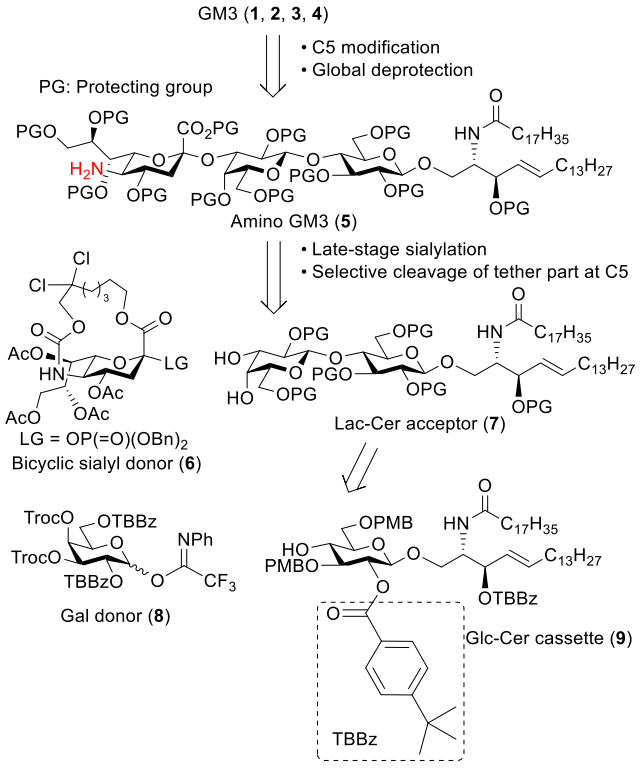
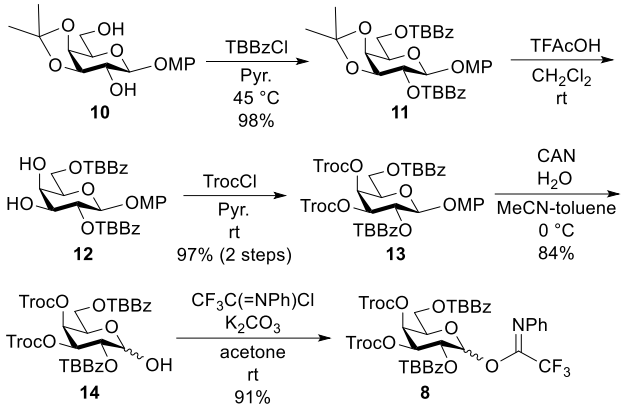
(Figure 1). Furthermore, ^{19}F solid-state NMR analysis of the GM3 analog **4** was carried out in a cholesterol-enriched model membrane[8-10] to investigate the dynamics of gangliosides in lipid rafts.

Results and discussion

As shown in Scheme 1, we designed the retrosynthetic strategy for GM3 and its analogs (**1**-**4**) based on the direct sialylation of a lactosylceramide (LacCer) acceptor with the bicyclic sialic acid donor, and subsequent C5-modifications. In this approach, the GM3 analogs were expected to be accessible from the corresponding C5-amino precursor **5**. The disconnection of the C5-amino compound **5** at the α(2,3)-glycosidic linkage of the sialyl galactose (NeuGal) sequence could be accessed using the bicyclic sialyl donor[7] and the LacCer acceptor **7**. However, the ceramide moiety of **7** would adversely affect its solubility in organic solvents due to its propensity for hydrophobic aggregation, which would impede the glycosidation reaction. To improve solubility, we incorporated multiple *p*-*tert*-butylbenzoyl (TBBz) groups in LacCer, based on our recent studies on the use of the TBBz group for improving solubility in organic solvents.[11] The tertiary butyl moiety of TBBz group would impede π-π or CH-π stacking by its bulikiness between the molecules, thereby supressing the aggregation of LacCer. The LacCer acceptor **7** could be synthesized from the 2,6-di-*O*-TBBz-Gal donor **8** and the glucosylceramide (GlcCer)



Scheme 1 Retrosynthetic analysis of the GM3 analogs.



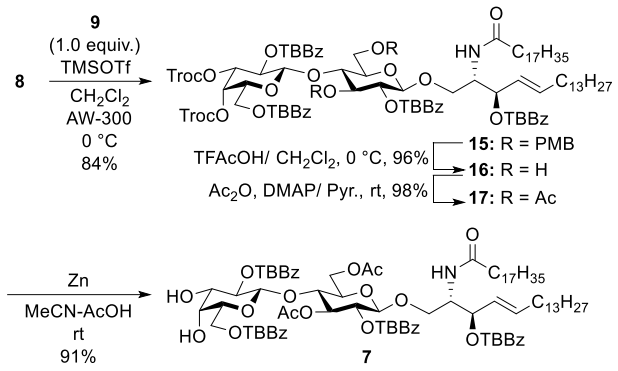
Scheme 2 Synthesis of Gal donor **8**.

9[11,12] bearing two TBBz groups. To achieve the GM3 construction without affecting the olefin moiety of Cer, we chose the dibenzyl phosphate sialyl donor **6**,[7] which can undergo facile activation with TMSOTf.

First, Gal donor **8** was prepared from **10**[13] (Scheme 2). The hydroxyl groups at the 2 and 6 positions of **10** were protected with the TBBz groups by treatment with TBBzCl in pyridine at 45 °C to produce **11** in 98% yield. Subsequent removal of an acetonide group in **11** using TFAcOH provided the 3,4-diol **12**, which was then reacted with TrocCl to furnish 3,4-di-*O*-Troc-Gal **13** in 97% yield over two steps. Next, the *p*-methoxyphenyl (MP) group at the anomeric position of **13** was removed using CAN and H_2O in MeCN/toluene[14] to afford the hemiacetal **14** in 84% yield. Finally, the introduction of the *N*-(phenyl)trifluoroacetimidoyl group[15] into the anomeric hydroxyl of **14** yielded Gal donor **8** in 91% yield as an anomeric mixture.

Next, we turned attention to the construction of LacCer acceptor **7** (Scheme 3). With the Gal donor **8** (1.0 equiv.) in hand, we carried out the glycosylation with the GlcCer acceptor **9** in the presence of a catalytic amount of TMSOTf in CH_2Cl_2 at 0 °C, to afford LacCer framework **15** in 84% yield. Next, the *p*-methoxybenzyl (PMB) groups in **15** were removed under acidic conditions, which resulted in the formation of diol **16** in high yield. Subsequently, the 3,6-hydroxyl groups of the Glc moiety were protected with acyl groups. Initially, we examined the introduction of TBBz group onto the diols. However, the reaction was highly sluggish, likely due to the bulkiness of TBBz group. Instead, we introduced the less bulky Ac groups to give **17** in 98% yield. Finally, LacCer **17** was treated with zinc powder in AcOH to remove the two Troc groups, thus affording the LacCer acceptor **7** carrying the unprotected hydroxyl groups at the 3 and 4 positions, in 91% yield.

Next, we examined the glycosidation of the bicyclic sialyl donor **6** and LacCer acceptor **7** to assemble the GM3 framework. As expected, the LacCer acceptor **7** bearing the TBBz groups was highly soluble in CH_2Cl_2. In addition, we confirmed that **7** was completely soluble in CH_2Cl_2 at temperatures as low as −60 °C. The conditions studied for the sialylation are presented in Table 1. Molecular sieves (3Å) were not used in this reaction, as their use induced precipitation of the LacCer acceptor **7** at low



Scheme 3 Synthesis of LacCer acceptor **7**.

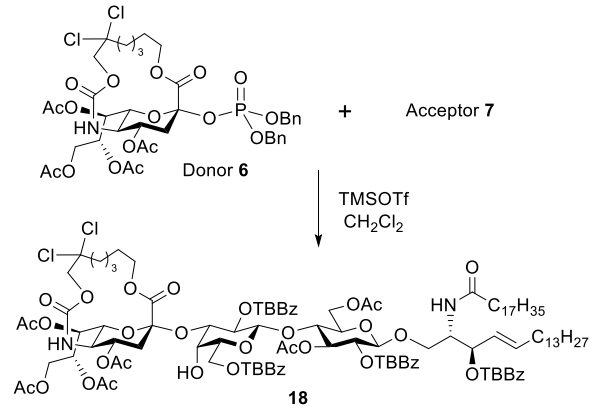
temperatures. First, phosphate donor **6** was reacted with an equimolar amount of the LacCer acceptor **7** at −40 °C and yielded GM3 framework **18** in 33% yield with complete stereo- and regioselectivity (entry 1). However, 64% of acceptor remained unreacted due to the degradation of the bicyclic sialyl donor into the 2,3-ene derivative *via* elimination. With the use of 3.0 equiv of donor **6** at same temperature, the coupling yield increased to 62% (entry 2). Furthermore, the reaction at −60 °C dramatically improved the yield to afford the GM3 framework **18** in 87% yield (entry 3). These results indicated that the bicyclic sialyl donor provided higher coupling yields at lower temperatures due to the decreased formation of 2,3-ene byproduct, which is consistent with our previous report.[7]

For carrying out various C5-modifications of the sialosyl moiety, the selective cleavage of the 16-membered ring of GM3 derivative **18** at the 2,2-dichloroethoxy carbamoyl position was attempted (Scheme 4) using Zn and acetic acid at room temperature, based on our previous report.[7] However, a significant portion of **18** remained unconsumed, and the corresponding C5-amino sialoside **5** was obtained in a moderate yield. Further investigation revealed that this reaction was effectively accelerated by controlled microwave heating.[16] The treatment of the GM3 derivative **18** with Zn in AcOH under microwave irradiation for 30 min at 40 °C, successfully afforded C5-amino GM3 **5**. C5-Amino GM3 **5** was sequentially treated with Ac_2O and DMAP in pyridine to produce the fully protected C5-NHAc-GM3 **19** in 84% yield over two steps. The reaction of GM3 **5** with acetoxyacetyl chloride in the presence of NEt_3 in CH_2Cl_2 allowed the selective introduction of the acetoxyacetyl group at C5-amino position to afford the C5-glycolyl derivative **20** in 94% yield over two steps. Similarly, amine **5** was reacted with $(CH_3{}^{13}CO)_2O$, which was prepared from commercially available $CH_3{}^{13}CO_2H$ and EDC·HCl (see Experimental section), in the presence of NEt_3, to afford C5-^{13}C-NHAc-GM3 **21** in 85% yield over two steps. Compound **19**, **20**, and **21** were then fully deprotected using 1M aq. NaOH to deliver C5-modified GM3s **1**, **2**, and **3**, respectively.

On the other hand, for the synthesis of C5-NHTFAc-GM3 **4**, GM3 **18** was first treated with 1M aq. NaOH in a mixture of THF and H_2O, which afforded amine **22** quantitatively (Scheme 5). The reaction of amine **22** with TFAcOMe in the presence of NEt_3 delivered C5-NHTFAc-GM3 **4** in 92% yield.

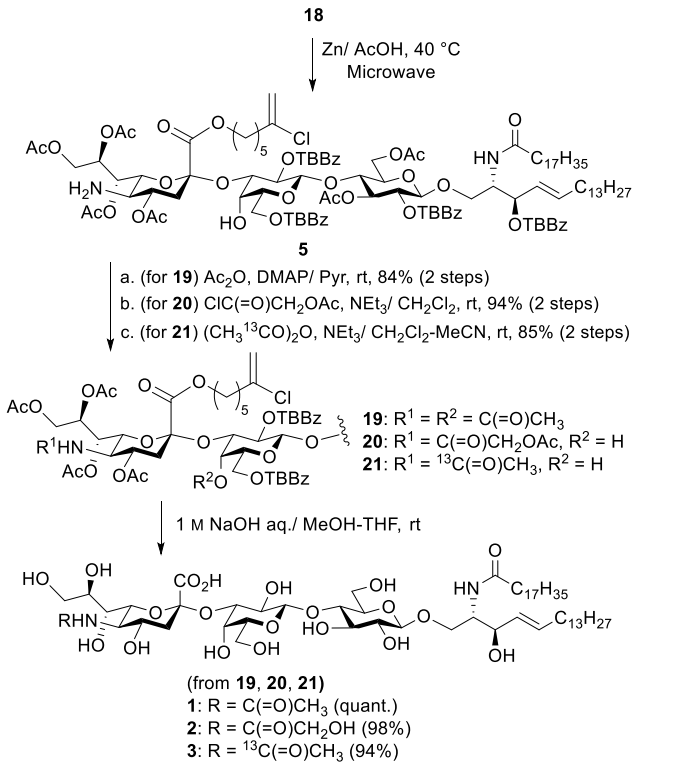
Next, we acquired the solid-state NMRs of the synthesized GM3 analogs in a lipid raft environment. The interaction of cholesterol with glycosphingolipids, including GM3, is known to play an important role in the formation of lipid rafts that regulate the functions of membrane proteins and signaling processes. We employed the C5-NHTFAc-GM3 **4** as a solid-state ^{19}F NMR probe to examine the effect of cholesterol on the

Table 1 Glycosidation of bicyclic sialyl donor with LacCer acceptor

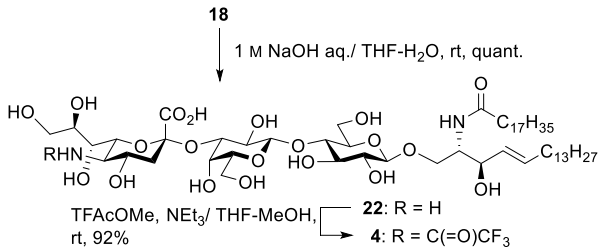


Entry	Donor **6** (equiv.)	Temp. (°C)	Time (h)	Unreacted acceptor **7**	Yield
1	1.0	−40	4	64%[a]	33%[a]
2	3.0	−40	4	35%	62%
3	3.0	−60	4	<10%	87%

[a] Calculated from ^{1}H NMR.



Scheme 4 C5-modification and global deprotection of target compounds.



Scheme 5 Synthesis of C5-NHTFAc-GM3 **4**.

Neu5Ac in the GM3 headgroup of the bilayer membrane. Without magic angle spinning, the chemical shift anisotropy (CSA) of CF_3 can be observed as a triplet due to the residual dipolar coupling with the neighboring ^{19}F atoms.[17,18] The coupling widths directly correlated with the wobbling and orientation of the Neu5Ac ring when the C-CF_3 vector on the amide was fixed on the Neu5Ac ring *via* hydrogen bonding.[19] It has been reported that dimyristoyl phosphatidylcholine (DMPC) can be used as a general co-lipid for diluting GM3 and cholesterol in the membrane system.[20,21]

Multi-lamellar vesicles comprising C5-NHTFAc-GM3/DMPC/cholesterol (7:60:33) and C5-NHTFAc-GM3/DMPC (10:90), with a GM3/DMPC ratio of 1:9 were successfully prepared, and their solid-state ^{19}F NMR was acquired. The residual dipolar coupling was clearly observed at 2.7 kHz in C5-NHTFAc-GM3/DMPC/cholesterol (7:60:33), while C5-NHTFAc-GM3/DMPC (10:90) membrane resonated slightly higher at 2.9 kHz (Figure 2). The data suggested that the Neu5Ac moiety of GM3, which is at a certain distance from the bilayer, is only affected slightly by membrane cholesterol, due to which only a small difference was observed in the CF_3 residual dipolar coupling.

Cholesterol preferentially interacts with sphingomyelin to form lipid domains with increased lipid ordering[22] with partial alterations to the conformation of the headgroup.[23] Ganglioside GM3 has a ceramide tail identical to that of sphingomyelin, while the headgroup is completely different. While GM3 is known to exhibit weaker interactions with cholesterol than that of sphingomyelin,[24] it is also able to form biologically-functional lipid domains with cholesterol.[25] The interaction of cholesterol with GM3 in the membrane is known to not only increase the ordering of the acyl chain but reduce the membrane thickness[26] likely through changes in the tilt angle of the headgroup.[27,28] Further, the involvement of the GM3 headgroup in the regulation of the membrane protein activity of integrin and EGF-receptor through interactions with the terminal sialic acid have been reported.[29] However, as more experimental parameters are essential for determining the exact angle,[20] we focused on the changes due to the interactions of cholesterol. The residual dipolar coupling (D_{obs}) is expressed using the order parameter S_{CF3} and the angle θ between the normal bilayer and that with the C-CF_3 vector, using equation (1) where D_0 is the dipolar coupling constant at the 90° edge (Figure 3).

$$D_{obs} = D_0 \cdot S_{CF3} \cdot \langle 3\,cos^2\,\theta - 1 \rangle \quad (1)$$

In a previous analysis of glucosylceramide, the reported order parameter of the glucose moiety *S* was around 0.3, and no significant difference was observed in the presence of cholesterol.[30] If the Neu5Ac residue is assumed to have similar order parameters of around 0.3, with an extended conformation in the DMPC membranes,[20] our data suggests that the Neu5Ac residue was slightly tilted in the presence of cholesterol, while the angular difference was expected to be within 5°. However, if the Neu5Ac moiety of GM3 receives a higher ordering effect from cholesterol, the values of the S_{CF3} term would increase, and thus the angular differences will be more significant.

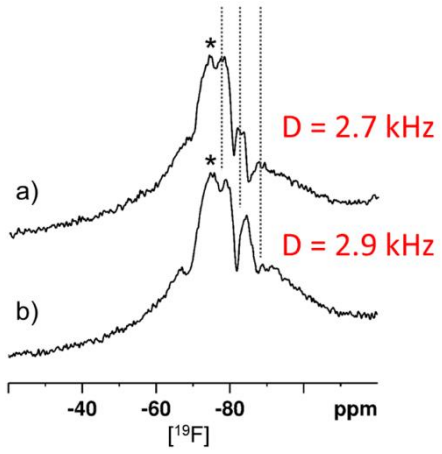


Fig. 2 ^{19}F Solid-state NMR spectra of C5-NHTFAc-GM3 in C5-NHTFAc-GM3/DMPC/cholesterol (7:60:33) (a); or C5-NHTFAc-GM3/DMPC (10:90) (b) vesicles. Due to the homo dipolar couplings of the three ^{19}F nuclei on a carbon, ^{19}F signal showed three peaks. Experiments were performed at 40 °C. *Symmetric signal from a part of the micelle or small vesicle. Baseline was distorted by acoustic ringing.

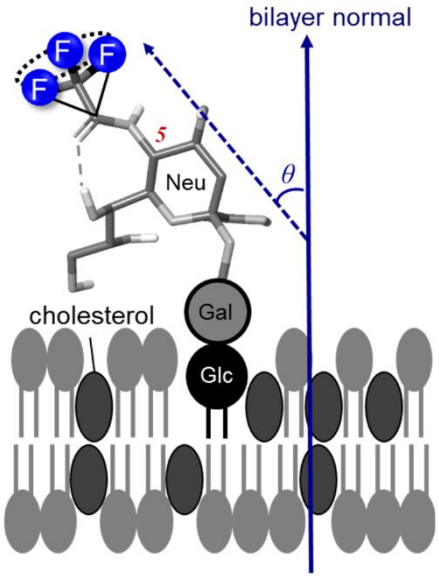


Fig. 3 Geometrical view of the C-CF_3 vector and bilayer normal. Glc; glucose, Gal; galactose, Neu; C5-NHTFAc-Neu5Ac.

Conclusions

We achieved the first direct sialylation of a glycolipid using a completely stereoselective sialidation method. The direct glycosidation of the bicyclic sialyl donor with the highly soluble LacCer acceptor bearing the TBBz groups provided efficient access to the GM3 framework. Subsequent C5-selective ring-opening of the sialoside was achieved with microwave assistance, which enabled various C5-modifications of GM3 in high yields. These results demonstrated the high efficiency of the late-stage sialylation method. This synthetic method would also be applicable for the synthesis of functionalized ganglioside probes such as those containing fluorescence-[11,25] or photoaffinity-labelled sialic acids.[31] Furthermore, the ^{19}F solid-state NMR analysis of the GM3 analogs on model membranes revealed the influence of cholesterol on glycan dynamics, demonstrating that our method could be useful for studying the glycan dynamics of gangliosides in lipid rafts.

Experimental section

General methods

All reactions were performed under an argon atmosphere. All chemicals were purchased from commercial suppliers and used without further purification. Molecular sieves were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and predried at 300 °C for 2 h in a muffle furnace and then dried in a flask at 300 °C for 2 h in vacuo prior to use. Dry solvents for reaction media (CH_2Cl_2, toluene, THF, CH_3CN, DMF, and pyridine) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan) and used without purification. TLC analyses were performed on Merck TLC plates (silica gel 60F254 on glass plate). Compound detection was either by exposure to UV light (253.6 nm) or by soaking in H_2SO_4 solution (10% in EtOH) or phosphomolybdic acid solution (20% in EtOH) followed by heating. Silica gel column chromatography separations were performed with a flash column chromatography system. Silica gel (80 mesh and 300 mesh; Fuji Silysia Co. (Aichi, Japan)) was used for flash column chromatography. The quantity of silica gel was typically 100 to 200 times the weight of the crude sample. Sephadex (Pharmacia LH-20) was used for size-exclusion chromatography. Solvent systems for chromatography are specified as v/v ratios. ^1H and ^{13}C NMR spectra were recorded on Avance III 500 and Avance III 800 spectrometers (Bruker, Billerica, MA, USA). Chemical shifts are expressed in ppm (δ) relative to Me_4Si signal (0.00 ppm). The sugar units are numbered using letters from a to c; Glc (a), Gal (b), and Neu (c). High-resolution mass spectrometry (ESI-TOF MS) data were obtained with a mass spectrometer (micrOTOF, Bruker). Optical rotations were measured with a high-sensitivity polarimeter (SEPA-300, Horiba (Kyoto, Japan)). Microwave assisted reactions were carried out using an Initiator+Eight microwave synthesizer (Biotage Japan, Tokyo, Japan) in sealed reaction vials (0.5-2 mL or 2-5 mL vial) under magnetic stirring (600 rpm) at 40 °C without pressure.

Synthetic procedure

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (1). To a solution of **19** (7.7 mg, 3.4 μmol) in THF/MeOH (343 μL/343 μL) was added 1 M NaOH aq. (68.5 μL, 68.5 μmol) at room temperature. After stirring for 3 days at ambient temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O/AcOH = 5:3:0.5:0.05), the reaction mixture was neutralized with Muromac C101 (H^+) and filtered through cotton. The combined filtrate and washings were concentrated. The resulting residue was purified by flash column chromatography on silica gel using $CHCl_3$/MeOH/H_2O (5:3:0.15 to 5:3:0.3) as the eluent to give **1** (4.3 mg, quant.): $[\alpha]_D$ –8.7° (*c* 0.4, MeOH); ^1H NMR (500 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 5.69 (m, 1 H, H-5^{Cer}), 5.45 (dd, 1 H, $J_{3,4}$ = 7.7 Hz, $J_{4,5}$ = 15.3 Hz, H-4^{Cer}), 4.42 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1^b), 4.30 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^a), 4.21 (dd, 1 H, $J_{1a,2}$ = 4.2 Hz, J_{gem} = 10.0 Hz, H-1a^{Cer}), 4.09 (t, 1 H, $J_{2,3}$ = 7.9 Hz, H-3^{Cer}), 4.02–3.31 (m, 21 H, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-4^c, H-5^c, H-6^c, H-7^c, H-8^c, H-9a^c, H-9b^c, H-1b^{Cer}, H-2^{Cer}), 2.85 (br dd, 1 H, H-3*eq*^{c}), 2.18 (t, 2 H, $COCH_2^{Cer}$), 2.04–2.00 (m, 5 H, H-6a^{Cer}, H-6b^{Cer}, Ac), 1.75 (s, 1 H, H-3*ax*^{c}), 1.59 (m, 2 H, CH_2^{Cer}), 1.43–1.15 (m, 50 H, 25 CH_2^{Cer}), 0.89 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 175.3, 174.9, 134.8, 130.1, 104.5, 103.6, 78.7, 76.2, 75.5, 75.2, 74.0, 72.5, 72.1, 69.3, 62.2, 61.1, 53.9, 53.3, 49.6, 37.0, 32.9, 32.5, 30.2, 30.1, 30.0, 29.9, 29.9, 29.8, 26.6, 23.2, 22.6, 14.3; HRMS (ESI) *m/z*: found $[M-H]^-$ 1179.7373, $C_{59}H_{108}N_2O_{21}$ calcd for $[M-H]^-$ 1179.7372.

(3,5-Dideoxy-5-hydroxyacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (2). To a solution of **20** (8.6 mg, 3.8 μmol) in MeOH/THF (380 μL/380 μL) was added 1 M NaOH aq. (84 μL, 84μmol) at room temperature. After stirring for 40 h at ambient temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/5% $CaCl_2$ aq. = 5:3:0.5), the reaction mixture was neutralized by Muromac C101 (H^+) and filtered through cotton. The combined filtrate and washings were concentrated. The resulting residue was purified by column chromatography on silica gel, using $CHCl_3$/MeOH/H_2O (5:3:0.1 to 5:3:0.3) as the eluent to give **2** (4.6 mg, 98%): $[\alpha]_D$ –13.5° (*c* 0.4, $CHCl_3$/MeOH = 1:1); ^1H NMR (500 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 5.66 (dt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 7.2 Hz, $J_{4,5}$ = 16.9 HZ, H-5^{Cer}), 5.41 (dd, 1 H, $J_{3,4}$ = 7.7 Hz, H-4^{Cer}), 4.39–3.27 (m, 27 H, H-1^a, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-1^b, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-4^c, H-5^c, H-6^c, H-7^c, H-8^c, H-9a^c, H-9b^c, 2 CH_2, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}), 2.83 (dd, 1 H, $J_{3eq,4}$ = 3.7 Hz, J_{gem} = 11.9 Hz, H-3*eq*^{c}), 2.14 (t, 2 H, $NHCO_2CH_2$), 2.01–1.97 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.72 (t, 1 H, $J_{3ax,4}$ = 11.7 Hz, H-3*ax*^{c}), 1.56–1.54 (m, 2 H, CH_2^{Cer}), 1.37–1.11 (m, 50 H, 25 CH_2^{Cer}), 0.87–0.84 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 176.7, 175.3, 134.9, 130.1, 104.5, 103.1, 80.3, 78.7, 76.9, 76.3, 75.6, 75.3, 74.0, 74.0, 72.5, 72.1, 69.9, 68.1, 64.1, 62.2, 62.1, 61.1, 52.9, 49.9, 37.0, 32.9, 32.5, 30.2, 30.2, 30.2, 30.1, 30.1, 30.0, 29.9, 29.9, 29.9, 29.8, 26.6, 23.2, 14.3; HRMS (ESI) *m/z*: found $[M-H]^-$ 1195.7322, $C_{59}H_{108}N_2O_{22}$ calcd for $[M-H]^-$ 1195.7321.

[5-(1-^{13}C)Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (3). To a solution of **21** (6.2 mg, 2.8 μmol) in THF/MeOH (280 μL/280 μL) was added 1 M NaOH aq. (56 μL, 56 μmol) at room temperature. After stirring for 36 h at ambient temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/5% $CaCl_2$ aq. = 5:3:0.5), the reaction mixture was neutralized with Muromac C101 (H^+) and filtered through cotton. The combined filtrate and washings were concentrated. The resulting residue was purified by flash column chromatography on silica gel using ($CHCl_3$/MeOH/H_2O = 5:3:0.1 to 5:3:0.2) as the eluent to give **3** (3.1 mg, 94%): $[\alpha]_D$ –4.2° (*c* 0.5, $CHCl_3$/MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1:1) δ 5.66 (dt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 7.1 Hz, $J_{4,5}$ = 16.8 Hz, H-5^{Cer}), 5.41 (dd, 1 H, $J_{3,4}$ = 7.6 Hz, H-4^{Cer}), 4.38–3.27 (m, 25 H, H-1^a, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-1^b, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-4^c, H-5^c, H-6^c, H-7^c, H-8^c, H-9a^c, H-9b^c, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}), 2.81 (dd, 1 H, $J_{3eq,4}$ = 2.4 Hz, J_{gem} = 12.4 Hz, H-3*eq*^{c}), 2.14 (t, 2 H, NHCOCH_2), 2.01–1.97 (m, 5 H, H-6a^{Cer}, H-6b^{Cer}, Ac), 1.70 (t, 1 H, $J_{3ax,4}$ = 12.8 Hz, H-3*ax*^{c}), 1.57–1.55 (m, 2 H, CH_2^{Cer}), 1.39–1.12 (m, 50 H, 25 CH_2^{Cer}), 0.87–0.81 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD = 1:1) δ 175.3, 174.9 (^{13}*C*OCH$_3$), 134.9, 130.1, 104.5, 103.6, 80.3, 78.7, 76.9, 76.2, 75.5, 74.2, 74.0, 72.5, 69.9, 69.3, 68.3, 68.1, 62.2, 53.9, 53.3, 49.9, 49.7, 37.0, 32.9, 32.5, 30.2, 30.2, 30.1, 30.1, 30.0, 29.9, 29.9, 29.8, 26.6, 23.2, 14.3; HRMS (ESI) *m/z*: found $[M-H]^-$ 1180.7404, $C_{58}{}^{13}CH_{108}N_2O_{21}$ calcd for $[M-H]^-$ 1180.7405.

(3,5-Dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (4). To a solution of **22** (1.5 mg, 1.3 μmol) in THF/MeOH (110 μL/30 μL) were added TFAcOMe (13 μL, 0.13 mmol) and triethylamine (37 μL, 0.26 mmol) at 0 °C. After stirring for 2.5 h at ambient temperature, to the reaction mixture were added TFAOMe (6.9 μL, 0.067 mmol) and triethylamine (18 μL, 0.13 mmol) at 0 °C. After stirring for 4 h at room temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/5% $CaCl_2$ aq. = 5:3:0.5), the mixture was concentrated. The residue was purified by flash column chromatography on silica gel using $CHCl_3$/MeOH/H_2O (5:3:0.15 to 5:3:0.5 to 5:3:1) and gel filtration column chromatography on Sephadex LH-20 using MeOH as the eluent to give **4** (1.5 mg, 92%): $[\alpha]_D$ –8.1° (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1:1) δ 5.65 (dt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 7.2 Hz, $J_{4,5}$ = 16.9 Hz, H-5^{Cer}), 5.42 (dd, 1 H, $J_{3,4}$ = 7.8 Hz, H-4^{Cer}), 4.39–3.28 (m, 25 H, H-1^a, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-1^b, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-4^c, H-5^c, H-6^c, H-7^c, H-8^c, H-9a^c, H-9b^c, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}), 2.80 (dd, 1 H, $J_{3eq,4}$ = 4.7 Hz, J_{gem} = 12.5 Hz, H-3*eq*^{c}), 2.13 (t, 2 H, NHCOCH_2), 2.01–1.97 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.72 (t, 1 H, $J_{3ax,4}$ = 12.1 Hz, H-3*ax*^{c}), 1.57–1.54 (m, 2 H, CH_2^{Cer}), 1.33–1.08 (m, 50 H, 25 CH_2^{Cer}), 0.87–0.84 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD = 1:1) δ 176.0, 176.0, 162.8 (q, $^2J_{C,F}$ = 37.5 Hz, *C*(O)CF_3), 142.9, 137.2, 135.0, 131.4, 115.9 (q, $^1J_{C,F}$ = 262.5 Hz, C(O)*C*F$_3$), 105.1, 104.5, 102.7, 101.1, 80.8, 77.6, 77.0, 76.5, 76.2, 74.8, 73.5, 73.0, 72.7, 70.9, 70.1, 69.9, 69.5, 68.8, 65.9, 65.4, 64.6, 62.7, 61.8, 54.7, 54.0, 42.2, 37.4, 33.5, 33.1, 33.1, 30.9, 30.9, 30.8, 30.8, 30.7, 30.6, 30.6, 30.5, 30.5, 30.4, 30.4, 30.2; HRMS (ESI) *m/z*: found $[M-H]^-$ 1233.7092, $C_{59}H_{105}F_3N_2O_{21}$ calcd for $[M-H]^-$ 1233.7089.

(2,6-Di-*O*-*p*-*tert*-butylbenzoyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (7). To a solution of **17** (115 mg, 58.7 μmol) in MeCN/AcOH (2.4 mL/0.6 mL) was added Zn (499 mg, 7.63 mmol) at room temperature. After stirring for 6.5 h at ambient temperature as the reaction was monitored by TLC (toluene/acetone = 9:1), the reaction mixture was filtered through a pad of Celite, and the pad was washed with EtOAc. The combined filtrate and washings were extracted with EtOAc, and washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel using toluene/acetone (10:1 to 4:1) as the eluent to give **7** (86.7 mg, 91%): $[\alpha]_D$ +7.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, 2 H, Ar), 7.96 (d, 2 H, Ar), 7.91–7.89 (m, 6 H, Ar), 7.52 (d, 2 H, Ar), 7.45–7.41 (m, 6 H, Ar), 5.78 (m, 1 H, H-5^{Cer}), 5.65 (d, 1 H, $J_{NH,2}$ = 9.3 Hz, NH), 5.47–5.36 (m, 3 H, H-3^a, H-3^{Cer}, H-4^{Cer}), 5.18–5.14 (m, 2 H, H-2^b, H-2^a), 4.72 (dd, 1 H, $J_{5,6a}$ = 6.5 Hz, J_{gem} = 11.4 Hz, H-6a^b), 4.52 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1^b), 4.46–4.39 (m, 2 H, H-1^a, H-6b^b), 4.36 (m, 1 H, H-2^{Cer}), 4.23–4.16 (m, 2 H, H-6a^a, H-6b^a), 4.01–3.97 (m, 2 H, H-4^b, H-1a^{Cer}), 3.86–3.76 (m, 3 H, H-4^a, H-3^b, H-5^b), 3.54 (m, 1 H, H-5^a), 3.48 (dd, 1 H, $J_{1b,2}$ = 4.0 Hz, J_{gem} = 9.8 Hz, H-1b^{Cer}), 3.33 (d, 1 H, $J_{3,OH}$ = 6.5 Hz, OH-3^b), 3.05 (d, 1 H, $J_{4,OH}$ = 4.2 Hz, OH-4^b), 2.00 (s, 3 H, Ac), 1.97–1.85 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.84 (s, 3 H, Ac), 1.82–1.80 (m, 2 H, $COCH_2^{Cer}$), 1.45–1.14 (m, 88 H, 4 *t*-Bu, 26 CH_2^{Cer}), 0.88 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 170.7, 170.5, 167.2, 166.9, 165.6, 165.4, 157.7, 157.7, 157.7, 156.8, 137.6, 130.2, 130.0, 130.0, 129.8, 127.9, 127.0, 126.7, 126.6, 126.0, 126.0, 125.9, 125.7, 125.3, 101.1, 101.1, 75.9, 74.3, 74.2, 73.3, 73.2, 72.8, 72.3, 68.7, 67.9, 62.7, 62.5, 50.7, 36.9, 35.5, 35.5, 35.4, 32.6, 32.3, 31.5, 31.4, 30.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.7, 29.6, 29.3, 25.9, 23.0, 21.2, 20.9, 14.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1637.0146, $C_{96}H_{143}NO_{19}$ calcd for $[M+Na]^+$ 1637.0147.

2,6-Di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (8). To a solution of **14** (735 mg, 0.863 mmol) in acetone (17.3 mL) were added $CF_3C(NPh)Cl$ (281 μL, 1.73 mmol) and K_2CO_3 (596 mg, 4.31 mmol) at 0 °C. After stirring for 1 h at room temperature, as the reaction was monitored by TLC (toluene), the solution was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on silica gel, using toluene as eluent, to give **8** (805 mg, 91%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97–7.93 (m, 4 H, Ar), 7.48–7.47 (m, 4 H, Ar), 7.10–7.07 (m, 2 H, Ar), 7.02–6.99 (m, 1 H, Ar), 6.82 (m, 1 H, Ar), 6.37 (m, 2 H, Ar, H-1), 5.72 (m, 2 H, H-3, H-4), 5.62 (dd, 1 H, $J_{1,2}$ = 2.8 Hz, $J_{2,3}$ = 10.7 Hz, H-2), 4.86–4.69 (m, 4 H, 2 CH_2CCl_3), 4.65–4.40 (m, 3 H, H-5, H-6a, H-6b), 1.36 (s, 9 H, *t*-Bu), 1.35 (s, 9 H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 165.4, 158.1, 157.6, 154.4, 153.5, 143.0, 130.2, 130.0, 128.9, 126.7, 126.1, 125.9, 125.9, 124.7, 119.4, 94.4, 94.2, 92.9, 72.7, 72.5, 69.2, 67.3, 61.6, 35.6,

35.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1042.0677, $C_{42}H_{42}Cl_6F_3NO_{12}$ calcd for $[M+Na]^+$ 1042.0682.

***p*-Methoxyphenyl 2,6-di-*O*-*p*-*tert*-butylbenzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (11)**. To a solution of **10** (984 mg, 3.01 mmol) in pyridine (30.1 mL) was added *p*-*tert*-butylbenzoyl chloride (2.73 mL, 15.1 mmol) at 0 °C. After stirring for 4 h at 45 °C, as the reaction was monitored by TLC (*n*-hexane/EtOAc = 4:1), MeOH was added to the reaction mixture at 0 °C, and the solution 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 purified by column chromatography on silica gel, using *n*-hexane/EtOAc (6:1) as the eluent, to give **11** (1.91 g, 98%): $[\alpha]_D$ +15.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01–7.98 (m, 4 H, Ar), 7.49–7.44 (m, 4 H, Ar), 6.94–6.91 (m, 2 H, Ar), 6.65–6.62 (m, 2 H, Ar), 5.49 (dd, 1 H, $J_{2,3}$ = 7.3 Hz, $J_{1,2}$ = 7.7 Hz, H-2), 4.95 (d, 1 H, H-1), 4.75 (dd, 1 H, $J_{5,6a}$ = 4.1 Hz, J_{gem} = 11.7 Hz, H-6a), 4.63 (dd, 1 H, $J_{5,6b}$ = 8.3 Hz, H-6b), 4.44 (dd, 1 H, $J_{3,4}$ = 5.7 Hz, H-3), 4.35 (dd, 1 H, $J_{4,5}$ = 2.1 Hz, H-4), 4.29 (m, 1 H, H-5), 3.70 (s, 3 H, OMe), 1.68 (s, 3 H, Me), 1.39 (s, 3 H, Me), 1.36 (s, 9 H, *t*-Bu), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 165.7, 157.3, 157.2, 155.8, 151.6, 130.1, 130.0, 127.4, 127.3, 125.8, 125.7, 119.2, 114.7, 111.5, 100.6, 73.8, 73.4, 71.7, 63.8, 55.9, 35.5, 35.4, 31.5, 31.5, 27.9, 26.7; HRMS (ESI) *m/z*: found $[M+Na]^+$ 669.3032, $C_{38}H_{46}O_9$ calcd for $[M+Na]^+$ 669.3034.

***p*-Methoxyphenyl 2,6-di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-galactopyranoside (13)**. To a solution of **11** (1.90 g, 2.93 mmol) in CH_2Cl_2 (40.0 mL) was added TFAcOH (20.0 mL) at 0 °C. After stirring for 10 min at room temperature, as the reaction was monitored by TLC (*n*-hexane/EtOAc = 3:2), satd $NaHCO_3$ was added to the reaction mixture. The mixture was diluted with $CHCl_3$ and washed with brine, dried over Na_2SO_4, and concentrated. The residue was exposed to high vacuum for 2 h. Next, to a solution of the resulting residue in pyridine (29.3 mL) was added 2,2,2-trichloroethyl chloroformate (982 µL, 7.33 mmol) at 0 °C. After stirring for 30 min at room temperature, as the reaction was monitored by TLC (*n*-hexane/EtOAc = 3:2), MeOH was added to the reaction mixture. The solution 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 purified by column chromatography on silica gel, using *n*-hexane/EtOAc (7:1) as the eluent, to give **13** (2.73 g, 97%, two steps): $[\alpha]_D$ +15.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96–7.94 (m, 4 H, Ar), 7.49–7.44 (m, 4 H, Ar), 6.94–6.92 (m, 2 H, Ar), 6.71–6.69 (m, 2 H, Ar), 5.85 (dd, 1 H, $J_{2,3}$ = 7.7 Hz, $J_{1,2}$ = 8.0 Hz, H-2), 5.57 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4), 5.28 (dd, 1 H, H-3), 5.10 (d, 1 H, H-1), 4.86–4.79 (m, 2 H, CH_2CCl_3), 4.71–4.62 (m, 3 H, CH_2CCl_3, H-6a), 4.49 (dd, 1 H, $J_{5,6b}$ = 6.6 Hz, J_{gem} = 11.3 Hz, H-6b), 4.24 (t, 1 H, H-5), 3.72 (s, 3 H, OCH_3), 1.35 (s, 9 H, *t*-Bu), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 165.1, 157.7, 157.6, 156.2, 154.5, 153.6, 153.5, 151.3, 130.1, 130.1, 126.8, 126.7, 125.9, 125.8, 119.5, 114.9, 101.5, 114.9, 101.5, 94.5, 94.3, 94.1, 76.0, 72.2, 71.1, 69.4, 61.6, 55.9, 35.5, 35.5, 31.5, 31.4, 30.0; HRMS (ESI) *m/z*: found $[M+Na]^+$ 977.0801, $C_{41}H_{44}Cl_6O_{13}$ calcd for $[M+Na]^+$ 977.0805.

2,6-Di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-D-galactopyranose (14). To a solution of **13** (1.00 g, 1.04 mmol) in MeCN/toluene/H_2O (9.0 mL/7.5 mL/4.5 mL) was added CAN (5.70 g, 10.4 mmol) at 0 °C. After stirring for 5 h at 0 °C as the reaction was monitored by TLC (toluene/acetone = 10:1), the reaction mixture was diluted with EtOAc and washed with H_2O, satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel using toluene/acetone (60:1) as the eluent, to give **14** (746 mg, 84%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96–7.93 (m, 4 H, Ar), 7.47–7.42 (m, 4 H, Ar), 5.78 (t, 1 H, $J_{1,2}$ = $J_{1,OH}$ = 3.5 Hz, H-1), 5.66–5.63 (m, 2 H, H-3, H-4), 5.45 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2), 4.84–4.81 (m, 2 H, CH_2CCl_3), 4.76–4.64 (m, 3 H, CH_2CCl_3, H-5), 4.58 (dd, 1 H, $J_{5,6a}$ = 6.0 Hz, J_{gem} = 11.2 Hz, H-6a), 4.36 (dd, 1 H, $J_{5,6b}$ = 7.6 Hz, H-6b), 3.72 (d, 1 H, OH), 1.33 (s, 9 H, *t*-Bu), 1.32 (s, 9 H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 166.4, 166.0, 157.7, 157.5, 154.5, 153.6, 130.3, 130.2, 130.0, 126.8, 126.6, 125.8, 125.8, 96.6, 94.6, 94.3, 91.0, 73.5, 72.6, 69.3, 66.2, 61.8, 60.8, 35.5, 31.4, 31.4, 21.4, 14.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 871.0388, $C_{34}H_{38}Cl_6O_{12}$ calcd for $[M+Na]^+$ 871.0387.

[2,6-Di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-galactopyranosyl]-(1→4)-(2-*O*-*p*-*tert*-butylbenzoyl-3,6-di-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (15). MS AW-300 (1.13 g) were added to a solution of **8** (772 mg, 0.755 mmol) and **9** (973 mg, 0.755 mmol) in CH_2Cl_2 (30.2 mL) at ambient temperature. After stirring for 1 h at 0 °C, TMSOTf (27.0 µL, 0.151 mmol) was added to the mixture at 0 °C. The reaction mixture was stirred for 4.5 h at 0 °C as the reaction was monitored by TLC (*n*-hexane/EtOAc = 2:1). The reaction mixture was quenched with satd $NaHCO_3$ and filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography on silica gel, using *n*-hexane/EtOAc (21:2) as the eluent, to give **15** (1.34 g, 84%): $[\alpha]_D$ +7.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93–7.85 (m, 8 H, Ar), 7.48–7.39 (m, 8 H, Ar), 7.21 (d, 2 H, Ar), 7.15 (d, 1 H, Ar), 6.91 (d, 2 H, Ar), 6.67 (d, 2 H, Ar), 5.79 (m, 1 H, H-5^{Cer}), 5.72 (d, 1 H, $J_{NH,2}$ = 9.1 Hz, NH), 5.58 (dd, 1 H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.1 Hz, H-2^b), 5.49 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 7.1 Hz, H-3^{Cer}), 5.44–5.40 (m, 2 H, H-4^b, H-4^{Cer}), 5.20 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 8.6 Hz, H-2^a), 5.05 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3^b), 4.85–4.54 (m, 8 H, H-1^b, H-6a^a, 2 CH_2CCl_3, PhCH_2), 4.37–4.31 (m, 3 H, H-6a^b, H-1^a, H-2^{Cer}), 4.26–4.22 (m, 1 H, H-6b^b), 4.15–4.11 (m, 2 H, H-4^a, H-5^a), 4.02 (m, 1 H, H-1a^{Cer}), 3.82–3.75 (m, 5 H, H-5^b, H-3^a, OMe), 3.68 (s, 3 H, OMe), 3.59 (d, 1 H, PhCH_2), 3.48 (m, 1 H, H-1b^{Cer}), 3,41 (d, 1 H, PhCH_2), 3.27 (m, 1 H, H-6b^a), 1.96–1.93 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.77–1.74 (m, 2 H, $COCH_2^{Cer}$), 1.35–1.22 (m, 88 H, 4 *t*-Bu, 26 CH_2^{Cer}), 0.88 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 166.0, 165.6, 165.4, 164.9, 159.8, 159.3, 157.7, 157.4, 157.3, 156.8, 157.3, 156.8, 154.5, 153.5, 137.4, 130.8, 130.5, 130.1, 130.0, 130.0, 129.9, 128.0, 127.2, 126.8, 126.5, 125.9, 125.8, 125.6, 125.3, 114.3, 113.9, 101.6, 100.6, 94.6, 94.1, 80.0, 76.0, 75.2, 74.9, 74.6, 73.8, 73.6, 72.2, 70.5, 69.8, 67.7, 60.9, 55.6, 55.5, 50.7, 36.8, 35.5, 35.5,

35.4, 32.7, 32.3, 31.5, 31.4, 30.1, 30.0, 30.0, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.4, 25.9, 23.0, 14.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 2140.9167, $C_{114}H_{157}Cl_6NO_{23}$ calcd for $[M+Na]^+$ 2140.9170.

[2,6-Di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-galactopyranosyl]-(1→4)-(2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (16). To a solution of **15** (1.17 g, 0.549 mmol) in CH_2Cl_2 (14.7 mL) was added TFAcOH (7.3 mL) 0 °C. After stirring for 1.5 h at 0 °C, as the reaction was monitored by TLC (toluene/acetone = 9:1), satd $NaHCO_3$ was added to the reaction mixture. The mixture was diluted with $CHCl_3$ and washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using toluene/acetone (25:1) as the eluent, to give **16** (990 mg, 96%): $[\alpha]_D$ +24.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (m, 4 H, Ar), 7.95 (d, 2 H, Ar), 7.90 (d, 2 H, Ar), 7.45 (m, 6 H, Ar), 7.39 (d, 2 H, Ar), 5.87 (m, 1 H, H-5^{Cer}), 5.81 (d, 1 H, $J_{NH,2}$ = 9.7 Hz, NH), 5.68 (dd, 1 H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.4 Hz, H-2^b), 5.57 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.7 Hz, H-3^{Cer}), 5.52 (d, 1 H, $J_{3,4}$ = 3.6 Hz, H-4^b) 5.43 (dd, 1 H, $J_{4,5}$ = 15.4 Hz, H-4^{Cer}), 5.22 (dd, 1 H, H-3^b), 5.14 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 8.6 Hz, H-2^a), 4.89 (d, 1 H, H-1^b), 4.79 (m, 2 H, CH_2CCl_3), 4.69 (d, 1 H, CH_2CCl_3), 4.64 (dd, 1 H, $J_{5,6a}$ = 5.5 Hz, J_{gem} = 11.5 Hz, H-6a^b), 4.58 (d, 1 H, CH_2CCl_3), 4.43–4.37 (m, 3 H, H-1^a, H-6b^b, H-2^{Cer}), 4.24 (t, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 6.7 Hz, H-5^b), 3.98–3.91 (m, 3 H, H-3^a, H-4^a, H-5^a), 3.84 (d, 1 H, J_{gem} = 8.0 Hz, H-1a^{Cer}), 3.47 (dd, 1 H, $J_{1b,2}$ = 3.6 Hz, J_{gem} = 9.3 Hz, H-1b^{Cer}), 3.38 (m, 1 H, H-6a^a), 3.16–3.14 (m, 2 H, H-6b^a, OH-3^a), 2.90 (dd, 1 H, $J_{OH,6b}$ = 3.9 Hz, $J_{OH,6a}$ = 10.8 Hz, OH-6^a), 1.99–1.94 (m, 4 H, H-6a^{Cer}, H-6b^{Cer}, $COCH_2^{Cer}$), 1.50–1.42 (m, 2 H, CH_2^{Cer}), 1.37–1.17 (m, 86 H, 4 *t*-Bu, 25 CH_2^{Cer}), 0.88 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 166.4, 166.2, 166.1, 165.0, 157.8, 157.5, 157.3, 157.3, 154.3, 153.5, 138.6, 130.2, 130.2, 130.0, 129.8, 127.7, 127.5, 126.5, 126.2, 125.9, 125.8, 125.8, 125.3, 102.1, 100.1, 94.4, 94.1, 80.5, 75.8, 74.3, 74.2, 73.9, 72.6, 72.4, 71.7, 69.1, 66.4, 61.6, 60.0, 50.7, 37.1, 35.5, 35.5, 35.5, 35.4, 32.7, 32.3, 31.4, 30.1, 30.1, 30.0, 30.0, 29.9, 29.8, 29.7, 29.7, 29.6, 29.3, 26.0, 23.0, 14.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1900.8015, $C_{98}H_{141}Cl_6NO_{21}$ calcd for $[M+Na]^+$ 1900.8019.

[2,6-Di-*O*-*p*-*tert*-butylbenzoyl-3,4-di-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-galactopyranosyl]-(1→4)-(3,6-di-*O*-acetyl-2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (17). To a solution of **16** (126 mg, 66.8 μmol) in pyridine (3.3 mL) were added Ac_2O (15.8 μL, 167 μmol) and 4-dimethylaminopyridine (1.6 mg, 13 μmol) at 0 °C. After stirring for 3 days at room temperature, as the reaction was monitored by TLC (toluene/acetone = 9:1), MeOH was added to the reaction mixture, and the solution 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 purified by column chromatography on silica gel, using toluene/acetone (35:1) as the eluent, to give **17** (128 mg, 98%): $[\alpha]_D$ +14.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96–7.88 (m, 8 H, Ar), 7.51–7.41 (m, 8 H, Ar), 5.77 (m, 1 H, H-5^{Cer}), 5.63 (d, 1 H, $J_{NH,2}$ = 9.3 Hz, NH), 5.53 (dd, 1 H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.3 Hz, H-2^b), 5.50 (d, 1 H, $J_{3,4}$ = 3.2 Hz, H-4^b), 5.46–5.37 (m, 3 H, H-3^a, H-3^{Cer}, H-4^{Cer}), 5.18 (dd, 1 H, H-3^b), 5.14 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 8.0 Hz, H-2^a), 4.84 (d, 1 H, J_{gem} = 11.9 Hz, CH_2CCl_3), 4.75 (d, 1 H, CH_2CCl_3), 4.72 (d, 1 H, H-1^b), 4.56 (d, 1 H, J_{gem} = 11.8 Hz, CH_2CCl_3), 4.62–4.55 (m, 2 H, CH_2CCl_3, H-1a^{Cer}), 4.47 (d, 1 H, H-1^a), 4.39–4.35 (m, 2 H, H-6a^b, H-2^{Cer}), 4.18–4.10 (m, 2 H, H-6a^a, H-1b^{Cer}), 4.06 (dd, 1 H, $J_{5,6b}$ = 4.2 Hz, J_{gem} = 12.0 Hz, H-6b^a), 3.99 (m, 1 H, H-6b^b), 3.90 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4^a), 3.54 (m, 1 H, H-5^a), 3.48 (dd, 1 H, $J_{5,6a}$ = 3.9 Hz, $J_{5,6b}$ = 9.8 Hz, H-5^b), 2.02 (s, 3 H, Ac), 1.95 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.81 (m, 2 H, $COCH_2^{Cer}$), 1.75 (s, 3 H, Ac), 1.47–1.14 (m, 88 H, 4 *t*-Bu, 26 CH_2^{Cer}), 0.88 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 170.5, 170.1, 166.2, 165.6, 165.4, 164.6, 157.8, 157.7, 157.6, 156.8, 154.3, 153.5, 137.5, 130.2, 130.0, 130.0, 129.8, 127.9, 126.6, 126.5, 126.2, 126.0, 125.9, 125.9, 125.6, 125.3, 101.2, 101.0, 94.5, 94.0, 76.0, 75.8, 74.2, 73.0, 72.4, 71.9, 70.8, 69.5, 67.9, 62.1, 61.0, 50.7, 36.8, 35.5, 35.5, 35.5, 35.4, 32.6, 32.2, 31.4, 31.4, 30.0, 30.0, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.3, 25.9, 23.0, 21.1, 20.8, 14.4; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1984.8232, $C_{102}H_{145}Cl_6NO_{23}$ calcd for $[M+Na]^+$ 1984.8231.

Compound 18. Sialyl donor **6** (33.6 mg, 37.1 μmol) and Lac-Cer acceptor **7** (20.0 mg, 12.4 μmol) were dissolved in CH_2Cl_2 (0.5 mL) at ambient temperature. TMSOTf (9.0 μL, 50 μmol) was added to the mixture at –60 °C. The reaction mixture was stirred for 4 h at –60 °C as the reaction was monitored by TLC (toluene/acetone = 6:1, developed twice). The reaction mixture was quenched with satd $NaHCO_3$, filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$ and washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography on silica gel, using *n*-hexane/acetone (5.5:1) as the eluent, to give **18** (24.1 mg, 87%): $[\alpha]_D$ +20.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3NO_2, 90 °C) δ 8.01 (d, 4 H, Ar), 7.96 (d, 4 H, Ar), 7.64 (m, 4 H, Ar), 7.57 (d, 4 H, Ar), 5.83–5.78 (m, 2 H, H-5^{Cer}, NH^{Cer}), 5.54–5.39 (m, 7 H, H-3^a, H-4^c, H-7^c, H-8^c, NH^c, H-3^{Cer}, H-4^{Cer}), 5.28 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.9 Hz, H-2^b), 5.16 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.5 Hz, H-2^a), 4.87 (d, 1 H, H-1^b), 4.72 (d, 1 H, H-1^a), 4.69 (dd, 1 H, $J_{5,6a}$ = 6.2 Hz, J_{gem} = 11.2 Hz, H-6a^b), 4.63–4.48 (m, 7 H, H-3^b, H-6b^b, H-6^c, H-9a^c, CO_2CH_2, NH(CO)OCH_2, H-1a^{Cer}), 4.38–4.31 (m, 2 H, H-6a^a, H-2^{Cer}), 4.25 (dd, 1 H, $J_{5,6b}$ = 5.4 Hz, J_{gem} = 12.0 Hz, H-6b^a), 4.15 (dd, 1 H, $J_{8,9b}$ = 5.6 Hz, J_{gem} = 12.5 Hz, H-9b^c), 4.08–4.02 (m, 4 H, H-4^a, H-4^b, H-5^b, H-1a^{Cer}), 3.98 (m, 1 H, $COCH_2$), 3.68 (m, 2 H, H-5^a, H-1b^{Cer}), 3.01 (br s, 1 H, OH-4^b), 2.84 (m, 1 H, H-5^c), 2.71 (dd, 1 H, $J_{2,3}$ = 5.3 Hz, J_{gem} = 12.7 Hz, H-3*eq*^{c}), 2.41 (m, 1 H, $CCl_2CH_2CH_2$), 2.27 (m, 1 H, $CCl_2CH_2CH_2$), 2.12 (s, 3 H, Ac), 2.05–1.92 (m, 11 H, H-6a^{Cer}, H-6b^{Cer}, 3 Ac), 1.94 (m, 2 H, $COCH_2^{Cer}$), 1.90–1.26 (m, 101 H, H-3*ax*^{c}, 3 CH_2, 26 CH_2^{Cer}, 2 Ac, 4 *t*-Bu), 1.00–0.91 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, CD_3NO_2, 90 °C) δ 172.4 (CO), 172.4 (CO), 172.0 (CO), 172.0 (CO), 171.8 (CO), 171.6 (CO), 169.8 (C-1^c), 168.0 (CO), 167.2 (CO), 167.1 (CO), 167.0 (CO), 159.4 (CO), 159.3 (CO), 159.1 (CO), 158.8 (CO), 138.1 (C-5^{Cer}), 131.5 (Ar), 131.3 (Ar), 131.2 (Ar), 131.0 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 128.8 (Ar), 127.4 (Ar), 127.3 (Ar), 127.1 (Ar), 126.7 (C-4^{Cer}), 102.8 (C-1^b), 102.6 (C-1^a), 99.9 (C-2^c), 91.8 (CCl_2), 76.1 (CH), 75.8 (CH), 75.1 (CH), 74.7 (CH), 74.3 (CH), 74.2 (CH),

72.7 (C-6^c), 72.5 (CH), 71.7 (NH(CO)OCH_2), 70.9 (C-8^c), 70.4 (C-7^c), 69.6 (CH), 69.3 (C-4^c), 66.9 (CO_2CH_2), 64.3 (C-6^a), 64.1 (C-6^b), 63.4 (C-9^c), 54.5 (C-5^c), 52.8 (C-2^{Cer}), 46.7 ($CCl_2CH_2CH_2$), 38.4 (C-3^c), 36.6, 36.5, 33.6, 33.3, 32.0, 32.0, 31.9, 31.1, 31.0, 31.0, 30.9, 30.8, 30.7, 30.5, 30.5, 28.5, 27.2, 25.9, 24.3, 24.0, 21.8, 21.5, 21.2, 21.2, 21.1, 14.7; HRMS (ESI) *m/z*: found $[M+Na]^+$ 2262.1473, $C_{121}H_{176}Cl_2N_2O_{32}$ calcd for $[M+Na]^+$ 2262.1475.

(6-Chloro-6-hepten-1-yl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4-*O*-acetyl-2,6-di-*O*-*p*-*tert*-butylbenzoyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (19). To a solution of **18** (17.0 mg, 7.58 μmol) in AcOH (1.9 mL) was added zinc nanopowder (Sigma–Aldrich, <50 nm particle size) (397 mg, 6.07 mmol) at room temperature. The vial was sealed and irradiated by microwave for 10 min for three times (total 30 min) under magnetic stirring at 40 °C, as the reaction was monitored every 10 min by TLC (toluene/EtOAc = 1:1). The reaction mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were diluted with $CHCl_3$, and washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was exposed to high vacuum for 2 h. To the resulting residue in pyridine (0.76 mL) were added Ac_2O (22.3 μL, 236 μmol) and DMAP (0.1 mg, 0.8 μmol) at 0 °C. After stirring for 7 h at room temperature, as the reaction was monitored by TLC (toluene/EtOAc = 2:1, developed twice), MeOH was added to the reaction mixture at 0 °C, and the solution 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 purified by column chromatography on silica gel, using *n*-hexane/acetone (4:1) as the eluent, to give **19** (14.2 mg, 84%, two steps): $[\alpha]_D$ +21.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, 2 H, Ar), 7.99 (d, 2 H, Ar), 7.89 (m, 4 H, Ar), 7.49 (m, 4 H, Ar), 7.42 (m, 4 H, Ar), 5.75 (m, 1 H, H-5^{Cer}), 5.63 (d, 1 H, $J_{NH,2}$ = 9.3 Hz, NH^{Cer}), 5.58 (m, 1 H, H-8^c), 5.44–5.34 (m, 3 H, H-3^a, H-3^{Cer}, H-4^{Cer}), 5.28 (dd, 1 H, $J_{6,7}$ = 2.7 Hz, $J_{7,8}$ = 9.7 Hz, H-7^c), 5.23 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.1 Hz, H-2^b), 5.16–5.11 (m, 4 H, H-2^a, H-4^b, 2 CH_2=C(Cl)CH_2), 4.93 (d, 1 H, $J_{NH,5}$ = 10.0 Hz, NH^c), 4.88 (m, 1 H, H-4^c), 4.80 (d, 1 H, H-1^b), 4.73 (dd, 1 H, $J_{3,4}$ = 3.1 Hz, H-3^b), 4.44–4.41 (m, 2 H, H-1^a, H-9a^c), 4.36–4.29 (m, 2 H, H-6a^b, H-2^{Cer}), 4.26–4.15 (m, 3 H, H-6a^a, H-6b^b, $COCH_2$), 4.11–3.95 (m, 6 H, H-4^a, H-6b^a, H-5^b, H-9b^c, $COCH_2$, H-1a^{Cer}), 3.74 (m, 1 H, H-5^c), 3.59 (dd, 1 H, $J_{5,6}$ = 10.7 Hz, H-6^c), 3.48–3.42 (m, 2 H, H-5^a, H-1b^{Cer}), 2.52 (dd, 1 H, $J_{3eq,4}$ = 4.5 Hz, J_{gem} = 12.4 Hz, H-3*eq*^{c}), 2.32 (t, 2 H, 2 CH_2=C(Cl)CH_2), 2.12 (s, 3 H, Ac), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 1.99 (s, 3 H, Ac), 1.95–1.92 (m, 5 H, H-6a^{Cer}, H-6b^{Cer}, Ac), 1.89 (s, 3 H, Ac), 1.84–1.74 (m, 5 H, CH_2, Ac), 1.70–1.12 (m, 98 H, H-3*ax*^{c}, 2 CH_2, 27 CH_2^{Cer}, Ac, 4 *t*-Bu), 0.89–0.86 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 171.0, 170.8, 170.6, 175.6, 170.3, 170.2, 170.1, 167.9, 166.1, 165.6, 165.4, 165.2, 157.5, 157.4, 157.0, 156.8, 143.0, 137.5, 130.6, 130.0, 130.0, 129.8, 127.9, 127.2, 127.1, 126.7, 125.9, 125.8, 125.6, 125.3, 112.4, 101.2, 101.2, 97.1, 75.3, 74.4, 73.0, 72.6, 72.3, 71.9, 71.5, 71.1, 70.7, 69.8, 67.8, 66.8, 66.6, 62.2, 61.6, 50.7, 49.2, 39.1, 37.6, 36.8, 35.5, 35.5, 35.4, 32.6, 32.3, 31.5, 31.5, 31.4, 31.4, 30.1, 30.0, 29.9, 29.9, 29.8, 28.7, 29.6, 29.3, 28.2, 27.0, 25.9, 25.1, 23.6, 23.0, 21.8, 21.2, 21.1, 21.0, 20.9, 14.5; HRMS (ESI) *m/z*: found $[M+Na]^+$ 2268.2177, $C_{124}H_{181}ClN_2O_{32}$ calcd for $[M+Na]^+$ 2268.2178.

(6-Chloro-6-hepten-1-yl 5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2,6-di-*O*-*p*-*tert*-butylbenzoyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (20). To a solution of **18** (10.0 mg, 4.46 μmol) in AcOH (1.1 mL) was added zinc nanopowder (Sigma–Aldrich, <50 nm particle size) (233 mg, 3.57 mmol) at room temperature. The vial was sealed and irradiated by microwave continuously for 30 min under magnetic stirring at 40 °C. The reaction was monitored after 30 min by TLC (toluene/EtOAc = 1:1). The reaction mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were diluted with $CHCl_3$, and washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was exposed to high vacuum for 30 min. The resulting residue was dissolved in CH_2Cl_2 (450 μL) and to the solution were added acetoxyacetyl chloride (2.4 μL, 22 μmol) and triethylamine (6.2 μL, 45 μmol) at 0 °C. After stirring for 30 min at ambient temperature as the reaction was monitored by TLC (toluene/EtOAc = 1:1), the reaction mixture was diluted with $CHCl_3$, washed with 2 M HCl, H_2O, satd $NaHCO_3$, and brine, and dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using toluene/acetone (7:1) as the eluent, to give **20** (9.5 mg, 94%, two steps); $[\alpha]_D$ +15.9° (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–7.40 (m, 16 H, 4 Ar), 5.76 (dt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 7.1 Hz, $J_{4,5}$ = 16.7 Hz, H-5^{Cer}), 5.71 (d, 1 H, $J_{5,NH}$ = 10.3 Hz, NH-5^c), 5.65 (d, 1 H, $J_{2,NH}$ = 9.3 Hz, NH-2^{Cer}), 5.50 (m, 1 H, H-8^c), 5.44–5.34 (m, 3 H, H-3^a, H-3^{Cer}, H-4^{Cer}), 5.26 (dd, 1 H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 9.5 Hz, H-2^b), 5.19 (dd, 1 H, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 9.6 Hz, H-7^c), 5.16 (dd, 1 H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2^a), 5.12 (d, 2 H, J_{gem} = 9.8 Hz, 2 CH_2=C(Cl)CH_2), 4.85 (dt, 1 H, $J_{3eq,4}$ = 3.9 Hz, $J_{3ax,4}$ = $J_{4,5}$ = 10.3 Hz, H-4^c), 4.66–4.63 (m, 2 H, H-1^b, H-6a^b), 4.51 (d, 1 H, J_{gem} = 15.3 Hz, AcOCH_2), 4.47 (dd, 1 H, $J_{3,4}$ = 3.2 Hz, $J_{2,3}$ = 9.9 Hz, H-3^b), 4.45–4.39 (m, 2 H, H-1^a, H-6b^b), 4.36–4.32 (m, 2 H, H-9a^c, H-2^{Cer}), 4.23 (d, 1 H, AcOCH_2), 4.19–4.16 (m, 2 H, H-6a^a, CO_2CH_2), 4.10–4.05 (m, 3 H, H-1a^{Cer}, H-9b^c, H-6^c), 4.00–3.89 (m, 3 H, H-6b^a, H-4^a, CO_2CH_2), 3.86 (t, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, H-5^b), 3.80 (q, 1 H, $J_{5,6}$ = 10.3 Hz, H-5^c), 3.64 (br s, 1 H, H-4^b), 3.47–3.43 (m, 2 H, H-5^a, H-1a^{Cer}), 2.55–2.52 (m, 2 H, H-3*eq*^{c}, OH-4^b), 2.30 (t, 2 H, 2 CH_2=C(Cl)CH_2), 2.15–1.60 (7 s, 21 H, 7 Ac), 1.96–1.75 (m, 3 H, H-3*ax*^{c}, H-6a^{Cer}, H-1b^{Cer}), 1.64–1.08 (m, 96 H, 4 *t*-Bu, 30 CH_2), 0.89–0.86 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.7, 170.5, 170.3, 170.2, 170.0, 168.4, 167.7, 166.3, 165.2, 157.4, 156.6, 142.6, 137.3, 130.2, 129.8, 129.7, 129.6, 127.7, 127.1, 126.9, 126.6, 125.8, 125.7, 125.7, 125.4, 125.1, 112.4, 101.1, 101.0, 97.0, 77.7, 75.3, 74.1, 73.8, 72.8, 72.2, 72.1, 71.8, 70.4, 67.9, 67.9, 66.9, 66.8, 66.6, 62.9, 62.2, 50.5, 49.2, 38.9, 36.7, 35.3, 35.3, 35.2, 35.2, 32.4, 32.1, 31.3, 31.3, 31.2, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.1, 28.0, 27.0, 25.7, 24.7, 22.8, 21.4, 20.9, 20.8, 20.8, 20.6,

14.3; HRMS (ESI) *m/z*: found $[M+Na]^+$ 2284.2126, $C_{124}H_{181}ClN_2O_{33}$ calcd for $[M+Na]^+$ 2284.2127.

[6-Chloro-6-hepten-1-yl 5-(1-^{13}C)acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-(2,6-di-*O*-*p*-*tert*-butylbenzoyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (21). To a solution of **18** (10.0 mg, 4.45 μmol) in AcOH (1.1 mL) was added zinc nanopowder (Sigma–Aldrich, <50 nm particle size) (233 mg, 3.57 mmol) at room temperature. The vial was sealed and irradiated by microwave continuously for 30 min under magnetic stirring at 40 °C. The reaction was monitored after 30 min by TLC (toluene/EtOAc = 1:1). The reaction mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were diluted with $CHCl_3$, and washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The resulting amine **5** was exposed to high vacuum for 30 min.

(Pre-preparation of $(CH_3{}^{13}CO)_2O$) To a solution of $CH_3{}^{13}COOH$ (20.7 μL, 372 μmol) in CH_2Cl_2 (220 μL) was added EDC·HCl (68.4 mg, 372 μmol) at room temperature. After stirring for 1 h at room temperature, the reaction mixture containing $(CH_3{}^{13}CO)_2O$) was used for the acetylation reaction without any purification.

(Acetylation using $(CH_3{}^{13}CO)_2O$) Next, resulting amine **5** was dissolved in CH_2Cl_2/MeCN (550 μL/550 μL). To the solution was added the prepared solution of $(CH_3{}^{13}CO)_2O$ in CH_2Cl_2 and triethylamine (12 μL, 89 μmol) at 0 °C. After stirring for 9 h at room temperature as the reaction was monitored by TLC (toluene/EtOAc = 1:1), MeOH was added to the reaction mixture, and the solution was concentrated. The residue was purified by flash column chromatography on silica gel, using $CHCl_3$/MeOH (100:1) as the eluent, to give **21** (8.2 mg, 85%, two steps): $[\alpha]_D$ +28.5° (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–7.41 (m, 16 H, 4 Ar), 5.76 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 6.8$ Hz, $J_{4,5} = 14.8$ Hz, H-5^{Cer}), 5.65 (d, 1 H, $J_{2,NH} = 9.3$ Hz, NH-2^{Cer}), 5.49 (m, 1 H, H-8^c), 5.44–5.34 (m, 3 H, H-3^a, H-3^{Cer}, H-4^{Cer}), 5.28–5.24 (m, 2 H, H-2^b, H-7^c), 5.17 (dd, 1 H, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.9$ Hz, H-2^a), 5.12 (d, 2 H, $J_{gem} = 9.2$ Hz, 2 CH_2=C(Cl)CH$_2$), 4.94 (dd, 1 H, $J_{13C,NH} = 3.1$ Hz, $J_{5,NH} = 10.4$ Hz, NH-5^c), 4.74 (dt, 1 H, $J_{3eq,4} = 3.9$ Hz, $J_{3ax,4} = J_{4,5} = 10.4$ Hz, H-4^c), 4.66–4.63 (m, 2 H, H-1^b, H-6a^b), 4.47 (dd, 1 H, $J_{3,4} = 3.3$ Hz, $J_{2,3} = 9.9$ Hz, H-3^b), 4.44–4.40 (m, 2 H, H-1^a, H-6b^b), 4.36–4.32 (m, 2 H, H-9a^c, H-2^{Cer}), 4.18–4.14 (m, 2 H, H-6a^a, CO_2CH_2), 4.08–4.05 (m, 2 H, H-6b^a, H-9b^c), 4.00–3.98 (m, 2 H, H-6^c, H-1a^{Cer}), 3.95–3.89 (m, 2 H, H-4^a, CO_2CH_2), 3.85 (t, 1 H, $J_{5,6a} = J_{5,6b} = 6.7$ Hz, H-5^b), 3.80 (m, 1 H, $J_{5,6} = 10.4$ Hz, H-5^c), 3.64 (br s, 1 H, H-4^b), 3.46–3.42 (m, 2 H, H-5^a, H-1b^{Cer}), 2.52–2.49 (m, 2 H, H-3*eq*^{c}, OH-4^b), 2.30 (t, 2 H, 2 CH$_2$=C(Cl)CH_2), 2.08–1.60 (6 s, 18 H, 6 Ac), 1.96–1.78 (m, 3 H, H-3*ax*^{c}, H-6a^{Cer}, H-6b^{Cer}), 1.64–1.08 (96 H, 4 *t*-Bu, 30 CH$_2$), 0.89–0.86 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 172.5, 171.2, 170.8, 170.7, 170.4, 170.3 (^{13}*C*OCH$_3$), 170.2, 170.1, 170.0, 170.0, 168.3, 166.3, 165.4, 165.2, 164.8, 161.2, 157.4, 157.3, 156.8, 156.6, 142.6, 137.3, 130.2, 129.8, 129.7, 129.6, 127.7, 127.1, 126.9, 126.5, 125.8, 125.7, 125.6, 125.4, 125.1, 112.4, 101.1, 97.0, 77.7, 75.3, 74.1, 73.7, 72.9, 72.4, 72.1, 71.8, 70.8, 70.3, 70.2, 68.9, 67.8, 67.7, 66.9, 66.7, 66.5, 63.8, 62.6, 62.3, 50.5, 49.1, 38.9, 37.5, 36.7, 35.3, 35.3, 35.2, 32.4, 32.1, 31.8, 31.3, 31.2, 31.2, 30.2, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 28.0, 26.7, 25.7, 24.9, 24.7, 22.8, 21.4, 21.0, 20.9, 20.9, 20.8, 20.6, 14.3; HRMS (ESI) *m/z*: found $[M+Na]^+$ 2227.2108, $C_{121}{}^{13}CH_{179}ClN_2O_{31}$ calcd for $[M+Na]^+$ 2227.2106.

(5-Amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (22). To a solution of **21** (6.7 mg, 3.0 μmol) in THF/H_2O (115 μL/115 μL) was added 1 M NaOH aq. (33.0 μL, 33.0 μmol) at room temperature. After stirring for 6 d at ambient temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/5% $CaCl_2$ aq. = 5:3:0.5), the reaction mixture was neutralized with Muromac C101 (H^+) and filtered through cotton. The combined filtrate and washings were concentrated. The resulting residue was purified by flash column chromatography on silica gel, using $CHCl_3$/MeOH/H_2O (5:3:0.1 to 5:3:0.25) as the eluent, to give **22** (1.9 mg, quant.): $[\alpha]_D$ –11.7° (*c* 0.2, $CHCl_3$/MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1:1) δ 5.66 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 7.2$ Hz, $J_{4,5} = 16.8$ Hz, H-5^{Cer}), 5.42 (dd, 1 H, $J_{3,4} = 7.6$ Hz, H-4^{Cer}), 4.54–3.30 (m, 24 H, H-1^a, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-1^b, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-4^c, H-6^c, H-7^c, H-8^c, H-9a^c, H-9b^c, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}), 2.98 (near t, 1 H, H-5^c), 2.80–2.78 (near d, 1 H, H-3*eq*^{c}), 2.14 (t, 2 H, *J* = 7.7 Hz, NHCO$_2$CH_2), 2.01–1.97 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.72 (t, 1 H, $J_{3ax,4} = J_{gem} = 11.4$ Hz, H-3*ax*^{c}), 1.57–1.54 (m, 2 H, CH$_2{}^{Cer}$), 1.36–1.05 (m, 50 H, 25 CH$_2{}^{Cer}$), 0.87–0.84 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (200 MHz, $CDCl_3$/CD_3OD = 1:1) δ 175.3, 174.9, 134.8, 130.1, 104.4, 103.6, 80.5, 76.7, 76.1, 75.5, 75.3, 73.9, 72.4, 70.6, 69.3, 68.8, 67.8, 62.1, 61.3, 53.9, 53.5, 51.2, 49.8, 36.9, 32.9, 32.4, 30.2, 30.2, 30.2, 30.0, 29.9, 29.9, 29.8, 26.5, 26.1, 25.9, 23.1, 21.2; HRMS (ESI) *m/z*: found $[M-H]^-$ 1137.7262, $C_{57}H_{106}N_2O_{20}$ calcd for $[M-H]^-$ 1137.7266.

Solid state ^{19}F NMR measurement in bilayer membrane

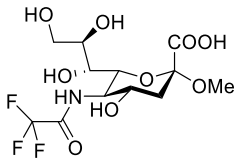
DMPC liposome containing GM3 derivative was prepared according to a previous report.[32] In brief, total 4.5 mg of lipids in the molar ratio of C5-NHTFAc-GM3/DMPC (10:90) or C5-NHTFAc-GM3/DMPC/cholesterol (7:60:33) were dissolved in $CHCl_3$/MeOH and the solvent was evaporated to obtain a homogeneous lipid film. After the removal of the residual solvent by keeping the sample in vacuo overnight, the film was hydrated with MilliQ, and the obtained suspension was subjected to freeze-thaw cycles. After five cycles, the suspension was lyophilized overnight and then hydrated with an equal weight of D_2O. Finally, the resultant suspension was subjected to 10 freeze-thaw cycles and transferred into a NMR rotor.

Solid-state ^{19}F NMR spectra were acquired by using BRUKER AVANCE600WB (^{19}F-frequency 564.7 MHz, Bruker, Billerica, MA, USA) equipped with 4-mm quadruple MAS probe (^{1}H/^{19}F/^{13}C/^{15}N) under a static condition. Around 15000 scans provided the spectra having sufficient resolution through Hahn echo pulse set with the 90° pulse width in 19 μs, and the relaxation delay 5 s. Chemical shifts were externally referenced

to 1% CF_3COOH in D_2O (−76.55 ppm). It is known that an axial rotation around the C-CF_3 bond, as well as a dipolar coupling within the CF_3 group, gives rise to the triplet signal under static conditions.[18] The splitting width (D_{obs}) can be theoretically expressed by Eq. (1) (*vide supra*)[17]where including the strength of the intrinsic dipolar interaction (D_0), modulation by a wobbling motion of C-CF_3 axis (S_{CF3}), and the angle between the outer magnetic field and C-CF_3 bond.

DFT optimization

The intrinsic dipolar splitting value D_0 between ^{19}F atoms can be estimated from the interatomic ^{19}F-^{19}F distance from the density functional theory (DFT)-optimized structure of the C5-TFAc-GM3. DFT optimization was carried out by Gaussian 09, Revision E.01. The key structure C5-NHTFAc-NeuOMe was used instead of the use of full size GM3 structure for DFT optimization to reduce computational time. Initial structure of C5-NHTFAc-Neu-OMe was created using a crystal structure, Neu5Ac-OMe methyl ester (CSD entry; VEFZIP).[19] The optimization which employed the Becke 3 parameter hybrid exchange functional and the Lee-Yang-Parr (LYP) correlation functional with 6-31G+(d,p) basis sets[33,34] afforded mean distances of 2.181 Å (in water). According to the previous report by Grage et al.,[17] the distance provided the intrinsic dipolar splitting D_0 of 7.7 kHz in 90° edge.



Structure of C5-NHTFAc-NeuOMe

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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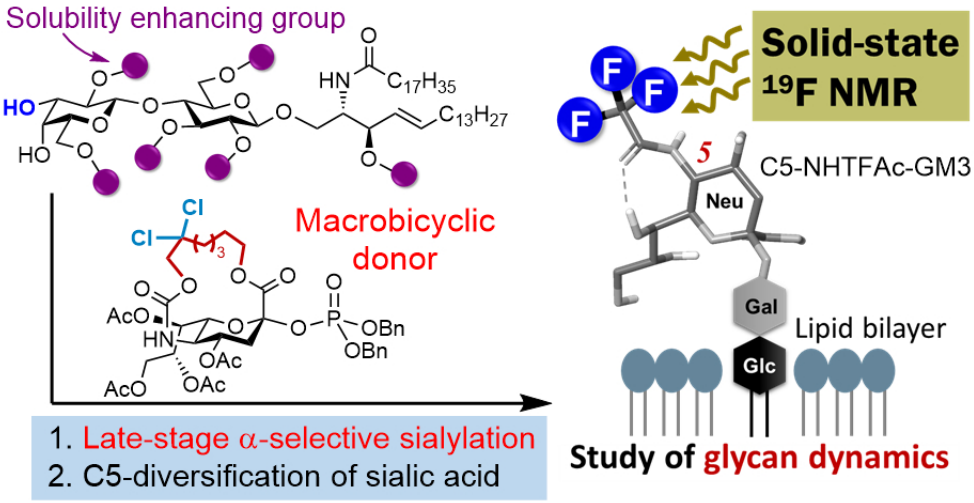
textual abstract for the contents pages

GM3 gangliosides have been synthesized *via* late-stage α-sialylation using a macro-bicyclic sialyl donor. ^{19}F solid-state NMR analysis of the C5-NHTFAc GM3 analog on model membrane revealed the influence of cholesterol on glycan dynamics.



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