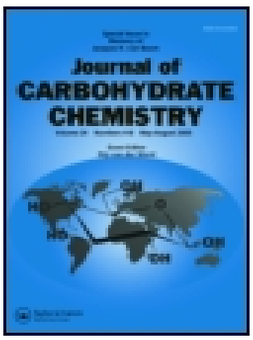

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Synthesis of ganglioside analogs containing fluorescently labeled GalNAc for single-molecule imaging

Machi Koikeda[a], Naoko Komura[b], Hide-Nori Tanaka[b], Akihiro Imamura[a], Hideharu Ishida[a,b], Makoto Kiso[a,c], and Hiromune Ando[b,c]

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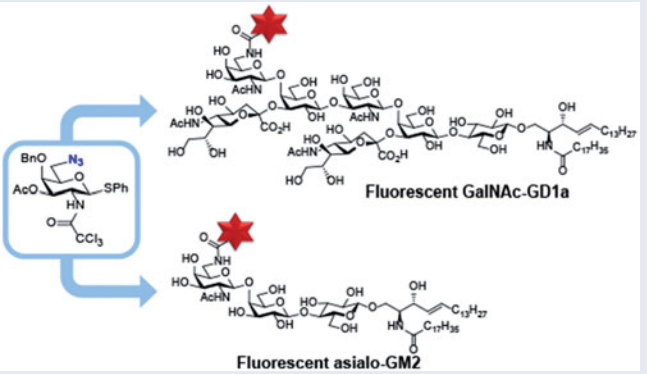
ABSTRACT

Gangliosides, glycosphingolipids containing sialic acid(s) in the glycan moieties, as major constituents of functional membrane domains such as lipid rafts participate in various biological processes. Previously, we developed fluorescent ganglioside analogs useful for single-molecule imaging of lipid raft formations. In this paper, we describe the development of a, 6-N_3-GalNTCAc donor and its application to the synthesis of fluorescent gangliosides containing fluorescent GalNAc residue(s) at the glycan terminus, namely, fluorescent analogs of GalNAc-GD1a and asialo-GM2.

GRAPHICAL ABSTRACT



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Lipid raft; single-molecule imaging; ganglioside; GalNAc-GD1a; asialo-GM2

Introduction

Gangliosides are sialic acid (Neu)-containing glycosphingolipids. Hundreds of different ganglioside species are widely distributed throughout the

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nervous system and participate in various biological processes, such as synaptic transmission, neuronal differentiation, and neuronal cell adhesion.[1] Gangliosides are present in cell plasma membranes (PMs) and function through both vertical interactions with proteins in the PMs of other cells (trans interactions) and lateral interactions with proteins in the PM of the same cell (cis interactions). The trans interactions of gangliosides have been studied thoroughly using X-ray crystallography and NMR spectroscopy, revealing the importance of the sialic acid residues.[2,3] However, the cis interactions of gangliosides remain poorly understood at the molecular level owing to the complexity of the processes involved, including transient segregation with other lipids and proteins into the membrane domains referred to as lipid rafts.[4,5]

Single-molecule imaging of fluorescently labeled membrane molecules is a powerful technique for elucidating their dynamic behavior, lateral interactions, and formation of lipid rafts.[6] Endogenous gangliosides in PMs have been indirectly detected using fluorescently tagged antibodies and ganglioside-binding proteins such as cholera toxin, which specifically binds to GM1. However, these biomolecules are generally multivalent and thus crosslink the gangliosides, altering their characteristics and behavior in the PMs. We previously developed fluorescent ganglioside analogs useful for single-molecule imaging. The fluorescent nature of these analogs permitted visualization of their dynamic behavior, transient lateral associations with lipid-raft-associated proteins and glycosylphosphatidylinositol-anchored proteins (CD59, ~40 ms lifetime), and lipid raft formation at the single-molecule level in the PMs.[7]

Our previous research into ganglioside probes revealed that the fluorescent dye must be introduced at the terminus of the glycan moiety of the ganglioside to maximally retain the biophysical and biochemical properties of the native ganglioside.[7,8] To synthesize the glycan-labeled gangliosides, we developed amino-modified saccharide units that permitted the site-selective conjugation of the unprotected gangliosides with a fluorescent dye, namely, 9-NHTFAc-sialyl α(2,3)Gal acceptor for GM3, 6-NHTFAc-Gal donor for GM1, and 6-N_3-GalN donor for GM2. From a synthetic perspective, the development of amine-modified GalN units was challenging due to the limited availability of suitable protecting groups for the amine moiety, although this was considered valuable because the chemistry of the GalN unit would be extendable to the synthesis of fluorescent glycans containing GlcNAc. Previously, we briefly reported the chemical synthesis of a fluorescent analog of ganglioside GM2 using the 6-N_3-GalN donor as the key intermediate.[7,8] Herein, we describe in detail the synthesis of the 6-N_3-GalN donor, in addition to the syntheses of new fluorescent analogs of a ganglioside related to Guillain–Barré syndrome, GalNAc-GD1a[9] and

asialo-GM2, which are expected to be useful for evaluating the role of the sialic acid residues of gangliosides in raft-related interactions.

Results and discussion

In our previous study,[7] we found that ganglioside analogs labeled at the glycan terminus exhibited behavior similar to that of native gangliosides in PMs and could be used for single-molecule imaging, whereas other analogs, such as those labeled at the central region of the glycan moiety or at the lipid moiety, displayed altered biophysical properties in PMs. We also demonstrated the necessity of using a highly hydrophilic fluorescent dye for the analogs to act in the same way as the native gangliosides in PMs. Based on these results, to synthesize a fluorescent analog of GM2, we introduced the fluorescent dye at an outermost position of the glycan moiety, the C6 position of GalNAc, and confirmed that the fluorescent GM2 analog exhibited similar biophysical properties to native GM2. Similarly, for GalNAc-GD1a and asialo-GM2, we selected the C6 position of GalNAc for labeling with a fluorescent dye (ATTO594) via an amide bond, affording the target molecules, 594-G6-GalNAc-GD1a (**1**) and 594-G6-asialo-GM2 (**2**) (Figure 1).

Retrosynthetic analysis of the GalNAc-GD1a probe was based on the synthesis of the natural ganglioside GalNAc-GD1a previously reported by our group (Scheme 1).[10] According to our previous report of fluorescent gangliosides, we elected to introduce the chemically unstable fluorescent dye in the final step of the synthesis. Therefore, the 6-OH group of the terminal GalNAc was replaced with an amino group to allow for incorporation of the fluorescent dye via an amide linkage in the final step. Thus, the target molecule was expected to be accessible from the corresponding aminoganglioside **3**. Compound **3** was then disconnected at the β-(1,4)-glycosidic linkage in the Gal-Glc sequence to provide the hexasaccharide

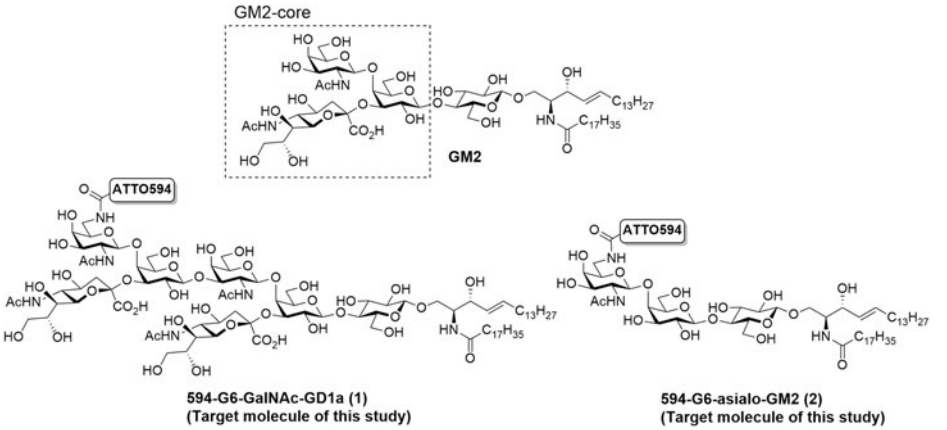
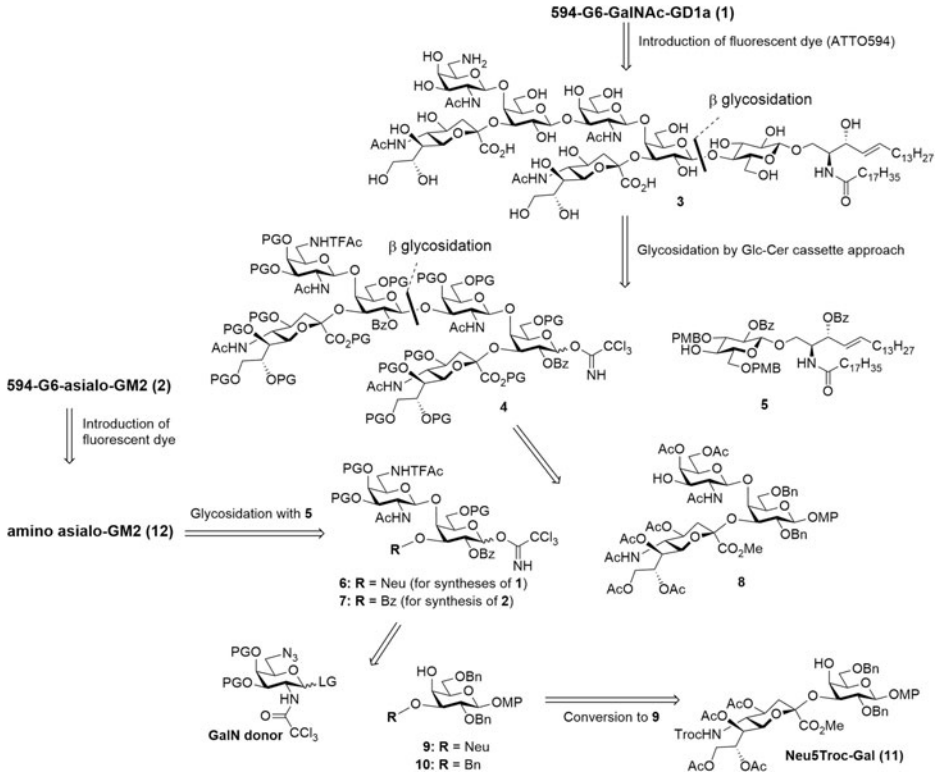


Figure 1. Structures of GM2 and fluorescent analogs of GalNAc-GD1a and asialo-GM2.

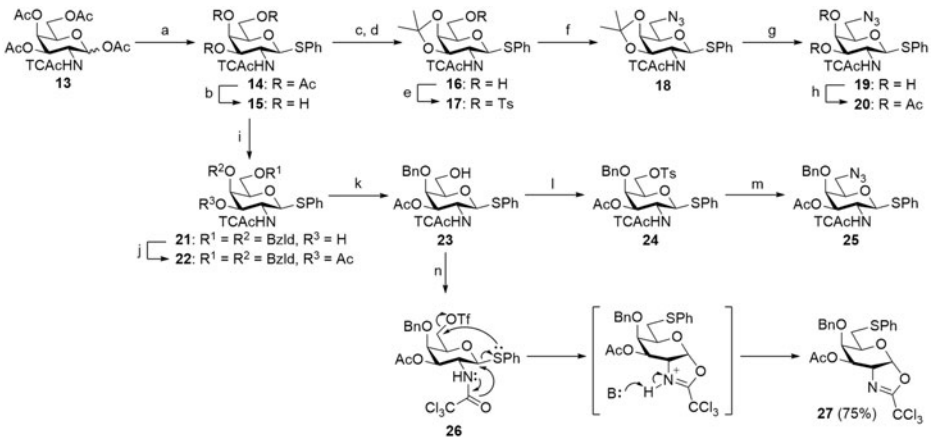


Scheme 1. Retrosynthetic analyses of the fluorescent analogs **1** and **2**.

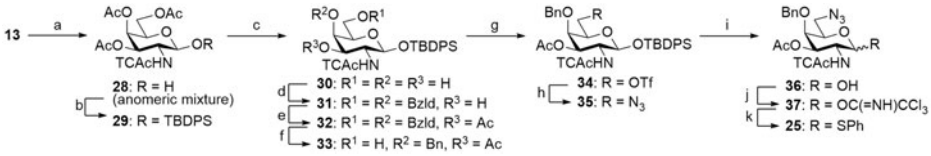
donor **4**, in which the amino group of the GalNAc residue was protected with a base-labile trifluoroacetyl (TFAc) group, and the Glc-Cer cassette **5**.[11–13] Disconnection of the hexasaccharide component **4**, which is the tandem sequence of a trisaccharide (referred to as GM2 core), gave the GM2 core donor **6** and the previously reported GM2 core acceptor **8**.[10] For the synthesis of **6**, we designed a GalN donor with the appropriate chemistry for the β-selective glycosylation with **9**[14] and subsequent conversion into 6-NHTFAc-GalNAc. Owing to the difficulty of the direct replacement of a hydroxyl group with NHTFAc, a stepwise introduction of the NHTFAc group involving the installation and reduction of an azide group followed by trifluoroacetylation was conceived. To achieve this design, we envisaged that a trichloroacetamide (NHTCAc) group at the C2 position could be exploited as both a neighboring group and a precursor of the desired acetamide group. Thus, the 6-azido-GalNTCAc donor could be used for the glycosylation of **9**, which could be followed by simultaneous selective reduction of the azide and trichloroacetamide groups and subsequent trifluoroacetylation, affording a suitably protected amino-containing GM2 core intermediate. A similar synthetic route was conceived for the

asialo-GM2 analog **2**, which started from the coupling of the 6-azido-GalNTCAc donor with Gal acceptor **10**.[15]

First, we focused on developing a 6-azido-GalNTCAc donor that allowed for efficient glycosylation and subsequent selective conversion into the desired 6-NHTFAc-GalNAc residue. We examined possible protecting group strategies for the C3 and C4 hydroxyl groups suitable for β-glycosylation (Scheme 2). The *N*-TCAc derivative **14**[16] was converted into triol **15**, which was subsequently transformed into **16** by regioselective acetonation of the 3,4-diol. Tosylation of the C6 hydroxyl group of **16** followed by treatment with NaN_3 and 18-crown-6 in *N*,*N*-dimethylacetamide (DMA) at 80 °C afforded 6-azido-GalNTCAc donor **18**. Subsequent acidic hydrolysis of the acetonide and acetylation furnished 3,4-di-*O*-acetyl-GalNTCAc donor **20**. To exploit the arming effect of an ether protecting group,[17,18] we also examined the synthesis of a 4-*O*-benzyl-GalNTCAc donor. In this case, triol **15** was converted into 6-OH derivative **23** via 4,6-benzylidenation[19], acetylation, and reductive ring cleavage of the benzylidene group using TESH and $PhBCl_2$. Next, a tosyl group was introduced at the 6-OH position to afford **24**, and subsequent treatment with *N*,*N*,*N*′,*N*′-tetramethylguanidinium azide ($TMGN_3$)[20] at 80 °C delivered 4-*O*-Bn donor **25** in a moderate yield (44%), which was accompanied by degradation of the starting material and desired product. Although the synthesis of **25** was also attempted via triflate **26**, the reaction of **23** with Tf_2O and 2,6-lutidine provided 6-SPh oxazoline **27** as the main product. Compound **27** might be produced immediately from **26** via intramolecular replacement of the



Scheme 2. Synthesis of GalN donors. (a) PhSH, $BF_3{\cdot}Et_2O/CH_2Cl_2$, rt, 65%; (b) NaOMe/MeOH, rt; (c) CSA/2,2-dimethoxypropane, rt; (d) MeOH-H_2O, reflux, 93% (3 steps); (e) TsCl/Pyr, rt, 92%; (f) NaN_3, 18-crown-6/DMA, 80 °C, 44%; (g) 80% AcOH aq., 50 °C; (h) Ac_2O, DMAP/Pyr, rt, quant. (2 steps); (i) BDA, CSA/MeCN-THF, rt, 94% (2 steps); (j) Ac_2O, DMAP/Pyr, rt, 94%; (k) TESH, $PhBCl_2$/CH_2Cl_2, MS4Å, −20 °C; (l) TsCl/Pyr, rt, 94% (2 steps); (m) $TMGN_3$/DMA, 80 °C, 44%; (n) Tf_2O, 2,6-lutidine/CH_2Cl_2, −20 °C, **27** (75%).



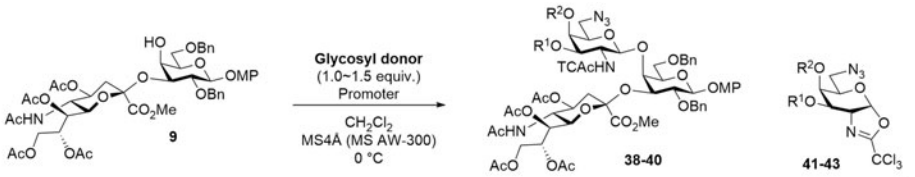
Scheme 3. Synthesis of GalN donors. (a) H_2NNH_2·AcOH/DMF, rt, 78%; (b) TBDPSCl, imidazole/DMF, 60 °C, 95%; (c) NaOMe/MeOH, rt; (d) BDA, *p*-TsOH/MeCN, rt; (e) Ac_2O/Pyr, rt; (f) TESH, $PhBCl_2/CH_2Cl_2$, MS4Å, −20 °C, 87% (4 steps); (g) Tf_2O, 2,6-lutidine/CH_2Cl_2, −20 °C; (h) NaN_3, 18-crown-6/DMA, 60 °C, 87% (2 steps); (i) TBAF, AcOH/THF, rt, 98%; (j) CCl_3CN, DBU/CH_2Cl_2, 0 °C, 73%; (k) PhSH, TMSOTf/CH_2Cl_2, MS AW-300, 0 °C, 69%.

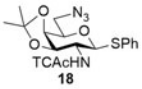
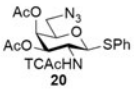
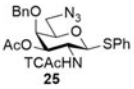
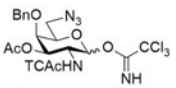
triflate at the C6 position by the nucleophilic sulfur atom at the C1 position, facilitated by the nucleophilic participation of the NHTCAc group, to form the oxazoline as depicted in Scheme 2.

Following these results, triflate **34** bearing a TBDPS ether moiety at the C1 position was synthesized and examined for azidation at the C6 position (Scheme 3). The 1-*O*-TBDPS derivative **33**, which was prepared from GalNTCAc tetraacetate **13**, was treated with Tf_2O and 2,6-lutidine to afford the 6-OTf derivative **34**. Next, triflate **34** was reacted with NaN_3 at 60 °C to afford **35** in an 81% yield over two steps. Azide **35** was readily converted into trichloroacetimidate **37** via TBDPS deprotection using TBAF in the presence of AcOH followed by treatment with CCl_3CN and DBU at 0 °C. Trichloroacetimidate **37** was further converted into thioglycoside **25** by reaction with PhSH in the presence of TMSOTf.

Next, 6-azido-GalNTCAc donors **18**, **20**, **25**, and **37** were used to glycosylate an equimolar amount of sialyl α(2,3)Gal (Neu-Gal) acceptor **9** at 0 °C in CH_2Cl_2 (Table 1). Attempted activation of 3,4-*O*-isopropylidene-protected donor **18** using *N*-iodosuccinimide (NIS) and TfOH was unsuccessful, unexpectedly generating oxazoline **41** as the predominant product (94%, entry 1). Reaction of 4-*O*-Ac-protected donor **20** under the same conditions afforded the corresponding trisaccharide **39** in a 42% yield and the oxazoline derivative **42** in a 57% yield (entry 2). The coupling yield increased to 76% upon using 1.5 equiv of donor **20** (entry 3). In contrast, the glycosylation reaction of 4-*O*-Bn GalNTCAc donor **25** (1.0 equiv) preferentially provided trisaccharide **40** in a 73% yield (entry 4), which increased to a 84% yield upon using 1.5 equiv of donor (entry 5). A comparable yield was obtained for trichloroacetimidate donor **37** in the presence of TMSOTf as promoter (entry 6). The glycosidation of **20** (entry 2, 80 min) proceeded more slowly than that of **25** (entry 4, 30 min). These results indicated that the C4 protecting group strongly affected the reactivity and coupling yields of 6-azido-GalNTCAc donors. The β-galactosaminidation reactions proceeded via the 1,3-oxazolenium intermediate, and thus competed with oxazoline formation. The 4-*O*-Bn group in **25** might stabilize the oxonium cation intermediate, decreasing the likelihood of forming

Table 1. Results of galactosaminidation to produce the GM2 core trisaccharide. [a] Conversion yield.

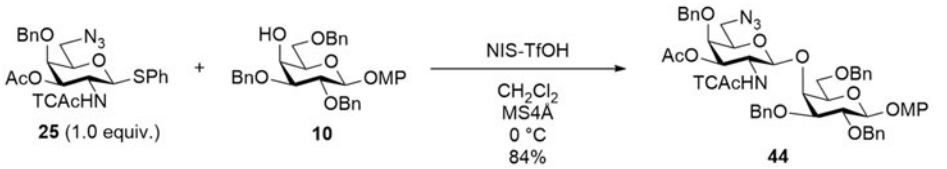


Entry	Glycosyl donor	Amount of donor	Promoter	Products (Yield)	
				Trisaccharide	Oxazoline[a]
1	 **18**	1.0 equiv.	NIS-TfOH	**38** (Trace)	**41** (94%)
2	 **20**	1.0 equiv.	NIS-TfOH	**39** (42%)	**42** (57%)
3	**20**	1.5 equiv.	NIS-TfOH	**39** (76%)	**42** (32%)
4	 **25**	1.0 equiv.	NIS-TfOH	**40** (73%)	**43** (Trace)
5	**25**	1.5 equiv.	NIS-TfOH	**40** (84%)	**43** (16%)
6	 **37 (α/β = 1/0.03)**	1.5 equiv.	TMSOTf	**40** (85%)	**43** (13%)

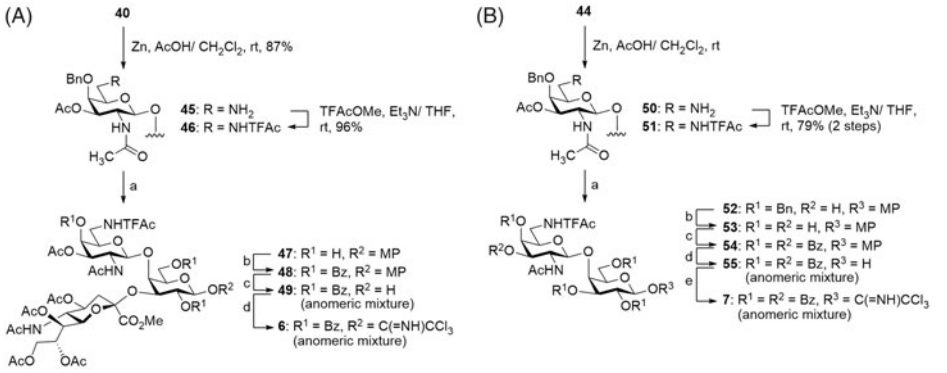
the 1,3-oxazolenium intermediate. Then, β-glycosylation might be directed by electrostatic interaction between the TCAc group and the anomeric center in the vicinity of the α face.

For the synthesis of asialo-GM2, equimolar amounts of 4-*O*-Bn thioglycoside **25** and Gal acceptor **10** were coupled to afford disaccharide **44** in an 84% yield (Scheme 4). The higher yield of this reaction compared with the reaction to form trisaccharide **40** (73%, Table 1, entry 4) may be attributable to reduced steric hindrance at the 4-OH group of the Gal moiety owing to the smaller substituent at the C3 position.

Next, the GM2 core **40** and the asialo-GM2 core **44** were converted into the corresponding glycosyl donors **6** and **7**, respectively (Scheme 5). For this step, we first addressed the simultaneous conversion of TCAc and azide groups in the GalN residues into acetyl (Ac) and amino groups, respectively. Treatment with Zn in AcOH was found to be the most efficient method for reducing trisaccharide **40** to 2-NHAc-6-NH_2 derivative **45** (87%). Next, the amino group in **45** was protected with the trifluoroacetyl group. The protecting groups in GM2 trisaccharide **46** were manipulated through hydrogenolysis of the three benzyl groups and subsequent



Scheme 4. Synthesis of the disaccharide moiety of asialo-GM2.

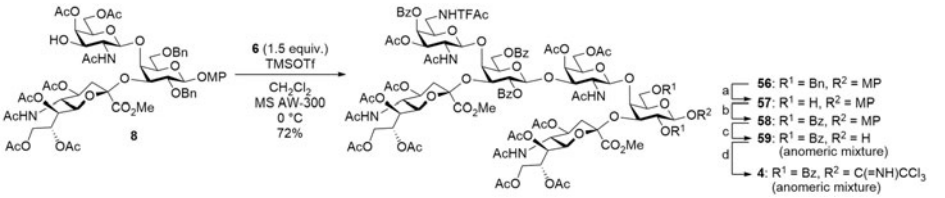


Scheme 5. (A) Synthesis of GM2 core donor. (a) H_2, $Pd(OH)_2$-C/EtOH, rt, 96%; (b) Bz_2O, DMAP/Pyr, rt, 91%; (c) CAN, H_2O/MeCN-toluene, 0 °C, 91%; (d) CCl_3CN, DBU/CH_2Cl_2, 0 °C, 79%. (B) Synthesis of asialo-GM2 core donor. (a) NaOMe/MeOH-CH_2Cl_2, rt; (b) H_2, $Pd(OH)_2$-C/1,4-dioxane, rt; (c) Bz_2O, DMAP/Pyr, rt, 89% (3 steps); (d) CAN, H_2O/MeCN-toluene, 0 °C, 82%; (e) CCl_3CN, DBU/CH_2Cl_2, 0 °C, 92%.

benzoylation to afford **48** (Scheme 5A). The cleavage of the MP group in **48** using cerium(IV) ammonium nitrate in an aqueous solvent mixture afforded the hemiacetal, which was transformed into the 6-NHTFAc-containing GM2 core trichloroacetimidate **6**. Similar procedures were used to efficiently convert disaccharide donor **44** into donor **7** for asialo-GM2 (Scheme 5B).

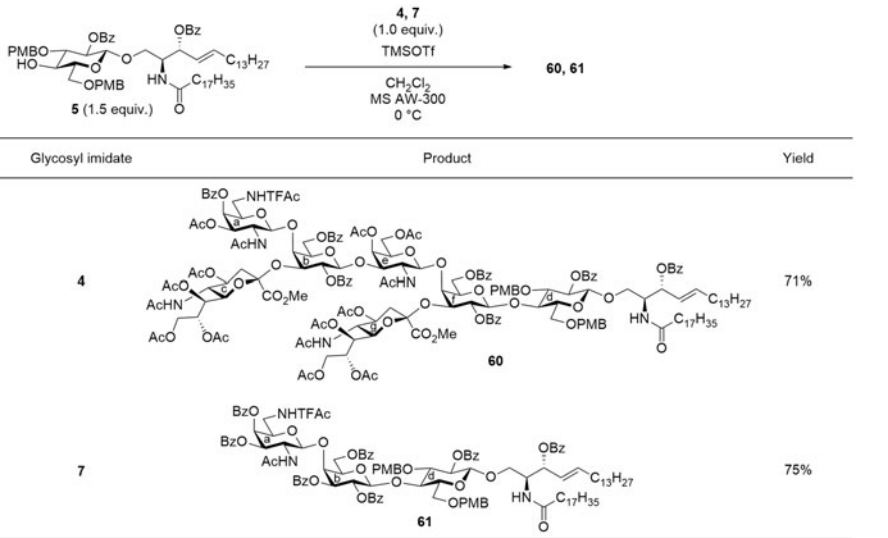
To our delight, the glycosylation of GM2 acceptor **8** with 6-NHTFAc-containing GM2 core donor **6**, promoted by TMSOTf in CH_2Cl_2 at 0 °C, afforded successfully GalNAc-GD1a hexasaccharide **56** in a 72% yield (Scheme 6). Subsequent hydrogenolysis of the benzyl groups followed by benzoylation delivered **58** in a high yield. To permit the final coupling with Glc-Cer cassette **5**, **58** was converted into trichloroacetimidate donor **4** in a similar manner as that with the GM2 donor **6**.

With glycosyl donors **4** and **7**, we constructed the glycolipid frameworks of GalNAc-GD1a and asialo-GM2. Thus, Glc-Cer acceptor **5** (1.5 equiv) were glycosylated with the two donors in the presence of TMSOTf in CH_2Cl_2, furnishing the protected GalNAc-GD1a **60** (71%) and asialo-GM2 **61** (75%) in high yields (Table 2).



Scheme 6. Synthesis of GalNAc-GD1a core donor. (a) H_2, $Pd(OH)_2$-C/1,4-dioxane, rt, 92%; (b) Bz_2O, DMAP/Pyr, rt, 95%; (c) CAN, H_2O/MeCN-toluene, 0 °C, 72%; (d) CCl_3CN, DBU/CH_2Cl_2, 0 °C, 82%.

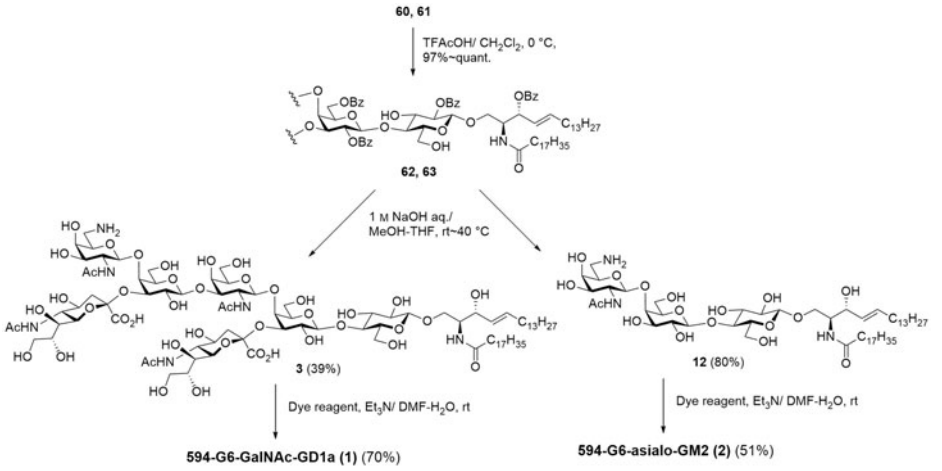
Table 2. Synthesis of ganglioside frameworks.



Finally, **60** and **61** were fully deprotected to afford the C6-amino derivatives **3** and **12**, respectively, which were then reacted with the activated ester of ATTO594 in DMF/H_2O, delivering the target molecules of 594-G6-GalNAc-GD1a (**1**) and 594-G6-asialo-GM2 (**2**) (Scheme 7).

Conclusion

We have developed a 6-N_3-GalNTCAc donor and applied it to the synthesis of fluorescent analogs of gangliosides GalNAc-GD1a and asialo-GM2. The combination of the 6-N_3 and C2-NHTCAc groups allowed single-step conversion into the 6-NHTFAc-GalNAc residue following the assembly of the GM2 core trisaccharide and asialo-GM2 disaccharide. The glycosylation reactions of GalN donors revealed the influence of the 4-OH protecting group on the coupling efficiency. Furthermore, the use of the 6-NHTFAc-GalNAc-containing saccharide units permitted fluorescent analogs of GalNAc-GD1a and asialo-GM2 to be successfully realized for the first time.



Scheme 7. Global deprotection and fluorescent labeling.

Biophysical experiments using these fluorescent probes are currently under way. These fluorescent analogs are expected to prove valuable for elucidating raft-associated neuropathies.

Experimental section

General procedures

^{1}H- and 13C-NMR spectra were recorded using Bruker Avance III 500 and Avance III 800 spectrometers. Chemical shifts are expressed in ppm (δ) relative to Me_4Si as an internal standard. High-resolution mass spectrometry (HRMS) was performed using a Bruker Daltonics micrOTOF (ESI-TOF) spectrometer. Specific rotations were determined using a Horiba SEPA-300 high-sensitivity polarimeter. Molecular sieves were purchased from FUJIFILM Wako Pure Chemical Corporation and dried at 300 °C for 2 h in a muffle furnace prior to use. Dry reaction solvents (CH_2Cl_2, toluene, THF, CH_3CN, DMF, and pyridine) were purchased from Kanto Chemical Co. Inc. and used without further purification. Thin-layer chromatography (TLC) was performed on Merck TLC plates (silica gel 60 F_{254} on glass plates), and analytes were detected by either UV illumination (253.6 nm) or soaking in an ethanolic solution of 10% H_2SO_4 followed by heating. Flash column chromatography was performed using silica gel (80 mesh and 300 mesh) manufactured by Fuji Silysia Co. The quantity of silica gel was typically estimated as 150–200 times the sample weight to be charged. The chromatographic solvent systems are specified by volume. Evaporation and condensation steps were performed under vacuum. ATTO594 *N*-succinimidyl ester was purchased from ATTO-Tec. The sugar units are numbered using letters from *a* to *g*; GalNAc-GD1a: GalNAc (*a*), Gal (*b*), and NeuAc

(*c*) in terminal GM2 core, GalNAc (*e*), Gal (*f*), and NeuAc (*g*) in inner GM2 core, Glc (*d*); asialo-GM1: GalNAc (*a*), Gal (*b*), and Glc (*d*) (e.g., see Table 2).

594-G6-GalNAc-GD1a (1)

Compound **3** (4.1 mg, 2.9 μmol) and ATTO594 *N*-succinimidyl ester (4.2 mg, 3.0 μmol) were dissolved in DMF/H_2O (180 μL/17 μL), and triethylamine (5.6 μL, 40 μmol) was added at room temperature. After the reaction had reached completion, as confirmed by TLC ($CHCl_3$/MeOH/H_2O = 5:6:2), the reaction mixture was purified by column chromatography on Sephadex LH-20, using $CHCl_3$/MeOH (1:1) followed by preparative TLC ($CHCl_3$/MeOH/H_2O = 5:5:1) to afford 594-G6-GalNAc-GD1a (**1**) (4.0 mg, 70%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.75–7.38 (m, 4 H, $Ar^{ATTO594}$), 7.33–5.88 (m, 6 H, $Ar^{ATTO594}$), 5.68 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 6.7$ Hz, $J_{4,5} = 15.3$ Hz, H-5^{Cer}), 5.44 (dd, 1 H, $J_{3,4} = 7.7$ Hz, H-4^{Cer}), 4.89-3.11 (m, 63 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-1^d, H-2^d, H-3^d, H-4^d, H-5^d, H-6a^d, H-6b^d, H-1^e, H-2^e, H-3^e, H-4^e, H-5^e, H-6a^e, H-6b^e, H-1^f, H-2^f, H-3^f, H-4^f, H-5^f, H-6a^f, H-6b^f, H-4^g, H-5^g, H-6^g, H-7^g, H-8^g, H-9a^g, H-9b^g, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}, 3 $NCH_2{}^{ATTO594}$, 2 $CH_2SO_3{}^{ATTO594}$), 2.76–2.64 (m, 5 H, H-3*eq*^{c}, H-3*eq*^{g}, $NMe^{ATTO594}$), 2.18–2.15 (m, 2 H, $NHCOCH_2{}^{Cer}$), 2.01–1.99 (m, 14 H, H-6a^{Cer}, H-6b^{Cer}, 4 Ac), 1.90-1.83 (m, 4 H, H-3*ax*^{c}, H-3*ax*^{g}, $NCH_2CH_2CH_2{}^{ATTO594}$), 1.69-1.49 (m, 14 H, $NHCOCH_2CH_2{}^{Cer}$, 4 $Me^{ATTO594}$), 1.39–1.29 (m, 58 H, 25 $CH_2{}^{Cer}$, $NCH_2CH_2{}^{ATTO594}$, 2 $NCH_2CH_3{}^{ATTO594}$), 0.91–0.88 (m, 6 H, 2 Me^{Cer}); ^{13}C NMR (200 MHz, CD_3OD) δ 175.9, 175.6, 175.6, 175.2, 175.1, 175.0, 174.7, 174.6, 171.0, 169.8, 161.5, 159.2, 154.9, 154.2, 154.1, 138.5, 138.5, 137.6, 135.1, 132.2, 131.6, 131.4, 131.2, 130.8, 129.5, 129.4, 128.8, 126.1, 126.0, 124.2, 122.8, 122.8, 115.0, 115.0, 106.5, 104.9, 104.5, 104.4, 104.2, 103.5, 103.3, 97.1, 83.0, 81.0, 79.6, 78.8, 76.5, 76.4, 76.3, 76.1, 75.8, 75.6, 75.3, 75.2, 75.1, 74.9, 74.2, 74.0, 73.5, 73.4, 73.0, 71.0, 71.0, 70.4, 70.0, 69.9, 69.7, 69.7, 69.6, 65.3, 64.9, 63.0, 61.8, 61.7, 61.7, 54.7, 54.2, 54.1, 53.9, 53.9, 53.8, 52.5, 49.8, 49.5, 49.4, 49.3, 47.8, 41.4, 41.4, 40.9, 38.8, 38.7, 38.6, 37.4, 33.9, 33.5, 33.1, 33.1, 32.7, 30.9, 30.9, 30.8, 30.8, 30.7, 30.7, 30.5, 30.5, 30.5, 30.4, 29.5, 29.4, 29.3, 29.3, 27.2, 23.8, 23.8, 23.6, 22.6, 14.5, 13.8, 13.7; HRMS (ESI) *m/z*: found $[M–3H]^{3–}$ 941.1016, $C_{133}H_{207}N_9O_{52}S_2$ calcd for $[M–3H]^{3–}$ 941.1018.

594-G6-asialo-GM2 (2)

Compound **12** (3.2 mg, 2.9 μmol) and ATTO594 *N*-succinimidyl ester (6.2 mg, 4.5 μmol) were dissolved in DMF/H_2O (270 μL/27 μL), and

triethylamine (12.2 µL, 87.5 µmol) was added in two portions over 2.5 h at room temperature. After the reaction had reached completion, as confirmed by TLC ($CHCl_3$/MeOH/H_2O = 5:4:1), the reaction mixture was purified by column chromatography on Sephadex LH-20, using $CHCl_3$/MeOH (1:1) followed by preparative TLC ($CHCl_3$/MeOH/H_2O = 5:2:0.1) to afford 594-G6-asialo-GM2 (**2**) (2.8 mg, 51%): ^{1}H NMR (800 MHz, CD_3OD) δ 7.73-5.89 (m, 10 H, $Ar^{ATTO594}$), 5.70 (m, 1 H, H-5^{Cer}), 5.44 (m, 1 H, H-4^{Cer}), 4.59-3.21 (m, 35 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-5b, H-6a^b, H-6b^b, H-1^d, H-2^d, H-3^d, H-4^d, H-5^d, H-6a^d, H-6b^d, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}, $3NCH_2{}^{ATTO594}$, $2CH_2SO_3{}^{ATTO594)}$, 2.73-2.64 (2 s, 3 H, $NMe^{ATTO594}$), 2.17-2.03 (m, 7 H, H-6a^{Cer}, H-6b^{Cer}, $NHCOCH_2{}^{Cer}$, Ac), 1.76–1.29 (m, 74 H, $26CH_2{}^{Cer}$, $NCH_2CH_2{}^{ATTO594}$, $NCH_2CH_2CH_2{}^{ATTO594}$, $2NCH_2CH_3{}^{ATTO594}$, $4\,Me^{ATTO594}$), 0.91–0.89 (m, 6 H, $2\,Me^{Cer}$); ^{13}C NMR (200 MHz, CD_3OD) δ 175.9, 175.4, 175.3, 175.1, 175.0, 171.5, 171.2, 159.2, 155.1, 154.9, 154.2, 154.1, 138.4, 138.3, 137.5, 137.4, 135.1, 132.1, 132.0, 131.9, 131.7, 131.4, 131.2, 131.0, 130.8, 130.3, 129.4, 128.8, 126.1, 126.0, 124.4, 124.1, 124.1, 122.9, 122.9, 115.1, 115.0, 114.9, 105.1, 104.4, 104.3, 104.1, 97.0, 80.8, 80.8, 78.2, 78.0, 76.4, 76.2, 76.1, 74.9, 74.8, 74.4, 74.4, 74.3, 72.9, 72.6, 72.5, 69.8, 69.8, 61.8, 61.7, 61.7, 55.1, 55.1, 54.6, 54.2, 53.8, 53.8, 47.3, 41.3, 41.3, 38.7, 37.4, 33.6, 33.5, 33.1, 33.1, 32.6, 30.9, 30.9, 30.8, 30.8, 30.8, 30.7, 30.7, 30.5, 30.5, 30.5, 30.4, 29.5, 29.4, 29.3, 27.2, 23.9, 23.8, 23.1, 14.5, 13.8, 13.7, 13.7; HRMS (ESI) *m*/*z*: found $[M–H]^-$ 1877.9969, $C_{97}H_{149}N_6O_{26}S_2$ calcd for $[M–H]^-$ 1877.9968.

4-Methoxyphenyl (3-O-acetyl-6-azido-4-O-benzyl-2,6-dideoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]-2,6-di-O-benzyl-β-D-galactopyranoside (40)

To a solution of **9** (81.9 mg, 87.1 µmol) and **25** (75.7 mg, 132 µmol) in CH_2Cl_2 (4.4 mL) were added 4 Å molecular sieves (224 mg) at room temperature under an Ar atmosphere, and the resulting mixture was stirred for 30 min. NIS (51.3 mg, 228 µmol) and TfOH (2.1 µL, 24 µmol) were then added to the mixture at 0 °C. After stirring for 1 h at 0 °C, as the reaction was monitored by TLC (*n*-hexane/AcOEt = 4:1, developed twice), the solution was filtered through a Celite pad and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, washed with saturated $NaHCO_3$, $Na_2S_2O_3$, and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using toluene/acetone (5:1) to afford **40** (102 mg, 84%): $[\alpha]_D$ −17.0° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–6.78 (m, 20 H, NH-2^a,

Ar), 5.24-5.18 (m, 5 H, H-3^a, H-4^c, H-7^c, H-8^c, NH-5^c), 5.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1^a), 5.04–4.92 (2d, 2 H, CH_2Ph), 4.87 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1^b), 4.64–4.50 (m, 5 H, H-2^a, CH_2Ph), 4.14 (m, 1 H, H-5^a), 4.10 (d, 1 H, $J_{3,4}$ = 2.1 Hz, H-4^a), 4.06–4.00 (m, 3 H, H-3^b, H-9a^c, H-9b^c), 3.94 (s, 3 H, COOMe), 3.93–3.79 (m, 6 H, H-2^b, H-4^b, H-6a^b, H-6b^b, H-5^c, H-6^c), 3.77 (s, 3 H, OMe), 3.69 (m, 1 H, H-5^b), 3.51 (dd, 1 H, $J_{5,6a}$ = 7.3 Hz, J_{gem} = 12.7 Hz, H-6a^a), 3.08 (dd, 1 H, $J_{5,6b}$ = 5.3 Hz, H-6b^a), 2.29 (dd, 1 H, $J_{3ax,4}$ = 11.5 Hz, J_{gem} = 14.3 Hz, H-3*ax*^{c}), 2.14–2.09 (m, 7 H, H-3*eq*^{c}, 2 Ac), 2.01-1.88 (4 s, 12 H, 4 Ac); 13C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.6, 170.4, 169.7, 169.4, 168.6, 162.0, 155.4, 151.4, 138.5, 138.2, 137.9, 128.6, 128.4, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 118.7, 114.6, 103.3, 100.9, 99.5, 92.9, 77.5, 77.3, 77.2, 76.9, 75.3, 75.3, 75.0, 74.6, 74.4, 74.3, 73.9, 73.2, 72.0, 70.3, 69.3, 67.7, 66.6, 62.0, 55.7, 53.5, 53.0, 51.1, 49.4, 35.7, 23.3, 21.1, 21.1, 20.9, 20.8, 20.7; HRMS (ESI) *m/z* found $[M + Na]^+$ 1424.3684, $C_{64}H_{74}Cl_3N_5O_{24}$ calcd for $[M + Na]^+$ 1424.3682.

4-Methoxyphenyl (3-O-acetyl-6-azido-4-O-benzyl-2,6-dideoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (44)

To a solution of **10** (62.1 mg, 111 μmol) and **25** (63.8 mg, 111 μmol) in CH_2Cl_2 (4.4 mL) were added 4 Å molecular sieves (225 mg) at room temperature under an Ar atmosphere, and the resulting mixture was stirred for 30 min. NIS (37.6 mg, 167 μmol) and TfOH (1.5 μL, 17 μmol) were then added to the mixture at 0 °C. After stirring for 2 h at 0 °C, as the reaction was monitored by TLC (toluene/AcOEt = 9:1, developed twice), the solution was filtered through a Celite pad and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, washed with saturated $NaHCO_3$, $Na_2S_2O_3$, and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using toluene/AcOEt (19:2) to afford **44** (95.6 mg, 84%): $[\alpha]_D$ −2.4° (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–6.74 (m, 24 H, Ar), 6.94 (d, 1 H, $J_{2,NH}$ = 8.0 Hz, NH-2^a), 5.05–5.00 (m, 2 H, H-3^a, CH_2Ph), 4.89 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1^a), 4.86–4.84 (m, 2 H, CH_2Ph), 4.80 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1^b), 4.76–4.49 (m, 5 H, CH_2Ph), 4.35 (m, 1 H, H-2^a), 4.00 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-4^b), 3.88 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-2^b), 3.80–3.72 (m, 6 H, H-4^a, H-6a^b, H-6b^b, OMe), 3.66 (m, 1 H, H-5^b), 3.62 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3^b), 3.50 (m, 1 H, H-5^a), 3.43 (dd, 1 H, $J_{5,6a}$ = 7.2 Hz, J_{gem} = 12.4 Hz, H-6a^a), 3.13 (dd, 1 H, $J_{5,6b}$ = 9.5 Hz, H-6b^a), 2.04 (s, 3 H, Ac); 13C NMR (125 MHz, $CDCl_3$) δ 170.6, 161.8, 155.3, 151.5, 138.7, 138.5, 137.8, 137.6, 129.0, 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 127.9, 127.8, 127.7, 118.6, 114.6, 103.1, 101.1, 92.5, 81.5, 79.8, 75.4, 75.1, 74.8, 74.5, 74.0,

73.9, 73.8, 73.7, 70.0, 55.7, 53.0, 50.9, 20.9; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1041.2618, $C_{51}H_{53}Cl_3N_4O_{12}$ calcd for $[M+Na]^+$ 1041.2618.

4-Methoxyphenyl (2-acetamido-3-O-acetyl-4-O-benzoyl-2,6-dideoxy-6-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-{[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]-2,6-di-O-benzoyl-β-D-galactopyranosyl}-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]-2,6-di-O-benzyl-β-D-galactopyranoside (56)

To a solution of **6** (323 mg, 254 μmol) and **8** (254 mg, 175 μmol) in CH_2Cl_2 (8.8 mL) were added 4 Å molecular sieves (AW-300, 1.71 g) at room temperature under an Ar atmosphere, and the resulting mixture was stirred for 1 h. TMSOTf (3.8 μL, 21 μmol) was then added to the mixture at 0 °C. After stirring for 2 h at 0 °C, as the reaction was monitored by TLC (toluene/acetone = 1:1), the reaction mixture was filtered through a Celite pad and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, washed with saturated Na_2CO_3 and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Sephadex LH-20, using $CHCl_3$/MeOH (1:1) followed by flash column chromatography on silica gel, using $CHCl_3$/MeOH (20:1) to afford **56** (318 mg, 72%): $[\alpha]_D$ +8.9° (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3CN) δ 8.15–6.79 (m, 30 H, NH-6^a, Ar), 6.43 (d, 1 H, $J_{2,NH}$ = 9.1 Hz, NH-2^a), 6.10–6.07 (m, 3 H, NH-5^c, NH-2^e, NH-5^g), 5.51 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-4^a), 5.44 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-4^e), 5.31 (dd, 1 H, $J_{2,3}$ = 11.2 Hz, H-3^a), 5.24–5.22 (m, 2 H, H-1^b, H-7^{Neu}), 5.18–5.11 (m, 3 H, H-2^b, H-8^c, H-8^g), 5.01 (m, 1 H, H-4^{Neu}), 4.98 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1^a), 4.95–4.88 (m, 2 H, H-1^f, H-4^{Neu}), 4.90 (dd, 1 H, $J_{6,7}$ = 3.0 Hz, $J_{7,8}$ = 7.7 Hz, H-7^{Neu}), 4.77 (d, 1 H, C*H*$_2$Ph), 4.65 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1^e), 4.62 (dd, 1 H, $J_{5,6a}$ = 5.3 Hz, J_{gem} = 11.5 Hz, H-6a^b), 4.57–4.53 (m, 2 H, H-3^b, C*H*$_2$Ph), 4.51–4.43 (m, 3 H, H-6b^b, C*H*$_2$Ph), 4.36 (t, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 6.7 Hz, H-5^a), 4.30–4.24 (m, 2 H, H-2^a, H-3^f), 4.14 (dd, 1 H, $J_{2,3}$ = 12.3 Hz, H-3^e), 4.08–3.72 (m, 16 H, H-4^b, H-5^b, H-5^c, H-6^c, H-9a^c, H-9b^c, H-2^e, H-5^e, H-6a^e, H-6b^e, H-4^f, H-5^f, H-5^g, H-6^g, H-9a^g, H-9b^g), 3.78–3.77 (2 s, 6 H, 2 COOMe), 3.72 (s, 3 H, OMe), 3.63–3.55 (m, 2 H, H-6a^f, H-6b^f), 3.46–3.35 (m, 3 H, H-6a^a, H-6b^a, H-2^f), 2.40 (dd, 1 H, $J_{3eq,4}$ = 4.9 Hz, J_{gem} = 13.5 Hz, H-3*eq*^{Neu}), 2.11–1.17 (m, 48 H, H-3*eq*^{Neu}, H-3*ax*^{c}, H-3*ax*^{g}, 15 Ac); ^{13}C NMR (125 MHz, CD_3CN) δ 171.6, 171.4, 171.3, 171.2, 171.1, 171.0, 170.9, 170.9, 170.8, 170.6, 170.6, 170.5, 170.4, 169.6, 169.4, 167.2, 166.8, 165.1, 158.4, 158.1, 157.8, 157.5, 156.3, 152.5, 139.9, 139.8, 134.7, 134.3, 134.3, 131.2, 130.9, 130.7, 130.7, 130.6, 130.2, 129.9, 129.7, 129.6, 129.2, 129.1,

128.5, 128.4, 119.1, 118.6, 115.7, 115.5, 103.3, 103.1, 102.7, 100.5, 100.3, 79.4, 79.1, 78.9, 75.9, 75.8, 74.3, 74.2, 73.6, 72.8, 72.6, 72.5, 72.2, 72.0, 71.3, 71.3, 71.0, 70.7, 70.6, 70.5, 69.3, 69.2, 68.7, 67.6, 65.0, 64.3, 62.8, 62.6, 56.2, 54.0, 53.9, 52.3, 51.4, 49.4, 48.9, 40.4, 36.2, 35.8, 32.6, 30.0, 23.3, 23.1, 23.1, 21.5, 21.5, 21.2, 21.0, 21.0, 21.0, 20.9, 14.4; HRMS (ESI) *m*/*z*: found $[M + Na]^+$ 2536.8154, $C_{118}H_{138}F_3N_5O_{52}$ calcd for $[M + Na]^+$ 2536.8152.

(2-Acetamido-3-O-acetyl-4-O-benzoyl-2,6-dideoxy-6-trifluoroacetamido-β-D-galacto-pyranosyl)-(1→4)-{[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]-2,6-di-O-benzoyl-β-D-galacto-pyranosyl}-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-{[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)]-2,6-di-O-benzoyl-β-D-galactopyranosyl}-(1→4)-(2-O-benzoyl-3,6-di-O-p-methoxybenzyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (60)

To a solution of **5** (69.9 mg, 59.4 μmol) and **4** (102 mg, 39.4 μmol) in CH_2Cl_2 (2.5 mL) were added 4 Å molecular sieves (AW-300, 487 mg) at room temperature under an Ar atmosphere, and the resulting mixture was stirred for 1 h. TMSOTf (0.8 μL, 4 μmol) was then added to the mixture at 0 °C. After stirring for 5 h at 0 °C, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 15:1, developed twice), triethylamine was added. The reaction mixture was filtered through a Celite pad and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, washed with saturated Na_2CO_3 and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel, using $CHCl_3$/MeOH (30:1) to afford **60** (101 mg, 71%): $[\alpha]_D$ +25.7° (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15–6.60 (m, 44 H, NH-6^a, Ar), 6.22 (d, 1 H, $J_{2,NH}$ = 7.6 Hz, NH-2^a), 5.76 (dt, 1 H, $J_{5,6a} = J_{5,6b}$ = 6.8 Hz, $J_{4,5}$ = 15.2 Hz, H-5^{Cer}), 5.70–5.68 (m, 2 H, H-3^a, NH-2^{Cer}), 5.64 (m, 1 H, H-8^{Neu}), 5.52 (d, 1 H, $J_{2,NH}$ = 6.5 Hz, NH-2^e), 5.49–5.44 (m, 3 H, H-4^e, H-3^{Cer}, H-7^{Neu}), 5.41–5.31 (m, 3 H, H-2^b, H-4^{Cer}, H-8^{Neu}), 5.27–5.24 (m, 2 H, H-2^f, H-7^{Neu}), 5.18–5.11 (m, 4 H, H-1^a, H-4^a, H-2^d, H-1^e), 5.07 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^f), 5.07 (m, 1 H, NH-5^{Neu}), 5.00 (dd, 1 H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 10.8 Hz, H-3^e), 4.96–4.90 (m, 2 H, H-4^{Neu}, NH-5^{Neu}), 4.82 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1^b), 4.79–4.73 (m, 2 H, H-4^{Neu}, CH_2Ar), 4.69 (dd, 1 H, $J_{5,6a}$ = 5.3 Hz, J_{gem} = 11.5 Hz, H-6a^b), 4.59 (dd, 1 H, $J_{5,6a}$ = 6.4 Hz, J_{gem} = 11.0 Hz, H-6a^f), 4.53 (d, 1 H, CH_2Ar), 4.43–4.39 (m, 2 H, H-6b^b, H-3^f), 4.36–4.28 (m, 2 H, H-3^b, H-2^{Cer}), 4.35 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^d), 4.24 (near dd, 1 H, H-9^{Neu}), 4.17–4.13 (m, 2 H, H-5^a, H-9^{Neu}), 4.10-3.66 (m, 20 H, H-2^a, H-4^b, H-5^b, H-5^c, H-6^c, H-3^d, H-4^d, H-5^e, H-6a^e, H-6b^e, H-4^f,

H-5^{f}, H-6b^{f}, H-5^{g}, H-6^{g}, H-9^{Neu}, H-9^{Neu}, H-1a^{Cer}, CH_2Ar), 3.81–3.73 (2 s, 6 H, 2 COOMe), 3.69–3.56 (2 s, 6 H, 2 OMe), 3.51 (d, 1 H, J_{gem} = 9.8 Hz, H-6a^{d}), 3.48-3.41 (m, 2 H, H-5^{d}, H-1b^{Cer}), 3.39-3.32 (m, 2 H, H-6a^{a}, H-6b^{d}), 3.26 (m, 1 H, H-6b^{a}), 3.15 (m, 1 H, H-2^{e}), 2.76 (dd, 1 H, $J_{3eq,4}$ = 4.0 Hz, J_{gem} = 12.8 Hz, H-3*eq*^{Neu}), 2.36 (dd, 1 H, $J_{3eq,4}$ = 4.5 Hz, J_{gem} = 13.2 Hz, H-3*eq*^{Neu}), 2.17-1.44 (15 s, 45 H, 15 Ac), 1.96-1.90 (m, 3 H, H-6a^{Cer}, H-6b^{Cer}, H-3*ax*^{Neu}), 1.73-1.65 (m, 3 H, H-3*ax*^{Neu}, NHCOCH_2^{Cer}), 1.33-1.14 (m, 50 H, 25 CH_2^{Cer}), 1.06 (m, 2 H, NHCOCH$_2$CH_2^{Cer}), 0.89-0.83 (m, 6 H, 2 Me^{Cer}); 13C NMR (125 MHz, $CDCl_3$) δ 172.7, 172.0, 171.2, 170.9, 170.8, 170.7, 170.6, 170.5, 170.5, 170.4, 170.3, 170.1, 169.8, 168.4, 168.2, 167.0, 166.7, 165.6, 165.3, 165.0, 164.4, 159.0, 158.9, 157.7, 157.4, 137.1, 133.9, 133.5, 133.4, 133.3, 133.0, 132.8, 130.5, 130.4, 130.4, 130.3, 130.2, 130.1, 130.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.1, 128.9, 128.6, 128.4, 128.3, 124.9, 117.0, 114.7, 113.6, 113.6, 101.2, 100.9, 100.8, 100.7, 98.6, 98.5, 97.7, 80.9, 76.8, 75.9, 75.2, 74.7, 74.0, 73.5, 73.4, 72.9, 72.8, 72.3, 72.0, 71.9, 71.5, 71.1, 70.6, 70.3, 70.1, 69.6, 69.1, 69.0, 68.9, 67.5, 67.2, 67.2, 66.7, 66.5, 64.3, 63.5, 63.2, 62.6, 62.2, 55.3, 55.2, 55.0, 53.3, 52.7, 52.4, 50.6, 49.3, 39.9, 36.9, 36.5, 36.4, 32.4, 32.0, 30.1, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 29.1, 25.6, 23.4, 23.3, 23.2, 23.2, 22.8, 21.4, 21.3, 20.9, 20.9, 20.8, 20.8, 20.5, 20.3, 20.2, 14.2; HRMS (ESI) *m*/*z*: found $[M + 2Na]^{2+}$ 1819.7374, $C_{183}H_{231}F_3N_6O_{64}$ calcd for $[M + 2Na]^{2+}$ 1819.7371.

(2-Acetamido-3,4-di-O-benzoyl-2,6-dideoxy-6-trifluoroacetamido-β-D-galacto-pyranosyl)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-O-benzoyl-3,6-di-O-p-methoxybenzyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (61)

To a solution of **5** (74.7 mg, 63.5 μmol) and **7** (48.4 mg, 41.9 μmol) in CH_2Cl_2 (2.2 mL) were added 4 Å molecular sieves (AW-300, 146 mg) at room temperature under an Ar atmosphere, and the resulting mixture was stirred for 1 h. TMSOTf (0.8 μL, 4 μmol) was then added to the mixture at 0 °C. After stirring for 75 min at 0 °C, as the reaction was monitored by TLC (toluene/acetone = 4:1), triethylamine was added. The reaction mixture was filtered through a Celite pad and the pad was washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, washed with saturated Na_2CO_3 and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel, using toluene/acetone (7:2) to afford **61** (67.6 mg, 75%): $[\alpha]_D$ +43.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07-6.56 (m, 44 H, NH-6^{a}, Ar), 5.79 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 6.8$ Hz, $J_{4,5} = 15.2$ Hz, H-5^{Cer}), 5.71-5.68 (m, 2 H, H-2^{b}, NH-2^{Cer}), 5.55 (d, 1 H, $J_{3,4} = 3.0$ Hz, H-4^{a}), 5.50 (t, 1 H,

$J_{2,3} = J_{3,4} = 7.1$ Hz, H-3^{Cer}), 5.42 (dd, 1 H, H-4^{Cer}), 5.33 (dd, 1 H, $J_{3,4} = 3.3$ Hz, $J_{2,3} = 11.3$ Hz, H-3^{a}), 5.30 (d, 1 H, $J_{2,NH} = 5.3$ Hz, NH-2^{a}), 5.23-5.17 (m, 2 H, H-3^{b}, H-2^{d}), 4.94 (d, 1 H, CH_2Ar), 4.90 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{b}), 4.83 (m, 1 H, H-6a^{b}), 4.82 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{a}), 4.65–4.54 (2 d, 2 H, CH_2Ar), 4.50 (d, 1 H, $J_{3,4} = 2.2$ Hz, H-4^{b}), 4.45 (m, 1 H, H-2^{a}), 4.38 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{d}), 4.37 (m, 1 H, H-2^{Cer}), 4.21-4.14 (m, 3 H, H-6b^{b}, H-4^{d}, CH_2Ar), 4.04 (dd, 1 H, $J_{1a,2} = 3.0$ Hz, $J_{gem} = 9.9$ Hz, H-1a^{Cer}), 3.84 (t, 1 H, $J_{5,6a} = J_{5,6b} = 5.8$ Hz, H-5^{b}), 3.80-3.76 (m, 2 H, H-5^{a}, H-3^{d}), 3.72 (s, 3 H, OMe), 3.64 (dd, 1 H, $J_{5,6a} = 3.3$ Hz, $J_{gem} = 11.2$ Hz, H-6a^{d}), 3.50 (dd, 1 H, $J_{1b,2} = 3.9$ Hz, H-1b^{Cer}), 3.46 (s, 3 H, OMe), 3.46–3.35 (m, 2 H, H-6a^{a}, H-6b^{d}), 3.30–3.24 (m, 2 H, H-6a^{b}, H-5^{d}), 2.01 (s, 3 H, Ac), 1.98–1.94 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.73 (t, 2 H, NHCOCH_2^{Cer}), 1.43–1.07 (m, 52 H, 26 CH_2^{Cer}), 0.89–0.86 (m, 6 H, 2 Me^{Cer}); 13C NMR (125 MHz, $CDCl_3$) δ 172.8, 170.5, 166.9, 166.6, 166.1, 165.9, 165.4, 165.3, 165.3, 159.6, 158.9, 158.0, 157.7, 137.3, 134.2, 133.9, 133.5, 133.3, 132.9, 130.7, 130.5, 130.2, 130.2, 129.9, 129.8, 129.8, 129.7, 129.6, 129.3, 129.0, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 125.0, 117.0, 114.7, 114.1, 113.5, 101.3, 101.2, 100.4, 79.8, 75.2, 74.7, 74.6, 74.6, 73.7, 73.5, 73.4, 72.4, 71.2, 70.6, 70.1, 68.9, 67.5, 64.1, 55.3, 54.9, 51.9, 50.6, 40.1, 36.5, 32.4, 32.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.1, 25.6, 23.4, 22.8, 14.2; HRMS (ESI) *m/z*: found $[M + Na]^+$ 2179.0145, $C_{123}H_{148}F_3N_3O_{27}$ calcd for $[M + Na]^+$ 2179.0145.

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