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Systematic synthesis and MAG-binding activity of novel sulfated GM1b analogues as mimics of Chol-1 (α-series) gangliosides: highly active ligands for neural siglecs☆

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Abstract

Systematic synthesis and myelin-associated glycoprotein (MAG)-binding activity of novel sulfated GM1b analogues structurally related to Chol-1 (α-series) gangliosides, high-affinity ligands for neural siglecs, are described. The suitably protected gangliotriose derivatives, 2-(trimethylsilyl)ethyl 2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside and 2-(trimethylsilyl)ethyl 2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside were each glycosylated with α-NeuAc-(2→3)-galactose donor to give the corresponding pentasaccharides in 94% (β1,3 glycoside only) and 90% (β1,3:β1,4 = 2:1), respectively. After proper manipulation of the protecting groups, the pentasaccharides were converted into three novel sulfated GM1b gangliosides by the successive introduction of the ceramide and sulfo groups, followed by complete deprotection. Among the synthetic gangliosides, GSC-338 (II^3III^6-disulfate of *iso*-GM1b) was surprisingly found to be the most potent MAG binding structure tested to date.

Keywords: Sulfated GM1b gangliosides; Neural siglecs; Sialic acid

1. Introduction

Siglecs[2,3] (sialic acid-binding Ig-like lectins), such as the macrophage receptor sialoadhesin (Siglec-1), the B-cell antigen (CD22, Siglec-2), the marker of myelomonocytic cells (CD33, Siglec-3), the myelin-associated glycoprotein (MAG, Siglec-4a), and its avian homologue, Schwann cell myelin protein (SMP, Siglec-4b), are a family of sialic acid-dependent adhesion molecules belong to the immunoglobulin superfamily, previously termed sialoadhesins or I-type lectins. These molecules contain an N-terminal V-type immunoglobulin (Ig) domain in common, which is required for sialic acid binding, a variable number of C-type Ig domains, a single transmembrane domain, and a short cytoplasmic tail. A nervous system siglec, MAG is a quantitatively minor component of central and peripheral nervous system myelin, and has been hypothesized to be implicated in myelin-axon interactions.[4] In particular, MAG has been shown to be a major neurite outgrowth inhibitor.[5–7]

In the course of study on sialic acid specificity of siglec binding with a series of gangliosides, we have demonstrated,[8–10] in the first time that a cholinergic neuron-specific polysialoganglioside GQ1bα[11,12] (Fig. 1) is a remarkably high affinity ligand for the neural siglecs, MAG and SMP. A family of α-series gangliosides, GT1aα[13,14] and GD1α[15] (Fig. 1), have also been found to be high-affinity ligands for the neural siglecs. The hierarchy of binding strength of a series of gang-

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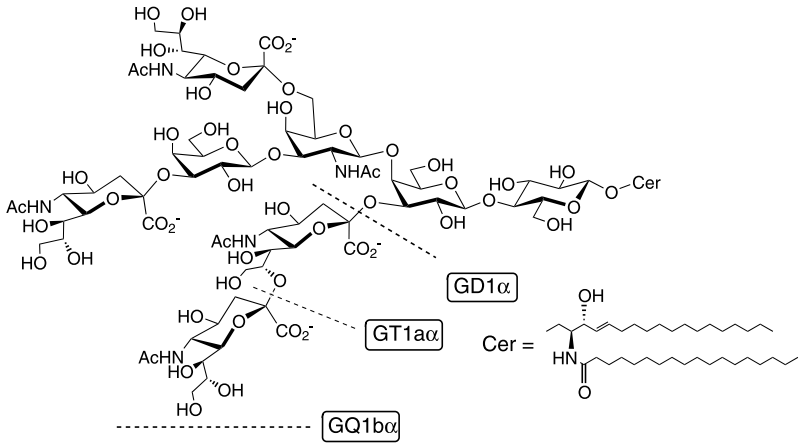


Fig. 1. Chol-1 (α-series) gangliosides.

liosides with MAG were GQ1bα > GT1aα, GD1α > GT1b, GD1a > GM3, GM4 (GQ1b, GD1b, GM1 and GD3 failed to support binding). These results indicate that not only the terminal NeuAcα(2→3)Gal structure, which is essential for MAG binding,[9] but also the internal sialic acids α2,6-linked to GalNAc (III^6) and α2,3-linked to another Gal (II^3) play important roles for displaying enhanced avidity for neural siglec-binding. Our recent study testing the role of the exocyclic glycerol chain hydroxyls on the α2,6-NeuAc residue of GD1α using its deoxy NeuAc analogues[15] demonstrated that each hydroxyl on the exocyclic glycerol chain is not strictly required for enhanced siglec recognition. In fact, all of the GD1α analogues tested could support the adhesion as strong as the parent GD1α, raising the possibility that the internal sialic acid may be replaced by other anionic substituents as demonstrated in the study on selectin ligands.[16–18] To corroborate this hypothesis we undertook, selectively, for the first time, the total synthesis of the novel III^6-sulfate [**B**; GSC-335] of ganglioside GM1b [**A**] (a GD1α analogue), the corresponding II^3III^6-disulfate [**C**; GSC-454] (a GT1aα analogue), and a novel series (we call tentatively *iso*-ganglio-series) ganglioside [**D**; GSC-338] (Fig. 2), and examined their bindings by MAG.

2. Results and discussion

The most important problems in the synthesis of the target compounds [**B**–**C**] are: (a) selective protection of the II^3 and III^6 hydroxyl groups that undergo regioselective sulfations; (b) efficient construction of the terminal NeuAcα(2→3)Gal structure linked to O-3 or O-4 of the GalNAc residue; and (c) facile introduction of the ceramide moiety. For the selective protection of the II^3 and III^6 hydroxyl groups, we selected the suitably protected ganglio-triose derivatives **5** and **8**[19] as the key intermediates (Scheme 1). Coupling of the galactosamine donor **2** with the benyl protected lactose acceptor **3**[20] gave **4** in 98% yield, which was then converted to **5**. Treatment of **5** or **8** with levulinic anhydride in pyridine in the presence of 4-dimethylaminopyridine (DMAP) gave the III^6-levulinoyl (**6**) and II^3III^6-dilevulinoyl (**9**) derivatives in 92 and 90% yields, respectively. The 3,4-*O*-isopropylidene group in **6** or **9** was then cleaved by treatment with 80% acetic acid at 40 °C to afford the desired glycosyl acceptors **7** and **10** in high yields. Dimethyl(methylthio)sulfonium triflate (DMTST)-promoted glycosylation[21] of **7** or **10** with a sialyl-α(2→3)-galactose donor **11**[22] was achieved (Scheme 1) β-stereoselectively to afford the protected pentasaccharide **12** (94%), and **13** (61%) accompanied by **14** (29%), respectively. Although it was expected that the β(1→3)-linked product (**13**) could be formed with high regioselectivity at O-3 of Gal, the β(1→4)-linked isomer **14** was obtained in about 30% yield. It may result from the steric hindrance and electronic effect of the levulinoyl group at II^3. The structures of **12**–**14**, were determined

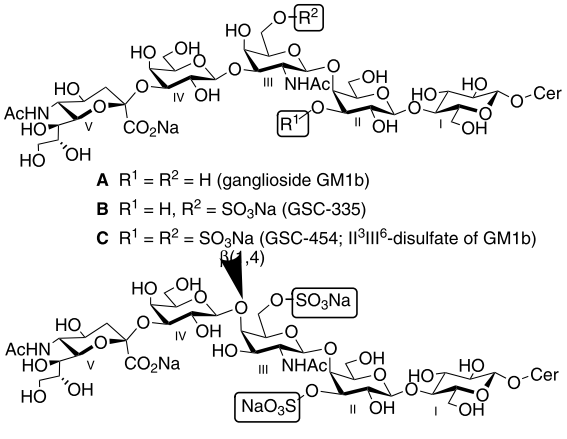
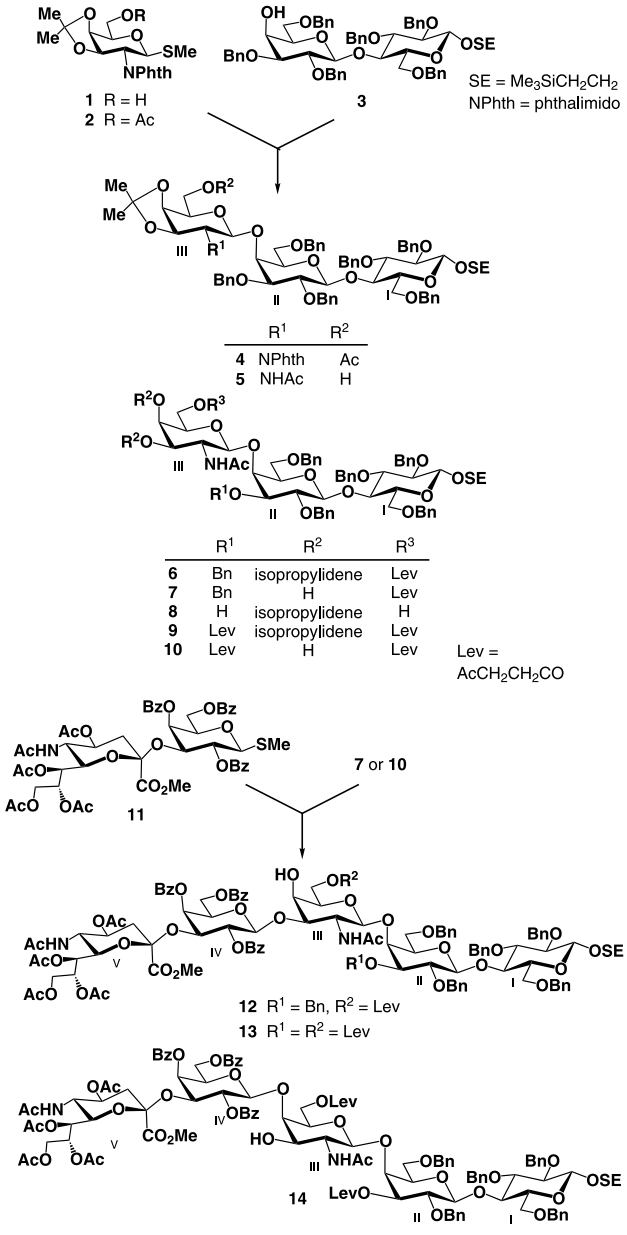


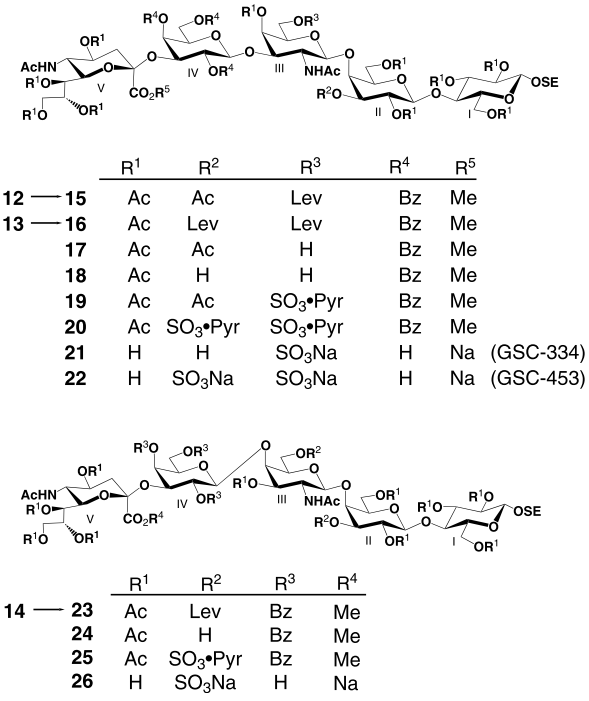
Fig. 2. Structure of ganglioside GM1b (**A**) and novel sulfated GM1b analogues (**B**–**D**) as the mimics of Chol-1 (α-series) gangliosides.



Scheme 1.

by the ^{1}H NMR analysis of the peracylated derivatives **15**, **16**, and **23**, respectively.

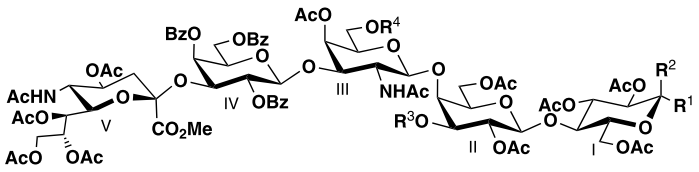
Hydrogenolytic removal of the benzyl groups in **12**, **13** and **14**, followed by acetylation, gave **15**, **16**, and **23**, respectively. The levulinoyl groups in **15**, **16**, and **23** were selectively removed by treatment with hydrazine monoacetate in ethanol to give **17**, **18**, and **24**, which were then sulfated with a sulfur trioxide–pyridine complex in DMF, yielding **19**, **20**, and **25**. Removal of all protective groups under basic conditions furnished the corresponding sulfated GM1b pentasaccharides **21** (GSC-334), **22** (GSC-453) and **26** in which the reducing end is protected by the 2-(trimethylsilyl)ethyl (SE) group (Scheme 2).



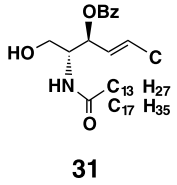
Scheme 2.

Selective cleavage of the 2-(trimethylsilyl)ethyl group in **15**, **16**, **23**, and treatment[23] of the resulting **27**, **28**, and **38** with trichloroacetonitrile and DBU, afforded a series of imidate derivatives **29**, **30**, and **39** (Scheme 3). The direct glycosylation of the ceramide derivative **31**[24] with **29**, **30**, and **39** was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and 4Å molecular sieves (AW-300) in dichloromethane to yield the protected gangliosides **32**, **33**, and **40** in about 40% yields. Most of the unreacted glycosyl donor (1-OH form) and acceptor **31** were recovered. This result is comparable to that obtained by an efficient β-glycosidation procedure using the 2-*O*-pivaloyl group as an auxiliary.[25] Therefore, the yields of ~40% is satisfactory considering the additional steps in the azidosphingosine procedure, which has been employed for various ganglioside synthesis.[26,27] The levulinoyl groups in **32**, **33**, and **40** were selectively removed by treatment with hydrazine monoacetate in ethanol to give **34**, **35**, and **41**, respectively, which were then sulfated with a sulfur trioxide–pyridine complex in DMF. Finally, removal of all protective groups under basic conditions furnished the target compounds **B**–**D** in high yields (Scheme 3 and Fig. 2).

In binding experiments with MAG-transfected COS cells[28] for the immobilized synthetic gangliosides, it was demonstrated that the compound **D** (GSC-338; $II^{3}III^{6}$-disulfo of *iso*-GM1b) was surprisingly found to be the most potent siglec-binding structure tested to date (Fig. 3). It was 10-fold more potent than GT1aα in supporting MAG and SMP binding, and fourfold more potent



	R^1	R^2	R^3	R^4
15 → 27	H , OH		Ac	Lev
16 → 28	H , OH		Lev	Lev
29	H	X	Ac	Lev
30	H	X	Lev	Lev
32	Y	H	Ac	Lev
33	Y	H	Lev	Lev
34	Y	H	Ac	H
35	Y	H	H	H
36	Y	H	Ac	SO_3•Pyr → B (GSC-335)
37	Y	H	SO_3•Pyr	SO_3•Pyr → C (GSC-454)



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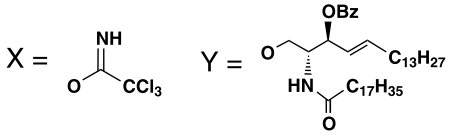

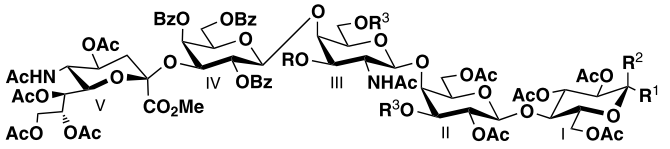

	R^1	R^2	R^3
23 → 38	H , OH		Lev
39	H	X	Lev
40	Y	H	Lev
41	Y	H	H
42	Y	H	SO_3•Pyr → D (GSC-338)

Scheme 3.

than GQ1bα, which was the highest affinity ligand for MAG so far.[8–10] The III^6-monosulfate **B** (GSC-335) showed almost the same potency as GD1α or GT1aα, and it was fourfold more potent than GM1b. Other sulfated glycolipids, including sulfatide and sulfoglucuronyl glycolipids, do not support adhesion of MAG-transfected COS cells.[28]

The validity of the MAG-Fc binding assay[29] was demonstrated using 10 gangliosides that had been tested previously for MAG binding using MAG-transfected COS cells.[28] The rank-order and relative affinity of the different gangliosides were the same in the two assays (Fig. 4). The absolute binding affinity of each ganglioside was modestly lower in the MAG-Fc assay compared to the cell binding assay, most likely due to the lower degree of multivalency. Nevertheless, enhanced MAG recognition of the α-series gangliosides was clear in both assays.

The data in Fig. 5 demonstrate very highly enhanced MAG-Fc binding to GSC-338. Fitting the data to a rectangular hyperbola reveals apparent K_D values of 38 pmol/well for GQ1bα, 7.4 pmol/well for GSC-454 (compound **C**) and 3.1 pmol/well for GSC-338, suggesting that GSC-338 is over 12-fold more potent than GQ1bα, itself a high affinity MAG ligand. Whereas GQ1bα and GSC-338 supported similar maximum binding (AU max = 2.5 under these conditions), GSC-454 supported only a quarter of that (AU max = 0.64). The reason for the lowered maximum binding to GSC-454 is not known, but may reflect a different physical

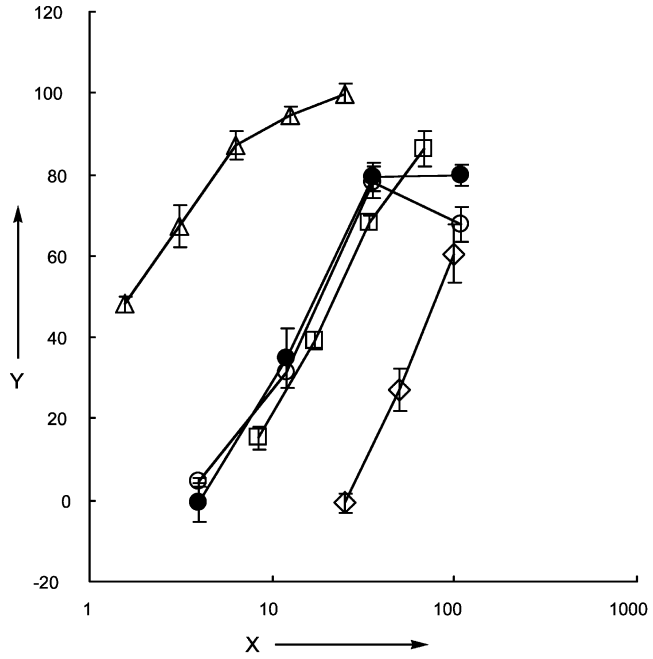

Fig. 3. MAG-mediated cell adhesion to the newly synthesized III^6-sulfate of GM1b (GSC-335) and II^{3}III^6-disulfate of *iso*-GM1b (GSC-338) analogues and genuine gangliosides. X: ganglioside added (pmol per well), Y: adhesion of MAG-transfected COS cells (% of cells added). △: GSC-338, ●: GT1aα, ○: GD1α, □: GSC-335, ◇: GM1b. For analytical procedure see ref. [8,28].

distribution of the ganglioside on the microwell. However, the apparent K_D of GSC-454 (7.4 pmol/well) reveals high affinity, although still >twofold less avid than for GSC-338.

These results indicate that the internal sialic acids α2,6-linked to GalNAc (III^6) and α2,3-linked to Gal (II^3) could be substituted by the sulfate groups to retain or greatly enhance the siglec-mediated cell adhesion. Systematic investigation for the specific counter-receptor of siglecs may open new perspectives on development of novel therapeutic agents.

3. Experimental

3.1. Synthesis

3.1.1. General methods. Optical rotations were determined with a HORIBA SEPA-300 polarimeter at 25 °C, and IR spectra were recorded with a JASCO IRA-100 spectrophotometer. ^{1}H NMR spectra were recorded at 400 or 500 MHz with Varian UNITY Inova-400- and 500-MHz spectrometers, respectively. Preparative chromatography was performed on silica gel (Fuji Silysia Co., 300 mesh) with the solvent systems specified. Concentrations were conducted in vacuo.

3.1.2. Methyl 6-*O*-acetyl-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-1-thio-β-D-galactopyranoside (2). To a stirred solution of methyl 2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-1-thio-β-D-galactopyranoside[30] (**1**; 310 mg, 0.82 mmol) in pyridine (3 mL) was added acetic anhydride (1.5 mL) at 0 °C. The mixture was stirred for 3 h at room temperature (rt), and MeOH (1.5 mL) was added. The solution was concentrated to a syrup which was extracted with chloroform. The extract was succes-

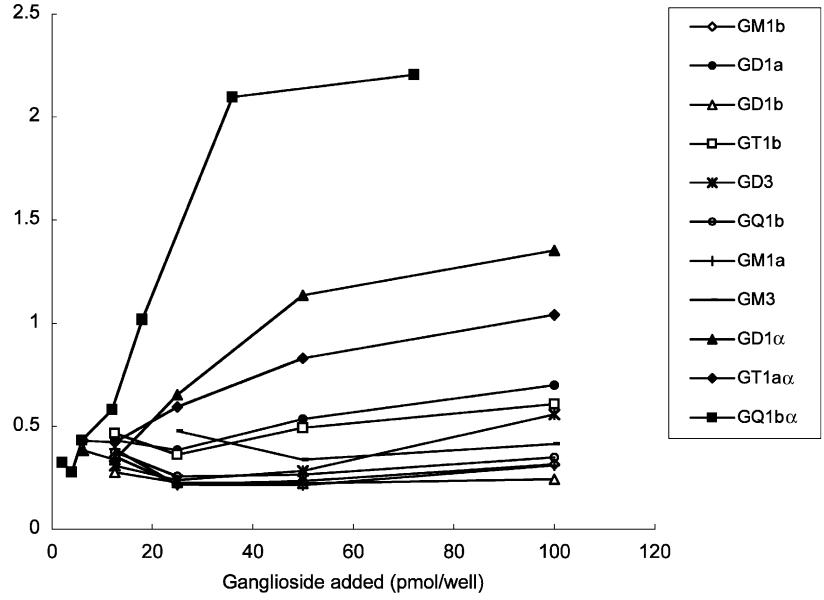

Fig. 4. MAG-Fc binding to various natural and synthetic gangliosides. Natural gangliosides GM3, GD3, GM1, GD1a, GD1b, GT1b, and GQ1b and synthetic gangliosides GD1α, GT1aα and GQ1bα were adsorbed on microwells at the indicated input concentrations (pmol/well) and MAG-Fc binding was determined as described in Section 3, and are plotted as the absorbance at 405 nm (y-axis) vs. the pmol ganglioside added per well. Data are presented as the mean ± S.E.M. for quintuplicate determinations.

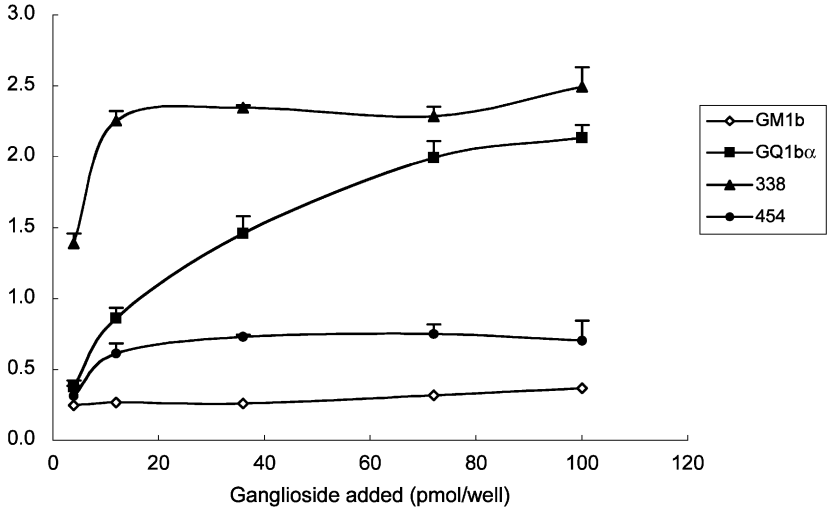

Fig. 5. MAG-Fc binding to GSC-338 (compound D), GSC-454 (compound C), GQ1bα, and GM1 (background). Gangliosides were adsorbed on microwell plates at the indicated input concentrations (pmol/well) and MAG-Fc binding was determined as described in Section 3, and are plotted as the absorbance at 405 nm (y-axis) vs. the pmol ganglioside added per well. Data are presented as the mean ± S.E.M. for quadruplicate determinations. Data are representative of three separate experiments. Data points for each gangliosides were fitted to a rectangular hyperbola (solid lines) to calculate apparent K_D and maximum binding (see text).

sively washed with 2 M HCl, water, M Na_2CO_3, and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:2 ethyl acetate–hexane) of the residue on silica gel gave **2** (344 mg, quant) as an amorphous mass. $[\alpha]_D$ +64.1° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.33 and 1.64 (2 s, 6 H, Me_2C), 2.12 and 2.15 (2 s, 6 H, AcO and SMe), 4.20 (m, 1 H, H-5), 4.27 (dd, 1 H, $J_{3,4}$ 5.0, $J_{4,5}$ 2.2 Hz, H-4), 3.37 (dd, 1 H, $J_{1,2}$ 10.5, $J_{2,3}$ 8.9 Hz, H-2), 4.40 (dd, 1 H, J_{gem} 11.3, $J_{5,6}$ 4.5 Hz, H-6), 4.42 (dd, 1 H, J_{gem} 11.3, $J_{5,6}$ 6.7 Hz, H-6′), 4.84 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 5.0 Hz, H-3), 5.08 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), and 7.72–7.85 (m, 4 H, Ph). Anal. Calcd for $C_{20}H_{23}NO_7Si$ (421.47): C, 57.00; H, 5.50; N, 3.32. Found: C, 56.99; H, 5.41; N, 3.22.

3.1.3. 2-(Trimethylsilyl)ethyl 6-*O*-acetyl-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4). To a solution of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[20] (**3**; 583 mg, 0.59 mmol) and **2** (300 mg, 0.71 mmol) in dichloromethane (7 mL) was added 4Å molecular sieves (1.0 g), and the mixture was stirred for 10 h at rt, then cooled to −15 °C. To the mixture were added *N*-iodosuccinimide (NIS; 240 mg, 1.07 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 14 μL, 0.11 mmol), and the stirring was continued for 2 h at −15 °C. After completion of the reaction, the precipitate was filtered off and washed with chloroform. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3 and M $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated. Column chromatography (1:2 ethyl acetate–hexane) of the residue on silica gel gave **4** (795 mg, 98%) as an amorphous mass. $[\alpha]_D$ +24.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 1.35 and 1.52 (2 s, 6 H, Me_2C), 2.03 (s, 3 H, AcO), 3.00 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.5 Hz, H-2^{II}), 3.11 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 2.7 Hz, H-3^{II}), 3.27 (m, 1 H, H-5^{I}), 3.31 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^{I}), 3.53 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 3.68 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{II}), 3.78 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 4.14 (m, 1 H, H-5^{III}), 4.18 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{II}), 4.29 (m, 1 H, H-4^{III}), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.39 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 6.9 Hz, H-6^{III}), 4.43 (t, 1 H, $J_{1,2} = J_{2,3}$ 8.9 Hz, H-2^{III}), 4.51 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 5.3 Hz, H-6′^{III}), 5.10 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 5.3 Hz, H-3^{III}), 5.18 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1^{III}), and 6.95–7.86 (m, 34 H, 7 Ph). Anal. Calcd for $C_{78}H_{89}NO_{18}Si$ (1356.65): C, 69.06; H, 6.61; N, 1.03. Found: C, 68.77; H, 6.60; N, 0.92.

3.1.4. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). A solution of **4** (795 mg, 0.59 mmol) in ethanol (20 mL) and hydrazine monohydrate (0.70 mL) was heated for 5 h under reflux. After cooling, insolubles were filtered off and washed with EtOH. The filtrate and washings were combined and evaporated to dryness, the residue was treated with acetic anhydride (1.5 mL) and MeOH (20 mL) for 8 h at rt, and the solution was concentrated. Column chromatography (50:1 chloroform–methanol) of the residue on silica gel gave **5** (710 mg, 98%) as an amorphous mass. $[\alpha]_D$ +32.5° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.04

(m, 2 H, $Me_3SiCH_2CH_2$), 1.31 and 1.50 (2 s, 6 H, Me_2C), 1.71 (s, 3 H, AcN), 3.28 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 2.5 Hz, H-3^{II}), 3.37 (m, 1 H, H-5^{I}), 3.39 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.3 Hz, H-2^{I}), 3.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^{II}), 3.52 (m, 1 H, H-2^{III}), 3.55 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3^{I}), 3.70 (m, 1 H, H-5^{II}), 3.83 (m, 1 H, H-5^{III}), 3.89 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4^{I}), 3.91 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{II}), 4.06 (dd, 1 H, $J_{3,4}$ 5.3, $J_{4,5}$ 2.1 Hz, H-4^{III}), 4.32 (dd, 1 H, $J_{2,3}$ 8.5, $J_{3,4}$ 5.3 Hz, H-3^{III}), 4.36 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.38 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{I}), 4.65 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}), 5.53 (d, 1 H, $J_{2,NH}$ 7.3 Hz, NH), and 7.20–7.43 (m, 30 H, 6 Ph). Anal. Calcd for $C_{70}H_{87}NO_{16}Si$ (1226.54): C, 68.55; H, 7.15; N, 1.14. Found: C, 68.39; H, 6.95; N, 1.06.

3.1.5. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). To a solution of **5** (1.5 g, 1.22 mmol) in pyridine (5 mL) and dichloromethane (10 mL) were added levulinic anhydride (0.81 g, 3.66 mmol) and 4-dimethylaminopyridine (80 mg, 0.61 mmol). The mixture was stirred for 4 h at rt, and MeOH (5 mL) was added. The solution was concentrated, then extracted with chloroform. The extract was succesively washed with 2 M HCl, water, M Na_2CO_3, and water, dried (Na_2SO_4) and concentrated. Column chromatography (50:1 chloroform–methanol) of the residue on silica gel gave **6** (1.50 g, 92%) as an amorphous mass. $[\alpha]_D$ +33.5° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.03 (m, 2 H, $Me_3SiCH_2CH_2$), 1.33 and 1.54 (2 s, 6 H, Me_2C), 1.72 (s, 3 H, AcN), 2.16 (s, 3 H, $CH_3COCH_2CH_2$), 2.44–2.77 (m, 4 H, $CH_3COCH_2CH_2$), 3.36 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.2 Hz, H-2^{I}), 3.41 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 3.4 Hz, H-3^{II}), 3.51 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.6 Hz, H-2^{II}), 3.54 (t, 1 H, $J_{1,2} = J_{2,3}$ 8.7 Hz, H-2^{III}), 3.56 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3^{I}), 3.92 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{I}), 3.95 (m, 1 H, H-5^{III}), 4.08 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{II}), 4.09 (dd, 1 H, $J_{3,4}$ 5.3, $J_{4,5}$ 2.3 Hz, H-4^{III}), 4.18 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 5.3 Hz, H-3^{III}), 4.33 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 7.1 Hz, H-6^{III}), 4.38 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.42 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{II}), 4.44 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 5.2 Hz, H-6′^{III}), 4.78 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}), 5.53 (d, 1 H, $J_{2,NH}$ 7.3 Hz, NH), and 7.19–7.47 (m, 30 H, 6 Ph). Anal. Calcd for $C_{75}H_{93}NO_{18}Si$ (1324.64): C, 68.01; H, 7.08; N, 1.06. Found: C, 67.72; H, 6.98; N, 1.04.

3.1.6. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). A solution of **6** (1.5 g, 1.13 mmol) in 80% aq acetic acid (15 mL) was heated, with stirring, for 48 h at 40 °C, then concentrated. Column chromatography (50:1 chloroform–methanol) of the residue on silica gel gave **7** (1.20 g, 83%) as an amorphous mass. $[\alpha]_D$ +17.4° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.03 (m, 2 H, $Me_3SiCH_2CH_2$), 1.51 (s, 3 H, AcN), 2.17 (s, 3 H, $CH_3COCH_2CH_2$), 2.40–2.80 (m, 4 H, $CH_3COCH_2CH_2$), 3.37 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^{I}), 3.38 (m, 1 H, H-5^{I}), 3.44 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 3.2 Hz, H-3^{II}), 3.50 (m, 1 H, H-2^{III}), 3.56 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 3.66 (m, 1 H, H-5^{III}), 3.74 (m, 1 H, H-2^{II}), 3.83 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{II}), 3.94 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 4.11 (m, 1 H, H-4^{III}), 4.34 (dd, 1 H, J_{gem} 11.7, $J_{5,6}$ 4.8 Hz, H-6^{III}), 4.38 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.46 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1^{III}), 4.52 (dd, 1 H, J_{gem} 11.7, $J_{5,6}$ 7.7 Hz, H-6′^{III}), and 7.21–7.47 (m, 30 H, 6 Ph). Anal. Calcd for $C_{72}H_{89}N_3O_{18}Si$ (1284.58): C, 67.32; H, 6.98; N, 1.09. Found: C, 67.22; H, 6.96; N, 0.87.

3.1.7. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-levulinoyl-β-D-galacto-pyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). To a solution of 2-(trimethylsilyl)ethyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[14] (**8**; 1.0g, 0.88 mmol) in pyridine (10 mL) was added levulinic anhydride (0.75 g, 3.52 mmol) and 4-dimethylaminopyridine (54 mg, 0.44 mmol), then workup as described for **6**. Column chromatography (ethyl acetate) of the residue on silica gel gave **9** (1.05 g, 90%) as an amorphous mass. $[\alpha]_D$ +31.8° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.04 (m, 2 H, $Me_3SiCH_2CH_2$), 1.36 and 1.56 (2 s, 6 H, Me_2C), 1.95 (s, 3 H, AcN), 2.15 and 2.22 (2 s, 6 H, 2 $CH_3COCH_2CH_2$), 2.37–2.76 (m, 8 H, 2 $CH_3COCH_2CH_2$), 3.02 (m, 1 H, H-2^{III}), 3.34 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.2 Hz, H-2^{I}), 3.40 (m, 1 H, H-5^{I}), 3.59 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3^{I}), 3.64 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{II}), 3.91 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{I}), 4.00 (m, 1 H, H-5^{III}), 4.11 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4^{II}), 4.16 (dd, 1 H, $J_{3,4}$ 5.0, $J_{4,5}$ 1.9 Hz, H-4^{III}), 4.33 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 7.6 Hz, H-6^{III}), 4.40 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{I}), 4.42 (dd, 1 H, J_{gem} 11.4, $J_{5,6}$ 6.6 Hz, H-6′^{III}), 4.46 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.57 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}), 4.59 (dd, 1 H, $J_{2,3}$ 8.5, $J_{3,4}$ 5.0 Hz, H-3^{III}), 4.63 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.0 Hz, H-3^{II}), and 7.12–7.53 (m, 25 H, 5 Ph). Anal. Calcd for $C_{73}H_{93}NO_{20}Si$ (1332.62): C, 65.80; H, 7.03; N, 1.05. Found: C, 65.63; H, 6.79; N, 0.85.

3.1.8. 2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10). A solution of **9** (1.0 g, 0.75 mmol) in 80% aq acetic acid (15 mL) was heated with stirring for 24 h at 40 °C, then concentrated. Column chromatography (40:1 chloroform–methanol) of the residue on silica gel gave **10** (900 mg, 93%) as an

amorphous mass. $[\alpha]_D$ +21.2° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.03 (m, 2 H, $Me_3SiCH_2CH_2$), 1.93 (s, 3 H, AcN), 2.14 and 2.19 (2 s, 6 H, 2 $CH_3COCH_2CH_2$), 2.43–2.73 (m, 8 H, 2 $CH_3COCH_2CH_2$), 3.04 (m, 1 H, H-2^{III}), 3.37 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2^{I}), 3.57 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 10.3 Hz, H-2^{II}), 3.59 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3^{I}), 3.73 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{III}), 3.74 (m, 1 H, H-5^{III}), 3.87 (m, 1 H, H-3^{III}), 3.96 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4^{I}), 4.04 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{II}), 4.27 (dd, 1 H, J_{gem} 11.2, $J_{5,6}$ 6.9 Hz, H-6^{III}), 4.39 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.45 (dd, 1 H, J_{gem} 11.2, $J_{5,6}$ 5.7 Hz, H-$6'^{III}$), 4.48 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{II}), 4.64 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.84 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.2 Hz, H-3^{II}), and 7.20–7.47 (m, 25 H, 5 Ph). Anal. Calcd for $C_{70}H_{89}NO_{20}Si$ (1292.56): C, 65.05; H, 6.94; N, 1.08. Found: C, 64.77; H, 6.74; N, 1.00.

3.1.9. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12). To a solution of **7** (938 mg, 0.73 mmol) and methyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (**11**; 1.27 g, 1.24 mmol) in dichloromethane (15 mL) were added 4Å molecular sieves (3.0 g), and the mixture was stirred for 6 h at rt, then cooled to 0 °C. Dimethyl(methylthio)sulfonium triflate (DMTST; 1.12 g, 2.48 mmol) was added, and the mixture was stirred for 48 h at 7 °C, then filtered. The precipitates were removed by filtration and washed thoroughly with chloroform. The combined filtrate and washings was successively washed with M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 chloroform–methanol) of the residue on silica gel gave **12** (1.55 g, 94%) as an amorphous mass. $[\alpha]_D$ +41.6° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 0.93, 1.52, 1.77, 1.90, 2.08, 2.12, and 2.20 (7 s, 21 H, 2 AcN, 4 AcO, and $CH_3COCH_2CH_2$), 1.60 (t, 1 H, $J_{gem} = J_{3ax,4}$ 11.2 Hz, H-$3^{V}ax$), 2.39–2.67 (m, 5 H, H-$3^{V}eq$ and $CH_3COCH_2CH_2$), 3.30 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 2.7 Hz, H-3^{II}), 3.34 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.0 Hz, H-2^{I}), 3.41 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.6 Hz, H-2^{II}), 3.46 (t, 1 H, H-2^{III}), 3.53 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3^{I}), 3.61 (dd, 1 H, $J_{5,6}$ 10.3, $J_{6,7}$ 2.7 Hz, H-6^{V}), 3.76 (q, 1 H, $J_{4,5}$ $J_{5,6}$ $J_{5,NH}$ 10.3 Hz, H-5^{V}), 3.85 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4^{I}), 3.85 (s, 3 H, MeO), 3.98 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{II}), 4.03 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 5.3 Hz, H-9^{V}), 4.11 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{III}), 4.29 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 2.5 Hz, H-$9'^{V}$), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.37 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.43 (dd, 1 H, $J_{2,3}$ 8.5, $J_{3,4}$ 2.5 Hz, H-3^{III}), 4.79 (m, 1 H, H-4^{V}), 4.87 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.97 (d, 1 H, $J_{5,NH}$ 10.3 Hz, NH), 5.00 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 5.04 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{III}), 5.21 (d, 1 H, $J_{2,NH}$ 7.3 Hz, NH), 5.24 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^{V}), 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.47 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.61 (m, 1 H, H-8^{V}), and 7.16–8.12 (m, 45 H, 9 Ph). Anal. Calcd for $C_{119}H_{138}N_2O_{38}Si$ (2232.48): C, 64.02; H, 6.23; N, 1.25. Found: C, 63.92; H, 5.99; N, 1.16.

3.1.10. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13). Glycosylation of **10** (300 mg, 0.23 mmol) with **11** (393 mg, 0.39 mmol) in dichloromethane (7 mL) in the presence of DMTST (414 mg, 0.98 mmol) and 4Å MS (1.0 g) for 24 h at 0 °C, then work up as described for **12**, gave **13** (317 mg, 61%), accompanied by position-isomer **14** (150 mg, 29%) as an amorphous mass. Compound **13**: $[\alpha]_D$ +41.0° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 1.11, 1.49, 1.76, 1.90, 2.07, 2.11, 2.19, and 2.20 (8 s, 24 H, 2 AcN, 4 AcO, and 2 $CH_3COCH_2CH_2$), 1.61 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-$3^{V}ax$), 2.31–2.68 (m, 9 H, H-$3^{V}eq$ and 2 $CH_3COCH_2CH_2$), 3.32 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2^{I}), 3.53 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.3 Hz, H-2^{II}), 3.56 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3^{I}), 3.61 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.7 Hz, H-6^{V}), 3.70 (m, 1 H, H-2^{III}), 3.75 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ 10.3 Hz, H-5^{V}), 3.85 (s, 3 H, MeO), 3.88 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{I}), 3.96 (dd, 1 H, J_{gem} 12.8, $J_{8,9}$ 5.9 Hz, H-9^{V}), 4.03 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{II}), 4.08 (m, 1 H, H-4^{III}), 4.25 (dd, 1 H, J_{gem} 12.8, $J_{8,9}$ 2.7 Hz, H-$9'^{V}$), 4.37 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.49 (m, 1 H, H-3^{III}), 4.65 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.7 Hz, H-3^{II}), 4.79 (m, 1 H, H-4^{V}), 4.86 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.97 (d, 1 H, $J_{5,NH}$ 10.3 Hz, NH), 5.02 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.34 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.48 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.52 (d, 1 H, $J_{2,NH}$ 7.8 Hz, NH), 5.59 (m, 1 H, H-8^{V}), and 7.15–8.13 (m, 45 H, 9 Ph). Anal. Calcd for $C_{117}H_{138}N_2O_{40}Si$ (2240.45): C, 62.72; H, 6.21; N, 1.25. Found: C, 62.72; H, 5.93; N, 1.02.

3.1.11. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (14). $[\alpha]_D$ +27.8° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.66 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz,

H-3^{V}ax), 1.90, 1.91, 2.06, 2.06, 2.07, 2.14, 2.16, and 2.19 (8 s, 24 H, 2 AcN, 4 AcO, and 2 CH_3COCH$_2$CH$_2$), 2.31–2.68 (m, 9 H, H-3^{V}eq and 2 CH$_3$COCH_2CH_2), 3.35 (m, 1 H, H-2^{I}), 3.44 (m, 1 H, H-2^{II}), 3.50 (m, 1 H, H-2^{III}), 3.55 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3^{I}), 3.81 (m, 1 H, H-5^{V}), 3.82 (s, 3 H, MeO), 3.86 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{I}), 4.10 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4^{II}), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.54 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.0 Hz, H-3^{II}), 4.80 (m, 1 H, H-4^{V}), 4.92 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 5.24 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.3 Hz, H-7^{V}), 5.40 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.51 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2^{IV}), 5.60 (m, 1 H, H-8^{V}), 6.03 (d, 1 H, $J_{2,NH}$ 8.2 Hz, NH), and 7.15–8.14 (m, 45 H, 9 Ph). Anal. Calcd for $C_{117}H_{138}N_2O_{40}Si$ (2240.45): C, 62.72; H, 6.21; N, 1.25. Found: C, 62.69; H, 5.92; N, 1.18.

3.1.12. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (15). A solution of **12** (1.14 g, 0.51 mmol) in ethanol (30 mL) was hydrogenated in the presence of 20% Pd(OH)$_2$ (1.1 g) for 48 h at 40 °C. The catalyst was removed by filtration, and the solution was concentrated. The residue was treated with acetic anhydride (5 mL) and pyridine (10 mL) for 24 h at rt The mixture was concentrated, and a solution of the residue in chloroform was successively washed with 2 M HCl and M Na_2CO_3, dried (Na_2SO_4) and concentrated. Column chromatography (40:1 chloroform–methanol) of the residue on silica gel gave **15** (850 mg, 83%) as an amorphous mass. $[\alpha]_D$ +30.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, Me$_3$SiCH_2CH$_2$), 1.54, 1.55, 1.66, 1.66, 1.78, 1.91, 2.00, 2.01, 2.05, 2.07, 2.07, 2.09, 2.13, and 2.14 (14 s, 42 H, 2 AcN, 11 AcO, and CH_3COCH$_2$CH$_2$), 2.45 (dd, 1 H, J_{gem} 12.7, $J_{3eq,4}$ 4.5 Hz, H-3^{V}eq), 2.48–2.72 (m, 4 H, CH$_3$COCH_2CH_2), 2.98 (m, 1 H, H-2^{III}), 3.59 (dd, 1 H, $J_{5,6}$ 10.7, $J_{6,7}$ 2.9 Hz, H-6^{V}), 3.73 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.77 (m, 1 H, H-5^{V}), 3.82 (s, 3 H, MeO), 4.01 (dd, 1 H, J_{gem} 12.8, $J_{8,9}$ 5.7 Hz, H-9^{V}), 4.10 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{II}), 4.27 (dd, 1 H, J_{gem} 12.8, $J_{8,9}$ 2.4 Hz, H-9′^{V}), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.80 (m, 1 H, H-4^{V}), 4.83 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.7 Hz, H-3^{II}), 4.84 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.89 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.96 (d, 1 H, $J_{5,NH}$ 10.1 Hz, NH), 4.99 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.02 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 3.7 Hz, H-3^{III}), 5.04 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.3 Hz, H-2^{II}), 5.04 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.12 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.22 (dd, 1 H, $J_{6,7}$ 2.9, $J_{7,8}$ 9.7 Hz, H-7^{V}), 5.34 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.8 Hz, H-2^{IV}), 5.46 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4^{III}), 5.61 (m, 1 H, H-8^{V}), 6.24 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.27–8.19 (m, 15 H, 3 Ph). Anal. Calcd for $C_{91}H_{116}N_2O_{45}Si$ (1985.98): C, 55.04; H, 5.89; N, 1.41. Found: C, 54.92; H, 5.62; N, 1.30.

3.1.13. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galacto-pyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16). Removal of the benzyl groups and subsequent O-acetylation of **13** (500 mg, 0.22 mmol), as described for **15**, gave **16** (445 mg, quant) as an amorphous mass. $[\alpha]_D$ +10.6° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (m, 2 H, Me$_3$SiCH_2CH$_2$), 1.55, 1.57, 1.77, 1.91, 2.01, 2.05, 2.06, 2.06, 2.07, 2.09, 2.09, 2.13, 2.13, and 2.15 (14 s, 42 H, 2 AcN, 10 AcO, and 2 CH_3COCH$_2$CH$_2$), 1.61 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^{V}ax), 2.42–2.80 (m, 9 H, H-3^{V}eq and 2 CH$_3$COCH_2CH_2), 3.03 (m, 1 H, H-2^{III}), 3.60 (dd, 1 H, $J_{5,6}$ 10.5, $J_{6,7}$ 2.7 Hz H-6^{V}), 3.74 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz H-4^{I}), 3.81 (s, 3 H, MeO), 4.03 (dd, 1 H, J_{gem} 12.3, $J_{8,9}$ 5.5 Hz, H-9^{V}), 4.09 (d, 1 H, $J_{3,4}$ 3.2 Hz H-4^{II}), 4.27 (dd, 1 H, J_{gem} 12.3, $J_{8,9}$ 2.3 Hz, H-9′^{V}), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.80 (m, 1 H, H-4^{V}), 4.84 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.85 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.2 Hz, H-3^{II}), 4.88 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.98 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.00 (m, 1 H, H-3^{III}), 5.06 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.2 Hz, H-2^{II}), 5.10 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.14 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.23 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.47 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{III}), 5.60 (m, 1 H, H-8^{V}), 6.03 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.39–8.19 (m, 15 H, 3 Ph). Anal. Calcd for $C_{94}H_{120}N_2O_{46}Si$ (2042.05): C, 55.29; H, 5.92; N, 1.37. Found: C, 55.25; H, 5.64; N, 1.28.

3.1.14. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (17). To a solution of **15** (60 mg, 0.030 mmol) in ethanol (3 mL) was added hydrazine mono-acetate (20 mg, 0.15 mmol), and the mixture was stirred for 45 min at rt, then concentrated. Column chromatography (30:1 chloroform–methanol) of the residue on silica gel gave **17** (47 mg, 82%) as an amorphous mass. $[\alpha]_D$ +15.3° (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 2 H, Me$_3$SiCH_2CH$_2$), 1.33, 1.54, 1.76, 1.89, 1.98, 2.01, 2.03, 2.03, 2.03, 2.05, 2.08, 2.08, and 2.14 (13 s, 39

H, 2 AcN and 11 AcO), 1.59 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.7 Hz, H-3^{V}ax), 2.43 (dd, 1 H, J_{gem} 12.7, $J_{3eq,4}$ 4.7 Hz, H-3^{V}eq), 3.05 (m, 1 H, H-2^{III}), 3.58 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.7 Hz, H-6^{V}), 3.71 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4^{I}), 3.74 (m, 1 H, H-5^{V}), 3.79 (s, 3 H, MeO), 4.01 (d, 1 H, $J_{3,4}$ 2.6 Hz, H-4^{II}), 4.03 (dd, 1 H, J_{gem} 12.5, $J_{8,9}$ 4.9 Hz, H-9^{V}), 4.25 (dd, 1 H, J_{gem} 12.5, $J_{8,9}$ 2.2 Hz, H-9′^{V}), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.44 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.77 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 2.6 Hz, H-3^{II}), 4.78 (m, 1 H, H-4^{V}), 4.84 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.87 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^{I}), 4.98 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.4 Hz, H-3^{III}), 4.99 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 5.04 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ = 10.4 Hz, H-2^{II}), 5.05 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.13 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3^{I}), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^{V}), 5.30 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.39 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{III}), 5.39 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.59 (m, 1 H, H-8^{V}), 6.11 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.26–8.18 (m, 15 H, 3 Ph). Anal. Calcd for $C_{86}H_{110}N_2O_{43}Si$ (1887.88): C, 54.71; H, 5.87; N, 1.48. Found: C, 54.56; H, 5.68; N, 1.42.

3.1.15. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (18). Selective removal of the levulinoyl group in **16** (100 mg, 0.048 mmol) with hydrazine monoacetate (46 mg, 0.48 mmol) in ethanol (3 mL) and workup as described for **17**, gave **18** (73 mg, 81%) as an amorphous mass. $[\alpha]_D$ +29.9° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 2 H, $Me_3SiCH_2CH_2$), 1.39, 1.57, 1.77, 1.90, 2.02, 2.03, 2.06, 2.06, 2.08, 2.08, 2.09, and 2.16 (12 s, 36 H, 2 AcN and 10 AcO), 1.59 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-3^{V}ax), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^{V}eq), 3.22 (m, 1 H, H-2^{III}), 3.46 (m, 1 H, H-3^{II}), 3.60 (dd, 1 H, $J_{5,6}$ 12.0, $J_{6,7}$ 3.0 Hz, H-6^{V}), 3.72 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.77 (m, 1 H, H-5^{V}), 3.81 (s, 3 H, MeO), 4.00 (m, 1 H, H-4^{II}), 4.01 (m, 1 H, H-9^{V}), 4.28 (m, 1 H, H-9′^{V}), 4.29 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.38 (m, 1 H, H-3^{III}), 4.44 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.70 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ = 8.9 Hz, H-2^{II}), 4.78 (m, 1 H, H-4^{V}), 4.86 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.87 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.98 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.02 (d, 1 H, $J_{5,NH}$ 10.0 Hz, NH), 5.13 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.30 (dd, 1 H, $J_{6,7}$ 3.0, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.41 (d, 1 H, $J_{3,4}$ 2.3 Hz, H-4^{III}), 5.43 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.8 Hz, H-2^{IV}), 5.54 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.62 (m, 1 H, H-8^{V}), 5.98 (d, 1 H, $J_{2,NH}$ 6.4 Hz, NH ^{III}), and 7.40–8.19 (m, 15 H, 3 Ph). Anal. Calcd for $C_{84}H_{108}N_2O_{42}Si$ (1845.85): C, 54.66; H, 5.90; N, 1.52. Found: C, 54.61; H, 5.84; N, 1.33.

3.1.16. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside pyridinium salt (19). To a solution of **17** (47 mg, 0.025 mmol) in *N*,*N*-dimethylformamide (DMF; 1 mL) was added sulfur trioxide–pyridine complex (20 mg, 0.13 mmol), and the mixture was stirred for 3 h at rt Methanol (1 mL) was added, and the mixture was concentrated. Column chromatography (1:1 chloroform–methanol) of the residue on Sephadex LH-20 gave the crude product, and this was purified by column chromatography (8:1 chloroform–methanol) on silica gel gave **19** (51 mg, quant) as an amorphous mass. $[\alpha]_D$ +15.9° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 0.88 (m, 2 H, $Me_3SiCH_2CH_2$), 1.52, 1.75, 1.90, 1.98, 1.99, 2.00, 2.01, 2.03, 2.04, 2.05, 2.07, 2.07, and 2.12 (13 s, 39 H, 2 AcN and 11 AcO), 1.60 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^{V}ax), 2.42 (m, 1 H, H-3^{V}eq), 3.15 (m, 1 H, H-2^{III}), 3.59 (dd, 1 H, $J_{5,6}$ 10.7, $J_{6,7}$ 2.5 Hz, H-6^{V}), 3.72 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.75 (m, 1 H, H-5^{V}), 3.78 (s, 3 H, MeO), 3.98 (m, 1 H, H-9^{V}), 4.12 (m, 2 H, H-5^{III} and H-6^{III}), 4.16 (m, 1 H, H-4^{II}), 4.32 (m, 1 H, H-9′^{V}), 4.40 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.58 (m, 1 H, H-6′^{III}), 4.80 (m, 1 H, H-4^{V}), 4.83 (m, 2 H, H-3^{II} and H-3^{IV}), 4.85 (m, 2 H, H-3^{II} and H-3^{IV}), 4.88 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.89 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.90 (m, 1 H, H-3^{III}), 4.98 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ = 10.5 Hz, H-2^{II}), 5.01 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{IV}), 5.15 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.20 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 9.6 Hz, H-7^{V}), 5.36 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.37 (m, 1 H, H-2^{IV}), 5.44 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{III}), 5.56 (m, 1 H, H-8^{V}), and 7.36–8.19 (m, 19 H, 4 Ph). Anal. Calcd for $C_{91}H_{114}N_3O_{46}SSi$ (2046.04): C, 53.42; H, 5.62; N, 2.05. Found: C, 53.40; H, 5.45; N, 1.96.

3.1.17. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside dipyridinium salt (20). To a solution of **18** (70 mg, 0.038 mmol) in DMF (2 mL) was added sulfur trioxide-pyridine complex (98 mg, 0.60 mmol), and the mixture was stirred for 48 h at 40 °C workup as described for **19** gave **20** (48 mg, 59%) as an amorphous mass. $[\alpha]_D$ +19.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 0.90 (m, 2 H, $Me_3SiCH_2CH_2$), 1.38, 1.54, 1.72, 1.86, 1.99, 2.03, 2.04, 2.04, 2.08, 2.09, 2.10, and 2.14 (12 s, 36 H, 2 AcN and 10 AcO), 1.41 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-3^{V}ax), 2.41 (dd, 1 H, J_{gem} 12.6, $J_{3ax,4}$ 4.6 Hz, H-3^{V}eq), 3.70 (dd,

1 H, $J_{5,6}$ 10.1, $J_{6,7}$ 2.7 Hz, H-6^{V}), 3.78 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.80 (s, 3 H, MeO), 4.08 (m, 1 H, H-3^{III}), 4.12 (m, 1 H, H-2^{III}), 4.18 (dd, 1 H, J_{gem} 11.0, $J_{5,6}$ 8.0 Hz, H-6^{III}), 4.25 (m, 1 H, H-9^{V}), 4.28 (m, 1 H, H-4^{II}), 4.31 (m, 1 H, H-5^{III}), 4.38 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.0 Hz, H-3^{II}), 4.49 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.52 (dd, 1 H, J_{gem} 11.0, $J_{5,6}$ 6.0 Hz, H-6′^{III}), 4.56 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.66 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.77 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2^{I}), 4.78 (m, 1 H, H-4^{V}), 4.80 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ = 10.1 Hz, H-2^{II}), 4.94 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 3.0 Hz, H-3^{IV}), 5.07 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3^{I}), 5.13 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 5.21 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.35 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.0 Hz, H-2^{IV}), 5.44 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4^{IV}), 5.59 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{III}), 5.64 (m, 1 H, H-8^{V}), and 7.47–8.19 (m, 23 H, 5 Ph). Anal. Calcd for $C_{94}H_{116}N_4O_{48}S_2Si$ (2162.16): C, 52.22; H, 5.41; N, 2.59. Found: C, 52.18; H, 5.21; N, 2.39.

3.1.18. 2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside disodium salt (21). To a solution **19** (88 mg, 0.044 mmol) in methanol was added sodium methoxide (10 mg). The mixture was stirred for 48 h at rt, and water (0.5 mL) was then added. After completion of the reaction (24 h), the mixture was concentrated. Column chromatography (methanol) of the residue on Sephadex LH-20 gave **21** (54 mg, quant) as an amorphous solid: $[\alpha]_D$ −13.6° (*c* 0.6 CH_3OH); ^{1}H NMR (CD_3OD): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.76 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^{V}ax), 2.00 and 2.01 (2 s, 6 H, 2 AcN), 2.72 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.6 Hz, H-3^{V}eq), 4.40 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.43 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{II}), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), and 4.65 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{III}). Anal. Calcd for $C_{42}H_{72}N_2Na_2O_{32}SSi$ (1223.15): C, 41.24; H, 5.93; N, 2.29. Found: C, 41.11; H, 5.89; N, 2.01.

3.1.19. 2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside trisodium salt (22). To a solution **20** (47 mg, 0.022 mmol) in methanol was added sodium methoxide (10 mg). Workup as described for **21** gave **22** (28 mg, quant) as an amorphous solid: $[\alpha]_D$ −2.8° (*c* 0.6 CH_3OH); ^{1}H NMR (CD_3OD): δ 1.02 (m, 2 H, $Me_3SiCH_2CH_2$), 1.90 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3^{V}ax), 2.01 and 2.06 (2 s, 6 H, 2 AcN), 2.78 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.1 Hz, H-3^{V}eq), 4.30 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.44 (d, 2 H, $J_{1,2}$ 7.8 Hz, H-1^{II} and H-1^{IV}), and 4.70 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}). Anal. Calcd for $C_{42}H_{71}N_2Na_3O_{35}S_2Si$ (1325.20): C, 38.07; H, 5.40; N, 2.11. Found: C, 37.96; H, 5.30; N, 1.89.

3.1.20. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galacto-pyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (23). Removal of the benzyl groups in **14** (350 mg, 0.16 mmol) and subsequent O-acetylation, as described for **15**, gave **23** (290 mg, 91%) as an amorphous mass. $[\alpha]_D$ +7.6° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.91 (m, 2 H, $Me_3SiCH_2CH_2$), 1.56, 1.78, 1.80, 1.94, 2.05, 2.05, 2.06, 2.09, 2.11, 2.12, 2.14, 2.14, 2.16, and 2.21 (14 s, 42 H, 2 AcN, 10 AcO, and 2 $CH_3COCH_2CH_2$), 1.72 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^{V}ax), 2.42–2.70 (m, 9 H, H-3^{V}eq and 2 $CH_3COCH_2CH_2$), 2.77 (m, 1 H, H-2^{III}), 3.64 (m, 1 H, H-6^{V}), 3.75 (m, 1 H, H-4^{I}), 3.77 (m, 1 H, H-5^{V}), 3.86 (s, 3 H, MeO), 4.06 (m, 1 H, H-4^{II}), 4.07 (dd, 1 H, J_{gem} 11.2, $J_{8,9}$ 5.5 Hz, H-9^{V}), 4.25 (dd, 1 H, J_{gem} 11.2, $J_{8,9}$ 6.9 Hz, H-9′^{V}), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.83 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 2.8 Hz, H-3^{II}), 4.86 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{IV}), 4.89 (m, 1 H, H-3^{III}), 4.91 (m, 1 H, H-4^{V}), 4.95 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.96 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 1.8 Hz, H-3^{IV}), 4.98 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.04 (m, 1 H, H-4^{III}), 5.08 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2^{II}), 5.21 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.30 (dd, 1 H, $J_{6,7}$ 1.8, $J_{7,8}$ 10.1 Hz, H-7^{V}), 5.39 (d, 1 H, $J_{3,4}$ 1.6 Hz, H-4^{IV}), 5.41 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 9.7 Hz, H-2^{IV}), 5.66 (m, 1 H, H-8^{V}), and 7.40–8.31 (m, 15 H, 3 Ph). Anal. Calcd for $C_{94}H_{120}N_2O_{46}Si$ (2042.05): C, 55.29; H, 5.92; N, 1.37. Found: C, 55.04; H, 5.72; N, 1.34.

3.1.21. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (24). Selective removal of the levulinoyl group in **23** (70 mg, 0.034 mmol) with hydrazine monoacetate (33 mg, 0.34 mmol) in ethanol (3 mL) and workup as described for **19** gave **24** (36 mg, 58%) as an amorphous mass. $[\alpha]_D$ +18.0° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 2 H, $Me_3SiCH_2CH_2$), 1.53, 1.78, 1.80, 1.93, 2.05, 2.06, 2.07, 2.11, 2.12, 2.18, 2.20, and 2.21 (12 s, 36 H, 2 AcN and 10 AcO), 1.72 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^{V}ax), 2.46 (m, 1 H, H-3^{V}eq), 2.78 (m, 1 H, H-2^{III}), 3.48 (m, 1 H, H-3^{II}), 3.65 (dd, 1 H, $J_{5,6}$ 10.5, $J_{6,7}$ 2.5 Hz, H-6^{V}), 3.73 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.84 (m, 1 H, H-5^{V}), 3.88 (s, 3 H, MeO), 3.97 (m, 1 H, H-4^{II}), 4.07 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 3.0 Hz, H-9^{V}), 4.14 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 3.3 Hz, H-9′^{V}), 4.19 (d,

1 H, $J_{3,4}$ 2.3 Hz, H-4^{III}), 4.27 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.64 (d, 1 H, $J_{2,NH}$ 6.4 Hz, NH), 4.70 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.3 Hz, H-2^{II}), 4.87 (m, 1 H, H-4^{V}), 4.88 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 4.90 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.4 Hz, H-3^{IV}), 4.93 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 5.05 (d, 1 H, $J_{5,NH}$ 10.0 Hz, NH), 5.20 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.30 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.36 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{IV}), 5.37 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 2.3 Hz, H-3^{III}), 5.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{III}), 5.48 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.66 (m, 1 H, H-8^{V}), and 7.29–8.35 (m, 15 H, 3 Ph). Anal. Calcd for $C_{84}H_{108}N_2O_{42}Si$ (1845.85): C, 54.66; H, 5.90; N, 1.52. Found: C, 54.39; H, 5.85; N, 1.24.

3.1.22. 2-(Trimethylsilyl)ethyl methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside dipyridinium salt (25). To a solution of **24** (36 mg, 0.019 mmol) in DMF (1 mL) was added sulfur trioxide–pyridine complex (25 mg, 0.15 mmol), and the mixture was stirred for 3 h at rt Workup as described for **19** gave **25** (42 mg, quant) as an amorphous mass. $[\alpha]_D$ +13.9° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 0.93 (m, 2 H, $Me_3SiCH_2CH_2$), 1.60 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-3^{V}ax), 1.67, 1.77, 1.77, 1.91, 1.03, 2.04, 2.05, 2.09, 2.11, 2.12, 2.12, and 2.17 (12 s, 36 H, 2 AcN and 10 AcO), 2.50 (dd, 1 H, J_{gem} 12.6, $J_{3ax,4}$ 4.8 Hz, H-3^{V}eq), 3.82 (m, 1 H, H-5^{V}), 3.82 (s, 3 H, MeO), 4.11 (dd, 1 H, J_{gem} 11.6, $J_{5,6}$ 5.5 Hz, H-9^{V}), 4.19 (m, 1 H, H-9^{V}), 4.21 (m, 1 H, H-4^{II}), 4.30 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{III}), 4.49 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.56 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.86 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.6 Hz, H-2^{I}), 4.91 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ = 10.1 Hz, H-2^{II}), 4.96 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.99 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 5.13 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3^{I}), 5.30 (dd, 1 H, $J_{6,7}$ 2.8, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.43 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.46 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.61 (m, 1 H, H-8^{V}), and 7.43–8.28 (m, 23 H, 5 Ph). Anal. Calcd for $C_{94}H_{116}N_4O_{48}S_2Si$ (2162.16): C, 52.22; H, 5.41; N, 2.59. Found: C, 51.99; H, 5.25; N, 2.59.

3.1.23. 2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside trisodium salt (26). To a solution **25** (30 mg, 0.015 mmol) in methanol was added sodium methoxide (10 mg). Workup as described for **21** gave **26** (19 mg, quant) as an amorphous solid: $[\alpha]_D$ −14.8° (*c* 0.4 CH_3OH); ^{1}H NMR (CD_3OD): δ 1.00 (m, 2 H, $Me_3SiCH_2CH_2$), 1.85 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3^{V}ax), 2.01 and 2.07 (2 s, 6 H, 2 AcN), 2.78 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.1 Hz, H-3^{V}eq), 4.35 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{I}), 4.42 (m, 2 H, H-1^{II} and H-1^{IV}), and 4.68 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{III}). Anal. Calcd for $C_{42}H_{71}N_2Na_3O_{35}S_2Si$ (1325.20): C, 38.07; H, 5.40; N, 2.11. Found: C, 38.03; H, 5.38; N, 2.01.

3.1.24. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (27). To a solution of **15** (220 mg, 0.11 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (1.5 mL) at 0 °C. The mixture was stirred for 3 h at rt and then concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **27** (208 mg, quant) as an amorphous mass. Anal. Calcd for $C_{86}H_{104}N_2O_{45}$ (1885.75): C, 54.78; H, 5.56; N, 1.49. Found: C, 54.48; H, 5.34; N, 1.33.

3.1.25. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (28). Selective removal of the 2-(trimethylsilyl)ethyl group of **16** (400 mg, 0.18 mmol) with trifluoroacetic acid (1.5 mL) in dichloromethane (3 mL), then workup as described for **27**, gave **28** (360 mg, 95%) as an amorphous mass. Anal. Calcd for $C_{89}H_{108}N_2O_{46}$ (1941.81): C, 55.05; H, 5.61; N, 1.44. Found: C, 54.86; H, 5.59; N, 1.29

3.1.26. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranose (38). Selective removal of the 2-(trimethylsilyl)ethyl group of **23** (220 mg, 0.10 mmol) with trifluoroacetic acid (1.5 mL) in dichloromethane (3 mL), then workup as described for **27**, gave **38** (206 mg, 99%) as an amorphous mass. Anal. Calcd for $C_{89}H_{108}N_2O_{46}$ (1941.81): C, 55.05; H, 5.61; N, 1.44. Found: C, 54.94; H, 5.58; N, 1.32

3.1.27. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-

galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (29). To a solution of **27** (208 mg, 0.11 mmol) in dichloromethane (2 mL) and trichloroacetonitrile (325 μL, 2.2 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 20 μL, 0.13 mmol) at 0 °C, and the mixture was stirred for 30 min at 0 °C, then concentrated. Column chromatography (20:1 chloroform–methanol) of the residue on silica gel gave **29** (211 mg, 94%) as an amorphous mass. $[\alpha]_D$ +33.6° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.55, 1.56, 1.71, 1.77, 1.91, 2.03, 2.04, 2.05, 2.06, 2.07, 2.08, 2.09, 2.10, and 2.14 (14 s, 42 H, 2 AcN, 11 AcO, and CH_3COCH$_2$CH$_2$), 1.61 (t, 1 H, $J_{gem} = J_{3ax,4}$ 12.8 Hz, H-3^V*ax*), 2.44 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3^V*eq*), 2.48–2.73 (m, 4 H, CH$_3$COCH_2CH_2), 3.00 (m, 1 H, H-2^{III}), 3.82 (s, 3 H, MeO), 4.38 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.78 (m, 1 H, H-4^V), 4.95 (d, 1 H, $J_{5,NH}$ 10.3 Hz, NH), 4.99 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{IV}), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 10.6 Hz, H-7^V), 5.35 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.9 Hz, H-2^{IV}), 5.45 (d, 1 H, $J_{3,4}$ 2.2 Hz, H-4^{III}), 5.61 (m, 1 H, H-8^V), 6.22 (d, 1 H, $J_{2,NH}$ 6.6 Hz, Hz NH), 6.49 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^I), 7.27–8.19 (m, 15 H, 3 Ph), and 8.66 (s, 1 H, C=NH). Anal. Calcd for $C_{88}H_{104}Cl_3N_3O_{45}$ (2030.14): C, 52.06; H, 5.16; N, 2.07. Found: C, 52.04; H, 4.96; N, 2.05.

3.1.28. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (30). Treatment of **28** (120 mg, 0.064 mmol) with trichloroacetonitrile (273 μL, 1.92 mmol) and DBU (11 μL, 0.077 mmol) in dichloromethane (2 mL), then workup as described for **29** gave **30** (130 mg, quant) as an amorphous mass. ^{1}H NMR ($CDCl_3$): δ 1.55, 1.56, 1.63, 1.78, 1.91, 2.03, 2.04, 2.06, 2.07, 2.09, 2.09, 2.13, 2.13, and 2.16 (14 s, 42 H, 2 AcN, 10 AcO, and 2 CH_3COCH$_2$CH$_2$), 2.42–2.79 (m, 9 H, H-3^V*eq* and 2 CH$_3$COCH_2CH_2), 3.00 (m, 1 H, H-2^{III}), 3.59 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.7 Hz H-6^V), 3.82 (s, 3 H, MeO), 4.03 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 5.5 Hz, H-9^V), 4.09 (d, 1 H, $J_{3,4}$ 2.7 Hz H-4^{II}), 4.27 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 2.3 Hz, H-9′^{V}), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.80 (m, 1 H, H-4^V), 4.83 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.84 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.7 Hz, H-3^{II}), 4.96 (d, 1 H, $J_{5,NH}$ 10.1 Hz, NH), 4.98 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.01 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.4 Hz, H-3^{IV}), 5.05 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2^I), 5.07 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.3 Hz, H-2^{II}), 5.09 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{III}), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.8 Hz, H-7^V), 5.35 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.47 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{III}), 5.48 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.3 Hz, H-3^I), 5.59 (m, 1 H, H-8^V), 6.35 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), 6.49 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^I), 7.27–8.18 (m, 15 H, 3 Ph), and 8.66 (s, 1 H, C=NH). Anal. Calcd for $C_{91}H_{108}Cl_3N_3O_{46}$ (2086.20): C, 52.39; H, 5.22; N, 2.01. Found: C, 52.20; H, 5.15; N, 1.81.

3.1.29. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (32). To a solution of **29** (300 mg, 0.15 mmol) and (2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (**31**; 198 mg, 0.30 mmol) in dichloromethane (4 mL) were added 4Å molecular sieves (AW-300; 1.5 g), and the mixture was stirred for 12 h at rt, then cooled to 0 °C. TMSOTf (6 μL, 0.030 mmol) was added, and the mixture was stirred for 30 h at 10 °C, then filtered. The insoluble materials were washed with chloroform, and the combined filtrate and washings was washed with M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (25:1 chloroform–methanol) of the residue on silica gel gave **32** (140 mg, 37%) as an amorphous mass. $[\alpha]_D$ +18.9° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.7 Hz, 2 *Me*CH$_2$), 1.26 (s, 52 H, 26 CH_2), 1.53, 1.54, 1.77, 1.91, 1.92, 1.97, 2.01, 2.04, 2.05, 2.06, 2.08, 2.09, 2.13, and 2.14 (14 s, 42 H, 2 AcN, 11 AcO, and CH_3COCH$_2$CH$_2$), 1.61 (dd, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-3^V*ax*), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^V*eq*), 2.48–2.72 (m, 4 H, CH$_3$COCH_2CH_2), 2.98 (m, 1 H, H-2^{III}), 3.59 (dd, 1 H, $J_{5,6}$ 10.7, $J_{6,7}$ 2.7 Hz, H-6^V), 3.61 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 4.6 Hz, Cer-1), 3.77 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.77 (m, 1 H, H-5^V), 3.82 (s, 3 H, MeO), 3.99 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 3.3 Hz, Cer-1′), 4.02 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 5.8 Hz, H-9^V), 4.10 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{II}), 4.12 (m, 1 H, H-5^{III}), 4.20 (dd, 1 H, J_{gem} 11.1, $J_{5,6}$ 4.0 Hz, H-6^{III}), 4.27 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 2.3 Hz, H-9′^{V}), 4.33 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^I), 4.46 (m, 1 H, Cer-2), 4.48 (dd, 1 H, J_{gem} 11.1, $J_{5,6}$ 5.2 Hz, H-6′^{III}), 4.80 (m, 1 H, H-4^V), 4.81 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.5 Hz, H-3^{II}), 4.84 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}),4.89 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^I), 4.93 (d, 1 H, $J_{5,NH}$ 8.5 Hz, NH), 4.98 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.04 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 4.2 Hz, H-3^{III}), 5.00 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ = 10.3 Hz, H-2^{II}), 5.04 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.12 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^I), 5.21 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^V), 5.34 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.45 (m, 1 H, Cer-4), 5.46 (d, 1 H, $J_{3,4}$ 4.2 Hz, H-4^{III}), 5.53 (m, 1 H, Cer-3), 5.60 (m, 1 H, H-8^V), 5.74 (m, 1 H, $J_{2,NH}$ 9.2 Hz, Cer-NH), 5.87 (m, 1 H, Cer-5), 5.97 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.38–8.19 (m, 20 H, 4 Ph). Anal. Calcd for $C_{129}H_{177}N_3O_{48}$

(2537.81): C, 61.05; H, 7.03; N, 1.16. Found: C, 60.76; H, 6.94; N, 1.61.

3.1.30. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (33). Glycosylation of **31** (85 mg, 0.13 mmol) with **30** (130 mg, 0.065 mmol) in dichloromethane (2 mL) in the presence of TMSOTf (2.4 μL, 0.013 mmol) and AW-300 (1.5 g) for 24 h at 10 °C, then workup as described for **32**, gave **33** (58 mg, 36%) as an amorphous mass. $[\alpha]_D$ +14.6° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.25 (s, 52 H, 26 CH_2), 1.55, 1.77, 1.91, 1.92, 2.01, 2.02, 2.04, 2.05, 2.08, 2.09, 2.13, 2.13, 2.13, and 2.15 (14 s, 42 H, 2 AcN, 10 AcO, and 2 $CH_3COCH_2CH_2$), 1.61 (dd, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^V*ax*), 2.41–2.79 (m, 9 H, H-3^{V}eq and 2 $CH_3COCH_2CH_2$), 3.01 (m, 1 H, H-2^{III}), 3.60 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.7 Hz, H-6^V), 3.62 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 4.4 Hz, Cer-1), 3.70 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.75 (m, 1 H, H-5^V), 3.81 (s, 3 H, MeO), 3.99 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 3.7 Hz, Cer-1′), 4.03 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 5.1 Hz, H-9^V), 4.08 (d, 1 H, $J_{3,4}$ 2.1 Hz, H-4^{II}), 4.26 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 2.3 Hz, H-9′^{V}), 4.32 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^I), 4.47 (m, 1 H, Cer-2), 4.78 (m, 1 H, H-4^V), 4.82 (m, 1 H, H-3^{II}), 4.83 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.89 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^I), 4.97 (d, 1 H, $J_{5,NH}$ 8.5 Hz, NH), 4.98 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 4.99 (m, 1 H, NH), 5.00 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.2 Hz, H-3^{III}), 5.03 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.3 Hz, H-2^{II}), 5.08 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.12 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^I), 5.22 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^V), 5.34 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.38 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.45 (m, 1 H, Cer-4), 5.46 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{III}), 5.53 (m, 1 H, Cer-3), 5.58 (m, 1 H, H-8^V), 5.75 (m, 1 H, $J_{2,NH}$ 9.2 Hz, Cer-NH), 5.87 (m, 1 H, Cer-5), 6.21 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.39–8.20 (m, 20 H, 4 Ph). Anal. Calcd for $C_{132}H_{181}N_3O_{49}$ (2593.87): C, 61.12; H, 7.03; N, 1.62. Found: C, 61.09; H, 6.93; N, 1.41.

3.1.31. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (34). Selective removal of the levulinoyl group in **32** (140 mg, 0.055 mmol) with hydrazine monoacetate (52 mg, 0.55 mmol) in ethanol (2 mL), and workup as described for **17**, gave **34** (116 mg, 87%) as an amorphous mass. $[\alpha]_D$ +15.7° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.8 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.31, 1.55, 1.77, 1.91, 1.97, 2.02, 2.03, 2.04, 2.05, 2.06, 2.08, 2.09, and 2.16 (13 s, 39 H, 2 AcN and 11 AcO), 1.60 (dd, 1 H, $J_{gem} = J_{3ax,4}$ 12.6 Hz, H-3^V*ax*), 2.44 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^V*eq*), 3.01 (m, 1 H, H-5^{III}), 3.05 (m, 1 H, H-2^{III}), 3.26 (m, 1 H, H-6^{III}), 3.43 (m, 1 H, H-6′^{III}), 3.59 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 2.7 Hz, H-6^V), 3.62 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 4.6 Hz, Cer-1), 3.72 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.77 (m, 1 H, H-5^V), 3.80 (s, 3 H, MeO), 3.99 (dd, 1 H, J_{gem} 10.3, $J_{1,2}$ 3.7 Hz, Cer-1′), 4.02 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{II}), 4.05 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 5.3 Hz, H-9^V), 4.26 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 2.3 Hz, H-9′^{V}), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.42 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^I), 4.47 (m, 1 H, Cer-2), 4.77 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 2.5 Hz, H-3^{II}), 4.80 (m, 1 H, H-4^V), 4.85 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.88 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^I), 4.98 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.7 Hz, H-3^{III}), 5.00 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.02 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.0 Hz, H-2^{II}), 5.06 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.14 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^I), 5.23 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^V), 5.33 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.40 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4^{III}), 5.43 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 10.3 Hz, H-2^{IV}), 5.45 (m, 1 H, Cer-4), 5.53 (m, 1 H, Cer-3), 5.60 (m, 1 H, H-8^V), 5.75 (m, 1 H, $J_{2,NH}$ 9.2 Hz, Cer-NH), 5.86 (m, 1 H, Cer-5), 5.96 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.28–8.20 (m, 20 H, 4 Ph). Anal. Calcd for $C_{124}H_{171}N_3O_{46}$ (2439.71): C, 61.05; H, 7.07; N, 1.72. Found: C, 60.84; H, 6.97; N, 1.57.

3.1.32. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (35). Selective removal of the levulinoyl group in **33** (58 mg, 0.022 mmol) with hydrazine monoacetate (21 mg, 0.22 mmol) in ethanol (2 mL), and workup as described for **17**, gave **35** (46 mg, 88%) as an amorphous mass. $[\alpha]_D$ +37.8° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.38, 1.57, 1.78, 1.90, 1.93, 2.03, 2.03, 2.04, 2.07, 2.09, 2.10, and 2.16 (12 s, 36 H, 2 AcN and 10 AcO), 2.45 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.6 Hz, H-3^V*eq*), 3.22 (m, 1 H, H-2^{III}), 3.42 (m, 1 H, H-3^{II}), 3.59 (dd, 1 H, $J_{5,6}$ 10.8, $J_{6,7}$ 3.0 Hz, H-6^V), 3.70 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.80 (m, 1 H, H-5^V), 3.81 (s, 3 H, MeO), 4.00 (s, 1 H, H-4^{II}), 4.02 (dd, 1 H, J_{gem} 12.6, $J_{8,9}$ 6.2 Hz, H-9^V), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.32 (m, 1 H, H-9′^{V}), 4.37 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.0 Hz, H-3^{III}), 4.41 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^I), 4.67 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.2 Hz, H-2^{II}), 4.80 (m, 1 H, H-4^V), 4.87 (dd, 1 H, $J_{1,2}$ 8.0,

$J_{2,3}$ 9.4 Hz, H-2^{I}), 4.88 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.95 (d, 1 H, $J_{5,NH}$ 9.6 Hz, NH),4.97 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{IV}), 5.12 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.23 (dd, 1 H, $J_{6,7}$ 3.0, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.35 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.42 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4^{III}), 5.43 (dd, 1 H, $J_{1,2}$ 7.6, $J_{2,3}$ 9.8 Hz, H-2^{IV}), 5.45 (m, 1 H, Cer-4), 5.54 (m, 1 H, Cer-3), 5.54 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.62 (m, 1 H, H-8^{V}), 5.75 (m, 1 H, $J_{2,NH}$ 9.4 Hz, Cer-NH), 5.86 (m, 1 H, Cer-5), 5.92 (d, 1 H, $J_{2,NH}$ 6.6 Hz, NH), and 7.27–8.20 (m, 20 H, 4 Ph). Anal. Calcd for $C_{122}H_{169}N_3O_{45}$ (2397.67): C, 61.12; H, 7.10; N, 1.75. Found: C, 60.82; H, 6.91; N, 1.70.

3.1.33. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol pyridinium salt (36). To a solution of **34** (110 mg, 0.045 mmol) in DMF (2 mL) was added sulfur trioxide–pyridine complex (37 mg, 0.22 mmol), and the mixture was stirred for 3 h at rt. Workup as described for **19** gave **36** (83 mg, 71%) as an amorphous mass. $[\alpha]_D$ +12.8° (*c* 1.8, $CHCl_3$); ^{1}H NMR (CD_3OD–D_2O): δ 0.89 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.50, 1.55, 1.75, 1.76, 1.91, 1.94, 1.98, 2.02, 2.04, 2.06, 2.09, 2.12, and 2.15 (13 s, 39 H, 2 AcN and 11 AcO), 1.55 (m, 1 H, H-$3^{V}ax$), 2.45 (dd, 1 H, J_{gem} 12.5, $J_{3eq,4}$ 4.4 Hz, H-$3^{V}eq$), 3.35 (m, 1 H, H-2^{III}), 3.63 (dd, 1 H, $J_{5,6}$ 11.2, $J_{6,7}$ 2.7 Hz, H-6^{V}), 3.66 (dd, 1 H, J_{gem} 9.9, $J_{1,2}$ 4.0 Hz, Cer-1), 3.70 (m, 1 H, H-5^{V}), 3.72 (m, 1 H, H-4^{I}), 3.81 (s, 3 H, MeO), 3.98 (dd, 1 H, J_{gem} 9.9, $J_{1,2}$ 4.6 Hz, Cer-1′), 4.02 (dd, 1 H, J_{gem} 12.2, $J_{8,9}$ 6.6 Hz, H-9^{V}), 4.08 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{II}), 4.27 (dd, 1 H, J_{gem} 12.2, $J_{8,9}$ 2.6 Hz, H-$9'^{V}$), 4.33 (m, 1 H, H-1^{II}), 4.43 (m, 1 H, Cer-2), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.76 (m, 1 H, H-4^{V}), 4.87 (m, 2 H, H-3^{II} and H-3^{IV}), 4.89 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2^{I}), 4.92 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 4.95 (m, 1 H, H-3^{III}), 5.00 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.0 Hz, H-2^{II}), 5.02 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 5.11 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3^{I}), 5.20 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.6 Hz, H-7^{V}), 5.38 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.41 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.45 (m, 1 H, Cer-4), 5.48 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4^{III}), 5.54 (m, 1 H, Cer-3), 5.60 (m, 1 H, H-8^{V}), 5.89 (m, 1 H, Cer-5), and 7.25–8.19 (m, 24 H, 5 Ph). Anal. Calcd for $C_{129}H_{175}N_4O_{49}S$ (2597.86): C, 59.64; H, 6.79; N, 2.16. Found: C, 59.41; H, 6.51; N, 1.87.

3.1.34. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol dipyridine salt (37). To a solution of **35** (46 mg, 0.019 mmol) in DMF (2.0 mL) was added sulfur trioxide–pyridine complex (31 mg, 0.19 mmol), and the mixture was stirred for 3 h at rt. Workup as described for **19** gave **37** (33 mg, 64%) as an amorphous mass. $[\alpha]_D$ +22.6° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.40, 1.57, 1.72, 1.86, 1.97, 2.01, 2.01, 2.04, 2.05, 2.08, 2.11, and 2.13 (12 s, 36 H, 2 AcN and 10 AcO), 2.40 (dd, 1 H, J_{gem} 12.6, $J_{3eq,4}$ 4.8 Hz, H-$3^{V}eq$), 3.67 (dd, 1 H, $J_{5,6}$ 11.0, $J_{6,7}$ 2.5 Hz, H-6^{V}), 3.76 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 3.78 (s, 3 H, MeO), 4.00 (s, 1 H, H-4^{II}), 4.01 (dd, 1 H, J_{gem} 12.4, $J_{8,9}$ 5.3 Hz, H-9^{V}), 4.25 (m, 1 H, H-$9'^{V}$), 4.31 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-4^{II}), 4.71 (m, 1 H, H-3^{III}), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.50 (m, 1 H, H-3^{II}),4.61 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{I}), 4.77 (m, 1 H, H-4^{V}), 4.82 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2^{I}), 4.86 (m, 1 H, H-2^{II}), 4.92 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.0 Hz, H-3^{IV}), 5.08 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 5.09 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^{I}), 5.23 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 9.8 Hz, H-7^{V}), 5.35 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.45 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-4^{IV}), 5.49 (m, 1 H, Cer-3), 5.53 (s, 1 H, H-4^{III}), 5.55 (m, 1 H, Cer-4), 5.60 (m, 1 H, H-8^{V}), 5.88 (m, 1 H, Cer-5), and 7.35–8.08 (m, 28 H, 6 Ph). Anal. Calcd for $C_{132}H_{177}N_5O_{51}S_2$ (2713.98): C, 58.42; H, 6.57; N, 2.58. Found: C, 58.18; H, 6.55; N, 2.58.

3.1.35. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-D-glucopyranosyl trichloroacetimidate (39). Treatment of **38** (206 mg, 0.11 mmol) with trichloroacetonitrile (469 mL, 3.18 mmol) and DBU (19 mL, 0.13 mmol) in dichloromethane (2 mL), then workup as described for **29**, gave **39** (184 mg, 83%) as an amorphous mass. The anomeric ratio (α:β) was estimated as 1:1 from the ratio of the intensities of the anomeric proton signals of galactose unit in the ^{1}H NMR spectrum. Anal. Calcd for $C_{91}H_{108}Cl_3N_3O_{46}$ (2086.20): C, 52.39; H, 5.22; N, 2.01. Found: C, 52.23; H, 4.99; N, 1.84.

3.1.36. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (40). Glycosylation of **31** (118 mg, 0.18 mmol) with **39** (184 mg, 0.088

mmol) in dichloromethane (3 mL) in the presence of TMSOTf (3.4 mL, 0.017 mmol) and AW-300 (1.0 g) for 15 h at 10 °C, then workup as described for **32**, gave **40** (75 mg, 33%) as an amorphous mass. $[\alpha]_D$ +17.8° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.55, 1.76, 1.80, 1.93, 1.94, 2.03, 2.05, 2.06, 2.12, 2.13, 2.14, 2.15, 2.16, and 2.22 (14 s, 42 H, 2 AcN, 10 AcO, and 2 CH_3COCH_2CH_2), 1.72 (dd, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^V*ax*), 2.41–2.70 (m, 9 H, H-3^{V}eq and 2 CH_3COCH_2CH_2), 2.75 (m, 1 H, H-2^{III}), 3.64 (dd, 1 H, $J_{5,6}$ 10.6, $J_{6,7}$ 2.7 Hz, H-6^V), 3.72 (m, 1 H, H-4^I), 3.82 (m, 1 H, H-5^V), 3.86 (s, 3 H, MeO), 4.06 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{II}), 4.08 (dd, 1 H, J_{gem} 12.0, $J_{8,9}$ 4.6 Hz, H-9^V), 4.16 (m, 1 H, H-9′^{V}), 4.32 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^I), 4.81 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 2.7 Hz, H-3^{II}), 4.83 (m, 1 H, H-3^{IV}), 4.86 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 4.88 (m, 1 H, H-4^V), 4.94 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}),4.96 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^I), 5.05 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.3 Hz, H-2^{II}), 5.20 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^I), 5.30 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 9.8 Hz, H-7^V), 5.38 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4^{IV}), 5.40 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2^{IV}), 5.45 (m, 1 H, Cer-4), 5.55 (m, 1 H, Cer-3), 5.66 (m, 1 H, H-8^V), 5.77 (d, 1 H, $J_{2,NH}$ 9.2 Hz, Cer-NH), 5.83 (d, 1 H, $J_{2,NH}$ 8.9 Hz, NH), 5.87 (m, 1 H, Cer-5), and 7.40–8.34 (m, 20 H, 4 Ph). Anal. Calcd for $C_{132}H_{181}N_3O_{49}$ (2593.87): C, 61.12; H, 7.03; N, 1.62. Found: C, 61.00; H, 6.82; N, 1.36.

3.1.37. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (41). Selective removal of the levulinoyl group in **40** (75 mg, 0.029 mmol) with hydrazine monoacetate (27 mg, 0.29 mmol) in ethanol (2 mL), and workup as described for **37**, gave **39** (35 mg, 50%) as an amorphous mass. $[\alpha]_D$ +22.5° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.9 Hz, 2 *Me*CH_2), 1.25 (s, 52 H, 26 CH_2), 1.52, 1.78, 1.80, 1.93, 1.94, 2.04, 2.05, 2.05, 2.13, 2.18, 2.20, and 2.21 (12 s, 36 H, 2 AcN and 10 AcO), 1.72 (dd, 1 H, $J_{gem} = J_{3ax,4}$ 12.4 Hz, H-3^V*ax*), 2.47 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.6 Hz, H-3^V*eq*), 2.77 (m, 1 H, H-2^{III}), 3.41 (m, 1 H, H-3^{II}), 3.64 (m, 1 H, H-6^V), 3.70 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^I), 3.84 (m, 1 H, H-5^V), 3.89 (s, 3 H, MeO), 3.96 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{II}), 4.07 (dd, 1 H, J_{gem} 12.2, $J_{8,9}$ 4.7 Hz, H-9^V), 4.13 (dd, 1 H, J_{gem} 12.2, $J_{8,9}$ 2.5 Hz, H-9′^{V}), 4.18 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4^{III}), 4.23 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^I), 4.56 (d, 1 H, $J_{2,NH}$ 6.4 Hz, NH), 4.67 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^{II}), 4.86 (m, 1 H, H-4^V), 4.88 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 4.91 (dd, 1 H, $J_{2,3}$ 9.4, $J_{3,4}$ 3.2 Hz, H-3^{IV}), 4.94 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^I), 5.18 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.4 Hz, H-3^I), 5.30 (dd, 1 H, $J_{6,7}$ 2.7, $J_{7,8}$ 10.0 Hz, H-7^V), 5.32 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 2.5 Hz, H-3^{III}), 5.36 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4^{IV}), 5.41 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.45 (m, 1 H, Cer-4), 5.47 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2^{IV}), 5.54 (m, 1 H, Cer-3), 5.66 (m, 1 H, H-8^V), 5.77 (m, 1 H, $J_{2,NH}$ 9.2 Hz, Cer-NH), 5.87 (m, 1 H, Cer-5), and 7.40–8.35 (m, 20 H, 4 Ph). Anal. Calcd for $C_{122}H_{169}N_3O_{45}$ (2397.67): C, 61.12; H, 7.10; N, 1.75. Found: C, 61.08; H, 6.97; N, 1.59.

3.1.38. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-acetyl-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol dipyridinium salt (42). To a solution of **41** (34 mg, 0.014 mmol) in DMF (1.5 mL) was added sulfur trioxide–pyridine complex (18 mg, 0.11 mmol), and the mixture was stirred for 3 h at rt. Workup as described for **36** gave **42** (33 mg, 87%) as an amorphous mass. $[\alpha]_D$ +14.9° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 6 H, $J_{Me,CH2}$ 6.7 Hz, 2 *Me*CH_2), 1.26 (s, 52 H, 26 CH_2), 1.60, 1.76, 1.84, 1.92, 1.95, 2.04, 2.05, 2.07, 2.09, 2.13, 2.15, and 2.20 (12 s, 36 H, 2 AcN and 10 AcO), 2.47 (dd, 1 H, J_{gem} 12.4, $J_{3eq,4}$ 4.3 Hz, H-3^V*eq*), 2.85 (m, 1 H, H-2^{III}), 3.83 (s, 3 H, MeO), 4.36 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{II}), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^I), 4.78 (m, 1 H, H-4^V), 4.90 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{IV}), 5.11 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3^I), 5.20 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 5.25 (m, 1 H, H-7^V), 5.36 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 9.4 Hz, H-2^{IV}), 5.37 (m, 1 H, H-4^{IV}), 5.46 (m, 1 H, Cer-4), 5.55 (m, 1 H, Cer-3), 5.57 (m, 1 H, H-8^V), 5.89 (m, 1 H, Cer-5), and 7.40–8.26 (m, 28 H, 6 Ph). Anal. Calcd for $C_{132}H_{177}N_5O_{51}S_2$ (2713.98): C, 58.42; H, 6.57; N, 2.58. Found: C, 58.23; H, 6.41; N, 2.53.

3.1.39. 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol disodium salt (B, GSC-335). To a solution **36** (83 mg, 28 mmol) in methanol (2 mL) and dioxane (1 mL) was added sodium methoxide (10 mg); then water (0.5 mL) was added. After completion of the reaction (24 h), the mixture was concentrated. Column chromatography (5:4:0.7:0.07 $CHCl_3$–MeOH–H_2O–Et_3N) of the residue on Sephadex LH-20 gave **B** (GSC-335) (53 mg, quant) as an amorphous mass. $[\alpha]_D$ +14.1° (*c* 0.2, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^1H NMR ($(CD_3)_2SO$–D_2O): δ 0.85 (t, 6 H, $J_{Me,CH2}$ 7.0 Hz, 2 *Me*CH_2), 1.23 (s, 52 H, 26 CH_2), 1.40 (m, 2 H, COCH_2CH_2), 1.80 and 1.90 (2 s, 6 H, 2 AcN), 2.74 (m, 1 H, H-3^V*eq*), 4.17 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1^I), 4.19

(d, 1 H, $J_{1,2}$ 7.0 Hz, H-1^{II}), 4.25 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1^{IV}), 4.52 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{III}), 5.33 (m, 1 H, Cer-4), and 5.52 (m, 1 H, Cer-5).

FABMS (negative-ion mode, triethanolamine matrix): *m/z* 1646.9 $[M-Na]^-$, 1624.9 $[M-2Na]^-$, 1333.8 $[M-Na-NeuAc]^-$, 1171.8 $[1333.8-Gal]^-$, 888.7 [lactosyl ceramide]$^-$, 726.7 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{34}S$ [M−Na]: 1646.8076; found: 1646.86.

3.1.40. 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol trisodium salt (C, GSC-454). To a solution of **37** (33 mg, 0.012 mmol) in methanol (3.0 mL) was added sodium methoxide (10 mg), and workup as described for **B**, gave **C** (GSC-454) (21 mg, quant) as an amorphous mass. $[\alpha]_D$ +0.3° (*c* 0.6, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^{1}H NMR ($(CD_3)_2SO$–D_2O): δ 0.83 (t, 6 H, $J_{Me,CH2}$ 7.0 Hz, 2 *Me*CH_2), 1.22 (s, 52 H, 26 CH_2), 1.41 (m, 2 H, $COCH_2CH_2$), 1.85 and 1.92 (2 s, 6 H, 2 AcN), 2.74 (m, 1 H, H-3^V*eq*), 4.08 (m, 1 H, H-1^I), 4.28 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1^{II}), 4.31 (m, 1 H, H-1^{IV}), 4.50 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{III}), 5.27 (m, 1 H, Cer-4), and 5.54 (m, 1 H, Cer-5).

FABMS (negative-ion mode, triethanolamine matrix): *m/z* 1726.8 $[M-2Na]^-$, 1625.0 $[M-2Na-SO_3Na+H]^-$, 1435.8 $[M-Na-NeuAc]^-$, 1333.8 $[1435.8-SO_3Na+H]^-$, 1171.7 $[1333.8-Gal]^-$, 968.7 [3′-*O*-sulfo lactosyl ceramide]$^-$, 726.6 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{37}S_2$ (*M*−2Na): 1726.7644; found: 1726.80.

3.1.41. 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl-(1→4)-3-*O*-sulfo-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol trisodium salt (D, GSC-338). To a solution of **42** (33 mg, 0.012 mmol) in methanol (4 mL) was added sodium methoxide (10 mg), and workup as described for **B**, gave (**D**, GSC-338) (21 mg, quant) as an amorphous mass. $[\alpha]_D$ −12.7° (*c* 0.2, 5:4:0.7 $CHCl_3$–MeOH–H_2O); ^{1}H NMR ($(CD_3)_2SO$–D_2O): δ 0.85 (t, 6 H, $J_{Me,CH2}$ 7.0 Hz, 2 *Me*CH_2), 1.23 (s, 52 H, 26 CH_2), 1.44 (m, 2 H, $COCH_2CH_2$), 1.87 and 1.90 (2 s, 6 H, 2 AcN), 2.71 (m, 1 H, H-3^V*eq*), 4.17 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^I), 4.23 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{II}), 4.29 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{IV}), 4.42 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{III}), 5.32 (m, 1 H, Cer-4), and 5.52 (m, 1 H, Cer-5).

FABMS (negative-ion mode, triethanolamine matrix): *m/z* 1726.9 $[M-2Na]^-$, 1625.0 $[M-2Na-SO_3Na+H]^-$, 1435.8 $[M-Na-NeuAc]^-$, 1333.7 $[1435.8-SO_3Na+H]^-$, 1171.8 $[1333.7-Gal]^-$, 968.7 [3′-*O*-sulfo lactosyl ceramide]$^-$, 726.5 [glucosyl ceramide]$^-$, 564.6 [ceramide]$^-$; calcd for $C_{73}H_{129}N_3NaO_{37}S_2$ [M−2Na]: 1726.7644; found: 1726.88.

4. MAG-glycolipid binding assays

MAG binding was quantified by measuring the adhesion of MAG-transfected cells or the binding or MAG-Fc chimeric protein to glycolipids adsorbed on multiwell plates. To simulate a membrane monolayer, glycosphingolipids were stably co-adsorbed with phosphatidylcholine and cholesterol onto the bottom of 96-well polystyrene multiwell plates as described in detail elsewhere.[28] Briefly, lipids were dissolved at the desired concentrations in ethanol containing 1 μM phosphatidylcholine and 4 μM cholesterol. An equal volume of water was added, and 50 μL of the solution was placed in each well of a 96-well multiwell plate (polystyrene, Corning-Costar #9017). Plates were left uncovered for 90 min to allow partial evaporation of the ethanol. This results in the lipids adsorbing onto the plastic surface as a monolayer, where they are stable in aqueous solutions. Prior studies indicated that co-adsorption of glycolipids with phosphatidylcholine and cholesterol resulted in equivalent adsorption efficiency (~ 50% of the added ganglioside), higher adsorption stability and enhanced recognition of immobilized glycolipids.[28] After ganglioside adsorption, the wells were washed with water and blocked by addition of 200 μL of BSA–PBS (1 mg/mL bovine serum albumin in Dulbecco's phosphate buffered saline) for 30 min at 37 °C.

To ensure that the different gangliosides tested adsorbed equivalently control experiments were performed, followed by lipid recovery and quantification. Synthetic and natural gangliosides were adsorbed at an input concentration of 200 pmol/well, blocked with BSA-containing buffer, then incubated with untransfected COS-1 cells (50,000 cells/well) for 45 min at 37 °C. The cells, which were non-adherent, were detached by controlled centrifugation in a custom-designed Plexiglas adhesion chamber.[28] After cell detachment, gangliosides were recovered from the microwell surfaces by washing each well with water then incubating with 200 μL of butanol. The butanol extract was evaporated, and the recovered gangliosides dissolved in a small volume of solvent and analyzed by quantitative thin-layer chromatography using resorcinol reagent for ganglioside detection.[31] Adsorption efficiency and stability among the different gangliosides tested varied over a twofold range. Most gangliosides (e.g., GM1a, GD1a) were recovered at 50% of the amount added. GSC335 was recovered at 35% and GSC338 at 26% of the amount added. Results are reported as cell adhesion or chimeric protein binding as a function of glycolipid added to the

well without correcting for adsorption efficiency over this range.

Adhesion of MAG-transfected COS-1 cells to immobilized glycolipids in microwell plates was determined as described previously.[28] Briefly, COS-1 cells were transiently transfected with an expression vector bearing the full length long form of rat MAG. After culturing to allow expression (48–72 h) cells were collected in an EDTA-containing buffer, washed by centrifugation, resuspended in buffer, and added at 50,000 cells/200 μL buffer to each well of glycolipid-adsorbed 96-well microplates. After 45 min to allow adhesion to proceed, the plate was immersed, inverted while immersed, placed in a custom-designed adhesion chamber, and subjected to a centrifugal detachment force of 110*g* for 10 min to remove non-adherent cells. The adhesion chamber was immersed in buffer, the plate recovered and righted, and adherent cells quantified by lysis (using Triton X-100) and analysis of released lactate dehydrogenase activity using a kinetic 96-well plate reader. Non-transfected COS-1 cells, or cells transfected with control expression vector, did not adhere to any glycolipid tested (Ref. 8 and data not shown).

Binding of MAG-Fc chimeric protein to immobilized glycolipids was determined as follows. An expression vector coding for a chimeric protein consisting of the entire extracellular domain of MAG fused via a three amino acid bridge (TGK) to the human Fc domain was produced using the 'pIgPlus' vector (Novagen, Madison, WI) as described previously.[29] The resulting construct was sequenced to confirm its identity, transfected into COS cells, and the resulting MAG-Fc chimera was purified from the culture medium using Protein G affinity chromatography. Binding of the chimera to microwell-adsorbed gangliosides was determined using a modified ELISA assay. After ganglioside adsorption and blocking (see above), wells were washed with PBS, and 50 μL of BSA–PBS containing 100 ng of MAG-Fc were added to each well (a concentration determined experimentally to result in half-maximal adhesion to a saturating concentration of the well-defined MAG ligand, ganglioside GD1a). After 1 h at ambient temperature, the wells were washed with PBS, and 50 μL of BSA–PBS containing 15 ng of biotinylated goat *anti*-human IgG (cat. no. H10115, F(ab′)2 Fc specific, Caltag Labs, Burlingame, CA) were added to each well. After 45 min at ambient temperature, the wells were washed with PBS, and 50 μL of BSA–PBS containing 17 ng of streptavidin–alkaline phosphatase (cat. no. 016-050-084, Jackson Immunoresearch Labs, West Grove, PA) were added. Finally, the wells were washed with enzyme buffer (100 mM Tris–HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5), 100 μL of enzyme buffer containing 2 mg/mL *p*-nitrophenylphosphate were added, and color development at 405 nm was determined kinetically using a microplate reader (Benchmark, Bio-Rad Labs, Hercules, CA). The data were fitted to rectangular hyperbolae with the ganglioside added per well as the 'x' value and the absorbance at 405 as the 'y' value, to provide an 'apparent K_D' for comparison of ganglioside potencies in supporting MAG-Fc binding.

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