Studies on Selectin Blockers. 4. Structure−Function Relationships of Sulfated Sialyl Lewis X Hexasaccharide Ceramides toward E-, P-, and L-Selectin Binding

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In order to clarify the real ligand structure of L-selectin proposed by Rosen et al., we first synthesized 6-sulfated sLe^x hexasaccharide ceramide **1**, 6′-sulfated sLe^x hexasaccharide ceramide **2**, and 6,6′-disulfated sLe^x hexasaccharide ceramide **3** and examined their binding avidities for L-selectin. As a result, we found that the 6′-sulfated sLe^x hexasaccharide ceramides **1**−**3** have similar binding avidities to L-selectin and their binding to L-selectin appeared somewhat stronger than that of sLe^x. For P-selectin, the sulfated sLe^x derivatives **1**−**3** showed a similar avidity to sLe^x. On the other hand, 6-sulfated sLe^x **2** was recognized to E-selectin and the binding avidity was apparently weak as compared to that of sLe^x hexasaccharide ceramide. Surprisingly, 6′-sulfated and 6,6′-disulfated sLe^{x}s **1** and **3** did not bind to E-selectin at all. We constructed the E-selectin−sLe^x complex model and investigated the binding mode. Namely, the galactose 6′-position was directed toward the negatively charged residues, Glu80 and Asp100. Our results with E-selectin indicate that the replacement of 6′-OH position from anionic charged group to cationic charged one, e.g., amino groups, could have a marked affect on E-selectin recognition. These results could provide useful information for the drug design of selectin blockers.

Introduction

Selectins (E-, P-, and L-selectin)[1−3] are a family of calcium-dependent adhesion molecules that can mediate spatially and temporally a specific rolling interaction between leukocytes and vascular endothelium prior to leukocyte extravasation. Although each selectin may have its own optimum carbohydrate ligand, it has been shown that all of these selectins can recognize oligosaccharides such as sialyl Lewis^x (sLe^x) and sialyl Lewis^a (sLe^a) that are linked to glycoproteins and glycolipids.[4] Their involvement in inflammatory diseases makes the selectins attractive targets for the therapy of these diseases.[5] Therefore, it is very important to identify the real ligand for each selectin. The analysis of the interaction between selectins and ligands is crucial to design a useful selectin blocker. Recently, three high endothelial venule (HEV) associated ligands for L-selectin have been identified as mucin-like O-linked glycoproteins (GlyCAM-1,[6] CD34,[7] MadCAM-1[8]), and one of these, GlyCAM-1, is well characterized among the three L-selectin ligands. Recently, Rosen et al.[9] have isolated two sulfated sLe^{x}s, Siaα2,3(6-SO_3)Galβ1,4-(Fucα1,3)GlcNAc (6′-sulfated sLe^x) and Siaα2,3Galβ1,4-(Fucα1,3) (6-SO_3)GlcNAc (6-sulfated sLe^x), by a mild hydrolysis of GlyCAM-1 and speculated that these sulfated sLe^{x}s might be structural determinants for binding to L-selectin. Additionally, Rosen proposed that 6,6′-disulfated sLe^x could also be a determinant on GlyCAM-1. In order to clarify the real ligand structure of L-selectin proposed by Rosen et al., we first synthesized 6-sulfated sLe^x, 6′-sulfated sLe^x, and 6,6′-disulfated sLe^x hexasaccharide ceramide and examined their binding avidity for L-selectin.

On the other hand, it has been known that L- and P-selectins can efficiently bind to sulfated carbohydrates such as sulfatides, fucoidan, and heparin.[10] In addition, recently, we have reported that sulfated Lewis^x analogs have potent inhibitory activities toward sLe^x pentasaccharide to E-, P-, and L-selectin bindings, respectively.[11] These data suggest that the 6-sulfated sLe^x, 6′-sulfated sLe^x, and 6,6′-disulfated sLe^x could also strongly bind to both E- and P-selectins. However, there is little information about the structure−function relationships of sulfated sLe^x toward E-, P-, and L-selectins binding.

In this report, we describe the first synthesis of 6-sulfated sLe^x , 6′-sulfated sLe^x, and 6,6′-disulfated sLe^x hexasaccharide ceramide, respectively, and their binding avidities toward E-, P-, and L-selectins.

Results and Discussion

Chemistry: Synthesis of 6′-Sulfated sLe^x Hexasaccharide Ceramide 1, 6-Sulfated sLe^x Hexasaccharide Ceramide 2, and 6,6′-Disulfated sLe^x Hexasaccharide Ceramide 3. Compounds **1**−**3** (Chart 1) were synthesized according to a published paper.[12] For the synthesis of compound **1** (Scheme 1), selective removal of the key intermediate **4** gave the corresponding 1-hydroxy compound, followed by the treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) for 2 h at 0 °C to afford the corresponding α-trichloroacetimidates **7** in a 91% yield. The glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-

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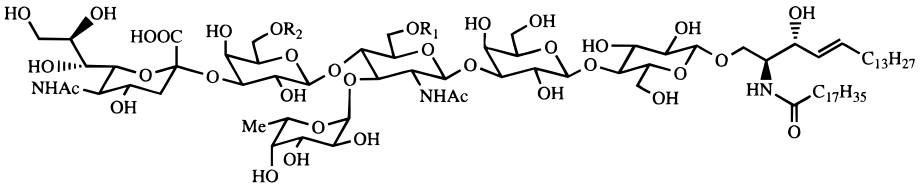
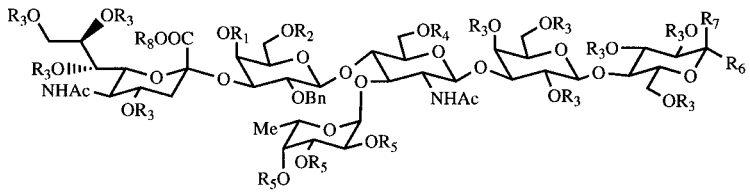
Chart 1

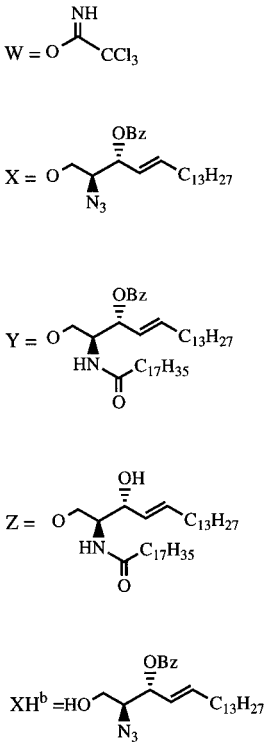


	R1	R2
1	H	SO_3Na
2	SO_3Na	H
3	SO_3Na	SO_3Na
sLe^x	H	H

Scheme 1[a]



		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
a	**4**	Bz	Lev	Ac	Ac	Ac	SE	H	Me
b	**7**	Bz	Lev	Ac	Ac	Ac	H	W	Me
c	**8**	Bz	Lev	Ac	Ac	Ac	X	H	Me
d	**9**	Bz	Lev	Ac	Ac	Ac	Y	H	Me
e	**16**	Bz	H	Ac	Ac	Ac	Y	H	Me
f	**17**	Bz	SO_3/Pyr	Ac	Ac	Ac	Y	H	Me
	1	H	SO_3Na	H	H	H	Z	H	H
a	**5**	Ac	Ac	Ac	Lev	Ac	SE	H	Me
b	**10**	Ac	Ac	Ac	Lev	Ac	H	W	Me
c	**11**	Ac	Ac	Ac	Lev	Ac	X	H	Me
d	**12**	Ac	Ac	Ac	Lev	Ac	Y	H	Me
e	**18**	Ac	Ac	Ac	H	Ac	Y	H	Me
f	**19**	Ac	Ac	Ac	SO_3/Pyr	Ac	Y	H	Me
	2	H	H	H	SO_3Na	H	Z	H	H
a	**6**	Bz	Lev	Ac	Lev	Ac	SE	H	Me
b	**13**	Bz	Lev	Ac	Lev	Ac	H	W	Me
c	**14**	Bz	Lev	Ac	Lev	Ac	X	H	Me
d	**15**	Bz	Lev	Ac	Lev	Ac	Y	H	Me
e	**20**	Bz	H	Ac	H	Ac	Y	H	Me
f	**21**	Bz	SO_3/Pyr	Ac	SO_3/Pyr	Ac	Y	H	Me
	3	H	SO_3Na	H	SO_3Na	H	Z	H	H
	sLe^x	H	H	H	H	H	Z	H	H



[a] Reagent and conditions: (a) CF_3CO_2H, CH_2Cl_2, CCl_3CN, DBU (**7**, 91%; **10**, 98%; **13**, 89%); (b) XH[b], BF_3·OEt_2, CH_2Cl_2 (**8**, 63%; **11**, 58%; **14**, 48%); (c) H_2S, pyridine, octadecanoic acid, WSC, CH_2Cl_2 (**9**, 54%; **12**, 60%, **15**, 60%); (d) NH_2NH_2, EtOH (**16**, 70%; **18**, 50%; **20**, 49%); (e) SO_3/pyridine, DMF (**17**, 98%; **19**, 91%; **21**, 91%); (f) NaOMe/MeOH (**1**, 98%; **2**, 98%; **3**, 98%).

octadecene-1,3-diol with **7** in CH_2Cl_2 for 5 h at 0 °C in the presence of boron trifluoride etherate and molecular sieves 4Å gave only the expected β-glycoside **8** in a 63% yield. Selective reduction of azido group in compound **8** with hydrogen sulfide in aqueous 83% pyridine for 72 h at 0 °C, followed by subsequent condensation with octadecanoic acid using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (WSC) in CH_2Cl_2 to afford a ganglioside derivative **9** in a 54% yield. Selective removal of the levulinoyl group from **9** was carried out in ethanol with hydrazine monoacetate at room temperature to give **16** in a 70% yield. Next, the treatment of compound **16** with sulfur trioxide−pyridine complex in DMF for 4 h at room temperature afforded

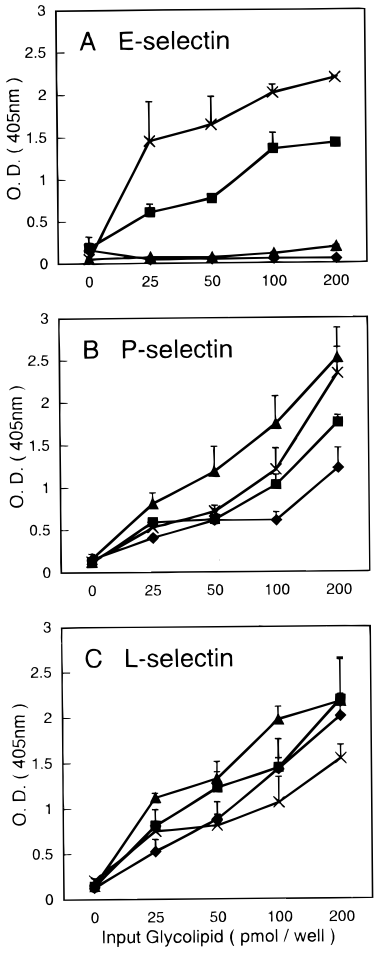


Figure 1. Selectin−Rg binding to A sulfoLe^x. Microtiter plate wells (96 wells) were coated with sLe^x (×), 6′-sulfoLe^x **1** (◆), 6-sulfoLe^x **2** (■) or 6,6′-sulfoLe^x **3** (▲) and blocked with 5% BSA (bovine serum albumin). The complex solution of biotinylated goat anti-human IgG(Fc), alkaline phosphatase-streptavidin, and selectin IgG chimera (E, P, or L) was added to the microtiter wells and allowed to react for 45 min at 37 °C. After each well was washed, the wells were developed for 1−2 h at room temperature by *p*-nitrophenyl phosphate, and absorbance at 405 nm was measured.

the corresponding sulfate **17** as a pyridine salt in a 98% yield. O-Deacylation of **17** with sodium methoxide in methanol, followed by subsequent saponification of the sialate methyl ester, yielded the desired sulfated sLe^x **1** in almost quantitative yield as a sodium salt. Compounds **2** and **3** were synthesized from the corresponding intermediates **5** and **6** by a similar method.

Biological Aspects: Selectins−Immunogloblin Binding to Plate-Coated Sulfated sLe^x Hexasaccharide Ceramides 1−3. The construction of selectin−immunoglobulin was carried out according to the previous paper.[13]

As shown in Figure 1, E-, P-, and L-selectins all recognized sLe^x hexasaccharide ceramide. This result agrees well with the accepted theory that E-selectin recognizes sLe^x and related oligosaccharides. Next, we found that the 6′-sulfated sLe^x hexasaccharide ceramides **1**, 6-sulfated sLe^x hexasaccharide ceramides **2**, and 6,6′-disulfated sLe^x hexasaccharide ceramides **3** have similar binding avidities for L-selectin. Their binding to L-selectin seemed to be little bit stronger than that of sLe^x. Although, from our data, it is not clear which sulfated sLe^x is a real ligand for L-selectin, the introduction of sulfo group at the 6-OH and/or 6′-OH positions made their binding to L-selectin favorable.

On the other hand, P-selectin−immunoglobulin also bound to sulfated sLe^x derivatives **1**−**3** with similar avidity as sLe^x. In addition, although the binding order of sulfated sLe^{x}s (**1**−**3**) to P-selectin was not distinct, their binding avidities increased in the order **3, 2, 1**. Recently, it has been reported that P-selectin binds to P-selectin glycoprotein ligand 1 (PSGL-1) expressed on neutrophil.[14] It is of interest that PSGL-1 synthesized in the presence of the sulfation inhibitors weakly binds to P-selectin,[15] and within the amino-terminal 20 residues, mutation of the tyrosines to phenylalanine abolishes binding. These data suggest that the sulfated PSGL-1 plays an important role to be well recognized by P-selectin. It is not clear if the P-selectin binding ligand (PSGL-1) contains the sulfated sLe^x or not. Our data suggest that sulfated sLe^{x}s **1**−**3** could be an intact ligand for P-selectin.

In contrast to the results with P- and L-selectins, sulfated sLe^x analogs **1**−**3** differed markedly in their binding activity with E-selectin. Surprisingly, 6′-sulfated and 6,6′-disulfated sLe^{x}s **1** and **3** did not bind to E-selectin at all. On the other hand, 6- sulfated sLe^x **2** was recognized with E-selectin and the binding avidity was apparently weak from that of sLe^x hexasaccharide ceramide. Our data suggest that the 6′-OH group of galactose of sLe^x would be a critically functional group recognized with E-selectin. On the contrary, it seems that the 6-OH group of glucosamine of sLe^x is not essential for binding to E-selectin. In order to clarify the importance of the 6′-OH group on sLe^x for binding to E-selectin, we constructed the molecular modeling of E-selectin−sLe^x complex based on the model reported previously[16] and investigated the conformational analysis around the 6′-OH group on sLe^x (Figure 2). Resultly, we got a similar model reported previously. Namely, it is the model of the E-selectin−carbohydrate complex in which the galactose 6′-position is directed toward the negatively charged residues, Glu80 and Asp100. If the compounds **1** and **3** bind in this mode, the modification of 6-OH group on sLe^x with some negatively charged groups e.g., sulfo group, carboxyl group, would be unfavorable for the binding to E-selectin. Our results with E-selectin indicate that the replacement of 6′-OH position from anionic charged group to cationic charged one, e.g., amino groups, could have a marked affect on E-selectin recognition.

These results will give an important information for the drug design of selectin blockers. There might be two possibilities for the reason why the 6′-OH position of sLe^x is critical for the binding to E-selectin. One possibility is that the 6′-OH group plays an important role in the binding to E-selectin. Another one is that the steric hindrance and/or the electric repulsion of 6′-sulfo group will make the insertion of ligand to E-selectin pocket difficult. To clarify our hypothesis, we are now trying to make 6′-amino sLe^x hexasaccharide ceramides which has a positive charged group at 6′-position of sLe^x.

Experimental Section

Construction, Expression, and Purification of Selectin−Immunogloblin Fusion Protein. Selectin-immunoglobrin fusion proteins (selectin−Ig) used in ELISA assays are recombinant chimeric molecules containing the lectin domain,

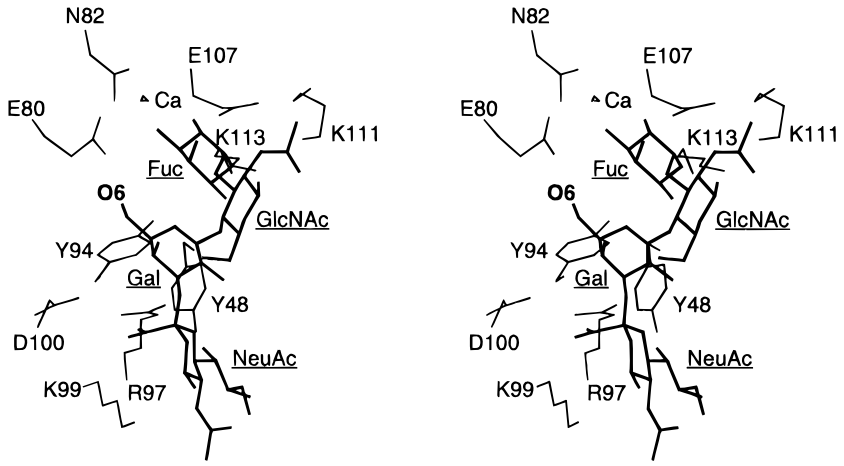


Figure 2. Stereorepresentation of the model of E-selectin−sLe^x complex. Thick and thin lines represent the structure of sLe^x and E-selectin, respectively. For E-selectin, only the side chains of the residues that contacted with the ligand are shown for clarity. Note that the 6-position of the galactose in sLe^x was directed toward the acidic residues Glu80 and Asp100.

epidermal growth factor domain, and one (L-selectin−Ig), two (P-selectin−Ig), or six (E-selectin−Ig) complement regulatory repeats coupled to the hinge, CH_2, and CH_3 regions of human IgG1. Corresponding E-selectin cDNA and P-selectin cDNA domains were amplified from HUVEC m-RNA by RT-PCR. L-Selectin domain was amplified from Jurkat cells m-RNA by RT-PCR. Selectin cDNAs were fused to the hinge and Fc region of human IgG1 heavy chain. Selectin−Ig was expressed in COS7(American Type Culture Collection CRL 1651) cells by transient transfection with selectin−Ig cDNA in pCDM8 vector (Funakoshi Co.) or expressed in CHO cells by stable transfection with selectin−Ig cDNA in pCIneo vector (Stratagene) by the lipofectamine (Gibco BRL). Selectin−Igs were affinity-purified from culture media using protein A silica gel (Nihon Gaishi Co.).

Selectin−Immunogloblin Binding to Plate-Coated Sulfated sLe^x Hexasaccharide Ceramides 1−3. A solution of sialyl Lewis^x hexasaccharide ceramide in 1:1 mixture of methanol and distilled water was pipetted into microtiter plate wells (96 wells) at 200, 100, 50, 25, or 0 pmol/well and adsorbed by evaporating the solvent. The wells were blocked with 5% BSA (bovine serum albumin)−50 mM imidazole buffer (pH7.2) supplemented with 1 mM $CaCl_2$ for 1 h at room temperature and washed with 50 mM imidazole buffer (pH7.2) after the blocking solution was discarded.

Separately, a mixture of 1:500 dilution of biotinylated goat anti-human IgG(Fc), 1:500 dilution of alkaline phosphatase-streptavidin, and E-selectin IgG chimera (2 μg/ml), P-selectin IgG chimera (20 μg/mL), or L-selectin IgG chimera (20 μg/mL) in 1% BSA- 50 mM imidazole buffer (pH 7.2) supplemented with 1 mM $CaCl_2$ was incubated for 30 min at room temperature to form a complex. The complex solution was then added to the above microtiter wells at 50 μl/well and allowed to react for 45 min at 37 °C. The wells were washed three times with 50 mM imidazole buffer (pH 7.2) and distilled water, respectively, and developed for 1−2 h at room temperature by adding 1 mg/mL of *p*-nitrophenyl phosphate and 0.01% of $MgCl_2$ in 1 M diethanolamine (pH 9.8) at 50 μL/well. The absorbance at 405 nm was measured (Figure 1).

Construction of the Model of the Complex of E-Selectin and sLex. All of the calculations were performed using AMBER 4.0.[17] The force-field parameters for carbohydrates were derived from GLYCAM_93, a general force field for carbohydrates suitable to AMBER, which takes account of exoanomeric effect.[18] The parameters for calcium ion were taken from Hamaguchi et al.[19] and Maynard et al.[20] Graphic manipulations and representations were performed by MidasPlus 2.0.[21] The crystal structures of the proteins used here were obtained from Protein Data Bank.[22] The electrostatic potential map of E-selectin was calculated with ESP program included in MidasPlus package.

The model of the complex of lectin-like domain of E-selectin and sLe^x was constructed in the mannar similar to that reported by Kogan et al.[23] First of all, the structure of sLe^x was constructed using the glycosidic torsion angles suggested by the NMR experiment of sLe^x with soluble E-selectin.[24,25] This initial structure of sLe^x was then placed on E-selectin, based on the crystal structure of rat mannose-binding protein (rMBP)−mannose complex.[26] rMBP is a calcium-dependent lectin homologous to E-selectin, and the structures of both proteins are very similar to each other.[27] The structure of the calcium ion and calcium-coordinated residues in the rMBP complex was first superposed on the equivalent calcium ion and metal ligands in the crystal structure of the lectin-like domain of E-selectin.[27] The mannose in the rMBP complex was then used as a template for the docking of sLe^x on E-selectin. That is, 2- and 3-hydroxyl groups of fucose in sLe^x were superposed on 4- and 3-hydroxyl group of the mannose, repectively. Finally, the coordinates of the rMBP complex was removed. The constructed model was then minimized until the root mean square of gradients was below 0.05 kcal/(mol·Å). During the minimization, sLe^x, the calcium ion, and the ligand binding site on E-selectin (Ala9−Tyr12, Tyr44−Gly52, Trp76−Leu114) were allowed to move. In the minimization, we used a nonbonded cutoff of 9 Å and a distance-dependent dielectric constant $\epsilon = 4r$.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*β*D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-*β*D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*D-galactopyranosyl)-(1−4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (7).** To a solution of **4**[10] (72.6 mg, 0.034 mM) in CH_2Cl_2 (4.5 mL), cooled to 0 °C, was added CF_3CO_2H (1.25 mL), and the mixture was stirred for 2 h at room temperature and concentrated. The product was purified by chromatography on a column of silica gel with AcOEt:hexane (4:1) to give the 1-hydroxy compound (63 mg). To a solution of this in CH_2Cl_2 (0.8 mL), cooled to 0 °C, were added trichloroacetonitrile (92 μL, 0.92 μM) and DBU (5.5 μL, 36 μM), the mixture was stirred for 2 h at 0 °C, and the progress of the reaction was monitored by TLC. The mixture was concentrated. Column chromatography (AcOEt:hexane, 4:1) of the residue on silica gel gave **7** (67 mg, 91%) as an amorphous mass: $[\alpha]_D$ +6.6° (*c* 1.4, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (d, J = 6.6 Hz, 3H, H-6f), 1.57−2.23 (13s, 51H, 14AcO, 2AcN, *Me*$COCH_2CH_2$), 2.38 (dd, J = 12.5 Hz, 4.4 Hz, 1H, H-3eq), 2.62−2.86 (m, 4H, Me$COCH_2CH_2$), 3.87 (s, 3H, MeO), 6.47 (d, J = 3.5 Hz, 1H, H-1a), 7.28−8.17 (m, 10H, 2Ph), 8.66 (s, 1H, C=NH). Anal. Calcd for ($C_{93}H_{116}N_3O_{51}Cl_3$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*β*D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-*β*D-glucopyranosyl)-**

(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1−1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (8). To a solution of **7** (83.6 mg, 0.038 mM) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (22, 32.5 mg, 76 μM) in dry CH_2Cl_2 (1 mL) was added MS-4Å (AW-300, 300 mg), and the mixture was stirred for 5 h at room temperature and then cooled to 0 °C. $BF_3{\cdot}OEt_2$ (10 mL, 37 μM) was added, and the mixture was stirred for a further 5 h at 0 °C. The solids were filtered off and washed with CH_2Cl_2. The combined filtrate and washings was washed with 1 M Na_2CO_3 and water, dried with Na_2SO_4, and concentrated. Column chromatography (AcOEt:hexane, 3:1) of the residue on silica gel gave **8** (59.5 mg, 63%) as an amorphous mass: $[\alpha]_D$ −7.5° (*c* 1.2, $CHCl_3$); IR (KBr) 3300, 2100, 1750, 1680, 1540, 1230, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.58, 1.78 (2s, 6H, 2AcN), 1.85−2.23 (15s, 45H, 14AcO, *Me*$COCH_2$-CH_2), 2.38 (dd, *J* = 12.6, 4.2 Hz, 1H, H-3eq), 2.62−2.81 (m, 4H, MeCOCH_2CH_2), 3.87 (s, 3H, MeO), 4.46 (d, *J* = 7.0 Hz, 1H, H-1a), 5.92 (dt, *J* = 14.7, 6.8 Hz, 1H, H-5 of sphingosine), 7.28−8.17 (m, 15H, 3Ph). Anal. Calcd for ($C_{116}H_{153}N_5O_{53}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1−1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (9).** Hydrogen sulfide was bubbled through a stirred solution of **8** (72.5 mg, 29 μM) in aqueous 83% pyridine (10 mL) for 3 days at 0 °C, with the progress of the reaction being monitored by TLC. The mixture was concentrated, and the residue was stirred with octadecanoic acid (17 mg, 60 μM) and 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (WSC, 17 mg, 89 μM) in dry CH_2Cl_2 (2 mL) for 12 h at room temperature. After completion of the reaction, CH_2Cl_2 (30 mL) was added to the mixture, and the solution was washed with water, dried with Na_2SO_4, and concentrated to a syrup that was chromatographed on a column of silica gel with AcOEt:hexane (4:1) to give **9** (43.2 mg, 54%) as an amorphous mass: $[\alpha]_D$ −5.1° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 6H, 2MeCH_2), 1.26 (s, 52H, 26H_2), 1.57, 1.78 (2s, 6H, 2AcN), 1.85−2.23 (15s, 45H, 14AcO, *Me*$COCH_2CH_2$), 2.38 (dd, *J* = 12.5, 4.2 Hz, 1H, H-3eq), 2.62−2.81 (m, 4H, MeCOCH_2CH_2), 3.87 (s, 3H, MeO), 5.87 (dt, *J* = 13.7, 6.8 Hz, 1H, H-5 of sphingosine), 7.27−8.17 (m, 15H, 3Ph). Anal. Calcd for ($C_{134}H_{189}N_3O_{54}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (10).** Selective removal of the 2-(trimethylsilyl)ethyl group in **5**[10] (220.5 mg, 0.11 μM) with CF_3CO_2H (4 mL) in CH_2Cl_2 (12 mL) for 2.5 h at room temperature and subsequent reaction of the product with trichloroacetonitrile (0.32 μL, 3.2 mM) in CH_2Cl_2 (1.8 mL) in the oresence of DBU (19 μL, 0.13 mM) for 2 h at 0 °C as described for **7** gave **10** (218.7 mg, 98%) as an amorphous mass: $[\alpha]_D$ +8.3° (*c* 1.7, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.24 (d, *J* = 7.1 Hz, 3H, H-6f), 1.49, 1.78 (2s, 6H, 2AcN), 1.91−2.20 (16s, 48H, 15AcO, *Me*$COCH_2CH_2$), 2.51 (dd, *J* = 12.8, 4.4 Hz, 1H, H-3eq), 2.69−2.79 (m, 4H, MeCOCH_2CH_2), 3.86 (s, 3H, MeO), 6.47 (d, *J* = 3.7 Hz, 1H, H-1a), 7.30−8.15 (m, 5H, Ph), 8.67 (s, 1H, C=NH). Anal. Calcd for ($C_{88}H_{114}N_3O_{51}Cl_3$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(4,6-di-O-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-levulinoyl-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1−1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (11).** Coupling of **10** (218.7 mg, 0.1 mM) with **22** (88 mg, 0.21 mM) in CH_2Cl_2 (2 mL) in the presence of $BF_3{\cdot}OEt_2$ (28 μL, 0.1 mM) and MS-4Å (AW-300, 600 mg) as described for **8** gave **11** (142 mg, 58%) as an amorphous mass: $[\alpha]_D$ −18.6° (*c* 1.2, $CHCl_3$); IR (KBr) 3300, 2100, 1750, 1680, 1540, 1230, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.58, 1.78 (2s, 6H, 2AcN), 1.90−2.20 (16s, 48H, 15AcO, *Me*$COCH_2$-CH_2), 2.51 (dd, *J* = 12.6, 4.6 Hz, 1H, H-3eq), 3.86 (s, 3H, MeO), 4.49 (d, *J* = 7.7, 1H, H-1a), 5.91 (dt, *J* = 14.1, 7.0 Hz, 1H, H-5 of sphingosine), 7.30−8.15 (m, 10H, 2Ph). Anal. Calcd for ($C_{111}H_{151}N_5O_{53}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(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 (12).** Selective reduction of the azido group in **11** (142 mg, 59 μM) with H_2S in aqueous 83% pyridine (20 mL) followed by coupling of the product with octadecanoic acid (34 mg, 0.12 mM) in the presence of WSC (34 mg, 0.18 mM) and workup as described for **9** gave **12** (91.5 mg, 60%) as an amorphous mass: $[\alpha]_D$ −9.8° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 6H, 2MeCH_2), 1.26 (s, 52H, 26H_2), 1.49, 1.78 (2s, 6H, 2AcN), 1.89−2.20 (16s, 48H, 15AcO, *Me*$COCH_2CH_2$), 2.51 (dd, *J* = 12.3 Hz, 4.2 Hz, 1H, H-3eq), 2.63−2.81 (m, 4H, MeCOCH_2CH_2), 3.86 (s, 3H, MeO), 5.07 (d, *J* = 2.9 Hz, 1H, H-1f), 5.86 (dt, *J* = 14.7 Hz, 7.3 Hz, 1H, H-5 of sphingosine), 7.30−8.14 (m, 10H, 2Ph). Anal. Calcd for ($C_{129}H_{187}N_3O_{54}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (13).** Selective removal of the 2-(trimethylsilyl)ethyl group in **6**[10] (109 mg, 0.05 μM) with CF_3CO_2H (2 mL) in CH_2Cl_2 (6 mL) for 2.5 h at room temperature and subsequent reaction of the product with trichloroacetonitrile (0.14 mL, 1.4 mM) in CH_2Cl_2 (0.9 mL) in the presence of DBU (8.5 μL, 0.057 mM) for 2 h at 0 °C as described for **7** gave **13** (99.5 mg, 89%) as an amorphous mass: $[\alpha]_D$ +8.3° (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.19 (d, *J* = 6.4 Hz, 3H, H-6f), 1.58, 1.78 (2s, 6H, 2AcN), 1.85−2.32 (15s, 45H, 13AcO, 2*Me*$COCH_2$-CH_2), 2.61−2.85 (m, 8H, 2MeCOCH2CH2), 3.87 (s, 3H, MeO), 5.64 (m, 1H, H-8e), 6.47 (d, *J* = 3.5 Hz, 1H, H-1a), 7.28−8.17 (m, 10H, 2Ph), 8.65 (s, 1H, C=NH). Anal. Calcd for ($C_{96}H_{120}N_3O_{52}Cl_3$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-levulinoyl-β-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1−1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (14).** Coupling of **13** (99.5 mg, 0.044 mM) with **22** (38 mg, 0.089 mM) in CH_2Cl_2 (1 mL) in the presence of $BF_3{\cdot}OEt_2$ (12 μL, 0.044 mM) and MS-4Å (AW-300, 300 mg) as described for **8** gave **11** (53 mg, 48%) as an amorphous mass: $[\alpha]_D$ −12.8° (*c* 1.1, $CHCl_3$); IR (KBr) 3350, 2100, 1750, 1690, 1540, 1220, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 3H, *Me*CH_2), 1.24 (s, 22H, 11CH_2), 1.58, 1.78 (2s, 6H, 2AcN), 1.85−2.23 (15s, 45H, 13AcO, 2*Me*$COCH_2CH_2$), 2.38 (dd, *J* = 12.3, 4.8 Hz, 1H, H-3eq), 2.63−2.78 (m, 8H, 2MeCOCH_2CH_2), 3.87 (s, 3H, MeO), 4.43 (d, *J* = 7.6 Hz, 1H, H-1a), 5.91 (dt, *J* = 13.6 Hz, 6.8 Hz, 1H, H-5 of sphingosine), 7.28−8.17 (m, 15H, 3Ph). Anal. Calcd for ($C_{119}H_{157}N_5O_{54}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−**

3)]-*O*-(2-acetamido-2-deoxy-6-*O*-levulinoyl-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 (15). Selective reduction of the azido group in **14** (53 mg, 21 μM) with H_2S in aqueous 83% pyridine (10 mL) followed by coupling of the product with octadecanoic acid (12 mg, 0.042 mM) in the presence of DBU (12 mg, 0.063 mM) and workup as described for **9** gave **15** (34.5 mg, 60%) as an amorphous mass: $[\alpha]_D$ −6.0° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.58, 1.78 (2s, 6H, 2AcN), 1.85−2.24 (15s, 45H, 13AcO, $2MeCOCH_2CH_2$), 2.38 (dd, J = 12.8, 4.8 Hz, 1H, H-3eq), 2.61−2.78 (m, 8H, $2MeCOCH_2CH_2$), 3.87 (s, 3H, MeO), 5.86 (dt, J = 14.4, 7.2 Hz, 1H, H-5 of sphingosine), 7.27−8.17 (m, 15H, 3Ph). Anal. Calcd for ($C_{137}H_{193}N_3O_{55}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 (16).** To a solution of **9** (10.7 mg, 0.0039 mM) in EtOH (0.3 mL) was added hydrazine monoacetate (0.4 mg, 0.0043 mM), and the mixture was stirred for 30 min at room temperature and then concentrated. Column chromatography (CH_2Cl_2:MeOH, 20:1) of the residue on silica gel gave **16** (7.0 mg, 70%) as an amorphous mass: $[\alpha]_D$ −6.4° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.54, 1.78 (2s, 6H, 2AcN), 1.86−2.22 (14s, 42H, 14AcO), 2.38 (dd, J = 12.1, 4.2 Hz, 1H, H-3eq), 3.77 (s, 3H, MeO), 5.86 (dt, J = 13.4, 6.7 Hz, 1H, H-5 of sphingosine), 7.27−8.18 (m, 15H, 3Ph). Anal. Calcd for ($C_{127}H_{183}N_3O_{52}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 Pyridine Salt (17).** To a solution of **16** (23.5 mg, 0.0089 mM) in DMF (0.5 mL) was added sulfur trioxide−pyridine complex (7 mg, 0.044 mM), and the mixture was stirred for 4 h at room temperature and then concentrated. Column chromatography ($CHCl_3$:MeOH, 5:1) of the residue on Sephadex LH-20 (20 g) gave the crude product, and this was purified by column chromatography on silica gel (20 g) with CH_2Cl_2:MeOH (15:1) to give **17** (24.2 mg, 98%) as an amorphous mass: $[\alpha]_D$ −3.3° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.55, 1.78 (2s, 6H, 2AcN), 1.88−2.24 (14s, 42H, 14AcO), 3.82 (s, 3H, MeO), 5.86 (dt, J = 14.7, 7.3 Hz, 1H, H-5 of sphingosine), 7.27−8.15 (m, 20H, 3Ph, C_5H_5N). Anal. Calcd for ($C_{134}H_{187}N_4O_{55}S$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 (18).** Selective hydrolysis of the levulinoyl group in **12** (91.1 mg, 0.034mM) with hydrazine monoacetate (3.8 mg, 0.041 mM) in EtOH (2.1 mL) and workup as described for **16** gave **18** (44.5 mg, 50%) as an amorphous mass: $[\alpha]_D$ −13.0° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.48, 1.78 (2s, 6H, 2AcN), 1.88−2.21 (15s, 45H, 15AcO), 2.53 (dd, J = 12.3, 4.4 Hz, 1H, H-3eq), 3.87 (s, 3H, MeO), 5.87 (dt, J = 13.4, 6.7 Hz, 1H, H-5 of sphingosine), 7.27−8.18 (m, 10H, 2Ph). Anal. Calcd for ($C_{124}H_{181}N_3O_{52}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl -*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-sulfo-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 Pyridine Salt (19).** To a solution of **18** (29 mg, 0.011 mM) in DMF (0.5 mL) was added sulfur trioxide−pyridine complex (9 mg, 0.057 mM), the mixture was stirred for 2 h at room temperature, and workup as described for **17** gave **19** (28 mg, 91%) as an amorphous mass: $[\alpha]_D$ −18.7° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.48, 1.80 (2s, 6H, 2AcN), 1.85−2.18 (15s, 45H, 15AcO), 3.80 (s, 3H, MeO), 5.86 (dt, J = 13.2, 6.6 Hz, 1H, H-5 of sphingosine), 7.27−8.24 (m, 15H, 2Ph, C_5H_5N). Anal. Calcd for ($C_{129}H_{185}N_4O_{55}S$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 (20).** Selective hydrolysis of the levulinoyl groups in **15** (34.2 mg, 0.012 mM) with hydrazine monoacetate (2.7 mg, 0.029 mM) in EtOH (0.7 mL) for 3 h at room temperature and workup as described for **16** gave **20** (16 mg, 49%) as an amorphous mass: $[\alpha]_D$ −6.3° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.55, 1.78 (2s, 6H, 2AcN), 1.82−2.24 (13s, 39H, 13AcO), 3.77 (s, 3H, MeO), 5.86 (dt, J = 14.8, 7.4 Hz, 1H, H-5 of sphingosine), 7.32−8.30 (m, 15H, 3Ph). Anal. Calcd for ($C_{127}H_{181}N_3O_{51}$) C, H, N.

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(2,4-di-*O*-benzoyl-6-*O*-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-*β*-D-glucopyranosyl)-(1−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(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 Pyridine Salt (21).** To a solution of **20** (10.5 mg, 0.004 mM) in DMF (0.5 mL) was added sulfur trioxide−pyridine complex (6.4 mg, 0.040 mM), the mixture was stirred for 2 h at room temperature, and workup as described for **17** gave **21** (10.6 mg, 91%) as an amorphous mass: $[\alpha]_D$ −8.0° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 6H, $2MeCH_2$), 1.26 (s, 52H, $26H_2$), 1.60−2.24 (15s, 45H, 13AcO, 2AcN), 3.78 (s, 3H, MeO), 5.86 (dt, J = 14.7, 7.3 Hz, 1H, H-5 of sphingosine), 7.26−8.25 (m, 25H, 3Ph, $2C_5H_5N$). Anal. Calcd for ($C_{137}H_{189}N_5O_{57}S_2$) C, H, N.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-(6-*O*-sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-*β*-D-glucopyranosyl-(1−1)-(2*S*,3*R*,4*E*)- 2-octadecanamido-4-octadecene-1,3-diol Disodium Salt (1).** To a solution of **17** (24.2 mg, 0.0087 mM) in MeOH (2 mL) and 1,4-dioxane (1 mL) was added NaOMe (600 mg, 28% NaOMe in MeOH), the mixture was stirred for 48 h at 20 °C, and then water (1 mL) was added. After completion of the reaction (24 h), the mixture was concentrated. Column chromatography ($CHCl_3$:MeOH:H_2O:Et_3N, 5:4:0.7:0.07) of the residue on Sephadex (40 g) gave **1** (15.5 mg, 98%) as an amorphous mass: $[\alpha]_D$ −5.7° (*c* 0.5, $CHCl_3$:MeOH:H_2O, 5:4:0.7); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 6H, $2MeCH_2$), 1.27 (s, 52H, $26H_2$), 2.31 (dd, J = 11.0, 4.0 Hz, 1H, H-3eq), 5.46 (dd, J = 6.8, 15.9 Hz, H-4 of sphingosine), 5.69 (dt, J = 7.7 Hz, 1H, H-5 of sphingosine); IR (KBr) 3600−3200, 2920, 2850, 1700, 1660, 1550 cm^{-1}; the mass spectrum of **1** (negative ion mode) showed the base peak at m/z 1794 $(M - Na)^-$ and 1771 $(M - 2Na)^{2-}$. The R_f value of **1** was 0.12 ($CHCl_3$:MeOH:H_2O).

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-*β*-D-glucopyranosyl)-(1−3)-*O*-*β*-D-**

galactopyranosyl-(1−4)-*O*-*β*-D-glucopyranosyl-(1−1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol Disodium Salt (2). To a solution of **19** (28 mg, 0.01 mM) in MeOH (2 mL) and 1,4-dioxane (1 mL) was added NaOMe (600 mg, 28% NaOMe in MeOH), and workup as described for **1** gave **2** (18.2 mg, 98%) as an amorphous mass: $[\alpha]_D$ −8.7° (*c* 0.6, $CHCl_3$:MeOH:H_2O, 5:4:0.7); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 6H, $2MeCH_2$), 1.28 (s, 52H, $26H_2$), 2.31 (m, 1H, H-3eq), 5.46 (m, H-4 of sphingosine), 5.69 (dt, J = 15.5, 7.0 Hz, 1H, H-5 of sphingosine); IR (KBr) 3600−3200, 2920, 2850, 1700, 1660, 1550 cm^{-1}; the mass spectrum of **2** (negative ion mode) showed the base peak at m/z 1794 $(M - Na)^-$ and 1771 $(M - 2Na)^{2-}$. The R_f value of **2** was 0.09 ($CHCl_3$:MeOH:H_2O).

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*2-nonulopyranosylonic acid)-(2−3)-*O*-(6-*O*-sulfo-*β*-D-galactopyranosyl-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)]-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-*β*-D-glucopyranosyl)-(1−3)-*O*-*β*-D-galactopyranosyl-(1−4)-*O*-*β*-D-glucopyranosyl-(1−1)-(2*S*,3*R*,4*E*)- 2-octadecanamido-4-octadecene-1,3-diol Disodium Salt (3).** To a solution of **21** (10.6 mg, 0.0036 mM) in MeOH (2 mL) and 1,4-dioxane (1 mL) was added NaOMe (600 mg, 28% NaOMe in MeOH), and workup as described for **1** gave **3** (6.8 mg, 98%) as an amorphous mass: $[\alpha]_D$ −10.3° (*c* 0.2, $CHCl_3$:MeOH:H_2O, 5:4:0.7); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.8, 6H, $2MeCH_2$), 1.27 (s, 52H, $26H_2$), 1.97, 2.03 (2s, 6H, 2AcN), 2.30 (m, 1H, H-3eq), 5.45 (m, H-4 of sphingosine), 5.70 (dt, J = 14.5, 6.8 Hz, 1H, H-5 of sphingosine); IR (KBr) 3600−3250, 2920, 2850, 1700, 1660, 1550 cm^{-1}; the mass spectrum of **3** (negative ion mode) showed the base peak at m/z 1895 $(M - Na)^-$. The R_f value of **3** was 0.07 ($CHCl_3$:MeOH:H_2O).

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