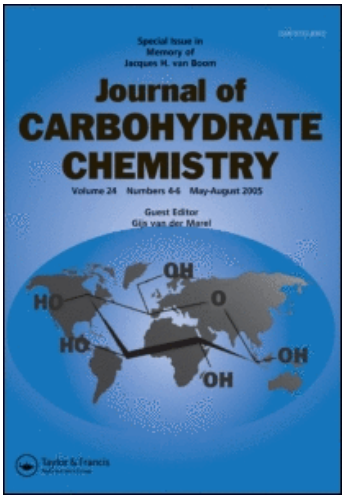

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Synthetic Studies on Sialoglycoconjugates 70: Synthesis of Sialyl and Sulfo Lewis × Analogs Containing a Ceramide or 2-(Tetradecyl)hexadecyl Residue

Akira Hasegawa[a]; Kenichi Ito[a]; Hideharu Ishida[a]; Makoto Kiso[a]
[a] Department of Applied Bioorganic Chemistry, Gifu University, Gifu, Japan

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SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 70: SYNTHESIS OF SIALYL AND SULFO LEWIS X ANALOGS CONTAINING A CERAMIDE OR 2-(TETRADECYL)HEXADECYL RESIDUE

Akira Hasegawa, Kenichi Ito, Hideharu Ishida and Makoto Kiso

Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11, Japan

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ABSTRACT

Four sialyl and sulfo Le^x analogs containing glucose in place of *N*-acetylglucosamine, and a ceramide or 2-(tetradecyl)hexadecyl residue, have been synthesized. Condensation of *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-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,4-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**1**) with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**2**) or 2-(tetradecyl)-hexadecyl-1-ol (**3**) gave the corresponding β-glycosides **4** and **7**. Compound **4** was converted into the ganglioside **6** *via* selective reduction of the azido group, coupling with octadecanoic acid, *O*-deacylation, and saponification of the methyl ester group. Hydrolysis of the *O*-acyl groups in **7** followed by saponification of the methyl ester, gave sialyl Le^x ganglioside analog **8** containing a branched fatty alkyl residue. On the other hand, glycosylation of *O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**13**), prepared from 2-(trimethylsilyl)ethyl *O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**9**) *via* selective 3-*O*-levulinylation, acetylation, removal of the 2-(trimethylsilyl)ethyl group, with **2** or **3**, gave the desired β-glycosides **14** and **19**. Selective reduction of the azido group in **14** followed by coupling with octadecanoic acid gave the ceramide derivative **16**. Removal of the levulinyl group in **16** and **19**, treatment with sulfur trioxide-

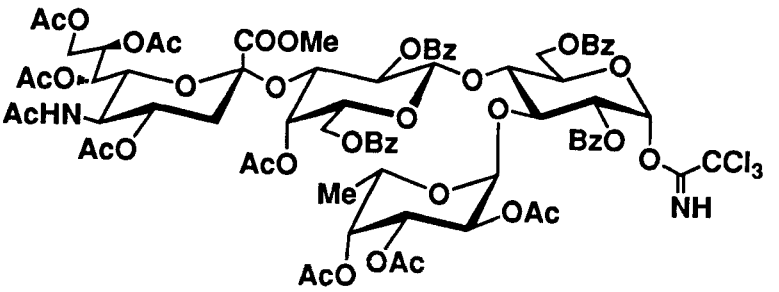
pyridine complex and subsequent hydrolysis of the protecting groups yielded the corresponding sulfo Le^x analogs **18** and **21**.

INTRODUCTION

There is now general agreement that all three selectins[1-4] [E-selectin (ELAM-1), L-selectin (LECAM-1) and P-selectin (PADGEM)] can recognize sialyl Lewis^x, sLe^x, α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-[α-L-Fuc-(1→3)]-β-GlcNAc; sialyl Lewis^a, sLe^a, α-Neu5Ac-(2→3)-β-D-Gal-(1→3)-[α-L-Fuc-(1→4)]-β-GlcNAc, which are found as the terminal carbohydrate structure of both cell-membrane glycolipids and glycoproteins, and the related oligosaccharides.[5-7] Recently, replacement of sialic acid with sulfate has received significant attention. For some time, it has been known that L- and P-selectin bind to fucoidan, sulphatides, a sulphated glucuronic acid (HNK-1) epitope and heparin.[8-11] Feizi's group[12,13] reported that E- and L-selectin can bind to sulfo-Le^x-like structures isolated from an ovarian cyst adenoma. In view of these facts, it is of interest to synthesize Le^x analogs containing sulfate in place of sialic acid for progress toward the goal of elucidating the structural features of this carbohydrate ligand required for selectin recognition. As a part of our continuing efforts along this line, we describe here the synthesis of sialo- and sulfo-Le^x analogs containing a ceramide, and also a branched alkyl residue in order to clarify the role of the ceramide moiety for selectin recognition.

RESULTS AND DISCUSSION

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-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,4-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate[14] (**1**) and *O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**13**) were selected as the glycosyl donors, while (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-



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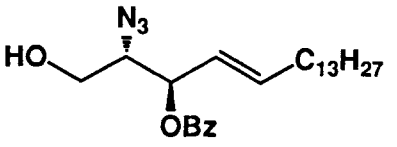

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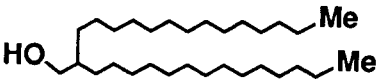

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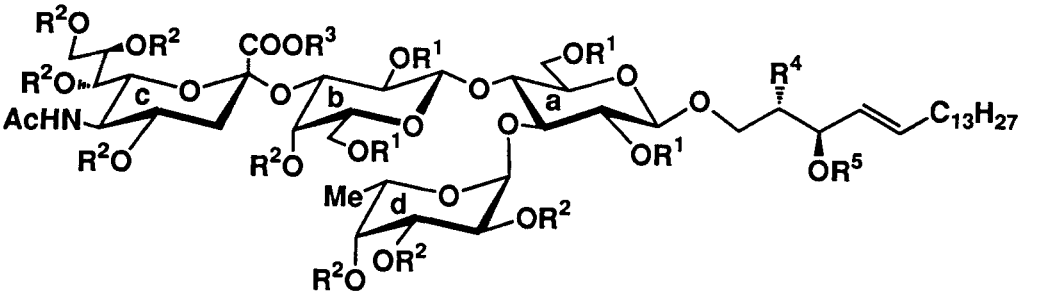
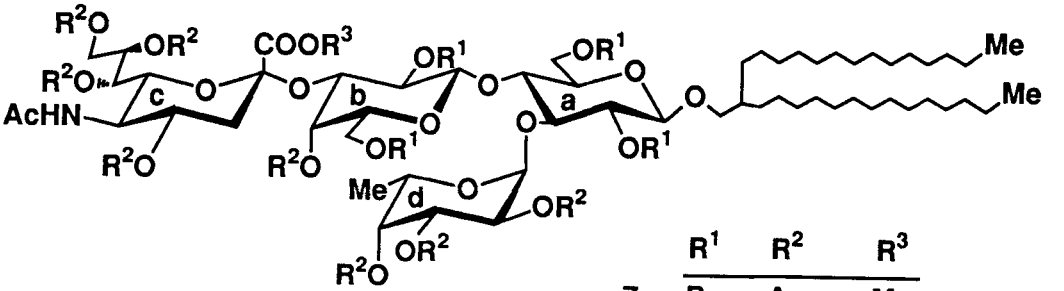


	R^1	R^2	R^3	R^4	R^5
4	Bz	Ac	Me	N_3	Bz
5	Bz	Ac	Me	$NHCO(CH_2)_{16}Me$	Bz
6	H	H	H	$NHCO(CH_2)_{16}Me$	H



	R^1	R^2	R^3
7	Bz	Ac	Me
8	H	H	H

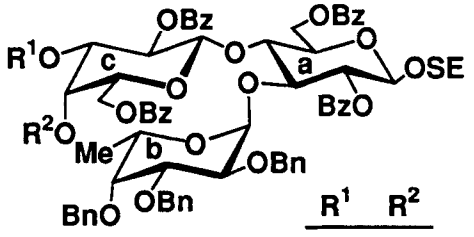
Bz = benzoyl

1,3-diol[15] (**2**) and 2-(tetradecyl)hexadecyl-1-ol[16] (**3**) served as the acceptors in the synthesis of the target sialo- and sulfo-Le^x analogs.

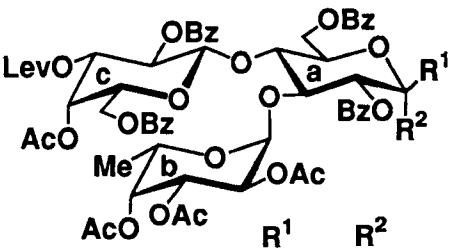
Glycosylation[17] of **2** or **3** with **1**, in dichloromethane in the presence of boron trifluoride etherate and molecular sieves 4Å, gave exclusively the β-glycosides **4** and **7** in 68 and 66%, respectively. Selective reduction[18] of the azido group in **4** with hydrogen sulfide in aq 83% pyridine, and subsequent condensation with octadecanoic acid, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) in dichloromethane furnished a good yield of the acylated ganglioside analog **5** in 85% yield. *O*-Deacylation of **5** or **7** with sodium methoxide in methanol, with subsequent saponification of the methyl ester group, gave the desired sLe^x ganglioside analogs **6** and **8** in quantitative yield, respectively. The ^{1}H NMR data for the Glc unit in **6** [δ 4.17 ($J_{1,2}$ = 7.7 Hz, H-1)] and **8** [δ 4.16 ($J_{1,2}$ = 7.9 Hz)] established the anomeric configuration to be β.

Treatment of 2-(trimethylsilyl)ethyl *O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,6-di-*O*-benzoyl-β-D-glucopyranoside[14] (**9**) with levulinic anhydride in pyridine-dichloromethane in the presence of 4-dimethylaminopyridine for 3 h at -50 °C gave the expected 3-*O*-levulinyl derivative **10** in 88% yield. A significant signal in the ^{1}H NMR spectrum of **10** was a one-proton doublet of doublets at δ 5.04 ($J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.1 Hz), indicating the levulinylated position to be at the C-3 hydroxyl of the Gal residue. Catalytic hydrogenolysis of the benzyl groups in **10** in ethanol-acetic acid and subsequent *O*-acetylation gave the per-*O*-acyl compound **11** in quantitative yield. Treatment[19] of **11** with trifluoroacetic acid in dichloromethane gave the 1-hydroxy compound **12** in 92% yield, which was reacted with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the α-trichloroacetimidate **13** in 95% yield. The ^{1}H NMR data for the Glc unit in **13** [δ 6.45 ($J_{1,2}$ = 3.7 Hz), 8.46 (C = NH)] established the anomeric configuration of the imidate.

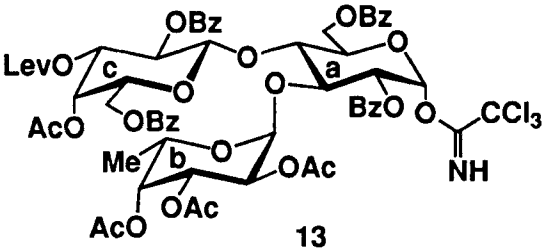
Glycosylation of **2** or **3** with **13** thus obtained, in essentially the same way as described for the synthesis of **4**, gave the desired β-glycosides **14** and **19** in 85 and 69% yields, respectively. Selective reduction of the azido group in **14**, and subsequent condensation with octadecanoic acid, as described above, afforded the per-*O*-acylated



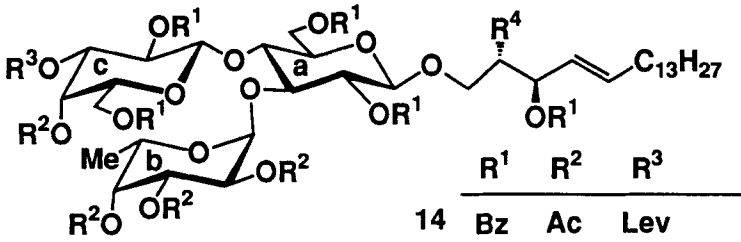
	R^1	R^2
9	H	H
10	Lev	H



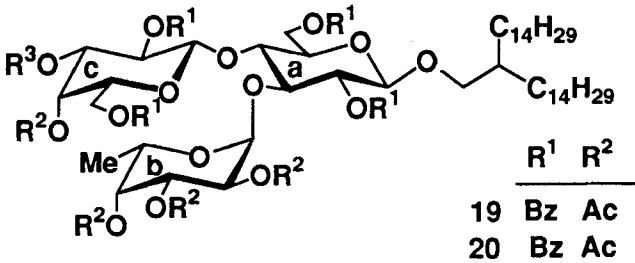
	R^1	R^2
11	OSE	H
12	OH, H	



13



	R^1	R^2	R^3	R^4
14	Bz	Ac	Lev	N_3
15	Bz	Ac	Lev	NH_2
16	Bz	Ac	Lev	$NHCO(CH_2)_{16}Me$
17	Bz	Ac	H	$NHCO(CH_2)_{16}Me$
18	H	H	SO_3Na	$NHCO(CH_2)_{16}Me$



	R^1	R^2	R^3
19	Bz	Ac	Lev
20	Bz	Ac	H
21	H	H	SO_3Na

Lev = $CH_3\overset{O}{\overset{\|}{C}}CH_2CH_2\overset{O}{\overset{\|}{C}}-$

Le^x sphingolipid **16** in good yield. Treatment of **16** or **19** in ethanol-tetrahydrofuran with hydrazine monoacetate at room temperature gave the 3-hydroxy derivatives **17** and **20** in high yields, respectively. Treatment of compounds **17** or **20** with sulfur trioxide-pyridine complex in *N,N*-dimethylformamide for one h at room temperature and subsequent *O*-deacylation with sodium methoxide in methanol yielded the desired sulfo Le^x analogs **18** and **21** as their sodium salts in good yields.

These gangliosides and sulfo Le^x derivatives were tested[20] by Dr. B. K. Brandley of Glycomed, Inc., Alameda, CA, USA, according to his published method.[6] In this system, all three selectins binded efficiently to compound **21** with a sulfate in place of sialic acid. For E-selectin, binding to **21** appeared to be equivalent to that of sLe^x ganglioside, while for L- and P-selectins, binding to **21** showed characteristics distinct from sLe^x ganglioside. Interestingly, the selectin binding to sulfo Le^x lipid **21** containing a branched alkyl residue was effectively distinct from that of the sulfo Le^x ceramide **18**, indicating that lipid aglycon can effect for selectin recognition.

EXPERIMENTAL

General Procedures. Specific rotations were determined with a Union PM-201 polarimeter at 25 °C and IR spectra were recorded with a Jasco A-100 spectrophotometer. ^1H NMR spectra were recorded with a JEOL JNM-GX 270 spectrometer. Electroscopy mass spectra were recorded on an API-III triple quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments, Thornhill, Canada) fitted with an atmospheric pressure ionization source. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

***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-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2,6-di-*O*-benzoyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (4).** To a solution of the

trichloroacetimidate[14] (**1**, 140 mg, 0.083 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[15] (**2**, 71 mg, 0.17 mmol) in dry CH_2Cl_2 (2 mL) were added molecular sieves 4Å [MS-4Å (AW-300), 400 mg] and the mixture was stirred for 6 h at room temperature, then cooled to 0 °C. Boron trifluoride etherate (21 μL, 0.17 mmol) was added and the mixture was stirred for 5 h at 0 °C then filtered. Dichloromethane (50 mL) was added, and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), then concentrated. Column chromatography (3:2 EtOAc-hexane) of the residue on silica gel (60 g) gave **4** (110 mg, 68%) as an amorphous mass: $[\alpha]_D$ -9.2° (*c* 1.0, $CHCl_3$); IR (KBr) 3400 (NH), 2100 (N_3), 1740 and 1230 (ester), 1680 and 1550 (amide), and 740 and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.33 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6d), 1.43-2.20 (9s, 27H, 8AcO, AcN), 2.51 (dd, 1H, Jgem = 12.5 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3c-*eq*), 3.76 (s, 3H, MeO), 4.45 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1a), and 7.26-8.40 (m, 25H, 5Ph).

Anal. Calcd for $C_{99}H_{120}N_4O_{37}$ (1958.0): C, 60.73; H, 6.18; N, 2.86. Found: C, 60.57; H, 5.99; N, 2.93.

***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-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (5).** Hydrogen sulfide gas was bubbled through a solution of **4** (110 mg, 0.056 mmol) in aq 83% pyridine (5 mL) for 48 h while the solution was stirred at 0 °C. The mixture was concentrated to a syrup, which was used without purification. A solution of the residue in dry CH_2Cl_2 (2 mL) was treated with octadecanoic acid (32 mg, 0.11 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 32 mg, 0.17 mmol), and the mixture was stirred for 8 h at room temperature. Dichloromethane (30 mL) was added and the mixture was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 acetone-hexane) of the residue on silica gel (20 g) gave **5** (105 mg, 85%) as an amorphous mass: $[\alpha]_D$ -3.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6d), 1.41-2.23 (9s, 27H, 8AcO, AcN), 2.51 (dd, 1H, J_{gem} = 12.5 Hz,

$J_{3eq,4}$ = 4.9 Hz, H-3c-*eq*), 3.75 (s, 3H, MeO), 4.39 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1a), 5.64 (m, 1H, H-8c), 5.72 (dt, 1H, $J_{4,5}$ = 14.4 Hz, $J_{5,6}$ = $J_{5',6}$ = 7.0 Hz, H-5 of ceramide), and 7.23-8.19 (m, 25H, 5Ph).

Anal. Calcd for $C_{117}H_{156}N_2O_{38}$ (2198.5): C, 63.92; H, 7.15; N, 1.27. Found: C, 63.79; H, 6.92; N, 1.21.

***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*-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (6).** To a solution of **5** (105 mg, 0.048 mmol) in MeOH (5 mL) was added NaOMe (10 mg) and the mixture was stirred for 24 h at 40 °C. Potassium hydroxide (0.2 M, 5 mL) was added and the mixture was stirred an additional 6 h at room temperature, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 $CHCl_3$-MeOH. The filtrate and washings were combined and concentrated. Column chromatography (5:4:0.7 $CHCl_3$-MeOH-H_2O) of the residue on Sephadex LH-20 (30 g) gave **6** (63.3 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -21.5° (*c* 1.1, 1:1 $CHCl_3$-MeOH); ^1H NMR (Me_2SO-*d*6) δ 0.85 (t, 6H, 2*Me*CH_2), 1.00 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6d), 1.24 (s, 52H, 26CH_2), 1.89 (s, 3H, AcN), 2.76 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.7 Hz, H-3c-*eq*), 4.17 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.30 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1b), 5.22 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1d), 5.36 (dd, 1H, $J_{3,4}$ = 6.6 Hz, $J_{4,5}$ = 15.2 Hz, H-4 of ceramide), and 5.56 (dt, 1H, $J_{5,6}$ = $J_{5',6}$ = 6.6 Hz, H-5 of ceramide).

Anal. Calcd for $C_{65}H_{118}N_2O_{25}$ (1327.7): C, 61.88; H, 5.99; N, 2.11. Found: C, 61.70; H, 5.84; N, 2.08.

2-(Tetradecyl)hexadecyl *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-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,6-di-*O*-benzoyl-β-D-glucopyranoside (7). Coupling of **1** (65.3 mg, 0.04 mmol) and 2-(tetradecyl) hexadecyl-1-ol[16] (**3**, 34 mg, 0.077 mmol), as described for **4**, gave **7** (51 mg, 66%) as an amorphous mass: $[\alpha]_D$ -0.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88-1.26 (m, 58H, 2Me, 26CH_2), 1.33 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6d), 1.43-2.20 (9s, 27H, 8AcO, AcN), 2.52 (dd, 1H, J_{gem} = 12.5 Hz, H-3*eq*,4 = 4.6 Hz, H-3c-*eq*), 3.07, 3.52 (2dd,

2H, J_{gem} = 9.4 Hz, H-1, H-1' of fatty alkyl), 3.77 (s, 3H, MeO), 4.33 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 5.28 (d, 1H, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 10.5 Hz, H-7c), 5.46 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1d), 5.63 (m, 1H, H-8c), and 7.27-8.22 (m, 20H, 4Ph).

Anal. Calcd for $C_{104}H_{143}NO_{35}$ (1967.3): C, 63.50; H, 7.33; N, 0.71. Found: C, 63.30; H, 7.30; N, 0.83.

2-(Tetradecyl)hexadecyl *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)]-β-D-glucopyranoside (8). Deacylation and saponification of **7** (105 mg, 0.048 mmol), as described for **6**, yielded **8** (64 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -31.5° (*c* 1.4, 1:1 $CHCl_3$-MeOH), ^1H NMR (Me_2SO-*d6*) δ 0.86 (t, 6H, 2*Me*CH_2), 0.91 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6d), 1.25 (s, 52H, 26CH_2), 1.89 (s, 3H, AcN), 2.85 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.9 Hz, H-3c-*eq*), 4.16 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.39 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1b), and 5.16 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1d).

Anal. Calcd for $C_{59}H_{109}NO_{23}$ (1200.5): C, 59.03; H, 9.15; N, 1.17. Found: C, 58.99; H, 9.03; N, 1.22.

2-(Trimethylsilyl)ethyl *O*-(2,6-Di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,6-di-*O*-benzoyl-β-D-glucopyranoside (10). To a solution of 2-(trimethylsilyl)ethyl *O*-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,6-di-*O*-benzoyl-β-D-glucopyranoside[14] (**9**, 450 mg, 0.35 mmol) and 4-dimethylaminopyridine (DMAP, 5 mg) in pyridine (5 mL) and CH_2Cl_2 (2 mL), cooled to -50 °C, was added a solution of levulinic anhydride (150 mg, 0.71 mmol) in CH_2Cl_2 (2 mL) and the mixture was stirred for 3 h. Methanol (1 mL) was added to the mixture, concentrated then extracted with CH_2Cl_2. The extract was successively washed with 2M HCl, M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 EtOAc-hexane) of the residue on silica gel (100 g) gave **10** (426 mg, 88%) as an amorphous mass: $[\alpha]_D$ -5.5° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.44 (m, 2H, $Me_3SiCH_2CH_2O$), 1.56 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 2.33 (s, 3H, AcO), 2.64, 2.90 (2m, 4H, $MeCOCH_2CH_2$), 4.97 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1b), 5.04 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.1 Hz, H-3c), 5.77 (dd, 1H, $J_{1,2}$ = 7.9 Hz, H-2c), and 7.14-8.31 (m, 35H, 7Ph).

Anal. Calcd for $C_{77}H_{84}O_{21}Si$ (1373.6): C, 67.33; H, 6.16. Found: C, 67.33; H, 6.00.

2-(Trimethylsilyl)ethyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,4-di-*O*-benzoyl-β-D-glucopyranoside (11). A solution of **10** (223 mg, 0.16 mmol) in EtOH (8 mL) and AcOH (2 mL) was hydrogenolyzed in the presence of 10% Pd-C (300 mg) for 12 h at 45 °C; the progress of the reaction was monitored by TLC. The precipitate was filtered off and washed with EtOH. The combined filtrate and washings was concentrated. The residue was acetylated with Ac_2O (2 mL)-pyridine (3 mL) in the presence of DMAP (5 mg) for 10 h at room temperature. Methanol (1 mL) was added to the mixture, and this was concentrated then extracted with CH_2Cl_2. The extract was washed with 2M HCl, M Na_2CO_3 and water, dried (Na_2SO_4) then concentrated. Column chromatography (1:3 EtOAc-hexane) of the residue on silica gel (60 g) gave **11** (210 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -17.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (m, 2H, $Me_3SiCH_2CH_2O$), 1.57 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 2.06-2.42 (5s, 15H, 5AcO), 2.56, 2.73 (2m, 4H, $MeCOCH_2CH_2$), 4.61 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 5.26 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.7 Hz, H-3c), and 7.16-8.34 (m, 20H, 4Ph).

Anal. Calcd for $C_{64}H_{74}O_{25}Si$ (1271.4): C, 60.46; H, 5.87. Found: C, 60.33; H, 5.75.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-2,6-di-*O*-benzoyl-β-D-glucopyranose (12).** A solution of **11** (170 mg, 0.13 mmol) in CH_2Cl_2 (1 mL) and CF_3CO_2H (2 mL) was stirred for 2 h at room temperature. Ethyl acetate (1 mL) was added and concentrated to a syrup which was chromatographed on a column of silica gel (20 g) with 1:2 EtOAc-hexane to give **12** (144 mg, 92%) as an amorphous mass: $[\alpha]_D$ +11.2° (*c* 0.8, after 5 h in $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.26 (d, 3H, $J_{5,6}$ = 7.1 Hz, H-6b), 1.64-2.17 (5s, 15H, 5AcO), 2.33, 2.53 (2m, 4H, $MeCOCH_2CH_2$), 4.11 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1a), 5.22 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1b), 5.73 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4c), and 7.16-8.10 (m, 20H, 4Ph).

Anal. Calcd for $C_{59}H_{62}O_{25}$ (1171.1): C, 60.51; H, 5.34. Found: C, 60.27; H, 5.08.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranosyltrichloroacetimidate (13).** To a solution of **12** (653 mg, 0.59 mmol) in CH_2Cl_2 (9 mL) and trichloroacetonitrile (1.8 mL, 17.7 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 41.8 μL, 0.29 mmol), and the mixture was stirred for 2 h at 0 °C then concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (100 g) gave **13** (700 mg, 95%) as an amorphous mass: $[\alpha]_D$ +57.0° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.40 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6b), 1.71-2.17 (5s, 15H, 5AcO), 2.37, 2.53 (2m, 4H, MeCOCH_2CH_2), 4.83 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1c), 5.26 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.4 Hz, H-2a), 5.35 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 2.9 Hz, H-3c), 5.65 (dd, 1H, H-2c), 5.75 (d, 1H, H-4c), 6.48 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1a), 7.38-8.09 (m, 20H, 4Ph), and 8.46 (s, 1H, NH).

Anal. Calcd for $C_{61}H_{62}Cl_3NO_{25}$ (1315.2): C, 55.69; H, 4.75; N, 1.06. Found: C, 55.58; H, 4.67; N, 1.09.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (14).** To a solution of **13** (233 mg, 0.17 mmol) and **2** (149 mg, 0.35 mmol) in CH_2Cl_2 (2 mL) was added MS-4Å (400 mg), and the mixture was stirred for 6 h at room temperature then cooled to 0 °C. Boron trifluoride etherate (0.043 mL) was added to the mixture, and this was stirred for 5 h at 0 °C. The precipitate was filtered off and washed with CH_2Cl_2. The filtrate and washings were combined, and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (1:4 EtOAc-hexane) of the residue on silica gel (60 g) gave **14** (230 mg, 85%) as an amorphous mass: $[\alpha]_D$ +4.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, *Me*CH_2), 1.25 (s, 22H, 11CH_2), 1.40 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.89-2.25 (5s, 15H, 5AcO), 2.38, 2.56 (2m, 4H,

MeCO*CH2CH2*), 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1a), 4.75 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1c), 5.26 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1b), and 7.27-8.15 (m, 25H, 5Ph).

Anal. Calcd for $C_{84}H_{99}N_3O_{27}$ (1582.7): C, 63.75; H, 6.31; N, 3.54. Found: C, 63.70; H, 6.28; N, 3.34.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (16).** Hydrogen sulfide was bubbled through a solution of **14** (130 mg, 0.082 mmol) in aq 83% pyridine (5 mL) for 48 h while the solution was stirred at 0 °C. The mixture was concentrated to a syrup, which was dissolved in dry CH_2Cl_2 (2 mL). Octadecanoic acid (47 mg, 0.164 mmol) and WSC (47 mg, 0.246 mmol) were added to the solution, and the mixture was stirred for 8 h at room temperature. Dichloromethane (50 mL) was added, and the solution was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (1:3 acetone-hexane) of the residue on silica gel (30 g) gave **16** (112 mg, 75%) as an amorphous mass: $[\alpha]_D$ -23.5° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6b), 1.88-2.23 (5s, 15H, 5AcO), 2.55, 3.44 (2m, 4H, MeCO*CH2CH2*), 4.38 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.69 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1c), 5.05 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), 5.74 (dt, 1H, $J_{4,5}$ = 14.5 Hz, $J_{5,6}$ = $J_{5',6}$ = 7.1 Hz, H-5 of ceramide), and 7.22-8.11 (m, 25H, 5Ph).

Anal. Calcd for $C_{102}H_{135}NO_{28}$ (1823.2): C, 67.20; H, 7.46; N, 0.77. Found: C, 67.29; H, 7.48; N, 0.85.

***O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2,6-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (17).** To a solution of **16** (144 mg, 0.13 mmol) in EtOH (2 mL) and tetrahydrofuran (THF, 0.5 mL) was added hydrazine monoacetate (8.4 mg, 0.12 mmol), and the mixture was stirred for 10 h at room temperature then concentrated. Column chromatography (1:2 EtOAc-hexane) of the residue on silica gel (30 g) gave **17** (74.5 mg, 70%) as an amorphous mass: $[\alpha]_D$ -46.1° (*c* 0.9, $CHCl_3$);

^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 1.88-2.24 (4s, 12H, 4AcO), 4.38 (bs, 1H, OH), 4.39 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 5.75 (dt, 1H, $J_{4,5}$ = 13.9 Hz, $J_{5,6}$ = $J_{5',6}$ = 6.7 Hz, H-5 of ceramide), and 7.22-8.15 (m, 25H, 5Ph).

Anal. Calcd for $C_{97}H_{129}NO_{26}$ (1725.1): C, 67.54; H, 7.54; N, 0.81. Found: C, 67.29; H, 7.48; N, 0.71.

***O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-*O*-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol sodium salt (18).** To a solution of **17** (74.5 mg, 0.04 mmol) in *N*,*N*-dimethylformamido (DMF, 2 mL) was added sulfur trioxide-pyridine complex (34 mg, 0.2 mmol) and the mixture was stirred for one h at room temperature; the course of the reaction was monitored by TLC. Methanol (0.5 mL) was added, and the mixture was concentrated at 25 °C. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel (30 g) gave the pyridine salt (75 mg, 94%) as an amorphous mass: $[\alpha]_D$ -8.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, 2*Me*CH_2), 1.26 (s, 52H, 26CH_2), 1.79-2.12 (4s, 12H, 4AcO), 4.38 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 5.73 (dd, 1H, H-3c), and 7.11-8.08 (m, 30H, 5Ph, C_5H_5N). To a solution of the pyridine salt (75 mg, 0.04 mmol) in MeOH (2 mL) and THF (1 mL) was added sodium methoxide (5 mg) and the mixture was stirred for 24 h at room temperature then concentrated at 25 °C. Column chromatography (5:4:0.7 $CHCl_3$-MeOH-H_2O) of the residue on Sephadex LH-20 (30 g) gave **18** (46.2 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -4.7° (*c* 1.0, 1:1 $CHCl_3$-MeOH); ^{1}H NMR (Me_2SO-*d*6) δ 0.85 (t, 6H, 2*Me*CH_2), 1.01 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.24 (s, 52H, 26CH_2), 4.16 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.39 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1c), 5.24 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1b), 5.36 (dd, 1H, $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 15.6 Hz, H-4 of ceramide), and 5.52 (dt, 1H, $J_{5,6}$ = $J_{5',6}$ = 6.9 Hz, H-5 of ceramide). The mass spectrum of **18** (negative ion mode) showed the base peak at *m/z* 1154.4 $(M-H)^-$.

2-(Tetradecyl)hexadecyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-levulinyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranoside (19). To a

solution of **13** (700 mg, 0.51 mmol) and 2-(tetradecyl)hexadecanol **3** (363 mg, 0.81 mmol) in CH_2Cl_2 (8.8 mL) was added MS-4Å (1.7 g) and the mixture was stirred for 6 h at room temperature then cooled to 0 °C. Boron trifluoride etherate (0.13 mL, 1.03 mmol) was added to the mixture, and this was stirred for 5 h at room temperature. A workup as described for **14** gave **19** (585 mg, 69%) as an amorphous mass: $[\alpha]_D$ -67.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88-1.43 (m, 58H, 2*Me*, 26CH_2), 1.82-2.15 (5s, 15H, 4AcO, Ac), 2.34, 3.67 (2dd, 2H, J_{gem} = 9.3 Hz, H-1 and H-1' of alkyl residue), 4.37 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.79 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1c), 5.22 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.8 Hz, H-3c), 5.47 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1b), 5.53 (dd, 1H, H-2c), 5.72 (dd, 1H, H-4c), and 7.35-8.13 (m, 20H, 4Ph).

Anal. Calcd for $C_{89}H_{122}O_{25}$ (1591.9): C, 67.15; H, 7.72. Found: C, 67.11; H, 7.67.

2-(Tetradecyl)hexadecyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-benzoyl-β-D-glucopyranoside (20). To a solution of **19** (583.5 mg, 0.36 mmol) in EtOH (15 mL) was added hydrazine monoacetate (168 mg, 0.18 mmol) and the mixture was stirred for one h at room temperature then concentrated. Column chromatography (1:3 EtOAc-hexane) of the residue gave **20** (545 mg, quantitative) as an amorphous mass: $[\alpha]_D$ -25.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85-1.33 (m, 58H, 2*Me*, 26CH_2), 1.59-2.11 (4s, 12H, 4AcO), 3.09, 3.79 (2dd, 2H, J_{gem} = 9.3 Hz, H-1 and H-1' of alkyl residue), 4.38 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), 4.63 (bs, 1H, OH), 4.67 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1c), 5.60 (d, 1H, $J_{1,2}$ = 2.9 Hz, H-1b), and 7.33-8.15 (m, 20H, 4Ph).

Anal. Calcd for $C_{84}H_{116}O_{23}$ (1493.8): C, 67.54; H, 7.83. Found: C, 67.30; H, 7.53.

2-(Tetradecyl)hexadecyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-β-D-glucopyranoside sodium salt (21). To a solution of **20** (78 mg, 0.05 mmol) in DMF (0.1 mL) was added sulfur trioxide-pyridine complex (41 mg, 0.26 mmol) and the mixture was stirred for one h at room temperature. A similar workup as described for **18** gave the pyridine salt (75 mg, 87%) as an amorphous mass: $[\alpha]_D$ -2.2° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85-1.31 (m, 58H, 2*Me*, 26CH_2), 1.38 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6b), 1.74-2.35

(4s, 12H, 4AcO), 4.37 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1a), and 7.09-8.05 (m, 25H, 4Ph, C_5H_5N). To a solution of the pyridine salt (75 mg, 0.05 mmol) in MeOH (2 mL) and THF (1 mL) was added sodium methoxide (5 mg) and the mixture was stirred for 24 h at room temperature then concentrated at 25 °C. Column chromatography (5:4:0.7 $CHCl_3$-MeOH-H_2O) of the residue on Sephadex LH-20 (20 g) gave **21** (42 mg, 92%) as an amorphous mass: $[\alpha]_D$ -30.0° (*c* 0.8, 1:1 $CHCl_3$-MeOH); ^1H NMR (C_5D_5N) δ 0.85-1.31 (m, 58H, 2*Me*, 26CH_2), 1.54 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6b), 5.06 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1b), 5.21 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.8 Hz, H-3c), 5.41 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1a), and 5.48 (d, 1H, $J_{1,2}$ = 6.7 Hz, H-1c). The mass spectrum of **21** (negative ion mode) showed the base peak at *m/z* 988.3 $(M-H)^-$.

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