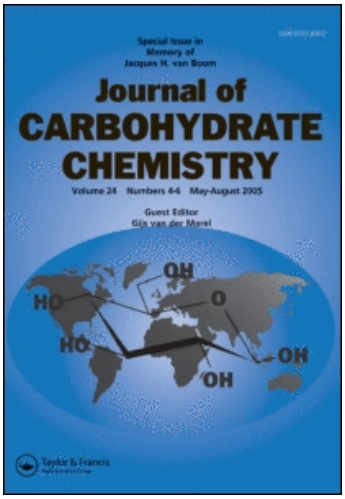

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Synthetic Studies on Selectin Ligands/Inhibitors: A Systematic Synthesis of Sulfatide and Its Higher Congeners Carrying 2-(Tetradecyl)Hexadecyl Group as a Ceramide Substitute

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SYNTHETIC STUDIES ON SELECTIN LIGANDS/INHIBITORS: A SYSTEMATIC SYNTHESIS OF SULFATIDE AND ITS HIGHER CONGENERS CARRYING 2-(TETRADECYL)HEXADECYL GROUP AS A CERAMIDE SUBSTITUTE[1]

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ABSTRACT

A systematic synthesis of sulfatide (**I**) and novel sulfatide analogs (**II-VI**) carrying 2-(tetradecyl)hexadecyl group as a ceramide substitute is described. The 3-*O*-, 4-*O*- and 3,4-di-*O*-levulinoyl derivatives of galactopyranosyl trichloroacetimidates (**1**, **12**, and **13**) were coupled with (2*S*,3*R*,4*E*)-3-*O*-acetyl-2-octadecanamido-4-octadecene-1,3-diol or 2-(tetradecyl)hexadecan-1-ol. The resulting glycolipids (**2**, **4**, **14**, and **15**) were each transformed, by selective removal of the levulinoyl group(s), and successive sulfation and de-*O*-acylation, into the 3-sulfates (**I**, **II**), 4-sulfate (**III**), and 3,4-disulfate (**IV**). The 6-sulfate (**V**) was prepared from 2-(tetradecyl)hexadecyl β-D-galactopyranoside (**21**) *via* the 6-*O*-*t*-butyldimethylsilyl derivative, while the 3'-sulfate of 2-(tetradecyl)hexadecyl β-D-lactoside (**VI**) was synthesized from 2-(trimethylsilyl)ethyl 3'-*O*-benzyl-β-D-lactoside (**26**). The structures of the sulfated glycolipids (**I-VI**) were characterized by ion-spray MS, MS/MS, and ^{1}H NMR spectrometry.

INTRODUCTION

Sulfatide, one of the major acidic glycosphingolipids in mammalian tissues, was first isolated[2] from brain and characterized[3] as cerebroside 3-sulfate, which usually shows structural variations in the ceramide moiety. Sulfatide is an early marker for the differentiation of oligodendrocytes and Schwann cells and becomes highly enriched in the myelin.[4] It was found that the adhesive glycoproteins laminin, thrombospondin, and von Willebrand factor bind specifically and with high affinity to sulfatide, strongly suggesting a possible function of sulfatide in cell adhesion.[5] Sulfatide is also a good ligand for L- and P-selectins,[6,7] which are a family of carbohydrate-binding proteins[8] involved in leukocyte trafficking, thrombosis, and inflammation.

We have succeeded[9] in the systematic synthesis of gangliosides, another major acidic glycosphingolipids, and demonstrated[10] that the sialyl Le^x determinant is a minimal effective structure for selectin binding. It has also been found that E- and L-selectins strongly bind to the sulphated Le^x/Le^a structures,[10-12] indicating that the sialic acid moiety could be replaced by the sulfate.

In this report, as a part of our study to clarify the biological functions of sialo- and sulfoglycolipids, a systematic synthesis of sulfatide and novel sulfatide analogs carrying 2-(tetradecyl)hexadecyl group as a ceramide substitute is described.

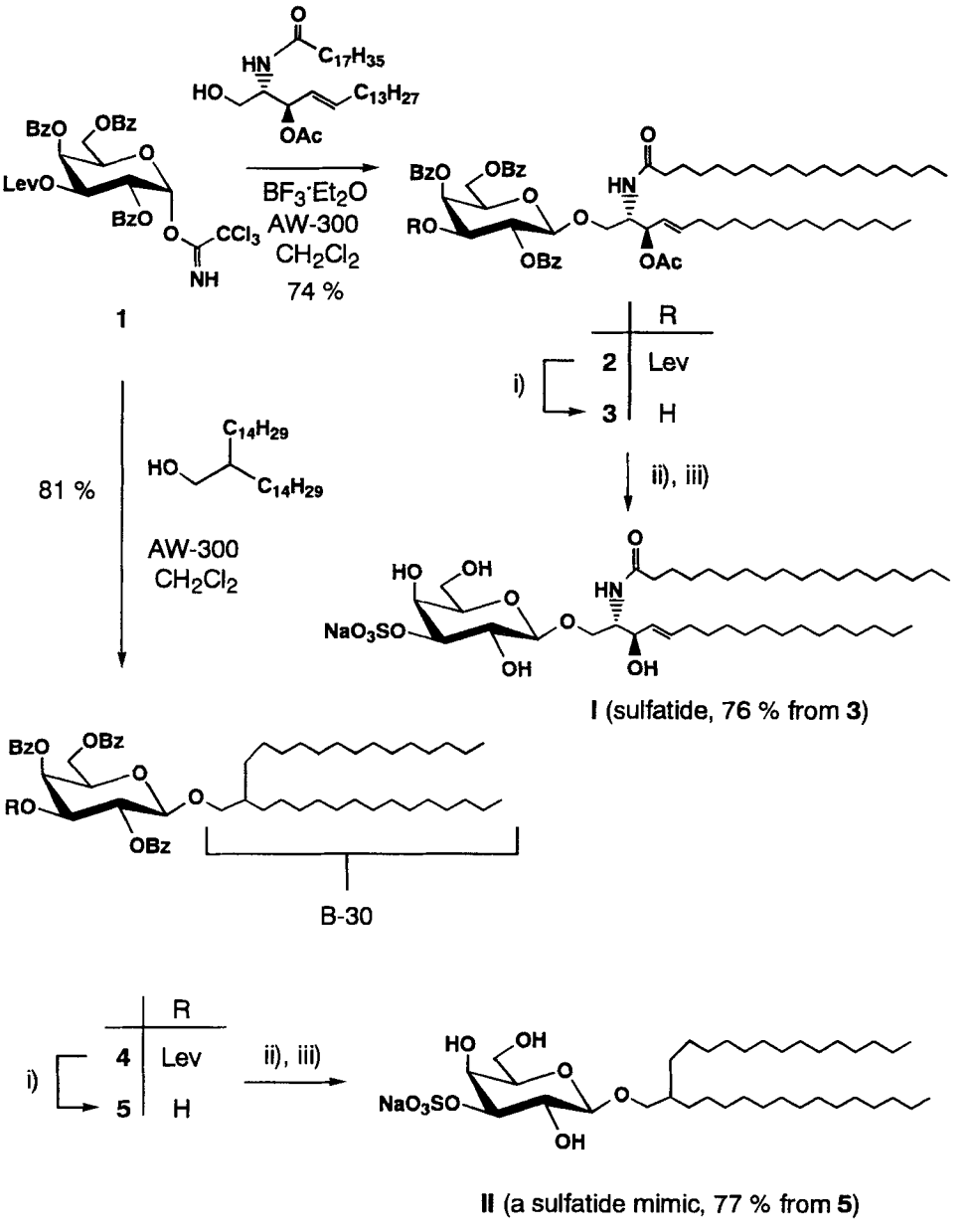
RESULTS AND DISCUSSION

The synthesis of 1-*O*-(β-D-galactopyranosyl 3- or 6-sulfate)-*N*-octadecanoyl-DL-dihydrosphingosine has been achieved by Flowers[13] using the acetyl protected galactose derivatives in which *OH*-3 or *OH*-6 remained unprotected. In the present study, the levulinoyl, *t*-butyldimethylsilyl, benzyl, benzoyl, and 2-(trimethylsilyl)ethyl groups were successfully employed for the selective protections of the sugar hydroxyl groups to achieve the systematic synthesis of a series of positional isomers of sulfatides.

2,4,6-Tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl trichloroacetimidate[14] (**1**) was coupled with (2*S*,3*R*,4*E*)-3-*O*-acetyl-2-octadecanamido-4-octadecene-1,3-diol[15] or 2-(tetradecyl)hexadecan-1-ol (B30-OH)[16] in the presence of molecular sieves AW-300 (MS AW-300) in dichloromethane (CH_2Cl_2) to give the corresponding β-glycosides **2** (74%) and **4** (81%), respectively (Scheme 1). Selective removal of the levulinoyl (Lev) group at *O*-3 in the galactose moiety with hydrazine monoacetate gave **3** (98%) and **5** (92%), which were then treated with sulfur trioxide-pyridine complex in *N*,*N*-dimethylformamide (DMF). The resulting 3-sulfates were each treated with sodium methoxide (NaOMe), followed by chromatographic purification, to afford the desired sulfatide **I** (76% from **3**) and a sulfatide mimic **II** (77% from **5**) as colorless solids. None of the positional isomers arising from the benzoyl migration was isolated.

The structures of **I** and **II** thus obtained were characterized by ion-spray MS, MS/MS, and ^{1}H NMR spectrometry. The molecular ions for **I** and **II** were clearly detected both in negative (*m/z* 806.5 $[M\text{-}Na]^-$ for **I**, *m/z* 680.0 $[M\text{-}Na]^-$ for **II**) and in positive (*m/z* 808.5 $[M\text{-}Na+2H]^+$ for **I**, *m/z* 681.7 $[M\text{-}Na+2H]^+$ for **II**) ion modes, respectively. In the positive-ion MS/MS fragmentation spectra, five significant daughter ions (*m/z* 728.6, 710.5, 548.3, 281.0, 264.0 for **I**, *m/z* 601.6, 421.4, 242.3, 163.1, 144.5 for **II**) were detected, providing unambiguous evidence for the sulfatide structures as shown in Figure 1, (A) and (B). In the ^{1}H NMR spectra (500 MHz) of **I** and **II** in DMSO-d_6 at 50 °C, both the *C*-3 ring protons in the galactose moiety were detected at δ 3.9-4.0 ppm, indicative of the 3-sulfate structure.

For the synthesis of 4-sulfate (**III**) and 3,4-disulfate (**IV**), the 4-*O*-levulinoyl (**8**) and 3,4-di-*O*-levulinoyl (**9**) derivatives were prepared either by regioselective 3-*O*-benzoylation of 2-(trimethylsilyl)ethyl 2,6-di-*O*-benzoyl-β-D-galactopyranoside (**6**) and then 4-*O*-levulinoylation, or by 3,4-di-*O*-levulinoylation of **6**, respectively (Scheme 2). Removal of the 2-(trimethylsilyl)ethyl group at *O*-1 and trichloroacetimidate formation gave **12** and **13**, which were then coupled with 2-(tetradecyl)hexadecan-1-ol to afford **14** (78%) and **15** (75%), respectively. The levulinoyl group(s) at *O*-4 or *O*-3,4 in **14** and **15** were selectively removed, and the resulting 4-hydroxy (**16**) or 3,4-dihydroxy



Scheme 1. i) NH_2NH_2•AcOH, EtOH-THF (**2**→**3**, 98 %; **4**→**5**, 92 %); ii) SO_3•pyr., DMF; iii) NaOMe (Lev = $CH_3COCH_2CH_2CO$)

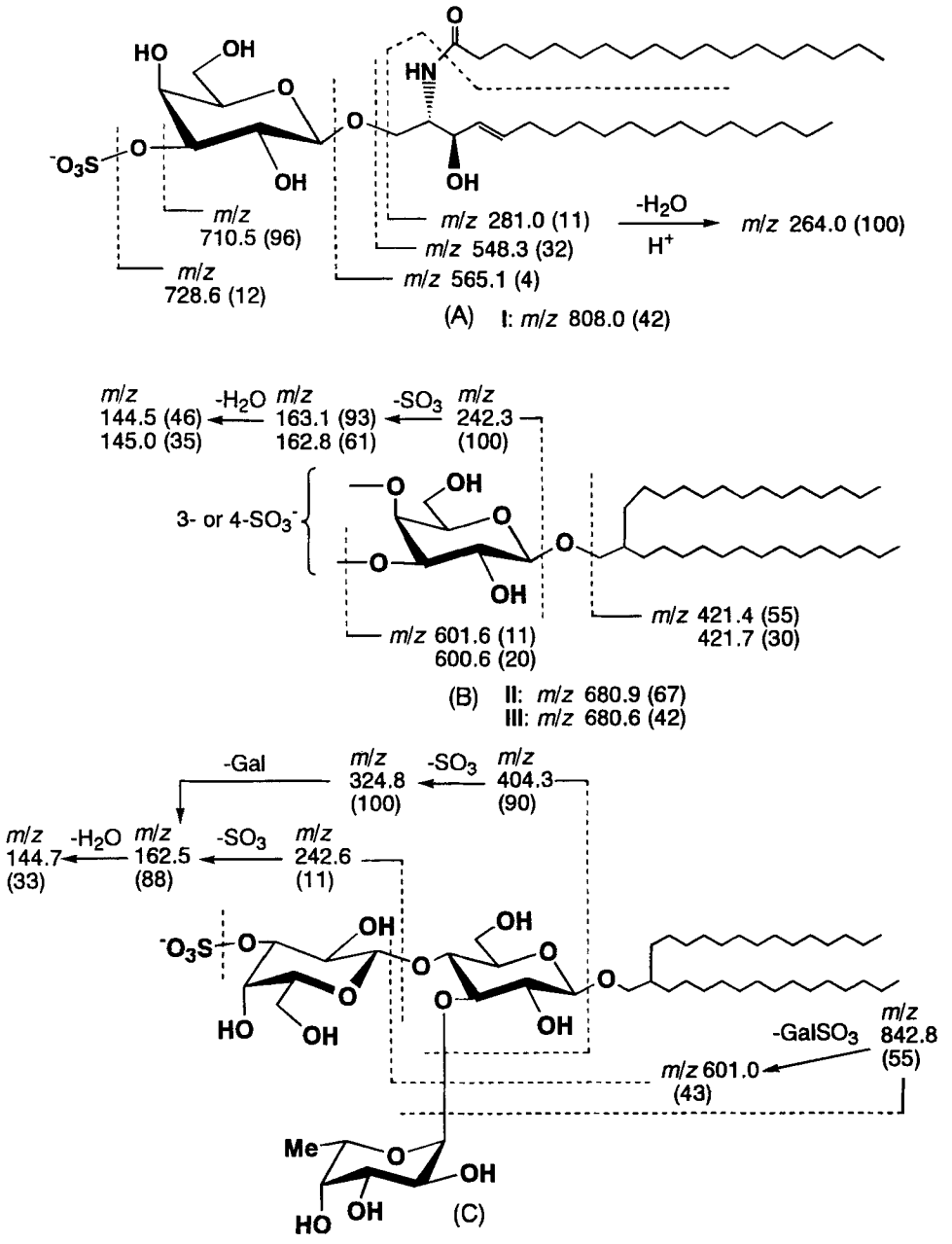
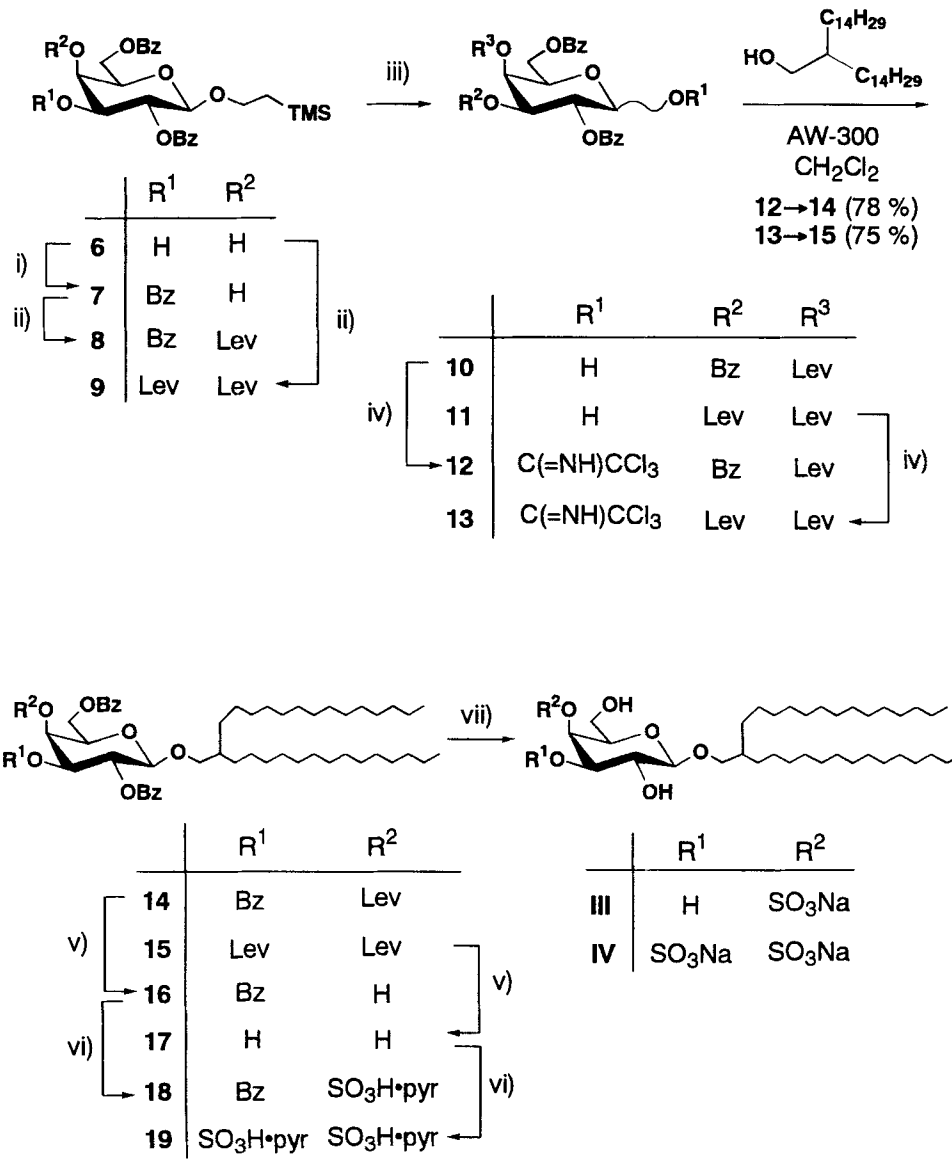


Figure 1. Positive-ion ion-spray MS/MS fragmentations. (A) Compound **I**; daughter ions for P=*m/z* 808.5. (B) Compound **II** and **III**; daughter ions for P=*m/z* 681.7 and *m/z* 681.5, respectively. (C) GSC-150 (ref. 17); daughter ions for P=*m/z* 989.7. The relative intensities (%) are shown in the parentheses.



	R^1	R^2
6	H	H
7	Bz	H
8	Bz	Lev
9	Lev	Lev

	R^1	R^2	R^3
10	H	Bz	Lev
11	H	Lev	Lev
12	$C(=NH)CCl_3$	Bz	Lev
13	$C(=NH)CCl_3$	Lev	Lev

	R^1	R^2
14	Bz	Lev
15	Lev	Lev
16	Bz	H
17	H	H
18	Bz	SO_3H•pyr
19	SO_3H•pyr	SO_3H•pyr

	R^1	R^2
III	H	SO_3Na
IV	SO_3Na	SO_3Na

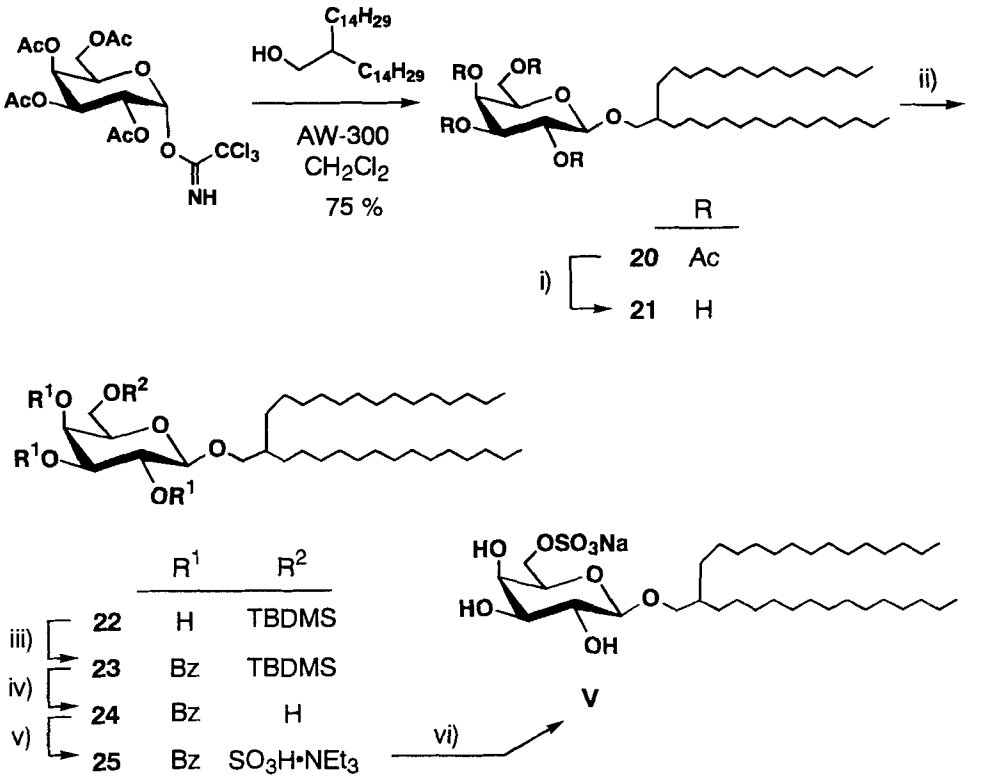
Scheme 2. i) BzCl, pyr., -50 °C (88 %); ii) Lev_2O, pyr., DMAP (**7**→**8**, 90 %; **6**→**9**, 81 %); iii) TFA, CH_2Cl_2 (**8**→**10**, 95 %; **9**→**11**, 83 %); iv) Cl_3CCN, DBU, CH_2Cl_2 (**10**→**12**, 91 %; **11**→**13**, 87 %); v) NH_2NH_2•AcOH, EtOH (92 %); vi) SO_3•pyr., DMF; vii) NaOMe, MeOH-THF

(**17**) compounds were each sulfated as described for **I** and **II** to give 4-*O*-sulfo (**18**) and 3,4-di-*O*-sulfo (**19**) derivatives in high yields. Treatment of **18** and **19** with NaOMe, and the following quick purification on a column of alkylated silica gel afforded 4-sulfate (**III**) and 3,4-disulfate (**IV**) in good yields.

In the ^{1}H NMR spectrum of **III** in pyridine-d_5, a significant narrow doublet of *H*-4 in the galactose moiety appeared at δ 5.64 characteristic of 4-sulfate, while in the spectrum of **IV** in DMSO-d_6 at 59 °C, both a one-proton doublet of doublets (*H*-3) and a narrow doublet (*H*-4) were clearly observed at δ 4.14 and 4.55, characteristic of the 3,4-disulfate structure. However, the positive-ion ion-spray MS and MS/MS of **III** were very similar to those of 3-sulfate (**II**) as shown in Figure 1, (B), indicating the difficulty of clear distinction between 3- and 4-sulfates by MS . The common fragment patterns observed for **II** and **III** were also found for the sulfated Le^x lipid (GSC-150),[17] a potent selectin ligand/inhibitor,[18] as shown in Figure 1, (C).

On the other hand, the ion-spray MS spectrum of **IV** in the negative-ion mode gave a single molecular ion peak at *m/z* 379.2 $[M-2Na]^{2-}$, from which eight significant daughter ions (*m/z* 660.7, 240.9, 222.4, 159.6, 152.6, 138.8, 96.7, 79.7) were produced as shown in Figure 2, (A). The ion at *m/z* 660.7 [P'] corresponds to the unsaturated monosulfate structure which was formed by the cleavage of HSO_4^- (*m/z* 96.7) from the parent ion [P = *m/z* 379]. The ions at *m/z* 152.6 and 138.8 are thought to be the ring-opening fragment ions[19] such as ^-O_3SO-CH=CH-CH_2OH and ^-O_3SO-CH=CHOH, respectively, which may give the evidence to distinguish between the 3- and 4-sulfate structures. The other daughter ions arise from the cleavage of the glycoside bond or the SO_3^- group.

The synthesis of 6-sulfate (**V**) was achieved according to Scheme 3. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate was coupled with 2-(tetradecyl)hexadecan-1-ol, and the resulting **20** (75%) was converted, by treatment with NaOMe, to crystalline **21**. The regioselective protection of the primary hydroxyl in **21** by the *t*-butyldimethylsilyl (TBDMS) group, and successive benzoylation of the remaining hydroxyls and selective removal of the TBDMS group afforded **24**, which was then sulfated to give **25** and converted to the desired 6-sulfate (**V**) as described for



Scheme 3. i) NaOMe, MeOH; ii) TBDMSCl, pyr., CH_2Cl_2; iii) BzCl, pyr., rt; iv) 80 % aq AcOH, 50 °C (93 %); v) SO_3•pyr., DMF then NEt_3; vi) NaOMe, MeOH.

I-IV. In the negative-ion ion-spray MS of **V**, a single molecular ion peak was detected at *m/z* 679.1 $[M-Na]^-$, which, on MS/MS fragmentation, gave four characteristic daughter ions at *m/z* 240.7, 180.6, 151.0, and 96.7 as shown in Figure 2, (B), providing the distinct evidence for the desired 6-sulfate structure.

The 3'-sulfate of 2-(tetradecyl)hexadecyl β-D-lactoside (**VI**), a mimic of the naturally occurring lactosyl ceramide 3'-sulfate,[20] was synthesized from 2-(trimethylsilyl)ethyl 3'-*O*-benzyl-β-D-lactoside[21] (**26**) as shown in Scheme 4. Compound **26** was converted, by benzoylation, selective removal of the TMS ethyl group and trichloroacetimidate formation, into **29**, which was then coupled with 2-

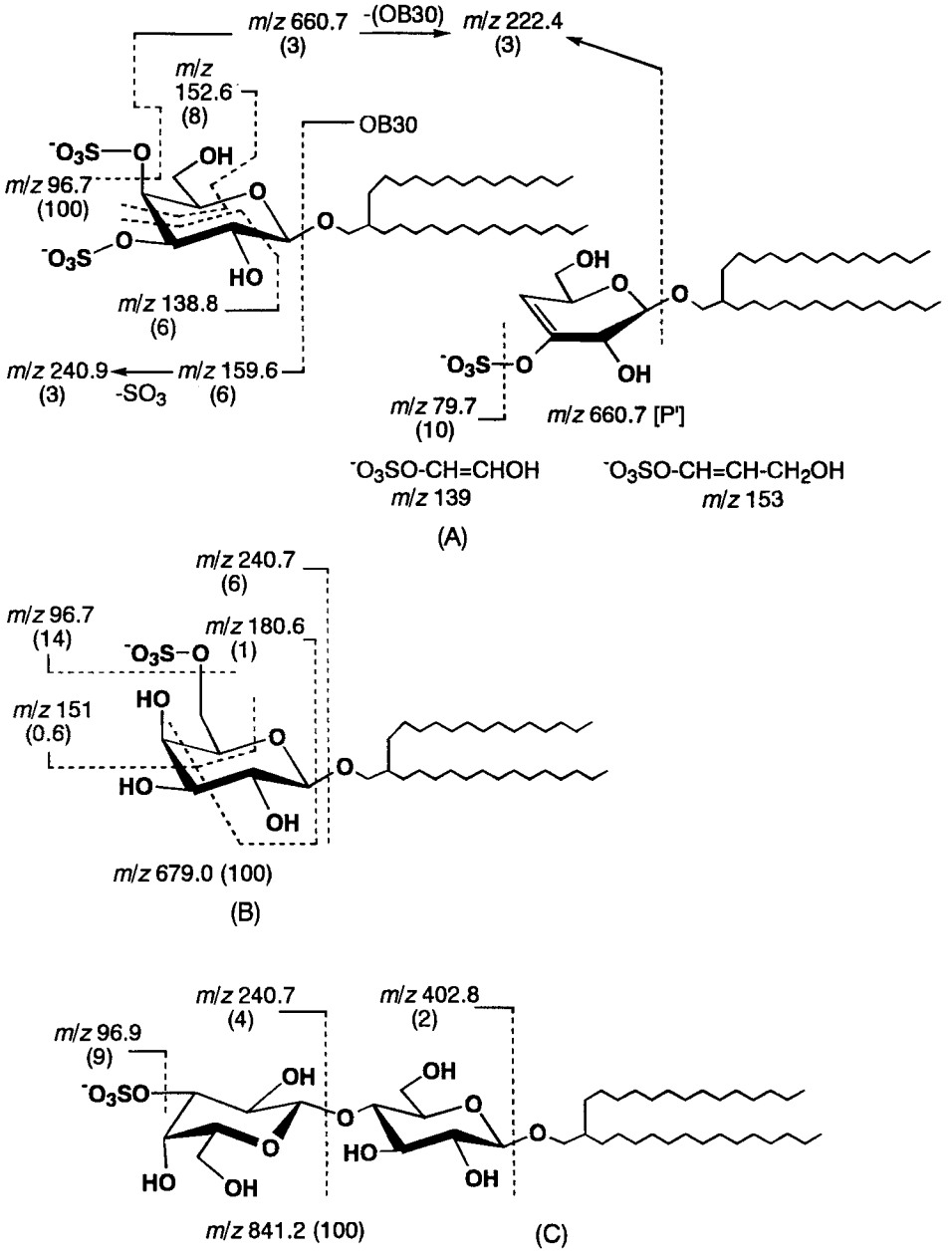
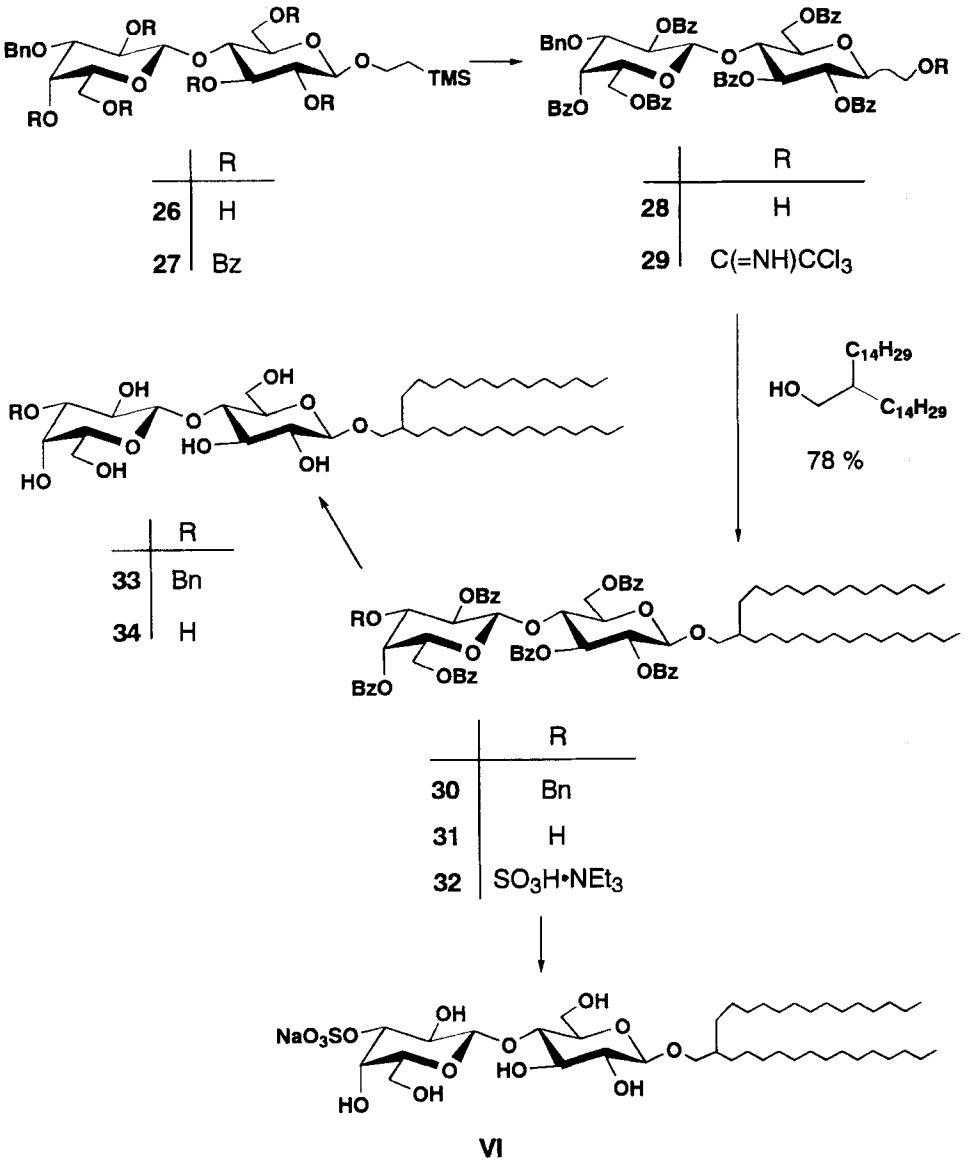


Figure 2. Negative-ion ion-spray MS/MS fragmentations. (A) Compound **IV**; daughter ions for P=*m/z* 379. (B) Compound **V**; daughter ions for P=*m/z* 679. (C) Compound **VI**; daughter ions for P=*m/z* 841. The relative intensities (%) are shown in the parentheses.



Scheme 4.

(tetradecyl)hexadecan-1-ol to give **30** in 78% yield. Hydrogenolytic removal of the benzyl group at *O*-3' and treatment of **31** with sulfur trioxide-pyridine complex in DMF, followed by triethylamine treatment, gave the triethylamine salt **32** in high yield. In the ^{1}H NMR spectrum of **32**, the *H*-3' ring proton was detected at δ 4.71 ppm (dd,

$J_{2,3}$ = 9.7, $J_{3,4}$ = 3.1 Hz) showing that the sulfation took place at *OH*-3' without acyl migration. Finally, all of the benzoyl groups were removed by treatment with NaOMe, and the reaction mixture was processed as described for **II** to afford **VI** as an amorphous mass. The most significant signal in the ^{1}H NMR spectrum of **VI** in DMSO-d_6 at 50 °C was a one-proton doublet of doublets at δ 4.00 ($J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.4 Hz, H-3'), indicating the desired 3'-sulfate structure.

The ion-spray MS of **VI** in negative-ion mode showed a single molecular ion peak at *m/z* 841.3 $[M-Na]^-$, which gave three significant daughter ions at *m/z* 402.8, 240.7 and 96.9 as shown in Figure 2, (C). De-*O*-benzoylation of **30** gave **33** which was hydrogenolyzed to afford **34**. A variety of sulfatide analogs including monosulfate **II**, **VI**, 3,6-disulfate and 3,4,6-trisulfate of **21**, and 3',6'-disulfate of **34** have also been synthesized starting from **21** and **34**, respectively, *via* the dibutylstannylene acetal intermediates as described in a separate paper by Ikami et al.[22]

Sulfated carbohydrates, including sulfatides, appear to have binding activity for selectins. The specificity of binding, in general, seems to favor L- and P-selectins. In the ELISA assay using L-selectin-IgG chimera, sulfatide showed the most active binding among the various sulfated or non-sulfated glycolipids tested.[23] All of the active glycolipids carried the non-reducing terminal galactose residue sulfated at 3 position, suggesting that the terminal HO3S-3Galβ1- structure is essential for the binding of L-selectin. In fact, the synthetic sulfatide analogs **II**, **VI**, and 3,6-di-*O*-/3,4,6-tri-*O*-sulfo derivatives[22] of **21** bound more preferentially to the chimera than the others. It was also demonstrated that those sulfatide analogs having the HO3S-3Galβ1- structure were highly protective against lung injury[24] and kidney inflammation in rats.[25]

In conclusion, a systematic synthesis of sulfatide (**I**) and its novel analogs (**II-VI**) carrying 2-(tetradecyl)hexadecyl group as a ceramide substitute has been achieved by the selective protections of the sugar hydroxyls with the levulinoyl, *t*-butyldimethylsilyl, benzyl, benzoyl and 2-(trimethylsilyl)ethyl groups. The structures of the resulting sulfated glycolipids were determined by ^{1}H NMR, ion-spray MS and MS/MS spectra. Synthetic sulfatides and related glycolipids may be useful not only as cell adhesion probes but also as therapeutic agents.

EXPERIMENTAL

General methods. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Union PM-201 polarimeter at 25 °C. ^{1}H NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) or Varian Unity Inova 500 (500 MHz) spectrometer. Ion-spray mass spectra were recorded on an API-III triple quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments) fitted with an atmospheric pressure ionization source, and the spectrometer was operated both in positive and negative-ion modes as described in ref. 26. The samples were dissolved in CH_3CN and diluted with 0.1% trifluoroacetic acid (TFA)-50% CH_3CN (positive-ion mode) or 50% CH_3CN (negative-ion mode), respectively. Infusion rate was 5 μL/min.

All reactions were monitored by TLC (Merck silica gel aluminum plates 60F-254) and preparative column chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) or Merck silica gel 60 silanized (70-230 mesh, No.7719) with the solvent systems specified.

(2*S*,3*R*,4*E*)-3-*O*-Acetyl-1-*O*-(2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-β-D-galactopyranosyl)-2-octadecanamido-4-octadecene-1,3-diol (2). To a solution of imidate[14] **1** (92 mg) and (2*S*,3*R*,4*E*)-3-*O*-acetyl-2-octadecanamido-4-octadecene-1,3-diol[15] (53.8 mg) in CH_2Cl_2 (0.85 mL) was added powdered MS AW-300 (100 mg) and the mixture was stirred for 4 h at room temperature under N_2, and then cooled to 0 °C. Boron trifluoride etherate (13 μL) was added and the stirring was continued for 4 h at 0 °C. The solids were filtered off with Celite and washed with CH_2Cl_2. The combined filtrate and washings were washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (9:1 hexane-EtOAc) of the residue on silica gel gave the title compound **2** (77.3 mg, 74%, based on ceramide) as a syrup: $[\alpha]_D$ +11° (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$ of ceramide), 1.0-1.45, 1.76 (m, 50H+2H, -C*H*$_2$-), 1.92, 2.01 (2s, 6H, COC*H*$_3$), 1.85-2.1 (m, 2H, -CH=CH-C*H*$_2$-), 2.25-2.7 (m, 6H, -COC*H*$_2$- of Lev), 3.58, 4.11 (2dd, 2H, H-1), 4.70 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 5.25-5.85 (m, 7H, H-3,4,5, H-2',3',4', and NH), 7.3-8.2 (m, 15H, 3PhCO).

Anal. Calcd for $C_{70}H_{101}NO_{14}$ (1180.57): C, 71.22; H, 8.62; N, 1.19. Found: C, 71.36; H, 8.50; N, 1.15.

(2*S*,3*R*,4*E*)-3-*O*-Acetyl-1-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-2-octadecanamido-4-octadecene-1,3-diol (3) and (2*S*,3*R*,4*E*)-2-Octadecanamido-1-*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-4-octadecene-1,3-diol sodium salt (I). To a solution of **2** (75 mg) in 5:1 EtOH-THF (3 mL) was added hydrazine monoacetate (7 mg) and the mixture was stirred for 1.5 h at room temperature, the reaction being monitored by TLC (50:1 CH_2Cl_2-MeOH) and then concentrated. Column chromatography (150:1 CH_2Cl_2-MeOH) of the residue on silica gel gave **3** (67.4 mg, 98%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$ of ceramide), 1.0-1.5, 1.76 (m, 50H+2H, -C*H*$_2$-), 1.96 (s, 3H, COC*H*$_3$), 1.85-2.1 (m, 2H, -CH=CH-C*H*$_2$-), 3.10 (brs, 1H, OH-3'), 3.58, 4.05 (2dd, 2H, H-1), 4.1-4.35 (m, 3H, H-2,3',5'), 4.39, 4.55 (2dd, 2H, H-6'), 4.64 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 5.23-5.42, 5.6-5.8 (m, 6H, H-3,4,5, H-2',4', and NH), 7.35-8.2 (m, 15H, 3PhCO).

To a solution of **3** (54 mg) in DMF (1 mL) was added sulfur trioxide-pyridine complex (40 mg), and the mixture was stirred for 3 h at room temperature until the starting material **3** completely disappeared on TLC (50:1 CH_2Cl_2-MeOH). The resulting 3-sulfate showed a single spot on TLC (R_f 0.47, 8:1 CH_2Cl_2-MeOH). Methanol (1 mL) was added and the reaction mixture was treated with NaOMe (1 mL of 25 wt. % soln in MeOH) overnight at room temperature, then concentrated. The deprotected single product (R_f 0.4, 4:1 CH_2Cl_2-MeOH) was purified on columns of Merck silica gel 60 silanized, 70-230 mesh (1:1 H_2O-MeOH, MeOH and then 1:1 MeOH-CH_2Cl_2) and Wakogel C-200 packed with Sephadex LH-20 (10:1 CH_2Cl_2-MeOH) to give **I** (41.4 mg, 76% from **3**) as a solid: mp 217-219 °C (decomp); ^{1}H NMR (DMSO-*d*$_6$+D_2O, 50 °C) δ 0.85 (t, 6H, 2×C*H*$_3$), 1.0-1.4 and 1.44 (m, 50H+2H, -C*H*$_2$-), 1.92 (m, 2H, -CH=CH-C*H*$_2$-), 2.03 (~t, 2H, -COC*H*$_2$-), 3.75 (m, 1H, H-1a), 3.8-4.0 (m, 4H, H-1b, H-3, H-3', H-4'), 4.16 (d, 1H, $J_{1',2'}$ = 7.3 Hz, H-1'), 5.36 (~dd, 1H, $J_{3,4}$ = 7, $J_{4,5}$ = 15 Hz, H-4), 5.54 (m, 1H, $J_{5,6}$ = 7 Hz, H-5), 7.42 (d, 1H, $J_{NH,2}$ = 9 Hz, NH); ion-spray MS (negative-ion mode) *m/z* 806.5

$[M - Na]^-$, (positive-ion mode) *m/z* 808.5 $[M - Na + 2H]^+$; MS/MS (positive-ion mode, P = *m/z* 808.5) *m/z* 808.0 $[M - Na + H]^+$ (42), 728.6 $[P - SO_3]^+$ (12), 710.5 $[P - SO_4]^+$ (96), 548.3 $[P - SO_3Gal]^+$ (32) (ceramide fragment), 281.0 $[548.3\text{-}C_{17}H_{35}CO]^+$ (11) (sphingosine fragment), and 264.0 $[548.3\text{-}C_{17}H_{35}CO - H_2O]^+$ (100). Calcd for $C_{42}H_{80}NO_{11}S$ (M-Na): 808.2. Also see Figure 1, (A).

2-(Tetradecyl)hexadecyl 2,4,6-Tri-*O*-benzoyl-3-*O*-levulinoyl-β-D-galactopyranoside (4). To a solution of imidate **1** (321 mg) and 2-(tetradecyl)hexadecan-1-ol (240 mg, 1.2 equiv) in CH_2Cl_2 (7 mL) was added powdered MS AW-300 (400 mg), and the mixture was stirred overnight at room temperature under N_2 gas, then worked up as described for **2**. Column chromatography (8:1 hexane-EtOAc) on silica gel afforded **4** (357 mg, 81%) as a syrup: $[\alpha]_D$ +15° (*c* 1.2, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, C*H*$_3$CH$_2$-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×C*H*$_2$, and C*H*), 1.90 (s, 3H, MeCO), 2.3-2.6 (m, 4H, COC*H*$_2$C*H*$_2$CO), 3.33, 3.82 (2dd, 2H, J_{gem} = 9.5, J_{vic} = 7, 4.9 Hz, OC*H*$_2$CH), 4.23 (~t, 1H, H-5), 4.38, 4.64 (2dd, 2H, J_{gem} = 11, $J_{5,6}$ = 6.4, 6.8 Hz, H-6), 4.69 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.38 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.3 Hz, H-3), 5.60 (dd, 1H, H-2), 5.83 (~d, 1H, $J_{4,5}$ = ~0 Hz, H-4), 7.4-8.2 (m, 15H, 3×PhCO).

Anal. Calcd for $C_{62}H_{90}O_{11}$ (1011.39): C, 73.63; H, 8.97. Found: C, 73.45; H, 8.85.

2-(Tetradecyl)hexadecyl 2,4,6-Tri-*O*-benzoyl-β-D-galactopyranoside (5). To a solution of **4** (323 mg) in EtOH (2 mL) was added hydrazine mono acetate (40 mg), and the mixture was stirred for 1.5 h at room temperature; the reaction being monitored by TLC (2:1 hexane-EtOAc). The mixture was concentrated to a residue which was chromatographed (12:1 hexane-EtOAc) on a column of silica gel to give **5** (270 mg, 92%) as a syrup: $[\alpha]_D$ +1.3° (*c* 1.1, CH_2Cl_2); ν 3450 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$CH$_2$-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×C*H*$_2$, and C*H*), 2.98 (brs, 1H, OH-3), 3.34, 3.88 (2dd, 2H, J_{gem} = 9.5, J_{vic} = 7, 4.9 Hz, OC*H*$_2$CH), 4.05-4.2 (m, 2H, H-3,5), 4.41, 4.60 (2dd, 2H, J_{gem} = 11, $J_{5,6}$ = 7, 6.2 Hz, H-6), 4.65 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 5.38 (dd, 1H, $J_{2,3}$ = 10 Hz, H-2), 5.77 (~d, 1H, $J_{3,4}$ = 3, $J_{4,5}$ = ~0 Hz, H-4), 7.35-8.2 (m, 15H, 3×PhCO).

Anal. Calcd for $C_{57}H_{84}O_9$ (913.29): C, 74.96; H, 9.27. Found: C, 74.72; H, 9.21.

2-(Tetradecyl)hexadecyl 3-*O*-Sulfo-β-D-galactopyranoside sodium salt (II). To a solution of **5** (364 mg) in DMF (1 mL) was added sulfur trioxide-pyridine complex (318 mg, 5 equiv), and the mixture was stirred for 3.5 h at room temperature as described for **I**. The resulting 3-sulfate showed a single spot on TLC (R_f 0.4. 8:1 CH_2Cl_2-MeOH). Methanol (1 mL) was added and the reaction mixture was stirred with NaOMe (2 mL of 25 wt. % soln in MeOH) overnight at room temperature to give a single spot on TLC (4:1 CH_2Cl_2-MeOH), then concentrated. The residue was chromatographed on a column of Merck silica gel 60 silanized (70-230 mesh) with (a) 1:1 H_2O-MeOH and (b) 1:1 CH_2Cl_2-MeOH. Eluent (b) gave the pure title compound **II** (216 mg, 77% from **5**) as a solid: mp 183-185 °C; ^1H NMR (DMSO-d_6, 50 °C) δ 0.85 (t, 6H, CH_3CH_2-), 1.24 (m, 53H, 26×CH_2), 1.49 (m, 1H, CH), 3.29, 3.60 (2dd, 2H, J_{gem} = 9.6, J_{vic} = 6 Hz, OCH_2CH), 3.34 (~t, 1H, H-5), 3.40-3.46 (m, 2H, H-2, H-6a), 3.53 (dt, 1H, J_{gem} = 11, $J_{5,6}$ = $J_{6,OH}$ = 6 Hz, H-6b), 3.91 (m, 1H, $J_{4,OH}$ = 5 Hz, H-4), 3.93 (dd, 1H, $J_{2,3}$ = 9.6, $J_{3,4}$ = 3.4 Hz, H-3), 4.11 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.32 (d, 1H, OH-4), 4.42 (t, 1H, OH-6), 4.78 (d, 1H, OH-2); (DMSO-d_6+D_2O, 50 °C) Complete disappearance of the peaks at δ 4.32, 4.42 and 4.78 (OH-4,6,2), ring protons δ 3.92-3.96 (m, 2H, H-3,4), 4.12 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1); ion-spray MS (negative-ion mode) *m/z* 680.0 $[M - Na]^-$, (positive-ion mode) *m/z* 681.7 $[M - Na + 2H]^+$ (base peak), 722.6 $[M - Na + 2H + MeCN]^+$; MS/MS (positive-ion mode, P = *m/z* 681.7) *m/z* 680.9 $[M - Na + H]^+$ (67), 601.6 $[M - Na + 2H - SO_3]^+$ (11), 421.4 [$C_{30}H_{61}$; 2-(tetradecyl)hexadecyl fragment]$^+$ (55), 242.3 $[680.9 - C_{30}H_{61}O]^+$ (100), 163.1 $[242.3 - SO_3]^+$ (93), 144.5 $[163.1\text{-}H_2O]^+$ (46). Calcd for $C_{36}H_{71}O_9S$ (M-Na): 680.0. Also see Figure 1, (B).

2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzoyl-β-D-galactopyranoside (7) and 2-(Trimethylsilyl)ethyl 2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-β-D-galactopyranoside (8). To a solution of **6** (3.0 g) in pyridine (1 mL) and CH_2Cl_2 (10 mL) was added benzoyl chloride (0.9 mL) in CH_2Cl_2 (9 mL) and the

mixture was stirred for 2 h at -50 °C. After completion of the reaction (TLC, 1:1 EtOAc-hexane), MeOH (1 mL) was added and the mixture was extracted with CH_2Cl_2. The extract was washed with M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (10:1 hexane-EtOAc) of the residue on silica gel gave **7** (3.2 g, 88%): ^1H NMR ($CDCl_3$) δ 3.20 (brs, 1H, OH-4), 4.50 (d, 1H, $J_{3,4}$ = 3, $J_{4,5}$ = ~0 Hz, H-4), 4.88 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.48 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3), 5.91 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10 Hz, H-2).

To a solution of **7** (2.5 g) in pyridine (10 mL) were added levulinic anhydride (1.6 g) and a catalytic amount of 4-dimethylaminopyridine (DMAP), and the mixture was stirred for 1 h at room temperature. Methanol was added and the mixture was concentrated. The residue was taken up in CH_2Cl_2 and washed with M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (5:1 hexane-EtOAc) of the residue on silica gel afforded **8** (3.2 g, 90%) as a syrup: $[\alpha]_D$ +28° (*c* 1.1, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 2.15 (s, 3H, MeCO), 2.7-2.9 (m, 4H, $COCH_2CH_2CO$), 4.90 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.58 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3), 5.79 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10 Hz, H-2), 5.86 (d, 1H, $J_{3,4}$ = 3, $J_{4,5}$ = ~0 Hz, H-4).

Anal. Calcd for $C_{37}H_{42}O_{11}Si$ (690.8): C, 64.33; H, 6.13. Found: C, 64.56; H, 6.20.

2-(Trimethylsilyl)ethyl 2,6-Di-*O*-benzoyl-3,4-di-*O*-levulinoyl-β-D-galactopyranoside (9). To a solution of **6** (913 mg) in pyridine (5 mL) were added levulinic anhydride (1.4 g) and a catalytic amount of DMAP, and the mixture was stirred overnight at room temperature. Work-up as described for **8** and column chromatography (300:1 CH_2Cl_2-MeOH) gave **9** (1.06 g, 81%) as a syrup: $[\alpha]_D$ +7.6° (*c* 1.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 2.09, 2.28 (2s, 6H, 2×MeCO), 4.47, 4.67 (2dd, 2H, J_{gem} = 11, $J_{5,6}$ = 6.8, 6.6 Hz, H-6), 4.79 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 5.33 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.5 Hz, H-3), 5.59 (dd, 1H, $J_{2,3}$ = 10, $J_{1,2}$ = 8 Hz, H-2), 5.68 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4).

Anal. Calcd for $C_{35}H_{44}O_{12}Si$ (684.81): C, 61.39; H, 6.48. Found: C, 61.17; H, 6.26.

2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-D-galactopyranose (10) and 2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-α-D-galactopyranosyl trichloroacetimidate (12). To a solution of **8** (5.02 g) in CH_2Cl_2 (100 mL), cooled to 0 °C, was added TFA (5 mL), and the mixture was stirred for 4 h at room temperature. Ethyl acetate was added and the mixture was concentrated. Column chromatography (1:1 hexane-EtOAc) of the residue on silica gel gave **10** (4.1 g, 95%): $[\alpha]_D$ +30° (*c* 2.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H, MeCO), 2.6-2.8 (m, 4H, $COCH_2CH_2CO$), complete disappearance of the peaks at δ 0.9-1.1 (m, 2H, CH_2SiMe_3), 3.67, 4.13 (2m, 2H, $CH_2CH_2SiMe_3$).

To a mixture of **10** (200 mg) and Drierite (100 mg) in CH_2Cl_2 (2 mL) were added trichoroacetonitrile (Cl_3CCN, 0.11 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.03 mL) at -5 °C, and the mixture was stirred for 1.5 h at room temperature, then concentrated. Column chromatography (1:1 hexane-EtOAc) of the residue on silica gel afforded **12** (226 mg, 91%): $[\alpha]_D$ +91° (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, MeCO), 2.6-2.8 (m, 4H, $COCH_2CH_2CO$), 4.42-4.54 (2dd, 2H, J_{gem} = 11.4, $J_{5,6}$ = 7.0, 6.2 Hz, H-6), 4.75 (~t, 1H, H-5), 5.85, 5.90, 5.96 (3dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.4, $J_{4,5}$ = 0.9 Hz, H-2,3,4), 6.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 8.60 (s, 1H, C=NH).

Anal. Calcd for $C_{34}H_{30}Cl_3NO_{11}$ (735.0): C, 55.56; H, 4.11; N, 1.91. Found: C, 55.42; H, 4.20; N, 1.85.

2,6-Di-*O*-benzoyl-3,4-di-*O*-levulinoyl-D-galactopyranose (11) and 2,6-Di-*O*-benzoyl-3,4-di-*O*-levulinyl-α-D-galactopyranosyl trichloroacetimidate (13). A solution of **9** (2.02 g) in CH_2Cl_2 (3 mL) was treated with TFA (3 mL) as described for **10**. The resulting product was purified by chromatography on a column of silica gel (5:1 hexane-EtOAc) to give **11** (1.41 g, 83%): $[\alpha]_D$ +63° (*c* 1.0, CH_2Cl_2).

To a mixture of **11** (300 mg) and Drierite (200 mg) in CH_2Cl_2 (2 mL) were added Cl_3CCN (0.13 mL) and DBU (0.04 mL), and the mixture was stirred for 2 h at room temperature. Work-up as described for **12** and column chromatography (5:1 hexane-EtOAc) afforded **13** (324 mg, 87%): $[\alpha]_D$ +80° (*c* 0.9, CH_2Cl_2); ^1H NMR

($CDCl_3$) δ 2.04, 2.18 (2s, 6H, 2×MeCO), 2.4-2.9 (m, 8H, 2×COC*H*$_2$C*H*$_2$CO), 4.37, 4.48 (2dd, 2H, J_{gem} = 11.4, $J_{5,6}$ = 6.8, 6.2 Hz, H-6), 4.65 (~t, 1H, H-5), 6.76 (~s, 1H, H-1), 8.58 (s, 1H, C=NH).

Anal. Calcd for $C_{32}H_{32}Cl_3NO_{12}$ (728.96): C, 52.73; H, 4.42; N, 1.92. Found: C, 52.48; H, 4.15; N, 1.82.

2-(Tetradecyl)hexadecyl 2,3,6-Tri-*O*-benzoyl-4-*O*-levulinoyl-β-D-galactopyranoside (14) and 2-(Tetradecyl)hexadecyl 2,3,6-Tri-*O*-benzoyl-β-D-galactopyranoside (16). A mixture of **12** (845 mg) and 2-(tetradecyl)hexadecan-1-ol (774 mg) in CH_2Cl_2 (8 mL) was stirred overnight with powdered MS AW-300 (850 mg) at room temperature and worked up as described for **4**. Column chromatography (4:1 hexane-EtOAc) on silica gel gave **14** (902 mg, 78%) as a syrup: $[\alpha]_D$ -2.8° (*c* 0.4, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$CH$_2$-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×C*H*$_2$, and C*H*), 2.10 (s, 3H, MeCO), 2.6-2.8 (m, 4H, COC*H*$_2$C*H*$_2$CO), 3.34, 3.87 (2dd, 2H, J_{gem} = 9.3, J_{vic} = 6.5, 5.0 Hz, OC*H*$_2$CH), 4.19 (~t, 1H, H-5), 4.38, 4.64 (2dd, 2H, J_{gem} = 11, $J_{5,6}$ = 7.2, 6.6 Hz, H-6), 4.69 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.46 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.3 Hz, H-3), 5.68 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.4 Hz, H-2), 5.74 (~d, 1H, $J_{3,4}$ = 3.3, $J_{4,5}$ = 0~1 Hz, H-4).

To a solution of **14** (745 mg) in EtOH (6 mL) was added hydrazine mono acetate (82.7 mg), and the mixture was stirred for 1.5 h at room temperature. Work-up and column chromatography as described for **5** gave **16** (620 mg, 92%): $[\alpha]_D$ +24° (*c* 0.7, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.45 (~t, 1H, H-5), 4.34 (~d, 1H, $J_{3,4}$ = 3.3, $J_{4,5}$ = ~0 Hz, H-4), 5.35 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.3 Hz, H-3), 5.76 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10.3 Hz, H-2), 7.3-8.1 (m, 15H, 3×PhCO).

Anal. Calcd for $C_{57}H_{84}O_9$ (913.29): C, 74.96; H, 9.27. Found: C, 75.21; H, 9.26.

2-(Tetradecyl)hexadecyl 2,6-Di-*O*-benzoyl-3,4-di-*O*-levulinoyl-β-D-galactopyranoside (15) and 2-(Tetradecyl)hexadecyl 2,6-Di-*O*-benzoyl-β-D-galactopyranoside (17). A mixture of **13** (323.7 mg) and 2-(tetradecyl)hexadecan-1-ol (170 mg) in CH_2Cl_2 (3 mL) was stirred overnight with

powdered MS AW-300 (300 mg) at room temperature as described for **14**. The product was purified by column chromatography (3:1 hexane-EtOAc) on silica gel to afford **15** (334 mg, 75%): $[\alpha]_D$ -1.4° (*c* 0.9, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×CH_2, and *CH*), 1.99, 2.18 (2s, 6H, MeCO), 3.29 3.85 (2dd, 2H, J_{gem} = 9.3, J_{vic} = 6.7, 5.0 Hz, OCH_2CH), 4.12 (~t, 1H, H-5), 4.36, 4.57 (2dd, 2H, J_{gem} = 11.2, $J_{5,6}$ = 7.0 Hz, H-6), 4.62 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.23 (dd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, H-3), 5.50 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.4 Hz, H-2), 5.59 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4).

Removal of the levulinoyl groups in **15** was performed by treatment of **15** (348 mg) with hydrazine monoacetate (77 mg) in EtOH (4 mL) as described for **16**. The product was purified by chromatography (200:1 CH_2Cl_2-MeOH) on a column of silica gel to give **17** (257 mg, 92%): $[\alpha]_D$ -5.8° (*c* 1.1, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 3.93 (dd, 1H, $J_{3,4}$ = 3.3 Hz, H-3), 4.15 (~d, 1H, H-4), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.70, 4.78 (2dd, 2H, J_{gem} = 12, J_{vic} = 6.8, 6.2 Hz, H-6), 5.31 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 10 Hz, H-2).

Anal. Calcd for $C_{50}H_{80}O_8$ (809.18): C, 74.22; H, 9.97. Found: C, 73.94; H, 9.74.

2-(Tetradecyl)hexadecyl 2,3,6-Tri-*O*-benzoyl-4-*O*-sulfo-β-D-galactopyranoside pyridinium salt (18) and 2-(Tetradecyl)hexadecyl 4-*O*-Sulfo-β-D-galactopyranoside sodium salt (III). To a solution of **16** (198 mg) in DMF (1.5 mL) was added sulfur trioxide-pyridine complex (146 mg) and the mixture was stirred at room temperature, the reaction being monitored by TLC (8:1 CH_2Cl_2-MeOH). Methanol (1 mL) was added and the mixture was concentrated to a residue, which was chromatographed (50:1~10:1 CH_2Cl_2-MeOH) on a column of silica gel packed with Sephadex LH-20 to give **18** (185 mg, 80%): $[\alpha]_D$ +22° (*c* 0.4, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×CH_2, and *CH*), 3.31, 3.79 (2dd, 2H, OCH_2CH), 4.74 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 5.34 (brd, 1H, $J_{3,4}$ = 3 Hz, H-4), 5.47 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3 Hz, H-3), 5.68 (dd, 1H, $J_{1,2}$ = 8, $J_{2,3}$ = 10 Hz, H-2).

To a solution of **18** (440 mg) in THF (7 mL) was added NaOMe (2 mL of 25 wt. % soln in MeOH), and the mixture was stirred overnight at room temperature, the reaction being monitored by TLC (4:1 CH_2Cl_2-MeOH), then processed as described for **II** to afford the title compound **III** (265 mg, 85%) as an amorphous mass: mp 207-209 °C (decomp); ^1H NMR (pyridine-d_5) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×CH_2, and CH), 3.58, 4.06 (2dd, 2H, J_{gem} = 9, J_{vic} = 6, 5.5 Hz, OCH_2CH), 4.13, 4.24 (2dd, 2H, J_{gem} = 11.4, $J_{5,6}$ = 6.4, 5.7 Hz, H-6), 4.30 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.1 Hz, H-3), 4.44 (dd, 1H, $J_{1,2}$ = 7.5, $J_{2,3}$ = 10 Hz, H-2), 4.68 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 5.64 (d, 1H, $J_{3,4}$ = 3.1, $J_{4,5}$ = ~0 Hz, H-4); ion-spray MS; (positive-ion mode) *m/z* 681.5 $[M - Na + 2H]^+$ (base peak), 722.6 $[M - Na + 2H + MeCN]^+$; MS/MS (positive-ion mode, P = *m/z* 681.5) *m/z* 680.6 $[M - Na + H]^+$ (42), 600.6 $[P - SO_3]^+$ (20), 421.7 [$C_{30}H_{61}$; 2-(tetradecyl)hexadecyl fragment]$^+$ (30), 242.3 $[P - C_{30}H_{61}O; GalSO_3]^+$ (100), 162.8 $[242.3' - SO_3]^+$ (61), 145.0 $[162.8 - H_2O]^+$ (35). Calcd for $C_{36}H_{71}O_9S$ (M-Na): 680.0.

2-(Tetradecyl)hexadecyl 3,4-Di-*O*-sulfo-β-D-galactopyranoside sodium salt (IV). To a solution of **17** (256 mg) in DMF (0.75 mL) was added sulfur trioxide-pyridine complex (510 mg) and the mixture was stirred for overnight at room temperature to give a single spot of **19** on TLC (6:1 CH_2Cl_2-MeOH). Methanol (1 mL) and THF (1 mL) were added and then the mixture was treated with NaOMe (4 mL of 25 wt. % soln in MeOH) overnight at room temperature to give a single spot on TLC (3.5:1:0.1 CH_2Cl_2-MeOH-H_2O), then concentrated. Column chromatography of the residue on Merck silica gel 60 silanized (70-230 mesh) with (a) 1:1 H_2O-MeOH and (b) 1:1 CH_2Cl_2-MeOH. Eluent (b) gave the pure title compound **IV** (151 mg, 63%): mp 225-227 °C (decomp); ^1H NMR (DMSO-d_6, 59 °C) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×CH_2, and CH), 4.14 (dd, 1H, $J_{2,3}$ = 9.9, $J_{3,4}$ = 3.3 Hz, H-3), 4.19 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.55 (d, 1H, $J_{3,4}$ = 3.3, $J_{4,5}$ = ~0 Hz, H-4); ion-spray MS (negative-ion mode) *m/z* 379.2 $[M - 2Na]^{2-}$; MS/MS (negative-ion mode, P = *m/z* 379) *m/z* 660.7 (3), 240.9 (3), 222.4 (3), 159.6 (6), 152.6 (8), 138.8 (6), 96.7 (100), 79.7 (10). Calcd for $C_{36}H_{70}O_{12}S_2$ (M-2Na): 759.0. Also see Figure 2, (A).

2-(Tetradecyl)hexadecyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (20) and 2-(Tetradecyl)hexadecyl β-D-Galactopyranoside (21). To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (1.0 g) and 2-(tetradecyl)hexadecan-1-ol (1.07 g) in CH_2Cl_2 (30 mL) was added powdered MS AW-300 (1.8 g), and the mixture was stirred overnight at room temperature under N_2 gas, then worked up as described for **4**. Column chromatography (8:1 hexane-EtOAc) on silica gel gave **20** (1.48 g, 80%) as a syrup: $[\alpha]_D$ -75.3° (*c* 0.9, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$CH$_2$-), 1.0-1.7 (m, 53H, 26×C*H*$_2$, and C*H*), 1.98, 2.03, 2.04, 2.14 (4s, 12H, AcO), 3.31, 3.85 (2dd, 2H, J_{gem} = 9.3, J_{vic} = 6.6, 4.9 Hz, OC*H*$_2$CH), 3.91 (~t, 1H, H-5), 4.15, 4.19 (2dd, 2H, J_{gem} = 11, $J_{5,6}$ = 7.0, 6.8 Hz, H-6), 4.43 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.03 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.4 Hz, H-3), 5.21 (dd, 1H, H-2), 5.40 (~d, 1H, H-4).

To a solution of **20** (1.0 g) in anhydrous MeOH (30 mL) was added catalytic amount of NaOMe, and the mixture was refluxed until the reaction was complete (~1 h). The solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings were concentrated. The residual solid gave crystalline **21** from hot MeOH: mp 77-78 °C; $[\alpha]_D$ -2.8° (*c* 1.4, 1:1 $CHCl_3$-MeOH). For further characterization, see ref. 21.

Anal. Calcd for $C_{36}H_{72}O_6$ (600.96): C, 71.95 ; H, 12.08. Found: C, 71.81; H, 11.94.

2-(Tetradecyl)hexadecyl 6-*O*-*t*-Butyldimethylsilyl-β-D-galactopyranoside (22), and 2-(Tetradecyl)hexadecyl 2,3,4-Tri-*O*-benzoyl-6-*O*-*t*-butyldimethylsilyl-β-D-galactopyranoside (23) and 2,3,4-Tri-*O*-benzoyl-β-D-galactopyranoside (24). To a solution of **21** (260 mg) in 1:1 pyridine-CH_2Cl_2 (4 mL) was added *t*-butyldimethylsilyl chloride (TBDMSCl, 94 mg), and the mixture was stirred for 6 h at room temperature. Methanol was added and the solvent was evaporated. The residue was taken up in chloroform, and washed successively with 2M HCl and water, dried and concentrated. The residue was chromatographed (10:1 CH_2Cl_2-MeOH) on a column of silica gel to give **22** (293 g, quantitative) as an amorphous mass: $[\alpha]_D$ -11.8° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.08 (s, 6H,

Me_2Si), 0.88 (t, 6H, CH_3CH_2-), 0.89 (s, 9H, Me_3C), 1.0-1.7 (m, 53H, 26×CH_2, and CH), 3.83, 3.90 (2dd, 2H, J_{gem} = 11, J_{vic} = 5.9, 5.3 Hz, H-6), 4.0 (~d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 4.17 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1).

To a stirred solution of **22** (162 mg) in pyridine (2 mL) was added benzoyl chloride (0.26 mL) and the stirring was continued overnight at room temperature. Work-up and column chromatography (50:1 hexane-EtOAc) on silica gel gave **23** (228 g, 98%): ^1H NMR ($CDCl_3$) δ 4.74 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.60 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.76 (dd, 1H, H-2), 5.95 (d, 1H, H-4), 7.20-8.10 (m, 15H, Ph).

A mixture of **23** (200 mg) and 80% aqueous acetic acid (20 mL) was stirred for 40 h at 50 °C, and the solvent was evaporated. The residue was chromatographed (6:1 hexane-EtOAc) on a column of silica gel to afford **24** (165 mg, 93%) as a syrup: $[\alpha]_D$ +104° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.6 (m, 53H, 26×CH_2, and CH), 3.36, 3.92 (2dd, 2H, J_{gem} = 10, J_{vic} = 7.0, 5.0 Hz, OCH_2CH), 3.67, 3.84 (2dd, 2H, J_{gem} = 12, $J_{5,6}$ = 7.2, 6.4 Hz, H-6), 4.05 (~t, 1H, H-5), 4.78 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.62 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.5 Hz, H-3), 5.83-5.90 (m, 2H, H-2,4), 7.19-7.62 (m, 9H, Ph(*m*,*p*)), 7.80-8.13 (m, 6H, Ph(*o*)).

Anal. Calcd for $C_{57}H_{84}O_9$ (913.29): C, 74.96; H, 9.27. Found: C, 74.80; H, 9.21.

2-(Tetradecyl)hexadecyl 2,3,4-Tri-*O*-benzoyl-6-*O*-sulfo-β-D-galactopyranoside triethylammonium salt (25) and 2-(Tetradecyl)hexadecyl 6-*O*-Sulfo-β-D-galactopyranoside sodium salt (V). To a solution of **24** (150 mg) in DMF (2 mL) was added sulfur trioxide pyridine complex (157 mg) and the mixture was stirred for 13 h at room temperature. The resulting 6-*O*-sulfate showed a single spot on TLC (R_f 0.43, 5:1 CH_2Cl_2-MeOH). Triethylamine (0.5 mL) was added and the mixture was concentrated to a residue, which was chromatographed (50:1 CH_2Cl_2-MeOH) on a column of silica gel to give **25** (180 mg, quantitative): $[\alpha]_D$ +33° (*c* 1.0, 1:1 $CHCl_3$-MeOH); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 0.9-1.6 (m, 53H, 26×CH_2, and CH), 4.18, 4.40 (2dd, 2H, J_{gem} = 12.5 Hz, $J_{5,6}$ =

9.9, 5.3 Hz, H-6), 4.74 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.52 (dd, 1H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 5.73 (dd, 1H, H-2), 5.97 (~d, 1H, H-4), 7.21-8.03 (m, 15H, Ph).

To a solution of **25** (180 mg) in MeOH was added NaOMe (1 mL of 25 wt. % soln in MeOH) and the mixture was processed as described for **II** to give **V** (115 mg, 87%) as a solid: mp 128-130 °C; ion-spray MS (negative-ion mode) *m/z* 679.1 [M - Na]$^-$; MS/MS (negative-ion mode, P = *m/z* 679) *m/z* 240.7 [P - $C_{30}H_{61}O]^-$, 180.6 [P - $C_{30}H_{61}O$ - HOCH = CHOH]$^-$, 151.0 (O = C = $CHCH_2OSO_3^-$), 96.7 (HSO_4^-). The average mass for $C_{36}H_{71}O_9S$ was 680.16. Also see Figure 2, (B).

2-(Trimethylsilyl)ethyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (27). To a solution of **26**[21] (1.0 g) in pyridine (5 mL), cooled to 0 °C, was added benzoyl chloride (2.2 mL) and the mixture was stirred for 24 h at room temperature. After completion of the reaction, MeOH (1 mL) was added and the mixture was extracted with CH_2Cl_2. The extract was washed with M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (3:1 hexane-EtOAc) of the residue on silica gel gave **27** (2.0 g, 90%): $[\alpha]_D$ +52° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.84 (m, 2H, OCH_2CH_2Si), 4.38, 4.10 (2d, 2H, Jgem = 12.8 Hz, CH_2Ph), 4.63 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.73 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 5.44 (dd, 1H, $J_{2,3}$ = 10 Hz, H-2), 5.47 (dd, 1H, $J_{2',3'}$ = 10 Hz, H-2') 5.63 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4'), 5.75 (t, 1H, J =10 Hz, H-3), 7.0 - 8.1 (m 35H, 7Ph).

Anal. Calcd for $C_{66}H_{64}O_{17}Si$ (1157.31): C, 68.50; H, 5.57. Found: C, 68.46; H, 5.55.

***O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranose (28) and *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (29).** A solution of **27** (1.9 g) in CH_2Cl_2 (5 mL) was treated with TFA (5 mL) as described for **10**. The resulting product was purified by chromatography on a column of silica gel (5:1 hexane-EtOAc) to give **28** (1.46 g, 86%): $[\alpha]_D$ +74° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) Complete

disappearance of the peaks at δ 0.9-1.1 (m, 2H, CH_2SiMe_3), 3.67, 4.13 (2m, 2H, $CH_2CH_2SiMe_3$).

To a solution of **28** (1.7 g) in CH_2Cl_2 (5 mL) were added Cl_3CCN (0.3 mL) and DBU (0.1 mL), and the mixture was stirred for 1 h at room temperature. Work-up as described for **12** and column chromatography (6:1 hexane-EtOAc) afforded **29** (1.25 g, 66%): $[\alpha]_D$ +76° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.6-3.8 (m, 3H, H-3', 5, 5'), 4.3, 4.5 (m, 4H, H-6a,b, 6'a,b), 4.38, 4.62 (2d, 2H, J_{gem} = 12.8, CH_2Ph), 4.72 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 5.54 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 10 Hz , H-2), 5.67 (d, 1H, $J_{3',4'}$ = 3.3 Hz, H-4'), 6.13 (dd, 1H, $J_{2',3'}$ = 10 Hz, $J_{1',2'}$ = 8 Hz, H-2'), 6.69 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 8.55 (s, 1H, C=NH).

Anal. Calcd for $C_{63}H_{52}Cl_3NO_{17}$ (1201.46): C, 62.98; H, 4.36; N, 1.17. Found: C, 62.76; H, 4.25; N, 1.12.

2-(Tetradecyl)hexadecyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (30) and 2-(Tetradecyl)hexadecyl *O*-(2,4,6-Tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (31). A mixture of **29** (300 mg) and 2-(tetradecyl)hexadecan-1-ol (300 mg) in CH_2Cl_2 (3 mL) and benzene (3 mL) was stirred overnight with powdered MS AW-300 (600 mg) at room temperature and worked up as described for **4**. Column chromatography (10:1 hexane-EtOAc) on silica gel gave **30** (286 mg, 78%) as a syrup: $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 1.0-1.4, 1.4-1.6 (m, 53H, 26×CH_2 and CH), 3.25, 3.47 (2dd, 2H, J_{gem} = 9.3, J_{vic} = 6.5, 5.0 Hz, OCH_2CH), 4.37, 4.60 (2d, 2H, J_{gem} = 12.8 Hz, CH_2Ph), 4.61, 4.62 (2d, 2H, $J_{1,2}$ = 8 Hz, H-1,1'),5.44, 5.47 (2dd, 2H, $J_{2,3}$ = 10 Hz, H-2, 2'), 5.63 (~d, 1H, $J_{3,4}$ = 3.1 Hz, H-4'), 5.76 (t, 1H, J = 10 Hz, H-3), 7.0-8.1 (m, 35H, 7Ph).

Anal. Calcd for $C_{91}H_{112}O_{17}$ (1477.88): C, 73.96; H, 7.64. Found: C, 73.85; H, 7.48.

Compound **30** (250 mg) in THF (5 mL) was hydrogenolyzed in the presence of 10% palladium carbon (250 mg) for 10 h at room temperature. The catalyst was filtered off and washed with THF. The combined filtrate and washings were

concentrated to dryness. Column chromatography (50:1 CH_2Cl_2-MeOH) of the residue on silica gel gave **31** (226 mg, 92%): $[\alpha]_D$ -1° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$CH$_2$-), 1.0-1.4, 1.4-1.6 (m, 53H, 26×C*H*$_2$ and C*H*), 3.25, 3.47 (2dd, 2H, J_{gem} = 9.3, J_{vic} = 6.5, 5.0 Hz, OC*H*$_2$CH), 4.63 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.73 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 5.30 (dd, 1H, $J_{2,3}$ = 10 Hz, H-2), 5.47 (dd, 1H, $J_{2',3'}$ = 10 Hz, H-2'), 5.48 (~d, 1H, $J_{3',4'}$ = 3.1, $J_{4',5'}$ = ~0 Hz, H-4'), 5.74 (t, 1H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3), 7.0-8.1 (m, 30H, 6×PhCO).

Anal. Calcd for $C_{84}H_{106}O_{17}$ (1387.7): C, 72.70; H, 7.70. Found: C, 72.66; H, 7.58.

2-(Tetradecyl)hexadecyl *O*-(2,4,6-Tri-*O*-benzoyl-3-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside triethylanmonium salt (32) and 2-(Tetradecyl)hexadecyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (VI). To a solution of **31** (470 mg) in DMF (2 mL) was added sulfur trioxide pyridine complex (110 mg) and the mixture was stirred at room temperature, the reaction being monitored by TLC (8:1 CH_2Cl_2-MeOH). Triethylamine (1 mL) was added and the mixture was concentrated to a residue, which was chromatographed (125:1 CH_2Cl_2-MeOH) on a column of silica gel to give **32** (580 mg, quant): $[\alpha]_D$ +19° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, C*H*$_3$CH$_2$-), 0.90 (m, 9H, 3×NCH$_2$C*H*$_3$), 1.0-1.4, 1.4-1.6 (m, 53H, 26×C*H*$_2$, and C*H*), 2.73 (m, 6H, 3×NC*H*$_2$CH$_3$), 3.20, 3.37 (2dd, 2H, J_{gem} = 10.9, J_{vic} = 6.8 Hz, OC*H*$_2$CH), 4.61 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.71 (dd, 1H, $J_{2',3'}$ = 10, $J_{3',4'}$ = 3.1 Hz, H-3'), 4.80 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 5.40 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 5.51 (dd, 1H, $J_{2',3'}$ = 10 Hz, H-2'), 5.85 (~d, 1H, $J_{3',4'}$ = 3.1, $J_{4',5'}$ = ~0 Hz, H-4'), 5.75 (t, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 6.9-8.1 (m, 30H, 6×PhCO).

Anal. Calcd for $C_{90}H_{121}NO_{20}S$ (1569.01): C, 68.90; H, 7.77, N, 0.89. Found: C, 68.69; H, 7.54, N, 0.78.

To a solution of **32** (500 mg) in MeOH (15 mL) was added NaOMe (2 mL of 25 wt. % soln in MeOH), and the mixture was stirred overnight at room temperature, the reaction being monitored by TLC (4:1 CH_2Cl_2-MeOH), then processed as

described for **II** to afford the title compound **VI** (215 mg, 78%) as an amorphous mass: mp 247 °C (decomp); ^{1}H NMR (DMSO-d_6: D_2O = 49:1, 50 °C) δ 0.86 (t, 6H, CH_3CH_2-), 0.9-1.4, 1.4-1.6 (m, 53H, 26×CH_2 and CH), 3.90 (d, 1H, $J_{3',4'}$ = 3.4 Hz, H-4'), 4.00 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.12 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.31 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'); ion spray MS (negative-ion mode) *m/z* 841.3 [M - Na]$^-$; MS/MS (negative-ion mode, P = *m/z* 841) *m/z* 402.8 [P - $C_{30}H_{61}O$]$^-$, 240.7 [P - $C_{30}H_{61}O$ - Glc]$^-$, 96.9 $(HSO_4)^-$. Also see Figure 2, (C).

2-(Tetradecyl)hexadecyl *O*-(-3-*O*-Benzyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (33). To a solution of **30** (390 mg) in MeOH (5 mL) and THF (5 mL) was added NaOMe (2 mL of 25 wt. % soln in MeOH), and the mixture was stirred overnight at room temperature. After completion of the reaction, the solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (30:1 CH_2Cl_2-MeOH) of the residue on silica gel gave **33** (210 mg, 93%) as a syrup: $[\alpha]_D$ +0.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, CH_3CH_2-), 0.90 (m, 9H, 3×NCH_2CH_3), 1.0-1.4, 1.4-1.6 (m, 53H, 26×CH_2 and CH), 2.73 (m, 6H, 3×NCH_2CH_3), 4.22 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.67, 4.74 (2d, J_{gem} = 12.2 Hz, CH_2Ph), 7.3-7.4 (m, 5H, *Ph*CH_2).

Anal. Calcd for $C_{49}H_{88}O_{11}$ (853.23): C, 68.98; H, 10.40. Found: C, 68.95; H, 10.27.

2-(Tetradecyl)hexadecyl *O*-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranoside (34). Compound **33** (210 mg) in EtOH (5 mL) and THF (5 mL) was hydrogenolyzed in the presence of 10% palladium carbon (200 mg) for 20 h at room temperature. The catalyst was filtered off and washed with THF. The combined filtrate and washings were concentrated to dryness. Column chromatography (20:1 CH_2Cl_2-MeOH) of the residue on silica gel gave **34** (153 mg, 82%): mp 207-210 °C; for ^{1}H and ^{13}C NMR data, see ref. 21.

Anal. Calcd for $C_{42}H_{82}O_{11}$ (763.11): C, 66.11; H, 10.83. Found: C, 65.94; H, 10.82.

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