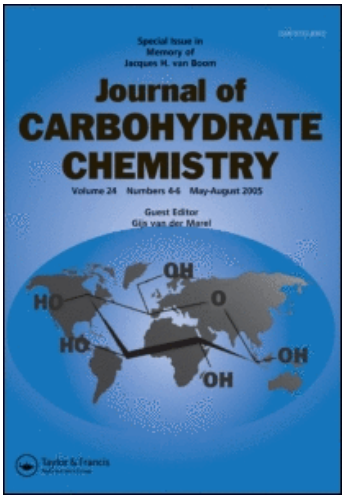

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Synthetic Studies on Selectin Ligands/Inhibitors: One-Pot Synthesis of the Mono- and Oligo-Sulfated 2-(Tetradecyl)Hexadecyl β-D-Galacto- and Lactopyranosides as the Sulfatide Mimetics

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SYNTHETIC STUDIES ON SELECTIN LIGANDS/INHIBITORS: ONE-POT SYNTHESIS OF THE MONO- AND OLIGO-SULFATED 2-(TETRADECYL)HEXADECYL β-D-GALACTO- AND LACTOPYRANOSIDES AS THE SULFATIDE MIMETICS[1]

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ABSTRACT

Regioselective sulfation through the dibutylstannylene acetals was applied to the key step to prepare a number of sulfated saccharides which are active as the inhibitors of L- and P-selectin. The number and the positions of the sulfate groups were confirmed by NMR and MS analyses. Using this methodology, our target sulfated glycolipids (**6-9**, **12-14**) were conveniently synthesized in one-pot from free 2-(tetradecyl)hexadecyl β-D-galactopyranoside **5** and lactoside **11**.

INTRODUCTION

Cell adhesion molecules (CAMs) play a crucial role in the inflammatory conditions and several immune system disorders by recruiting leukocytes to the injured area.[2] The selectins are a family of CAMs comprised of three structurally related carbohydrate-binding proteins [E-selectin (ELAM-1), L-selectin (LECAM-1) and P-selectin (GMP-140, PADGEM)].[3-6] The selectin family appears to be involved in the earliest events of the acute inflammatory response, and the selectin-dependent adhesion-promoting process is thought to be responsible for the transient "rolling" phenomenon of leukocytes along the endothelial surfaces.[7,8] A number of recent reports have focused on the identification of carbohydrate ligand for the selectin family.[9-14] It has also been known that L- and P-selectin bind to sulfated carbohydrates such as sulfatides, fucoidan, a sulfated glucuronic acid (HNK-1) epitope and heparin.[15-18] In particular, sulfatide (ceramide is linked to galactose containing a sulfate group on position 3 of the pyranoside ring) and synthetic sulfatides[1] bind avidly to L-selectin.[19] They have also shown highly protective effects against selectin-dependent inflammatory lung injury.[20] In view of these facts, we here describe an effective method for the synthesis of mono- and oligo-sulfated glycolipids as selectin inhibitors, by employing the stannylene mediated regioselective sulfation[21,22] on the galactoside and lactoside with a "branched anchor", 2-(tetradecyl)hexadecyl group.[23,24]

RESULTS AND DISCUSSION

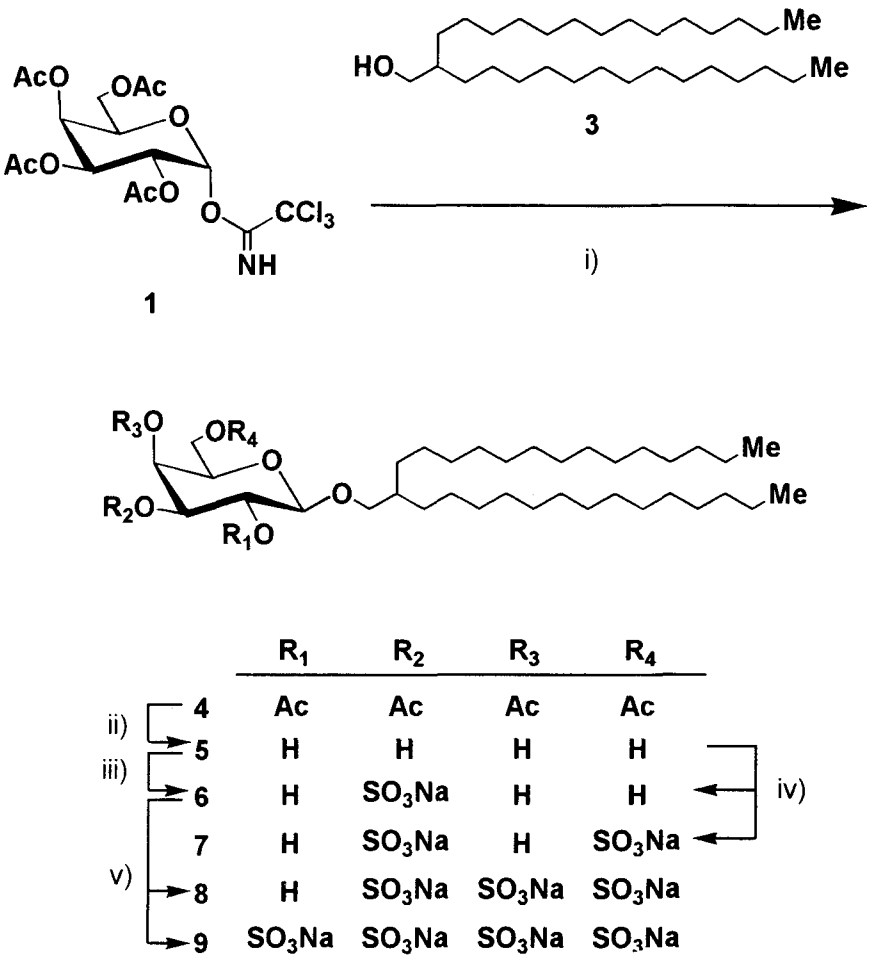
2,3,4,6-Tetra-*O*- acetyl-α-D-galactopyranosyl trichloroacetimidate[1] (**1**) and *O*-(2,3,4,6-tetra-*O*- acetyl-β-D-galactopyranosyl) - (1→4) -2,3,6-tri-*O*- acetyl-α-D-glucopyranosyl trichloroacetimidate (**2**) were each coupled with 2-(tetradecyl)-hexadecan-1-ol (**3**) in dichloromethane in the presence of boron trifluoride etherate and molecular sieves (MS AW 300), to give exclusively the β-glycosides **4**[1] and **10** in 67 and 61% yield, respectively. *O*-Deacylation of **4** or **10** with sodium hydroxide in methanol gave the desired parent glycolipids **5** and **11** in which all

hydroxy groups are unprotected. The structures of **5** and **11** were confirmed by ^{1}H and ^{13}C NMR, and FAB-MS analyses.

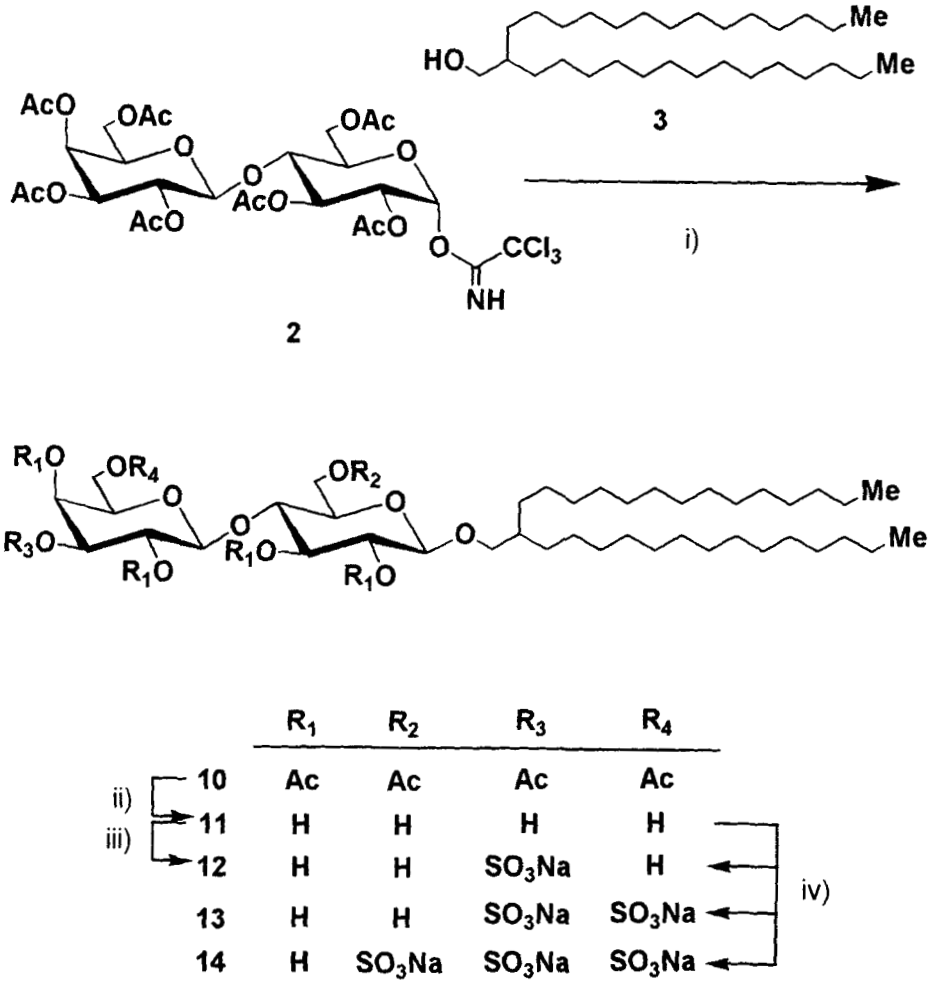
The regioselective, one-pot sulfations for **5** and **11** were achieved by treatment of the corresponding stannylene intermediates with sulfur trioxide/ trimethylamine complex. 2-(Tetradecyl)hexadecyl β-D-galactopyranoside (**5**) was converted, by stirring with dibutyltin oxide in dry toluene, to the stannylene acetal which was then sulfated with 1.0 equivalent of sulfur trioxide/trimethylamine complex in 1:1 DMF-THF solution to give only 3-sulfated galactoside **6** in 68% yield, exclusively. The use of 2.2 equivalents of sulfur trioxide/trimethylamine complex afforded 3-sulfated galactoside **6** and 3,6-disulfated galactoside **7** in 34 and 66% yield, respectively. Further sulfation of 3-sulfated galactoside **6** using 2.5 equivalents of sulfur trioxide/trimethylamine complex afforded 3,4,6-trisulfated galactoside **8** and 2,3,4,6-tetrasulfated galactoside **9** in 44 and 29% yield, respectively (Scheme 1). The sulfated products could be easily separated on a column of silica gel and isolated as sodium salts by cation exchange resin.

The similar, one-pot sulfation of 2-(tetradecyl)hexadecyl *O*-(β-D-galacto-pyranosyl)-(1→4)-β-D-glucopyranoside (**11**) using 1.0 equivalent of sulfur trioxide/ trimethylamine complex, as just described for galactoside **5**, gave only 3'-sulfated lactoside **12** in 66% yield. The use of 2.2 equivalents of sulfur trioxide/ trimethylamine complex afforded 3'-sulfated lactoside **12**, 3',6'-disulfated lactoside **13** and 6,3',6'-trisulfated lactoside **14** in molar ratio 1 : 2 : 1 (Scheme 2).

The structures of the sulfated products were determined by NMR and MS analyses. The ^{1}H and ^{13}C NMR has also been used to locate the positions of sulfate groups.[25-27] Comparison of the ^{1}H NMR data (Table 1) of the sulfated galactosides **6**, **7**, **8** and **9** with those of the unsulfated precursor glycolipid **5** demonstrated that the sulfate groups deshield the geminal and vicinal protons. The secondary sulfate groups in sulfated derivatives caused α effects of 0.7 - 0.8 ppm, whereas the primary sulfate groups showed 0.4 - 0.5 ppm for this effects. The β effects were 0.1 - 0.4 ppm depending on the axial or equatorial orientation of the vicinal proton. The H-4 signal of the 3-sulfate **6** was shifted more than the H-2



Scheme 1. i) $BF_3 \cdot Et_2O$, CH_2Cl_2 (**1**→**4**, 67%).
ii) NaOH, MeOH-THF (**4**→**5**, 98%).
iii) Bu_2SnO(1.1 equiv), toluene,
$SO_3 \cdot NMe_3$(1.0 equiv), DMF-THF (**5**→**6**, 68%).
iv) Bu_2SnO(1.1 equiv), toluene, $SO_3 \cdot NMe_3$(2.2 equiv),
DMF-THF (**5**→**6**, 34%; **5**→**7**, 66%).
v) $SO_3 \cdot NMe_3$(2.5 equiv), DMF-THF (**6**→**8**, 44%; **6**→**9**, 29%).



		R_1	R_2	R_3	R_4
	10	Ac	Ac	Ac	Ac
ii)	**11**	H	H	H	H
iii)	**12**	H	H	SO_3Na	H
iv)	**13**	H	H	SO_3Na	SO_3Na
iv)	**14**	H	SO_3Na	SO_3Na	SO_3Na

Scheme 2. i) $BF_3 \cdot Et_2O$, CH_2Cl_2 (**2**→**10**, 61%).
ii) NaOH, MeOH (**10**→**11**, 93%).
iii) Bu_2SnO(1.1 equiv), toluene,
$SO_3 \cdot NMe_3$(1.0 equiv), DMF (**11**→**12**, 66%).
iv) Bu_2SnO(1.1 equiv), toluene,
$SO_3 \cdot NMe_3$(2.2 equiv), DMF (**11**→**12**, 24%; **11**→**13**, 48%;
11→**14**, 19%).

Table 1. ^{1}H NMR Chemical Shifts[a] of the Unsulfated and Sulfated Galactopyranosides **5 - 9**.

Compound	Chemical Shift (and Shift Relative to **5**)						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6'
5 (unsulfated)	4.18	3.52	3.45	3.85	3.49	3.75	3.75
6 (3-sulfate)	4.28	3.70	**4.21**	4.25	3.53	3.74	3.74
	(+0.10)	(+0.18)	(+0.76)	(+0.40)	(+0.04)	(-0.01)	(-0.01)
7 (3,6-disulfate)	4.30	3.70	**4.25**	4.27	3.82	**4.16**	**4.22**
	(+0.12)	(+0.18)	(+0.80)	(+0.42)	(+0.33)	(+0.41)	(+0.47)
8 (3,4,6-trisulfate)	4.45	3.57	**4.40**	**5.00**	4.00	**4.18**	**4.23**
	(+0.27)	(+0.05)	(+0.95)	(+1.15)	(+0.51)	(+0.43)	(+0.48)
9 (2,3,4,6-tetrasulfate)	4.60	**4.40**	**4.55**	**5.10**	4.10	**4.20**	**4.25**
	(+0.42)	(+0.88)	(+1.10)	(+1.25)	(+0.61)	(+0.45)	(+0.45)

a. Recorded in CD_3OD with TMS as an internal standard.

signal. The resonance for an equatorial proton next to an equatorial sulfate group was shifted more downfield as compared to an axial proton. Downfield shifts of the H-5 signals were also observed in the 3,4,6-trisulfate **8** and 2,3,4,6-tetrasulfate **9**. Comparison of the ^{13}C NMR data (Table 2) of the sulfated galactosides **6**, **7**, **8** and **9** with those of the parent glycolipid **5** demonstrated that the specific downfield shifts of 4 - 7 ppm for the signals of the α-carbon atoms bearing the sulfate groups were observed, while the β-carbon atoms were upfield 2 - 4 ppm.

The ^{1}H and ^{13}C NMR data of sulfated lactosides **12**, **13** and **14** could be assigned completely by 1D decoupling, by 2D (^{1}H,^{1}H) COSY, and by 2D (^{13}C,^{1}H) COSY experiments. The signals of the geminal protons were shifted downfield 0.3 - 0.4 ppm by a primary sulfate group (**13** and **14**) and 0.6 - 0.7 ppm by a secondary sulfate group (**12**, **13** and **14**) (Table 3). The signals of the α-carbon atoms were shifted downfield 4 - 5 ppm by a primary sulfate group (**13** and **14**) and 5 - 6 ppm by a secondary sulfate group (**12**, **13** and **14**) (Table 4). These data were in agreement with previous observations.[25-27] Furthermore negative FAB mass spectrometry gave $(M-Na)^-$ ions as the base peaks, confirming the number of the sulfate groups in the molecules.

The above results indicated that the combination of NMR and MS analyses is helpful in assigning the number and positions of sulfate groups in oligosaccharides.

In conclusion, a systematic synthesis of the mono- and oligo-sulfated glycolipids (**6-9**, **12-14**) carrying the 2-(tetradecyl)hexadecyl group as a substitute for ceramide has been achieved by the regioselective, one-pot sulfation through the stannylene acetals with a certain amount of sulfur trioxide/trimethylamine complex. The structures of the resulting sulfated glycolipids were determined by NMR and MS analyses. These glycolipids may be useful as the effective therapeutic agents against selectin-dependent inflammation.

EXPERIMENTAL

General methods. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured

Table 2. ^{13}C NMR Chemical Shifts[a] of the Unsulfated and Sulfated Galactopyranosides **5** - **9**.

Compound	Chemical Shift (and Shift Relative to **5**)					
	C-1	C-2	C-3	C-4	C-5	C-6
5 (unsulfated)	105.4	72.8	75.2	70.4	76.6	62.5
6 (3-sulfate)	105.0	70.7	**82.2**	68.5	76.0	62.2
	(-0.4)	(-2.1)	(+7.0)	(-1.9)	(-0.6)	(-0.3)
7 (3,6-disulfate)	104.3	69.9	**81.3**	67.2	72.6	**66.3**
	(-1.1)	(-2.9)	(+6.1)	(-3.2)	(-4.0)	(+3.8)
8 (3,4,6-trisulfate)	104.1	70.0	**78.6**	**75.8**	72.5	**67.3**
	(-1.3)	(-2.8)	(+3.4)	(+5.4)	(-4.1)	(+4.8)
9 (2,3,4,6-tetrasulfate)	102.6	**76.3**	**76.9**	**76.1**	72.6	**67.4**
	(-2.8)	(+3.5)	(+1.7)	(+5.7)	(-4.0)	(+4.9)

a. Recorded in CD_3OD with TMS as an internal standard.

Table 3. [1] H NMR Chemical Shifts [a] of the Unsulfated and Sulfated Lactosides **11 - 14**.

Compound	Chemical Shift (and Shift Relative to **11**)													
	Glucose							Galactose						
	H-1a	H-2a	H-3a	H-4a	H-5a	H-6a	H-6'a	H-1b	H-2b	H-3b	H-4b	H-5b	H-6b	H-6'b
11 (unsulfated)	4.22	3.18	3.38	3.38	3.32	3.58	3.76	4.29	3.42	3.40	3.71	3.54	3.56	3.56
12 (3'-sulfate)	4.13	3.00	3.36	3.38	3.26	3.57	3.76	4.33	3.52	**4.01**	3.89	3.52	3.52	3.52
	(-0.09)	(-0.18)	(-0.02)	(+0.00)	(-0.06)	(-0.01)	(+0.00)	(+0.04)	(+0.10)	(+0.61)	(+0.18)	(-0.02)	(-0.04)	(-0.04)
13 (3',6'-disulfate)	4.15	3.04	3.36	3.35	3.25	3.61	3.73	4.36	3.54	**4.02**	3.94	3.75	**3.88**	**3.88**
	(-0.07)	(-0.14)	(-0.02)	(-0.03)	(-0.07)	(+0.03)	(-0.03)	(+0.07)	(+0.12)	(+0.62)	(+0.23)	(+0.21)	(+0.32)	(+0.32)
14 (6,3',6'-trisulfate)	4.18	3.07	3.36	3.34	3.48	**3.90**	**4.08**	4.43	3.47	**4.05**	3.97	3.72	**3.90**	**3.90**
	(-0.04)	(-0.11)	(-0.02)	(-0.04)	(+0.16)	(+0.32)	(+0.34)	(+0.14)	(+0.05)	(+0.65)	(+0.26)	(+0.18)	(+0.34)	(+0.34)

a. Recorded in DMSO-d_6 with TMS as an internal standard.

Table 4. ^{13}C NMR Chemical Shifts [a] of the Unsulfated and Sulfated Lactosides **11** - **14**.

Compound	Chemical Shift (and Shift Relative to **11**)											
	Glucose						Galactose					
	C-1a	C-2a	C-3a	C-4a	C-5a	C-6a	C-1b	C-2b	C-3b	C-4b	C-5b	C-6b
11 (unsulfated)	102.9	73.0	75.0	80.8	74.7	60.2	103.8	70.5	73.1	68.0	75.4	60.6
12 (3'-sulfate)	102.9	73.1	74.9	81.1	74.7	60.5	103.7	69.3	**78.7**	66.5	75.3	60.0
	(+0.0)	(+0.1)	(-0.1)	(+0.3)	(+0.0)	(+0.3)	(-0.1)	(-1.2)	(+5.6)	(-1.5)	(-0.1)	(-0.6)
13 (3',6'-disulfate)	103.1	73.5	75.2	80.5	75.1	60.9	103.9	68.9	**79.1**	66.8	73.3	**65.0**
	(+0.2)	(+0.5)	(+0.2)	(-0.3)	(+0.4)	(+0.7)	(+0.1)	(-1.6)	(+6.0)	(-1.2)	(-2.1)	(+4.4)
14 (6,3',6'-trisulfate)	103.0	73.1	74.9	80.3	73.1	**65.2**	103.5	69.1	**78.6**	66.7	73.3	**65.0**
	(+0.1)	(+0.1)	(-0.1)	(-0.5)	(-1.6)	(+4.5)	(-0.3)	(-1.4)	(+5.5)	(-1.3)	(-2.1)	(+4.4)

a. Recorded in DMSO-d_6 with TMS as an internal standard.

on a Horiba SEPA-300 polarimeter in a 10 cm cell at 25 ℃. IR spectra were recorded with a Perkin-Elmer 1600 spectrometer. NMR spectra were recorded on a Jeol JNM-GX 270 spectrometer (270 MHz for ^{1}H and 68 MHz for ^{13}C). Chemical shifts were expressed in parts per million downfield from TMS. FAB-MS were recorded on a Jeol JMS-SX 120A mass spectrometer/JMA-DA7000 data system. Each sample was mixed with a glycerol matrix (LRMS) and PEG 600 or PEG 1000 matrix (HRMS) on a target. The ion accelerating voltage was 8.0 kV and the primary beam for the bombardment was 6.0 keV of xenon. Thin-layer chromatography was run on Merck Kieselgel 60 F_{254} with detection by UV and spraying 6N H_2SO_4, then heating about 2 min at 300 ℃. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Tetradecyl)hexadecyl β-D-Galactopyranoside (5). To a solution of **4** (940 mg, 1.22 mmol) in MeOH (5 mL) and THF (5 mL) was added 4N NaOH (2.44 mL, 9.78 mmol) and the mixture was stirred at room temperature until deacetylation was complete (2 h). Purification by precipitation with water yielded **5** (716 mg, 98%) as an amorphous mass: mp 78 ℃; $[\alpha]_D$ -6.0° (*c* 0.3, MeOH); R_f 0.58 (4:1 $CHCl_3$-MeOH); ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me.CH_2}$ = 6.9 Hz, 2*Me* CH_2), 1.30 (s, 52H, 26CH_2), 1.55-1.65 (m, 1H, CH of fatty alkyl), 3.42 (dd, 1H, J_{vic} = 3.5, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.45 (dd, 1H, $J_{3,4}$ = 3.5 Hz, H-3), 3.49 (dt, 1H, $J_{5,6}$ = 5.9 Hz, H-5), 3.52 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 3.75 (d, 2H, H-6), 3.80 (dd, 1H, J_{vic} = 5.9 Hz, H-1' of fatty alkyl), 3.85 (dd, 1H, $J_{4,5}$ = 1.0 Hz, H-4), 4.18 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1); ^{13}C NMR (CD_3OD) δ 14.3 (CH_3), 23.6, 27.8, 30.4, 30.6, 30.7, 31.0, 32.3 and 33.0 (CH_2), 39.6 (CH), 62.5 (C-6), 70.4 (C-4), 72.8 (C-2), 74.1 (OCH_2), 75.2 (C-3), 76.6 (C-5), 105.4 (C-1). LRMS (FAB negative) : *m/z* 599.5 [100% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{71}O_6$ $(M-H)^-$: 599.5251. Found: 599.5249.

2-(Tetradecyl)hexadecyl 3-*O*-Sulfo-β-D-galactopyranoside sodium salt (6) and **2-(Tetradecyl)hexadecyl 3,6-Di-*O*-sulfo-β-D-galactopyranoside disodium salt (7).** Compound **5** (250 mg, 0.417 mmol) and dibutyltin oxide (114 mg,

0.458 mmol) were stirred in refluxing toluene (40 mL) for 24 h with continuous removal of water, and concentrated. To a solution of the stannyl complex in DMF (4 mL) and THF (4 mL) was added sulfur trioxide/trimethylamine complex (127 mg, 0.917 mmol) and the mixture was stirred for 12 h at room temperature, then concentrated. The residue was chromatographed (3:17 $CHCl_3$-MeOH) on silica gel (40 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH), to give **6** (99 mg, 34%) and **7** (221 mg, 66%) as amorphous masses.

Compound **6** had $[\alpha]_D$ +0.8° (*c* 0.3, MeOH) ; Rf 0.55 (8:5:1 $CHCl_3$-MeOH -H_2O); ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2*Me* CH_2), 1.30 (s, 52H, 26CH_2), 1.55-1.65 (m, 1H, CH of fatty alkyl), 3.42 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.53 (t, 1H, $J_{5,6}$ = 6.4 Hz, H-5), 3.70 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 3.74 (d, 2H, H-6), 3.80 (dd, 1H, J_{vic} = 5.9 Hz, H-1' of fatty alkyl), 4.21 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 4.25 (d, 1H, H-4), 4.28 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1) ; ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.6, 27.6, 27.7, 30.3, 30.6, 31.0, 32.0 and 32.9 (CH_2), 39.4 (CH), 62.2 (C-6), 68.5 (C-4), 70.7 (C-2), 74.0 (OCH_2), 76.0 (C-5), 82.2 (C-3), 105.0 (C-1). LRMS (FAB negative): *m/z* 679.5 [100% $(M\text{-}Na)^-$], 701.5[4% $(M\text{-}H)^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{71}O_9S$ $(M\text{-}Na)^-$: 679.4819. Found: 679.4792.

Compound **7** had $[\alpha]_D$ +0.3° (*c* 0.3, 5:1 H_2O-MeOH) ; Rf 0.46 (8:5:1 $CHCl_3$-MeOH-H_2O); ^{1}H NMR (1:1 CD_3OD-D_2O) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2*Me* CH_2), 1.30 (s, 52H, 26CH_2), 1.55-1.65 (m, 1H, CH of fatty alkyl), 3.41 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.8 Hz, H-1 of fatty alkyl), 3.70 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 3.78 (dd, 1H, J_{vic} = 6.4 Hz, H-1' of fatty alkyl), 3.82 (dd, 1H, $J_{5,6}$ = 6.4, $J_{5,6'}$ = 6.1 Hz, H-5), 4.16 (dd, 1H, J_{gem} = 10.3 Hz, H-6), 4.22 (dd, 1H, H-6'), 4.25 (d, 1H, H-3), 4.27 (near s ,1H, H-4), 4.30 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1) ; ^{13}C NMR (1:1 CD_3OD-D_2O) δ 14.7 (CH_3), 23.6, 27.2, 30.4, 30.6, 30.8, 30.9, 31.1 and 32.9 (CH_2), 38.8 (CH), 66.3 (C-6), 67.2 (C-4), 69.9 (C-2), 72.6 (C-5), 74.6 (OCH_2), 81.3 (C-3), 104.3 (C-1). LRMS (FAB negative): *m/z* 781.4 [100% $(M\text{-}Na)^-$], 803.4 [4% $(M\text{-}H)^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{70}O_{12}S_2Na$ $(M\text{-}Na)^-$: 781.4206. Found : 781.4236.

2-(Tetradecyl)hexadecyl 3,4,6-Tri-*O*-sulfo-β-D-galactopyranoside tri-sodium salt (8) and **2-(Tetradecyl)hexadecyl 2,3,4,6-Tetra-*O*-sulfo-β-D-galacto-pyranoside tetrasodium salt (9).** A mixture of **6** (100 mg, 0.142 mmol) and sulfur trioxide/trimethylamine complex (88.7 mg, 0.356 mmol) in DMF (1 mL) and THF (1 mL) was stirred for 14 h at room temperature, then concentrated. The residue was chromatographed (8:5:1 $CHCl_3$-MeOH-H_2O) on silica gel (30 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH) to give **8** (57.0 mg, 44%) and **9** (41.4 mg, 29%) as amorphous masses.

Compound **8** had $[\alpha]_D$ +1.5° (*c* 1.0, 10:1 H_2O-MeOH) ; Rf 0.32 (8:5:1 $CHCl_3$-MeOH-H_2O) ; ^1H NMR (1:1 CD_3OD-D_2O) δ 0.84 (t, 6H, J_{Me,CH_2} = 6.8 Hz, 2*Me* CH_2), 1.30 (s, 52H, 26CH_2), 1.55-1.65 (m, 1H, CH of fatty alkyl), 3.42 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.3 Hz, H-1 of fatty alkyl), 3.57 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 3.78 (dd, 1H, J_{vic} = 6.4 Hz, H-1' of fatty alkyl), 4.00 (dd, 1H, $J_{5,6}$ = 6.4, $J_{5,6'}$ = 4.9 Hz, H-5), 4.18 (dd, 1H, J_{gem} = 10.9 Hz, H-6), 4.23 (dd, 1H, H-6'), 4.40 (d, 1H, H-3), 4.45 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.00 (near s, 1H, H-4) ; ^{13}C NMR (1:1 CD_3OD-D_2O) δ 14.7 (CH_3), 23.6, 26.7, 27.0, 30.2, 30.5, 30.7, 30.9 and 32.9 (CH_2), 38.6 (CH), 67.3 (C-6), 70.0 (C-2), 72.5 (C-5), 75.1 (OCH_2), 75.8 (C-4), 78.6 (C-3), 104.1 (C-1). LRMS (FAB negative): *m/z* 883.4 [100% $(M-Na)^-$], 905.4 [4% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{69}O_{15}S_3Na_2$ $(M-Na)^-$: 883.3594. Found: 883.3568.

Compound **9** had $[\alpha]_D$ +0.5° (*c* 0.3, H_2O); Rf 0.16 (8:5:1 $CHCl_3$-MeOH-H_2O); ^1H NMR (1:1 CD_3OD-D_2O) δ 0.88 (t, 6H, J_{Me,CH_2} = 6.8 Hz, 2*Me* CH_2), 1.28 (s, 52H, 26CH_2), 1.60-1.70 (m, 1H, CH of fatty alkyl), 3.48 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.3 Hz, H-1 of fatty alkyl), 3.80 (dd, 1H, J_{vic} = 6.4 Hz, H-1' of fatty alkyl), 4.10 (dd, 1H, $J_{5,6}$ = 6.9, $J_{5,6'}$ = 5.4 Hz, H-5), 4.20 (dd, 1H, J_{gem} = 9.9 Hz, H-6), 4.25 (dd, 1H, H-6'), 4.40 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 4.55 (dd, 1H, $J_{3,4}$ = 2.0 Hz, H-3), 4.60 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1), 5.10 (d, 1H, H-4) ; ^{13}C NMR (1:1 CD_3OD-D_2O) δ 14.7 (CH_3), 23.6, 26.8, 27.0, 30.2, 30.4, 30.6, 30.8 and 32.9 (CH_2), 38.5 (CH), 67.4 (C-6), 72.6 (C-5), 75.3 (OCH_2), 76.1 (C-4), 76.3 (C-2), 76.9 (C-3), 102.6 (C-1). LRMS (FAB negative) : *m/z* 985.3 [68% $(M-Na)^-$].

HRMS (FAB negative) Calcd for $C_{36}H_{68}O_{18}S_4Na_3$ $(M-Na)^-$: 985.2982. Found: 985.2982.

2-(Tetradecyl)hexadecyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (10). To a solution of the trichloroacetimidate **2** (930 mg, 1.19 mmol) and 2-(tetradecyl)hexadecan-1-ol (**3**, 522 mg, 1.19 mmol) in dry CH_2Cl_2 (3 mL) were added powdered molecular sieves (MS AW 300, 1.8 g) and the mixture was stirred for 6 h at room temperature, then cooled to 0 ℃. Boron trifluoride etherate (150 μL, 1.19 mmol) was added and the mixture was stirred for 2 h at room temperature. The precipitates were filtered off and washed with CH_2Cl_2. The filtrate and washings were combined and the solution was successively washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), then concentrated. Column chromatography (2:3 EtOAc-hexane) of the residue on silica gel (30 g) gave **10** (765 mg, 61%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2*Me* CH_2), 1.30 (s, 52H, 26CH_2), 1.5-1.6 (m, 1H, CH of fatty alkyl), 1.97, 2.02, 2.05, 2.06, 2.07, 2.12 and 2.15 (7s, 21H, 7AcO), 3.27 and 3.76 (2dd, 2H, J_{vic} = 6.4, J_{gem} = 9.4 Hz, H-1 and H-1' of fatty alkyl), 3.59 (ddd, 1H, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 2.0 Hz, H-5a), 3.79 (t, 1H, $J_{4,5}$ = 9.9 Hz, H-4a), 3.87 (t, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.9 Hz, H-5b), 4.08 (dd, 1H, J_{gem} = 11.4 Hz, H-6b), 4.10 (dd, 1H, J_{gem} = 11.4 Hz, H-6a), 4.14 (dd, 1H, H-6'b), 4.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1a), 4.48 (dd, 1H, H-6'a), 4.48 (d, 1H, $J_{1,2}$ = 7.9Hz, H-1b), 4.89 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2a), 4.95 (dd, 1H, $J_{3,4}$ = 3.5 Hz, H-3b), 5.11 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2b), 5.19 (t, 1H, $J_{3,4}$ = 9.9 Hz, H-3a), 5.35 (d, 1H, H-4b). LRMS (FAB positive): *m/z* 619.2 [100% $(M\text{-aglycon})^+$], 1057.7 [14% $(M+H)^+$]. HRMS (FAB positive) Calcd for $C_{56}H_{97}O_{18}$ $(M+H)^+$: 1057.6675. Found: 1057.6676.

2-(Tetradecyl)hexadecyl *O*-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranoside (11). A solution of **10** (465 mg, 0.440 mmol) in MeOH (15 mL) was treated with 4N NaOH (1.00 mL, 4.01 mmol) as described for **5**. The resulting product was purified by precipitation with MeOH yielded **11** (312mg, 93.0%) as an amorphous mass: mp 207 ℃ ; $[\alpha]_D$ -9.8° (*c* 0.3, THF) ; Rf 0.63 (13:6:1 $CHCl_3$-MeOH-H_2O) ; ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), see Table 3 and

Table 4 LRMS (FAB negative): *m/z* 761.6 [100% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{81}O_{11}$ $(M-H)^-$: 761.5779. Found: 761.5779.

2-(Tetradecyl)hexadecyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (12), 2-(Tetradecyl)hexadecyl *O*-(3,6-Di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside disodium salt (13) and **2-(Tetradecyl)hexadecyl *O*-(3,6-Di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-6-*O*-sulfo-β-D-glucopyranoside trisodium salt (14).** Compound **11** (115 mg, 0.151 mmol) and dibutyltin oxide (41.3 mg, 0.166 mmol) were stirred in refluxing toluene (20 mL) for 24 h with continuous removal of water, and concentrated. A solution of the stannyl complex in DMF (4 mL) was treated with sulfur trioxide/ trimethylamine complex (46.2 mg, 0.332 mmol) as described for **6** and **7**. The resulting residue was chromatographed (13:6:1 $CHCl_3$-MeOH-H_2O) on silica gel (30 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH) to give **12** (31.0 mg, 24%), **13** (70.0 mg, 48%) and **14** (30.3 mg, 19%) as amorphous masses.

Compound **12** had $[\alpha]_D$ -2.9° (*c* 0.2, 5:1 MeOH-H_2O); R_f 0.45 (13:6:1 $CHCl_3$-MeOH-H_2O); ^1H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), see Table 3 and Table 4. LRMS (FAB negative): *m/z* 841.5 [100% $(M-Na)^-$], 863.5 [8% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{81}O_{14}S$ $(M-Na)^-$: 841.5347. Found: 841.5328.

Compound **13** had $[\alpha]_D$ -3.7° (*c* 0.3, 1:1 MeOH-H_2O); R_f 0.27 (13:6:1 $CHCl_3$-MeOH-H_2O); ^1H NMR (5:1 DMSO-d_6-CD_3OD) and ^{13}C NMR (5:1 DMSO-d_6-CD_3OD), see Table 3 and Table 4. LRMS (FAB negative): *m/z* 943.5 [100% $(M-Na)^-$], 965.5 [13% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{80}O_{17}S_2Na$ $(M-Na)^-$: 943.4735. Found: 943.4706.

Compound **14** had $[\alpha]_D$ -3.8° (*c* 0.3, 1:1 MeOH-H_2O); R_f 0.13 (13:6:1 $CHCl_3$-MeOH-H_2O); ^1H NMR (5:1 DMSO-d_6-CD_3OD) and ^{13}C NMR (5:1 DMSO-d_6-CD_3OD), see Table 3 and Table 4. LRMS (FAB negative): *m/z* 1045.4 [71% $(M-Na)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{79}O_{20}S_3Na_2$ $(M-Na)^-$: 1045.4122. Found: 1045.4115.

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