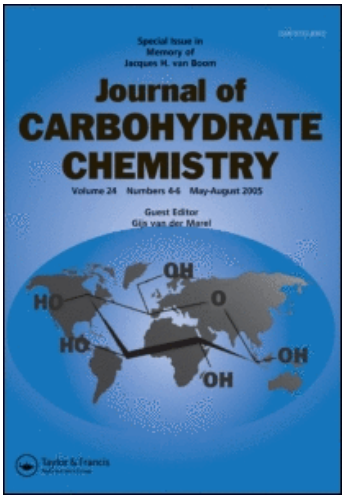

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Synthetic Studies on Selectin Ligands/Inhibitors: Synthesis and Biological Activity of the Sulfated and Phosphorylated Multivalent β-D-Galactopyranosides Containing Fatty Alkyl Residues

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SYNTHETIC STUDIES ON SELECTIN LIGANDS/INHIBITORS: SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE SULFATED AND PHOSPHORYLATED MULTIVALENT β-D-GALACTOPYRANOSIDES CONTAINING FATTY ALKYL RESIDUES[1,2]

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ABSTRACT

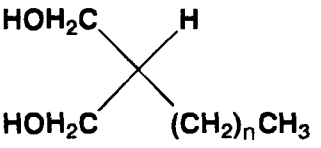
Ten sulfated and three phosphorylated β-D-galactopyranoside dimers and one sulfated β-D-galactopyranoside trimer containing fatty alkyl residues in place of ceramide have been synthesized. The coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**) with branched fatty alkyl diols and a triol (**1a-1j**) using mercury bromide as an activating agent gave the corresponding parent glycolipids (**4a-4j**) in good yields. Regioselective sulfation of these parent glycolipids through the dibutylstannylene acetals produced the target sulfated glycolipids, 3-sulfate (**5a-5j**) while stepwise phosphorylation with dibenzyloxy(diisopropylamino)phosphine gave the phosphorylated glycolipids, 3,4-bisphosphate (**9e, g, i**). The synthetic glycolipids

were assayed for their ability to block the adhesion of HL-60 cells to immobilized P-, L- and E-selectin.

INTRODUCTION

In recent years, many cell adhesion molecules (CAMs) have been identified which concern direct cell contact between cells per se or cell and the exo-cellular matrix.[3] These CAMs are classified into groups of the selectin family, immunoglobulin super family, integrin family, CD44 and the like, based on their structural characteristics.[3,4] Many of the CAMs belonging to the selectin family are involved in immunophlogistic reactions.[4] At the present time, three structurally related carbohydrate-binding proteins [E-selectin (ELAM-1), L-selectin (LECAM-1) and P-selectin (GMP-140, PADGEM)] are known to belong to the selectin family.[5-8] Among them, L-selectin is expressed on a lymphocyte, neutrocyte and monocyte, and it has been considered to mediate homing of the lymphocyte and adhesion of an inflamed region to vascular endothelium cells.[7,9,10] E-selectin is a protein that is expressed on inflammatory vascular endothelium cells by stimulation of inflammatory cytokine, and mediates the cell adhesion of a neutrocyte and monocyte.[5,9,10] P-selectin is expressed on activated vascular endothelium cells and activated platelets, and mediates cell adhesion between a platelet and a leukocyte or the vascular endothelium cell and a leukocyte.[6,9,10] It has been clarified that these selectins roll over the surface of vascular endothelium cells of the leukocyte rather than undergoing the powerful cell adhesion action in which rolling occurs prior to cell adhesion.[11,12] Recently, saccharide ligands recognizing these selectins have been elucidated on a molecular level.[13-19] Particularly, it has been found that sialyl Lewis X [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc](sLe^X) and sialyl Lewis A [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc](sLe^a) are common ligands of the E-, L- and P-selectins,[14,17,20,21] and various derivatives including multivalent sLe^X have been synthesized.[22-25] Furthermore, it has been found that sulfated carbohydrates such as sulfatides, fucoidan, a sulfated glucuronic acid (HNK-1) epitope, heparin and synthetic sulfated glycopolymers strongly bind to the P- and L-selectins.[26-31] In particular, sulfatide (ceramide is linked to galactose containing a sulfate group at C-3 of the pyranoside ring) and synthetic sulfatides strongly bind to

Type I



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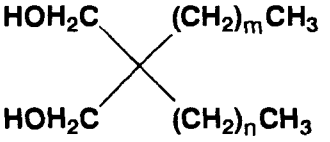
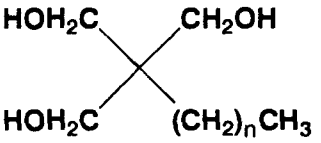
1b	13
1c	15
1d	21

Type II

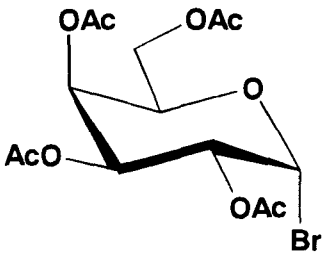


Series	m	n
1e	2	2
1f	13	13
1g	5	15
1h	9	15
1i	15	15

Type III



Series	n
1j	13



2

L-selectin[30] and have highly protective effects against selectin-dependent inflammatory lung injury.[32]

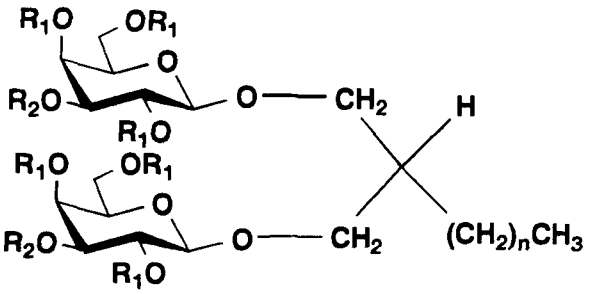
In this report, as a part of our study to design new selectin inhibitors,[33] a systematic synthesis and in vitro activity of novel sulfated and phosphorylated multivalent β-D-galactopyranosides containing fatty alkyl residues in place of ceramide are described.

RESULTS AND DISCUSSION

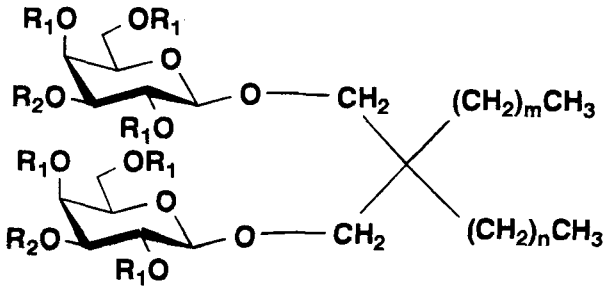
Synthesis. For the synthesis of the target glycolipids, we employed 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**) as the glycosyl donor, and 2,2-substituted 1,3-propanediols (**1a-i**) and triol (**1j**) as the glycosyl acceptors. The glycosylation of **1a** with **2** in dichloromethane in the presence of mercury bromide gave exclusively the β-glycosides (**3a**) in 63% yield. Significant signals in the ^{1}H NMR spectrum of **3a** were two-proton doublets at δ 4.40 and 4.41 ($J_{1,2}$ = 7.8 Hz, for 2×H-1), showing the newly formed glycosidic linkages to be β. Similarly, glycosylation of **1b-j** gave the corresponding β-glycolipids (**3b-j**). *O*-Deacylation of **3a-j** with aqueous sodium hydroxide in methanol-THF solution gave quantitative yields of the desired parent glycolipids (**4a-j**) in which all hydroxy groups are unprotected.

The regioselective sulfation of **4a-j** was achieved by treatment of the corresponding stannyl intermediates with the sulfur trioxide/trimethylamine complex, according to a published procedure.[34-36] 2-Decyl-1,3-bis[(β-D-galactopyranosyl)oxy]-propane (**4a**) was converted to the stannylene acetal by stirring with dibutyltin oxide in dry MeOH. Sulfation of the stannyl complex in DMF and THF using 2.4 equivalents of the sulfur trioxide/trimethylamine complex gave bis 3-sulfated galactoside **5a** in 60% yield. Using a similar procedure, the regioselective sulfation of **4b-j** gave the corresponding sulfated glycolipids (**5b-j**). The sulfated products could be easily separated on a column of silica gel and isolated as sodium salts using a cation exchange resin.

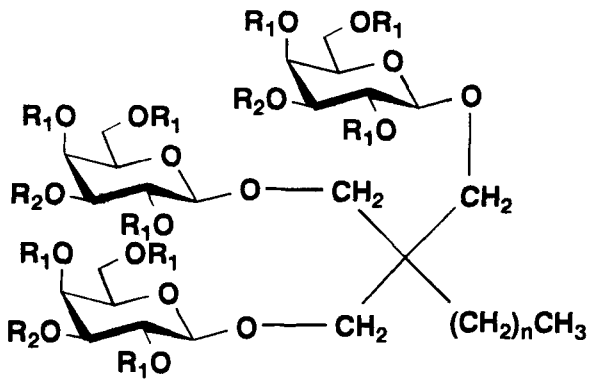
The structures of the sulfated compounds were confirmed by NMR[37,38] and MS analyses. A comparison of the ^{1}H NMR data of the sulfated galactoside (**5a-j**) with those of the parent compound (**4a-j**) demonstrated that the sulfate group deshielded



		R_1	R_2
	3a-d	**Ac**	**Ac**
i)	**4a-d**	**H**	**H**
ii)	**5a-d**	**H**	**SO_3Na**



		R_1	R_2
	3e-i	**Ac**	**Ac**
i)	**4e-i**	**H**	**H**
ii)	**5e-i**	**H**	**SO_3Na**



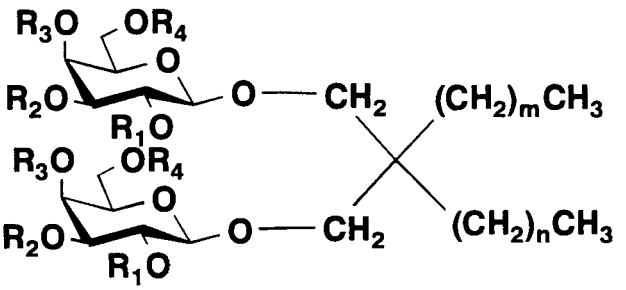
		R_1	R_2
	3j	**Ac**	**Ac**
i)	**4j**	**H**	**H**
ii)	**5j**	**H**	**SO_3Na**

Scheme 1. i) aq.NaOH, MeOH-THF (**a**, 99%; **b**, 94%; **c**, 97%; **d**, 92%; **e**, 94%; **f**, 97%; **g**, 92%; **h**, 89%; **i**, 93%; **j**, 97%). ii) Bu_2SnO, MeOH, SO_3•NMe_3, DMF-THF (**a**, 60%; **b**, 68%; **c**, 67%; **d**, 60%; **e**, 57%; **f**, 64%; **g**, 59%; **h**, 62%; **i**, 67%; **j**, 74%) .

the geminal and vicinal protons. The sulfate groups in the sulfated derivatives caused α effects of 0.7 - 0.8 ppm and β effects of 0.1 - 0.5 ppm. On the other hand, negative FAB mass spectrometry gave the $(M-Na)^-$ ion as the base peak, confirming the number of sulfate groups in the molecule. These results indicated that the NMR and MS analyses would be helpful in assigning the number of sulfate groups and their positions in the oligosaccharides.

The phosphorylation of **4e,g,i** was achieved by treatment with dibenzyloxy(diisopropylamino)phosphine.[39] Acetonation of **4e** in acetone with 2,2-dimethoxypropane gave the 3,4-*O*-isopropylidene derivative (**6e**) in 72% yield. Protection of HO-6 and HO-2 with benzyl bromide gave compound **7e** in 79% yield. Hydrolysis of the isopropylidene group of **7e** with aqueous 90% trifluoroacetic acid in dichloromethane at 0 °C, then treatment with dibenzyloxy(diisopropylamino)phosphine and 1*H*-tetrazole in acetonitrile-dichloromethane solution, and further oxidation with catalytic $RuCl_3$ and $NaIO_4$ gave **8e** in 86% yield. Finally, catalytic hydrogenolysis of **8e** with 10% Pd-C in buffered solution followed by treatment with a cation exchange resin gave bis 3,4-bisphosphorylated galactoside (**9e**) in 95% yield. Using a similar method, the phosphorylation of **4g,i** gave the corresponding phosphorylated glycolipids (**9g,i**).

Biological activity. The inhibitory activity of the target glycolipids *in vitro* was measured in binding assays of HL-60 cells (sLe^X expressing) to recombinant human selectin-IgG fusion proteins on plates.[22,23] Several synthesized glycolipids were able to inhibit HL-60 cells binding to the selectin fusion proteins with greater potency than the sLe^X tetrasaccharide itself (Table 1). The compounds **5e**, **5f** and **5i** were significantly more potent than sLe^X, **5a**, **5b** and **5j** in blocking adhesion to P-selectin, the trend being **5i**>**5f**>**5e**. Essentially the same trend was observed with L-selectin. These data indicated that the attachment of a branched fatty-alkyl residue[40] to 3-sulfated β-D-galactopyranoside was important for binding to the P- and L-selectins. In addition, when a branched fatty alkyl residue was long, there was greater potency of the blocking adhesion to the P- and L-selectins. On the other hand, 3,4-bisphosphate (**9e, 9g** and **9i**) were less potent than 3-sulfate (**5e** and **5i**) to the P- and L-selectins, but more potent to the E-selectin. Details of this biological investigation will be reported in the near future.



		R_1	R_2	R_3	R_4
	4e,g,i	**H**	**H**	**H**	**H**
i)	**6e,g,i**	**H**	**isopropylidene**		**H**
ii)	**7e,g,i**	**Bn**	**isopropylidene**		**Bn**
iii)	**8e,g,i**	**Bn**	**$PO(OBn)_2$**	**$PO(OBn)_2$**	**Bn**
iv)	**9e,g,i**	**H**	**$PO(ONa)_2$**	**$PO(ONa)_2$**	**H**

Scheme 2. i) 2,2-dimethoxypropane, H_2SO_4, acetone (**e**, 72%; **g**, 60%; **i**, 56%).
ii) benzyl bromide, DMF (**e**, 79%; **g**, 75%; **i**, 73%).
iii) dibenzyloxy(diisopropylamino)phosphine, CH_2Cl_2 (**e**, 86%; **g**, 79%; **i**, 79%).
iv) Pd-C, MeOH-buffered Sol. (**e**, 95%; **g**, 88%; **i**, 86%).

EXPERIMENTAL

General methods. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. NMR spectra were recorded on a Jeol JNM-GX 270 spectrometer (270 MHz for ^1H and 68 MHz for ^{13}C). Chemical shifts were expressed in parts per million downfield from TMS. FAB-MS were

Table 1. Inhibition activity of target compounds

	% inhibition at 0.3 mM		
	P-selectin	L-selectin	E-selectin
sLe^x	3	0	0
5a	4	0	0
5b	0	0	0
5c	Nd	nd	nd
5d	Nd	nd	nd
5e	24	17	0
5f	43	36	0
5g	Nd	nd	nd
5h	Nd	nd	nd
5i	81	79	1
5j	0	0	0
9e	0	0	4
9g	0	0	5
9i	0	0	11

nd : not determined the compound causes cell lysis and prevents a determination of its inhibitory activity in this assay.

recorded on a Jeol JMS-SX 120A mass spectrometer/JMA-DA7000 data system. Each sample was mixed with a glycerol or *m*-nitrobenzyl alcohol matrix 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 for about 2 min at 300 °C. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the specified solvent systems. Concentrations were conducted *in vacuo*.

2-Decyl-1,3-bis[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxy]propane (3a) and 2-Decyl-1,3-bis[(β-D-galactopyranosyl)oxy]propane (4a). A suspension

of anhydrous $CaSO_4$ (10 g), 1,2-dichloroethane (50 mL), mercury oxide (3.00 g, 14 mmol), and 2-decyl 1,3-propanediol (**1a**, 1.50 g, 6.9 mmol) was stirred under argon for 2 h. The reaction mixture was then cooled to 0 °C, and a suspension of **2** (8.55 g, 21 mmol), 1,2-dichloroethane (50 mL) and mercury bromide (150 mg, 0.42 mmol) was added. The reaction mixture was then stirred at 0 °C overnight, and filtered through Celite. The filtrate was washed with water (100 mL), 0.1 M HCl (50 mL), water (50 mL), saturated $NaHCO_3$ (50 mL), water (50 mL) and then dried (Na_2SO_4) and concentrated. Column chromatography (2:1 *n*-hexane-EtOAc) of the residue on silica gel (300 g) gave **3a** (3.82 g, 63 %) as a syrup: ^{1}H NMR($CDCl_3$) δ 0.88 (t, 3H, $J_{Me,CH2}$ = 6.8 Hz, *Me*CH_2), 1.26 (s, 18H, 9×CH_2), 1.82 (m, 1H, CH of fatty alkyl), 1.99, 2.04, 2.05 and 2.16 (4s, 24H, 8×AcO), 3.37-3.51 (m, 2H, CH_2O of fatty alkyl), 3.75-3.97 (m, 4H, CH_2O of fatty alkyl and 2×H-5), 4.17 (m, 4H, 2×H-6 and H-6'), 4.40, 4.41 (2d, 2H, $J_{1,2}$ = 7.8 Hz, 2×H-1), 5.02 (dd, 2H, $J_{2,3}$ = 10.8, $J_{3,4}$ = 3.4 Hz, 2×H-3), 5.14-5.27 (m, 2H, 2×H-2), 5.39 (dd, 2H, $J_{4,5}$ = 1.0 Hz, 2×H-4).

Anal. Calcd for $C_{41}H_{64}O_{20}$: C, 56.16; H, 7.36. Found: C, 56.22; H, 7.15.

To a solution of **3a** (1.28 g, 1.5 mmol) in MeOH (20 mL) and THF (10 mL) was added 4N NaOH (5 mL, 20 mmol) and the mixture was stirred at room temperature until deacetylation was complete (2 h). Purification by precipitation with water yielded **4a** (784 mg, 99%) as an amorphous mass: mp 225 °C; ^{1}H NMR(CD_3OD) δ 0.89 (t, 3H, $J_{Me,CH2}$ = 6.8 Hz, *Me*CH_2), 1.28 (s, 18H, 9×CH_2), 1.86 (m, 1H, CH of fatty alkyl), 3.42-3.52 (m, 6H, 2×H-2, H-3 and H-5), 3.63 (m, 2H, CH_2O of fatty alkyl), 3.73 (m, 4H, 2×H-6 and H-6'), 3.82 (d, 2H, $J_{3,4}$ = 3.1 Hz, 2×H-4), 3.89 (m, 2H, CH_2O of fatty alkyl), 4.24 (d, 2H, $J_{1,2}$ = 7.3 Hz, 2×H-1). MS(FAB negative) *m/z* : 539.6 [100% $(M-H)^-$].

Anal. Calcd for $C_{25}H_{48}O_{12}$: C, 55.54; H, 8.95. Found: C, 55.38; H, 8.85.

2-Decyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5a). A solution of **4a** (105 mg, 0.19 mmol) and dibutyltin oxide (116 mg, 0.47 mmol) was stirred in refluxing dry MeOH (5 mL) for 24 h with continuous removal of water, and then concentrated. To a solution of stannyl complex in DMF (2 mL) and THF (2 mL) was added sulfar trioxide/trimethylamine complex (64.9 mg, 0.47 mmol) and the mixture was stirred for 12 h at room temperature, then concentrated. The residue was chromatographed (8:5:1 $CHCl_3$-MeOH-H_2O) on silica gel (20 g) and

loaded onto a cation exchange resin column (AG50W-X8, sodium form, 3×20 cm, MeOH), to give **5a** (86 mg, 60%) as an amorphous mass: ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, $J_{Me,CH2}$ = 6.9 Hz, *Me*CH_2), 1.29 (s, 18H, 9×CH_2), 1.89 (m, 1H, CH of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.63 (m, 2H, CH_2O of fatty alkyl), 3.68 (m, 2H, 2×H-2), 3.74 (m, 4H, 2×H-6 and H-6'), 3.85 (m, 2H, CH_2O of fatty alkyl), 4.25 (dd, 2H, $J_{2,3}$ = 7.4, $J_{3,4}$ = 3.5Hz, 2×H-3), 4.25 (d, 2H, 2×H-4), 4.34 (d, 2H, $J_{1,2}$ = 7.9Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.7, 28.2, 29.4, 30.4, 30.7, 30.8, 31.1 and 33.0 (CH_2), 40.7 (CH), 62.4 (C-6), 68.7 (C-4), 70.8 (C-2), 71.5 (OCH_2), 76.2 (C-5), 82.1 (C-3), 105.1 and 105.2 (C-1). MS(FAB negative) *m/z* : 721.2 [100% $(M-Na)^-$], 743.2 [20% $(M-H)^-$].

Anal. Calcd for $C_{25}H_{46}O_{18}S_2Na_2$: C, 40.32; H, 6.23. Found: C, 40.19; H, 6.28.

Other 2-Alkyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salts (5b-d). Compounds **5b-d** were prepared via **3b-d** and **4b-d** by the same sequence as described for **5a**.

2-Tetradecyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5b). ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, $J_{Me,CH2}$ = 6.9 Hz, *Me*CH_2), 1.29 (s, 26H, 13×CH_2), 1.88 (m, 1H, CH of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.65 (m, 2H, CH_2O of fatty alkyl), 3.68 (m, 2H, 2×H-2), 3.71 (m, 4H, 2×H-6 and H-6'), 3.90 (m, 2H, CH_2O of fatty alkyl), 4.23 (dd, 2H, $J_{2,3}$ = 7.4, $J_{3,4}$ = 3.4Hz, 2×H-3), 4.25 (d, 2H, 2×H-4), 4.35 (d, 2H, $J_{1,2}$ = 7.8Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 15.4 (CH_3), 24.5, 29.1, 30.4, 31.5, 31.8, 32.0 and 34.0 (CH_2), 41.4 (CH), 63.2 (C-6), 69.5 (C-4), 71.5 and 72.2 (OCH_2), 71.6 (C-2), 76.7 (C-5), 82.7 (C-3), 105.8 and 106.0 (C-1). MS(FAB negative) *m/z*: 777.3 [100% $(M-Na)^-$], 799.3 [5% $(M-H)^-$].

Anal. Calcd for $C_{29}H_{54}O_{18}S_2Na_2$: C, 43.48; H, 6.80. Found: C, 43.22; H, 6.67.

2-Hexadecyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5c). ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, $J_{Me,CH2}$ = 6.9 Hz, *Me*CH_2), 1.29 (s, 30H, 15×CH_2), 1.89 (m, 1H, CH of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.65 (m, 2H, CH_2O of fatty alkyl), 3.69 (m, 2H, 2×H-2), 3.72 (m, 4H, 2×H-6 and H-6'), 3.90 (m, 2H, CH_2O of fatty alkyl), 4.23 (dd, 2H, $J_{2,3}$ = 7.2, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.24 (d, 2H, 2×H-4), 4.34 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1); ^{13}C NMR

(CD_3OD) δ 14.4 (CH_3),23.7, 28.2, 29.5, 30.4, 30.8, 31.1 and 33.0 (CH_2), 40.7 (CH), 62.4 (C-6), 68.7 (C-4), 70.8 (C-2), 71.5 (OCH_2), 76.2 (C-5), 82.1 (C-3), 105.1 and 105.2 (C-1). MS(FAB negative) *m/z*: 805.3 [100% $(M-Na)^-$].

Anal. Calcd for $C_{31}H_{58}O_{18}S_2Na_2$: C, 44.92; H, 7.05. Found: C, 44.86; H, 7.12.

2-Docosanyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5d). ^1H NMR(CD_3OD) δ 0.90 (t, 3H, $J_{Me,CH2}$ = 6.9 Hz, *Me*CH_2), 1.29 (s, 42H, 21×CH_2), 1.93 (m, 1H, CH of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.63 (m, 2H, CH_2O of fatty alkyl), 3.70 (m, 2H, 2×H-2), 3.76 (m, 4H, 2×H-6 and H-6'), 3.91 (m, 2H, CH_2O of fatty alkyl), 4.25 (dd, 2H, $J_{2,3}$ = 7.4, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.24 (d, 2H, 2×H-4), 4.39 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 14.7 (CH_3), 23.8, 28.2, 29.5, 30.4, 30.6, 30.7, 30.8, 31.2 and 33.1 (CH_2), 40.7 (CH), 62.4 (C-6), 68.7 (C-4), 70.9 (C-2), 71.3 and 71.8 (OCH_2), 76.2 (C-5), 82.2 (C-3), 105.0 and 105.1 (C-1). MS(FAB negative) *m/z*: 889.4 [100% $(M-Na)^-$].

Anal. Calcd for $C_{37}H_{70}O_{18}S_2Na_2$: C, 48.67; H, 7.73. Found: C, 48.54; H, 7.58.

2,2-Dihexadecyl-1,3-bis[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxy]-propane (3i) and 2,2-Dihexadecyl-1,3-bis[(β-D-galactopyranosyl)oxy]propane (4i). A solution of **2** (4.70 g, 11 mmol) and 2,2-dihexadecyl 1,3-propanediol (**1i**, 2.00 g, 3.8 mmol) in dry 1,2-dichloroethane (100 mL) was treated as described for **3a**. The resulting product was purified by chromatography (3:1 *n*-hexane-EtOAc) on a column of silica gel (300 g) to give **3i** (2.80 g, 62 %) as a syrup; ^1H NMR($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.25 (s, 60H, 30×CH_2), 1.99, 2.05, 2.06 and 2.16 (4s, 24H, 8×AcO), 3.23 and 3.65 (2d, 4H, J_{gem} = 9.8Hz, CH_2O of fatty alkyl), 3.91 (m, 2H, 2×H-5), 4.18 (m, 4H, 2×H-6, H-6'), 4.37 (d, 2H, $J_{1,2}$ = 7.8 Hz, 2×H-1), 5.01 (dd, 2H, $J_{3,4}$ = 3.4 Hz, 2×H-3), 5.19 (dd, 2H, $J_{2,3}$ = 10.8 Hz, 2×H-2), 5.39 (dd, 2H, $J_{4,5}$ = 1.0 Hz, 2×H-4).

Anal. Calcd for $C_{63}H_{108}O_{20}$: C, 63.83; H, 9.18. Found: C, 64.12; H, 9.27.

A solution of **3i** (2.00 g, 1.7 mmol) in MeOH (50 mL) and THF (50 mL) was treated with 4N NaOH (5 mL, 20 mmol) as described for **4a**. The resulting product was purified by precipitation with MeOH yielding **4i** (1.33 g, 93%) as an amorphous mass: mp 110 °C; ^1H NMR (CD_3OD) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.28 (s, 60H, 30×CH_2), 3.43-3.57 (m, 8H, CH_2O of fatty alkyl, 2×H-2, H-3 and H-5), 3.71-3.77 (m, 6H, CH_2O of fatty alkyl, 2×H-6 and H-6'), 3.85 (d, 2H, $J_{3,4}$ = 3.0

Hz, 2×H-4), 4.29 (d, 2H, $J_{1,2}$ = 7.3 Hz, 2×H-1). MS(FAB negative) *m/z* : 848.2 [100% $(M-H)^-$].

Anal. Calcd for $C_{47}H_{92}O_{12}$: C, 66.47; H, 10.92. Found: C, 66.61; H, 11.20.

2,2-Dihexadecyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5i). A solution of **4i** (106 mg, 0.13 mmol) and dibutyltinn oxide (74.7 mg, 0.30 mmol) in dry MeOH (5 mL) were treated with sulfar trioxide/trimethylamine complex (41.8 mg, 0.30 mmol) as described for **5a**. The resulting product was purified by chromatography (8:5:1 $CHCl_3$-MeOH-H_2O) on a column of silica gel and a cation exchange resin to give **5i** (89 mg, 67 %) as an amorphous mass: ^{1}H NMR (CD_3OD) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×*Me*CH_2), 1.30 (s, 60H, 30×CH_2), 3.53 (d, 2H, J_{gem} = 9.9 Hz, CH_2O of fatty alkyl), 3.60 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.69 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.70 (d, 2H, CH_2O of fatty alkyl), 3.79 (d, 4H, 2×H-6 and H-6'), 4.23 (dd, 2H, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.34 (d, 2H, 2×H-4), 4.46 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 14.8 (CH_3), 23.0, 23.7, 30.4, 30.6, 30.7, 30.8, 30.9, 31.3 and 33.0 (CH_2), 41.9 (OCH_2*C*), 61.4 (C-6), 67.9 (C-4), 70.4 (C-2), 72.7 (OCH_2), 75.2 (C-5), 81.9 (C-3), 104.7 (C-1). MS(FAB negative) *m/z*: 1029.5 [100% $(M-Na)^-$], 1051.5 [5% $(M-H)^-$].

Anal. Calcd for $C_{47}H_{90}O_{18}S_2Na_2$: C, 53.59; H, 8.61. Found: C, 53.61; H, 8.78.

Other 2,2-Dialkyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salts (5e-h). Compounds **5e-h** were prepared via **3e-h** and **4e-h** by the same sequence as described for **5i**.

2,2-Dipropyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5e). ^{1}H NMR (CD_3OD) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×*Me*CH_2), 1.35 (s, 8H, 4×CH_2), 3.49 (d, 2H, J_{gem} = 9.4 Hz, CH_2O of fatty alkyl), 3.54 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.70 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.74 (d, 2H, CH_2O of fatty alkyl), 3.74 (d, 4H, 2×H-6 and H-6'), 4.23 (dd, 2H, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.25 (d, 2H, 2×H-4), 4.35 (d, 2H, $J_{1,2}$ = 7.9Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 15.3 (CH_3), 16.9, 34.7 (CH_2), 42.2 (OCH_2*C*), 62.4 (C-6), 68.7 (C-4), 70.9 (C-2), 73.3 (OCH_2), 76.2 (C-5), 82.1 (C-3), 105.4 (C-1). MS(FAB negative) *m/z*: 665.1 [100% $(M-Na)^-$], 687.1 [23% $(M-H)^-$].

Anal. Calcd for $C_{21}H_{38}O_{18}S_2Na_2$: C, 36.63; H, 5.56. Found: C, 36.41; H, 5.55.

2,2-Ditetradecyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5f). ^{1}H NMR (CD_3OD) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.28 (s, 52H, 26×CH_2), 3.49 (d, 2H, J_{gem} = 9.4 Hz, CH_2O of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.70 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.72 (d, 2H, CH_2O of fatty alkyl), 3.74 (d, 4H, 2×H-6 and H-6'), 4.24 (dd, 2H, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.25 (d, 2H, 2×H-4), 4.36 (d, 2H, $J_{1,2}$ = 7.8Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 15.4 (CH_3), 24.3, 24.8, 31.5, 31.9, 32.0, 32.5 and 34.1 (CH_2), 42.7 (OCH_2C), 63.1 (C-6), 69.5 (C-4), 71.6 (C-2), 74.6 (OCH_2), 76.9 (C-5), 82.7 (C-3), 106.1 (C-1). MS(FAB negative) *m/z*: 973.5 [100 % $(M-Na)^-$], 995.5 [16 % $(M-H)^-$].

Anal. Calcd for $C_{43}H_{82}O_{18}S_2Na_2$: C, 51.79; H, 8.29. Found: C, 51.68; H, 8.37.

2-Hexadecyl-2-hexyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5g). ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×*Me*CH_2), 1.29 (s, 40H, 20×CH_2), 3.51 (d, 2H, J_{gem} = 9.4 Hz, CH_2O of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 6.4Hz, 2×H-5), 3.70 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.72 (d, 2H, CH_2O of fatty alkyl), 3.75 (d, 4H, 2×H-6 and H-6'), 4.24 (dd, 2H, $J_{3,4}$ = 3.0 Hz, 2×H-3), 4.26 (d, 2H, 2×H-4), 4.37 (d, 2H, $J_{1,2}$ = 7.9Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.5, 23.7, 30.4, 30.6, 30.7, 31.3, 31.6, 31.9 and 33.0 (CH_2), 42.0 (OCH_2C), 62.3 (C-6), 68.7 (C-4), 70.8 (C-2), 73.3 (OCH_2), 76.1 (C-5), 82.0 (C-3), 105.3 (C-1). MS(FAB negative) *m/z*: 889.4 [100% $(M-Na)^-$], 911.4 [6% $(M-H)^-$].

Anal. Calcd for $C_{37}H_{70}O_{18}S_2Na_2$: C, 48.67; H, 7.73. Found: C, 48.85; H, 7.81.

2-Decyl-2-hexadecyl-1,3-bis[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Disodium Salt (5h). ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×*Me*CH_2), 1.29 (s, 48H, 24×CH_2), 3.50 (d, 2H, J_{gem} = 9.4 Hz, CH_2O of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 6.4Hz, 2×H-5), 3.70 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.72 (d, 2H, CH_2O of fatty alkyl), 3.75 (d, 4H, 2×H-6 and H-6'), 4.23 (dd, 2H, $J_{3,4}$ = 3.0 Hz, 2×H-3), 4.26 (d, 2H, 2×H-4), 4.36 (d, 2H, $J_{1,2}$ = 7.9Hz, 2×H-1); ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.4, 23.7, 30.4, 30.5, 30.6, 30.8, 31.5, 31.6, 31.8 and 33.0 (CH_2), 42.0 (OCH_2C), 62.3 (C-6), 68.7 (C-4), 70.8 (C-2), 73.3 (OCH_2), 76.1 (C-5), 82.0 (C-3), 105.3 (C-1). MS(FAB negative) *m/z*: 945.4 [100% $(M-Na)^-$], 967.4 [4% $(M-H)^-$].

Anal. Calcd for $C_{41}H_{78}O_{18}S_2Na_2$: C, 50.81; H, 8.11. Found: C, 50.55; H, 8.10.

2-Tetradecyl-1,2,3-tris[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxy]-propane (3j) and 2-Tetradecyl-1,2,3-tris[(β-D-galactopyranosyl)oxy]propane (4j).

A solution of **2** (1.57 g, 3.8 mmol) and 2-tetradecyl 1,2,3-propanetriol (**1j**, 100 mg, 0.33 mmol) in dry 1,2-dichloroethane (10 mL) was treated as described for **3a**. The resulting product was purified by chromatography (2:3 *n*-hexane-EtOAc) on a column of silica gel (40 g) to give **3j** (251 mg, 58%) as a syrup: ^{1}H NMR($CDCl_3$) δ 0.88 (t, 3H, $J_{Me,CH2}$ = 6.8 Hz, *Me*CH_2), 1.27 (s, 26H, 13 × CH_2), 1.99, 2.05, 2.06 and 2.16 (4s, 36H, 12 × AcO), 3.34 and 3.72 (2d, 6H, J_{gem} = 9.8Hz, CH_2O of fatty alkyl), 3.90 (t, $J_{5,6}$ = 6.8 Hz, 3H, 3 × H-5), 4.15 (d, 6H, 3 × H-6 and H-6'), 4.38 (d, 3H, $J_{1,2}$ = 7.8 Hz, 3 × H-1), 5.02 (dd, 3H, $J_{3,4}$ = 3.4 Hz, 3 × H-3), 5.17 (dd, 3H, $J_{2,3}$ = 10.3 Hz, 3 × H-2), 5.39 (d, 2H, 3 × H-4).

Anal. Calcd for $C_{60}H_{92}O_{30}$: C, 55.72; H, 7.17. Found: C, 55.75; H, 7.28.

A solution of **3j** (231 mg, 0.18 mmol) in MeOH (3 mL) was treated with 4N NaOH (1 mL, 4.0 mmol) as described for **4a**. The resulting product was purified by precipitation with MeOH yielding **4j** (136 mg, 97%) as an amorphous mass: mp 262 °C(dec); ^{1}H NMR(CD_3OD) δ 0.89 (t, 3H, $J_{Me,CH2}$ = 6.8 Hz, *Me*CH_2), 1.27 (s, 26H, 13 × CH_2), 3.44-3.61 (m, 12H, CH_2O of fatty alkyl, 3 × H-2, H-3 and H-5), 3.72-3.75 (m, 6H, 3 × H-6 and H-6'), 3.83 (m, 3H, 3 × H-4), 3.88 (d, 3H, J_{gem} = 9.8 Hz, CH_2O of fatty alkyl), 4.28 (d, 3H, $J_{1,2}$ = 7.3 Hz, 3 × H-1). MS(FAB negative) *m/z* : 787.4 [100% $(M-H)^-$].

Anal. Calcd for $C_{36}H_{68}O_{18}$: C, 54.81; H, 8.69. Found: C, 54.92; H, 8.82.

2-Tetradecyl-1,2,3-tris[(3-*O*-sulfo-β-D-galactopyranosyl)oxy]propane Trisodium Salt (5j). A solution of **4j** (60 mg, 0.076 mmol) and dibutyltinn oxide (68 mg, 0.28 mmol) in dry MeOH (3 mL) were treated as described for **5a**. The resulting product was purified by chromatography (6:4:1 $CHCl_3$-MeOH-H_2O) on a column of silica gel and a cation exchange resin to give **5j** (62 mg, 74%) as an amorphous mass: ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, $J_{Me,CH2}$ = 6.8 Hz, *Me*CH_2), 1.35 (s, 26H, 13 × CH_2), 3.55 (d, 3H, J_{gem} = 9.4 Hz, CH_2O of fatty alkyl), 3.55 (t, 2H, $J_{5,6}$ = 6.4Hz, 2 × H-5), 3.70 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2 × H-2), 3.72 (d, 3H, CH_2O of fatty alkyl), 3.75 (d, 4H, 2 × H-6 and H-6'), 4.23 (dd, 2H, $J_{3,4}$ = 3.0 Hz, 2 × H-3), 4.26 (d, 2H, 2 × H-4), 4.35 (d, 2H, $J_{1,2}$ = 7.8Hz, 2 × H-1); ^{13}C NMR (CD_3OD) δ 15.3 (CH_3), 24.6, 24.9, 25.1, 31.4, 31.8, 31.9, 32.0, 32.5, 32.8 and 34.1 (CH_2), 44.9 (OCH_2C), 63.2 (C-6), 69.5 (C-4),

71.5 (C-2), 72.6 (OCH_2), 76.9 (C-5), 82.7 (C-3), 106.2 (C-1). MS(FAB negative) *m/z*: 1071.3 [100% $(M-Na)^-$], 1093.3 [100% $(M-H)^-$].

Anal. Calcd for $C_{36}H_{66}O_{27}S_3Na_3$: C, 39.45; H, 6.07. Found: C, 39.27; H, 5.98.

2,2-Dipropyl-1,3-bis[(3,4-*O*-isopropylidene-β-D-galactopyranosyl)oxy]-propane (6e). Compound **4e** (500 mg, 1.0 mmol) was dissolved in acetone (100 mL), and 2,2-dimethoxypropane (1 mL) and H_2SO_4 (20 μL) were added. The mixture was stirred for 16 h at room temperature after which Na_2CO_3 was added to neutralize the solution. The solvents were evaporated and the residue was chromatographed (10:1 $CHCl_3$-MeOH) on silica gel (40 g) to give **6e** (420 mg, 72%) as a syrup: ^1H NMR (CD_3OD) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.32 (s, 8H, 4×CH_2), 1.32 and 1.48 (2s, 12H, 4×CH_3), 3.41 (dd, 2H, $J_{2,3}$ = 7.3 Hz, 2×H-2), 3.43, 3.72 (2d, 4H, J_{gem} = 9.8 Hz, CH_2O of fatty alkyl), 3.76 (m, 4H, 2×H-6 and H-6'), 3.82 (m, 2H, 2×H-5), 4.00 (dd, 2H, $J_{3,4}$ = 5.4 Hz, 2×H-3), 4.18 (dd, 2H, $J_{4,5}$ = 2.0Hz, 2×H-4), 4.25 (d, 2H, $J_{1,2}$ = 8.3Hz, 2×H-1). MS(FAB negative) *m/z* : 563.3 [100% $(M-H)^-$].

Anal. Calcd for $C_{27}H_{48}O_{12}$: C, 57.43; H, 8.57. Found: C, 57.53; H, 8.68.

2,2-Dipropyl-1,3-bis[(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)oxy]propane (7e). Compound **6e** (380 mg, 0.67 mmol) was added to a suspension of NaH (214 mg, 5.4 mmol) in *N,N*-dimethylformamide (5 mL) at 0 °C. The suspension was stirred at that temperature for 30 min and then benzyl bromide (0.64 mL, 5.4 mmol) was added. The solution was allowed to warm slowly to room temperature. After 16 h, the reaction was quenched by addition of MeOH (2 mL) at 0 °C, diluted with $CHCl_3$ (50 mL) and washed twice with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (4:1 *n*-hexane-EtOAc) on silica gel (40 g) to give **7e** (490 mg, 79%) as a syrup: ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, $J_{Me,CH2}$ = 6.6 Hz, 2×*Me*CH_2), 1.30 (s, 8H, 4×CH_2), 1.31 (s, 12H, 4×CH_3), 3.34 (m, 2H, 2×H-2), 3.36 (d, 2H, J_{gem} = 9.8 Hz, CH_2O of fatty alkyl), 3.67 (m, 4H, 2×H-6 and H-6'), 3.71 (m, 2H, 2×H-5), 3.75 (d, 2H, CH_2O of fatty alkyl), 4.05 (m, 4H, 2×H-3 and H-4), 4.22 (d, 2H, $J_{1,2}$ = 8.3Hz, 2×H-1), 4.51 (d, 2H, J_{gem} = 11.8 Hz, 2×PhCH), 4.59 (d, 2H, J_{gem} = 11.8 Hz, 2×PhCH), 4.76 (d, 2H, J_{gem} = 11.7 Hz, 2×PhCH), 4.83 (d, 2H, J_{gem} = 11.7 Hz, 2×PhCH), 7.18-7.39 (m, 20H, 4×Ph).

Anal. Calcd for $C_{55}H_{72}O_{12}$: C, 71.40; H, 7.84. Found: C, 71.43; H, 7.72.

2,2-Dipropyl-1,3-bis[(2,6-di-*O*-benzyl-3,4-di-*O*-dibenzylphosphono-β-D-galactopyranosyl)oxy]propane (8e). To a solution of compound **7e** (153 mg, 0.17 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added aq 90% trifluoroacetic acid (0.2 mL). After 30 min toluene (10 mL) and EtOAc (10 mL) were added and removed *in vacuo*. A solution of CH_2Cl_2 (2 mL), acetonitrile (2 mL), 1*H*-tetrazole (64 mg, 0.92 mmol) and dibenzyloxy(diisopropylamino)phosphine (425 mg, 1.2 mmol) was added at room temperature with stirring. After 12 h, water (5 mL), $RuCl_3 \cdot 3H_2O$ (2 mg, 0.01 mmol), and $NaIO_4$ (263 mg, 1.2 mmol) were added and the mixture was vigorously stirred for 1 h. Then the mixture was diluted with CH_2Cl_2 (10 mL) and washed twice with water (10 mL). The aqueous phase was extracted twice with CH_2Cl_2 (10 mL), the organic phases were combined and dried (Na_2SO_4), and the solvent was evaporated. The residue was chromatographed (1:1 *n*-hexane-EtOAc) on silica gel (40 g) to give **8e** (270 mg, 86%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 6H, $J_{Me,CH2}$ = 6.6 Hz, 2× *Me*CH_2), 1.26 (s, 8H, 4×CH_2), 3.25 (d, 2H, J_{gem} = 9.8 Hz, CH_2O of fatty alkyl), 3.37 (t, 2H, $J_{5,6}$ = 6.3Hz, 2×H-5), 3.46-3.63 (m, 6H, 2×H-2, H-6 and H-6'), 3.71 (d, 2H, CH_2O of fatty alkyl), 4.15 (d, 2H, $J_{1,2}$ = 7.8Hz, 2×H-1), 4.30 (d, 2H, J_{gem} = 12.2 Hz, 2×PhCH), 4.40 (m, 2H, 2×H-3), 4.44 (d, 2H, J_{gem} = 12.2 Hz, 2×PhCH), 4.58 (d, 2H, J_{gem} = 12.2 Hz, 2×PhCH), 4.82 (m, 4H, 2×$PhCH_2$), 4.92-5.05 (m, 16H, 7× $PhCH_2$, 2×H-4), 7.04-7.33 (m, 60H, 12×Ph).

Anal. Calcd for $C_{105}H_{116}O_{24}P_4$: C, 66.87; H, 6.20. Found: C, 66.69; H, 6.20.

2,2-Dipropyl-1,3-bis[(3,4-bisphospho-β-D-galactopyranosyl)oxy]propane Tetrasodium Salt (9e). Compound **8e** (141 mg, 0.075 mmol) was dissolved in MeOH (5 mL) and buffered aq AcOH-NaOAc (1 mL, pH 5, 0.5 M), and the mixture was treated with 10% Pd-C (28 mg) and H_2 at atmospheric pressure with stirring at room temperature until reduction was complete (2 h). The mixture was filtered (Celite) and partially evaporated, and the solution was loaded onto a cation exchange resin column (WK-10, sodium form, 1×4 cm, MeOH), to give **9e** (70 mg, 95%) as amorphous mass: ^{1}H NMR(D_2O) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.25 (s, 8H, 4× CH_2), 3.46 (d, 2H, J_{gem} = 9.9 Hz, CH_2O of fatty alkyl), 3.63 (m, 2H, 2×H-2), 3.68 (m, 2H, 2×H-5), 3.70 (m, 4H, 2×H-6 and H-6'), 3.72 (d, 2H, CH_2O of fatty alkyl), 4.11 (ddd, 2H, $J_{2,3}$ = 6.9, $J_{3,4}$ = 3.0, $J_{3,P}$ = 9.9 Hz, 2×H-3), 4.41 (d, 2H, $J_{1,2}$ = 7.9Hz,

2×H-1), 4.59 (dd, 2H, $J_{4,P}$ = 10.9 Hz, 2×H-4); ^{13}C NMR (D_2O) δ 15.1 (CH_3), 16,5 and 34.2 (CH_2), 41.8 (OCH_2C), 60.2 (C-6), 71.3 (C-4), 71.3 (C-2), 73.8 (OCH_2), 74.7 (C-5), 76.8 (C-3), 104.8 (C-1).

Anal. Calcd for $C_{21}H_{36}O_{24}P_4Na_8$: C, 25.73; H, 3.70. Found: C, 25.43; H, 3.89.

Other 2,2-Dialkyl-1,3-bis[(3,4-bisphospho-β-D-galactopyranosyl)oxy]-propane Tetrasodium Salts (9g,i). Compounds **9g,i** were prepared via **6g,i**, **7g,i** and **8g,i** by the same sequence as described for **9e**.

2-Hexadecyl-2-hexyl-1,3-bis[(3,4-bisphospho-β-D-galactopyranosyl)oxy]-propane Tetrasodium Salt (9g). ^{1}H NMR (D_2O) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2 ×$MeCH_2$), 1.28 (s, 40H, 20×CH_2), 3.48 (m, 2H, CH_2O of fatty alkyl), 3.65 (m, 2H, 2×H-2), 3.68 (t, 2H, $J_{5,6}$ = 6.4Hz, 2×H-5), 3.70 (m, 6H, CH_2O of fatty alkyl, 2× H-6 and H-6'), 4.15 (ddd, 2H, $J_{2,3}$ = 6.9, $J_{3,4}$ = 3.0, $J_{3,P}$ = 9.8 Hz, 2×H-3), 4.47 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1), 4.62 (d, 2H, $J_{4,P}$ = 10.9 Hz, 2×H-4); ^{13}C NMR (D_2O) δ 14.8 (CH_3), 23.4, 23.5, 30.1, 30.2, 30.4, 30.6, 30.8, 32.7 and 32.8 (CH_2), 41.7 (OCH_2C), 60.0 (C-6), 71.2 (C-4), 71.2 (C-2), 73.2 (OCH_2), 74.5 (C-5), 76.8 (C-3), 104.6 (C-1).

Anal. Calcd for $C_{37}H_{68}O_{24}P_4Na_8$: C, 36.89; H, 5.69. Found: C, 36.66; H, 5.51.

2,2-Dihexadecyl-1,3-bis[(3,4-bisphospho-β-D-galactopyranosyl)oxy]-propane Tetrasodium Salt (9i). ^{1}H NMR(D_2O) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2× $MeCH_2$), 1.28 (s, 60H, 30×CH_2), 3.49 (m, 2H, CH_2O of fatty alkyl), 3.62 (m, 2H, 2 ×H-2), 3.65 (m, 2H, CH_2O of fatty alkyl), 3.69 (m, 2H, 2×H-5), 3.72 (m, 4H, 2× H-6 and H-6'), 4.13 (ddd, 2H, $J_{2,3}$ = 6.4, $J_{3,4}$ = 3.0, $J_{3,P}$ = 9.8 Hz, 2×H-3), 4.40 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1), 4.62 (dd, 2H, $J_{4,P}$ = 10.9 Hz, 2×H-4). ^{13}C NMR (D_2O) δ 14.4 (CH_3), 22.6, 23.3, 30.1, 30.2, 30.3, 30.4, 30.5, 30.6, 30.7 and 32.6 (CH_2), 41.6 (OCH_2C), 60.0 (C-6), 71.2 (C-2), 71.3 (C-4), 73.0 (OCH_2), 74.6 (C-5), 76.8 (C-3), 104.7 (C-1).

Anal. Calcd for $C_{47}H_{88}O_{24}P_4Na_8$: C, 41.97; H, 6.59. Found: C, 41.71; H, 6.32.

In vitro biological activity. The activity of compounds *in vitro* was measured in adhesion assays as the inhibition of the binding of promyelocytic leukemia HL-60 cells to immobilized recombinant selectin-IgG fusion proteins.[22,23] Briefly, P-selectin-IgG was immobilized onto microtiter plate wells (96 wells; Nunc Maxisorp) by adding 20

ng of the purified protein to each well in a final volume of 100 μL PBS(+) and incubated overnight at 4 °C. The excess coating solution was removed by aspirating, and non-specific binding sites were blocked by a 1 h incubation with PBS(+) containing 1% BSA (w/v) at room temperature. After aspirating the blocking solution, 100 μL of the test compound was dissolved in RPMI 1640 supplemented 10% FBS, and 100 μL of HL-60 cells (10^6 cell/mL suspended in the binding buffer) were added to each well. The plate was centrifuged at 500 rpm for 2 min at room temperature and the wells were carefully filled with the binding buffer. The plate was sealed with acetate sealing tape, being careful to displace any trapped air bubbles. Non-adherent HL-60 cells were removed by inverting the plate, centrifuging at 500 rpm for 10 min, removing the acetate film and aspirating the binding buffer. The amount of bound cells was quantified by the WST-1 assay (Dojin Chemicals, Japan).[41] Inhibition of L- or E-selectin binding was carried out as described above, using immobilized L- (100 ng) or E-selectin-IgG (40 ng).

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