Synthetic Studies on Selectin Ligands/Inhibitors.[1] Synthesis and Biological Evaluation of Sulfated and Phosphorylated β-D-Galacto- and Lactopyranosides Containing Fatty-Alkyl Residues of Different Carbon Chain Lengths

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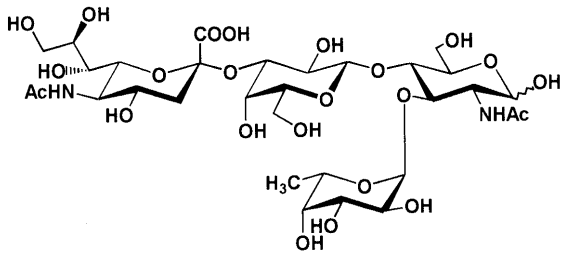
Received November 11, 1997; accepted December 23, 1997

To investigate the biological selectin-ligand interactions, fourteen sulfated and eight phosphorylated β-D-galacto- and lactopyranosides containing branched fatty-alkyl residues in place of the ceramide have been synthesized. Regioselective sulfation of the parent glycolipids through the dibutylstannylene acetal with a certain amount of sulfur trioxide–trimethylamine complex produced the target sulfated glycolipids, while stepwise phosphorylation by treatment of the properly protected diol with dibenzyloxy(diisopropylamino)phosphine gave the phosphorylated glycolipids. The synthetic glycolipids showed an interesting mode of inhibition of the binding of HL-60 cells to immobilized P-, L- and E-selectins during *in vitro* experiments. In addition, using computer modeling techniques, we examined the molecular basis for the ligand–selectin complex formation. These glycolipids may be useful as therapeutic agents against selectin-dependent inflammation.

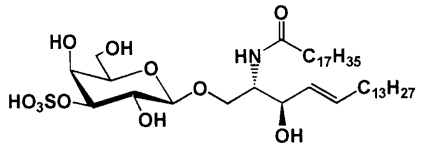
Key words selectin; sialyl Lewis X; sulfatide; cell adhesion; inflammatory disease

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 endothelial surfaces.[4] At the present time, three structurally related carbohydrate-binding proteins [E-selectin (ELAM-1), P-selectin (GMP-140, PADGEM) and L-selectin (LECAM-1)] are known to belong to the selectin family.[5] Among them, E- and P-selectins are induced on the endothelial surface in response to inflammatory signals, while L-selectin is constitutively expressed on all classes of circulating leukocytes and interacts with cognate ligands on endothelial cells.[4a] They share a common mosaic structure consisting of an N-terminal C-type lectin domain (carbohydrate binding site), a single epidermal growth factor domain, a discrete number of sequence modules similar to those found in regulatory proteins that bind the complement, a transmembrane domain and a C-terminal cytoplasmic domain.[4a] A number of recent reports have focused on the identification of carbohydrate ligands for the selectin family.[6] There is now general agreement that all three selectins can recognize sialyl Lewis X [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc](sLe^x, Fig. 1), sialyl Lewis A [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc] (sLe^a) and the structually related Lewis blood group oligosaccharides,[6e,f] and therefore, much attention has been focused on sLe^x by many groups.[7] Nevertheless, each selectin may have its own optimum carbohydrate ligands.[6e-g] The L- and P-selectins can efficiently bind to sulfated carbohydrates such as sulfatides, fucoidan, a sulfated glucuronic acid (HNK-1) epitope, heparin and sulfo Lewis X analogs.[8] In particular, sulfatide (Fig. 1) and synthetic sulfatides[9] strongly bind to L-selectin[10] and have highly protective effects against selectin-dependent inflammatory lung injury.[11]

In view of these facts, as a part of our search for selectin inhibitors,[12] we describe herein the systematic synthesis and biological evaluation of novel sulfated and phosphorylated β-D-galactopyranosides and β-D-lactopyranosides containing branched fatty-alkyl residues in place of ceramide.



sLe^x



sulfatide

Fig. 1. Chemical Structures of sLe^x and Sulfatide

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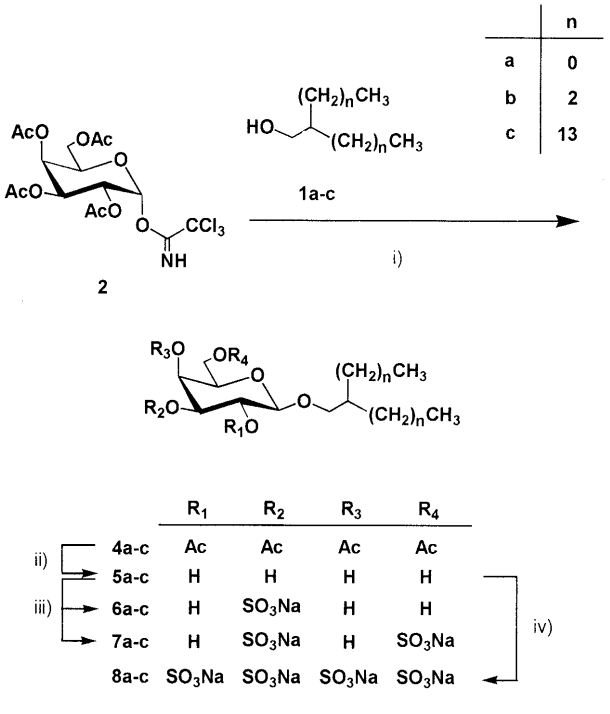
Chemistry

For the synthesis of the target glycolipids, we employed 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**2**)[9,12a] and *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**3**)[12a] as the glycosyl donors, and 2-branched fatty-alkyl alcohols (**1a—c**) as the glycosyl acceptors. The glycosylation of **1b** with **2** in dichloromethane in the presence of boron trifluoride etherate and molecular sieves (MS AW 300) exclusively gave the β-glycosides **4b** in 72% yield. The significant signal in the ^{1}H-NMR spectrum of **4b** was the one-proton doublet at δ 4.42 ($J_{1,2}$ = 7.8 Hz, H-1), showing the newly formed glycosidic linkage to be β. In essentially the same way, glycosylation of **1a, c** with **2**, and **1a—c** with **3** gave the corresponding β-glycosides (**4a, c** and **9a—c**) in good yields, respectively. The *O*-deacylation of **4a—c** or **9a—c** with sodium hydroxide in methanol yielded the desired parent glycolipids **5a—c** and **10a—c**, in which all the hydroxy groups are unprotected. The ^{1}H- and ^{13}C-NMR data, and FAB-MS data of the products thus obtained are consistent with the assigned structures.[12a]

The regioselective, one-pot sulfations for **5a—c** and **10a—c** were achieved by treatment of the corresponding stannylene intermediates with the sulfur trioxide–trimethylamine complex.[12a,13] 2-(Tetradecyl)hexadecyl β-D-galactopyranoside (**5c**) was converted by stirring with dibutyltin oxide in dry toluene to the stannylene acetal, which was then sulfated with 2.2 eq of the sulfur trioxide–trimethylamine complex in 1 : 1 *N,N*-dimethyl formamide (DMF)–tetrahydrofuran (THF) solution to give 3-sulfated galactoside **6c** and 3,6-disulfated galactoside **7c** in 34% and 66% yield, respectively (Chart 1). Similarly, regioselective sulfations of **5a, b** with 2.2 eq of sulfur trioxide–trimethylamine gave the corresponding sulfated β-glycosides (**6a, b** and **7a, b**) in good yields. The sulfated products could be easily separated on a column of silica gel and isolated as sodium salts by using a cation exchange resin.

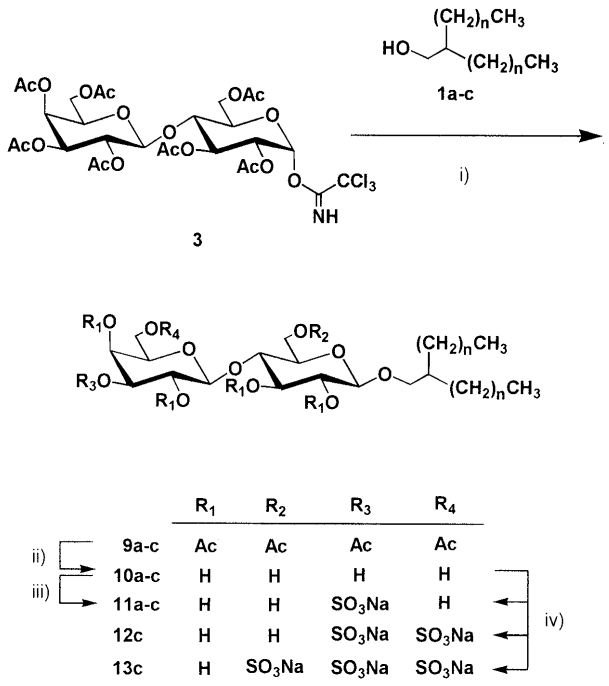
A similar, one-pot sulfation of alkyl β-D-lactopyranosides **10a—c** using 1.1 eq of the sulfur trioxide–trimethylamine complex, as just described for the galactosides **5a—c**, gave only the 3′-sulfated lactosides **11a—c** in 77%, 74% and 66% yields, respectively. The use of 2.2 eq of the sulfur trioxide-trimethylamine complex with 2-(tetradecyl)hexadecyl β-D-lactopyranoside (**10c**) afforded the 3′-sulfated lactoside **11c**, 3′,6′-disulfated lactoside **12c** and 6,3′,6′-trisulfated lactoside **13c** in the molar ratio of 1 : 2 : 1 (Chart 2).

The structures of the sulfated products were determined by NMR and MS analyses. The ^{1}H- and ^{13}C-NMR spectra have also been used to locate the positions of the sulfate groups. Comparison of the ^{1}H-NMR data of the sulfated galactosides **6a—c** and **7a—c** with those of the unsulfated precursor glycolipids **5a—c** demonstrated that the sulfate groups deshield the geminal and vicinal protons. The secondary sulfate groups in the sulfated derivatives caused



i) BF_3•Et_2O, CH_2Cl_2 (**2**→**4a**, 69%; **2**→**4b**, 72%; **2**→**4c**, 67%).
ii) NaOH, MeOH-THF (**4a**→**5a**, 98%; **4b**→**5b**, 92%; **4c**→**5c**, 98%).
iii) Bu_2SnO(1.1 equiv), toluene, SO_3•NMe_3(2.2 equiv), DMF-THF (**5a**→**6a**, 31%; **5a**→**7a**, 61%; **5b**→**6b**, 32%; **5b**→**7b**, 57%; **5c**→**6c**, 34%; **5c**→**7c**, 66%).
iv) SO_3•NMe_3(8.0 equiv), DMF-THF (**5a**→**8a**, 65%; **5b**→**8b**, 62%; **5c**→**8c**, 61%).

Chart 1



i) BF_3• Et_2O, CH_2Cl_2 (**3**→**9a**, 60%; **3**→**9b**, 69%; **3**→**9c**, 61%).
ii) NaOH, MeOH (**9a**→**10a**, 86%; **9b**→**10b**, 94%; **9c**→**10c**, 93%).
iii) Bu_2SnO(1.1 equiv), toluene, SO_3•NMe_3(1.1 equiv), DMF (**10a**→**11a**, 77%; **10b**→**11b**, 74%; **10c**→**11c**, 66%).
iv) Bu_2SnO(1.1 equiv), toluene, SO_3•NMe_3(2.2 equiv), DMF (**10c**→**11c**, 24%; **10c**→**12c**, 48%; **10c**→**13c**, 19%).

Chart 2

α effects of 0.7—0.8 ppm, whereas the primary sulfate groups showed α effects of 0.4—0.5 ppm. The β effects were 0.1—0.4 ppm depending on the axial or equatorial orientation of the vicinal proton. The H-4 signals of the 3-sulfated **6a—c** were shifted more than the H-2 signals. The resonance of an equatorial proton next to an equatorial sulfate group was shifted further downfield as compared to that of an axial proton. Comparison of the ^{13}C-NMR data of the sulfated galactosides **6b, c** and **7b, c** with those of the parent glycolipid **5b, c** demonstrated that 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 shifted upfield by 2—4 ppm. These data are in agreement with previous observations.[12a,14]

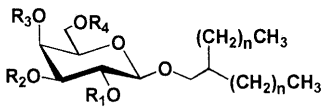
Similarly, the ^{1}H- and ^{13}C-NMR data of the sulfated lactosides **11a—c**, **12c** and **13c** could be completely assigned with the aid of 1D decoupling, 2D (^{1}H, ^{1}H) correlation spectroscopy (COSY), and 2D (^{13}C, ^{1}H) COSY experiments. Furthermore, negative FAB mass spectrometry gave $(M-Na)^-$ ions as the base peaks, thus confirming the number of sulfate groups in the molecules.

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

The phosphorylation of **5a—c** and **10b, c** was achieved by selective protection with dibenzyloxy(diisopropylamino)phosphine.[15] Acetonation of **5a** in acetone with H_2SO_4 gave the 3,4-*O*-isopropylidene derivative **14a** in 64% yield. Protection of 6-OH and 2-OH with benzyl bromide gave compound **15a** in 87% yield. Hydrolysis of the isopropylidene group of **15a** 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 **16a** in 90% yield. Finally, the catalytic hydrogenolysis of **16a** with 10% Pd–C in buffered solution and sequential treatment with a cation exchange resin gave the 3,4-bisphosphorylated galactoside **17a** in 96% yield (Chart 3). Using a similar method (Charts 3 and 4), the phosphorylation of **5b, c** and **10b, c** gave the corresponding target glycolipids (**17b, c** and **23b, c**).

Biological Results and Discussion

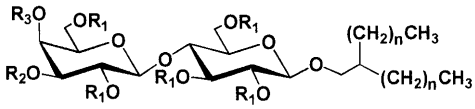
***In Vitro* Inhibitory Activity** The activity of the target glycolipids *in vitro* was measured in adhesion assays in terms of the inhibition of the binding of HL-60 cells (sLe^x expressing) to recombinant human selectin-IgG fusion proteins on plates.[7c,d] Several of the synthesized glycolipids were able to inhibit HL-60 cell binding to the selectin fusion proteins with greater potency than the sLe^x tetrasaccharide (Table 1). In particular, compounds **7c** and **8c** were both significantly more potent than sLe^x and phosphorylated β-D-galactopyranosides in blocking adhesion to the P- and L-selectins. In addition, when the branched fatty-alkyl residue was long, there was greater potency of the blocking adhesion to the P- and L-selectins. These data indicated that the sulfate groups on positions 3 and 6 of the galactopyranoside, and the attachment of a long branched fatty-alkyl residue to β-D-galactopyranoside were important for binding to the P- and L-selectins. On the other hand, 3,4-bisphosphorylated β-D-galactopy-



		R_1	R_2	R_3	R_4	
i)	**5a-c**	H	H	H	H	
ii)	**14a-c**	H	isopropylidene		H	
ii)	**15a-c**	Bn	isopropylidene		Bn	
iii)	**16a-c**	Bn	$PO(OBn)_2$	$PO(OBn)_2$	Bn	v)
iv)	**17a-c**	H	$PO(ONa)_2$	$PO(ONa)_2$	H	
	18a-c	$PO(OBn)_2$	$PO(OBn)_2$	$PO(OBn)_2$	$PO(OBn)_2$	
	19a-c	$PO(ONa)_2$	$PO(ONa)_2$	$PO(ONa)_2$	$PO(ONa)_2$	vi)

i) H_2SO_4, acetone
(**5a→14a**, 64%; **5b→14b**, 77%; **5c→14c**, 57%).
ii) benzyl bromide, DMF
(**14a→15a**, 87%; **14b→15b**, 89%; **14c→15c**, 90%).
iii) dibenzyloxy(diisopropylamino)phosphine, CH_2Cl_2
(**15a→16a**, 90%; **15b→16b**, 98%; **15c→16c**, 96%).
iv) Pd-C, MeOH-buffered sol.
(**16a→17a**, 96%; **16b→17b**, 99%; **16c→17c**, 87%).
v) dibenzyloxy(diisopropylamino)phosphine, CH_2Cl_2
(**5a→18a**, 67%; **5b→18b**, 64%; **5c→18c**, 66%).
vi) Pd-C, MeOH-buffered sol.
(**18a→19a**, 86%; **18b→19b**, 87%; **18c→19c**, 71%).

Chart 3



		R_1	R_2	R_3
i)	**10b,c**	H	H	H
ii)	**20b,c**	H	isopropylidene	
ii)	**21b,c**	Bn	isopropylidene	
iii)	**22b,c**	Bn	$PO(OBn)_2$	$PO(OBn)_2$
iv)	**23b,c**	H	$PO(ONa)_2$	$PO(ONa)_2$

i) 2,2-dimethoxypropane, H_2SO_4, acetone
(**10b→20b**, 55%; **10c→20c**, 51%).
ii) benzyl bromide, DMF
(**20b→21b**, 89%; **20c→21c**, 86%).
iii) dibenzyloxy(diisopropylamino)phosphine, CH_2Cl_2
(**21b→22b**, 93%; **21c→22c**, 94%).
iv) Pd-C, MeOH-buffered sol.
(**22b→23b**, 92%; **22c→23c**, 87%).

Chart 4

Table 1. Inhibitory Activity of Target Compounds

	% inhibition at 0.3 mM		
	P-Selectin	L-Selectin	E-Selectin
SLe^x	3±6	0±5	0±4
6a	1±1	13±7	0±2
6b	3±1	0±2	0±5
6c	3±6	27±7	4±3
7a	4±1	14±6	0±6
7b	3±4	13±4	0±5
7c	65±3	94±1	11±4
8a	5±1	12±7	8±4
8b	0±4	21±3	9±5
8c	55±5	76±6	14±2
11a	0±3	24±3	7±3
11b	0±1	21±4	12±1
11c	nd	nd	nd
12c	37±7	54±2	29±4
13c	29±6	65±3	28±7
17a	0±4	0±2	17±2
17b	13±3	0±5	20±4
17c	24±5	0±2	20±3
19a	5±1	13±3	4±2
19b	1±1	11±6	2±4
19c	8±6	11±5	21±3
23b	12±6	0±5	22±4
23c	16±5	0±5	46±5

Each value represents the mean±S.E. of 5 wells. nd: not determined because the compound causes cell lysis, which prevents determination of its inhibitory activity in this assay.

ranosides (**17a—c**) and 3′,4′-bisphosphorylated β-D-lactopyranosides (**23b, c**) were less potent than the sulfated glycolipids toward the P- and L-selectins but more potent toward E-selectin.

Ligand–Selectin Complex Models Studies of ligand–selectin complex modeling have focused on sLe^x[16] and its analog.[17] However, it is now widely accepted that many kinds of carbohydrates (*e.g.*, sLe^x) and glycolipids (*e.g.*, sulfatide) are bound to the three known selectins.[6f] Since these ligands have different selectin binding profiles, it has been proposed that these binding sites are distinct and/or partially overlap.[8c,d] A property of glycolipid binding to the selectins is L- and P-selectin specificity.[6f] Our compounds **7c** and **8c** showed the same trend. We assumed that this is caused by the long fatty-alkyl residue. Recently the complex of E-selectin and sLe^x analog with a long fatty-alkyl residue has been reported.[17b] However, the interaction modes between a sulfated galactose containing a long fatty-alkyl residue and the three selectins have not been resolved yet. Therefore we constructed **7c**-selectin complex models by a novel method. From the complex model (Figs. 2 and 3), the fatty-alkyl residue of compound **7c** is situated on the hydrophobic regions consisting of Ala108, His110, Cys109, Trp12, Leu112 and Met10 and the alkyl group on Lys87, Glu88, Gln13, Lys111, Asn 11 and Arg14, while the galactose residue is situated on the carbohydrate recognition site of the lectin domain. This position of the galactose residue is important for explaining the overlapping binding site for sLe^x and sulfatide. The sulfate group at position 3 of the galactose residue bound Ca^{2+} and the 6-sulfate group bound Lys111 and Lys113 (Fig. 3a). The 4-OH formed a hydrogen bond with NH_2 of Asn105. This model did not have interactions with Tyr48 and Tyr94. This model suggested that Lys111 and Lys113 are important and Tyr48 and Tyr94 are not important for binding L-selectin, in accordance with the experimental results on binding of sulfatide and variant P-selectin mutants.[16b] Moreover, we discovered that the second lowest energy conformation of the galactose residue is the overturned pyranose ring, binding the 3-sulfate group to Lys111 and Lys113, and the 6-sulfate group to Ca^{2+} (Fig. 3b). The difference in the interaction energy is very small. If the sulfate group is linked to the 3-OH of the galactose, which is sulfatide-like, the 3-sulfate group is able to interact with Ca^{2+} or alternatively with Lys111 and Lys113. This model can explain why the sulfatide binding to L-selectin is Ca^{2+}-independent.[8c,d] Furthermore, the fatty-alkyl residue of compound **7c** shows the best fit in binding to L-selectin, because the No.108 residue is Ala in L-selectin. As a result, the fatty-alkyl residue completely covers Ala108, affording maximal hydrophobic interaction (Fig. 4a). In the case of P-selectin, the fatty-alkyl residue makes a detour to His108, which is more bulky than Ala. The His108 is then buried by the fatty-alkyl residue to enhance the hydrophobic interaction for ligand-protein binding (Fig. 4b). In E-selectin, changing to Arg instead of Ala, the fatty-alkyl residue takes the most roundabout route for binding to selectin: the side-chain of Arg108 is folded and fixed, and its guanidine moiety is buried by the fatty-alkyl residue of the ligand (Fig. 4c). This formation is unfavorable for binding during the ligand–protein interaction. Therefore, we postulate that compound **7c**–E-selectin binding is disallowed. As already mentioned, the residue 108 in this hydrophobic interaction site is critical for L- and P-selectin specific binding.

Experimental

Melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin Elmer 1600 spectrometer. NMR spectra were recorded on a JEOL JNM-GSX 270 spectrometer (270 MHz for ^1H and 68 MHz for ^{13}C). Chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS). FAB-MS were recorded on a JEOL JMS-SX 102A mass spectrometer/JMA-DA7000 data system. Each sample was mixed with a glycerol or *m*-nitrobenzyl alcohol matrix [low-resolution MS (LRMS)] and PEG 600 or PEG 1000 matrix [high-resolution MS (HRMS)] on a target. The ion accelerating voltage was 8.0 kV, and the primary beam for the bombardment was 6.0 keV xenon. Thin-layer chromatography was run on Merck Kieselgel 60 F_{254} and RP-18 F_{254} with detection by UV and spraying with 6 N H_2SO_4, followed by 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. Solutions were concentrated *in vacuo*.

2-(Propyl)pentyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (4b) and 2-(Propyl)pentyl β-D-Galactopyranoside (5b) Powdered molecular sieves (MS AW 300, 2.0 g) were added to a solution of the trichloroacetimidate **2** (3.00 g, 6.09 mmol) and 2-(propyl)pentane-1-ol (**1b**, 793 mg, 6.09 mmol) in dry CH_2Cl_2 (3 ml), and the mixture was stirred for 6 h at room temperature, then cooled to 0 °C. Boron trifluoride etherate (768 μl, 6.09 mmol) was added and the whole 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 (*n*-hexane–EtOAc 3 : 1) of the residue on silica gel (100 g) gave **4b** (2.01 g, 72%) as a syrup. ^1H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J_{Me,CH_2}=6.8 Hz, 2MeCH$_2$), 1.28 (8H, s, 4CH$_2$), 1.57 (1H, m, CH of fatty alkyl), 1.99, 2.04, 2.05 and 2.15 (12H, 4s,

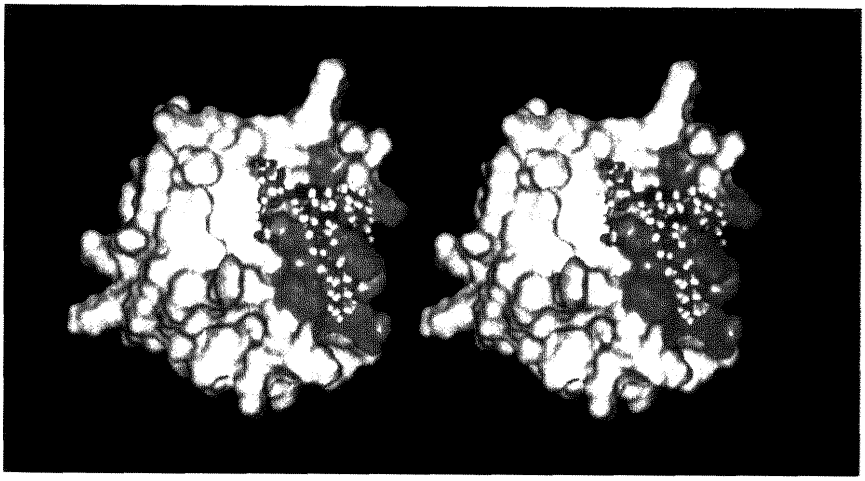


Fig. 2. Stereoview of Compound **7c**–L-Selectin Complex Model

The structure of the ligand is shown as a stick model, colored yellow, red, green and white for S, O, C and H atoms, respectively. L-Selectin is shown as a Connolly surface model, while Ca^{2+} is colored magenta, the hydrophobic regions close to the fatty-alkyl residue of ligand are colored cyan, the residue 108 (see Fig. 3) is colored blue and Tyr 48 and Tyr 94 are colored yellow.

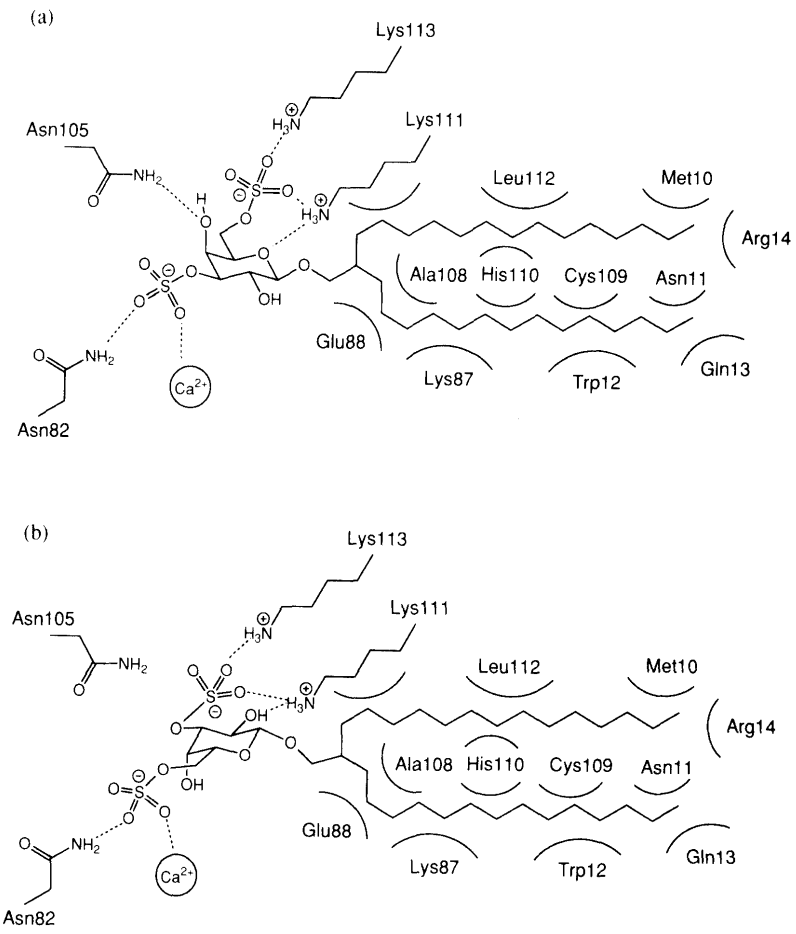


Fig. 3. Schematic Representation of the Binding Mode of Compound **7c** and L-Selectin

(a) The lowest interaction energy binding mode (equivalent to Fig. 2). The 3-sulfate group on the galactose residue interacts with Ca^{2+} and NH_2 of Asn 82. The 6-sulfate group interacts with NH_3 of Lys 111 and 113. The fatty-alkyl residue of the ligand interacts with hydrophobic regions consisting of residues 10, 11, 12, 13, 87, 88, 108, 109,110,111 and 112. The broken lines and arched lines show electrostatic interaction and hydrophobic interactions, respectively. (b) The second-lowest interaction energy binding mode. The galactose residue is turned over, and the 3 and 6-sulfate groups have swapped each binding residues. The difference between the two interaction energies is 3.33 kcal/mol.

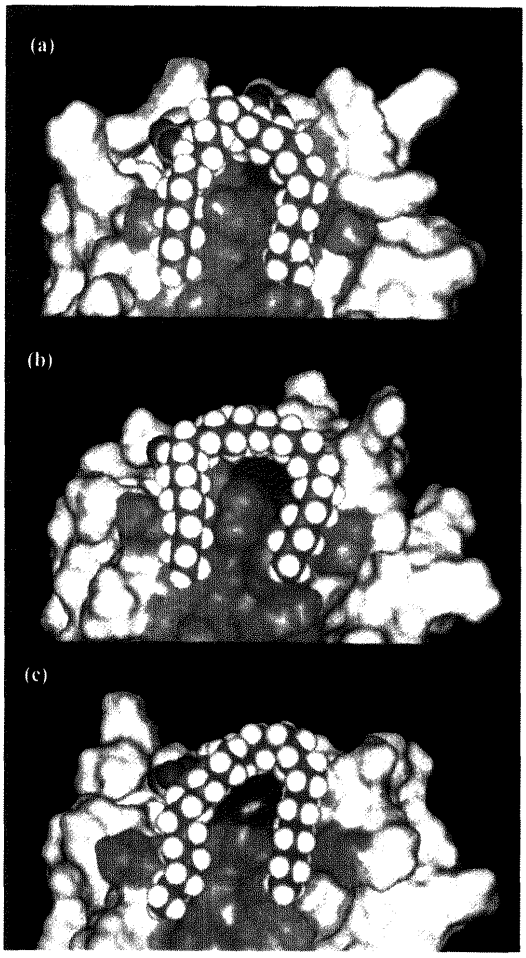


Fig. 4. Detailed Views of the Interaction between the Fatty-Alkyl Residue of Ligand and L-Selectin (a), P-Selectin (b) and E-Selectin (c)

The structure of the ligand is a CPK model, colored yellow, red, green and white for S, O, C and H atoms, respectively. Selectins are shown as Connolly surface models, and Ca^{2+} is colored magenta, the hydrophobic regions close to the fatty-alkyl residue of the ligand are colored cyan, and residue 108 is colored blue. Each conformation of the fatty-alkyl residue is different owing to the difference of residue 108.

4AcO), 3.28 (1H, dd, $J_{vic}=6.4$, $J_{gem}=9.3$ Hz, H-1 of fatty alkyl), 3.84 (1H, dd, $J_{vic}=4.9$ Hz, H-1′ of fatty alkyl), 3.89 (1H, t, $J_{5,6}=J_{5,6'}=6.8$ Hz, H-5), 4.12 (1H, dd, $J_{gem}=11.2$ Hz, H-6), 4.20 (1H, dd, H-6′), 4.42 (1H, d, $J_{1,2}=7.8$ Hz, H-1), 5.02 (1H, dd, $J_{3,4}=3.4$ Hz, H-3), 5.22 (1H, dd, $J_{2,3}=10.3$ Hz, H-2), 5.39 (1H, dd, $J_{4,5}<1.0$ Hz, H-4). LRMS (CI iso-C_4H_{10} positive) *m/z*: 331 [100% (M−aglycon)$^+$].

A solution of **4b** (2.00 g, 4.34 mmol) in MeOH (10 ml) was treated with 4 N NaOH (8.69 ml, 34.8 mmol) and the mixture was stirred at room temperature until deacetylation was complete (2 h). Purification by precipitation with water yielded **5b** (1.17 g, 92%) as an amorphous mass, mp 77 °C. *Rf* 0.79 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3406 (OH), 1062. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, $J_{Me,CH_2}=6.8$ Hz, 2MeCH$_2$), 1.30 (8H, s, 4CH$_2$), 1.58 (1H, m, CH of fatty alkyl), 3.40 (1H, dd, $J_{vic}=5.9$, $J_{gem}=9.8$ Hz, H-1 of fatty alkyl), 3.44 (1H, dd, $J_{3,4}=2.9$ Hz, H-3), 3.48 (1H, dt, $J_{5,6}=J_{5,6'}=6.8$ Hz, H-5), 3.51 (1H, dd, $J_{2,3}=10.7$ Hz, H-2), 3.73 (2H, d, H-6 and H-6′), 3.82 (1H, dd, $J_{vic}=5.9$ Hz, H-1′ of fatty alkyl), 3.83 (1H, dd, $J_{4,5}=1.0$ Hz, H-4), 4.17 (1H, d, $J_{1,2}=7.3$ Hz, H-1). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 20.9 and 34.7 (CH_2), 39.2 (CH), 62.4 (C-6), 70.3 (C-4), 72.6 (C-2), 73.8 (OCH_2), 75.1 (C-3), 76.5 (C-5), 105.4 (C-1). LRMS (FAB negative) *m/z* : 291 [100% (M−H)$^-$].

Other Alkyl β-D-Galactopyranosides (5a, c) and Lactopyranosides (10a—c) Compounds **5a, c** and **10a—c** were prepared *via* **4a, c** and **10a—c** by means of the same sequence as described for **5b**.

2-(Methyl)propyl β-D-Galactopyranoside (**5a**): mp 96 °C. *Rf* 0.60 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3417 (OH), 1070. ^{1}H-NMR (CD_3OD) δ: 0.93 and 0.94 (6H, 2d, $J_{Me,CH}=6.8$ Hz, 2MeCH), 1.85 (1H, m, CH of fatty alkyl), 3.29 (1H, dd, $J_{vic}=6.3$, $J_{gem}=9.8$ Hz, H-1 of fatty alkyl), 3.44 (1H, dd, $J_{3,4}=3.0$ Hz, H-3), 3.48 (1H, dt, $J_{5,6}=J_{5,6'}=5.4$ Hz, H-5), 3.51 (1H, dd, $J_{2,3}=9.8$ Hz, H-2), 3.67 (1H, dd, $J_{vic}=6.8$ Hz, H-1′of fatty alkyl), 3.73 (2H, d, H-6 and H-6′), 3.82 (1H, dd, $J_{4,5}=1.0$ Hz, H-4), 4.19 (1H, d, $J_{1,2}=6.9$ Hz, H-1). LRMS (FAB negative) *m/z*: 235 [100% (M−H)$^-$].

2-(Tetradecyl)hexadecyl β-D-Galactopyranoside (**5c**): mp 78 °C. *Rf* 0.58 ($CHCl_3$–MeOH 4:1). IR (KBr) cm^{-1}: 3412 (OH), 2919, 1065. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, $J_{Me,CH_2}=6.9$ Hz, 2MeCH$_2$), 1.30 (52H, s, 26CH$_2$), 1.60 (1H, m, CH of fatty alkyl), 3.42 (1H, dd, $J_{vic}=3.5$, $J_{gem}=9.4$ Hz, H-1 of fatty alkyl), 3.45 (1H, dd, $J_{3,4}=3.5$ Hz, H-3), 3.49 (1H, dt, $J_{5,6}=J_{5,6'}=5.9$ Hz, H-5), 3.52 (1H, dd, $J_{2,3}=9.4$ Hz, H-2), 3.75 (2H, d, H-6 and H-6′), 3.80 (1H, dd, $J_{vic}=5.9$ Hz, H-1′ of fatty alkyl), 3.85 (1H, dd, $J_{4,5}=1.0$ Hz, H-4), 4.18 (1H, d, $J_{1,2}=6.9$ 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)$^-$].

2-(Methyl)propyl *O*-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranoside (**10a**): mp 167 °C. *Rf* 0.48 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3424 (OH), 1065. ^{1}H-NMR (CD_3OD) δ: 0.92 and 0.94 (6H, 2d, $J_{Me,CH}=2.9$ Hz, 2MeCH), 1.92 (1H, m, CH of fatty alkyl), 3.25 (1H, m, H-2a), 3.30 (1H, m, H-1 of fatty alkyl), 3.38 (1H, m, H-5a), 3.48 (1H, m, H-3b), 3.50 (1H, m, H-3a), 3.54 (1H, m, H-2b), 3.56 (1H, m, H-4a), 3.57 (1H, m, H-5b), 3.66 (1H, m, H-1′ of fatty alkyl), 3.68 (1H, dd, $J_{5,6}=4.4$, $J_{gem}=11.4$ Hz, H-6b), 3.78 (1H, dd, $J_{5,6'}=7.5$ Hz, H-6′b), 3.81 (1H, m, H-4b), 3.83 (1H, dd, $J_{5,6}=2.9$, $J_{gem}=12.3$ Hz, H-6a), 3.89 (1H, m, H-6′a), 4.27 (1H, d, $J_{1,2}=7.9$ Hz, H-1a), 4.36 (1H, d, $J_{1,2}=7.4$ Hz, H-1b). ^{13}C-NMR (CD_3OD) δ: 19.7 (CH_3), 29.7 (CH), 61.9 (C-6a), 62.5 (C-6b), 70.3 (C-4b), 72.6 (C-2b), 74.8 (C-2a), 74.8 (C-3b), 76.4 (C-5a), 76.5 (C-3a), 77.1 (C-5b), 77.6 (OCH_2), 80.7 (C-4a), 104.5 (C-1a), 105.1 (C-1b). LRMS (FAB negative) *m/z*: 397 [100% (M−H)$^-$].

2-(Propyl)pentyl *O*-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranoside (**10b**): mp 186 °C. *Rf* 0.59 ($CHCl_3$–MeOH–H_2O 16:8:1). IR (KBr) cm^{-1}: 3429 (OH), 1063. ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, $J_{Me,CH_2}=6.9$ Hz, 2MeCH$_2$), 1.33 (8H, m, 4CH$_2$), 1.63 (1H, m, CH of fatty alkyl), 3.24 (1H, dd, $J_{2,3}=8.4$ Hz, H-2a), 3.37 (1H, m, H-5a), 3.38 (1H, dd, $J_{vic}=5.9$, $J_{gem}=9.4$ Hz, H-1 of fatty alkyl), 3.47 (1H, m, H-3b), 3.48 (1H, m, H-3a), 3.54 (1H, t, $J_{2,3}=7.4$ Hz, H-2b), 3.55 (1H, m, H-4a), 3.56 (1H, m, H-5b), 3.68 (1H, dd, $J_{5,6}=3.0$, $J_{gem}=11.4$ Hz, H-6b), 3.77 (1H, dd, $J_{5,6'}=8.4$ Hz, H-6′b), 3.79 (1H, m, H-1′ of fatty alkyl), 3.81 (1H, m, H-4b), 3.83 (1H, dd, $J_{5,6}=3.0$, $J_{gem}=12.4$ Hz, H-6a), 3.90 (1H, m, H-6′a), 4.25 (1H, d, $J_{1,2}=7.9$ Hz, H-1a), 4.36 (1H, d, $J_{1,2}=7.4$ Hz, H-1b). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 20.1 and 34.7 (CH_2), 39.2 (CH), 62.0 (C-6a), 62.5 (C-6b), 70.3 (C-4b), 72.6 (C-2b), 74.0 (OCH_2), 74.8 (C-2a), 74.8 (C-3b), 76.4 (C-5a), 76.5 (C-3a), 77.1 (C-5b), 80.7 (C-4a), 104.7 (C-1a), 105.1 (C-1b). LRMS (FAB negative) *m/z* : 453 [100% (M−H)$^-$].

2-(Tetradecyl)hexadecyl *O*-(β-D-Galactopyranosyl)-(1→4)-β-D-glucopyranoside (**10c**): mp 207 °C. *Rf* 0.63 ($CHCl_3$–MeOH–H_2O 13:6:1). IR (KBr) cm^{-1}: 3424 (OH), 2921, 1064. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (6H, t, $J_{Me,CH_2}=6.4$ Hz, 2MeCH$_2$), 1.24 (52H, s, 26CH$_2$), 1.51 (1H, m, CH of fatty alkyl), 3.18 (1H, t, $J_{2,3}=7.9$ Hz, H-2a), 3.32 (1H, m, H-5a), 3.33 (1H, m, H-1 of fatty alkyl), 3.38 (2H, m, H-3a and H-4a), 3.40 (1H, m, H-3b), 3.42 (1H, m, H-2b), 3.54 (1H, m, H-5b), 3.56 (2H, m, H-6b and H-6′b), 3.58 (1H, m, H-6a), 3.71 (1H, m, H-4b), 3.72 (1H, m, H-1′ of fatty alkyl), 3.76 (1H, m, H-6′a), 4.22 (1H, d, $J_{1,2}=7.9$ Hz, H-1a), 4.29 (1H, d, $J_{1,2}=7.4$ Hz, H-1b). ^{13}C-NMR (DMSO-d_6) δ: 13.8 (CH_3), 22.0, 25.9, 26.0, 28.6, 28.9, 29.3, 30.3, 30.4 and 31.2 (CH_2), 37.4 (CH), 60.2 (C-6a), 60.6 (C-6b), 68.0 (C-4b), 70.5 (C-2b), 71.9 (OCH_2), 73.0 (C-2a), 73.1 (C-3b), 74.7 (C-5a), 75.0 (C-3a), 75.4 (C-5b), 80.8 (C-4a), 102.9 (C-1a), 103.8 (C-1b). LRMS (FAB negative) *m/z*: 761.6 [100% (M−H)$^-$].

2-(Tetradecyl)hexadecyl 3-*O*-Sulfo-β-D-galactopyranoside Sodium Salt (6c) and 2-(Tetradecyl)hexadecyl 3,6-Di-*O*-sulfo-β-D-galactopyranoside Disodium Salt (7c) Compound **5c** (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. A solution of the stannyl complex in DMF (4 ml) and THF (4 ml) was treated with 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 ($CHCl_3$–MeOH 17:3) on silica gel (40 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1 × 4 cm, MeOH), to give **6c** (99 mg, 34%) and **7c** (221 mg, 66%) as amorphous masses.

Compound **6c** had *Rf* 0.55 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3424, 2918, 1252 (SO_2), 1064 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.90 (6H, t, $J_{Me,CH_2}=6.8$ Hz, 2MeCH$_2$), 1.30 (52H, s, 26CH$_2$), 1.60 (1H, m, CH of fatty alkyl), 3.42 (1H, dd, $J_{vic}=5.9$, $J_{gem}=9.4$ Hz, H-1 of fatty alkyl), 3.53 (1H, t, $J_{5,6}=6.4$ Hz, H-5), 3.70 (1H, dd, $J_{2,3}=9.3$ Hz, H-2), 3.74 (2H, d, H-6), 3.80 (1H, dd, $J_{vic}=5.9$ Hz, H-1′ of fatty alkyl), 4.21 (1H, dd, $J_{3,4}=3.4$ Hz, H-3), 4.25 (1H, d, H-4), 4.28 (1H, d, $J_{1,2}=7.8$ 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−Na)$^-$], 701.5 [4% (M−H)$^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{71}O_9S$ (M−Na)$^-$: 679.4819. Found 679.4792.

Compound **7c** had *Rf* 0.46 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3450, 2922, 1261 (SO_2), 1026 (SO_2). ^{1}H-NMR (CD_3OD–D_2O 1:1) δ: 0.90 (6H, t, $J_{Me,CH_2}=6.8$ Hz, 2MeCH$_2$), 1.30 (52H, s, 26CH$_2$), 1.60 (1H, m, CH of fatty alkyl), 3.41 (1H, dd, $J_{vic}=5.9$, $J_{gem}=9.8$ Hz, H-1 of fatty alkyl), 3.70 (1H, dd, $J_{2,3}=9.3$ Hz, H-2), 3.78 (1H, dd, $J_{vic}=6.4$ Hz, H-1′ of fatty alkyl), 3.82 (1H, dd, $J_{5,6}=6.4$, $J_{5,6'}=6.1$ Hz, H-5), 4.16 (1H, dd, $J_{gem}=10.3$ Hz, H-6), 4.22 (1H, dd, H-6′), 4.25 (1H, d, H-3), 4.27 (1H, near s, H-4), 4.30 (1H, d, $J_{1,2}=7.8$ Hz, H-1). ^{13}C-NMR (CD_3OD–D_2O 1:1) 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−Na)$^-$], 803.4 [4% (M−H)$^-$]. HRMS (FAB negative) Calcd for $C_{36}H_{70}O_{12}NaS_2$ (M−Na)$^-$: 781.4206. Found 781.4236.

Other Alkyl 3-Sulfated and 3,6-Disulfated β-D-Galactopyranosides (6a, b and 7a, b) Compounds **6a, b** and **7a, b** were prepared in one-pot from **5a, b** *via* the same sequence as described for **6c** and **7c**.

2-(Methyl)propyl 3-*O*-Sulfo-β-D-galactopyranoside Sodium Salt (**6a**): *Rf* 0.42 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3420, 1244 (SO_2), 1020 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.92 (6H, d, $J_{Me,CH}=6.8$ Hz, 2MeCH), 1.90 (1H, m, CH of fatty alkyl), 3.31 (1H, dd, $J_{vic}=7.8$, $J_{gem}=9.8$ Hz, H-1 of fatty alkyl), 3.53 (1H, dt, $J_{5,6}=J_{5,6'}=6.3$ Hz, H-5), 3.66 (1H, dd, $J_{vic}=5.9$ Hz, H-1′ of fatty alkyl), 3.69 (1H, dd, $J_{2,3}=8.8$ Hz, H-2), 3.73 (2H, d, H-6 and H-6′), 4.21 (1H, dd, $J_{3,4}=3.4$ Hz, H-3), 4.24 (1H, dd, $J_{4,5}=1.0$ Hz, H-4), 4.29 (1H, d, $J_{1,2}=7.8$ Hz, H-1). LRMS (FAB negative) *m/z*: 315 [100% (M−Na)$^-$], 337 [13% (M−H)$^-$]. HRMS (FAB negative) Calcd for $C_{10}H_{19}O_9S$ (M−Na)$^-$: 315.0750. Found: 315.0731.

2-(Methyl)propyl 3,6-Di-*O*-sulfo-β-D-galactopyranoside Disodium Salt (**7a**): *Rf* 0.30 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3450, 1252 (SO_2), 1016 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.91 and 0.92 (6H, 2d, $J_{Me,CH}=6.4$ Hz, 2MeCH), 1.90 (1H, m, CH of fatty alkyl), 3.31 (1H, dd, $J_{vic}=6.8$, $J_{gem}=9.3$ Hz, H-1 of fatty alkyl), 3.65 (1H, dd, $J_{vic}=6.8$ Hz, H-1′ of fatty alkyl), 3.69 (1H, dd, $J_{2,3}=9.3$ Hz, H-2), 3.81 (1H, t, $J_{5,6}=J_{5,6'}=6.4$ Hz, H-5), 4.14 (1H, dd, $J_{gem}=10.3$ Hz, H-6), 4.20 (1H,

dd, H-6′), 4.23 (1H, d, H-3), 4.26 (1H, near s, H-4), 4.31 (1H, d, $J_{1,2}$=7.8 Hz, H-1). LRMS (FAB negative) *m/z*: 417 [87% $(M-Na)^-$], 439 [4% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{10}H_{18}NaO_{12}S_2$ $(M-Na)^-$: 417.0137. Found: 417.0160.

2-(Propyl)pentyl 3-*O*-Sulfo-β-D-galactopyranoside Sodium Salt (**6b**): *Rf* 0.45 ($CHCl_3$–MeOH–H_2O 16:8:1). IR (KBr) cm^{-1}: 3448, 1245 (SO_2), 1019 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, t, J_{Me,CH_2}=6.8 Hz, 2MeCH$_2$), 1.30 (8H, s, 4CH$_2$), 1.65 (1H, m, CH of fatty alkyl), 3.41 (1H, dd, J_{vic}=5.9, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.52 (1H, dt, $J_{5,6}=J_{5,6'}$=6.3 Hz, H-5), 3.69 (1H, dd, $J_{2,3}$=9.3 Hz, H-2), 3.73 (2H, d, H-6 and H-6′), 3.81 (1H, dd, J_{vic}=6.4 Hz, H-1′ of fatty alkyl), 4.21 (1H, dd, $J_{3,4}$=3.4 Hz, H-3), 4.24 (1H, dd, $J_{4,5}$=1.0 Hz, H-4), 4.27 (1H, d, $J_{1,2}$=7.8 Hz, H-1). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 20.9 and 34.7 (CH_2), 39.2 (CH), 62.3 (C-6), 68.5 (C-4), 70.8 (C-2), 73.9 (OCH_2), 76.2 (C-5), 82.4 (C-3), 105.1 (C-1). LRMS (FAB negative) *m/z*: 371 [100% $(M-Na)^-$], 393 [5% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{14}H_{27}O_9S$ $(M-Na)^-$: 371.1372. Found: 371.1376.

2-(Propyl)pentyl 3,6-Di-*O*-sulfo-β-D-galactopyranoside Disodium Salt (**7b**): *Rf* 0.38 ($CHCl_3$–MeOH–H_2O 16:8:1). IR (KBr) cm^{-1}: 3468, 1248 (SO_2), 1020 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, t, J_{Me,CH_2}=6.8 Hz, 2MeCH$_2$), 1.30 (8H, s, 4CH$_2$), 1.65 (1H, m, CH of fatty alkyl), 3.41 (1H, dd, J_{vic}=5.4, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.68 (1H, dd, $J_{2,3}$=8.8 Hz, H-2), 3.78 (1H, t, $J_{5,6}=J_{5,6'}$=6.4 Hz, H-5), 3.79 (1H, dd, J_{vic}=5.9 Hz, H-1′ of fatty alkyl), 4.13 (1H, dd, J_{gem}=10.3 Hz, H-6), 4.21 (1H, dd, H-6′), 4.22 (1H, dd, $J_{3,4}$=1.0 Hz, H-3), 4.26 (1H, d, H-4), 4.29 (1H, d, $J_{1,2}$=7.8 Hz, H-1). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 21.0 and 34.7 (CH_2), 39.2 (CH), 67.6 (C-6), 68.5 (C-4), 70.7 (C-2), 73.9 (OCH_2), 73.9 (C-5), 81.8 (C-3), 105.0 (C-1). LRMS (FAB negative) *m/z*: 473 [100% $(M-Na)^-$], 495 [7% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{14}H_{26}NaO_{12}S_2$ $(M-Na)^-$: 473.0763. Found : 473.0781.

2-(Tetradecyl)hexadecyl 2,3,4,6-Tetra-*O*-sulfo-β-D-galactopyranoside Tetrasodium Salt (8c) A mixture of **5c** (100 mg, 0.166 mmol) and sulfur trioxide–trimethylamine complex (185 mg, 1.33 mmol) in DMF (1 ml) and THF (1 ml) was stirred for 24 h at room temperature, then concentrated. The residue was chromatographed ($CHCl_3$–MeOH–H_2O 8:5:1) on silica gel (30 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH), to give **8c** (102 mg, 61%) as an amorphous mass. *Rf* 0.16 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3448, 2921, 1260 (SO_2), 1020 (SO_2). ^{1}H-NMR (CD_3OD–D_2O 1:1) δ: 0.88 (6H, t, J_{Me,CH_2}=6.8 Hz, 2MeCH$_2$), 1.28 (52H, s, 26CH$_2$), 1.65 (1H, m, CH of fatty alkyl), 3.48 (1H, dd, J_{vic}=5.9, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.80 (1H, dd, J_{vic}=6.4 Hz, H-1′ of fatty alkyl), 4.10 (1H, dd, $J_{5,6}$=6.9, $J_{5,6'}$=5.4 Hz, H-5), 4.20 (1H, dd, J_{gem}=9.9 Hz, H-6), 4.25 (1H, dd, H-6′), 4.40 (1H, dd, $J_{2,3}$=9.4 Hz, H-2), 4.55 (1H, dd, $J_{3,4}$=2.0 Hz, H-3), 4.60 (1H, d, $J_{1,2}$=7.4 Hz, H-1), 5.10 (1H, d, H-4). ^{13}C-NMR (CD_3OD–D_2O 1:1) 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 $C_{36}H_{68}Na_3O_{18}S_4$ $(M-Na)^-$: 985.2982. Found: 985.2982.

Other Alkyl 2,3,4,6-Tetrasulfated β-D-Galactopyranosides (8a, b) Compounds **8a** and **8b** were prepared from **5a** and **5b** *via* the same sequence as described for **8c**.

2-(Methyl)propyl 2,3,4,6-Tetra-*O*-sulfo-β-D-galactopyranoside Tetrasodium Salt (**8a**): *Rf* 0.10 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3448, 1255 (SO_2), 1020 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.92 and 0.94 (6H, 2d, $J_{Me,CH}$=6.8 Hz, 2MeCH), 1.90 (1H, m, CH of fatty alkyl), 3.32 (1H, dd, J_{vic}=6.9, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.67 (1H, dd, J_{vic}=6.4 Hz, H-1′ of fatty alkyl), 4.01 (1H, dd, $J_{5,6}$=7.3, $J_{5,6'}$=3.9 Hz, H-5), 4.21 (1H, dd, J_{gem}=11.7 Hz, H-6), 4.38 (1H, dd, H-6′), 4.44 (1H, d, H-3), 4.49 (1H, d, $J_{1,2}$=3.9 Hz, H-1), 4.51 (1H, dd, $J_{2,3}$=8.3 Hz, H-2), 5.09 (1H, near s, H-4). LRMS (FAB negative) *m/z*: 621 [100% $(M-Na)^-$]. HRMS (FAB negative) Calcd for $C_{10}H_{16}Na_3O_{18}S_4$ $(M-Na)^-$: 620.8913. Found: 620.8932.

2-(Propyl)pentyl 2,3,4,6-Tetra-*O*-sulfo-β-D-galactopyranoside Tetrasodium Salt (**8b**): *Rf* 0.14 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3456, 1256 (SO_2), 1019 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH$_2$), 1.35 (8H, s, 4CH$_2$), 1.65 (1H, m, CH of fatty alkyl), 3.43 (1H, dd, J_{vic}=6.4, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.79 (1H, dd, J_{vic}=5.9 Hz, H-1′ of fatty alkyl), 4.00 (1H, dd, $J_{5,6}$=7.3, $J_{5,6'}$=4.4 Hz, H-5), 4.20 (1H, dd, J_{gem}=11.2 Hz, H-6), 4.38 (1H, dd, H-6′), 4.45 (1H, m, H-2), 4.45 (1H, m, H-3), 4.47 (1H, d, $J_{1,2}$=7.4 Hz, H-1), 5.10 (1H, near s, H-4). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 20.9 and 34.4 (CH_2), 39.0 (CH), 68.4 (C-6), 73.5 (C-5), 73.8 (OCH_2), 76.1 (C-4), 76.6 (C-2), 77.6 (C-3), 103.0 (C-1). LRMS (FAB negative) *m/z*: 677 [100% $(M-Na)^-$]. HRMS (FAB negative) Calcd for $C_{14}H_{24}Na_3O_{18}S_4$ $(M-Na)^-$: 676.9539. Found: 676.9558.

2-(Methyl)propyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Sodium Salt (11a) Compound **10a** (99.6 mg, 0.250 mmol) and dibutyltin oxide (68.4 mg, 0.275 mmol) were stirred in refluxing toluene (5 ml) for 16 h with continuous removal of water, and concentrated. A solution of the stannyl complex in DMF (5 ml) was treated with sulfur trioxide–trimethylamine complex (41.7 mg, 0.300 mmol) as described for **6c**. The resulting residue was chromatographed ($CHCl_3$–MeOH–H_2O 8:5:1) on silica gel (25 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH) to give **10a** (96.0 mg, 77%) as an amorphous mass. *Rf* 0.33 ($CHCl_3$–MeOH–H_2O 8:5:1). IR (KBr) cm^{-1}: 3460, 1252 (SO_2), 1004 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.93 and 0.94 (6H, 2d, $J_{Me,CH}$=6.9 Hz, 2MeCH), 1.90 (1H, m, CH of fatty alkyl), 3.25 (1H, dd, $J_{2,3}$=8.9 Hz, H-2a), 3.30 (1H, m, H-1 of fatty alkyl), 3.37 (1H, m, H-5a), 3.53 (1H, t, $J_{3,4}$=8.9 Hz, H-3a), 3.65 (3H, m, H-4a, H-5b and H-1′ of fatty alkyl), 3.70 (1H, m, H-2b), 3.74 (1H, m, H-6b), 3.78 (1H, m, H-6′b), 3.88 (2H, m, H-6a and H-6a′), 4.23 (1H, m, H-4b), 4.25 (1H, m, H-3b), 4.28 (1H, d, $J_{1,2}$=7.8 Hz, H-1a), 4.48 (1H, d, $J_{1,2}$=7.4 Hz, H-1b). ^{13}C-NMR (CD_3OD) δ: 19.6 (CH_3), 29.7 (CH), 62.0 (C-6a), 62.4 (C-6b), 68.6 (C-4b), 70.9 (C-2b), 74.8 (C-2a), 76.4 (C-5a), 76.4 (C-3a), 76.8 (C-5b), 77.6 (OCH_2), 81.2 (C-4a), 81.8 (C-3b), 104.5 (C-1a), 105.0 (C-1b). LRMS (FAB negative) *m/z*: 477 [100% $(M-Na)^-$], 499 [11% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{16}H_{29}O_{14}S$ $(M-Na)^-$: 477.1278. Found: 477.1292.

2-(Propyl)pentyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Sodium Salt (11b) Compound **11b** was prepared from **10b** *via* the same sequence as described for **11a**. *Rf* 0.45 ($CHCl_3$–MeOH–H_2O 16:8:1). IR (KBr) cm^{-1}: 3442, 1249 (SO_2), 1033 (SO_2). ^{1}H-NMR (CD_3OD) δ: 0.89 (6H, t, J_{Me,CH_2}=6.9 Hz, 2MeCH$_2$), 1.31 (8H, s, 4CH$_2$), 1.61 (1H, m, CH of fatty alkyl), 3.17 (1H, t, $J_{2,3}$=7.9 Hz, H-2a), 3.32 (1H, m, H-5a), 3.37 (1H, dd, J_{vic}=5.9, J_{gem}=9.9 Hz, H-1 of fatty alkyl), 3.45 (1H, m, H-3a), 3.50 (1H, m, H-4a), 3.55 (1H, m, H-5b), 3.63 (1H, dd, $J_{2,3}$=9.9 Hz, H-2b), 3.65 (2H, m, J_{gem}=11.2 Hz, H-6b and H-6b′), 3.78 (1H, dd, J_{vic}=5.9 Hz, H-1′ of fatty alkyl), 3.79 (1H, dd, $J_{5,6}$=3.0, J_{gem}=12.4 Hz, H-6a), 3.86 (1H, dd, $J_{5,6'}$=3.0 Hz, H-6′a), 4.14 (1H, d, H-4b), 4.18 (1H, dd, $J_{3,4}$=3.4 Hz, H-3b), 4.23 (1H, d, $J_{1,2}$=7.9 Hz, H-1a), 4.44 (1H, d, $J_{1,2}$=7.4 Hz, H-1b). ^{13}C-NMR (CD_3OD) δ: 15.1 (CH_3), 20.9 and 34.6 (CH_2), 39.0 (CH), 62.0 (C-6a), 62.1 (C-6b), 68.3 (C-4b), 70.8 (C-2b), 73.8 (OCH_2), 74.8 (C-2a), 76.4 (C-5a), 76.5 (C-3a), 76.8 (C-5b), 81.2 (C-3b), 81.8 (C-4a), 104.6 (C-1a), 105.1 (C-1b). LRMS (FAB negative) *m/z*: 533 [100% $(M-Na)^-$], 555 [5% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{20}H_{37}O_{14}S$ $(M-Na)^-$: 533.1904. Found: 533.1933.

2-(Tetradecyl)hexadecyl *O*-(3-*O*-Sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Sodium Salt (11c), 2-(Tetradecyl)hexadecyl *O*-(3,6-Di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Disodium Salt (12c) and 2-(Tetradecyl)hexadecyl *O*-(3,6-Di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-6-*O*-sulfo-β-D-glucopyranoside Trisodium Salt (13c) Compound **10c** (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 **6c**. The resulting residue was chromatographed ($CHCl_3$–MeOH–H_2O 13:6:1) on silica gel (30 g) and loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1×4 cm, MeOH) to give **11c** (31.0 mg, 24%), **12c** (70.0 mg, 48%) and **13c** (30.3 mg, 19%) as amorphous masses.

Compound **11c** had *Rf* 0.45 ($CHCl_3$–MeOH–H_2O 13:6:1). IR (KBr) cm^{-1}: 3464, 2922, 1243 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 0.85 (6H, t, J_{Me,CH_2}=6.9 Hz, 2MeCH$_2$), 1.24 (52H, s, 26CH$_2$), 1.50 (1H, m, CH of fatty alkyl), 3.00 (1H, t, $J_{2,3}$=7.4 Hz, H-2a), 3.26 (1H, m, H-5a), 3.29 (1H, m, H-1 of fatty alkyl), 3.36 (1H, m, H-3a), 3.38 (1H, m, H-4a), 3.52 (4H, m, H-2b, H-5b, H-6b and H-6b′), 3.57 (1H, m, H-6a), 3.65 (1H, m, H-1′ of fatty alkyl), 3.76 (1H, m, H-6′a), 3.89 (1H, d, H-4b), 4.01 (1H, dd, $J_{3,4}$=3.4 Hz, H-3b), 4.13 (1H, d, $J_{1,2}$=7.4 Hz, H-1a), 4.33 (1H, d, $J_{1,2}$=7.8 Hz, H-1b). ^{13}C-NMR (DMSO-d_6) δ: 13.8 (CH_3), 22.0, 25.9, 28.6, 28.9, 29.3, 30.3 and 31.2 (CH_2), 37.5 (CH), 60.0 (C-6b), 60.5 (C-6a), 66.5 (C-4b), 69.3 (C-2b), 72.0 (OCH_2), 73.1 (C-2a), 74.7 (C-5a), 74.9 (C-3a), 75.3 (C-5b), 78.7 (C-3b), 81.1 (C-4a), 102.9 (C-1a), 103.7 (C-1b). 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 **12c** had *Rf* 0.27 ($CHCl_3$–MeOH–H_2O 13:6:1). IR (KBr) cm^{-1}: 3450, 2923, 1252 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (6H, t, J_{Me,CH_2}=6.9 Hz, 2MeCH_2), 1.25 (52H, s, 26CH_2), 1.52 (1H, m, CH of fatty alkyl), 3.04 (1H, t, $J_{2,3}$=7.9 Hz, H-2a), 3.25 (1H, m, H-5a), 3.32 (1H, m, H-1 of fatty alkyl), 3.35 (1H, m, H-4a), 3.36 (1H, m, H-3a), 3.54 (1H, dd, $J_{2,3}$=9.9 Hz, H-2b), 3.61 (1H, m, H-6a), 3.66 (1H, m, H-1′ of fatty alkyl), 3.73 (1H, m, H-6′a), 3.75 (1H, m, H-5b), 3.88 (2H, m, H-6b and H-6b′), 3.94 (1H, d, H-4b), 4.02 (1H, dd, $J_{3,4}$=2.9 Hz, H-3b), 4.15 (1H, d, $J_{1,2}$=7.9 Hz, H-1a), 4.36 (1H, d, $J_{1,2}$=7.9 Hz, H-1b). ^{13}C-NMR (DMSO-d_6) δ: 13.9 (CH_3), 22.2, 26.2, 28.8, 29.1, 29.5, 30.5 and 31.4 (CH_2), 37.7 (CH), 60.9 (C-6a), 65.0 (C-6b), 66.8 (C-4b), 68.9 (C-2b), 72.2 (OCH_2), 73.3 (C-5b), 73.5 (C-2a), 75.1 (C-5a), 75.2 (C-3a), 79.1 (C-3b), 80.5 (C-4a), 103.1 (C-1a), 103.9 (C-1b). LRMS (FAB negative) *m/z* : 943.5 [100% $(M-Na)^-$], 965.5 [13% $(M-H)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{80}NaO_{17}S_2$ $(M-Na)^-$: 943.4735. Found: 943.4706.

Compound **13c**: *Rf* 0.13 ($CHCl_3$–MeOH–H_2O 13:6:1). IR (KBr) cm^{-1}: 3450, 2924, 1254 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH_2), 1.25 (52H, s, 26CH_2), 1.53 (1H, m, CH of fatty alkyl), 3.07 (1H, t, $J_{2,3}$=7.8 Hz, H-2a), 3.28 (1H, m, H-1 of fatty alkyl), 3.34 (1H, m, H-4a), 3.36 (1H, m, H-3a), 3.47 (1H, m, H-2b), 3.48 (1H, m, H-5a), 3.72 (1H, t, $J_{5,6}$=$J_{5,6'}$=6.9 Hz, H-5b), 3.78 (1H, dd, J_{vic}=6.8 Hz, H-1′ of fatty alkyl), 3.90 (3H, m, H-6a, H-6b and H-6b′), 3.97 (1H, m, H-4b), 4.05 (1H, m, H-3b), 4.08 (1H, m, H-6′a), 4.18 (1H, d, $J_{1,2}$=7.8 Hz, H-1a), 4.43 (1H, d, $J_{1,2}$=8.3 Hz, H-1b). ^{13}C-NMR (DMSO-d_6) δ: 13.9 (CH_3), 22.2, 26.2, 26.3, 28.8, 29.2, 29.5, 30.6 and 31.4 (CH_2), 37.8 (CH), 65.0 (C-6b), 65.2 (C-6a), 66.7 (C-4b), 69.1 (C-2b), 72.4 (OCH_2), 73.1 (C-2a), 73.1 (C-5a), 73.3 (C-5b), 74.9 (C-3a), 78.6 (C-3b), 80.3 (C-4a), 103.0 (C-1a), 103.5 (C-1b). LRMS (FAB negative) *m/z*: 1045.4 [71% $(M-Na)^-$]. HRMS (FAB negative) Calcd for $C_{42}H_{79}Na_2O_{20}S_3$ $(M-Na)^-$: 1045.4122. Found: 1045.4115.

2-(Methyl)propyl 3,4-*O*-Isopropylidene-β-D-galactopyranoside (14a) Compound **5a** (200 mg, 0.847 mmol) was dissolved in acetone (20 ml) and H_2SO_4 (10 μl) was added. The mixture was stirred for 18 h at room temperature, after which Na_2CO_3 was added to neutralize the solution. The solvents were evaporated and the residue was chromatographed (*n*-hexane–EtOAc 2:3) on silica gel (30 g) to give **14a** (149 mg, 64%) as a syrup. ^{1}H-NMR (CD_3OD) δ: 0.92 and 0.93 (6H, 2d, $J_{Me,CH}$=6.8 Hz, 2MeCH), 1.32 and 1.47 (6H, 2s, 2CH_3), 1.89 (1H, m, CH of fatty alkyl), 3.28 (1H, dd, J_{vic}=6.8, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.40 (1H, dd, $J_{2,3}$=5.4 Hz, H-2), 3.67 (1H, dd, J_{gem}=6.8 Hz, H-1′ of fatty alkyl), 3.77 (2H, m, H-6 and H-6′), 3.83 (1H, m, H-5), 4.00 (1H, dd, $J_{3,4}$=5.4 Hz, H-3), 4.19 (2H, dd, $J_{4,5}$=2.0 Hz, H-4), 4.20 (2H, d, $J_{1,2}$=8.3 Hz, H-1).

2-(Methyl)propyl 2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (15a) Compound **14a** (127 mg, 0.460 mmol) was added to a suspension of NaH (73.5 mg, 1.84 mmol) in *N*,*N*-dimethylformamide (2 ml) at 0 °C. The suspension was stirred at that temperature for 30 min and then benzyl bromide (0.218 ml, 1.84 mmol) was added. The solution was allowed to warm slowly to room temperature. After 12 h, the reaction was quenched by addition of MeOH (1 ml) at 0 °C, then the mixture was diluted with $CHCl_3$ (10 ml), washed twice with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (*n*-hexane–EtOAc 5:1) on silica gel (30 g) to give **15a** (182 mg, 87%) as a syrup. ^{1}H-NMR ($CDCl_3$) δ: 0.96 and 0.97 (6H, 2d, $J_{Me,CH}$=6.8 Hz, 2MeCH), 1.32 and 1.34 (4H, 2s, 2CH_2), 1.96 (1H, m, CH of fatty alkyl), 3.25 (1H, dd, J_{vic}=7.0, J_{gem}=9.2 Hz, H-1 of fatty alkyl), 3.38 (1H, m, H-2), 3.76 (1H, dd, J_{vic}=6.4 Hz, H-1′ of fatty alkyl), 3.80 (2H, m, H-6 and H-6′), 3.91 (1H, m, H-5), 4.14 (2H, m, H-3 and H-4), 4.29 (1H, d, $J_{1,2}$=7.8 Hz, H-1), 4.56, 4.65, 4.78 and 4.87 (4H, 4d, J_{gem}=11.7 Hz, 4PhCH), 7.22—7.42 (10H, m, 2Ph).

2-(Methyl)propyl 2,6-Di-*O*-benzyl-3,4-di-*O*-dibenzylphosphono-β-D-galactopyranoside (16a) A solution of compound **15a** (178 mg, 0.390 mmol) in CH_2Cl_2 (4 ml) at 0 °C was treated with aqueous 90% trifluoroacetic acid (0.6 ml). After 30 min, toluene (10 ml) and EtOAc (10 ml) were added and then removed *in vacuo*. A solution of CH_2Cl_2 (2 ml), acetonitrile (2 ml), 1*H*-tetrazole (81.9 mg, 1.17 mmol) and dibenzyloxy(diisopropylamino)phosphine (539 mg, 1.56 mmol) was added at room temperature with stirring. After 12 h, water (10 ml), $RuCl_3 \cdot 3H_2O$ (2 mg, 0.01 mmol), and $NaIO_4$ (334 mg, 1.56 mmol) were added and the mixture was vigorously stirred for 2 h. Then it 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 (*n*-hexane–EtOAc 2:1) on silica gel (40 g) to give **16a** (328 mg, 90%) as a syrup. ^{1}H-NMR ($CDCl_3$) δ: 0.92 and 0.94 (6H, 2d, $J_{Me,CH}$=6.8 Hz, 2MeCH), 1.92 (1H, m, CH of fatty alkyl), 3.26 (1H, dd, J_{vic}=7.3, J_{gem}=9.3 Hz, H-1 of fatty alkyl), 3.60—3.69 (4H, m, H-2, H-5, H-6 and H-6′), 3.74 (1H, dd, J_{vic}=6.4 Hz, H-1′ of fatty alkyl), 4.39 (1H, d, $J_{1,2}$=7.8 Hz, H-1), 4.35, 4.42, 4.47 and 4.62 (4H, 4d, J_{gem}=11.7 Hz, 4PhCH), 4.85—5.08 (10H, m, H-3, H-4 and 4PhCH_2), 7.11—7.36 (30H, m, 6Ph).

2-(Methyl)propyl 3,4-Bisphospho-β-D-galactopyranoside Tetrasodium Salt (17a) Compound **16a** (250 mg, 0.267 mmol) was dissolved in MeOH (3 ml) and buffered aqueous AcOH–NaOAc (1 ml, pH 5, 0.5 M), and the mixture was treated with 10% Pd–C (18 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 **17a** (124 mg, 96%) as an amorphous mass. IR (KBr) cm^{-1}: 3443, 1074 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.96 and 0.97 (6H, 2d, $J_{Me,CH}$=6.9 Hz, 2MeCH), 1.95 (1H, m, CH of fatty alkyl), 3.50 (1H, dd, J_{vic}=6.9, J_{gem}=9.4 Hz, H-1 of fatty alkyl), 3.63 (2H, m, H-2 and H-1′ of fatty alkyl), 3.68 (1H, m, H-6), 3.78 (2H, m, H-5 and H-6′), 4.20 (1H, ddd, $J_{2,3}$=6.9, $J_{3,4}$=3.0, $J_{3,P}$=9.9 Hz, H-3), 4.54 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.67 (1H, dd, $J_{4,P}$=10.4 Hz, H-4). ^{13}C-NMR (D_2O) δ: 20.5 (CH_3), 29.8 (CH), 61.5 (C-6), 72.1 (C-2), 72.6 (C-4), 76.0 (C-5), 78.0 (C-3), 79.1 (OCH_2), 104.8 (C-1). *Anal.* Calcd for $C_{10}H_{18}Na_4O_{12}P_2$: C, 24.81; H, 3.75. Found: C, 24.60; H, 3.77.

Other Alkyl 3,4-Bis phosphorylated β-D-Galactopyranosides (17b, c) and Lactopyranosides (23b, c) Compounds **17b, c** and **23b, c** were prepared *via* **14b, c—16b, c** and **20b, c—22b, c** by means of the same sequence as described for **17a**.

2-(Propyl)pentyl 3,4-Bisphospho-β-D-galactopyranoside Tetrasodium Salt (**17b**): IR (KBr) cm^{-1}: 3424, 1075 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.93 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH_2), 1.36 (8H, s, 4CH_2), 1.71 (1H, m, CH of fatty alkyl), 3.62 (1H, dd, J_{vic}=5.9, J_{gem}=9.9 Hz, H-1 of fatty alkyl), 3.74 (1H, dd, $J_{2,3}$=10.4 Hz, H-2), 3.78 (1H, m, H-6), 3.83 (1H, m, H-5), 3.88 (2H, m, H-6′ and H-1′ of fatty alkyl), 4.20 (1H, ddd, $J_{3,4}$=3.5, $J_{3,P}$=8.9 Hz, H-3), 4.53 (2H, d, $J_{1,2}$=7.9 Hz, H-1), 4.69 (2H, dd, $J_{4,P}$=10.9 Hz, H-4). ^{13}C-NMR (D_2O) δ: 15.7 (CH_3), 21.2 and 34.6 (CH_2), 38.9 (CH), 61.5 (C-6), 72.0 (C-2), 72.6 (C-4), 75.8 (OCH_2), 75.8 (C-5), 78.1 (C-3), 105.0 (C-1). *Anal.* Calcd for $C_{14}H_{26}Na_4O_{12}P_2$: C, 31.12; H, 4.85. Found: C, 30.87; H, 4.78.

2-(Tetradecyl)hexadecyl 3,4-Bisphospho-β-D-galactopyranoside Tetrasodium Salt (**17c**): IR (KBr) cm^{-1}: 3444, 2923, 1075 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.95 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH_2), 1.33 (52H, s, 26CH_2), 1.83 (1H, m, CH of fatty alkyl), 3.48 (1H, m, H-1 of fatty alkyl), 3.63 (1H, m, H-5), 3.73 (1H, m, H-2), 3.78 (2H, m, H-6 and H-1′ of fatty alkyl), 3.88 (1H, m, H-6′), 4.23 (1H, m, H-3), 4.40 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.73 (1H, m, H-4). ^{13}C-NMR (D_2O) δ: 15.8 (CH_3), 24.6, 28.0, 28.1, 31.2, 31.4, 31.5, 31.7 and 34.1 (CH_2), 39.8 (CH), 61.1 (C-6), 72.1 (C-2), 72.3 (C-4), 75.7 (OCH_2), 75.9 (C-5), 78.0 (C-3), 105.6 (C-1). *Anal.* Calcd for $C_{36}H_{70}Na_4O_{12}P_2$: C, 50.94; H, 8.31. Found: C, 50.87; H, 8.29.

2-(Propyl)pentyl *O*-(3,4-Bisphospho-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Tetrasodium Salt (**23b**): *Rf* 0.48 (MeOH–H_2O 1:2). IR (KBr) cm^{-1}: 3424, 1090 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.90 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH_2), 1.32 (8H, s, 4CH_2), 1.66 (1H, m, CH of fatty alkyl), 3.29 (1H, dd, $J_{2,3}$=8.9 Hz, H-2a), 3.45 (1H, m, H-5a), 3.51 (1H, m, H-1 of fatty alkyl), 3.59 (1H, m, H-3a), 3.62 (1H, m, H-4a), 3.72 (1H, dd, $J_{5,6}$=2.0, J_{gem}=7.9 Hz, H-6b), 3.78 (2H, m, H-2b and H-5b), 3.82 (2H, m, H-6′b and H-1′ of fatty alkyl), 3.87 (1H, dd, $J_{5,6}$=4.5, J_{gem}=12.4 Hz, H-6a), 3.94 (1H, dd, $J_{5,6'}$=2.5 Hz, H-6′a), 4.15 (1H, dt, $J_{2,3}$=$J_{3,P}$=8.9, $J_{3,4}$=3.0 Hz, H-3b), 4.36 (1H, d, $J_{1,2}$=7.9 Hz, H-1a), 4.53 (1H, d, $J_{1,2}$=7.9 Hz, H-1b), 4.57 (1H, dd, $J_{4,P}$=10.4 Hz, H-4b). ^{13}C-NMR (D_2O) δ: 14.6 (CH_3), 20.4 and 34.0 (CH_2), 38.3 (CH), 60.5 (C-6b), 61.4 (C-6a), 71.7 (C-4b), 71.8 (C-2b), 74.2 (C-2a), 74.5 (OCH_2), 75.5 (C-5b), 75.7 (C-3a), 75.9 (C-5a), 76.2 (C-3b), 80.2 (C-4a), 104.0 (C-1a), 104.4 (C-1b). *Anal.* Calcd for $C_{20}H_{36}Na_4O_{17}P_2$: C, 34.20; H, 5.17. Found: C, 33.99; H, 5.20.

2-(Tetradecyl)hexadecyl *O*-(3,4-Bisphospho-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside Tetrasodium Salt (**23c**): IR (KBr) cm^{-1}: 3456, 2924, 1082 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.87 (6H, t, J_{Me,CH_2}=6.4 Hz, 2MeCH_2), 1.27 (52H, s, 26CH_2), 1.82 (1H, m, CH of fatty alkyl), 3.26 (1H, t, $J_{2,3}$=7.9 Hz, H-2a), 3.43 (2H, m, H-5a and H-1 of fatty alkyl), 3.56 (1H, m, H-3a), 3.61 (1H, m, H-4a), 3.65 (1H, m, H-2b), 3.69 (2H, m, H-5b and H-6b), 3.79 (4H, m, H-6a, H-6′a, H-6′b and H-1′ of fatty alkyl), 4.15 (1H, m, H-3b), 4.28 (1H, m, H-1a), 4.54

(1H, m, H-1b), 4.62 (1H, m, H-4b). ^{13}C-NMR (D_2O) δ: 14.5 (CH_3), 23.3, 27.1, 30.2, 30.4, 30.5, 30.6, 31.1 and 32.7 (CH_2), 38.7 (CH), 60.8 (C-6b), 61.4 (C-6a), 71.3 (C-2b), 71.7 (C-4b), 74.0 (C-2a), 74.3 (OCH_2), 75.2 (C-5b), 75.5 (C-3a), 75.6 (C-5a), 76.4 (C-3b), 79.9 (C-4a), 103.9 (C-1a), 104.0 (C-1b). *Anal.* Calcd for $C_{40}H_{76}Na_4O_{17}P_2$: C, 48.88; H, 7.79. Found: C, 48.59; H, 7.71.

2-(Methyl)propyl 2,3,4,6-Tetra-*O*-dibenzylphosphono-β-D-galactopyranoside (18a) 1*H*-Tetrazole (356 mg, 5.08 mmol) and dibenzyloxy-(diisopropylamino)phosphine (1.17 g, 3.39 mmol) were added to a solution of compound **5a** (100 mg, 0.423 mmol) in CH_2Cl_2 (2.5 ml) and acetonitrile (2.5 ml) at room temperature with stirring. After 12 h, water (10 ml), $RuCl_3 \cdot 3H_2O$ (2 mg, 0.01 mmol), and $NaIO_4$ (724 mg, 3.39 mmol) were added and the mixture was vigorously stirred for 1 h. Then it 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 *n*-hexane–EtOAc 2 : 1) on silica gel (40 g) to give **8e** (361 mg, 67%) as a syrup. ^{1}H-NMR (C_6D_6) δ: 0.96 and 0.97 (6H, 2d, $J_{Me,CH}$ = 6.4 Hz, 2MeCH), 1.96 (1H, m, CH of fatty alkyl), 3.25 (1H, dd, J_{vic} = 6.4, J_{gem} = 8.8 Hz, H-1 of fatty alkyl), 3.47 (1H, m, H-5), 3.72 (1H, dd, J_{vic} = 7.4 Hz, H-1′ of fatty alkyl), 4.37 (1H, d, $J_{1,2}$ = 7.8Hz, H-1), 4.59 (2H, m, H-6 and H-6′), 4.86 (1H, m, H-2), 5.16—5.63 (18H, m, H-3, H-4 and 8PhCH$_2$), 7.03—7.62 (40H, m, 8Ph).

2-(Methyl)propyl 2,3,4,6-Tetrakisphospho-β-D-galactopyranoside Octasodium Salt (19a) Compound **18a** (88.6 mg, 69.3 μmol) was dissolved in MeOH (2 ml) and buffered aqueous AcOH–NaOAc (1 ml, pH 5, 0.5 M), and the mixture was treated with 10% Pd–C (50 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 **19a** (43.8 mg, 86%) as an amorphous mass. IR (KBr) cm^{-1}: 3425, 1107 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.97 and 0.99 (6H, 2d, $J_{Me,CH}$ = 6.7 Hz, 2MeCH), 1.97 (1H, m, CH of fatty alkyl), 3.55 (1H, dd, J_{vic} = 6.7, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.75 (1H, dd, J_{vic} = 6.7 Hz, H-1′ of fatty alkyl), 3.93 (1H, m, H-5), 3.96 (1H, m, H-6), 4.03 (1H, m, H-6′), 4.23 (1H, m, H-3), 4.27 (1H, m, H-2), 4.61 (1H, d, $J_{1,2}$ = 6.9 Hz, H-1), 4.63 (1H, dd, $J_{3,4}$ = 2.0, $J_{4,P}$ = 8.9 Hz, H-4). ^{13}C-NMR (D_2O) δ: 20.7 (CH_3), 29.8 (CH), 65.8 (C-6), 75.0 (C-4), 76.3 (C-5), 76.7 (C-2), 77.6 (C-3), 79.3 (OCH_2), 104.4 (C-1). *Anal.* Calcd for $C_{10}H_{16}Na_8O_{18}P_4$: C, 16.41; H, 2.20. Found: C, 16.22; H, 2.01.

Other Alkyl 2,3,4,6-Tetraphosphorylated β-D-Galactopyranosides (19b, c) Compounds **19b** and **19c** were prepared *via* **18b** and **18c** by means of the same sequence as described for **19a**.

2-(Propyl)pentyl 2,3,4,6-Tetrakisphospho-β-D-galactopyranoside Octasodium salt (**19b**): IR (KBr) cm^{-1}: 3424, 1108 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.93 (6H, t, J_{Me,CH_2} = 6.4 Hz, 2MeCH$_2$), 1.38 (8H, s, 4CH$_2$), 1.76 (1H, m, CH of fatty alkyl), 3.64 (1H, dd, J_{vic} = 6.4, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.89 (1H, dd, J_{vic} = 6.4 Hz, H-1′ of fatty alkyl), 3.90 (1H, m, H-5), 4.01 (1H, m, H-6), 4.03 (1H, m, H-6′), 4.21 (2H, ddd, $J_{2,3}$ = 6.9, $J_{3,4}$ = 2.0, $J_{3,P}$ = 11.9 Hz, H-3), 4.26 (1H, dt, $J_{2,3}$ = $J_{2,P}$ = 9.9 Hz, H-2), 4.57 (2H, dd, $J_{4,P}$ = 8.9 Hz, H-4), 4.58 (1H, d, $J_{1,2}$ = 7.4Hz, H-1). ^{13}C-NMR (D_2O) δ: 15.9 (CH_3), 21.2 and 34.4 (CH_2), 39.0 (CH), 66.2 (C-6), 74.9 (C-4), 76.2 (OCH_2), 76.7 (C-2), 76.8 (C-5), 77.8 (C-3), 104.8 (C-1). *Anal.* Calcd for $C_{14}H_{24}Na_8O_{18}P_4$: C, 21.34; H, 3.07. Found: C, 21.23; H, 2.99.

2-(Tetradecyl)hexadecyl 2,3,4,6-Tetrakisphospho-β-D-galactopyranoside Octasodium Salt (**19c**): IR (KBr) cm^{-1}: 3418, 2924, 1074 (P-O-C). ^{1}H-NMR (D_2O) δ: 0.94 (6H, t, J_{Me,CH_2} = 6.4 Hz, 2MeCH$_2$), 1.36 (52H, s, 26CH$_2$), 1.73 (1H, m, CH of fatty alkyl), 3.59 (1H, m, H-1 of fatty alkyl), 3.89 (1H, m, H-1′ of fatty alkyl), 3.94 (2H, m, H-5 and H-6), 4.05 (1H, m, H-6′), 4.26 (1H, m, H-3), 4.28 (1H, m, H-2), 4.62 (1H, d, $J_{1,2}$ = 7.9Hz, H-1), 4.64 (1H, m, H-4). ^{13}C-NMR (D_2O) δ: 15.9 (CH_3), 24.7, 28.0, 31.4, 31.6, 31.9, 32.2, 32.4 and 34.0 (CH_2), 39.6 (CH), 64.6 (C-6), 74.6 (C-4), 75.4 (C-5), 76.7 (C-2), 76.9 (OCH_2), 77.6 (C-3), 104.7 (C-1). *Anal.* Calcd for $C_{34}H_{64}Na_8O_{18}P_4$: C, 38.21; H, 6.04. Found: C, 38.02; H, 6.03.

Biological Assays *In Vitro* Inhibitory Activity: The activity of the compounds *in vitro* was measured in adhesion assays, in terms of the inhibition of binding of promyelocytic leukemia HL-60 cells to immobilized recombinant selectin-IgG fusion proteins.[7c,d] 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 of phosphate buffered saline (PBS)(+) and incubated overnight at 4 °C. The excess coating solution was removed by aspiration, and non-specific binding sites were blocked by a 1 h incubation with PBS(+) containing 1% bovine serum albumin (BSA) (w/v) at room temperature. After aspiration of the blocking solution, 100 μl of the test compound was dissolved in the culture medium (RPMI 1640 Medium "Nissui"), then 100 μl of HL-60 cells (10^6 cell/ml suspended in the binding buffer) was 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 carefully sealed with acetate sealing tape, so as to avoid trapping any 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 then aspirating the binding buffer. The amount of bound cells was quantified by the WST-1 assay method (Dojin Chemicals, Japan).[18] Inhibition of L- or E-selectin binding was carried out as described above using immobilized L- (100 ng) or E-selectin-IgG (40 ng).

Molecular Modeling Coordinates for the C-type lectin-like domain of the E- and L-selectins were obtained from the Brookhaven Protein Data Bank (1ESL and 1KJB, respectively).[19] The P-selectin coordinates were generated on the basis of the E-selectin crystal structure and homology, because P-selectin exhibits better structural homology with E-selectin than with rat mannose binding protein (MBP).[20] In order to determine the binding site of the fatty-alkyl residue on each selectin, a docking study of ligand-protein was performed using over 8000 conformations of ligands and fixed protein coordinates by DOCK 3.0.[21] From the docking results, the specific binding site of the fatty-alkyl residue for the L- and P-selectins, but not the E-selectin, was found near the carbohydrate recognition site on the selectin. On the basis of the coordinates of the fatty-alkyl residue and 3,6-disulfated galactose calculated by the docking study, the compound **7c**–L-selectin complex model was constructed. After minimization of compound **7c**, a search procedure for optimizing the conformation of the ligand was performed. First, the 3,6-disulfated galactose residue of the ligand was optimized by a torsional random sampling search, followed by minimization. During this calculation, the protein and fatty-alkyl residue of the ligand were fixed. Next, the fatty-alkyl residue of the ligand was optimized by a high-temperature molecular dynamics (MD) calculation. During the MD calculation, the fatty-alkyl residue of the ligand and the protein side-chain close to it were allowed to move. Finally, the overall ligand was minimized. In the same way, compound **7c** complex models were constructed for the E- and P-selectins. A distance-dependent dielectric constant was used, $\varepsilon = 4r$. Molecular modeling was performed using the software package QUANTA/CHARMm, and molecular graphics were performed using the software Insight II, both from Molecular Simulation Inc., running on Silicon Graphics Indigo 2 workstation (R4400 Extreme).

Acknowledgment The authors wish to thank Dr. B. Seed for providing the plasmids of the three selectins.

References and Notes

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