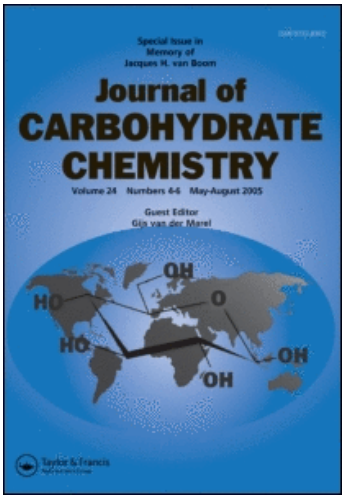

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthetic Studies on Selectin Ligands/Inhibitors: Synthesis and Inhibitory Activity of 2-*O*-Fucosyl Sulfatides Containing 2-Branched Fatty Alkyl Residues in Place of Ceramide

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To cite this Article Ikami, Takao , Kakigami, Takuji , Baba, Kunihisa , Hamajima, Hitoshi , Jomori, Takahito , Usui, Toshinao , Suzuki, Yasuo , Tanaka, Harunari , Ishida, Hideharu , Hasegawa, Akira and Kiso, Makoto(1998) 'Synthetic Studies on Selectin Ligands/Inhibitors: Synthesis and Inhibitory Activity of 2-*O*-Fucosyl Sulfatides Containing 2-Branched Fatty Alkyl Residues in Place of Ceramide', Journal of Carbohydrate Chemistry, 17: 3, 453 — 470
To link to this Article: DOI: 10.1080/07328309808002905
URL: http://dx.doi.org/10.1080/07328309808002905

J. CARBOHYDRATE CHEMISTRY, 17(3), 453-470 (1998)

SYNTHETIC STUDIES ON SELECTIN LIGANDS/INHIBITORS: SYNTHESIS AND INHIBITORY ACTIVITY OF 2-*O*-FUCOSYL SULFATIDES CONTAINING 2-BRANCHED FATTY ALKYL RESIDUES IN PLACE OF CERAMIDE[1]

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Received August 5, 1997 - Final Form December 8, 1997

ABSTRACT

To investigate the biological selectin-ligand interactions, four sulfated 2-*O*-α-L-fucopyranosyl β-D-galactopyranosides containing 2-branched fatty-alkyl residues in place of ceramide have been systematically synthesized. The target glycolipids were assayed for their ability to block the adhesion of HL-60 cells to immobilized P-, L- and E-selectin. Among them, 2- *O*-α-L-fucopyranosyl sulfatide, which is anchored with 2-(tetradecyl) hexadecyl residue showed significantly more potency of the blocking adhesion to P- and L-selectins.

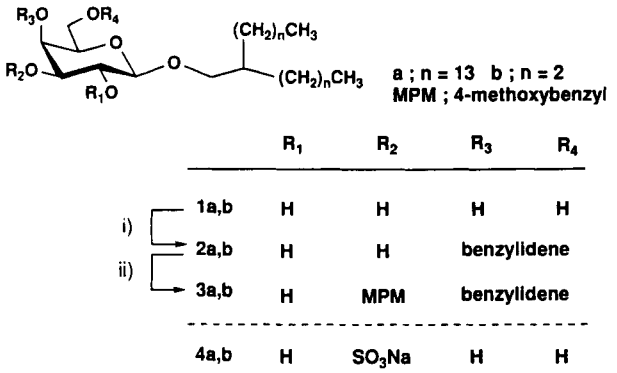
INTRODUCTION

The selectins are a family of adhesion receptors implicated in the initial interaction between leukocytes and vascular endothelium leading to an inflammatory response.[2,3] 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.[4-7] There is now general agreement that all three selectins can efficiently recognize sialyl Lewis X [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc](sLe^x) and sialyl Lewis A [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc](sLe^a),[8,9] and therefore, much attention has focused on sLe^x by many laboratories.[9] On the other hand, sulfatide, one of the major acidic glycosphingolipids in mammalian tissues, is found to be a good ligand for L- and P-selectin[10-12] and shows highly protective effects against selectin-dependent inflammatory lung injury.[13] In particular, it is found that the non-reducing terminal SO_3H-Galβ1- structure is essential for the binding to L-selectin.[12] Although many mimetics[14,15] as well as analogues[16-18] of sLe^x have been designed and synthesized as potent anti-inflammatory agents, few attempts have been made to develop a selectin blocker derived from sulfatide. It is also reported that the fucose moiety is an essential component for sLe^x to be recognized by selectins[19] and that fucoidan, the sulfated fucose polymer, binds to L-selectin and inhibits L-selectin mediated lymphocyte adhesion to the lymphnade endothelial venules.[20]

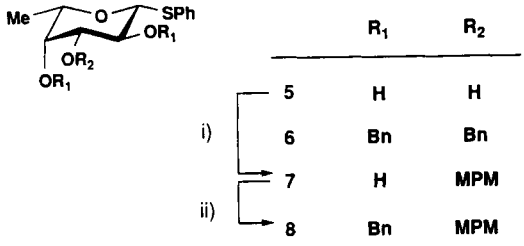
In view of these facts, as a part of our continuing efforts on the chemical modification of sulfatide,[21] and for the development of the selectin inhibitors,[22,23] we describe herein the systematic synthesis and in vitro activity of novel sulfated 2-*O*-α-L-fucopyranosyl β-D-galactopyranosides containing 2-branched fatty alkyl residues in place of ceramide.

RESULTS AND DISCUSSION

Synthesis. For the synthesis of the target glycolipids, we selected 2-(tetradecyl)hexadecyl β-D-galactopyranoside (**1a**)[22,24] and 2-(propyl)pentyl β-D-galactopyranoside (**1b**) as the starting materials. Treatment of **1a** and **1b** with

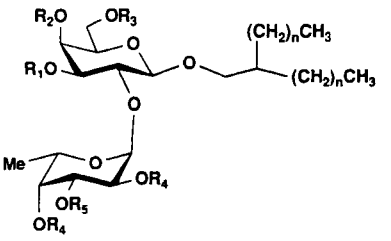


Scheme 1. i) benzaldehyde dimethyl acetal, H_2SO_4, THF (**1a**→**2a**, 77%; **1b**→**2b**, 76%).
ii) Bu_2SnO, 4-methoxybenzyl chloride, Bu_4NI, THF (**2a**→**3a**, 74%; **2b**→**3b**, 74%).



Scheme 2. i) Bu_2SnO, MeOH, 4-methoxybenzyl chloride, Bu_4NI, THF (**5**→**7**, 59%). ii) benzyl bromide, NaH, DMF (**7**→**8**, 65%).

benzaldehyde dimethyl acetal in THF containing H_2SO_4 afforded the 4,6-*O*-benzylidene derivatives **2a** and **2b** in 77 and 76% yield, respectively. Regioselective 3-*O*-4-methoxybenzylation of **2a** and **2b** was achieved by treatment of the corresponding stannylene intermediates with 4-methoxybenzyl chloride, according to a published procedure,[25] to give **3a** and **3b**, exclusively in 74% yield. The H-3 proton in the ^1H NMR spectra of **3a** and **3b** appeared at δ 3.47 ($J_{2,3}$ = 9.9, $J_{3,4}$ = 3.5 Hz in **3a**) and δ 3.50 ($J_{2,3}$ = 9.9, $J_{3,4}$ = 3.4 Hz in **3b**) indicating the 4-methoxybenzylated position to be *O*-3. The glycosylation of **3a** and **3b** with **6**[26] or **8** derived from **5**[26]



		R_1	R_2	R_3	R_4	R_5
i)	9a,b	MPM	benzylidene		Bn	Bn
ii)	10a,b	H	benzylidene		Bn	Bn
iii)	11a,b	SO_3Na	benzylidene		Bn	Bn
	12a,b	SO_3Na	H	H	H	H
i)	13a,b	MPM	benzylidene		Bn	MPM
ii)	14a,b	H	benzylidene		Bn	H
iii)	15a,b	SO_3Na	benzylidene		Bn	SO_3Na
	16a,b	SO_3Na	H	H	H	SO_3Na

Scheme 3. i) DDQ, CH_2Cl_2-H_2O (**9a**→**10a**, 46%; **9b**→**10b**, 40%; **13a**→**14a**, 41%; **13b**→**14b**, 57%). ii) sulfur trioxide-pyridine complex, DMF (**10a**→**11a**, 93%; **10b**→**11b**, 90%; **14a**→**15a**, 91%; **14b**→**15b**, 95%). iii) Pd/C, MeOH-THF (**11a**→**12a**, 67%; **11b**→**12b**, 73%; **15a**→**16a**, 63%; **15b**→**16b**, 82%).

was performed in the presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH)[27,28] and molecular sieves 4A (MS-4A) in toluene for 1 h at -20 ℃ to afford the corresponding disaccharides **9a** (82%), **9b** (79%), **13a** (78%) and **13b** (71%), respectively. Significant signals in the ^{1}H NMR spectra of **9a,b** and **13a,b** were one-proton doublets at δ 5.68 ($J_{1,2}$ = 2.0 Hz, H-1 of Fuc in **9a**), 5.68 ($J_{1,2}$ = 2.4 Hz, H-1 of Fuc in **9b**), 5.68 ($J_{1,2}$ = 1.8 Hz, H-1 of Fuc in **13a**) and 5.67 ($J_{1,2}$ = 2.0 Hz, H-1 of Fuc in **13b**), showing characteristics of the α-L-fucopyranosyl unit. Selective removal of the 4-methoxybenzyl groups at *O*-3 in **9a,b** and **13a,b** in dichloromethane-water solution in the presence of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[29] for 1-3 h at room temperature gave **10a,b** and **14a,b**.

Table 1. Inhibition activity of target compounds

	% inhibition at 0.3 mM		
	P-selectin	L-selectin	E-selectin
sLe^x	3	0	0
4a	23	27	0
4b	3	0	0
12a	100	99	0
12b	45	11	2
16a	76	69	8
16b	16	33	2

The sulfation of compounds **10a,b** and **14a,b** with a sulfur trioxide-pyridine complex in DMF for 2-4 h at room temperature afforded the corresponding sulfates **11a** (93%), **11b** (90%), **15a** (91%) and **15b** (95%), respectively. Reductive removal of the benzylidene and benzyl groups in **11a,b** and **15a,b** and sequential treatment by a cation exchange resin afforded the desired sulfated 2-*O*-α-L-fucopyranosyl β-D-galactopyranosides **12a** (67%), **12b** (73%), **16a** (63%) and **16b** (82%), respectively. FABMS spectra (negative ion mode) showed the base peaks at *m/z* (M-Na)$^-$ 825.6 for **12a**, 517.3 for **12b**, 927.5 for **16a** and 619.1 for **16b**.

Biological activity. The activity of the target glycolipids in vitro was measured in adhesion assays as the inhibition of the binding of HL-60 cells (sLe^x expressing) to recombinant human selectin-IgG fusion proteins on plates.[16,17] Several of the 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 results demonstrated that compounds **12a** and **16a** were each significantly more potent than sLe^x and the corresponding non-fucosylated β-D-galactopyranosides **4a** in blocking adhesion to 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. The same trend was obtained in our previous work for the development of the selectin inhibitors.[23] These data indicated that the fucose

moiety and the attachment of a branched long fatty alkyl residue to β-D-galactopyranoside were important for binding to the P- and L-selectins.

EXPERIMENTAL

General methods. Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. NMR spectra were recorded on a Jeol JNM-GSX 270 spectrometer (270 MHz for ^{1}H and 68 MHz for ^{13}C). Chemical shifts were expressed in parts per million downfield from TMS. FAB-MS spectra were 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 with 6N H_2SO_4, then heating for about 2 min at 300 ℃. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the specified solvent systems. Concentrations were conducted *in vacuo*.

2-(Tetradecyl)hexadecyl 4,6-*O*-Benzylidene-β-D-galactopyranoside (2a) and 2-(Tetradecyl)hexadecyl 4,6-*O*-Benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (3a). 2-(Tetradecyl)hexadecyl β-D-galactopyranoside[22,23] (**1a**; 39.2 g, 65.2 mmol) was dissolved in THF (600 mL), and benzylaldehyde dimethyl acetal (19.9 g, 131mmol) and H_2SO_4 (2 mL) were added. The mixture was stirred for 20 h at room temperature under Ar gas after which Na_2CO_3 was added to neutralize the solution. The solvents were evaporated and the residue was chromatographed (3:2 *n*-hexane-EtOAc) on silica gel (400 g) to give **2a** (34.6 g, 77%) as an amorphous mass: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.25 (s, 52H, 26×CH_2), 1.64 (m, 1H, CH of fatty alkyl), 2.38 (d, 1H, $J_{2\text{-}H,2\text{-}OH}$ = 1.5 Hz, OH-2), 2.50 (d, 1H, $J_{3\text{-}H,3\text{-}OH}$ = 7.8 Hz, OH-3), 3.33 (dd, 1H, J_{vic} = 6.3, J_{gem} = 9.3 Hz, H-1 of fatty alkyl), 3.47 (dd, 1H, $J_{5,6}$ = 2.0, $J_{5,6'}$ = 1.0 Hz, H-5 of Gal), 3.70 (dt, 1H, $J_{3,4}$ = 3.9 Hz, H-3), 3.76 (ddd, 1H, $J_{2,3}$ = 3.9 Hz, H-2), 3.88 (dd, 1H, J_{vic} = 5.4 Hz, H-1' of fatty alkyl), 4.09 (dd, 1H, J_{gem} = 12.2 Hz, H-6 of Gal), 4.21 (d, 1H, H-4), 4.24 (d, 1H,

$J_{1,2}$ = 7.8Hz, H-1), 4.34 (dd, 1H, H-6' of Gal), 5.56 (s, 1H, PhC*H*), 7.26-7.53 (m, 5H, Ph).

Anal. Calcd for $C_{43}H_{76}O_6$: C, 74.95; H, 11.12. Found: C, 74.89; H, 11.20.

Compound **2a** (34.5 g, 50.1 mmol) and dibutyltin oxide (13.8 g, 50.1 mmol) were stirred in refluxing toluene (500 mL) for 24 h with continuous removal of water, and concentrated. To a solution of stannyl complex in THF (500 mL) were added tetra *n*-butyl ammonium iodide (23.0 g, 62.3 mmol) and 4-methoxybenzyl chloride (11.8 g, 75.1 mmol) and the mixture was stirred for 24 h at reflux, then concentrated. The residue was chromatographed (3:1 *n*-hexane-EtOAc) on silica gel (400 g) to give **3a** (30.0 g, 74%) as an amorphous mass: R_f 0.72 (10:1 $CHCl_3$-MeOH); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.25 (s, 52H, 26×CH_2), 1.63 (m, 1H, CH of fatty alkyl), 2.33 (s, 1H, OH-2), 3.33 (m, 2H, H-1 of fatty alkyl and H-5), 3.47 (dd, 1H, $J_{2,3}$ = 9.9, $J_{3,4}$ = 3.5 Hz, H-3), 3.79 (s, 3H, OCH_3), 3.85 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.4 Hz, H-1' of fatty alkyl), 3.99 (m, 2H, H-2 and H-6), 4.09 (d, 1H, H-4), 4.23 (d, 1H, $J_{1,2}$ = 7.9Hz, H-1), 4.29 (dd, 1H, $J_{5,6'}$ = 1.0, J_{gem} = 11.4 Hz, H-6' of Gal), 4.70 (s, 2H, MeOPhC*H*$_2$), 5.45 (s, 1H, PhC*H*), 6.79-7.54 (m, 9H, aromatic). MS (FAB positive) *m/z* 807.7[100% $(M-H)^+$].

Anal. Calcd for $C_{51}H_{84}O_7$: C, 75.70; H, 10.46. Found: C, 75.93; H, 10.51.

2-(Propyl)pentyl 4,6-*O*-Benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (3b). Compound **3b** was prepared via **1b** and **2b** by the same sequence as described for **3a**: R_f 0.86 (20:1 $CHCl_3$-MeOH); ^1H NMR ($CDCl_3$) δ 0.91 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.34 (m, 8H, 4×CH_2), 1.64 (m, 1H, CH of fatty alkyl), 3.38 (m, 2H, H-1 of fatty alkyl and H-5), 3.50 (dd, 1H, $J_{2,3}$ = 9.9, $J_{3,4}$ = 3.4 Hz, H-3), 3.76 (s, 3H, OCH_3), 3.81 (dd, 1H, J_{vic} = 5.9, J_{gem} = 9.4 Hz, H-1' of fatty alkyl), 4.00 (m, 2H, H-2 and H-6), 4.09 (d, 1H, H-4), 4.26 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.29 (dd, 1H, $J_{5,6'}$ = 1.0, J_{gem} = 11.8 Hz, H-6' of Gal), 4.65 (s, 2H, MeOPhC*H*$_2$), 5.50 (s, 1H, PhC*H*), 6.82-7.52 (m, 9H, aromatic). MS (FAB positive) *m/z* 499.3 [100% $(M-H)^+$].

Anal. Calcd for $C_{29}H_{40}O_7$: C, 69.58; H, 8.05. Found: C, 69.55; H, 8.12.

Phenyl 3-*O*-(4-Methoxybenzyl)-1-thio-β-L-fucopyranoside (7). Phenyl 1-thio-β-L-fucopyranoside[26] (**5**; 10.0 g, 39.0 mmol) and dibutyltin oxide (9.71 g, 39.0

mmol) were stirred in refluxing dry MeOH (220 mL) for 24 h, and concentrated. To a solution of stannyl complex in THF (120 mL) were added tetra *n*-butyl ammonium iodide (21.6 g, 58.5 mmol) and 4-methoxybenzyl chloride (9.16 g, 58.5 mmol) and the mixture was stirred for 24 h at reflux, then concentrated. The residue was chromatographed (2:1 *n*-hexane-EtOAc) on silica gel (200 g) to give **7** (8.64 g, 59%) as a syrup: R_f 0.53 (10:1 $CHCl_3$-MeOH); ^1H NMR (CD_3OD) δ 1.25 (d, 3H, $J_{5,6}$ = 6.8 Hz, CH_3 of Fuc), 3.38 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 3.58 (q, 1H, H-5), 3.71 (t, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 3.76 (s, 3H, OCH_3), 3.78 (d, 1H, H-4), 4.56 (d, 1H, $J_{1,2}$ = 9.4 Hz, H-1), 4.56 and 4.64 (2d, 2H, J_{gem} = 9.4 Hz, MeOPhCH_2), 6.84-7.53 (m, 9H, aromatic). MS (FAB positive) *m/z* 377.1[100% $(M+H)^+$].

Anal. Calcd for $C_{20}H_{24}O_5S$: C, 63.81; H, 6.43. Found: C, 63.66; H, 6.41.

Phenyl 2,4-Di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (8). To a solution of **7** (2.62 g, 6.96 mmol) in DMF (30 mL) was added NaH (1.11 g, 27.8 mmol), the mixture was stirred for 30 min at 0 ℃, and benzyl bromide (4.76 g, 27.8 mmol) was added. After 2 h, excess NaH was destroyed by addition of MeOH (5 mL), the mixture was partitioned between $CHCl_3$ and water, and the organic phase was washed with 4 portions of water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (5:1 *n*-hexane-EtOAc) on silica gel (200 g) to give **8** (2.51 g, 65%) as an amorphous mass: R_f 0.73 (2:1 *n*-hexane-AcOEt); ^1H NMR (CD_3OD) δ 1.24 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 3.65 (m, 2H, H-3 and H-5), 3.78 (m, 4H, H-4 and OCH_3), 3.80 (t, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 4.65 (d, 1H, $J_{1,2}$ = 9.4 Hz, H-1), 4.60-4.96 (m, 6H, MeOPhCH_2 and 2×PhCH_2), 6.83-7.55 (m, 19H, aromatic). MS (FAB positive) *m/z* 557.3[100% $(M+H)^+$].

Anal. Calcd for $C_{34}H_{56}O_5S$: C, 73.35; H, 6.52. Found: C, 73.51; H, 6.53.

2-(Tetradecyl)hexadecyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (9a). To a solution of **3a** (2.00 g, 2.47 mmol) and **6** (2.60 g, 4.94 mmol) in dry toluene (30 mL) was added powdered MS-4A (10 g), and the mixture was stirred for 24 h at room temperature, then cooled to -20 ℃. *N*-Iodosuccinimide (NIS, 2.22 g, 9.89 mmol) and trifluoromethanesulfonic acid (TfOH, 87.5 μL, 0.989 mmol) were added to the mixture, and this was stirred for 1 h at -20 ℃ and neutralized with Et_3N. After

dilution with toluene (100 mL), the solids were collected and washed with toluene, and the combined filtrate and washings were washed with water, dried (Na_2SO_4) and concentrated. The residue was chromatographed (4:1 *n*-hexane-EtOAc) on silica gel (120 g) to give **9a** (2.50 g, 82%) as an amorphous mass: R_f 0.64 (3:1 *n*-hexane-EtOAc); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.14 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.25 (s, 52H, 26×CH_2), 1.50 (m, 1H, CH of fatty alkyl), 3.25 (m, 1H, H-1 of fatty alkyl), 3.28 (m, 1H, H-5 of Gal), 3.67 (m, 1H, H-3 of Fuc), 3.72 (s, 3H, OCH_3), 3.77 (m, 1H, H-3 of Gal), 3.82 (m, 1H, H-1' of fatty alkyl), 3.98 (m, 1H, H-2 of Fuc), 4.00 (m, 1H, H-4 of Fuc), 4.10 (m, 2H, H-6 of Gal), 4.10 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 of Gal), 4.21 (dd, 1H, $J_{2,3}$ = 8.9 Hz, H-2 of Gal), 4.47 (q, 1H, H-5 of Fuc), 4.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 of Gal), 4.52-4.96 (m, 8H, MeOPhC*H*$_2$ and 3×PhC*H*$_2$), 5.38 (s, 1H, PhC*H*), 5.68 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1 of Fuc), 6.74-7.51 (m, 24H, aromatic). MS (FAB positive) *m/z* 807.7[100% (M-Fuc unit)$^+$], 1223.8[25% (M-H)$^+$].

Anal. Calcd for $C_{78}H_{112}O_{11}$: C, 76.43; H, 9.21. Found: C, 76.69; H, 9.38.

2-(Propyl)pentyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (9b). To a solution of **3b** (2.50 g, 4.99 mmol) and **6** (5.26 g, 9.99 mmol) in dry toluene (30 mL) was added powdered MS-4A (10 g), and the mixture was stirred for 24 h at room temperature, then cooled to -20 ℃. NIS (4.49 g, 20.0 mmol) and TfOH (177 μL, 2.00 mmol) were added to the mixture, and this was stirred for 1 h at -20 ℃. Workup was as described for **9a**. The resulting residue was chromatographed (5:2 *n*-hexane-EtOAc) on silica gel (200 g) to give **9b** (3.60 g, 79%) as an amorphous mass: R_f 0.48 (2:1 *n*-hexane-EtOAc); ^1H NMR ($CDCl_3$) δ 0.85 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.10 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.24 (m, 8H, 4×CH_2), 1.50 (m, 1H, CH of fatty alkyl), 3.25 (dd, 1H, J_{vic} = 5.5, J_{gem} = 9.5 Hz, H-1 of fatty alkyl), 3.32 (m, 1H, H-5 of Gal), 3.76 (s, 3H, OCH_3), 4.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 of Gal), 4.56-4.96 (m, 8H, MeOPhC*H*$_2$ and 3×PhC*H*$_2$), 5.41 (s, 1H, PhC*H*), 5.68 (d, 1H, $J_{1,2}$ = 2.4 Hz, H-1 of Fuc), 6.75-7.54 (m, 24H, aromatic). MS (FAB positive) *m/z* 499.3[100% (M-Fuc unit)$^+$], 915.5[13% (M-H)$^+$].

Anal. Calcd for $C_{56}H_{68}O_{11}$: C, 73.34; H, 7.47. Found: C, 73.45; H, 7.53.

2-(Tetradecyl)hexadecyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside (10a). To a solution of **9a** (1.50 g, 1.22 mmol) in CH_2Cl_2 (15 mL) and water (1 mL) was added 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 306 mg, 1.35 mmol), and the mixture was stirred for 2 h at room temperature, then concentrated. The residue was chromatographed (3:1 *n*-hexane-EtOAc) on silica gel (40 g) to give **10a** (628 mg, 46%) as an amorphous mass: R_f 0.50 (3:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.12 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.26 (s, 52H, 26×CH_2), 1.54 (m, 1H, CH of fatty alkyl), 3.30 (m, 1H, H-1 of fatty alkyl), 3.41 (m, 1H, H-5 of Gal), 4.60-4.99 (m, 6H, 3×PhC*H*$_2$), 5.39 (d, 1H, $J_{1,2}$ = 2.4 Hz, H-1 of Fuc), 5.51 (s, 1H, PhC*H*), 7.21-7.55 (m, 20H, Ph). MS (FAB positive) *m/z* 1103.8[100% $(M-H)^+$].

Anal. Calcd for $C_{70}H_{104}O_{10}$: C, 76.05; H, 9.48. Found: C, 76.12; H, 9.41.

2-(Propyl)pentyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside (10b). A solution of **9b** (540 mg, 0.589 mmol) in CH_2Cl_2 (5 mL) and water (2.5 mL) was treated with DDQ (134 mg, 0.589 mmol) as described for **10a**. The resulting residue was chromatographed (5:2 *n*-hexane-EtOAc) on silica gel (40 g) to give **10b** (190 mg, 40%) as an amorphous mass: R_f 0.33 (2:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.11 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.26 (m, 8H, 4×CH_2), 1.56 (m, 1H, CH of fatty alkyl), 3.25 (dd, 1H, J_{vic} = 6.0, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.41 (m, 1H, H-5 of Gal), 3.66 (d, 1H, $J_{3,4}$ = 1.5 Hz, H-4 of Fuc), 3.82 (dd, 1H, J_{vic} = 5.8 Hz, H-1' of fatty alkyl), 3.88 (m, 2H, H-2 and H-3 of Gal), 3.98 (m, 1H, H-3 of Fuc), 4.06 (m, 2H, H-6 of Gal and H-2 of Fuc), 4.20 (m, 1H, H-4 of Gal), 4.25 (m, 1H, H-5 of Fuc), 4.34 (m, 1H, H-1 and H-6' of Gal), 4.63-4.99 (m, 6H, 3×PhC*H*$_2$), 5.40 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1 of Fuc), 5.55 (s, 1H, PhC*H*), 7.23-7.55 (m, 20H, Ph). MS (FAB positive) *m/z* 795.4[100% $(M-H)^+$].

Anal. Calcd for $C_{48}H_{60}O_{10}$: C, 72.34; H, 7.59. Found: C, 72.28; H, 7.56.

2-(Tetradecyl)hexadecyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-sulfo-β-D-galactopyranoside Sodium Salt (11a) and 2-(Tetradecyl)hexadecyl *O*-(α-L-Fucopyranosyl)-(1→2)-3-*O*-sulfo-β-D-galactopyranoside Sodium Salt (12a). To a solution of **10a** (200 mg, 0.181 mmol)

in DMF (1 mL) was added sulfur trioxide-pyridine complex (144 mg, 0.904 mmol) and the mixture was stirred for 4 h at room temperature. MeOH (2 mL), THF (2 mL) and NaOMe (51.4 mg, 0.904 mmol) were added, and the mixture was stirred for 1 h at room temperature and then concentrated. TLC showed the disappearance of starting material and formation of a new spot (R_f origin, 2:1 *n*-hexane-EtOAc, 0.39, 20:1 EtOAc-MeOH). The residue was chromatographed (20:1 EtOAc-MeOH) on silica gel (40 g) to give **11a** (202 mg, 93%) as an amorphous mass: ^{1}H NMR (CD_3OD) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.09 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.26 (s, 52H, 26×CH_2), 1.62 (m, 1H, CH of fatty alkyl), 3.45 (dd, 1H, J_{vic} = 5.4, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.57 (m, 1H, H-5 of Gal), 3.87 (dd, 1H, J_{vic} = 5.8 Hz, H-1' of fatty alkyl), 4.52-4.98 (m, 6H, 3×PhC*H*$_2$), 5.49 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1 of Fuc), 5.54 (s, 1H, PhC*H*), 7.17-7.51 (m, 20H, Ph).

Anal. Calcd for $C_{70}H_{103}O_{13}SNa$: C, 69.62; H, 8.60. Found: C, 69.37; H, 8.62.

Subsequently, compound **11a** (193 mg, 0.160 mmol) was dissolved in MeOH (1 mL) and THF (1 mL), and the mixture was treated with 10% Pd-C (200 mg) and H_2 at atmospheric pressure with stirring at room temperature until reduction was complete (24 h). The mixture was filtered (Celite) and partially concentrated, and the solution was loaded onto a cation exchange resin column (WK-10, sodium form, 1× 4 cm, MeOH), to give **12a** (90.9 mg, 67%) as an amorphous mass: ^{1}H NMR (CD_3OD+D_2O) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×*Me*CH_2), 1.20 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.29 (s, 52H, 26×CH_2), 1.60 (m, 1H, CH of fatty alkyl), 3.45 (m, 1H, H-1 of fatty alkyl), 3.64 (t, 1H, $J_{5,6}$ = 6.4 Hz, H-5 of Gal), 3.72 (m, 1H, H-4 of Fuc), 3.75 (m, 1H, H-3 of Fuc), 3.75 (m, 2H, H-6 and H-6' of Gal), 3.75 (m, 1H, H-1' of fatty alkyl), 3.80 (m, 1H, H-2 of Fuc), 3.70 (m, 1H, H-2 of Gal), 4.33 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 of Gal), 4.38 (m, 1H, H-5 of Fuc), 4.41 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1 of Gal), 4.48 (m, 1H, H-3 of Gal), 5.26 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1 of Fuc); ^{13}C NMR (CD_3OD+D_2O) δ 14.7 (CH_3), 16.9 (CH_3 of Fuc), 23.6, 27.2, 27.5, 30.4, 30.6, 30.7, 30.8, 30.9, 31.7 and 32.9 (CH_2), 39.3 (CH), 61.2 (C-6 of Gal), 67.5 (C-5 of Fuc), 67.9 (C-4 of Gal), 69.7 (C-2 of Fuc), 71.3 (C-3 of Fuc), 74.2 (OCH_2), 74.4 (C-4 of Fuc), 74.6 (C-2 of Gal), 75.0 (C-5 of Gal), 82.5 (C-3 of Gal), 100.0 (C-1 of Fuc), 103.0 (C-1 of Gal). MS (FAB negative) *m/z* 825.6[100% $(M-Na)^-$], 847.6[8% $(M-H)^-$].

Anal. Calcd for $C_{42}H_{81}O_{13}SNa$: C, 59.41; H, 9.61. Found: C, 59.28; H, 9.45.

2-(Propyl)pentyl *O*-(α-L-Fucopyranosyl)-(1→2)-3-*O*-sulfo-β-D-galactopyranoside Sodium Salt (12b). Compound **12b** was prepared via **10b** and **11b** by the same sequence as described for **12a**: ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.18 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.32 (m, 8H, 4×CH_2), 1.60 (m, 1H, CH of fatty alkyl), 3.41 (dd, 1H, J_{vic} = 5.4, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.54 (t, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.4 Hz, H-5 of Gal), 3.62 (m, 1H, H-4 of Fuc), 3.74 (d, 2H, H-6 and H-6' of Gal), 3.76 (m, 2H, H-2 and H-3 of Fuc), 3.82 (dd, 1H, J_{vic} = 6.4 Hz, H-1' of fatty alkyl), 3.89 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2 of Gal), 4.25 (d, 1H, H-4 of Gal), 4.38 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1 of Gal), 4.38 (q, 1H, H-5 of Fuc), 4.43 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3 of Gal), 5.35 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1 of Fuc); ^{13}C NMR (CD_3OD) δ 14.8 (CH_3), 16.8 (CH_3 of Fuc), 20.9, 21.1, 34.8 and 34.9 (CH_2), 39.4 (CH), 62.3 (C-6 of Gal), 67.5 (C-5 of Fuc), 68.9 (C-4 of Gal), 70.2 (C-2 of Fuc), 71.6 (C-3 of Fuc), 73.7 (C-4 of Fuc), 73.8 (OCH_2), 74.0 (C-2 of Gal), 76.2 (C-5 of Gal), 82.8 (C-3 of Gal), 100.0 (C-1 of Fuc), 103.9 (C-1 of Gal). MS (FAB negative) *m/z* 517.3[100% $(M-Na)^-$], 539.2[13% $(M-H)^-$].

Anal. Calcd for $C_{20}H_{37}O_{13}SNa$: C, 44.44; H, 6.90. Found: C, 44.26; H, 6.97.

2-(Tetradecyl)hexadecyl *O*-(2,4-Di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (13a). To a solution of **3a** (1.00 g, 1.24 mmol) and **8** (1.38 g, 2.47 mmol) in dry toluene (30 mL) was added powdered MS-4A (5 g), and the mixture was stirred for 24 h at room temperature, then cooled to -20 ℃. NIS (1.11 g, 4.34 mmol) and TfOH (42.9 μL, 0.494mmol) were added to the mixture, and this was stirred for 1 h at -20 ℃. Workup was as described for **9a**. The resulting residue was chromatographed (4:1 *n*-hexane-EtOAc) on silica gel (80 g) to give **13a** (1.20 g, 78%) as an amorphous mass: R_f 0.57 (3:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.12 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.24 (s, 52H, 26×CH_2), 1.49 (m, 1H, CH of fatty alkyl), 3.23 (m, 1H, H-1 of fatty alkyl), 3.30 (m, 1H, H-5 of Gal), 3.68 (m, 1H, H-3 of Fuc), 3.73 and 3.78 (2s, 6H, 2×OCH_3), 3.80 (m, 1H, H-3 of Gal), 3.82 (m, 1H, H-1' of fatty alkyl), 3.99 (m, 2H, H-2 and H-4 of Fuc), 4.00 (m, 1H, H-6 of Gal), 4.12 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 of

Gal), 4.26 (dd, 1H, $J_{5,6'}$ = 5.8, J_{gem} = 12.0 Hz, H-6' of Gal), 4.30 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2 of Gal), 4.42 (m, 1H, H-5 of Fuc), 4.43 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1 of Gal), 4.45-4.95 (m, 8H, 2×MeOPhCH_2 and 2×PhCH_2), 5.40 (s, 1H, PhC*H*), 5.68 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1 of Fuc), 6.66-7.54 (m, 23H, aromatic). MS (FAB positive) *m/z* 807.7[100% (M-Fuc unit)$^+$], 1253.8[26% (M-H)$^+$].

Anal. Calcd for $C_{79}H_{114}O_{12}$: C, 75.56; H, 9.15. Found: C, 75.72; H, 9.00.

2-(Propyl)pentyl *O*-(2,4-Di-*O*-benzyl-3-*O*-(4-methoxybenzyl)-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (13b). To a solution of **3b** (900 mg, 1.80 mmol) and **8** (1.20 g, 2.16 mmol) in dry toluene (8 mL) was added powdered MS-4A (5 g), and the mixture was stirred for 24 h at room temperature, then cooled to -20 ℃. NIS (970 mg, 4.31 mmol) and TfOH (38.0 μL, 0.431mmol) were added to the mixture which was stirred for 1 h at -20 ℃. Workup was as described for **9b**. The resulting residue was chromatographed (3:1 *n*-hexane-EtOAc) on silica gel (80 g) to give **13b** (1.20 g, 71%) as an amorphous mass: R_f 0.83 (1:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.09 (d, 3H, $J_{5,6}$ = 6.8 Hz, CH_3 of Fuc), 1.25 (m, 8H, 4×CH_2), 1.52 (m, 1H, CH of fatty alkyl), 3.23 (dd, 1H, J_{vic} = 5.8, J_{gem} = 9.2 Hz, H-1 of fatty alkyl), 3.48 (m, 1H, H-5 of Gal), 3.75 and 3.80 (2s, 6H, 2×OCH_3), 4.43 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1 of Gal), 4.44-4.95 (m, 8H, 2×MeOPhCH_2 and 2×PhCH_2), 5.40 (s, 1H, PhC*H*), 5.67 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1 of Fuc), 6.74-7.53 (m, 23H, aromatic). MS (FAB positive) *m/z* 499.3[100% (M-Fuc unit)$^+$], 945.5[21% (M-H)$^+$].

Anal. Calcd for $C_{57}H_{70}O_{12}$: C, 72.28; H, 7.45. Found: C, 72.31; H, 7.21.

2-(Tetradecyl)hexadecyl *O*-(2,4-Di-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside (14a). A solution of **13a** (1.00 g, 0.796 mmol) in CH_2Cl_2 (12 mL) and water (1 mL) was treated with DDQ (477 mg, 2.10 mmol) as described for **10a**. The resulting residue was chromatographed (3:1 *n*-hexane-EtOAc) on silica gel (40 g) to give **14a** (334 mg, 41%) as an amorphous mass: R_f 0.18 (3:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.09 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.23 (s, 52H, 26×CH_2), 1.53 (m, 1H, CH of fatty alkyl), 3.35 (m, 1H, H-1 of fatty alkyl), 3.41 (m, 1H, H-5 of

Gal), 4.55-4.96 (m, 4H, 2×PhC*H*$_2$), 5.57 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1 of Fuc), 5.59 (s, 1H, PhC*H*), 7.20-7.58 (m, 15H, Ph). MS (FAB positive) *m/z* 1013.7[100% $(M-H)^+$].

Anal. Calcd for $C_{63}H_{98}O_{10}$: C, 74.52; H, 9.73. Found: C, 74.49; H, 9.88.

2-(Propyl)pentyl *O*-(2,4-Di-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranoside (14b). A solution of **13b** (764 mg, 0.807 mmol) in CH_2Cl_2 (7.5 mL) and water (0.5 mL) was treated with DDQ (439 mg, 1.93 mmol) as described for **10b**. The resulting residue was chromatographed (2:1 *n*-hexane-EtOAc) on silica gel (40 g) to give **14b** (327 mg, 57%) as an amorphous mass: R_f 0.33 (2:1 *n*-hexane-EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.0 Hz, 2×*Me*CH_2), 1.05 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.23 (m, 8H, 4×CH_2), 1.59 (m, 1H, CH of fatty alkyl), 3.37 (dd, 1H, J_{vic} = 5.4, J_{gem} = 9.8 Hz, H-1 of fatty alkyl), 3.53 (m, 1H, H-5 of Gal), 3.59 (d, 1H, H-4 of Fuc), 3.81 (m, 4H, H-1' of fatty alkyl, H-2 of Fuc, H-2 and H-3 of Gal), 4.05 (dd, 1H, $J_{2,3}$ = 6.9, $J_{3,4}$ = 3.5 Hz, H-3 of Fuc), 4.16 (m, 3H, H-4, H-6 andH-6' of Gal), 4.39 (m, 2H, H-1 of Gal and H-5 of Fuc), 4.57-4.95 (m, 4H, 2×PhC*H*$_2$), 5.57 (d, 1H, $J_{1,2}$ = 2.6 Hz, H-1 of Fuc), 5.60 (s, 1H, PhC*H*), 7.22-7.58 (m, 15H, Ph). MS (FAB positive) *m/z* 705.4[100% $(M-H)^+$].

Anal. Calcd for $C_{41}H_{54}O_{10}$: C, 69.67; H, 7.70. Found: C, 69.50; H, 7.54.

2-(Tetradecyl)hexadecyl *O*-(2,4-Di-*O*-benzyl-3-*O*-sulfo-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-3-*O*-sulfo-β-D-galactopyranoside Disodium Salt (15a) and 2-(Tetradecyl)hexadecyl *O*-(3-*O*-Sulfo-α-L-fucopyranosyl)-(1→2)-3-*O*-sulfo-β-D-galactopyranoside Disodium Salt (16a). To a solution of **14a** (150 mg, 0.148 mmol) in DMF (2 mL) was added sulfur trioxide-pyridine complex (118 mg, 0.739 mmol) and the mixture was stirred for 4 h at room temperature. MeOH (2 mL), THF (2 mL) and NaOMe (39.9 mg, 0.739 mmol) were added. Workup was as described for **11a**. TLC showed the disappearance of starting material and formation of a new spot (R_f origin, 1:1 *n*-hexane-EtOAc, 0.67, 5:1 EtOAc-MeOH). The resulting residue was chromatographed (7:1 EtOAc-MeOH) on silica gel (20 g) to give **15a** (163 mg, 91%) as an amorphous mass: ^{1}H NMR (CD_3OD) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.8 Hz, 2×*Me*CH_2), 1.05 (d, 3H, $J_{5,6}$ = 6.3 Hz, CH_3 of Fuc), 1.27 (s, 52H, 26×CH_2), 1.62 (m, 1H, CH of fatty alkyl), 3.47 (dd, 1H, J_{vic} = 5.4, J_{gem} = 9.3 Hz, H-1 of fatty alkyl), 3.57 (m, 1H, H-5 of Gal), 3.87 (dd, 1H, J_{vic} = 5.8 Hz, H-1' of fatty alkyl), 4.52-4.98

(m, 4H, 2×PhCH_2), 5.56 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1 of Fuc), 5.58 (s, 1H, PhC*H*), 7.19-7.51 (m, 15H, Ph).

Anal. Calcd for $C_{63}H_{96}O_{16}S_2Na_2$: C, 62.05; H, 7.93. Found: C, 61.79; H, 7.88.

Subsequently, compound **15a** (159 mg, 0.130 mmol) was dissolved in MeOH (1 mL) and THF (1 mL), and the mixture was treated with 10% Pd-C (150 mg) and H_2 at atmospheric pressure with stirring at room temperature. Workup was as described for **12a** gave **16a** (78.0 mg, 63%) as an amorphous mass: ^{1}H NMR (CD_3OD +D_2O) δ 0.89 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.22 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.29 (s, 52H, 26×CH_2), 1.64 (m, 1H, CH of fatty alkyl), 3.45 (m, 1H, H-1 of fatty alkyl), 3.64 (m, 1H, H-5 of Gal), 3.65 (m, 1H, H-1' of fatty alkyl), 3.70 (m, 2H, H-6 and H-6' of Gal), 3.80 (m, 1H, H-2 of Gal), 3.93 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2 of Fuc), 4.10 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 of Fuc), 4.36 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4 of Gal), 4.38 (m, 1H, H-5 of Fuc), 4.49 (m, 1H, H-3 of Fuc), 4.51 (m, 1H, H-1 of Gal), 4.53 (m, 1H, H-3 of Gal), 5.25 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1 of Fuc); ^{13}C NMR (CD_3OD + D_2O) δ 14.7 (CH_3), 16.7 (CH_3 of Fuc), 23.6, 26.9, 27.3, 30.3, 30.5, 30.6, 30.7, 31.4 and 32.9 (CH_2), 39.2 (CH), 60.9 (C-6 of Gal), 67.4 (C-5 of Fuc), 67.5 (C-2 of Fuc), 67.6 (C-4 of Gal), 71.4 (C-4 of Fuc), 74.3 (OCH_2), 74.7 (C-5 of Gal), 75.4 (C-2 of Gal), 79.0 (C-3 of Fuc), 82.3 (C-3 of Gal), 100.5 (C-1 of Fuc), 103.0 (C-1 of Gal). MS (FAB negative) *m/z* 927.5[100% $(M-Na)^-$], 949.5[5% $(M-H)^-$].

Anal. Calcd for $C_{42}H_{80}O_{16}S_2Na_2$: C, 53.04; H, 8.48. Found: C, 52.86; H, 8.72.

2-(Propyl)pentyl *O*-(3-*O*-Sulfo-α-L-fucopyranosyl)-(1→2)-3-*O*-sulfo-β-D-galactopyranoside Disodium Salt (16b). Compound **16b** was prepared via **14b** and **15b** by the same sequence as described for **16a**: ^{1}H NMR (CD_3OD) δ 0.90 (t, 6H, $J_{Me,CH2}$ = 6.4 Hz, 2×*Me*CH_2), 1.19 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3 of Fuc), 1.35 (m, 8H, 4×CH_2), 1.64 (m, 1H, CH of fatty alkyl), 3.45 (dd, 1H, J_{vic} = 4.9, J_{gem} = 9.4 Hz, H-1 of fatty alkyl), 3.54 (t, 1H, $J_{5,6}$ = 5.9 Hz, H-5 of Gal), 3.74 (d, 2H, H-6 and H-6' of Gal), 3.80 (dd, 1H, J_{vic} = 6.9 Hz, H-1' of fatty alkyl), 3.93 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2 of Gal), 3.95 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2 of Fuc), 4.05 (d, 1H, H-4 of Fuc), 4.29 (d, 1H, H-4 of Gal), 4.41 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1 of Gal), 4.45 (m, 1H, H-5 of Fuc), 4.46 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3 of Gal), 4.49 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3 of Fuc), 5.36 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1 of Fuc); ^{13}C NMR (CD_3OD) δ 14.9 (CH_3), 16.8 (CH_3

of Fuc), 20.8, 21.1, 34.7 and 34.9 (CH_2), 39.4 (CH), 62.3 (C-6 of Gal), 67.4 (C-5 of Fuc), 68.0 (C-2 of Fuc), 68.9 (C-4 of Gal), 72.0 (C-4 of Fuc), 73.9 (C-2 of Gal), 74.1 (OCH_2), 76.2 (C-5 of Gal), 79.2 (C-3 of Fuc), 82.8 (C-3 of Gal), 100.3 (C-1 of Fuc), 103.7 (C-1 of Gal). MS(FAB negative) *m/z* 619.1[100% $(M-Na)^-$], 641.1[4% $(M-H)^-$].

Anal. Calcd for $C_{20}H_{36}O_{16}S_2Na_2$: C, 37.38; H, 5.65. Found: C, 37.11; H, 5.54.

In vitro biological activity. The activity of the compounds *in vitro* was measured in adhesion assays as the inhibition of binding of promyelocytic leukemia HL-60 cells to immobilized recombinant selectin-IgG fusion proteins.[16,17] 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 ℃. 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, 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).[30] Inhibition of L- or E-selectin binding was carried out as described above, using immobilized L- (100 ng) or E-selectin-IgG (40 ng).

ACKNOWLEDGMENT

The authors wish to thank Dr. B. Seed for kindly donating the plasmids of the three selectins.

REFERENCES

1. Synthetic Studies on Sialoglycoconjugates, Part 103. For Part 102, see T. Ikami, N. Tomiya, T. Morimoto, N. Iwata, R. Yamashita, T. Jomori, T.

Usui, Y. Suzuki, H. Tanaka, D. Miyamoto, H. Ishida, A. Hasegawa and M. Kiso, *J. Carbohydr. Chem.*, in press (HASEGAWA MEMORIAL ISSUE).
2. B. A. Imhof and D. Duron, *Adv. Immunol.*, **58**, 345 (1995).
3. T. A. Springer, *Nature*, **346**, 425 (1990).
4. M. P. Bevilacqua, S. Stengelin, M. A. Gimbrone, Jr. and B. Seed, *Science*, **243**, 1160 (1989).
5. G. I. Johnston, R. G. Cook and R. P. McEver, *Cell*, **56**, 1033 (1989).
6. L. A. Lasky, M. S. Singer, T. A. Yednock, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel and S. D. Rosen, *Cell*, **56**, 1045 (1989).
7. T. F. Tedder, C. M. Isaacs, E. J. Ernst, G. D. Demetri, D. A. Adler and C. M. Disteche, *J. Exp. Med.*, **170**, 123 (1989).
8. C. Foxall, S. R. Watson, D. Dowbenko, C. Fennie, L. A. Lasky, M. Kiso, A. Hasegawa, D. Asa and B. Brandley, *J. Cell Biol.*, **117**, 895 (1992).
9. A. Varki, *Proc. Natl. Acad. Sci. USA*, **91**, 7390 (1994).
10. S. R. Watson, Y. Imai, C. Fennie, J. S. Geoffroy, S. D. Rosen and L. A. Lasky, *J. Cell Biol.*, **110**, 2221 (1990).
11. J. Bajorah, D. Hollenbaugh, G. King, W. Harte Jr., D. C. Eustice, R. P. Darveau and A. Aruffo, *Biochemistry*, **33**, 1332 (1994).
12. Y. Suzuki, Y. Toda, T. Tamatani, T. Watanabe, T. Suzuki, T. Nakao, K. Murase, M. Kiso, A. Hasegawa, K. T. Aritomi, I. Ishizuka and M. Miyasaka, *Biochem. Biophys. Res. Commun.*, **190**, 426 (1993).
13. M. S. Mulligan, M. Miyasaka, M. Suzuki, H. Kawashima, M. Iizuka, A. Hasegawa, M. Kiso, R. L. Warner and P. A. Ward, *Int. Immunol.*, **7**, 1107 (1995).
14. B. N. N. Rao, M. B. Anderson, J. H. Musser, J. H. Gilbert, M. E. Schaefer, C. Foxall and B. K. Brandley, *J. Biol. Chem.*, **269**, 19663 (1994).
15. T. P. Kogan, B. Dupre, K. M. Keller, I. L. Scott, H. Bui, R. V. Market, P. J. Beck, J. A. Voytus, B. M. Revelle and D. Scott, *J. Med. Chem.*, **38**, 4976 (1995).
16. J. K. Welply, S. Z. Abbas, P. Scudder, J. L. Keene, K. Broschat, S. Casnocha, C. Gorka, C. Steininger, S. C. Howard, J. J. Schmuke, M. Graneto, J. M. Rotsaert, I. D. Manger and G. S. Jacob, *Glycobiology*, **4**, 259 (1994).
17. G. Kretzschmar, U. Sprengard, H. Kunz, E. Bartnik, W. Schmidt, A. Toepfer, B. Horsch, M. Krause and D. Seiffge, *Tetrahedron*, **51**, 13015 (1995).
18. S. A. DeFrees, W. Kosch, W. Way, J. C. Paulson, S. Sabesan, R. L. Halcomb, D.-H. Huang, Y. Ichikawa and C.-H. Wong, *J. Am. Chem. Soc.*, **117**, 66 (1995).
19. B. K. Brandley, M. Kiso, S. Abbas, P. Nikrad, O. Srivasatava, C. Foxall, Y. Oda and A. Hasegawa, *Glycobiology*, **3**, 633 (1993).
20. K. Ley, G. Linnemann, M. Meinen, L. M. Stoolman and P. Gaehtgens, *Blood*, **81**, 177 (1993).

21. H. Ishida, S. Sago, T. Ikami, M. Kiso and A. Hasegawa, *Biosci. Biotechnol. Biochem.*, **61**, 1615 (1997)..
22. T. Ikami, H. Hamajima, T. Usui, T. Mitani, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **16**, 859 (1997).
23. T. Ikami, N. Tomiya, T. Morimoto, N. Iwata, T. Jomori, T. Usui, Y. Suzuki, H. Tanaka, D. Miyamoto, H. Ishida, A. Hasegawa and M. Kiso, *Chem. Pharm. Bull.*, **45**, 1726 (1997).
24. E. Tanahashi, K. Murase, M. Shibuya, Y. Igarashi, H. Ishida, A. Hasegawa and M. Kiso, *J. Carbohydr. Chem.*, **16**, 831 (1997).
25. A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **200**, 269 (1990).
26. S. Komba, H. Ishida, M. Kiso and A. Hasegawa, *Bioorg. Med. Chem.*, **4**, 1833 (1996).
27. G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990).
28. P. Konradsson, D. R. Mootoo, R. E. McDevitt and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, 270 (1990).
29. Y. Oikawa, T. Yoshioka and O. Yonamitsu, *Tetrahedron Lett.*, **23**, 885 (1982).
30. M. Ishiyama, M. Shiga, M. Mizoguchi and P. He, *Chem. Pharm. Bull.*, **41**, 1118 (1993).