SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE NOVEL SULFATED AND PHOSPHORYLATED BIVALENT β-D-GALACTOPYRANOSIDES CONTAINING FATTY-ALKYL RESIDUES

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Novel sulfated and phosphorylated β-D-galactopyranoside dimers containing fatty-alkyl residues in place of ceramide have been synthesized. The synthetic glycolipids showed an interesting inhibition of the binding of HL-60 cells to immobilized P-, L-, and E-selectins in *in vitro* experiments. These glycolipids may be useful as effective therapeutic agents against selectin-dependent inflammation.

KEY WORDS selectin; sialyl Lewis X; sulfatide; cell adhesion

The selectins[1] are a family of three structurally related carbohydrate-binding proteins [E-selectin (ELAM-1), L-selectin (LECAM-1), and P-selectin (GMP-140, PADGEM)] that appear to be involved in the earliest events of acute inflammatory response, and the selectin-dependent adhesion-promoting process is thought to be responsible for the transient "rolling" phenomenon of leukocytes along the endothelial surfaces.[2] Recently, saccharide ligands recognizing these selectins have been elucidated at the molecular level.[3] Particularly, it has been found that sialyl Lewis X, [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc](sLe^x) and sialyl Lewis A, [Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc](sLe^a) are common ligands of E-, L- and P-selectins. However, sLe^x and synthetic monomeric sLe^x analogs bind weakly to all the selectins.[4] Further, it has been found that a sulfated carbohydrates such as sulfatides, fucoidan, a sulfated glucuronic acid (HNK-1) epitope and heparin strongly bind to the P- and L-selectins. In particular, sulfatide (ceramide is linked to galactose containing a sulfate group on position 3 of the pyranoside ring) and synthetic sulfatides bind avidly to L-selectin.[5] They have also shown highly protective effects against selectin-dependent inflammatory lung injury.[6] In this report, as a part of our study to design new selectin inhibitors, a systematic synthesis and the *in vitro* activity of novel sulfated and phosphorylated bivalent β-D-galactopyranosides containing fatty-alkyl residues in place of ceramide are described.

For the synthesis of the target glycolipids, we employed 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) as the glycosyl donor, and 2,2-substituted 1,3-propanediol **2a-d** as the glycosyl acceptors. The glycosylation of **2a-d** with **1** gave exclusively β-glycosides **3a-d**. Significant signals in the ^{1}H-NMR spectrum of **3a-d** were two-proton doublets at δ 4.3-4.4 ($J_{1,2}$ = 7.0-7.8 Hz, for 2×H-1), showing the newly formed glycosidic linkages to be β. *O*-Deacylation of **3a-d** gave the desired parent glycolipids **4a-d** in which all hydroxy groups are unprotected. Regioselective sulfation of **4a-d** was achieved by treatment of the corresponding stannyl intermediate with the sulfur trioxide/trimethylamine complex, according to a published procedure.[7] Compounds **4a-d** were converted to the stannylene acetal by stirring with dibutyltin oxide, and then sulfation of the stannyl complex using a certain amount of the sulfur trioxide/trimethylamine complex gave bis 3-sulfated galactosides **5a-d**. The structure of the sulfated compounds was confirmed by NMR and MS analyses. Phosphorylation of **4d** was achieved by treatment of the properly protected diol with dibenzyloxy(diisopropylamino)phoshine.[8] Acetonation of **4d** gave the 3,4-*O*-isopropylidene derivative **6d**. Protection of HO-6 and HO-2 with benzyl bromide gave compound **7d**. Hydrolysis of the isopropylidene group of **7d** with aqueous 90% trifruoroacetic acid, treatment with dibenzyloxy (diisopropylamino)phoshine and 1*H*-tetrazole, and further oxidation with catalytic $RuCl_3$ and $NaIO_4$ gave

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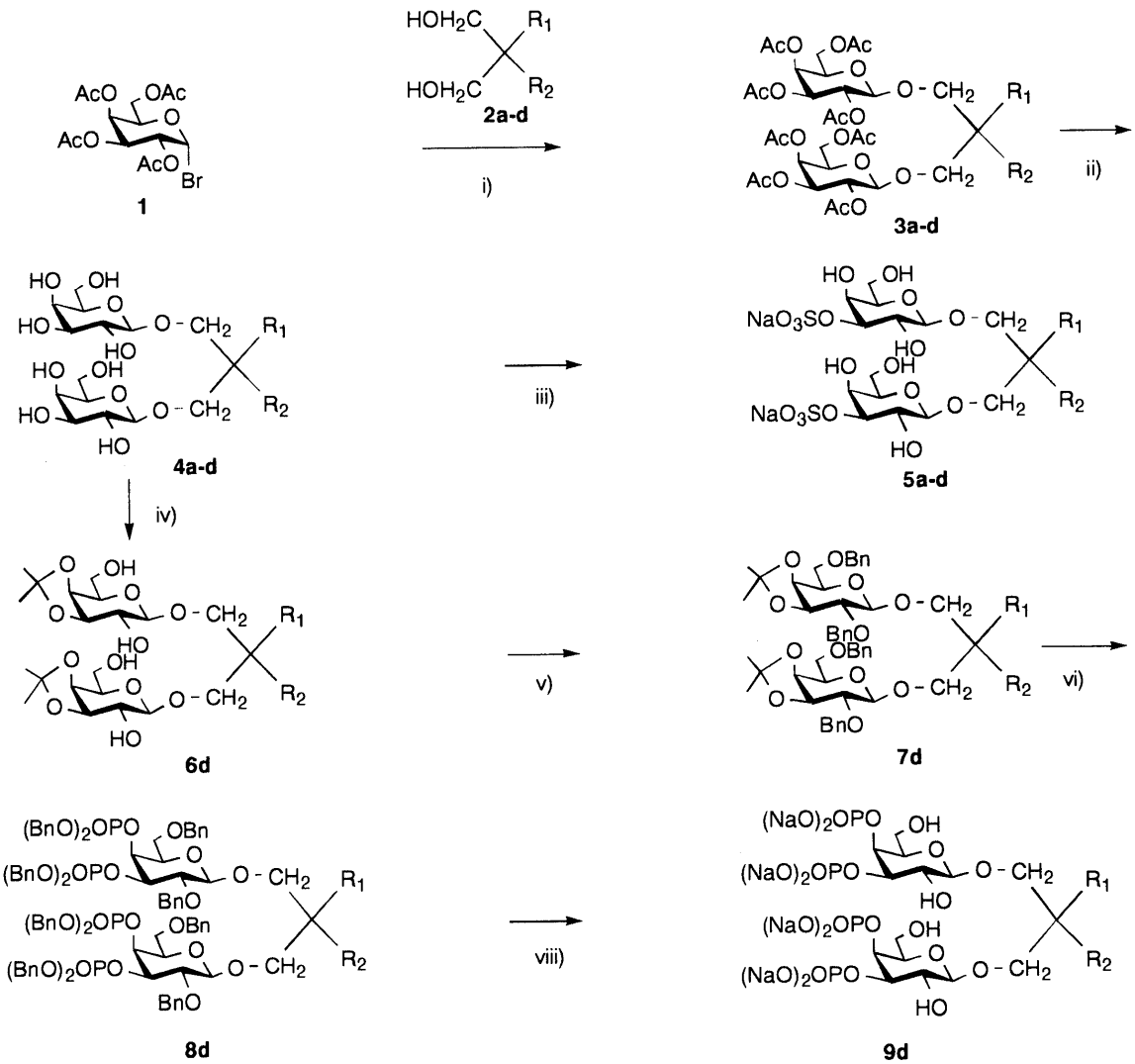
derivative **8d**. Finally, catalytic hydrogenolysis of **8d** with 10% Pd-C and sequential treatment by cation exchange resin gave bis 3,4-bisphosphorylated galactoside **9d**.

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. The results demonstrated (Table 1) that compounds **5b**, **5c**, and **5d**[9] were each significantly more potent than the sLe^x tetrasaccharide itself and **5a** in blocking adhesion to P-selectin, with the trend being **5d** > **5c** > **5b**. Essentially the same trend was observed with L-selectin. These data indicate that the attachment of a branched fatty-alkyl residue to 3-sulfated β-D-galactopyranoside is important for binding to the P- and L-selectins. In addition, when the branched fatty-alkyl residue is long, there is greater potency of the blocking adhesion to the P- and L-selectins. On the other hand, 3,4-bisphosphate **9d**[10] is less potent than 3-sulfate **5d** to the P- and L-selectins but interestingly is more potent toward the E-selectin.



a R_1=H, R_2=$(CH_2)_{13}CH_3$; **b** R_1,R_2=$(CH_2)_2CH_3$; **c** R_1,R_2=$(CH_2)_{13}CH_3$; **d** R_1,R_2=$(CH_3)_{15}CH_3$

Chart 1. i) $HgBr_2$, CH_2Cl_2. ii) NaOH, MeOH-THF. iii) Bu_2SnO, MeOH, $SO_3 \cdot NMe_3$, DMF-THF. iv) 2,2-dimethoxypropane, H_2SO_4, acetone. v) benzyl bromide, DMF. vi) 90% CF_3COOH, CH_2Cl_2. vii) dibenzyloxy(diisopropylamino)phosphine, CH_2Cl_2. viii) 10% Pd-C, MeOH-buffered Sol.

Table 1. Inhibition Activity of Target Compounds

	% inhibition at 0.3 mM		
	P-selectin	L-selectin	E-selectin
sLe^x	3	0	0
5a	0	0	0
5b	24	17	0
5c	43	36	0
5d	81	79	1
9d	0	0	11

ACKOWLEDGEMENT

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

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9) 2,2-Dihexadecyl-1,3-bis((3-*O*-sulfo-β-D-galactopyranosyl)oxy)propane disodium salt (**5d**). FAB-MS (negative) *m/z*: 1029.5 [(M-Na)$^-$,100], 1051.5 [(M-H)$^-$,5]. ^{1}H-NMR (CD_3OD) δ: 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×$\underline{Me}CH_2$), 1.30 (s, 60H, 30×CH_2), 3.53 (d, 2H, J_{gem} = 9.9 Hz, CH_2O of fatty alkyl), 3.60 (t, 2H, $J_{5,6}$ = 5.9Hz, 2×H-5), 3.69 (dd, 2H, $J_{2,3}$ = 7.4 Hz, 2×H-2), 3.70 (d, 2H, CH_2O of fatty alkyl), 3.79 (d, 4H, 2×H-6, H-6'), 4.23 (dd, 2H, $J_{3,4}$ = 3.5 Hz, 2×H-3), 4.34 (d, 2H, 2×H-4), 4.46 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1). ^{13}C-NMR (CD_3OD) δ: 14.8 (CH_3), 23.0, 23.7, 30.4, 30.6, 30.7, 30.8, 30.9, 31.3 and 33.0 (CH_2), 41.9 ($OCH_2\underline{C}$), 61.4 (C-6), 67.9 (C-4), 70.4 (C-2), 72.7 (OCH_2), 75.2 (C-5), 81.9 (C-3), 104.7 (C-1).

10) 2,2-Dihexadecyl-1,3-bis((3,4-bisphospho-β-D-galactopyranosyl)oxy)propane tetrasodium salt (**9d**). ^{1}H-NMR (D_2O) δ: 0.88 (t, 6H, $J_{Me,CH2}$ = 6.9 Hz, 2×$\underline{Me}CH_2$), 1.28 (s, 60H, 30×CH_2), 3.49 (m, 2H, CH_2O of fatty alkyl), 3.62 (m, 2H, 2×H-2), 3.65 (m, 2H, CH_2O of fatty alkyl), 3.69 (m, 2H, 2×H-5), 3.72 (m, 4H, 2×H-6, H-6'), 4.13 (ddd, 2H, $J_{2,3}$ = 6.4, $J_{3,4}$ = 3.0, $J_{3,P}$ = 9.8 Hz, 2×H-3), 4.40 (d, 2H, $J_{1,2}$ = 7.4Hz, 2×H-1), 4.62 (dd, 2H, $J_{4,P}$ = 10.9 Hz, 2×H-4). ^{13}C-NMR (D_2O) δ: 14.4 (CH_3), 22.6, 23.3, 30.1, 30.2, 30.3, 30.4, 30.5, 30.6, 30.7 and 32.6 (CH_2), 41.6 ($OCH_2\underline{C}$), 60.0 (C-6), 71.2 (C-2), 71.3 (C-4), 73.0 (OCH_2), 74.6 (C-5), 76.8 (C-3), 104.7 (C-1).

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

(Received August 25, 1997; accepted September 10, 1997)