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Synthesis of a set of highly clustered monosulfated galactopyranosides

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Abstract

There are several biological events that are known to involve certain sulfated saccharides. In many such cases, however, clustered ligands have been shown to be more effective than monovalent saccharides. A set of 6-aminohexyl glycosides of 2,3,4 or 6-monosulfated galactose have been synthesized and linked to polyglutamic acid. Because of the bulky aglycon employed, the 2-OH group of the key compound, 6-benzyloxycarbonylaminohexyl 4,6-*O*-benzylidene-β-D-galactopyranoside was markedly less reactive than 3-OH. Thus, site-specific acetylation of 3-OH was readily carried out to obtain 2-*O*-sulfated galactosides, and even the direct sulfation of 3-OH afforded the 3-sulfate in a reasonable yield. On the other hand, the key compound was unexpectedly resistant to 2,3-*O*-dibenzylation or 2,3-*O*-dibenzoylation, both of which were meant for regioselective cleavage of 4,6-benzylidene to obtain the 4-sulfate.

Keywords: Aminohexyl galactoside; Galactose sulfates synthesis; Clustering effect; Polyglutamic acid

1. Introduction

Sulfated carbohydrates have great biological significance. They are found to bind a group of mammalian leukocyte adhesion molecules, selectins [1–3]. Among them, L-selectin (lymphocyte homing receptor) recognizes mucin-type oligosaccharides which are sialylated and sulfated [4,5]. Moreover, sulfated carbohydrates exhibit a potential to suppress the infectivity of some microbes and viruses, such as *Mycoplasma* [6], herpes viruses [7–9], myxoviruses, and retroviruses [10], which include the HIV virus. In the case of the HIV virus, sulfated carbohydrates or polyanions inhibit its growth at some distinctive stages in vitro, namely, its binding to CD4 molecule on T-cells [11], the formation of syncytia [12], and the activity of viral ribonuclease [13]. Interestingly, in many cases, ligand clustering is required for optimal activities [14–17].

In the present study, we started with 6-benzyloxycarbonylaminohexyl β-D-galactopyranoside so that sulfated galactose could be readily conjugated to a polypeptide backbone through the 6′-amino group. This large aglycon part, unfortunately, forced us to adjust the synthetic strategy because of diminished reactivity of the 2-OH. Thus, 2,3-dibenzylation or

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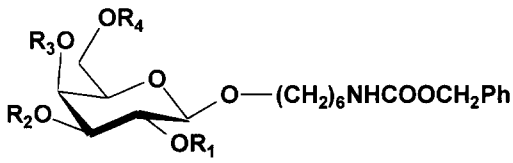
2,3-dibenzoylation was practically impossible from the 4,6-*O*-benzylidene derivative (**2**). In contrast, the reactivity of the 3-OH group of **2** was enhanced, as indicated by a 72% yield of 3-*O*-acetylated compound (**3**).

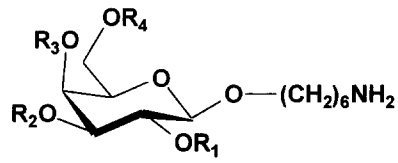
Polyglutamic acid, as employed here, appeared to be suitable in providing a desired range of glycoside clustering if the average molecular size of the polyglutamic acid is carefully chosen. The coupling of 6′-aminohexyl glycosides to polyglutamic acid could be achieved nearly quantitatively under anhydrous conditions.

2. Results and discussion

In the present series of syntheses, the 4,6-benzylidene derivative (**2**) of 6-benzyloxycarbonylaminohexyl β-D-galactopyranoside (**1**) was employed as the key compound for obtaining 2-, 3- and 4-*O*-sulfated galactosides. The reactivity of the 3-OH group of **2** was expected to be higher than that of 2-OH (which is rendered less reactive), because of ring hindrance. In addition, the large aglycon, the 6-benzyloxycarbonylaminohexyl group, seemed to decrease the reactivity of 2-OH even further, as indicated by the difficulty of dibenzylation at the 2- and 3-OH groups, as later described. Indeed, such dibenzylation could be readily performed with 3-fold excess of reagent, when the allyl group was used as the aglycon (data not shown). Limited acetylation of **2** produced predominantly the 3-*O*-acetyl derivative (**3**, 72%) and only a small amount of 2,3-di-*O*-acetyl derivative (24%). Compound **3** was sulfated to yield the 2-*O*-sulfated derivative (**4**).



Compound	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	H	PhCH<	
3	Ac	H	PhCH<	
4	Ac	SO_3	PhCH<	
5	H	SO_3	PhCH<	
6	SO_3	H	PhCH<	
7	H	Bz	PhCH<	
8	Ac	Bz	PhCH<	
9	Ac	Bz	H	Bn
10	Ac	Bz	SO_3	Bn
11	H	H	H	$(Ph)_3C$
12	Bz	Bz	Bz	$(Ph)_3C$
13	Bz	Bz	Bz	H
14	Bz	Bz	Bz	SO_3



Compound	R_1	R_2	R_3	R_4
15	SO_3	H	H	H
16	H	SO_3	H	H
17	H	H	SO_3	H
18	H	H	H	SO_3

This difference of reactivity between 3-OH and 2-OH of **2** was also utilized for sulfation at 3-OH. Direct sulfation of **2** without protection of the 2-OH group gave a good yield (60%) of the monosulfated derivatives, which contained predominantly the desired 3-*O*-sulfated, product. After removing the 2,3-*O*-disulfated galactose derivative by a Sephadex LH-20 column, crystallization of the monosulfated fraction from 95% EtOH afforded the pure 6-benzyloxycarbonylaminohexyl 4,6-*O*-benzylidene-β-D-galactopyranoside 3-sulfate (**6**, 38% from **2**), plus a mother liquor containing a significant amount of **6** and some contamination by the 2-*O*-sulfated compound.

To obtain the derivative having 4-OH free, regioselective cleavage of the 4,6-*O*-benzylidene group is possible. Compound **2**, however, was found to be surprisingly resistant to 2 and 3-OH dibenzylation, even when a > 20-fold excess of NaH and benzyl bromide were used. The yield of dibenzoylation product did not exceed 50% even with 20-fold excess of benzoyl chloride at 55 °C. Finally, the 2-*O*-acetyl-3-*O*-benzoyl derivative **8** was shown to be a satisfactory alternative intermediate for regioselective cleavage of the 4,6-benzylidene group. Interestingly, the reductive cleavage of compound **8** did not alter the apparent R_f value on TLC.

The intermediate with an open 6-OH group could

Table 1
Chemical shift of ring protons on ^{1}H NMR

Compound	[Chemical shifts (ppm)[a]]						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
[15]	4.35	4.02	3.65	3.79	3.57	3.61–3.57	3.53–3.48
[16]	4.48	3.63	4.28	4.23	3.79–3.68	3.79–3.68	3.79–3.68
[17]	4.41	3.49	3.81–3.71	4.64	3.81–3.71	3.81–3.71	3.81–3.71
[18]	4.41	3.50	3.66	3.96	3.93	4.20	4.18

[a]All data were obtained in deuterium oxide using acetone as the internal standard (2.225 ppm).

be produced efficiently by the previously established method [18] of tritylating the 6-OH group and deprotecting after benzoylation of 2,3,4-OH. This reactivity of 2-OH upon benzoylation may be attributed to the absence of the benzylidene group, which would cause ring hindrance.

Once a sulfate group had been introduced, column chromatography on Sephadex LH-20 was more effective than on silica gel. The former can be operated with more polar solvents in which sulfated compounds are readily soluble, and a better recovery is usually obtained. Moreover, the separation of inorganic sulfate or a disulfated compound was much easier than with silica gel. In the present work, the Sephadex LH-20 columns were eluted by water or water–methanol, to enhance hydrophobic interaction between the solutes and the column media for better separation.

The chemical shifts of ring protons of each mono-sulfated galactopyranoside in ^{1}H NMR are listed in Table 1. The effect of *O*-sulfation on the neighboring proton observed here was consistent with the previous report [18], confirming the identity of the products listed. In addition, negative ion FAB–mass analyses of compound **15–18** showed the M–Na signal at *m/z* 358.

It is noteworthy that a certain ionized form of the sulfate group is significantly more labile than the sodium form. Indeed, the 4-*O*- and 6-*O*-sulfated compounds underwent spontaneous and complete desulfation during the purification step at room temperature, even though they had been treated with an excess of $NaHCO_3$ and most of the sulfates were expected to be converted into the sodium form. Such desulfation might be explained by the presence of some residual sulfate groups in the pyridinium form, which can initiate an autolytic process. The pyridinium form is known to be more labile than the sodium form, and once a small amount of sulfate is released as sulfuric acid, it accelerates the hydrolysis even further. Consequently, the counter ion of sulfate was changed to sodium by successively passing the product through a column of H^+ form resin and a column of Na^+ form resin.

Compound **15–18** were eventually clustered on to a polyglutamic acid backbone in an anhydrous medium to form clustered glycosides. The polyglutamic acid used here was first fractionated on a Sephadex G-50 column (2.5 × 90 cm) eluted with water to obtain a relatively uniform molecular size (30–38 kDa). The degree of polymerization was estimated as described in Section 3. The coupling efficiency was almost quantitative with a 2-fold excess of 6-aminohexyl glycosides, as shown in Table 2. Formation of an amide bond between the 6-amino group of the aglycon and the γ carbonyl group of

Table 2
The coupling efficiency of each mono-sulfated galactopyranoside to polyglutamic acid

Compound	Occupied glutamic acid (%)
[15]	[Quantitative]
[16]	[91]
[17]	[99]
[18]	[92]

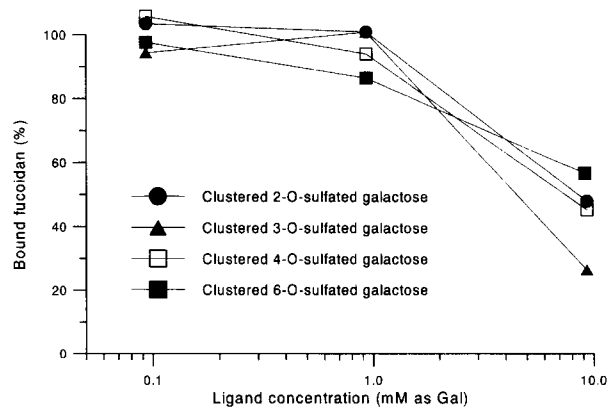


Fig. 1. Binding activity of synthesized ligands to L-selectin. The binding affinities of clustered galactose monosulfates were analyzed by their inhibitory potential to ^{125}I-labeled fucoidan.

glutamic acid was confirmed by ^{1}H NMR analyses: the downfield shift (0.34–0.35 ppm) of the methylene protons next to the 6-amino group, and the appearance of additional methyne (δ 4.26, 1 H) and methylene (δ 2.33, 2 H, 2.08, 1 H and 1.97, 1 H) from the glutamic acid residue.

The binding affinities of newly synthesized clustered ligands were examined as their inhibitory activities to the binding of ^{125}I-labeled fucoidan with mouse L-selectin [19]. All four structures exhibited significant affinities as shown by Fig. 1, whereas any of the monomeric counterparts were not active, even at the concentration of 100 mM (data not shown).

3. Experimental

General methods.—Melting points were determined with a Fisher–Johns apparatus and are uncorrected. All solvents were dried over 4 Å molecular sieves except for MeOH (3 Å molecular sieves) and $CHCl_3$ (Na_2SO_4) before use. ^{1}H NMR spectra were obtained with a Bruker AMX 300 spectrometer at 22 °C. The internal standards for ^{1}H NMR measurement were Me_4Si for $CDCl_3$ and CD_3OD, or acetone (2.225 ppm) for measurements in deuterium oxide. Elemental analyses were performed by Galbraith Lab., (Knoxville, TN). All evaporations were performed with a rotary evaporator. Thin layer chromatography was performed on Silica Gel 60 F_{254}-precoated aluminum sheets (EM Industries, Gibbstown, NJ) and compounds were detected by UV absorbance (254 nm), ninhydrin reaction, or charring. Charring was done after spraying with 15% H_2SO_4 in 50% EtOH. Preparative chromatography was performed with columns of Silica gel 60 (EM Industries) or Sephadex LH-20 (1.5 × 26 cm, Pharmacia Biotech., Uppsala, Sweden). The content of sulfate ion was determined by ion chromatography using an ION-120 column (Interaction Chemicals, CA) as described elsewhere [19]. The carbohydrate was quantified with the phenol–H_2SO_4 reaction, using galactose as standard. Hydrogenolysis was carried out with a Brown hydrogenator [20].

6 - Benzyloxycarbonylaminohexyl β - D - galactopyranoside (**1**).—Glycosidation of tetra-*O*-acetyl α-D-galactopyranosyl bromide (6.24 g, 15.5 mmol) with 6-benzyloxycarbonylaminohexanol (3.52 g, 14.0 mmol) was performed in 1:1 (v/v) toluene–nitromethane (60 mL) in the presence of mercury(II) bromide (5.59 g, 15.5 mmol) and mercury(II) cyanide (3.92 g, 15.5 mmol), which yielded 6-benzyloxycarbonylaminohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (R_f 0.38, 2:1 (v/v)) toluene–EtOAc as well as the partially *O*-deacetylated compounds during 18 h at 22 °C. Column chromatography on Sephadex LH-20 in 95% EtOH and deacetylation with 30 mM NaOMe in dry MeOH (20 mL) afforded the title compound, which was crystallized from 95% EtOH (2.2 g, 39%): mp 87.5–88.5 °C. R_f 0.42 in 10:2:1 (v/v) EtOAc–2-propanol–water; ^{1}H NMR (deuterium oxide–acetone): δ 7.4–7.1 (m, Ph), 4.90 (s, 2 H, O–CH_2–Ph), 4.17 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.71 (dd, $J_{3,4}$ 3.3 Hz, H-4), 3.75–3.67 (dt, O–CH_2–$(CH_2)_4$), 3.57 (dd, $J_{6,6'}$ 11.6 Hz, H-6), 3.55 (dd, H-6'), 3.46 (m, H-5), 3.42 (dd, $J_{2,3}$ 9.9 Hz, H-3), 3.24 (dd, H-2), 2.92 (dt, 2 H, $(CH_2)_4$–CH_2–NH), 1.5–1.1 (m, 8 H, $(CH_2)_4$).

6-Benzyloxycarbonylaminohexyl 4,6-O-benzylidene-β-D-galactopyranoside (**2**).—A mixture of **1** (1.78 g, 4.30 mmol), benzaldehyde (3.25 mL, 32.5 mmol) and formic acid (3.25 mL) was stirred at 22 °C for 1 h [21]. The title compound [R_f 0.23, 1:2 (v/v) toluene–EtOAc] crystallized out, when Et_2O (~ 25 mL) was added to the mixture at 4 °C. The crystal was washed with ~ 60 mL of Et_2O. The mother liquor was concentrated, mixed with 1 mL of formic acid and stirred for 30 min to allow further reaction. An additional **2** was obtained after addition of Et_2O to give a total yield of 3.55 mmol (82.6%); mp 153–155 °C; ^{1}H NMR ($CDCl_3$–Me_4Si): δ 7.5–7.3 (m, 10 H, Ph), 5.54 (s, 1 H, C*H*–Ph), 5.09 (s, 2 H, CH_2–Ph), 4.32 (dd, 1 H, $J_{6,6'}$ 12.4 Hz, H-6), 4.26 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.38 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-4), 4.07 (dd, 1 H, H-6'), 3.74 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 3.72–3.64 [m, 1 H, O–CH_2–$(CH_2)_4$], 3.68 (dd, 1 H, H-3), 3.53–3.46 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.46 (m, 1 H, H-5), 3.20 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$]. Anal. Calc. for $C_{27}H_{35}O_8N_1$: C, 64.70; H, 7.03; N, 2.79. Found: C, 64.42; H, 7.01; N, 2.76.

6-Benzyloxycarbonylaminohexyl 3-O-acetyl-4,6-O-benzylidene-β-D-galactopyranoside (**3**).—To a solution of **2** (1.50 g, 3.0 mmol) in 15 mL of dry pyridine, acetyl chloride (0.43 mL, 5.93 mmol) was added dropwise with stirring in an ice bath. After incubation at for 5 h 22 °C, the mixture was quenched with ice, diluted with $CHCl_3$ and sequentially washed with 0.5 M chilled H_2SO_4 and $NaHCO_3$. The residue was chromatographed on silica gel [2:1 (v/v) toluene–EtOAc] to yield **3** (1.14 g, 2.1 mmol, 72%); R_f 0.40 in 1:1 (v/v) toluene–EtOAc. ^{1}H NMR ($CDCl_3$–Me_4Si): δ 7.3–7.5 (m, 10 H, Ph), 5.54 (s, 1 H, C*H*–Ph), 5.09 (s, 2 H, CH_2–Ph), 4.86 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.7 Hz, H-3), 4.38 (dd, 1 H, H-4), 4.34

(d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.31 (dd, 1 H, $J_{6,6'}$ 12.4 Hz, H-6), 4.05 (dd, 1 H, H-6'), 4.00 (dd, 1 H, H-2), 3.72–3.64 [m, 1 H, O–CH_2–$(CH_2)_4$], 3.53–3.46 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.48 (m, 1 H, H-5), 3.20 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 2.13 (s, 3 H, CH_3CO), 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 3-O-acetyl-4,6-O-benzylidene-β-D-galactopyranoside 2-sulfate (**4**).—To a solution of **3** (1.14 g, 2.1 mmol) in 10 mL dry (1:1) DMF (Me_2NCHO)–pyridine, sulfur trioxide–pyridine complex (1.17 g, 7.4 mmol) was added to react for 7 h at 55 °C. The reaction was stopped by the addition of 16.8 mL of 1 M $NaHCO_3$ and the mixture was evaporated in vacuo. The syrup was chromatographed on a LH-20 column (1.5 × 26 cm), eluted with water to yield the desired product **4** (1.26 g, 89.5%); R_f 0.50 in 6:1 (v/v) EtOAc–MeOH; ^{1}H NMR (deuterium oxide–acetone): δ 7.5–7.3 (m, 10 H, Ph), 5.65 (s, 1 H, C*H*–Ph), 5.21 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.6 Hz, H-3), 5.06 (s, 2 H, CH_2–Ph), 4.66 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.49 (dd, 1 H, H-2), 4.44 (dd, 1 H, H-4), 4.18 (m, 2 H, H-6,6'), 3.92–3.84 [dt,1 H, O–CH_2–$(CH_2)_4$], 3.72 (m, 1 H, H-5), 3.71–3.62 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.10 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 4,6-O-benzylidene-β-D-galactopyranoside 2-sulfate (**5**).—Compound 4 (1.26 g) was dissolved in 10 mL dry MeOH and *O*-de-acetylated using 30 mM NaOMe at 22 °C during 17 h. The reaction was stopped by passing the solution through Dowex 50 × 8 (H^+) column to lower the pH to < 2 and then immediately neutralized with aq. $NaHCO_3$. The residue was concentrated and crystallized from 95% EtOH to afford 0.73 g of **5** (833 mg, 73%); R_f 0.38 in 6:1 (v/v) EtOAc–MeOH; mp 138–139 °C. Anal. Calc. for $C_{27}H_{34}O_{11}N_1S_1Na_1 \cdot 1.5H_2O$: C, 51.42; H, 5.75; N, 2.22. Found: C, 51.75; H, 5.88; N, 2.29.

6-Benzyloxycarbonylaminohexyl 4,6-O-benzylidene-β-D-galactopyranoside 3-sulfate (**6**).—Compound **2** (744 mg, 1.48 mmol) and sulfur trioxide–pyridine complex (897 mg, 5.64 mmol) were dissolved in dry 1:1 Me_2NCHO–pyridine (7.4 mL) and incubated for 1 h at 55 °C until complete sulfation was achieved as determined by TLC. After stopping the reaction by 11.3 mL of aq. 1 M $NaHCO_3$, the mixture was concentrated to a syrup and chromatographed on a Sephadex LH-20 column (2.2 × 46 cm) eluted with water. The fractions containing the title compound were collected, and treated with Dowex 50 × 8 (H^+) and Amberlite CG-50 (Na^+) sequentially to convert the counter ion into Na^+. Evaporation of the solution and crystallization from 95% EtOH yielded **6** (334 mg, 37.6%); R_f 0.30 in 6:1 (v/v) EtOAc–MeOH; mp 130–131.5 °C; ^{1}H NMR (deuterium oxide–acetone): δ 7.5–7.3 (m, 10 H, Ph), 5.69 (s, 1 H, CH–Ph), 5.06 (s, 2 H, CH_2–Ph), 4.64 (bd, 1 H, H-4), 4.54 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.41 (dd, 1 H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 3.6 Hz, H-3), 4.18 (m, 2 H, H-6,6'), 3.89 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.73 (dd, 1 H, H-2), 3.70–3.62 [m, 1 H, O–CH_2–$(CH_2)_4$], 3.69 (bs, 1 H, H-5), 3.08 [dt, 2 H, $(CH_2)_4$–CH_2–NH), 1.6–1.2 [m, 8 H, $(CH_2)_4$]. Anal. Calc. for $C_{27}H_{34}O_{11}N_1S_1Na_1 \cdot H_2O$: C, 52.17; H, 5.84; N, 2.25. Found: C, 52.16; H, 6.06; N, 2.32.

6-Benzyloxycarbonylaminohexyl 3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**7**).—To a stirred solution of compound **2** (0.84 g, 1.67 mmol) in dry pyridine (8 mL), benzoyl chloride (2.52 mL, 2.17 mmol) was added dropwise and reacted for 4 h at 22 °C to give the title compound [R_f 0.39, 2:1 (v/v) toluene–EtOAc]. The mixture was evaporated to remove most of the pyridine, diluted with $CHCl_3$ and washed sequentially with H_2SO_4 and $NaHCO_3$. After evaporation and silica gel column chromatography in 3:1 (v/v) toluene–EtOAc, **7** (0.79 g, 78.4%) was obtained; ^{1}H NMR: δ 8.0–7.3 (m, 15 H, Ph), 5.53 (s, 1 H, CH–Ph), 5.22 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.5 Hz, H-3), 5.07 (s, 2 H, CH_2–Ph), 4.52 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.51 (bd, 1 H, H-4), 4.36 (bd, 1 H, $J_{6,6'}$ ~ 12 Hz, H-6), 4.17 (dd, 1 H, H-2), 4.05 (bd, 1 H, H-6 ~), 3.96 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.63 (bs, 1 H, H-5), 3.58 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.16 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 2-O-acetyl-3-O-benzoyl-6-O-benzyl-β-D-galactopyranoside (**9**).—Acetyl chloride (0.32 mL, 4.72 mmol) was added dropwise to a stirred solution of **7** (0.79 g, 1.31 mmol) in dry pyridine (8 mL) and incubated at 22 °C for 2 h. The mixture was processed in the same way as compound **3** to afford compound **8** [R_f 0.49, 2:1 (v/v) toluene–EtOAc]. After concentration, sodium cyanoborohydride (0.79 g, 12.5 mmol), dissolved in dry tetrahydrofuran (8 mL), was added and the mixture was stirred on ice. To the stirred solution, HCl–saturated diethyl ether (~ 3 mL) was added dropwise until bubbling stopped [22]. The mixture was filtered through Celite, evaporated in vacuo, and diluted with $CHCl_3$. The organic layer was washed with $NaHCO_3$ and fractionated by silica gel column chromatography [4:1 (v/v) toluene–EtOAc] to give 0.48 g (0.739 mmol, 58.6%) of **9**; R_f 0.49 in 2:1 (v/v) toluene–EtOAc; ^{1}H NMR ($CDCl_3$–Me_4Si): δ 8.0–7.2 (m, 15 H, Ph), 5.48 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.2 Hz, H-2),

5.09 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 5.09 (s, 2 H, CO–O–CH_2–Ph), 4.58 and 4.57 (d, 2 H, J 12.2 Hz, Ph–CH_2–O), 4.51 (d, 1 H, H-1), 4.31 (bd, H-4), 3.93–3.86 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.79 (dd, 1 H, $J_{6,6'}$ 10.4 Hz, H-6), 3.78 (m, 1 H, H-5), 3.77 (dd, 1 H, H-6'), 3.53–3.46 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.20 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.97 (s, 3 H, CH_3CO), 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 2-O-acetyl-3-O-benzoyl-6-O-benzyl-β-D-galactopyranoside 4-sulfate (**10**).—Compound **9** (0.48 g, 0.74 mmol) was dissolved in 5 mL of dry Me_2NCHO–pyridine (1:1, v/v) and sulfur trioxide–pyridine complex (1.17 g, 7.42 mmol) was added. The solution was incubated for 3 h at 55 °C and the reaction was stopped by 15.8 mL of aq. 1 M $NaHCO_3$. The mixture was concentrated to a syrup, dissolved in MeOH, and filtered through a layer of Celite to remove inorganic salts. The residue was subjected to LH-20 column chromatography eluted with 1:1 (v/v) MeOH–water to give **10** (242 mg, 43.6%); R_f 0.46 in 1:1 (v/v) EtOAc–acetone; ^{1}H NMR (CD_3OD–Me_4Si): δ 8.1–7.2 (m, 15 H, Ph), 5.36 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.4 Hz, H-2), 5.19 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 5.05 (s, 2 H, CO–O–CH_2–Ph), 4.96 (bd, $J_{3,4}$ 3.2 Hz, H-4), 4.60 and 4.56 (d, 2 H, J 11.7 Hz, Ph–CH_2–O), 4.58 (d, 1 H, H-1), 3.97 [m, 1 H, H-5], 3.90–3.78 [m, 3 H, H-6,6', O–CH_2–$(CH_2)_4$], 3.54 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.09 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.95 (s, 3 H, CH_3CO), 1.6–1.2 [m, 8 H, $(CH_2)_4$]. Anal. Calc. for $C_{36}H_{42}O_{13}S_1Na_1 \cdot 4H_2O$: C, 51.36; H, 6.12; N, 1.70. Found: C, 51.69; H, 5.79; N, 2.10.

6-Benzyloxycarbonylaminohexyl 2,3,4-tri-O-tribenzoyl-6-O-triphenylmethyl-β-D-galactopyranoside (**12**).—To the stirred solution of compound **1** (1.24 g, 3.01 mmol) in dry pyridine (10 mL), triphenylmethyl chloride (1.51 g, 5.42 mmol) was added and maintained for 48 h at 22 °C to give **11** [R_f 0.31, 1:1 (v/v) toluene–acetone]. To the mixture, benzoyl chloride (2.09 mL, 18 mmol) was added dropwise. After reacting overnight, the mixture was quenched with ice, diluted with $CHCl_3$ and washed sequentially with H_2SO_4 and $NaHCO_3$. The organic layer was concentrated to a syrup and chromatographed on a silica gel column using 12:1 (v/v) toluene–EtOAc to yield the title compound (2.76 g, 95%); R_f 0.35 in 12:1 (v/v) toluene–EtOAc; ^{1}H NMR ($CDCl_3$–Me_4Si): δ 8.1–7.1 (m, 35 H, Ph), 6.04 (bd, H-4), 5.63 (dd, 1 H, $J_{1,2}$ 7.3, $J_{2,3}$ 10.5 Hz, H-2), 5.61 (dd, 1 H, H-3), 5.08 (s, 2 H, CH_2–Ph), 4.69 (d, 1 H, H-1), 4.04 (m, 1 H, H-5), 3.92 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.54–3.45 [m, 2 H, H-6,6'], 3.27 [bt, 1 H, O–CH_2–$(CH_2)_4$], 2.97 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside (**13**).—Compound **12** (2.76 g, 2.85 mmol) was refluxed in aq. 80% CH_3COOH for 25 min. After concentration in vacuo and silica gel chromatography [2:1 (v/v) toluene–EtOAc], the title compound was obtained (1.53 g, 75.2%); R_f 0.27 in 2:1 (v/v) toluene–EtOAc; ^{1}H NMR ($CDCl_3$–Me_4Si): δ 8.2–7.1 (m, 20 H, Ph), 5.83 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.5 Hz, H-2), 5.80 (bd, H-4), 5.59 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 5.08 (s, 2 H, CH_2–Ph), 4.77 (d, 1 H, H-1), 4.02 (m, 1 H, H-5), 3.96 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.83 (dd, 1 H, $J_{6,6'}$ 12.0, $J_{5,6}$ 6.4 Hz, H-6,6'), 3.65 (dd, 1 H, $J_{5,6'}$ 7.0 Hz, H-6'), 3.54 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.01 (bt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$].

6-Benzyloxycarbonylaminohexyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside 6-sulfate (**14**).—To a solution of **13** (1.23 g, 1.70 mmol) in dry Me_2NCHO–pyridine (1:1, 10 mL), sulfur trioxide–pyridine complex (1.09 g, 6.79 mmol) was added and the mixture was incubated for 1.5 h at 55 °C. The mixture was quenched with 13.6 mL 1 M $NaHCO_3$, concentrated in vacuo to a syrup, dissolved in MeOH and fractionated on a Sephadex LH-20 column eluted with 1:1 MeOH–water to give **14** (1.42 g, 99.0%); R_f 0.51 in 6:1 (v/v) EtOAc–MeOH; ^{1}H NMR (CD_3OD–Me_4Si): δ 8.1–7.2 (m, 20 H, Ph), 5.93 (bd, 1 H, H-4), 5.69 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-3), 5.66 (dd, 1 H, $J_{1,2}$ 7.0, $J_{2,3}$ 10.3 Hz, H-2), 5.06 (s, 2 H, CH_2–Ph), 4.96 (d, 1 H, H-1), 4.43 (bt, 1 H, H-5), 4.20 (dd, 1 H, $J_{6,6'}$ 10.6 Hz, H-6), 4.16 (dd, 1 H, H-6'), 3.97 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.61 [dt, 1 H, O–CH_2–$(CH_2)_4$], 2.93 [dt, 2 H, $(CH_2)_4$–CH_2–NH], 1.6–1.2 [m, 8 H, $(CH_2)_4$]. Anal. Calc. for $C_{41}H_{42}O_{14}N_1S_1Na_1 \cdot 1/2H_2O$: C, 58.85; H, 5.18; N, 1.67. Found: C, 58.66; H, 5.40; N, 1.70.

6-Aminohexyl β-D-galactopyranoside 2-sulfate (**15**).—A stirred solution of **6** (69 mg, 114 μmol) in CH_3COOH (1.4 mL) was hydrogenolyzed in the presence of palladium (10%) on activated carbon (61 mg) for 36 h at 22 °C. TLC showed the disappearance of UV absorption and the appearance of primary amino group as shown by the ninhydrin reaction [R_f 0.22, 3:2:1 (v/v) EtOAc–2-propanol–water]. The mixture was filtered through Celite, concentrated in vacuo and evaporated with MeOH several times to remove residual CH_3COOH to yield 98.3 μmol (85.8%, as determined by the phenol–H_2SO_4 reaction). The content of organic sulfate in the specimen was 1.00 mol per mol galactose by ion chromatogra-

phy analysis. ^{1}H NMR (deuterium oxide–acetone): δ 4.35 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.02 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 3.79 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 3.75 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.65 (dd, 1 H, H-3), 3.61–3.57 (dd, 1 H, H-6), 3.57 (m, 1 H, H-5), 3.53–3.48 (m, 1 H, H-6′), 3.49 [dt, 1 H, O–CH_2–$(CH_2)_4$], 2.81 [bt, 2 H, $(CH_2)_4$–CH_2–NH_2], 1.6–1.1 [m, 8 H, $(CH_2)_4$].

6-Aminohexyl β-D-galactopyranoside 3-sulfate (**16**).—Hydrogenolysis of **5** (69 mg, 115 μmol) was performed in CH_3COOH employing palladium (10%) on activated carbon (33 mg) as catalyst for 36 h at 22 °C. The mixture was processed as for **15** to yield 98 μmol (85.8%) of **16** [R_f 0.12, 3:2:1 (v/v) EtOAc–2-propanol–water], based on the phenol–H_2SO_4 reaction. The organic sulfate was 0.97 mol per mol galactose, as analyzed by ion chromatography. ^{1}H NMR (deuterium oxide–acetone): δ 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.28 (dd, 1 H, $J_{2,3}$ 9.1, $J_{3,4}$ 3.3 Hz, H-3), 4.23 (bd, 1 H, H-4), 3.92 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.79–3.68 [m, 4 H, H-6,6′,5, O–CH_2–$(CH_2)_4$], 3.62 (dd, 1 H, H-2), 2.97 [dt, 2 H, $(CH_2)_4$–CH_2–NH_2], 1.7–1.3 [m, 8 H, $(CH_2)_4$].

6-Aminohexyl β-D-galactopyranoside 4-sulfate (**17**).—Compound **10** (0.23 g, 306 μmol) was dissolved in dry MeOH (2 mL) and mixed with 20 μL of 3 M NaOMe. After reacting at 22 °C overnight, Dowex 50 × 8 (H^+) resin was added to remove sodium ions, and the resin was filtered off. The filtrate was immediately treated with Amberlite CG-50 (Na^+) to adjust the pH to 6 and subjected to chromatography on a Sephadex LH-20 column that was eluted with 1:1 (v/v) MeOH–water. The resultant compound [R_f 0.27, 10:2:1 (v/v) EtOAc–2-propanol–water] was concentrated, dissolved in MeOH and hydrogenolyzed in the presence of palladium (10%) on activated carbon to give the title compound, which was ninhydrin positive [90 mg, 76.9%, R_f 0.10, 3:2:1 (v/v) EtOAc–2-propanol–water]. The ion chromatography and phenol–H_2SO_4 analyses indicated the molar ratio of sulfate to galactose to be 0.98. ^{1}H NMR (deuterium oxide–acetone): δ 4.64 (bd, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 4.41 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.90 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.81–3.71 (m, 4 H, H-3, H-5, H-6,6′), 3.49 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 3.67 [dt, 1 H, O–CH_2–$(CH_2)_4$], 2.92 [dt, 2 H, $(CH_2)_4$–CH_2–NH_2], 1.7–1.3 [m, 8 H, $(CH_2)_4$].

6-Aminohexyl β-D-galactopyranoside 6-sulfate (**18**).—Compound **14** (439 mg, 0.53 mmol) in dry MeOH (2 mL) was treated with 30 mM NaOMe for 3 h at 22 °C. The reaction was stopped with Dowex 50 × 8 (H^+) and the pH was adjusted with Amberlite CG-50 (Na^+) immediately as that described. The residue was concentrated and fractionated on Sephadex LH-20 column eluted with 1:1 (v/v) MeOH–water. The resultant compound [0.264 g, 96.8%, R_f 0.30, 8:2:1 (v/v) EtOAc–2-propanol–water] was subjected to hydrogenolysis in 2:1 (v/v, 3 mL) MeOH–water in the presence of palladium (10%) on activated carbon (32 mg) to yield the title compound (0.447 mmol, 87.3%), which gave a ninhydrin-positive spot [R_f 0.14, 3:2:1 (v/v) EtOAc–2-propanol–water]. The ratio of sulfate to galactose was 1.00 by ion chromatography and phenol–H_2SO_4 reaction. ^{1}H NMR (deuterium oxide–acetone): δ 4.41 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.20 (dd, 1 H, $J_{6,6'}$ 10.7, $J_{5,6}$ 5.7 Hz, H-6), 4.18 (dd, 1 H, $J_{5,6'}$ 6.8 Hz, H-6′), 3.96 (bd, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 3.93 (bt, 1 H, H-5), 3.91 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.71 [dt, 1 H, O–CH_2–$(CH_2)_4$], 3.66 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-3), 3.50 (dd, 1 H, H-2), 2.82 [dt, 1 H, $(CH_2)_4$–CH_2–NH_2], 1.7–1.3 [m, 8 H, $(CH_2)_4$].

Polymerization of sulfated galactosides.—Poly-L-glutamic acid (Sigma Chemical, St. Louis, MO) was first fractionated on Sephadex G-50 (Pharmacia Biotech.) column (2.5 × 90 cm) eluted with water. The fractions containing 230–300 mer polypeptide were collected (514 mg) and decationized with Dowex 50x8 (H^+) for tyrosination at its amino-terminus, which was performed by succinimidyl *t*-butoxycarbonyl tyrosinate (110 mg, Sigma Chemical) in dry Me_2NCHO (5 mL), in the presence of Et_3N (0.83 ml). The reaction mixture was evaporated in vacuo, neutralized with NaOH and chromatographed on Bio-Gel P-4 column (1.5 × 24 cm, Bio-Rad Lab., Hercules, CA) eluted with water. The resultant compound was again decationized with Dowex 50 × 8 (H^+) for glycoside conjugation. The molecular size of polyglutamic acid was estimated by comparing the reactivity to fluorescamine with or without hydrolysis in 2 M NaOH for 16 h at 100 °C [23].

Each of the sulfated 6-aminohexyl β-galactosides (100 μmol) was conjugated to polyglutamic acid (50 μmol) in 0.5 mL Me_2NCHO–Me_2SO 1:1 (0.5 mL) in the presence of anhydrous hydroxybenztriazol (75 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (75 μmol) for 24 h at 22 °C. After the reaction, the mixture was concentrated in vacuo, and fractionated on a Bio-Gel P-4 column (1.5 × 24 cm, Bio-Rad Lab., Hercules, CA) eluted with water. The conjugation efficiency of sulfated galactoside shown in Table 2 was determined by the amount of sugar eluted at the void volume, assuming the recovery of polyglutamic acid to be 100%.

Binding analyses of compounds.—The affinities of each compound to mouse L-selectin were determined using a liquid phase system as described before [19]. Briefly, 0.4 μg of L-selectin-IgG chimeric protein was incubated with ^{125}I-tyrosinated fucoidan in the presence of inhibitor ligands, and the bound radioactivity was measured by precipitation with protein G bacteria (Omnisorb cells, Calbiochem, La Jolla, CA).

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