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Chemically Defined Sialoside Scaffolds for Investigation of Multivalent Interactions with Sialic Acid Binding Proteins†

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Four glycodendrons and a glycocluster were synthesized from carbohydrate building blocks to form paucivalent (di- to tetravalent) structures of controlled scaffold architectures. Enzymatic sialylation of the functionalized cluster and dendrons, terminated in lactose residues, generated a library of paucivalent synthetic sialosides displaying sialic acids with different dispositions. These newly constructed bioactive sialic acid-based structures were differentially recognized by sialoadhesin, a mammalian macrophage sialic acid binding protein. The binding of the sialosides to sialoadhesin was evaluated by an enzyme-linked immunosorbant assay to investigate the complementarity of scaffold structure and binding to sialoadhesin. Modulating the interaction between sialoadhesin and its sialic acid ligands has important implications in immunobiology.

Introduction

The importance of carbohydrate binding proteins[1−5] (CBPs or lectins) in regulating biological systems is widely recognized and has inspired numerous efforts to understand and manipulate these processes. Typically, the biologically relevant interactions involve multivalent interactions between multimeric or membrane-bound CBPs and multiple carbohydrate ligands. The carbohydrates responsible for mediating cellular adhesion and communication are often complex oligosaccharides attached to proteins or lipids at the cell surface. The large structural and conformational diversity of these complex oligosaccharides allows nature to create a variety of scaffolds onto which the terminal saccharide ligands, recognized by lectins, are attached. Carbohydrates provide unique recognition units and scaffolds for mediating both physiological and pathological processes ranging from fertilization to angiogenesis.

The idea of modulating protein−carbohydrate interactions using multivalent synthetic ligands has been explored by a number of groups.[6] Highly polyvalent ligands, such as polymers with pendent oligosaccharides[7] or neoglycoproteins with sugars attached to lysine side chains in the proteins by reductive amination with pendent sugars,[8] have been found to bind to a variety of CBPs with dramatically enhanced affinities. Attaining enhanced binding affinities with designed synthetic paucivalent (di- to decavalent) constructs has met with less success. Bundle et al.[9] demonstrated that rational design can be applied to the successful synthesis of dendritic structures for binding the pentameric Shigalike toxin with high affinity. Other approaches[10] have introduced structural diversity by sequential changes to the lengths and constitutions of the linker(s) between two or more

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carbohydrate ligands to determine the structural requirements of the paucivalent constructs to obtain high binding affinities.

In this article, we evaluate the structural requirements for recognition of paucivalent ligands by a mammalian sialic acid binding protein, sialoadhesin, a member of the Siglec[11] (sialic acid/immunoglobulin-like/lectin) family of cell adhesion receptors. The Siglecs are currently comprised of eleven human and seven murine family members, all of which bind sialic acid-containing[12] carbohydrates as ligands via an N-terminal immunoglobulin domain. All but one are expressed on the surfaces of white blood cells in a highly cell type specific fashion. These lectins typically bind their preferred sialoside ligand with low affinities ($K_d \sim 0.2-1.0$ mM), and their functions are believed to be modulated by interactions with sialic acids on glycoproteins on the same cell (cis interactions) and on adjacent cells (trans interactions). To date, Siglecs have been demonstrated to be involved in a gamut of functions, ranging from myeloid cell interactions to activation of B cells and regulation of neuronal cell growth and myelination.[13] Thus, the ability to alter the interaction between the Siglecs and their sialic acid ligands may have pharmaceutical implications for such diverse applications as modulation of immune function and nerve regeneration.

Sialoadhesin[14−18] (Siglec 1), a macrophage-restricted sialic acid binding receptor, mediates interactions with hemopoeitic cells, including granulocytes and lymphocytes. The crystal structure[19] of the N-terminal domain of sialoadhesin complexed to 3′-sialyllactose has been solved at 1.85 Å resolution. The sialic acid moiety is bound in a shallow pocket, stabilized by hydrogen bonding and van der Waals interactions, with no discernible interactions involving the lactose residue. Although the affinity of sialoadhesin for the monovalent sialoside[20] is approximately 1 mM, cell surface sialoadhesin mediates adhesion of cells, demonstrating the importance of multivalent interactions in its biological function.[21] Several synthetic multivalent sialoside constructs have been demonstrated to form stable binding interactions with sialoadhesin, including sialosides covalently linked to lysines on aggregated bovine serum albumin or attached to biotin and adsorbed to a streptavidin alkaline phosphatase conjugate.[22] However, because the spatial relationships between the sialosides in these constructs are not controlled, little concrete information has been obtained about the molecular requirements for high-affinity multivalent interactions.

We have been actively developing carbohydrate-based dendrons and dendrimers[23] for the multivalent display of saccharides using a suitably protected AB_2 trisaccharide as the monomer unit where reductive amination is used to couple the units together chemoselectively. Following nature's lead, we have turned to carbohydrate-based dendrons to create branched oligosaccharide structures whose overall conformations are dictated by the configuration of the trisaccharide monomer units. In particular, the α-linkage in the maltosyl component of dendrons **7** and **9** directs the two sialoside ligands toward the same face while the β-linkage of dendron **17** directs the two sialoside ligands toward opposite faces. Here, we report a chemo-enzymatic approach to a range of suitably branched, well-defined carbohydrate-based paucivalent sialosides,[24] designed to display, on their peripheries, ligands capable of interaction with sialoadhesin in a multivalent manner. Evaluation of the interaction of these sialosides with sialoadhesin in an enzyme-linked immunosorbant assay (ELISA)-type assay reveals differential structural requirements for optimal multivalent binding interactions.

Results and Discussion

Synthetic Strategy. Five sialylated α-2,3-linked *O*-glycans have been derived (Figure 1) from lactose-based monomers, clusters, and glycodendrons. In the formation of the multivalent lactosides, we first embarked on the synthesis of lactose-based targets that comprise different spatial arrangements of their peripheral lactose residues. Then, in the final stages, the deprotected lactose derivatives were treated with sialyltransferases[25−27] to afford the final sialylated compounds. Starting with the tetravalent sialocluster (Figure 1a) and proceeding to diva-

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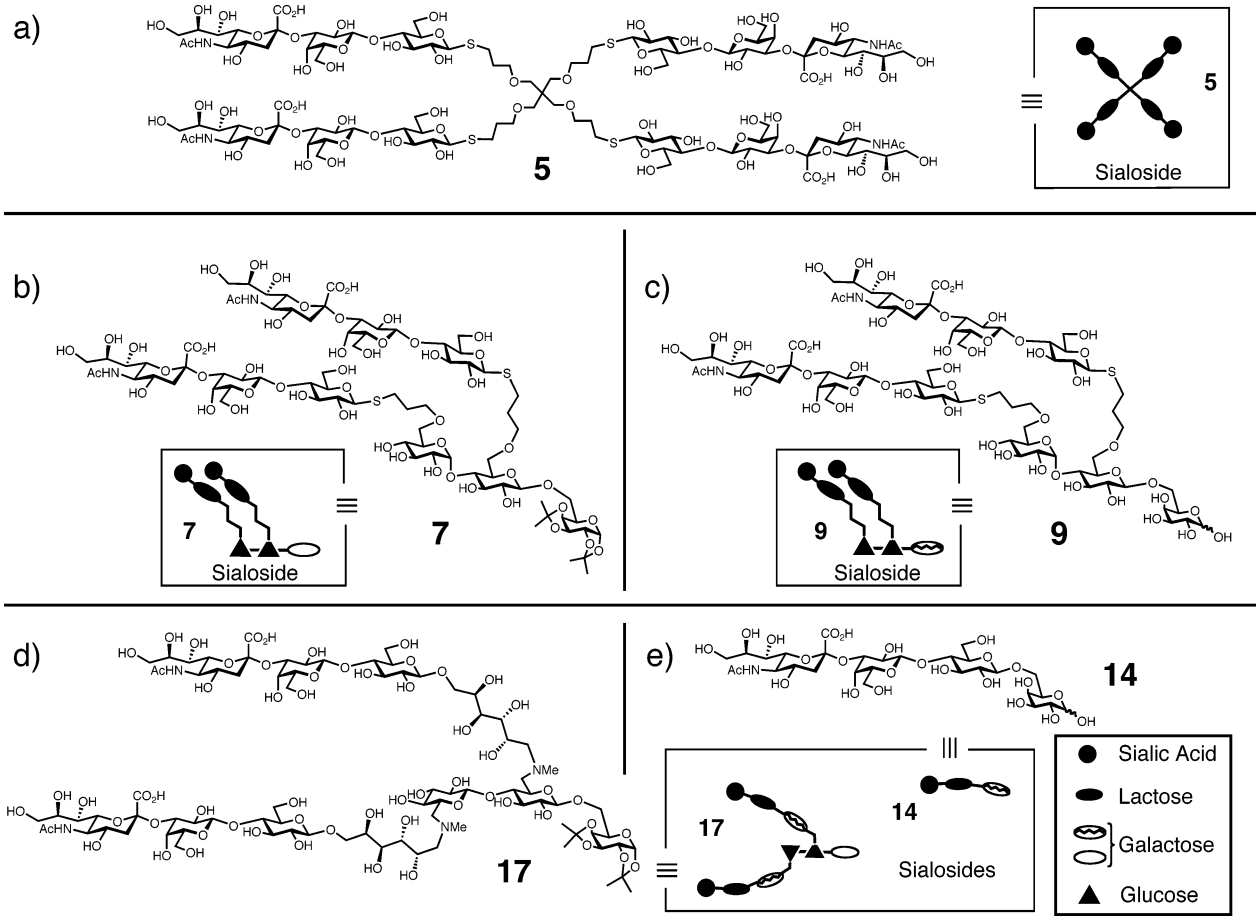

FIGURE 1. Five sialylated α-2,3-linked *O*-glycans derived from monomer (e), cluster (a), and glycodendron (b, c, d) scaffolds.

lent sialo-glycodendrons (Figure 1b−d) and a monovalent sialomonomer (Figure 1e), it was possible to present the sialic acid residues in precise orientations about these differing scaffolds.

Chemical Preparation of Lactose-Based Derivatives Followed by Enzymatic Preparation of Sialylated Derivatives. The lactose-based targets were synthesized employing photoaddition[28−30] and reductive amination[31−33] methodologies. These neutral lactose precursor oligosaccharides, prepared by chemical synthesis, were sialylated enzymatically with purified sialyltransferase to form the α-D-Neu5Ac-(2,3)-β-D-Gal-linked sialyloligosaccharides (Schemes 1−4).

The anti-Markovnikov photoaddition of peracetylated β-D-thiolactose[34] **2** to tetra-*O*-allyl pentaerythritol[35] **1**, a core[36,37] commonly used in the construction of glycoclusters, is outlined in Scheme 1. The thioether-linked acetyl-protected tetravalent cluster lactoside **3** was obtained in 45% yield upon irradiation of the reaction mixture with UV light from a mercury lamp. Following deacetylation under Zemplén conditions, the unprotected lactoside cluster **4** was converted into the sialylated derivative **5** upon transfer of the *N*-acetyl neuraminic acid (Neu5Ac) from the nucleotide donor (CMP-Neu5Ac)[25b] to the acceptor lactoside **4** with catalytic assistance from α-2,3-sialyltransferase fusion protein[25a] (ST3-fusion) to afford the sialylated **5** in 97% yield.

The syntheses of the sialylated maltosyl-based dendrons **7** and **9** from the lactosides **6** and **8**, respectively, are shown in Scheme 2. All of these dendrons contain, in their interior, a trisaccharide composed of a functionalized maltose residue linked to a protected galactose; indeed, the AB_2 monomer building block we developed previously.[23a] Emanating from the primary positions of

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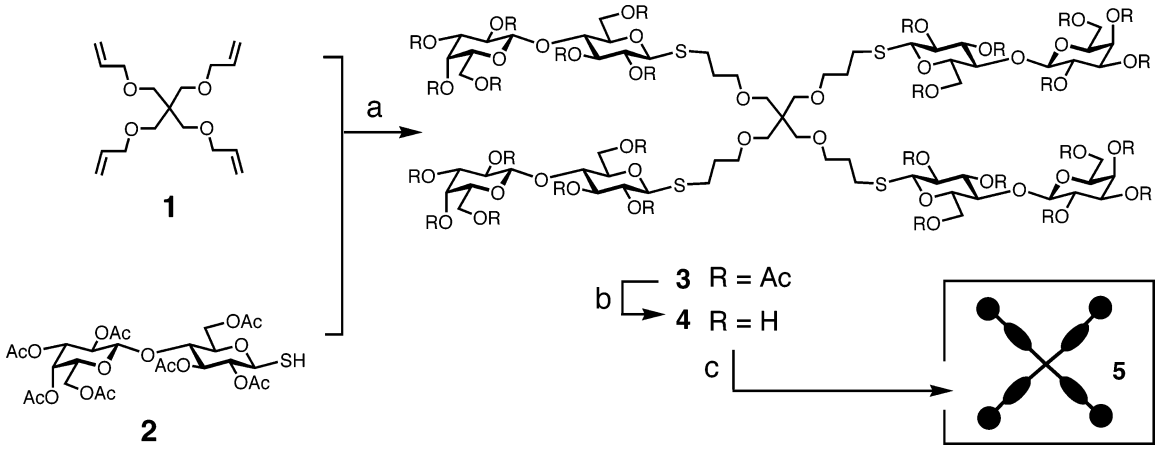
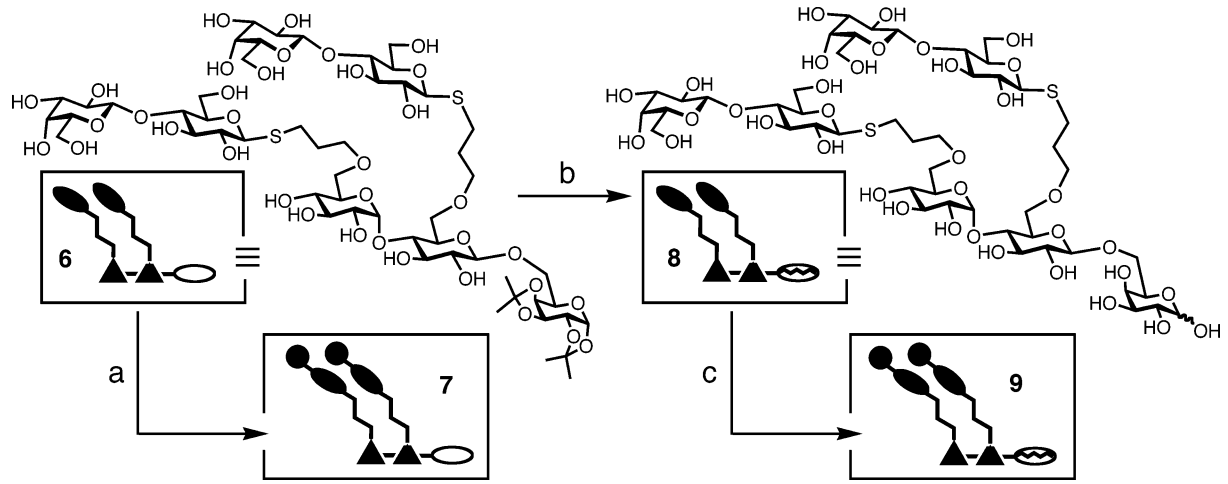
SCHEME 1. Synthesis of the Tetrasialoside 5[a]



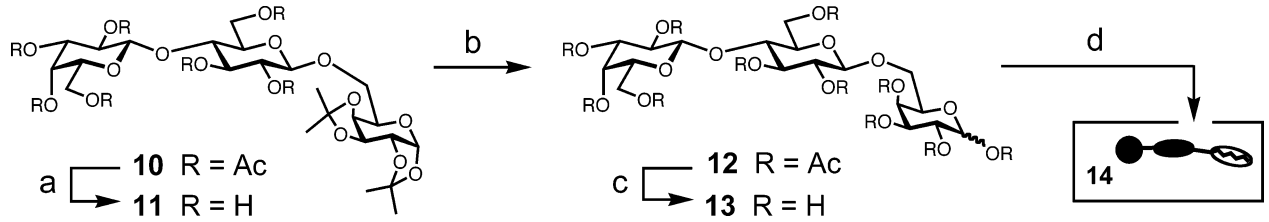
[a] Reagents and conditions: (a) MeOH, *hν*, rt, 5 h (45%); (b) NaOMe, MeMeOH, rt, 12 h (90%); (c) ST3-fusion, CMP-Neu5Ac, $MnCl_2$, cacodylate buffer, pH ~6−7, rt, 12 h (97%).

SCHEME 2. Syntheses of the Disialosides 7 and 9[a]



[a] Reagents and conditions: (a) ST3-Fusion, CMP-Neu5Ac, $MnCl_2$, cacodylate buffer, pH ~6−7, rt, 12 h (95%); (b) TFA, H_2O (9:1), rt, 5 min (quantitative); (c) ST3-fusion, CMP-Neu5Ac, $MnCl_2$, cacodylate buffer, pH ~6−7, rt, 12 h (97%).

SCHEME 3. Synthesis of Monomer Sialoside 14[a]



[a] Reagents and conditions: (a) NaOMe, MeOH, rt, 6 h (83%); (b) (i) TFA, H_2O (9:1), rt, 1 h, (ii) C_5H_5N, DMAP, Ac_2O, rt, 16 h (50%); (c) NaOMe, MeOH, rt, 3 h (74%); (d) ST3-fusion, CMP-Neu5Ac, $MnCl_2$, cacodylate buffer, pH ~6−7, rt, 12 h (96%).

the glucose residues are lactose-derived appendages. The syntheses of the lactosides **6** and **8** are based on unpublished research. The lactoside **8** was generated by trifluoroacetic acid-catalyzed hydrolysis of **6**. The lactosides **6** and **8** were converted to their sialylated derivatives **7** and **9**, respectively, using ST3-fusion enzymatic conditions similar to those employed in the preparation of the sialylated derivative **5**.

The synthesis of the sialylated lactose monomer **14** is depicted in Scheme 3. The lactose trisaccharide **10** was readily obtained from lactosyl trichloroacetimidate and diacetone-D-galactose, as described previously by Wong and co-workers.[38] Following deacetylation under Zemplén conditions, the acetylated trisaccharide **10** was deprotected to give **11** in 83% yield. The final three steps toward the synthesis of monomer **14** involved (1) trifluoroacetic acid-catalyzed hydrolysis of the trisaccharide **11**

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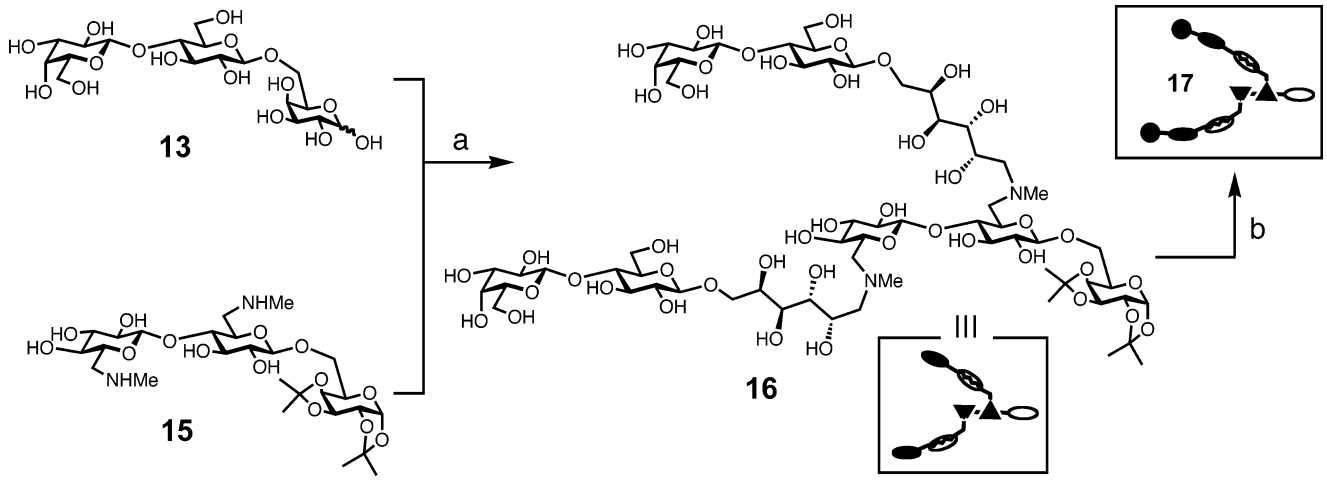
SCHEME 4. Synthesis of Disialoside 17[a]



[a] Reagents and conditions: (a) $NaCNBH_3$, MeOH, AcOH, 60 °C, 6 h (50%); (b) ST3-fusion, CMP-Neu5Ac, $MnCl_2$, cacodylate buffer, pH ~6−7, rt, 12 h (96%).

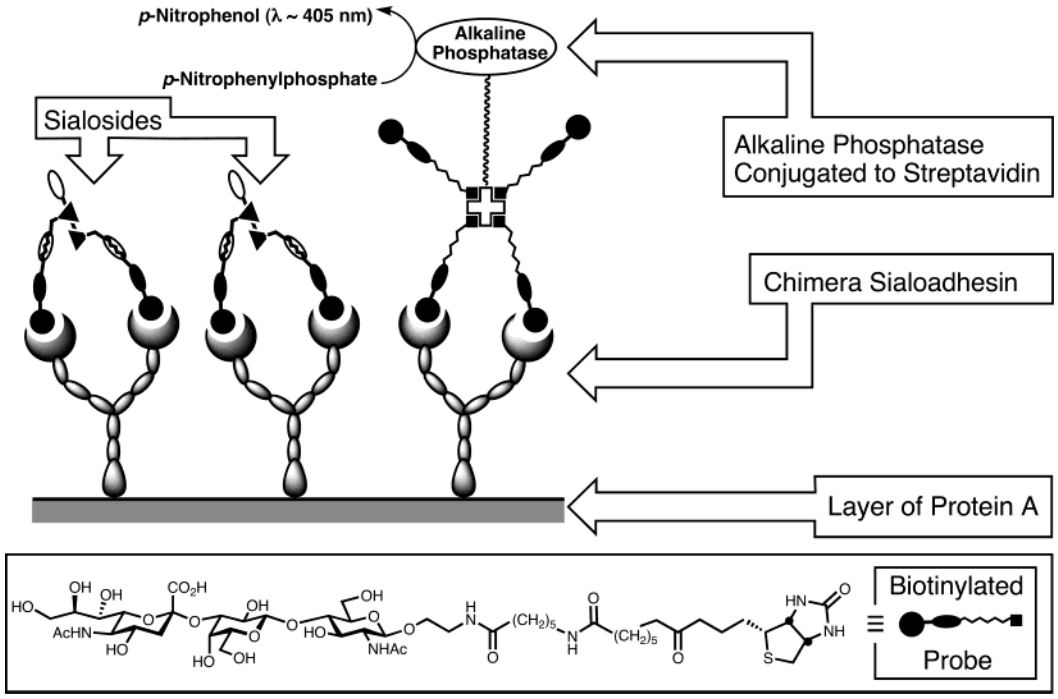


FIGURE 2. Competitive ELISA for evaluating sialyloligosaccharide binding to sialoadhesin.

and then reacetylation of **11** in 50% yield to obtain the pure trisaccharide **12**, (2) subjection to a final Zemplén deacetylation to afford the monomer lactoside **13**, and (3) treatment in the final step with ST3-fusion to yield (96%) the sialylated trisaccharide **14**. The lactose-based trisaccharide **13** was also used as the reducing sugar to couple, under reductive amination conditions, to the bismethylamino-cellobiosyl-based monomer **15,** previously prepared by our group,[23c] generating the bislactose dendron **16** in 50% yield. This final bislactose acceptor was sialylated (Scheme 4) under ST3-fusion enzymatic conditions to afford the sialylated dendron **17** in good yield.

Evaluating Sialyloligosaccharide Binding to Sialoadhesin. With the paucivalent sialyloligosaccharides **5**, **7**, **9**, **14**, and **17** in hand, we sought to evaluate their ability to exhibit enhanced binding to sialoadhesin in a multivalent binding assay. To this end, an ELISA,[39] employing a multivalent sialoside probe binding to a bivalent recombinant sialoadhesin−immunoglobulin chimera,[40] was used (Figure 2). The assay employs a sialoside probe, comprised of sialyllactose linked to biotin, bound to a streptavidin-alkaline phosphatase complex

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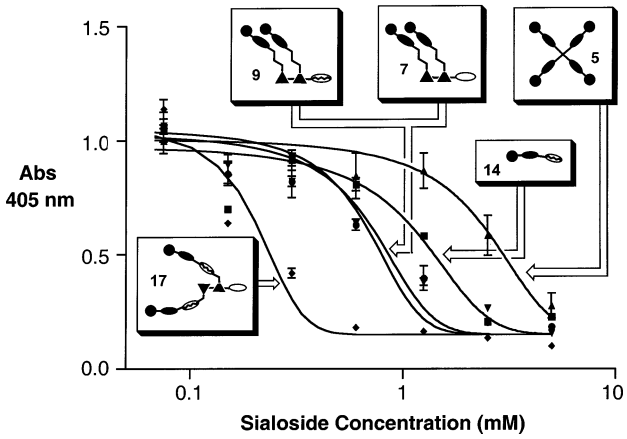

FIGURE 3. Inhibition curves from competition experiments carried out between the sialyloligosaccharides and the biotinylated sialyllactose for the sialoadhesin binding sites. Absorbances were measured at 405 nm.

TABLE 1. Comparison of Inhibitory Potencies (IC_{50} Values) of Sialosides 5, 7, 9, 14, and 17 in the Sialoadhesin Binding (ELISA) Assay[a]

sialoside	IC_{50}[b] (mM)	rel IC_{50}[c] (mM)
5	2.4	9.6
7	0.7	1.4
9	0.7	1.4
14	1.4	1.4
17	0.2	0.4

[a] In the ELISA assay, the evaluated sialoside competes with a biotinylated probe in binding to a bivalent recombinant sialoadhesin−immunoglobulin chimera. [b] The inhibition curve for each compound was fitted with a nonlinear regression formula of PRISM (GraphPad Software, Inc., San Diego, CA) to generate the IC_{50} values shown. [c] The relative IC_{50} values were obtained by multiplying the IC_{50} value by the number of sialic acid ligands present in the molecule.

(SA−AP). This probe was added to wells of a microtiter plate containing the recombinant sialoadhesin-immunoglobulin chimera adsorbed to protein A, coated on the surface of the wells. After washing, the probe bound to the sialoadhesin−immunoglobulin chimera is revealed upon addition of the *p*-nitrophenyl phosphate substrate for the alkaline phosphatase. Hydrolysis of the substrate yields *p*-nitrophenol (yellow), which is detected in an automated plate reader as an increase in absorbance at 405 nm.

The relative affinity of the sialyloligosaccharides for sialoadhesin was evaluated by comparing their ability to compete with the binding of the SA−AP probe, as shown in Figure 3 and summarized in Table 1. As a reference, the monovalent sialoside **14** exhibited an IC_{50} of 1.4 mM. Of the three disialosides investigated, the two maltosyl-based dendrons, **7** and **9**, had 2-fold increased potency (IC_{50} = 0.7 mM), while the cellobiosyl-based dendron, **17**, had nearly 7-fold increased potency (IC_{50} = 0.2 mM). In contrast, the tetravalent cluster **5** (IC_{50} = 2.4 mM) was less potent than the monomer and over 10 times less potent than the cellobiosyl-based dimer.

Conclusions

Several novel paucivalent sialyloligosaccharide ligands of sialoadhesin have been synthesized by chemo-enzymatic methods to investigate the influence of valency and ligand architecture on multivalent attachment and increased affinity. First, precursor lactose-based monomers, clusters, and dendrons were synthesized chemically by photoaddition and reductive amination strategies. Then, sialic acid was attached enzymatically to these lactose precursor compounds with the assistance of purified sialyltransferases to enable the specific formation of α-D-Neu5Ac-(2,3)-β-D-Gal linkages. The resulting new sialyloligosaccharides had molecular weights ranging from 796 to 2895 and were characterized by MALDI-TOF mass spectrometry and ^1H and ^{13}C NMR spectroscopies.

Capitalizing upon the natural complementarity that exists between biological Siglecs and sialyloligosaccharides, binding studies between sialoadhesin and the five novel sialyloligosaccharides have been conducted. These studies were exploratory efforts to help unravel the optimal conformation of the underlying scaffold for the optimal display of 2,3-linked sialic acids. Specifically, competitive ELISA was used to measure how much the synthetic sialyloligosaccharides could inhibit the biotinylated probe from binding to sialoadhesin.

The relative affinities of these sialosides for sialoadhesin, measured as inhibitor potency (Figure 3 and Table 1), correlated poorly with valency. Indeed, although the tetrasialoside **5**, based on a pentaerythritol-based cluster scaffold, had the highest valency, it was the least potent inhibitor of all the sialosides tested, including the monosialoside **14**. The reduced potency of this symmetrical tetravalent ligand could reflect the fact that the close spacing of the ligands sterically restricts their interaction with sialoadhesin relative to the monomer. Two of the divalent sialosides, the maltosyl-based dimers **7** and **9**, had a 2-fold increased potency relative to that of the monomer **14**. While these dimers were 2-fold more potent than the monovalent sialoside on a molar basis (IC_{50} values of 0.7 vs 1.4 mM), the apparent increase in the affinities of the dimers could be accounted for by the two sialoside ligands binding independently or even rebinding as a result of a higher effective local concentration of the second ligand once the first ligand is bound. In this regard, the 7-fold increase in binding of the cellobiosyl-based dimer **17** over the monomer **14** on a molar basis (IC_{50} values of 0.2 vs 1.4 mM) suggests that the two sialoside ligands bind cooperatively and may facilitate receptor clustering.

The increased potency of the divalent cellobiosyl-based sialoside **17** suggests that it has a preferred architecture for multivalent binding to sialoadhesin in the assay compared to the maltosyl-based sialosides, namely, **7** and **9**. These maltosyl-based dendrons share the same IC_{50} values, despite the presence or absence of acetonide protecting groups on their galactose units. This result suggests that the internal structure of the scaffolds does not influence the interaction with the sialoadhesin binding site. This outcome is perhaps not surprising in view of the shallow binding pocket for the terminal sialic acid revealed by the crystal structure.[19] The maltosyl and cellobiosyl building blocks differ in several respects. First, they differ in the configuration of the anomeric linkage between the two glucopyranose units in the core (α and β, respectively). Furthermore, the spacer group between the sialyllactose residues and the building block is a short propylene linker for dendrons **7** and **9** whereas it is a

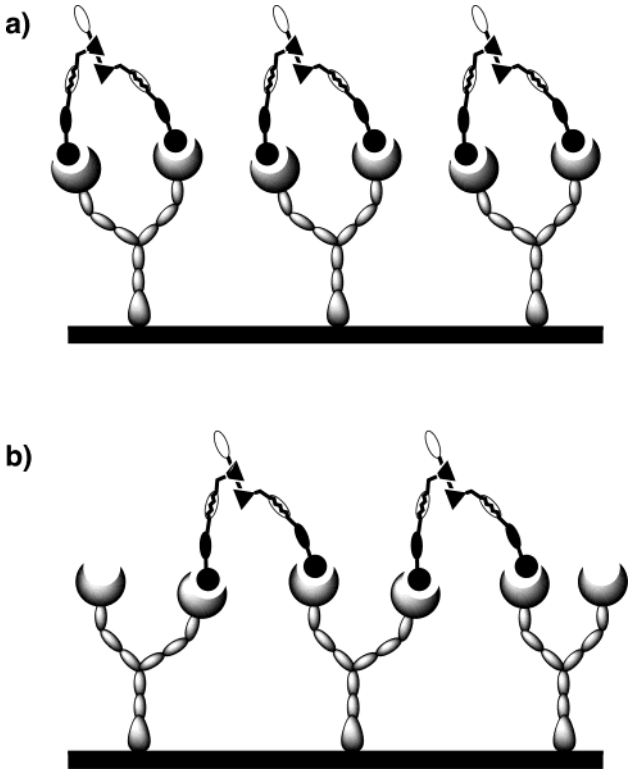

FIGURE 4. Two potential interactions of bivalent dendrons with sialoadhesin−Fc chimera molecules: The architecture of the dimer supports simultaneous binding to (a) sialoadhesin on both arms of the Fc-chimera or (b) adjacent sialoadhesin−Fc chimera molecules.

longer galactitol linker for dendron **17**. Such differences may be sufficient to provide the optimal spacing and architectural requirements for effective binding of the dendron **17** to sialoadhesin in the assay used to evaluate their potency.

It is noteworthy that the binding assay employs a bivalent sialoadhesin−Fc chimera, which is fixed to the surface of the plastic microwells by binding to protein A coated on the surface. Thus, the cellobiosyl−sialoside dimer could influence multivalent binding to sialoadhesin in two distinctly different ways (Figure 4). On one hand, the architecture of the dimer may allow simultaneous binding to the two sialoadhesin domains on the tips of a single sialoadhesin-Fc chimera (Figure 4a). Alternatively, it could allow binding to two adjacent sialoadhesin-Fc chimera molecules (Figure 4b) or indeed to a combination of both. We are actively pursuing the modeling of these sialyloligosaccharides in complexes with the chimera sialoadhesin receptor to help explain these results. In addition, we are elaborating the sialyloligosaccharides to larger dendritic structures such as glycodendrimers[6] in the hope of amplifying the multivalent effect in the inhibition results we witnessed with the ELISA binding studies.

Experimental Section

Pentaerythritol Tetrakis[(tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-thioglucopyranosyl)-3-propyl] Tetraether (3). Peracetylated *β*-thiolactose[34] (391 mg, 600 *μ*mol) and tetra-*O*-allyl-pentaerythritol[35] (15 mg, 50 *μ*mol) were dissolved in freshly distilled MeOH (10 mL). Argon was bubbled through the solution for 30 min to thoroughly degas the solvent. The vial was then filled with argon, sealed, and then irradiated with a Hg lamp, and the mixture was stirred for 5 h. The reaction mixture was concentrated to a smaller volume, and the resulting solution was purified using gel filtration chromatography (LH-20, MeOH) to give **3** (65 mg, 45%) after freeze-drying. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.73−1.82 (m, 8H), 1.95, 2.04, 2.05, 2.10, 2.14 (5s, 84H), 2.66−2.70 (m, 8H), 3.28, 3.32 (2t, J = 5.8 Hz, 16H), 3.63 (m, 4H), 3.77 (t, J = 9.4 Hz, 4H), 3.88 (m, 4H), 4.05−4.13 (m, 16H), 4.45−4.50 (m, 12H), 4.88 (m, 4H), 5.07−5.11 (m, 4H), 5.18−5.21 (t, J = 9.2 Hz, 4H), 5.33−5.34 (d, J = 2.6 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6 (3C), 20.7, 20.8, 27.4, 29.9, 60.6, 62.2, 66.5, 69.0, 69.3, 70.3, 70.5, 70.9, 73.7, 76.1, 76.5, 76.7, 83.7, 101.0, 169.0, 169.5, 169.6, 169.9, 170.0, 170.1 (2C), 170.2. MS (MALDI-TOF) m/z 2927.76 $[M + Na]^+$.

Pentaerythritol Tetrakis[(*β*-D-galactopyranosyl-(1→4)-*β*-D-thioglucopyranosyl)-3-propyl] Tetraether (4). NaOMe (1 mL, 0.5 M in MeOH, 0.5 mmol) was added to a methanolic (5 mL of MeOH) solution of the peracetylated derivative **3** (65 mg, 22 *μ*mol), and the mixture was left to stir at room temperature overnight. The solution was then neutralized with Amberlite IR-120 (H^+ form) ion-exchange resin, filtered, and concentrated to yield **4** (35 mg, 90%). ^{1}H NMR (D_2O, 500 MHz): δ 1.94 (bs, 8H), 2.70−2.85 (m, 8H), 3.39−3.99 (band of m, 64H), 4.46−4.47 (d, 4H, J = 7.6 Hz), 4.56−4.58 (d, 4H, J = 9.9 Hz). ^{13}C NMR (D_2O, 125 MHz): δ 26.7, 29.1, 60.2, 61.0, 68.5, 69.1, 69.9, 70.9, 72.0, 72.5, 75.3, 75.7, 78.1, 78.6, 85.3, 102.8, 170.1. MS (MALDI-TOF) m/z 1751.52 $[M + Na]^+$.

General Procedure for the Synthesis of Sialosides 5, 7, 9, 14, and 17. The lactoside (6.9 *μ*mol) and CMP-Neu5Ac (88 mg, 83 *μ*mol) were dissolved in H_2O (1.2 mL) containing ST3-fusion[25] (2.0 mL, 11 U), $MnCl_2$ (0.4 mL, 200 mM), and cacodylate buffer (0.4 mL, 500 mM, pH = 6.6). The pH of the reaction was maintained between 6 and 7, and the progress of the reaction was monitored by TLC (EtOAc/MeOH/AcOH/H_2O, 4:3:3:2). After 1 day, the reaction mixture was centrifuged to remove some of the excess of the fusion protein and the supernatant was further purified by gel filtration chromatography (Sephadex G25, 95% H_2O, 5% *n*BuOH) to afford the target sialoside.

Pentaerythritol Tetrakis[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-thioglucopyranosyl)-3-propyl] Tetraether (5). ^{1}H NMR (D_2O, 500 MHz): δ 1.66 (t, 4H, J = 12.1 Hz), 1.80 (t, 8H, J = 6.5 Hz), 2.60−2.70 (m, 24 H), 3.29−3.70 (band of m, 80 H), 3.81 (d, 4H, J = 2.7 Hz), 3.92 (d, 4H, J = 3.0 Hz), 3.96 (dd, 4H, J = 10.0 Hz), 4.32 (d, 4H, J = 8.1 Hz), 4.39 (d, 4H, J = 7.7 Hz). ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 26.6, 29.0, 39.4, 51.5, 60.1, 60.9, 62.4, 67.3, 67.9, 68.2, 68.9, 69.2, 69.4, 69.7, 71.6, 71.9, 72.7, 75.0, 75.3, 75.6, 77.9, 78.5, 85.2, 99.7, 102.4, 173.8, 174.8. MALDI-FTMS m/z: $[M + Na]^+$ calcd for $C_{109}H_{184}N_4O_{76}S_4Na$, 2917.0215; found, 2917.0215.

Bis[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-thioglucopyranosyl-3-propyl]-6,6′-*β*-maltosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (7). Selected ^{1}H NMR (D_2O, 500 MHz) data: δ 1.27, 1.35, 1.45, 1.46 (4s, 12H), 1.66 (t, 2H, J = 12.0 Hz), 1.82 (m, 4H), 1.89 (s, 6H), 2.62 (m, 6H), 3.23 (m, 3H), 3.33 (m, 1H), 3.90 (m, 2H), 3.92 (m, 2H), 4.40 (m, 5H), 5.19 (d, 1H, J = 3.7 Hz), 5.54 (d, 1H, J = 4.9 Hz). ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 23.1, 24.7, 24.8, 39.5, 51.5, 60.9, 62.4, 67.3, 67.9, 68.2, 69.2, 69.3, 69.4, 69.7, 69.8, 71.6, 71.9, 72.7, 75.0, 75.3, 75.6, 78.5, 85.3, 95.6, 99.6, 102.5, 109.8, 109.9, 173.7, 174.8. MALDI-FTMS m/z: $[M + Na]^+$ calcd for $C_{76}H_{126}N_2NaO_{52}S_2$, 1986.1662; found, 1986.1661.

Bis[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-thioglucopyranosyl-3-propyl]-6,6′-*β*-maltosyl-(1→6)-D-galactose (9). Selected ^{1}H NMR (D_2O, 500 MHz)

data: δ 1.66 (t, 2H, *J* = 12.0 Hz), 1.82 (m, 4H), 1.89 (s, 6H), 2.62 (m, 6H), 3.23 (m, 3H), 3.63 (m, 2H), 3.90 (m, 2H), 3.92 (m, 2H), 4.40 (m, 5H), 5.19 (d, 1H, *J* = 3.7). ^{13}C NMR (D_2O, 125 MHz): δ 13.9, 21.9, 26.6, 29.1, 29.5, 31.5, 31.6, 31.9, 37.5, 39.5, 51.5, 56.7, 60.1, 60.9, 62.4, 67.3, 67.9, 68.2, 69.1, 69.2, 69.3, 69.4, 69.6, 69.7, 71.6, 71.09, 72.7, 73.6, 75.0, 75.3, 75.6, 77.9, 78.5, 85.2, 92.2, 96.3, 99.8, 102.3, 102.4, 102.7, 113.7, 126.6, 173.7, 174.8. MALDI-FTMS *m/z*: $[M + Na]^+$ calcd for $C_{70}H_{118}N_2NaO_{52}S_2$, 1906.9993; found, 1907.0001.

***β*-D-Galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (11).** A solution of **10** (21.8 g, 24.8 mmol) and NaOMe (1.5 mL, 0.5 M in MeOH, 0.75 mmol) in MeOH (150 mL) was stirred for 6 h at room temperature. The solution was neutralized with Amberlite IR-120 (H^+ form) ion-exchange resin, filtered, and concentrated to afford **11** (12.02 g, 83%) as a foam. ^{1}H NMR (CD_3OD, 500 MHz): δ 1.29−1.49 (4s, 12H), 3.24 (dd, *J* = 7.8 Hz, *J* = 8.35 Hz), 3.40 (ddd, *J* = 2.3 Hz, *J* = 4.4 Hz, *J* = 9.8 Hz), 3.47 (t, 1H), 3.48−3.60 (m, 4H), 3.62 (m, 1H), 3.67 (dd, *J* = 4.6 Hz, *J* = 11.4 Hz, 1H), 3.74 (dd, *J* = 7.5 Hz, 1H), 3.78−3.81 (m, 1H), 3.80 (dd, *J* = 12.2 Hz, 1H), 3.88 (dd, 1H), 3.98−4.03 (m, 2H), 4.28 (dd, *J* = 1.3 Hz, *J* = 7.9 Hz, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 4.31 (d, *J* = 7.4 Hz, 1H), 4.33 (dd, *J* = 2.3 Hz, *J* = 5.0 Hz, 1H), 4.60 (dd, 1H), 5.48 (d, 1H). ^{13}C NMR (CD_3OD, 125 MHz): δ 24.5, 25.1, 26.3 (2C), 61.9, 62.4, 68.8, 69.8, 70.2, 71.8, 71.85, 72.35, 72.4, 74.5, 74.7, 76.0, 76.4, 77.0, 80.6, 97.6, 104.5, 105.0, 110.0, 110.4. FAB-MS *m/z*: 607.46 [M + Na]$^+$, 585.49 [M + H]$^+$, 569.42 [M − CH_3]$^+$. HR FAB-MS *m/z*: $[M + H]^+$ calcd for $C_{24}H_{41}O_{16}$, 585.2395; found, 585.2386.

2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-galactopyranose (12). Diacetonide **11** (11.16 g, 19.11 mmol) was dissolved in TFA/H_2O (100 mL, 9:1) and stirred at room temperature for 1 h. The solution was concentrated and then coevaporated with MeOH (200 mL). The resulting foam was dissolved in H_2O (50 mL) and freeze-dried. The crude product was then dissolved in C_5H_5N (50 mL) before adding DMAP (50 mg) and acetic anhydride (25 mL). The reaction was stirred at room temperature for 16 h before being poured into 1 M HCl (500 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 500 mL). The organic layer was then washed with saturated aqueous $NaHCO_3$ (2 × 500 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/EtOAc, 1:1) gave **12** (15.15 g, 50%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.92−2.16 (m, 33H), 3.25−3.50 (m, 2H), 3.67−3.72 (m, 1H), 3.73−3.79 (m, 1H), 3.85 (m, 1H), 3.97 (m, 0.6H), 4.00−4.14 (m, 3H), 4.22 (m, 0.4H), 4.41−4.49 (m, 3H), 4.79−4.84 (m, 1H), 4.90−4.96 (m, 1H), 5.02−5.18 (m, 3H), 5.25−5.30 (m, 1H), 5.32 (m, 1H), 5.38 (m, 0.6H), 5.46 (m, 0.4H), 5.66 (d, *J* = 2.41 Hz, 0.6H), 6.32 (d, *J* = 8.33 Hz, 0.4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.6−21.0 (11C), 60.9, 61.9, 66.2, 66.7, 67.0 + 67.6 (1C), 67.1 + 67.8 (1C), 69.2, 69.9 + 72.83 (1C), 70.8, 71.03 + 72.79 (1C), 71.08, 71.37 + 71.42 (1C), 72.71, 72.74, 76.1, 89.7 + 92.2 (1C), 101.0, 101.1, 169.1−170.5 (11C). FAB-MS *m/z* 989.12 $[M + Na]^+$. HRFAB-MS *m/z*: $[M + Na]^+$ calcd for $C_{40}H_{54}NaO_{27}$, 989.2750; found, 989.2748.

***β*-D-Galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-D-galactopyranose (13).** A solution of **12** (11.0 g, 11.39 mmol) and NaOMe (5 mL, 0.5 M in MeOH) in MeOH (150 mL) was stirred for 3 h at room temperature. Formation of a pale-yellow solid was observed. The reaction mixture was then filtered, and the filtrate was evaporated, affording more solid, which was filtered and washed with MeOH. After three recovery operations from the filtrate, **13** (4.235 g, 74%) was obtained as a pale-yellow powder. ^{1}H NMR (D_2O, 500 MHz): δ 3.20−4.15 (m, 12H), 4.33−4.48 (m, 2.67H), 5.15 (d, *J* = 3.8 Hz, 0.33H). ^{13}C NMR (D_2O, 125 MHz): δ 59.8, 60.8 (2C), 68.0−78.2 (12C), 69.3, 92.3 + 96.5 + 102.7 (1C), 102.2. ES-MS *m/z*: 1031.3 $[2M + Na]^+$, 527.0 $[M + Na]^+$, 505.1 $[M + H]^+$. HR MALDI-MS *m/z*: $[M + Na]^+$ calcd for $C_{18}H_{32}NaO_{16}$, 527.1588; found, 527.1591.

(5-Acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)−(2→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-D-galactopyranose (14). ^{1}H NMR (D_2O, 500 MHz): δ 1.65 (t, *J* = 12.0 Hz, 1H), 1.88 (s, 3H), 2.60 (dd, *J* = 4.1 Hz, *J* = 12.0 Hz, 1H), 3.20 (br t, 1H, H-2b), 3.45−3.93 (m, 22H), 3.95 (d, *J* = 3.3 Hz, 1H), 4.15 (br s, 1H), 4.38 (br s, 1H), 4.44 (br s, 1H), 5.15 (br s, 1H). ^{13}C NMR (D_2O, 125 MHz): δ 21.9, 22.0, 39.2, 39.4, 51.5, 52.1, 60.9, 62.4, 67.9, 68.6, 69.1, 69.4, 71.6, 72.4, 72.6, 72.7, 73.6, 74.0, 74.6, 75.0, 75.3, 78.0, 92.1 + 102.2 (1C), 96.2, 99.6, 102.3, 102.5, 173.7, 174.8. MALDI-FTMS *m/z*: $[M + Na]^+$ calcd for $C_{29}H_{49}NaO_{24}$, 818.2537; found, 818.2537. HRESI *m/z*: $[M - H]^-$ calcd for $C_{29}H_{48}O_{24}$, 794.2566; found, 794.2590.

Bis[*β*-lactosyl-(1→6)-D-galactit-1-yl]-6,6′-dideoxy-6,6′-(dimethylamino)-*β*-cellobiosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (16). Acetic acid (28 μL, 570 μmol) was added to a solution of the reducing sugar **13** (430 mg, 850 μmol), the bismethylamino monomer **15** (200 mg, 320 μmol), and sodium cyanoborohydride (131 mg, 2.1 mmol) in MeOH (20 mL). The reaction mixture was stirred and heated under reflux for 6 h. The mixture was allowed to cool to room temperature, concentrated, redissolved in H_2O (3 mL), and purified by preparative reverse-phase chromatography (15 g C-18 reverse-phase, MeOH/H_2O, 0:100 to 100:0) to afford pure **16** (254 mg, 50%). Selected ^{1}H NMR (CD_3OD, 500 MHz) data: δ 1.41, 1.42, 1.49, 1.62 (4s, 12H), 2.78 (br s, 3H), 2.92 (br s, 3H), 3.08 (m, 2H), 4.01 (m, 1H), 4.14−4.31 (m, 6H), 4.60 (dd, *J* = 2.2 Hz, *J* = 7.9 Hz, 1H), 5.51 (d, *J* = 4.9 Hz, 1H). ^{13}C NMR (D_2O, 125 MHz): δ 23.0, 23.8, 24.6, 24.8, 43.5 (2C), 58.4 (6C), 59.8, 60.8 (2C), 67.4, 68.3, 69.3, 69.7, 70.3, 70.4, 70.7, 70.9, 71.7, 72.3, 72.6, 72.9, 74.1, 75.2, 78.2 (3C), 95.6, 102.0, 102.4, 102.8, 109.8, 109.9, 115.0. ESMS *m/z* 1588.8 $[M + H]^+$, 794.7 $[M + 2H]^{2+}$.

Bis[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-D-galactit-1-yl]-6,6′-dideoxy-6,6′-(dimethylamino)-*β*-cellobiosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (17). Selected ^{1}H NMR (D_2O, 500 MHz) data: δ 1.25, 1.33, 1.44, 1.46 (4s, 12 H), 1.66 (d, *J* = 12.0 Hz, 2H), 2.60 (d, *J* = 12.0 Hz, 2H), 2.52−2.82 (m, 8H), 3.81 (d, *J* = 2.7 Hz, 2H), 3.92 (d, *J* = 3.0 Hz, 2H), 3.96 (d, *J* = 10.0 Hz, 2H), 4.41 (m, 6H), 5.53 (d, *J* = 4.9 Hz, 1H). ^{13}C NMR (D_2O, 125 MHz): δ 21.9 (2C), 31.6, 39.2, 39.4, 51.5, 52.1, 60.9, 62.4, 63.1, 67.1, 67.3, 67.9, 68.1, 68.4, 69.2, 69.4, 69.5, 70.0, 70.1, 71.6, 72.7, 74.6, 75.0, 75.3, 95.7, 96.2, 99.6, 102.0, 102.4, 102.5, 109.8, 109.9, 113.7, 173.7, 174.8. MALDI-FTMS *m/z*: $[M + H]^+$ calcd for $C_{84}H_{144}N_4O_{60}$, 2171.3433; found, 2171.3433.

General Method for ELISA. Protein A (1 μg/well) was immobilized on the wells of microtiter plates through incubation at 4 °C in hydrogen carbonate buffer (100 μL, 50 mM, pH 9.0). ELISA buffer (200 μL, 20 mM HEPES and 0.5% bovine serum albumin, 125 mM NaCl, pH 7.50) was added and remained for 1 h at room temperature to block the wells. After washing the wells with ELISA buffer (5 × 200 μL), mSiglec-1-Fc (0.25 μg/well) that had been treated first with Neuraminidase (*Vibrio cholerae*, 50 mU/mL, 4 mM $CaCl_2$, ELISA buffer) and then diluted with more ELISA buffer was added to the wells. After washing the wells with ELISA buffer (5 × 200 μL), the sialosides (50 μL, 5 mM−4 nM) were added to the wells. A solution of sialoside, SA−AP conjugate (50 mL, 1 μg/well), and biotinylated sialyllactose probe (0.5 μg/well) was added. After 20 min of incubation at room temperature, the wells were washed with ELISA buffer (5 × 200 μL) and developed with *p*-nitrophenyl phosphate (50 μL/well). A plate reader was used to read the absorbances of the wells at 405 nm. Wells without mSiglec-1-Fc were used as negative controls, and all assays were done in duplicate. To analyze the data, the inhibition curves used to generate IC_{50} values were fitted using a nonlinear regression formula.

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Supporting Information Available: NMR spectra for compounds **3**−**8**, **11**−**14**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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