Practical Synthesis of Starburst PAMAM α-Thiosialodendrimers for Probing Multivalent Carbohydrate−Lectin Binding Properties

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Synergistic cluster or multivalent effects are known to compensate for the weak binding constants of natural oligosaccharides via the cooperative combination of multiple carbohydrate−protein interactions.[1] Exploiting the concept of multivalency, sialic acid-containing polymers[2] and, more recently, sialodendrimers[3−5] have been shown to be potent inhibitors of the hemagglutination of human erythrocytes by influenza viruses. Glycodendrimers[6] are monodispersed macromolecules with covalently attached carbohydrate residues. Because glycodendrimers are a chemically well-defined series of structurally similar neoglycoconjugates differing in the number of sugar epitopes, they lend themsleves as useful tools in a systematic analysis of multivalency.

In fact, since the first reported synthesis of L-lysine-based α-thiosialodendrimers,[3] much scientific effort has gone into the preparation of other glycodendrimers. Ashton et al.[7,8] have reported glycodendrimers bearing up to 36 D-glucose residues. α-Thiosialodendrimers based on *N*-(3-aminopropyl)-1,3-propanediamine [3,3′-iminobis-(propylamine)][4] and gallic acid[5] cores have been previously described. Other work has stemmed from the attachment of various carbohydrate derivatives to Starburst PAMAM dendrimers. To date, disaccharide lactones of lactose and maltose have been conjugated to PAMAM dendrimers.[9] A T_N-antigen peptide[10] has been coupled to the amine-terminated dendrimers via amide bond formation. Last, an isothiocyanate coupling strategy[11−13] has been employed to conjugate PAMAM dendrimers to α-[11−13] and β-D-mannose,[11] β-D-glucose,[11] β-cellobiose,[11] and β-lactose[11] isothiocyanate derivatives.

Spherical Starburst PAMAM dendrimers (**1**−**4**)[14] are made of β-alanine repeating units and are commercially available in fairly large quantities. They represent the first successful synthesis of spherical, highly branched dendrimers up to generation 10 with a defined number of amine surface groups, and their structures are depicted in Figure 1. Due to their hydrophilic backbones, they could be used advantageously over other dendrimers for biological investigations. PAMAM-based glycodendrimers incorporating sialic acid residues have thus far not been reported. In keeping with the design of chemically well-defined multivalent sialosides and for purposes of comparison to previously described α-thiosialodendrimers,[4,5] the synthesis and relative binding properties of sialylated PAMAM dendrimers are presented herein. Such novel "nanostructures" should be helpful to better refine our understanding of multiple carbohydrate−protein interactions over poorly defined polymers.

Amine-terminated PAMAM cores **1**−**4** were conjugated to *p*-isothiocyanatophenyl α-sialoside **8** to give polythiourea derivatives. This approach to carbohydrate−dendrimer conjugation allowed minimal manipulation of the dendritic cores and proceeded smoothly.[11−13] Several methods for the preparation of glycosyl isothiocyanates have been reported.[15−17] While these procedures may proceed efficiently for most simple carbohydrates, sialic acid, with its unique anomeric configuration, does not lend itself easily to these techniques. Instead, liquid−liquid phase transfer catalysis (PTC)[18] was used for the synthesis of the key *p*-nitrophenyl 2-thio-α-sialoside **6**. Acetochloroneuraminic acid (**5**) was prepared according to published procedures.[19] This was then treated with tetra-*n*-butylammonium hydrogen sulfate (TBAHS, 1 equiv, 25 °C) as the catalyst with *p*-nitrothiophenol (1.5 equiv, 25 °C) in equal volumes of ethyl acetate and 1 M Na_2CO_3 to give thioglycoside **6** in 75% yield (Scheme 1). The reaction occurred with complete stereocontrol to give the glycoside shown (**6**) as judged by well-documented data for **6** (^{1}H NMR ($CDCl_3$) δ 2.77 for H-3eq).[20] The nitro group in sialic acid derivative **6** was efficiently reduced with tin(II) chloride in refluxing ethanol to give the

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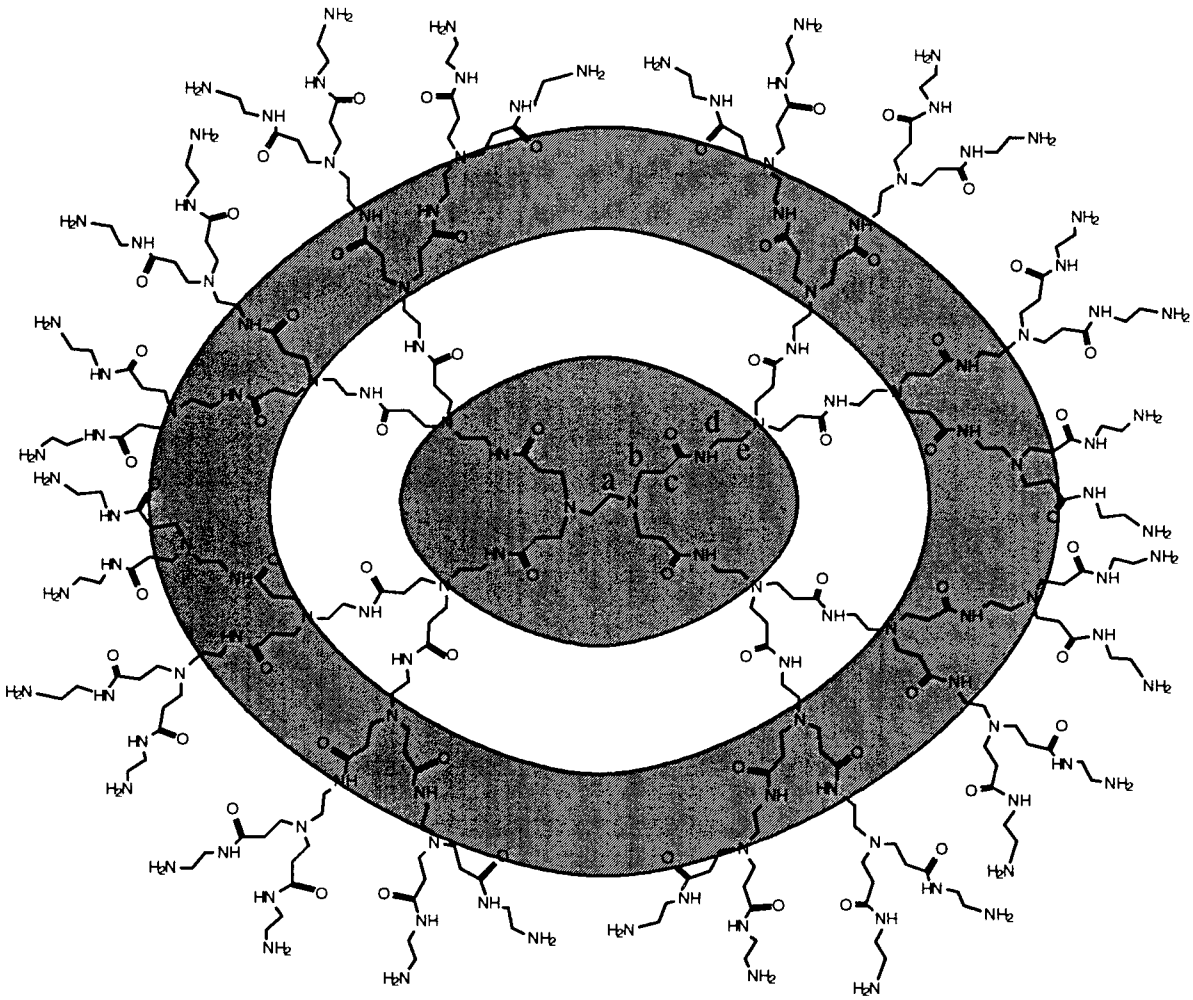

Figure 1. G0 PAMAM core with 4 surface amines **1**. G1 PAMAM core with 8 surface amines **2**. G2 PAMAM core with 16 surface amines **3**. G3 PAMAM core with 32 surface amines **4**.

corresponding *p*-aminophenyl thioglycoside **7** (2 h, 99%). Derivative **7** was treated with thiophosgene in dichloromethane (2 equiv, diisopropylethylamine) to provide *p*-isothiocyanatophenyl sialoside **8** in 87% yield. Isolated **8** was used for dendrimer conjugation.

Commercially available methanolic solutions of PAMAM dendrimers **1**−**4** were concentrated in vacuo. CH_2Cl_2 was then added and coevaporated with any residual MeOH three times under reduced pressure. The resulting residues were dissolved in CH_2Cl_2 for **1** or in DMF for **2**−**4**. Diisopropylethylamine (DIPEA, 1 equiv per amine functionality) was then added to the solution. Sialyl isothiocyanate derivative **8** (1.2 equiv per amine moiety) in CH_2Cl_2 was added dropwise to the solutions which were stirred overnight at room temperature (Scheme 2). The reaction mixtures were concentrated in vacuo, and the residues were dialyzed against a mixture of DMSO and H_2O in which the fully protected sialodendrimers were freely soluble (1:1, v/v, MW cutoff 2 kDa). PAMAM-based α-thiosialodendrimers **9a**−**12a** were obtained in excellent yields (71−100%) after freeze-drying.

Complete deprotection of sialodendrimers **9a**−**12a** by sequential ester hydrolysis ((i) NaOMe/MeOH, (ii) 0.05 M NaOH) followed by dialysis against DMSO/H_2O (1:1, v/v, MW cutoff 2 kDa) afforded water-soluble dendrimers **9b**−**12b** with 4, 8, 16, and 32 NeuAc residues, respectively (59−93%), after liophylization. The high field (500 MHz) ^{1}H NMR spectra confirmed complete incorporation of sialic acid residues (±2−3%). Key signals included the β-CH_2 of the dendritic core at δ 2.28 ppm and the NAc, H-3eq, and H-4 of the NeuAc moieties at δ 1.65, 2.70, and 4.69 ppm in DMSO-d_6, respectively.

To demonstrate the ability of these α-thiosialodendrimers to bind to the slug lectin from *Limax flavus* (LFA), turbidimetric analysis (nephelometry) was ini-

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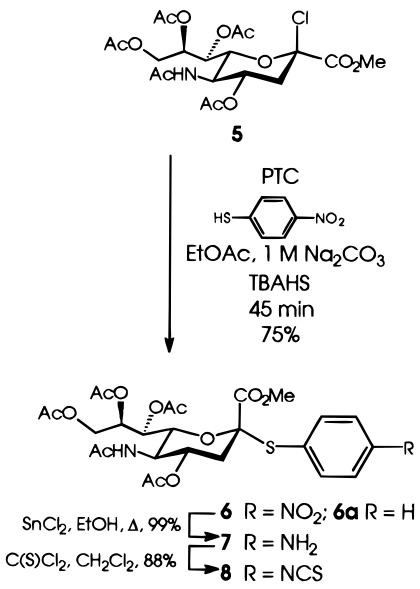
Scheme 1



tially performed. These microquantitative precipitation experiments confirmed the direct binding and cross-linking properties of the PAMAM-based α-thiosialodendrimers with LFA since nice and well-organized precipitates could be directly visualized with the naked eye and quantified as a function of time by optical density measurements at 490 nm (results not shown, see Experimental Section).

The relative efficiency of sialylated PAMAM dendrimers **9b**−**12b** to inhibit the binding of horseradish peroxidase (HRPO) labeled LFA to human α_1-acid glycoprotein (orosomucoid) was then determined by a competitive enzyme-linked lectin assay (ELLA). In this experiment, the IC_{50} of each sialodendrimer was measured, and the results are shown in Table 1. Phenyl 2-thio-α-sialoside (**6a**)[21] was used as monovalent standard.

In this spherical α-sialodendrimer series, an increase in multivalency resulted in a steady increase in inhibitory potential. Glycodendrimers with valencies of 4 (**9b**), 8 (**10b**), 16 (**11b**), and 32 (**12b**) exhibited IC_{50}'s of 69.2, 21.5, 2.89, and 1.13 nM, respectively (277, 172, 46.2, and 36.2 nM on a per sialoside residue basis, respectively, Table 1). This represents a 210-fold (6.7-fold/sialoside) jump in inhibitory potential over the monomeric analogue. Once again, the increased binding between carbohydrates and proteins may be attributed to an increase in multivalency as reflected by the well-known cluster effect.[1b] This is directly evident when comparing IC_{50} and/or relative potency as a function of dendrimer valency.

It should be noted that when compared to the previously reported tetravalent 3,3′-iminobis(propylamine)-based α-thiosialodendrimer,[4] in the same set of experiments, tetravalent sialylated PAMAM dendrimer **9b** exhibited a higher IC_{50} value. That is, at the second generation, the 3,3′-iminobis(propylamine)-based divergent series seems to be a better inhibitor of the carbohydrate−protein interaction studied. However, at higher generations, these spherical sialodendrimers appear to have structural organizations and/or aglycon spacer requirements more suitable than the 3,3′-iminobis(propylamine) α-thiosialodendrimers reported earlier[4] for the solid-phase inhibition of the binding of human α_1-acid glycoprotein to LFA. These results confirm that an amplification in carbohydrate−protein interactions is related to an increase in the valency of sugar residues in neoglycoconjugates. It is unlikely that the origin of this effect relied on the spanning of these novel sialodendrimers with two remotely positioned binding sites of this dimeric lectin, as might be the case with some high molecular weight glycopolymers. Alternatively, one may speculate that the higher permanent local concentration of the multivalent carbohydrate ligands is responsible for the higher avidity (not affinity) of these molecules, presumably by affecting their k_{on}/k_{off} ratios.

Experimental Methods

General Methods. Proton chemical shifts (δ) are given relative to internal dimethyl sulfoxide (2.49 ppm) for DMSO-d_6 solutions and to internal HOD (4.76 ppm) for D_2O solutions. Carbon chemical shifts are given relative to DMSO-d_6 (39.4 ppm). Assignments were based on COSY, HMQC, and DEPT experiments. Phenyl 2-thio-α-sialoside **6a** was obtained as previously described.[21] Human α_1-acid glycoprotein (orosomucoid) was purchased from Sigma Chemical Co., and the lectins LFA and peroxidase labeled LFAwere obtained from E-Y Laboratories (San Mateo, CA).

Preparation of Methyl (4-Nitrophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5,-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6). To a solution of freshly prepared acetochloroneuraminic acid **5** (3.30 g, 6.47 mmol) in EtOAc (60 mL) was added a solution of *p*-nitrothiophenol (1.21 g, 7.73 mmol) and TBAHS (2.20 g, 6.47 mmol) in 1 M Na_2CO_3 (60 mL). The mixture was stirred vigorously for 1 h at room temperature and next diluted with 125 mL each of EtOAc and of saturated $NaHCO_3$. The organic phase was separated and washed with saturated $NaHCO_3$ (2 × 100 mL) followed by saturated NaCl (100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes to EtOAc to give compound **6** as an off-white foam in 75% yield (3.05 g, 4.85 mmol): mp 167.0−173.0 °C; $[\alpha]_D$ 28.5° (c = 1.2, $CHCl_3$); lit.[21] mp 168.0−172.0 °C, $[\alpha]_D$ 27.6° (c = 1.0, MeOH).

Preparation of Methyl (4-Aminophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (7). *p*-Nitrophenyl derivative **6** (40.0 mg, 0.064 mmol) was suspended in absolute ethanol (10 mL) to which was added tin(II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$, 75.0 mg, 0.402 mmol). The reaction mixture was stirred at 70 °C for 2 h and then cooled and poured onto ice−water and the final pH adjusted to ∼8 with $NaHCO_3$. The resulting mixture was filtered and the clear filtrate extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed successively with saturated $NaHCO_3$ (20 mL), H_2O (20 mL), and saturated NaCl (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Title compound **7** was obtained as an off-white powder in 99% yield (37.7 mg, 0.063 mmol) and used directly in subsequent reactions without further purification: mp 96.0−101.0 °C; $[\alpha]_D$ 26.0° (c = 1.0, $CHCl_3$); lit.[21] mp 98.0−102.0 °C, $[\alpha]_D$ 26.1° (c = 1.270, MeOH).

Preparation of Methyl (4-Isothiocyanatophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (70). *p*-Aminophenyl derivative **7** (35.0 mg, 0.058 mmol) was dissolved in CH_2Cl_2 (20 mL) containing diisopropylethylamine (DIPEA, 18.7 mg, 0.145 mmol). Thiophosgene (16.7 mg, 0.145 mmol) was added and the solution stirred at 25 °C for 1 h. The solvent was evaporated under reduced pressure and the residue purified by silica gel column chromatography using a gradient of hexanes to EtOAc as eluent to afford **8** in 87% yield (32.6 mg, 0.051 mmol): mp 125.0−130.1 °C; $[\alpha]_D$ 26.3° (c = 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.85 (s, 3H, NAc), 2.00, 2.03, 2.04, 2.13 (4s, 12H, OAc's), 2.02 (m, 1H, H-3ax), 2.80 (dd, 1H, $J_{3ax,3eq}$ 12.8 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq), 3.57 (s, 3H, OCH_3), 3.78−3.90 (m, 2H, H-5, H-6), 3.96−

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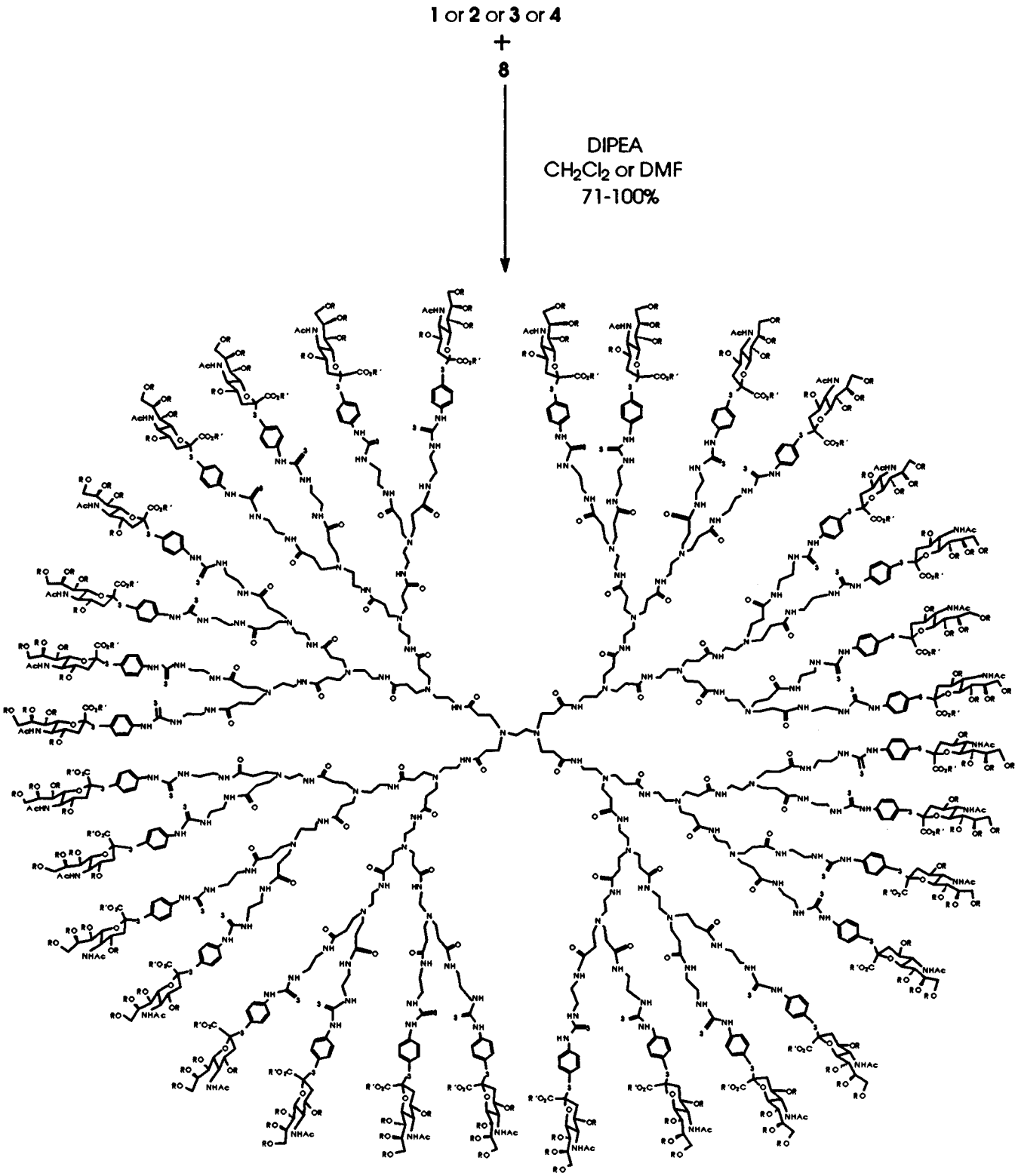
Scheme 2



i) NaOMe/MeOH
ii) 0.05 M NaOH, 80% → 4mer **9a** R = Ac, R′ = Me; **b** R = R′ = H

i) NaOMe/MeOH
ii) 0.05 M NaOH, 79% → 8mer **10a** R = Ac, R′ = Me; **b** R = R′ = H

i) NaOMe/MeOH
ii) 0.05 M NaOH, 59% → 16mer **11a** R = Ac, R′ = Me; **b** R = R′ = H

i) NaOMe/MeOH
ii) 0.05 M NaOH, 93% → 32mer **12a** R = Ac, R′ = Me; **b** R = R′ = H

Table 1. Inhibition of Binding of Human α_1-Acid Glycoprotein to *Limax flavus* by Sialodendrimers

compound	IC_{50} (nM)[a]	relative potency[a]
phenyl 2-thio-α-sialoside **6a**	242	1
PAMAM-based 4mer **9b**	69.2 (277)	3.5 (0.87)
PAMAM-based 8mer **10b**	21.5 (172)	11 (1.4)
PAMAM-based 16mer **11b**	2.89 (46.2)	84 (5.2)
PAMAM-based 32mer **12b**	1.13 (36.2)	210 (6.7)
3,3′-iminobis(propylamine)-based 4mer[4,5]	37.2 (149)	6.5 (1.6)

[a] Values in parentheses are based on a per sialoside residue.

4.11 (m, 1H, H-9), 4.31 (m, 1H, H-9′), 4.83 (ddd, 1H, $J_{3ax,4}$ 11.7 Hz, $J_{4,5}$ 10.3 Hz, H-4), 5.10 (d, 1H, $J_{NH,5}$ 9.9 Hz, NH), 5.23−5.27 (m, 2H, H-7, H-8), 7.16 (d, 2H, $J_{ortho,meta}$ 8.6 Hz, H-ortho), 7.45 (d, 1H, H-meta); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9 (OAc's), 23.1 (NAc), 38.1 (C-3), 49.3 (C-5), 52.8 (OCH_3), 60.3 (C-9), 62.0 (C-7), 67.5 (C-7), 69.2 (C-4), 69.4 (C-8), 74.5 (C-6), 126.0 (C-ortho), 127.9 (N═C═S), 132.9 (C-para), 137.1 (C-ipso), 137.2 (C-meta), 167.5−170.8 (C═O's); FAB-MS (pos.) calcd for $C_{27}H_{32}N_2O_{12}S$ 640.14; found 641.2 ($M^+ + 1$, 18.0). Anal. Calcd for $C_{27}H_{32}N_2O_{12}S$: C, 50.62; H, 5.03; N, 4.37. Found: C, 50.23; H, 5.10; N, 4.52.

Preparation of Peracetylated PAMAM-Based α-Thiosialodendrimers 9a, 10a, 11a, and 12a. Typical Procedure. A methanolic solution of amine-terminated tetravalent Starburst PAMAM dendritic core **1** (23.0 mg of a 36.02% (w/w) solution in MeOH, 0.016 mmol, Dendritech (Midland, MI)) was evaporated under reduced pressure. The resulting residue was redissolved in CH_2Cl_2 (5 mL) and the solution re-evaporated. This was repeated three times. Compound **1** was then dissolved in CH_2Cl_2 (5 mL), to this was added diisopropylethylamine (DIPEA, 8.3 mg, 0.064 mmol), and the solution was stirred at room temperature. Sialic acid isothiocyanato derivative **8** (50.0 mg, 0.078 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the stirred solution and the reaction left at 25 °C. After 20 h, the reaction mixture was concentrated in vacuo and then dialyzed against 1:1 DMSO/H_2O (MW cutoff 2 kDa). The resulting solution was lyophilized to give tetravalent PAMAM-based α-thiosialodendrimer **9a** as an off-white solid in 94% yield (47.2 mg, 0.015 mmol).

Fully protected α-thiosialodendrimers **10a**, **11a**, and **12a** were obtained in the same manner from Starburst PAMAM dendritic cores **2**, **3**, and **4** using DMF as the reaction solvent, in 71, 100, and 100% yields, respectively.

9a: ^{1}H NMR (DMSO-d_6) δ 1.65 (s, 12H, NAc), 1.77 (dd, 4H, *J* 12.1 Hz, H-3ax), 1.90, 1.99, 2.00, 2.02 (4s, 48H, OAc's), 2.28 (bs, 8H, b-CH_2), 2.52−2.99 (3m, 16H, a-CH_2, c-CH_2, H-3eq), 3.25 (m, 8H, d-CH_2), 3.52−3.60 (m, 8H, e-CH_2), 3.54 (s, 12H, OCH_3), 3.59−3.79 (2m, 8H, H-5, H-6), 4.09 (dd, 4H, $J_{8,9}$ 6.0 Hz, $J_{9,9'}$ 12.2 Hz, H-9), 4.28 (dd, 4H, $J_{8,9'}$ 2.5 Hz, H-9′), 4.69 (ddd, 4H, H-4), 5.13 (m, 4H, H-7), 5.19 (m, 4H, H-8), 7.38 (d, 8H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 8H, H-meta), 7.62 (d, 4H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 8.00 (bs, 4H, N*H*C(S)NHPh), 8.05 (bs, 4H, amide NH's), 9.86 (bs, 4H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 20.6, 20.8 (OAc's), 22.6 (NAc), 32.4 (C-b), 37.7 (C-3), 37.8 (C-d), 40.5 (C-a), 43.4 (C-e), 47.7 (C-5), 48.8 (C-c), 52.8 (OCH_3), 61.7 (C-9), 67.4 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 (C-para), 136.4 (C-ortho), 141.3 (C-ipso), 167.8−170.1 (C═O's), 180.4 (C═S).

10a: ^{1}H NMR (DMSO-d_6) δ 1.64 (s, 24H, NAc), 1.77 (dd, 8H, *J* 12.1 Hz, H-3ax), 1.90, 1.99, 2.00, 2.02 (4s, 96H, OAc's), 2.25 (bs, 12H, b-CH_2), 2.55−2.95 (2m, 24H, a-CH_2, b-CH_2, H-3eq), 3.25−3.49 (2m, 48H, c-CH_2, d-CH_2), 3.52−3.60 (m, 24H, e-CH_2), 3.54 (s, 24H, OCH_3), 3.59−3.79 (2m, 16H, H-5, H-6), 4.09 (m, 8H, H-9), 4.28 (dd, 8H, $J_{8,9'}$ 2.5 Hz, $J_{9,9'}$ 12.2 Hz, H-9′), 4.69 (ddd, 8H, H-4), 5.13 (m, 8H, H-7), 5.19 (m, 8H, H-8), 7.38 (d, 16H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 16H, H-meta), 7.62 (d, 8H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 8.00−8.20 (2m, 20H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 8H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 20.5, 20.7 (OAc's), 22.5 (NAc), 37.6 (C-3), 37.7 (C-d), 40.4 (C-a), 43.3 (C-e), 47.6 (C-5), 49.4 (C-c), 52.7 (OCH_3), 61.6 (C-9), 67.3 (C-7), 68.9 (C-8), 69.4 (C-4), 74.0 (C-6), 87.2 (C-2), 121.9 (C-meta), 122.7 (C-para), 136.3 (C-ortho), 141.2 (C-ipso), 167.7−170.0 (C═O's), 180.4 (C═S).

11a: ^{1}H NMR (DMSO-d_6) δ 1.64 (s, 48H, NAc), 1.77 (m, 16H, H-3ax), 1.89, 1.99, 2.00, 2.01 (4s, 192H, OAc's), 2.22 (bs, 56H, b-CH_2), 2.45−2.99 (m, 76H, a-CH_2, c-CH_2, H-3eq), 3.19−3.40 (2m, 56H, d-CH_2), 3.52−3.60 (m, 56H, e-CH_2), 3.53 (s, 48H, OCH_3), 3.59−3.79 (2m, 32H, H-5, H-6), 4.09 (m, 16H, H-9), 4.28 (m, 16H, H-9′), 4.69 (ddd, 16H, H-4), 5.13 (m, 16H, H-7), 5.19 (m, 16H, H-8), 7.38 (d, 32H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 32H, H-meta), 7.62 (d, 16H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 7.80−8.15 (3bs, 44H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 16H, NHC(S)-N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 20.6, 20.8 (OAc's), 22.6 (NAc), 37.7 (C-3), 37.8 (C-d), 40.5 (C-a), 43.4 (C-e), 47.7 (C-5), 52.8 (OCH_3), 61.7 (C-9), 67.4 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 (C-para), 1366.4 (C-ortho), 141.3 (C-ipso), 167.8−170.1 (C═O's), 180.4 (C═S).

12a: ^{1}H NMR (DMSO-d_6) δ 1.65 (s, 96H, NAc), 1.78 (m, 32H, H-3ax), 1.90, 2.00, 2.01, 2.02 (4s, 384H, OAc's), 2.25 (bs, 120H, b-CH_2), 2.52−2.85 (m, 156H, a-CH_2, c-CH_2, H-3eq), 3.00−3.60 (m, 240H, d-CH_2, e-CH_2), 3.56 (s, 96H, OCH_3), 3.59−3.79 (2m, 64H, H-5, H-6), 4.09 (m, 32H, H-9), 4.28 (m, 32H, H-9′), 4.69 (ddd, 32H, H-4), 5.13 (m, 32H, H-7), 5.19 (m, 32H, H-8), 7.38 (d, 64H, $J_{ortho,meta}$ 8.2 Hz, H-ortho), 7.54 (d, 64H, H-meta), 7.62 (d, 32H, $J_{5,NHAc}$ 9.5 Hz, N*H*Ac), 7.75−8.15 (m, 92H, N*H*C(S)NHPh, amide NH's), 9.90 (bs, 32H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 20.6, 20.7 (OAc's), 22.5 (NAc), 32.4 (C-b), 37.8 (C-3), 43.4 (C-e), 47.6 (C-5), 49.4 (C-c), 52.8 (OCH_3), 61.7 (C-9), 67.3 (C-7), 68.9 (C-8), 69.5 (C-4), 74.1 (C-6), 87.3 (C-2), 122.0 (C-meta), 122.2 (C-para), 136.4 (C-ortho), 141.3 (C-ipso), 167.7−170.1 (C═O's), 180.4 (C═S).

Preparation of Fully Deprotected PAMAM-Based α-Thiosialodendrimers 9b, 10b, 11b, and 12b. Typical Procedure. To peracetylated glycodendrimer **9b** (47.2 mg, 0.015 mmol) dissolved in DMSO (0.5 mL) was added 1 M NaOMe in MeOH (5 mL), and the solution was stirred at 25 °C. After 1 h, MeOH was evaporated under reduced pressure and 0.05 M NaOH (5 mL) added. After 1.5 h, the solution was neutralized with 1 M HCl and the solvent removed by lyophilization. The resulting residue was purified by dialysis against 1:1 DMSO/H_2O (MW cutoff 2 kDa) and then freeze-dried. Compound **9b** was isolated as a white, spongy solid in 80% yield (28.9 mg, 0.012 mmol).

Fully deprotected α-thiosialodendrimers **10b**, **11b**, and **12b** were obtained in the same manner from glycodendrimers **10a**, **11a**, and **12a** in 79, 100, and 100% yields, respectively.

9b: ^{1}H NMR (DMSO-d_6) δ 1.66 (dd, 4H, *J* 11.9 Hz, H-3ax), 1.84 (s, 12H, NAc), 2.25−2.59 (m, 12H, b-CH_2, H-3eq), 2.63−3.00 (m, 12H, a-CH_2, c-CH_2), 3.05−3.60 (m, 44H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.72, 5.18 (4bs, 16H, OH's), 7.22−8.15 (4m, 28H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.88−10.20 (4bs, 4H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 22.6 (NAc), 37.8 (C-d), 40.4 (C-3), 40.7 (C-a), 42.1 (C-c), 43.2 (C-e), 51.8 (C-5), 63.2 (C-9), 66.8 (C-7), 68.7 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.5, 122.2 (C-meta, C-para), 136.2 (C-ortho), 141.5 (C-ipso), 169.2−172.1 (C═O's), 180.4 (C═S).

10b: ^{1}H NMR (DMSO-d_6) δ 1.66 (m, 8H, H-3ax), 1.83 (s, 24H, NAc), 2.25−2.61 (m, 32H, b-CH_2, H-3eq), 2.63−3.00 (m, 28H, a-CH_2, c-CH_2), 3.05−3.68 (m, 104H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.98, 5.20 (4bs, 32H, OH's), 7.22−8.15 (4m, 60H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.88 (bs, 8H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 22.7 (NAc), 37.8 (C-d), 40.5 (C-3), 40.7 (C-a), 43.4 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.8 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.8 (C-meta), 136.4 (C-ortho), 169.3−171.6 (C═O's), 180.4 (C═S).

11b: ^{1}H NMR (DMSO-d_6) δ 1.66 (dd, 16H, *J* 12.0 Hz, H-3ax), 1.83 (s, 48H, NAc), 2.25−2.62 (m, 72H, b-CH_2, H-3eq), 2.63−3.00 (m, 60H, a-CH_2, c-CH_2), 3.05−3.70 (m, 224H, d-CH_2, e-CH_2, and NeuAc residues excluding above), 4.27, 4.59, 4.72, 5.18 (4bs, 64H, OH's), 7.22−8.15 (4m, 124H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.90 (bs, 16H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 22.7 (NAc), 37.9 (C-d), 40.5 (C-3), 40.7 (C-a), 43.3 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.7 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.8, (C-meta), 136.4 (C-ortho), 141.2 (C-ipso), 169.3−171.6 (C═O's), 180.4 (C═S).

12b: ^{1}H NMR (DMSO-d_6) δ 1.66 (dd, 32H, *J* 11.9 Hz, H-3ax), 1.84 (s, 96H, NAc), 2.25 (bs, 120H, b-CH_2), 2.50−2.80 (m, 154H, a-CH_2, c-CH_2, H-3eq), 3.05−3.70 (m, 464H, d-CH_2, e-CH_2, and

NeuAc residues excluding above), 4.27, 4.59, 5.18 (3bs, 128H, OH's), 7.22−8.15 (4m, 252H, amide NH's, N*H*C(S)NHPh, H-ortho, H-meta, NHAc), 9.95 (bs, 32H, NHC(S)N*H*Ph); ^{13}C NMR (DMSO-d_6) δ 22.7 (NAc), 32.5, 32.9 (C-b), 37.8 (C-d), 39.1 (C-3), 40.5 (C-a), 42.1 (C-c), 43.4 (C-e), 51.9 (C-5), 63.2 (C-9), 66.8 (C-7), 68.8 (C-8), 71.3 (C-4), 76.1 (C-6), 86.5 (C-2), 121.9, 122.5 (C-meta, C-para), 136.4 (C-ortho), 141.2 (C-ipso), 169.3−171.6 (C=O's), 180.4 (C=S).

Turbidimetric Analysis between the Lectin from *L. flavus* and PAMAM-Based α-Thiosialodendrimers 9b−12b. Turbidimetry experiments were performed in Linbro (Titertek) microtitration plates where 50 μL/well of stock lectin solutions prepared from *L. flavus* (1 mg/mL in PBS) were mixed with 50 μL each of glycodendrimers **9b**−**12b** (0.22, 0.24, 0.24, and 0.25 mg/mL in PBS, respectively, or 0.371 mM sialoside content each) and incubated at room temperature for up to 4 h. The turbidity of the solutions was monitored by reading the optical density (OD) at 490 nm at regular time intervals until no noticeable changes could be observed.

Competitive Inhibition ELLA Using Human α_1-Acid Glycoprotein and PAMAM-Based α-Thiosialodendrimers 9b−12b as Inhibtors. Linbro (Titertek) microtitration plates were coated with human α_1-acid glycoprotein (orosomucoid) at 100 μL/well of a stock solution of 10 μg/mL in 0.01 M phosphate buffer (pH 7.3) overnight. The wells were then washed three times with 300 μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). Similar washings with PBST were repeated after each incubation period. Wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h at 37 °C. After being washed, the wells were filled with 100 μL/well of inhibitor solutions and incubated again at 37 °C for 1 h. Inhibitors included phenyl 2-thio-α-sialoside **6a**[21] as a reference monomer, tetravalent 3,3′-iminobis(propylamine)-based sialodendrimer,[4,5] and PAMAM-based, spherical glycodendrimers **9b**−**12b**. Each inhibitor was added in serial 2-fold dilutions (60 μL/well) in PBS with the appropriate lectin−enzyme conjugate concentration (60 μL/well of 100-fold dilution of a 1 mg/mL stock solution of *L. flavus* in PBS) on Nunclon (Delta) microtiter plates and incubated at 37 °C for 1 h. These inhibitor solutions (100 μL) were transferred to the antigen-coated plates and incubated for a second hour. The plates were washed, and 50 μL/well of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/mL) in citrate-phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 μL/well of 1 M H_2SO_4 and the optical density measured at 410 nm relative to 570 nm. Percent inhibition was calculated as follows (eq 1):

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100 \quad (1)$$

IC_{50}'s were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed six times, and average values are reported in Table 1.

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