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Synthesis and Protein Binding Properties of T-Antigen Containing GlycoPAMAM Dendrimers

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Abstract—Allyl *O*-(β-D-galactopyranosyl)-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**8**) was prepared in excellent yield from the corresponding galactosyl bromide (**6**, **7**) and allyl 2-acetamido-4,6-benzylidene-2-deoxy-α-D-galactopyranoside (**5**) using $Hg(CN)_2$ as a promoter. Compound **5** was obtained from *N*-acetylglucosamine **1** following sequential protecting group strategy and C-4 epimerization as a key step. Carboxylic acid functionalized T-antigen derivative **15**, obtained by radical addition of 3-mercaptopropionic acid to allyl disaccharide **10**, was conjugated to PAMAM dendritic cores **13**–**16** by an efficient amide coupling strategy using TBTU. GlycoPAMAM dendrimers having T-antigen residues with 4, 8, 16 and 32 valencies (**17**–**20**) were obtained in 73 to 99% yields. Their protein binding properties were demonstrated using peanut lectin from *Arachis hypogaea* and a mouse monoclonal IgG antibody. The higher valency conjugates generated stronger binding interactions indicating a cluster effect. The inhibitory potential of these glycoPAMAM conjugates toward antibody-coating antigen interactions was enhanced up to 3800 times over that of the monomeric T-antigen residue (**10**).

Introduction

Cell surface carbohydrates are involved in critical biological phenomena such as cellular recognition, adhesion, cell growth regulation, pathogenic infection, cancer and xenotransplantation.[1,2] The investigation of novel carbohydrate-based biological interactions has established a new platform of rational drug design for the purpose of diagnostics and therapeutics. However it is known that individual carbohydrate–protein interactions are of low affinity.[3] Recent developments in the multiple expression of these interactions by means of cluster effects have been shown to improve the efficiency of binding.[4,5] This is a mimic of the multivalent nature of cell surface carbohydrates, found on glycoproteins, glycolipids, polysaccharides and proteoglycans where cooperativity can increase the overall binding affinity.

The requirement of multivalency for strong binding has stimulated the synthesis of several types of glycoconjugates with multiple carbohydrate binding sites, for example, glycopolymers and more recently, glycodendrimers.[6–9]

Moreover, highly conjugated polymeric materials have been incorporated into biologically active species to detect their role in biosystems.[10] These new types of conjugates have great potential in the development of biosensory devices for diagnostic purposes.

By virtue of glycodendrimers as chemically and geometrically well-defined monodisperse macromolecules, they lend themselves as useful tools for medicinal and pharmaceutical purposes.[11–14] Poly(amidoamine) (PAMAM) and related dendrimers[15,16] have been employed in various biochemical and medicinal applications. Examples are complexation with DNA for efficient transfection[17] and biological evaluation such as toxicity, immunogenicity and biodistribution to organs.[18] The combination of PAMAM dendrimers with different carbohydrate moieties has broadened their potential applications to biological systems.[19–23] In spite of potential biological applications, the systematic preparation and biological evaluation of PAMAM based T-antigen, βGal-(1-3)-αGalNAc, glycodendrimers have not been reported to date.

T-antigen (Thomsen–Friedenrich antigen) has been reported as a cancer-related epitope and as an important antigen for the detection and immunotherapy of carcinomas particularly relevant in breast cancer patients.[24–26] For pharmaceutical applications, T-antigen

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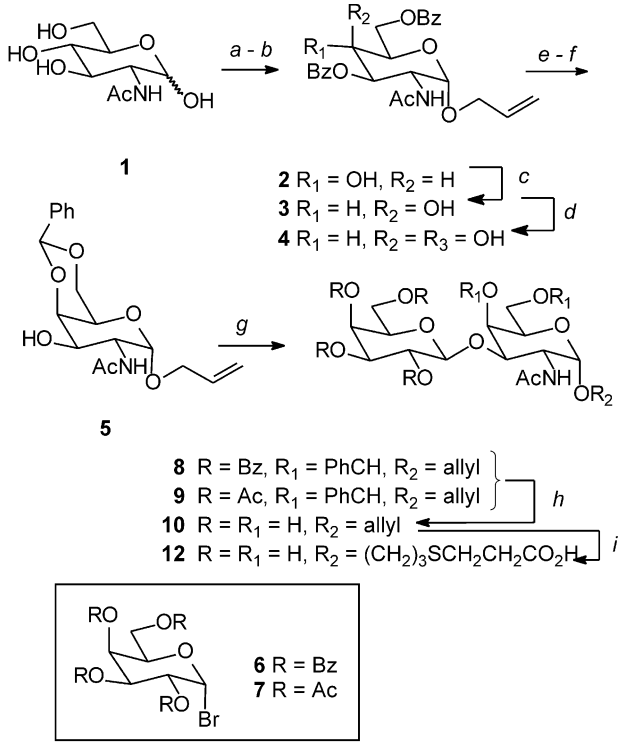
containing linear glycopolymers have been employed to develop solid-phase glycosyltransferase assays for high-throughput screening in drug discovery research.[27] Recently, we have reported that mouse monoclonal antibodies recognize breast cancer tissues selectively.[28] The corresponding vaccine was generated from a T-antigen-Bovine Serum Albumin (BSA) conjugate which did not contain the *O*-linked Ser/Thr residues of the natural T-antigen, thus demonstrating the sole carbohydrate binding requirement of our antibodies.[28] Consequently, multiple *O*-linked T-antigens deprived of O-Ser/Thr aglycons were synthesized. Recently, a new type of water soluble T-antigen containing linear conjugates that have high lipophilicity has been synthesized.[29] These biological and immunochemical results motivated the synthesis of chemically well-defined multivalent T-antigen-glycoPAMAM dendrimers reported here. Their binding properties against our mouse monoclonal IgG antibody were also evaluated.

Results and Discussion

For the synthesis of T-antigen (T-Ag), the glycosyl acceptor, 4,6-benzylidene-α-D-GalNAc (**5**) was prepared from GalNAc (**1**) by a C-4 epimerization method (Scheme 1).[32] In brief, selective benzoylation of **1** at the C-3 and 6 positions was performed at −60 °C to give compound **2** in 92% yield. Compound **2** was successively treated with Tf_2O (CH_2Cl_2/Py, −15 °C) and $NaNO_2$ (DMF, rt) to give compound **3** in 85% overall yield. De-benzoylation (NaOMe/MeOH) and benzylidene acetal formation of **3** ($PhCHO/ZnCl_2$ or $PhCH(OMe)_2$/p-TSA, o.n.) afforded compound **5** in 87% overall yield.



Scheme 1. (a) Allyl alcohol, BF_3OEt_2, reflux, 3 h, 65%; (b) BzCl (2.2 equiv), Py/$CHCl_3$, −60 °C, 92%; (c) (i) Tf_2O (1.5 equiv), Py (3.3 equiv)/CH_2Cl_2, −15 °C; (ii) $NaNO_2$ (10 equiv), DMF, 25 °C, 85% (2 steps); (d) NaOMe/MeOH; (e) MeONa, MeOH, quantitative; (f) PhCHO, $ZnCl_2$ (2 equiv) or $PhCH(OMe)_2$ (5 equiv), 25 °C, o.n., 87%; (g) peracylated Gal-Br [**4** (Bz) or **5** (Ac)] (1.5 equiv), $Hg(CN)_2$ (1.5 equiv), benzene/$MeNO_2$, 25 °C 78–98%; (h) (i) MeONa, MeOH; (ii) 60% aq AcOH, 60 °C, 93%; (i) $HSCH_2CH_2CO_2H$ (**11**, 1.1 equiv, UV (254 nm), deoxygenated water, 83%. BzCl = benzoyl chloride, Py = pyridine, Tf_2O = trifluoroacetic anhydride, Gal-Br = peracylated galactopyranosyl bromide, AcOH = acetic acid.

Compound **5** was coupled with different glycosyl donors (**6**, and **7**) in two strategies to give the desired T-Ag, **8**, **9** and **12** (Scheme 1). Perbenzoylated (or peracetylated) Gal-Br (**6** or **7**) were employed under Helferich conditions using $HgCN_2$ to give **8** and **9** in 98 and 78% yields, respectively. Successive deprotection (i. NaOMe/MeOH, ii. aq AcOH, 60 °C) afforded T-Ag **10** in 95% yield. Acid functionalization of **10** was accomplished using 3-mercaptopropionic acid (**11**) (254 nm, degassed water) to give compound **12** in 83% yield.

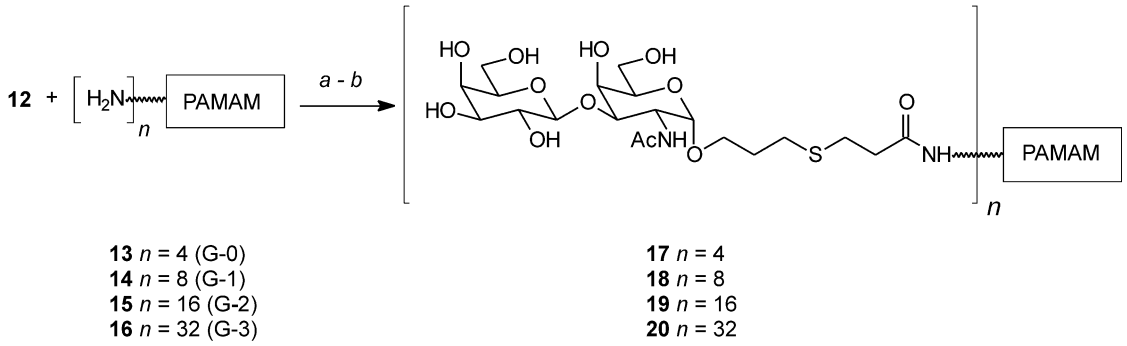
T-Ag-GlycoPAMAM dendrimers were prepared by amide bond formation between T-Ag-acid (**12**) and PAMAM dendritic cores (**13**, **14**, **15** and **16**). Briefly, commercially available PAMAM dendritic cores in MeOH were dried under vacuum and the the residue was dissolved in dimethyl sulfoxide (DMSO). To a slight excess of **12**, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetra-fluoroborate (TBTU) and *N*,*N*-diisopropylethylamine (DIPEA) (pH = 9) were successively added and the resulting solution was stirred overnight at room temperature (Scheme 2). The solution was lyophilized to dryness and the residue was purified by either gel permeation chromatography (P-2 or P-4, H_2O) or dialysis (H_2O, 2000 molecular cut-off) to afford glycoPAMAM conjugates, **17**, **18**, **19** and **20** having 4, 8, 16 and 32 T-Ag residues in 73, 81, 99 and 79% yields, respectively. Conjugation was judged complete based upon negative ninhydrin tests and high field ^{1}H NMR spectral data (D_2O) (sensisitivity ≤ 2–3%; ±1 residue/30). The ratios of the signals clearly confirmed the total incorporation of the T-antigen residues by comparing the relative integration of the two methylene signals of the dendritic cores (δ 2.80 ppm for **17** and **18**, 2.77 ppm for **19** and 3.05 ppm for **20**, respectively) to those of the two anomeric protons of the T-antigen (Fig. 1) (δ 4.96 ppm for H-1 and 4.56 ppm for H-1′).

Immunochemical assays with proteins (peanut lectin from arachis hypogaea and mouse monoclonal igg antibody). To demonstrate the binding ability of the conjugates toward proteins, turbidimetric analysis was initially performed with peanut lectin from *Arachis hypogaea* (Fig. 2). In the presence of the same amount of T-Ag, conjugate **20** ($n = 32$) showed the highest OD value (0.41) where other conjugates, **17**, **18** and **19** showed values of 0.17, 0.26, and 0.28, respectively. These microquantitative precipitation experiments first confirmed the direct binding (cross-linking) abilities of the glycoPAMAM conjugates with protein, thus generating glycodendrimer–protein complexes that could be

visualized with the naked eye and quantified as a function of time by optical density measurements.

The efficiency of these conjugates toward protein binding interactions were further substantiated by a solid-phase enzyme linked lectin assay (ELLA) where the glycoPAMAM conjugates were employed as coating antigens (Fig. 3). Clearly, and in spite of apparent increase in surface group hydrophilicity, the more substituted glycoPAMAMs generated species that bound better to the hydrophobic polystyrene plate surfaces. For instance, dendrimer **20** (32-mer) showed 18-fold better sensitivity when compared to that of **17** (4-mer). These results represent enhanced coating ability as the glycoPAMAMs' density increased.

The relative efficiency of the glycodendrimers to inhibit the binding of mouse monoclonal IgG antibody[28] to coating antigen (T-Ag-copolyacrylamide)[27,29] was determined using goat anti-mouse monoclonal IgG



13 n = 4 (G-0)
14 n = 8 (G-1)
15 n = 16 (G-2)
16 n = 32 (G-3)

17 n = 4
18 n = 8
19 n = 16
20 n = 32

Scheme 2. (a) TBTU, DIPEA, DMSO, 25 °C; (b) gel permeation chromatography (P-2, or P-4, water) or dialysis (2000 molecular weight cut-off) 73–99%. TBTU = O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, DIPEA = diisopropylethylamine, DMSO = dimethyl sulfoxide.

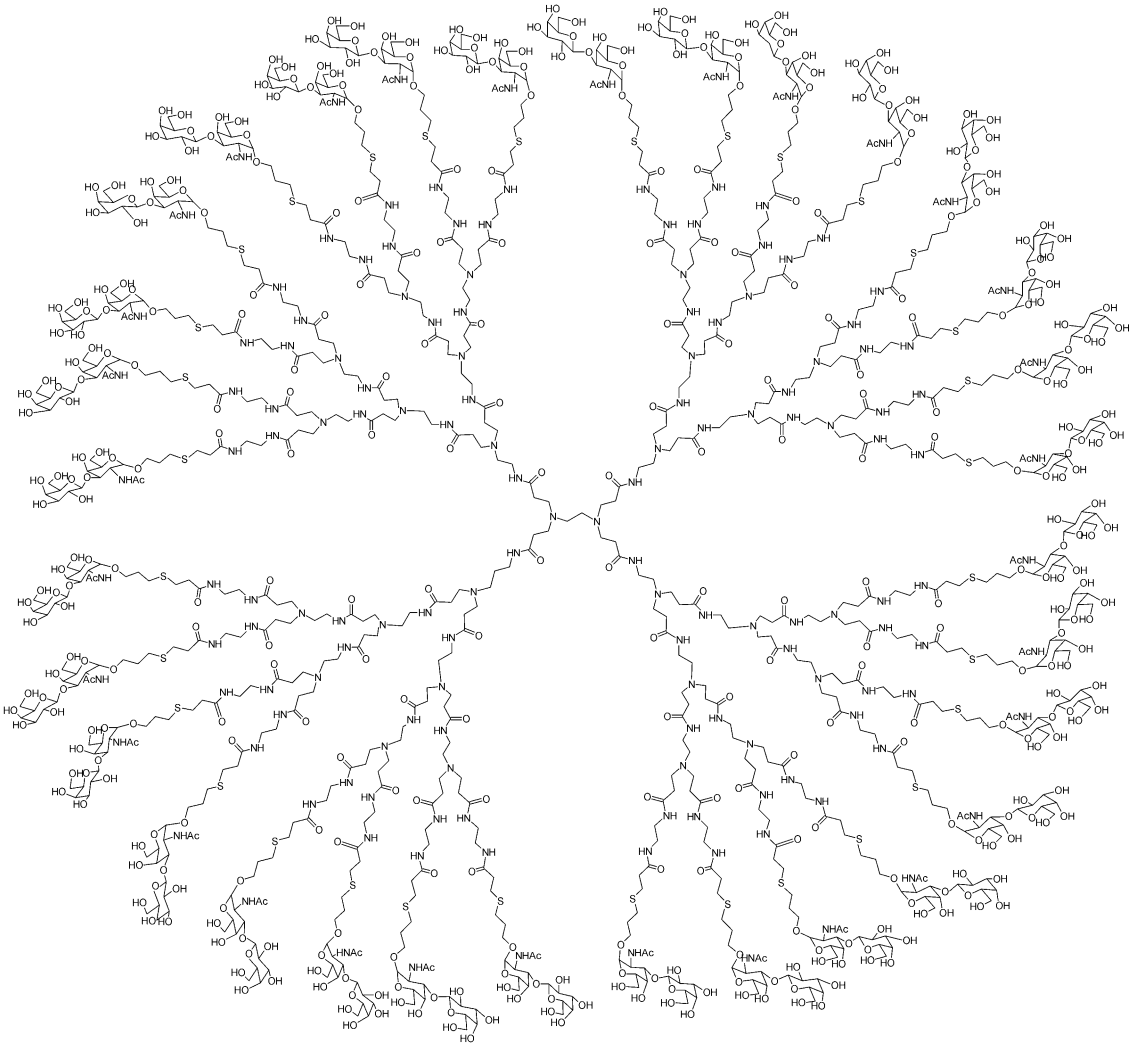


Figure 1. Structure of 32-valent glycoPAMAM dendrimer (**20**).

antibody by a competitive solid-phase enzyme linked immunosorbent assay (ELISA). Antibody (50 μL/well, 0.25 μmol in phosphate buffer saline containing tween (PBST) was pre-incubated with varying concentration (257–1.07 nmol) of glycoPAMAMs. This solution was added to a plate coated with T-Ag-copolyacrylamide. The inhibitory potential of each conjugate was measured at 410 nm. The degree of inhibition was proportional to the conjugate valencies and showed maximum inhibition when conjugate **20** (32 T-antigen surface residues) was used. These results are consistent with the evaluation using turbidimetric analysis and ELLA. The concentrations of conjugates to give 50% inhibition of the antibody-binding to T-Ag copolymer interaction were 5.0, 2.4, 1.4 and 0.6 nmol for conjugates **17**, **18**, **19** and **20** where monomeric T-Ag, **10** required 2.3 μmol. These values represent a 460, 960, 1700 and 3800 fold enhancement of inhibitory potentials over that of monomeric **10** (IC_{50} 2300 nM) when considered as a whole. However, each T-Ag epitope is, on average, only 20 fold more efficient than the monomer irrespective of the dendrimer valency (IC_{50}/valency). These results confirmed earlier findings[33,34] demonstrating that tetrameric T-Ag clusters may represent the optimum size for antibody-glycocluster inhibition.

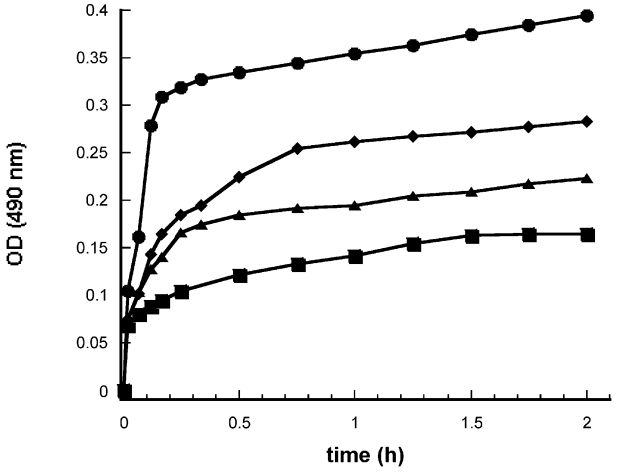


Figure 2. Turbidimetric analysis of glycoPAMAM dendrimers, **17** (■), **18** (▲), **19** (◆) and **20** (●), with peanut lectin from *Arachis hypogaea*.

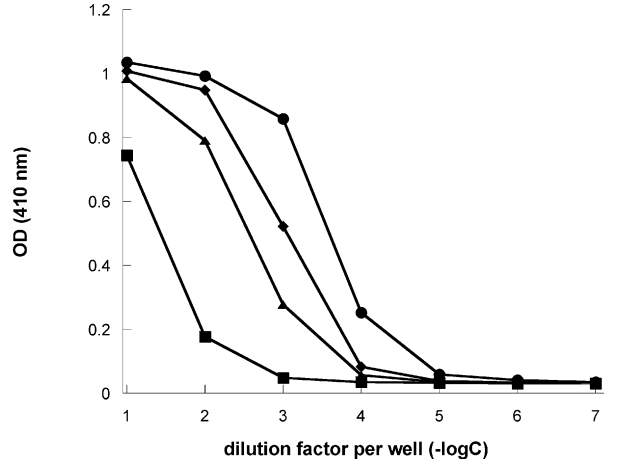


Figure 3. Enzyme linked lectin assays (ELLA) of peanut lectin to coated glycoPAMAM dendrimers, **17** (■), **18** (▲), **19** (◆) and **20** (●).

From a series of bioassays, the most remarkable finding is that artificial T-Ag glycoPAMAMs showed excellent protein binding potential in the presence of competitive antigens and are successfully employed as inhibitors for antibody-coating antigen interactions.

Conclusions

Chemically and geometrically well-defined T-Ag glycoPAMAM dendrimers with valencies of 4, 8, 16 and 32 were synthesized via efficient amide bond formation. Successive bioassays with peanut lectin from *Arachis hypogaea* which has potent anti-T activity analogous to our mouse monoclonal IgG antibody showed strong protein binding properties demonstrating an excellent cluster effect. GlycoPAMAMs are strong candidates for biological and immunochemical applications such as inhibition of cancer cell metastasis.[35]

Materials and Methods

General methods

Melting points were determined on a Gallenkamp apparatus. Optical rotation values were measured on a Perkin–Elmer 241 polarimeter and were run at 25 °C. Mass spectral data were obtained on a VG 7070-E spectrometer (CI, ether) and Krotas Concept IIH for FAB-MS (glycerol). Thin layer chromatography (TLC) was performed using silica gel 60 F-254 and column chromatography on silica gel 60. The ^1H and ^{13}C NMR spectra were obtained on a Bruker 500 MHz AMX NMR spectrometer. The chemical shifts of protons (δ) were given relative to internal chloroform (7.24 ppm) for $CDCl_3$ solutions, to internal DMSO (2.49 ppm) for DMSO-d_6 solutions, and to internal HOD (4.65 ppm) for D_2O solutions. ^{13}C chemical shifts were given relative to $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm). The assignments were based on COSY, HMQC, and DEPT experiments. Absorbance for the turbidimetric, ELLA, and ELISA tests were performed on a Dynatech MR 600 Microplate Reader. α-D-glucosamine was purchased from Sigma. Peanut lectin from *Arachis hypogaea* and labeled peanut lectin-peroxidase were purchased from Sigma (product number L 0881 and L 7759). Mouse monoclonal IgG antibodies were generated using bovine serum albumin (BSA)-T-antigen conjugates.[28] PAMAMs were kindly donated by Dr R. Spindler (Dendritech Inc., Midland, MI).

Allyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1-3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (8). A solution of allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**5**)[30] (1.67 g, 4.78 mmol) in a mixture of nitromethane/benzene (60 mL, 1:1 v/v) was stirred for 2 h at room temperature. To

ensure dryness, the solution was concentrated under reduced pressure. This process was repeated 3 times. The same volume of solvent was added and then concentrated until half the volume remained. The temperature was adjusted to 25 °C and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**6**)[31] (4.70 g, 7.13 mmol) and $Hg(CN)_2$ (1.80 g, 7.13 mmol) were added successively under N_2 atmosphere. The resulting solution was stirred at room temperature for 18 h. The solvent was removed under vacuum and the residue was dissolved in $CHCl_3$ (40 mL) and then filtered through a Celite pad. The filtrate was successively washed with 10% aqueous KI, saturated $NaHCO_3$, distilled water, and then dried over Na_2SO_4. After concentration, the residue was purified by silica gel column chromatography (benzene/EtOAc, 15:1) to give a white foamy solid in 98% yield (4.31 g, 4.67 mmol): mp 109.7–111.0 °C; R_f 0.59 (benzene/EtOAc, 1:2); $[\alpha]_D + 116.0°$ (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.06–7.19 (m, 25H, Ar), 5.98 (dd, 1H, $J_{34'} = 3.3$, $J_{45'} \leq 1$ Hz, H-4′), 5.85–5.78 (m, 2H, CH, H-2′), 5.60 (dd, 1H, $J_{23'} = 10.2$, $J_{34'} = 3.4$ Hz, H-3′), 5.48 (broad s, 1H, NH), 5.38 (s, 1H, CH), 5.23 (dd, 1H, $J_{gem} = 1.5$, $J_{trans} = 17.2$ Hz, CH), 5.16 (m, 2H, $J_{cis} = 8.0$ Hz, CH, H-1′), 5.10 (d, 1H, $J_{12} = 3.4$ Hz, H-1), 4.68 (dd, 1H, $J_{56'} = 6.9$, $J_{6ab'} = 11.4$ Hz, H-6′), 4.63–4.58 (m, 1H, H-2), 4.46–4.36 (m, 3H, H-4, H-5′, H-6′), 4.15–4.07 (m, 3H, 1H-6, CH, H-3), 3.96 (dd, 1H, $J_{gem} = 6.1$, $J_{ab} = 13.0$ Hz, CH), 3.75 (dd, 1H, $J_{56} = 1.4$, $J_{gem} = 12.4$ Hz, H-6), 3.51 (m, 1H, H-5), 1.40 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$) δ 170.0, 166.0, 165.6, 165.5, 165.0, 137.7, 133.7, 133.5, 133.4, 133.1, 130.0, 129.9, 129.8, 129.7, 129.4, 129.2, 128.9, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 126.2, 126.0 (31 C between 137.7 and 126.0), 117.7, 102.0, 100.9, 97.4, 75.8, 75.4, 71.8 (2 C), 70.2, 69.2, 68.7, 68.1, 63.0, 62.7, 48.4, 22.4; (+) FAB-MS (glycerol) *m*/*z* 928.3 (M + 1), 1856 (2M + 1). Anal. calcd for $C_{52}H_{49}O_{15}N_1$: C, 67.20; H, 5.31; N, 1.53. Found: C, 66.94; H, 5.27; N, 1.48.

Allyl *O*-(β-D-galactopyranosyl)-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (10). Compound **8** (or **9**) (1 g, 2.0 mmol) was dissolved in MeOH (10 mL) and MeONa/MeOH (cat.) was added to adjust the pH to 9. The resulting solution was stirred for 30 min at room temperature. Solvent was removed under vacuum and the residue was dissolved in water (10 mL), the aqueous layer was separated from the organic, and then lyophilized to give the de-benzoylated intermediate. Subsequently, this intermediate was dissolved in 60% aqueous acetic acid (15 mL) and the resulting solution was stirred for 1.5 h at 60 °C. Solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 11:6:1) to give white solid in 93% yield (0.79 g, 1.86 mmol): mp 230–232 °C; R_f 0.53 ($CHCl_3$/MeOH/H_2O); $[\alpha]_D + 120.0°$ (c 1, H_2O); ^1H NMR (D_2O) δ 6.08–6.01 (m, 1H, CH), 5.42 (dd, 1H, $J_{gem} = 1.6$, $J_{trans} = 17.3$ Hz, CH), 5.33 (dd, 1H, $J_{gem} = 1.7$, $J_{cis} = 10.4$ Hz, CH), 5.01 (d, 1H, $J_{12} = 3.8$ Hz, H-1), 4.53 (d, 1H, $J_{12'} = 7.8$ Hz, H-1′), 4.41 (dd, 1H, $J_{12} = 3.7$, $J_{23} = 11.2$ Hz, H-2), 4.31–4.27 (m, 2H, H-4, 1H of CH_2), 4.13–4.07 (m, 3H, H-3, H-5, 1H of CH_2), 3.98 (dd, 1H, $J_{34'} = 3.4$, $J_{45'} = 0.8$ Hz, H-4′), 3.86–3.78 (m, 4H, H-6, H-6′), 3.74–3.71 (m, 1H, H-5′), 3.69 (dd, 1H, $J_{23'} = 10.0$, $J_{34'} = 3.4$ Hz, H-3′), 3.59 (dd, 1H, $J_{12'} = 7.7$, $J_{23'} = 10.0$ Hz, H-2′), 2.09 (s, 3H, Ac); ^{13}C NMR (D_2O) δ 174.1, 133.2, 117.4, 104.2, 95.9, 76.8, 74.5, 72.1, 70.2, 70.1, 68.3, 68.1, 68.0, 60.7, 60.5, 48.1, 21.5; (+) FAB-MS (glycerol) *m*/*z*: 424.2 (M + 1). Anal. calcd for $C_{17}H_{29}O_{11}N_1$: C, 45.30; H, 6.92; N, 3.30. Found: C, 45.30; H, 6.93; N, 3.07

3-(2-Carboxyethylthio)propyl β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (12). To a solution of allyl β-D-Gal-(1-3)-α-D-GalNAc (**13**) (100 mg, 0.24 mmol) in deoxygenated distilled water (2.5 mL) was added 3-mercaptopropionic acid (**11**, 21 μL, 1 equiv) and the resulting solution was irradiated (254 nm) for 7 h under N_2 atmosphere. The reaction solution was then loaded onto an anion exchange resin column (Amberite IRA 400 OH^-) and washed with water. Eluent was then changed gradually to aqueous acetic acid with an increasing acetic acid gradient. The fractions containing **12** were collected and evaporated under reduced pressure followed by multiple coevaporation with ethanol. A small amount of water was added and lyophilized to afford a white spongy solid in 83% yield (105.9 mg, 2.0 mmol): mp 90.0–92.5 °C; $\alpha_D + 76.0°$ (c 1, H_2O); R_f 0.33 ($CHCl_3$/MeOH/H_2O, 10:9:1); ^1H NMR (D_2O) δ 4.96 (d, 1H, $J_{12} = 3.7$ Hz, H-1), 4.54 (d, 1H, $J_{12'} = 7.7$ Hz, H-1′), 4.39 (dd, 1H, $J_{12} = 3.7$, $J_{23} = 11.0$ Hz, H-2), 4.31 (broad d, 1H, H-4), 4.10 (dd, 1H, $J_{23} = 11.1$, $J_{34} = 3.1$ Hz, H-3), 4.07 (broad s, 1H, H-5), 3.98 (broad d, 1H, $J_{34'} = 3.5$ Hz, H-4′), 3.89–3.79 (m, 5H, H-6, H-6′, 1H of CH_2), 3.74–3.72 (m, 1H, H-5′), 3.69 (dd, 1H, $J_{23'} = 10.0$, $J_{34'} = 3.4$ Hz, H-3′), 3.65–3.57 (m, 2H, H-2′, 1H of CH_2), 2.89 (t, 2H, $J = 7.5$ Hz, CH_2), 2.80–2.73 (m, 4H, 2 CH_2), 2.10 (s, 3H, Ac), 2.01–1.96 (m, 2H, CH_2); ^{13}C NMR (D_2O) δ 174.0, 104.2, 96.7, 76.8, 74.5, 72.0, 70.1, 70.1, 68.2, 68.1, 66.0, 60.7, 60.5, 48.2, 34.2, 27.9, 27.6, 26.0, 21.5; (+) FAB-MS (glycerol) *m*/*z*: 530.3 (M + 1).

Synthesis of glycoPAMAM dendrimers

General procedure. PAMAM in MeOH (**13**, **14**, **15** and **16**, $n = 4$, 8, 16 and 32) was added to a round bottom flask (25 mL) and dried under vacuum. The residue was dissolved in DMSO and compound **12** (1.1 equiv per amino group), TBTU (1.2 equiv per acid) and DIPEA (pH = 9) were added. The resulting solution was stirred overnight at room temperature. The extent of coupling was confirmed by a negative ninhydrin test. DMSO was eliminated by lyophilization and the residue was purified by gel permeation chromatography (P-2, P-4, H_2O) or dialysis (2000 molecular weight cut-off). The product portion was collected and lyophilized to afford the corresponding glycoPAMAM dendrimers (**17**–**20**) in 73, 81, 99 and 79% yields, respectively.

Tetrameric glycoPAMAM dendrimer 17. PAMAM (**13**, $n = 4$) dendritic core (7.2 mg, 13.9 μmol) and T-antigen derivative (**12**, 32.4 mg, 61.2 μmol) were dissolved in DMSO (6 mL) and TBTU (23.6 mg, 73.5 μmol) and DIPEA (pH = 9) were added. After purification, glycoPAMAM dendrimer **17** was obtained in 73% yield (26 mg, 10.2 μmol). ^1H NMR (D_2O) δ 4.96 (d, 4H, $J_{12} = 3.8$

Hz, H-1), 4.55 (d, 4H, $J_{12'}=7.8$ Hz, H-1′), 4.40 (dd, 4H, $J_{12}=3.8$, $J_{23}=11.1$ Hz, H-2), 4.32 (broad d, 4H, $J_{34}=2.8$, $J_{45}\leq 1$ Hz, H-4), 4.10 (dd, 4H, $J_{23}=11.1$, $J_{34}=3.0$ Hz, H-3), 4.07 (broad t, 4H, $J_{45}\leq 1$, $J_{56}=6.2$ Hz, H-5), 3.99 (broad d, 4H, $J_{34'}=3.3$, $J_{45'}\leq 1$ Hz, $H_{-4'}$), 3.89–3.76 (multi, 20H, H-6, H-6′, CH_2), 3.75–3.72 (multi, 4H, H-5′), 3.70 (dd, 4H, $J_{23'}=9.9$, $J_{34'}=3.4$ Hz, H-3′), 3.66–3.58 (multi, 8H, CH_2, H-2′), 3.41 (broad s, 16H, CH_2, CH_2), 3.29 (broad t, 8H, CH_2), 2.89 (t, 8H, $J=6.9$ Hz, CH_2), 2.80 (s, 4H, CH_2), 2.77 (t, 8H, $J=7.1$ Hz, CH_2), 2.72 (t, 8H, $J=6.7$ Hz, CH_2), 2.62 (t, 8H, $J=6.9$ Hz, CH_2), 2.11 (s, 12H, CH_3), 2.01–1.95 (multi, 8H, CH_2); ^{13}C NMR (D_2O) δ 174.2, 174.0, 172.6 (12 C between 174.2 and 172.6), 104.2 (4 C), 96.8 (4 C), 76.8 (4 C), 74.5 (4 C), 72.1 (4 C), 70.2 (4 C), 70.1 (4 C), 68.2 (4 C), 68.1 (4 C), 66.0 (4 C), 60.7 (4 C), 60.6 (4 C), 48.6 (4 C), 48.3 (4 C), 38.4 (8 C), 38.0 (2 C), 35.3 (4 C), 29.9 (4 C), 28.0 (4 C), 27.6 (4 C), 26.7 (4 C), 21.6 (4 C).

Octameric glycoPAMAM dendrimer 18. PAMAM (**14**, $n=8$) dendritic core (8.37 mg, 5.85 μmol) and T-antigen derivative (**12**, 27.3 mg, 51.6 μmol) were dissolved in DMSO (5 mL) and TBTU (20 mg, 62.3 μmol) and DIPEA (pH = 9) were added. After purification, glycoPAMAM dendrimer **18** was obtained in 81% yield (26.1 mg, 4.7 μmol). ^{1}H NMR (D_2O) δ 4.96 (d, 8H, $J_{12}=3.7$ Hz, H-1), 4.55 (d, 8H, $J_{12'}=7.8$ Hz, H-1′), 4.39 (dd, 8H, $J_{12}=3.7$, $J_{23}=11.1$ Hz, H-2), 4.32 (d, 8H, $J_{34}=2.7$, $J_{45}\leq 1$ Hz, H-4), 4.10 (dd, 8H, $J_{23}=11.1$, $J_{34}=2.9$ Hz, H-3), 4.06 (broad t, 8H, $J_{56}=6.6$ Hz, H-5), 3.99 (d, 8H, $J_{34'}=3.3$ Hz, H-4′), 3.89–3.77 (multi, 40H, H-6, H-6′, CH_2), 3.74–3.69 (multi, 24H, H-5′, H-3′, CH_2), 3.66–3.52 (multi, 24H, H-2′, CH_2), 3.47 (broad t, 8H, CH_2), 3.41 (broad s, 32H, CH_2), 3.28 (broad t, 16H, CH_2), 2.90–2.83 (multi, 24H, CH_2), 2.80 (s, 4H, CH_2), 2.79–2.75 (multi, 32H, CH_2), 2.62 (t, 16H, $J=6.8$ Hz, CH_2), 2.10 (s, 24H, CH_3), 1.99 (multi, 16H, CH_2); ^{13}C NMR (D_2O) δ 174.2, 173.7, 173.3, 171.42 (28 C), 104.2 (8 C), 96.8 (8 C), 76.8 (8 C), 74.5 (8 C), 72.1 (8 C), 70.2 (8 C), 70.1 (8 C), 68.2 (8 C), 68.1 (8 C), 66.0 (8 C), 60.8 (8 C), 60.6 (8 C), 51.7 (4 C), 49.6 (4 C), 48.7 (8 C), 38.4, 38.0 (18 C between 38.4 and 38.0), 35.3 (8 C), 34.0 (4 C), 30.2 (8 C), 29.8 (8 C), 28.1 (8 C), 28.0 (4 C), 27.6 (8 C), 26.7 (8 C), 21.6 (8 C).

16-Mer glycoPAMAM dendrimer 22. PAMAM (**18**, $n=16$) dendritic core (11.5 mg, 3.52 μmol) and T-antigen derivative (**15**, 32.8 mg, 62.0 μmol) were dissolved in DMSO (10 mL) and TBTU (24.0 mg, 74.7 μmol) and DIPEA (pH = 9) were added. After purification, glycoPAMAM dendrimer **22** was obtained in 99% yield (40 mg, 3.5 μmol). ^{1}H NMR (D_2O) δ 4.96 (d, 16H, $J_{12}=3.7$ Hz, H-1), 4.56 (d, 16H, $J_{12'}=7.8$ Hz, H-1′), 4.40 (dd, 16H, $J_{12}=3.7$, $J_{23}=11.1$ Hz, H-2), 4.32 (d, 16H, $J_{34}=3.0$, $J_{45}\leq 1$ Hz, H-4), 4.10 (16H, $J_{23}=11.1$, $J_{34}=3.1$ Hz, H-3), 4.06 (broad t, 16H, $J_{56}=6.4$ Hz, H-5), 3.99 (d, 16H, $J_{34'}=3.3$ Hz, H-4′), 3.89–3.76 (multi, 80H, H-6, H-6′, CH_2), 3.75–3.69 (multi, 32H, H-3′, H-5′), 3.65–3.58 (multi, 64H, H-2′, CH_2), 3.41–3.30 (multi, 120H, CH_2) 3.28–3.06 (multi, 80H, CH_2), 2.89 (t, 32H, J = 7.0 Hz, CH_2), 2.79–2.75 (multi, 92H, CH_2), 2.62 (t, 32H, $J=7.0$ Hz, CH_2), 2.10 (s, 48H, CH_3), 2.09–1.95 (multi, 32H, CH_2); ^{13}C NMR (D_2O) δ 174.1, 173.9, 173.1, 172.3 (60 C), 104.2 (16 C), 102.8 (16 C), 76.9 (16 C), 74.7 (16 C), 72.1 (16 C), 70.4 (16 C), 70.2 (16 C), 68.2 (16 C), 68.2 (16 C), 66.0 (16 C), 60.8 (16 C), 60.57 (16 C), 53.9, 51.6, 49.3, 48.8 (40 C between 53.9 and 48.8), 49.3, 48.8, 48.3, 43.0, 38.4, 38.3 (44 C between 49.3 and 38.3), 48.8 (16 C), 35.3 (16 C), 31.50, 29.4, 28.05 (46 C between 31.5 and 28.0), 27.6 (16 C), 26.7 (16 C), 21.7 (16 C).

32 Mer glycoPAMAM dendrimer 20. PAMAM (**16**, $n=32$) dendritic core (12.4 mg, 1.80 μmol) and T-antigen derivative (**12**, 33.5 mg, 63.4 μmol) were dissolved in DMSO (8 mL) and TBTU (24.4 mg, 76.0 μmol) and DIPEA (pH = 9) were added. After purification, glycoPAMAM dendrimer **20** was obtained in 79% yield (32.2 mg, 1.38 μmol). ^{1}H NMR (D_2O) δ 4.96 (d, 32H, $J_{12}=3.7$ Hz, H-1), 4.56 (d, 32H, $J_{12'}=7.7$ Hz, H-1′), 4.40 (dd, 32H, $J_{12}=3.7$, $J_{23}=1.1$ Hz, H-2), 4.32 (broad d, 32H, $J_{34}=2.9$, $J_{45}\leq 1$ Hz, H-4), 4.10 (dd,32H, $J_{23}=11.1$, $J_{34}=3.0$ Hz, H-3), 4.06 (broad t, 32H, $J_{56}=6.1$ Hz, H-5), 3.99 (broad d, 32H, $J_{34'}=3.4$ Hz, H-4′), 3.95–3.79 (multi, 160H, H-6, H-6′, CH_2), 3.78–3.69 (multi, 64H, H-3′, H-5′), 3.65–3.58 (multi, 64H, H-2′, CH_2), 3.40 (broad s, 192H, CH_2), 2.92 (broad s, 112H, CH_2), 2.88 (t, 64H, $J=7.0$ Hz, CH_2), 2.77 (multi, 128H, CH_2), 2.62 (t, 64H, $J=7.0$ Hz, CH_2), 2.56–2.51 (broad s, 112H, CH_2), 2.10 (s, 96H, CH_3), 2.00–1.39 (multi, 64H, CH_2); ^{13}C NMR (D_2O) δ 174.2, 174.0, 173.9 (124 C), 104.20 (32 C), 96.8 (32 C), 76.8 (32 C), 74.6 (32 C), 72.1 (32 C), 70.2 (32 C), 70.1 (32 C), 68.2 (32 C), 68.1 (32 C), 66.0 (32 C), 60.7 (32 C), 60.55 (32 C), 50.9 (32 C), 48.7 (56 C), 48.3 (32 C), 38.2, 36.2 (98 C between 38.2 and 36.2), 35.3 (32 C), 32.1 (56 C), 28.1 (32 C), 27.6 (32 C), 26.7 (32 C), 21.7 (32 C).

Turbidimetric analysis between peanut lectin from *Arachis Hypogaea* and glycoPAMAM dendrimers (17, 18, 19 and 20). Turbidimetric experiments were performed in Linbro (Titertek) microtitration plates where 50 μL/well of stock lectin solutions prepared from peanut lectin (2 mg/mL in PBS) were mixed with 50 μL of a stock solution of glycodendrimers, **17**, **18**, **19** and **20** (2.1, 1, 0.54 and 0.27 nmol/well in PBS) and incubated at room temperature for 3 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 were observed.[36] Each test was performed in triplicate.

Enzyme linked lectin assays (ELLA) between peanut lectin and PAMAM based T-antigen dendrimers (17, 18, 19 and 20). Linbro (Titertek) microtitration plates were coated with 100 μL/well of glycoPAMAM dendrimers (**17**–**20**) in a stock solution (1.4 nmol in 0.01M PBS, pH = 7.3) and incubated overnight at room temperature. Previous studies with lactose-based dendrimers showed equimolar amount of dendrimers bound to the plates.[34] After washing with PBST and blocking with BSA (1% PBS), peanut lectin (10 μL of supernatant (1 mg/mL) in PBS) was added and incubated for 1 h at 37 °C. The plate was washed with PBST and the wells were filled with 100 μL/well of *Arachis hypogaea*-peroxidase supernatant (0.1 mg/mL) with a serial dilution by a

factor of 10 in PBST and incubated at 37 °C for 1 h. The plates were washed and 50 μL/well of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/4 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 H_2SO_4 (1 M, 50 μL/well) and the optical density was measured at 410 nm relative to 570 nm. A blank well contained citrate-phosphate buffer. Each test was performed in triplicate.

Competitive double sandwich inhibition ELISA using mouse monoclonal antibody JAA-F11 IgG3 and T-antigen dendrimers, 17, 18, 19 and 20 as inhibitors. Linbro (Titertek) microtiter plates were coated overnight with T-antigen containing co-polyacrylamide[28,29] with 100 μL of a polymer stock solution, [10 μg/mL in 0.01 M phosphate buffer (pH = 7.3)] at room temperature. Each well contained 1 μg/well of polymer which corresponds to 0.36 μg (0.85nmol) T-antigen. The wells were then washed 3 times with 400 μL of PBST (0.01 M phosphate buffer (pH = 7.3) containing 0.05% (v/v) Tween 20). BSA solution (1% in PBS 150 μL/well) was added to each well and incubated for 1 h at 37 °C. While, 50 μL of mouse monoclonal IgG antibody solution in PBST (10 times dilution of ascitic fluid, 0.25 μmol/50 μL) and 50 μL of inhibitor solution in PBST with varying concentrations from 275 to 1.07 nmol/well of T-antigen prepared by twofold serial dilution were mixed in Nunclon (Delta) microtiter plates and preincubated for 1 h at 37 °C. After excess BSA was washed out with with PBST, each well was filled with 100 μL/well of pre-incubated mouse IgG MAb/inhibitor solution and incubated again for 1 h at 37 °C. The wells were washed with PBST as described above and then filled with 100 μL of goat anti-mouse IgG in PBST (1,000 times dilution of ascitic fluid) followed by incubation for 1 h at 37 °C. The wells were washed with PBST and 50 μL of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg/4 mL of 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 the addition of 50 μL/well of 1 M aqueous sulfuric acid solution. Optical density was measured at 410 nm relative to 570 nm. The percent inhibition was calculated as follows:

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

IC_{50}s were calculated as the concentration required for 50% inhibition of the coating antigen. All test were performed in triplicate.

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