Synthesis of *N*,*N*′-bis(Acrylamido)acetic Acid-Based T-Antigen Glycodendrimers and Their Mouse Monoclonal IgG Antibody Binding Properties

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Abstract: Novel glycodendrimers based on *N*,*N*'-bis(acrylamido)acetic acid core with valencies between two and six were synthesized. The breast cancer-associated T-antigen carbohydrate marker, (*β*-Gal-(1-3)-α-GalNAc-OR), was then conjugated by (i) 1,4-conjugate addition of thiolated T-antigen to the *N*-acrylamido dendritic cores and by (ii) amide bond formation between an acid derivative of the T-antigen and the polyamino dendrimers. The protein-binding ability of these new glycodendrimers was fully demonstrated by turbidimetric analysis and by enzyme-linked immunosorbent assay (ELISA) using peanut lectin from *Arachis hypogaea* and a mouse monoclonal antibody (MAb) FAA-J11 (IgG3). When tested as inhibitors of binding between MAb and a polymeric form of the T-antigen (T-antigen-*co*-polyacrylamide) used as a coating antigen, di- (**17**), tetra- (**20**), hexa- (**21**), and tetravalent (**22**) dendrimers showed IC_{50} values of 174, 19, 48, and 18 nM, respectively. Two tetramers showed 120- to ∼128-fold increased inhibitory properties over the monovalent antigen **6** used as a standard (IC_{50} 2.3 mM). Heterobifunctional glycodendrimer bearing a biotin probe was also prepared for cancer cell labeling.

Introduction

The Thomsen-Friedenreich (T or TF) carbohydrate antigen (T-Ag), *β*-Gal-(1−3)-α-GalNAc, has been well-documented as a cancer-related marker[1] and as an important antigen for the detection and immunotherapy of carcinomas, particularly relevant to breast cancer. In related pharmaceutical applications, T-Ag-containing glycopolymers (Figure 1) have been employed in high-throughput screening toward the development of a solid-phase glycosyltransferase assay for drug discovery research.[2]

Mouse monoclonal antibodies, JAA-F11 (IgG3) and C5 (IgM) were also recently developed for successful immunohistochemical staining of breast adenocarcinomas.[3] These antibodies were obtained from T-antigen-BSA conjugates (Figure 1) that did not contain any peptide segment of the natural T-antigen present on mucins, thus demonstrating the thorough carbohydrate nature of the cancer marker. The expression of T-Ag has been proposed as a tool for the detection of tumors and as a criterion for prognosis.[4] Moreover, there is an increased expression of T-Ag in metastatic tumors, and a plant lectin (*Arachis hypogaea*) that recognizes T-Ag has been shown to bind to common sites of metastatic tumor growth.[5] Receptors for T-Ag adhesion have been proposed to be in the liver, the bone marrow, and the lymph nodes.

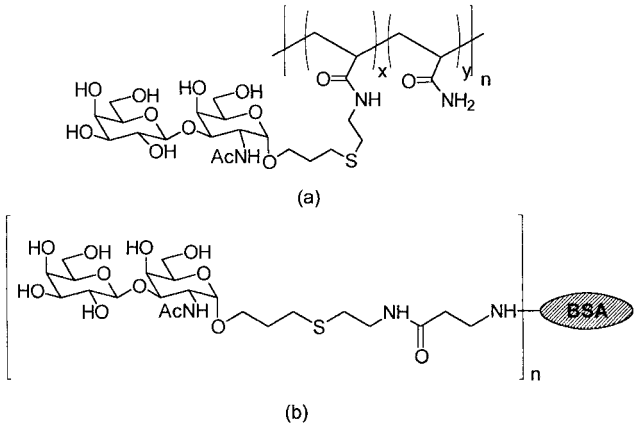

Figure 1. Structure of T-antigen bearing neoglycoconjugates.

With the necessary antibodies in hand, it therefore became of interest to synthesize nonimmunogenic, high-affinity T-Ag-containing glycodendrimers that could be used to block the potential metastatic sites of invasion tumors cells. These molecules might be particularly useful after surgery to protect

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(1) (a) Livingston, P. O.; Koganty, R.; Longenecker, B. M.; Lloyd, K. O.; Calves, M. *Vaccine Res.* **1992**, *1*, 99. (b) Springer, G. F.; Murthy, M. S.; Desai, P. R.; Scanlon, E. F. *Cancer* **1980**, *45*, 2949. (c) Itzkowitz, S. H.; Yuan, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res.* **1989**, *49*, 197. (d) Bray, J.; Leumieux, R. U.; McPherson, T. A. *J. Immunol.* **1981**, *126*, 1966. (e) Kichler, A.; Schuber, F. *Glycoconjugate J.* **1995**, *12*, 275. (f) Höppner, W.; Fischer, K.; Poschmann, A.; Paulsen, H. *Mol. Immunol.* **1985**, *22*, 1341. (g) Hung, P. Y. S.; Madej, M.; Koganty, R. R.; Longenecker, B. M. *Cancer Res.* **1990**, *50*, 4308. (h) Chen, Y.; Jain, R. K.; Chandrasekaran, E. V.; Matta, K. L. *Glycoconjugate J.* **1995**, *12*, 55.

(2) (a) Donovan, R. S.; Datti, A.; Baek, M. G.; Wu, Q. Q.; Sas, I. J.; Korczak, B.; Berger, E. G.; Roy, R.; Dennis, J. W. *Glycoconjugate J.* **1999**, *16*, 607. (b) Baek, M.-G.; Roy, R. *Biomacromolecules* **2000**, *1*, 768.

(3) Rittenhouse-Diakun, K.; Xia, Z.; Pickhardt, D.; Morey, P. S.; Baek, M. G.; Roy, R. *Hybridoma* **1998**, *17*, 165.

(4) Maclean, G. D.; Longenecker, B. M. *Semin. Cancer Biol.* **1991**, *2*, 433.

(5) Gabius, H.-J.; Schroter, C.; Gabius, S.; Brink, U.; Tietze, L.-F. *J. Histochem. Cytochem.* **1990**, *38*, 1625.

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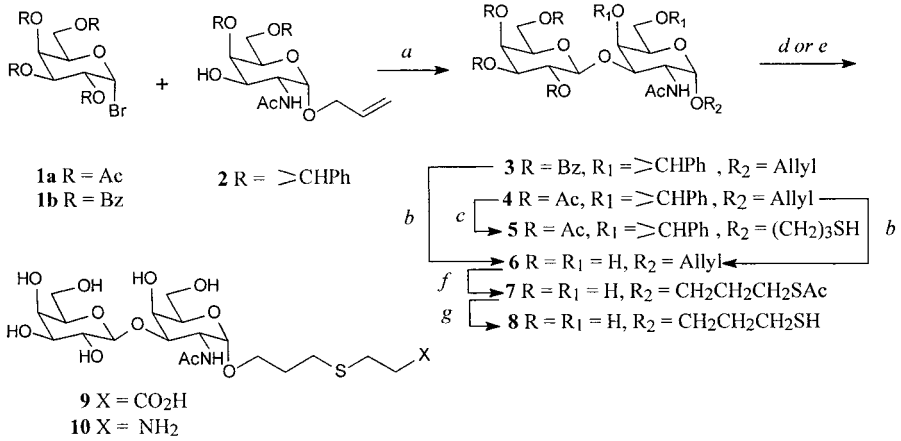
Scheme 1[a]



[a] $Hg(CN)_2$, PhH/CH_3NO_2, room temp, 98%; (b) (i) $CH_3O^-Na^+$/MeOH, (ii) aq AcOH, 60 °C, quant; (c) AcSH/AIBN, MeOH, reflux, 71%, then $CH_3O^-Na^+$/MeOH, quant; (d) $HSCH_2CH_2NH_2$ (**13**) or (e) $HSCH_2CH_2CO_2H$, *hν* (254 nm), water, 63 or 83%; (f) AcSH/AIBN, MeOH, heat, 71%; (g) $CH_3O^-Na^+$/MeOH, quant.

against the spreading of cancer cells that have evaded the sites of intervention. Toward this goal, we synthesized and evaluated the relative binding properties of a new type of T-antigen dendrimers (**17**, **20**, **21**, and **22**) with valencies varying between two and six on the basis of *N*,*N*′-bis(acrylamido)acetic acid (**11**) used as the seed molecule. The cluster effect of the T-antigen dendrimers was also evaluated toward the highly specific protein from the plant lectin of *A. hypogaea* and to our mouse MAb JAA-F11 in comparison to monomeric T-antigen (**6**).

Multivalent neoglycoconjugates are excellent tools for the understanding of multiple carbohydrate-protein binding interactions via cooperative association energies.[6] Several glycopolymers,[7−9] including our recently synthesized T-Ag copolyacrylamides,[2] showed strong inhibitory properties against a wide range of carbohydrate receptors. However, glyco-*co*-polymers have ill-defined chemical structures with random distribution of carbohydrates, and some condensation polymers are too dense.

Due to the potential applications in medicinal engineering, drug delivery, gene- and chemo-therapy, sensory devices, and as bioactive function carriers with immunochemical and pharmaceutical applications, monodisperse high-molecular-weight dendrimers have stimulated wide interest in the field of chemistry and biology.[10] Recently, novel classes of nonimmunogenic glycodendrimers have provided a better understanding of multiple-carbohydrate−protein interactions.[11−13]

As cell surface carbohydrates are involved in a wide variety of important biological phenomena, such as recognition of bacteria, viruses, hormones, and other cells; cell growth; regulation and differentiation; cancer metastasis; and cellular trafficking;[14] it can be stated that chemically well-defined glycoconjugates (glycodendrimers) provide a better understanding of biological recognition processes by virtue of well-organized carbohydrate valencies, shapes, and orientations in comparison to random copolymers. Many such glycodendrimers containing diverse carbohydrate ligands have been reported.[11−13]

Results and Discussion

Although several syntheses of the T-Ag with various aglycones exist,[15] we chose to synthesize the required allyl T-antigen (*β*-D-Gal-(1−3)-α-D-GalNAc−O-allyl) in large scale from **1** and **2** under classical Helferich glycosylation method in excellent yields ($PhH/MeNO_2$, $Hg(CN)_2$, 78 to ∼98%, respectively) (Scheme 1). The T-antigen derivatives **3** and **4** were deprotected under Zemplén conditions (NaOMe, MeOH), followed by aqueous acetic acid hydrolysis of the benzylidene acetal to afford **6** quantitatively. The thiol functionality was introduced onto the alkenyl moiety of **4** and **6** using photochemical activation with thioacetic acid. The resulting thioacetates were successively treated under Zemplén conditions to provide **5** and **8** (degassed MeOH, reflux, 82 and 71%, respectively). Other functional groups such as acid (**9**) and amine (**10**) were also introduced via sulfide linker formation using thiol derivatives such as 3-mercaptopropionic acid and cysteamine (**13**) (254 nm, degassed water, 63 and 83% yields, respectively).

Synthesis of Dendritic Cores Based on *N*,*N*′-Bis(acrylamido)acetic Acid. Due to its bifunctionality and its commercial accessibility, *N*,*N*′-bis(acrylamido)acetic acid (**11**) was chosen

(6) (a) Bundle, D. R.; Young, N. M. *Curr. Opin. Struct. Biol*. **1995**, *2*, 666. (b) Vyas, N. K. *Curr. Opin. Struct. Biol.* **1996**, *6*, 732. (c) Toone, E. J. *Curr. Opin. Struct. Biol*. **1991**, *4*, 719.

(7) (a) Roy, R. In *Carbohydrate Chemistry*; Boon, G.-J., Ed.; Blackie Academic & Professional: London, U.K. **1998**, p 243. (b) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692. (c) Roy, R. *Top. Curr. Chem*. **1997**, *187*, 241. (d) Roy, R. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79.

(8) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754.

(9) (a) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413. (b) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71.

(10) (a) Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr*. **1995**, *132*, 875. (b) Fréchet, J. M. J. *Science* **1994**, *263*, 1710. (c) Gensch, T.; Hofkens, J.; Heirmann, A.; Tsuda, K.; Verheijen, W.; Vosch, T.; Christ, T.; Basché, T.; Müller, K.; De Schryver, T. C. *Angew. Chem., Int. Ed. Engl*. **1999**, *38*, 3752. (d) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413.

(11) (a) Roy, R. *Polymer News* **1996**, *21*, 226. (b) Roy, R. *Carbohydr. Eur.* **1999**, *27*, 34. (b) Zanini, D.; Roy, R. *J. Am. Chem. Soc*. **1997**, *119*, 2088. (c) Zanini, D.; Roy, R. *J. Org. Chem*. **1996**, *61*, 7348.

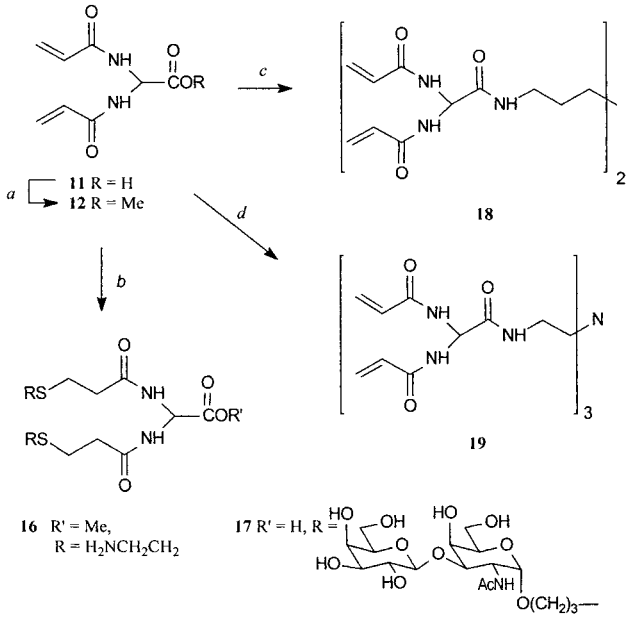
(12) Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 1953.

(13) Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen, T. M.; Spencer, N.; Stoddart, J. F.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429.

(14) (a) Varki, A. *Glycobiology* **1993**, *3*, 97.

(15) (a) Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 837. (b) Paulsen, H.; Peters, S.; Bielfeldt, T.; Meldal, M.; Bock, K. *Carbohydr. Res.* **1995**, *268*, 17. (c) Kunz, H.; Birnbach, S.; Wernig, P. *Carbohydr. Res.* **1990**, *202*, 207. (d) Kinzy, W.; Schmidt, R. R. *Carbohydr. Res.* **1987**, *166*, 265. (e) Qiu, D.; Gandhi, S. S.; Koganty, R. R. *Tetrahedron Lett.* **1996**, *37*, 595. (f) Lubineau, A.; Bienaymé, H. *Carbohydr. Res.* **1991**, *212*, 267. (g) Gambert, U.; Thiem, J. *Carbohydr. Res.* **1997**, *299*, 85. (h) Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, 231. (i) Bay, S.; Berthier-Vergnes, O.; Cantacuzene, D. *Carbohydr. Res.* **1997**, *298*, 153. (j) Roy, R.; Tropper, F. D.; Romanowska, A.; Jain, R. K.; Piskorz, C. F.; Matta, K. L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 911.

Scheme 2[a]



[a] $SOCl_2$/MeOH, −15 → 25 °C, 80%; (b) for **16**: (i) $HSCH_2CH_2NH_2$ (**13**), MeOH, DIPEA, overnight, 25 °C, (ii) OH^- resin, 93%; for **17**: (i) **8**, Et_3N, MeOH, 25 °C, N_2, quant; (c) $H_2N(CH)_6NH_2$ (**14**), HOBt/EDC/DIPEA, DMSO, overnight, 60%; (d) $N(CH_2CH_2NH_2)_3$ (**15**).

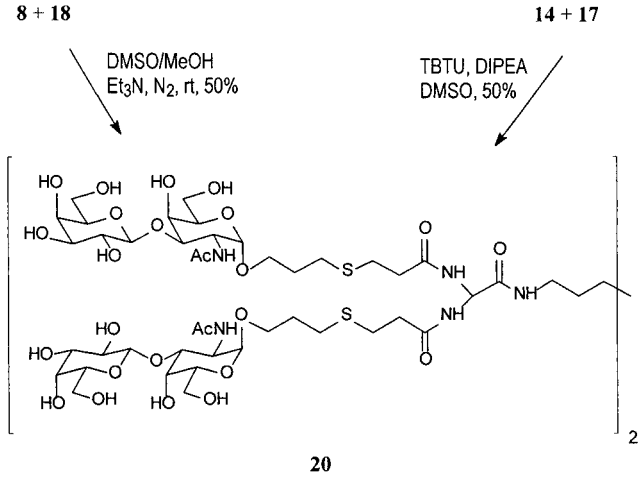
as a building block. Compound **11** was esterified to give methyl ester **12** ($SOCl_2$, MeOH, 80%). Subsequently, **12** was coupled with cysteamine **13** to provide a molecule with extended spacer length under mild condition to give bis-amino methyl ester **16** in an excellent yield [(i) **13**, DIPEA, MeOH; (ii) HO^-, Amberlite IRA-400, 93%] (Scheme 2).

Acid **11** was also successfully coupled to thiolated T-antigen **10** by a 1,4-conjugate addition to give divalent cluster **17** (Et_3N, degassed MeOH, quantitative). Construction of tetra- and hexavalent dendritic cores **18** and **19** were efficiently accomplished using a carbodiimide-hydroxybenzotriazole (EDC/HOBt) method (Scheme 2). Briefly, **11** was coupled with 1,6-hexamethylenediamine (**14**) or tris(2-ethylamino)amine (**15**) to afford the corresponding glycodendrimer precursors **18** and **19,** which were separated from the diisopropylcarbodiimide urea by precipitation (60 and 62%, respectively).

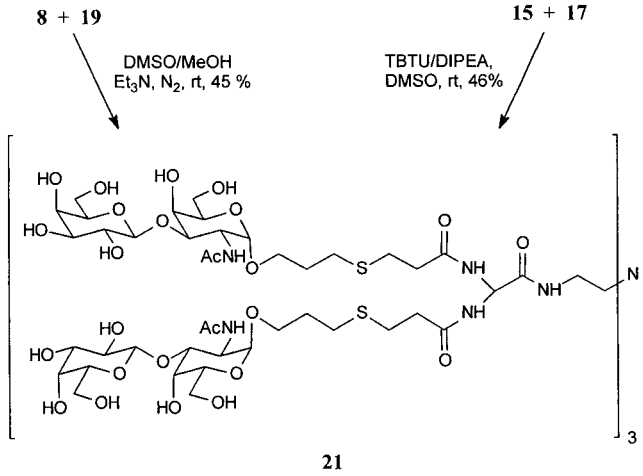
The synthesis of the small glycoclusters was based on the coupling of dendritic cores and the T-antigen derivatives by Michael-type addition of thiolated carbohydrates or by peptide coupling, depending on the functionality of each component, to give the corresponding glycodendrimers with valencies between four and six. Thus, tetravalent T-antigen dendrimer **20** was prepared as follows: Pre-built tetravalent acrylamido derivative **18** was treated with a slight excess of thiolated T-antigen **8** (Et_3N, degassed DMSO/MeOH, N_2). Alternatively, 1,6-hexamethylenediamine **14** was coupled to divalent T-antigen **17** (TBTU, DIPEA, DMSO) to give tethered T-Ag dendrimer **20** in 50% yield. Dendrimer **20** was isolated by gel permeation chromatography (GPC, Bio-gel P-2) using water as eluent. The hexavalent cluster **21** was obtained from either thiolated **8** and hexavalent core **19** (1,4-conjugate addition) or, alternatively, from trisamine **15** and acid **17** (amide coupling), as described above, in 45 and 46% yields, respectively (Scheme 4).

Complete couplings between the dendritic cores and the T-antigen were verified by ^1H NMR spectra for compounds **17**, **20**, and **21**. Disappearance of the acryloyl group at δ 6.35, 6.12, and 5.62 ppm (DMSO-d_6) and the emergence of new signals corresponding to the anomeric protons of the disaccharide (δ 4.96 and 4.55 ppm, D_2O) and the integration of these signals relative to methylene signals of the cores fully established the complete extent of T-antigen incorporation.

Scheme 3



Scheme 4



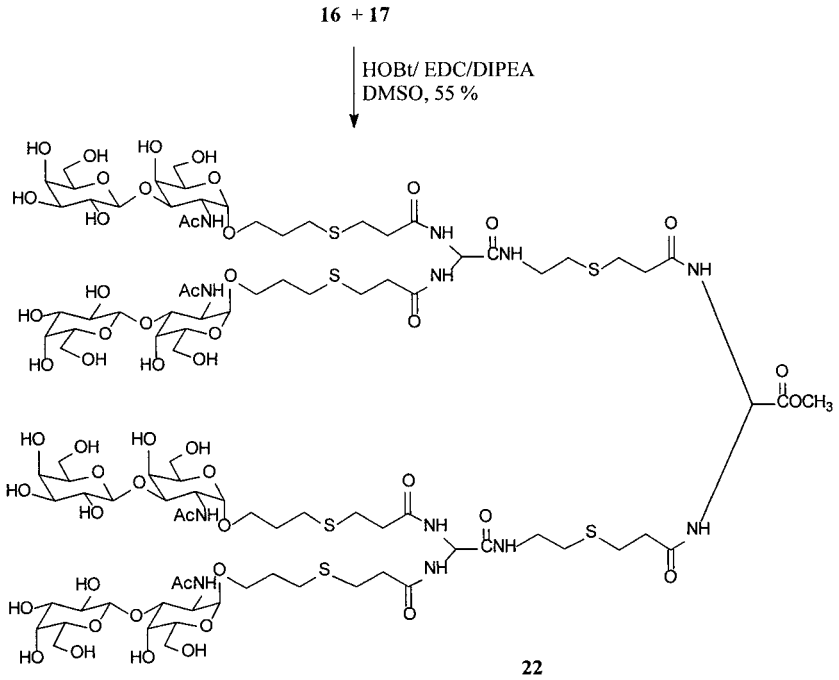
An additional tetravalent T-antigen cluster **22** was analogously obtained from diamine methyl ester **16** and divalent T-antigen **17** using carbodiimide coupling as above (55%) (Scheme 5). The strategy described for the efficient syntheses of the glycoclusters follows a convergent approach that was shown to be efficient to provide all the desired products in good yields. The chemical characterization of the dendritic structures was straightforward. Structural analysis of each dendrimer was performed by high-field NMR spectroscopy using the well-resolved doublets at δ 4.96 and 4.55 ppm, which correspond to the two anomeric protons relative to other signals, especially from the methylene or methine signals of the core.

For the purpose of broadening the potential application of these conjugates, a heterobifunctional glycocluster **24** was also prepared using divalent acid **17** and biotin derivative **23**[16] (TBTU, DIPEA, DMF, 66%) (Scheme 6). The resulting probe **24** was purified by GPC using Biogel P-4 in water. Such a labeled glycocluster can be used to screen T-Ag receptors in cancer cells using well-established streptavidin assays. Further investigation is under way to broaden the application and efficiency of the multi-functional glycoconjugates, and the results will be reported elsewhere.

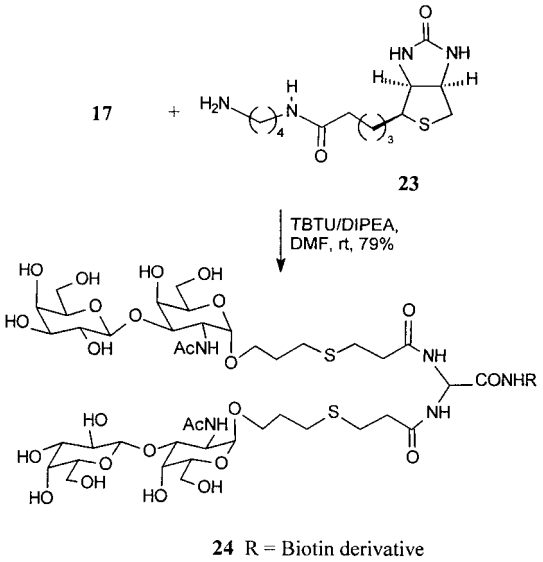
Turbidimetric Analysis of T-Antigen Dendrimers. *A. hypogaea*, having four noncovalently associated subunits (Mr

(16) Zanini, D. Ph.D. Dissertation, University of Ottawa, 1997.

Scheme 5



Scheme 6



= 120 000), is a plant having lectin with strong T-antigen (*β*-Gal-(1−3)-α-GalNAc)-binding specificity. It does not agglutinate normal human erythrocytes but does strongly agglutinate neuraminidase-treated erythrocytes and has potent anti-T activity similar to that of the anti-T antibody in human sera.[17] The relative binding ability of T-antigen dendrimers to peanut lectin was determined by a microturbidimetric analysis, which served to determine the cross-linking ability of the glycoclusters to form insoluble complexes with the tetrameric lectin.

The time course formation of precipitin complexes between peanut lectin and di- (**17**), tetra- (**20**), hexa-(**21**), and tetravalent (**22**) dendrimers is compared in Figure 2. In most cases, the maximum turbidity was obtained within 1 h. These microquantitative experiments demonstrated that the hexamer **21** showed the strongest/fastest ability to precipitate the lectin (OD = 0.43). Two tetramers, **20** and **22**, showed moderate OD values of 0.13, and 0.12, respectively. Dimer **17** showed the lowest value (OD = 0.1). The relative turbidities of di- and tetravalent dendrimers were up to only 50% when compared to the value of hexamer. These preliminary results clearly confirmed the multivalency effect for the binding to the receptor protein and also convinced us of its further applications in the fields of biology and immunology as a potential inhibitor for biological applications.

Enzyme-Linked Immunosorbent Assays (ELISA). The relative inhibitory potency of each dendrimer toward the mouse monoclonal antibody (MAb) JAA-F11[3] bound to the coated antigen (Gal-*β*-(1-3)-GalNAc-α-*O*-)-*co*-polyacrylamide (T-antigen/acrylamide, 1/10)[2] was determined by a competitive enzyme-linked immunosorbent assay (ELISA). Horseradish peroxidase-labeled goat−antimouse IgG was used for quantitative detection. The results for the inhibition of binding of JAA-F11 to the coated antigen are shown in Figure 3. In general, an increase in multivalency resulted in an increase in inhibitory potency. At low dendrimer concentrations, no inhibition was detected without the multivalency effect, regardless of dendrimer valencies. As the concentration of inhibitors increased, these effects were more significant.

Further addition resulted in the saturation of inhibition for all dendrimers. Among these series, two tetravalent dendrimers,

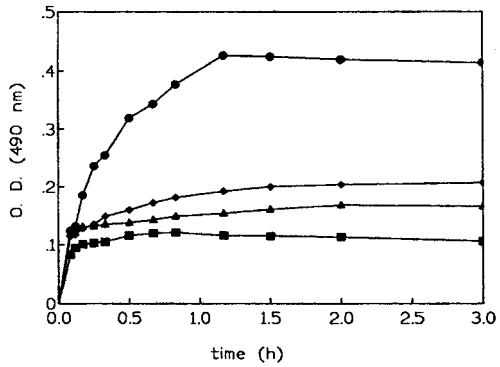


Figure 2. Time-course turbidimetric analysis of peanut lectin with T-antigen dendrimers **17** (■), **20** (▲), **21** (●), and **22** (◆).

(17) (a) Lotan, R *J. Biol. Chem*. **1975**, *250*, 8518. (b) London, J. *J. Immunol*. **1978**, *121*, 438.

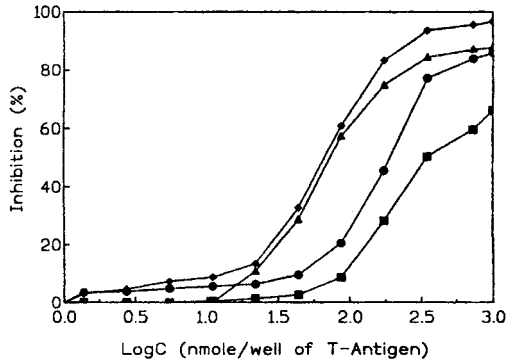


Figure 3. Inhibition of the binding of mouse monoclonal antibody (IgG3) to T-antigen-copolyacrylamide by dendrimers **17** (■), **20** (▲), **21** (●), and **22** (◆).

Table 1. Inhibitory Potencies (IC_{50}'s) of T-antigen Dendrimers to Mouse Monoclonal Antibody JAA-F11 (IgG3)

compd	IC_{50} (nM)[a]	rel potency[a]
6 (monomer)	2300	1
17 (dimer)	174 (347)	13.3 (6.6)
20 (tetramer)	19 (76)	120.5 (30.1)
21 (hexamer)	48 (288)	47.8 (8.0)
22 (tetramer)	18 (72)	128.1 (32.2)

[a] Values in parentheses are based on per T-antigen residue.

20 and **22**, showed the best results. The IC_{50} values were 19 and 18 nM, respectively. These values represent 120 to ~130-fold increased inhibition over that of the T-antigen monomer **6** (IC_{50} 2.3 mM), which was used as a reference (Table 1). On a per-T-antigen basis, each residue was ~30 times more potent than the corresponding monomer. Divalent cluster **17** had an intermediate inhibitory potency (IC_{50} 174 nM) 13 times more potent than **6**. Interestingly, hexavalent dendrimer **21** showed less inhibition (IC_{50} 48 nM) than the tetravalent ones, with a potency only 48-fold higher than that of **6** and 8 times more potent on a T-antigen basis. These results imply that not all of the carbohydrate residues are properly participating in the inhibition event. It may due to inappropriate conformational environments, such as insufficient length of linkers and lack of spatial freedom of each the carbohydrate components, resulting in insufficient participation in binding. As a result, unbound T-antigen residues reduce the overall inhibitory ability on a per-saccharide basis. This phenomenon has been previously observed with divalent IgG antibodies.[18] However, all dendrimers provided multivalent effect in terms of the inhibition of antibody (JAA-F11) binding to the coated antigen.

Conclusions

A new family of small glycodendrons (**17**, **22**) and glycodendrimers (**20**, **21**) based on a *N*,*N*′-bis(acrylamido)acetic acid core were synthesized in an efficient, convergent strategy. An important cancer marker, the T-antigen, was conjugated to the dendritic cores by either 1,4-conjugate addition of thiolated disaccharide derivatives to the acrylamido group or by amide bond formation between a T-antigen acid derivative (**9**) and multiamino dendritic cores to give the desired glycodendrimers. Turbidimetric analysis confirmed the binding abilities of glycodendrimers **17**, **20**, **21**, and **22** toward the plant lectin from *A. hypogaea*, forming insoluble cross-linked lattice complexes. Solid-phase competitive ELISA demonstrated strong inhibitory properties of ≤30-fold enhancement on a per-T-Ag basis (**20**, **22**) against a mouse monoclonal IgG antibody binding using a polymeric form of the T-Ag used as coating material (1 μg/well). These results further substantiate the enhanced carbohydrate affinity toward the antibody by means of a multivalency effect.

However, due care should be taken in the interpretation of the origin of the observed enhanced binding. Indeed, we have recently demonstrated that the increases in K_a of multivalent glycoforms are due to more positive entropy ($T\Delta S$) contributions that critically depended on the extent of both the valency of the glycoclusters and the protein receptors.[18,22] These results are further corroborated by several other studies on polymannosides binding toward concanavalin A.[23,24] Clearly, glycoforms have the added beneficial potential to form cross-linked complexes. The nature and stability of these are dependent on whether the proteins are themselves divalent (soluble complexes) or tetravalent or higher (insoluble).[25] So far, however, polymeric carbohydrates have almost always surpassed glycodendrimers, with the exception of hybrids containing dendronized polymers.[26]

Theses results open various medicinal and pharmaceutical applications for these conjugates to interrupt and modulate biological pathways of cancer cell metastasis.[19] In this manner, the applications of well-organized hyperbranched glycoconjugates can be expanded toward biological contributions such as immuno diagnostic and therapeutic purposes and in drug delivery systems.

Experimental Section

General Methods. The ^1H and ^{13}C NMR spectra were recorded on a Brüker 500 MHz NMR spectrometer. Proton chemical shifts (δ) are given relative to internal $CHCl_3$ (7.24 ppm) for $CDCl_3$ solutions, 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 $CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.4 ppm). Assignments were based on COSY, DEPT, and HMQC experiments. Mass spectra were obtained either on a VG 7070-E spectrometer (CI, ether) or on a Kratos Concept IIH spectrometer (FAB−MS, glycerol matrix). Optical rotation values were measured on a Perkin−Elmer 241 polarimeter and were run at 25 °C. Absorbance for the turbidimetric and ELISA assays were performed on a Dynatech MR 600 microplate reader. Peanut lectin from *A. hypogaea* and its peroxidase-labeled form were purchased from Sigma. Mouse monoclonal IgG antibodies were generated using bovine serum albumin (BSA)−T-antigen conjugates.[3] Thin-layer chromatography was performed on silica gel 60 F-254, and column chromatography was carried out on silica gel 60. Gel permeation chromatography (GPC) was performed using Biogel P-2, and P-4 using water as eluent.

Synthesis of Allyl (2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1-3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (3). A solution of allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**2**)[20] (1.67 g, 4.78 mmol) in a mixture of nitromethane/benzene (60 mL; 1:1, v:v) was stirred for a couple of hours at room temperature. To ensure dryness, the solution was concentrated under reduced pressure. This process was repeated three

(18) André, S.; Cejas Ortega, P. J.; Alamino Perez, M.; Roy, R.; Gabius, H.-J. *Glycobiology* **1999**, *9*, 1253.
(19) Déprez, B.; Gras-Masse, H.; Martinon, F.; Gomard, E.; Lévy, J.-P.; Tartar, A. *J. Med. Chem.* **1995**, *38*, 459.
(20) Lee, R. T.; Wong, T. C.; Lee, Y. C. *J. Carbohydr. Chem.* **1986**, *5*, 343.
(21) Kartha, K. P. R.; Jennings, H. J. *J. Carbohydr. Chem.* **1990**, *9*, 777.
(22) Dam, T. K.; Roy, R.; Das, S. K.; Oscoarson, S.; Brewer, C. F. *J. Biol. Chem.* **2000**, *275*, 14223.
(23) Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949.
(24) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286.
(25) Brewer, C. F. *Chemtracts Biochem. Mol. Biol.* **1996**, *6*, 165.
(26) Reuter, J. D.; Myc, A.; Hayes, M. M.; Gan, Z.; Roy, R.; Qin, D.; Yin, R.; Piehler, L. T.; Esfand, R.; Tomalia, D. A.; Baker, J. R., Jr. *Bioconjugate Chem.* **1999**, *10*, 271.

times. The same volume of solvent was added and then concentrated until one-half of the volume remained. The temperature was adjusted to 25 °C and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**1**)[21] (4.70 g, 7.13 mmol) and $Hg(CN)_2$ (1.80 g, 7.13 mmol) were added successively at N_2 atmosphere. The resulting solution was stirred at room temperature for 18 h. The solvent was removed under vacuum. 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 $NaHCO_3$, and distilled water, and then was dried over Na_2SO_4. After concentration, the residue was purified by silica gel column chromatography (benzene:ethyl acetate, 15:1) to give a white foamy solid in 98% yield (4.31 g, 4.67 mmol): mp 109.7 to ~111.0 °C; R_f 0.59 (benzene:EtOAc, 1:2); $[\alpha]_D + 116.0$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.06 to ~7.19 (m, 25H, Ar), 5.98 (dd, 1H, $J_{34}' = 3.3$, $J_{45}' \leq 1$ Hz, H-4′), 5.85 to ~5.78 (m, 2H, CH, H-2′), 5.60 (dd, 1H, $J_{23}' = 10.2$, $J_{34}' = 3.4$ Hz, H-3′), 5.48 (br 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 to ~4.58 (m, 1H, H-2), 4.46 to ~4.36 (m, 3H, H-4, H-5′, H-6′), 4.15 to ~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 (2C), 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 (2 M + 1); Anal. Calcd. for $C_{52}H_{49}O_{15}N$ (927.5): C, 67.20; H, 5.31; N, 1.53. Found: C, 66.84; H, 5.27; N, 1.48.

Preparation of Thiol Functionalized T-antigen Derivatives 5 and 8. These compounds were prepared in situ from their mother compounds **4** and **7** due to their readily oxidation into disulfide byproducts.

Allyl *O*-(β-D-galactopyranosyl)-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (6). Compound **3** (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 eliminated by rotary evaporator. The residue was dissolved in water (10 mL), and the aqueous layer was separated from the organic layer then was lyophilized to give de-benzoylated intermediate. Subsequently, this intermediate was dissolved in 60% aq acetic acid (15 mL), and the resulting solution was stirred for 1.5 h at 60 °C. The 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 a white solid in 93% yield (0.79 g, 1.86 mmol): mp 230 to ~232 °C; R_f 0.53 ($CHCl_3$/MeOH/H_2O); $[\alpha]_D + 120.0$ (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 6.08 to ~6.01 (m, 1H, CH), 5.42 (dd, 1H, $J_{gem} = 1.6$ Hz, $J_{trans} = 17.3$ Hz, CH), 5.33 (dd, 1H, $J_{gem} = 1.7$ Hz, $J_{cis} = 10.4$ 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, $J_{12} = 3.7$ Hz, $J_{23} = 11.2$ Hz, H-2), 4.31 to ~4.27 (m, 2H, H-4, 1H of CH_2), 4.13 to ~4.07 (m, 3H, H-3, H-5, 1H of CH_2), 3.98 (dd, 1H, $J_{34}' = 3.4$ Hz, $J_{45}' = 0.8$ Hz, H-4′), 3.86 to ~3.78 (m, 4H, H-6, H-6′), 3.7 to ~3.71 (m, 1H, H-5′), 3.69 (dd, 1H, $J_{23}' = 10.0$ Hz, $J_{34}' = 3.4$ Hz, H-3′), 3.59 (dd, 1H, $J_{12}' = 7.7$ Hz, $J_{23}' = 10.0$ 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; Anal. Calcd. for $C_{17}H_{29}O_{11}N$ (423.2): C, 45.30; H, 6.92; N, 3.30. Found: C, 45.30; H, 6.93; N, 3.07. (+) FAB−MS (glycerol) *m/z*: 424.2 (M + 1).

(3-Thioacetyl)propyl (β-D-galactopyranosyl)-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (7). To a solution of allyl β-D-Gal-(1-3)-α-D-GalNAc (**6**) (60 mg, 0.14 mmol) and thioacetic acid (63 μL, 0.85 mmol) in deoxygenated MeOH (3 mL) was added a catalytic amount of AIBN, and the resulting solution was refluxed for 1 day under an N_2 atmosphere. The solution was then evaporated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH:H_2O, 7:4:0.8) to afford an ivory-colored powder in 71% yield (50 mg, 0.1 mmol): mp 119.6 to ~122.5 °C; $[\alpha]_D + 83.0$ (*c* 1, H_2O); R_f 0.69 ($CHCl_3$:MeOH:H_2O, 10:5:1); ^{1}H NMR (D_2O) δ 4.95 (d, 1H, $J_{12} = 3.8$ Hz, H-1), 4.55 (d, 1H, $J_{12}' = 7.8$ Hz, H-1′), 4.39 (dd, 1H, $J_{12} = 3.8$ Hz, $J_{23} = 11.1$ Hz, H-2), 4.31 (d, 1H, $J_{34} = 3.1$ Hz, $J_{45} \leq 1$ Hz, H-4), 4.10 (dd, 1H, $J_{23} = 11.1$ Hz, $J_{34} = 3.1$ Hz, H-3), 4.06 to ~4.03 (m, 1H, H-5), 3.98 (d, 1H, $J_{34}' = 3.4$ Hz, H-4′), 3.86 to ~3.79 (m, 5H, H-6, H-6′, 1H of CH_2), 3.74 to ~3.72 (m, 1H, H-5′), 3.70 (dd, 1H, $J_{23}' = 10.0$ Hz, $J_{34}' = 3.3$ Hz, H-3′), 3.59 (dd, 1H, $J_{12}' = 7.8$ Hz, $J_{23}' = 9.9$ Hz, H-2′), 3.60 to ~3.56 (m, 1H, 1H of CH_2), 3.13 to ~3.04 (m, 2H, CH_2), 2.46 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.00 to ~1.94 (m, 2H, CH_2); ^{13}C NMR (D_2O) δ 201.4, 174.1, 104.2, 96.7, 76.8, 74.5, 72.0, 70.1, 70.1, 68.3, 68.1, 65.7, 60.7, 60.5, 48.2, 29.5, 27.8, 25.2, 21.5; Calcd. for $C_{19}H_{33}O_{12}NS$ (499.4). (+) FAB−MS (glycerol) *m/z*: 500.2 (M + 1).

3-(2-Carboxyethylthio)propyl β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (9). To a solution of allyl β-D-Gal-(1-3)-α-D-GalNAc (**6**) (100 mg, 0.24 mmol) in deoxygenated distilled water (2.5 mL) was added 3-mercaptopropionic acid (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. The eluent was then changed gradually to aq acetic acid with an increasing acetic acid gradient. The fractions containing **9** were collected and evaporated under reduced pressure, followed by multiple coevaporation with ethanol. A small amount of water was added and the fraction was lyophilized to afford a white spongy solid in 83% yield (105.9 mg, 2.0 mmol): mp 90.0 to ~92.5 °C; $[\alpha]_D + 76.0$ (*c* 1, H_2O); R_f 0.33 ($CHCl_3$:MeOH:H_2O, 10:9:1); ^{1}H 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, $J_{12} = 3.7$ Hz, $J_{23} = 11.0$ Hz, H-2), 4.31 (br d, 1H, H-4), 4.10 (dd, 1H, $J_{23} = 11.1$ Hz, $J_{34} = 3.1$ Hz, H-3), 4.07 (br s, 1H, H-5), 3.98 (br d, 1H, $J_{34}' = 3.5$ Hz, H-4′), 3.893.79 (m, 5H, H-6, H-6′, 1H of CH_2), 3.74 to ~3.72 (m, 1H, H-5′), 3.69 (dd, 1H, $J_{23}' = 10.0$ Hz, $J_{34}' = 3.4$ Hz, H-3′), 3.65 to ~3.57 (m, 2H, H-2′, 1H of CH_2), 2.89 (t, 2H, $J = 7.5$ Hz, CH_2), 2.80 to ~2.73 (m, 4H, 2 CH_2), 2.10 (s, 3H, Ac), 2.01 to ~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; Calcd. for $C_{20}H_{35}O_{13}NS$ (529.4); (+) FAB−MS (glycerol) *m/z*: 530.3 (M + 1).

3-(2-Aminoethylthio)propyl β-D-galactopyranosyl-(1-3)-2-acetamido-2-deoxy-α-D-galactopyranoside (10). A solution of allyl β-D-Gal-(1-3)-α-D-GalNAc (**6**) (50 mg, 0.12 mmol) and cysteamine hydrochloride (**13**) (12.5 mg, 0.11 mmol) in deoxygenated water (1 mL) was irradiated (254 nm) for 4.5 h under nitrogen atmosphere. The product was loaded onto a cation-exchange resin column (Dowex 50W, 100 mesh, NH_4^+ form) and washed with water. The eluent was then changed gradually to NH_4OH with an increasing concentration from 0 to 1.5 M with a linear gradient. The fractions containing the product were collected and evaporated under reduced pressure. A small amount of water was added and the fraction was lyophilized to afford a white spongy solid in 63% yield (36.9 mg, 74 μmol): mp 108.5 to ~109.6 °C; $[\alpha]_D + 74.0$ (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 4.97 (d, 1H, $J_{12} = 3.8$ Hz, H-1), 4.54 (d, 1H, $J_{12}' = 7.8$ Hz, H-1′), 4.40 (dd, 1H, $J_{12} = 3.8$ Hz, $J_{23} = 11.1$ Hz, H-2), 4.31 (d, 1H, $J_{34} = 3.0$ Hz, H-4), 4.10 (dd, 1H, $J_{23} = 11.1$ Hz, $J_{34} = 3.0$ Hz, H-3), 4.06 (m, 1H, H-5), 3.98 (d, 1H, $J_{34}' = 3.4$ Hz, H-4′), 3.90 to ~3.79 (m, 5H, H-6, H-6′, 1H of CH_2), 3.73 (m, 1H, H-5′), 3.69 (dd, 1H, $J_{23}' = 9.9$ Hz, $J_{34}' = 3.4$ Hz, H-3′), 3.64 (m, 1H, 1H of CH_2), 3.59 (dd, 1H, $J_{12}' = 7.8$ Hz, $J_{23}' = 9.9$ Hz, H-2′), 3.05 (m, 2H, CH_2), 2.82 (t, 2H, $J = 6.6$ Hz, CH_2), 2.76 (t, 2H, $J = 7.2$ Hz, CH_2), 2.10 (s, 3H, Ac), 1.98 (m, 2H, CH_2); ^{13}C NMR (D_2O) δ 174.0, 104.2, 96.7, 76.7, 74.5, 72.0, 70.1 (2C), 68.2, 68.1, 66.0, 60.7, 60.5, 48.2, 38.6, 30.8, 27.9, 27.2, 21.5; Anal. Calcd. for $C_{19}H_{36}O_{11}N_2S$ (500.2): C, 45.63; H, 7.31; N, 5.64. Found: C, 45.09; H, 6.95; N, 5.07. (+) FAB−MS (glycerol) *m/z*: 501.2 (M + 1).

Methyl *N*,*N*′-Bis(acrylamido)acetate (12). A solution of *N*,*N*′-bis-(acrylamido)acetic acid, **11** (2 g, 10.1 mmol) in MeOH was cooled to −15 °C. Thionyl chloride (884 μL, 12.1 mmol, 1.2 equivs) was added dropwise over a 1-h period. The resulting mixture was stirred another 1 h at the same temperature and 3 h at room temperature. The solution was condensed to one-half of the volume, then purified by flash silica gel column chromatography (CH_3Cl:MeOH, 10:2) to give a white solid, **11**, in 80% yield (1.7 g, 8.1 mmol): mp 229 to ~231 °C (soften temp), >350 °C; R_f 0.73 (EtOAc:MeOH, 24:1); ^{1}H NMR (DMSO-d_6) δ 9.08 (d, 2H, $J = 7.5$ Hz, 2 NH), 6.33 (dd, 2H, $J_{cis} = 10.2$, $J_{trans} = 17.1$ Hz), 6.14 (dd, 2H, $J_{gem} = 2.0$, $J_{trans} = 17.1$ Hz), 5.76 (t, 1H, $J = 7.6$ Hz), 5.66 (dd, 2H, $J_{gem} = 2.0$, $J_{cis} = 10.2$ Hz), 3.64 (s, 3H, CH_3); ^{13}C NMR

(DMSO-d_6) δ 168.7 (2C), 164.6, 130.7 (2C), 126.9 (2C), 56.1, 52.5; Calcd. for $C_9H_{12}O_4N_2$ (212.1), CI−MS (ether) *m*/*z* 213.1 (M + 1).

Preparation of Methyl Bis[*N*,*N*′-(2-(2-aminoethyl)thioethyl-amido)]acetate (16). To a solution of **12** (0.4 g, 1.89 mmol) in MeOH (30 mL) was added a solution of cysteamine, **13** (0.44 g, 3.87 mmol) and DIPEA (680 μL, 3.87 mmol) in MeOH (3 mL). The solution was stirred for 6 h at room temperature. After solvent evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH:H_2O, 10:6:1 and EtOAc:AcOH:H_2O, 3:2:2). The fractions containing **15** were collected and evaporated under reduced pressure. The residue was dissolved in 50 mL of distilled water and neutralized with OH^- resin (amberite IRA 400, OH^-). The solution was filtered, and the filtrate was lyophilized to give an amorphous hygroscopic solid, **15**, in 93% yield (0.64 g, 1.76 mmol): ^{1}H NMR (D_2O) δ 6.00 (br s, 1H, CH), 3.95 (s, 3H, CH_3), 3.37 (t, 4H, J = 6.7 Hz, $2CH_2$), 3.01 (t, 4H, J = 6.8 Hz, $2CH_2$), 2.99 (t, 4H, J = 6.8 Hz, $2CH_2$), 2.77 (t, 4H, J = 6.8 Hz, $2CH_2$); ^{13}C NMR (D_2O) δ 173.8 (2C), 169.3, 56.1, 53.2, 37.9 (2C), 34.4 (2C), 27.9 (2C), 25.8 (2C); Calcd. for $C_{13}H_{26}O_4N_4S_2$ (366.4), CI−MS (ether) *m*/*z*: 367.2 (M + 1).

Preparation of Divalent T-Antigen Dendrimer (17). A solution of **11** (23.8 mg, 0.12 mmol) and triethylamine (33.5 μL, 0.24 mmol) in degassed MeOH (5 mL) was saturated with N_2. Thiol **8** (150 mg, 0.3 mmol) which was prepared in-situ from **7** (NaOMe, MeOH) in degassed MeOH (1 mL) was added to the reaction flask using a syringe. The reaction was continued for 5 h under N_2 atmosphere. After evaporation of the solvent, the crude product was purified by silica gel column chromatography ($CHCl_3$:MeOH:H_2O, 10:8:2) to give **17** in quantitative yield: mp 162.8 to ∼164.7 °C; $[\alpha]_D$ + 80.7 (*c* 1, H_2O); R_f 0.26 ($CHCl_3$:MeOH:H_2O, 10:8:2); ^{1}H NMR (D_2O) δ 5.56 (s, 1H, CH), 4.96 (d, 2H, J_{12} = 3.8 Hz, 2H-1), 4.55 (d, 2H, J_{12}' = 7.8 Hz, 2H-1′), 4.40 (dd, 2H, J_{12} = 3.8, J_{23} = 11.1 Hz, 2H-2), 4.32 (d, 2H, J_{34} = 2.9 Hz, $J_{45} \leq$ 1, 2H-4), 4.10 (dd, 2H, J_{23} = 11.1, J_{34} = 3.1 Hz, 2H-3), 4.09 to ∼4.06 (br t, 2H, $J_{45} \leq$ 1, J_{56} = 6.4 Hz, 2H-5), 3.99 (d, 2H, J_{34}' = 3.4, $J_{45}' \leq$ 1 Hz, 2H-4′), 3.89 to ∼3.79 (m, 10H, 4H-6, 4H-6′, 2H-a), 3.75 to ∼3.72 (m, 2H, 2H-5′), 3.71 (dd, 2H, J_{23}' = 10.0, J_{34}' = 3.4 Hz, 2H-3), 3.65 to ∼3.60 (m, 2H, 2H-a), 3.59 (dd, 2H, J_{12}' = 7.8, J_{23}' = 10.0 Hz, 2H-2′), 2.90 (t, 4H, J = 7.1 Hz, $2CH_2$), 2.77 (t, 4H, J = 7.4 Hz, $2CH_2$), 2.67 (t, 4H, J = 6.9 Hz, $2CH_2$), 2.04 to ∼1.95 (m, 4H, $2CH_2$); ^{13}C NMR (D_2O) δ 174.3, 173.4 (2C), 172.6 (2C), 104.2 (2C), 96.7 (2C), 76.8 (2C), 74.5 (2C), 72.1 (2C), 70.2 (2C), 70.1 (2C), 68.3 (2C), 68.1 (2C), 66.1 (2C), 60.7 (2C), 60.5 (2C), 57.7, 48.2 (2C), 35.0 (2C), 28.0 (2C), 27.6 (2C), 26.4 (2C), 21.6 (2C); Calcd. for $C_{42}H_{72}O_{26}N_4S_2$ (1112.4), (+) FAB−MS (glycerol) *m*/z: 1135.6 (M + Na).

Synthesis of *N*,*N*′-Bis(acrylamido)acetic Acid-Based Dendritic Cores 18 and 19. Typical Procedure. To compound **11** (0.44 g, 2.22 mmol) in DMSO (40 mL) successively was added 1-hydroxybenzotriazole hydrate (HOBt) (0.45 g, 3.33 mmol), hexamethylenediamine, **14** (0.12 g, 1 mmol), and DIPEA (87.1 μL, 0.5 mmol) at 0 °C. The heterogeneous solution was stirred for 10 min, after which was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (0.98 g, 3.3 mmol). The suspension was allowed to reach room temperature and then was stirred for another 6 h. The solvent was removed by lyophilization, and the yellow residue was dissolved in a mixture of CH_2Cl_2:H_2O (1:1, v:v; 100 mL) and left in the refrigerator for 1 day. The precipitate was filtered, washed successively with MeOH and water, and dried under vacuum to give a solid in 60% yield (0.29 g, 1.33 mmol). Hexavalent dendritic core, **19**, was isolated as described above from trisamine, **15** and a seed molecule, **11**, as a light brown solid in 62% yield. Data for **18**: mp >350 °C; ^{1}H NMR (DMSO-d_6) δ 8.64 (d, 4H, J = 6.5 Hz, 4 NH), 8.02 (br s, 2H, 2 NH), 6.38 to ∼6.33 (m, 4H, 4CH), 6.10 (d, 4H, J_{trans} = 17.1, J_{gem} = 2.0 Hz, 4CH), 5.84 (br s, 2H, 2CH), 5.61 (d, 4H, J_{cis} = 9.9, J_{gem} = 1.9 Hz, 4CH), 3.04 (br s, 4H, $2CH_2$), 1.37 (br s, 4H, $2CH_2$), 1.21 (br s, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6) δ 167.4 (2C), 164.3 (4C), 131.2 (4C), 126.2 (4C), 57.1 (2C), 39.2 (2C), 38.8 (2C), 28.8 (2C); Calcd. for $C_{22}H_{32}O_6N_6$ (476.3), (+) FAB−MS (glycerol) *m*/z 951.4 (2 M). Data for **19**: ^{1}H NMR (DMSO-d_6) δ 8.71 (d, 6H, J = 7.6 Hz, 6 NH), 7.97 (m, 3H, 3 NH), 6.36 (dd, 6H, J_{cis} = 10.2, J_{trans} = 17.1 Hz, 6CH), 6.12 (dd, 6H, J_{gem} = 2.0, J_{trans} = 17.1 Hz, 6H of allylic CH_2), 5.88 (d, 3H, J = 7.7 Hz, 3CH), 5.62 (dd, 6H, J_{gem} = 1.9, J_{cis} = 10.3 Hz, 6H of allylic CH_2), 3.11 (br s, 6H, $3CH_2$), 2.53 (hidden, 6H, $3CH_2$); ^{13}C NMR (DMSO-d_6) δ 167.6 (3C), 164.5 (6C), 131.1 (6C), 126.3 (6C), 57.0 (3C), 52.8 (3C), 37.1 (3C); Calcd. for $C_{30}H_{42}O_9N_{10}$ (686.4), (+) FAB−MS (glycerol) *m*/z: 687.4 (M + 1).

Synthesis of Tetra- and Hexavalent T-Antigen Dendrimers (20 and 21). Typical Procedure 1. To a solution of **18** (5 mg, 10.5 μmol) and triethylamine (7 μL, 5 equivs) in degassed DMSO (1 mL) was added **8** (25 mg, 50 μmol) in degassed MeOH (1 mL), which was prepared in situ from **7** under N_2 atmosphere (NaOMe, MeOH). The resulting solution was stirred overnight at room temperature. After evaporation of the solvent under vacuum, the residue was purified by gel permeation chromatography (P-2) in water. The fractions containing pure **20** were collected and lyophilized to give a white solid in 50% yield (12 mg, 5.3 mmol). **Typical Procedure 2.** To a solution of **14** and **17** in DMSO was added DIPEA (pH = 9) and TBTU (1.2 equivs). The resulting solution was stirred overnight at room temperature. After solvent evaporation, the residue was purified as described above. The fraction containing the desired product was collected and lyophilized to give a white spongy solid in 50% yield. Hexavalent T-antigen dendrimer **21** was obtained as described above from **8**/**19** or **15**/**17** in 45 to ∼46% yields, respectively. Data for **20**: mp 149.2 to ∼153.5 °C; $[\alpha]_D$ + 69.2 (*c* 0.65, H_2O); ^{1}H NMR (D_2O) 4.97 (d, 4H, J_{12} = 3.8 Hz, H-1), 4.55 (d, 4H, J_{12}' = 7.8 Hz, H-1′), 3.89 to ∼3.79 (m, 20H, 8H-6, 8H-6′, $2CH_2$), 3.65 to ∼3.61 (m, 4H, $2CH_2$), 3.30 (t, 4H, J = 6.8 Hz, $2CH_2$), 2.91 (t, 8H, J = 6.9 Hz, $4CH_2$), 2.77 (t, 8H, J = 7.2 Hz, $4CH_2$), 2.69 (t, 8H, J = 6.9 Hz, $4CH_2$), 2.10 (s, 12H, Ac), 2.01 to ∼1.95 (m, 8H, $4CH_2$), 1.58 to ∼1.56 (m, 4H, $2CH_2$), 1.40 to ∼1.37 (m, 4H, $2CH_2$); ^{13}C NMR (D_2O) δ 174.0, 173.9 (6C between 174.0 and 173.9), 168.1 (4C), 104.2 (4C), 96.7 (4C), 76.8 (4C), 74.5 (4C), 72.1 (4C), 70.2 (4C), 70.1 (4C), 68.2 (4C), 68.1 (4C), 66.0 (4C), 60.7 (4C), 60.5 (4C), 56.9 (2C), 48.2 (4C), 39.1 (2C), 34.8 (4C), 28.0 (4C), 27.7 (2C), 27.6 (4C), 26.4 (4C), 25.1 (2C), 21.6 (4C); Calcd. for $C_{90}H_{156}O_{50}N_{10}S_4$ (2304.9), (+) FAB−MS (glycerol) *m*/z 2328.5 (M + Na). Data for **21**: mp 194 to ∼195 °C; $[\alpha]_D$ + 81.2 (*c* 0.85, H_2O); ^{1}H NMR (D_2O) δ 5.54 (s, 3H, 3CH), 4.96 (d, 6H, J_{12} = 3.7 Hz, H-1), 4.54 (d, 6H, J_{12}' = 7.7 Hz, H-1′), 3.86 to ∼3.78 (m, 30H, 12H-6, 12H-6′, $3CH_2$), 3.63 to ∼3.56 (m, 12H, 6H-2′, $3CH_2$), 2.88 (t, 12H, J = 6.9 Hz, $6CH_2$), 2.85 (t, 6H, J = 7.3 Hz, $3CH_2$), 2.75 (t, 12H, J = 7.1 Hz, $6CH_2$), 2.65 (t, 12H, J = 6.8 Hz, $6CH_2$), 2.56 (t, 6H, J = 7.2 Hz, $3CH_2$), 2.09 (s, 18H, Ac); ^{13}C NMR (D_2O) δ 174.1 (3C), 173.9 (6C), 173.6 (6C), 104.2 (6C), 96.7 (6C), 76.8 (6C), 74.5 (6C), 72.0 (6C), 70.2 (6C), 70.1 (6C), 68.3 (6C), 68.01 (6C), 66.0 (6C), 60.7 (6C), 60.5 (6C), 57.7 (3C), 48.2 (6C), 34.9 (6C), 33.8 (6C), 28.1 (6C), 27.6 (6C), 27.5 (3C), 26.4 (6C), 21.7 (6C); Calcd. for $C_{132}H_{228}O_{75}N_{16}S_6$ (3429.3), ES-MS (10 V) *m*/*z* 1144.4 (1/3 M + 1).

Preparation of Tetravalent T-Antigen Dendrimer (22). To a solution of **16** (2.1 mg, 5.8 μmol) and **17** (13 mg, 11.7 μmol) in DMSO (1 mL) was added HOBt (4 mg, 29.6 μmol), EDC (8.6 mg, 29.0 μmol), and DIPEA (100 μL) at 0 °C. The resulting solution was stirred for 40 min, then the temperature was allowed to rise to room temperature. The reaction was continued overnight. After solvent elimination, the residue was dissolved in a minimum amount of water and purified by gel permeation chromatography (P-4) in water. The fractions containing **22** were collected and lyophilized to give a white solid in 65% yield (9.2 mg, 7.61 μmol): mp 127.0 to ∼131.0 °C; $[\alpha]_D$ + 57.5 (*c* 0.8, H_2O); ^{1}H NMR (D_2O) δ 5.90 (s, 3H, 3CH), 4.95 (d, 4H, J_{12} = 3.7 Hz, H-1), 4.54 (d, 4H, J_{12}' = 7.8 Hz, H-1′), 3.87 (s, 3H, CH_3), 3.85 to ∼3.78 (m, 20H, 4H-6, 4H-6′, $2CH_2$), 3.64 to ∼3.60 (m, 4H, $2CH_2$), 3.51 (t, 4H, J = 6.6 Hz, $2CH_2$), 2.91 to ∼2.85 (m, 12H, $6CH_2$), 2.79 to ∼2.74 (m, 12H, $6CH_2$), 2.73 to ∼2.65 (m, 12H, $6CH_2$), 2.09 (s, 12H, AC), 2.00 to ∼1.94 (m, 8H, $4CH_2$); ^{13}C NMR (D_2O) δ 174.0, 173.9 (9C between 174.0 and 173.9), 168.4 (4C), 104.2 (4C), 96.7 (4C), 76.8 (4C), 74.5 (4C), 72.2 (4C), 70.1 (8C), 68.2 (4C), 68.1 (4C), 66.0 (4C), 60.7 (4C), 60.5 (4C), 56.8 (3C), 53.2, 48.2 (4C), 38.5 (2C), 34.8 (4C), 34.7 (2C), 29.9 (2C), 28.0 (4C), 27.6 (4C), 26.4 (4C), 26.1 (2C), 21.6 (4C); Calcd. for $C_{97}H_{166}O_{54}N_{12}S_6$ (2556.0), ES-MS (10 V) *m*/*z* 852.7 (1/3 M + 1).

Preparation of Biotin-Labeled Divalent T-Antigen Dendrimer (24). To a solution of divalent T-antigen **17** (16 mg, 14.4 μmol) and 4-aminobutanamide biotin derivative **23**[16] (4.53 mg, 1 equiv) in DMF (3 mL) was added TBTU (5.6 mg, 1.2 equivs) and DIPEA (pH = 8 to ∼9). The reaction mixture was stirred for 5 h at room temperature.

Completion of the reaction was confirmed by a negative Kaiser test. The solvent was removed under reduced pressure, and the residue was purified by gel permeation chromatography (P-4) in water. The fractions containing **24** were collected and lyophilized to give a white solid in 79% yield (16.1 mg, 11.4 μmol): mp 170 to ~172 °C; $[\alpha]_D$ + 22.2 (*c* 1.4, MeOH); ^{1}H NMR (D_2O) δ 4.95 (d, 2H, J_{12} = 3.7 Hz, H-1), 4.67 to ~4.65 (m, 1H, CH), 4.54 (d, 2H, $J_{12'}$ = 7.8 Hz, H-1′), 4.48 to ~4.45 (m, 1H, CH), 3.87 to ~3.78 (m, 10H, H-6, H-6′, CH_2), 3.63 to ~3.57 (m, 3H, H-2′, CH_2), 3.39 to ~3.35 (m, 1H, CH), 3.31 (br t, 2H, *J* = 6.3 Hz, CH_2), 3.24 (br t, 2H, *J* = 6.5 Hz, CH_2), 3.05 (dd, 1H, *J* = 5.0, *J* = 13.1 Hz, CH), 2.93 to ~2.81 (m, 5H, $2CH_2$, CH), 2.75 (t, 4H, *J* = 7.1 Hz, $2CH_2$), 2.68 (t, 4H, *J* = 6.8 Hz, $2CH_2$), 2.30 (t, 2H, *J* = 7.3 Hz, CH_2), 2.10 (s, 6H, $2CH_3$), 1.99 to ~1.93 (m, 4H, $2CH_2$), 1.81 to ~1.64 (m, 4H, $2CH_2$), 1.63 to ~1.50 (br s, 4H, $2CH_2$), 1.49 to ~1.42 (m, 2H, CH_2); ^{13}C NMR (D_2O) δ 176.1, 174.0, 173.9 (3C between 174.0 and 173.9), 168.2 (2C), 165.3, 104.2 (2C), 96.8 (2C), 76.9 (2C), 74.5 (2C), 72.1 (2C), 70.2 (4C), 68.3 (2C), 68.2 (2C), 66.0 (2C), 61.6, 60.7 (2C), 60.5 (2C), 59.8, 57.0, 54.9, 48.3 (2C), 39.3, 38.9, 38.4, 35.1, 34.8 (2C), 28.0 (2C), 27.6 (2C), 27.4, 26.4 (2C), 25.3 (2C), 24.7 (2C), 21.6 (2C); Calcd. for $C_{56}H_{96}O_{27}N_8S_3$ (1408.6), ES-MS (10 V) *m*/*z* 705.3 (1/2 M + 1).

Turbidimetric Analysis between Peanut Lectin and Di- (17), Tetra- (20, 22), and Hexavalent (21) T-Antigen Dendrimers. 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**, **20**, **21,** and **22** (11 nmol/well of T-antigen for all dendrimers) 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. Each test was performed in triplicate.

Competitive Double Sandwich Inhibition ELISA Using Mouse Monoclonal Antibody JAA-F11 IgG3 and T-Antigen Dendrimers, 17, 20, 21, and 22 as Inhibitors. Linbro (Titertek) microtiter plates were coated overnight with T-antigen containing *co*-polyacrylamide[2] at 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 corresponded to 0.36 μg (0.85 nmol) 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. At the same time, 50 μL of mouse monoclonal IgG antibodies solution in PBST (10 times dilution of ascitic fluid, 0.25 μmol/50 μL) and 50 μL of inhibitor solution in PBST with varying concentration from 138 to 2.15 nmol/well of T-antigen by 2-fold 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 preincubated 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 (1000 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 50 μL/well of 1 M aqueous sulfuric acid solution. Optical density was measured at 410 nm relative to 570 nm. Percent inhibitions were 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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