Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 3005–3011

BIOORGANIC & MEDICINAL CHEMISTRY

Simultaneous Binding of Mouse Monoclonal Antibody and Streptavidin to Heterobifunctional Dendritic L-Lysine Core Bearing T-Antigen Tumor Marker and Biotin

Myung-Gi Baek and René Roy*

Department of Chemistry, Centre for Research in Biopharmaceuticals, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

Received 13 March 2001; accepted 29 May 2001

Abstract—Thiolated T-antigen [Galβ-(1-3)-GalNAcα, T-Ag] (**6**), derived in situ from thioacetate **5** was coupled to *N*-chloroacetylated glycylglycyl L-lysine dendritic cores (**7**–**9**) using high yielding substitution reactions to afford di- (**10**), tetra- (**11**), and octa-valent (**12**) glycodendrimers in good yields (76–86%). Heterobifunctional conjugate **14** was prepared as a biosensor from tetravalent conjugate **11** and biotin hydrazide **13** using TBTU strategy. In a solid-phase double sandwich enzyme linked immunosorbent assays (ELISA), biotinylated conjugate **14** was shown to bind to streptavidin used as a coating material. Mouse monoclonal anti T-Ag antibody (IgG3) and horseradish peroxydase-labeled goat anti mouse IgG, used for quantification, were found to bind T-Ag tetramer **14** immobilized on the surface of the streptavin layer. A typical saturation curve was observed for **14** while non-biotinylated tetramer **11** showed no binding in the entire concentration range. These results demonstrate the availability of both haptens toward the T-Ag antibody and streptavidin receptors.

Introduction

Cell-surface carbohydrates derived from glycoconjugates mediate the associative interactions of enzymes, hormones, toxins, viruses, bacteria, lectins, and antibodies.[1–6] Carbohydrate–protein interactions are necessary for stimulation of immunological surveillances, protection, or clearance of senescent glycoproteins from circulation, inflammation, and microbial infections.[1–6] For a fundamental understanding of these interactions, their intrinsically low affinity has to be overcome.[7] In fact, nature utilizes multiple expression of carbohydrates on the cell surface that act cooperatively to increase the binding affinity toward their receptors.[6,8] To this end, glycopolymers[9] and glycodendrimers[10–12] have been successfully employed as potent multivalent antagonists.

The Thomson–Friedenrich carbohydrate antigen [T-Ag, Galβ-(1-3)-GalNAcα], has been known as a cancer related marker and is therefore an important antigen for the detection and immunotherapy of carcinomas, particularly relevant to breast cancer.[13] In related immunochemical applications, synthetic T-Ag containing glycoproteins have been used to develop mouse monoclonal antibodies (MAb), JAA-F11 (IgG3) and C5 (IgM) for immunohistochemical staining of breast adenocarcinomas.[9a] These antibodies were applied as receptors for several T-Ag conjugates including glycodendrimers.[10a–c] Glycopolymers bearing T-Ag have also been employed in high-throughput screening toward the development of solid-phase glycosyltransferase assays for drug discovery research.[9b]

The expression of T-Ag has been proposed as a tool for the detection of tumors and as a criteria for prognosis.[14] Moreover, there is an increased expression of T-Ag in metastatic tumors and a plant lectin (*Arachis hypogaea*) recognizing T-Ag has been shown to bind to common sites of metastatic tumor growth.[15] To date, only a few hypervalent T-Ag clusters have been prepared for biological and pharmaceutical applications.[9] These conjugates have architectures varying in size and carbohydrate orientations. Changes in their structure shapes for precise quantitative analysis of bioassays are necessary. In this respect, glycoclusters offer distinct advantages over random coil glycopolymers and glycoproteins. Moreover, they are unlikely to be immunogenic. The preparation of L-lysine-based dendritic polypeptides has attracted considerable attention due to

*Corresponding author. Tel.: +1-613-562-5800 x 6055; fax: +1-613-562-5170; e-mail: rroy@science.uottawa.ca

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00205-X

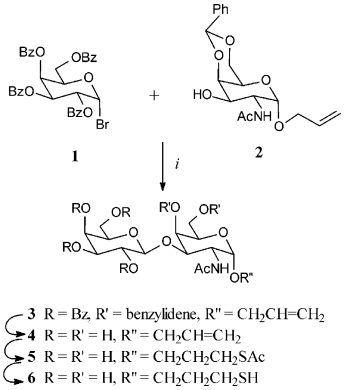
potential vaccine applications,[16–18] and few recent syntheses of glycodendrimers scaffolded onto hyperbranched L-lysine core have been described.[19,20]

The efficient synthesis of T-Ag containing L-lysine di- (**10**), tetra- (**11**), and octa-meric (**12**) dendrons is described herein. A biotin labeled heterobifunctional T-Ag tetrameric conjugate has been prepared using convenient peptide coupling methods. Its binding properties toward mouse MAb IgG3[9a] is described using streptavidin as a coating protein. It is anticipated that such biomacromolecules would constitute chemically well-defined, monodispersed, heterobifunctional ligands that can be used to detect T-Ag receptors (metastatic sites), thus providing improvement over ill-defined artificial polymers and glycoproteins.

Results and Discussion

Allyl T-antigen (**3**) was synthesized from per-benzoylated galactosyl bromide (**1**) and 4,6-*O*-benzylidene protected allyl GalNAc (**2**) which was prepared in 98% yield under classical Helferich glycosylation method according to a known procedure [$PhH/MeNO_2$, $Hg(CN)_2$][10a] (Scheme 1). The required disaccharide **3** was successfully deprotected under Zemplén conditions (MeONa/MeOH) followed by aqueous acetic acid hydrolysis for deacetalation. Fully deprotected T-Ag **4** was obtained in quantitative yield after aqueous work up and lyophilization. The thiol functional group was introduced onto the alkenyl group of **4** by radical activation (AIBN) with thioacetic acid to give thiacetate **5** in 71% yield. Compound **5** was then deprotected under Zemplén conditions (NaOMe, MeOH) to afford thiol **6** (Scheme 2).

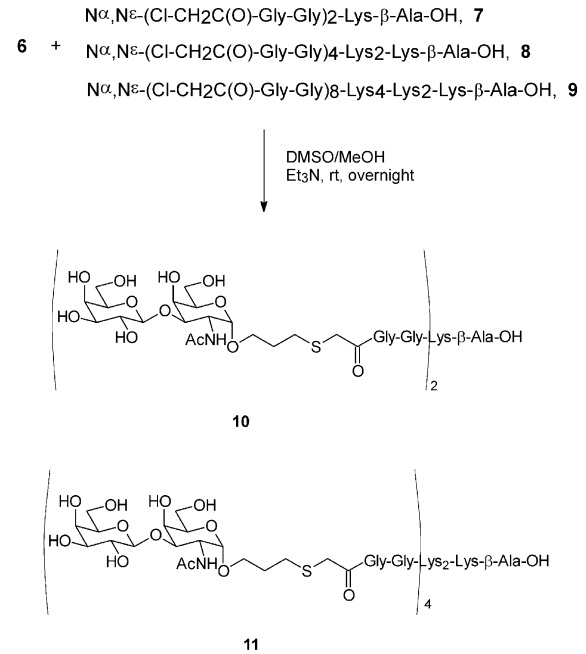
L-Lysine based T-Ag dendrons with valencies of two (**10**), four (**11**), and eight (**12**) were synthesized in 86, 76, and 79% yields, respectively.[11a,19] Each *N*-chloroacetyl group in the known lysine cores, **7**, **8**, and **9** was substituted by thiolated T-Ag **6** prepared in situ from thioacatate **5** under basic conditions. The T-Ag containing conjugates (**10**, **11**, and **12**) were then purified by gel permeation chromatography (Biogel P-2 or P-4, H_2O). Completion of conjugation was confirmed by ^1H, ^{13}C NMR, and MS spectral data. The ^1H NMR spectra systematically showed two anomeric protons of the β- and α-galactosides at δ 4.55 and 4.96 ppm and L-lysyl and β-alanyl methylene residues at 1.3–3.3 ppm. At room temperature (500 MHz), no distinctive signals could be observed for the L-lysyl α- or ε-branches in either the ^1H and ^{13}C NMR.

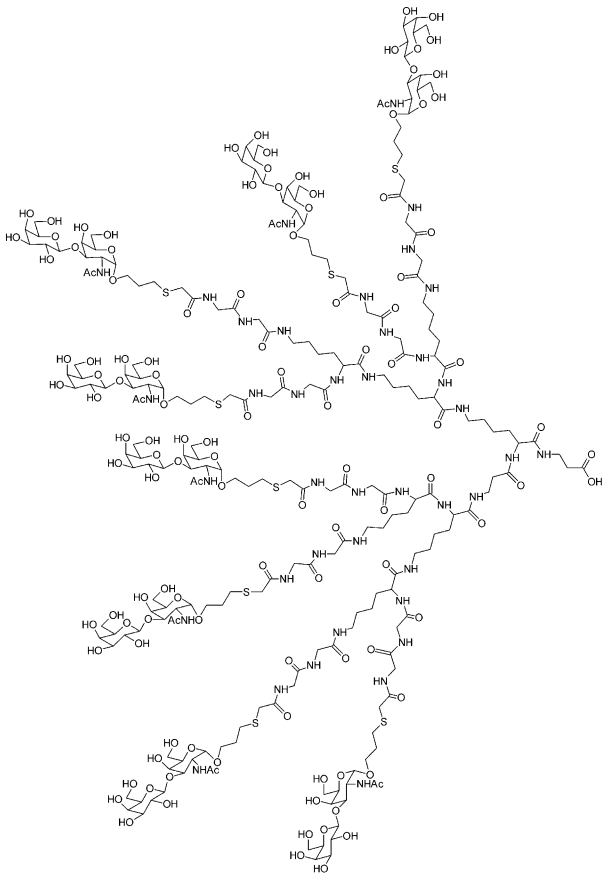


Scheme 1. Synthesis of β-D-Gal-(1-3)-α-D-GalNAc (T-antigen): (i) $Hg(CN)_2$, $PhH/MeNO_2$, rt, N_2; (ii) cat. MeONa/MeOH (1 M), 30 min; (iii) 60% aq AcOH, 60 °C, 1.5 h; (iv) AcSH, AIBN, MeOH, N_2, reflux, 12 h; (v) NaOMe, MeOH, rt, N_2.

For the purpose of broadening the potential application of these conjugates, biotin-labeled heterobifunctional T-Ag conjugate **14** was prepared using tetravalent **11** and biotin hydrazide **13** by a TBTU strategy in 92% yield after gel permeation chromatography (Biogel P-4, H_2O) (Scheme 3). High resolution ^1H and COSY-NMR spectra (500 MHz, D_2O) showed the key signals, which represent the glycoside (4.55 and 4.96 ppm for H-1s), L-lysyl dendritic core (1.65–1.51 ppm for lysyl δ-CH_2) and biotin hydrazide (4.68 and 4.50 ppm for protons in the ring system).

In previous work,[9a,b,10a–c] T-Ag containing dendritic and polymeric conjugates demonstrated strong binding interactions as inhibitors in biological tests which expanded their contribution as potential immunodiagnostic and therapeutic materials as well as for drug delivery systems. To evaluate the relative affinity of the above glycodendrimers toward mouse monoclonal antibody (mAb IgG), a competitive solid-phase inhibition experiment was set using a T-Ag-containing polymer, obtained by copolymerization of **4** with acrylamide,[10c] as a coating antigen. The relative inhibitory properties of **10**–**12** was evaluated as previously described using peroxidase-labeled goat anti-mouse antibody.[10a] The results indicated relative inhibitory potencies of ~50 (dimer **10**), 300 (tetramer **11**), and 200 (octamer **12**) when measured on a per saccharide basis compared to monomer **4** (IC_{50} 2.3 μM). These results indicate once more that in some cases, smaller glycoclusters are better inhibitors than larger one, presumably because of the increasing steric inaccessibility of the more crowded species.





12

Of all T-Ag dendrimers prepared so far,[10a,b] tetrameric structures were shown to bind with the strongest affinity when expressed on a per saccharide basis. Based on these results, heterobifunctional tetramer **14**, containing both T-Ag and biotin ligands, was prepared for the purpose of detecting cancer cell receptors, known to localize in the lymph nodes, lung, and liver where metastatic cells are often captured. Quantitative labeling measurements were performed using streptavidin as a general biosensor. The cooperative binding interactions between biotin and streptavidin were demonstrated via antigen (T-Ag)–antibody (mouse MAb–IgG3) interactions (Fig. 1). The assays were performed with con-

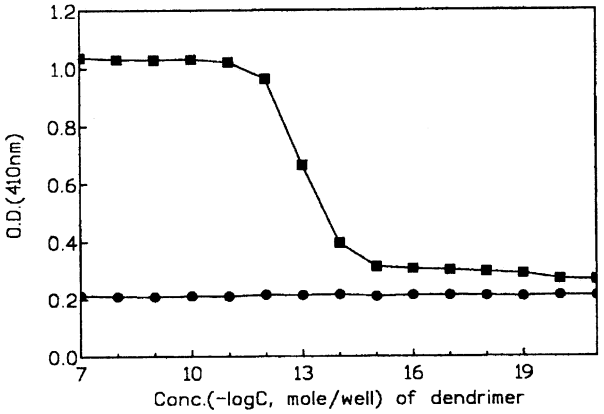
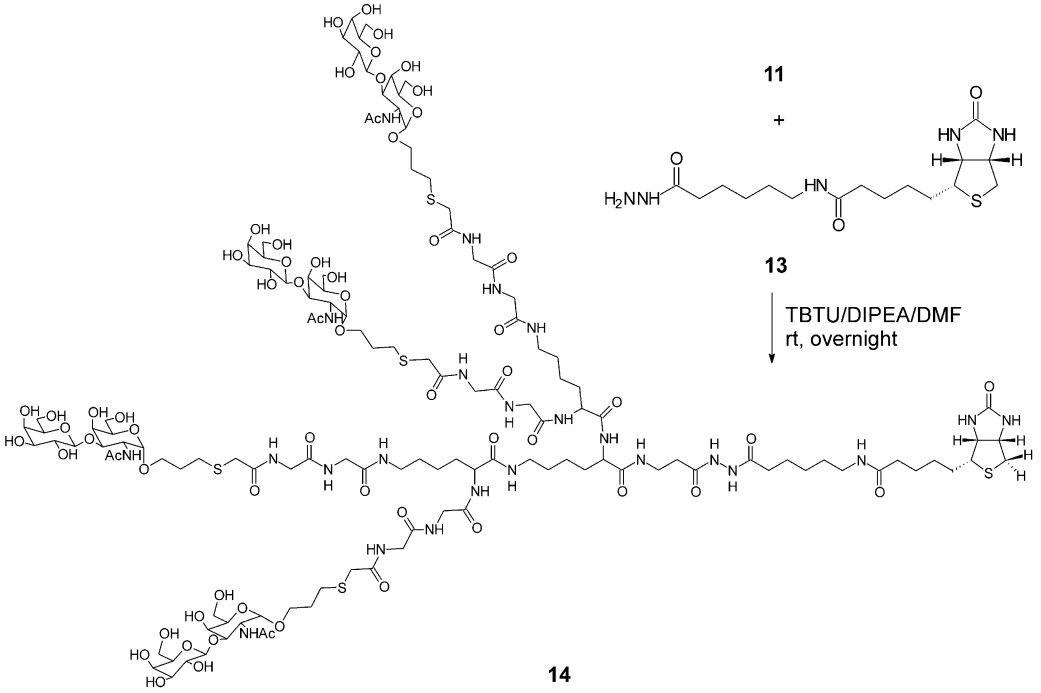


Figure 1. Solid-phase double sandwich enzyme linked immunosorbent assay (ELISA) illustrating the binding of biotin-labeled tetravalent T-Ag dendrimer **14** (■) to streptavidin used as coating material. The detection was based on mouse monoclonal antibody (IgG3) and horseradish peroxidase-labeled goat anti-mouse antibodies. T-antigen dendrimer **11** (●), deprived of biotin, was used as a negative control.



Scheme 3. Synthesis of biotin-labeled heterobifunctional tetravalent T-antigen dendron **14**.

jugate **11** as a reference negative control. Thus, coating microtiter plates with streptavidin, binding to **14** through the intermediacy of biotin, followed by detection of bound T-Ag with the mAb IgG indicated bifunctional ligand behavior.

The optical density (OD) values were dramatically increased from 0.2 to 1.2 in the concentration range between 1.5×10^{-10} and 1.5×10^{-12} mol/well. There was no further increase of OD even at higher concentration of conjugate **14**. Meanwhile, conjugate **11** showed flat OD values over the entire range of concentrations.

Conclusions

Chemically well-defined T-Ag-L-lysine dendrons having valencies of 2, 4, and 8 (**10**, **11**, and **12**) were synthesized by nucleophilic substitution of thiolated T-Ag derivative (**6**) to L-lysyl-containing dendritic cores. Although the chemistry represents yet another example of small glycoclusters,[19,20] using L-lysine core, it further demonstrates the usefulness of these 'glycotope mimetics' in evaluating factors that dictate multiple protein–carbohydrate interactions. Besides numerous multivalent lectin–carbohydrate interactions studies,[10] there is very scarce examples wherein these multiple interactions are studied in the context of antibodies, particularly of the case of divalent IgGs. The first example of glycodendrimer synthesis having biotin label to provide a heterobifunctional tetramer **14** was also demonstrated from the corresponding precursor **11** and biotin hydrazide (**13**) via efficient peptide bond formation. The doubly associative binding interactions between conjugate **14** and the coating streptavidin together with mouse monoclonal T-Ag–antibody recognition were fully demonstrated herein using conventional sandwich solid-phase immunoassays. The virtue of the T-Ag glycopolymer used as coating antigen in the present case and elsewhere[10a–c] should not be minimized since, not only it represents very effective, high avidity anchoring motif (nanomolar coating), as shown implicitly by its binding to the antibody in the ELISA format, but it also constitutes an efficient cell surface model.

Experimental

Materials and methods

α-D-Galactosamine, biotin hydrazide, streptavidin, and goat anti mouse monoclonal IgG antibody–horseradish peroxydase conjugates were purchased from Sigma. *N*-Chloroacetylated dendrimers were prepared as previously described.[17] The ^{1}H and ^{13}C NMR spectral data were obtained using a Brüker 500 MHz AMX NMR spectrometer. Proton chemical shifts (δ) were assigned relative to internal HOD (4.76 ppm) for D_2O solutions. Carbon chemical shifts were assigned relative to DMSO-d_6 (39.4 ppm). These assignments were based on COSY, HMQC and DEPT experiments. Mass spectral data were obtained on a Kratos Concept IIH spectrometer (FAB-MS, glycerol matrix). Gel permeation chromatography was performed using Biogel P-2 and P-4 columns using water as an eluent. Optical density values (ODs) for the ELISA were measured on a Dynatech MR600 microplate reader. Mouse MAb IgG3 was obtained from T-Ag–BSA glycoprotein by mice immunization.[9a]

Allyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (3). Allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranose (**2**, 1.67 g, 4.78 mmol) was dissolved in a mixture of anhydrous $MeNO_2$/PhH (60 mL, 1/1, v/v). To ensure dryness, the solution was concentrated under reduced pressure. The same volume of solvent mixture was added and then concentrated until approximately half the volume remained. The temperature was adjusted to 25 °C, then 1-bromo-2,3,4,6-tetrabenzoyl-α-D-galactopyranoside (**1**, 4.70 g, 7.13 mmol) and $Hg(CN)_2$ (1.80 g, 7.13 mmol) were added. The solution was stirred for 18 h under the same conditions. The solvent was removed under reduced pressure, then the residue was dissolved in $CHCl_3$ (40 mL) and filtered through a Celite pad. The filtrate was washed successively with 10% aq KI, saturated $NaHCO_3$ and water, and then dried over $MgSO_4$. After the solvent was evaporated, the residue was purified by silica gel column chromatography (PhH/EtOAc, 15:1) to give compound **3** in 98% yield (4.31 g, 4.67 mmol). Mp 109.7–111.0 °C, R_f 0.59 (PhH/EtOAc, 1/2), $[\alpha]_D + 116.0°$ (*c* 1, $CHCl_3$), (+) FAB-MS (glycerol): 928.3 (M + 1), 1856 [2(M + 1)]. ^{1}H NMR ($CDCl_3$) δ 8.06–7.19 (multi, 25H, Ar), 5.98 (dd, 1H, $J_{34'} = 3.3$ Hz, $J_{45'} \leq 1$ Hz, H-4′), 5.85–5.78 (multi, 2H, CH, H-2′), 5.60 (dd, 1H, $J_{23'} = 10.2$ Hz, $J_{34'} = 3.4$ Hz, H-3′), 5.48 (broad s, 1H, NH), 5.38 (s, 1H, PhCH), 5.23 (dd, 1H, $J_{gem} = 1.5$ Hz, $J_{trans} = 17.2$ Hz, CH), 5.16 (multi, 2H, $J_{cis} = 8.0$ Hz, CH, H-1′), 5.10 (d, 1H, $J_{12} = 3.4$ Hz, H-1), 4.68 (dd, 1H, $J_{56'} = 6.9$ Hz, $J_{6ab'} = 11.4$ Hz, H-6a′), 4.63–4.58 (multi, 1H, H-2), 4.46–4.36 (multi, 3H, H-4, H-3′, H-6b′), 4.15–4.07 (multi, 3H, 1H6a, CH, H-3), 3.96 (dd, 1H, $J_{gem} = 6.1$ Hz, $J_{ab} = 13.0$ Hz, CH), 3.75 (dd, 1H, $J_{56} = 1.4$ Hz, $J_{gem} = 12.4$ Hz, H6b), 3.51 (multi, 1H, H-3), 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 $C_{52}H_{49}O_{15}N$ (927.5): C, 67.20; H, 5.31; N, 1.53. Found: C, 66.84; H, 5.27; N, 1.48.

Allyl 3-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-galactopyranoside (4). To compound **3** (1.9 g, 2 mmol) in MeOH (20 mL) was added a catalytic amount of MeONa/MeOH (1 M) to adjust the pH to 9. The reaction mixture was stirred for 30 min at room temperature. The solution was neutralized with H^+ resin (Amberite IR-120) then, condensed under reduced pressure. The de-benzoylated intermediate was dissolved in 60% aqueous acetic acid (15 mL) and the resulting solution was stirred for 1.5 h at 60 °C. After evaporation, the residue was dissolved in water (10 mL)

and washed with $CHCl_3$ (20 mL). The solution was condensed and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 11:6:1) followed by evaporation of $CHCl_3$ and MeOH, and lyophilization to give T-antigen **4** in quantitative yield (0.9 g 2 mmol). Mp 230–232 °C, R_f 0.53 ($CHCl_3$/MeOH/H_2O), $[\alpha]_D$ + 120.0° (*c* 1, H_2O), (+) FAB-MS (glycerol): 424.2 (M + 1), ^{1}H NMR (D_2O) δ 6.08–6.01 (multi, 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), 4.31–4.27 (multi, 2H, H-4, CH), 4.13–4.07 (multi, 3H, H-3, H-5, CH), 3.98 (dd, 1H, J_{34} = 3.4 Hz, J_{45} = 0.8 Hz, H-4), 3.86–3.78 (multi, 4H, 2H-6, 2H-6), 3.74–3.71 (multi, 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; (+) FAB-MS (glycerol) *m*/*z* 424.2 (M + 1). 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.

3-(Thioacetyl)propyl 3-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-galactopyranoside (5). To compound **4** (60 mg, 0.14 mmol) and AcSH (63 μL, 0.85 mmol) in deoxygenated MeOH (3 mL) was added a catalytic amount of AIBN. The resulting solution was refluxed for 1 day under nitrogen atmosphere. The solution was then condensed under reduced pressure and the residue was dissolved in $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$, water and then dried over Na_2SO_4. The solution was condensed and the crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 7:4:0.8) to afford thioacetate **5** in 71% yield (50 mg, 0.1 mmol). Mp 119.6–122.5 °C, α_D + 83.0° (*c* 1, H_2O), RI_f 0.69 ($CHCl_3$/MeOH/H_2O, 10/5/1); (+) FAB-MS (glycerol): calcd for $C_{19}H_{33}O_{12}N_1S_1$, 499.17, found, 500.2 (M + 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} = 11.9 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), 4.06–4.03 (multi, 1H, H-5), 3.98 (d, 1H, $J_{34'}$ = 3.4 Hz, H-4′), 3.86–3.79 (multi, 5H, H-6, H-6′, CH), 3.74–3.72 (multi, 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′), 3.60–3.56 (multi, 1H, CH), 3.13–3.04 (multi, 2H, CH), 2.46 (s, 3H,-SAc), 2.10 (s, 3H, AcNH–), 2.00–1.94 (multi, 2H, CH): ^{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).

General procedure for T-antigen dendrons synthesis

L-Lysine dendritic cores (**7**, **8**, and **9**),[19b] prepared by solid-phase, peptide synthesis, were dissolved in deoxygenated DMSO under N_2 atmosphere. Thioacetylated T-antigen **5** (1.2 equiv per chloroacetyl group) was dissolved in deoxygenated methanol and CH_3ONa/MeOH (degassed, 0.3 M) was added dropwise until compound **5** was changed to thiol **6**. Once the reaction was completed, H^+ resin (IRA 120) was added to neutralize excess $CH_3O^-Na^+$ and the mixture was then filtered. The filtrate was directly transferred to the solution of lysine dendritic cores in DMSO and Et_3N was added to adjust the pH to 9. The solution was stirred overnight under the same conditions. After the solvent was evaporated, the residue was purified by gel permeation chromatography (P-2 or P-4, H_2O) to afford the desired T-Ag conjugates, **10**, **11**, and **12**.

β-D-Gal-(1-3)-α-D-GalNAc-$OCH_2CH_2CH_2SCH_2C$(O)-Gly-Gly_2-Lys-β-Ala-OH (10). T-Ag **6** (35 mg, 1.2 equiv per chloroacetyl group) and divalent dendritic lysine core **7** (20 mg, 32 μmol) were used. After purification by gel permeation chromatography (P-2), conjugate **10** was obtained in 86% yield (42.5 mg, 27.6 μmol). ^{1}H NMR (D_2O) δ 4.95 (d, 2H, J_{12} = 3.7 Hz, H-1), 4.54 (d, 2H, J_{12} = 7.8 Hz, H-1′), 4.39 (dd, 2H, J_{12} = 3.7 Hz, J_{23} = 11.0 Hz, H-2), 4.42–4.31 (multi, 4H, H-4, lysyl α-CH_2), 4.14–3.98 (multi, 14H, H-3, H-4′, H-5, glycyl CH_2), 3.89–3.79 (multi, 10H, H-6, H-6′, CH), 3.74–3.70 (multi, 4H, H-3′, H-5′), 3.69–3.60 (multi, 4H, H-2′, CH), 3.45 (broad s, 2H, β-alanyl β-CH_2), 3.07 (t, 2H, $J_{\delta\varepsilon}$ = 7.3 Hz, lysyl ε-CH_2), 2.81 (t, 4H, *J* = 7.2 Hz, CH_2S), 2.49 (broad t, 2H, β-alanyl α-CH_2), 2.09 (s, 6H, CH_3), 2.00–1.96 (multi, 4H, CH_2), 1.92–1.80 (multi, 2H, lysyl β-CH_2), 1.78–1.71 (multi, 2H, lysyl δ-CH_2), 1.51–1.42 (multi, 2H, lysyl γ-CH_2); ^{13}C NMR (D_2O) δ 174.0, 170.8, 104.2, 96.71, 76.9, 74.5, 72.1, 70.2, 68.3, 68.1, 65.9, 60.7, 60.5, 53.2, 48.2, 42.2, 41.9, 38.7, 36.4, 34.3, 30.0, 28.4, 27.6, 25.7, 21.6; (+) FAB-MS (glycerol) *m*/*z*; cacld for $C_{55}H_{93}O_{31}N_9S_2$, 1439.5, found, 1482 (M + Na + H_2O).

β-D-Gal-(1-3)-α-D-GalNAc-$OCH_2CH_2CH_2SCH_2C$(O)-Gly-Gly_4-Lys_2-Lys-β-Ala-OH (11). T-Ag **6** (34 mg, 1.2 equiv per chloroacetyl group) and tetravalent dendritic lysine core **8** (16.7 mg, 13.5 μmol) were used. After purification by gel permeation chromatography (P-4), conjugate **11** was obtained in 76% yield (30.1 mg, 10.3 μmol). ^{1}H 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 Hz, J_{23} = 11.1 Hz, H-2), 4.32 (d, 4H, J_{34} = 3.0 Hz, $J_{45} \leq$ 1 Hz, H-4), 4.37–4.35, 4.31–4.28 (multi, 3H, lysyl α-CH_2), 4.11–4.05 (multi, 20H, H-3, H-5, glycyl CH_2), 3.99 (multi, 8H, H-4′, glycyl CH_2), 3.89–3.79 (multi, 20H, H-6, H-6′, OCH_2), 3.75–3.69 (multi, 8H, H-3′, H-5′), 3.65–3.58 (multi, 8H, H-2′, OCH_2), 3.46–3.43 (multi, 2H, β-alanyl β-CH_2), 3.29–3.23 (multi, 6H, lysyl ε-CH_2), 2.83–2.77 (multi, 8H, SCH_2), 2.47–2.45 (t, 2H, *J* = 7.0 Hz, β-alanyl α-CH_2), 2.01 (s, 12H, CH_3), 2.02–1.95 (multi, 8H, CH_2), 1.93–1.71 (multi, 6H, lysyl β-CH_2), 1.60–1.57 (multi, 6H, lysyl δ-CH_2), 1.44–1.37 (multi, 6H, lysyl γ-CH_2); ^{13}C NMR (D_2O) δ 174.02, 170.54, 104.21, 96.7, 76.8, 74.5, 72.1, 70.2, 70.2, 68.3, 68.2, 65.9, 60.7, 60.6, 53.6, 53.3, 48.2, 42.4, 41.9, 38.6, 38.3, 36.2, 34.3, 30.1, 28.5, 27.7, 27.3, 21.9, 21.6; (+) FAB-MS (glycerol) *m*/*z*; cacld for $C_{111}H_{191}O_{61}N_{19}S_4$, 2895.1, found, 1448 (1/2M + 1), 724 (1/4M + 1).

β-D-Gal-(1-3)-α-D-GalNAc-$OCH_2CH_2CH_2SCH_2C$(O)-Gly-Gly_8-Lys_4-Lys_2-Lys-β-Ala-OH (12). T-Ag **6** (35 mg, 1.2 equiv per chloroacetyl group) and octavalent dendritic lysyne core **9** (20 mg, 8 μmol) were used. After

purification by gel permeation chromatography (P-4), conjugate **12** was obtained in 79% yield (39 mg, 6.3 μmol). ^{1}H NMR (D_2O) δ 4.94 (d, 8H, $J_{12}=3.7$ Hz, H-1), 4.53 (d, 8H, $J_{12'}=7.8$ Hz, H-1′), 4.38 (dd, 8H, $J_{12}=3.7$ Hz, $J_{23}=11.1$ Hz, H_{-2}), 4.30 (d, 8H, $J_{34}=3.0$ Hz, $J_{45}\leq 1$ Hz, H-4), 4.36–4.34 and 4.28–4.26 (multi, 7H, lysyl α-CH_2), 4.10–4.03 (multi, 32H, H-3, H-5, glycyl CH_2), 3.97 (multi, 24H, H-4′, glycyl CH_2), 3.87–3.80 (multi, 40H, H-6, H-6′, OCH_2), 3.79–3.69 (multi, 16H, H-3′, H-5′), 3.68–3.56 (multi, 16H, H-2′, OCH_2), 3.43 (broad s, 2H, β-alanyl β-CH_2), 3.27–3.25 (multi, 14H, lysyl ε-CH_2), 2.80–2.79 (multi, 16H, SCH_2), 2.44 (t, 2H, $J_{\alpha\beta}=7.0$ Hz, β-alanyl α-CH_2), 2.08 (s, 24H, CH_3), 1.98–1.96 (multi, 16H, CH_2), 1.85–1.78 (multi, 14H, lysyl β-CH_2), 1.59–1.57 (multi, 14H, lysyl δ-CH_2), 1.55–1.36 (multi, 14H, lysyl γ-CH_2); ^{13}C NMR (D_2O) δ 174.0, 170.5, 104.2, 96.7, 76.8, 74.5, 72.1, 70.2, 68.2, 68.1, 65.9, 60.7, 60.5, 53.6, 53.3, 48.2, 42.3, 42.0, 38.6, 36.4, 34.3, 30.2, 28.5, 27.7, 27.7, 21.9, 21.6; (+) FAB-MS (glycerol) *m*/*z*; cacld for $C_{232}H_{392}O_{121}N_{40}S_8$, 5930.4, found, 1507 (1/4M + Na).

β-D-Gal-(1-3)-α-D-GalNAc-$OCH_2CH_2CH_2SCH_2C$(O)-Gly-Gly_4-Lys_2-Lys-β-Ala-Biotin hydrazide (14). Tetravalent conjugate **11** (30 mg, 10.3 μmol) and biotin hydrazide **13** (3.8 mg, 10.3 μmol) were dissolved in DMF (3 mL). TBTU (4 mg, 1.2 equiv) and DIPEA (pH 9) were added and the resulting solution was stirred overnight at room temperature. Solvent was removed under reduced pressure and the residue was purified by gel permeation chromatography (P-4) to afford the heterobifunctional conjugate **14** in 92% yield (31 mg, 9.5 μmol). ^{1}H NMR (D_2O) δ 4.96 (d, 4H, $J_{12}=3.8$ Hz, H-1), 4.68 (dd, 1H, $J=4.5$ Hz, $J=8.0$ Hz, H-8), 4.55 (d, 4H, $J_{12'}=7.8$ Hz, H-1′), 4.50 (dd, 1H, $J=8.0$, 4.5 Hz, CH), 4.40 (dd, 4H, $J_{12}=3.7$ Hz, $J_{23}=11.1$ Hz, H-2), 4.31 (d, 4H, $J_{34}=2.9$ Hz, $J_{45}\leq 1$ Hz, H-4), 4.37, 4.34–4.25 (multi, 6H, lysyl α-CH_2), 4.11–4.05 (multi, 20H, H-3, H-5, glycyl CH_2), 3.99 (multi, 8H, H-4′, glycyl CH_2), 3.96–3.78 (multi, 20H, H-6, H-6′, OCH_2), 3.74–3.67 (multi, 8H, H-3′, H-5′), 3.65–3.58 (multi, 8H, H-2′, OCH_2), 3.57–3.49 (multi, 2H, β-alanyl β-CH_2), 3.29–3.19 (multi, 9H, CH, CH_2, lysyl ε-CH_2), 3.08–3.05 (dd, 1H, $J=5.0$ Hz, $J_{gem}=13.1$ Hz, CH), 2.87–2.79 (multi, 9H, SCH_2, CH), 2.62 (t, 2H, $J=6.7$ Hz, β-alanyl α-CH_2), 2.40 (t, 2H, $J=7.4$ Hz, CH_2), 2.32 (t, 2H, $J=7.2$ Hz, CH_2), 2.10 (s, 12H, CH_3), 2.00–1.97 (multi, 8H, CH_2), 1.93–1.66 (multi, 14H, CH_2, CH_2, CH_2, CH_2, lysyl β-CH_2), 1.65–1.51 (multi, 8H, lysyl δ-CH_2), 1.47–1.36 (multi, 12H, CH_2, CH_2, lysyl γ-CH_2); ^{13}C NMR (D_2O) δ 174.0, 170.5, 104.2, 96.7, 76.8, 74.5, 72.1, 70.2, 70.2, 68.2, 68.1, 65.9, 61.6, 60.7, 60.5, 59.8, 54.9, 53.6, 53.0, 48.2, 42.4, 41.9, 39.5, 38.6, 32.1, 34.3, 32.8, 30.2, 30.1, 28.5, 27.7, 27.6, 28.5, 27.6, 24.9, 24.7, 22.0, 21.6.

Competitive solid-phase inhibition of binding of mAb IgG to T-Ag copolyacrylamide by dendrimers 10, 11, and 12

These sets of experiments were essentially accomplished as described before.[10a] Briefly, 1 μg/well (~1 nmol T-Ag) of the copolymer was used as coating material in microtiter plates in PBS buffer. After usual washing and blocking with BSA, pre-incubated mixtures of the T-Ag dendrimers and mAb IgG were added 2-fold serial dilution from 1 to 200 nmol/well. Inhibition of binding was recorded my measuring residual mAb present using peroxidase-labeled goat anti-mouse antibody. Values were compared to monomer **4** (IC_{50} 2.3 μM). The results indicated relative inhibitory potency of ~50 (dimer **10**), 300 (tetramer **11**), and 200 (octamer **12**) when measured on a per saccharide basis.

Solid-phase double sandwich enzyme linked immunosorbent assay (ELISA) using streptavidin as coating material, mouse monoclonal IgG antibodies and tetrameric L-lysine based dendrons 11 and 14

Linbro (titertek) microtiter plates were coated overnight with 100 μL/well of a streptavidin stock solution (10 μg/mL in 0.01 M phosphate buffer, pH 7.3) at room temperature. Each well contained 1 μg/well of streptavidin. The wells were then washed three times with 400 μL/well of phosphate buffer (0.01 M, pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). The wells were blocked with 150 μL/well of 1% BSA in PBS for 1 h at 37 °C. After washing the excess BSA with PBST, the wells were filled with 100 μL/well of dendrimer solutions of varying concentration by serial 10-fold dilutions from 1.5×10^{-7} to 1.5×10^{-21} mol/well and incubated for 1 h at 37 °C. Dendrimers included conjugate **11** and biotin-labeled conjugate **14**. The wells were washed as described above and filled with 100 μL/well of mouse monoclonal IgG3 antibody solution in PBST (10 times dilution of supernatant, 0.5 μmol/100 μL). After incubation and washing, the wells were filled with 100 μL/well of goat-anti-mouse IgG-MAb horseradish peroxydase conjugate solution in PBST (1000 times dilution of supernatant) followed by incubation for 1 h at 37 °C. The wells 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 50 μL/well of aqueous sulfuric acid (1 M). Optical density was measured at 410 nm relative to 570 nm. All tests were performed in triplicate.

Acknowledgements

The authors thank Dr. D. Zanini for valuable assistance in the preparation of lysine dendrimers. We also thank the National Science and Engineering Research Council of Canada (NSERC) for financial support.

References and Notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97.
2. Hakomori, S. I. *Cancer Res.* **1985**, *45*, 2405.
3. Ankel, H.; Krishnamurti, C.; Besancon, F.; Stefarnos, S.; Falcott, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2528.
4. (a) Reutter, W.; Köttgen, E.; Bauer C.; Gerok, W. In *Sialic Acids, Chemistry, Metabolism and Function*; Springer-Verlag: New York, 1982; pp 5–50. (b) Goldstein, I. J.; Poretz, R. D. In

The Lectins. Properties, Functions and Applications in Biology and Medicine; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic: Orlando, 1986; pp 103–115.
5. Paulson, J. C. In *The Receptors*; Conn, M., Ed.; Academic: Orlando, 1985; Vol. 2, p 131.
6. (a) Lee, Y. C. *FASEB J*. **1992**, *6*, 3193. (b) Lis, H.; Sharon, N. *Chem. Rev*. **1998**, *98*, 637.
7. Toone, E. J. *Curr. Opin. Struct. Biol*. **1994**, *4*, 719.
8. Drickamer, K.; Taylor, M. E. *Annu. Rev. Cell Biol*. **1993**, *9*, 237.
9. (a) Rittenhouse-Diakun, K.; Xia, K.; Pickhardt, D.; Baek, M. G.; Roy, R. *Hybridoma* **1998**, *17*, 165. (b) Donovan, R.; Baek, M. G.; Wu, Q.; Sas, I.; Korczak, B.; Datti, A.; Berger, E.; Roy, R.; Dennis, J. W. *Glycoconjugate J*. **1999**, *16*, 607. (c) Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc*. **1996**, *118*, 3789. (d) Roy, R. *Trends Glycosci. Glycotechnol*. **1996**, *8*, 79. (e) Kiessling, L. L.; Pohl, N. L. *Chem. Biol*. **1996**, *3*, 71.
10. (a) Roy, R.; Baek, M. G.; Rittenhouse-Olson, K. *J. Am. Chem. Soc*. **2001**, *123*, 1809. (b) Baek, M. G.; Rittenhouse-Olson, K.; Roy, R. *Chem. Commun*. **2001**, 257. (c) Baek, M. G.; Roy, R. *Biomacromolecules* **2000**, *1*, 768. (d) Roy, R. *Carbohydr. Eur*. **1999**, 34. (e) Roy, R. *Curr. Opin. Struct. Biol*. **1996**, *6*, 692. (f) Roy, R. *Polymer News* **1996**, *21*, 226. (g) Allen, J. R.; Harris, C. R.; Danishefsky, S. J. *J. Am. Chem. Soc*. **2001**, *123*, 1890. (h) Bay, S.; Berthier-Vergnes, O.; Cantacuzene, D. *Carbohydr. Res*. **1997**, *298*, 153. (i) Bay, S.; Lo-Man, R.; Osinaga, E.; Nakada, H.; Leclerc, C.; Cantacuzene, D. *J. Peptide Res*. **1997**, *49*, 620. (j) Kunz, H.; Birnbach, S.; Wernig, P. *Carbohydr. Res*. **1990**, *202*, 207.
11. (a) Roy, R.; Zanini, D.; Meunier, S. J.; Romanowska, A. *J. Chem. Soc., Chem. Commun*. **1993**, 1869. (b) 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.
12. (a) André, S.; Cejas Ortega, P. J.; Perez, M. A.; Roy, R.; Gabius, H. J. *Glycobiology* **1999**, *9*, 1253. (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669.
13. (a) Springer, G. F.; Desai, P. R. *Biochem., Biophys. Res. Commun*. **1974**, *61*, 470. (b) Rahman, A. F.; Longenecker, B. M. *J. Immunol*. **1982**, *129*, 2021. (c) Chen, Y.; Jain, R. K.; Chandrasekaran, E. V.; Matta, K. L. *Glycoconjugate J*. **1995**, *12*, 55. (d) Singhal, A.; Fohn, M.; Hakomori, S.-I. *Cancer Res*. **1991**, *51*, 1406. (e) Itzkowitz, S. H.; Yuan, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res*. **1989**, *49*, 197. (f) Springer, G. F.; Murthy, M. S.; Desai, P. R.; Scanlon, E. F. *Cancer* **1980**, *45*, 2949. (g) Livingston, P. O.; Koganty, R.; Longenecker, B. M.; Lloyd, K. O.; Calves, M. *Vaccine Res*. **1992**, *1*, 99.
14. Maclean, G. D.; Longenecker, B. M. *Seminnar Cancer Biol*. **1991**, *2*, 433.
15. Gabius, H. J.; Schroter, C.; Gabius, S.; Brink, U.; Tietze, L. F. *J. Histochem. Cytochem*. **1990**, *38*, 1625.
16. (a) Kress, J.; Rosner, A.; Hirsch, A. *Chem. Eur. J*. **2000**, *6*, 247. (b) Voyer, N.; Lamothe, J. *Tetrahedron* **1995**, *51*, 9241.
17. (a) Denkewalter, R. G.; Kolc, J. F.; Lukasavage, W. J. US Patent 1983, 4, 410688; *Chem. Abstr*. **1984**, *100*, 103907 (b) Chang, R.; Tam, J. P. *J. Am. Chem. Soc*. **1994**, *116*, 6975. (c) Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A*. **1988**, *85*, 5409. (d) Yoshida, M.; Tam, J. P.; Merrifield, R. B. In *Peptide Chemistry; Proceedings of the 2nd Japan Symposium on Peptide Chemistry*; Yanaihara, N., Ed.; Escom: Leiden, 1989; pp 279–299.
18. Nardelli, B.; Lu, Y. A.; Shiu, D. R.; Delpierre-Delfoort, C.; Profy, A. T.; Tam, J. P. *J. Immunol. Methods* **1992**, *148*, 914.
19. (a) Zanini, D.; Roy, R. *Bioconjugate Chem*. **1997**, *8*, 187. (b) Roy, R.; Zanini, D.; Meunier, S.; Romanowska, A. *ACS Symp. Ser*. **1994**, *560*, 104. (c) Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem*. **1996**, *4*, 1949.
20. (a) Grandjean, C.; Gras-Masse, H.; Melnyk, O. *Chem. Eur. J*. **2001**, *7*, 230. (b) Grandjean, C.; Rommens, C.; Gras-Masse, H.; Melnyk, O. *Angew. Chem. Int. Ed*. **2000**, *39*, 1068. (c) Grandjean, C.; Rommens, C.; Gras-Masse, H.; Melnyk, O. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2967.