Synthesis and antibody binding properties of glycodendrimers bearing the tumor related T-antigen

Myung-Gi Baek,[a] Kate Rittenhouse-Olson,[b] and René Roy*[a]

[a] *Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, Ontario K1N 6N5 Canada*
[b] *Department of Microbiology and Immunology, Roswell Park Division, State University of New York at Buffalo, Buffalo, NY 14214. USA. E-Mail: rroy@science.uottawa.ca; Fax: +1-613-562-5170; Tel: +1-613-562-5800 ext 6055*

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Breast cancer marker T-antigen (Gal(β1-3)αGalNAc) was prepared as an allyl glycoside that was transformed into an active ester and coupled to a series of poly(amidoamine) dendrimers showing strong binding to mouse monoclonal IgG antibodies and serving as useful coating antigens in microtiter plates.

Poly(amidoamine) (PAMAM) dendrimers[1] are actually scrutinized as core structures bearing various pharmacophores for potential applications in biomedical fields such as DNA transfection,[2] inhibition of pathogenic infections,[3] and toxicity, immunogenicity and biodistribution to organs.[4] PAMAM have further broadened their potential usefulness to biological systems by combination with different carbohydrate moieties.[5–7] Chemically well defined monodisperse neoglycoconjugates with several covalently attached carbohydrate residues, can efficiently compensate for the usually weak affinities of individual haptens *via* cooperative binding interactions.[8] However, in spite of these potential biological applications, systematic preparation and biological evaluation of PAMAM-based T-antigen, Gal(β13)αGalNAc, glycodendrimers have not been reported to date.

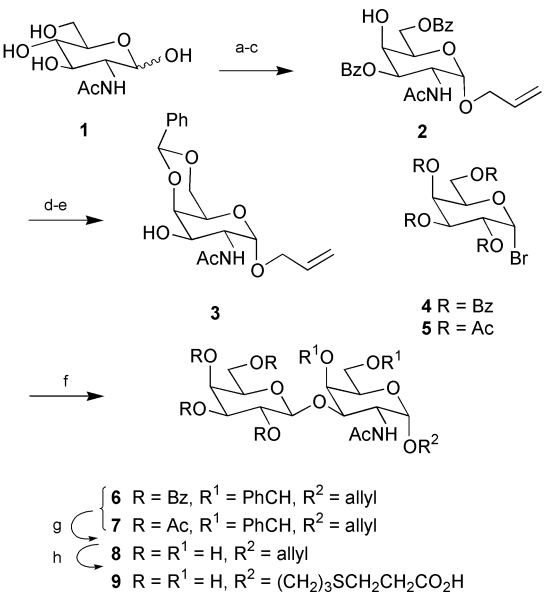
T-antigen (Thomsen–Friedenrich antigen) has been reported as a cancer-related epitope and as an important antigen for the detection and immunotherapy of carcinomas, particularly relevant in breast cancer.[9] For pharmaceutical applications, T-antigen containing linear glycopolymers have been employed in solid-phase glycosyl transferase assays for high-throughput screening in cancer drug discovery research.[10] Recently, we have reported that mouse monoclonal antibodies raised against the T-antigen recognised breast cancer tissues selectively.[11]

These biological and immunochemical results motivated the synthesis of well defined T-antigen-glycoPAMAMs that can be used as vaccines or as antibody targeting species. Their binding properties against mouse monoclonal IgG antibody has also been evaluated. The synthetic strategy for the construction of T-antigen-containing glycoPAMAMs was based on amide bond formation between amine terminated PAMAM cores **10**–**13** and acid functionalized T-antigen derivative **9**.

α-D-GalNAc derivative **2**† was prepared in 3 steps from *N*-acetylglucosamine (**1**) by a C-4 epimerization strategy using an adaptation of documented procedure (Scheme 1).[12] Compound **2** was debenzoylated under Zemplén conditions (NaOMe, MeOH) and subsequently exposed to either benzaldehyde–zinc chloride or benzaldehyde dimethyl acetal and a catalytic amount of toluene-*p*-sulfonic acid monohydrate to give 4,6-benzylidene protected glycosyl acceptor **3** in 87% yield after deprotection. Compound **3** was coupled with peracylated galactosyl bromide (**4** or **5**) using mercuric(II) cyanide as a promoter at rt to provide disaccharides **6** and **7** in 98 and 78% yields, respectively. Successive de-O-acylation (MeONa–MeOH) and de-acetalation (aq. AcOH, 60 °C) afforded the desired T-antigen **8** in 95% overall yield. Finally, radical initiated 3-mercaptopropionic acid addition to **8** in the presence of UV light (254 nm) under deoxygenated aqueous conditions gave acid functionalized **9** in 83% yield.

PAMAM cores **10**–**13** were conjugated *via* amide bond formation with a slight excess acid **9** (1.1 eq.) using TBTU‡ reagent (Scheme 2). The reaction mixtures were lyophilized to dryness and the residues were purified by either gel permeation chromatography (P-2 or P-4, H_2O) or dialysed against water (2000 molecular weight cut-off). After purification, T-antigen-glycoPAMAMs, **14**–**17** (Fig. 1) with 4, 8, 16, and 32 T-antigen residues were obtained in excellent yields (73, 81, 99, and 79%, respectively).

On the basis of negative ninhydrin tests, mass spectrometry (FAB-MS), and high field ^{1}H NMR spectral data, the extent of conjugations were shown to be complete. The ratios of the NMR signals, fully assigned using COSY and HMQC experiments (D_2O, sensisitivity $\leqslant 2 \sim 3\%$; ± 1 residue/30), 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 for **14** and **15**, 2.77 for **16** and 3.05 ppm for **17**, respectively) to those of the two anomeric protons of the T-antigen (δ 4.96 for H-1 and 4.56 ppm for H-1′ respectively).



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

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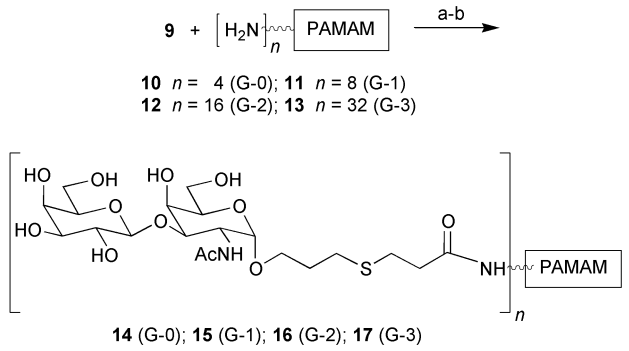

Scheme 2 (a) 1.1 eq **9** per amine, TBTU, DIPEA, DMSO, 25 °C, (b) gel permeation chromatography (P-2, or P-4, water) or dialysis (2000 molecular weight cut-off) 73–99%. DIPEA = diisopropylethylamine, DMSO = dimethylsulfoxide.

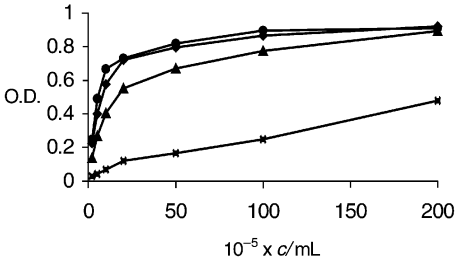

Fig. 1 ELISA of T-antigen-glycoPAMAMs, **14**(●), **15** (×), **16** (◆) and **17** (▲) with mouse monoclonal IgG antibody, goat anti-mouse IgG-horseradish peroxidase conjugate and ABTS-H_2O_2 as enzyme substrate. O.D. (410 nm) = optical density, ABTS = 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid). *C*: Relative concentration.

The binding properties of glycoPAMAMs to mouse monoclonal IgG antibody (MAb)[11] were initially evaluated by enzyme linked immunosorbent assay (ELISA) (Fig. 1). GlycoPAMAMs were used as coating antigens to determine their ability to capture antibodies on the surface of the microtiter plates. Goat anti-mouse MAb IgG-horseradish peroxidase (HRP) conjugate was used for quantitative detection. The ability of the mouse MAb to recognise all glycodendrimers was clearly demonstrated (Fig. 1). Compound **17**, having 32 T-antigen residues, was shown to be the best ligand. To obtain an optical density value of 0.6, 90 μmol of coating glycodendrimers was needed. This value represents 2- to 4-fold increased binding potentials over those of **15** (16-mer) and **16** (8-mer) (330 and 150 μmol, respectively) which where > 25-fold more potent than tetramer **14** (> 2 mmol).

The efficiency of these glycoPAMAMs for protein-binding interactions were further substantiated by competitive double sandwich inhibition where conjugates **14**–**17** were employed as inhibitors. T-antigen-*co*-polyacrylamide (T-antigen–acrylamide, 1 : 10)[13] was used as a coating antigen. To this end, the T-antigen polymer (1μg well^{-1}, 0.85 nmol of T-antigen in PBS) was deposited in the microtiter plates. In separated plates (Nunclon, Delta), mouse-MAb IgG (50 μL well^{-1}, 0.25 μmol in PBS-tween) and glycoPAMAM inhibitors (50 μL well^{-1} in PBS-tween with varying concentrations from 275 to 1.07 nmol well^{-1}) were preincubated. After blocking the microtiter plates with bovine serum albumin (BSA), the wells were filled with the mixture of antibody–glycoPAMAM inhibitors (100 μL well^{-1}) and the mixtures were then incubated for 1 h at 37 °C. For the quantitative detection of antibodies, goat anti-mouse MAb-HRP was then added, as above. Based on bulk conjugates, the glycoPAMAMs with highest carbohydrate density (**17**) exhibited the strongest inhibitions. Conjugates **14**–**17** showed IC_{50} values of 5.0, 2.4, 1.4 and 0.6 nmol, respectively where monomeric T-antigen **8** needed 2.3 μmol. The inhibitory abilities of these conjugates were thus 460, 960, 1700 and 3800 times higher than that of monomer **8**. Interestingly, when expressed on a per saccharide basis, all dendrimers were equivalent to one another (115 fold better than **8**), thus supporting previous observations that low density glycoPAMAMs are efficient inhibitors.[5]

In conclusion, geometrically well designed starburst T-antigen-glycoPAMAMs were efficiently synthesized with valencies between 4 to 32 using efficient peptide coupling strategy. A series of bioassays with mouse MAb IgG demonstrated that these conjugates strongly bound to the antibody. All conjugates were antigenetically active as previously demonstrated with analogous structures.[5–7] Moreover, some carcinoma cells have been reported to express T-antigen binding sites,[14] it is therefore possible that the compounds described herein may also find applications in specific cancer cell targeting.

Notes and references

† Physical data for representative compounds, NMR assignments are based on COSY and HMQC.

6: mp 109.7–111.0 °C; R_f = 0.59 (benzene–ethyl acetate, 1 : 2); $[\alpha]_D$ = +116.0° (*c* = 1.00, $CHCl_3$); (+) FAB-MS (glycerol) (*m/z*): 928.3 (M + 1); δ_H (500 MHz, $CDCl_3$): 5.16 (m, J_{cis} = 8.0 Hz, 2 H, CH, H1′), 5.10 (d, J_{12} = 3.4 Hz, 1 H, H1); δ_C (500 MHz, $CDCl_3$): 102.0 (C1′), 100.9 (CH), 97.4 (C1); Anal. Calcd. for $C_{52}H_{49}O_{15}N$ (927.49): C, 67.20; H, 5.31; N, 1.53. Found: C, 66.84; H, 5.27; N, 1.48%. **9**: 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); (+) FAB-MS (glycerol): 530.3 (M + 1); δ_H (ppm, D_2O): 4.96 (d, J_{12} = 3.7 Hz, 1 H, H1), 4.54 (d, $J_{1'2'}$ = 7.7 Hz, 1 H, H1′); δ_H (ppm, D_2O): 104.2 (C1′), 96.7 (C1). **17**: δ_H (500 MHz, D_2O): 4.96 (d, J_{12} = 3.7 Hz, 32 H, H1), 4.56 (d, $J_{1'2'}$ = 7.7 Hz, 32 H, H1′); δ_C (D_2O): 104.2 (C1′), 96.8 (C1). Anal. Calcd. for $C_{942}H_{1664}O_{444}N_{154}S_{32}$(23,278.7): C, 48.60; H, 7.20; N, 9.27. Found: C, 48.85; H, 7.21; N, 8.79%.

‡ TBTU = *O*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate.

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