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Bacteria Targeted By Human Natural Antibodies Using α-Gal Conjugated Receptor-specific Glycopolymers

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Abstract—Synthesis of polymerizable β-lactosyl, Galα1→3Gal and α-mannosyl acrylamide derivatives with either a hydrophobic aromatic spacer or a hydrophilic biocompatible oligoethoxyl spacer was accomplished. Radical terpolymerizations of β-lactosyl monomer, α-mannosyl monomer, and acrylamide were conducted in aqueous media with ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine as initiators. The resulting water soluble glycopolymers were further transformed efficiently by a recombinant α1→3 galactosyltransferase to afford mediators bearing Galα1→3Gal termini as xenoactive antigens and α-mannosyl termini as specific ligands for bacterial cells. The binding of the resulting multivalent glycopolymer to bacteria was tested by its ability to inhibit agglutination of yeast to *E. coli*. The binding of human natural anti-Gal antibodies to the α-Gal containing glycopolymers and a monovalent α-Gal-Man glycoconjugate was demonstrated by an ELISA inhibition assay.

Introduction

Carbohydrate structures containing a Galα1→3 Gal terminus, namely α-Gal epitope, have been identified as xenoactive antigens and are considered to be the major cause for hyperacute rejection in xenotransplantation.[1–6] Similar to ABO blood-mismatched organ allotransplants, transplants from species other than Old World primates are rejected within minutes by human natural antibodies, so-called anti-Gal. The anti-Gal antibody is reported at levels of 1–2% of total IgG and 3–8% of total IgM in human blood.[2] They bind specifically to an α-Gal epitope on the surface of the xenograft cells to initiate complement-mediated lysis of the cells.[7] Taking advantage of this humoral response from the human body, we report the design, synthesis, and binding of α-Gal epitope containing glycoconjugates for targeting bacterial cells with naturally occurring human anti α-Gal antibodies. The feasibility of using antibody targeting followed by mediated immune response against bacterial cells was proved by Bertozzi et al.[8,9] However, antibody based immunotherapy is often limited by immunogenicity. Development of humanized antibodies is a complex and difficult task. Recently the direct use of human natural antibodies such as anti-Gal for the specific deletion of target cells has become very attractive.[10,11] In this paper, we chose bacterial cells as a model system. Numerous bacterial strains produce surface lectins which are in the form of fimbriae (or pili) that are filamentous appendages of protein subunits. They are found to be responsible for cell–cell recognition and infectious disease. However, many bacteria undergo genetic changes to eschew the host immune system. In spite of these changes, the binding specificity of bacterial cell-surface lectins is conserved.[12] Many of the bacterial lectins exhibit distinct sugar specificities. Among the best characterized fimbrial lectins are the type 1 fimbrial lectins which are specific for α-mannosides.[13,14] The binding affinity of the mannose receptor to a mannose ligand falls in the millimolar range. Recently it has been found that the avidity of carbohydrate ligands binding to proteins can be increased dramatically when carbohydrate ligands are organized as multivalent clusters.[15–18] The mannose-containing glycopolymers and glycodendrimers have been reported to have ideal inhibitory effect of binding of yeast mannan to Concanavalin A and Pea lectins.[19] It was indicated that the well-defined glycodendrimers were not as effective as glycopolymer. We synthesized a new type of α-Gal epitope and mannose containing glycopolymers

Key words: α-Gal epitope; chemoenzymatic synthesis; bacteria; human natural anti-Gal antibodies.

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(Fig. 1). Binding of such glycopolymers to mannose specific bacteria, followed by treatment with human natural anti-Gal should result in complement-mediated lysis of the bacteria. The binding of the glycopolymers to bacteria was tested for inhibition against agglutination of yeast mannan (*S. cerevisiae*) to *E. coli*. Human natural anti-Gal antibody binding to the α-Gal containing glycopolymers was demonstrated by an ELISA inhibition assay.

Results and Discussion

Preparation of monomers bearing a hydrophilic spacer from lactose and mannose

Scheme 1 shows the synthesis of β-lactosyl acrylamide (**5**). We chose commercially available 2-[2-(2-chloroethoxy)-ethoxy]ethanol (**1**) as a spacer building block. Complete transformation of chloride to azide **2** was accomplished in DMF at 80°C according to a known procedure.[20] Glycosylation of **2** with α-lactosyl bromide heptaacetate using Köenigs–Knorr glycosylation method gave compound **3** in 82% yield. The desired β-configuration was confirmed by the coupling constant of anomeric proton (J_{12} = 7.8 Hz). The glycoside **3** was deacetylated by the Zemplén method to give water soluble **4** in good yield. Hydrogenation of compound **4**, followed by the treatment with acryloyl chloride to provide monomer precursor **5** in 71% yield.

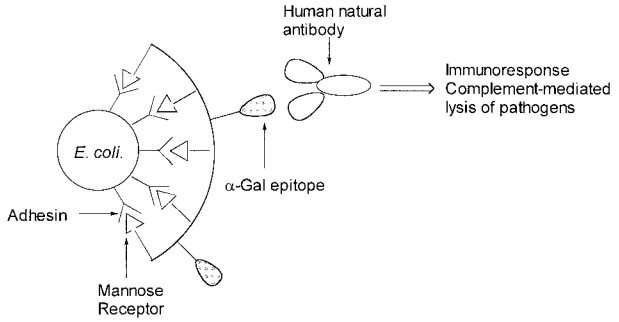
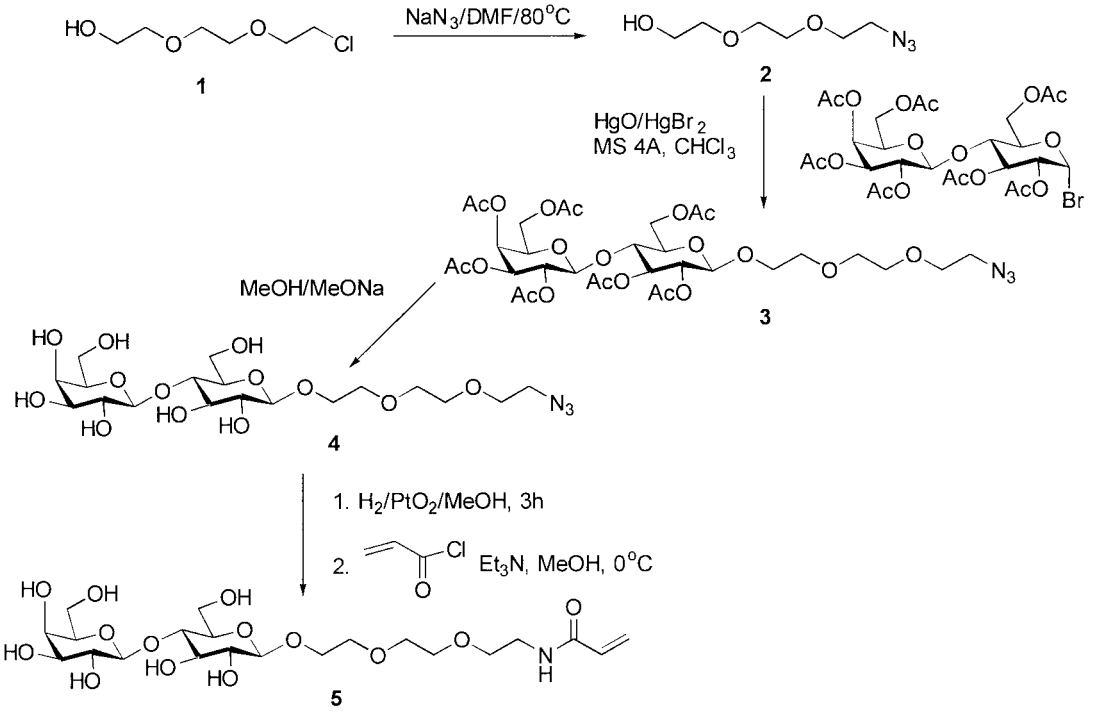


Figure 1. Bacteria targeted by human natural anti-Gal antibody thought α-Gal and mannose containing glycopolymer mediator.

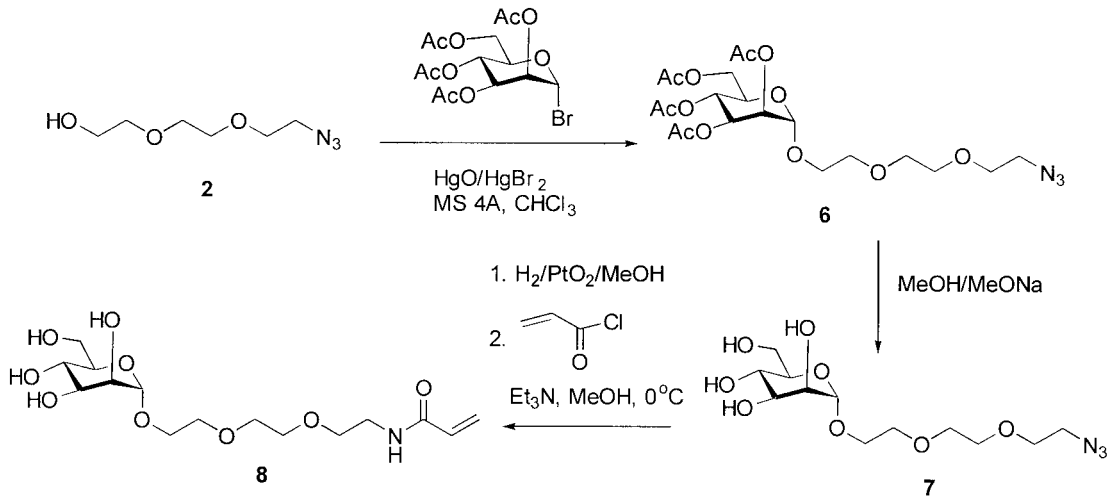
Scheme 2 shows a similar glycosylation approach toward the synthesis of α-mannosyl acrylamide (**8**). The glycosylation reaction provided compound **6** in moderate yield (49%). The α-configuration was determined by the anomeric proton coupling constant (J_{12} = 1.5 Hz) along with a characteristic feature that no carbon chemical shifts around 76 ppm (In peracetylated mannoside, C-5 chemical shift is 76 ppm for β-anomer, while 70 ppm for α-anomer). Comparing to α-lactosyl bromide heptaacetate, α-mannosyl bromide tetraacetate was more reactive. Consequently, more by-products were observed in this reaction.

Enzymatic synthesis of an α-Gal disaccharide containing monomer precursor

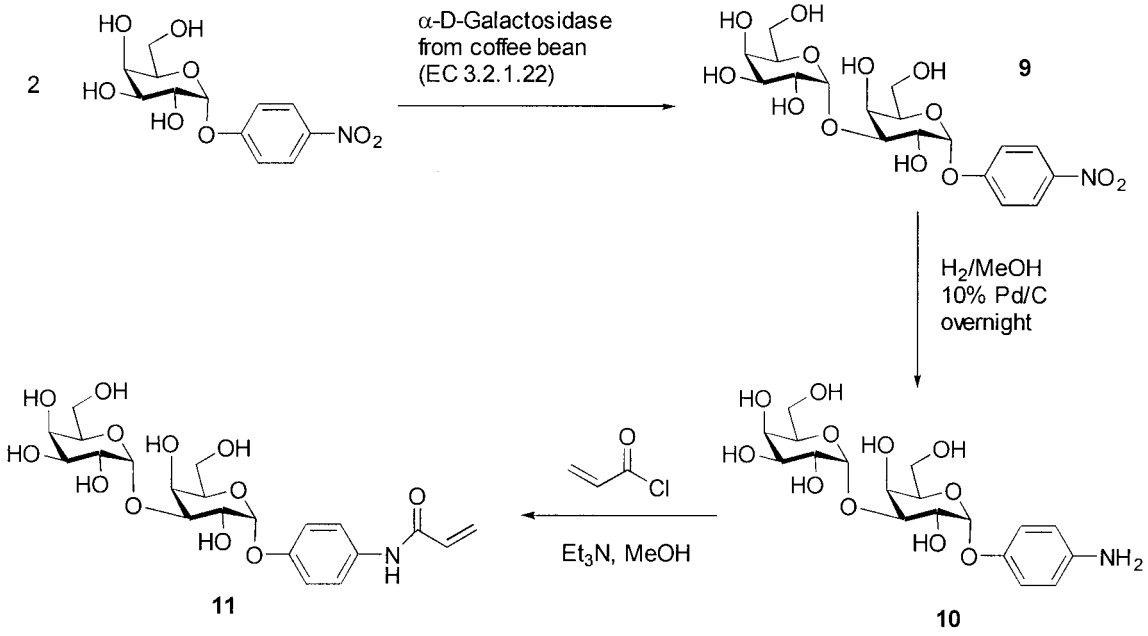
Synthesis of Galα1→3Gal acrylamide monomer precursor **11** was accomplished by a one-pot enzymatic transglycosylation reaction catalyzed by α-galactosidase from green coffee beans (Scheme 3).[21] Although the yield of this enzymatic reaction, like the conventional transglycosylation, is relatively low, the concise synthetic approach is still more attractive than the typical protecting-deprotecting chemical synthesis. Transformation of



Scheme 1.



Scheme 2.



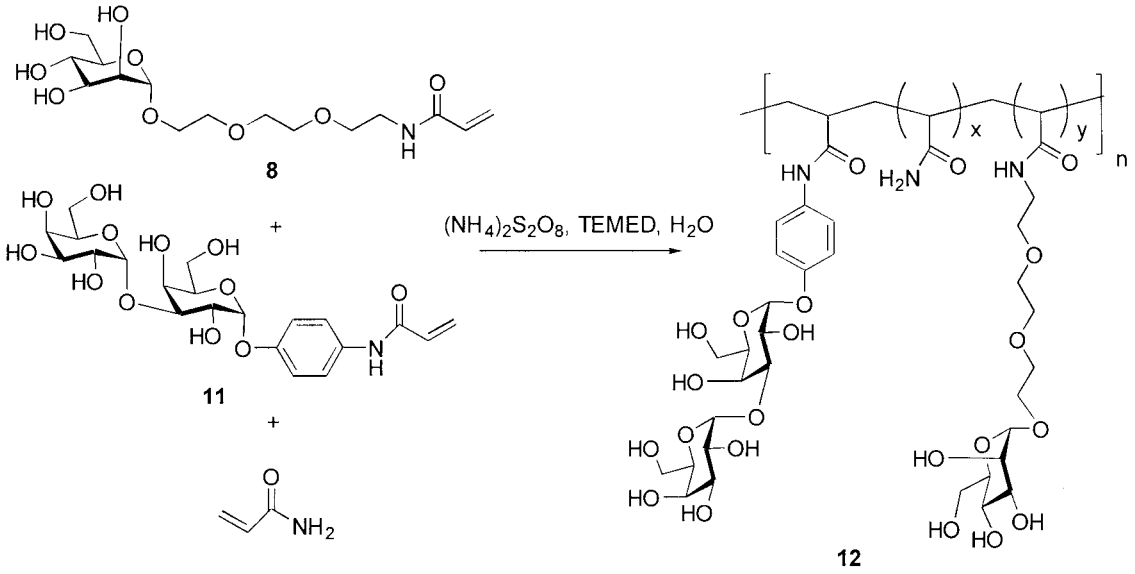
Scheme 3.

the nitro group of compound **9** to the *N*-acryloyl function was then effected by hydrogenation, followed by the treatment with acryloyl chloride in methanol to give compound **11**.

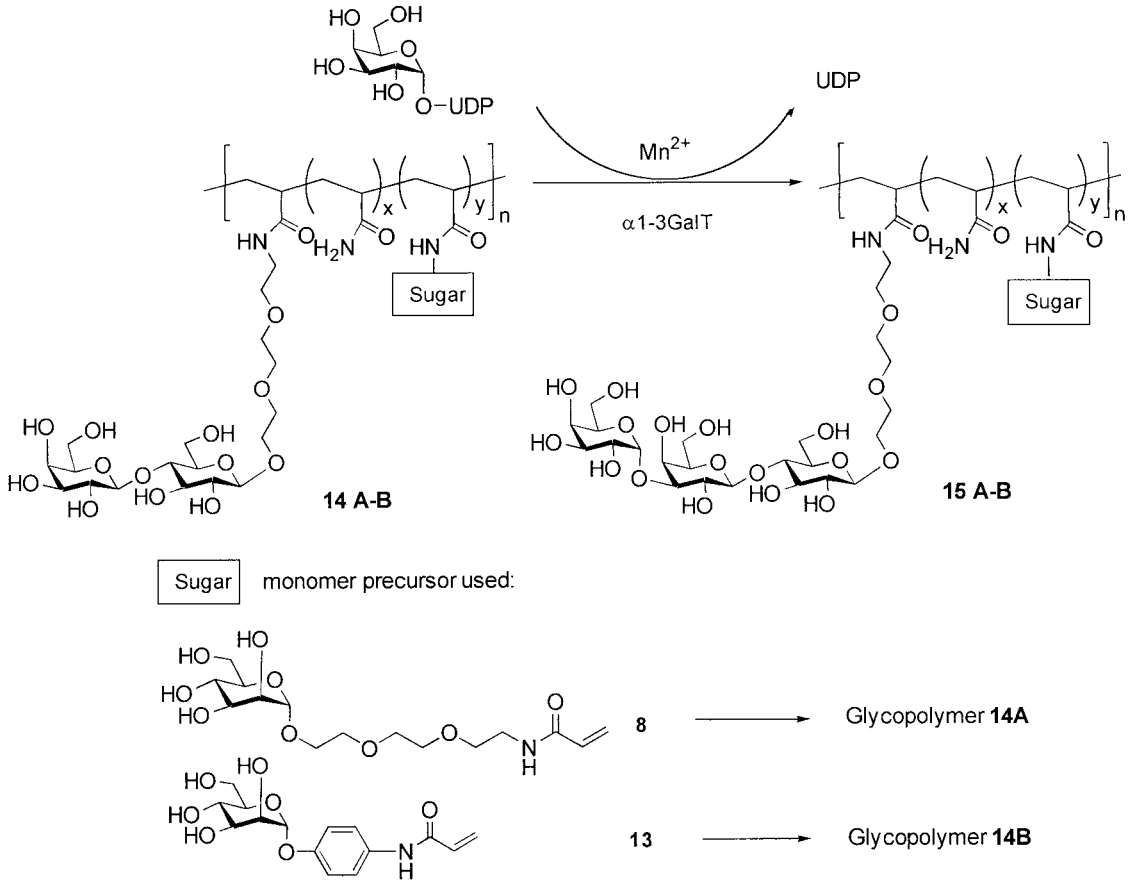
Radical terpolymerization and enzymatic preparation of glycopolymers containing lactose and mannose

The glycopolymers bearing α-Gal epitope were constructed through two different routes. The first one is to use the epitope **11** to copolymerize with mannose monomer precursor **8** and acrylamide in an established terpolymerization method[22] (Scheme 4). The polymerization was initiated by ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine in aqueous solution. Thin-layer chromatography was used to monitor the reaction. The final polymer was obtained as a white fluffy material after separation by Bio-gel P-2 permeation column and freeze-drying (76% yield). The ^{1}H NMR in D_2O verified the incorporation of all three monomers in the final glycopolymer **12**.

We have developed an efficient chemoenzymatic synthesis of α-Gal epitope using a recombinant α1-3 galactosyltransferase.[23] The second route is to use the recombinant enzyme to transfer a galactose from uridine 5′ diphosphate galactose (UDP-Gal) to a lactosyl acceptor in a glycopolymer containing other sugar moieties such as mannose which is not an acceptor of the enzyme (Scheme 5). To the best of our knowledge, this is the first example showing the selective modification of glycopolymers bearing different sugar moieties. Similar enzymatic transformations to the polymers bearing single type of sugar ligand have been reported.[24–26] In order to enhance the transfer efficiency, the biocompatible oligoethoxyl group was employed. This provided



Scheme 4.



Scheme 5.

the lactosyl acceptor side chains with adequate space away from the bulky polymer main chain. The enzyme then can bind to the acceptor completely for subsequent transformation. As anticipated, the enzymatic transformation generated the modified glycopolymers **15A–B** in nearly quantitative yield (confirmed by the integration ratio of anomeric protons). It is noteworthy that all the glycopolymers obtained from terpolymerization have a high molecular weight ($M_w > 870$ kD) with mean hydrodynamic diameter of polymer chain in aqueous solution greater than 69 nm at ambient temperature.

The choice of using aromatic **13** and non-aromatic **8** α-mannoside ligands for the glycopolymers was based on

the work done by Firon et al.[27,28] They found that *E. coli*. showed a hydrophobic region close to mannose-binding sites which favored aromatic α-mannosides such as *p*-nitrophenyl-α-D-mannopyranoside. They also noticed that *Salmonella* species possessed a binding site which was smaller than that of *E. coli*., and devoid of a hydrophobic region.

The ^{13}C NMR spectrum of glycopolymer **15B** is shown in Figure 2. The anomeric region is expanded and clearly shows the presence of lactose with 103.5 and 102.7 ppm, mannose with 99.1 ppm, and newly formed α-galactose anomeric carbon resonance of 96.0 ppm (Fig. 3).

Chemoenzymatic synthesis of monovalent α-Gal-mannose conjugate

As shown in Scheme 6, compound **19** with two single ligands of α-Gal epitope and α-mannoside was constructed for a control experiment comparing with the multivalent counterpart **15B**. Glycosylation of methyl 6-hydroxyhexanoate with α-lactosyl bromide heptaacetate using the same Köenigs–Knorr glycosylation condition as in Scheme 1 afforded compound **16**. The glycoside **16** was deacetylated by the Zemplén method, followed by saponification to give water soluble **17** with a free carboxylic group. The lactosyl derivative was then coupled with *p*-aminophenyl-α-D-mannopyranoside using diphenylphosphoryl azide (DPPA) as a promoter which was demonstrated to activate glycopeptide coupling in the presence of free hydroxyl groups.[29] The reaction was conducted in DMF at room temperature to give conjugate **18** in 66% yield. Enzymatic galactosylation of compound **18** using α1-3 galactosyltransferase provided the α-gal epitope containing conjugate **19**.

Binding studies of α-Gal-mannose glycoconjugates to bacteria

The α-Gal-mannose glycopolymers were tested for their ability to function as multivalent ligands for the binding of *E. coli* K-12 HB101 bacterial cells. This *E. coli* strain contains mannose-binding sites located on the surface of the bacteria, which can bind yeast (*Saccharomyces cerevisiae*), resulting in visible agglutination. To evaluate the activity of our glycopolymers **12** and **15A–B**, we compared their inhibitory effects on agglutination with several controls including the monovalent methyl-α-D-mannopyranoside, *p*-nitrophenyl α-D-mannopyranoside, α-Gal-mannose conjugate **19**, polyvalent mannose copolymers **15C–D**. The results indicated that the glycopolymers **12** and **15A–B** are effective in preventing agglutination at concentrations as low as 100 μM. Comparing to methyl-α-D-mannopyranoside, dramatic increase in the inhibitory efficacy was observed with polyvalent mannose. However, *p*-nitrophenyl α-D-mannopyranoside was found to be the most potent inhibitor. This compound showed strong inhibition at concentrations as low as 90 μM. Monovalent conjugate **19** and polymer **15B** bearing a hydrophobic aromatic ring had comparable inhibitory effects. The polyvalency here is unexpectedly overshadowed by van der Waals forces resulting from the packing of the aromatic ring againt a hydrophobic binding pocket on the surface of the bacteria. It is noteworthy that no inhibition was

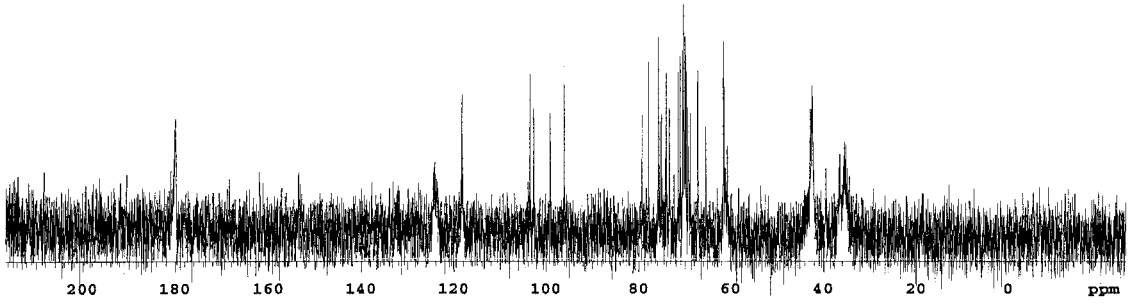


Figure 2. ^{13}C NMR spectrum of glycopolymer **15B** on D_2O at room temperature.

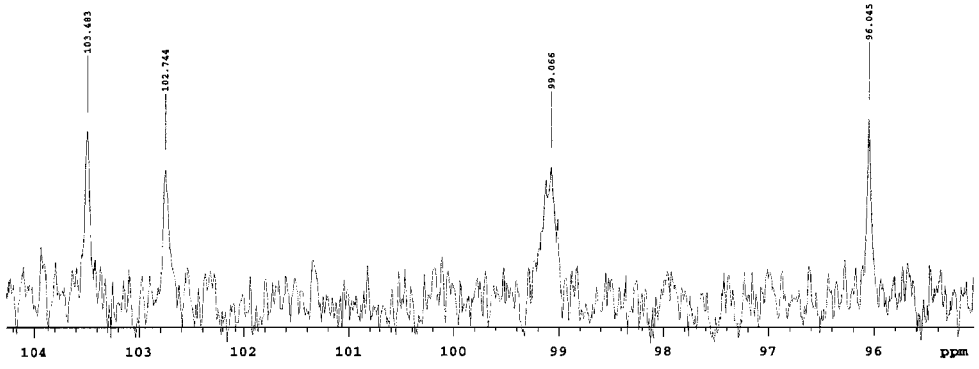
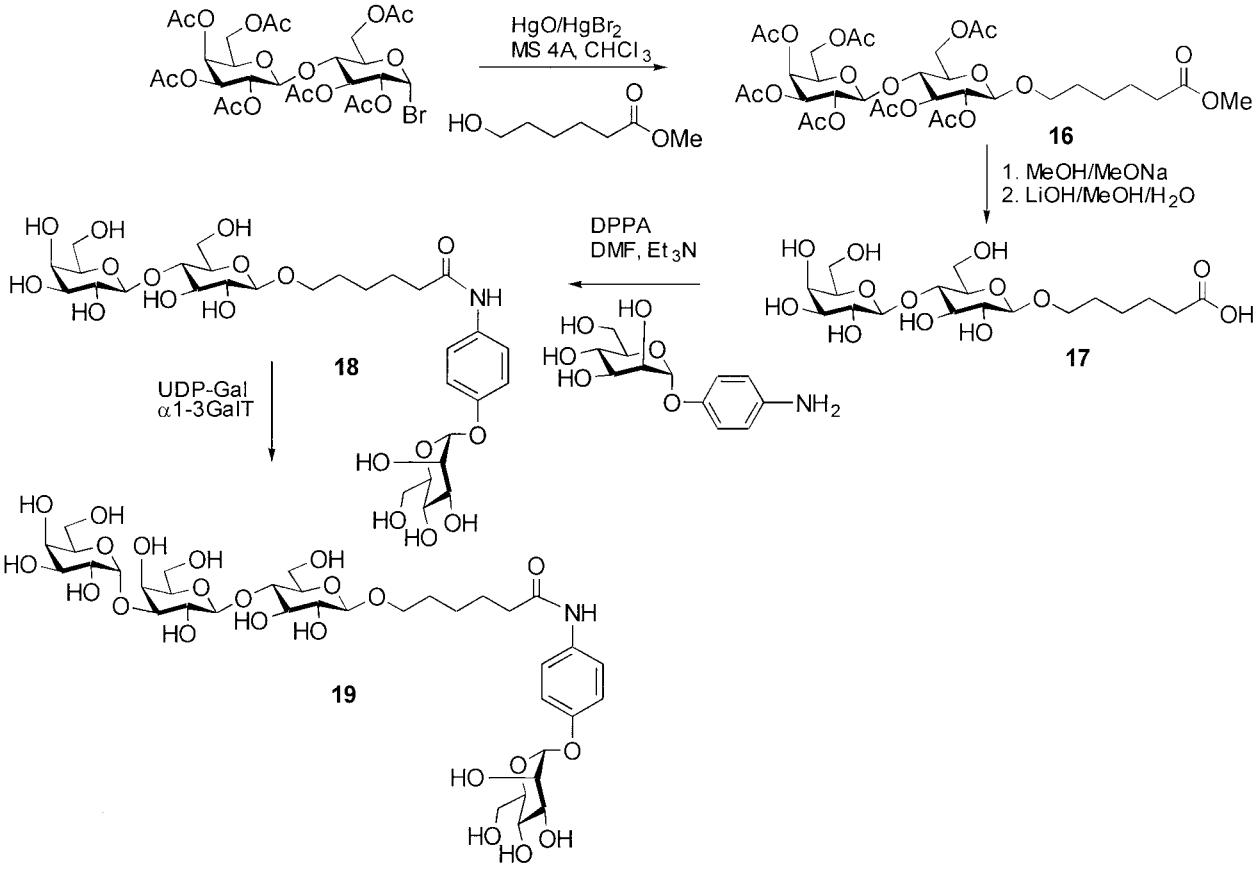


Figure 3. Expanded ^{13}C anomeric region of glycopolymer **15B**.



Scheme 6.

observed with *p*-nitrophenyl α-D-galactopyranoside. This indicates the mannose-binding sites are highly specific. α-Gal residue is not able to compete with mannose in binding to the bacterial mannose receptors (Table 1).

Binding studies of α-Gal-mannose glycoconjugates to human natural anti-Gal antibodies using an ELISA inhibition assay

To assess the activity of α-Gal-mannose glycoconjugates binding to the anti α-Gal antibodies, we applied a previously reported ELISA inhibition assay.[30] Mouse laminin having α-Gal epitopes were fixed on ELISA plate as solid-phase antigens. Test glycoconjugates were then incubated with human anti-Gal antibodies on the ELISA plate. The plate was washed and incubated with horseradish peroxidase (HRP) conjugated anti-human IgG antibody. After additional washing, the color was developed by HRP reaction using a chromogenic compound. Monovalent conjugate **19** and polymer **15B** were tested with two controls α-Gal epitope (Galα1→3Galβ1→4Glc) and mannose copolymer **15D**. As shown in Table 2, conjugate **19** and polymer **15B** bind effectively to human anti-Gal antibody. The polyvalency of α-Gal epitope on polymer **15B** was clearly demonstrated by comparing the inhibition of compound **19** with polymer **15B** at the same concentration of α-Gal epitopes. Polymer **15D** without having α-Gal epitope had no binding to human anti-Gal antibody. It can be concluded that no cross reactivity exists between mannose and human natural anti-Gal antibodies. More importantly, inhibition stem from steric stabilization[31] of antibody surface was not observed under our experimental conditions.

Table 1. Agglutination inhibition assays

Inhibitor	Inhibiting conc. (M)[a]
Methyl-α-D-mannopyranoside	1.5×10^{-2}
p-Nitrophenyl α-D-mannopyranoside	9.0×10^{-5}
p-Nitrophenyl α-D-galactopyranoside	$>2.0\times10^{-2}$
Compound **19**	1.0×10^{-4}
Polymer **12**	1.5×10^{-3}
Polymer **15A**	1.0×10^{-3}
Polymer **15B**	1.0×10^{-4}
Polymer **15C**	1.0×10^{-3}
Polymer **15D**	1.0×10^{-4}

[a] The molar concentration used in all polymers were mannose concentrations.

Conclusion

In summary, we have used chemoenzymatic synthesis to effectively construct monovalent and multivalent α-Gal conjugates containing mannose ligands. The α-Gal and mannose containing compounds were subsequently proved to be potent inhibitors against the bindings of both yeast mannan to *E. coli*., K-12 and human natural anti-Gal antibodies to mouse laminin. Cross reactivities of mannose binding to anti-Gal antibodies and α-Gal

Table 2. Binding of the human natural polyclonal anti-Gal antibody IgG to α-Gal-mannose glycoconjugates as measured by ELISA

Glycoconjugate	OD_{655}(% inhibition)
Laminin	0.799 (0%)
Galα1→3Galβ1→4Glc (0.1 mM)	0.427 (47%)
Galα1→3Galβ1-4Glc (1.0 nM)	0.279 (65%)
Compound **19** (0.1 nM)	0.386 (52%)
Compound **19** (1.0 nM)	0.278 (65%)
Polymer **15B**[a] (0.1 mM)	0.241 (70%)
Polymer **15B**[b] (1.0 mM)	0.183 (77%)
Polymer **15D**[b] (0.1 mM)	0.784 (2%)
Polymer **15D**[b] (1.0 mM)	0.729 (9%)

[a] The molar concentrations used in polymer **15B** were α-Gal trisaccharide concentrations.
[b] The molar concentrations used in polymer **15D** were mannose concentrations.

residue binding to bacteria were not observed. The model compounds with dual binding capacity will be viable ligands to redirect human natural immunity against bacterial pathogens coated with xenoactive epitopes. The sandwiched binding motif as well as bacterial killing assay will be further reported in due course.

Experimental

General

^{1}H and ^{13}C spectra were recorded on a 300 MHz GE Gemini and 500 MHz Varian Unity spectrometers. Mass spectra (FAB or ESI) were run on the mass spectrometry facility at the University of California, Riverside. Molecular weight of glycopolymers were measured by static light scattering using NICOMP model 370 submicron particle sizer. Thin-layer chromatography was conducted on Baker Si_{250F} silica gel TLC plates with a fluorescent indicator. Column chromatography was conducted with silica gel, grade 62, 60–200 mesh, 150 Å and Bio-Gel P-2. Dialysis was performed against deionized water using dialysis tubing (8000 MW cutoff). Mouse laminin (L2020) and human serum derived from sterile-filtered male AB plasma were obtained from Sigma Co., St. Louis, MO. *E. coli* K-12 HB101 was obtained from American Type Culture Collection (ATCC) Rockville, MD.

2-[2-(2-Azidoethoxy)ethoxy]ethoxyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(14)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (3). 2,3,4,6-Hepta-*O*-acetyl-α-lactosyl bromide (4.5 g, 6.4 mmol) and 2-[2-(2-azidoethoxy)-ethoxy]-ethanol (1.6 g) were added to a previously flame-dried flask containing 6 g MS 4 Å and 50 mL anhydrous $CHCl_3$. The resulting suspension was stirred for half an hour. HgO (1.4 g) and $HgBr_2$ (cat) were added to the suspension. The mixture was stirred in the dark at ambient temperature for 48 h. The resulting mixture was passed through a Celite packed glass funnel, and washed with saturated NaHCO3 and water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed (hexane:EtOAc, 1:1) to afford product **3** (4.2 g, 82%). **3**: ^{1}H NMR ($CDCl_3$) Selected δ 5.31 (d, J = 2.4 Hz, 1H), 5.16 (t, J = 9.3 Hz, 1H), 5.07 (dd, J = 7.8 Hz, 10.2, 1H), 4.85–4.94 (m, 2H), 4.54 (d, J = 7.8 Hz, 1H), 4.48–4.44 (m, 2H), 3.36–4.11 (m, 18 H) ^{13}C δ 170.3, 170.1, 170.0, 169.7, 169.6, 169.0, 101.0, 100.5, 76.2, 72.7, 72.6, 71.6, 70.9, 70.6, 70.6, 70.3, 69.9, 69.1, 69.0, 66.6, 61.9, 60.8, 50.6, 20.5-20.8. MS (m/z) 816 ($M + Na^+$); HRMS calcd for $C_{32}H_{47}N_3O_{20}Na^+$ 816.2651, found 816.2648.

2-[2-(2-Azidoethoxy)ethoxy]ethoxyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (4). To a solution of **3** (3 g) in anhydrous methanol (200 mL) at 0°C was added NaOMe until the pH was 10. The solution was stirred for 3 h. Dowex cation exchange resin (H form) was added to adjust the pH to 6–7 and filtered. The filtrate was evaporated to give a syrup **4** in quantitative yield. **4**: ^{1}H NMR (D_2O) δ4.35 (s, J = 8.1 Hz, 1H), 4.27 (d, J = 7.8 Hz, 1H), 3.32–3.91 (m, 23 H), 3.17 (t, J = 7.5 Hz, 1H). ^{13}C δ 103.4, 102.5, 78.9, 75.8, 75.2, 74.8, 73.3, 73.0, 71.4, 70.0, 70.0, 69.9, 69.6, 69.2, 69.0, 61.5, 60.5, 50.6.

2-[2-(2-*N*-acryloyl-aminoethoxy)ethoxy]ethoxyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (5). In the presence of PtO_2 (160 mg), **4** (1.6 g) in methanol (40 mL) was charged with hydrogen (50 lb/in^2) for 1 h. After the reaction, the Pt black was filtered and the filtrate was evaporated to give a residue. To a solution of the residue in cold MeOH (120 mL) was added TEA (2 mL) and acryloyl chloride (0.6 mL in 20 mL chloroform) dropwise at 0°C. The mixture was allowed to warm to room temperature and stirred for 2 h. The solution was washed with anionic resin (OH^-) and cationic resin (H^+) successively until the solution reached neutrality. The solution was concentrated and purified on a Bio-Gel P2 column and lyophilized to give **5** (1.2 g, 71%). **5**: ^{1}H NMR (D_2O) δ 6.04–6.12 (m, 1H), 5.58–5.61 (m, 2H), 4.27 (d, J = 8.0 Hz, 1H), 4.34 (d, J = 7.5 Hz, 1H), 3.15–3.91 (m, 23 H) ^{3}C δ(130.4, 128.0, 103.5, 102.7, 78.9, 76.0, 75.4, 74.9, 73.4, 73.1, 71.5, 70.3, 70.2, 70.1, 70.0, 69.3, 69.1, 61.6, 60.6, 39.6. HRFABMS calcd for $C_{21}H_{37}NO_{14}Na^+$ 550.2112, found 550.2104.

2-[2-(2-Azidoethoxy)ethoxy]ethoxyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (6). 2,3,4,6-Tetra-*O*-acetyl-α-mannosyl bromide (7 g, 17 mmol) and 2-[2-(2-azidoethoxy)ethoxy]ethanol **1** (3 g) were added to a previously flame-dried flask containing 10 g MS 4 Å and 100 mL anhydrous $CHCl_3$. The resulting suspension was stirred for half an hour. HgO (3.7 g) and $HgBr_2$ (cat) were added to the suspension. The mixture was stirred in the dark at ambient temperature for 48 h. The resulting mixture was passed through a Celite packed glass funnel, and washed with saturated $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed (hexane:EtOAc, 1:1) to afford compound **6** (4.2 g, 49%). **6**: ^{1}H NMR ($CDCl_3$) δ 5.21–5.34 (m, 3H), 4.83 (d, J = 1.5 Hz, 1H), 4.25 (dd, J = 8.5 Hz, 12.3 Hz, 1H), 4.00–4.07 (m, 2H), 3.74–3.80 (m, 1H), 3.67–3.61 (m, 9H), 3.36 (t, J = 8.5 Hz, 2H), 2.11 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H) ^{13}C δ(170.6, 169.9, 169.8, 169.6, 97.7, 70.7, 70.6, 70.0, 69.5, 69.0, 68.4, 67.3,

66.1, 62.4, 50.6, 20.6-20.8. MS (*m*/*z*) 528 ($M+Na^+$); HRMS calcd for $C_{20}H_{31}N_3O_{12}Na^+$ 528.1805, found 528.1853.

2-[2-(2-Azidoethoxy)ethoxy]ethoxyl-(-D-mannopyranoside (7). To a solution of **6** (3.6 g) in anhydrous methanol (200 mL) at 0°C was added NaOMe until pH was 10. The solution was stirred for 3 h. Dowex cation exchange resin (H form) was added to adjust the pH to 6–7 and filtered. The filtrate was evaporated to give a syrup **7** in quantitative yield. **7**: ^{1}H NMR (D_2O) δ(4.72 (s, 1H), 3.33–3.80 (m, 18 H) ^{13}C δ 100.5, 73.3, 71.1, 70.5, 70.2, 70.1, 70.1, 69.9, 67.3, 67, 50.7.

2-[2-(2-*N*-acryloyl-aminoethoxy)ethoxy]ethoxyl-(-D-mannopyranoside (8). In the presence of PtO_2 (98 mg), 7 (1.5 g) in methanol (40 mL) was charged with hydrogen (50 lb/in^2) for 3 h. After the reaction, the Pt black was filtered and the filtrate was evaporated to give a residue. To a solution of the residue in cold MeOH (120 mL) was added TEA (2 mL) and acryloyl chloride (0.5 mL in 20 mL chloroform) dropwise at 0°C. The mixture was allowed to warm to room temperature and stirred for 2 h. The solution was washed with anionic resin (OH^-) and cationic resin (H^+) successively until the solution reached neutrality. The solution was concentrated and purified on a Bio-Gel P2 column and lyophilized to give **8** (1.4 g, 88%). **8**: ^{1}H NMR (D_2O) δ(6.13–6.00 (m, 2H), 5.59 (d, $J=10.5$ Hz, 1H), 4.70 (s, 1H), 3.30–3.78 (m, 18 H) ^{13}C δ 130.4, 128.0, 100.5, 73.3, 71.1, 70.1, 70.6, 70.2, 70.1, 70.1, 69.4, 67.3, 66.9, 61.5, 39.6. HRFABMS calcd for $C_{15}H_{27}NO_9Na^+$ 388.1584, found 388.1589.

***p*-Aminophenyl 3-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (10).** In the presence of 10% Pd/C (20 mg), **9** obtained as described previously,[21] (220 mg, 0.48 mmol) was charged with hydrogen (50 lb/in^2) in methanol (25 mL) for overnight. After the reaction, the Pd black was filtered and the filtrate was evaporated to give a residue **10** (200 mg, 97%). **10**: ^{1}H NMR (D_2O) δ 6.87, 6.67 (2d, each 2H, $J=8.1$ Hz), 5.37 (d, $J=3.3$ Hz, 1H), 5.03 (d, $J=3.3$ Hz, 1H), 4.14–3.55 m, 12 H), ^{13}C δ 150.2, 141.4, 119.5, 118.3, 98.9, 95.6, 74.6, 71.8, 71.5, 69.8, 69.7, 68.7, 67.0, 65.9, 61.6, 61.5.

***N*-acryloyl-aminophenyl 3-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (11).** To a solution of compound **10** (100 mg, 0.23 mmol) in cold MeOH (20 mL) was added TEA (0.6 mL) and acryloyl chloride (0.2 mL) dropwise at 0°C. The mixture was allowed to warm to room temperature and stirred for 2 h. The solution was washed with anionic resin (OH^-) and cationic resin (H^+) successively until the solution reached neutrality. The solution was concentrated and purified on a Bio-Gel P-2 column and lyophilized to give **11** (49 mg, 44%). **11**: ^{1}H NMR (D_2O) δ 7.29, 7.03 (2d, each 2H, $J=8.5$ Hz), 6.28–6.14 (m, 2H), 5.70 (d, $J=10.0$ Hz, 1H), 5.53 (d, $J=4.0$ Hz, 1H), 5.06 (d, $J=4.0$ Hz, 1H), 4.15–3.57 (m, 12H) ^{13}C δ 130.2, 128.0, 123.5, 117.8, 97.4, 95.1, 74.1, 71.5, 71, 69.3, 69.2, 68.2, 66.5, 65.4, 61.2, 61.2. HRFABMS calcd for $C_{21}H_{29}NO_{12}Na^+$ 510.1587, found 510.1601.

Poly {acrylamide-*co*-2-[2-(2-*N*-acrylamidoethoxy)ethoxy]-ethoxyl-α-D-mannopyranoside-*co*-*N*-acrylamidophenyl 3-*O*-α-D-galactopyranosyl-α-D-galactopyranoside} (12). Compound **8** (70 mg), compound **11** (48 mg) and acrylamide (80 mg) were dissolved in deoxygenated dd water (2 mL). Ammonium persulfate (40 μL of a stock solution, 50 mg/mL) and TMEDA (5 μL) were then added. The reaction mixture was stirred under argon at 55°C overnight. The cooled reaction mixture was dialyzed (8000 MW cutoff) exhaustively against deionized water. The aqueous solution of the polymer was lyophilized to afford white fluffy powder **12** (150 mg, 76%). **12**: ^{1}H NMR (D_2O) δ 7.24 (s, 2H), 7.03 (d, $J=7.0$ Hz, aromatic proton), 5.55 (s, anomeric proton), 5.06 (s, anomeric proton), 4.73 (s, anomeric proton), 3.26–4.15 (m), 2.17 and 2.02 (2bs), 1.60 and 1.48 (2bs). The average incorporation molar ratio is 1/28/5 (Gal α1→3 Gal/acrylamide/mannose).

Poly {acrylamide-*co*-2-[2-(2-*N*-acrylamidoethoxy)ethoxy]-ethoxyl-α-D-mannopyranoside-*co*-2-[2-(2-*N*-acrylamidoethoxy)ethoxy]ethoxyl-β-lactoside} (14A). Compound **8** (330 mg), compound **5** (176 mg) and acrylamide (214 mg) were dissolved in deoxygenated dd water (6 mL). Ammonium persulfate (100 μL of a stock solution, 50 mg/mL) and TMEDA (10 μL) were then added. The reaction mixture was stirred under argon at 75°C for 4 h. The cooled reaction mixture was dialyzed (8000 MW cutoff) exhaustively against deionized water. The aqueous solution of the polymer was lyophilized to afford white fluffy powder **14A** (600 mg). **14A**: ^{1}H NMR (D_2O) δ 4.72 (s, mannose anomeric proton), 4.35 (d, $J=7.5$ Hz, anomeric proton), 4.28 (d, $J=7.8$ Hz, anomeric proton), 3.26–4.79 (m), 2.17 and 2.01 (2bs), 1.58 and 1.47 (2bs). The average incorporation molar ratio is 1:12:2 (lactose:acrylamide:mannose).

Poly {acrylamide-*co*-*N*-acryloyl-aminophenyl α-D-mannopyranoside-*co*-2-[2-(2-N-acrylamidoethoxy)ethoxy]ethoxyl-β-lactoside} (14B). Compound **13** obtained as described previously [19] (100 mg), compound **5** (162 mg) and acrylamide (131 mg) were dissolved in deoxygenated dd water (4 mL). Ammonium persulfate (40 μL of a stock solution, 50 mg/mL) and TMEDA (5 μL) were then added. The reaction mixture was stirred under argon at 90°C for 34 min. The cooled reaction mixture was dialyzed (8000 MW cutoff) exhaustively against deionized water. The aqueous solution of the polymer was lyophilized to afford the white fluffy powder **14B** (320 mg). **14B**: ^{1}H NMR (D_2O) (m, aromatic protons), 5.42 (bs, anomeric proton), 4.35 (d, $J=7.5$ Hz, anomeric proton), 4.28 (d, $J=8.0$ Hz, anomeric proton), 4.00–3.20 (m), 2.18 and 2.03 (2bs), 1.60 and 1.48 (2bs). The average incorporation molar ratio is 1:5.8:1 (lactose:acrylamide:mannose).

Polymer (15A). Acceptor glycopolymer **14A** (100 mg, 47 μmol of lactose), UDP-Gal (40 mg) and rα1-3GalT (10 unit) were incubated in a solution of Tris–HCl buffer (50 mM, pH 7) containing Mn^{2+} (10 mM) for 48 h at ambient temperature. The mixture was directly applied to Bio-Gel P-2 with water as the eluent. The fractions containing the glycopolymer were collected

and lyophilized to afford the white powder **15A** in quantitative yield. **15A**: Selected ^{13}C NMR (D_2O) 103.5, 102.7, 99.1 (mannose anomeric carbon) 96.0 (α-galactose anomeric carbon), 79.2 (C-4 of glucose), 77.8 (C-3 of β-galactose).

Polymer (15B). Acceptor glycopolymer **14B** (120 mg, 93 μmol of lactose), UDP-Gal (80 mg) and rα1-3GalT (15 unit) were incubated in a solution of Tris–HCl buffer (50 mM, pH 7) containing Mn^{2+} (10 mM) for 48 h at ambient temperature. The mixture was directly applied to Bio-Gel P-2 with water as the eluent. The fractions containing the glycopolymer were collected and lyophilized to afford the white powder **15B** in quantitative yield. **15B**: Selected ^{13}C NMR (D_2O) δ 103.4, 102.7, 100.4 (mannose anomeric carbon) 95.9 (α-galactose anomeric carbon), 79.1 (C-4 of glucose), 77.7 (C-3 of β-galactose).

Poly {acrylamide-*co*-2-[2-(2-*N*-acrylamidoethoxy)ethoxy]-ethoxyl-α-D-mannopyranoside} (15C). Copolymer **15C** was synthesized using compound **8** following the procedure described for terpolymer **14A**. The average incorporation molar ratio is 3:1 (acrylamide:mannose).

Poly {acrylamide-*co*-*N*-acryloyl-aminophenyl α-D-mannopyranoside} (15D). Copolymer **15D** was synthesized using compound **13** following the procedure described before.[8] The average incorporation molar ratio is 6:1 (acrylamide:mannose).

Poly {acrylamide-*co*-2-[2-(2-*N*-acrylamidoethoxy)ethoxy]-ethoxyl-β-lactoside} (15E). Copolymer **15E** was synthesized using compound **5** following the procedure described for terpolymer **14A**. The average incorporation molar ratio is 1:12 (lactose:acrylamide).

Polymer (15F). Copolymer **15F** was synthesized following the procedure described for polymer **15A**. The enzymatic reaction afforded white powder **15F** in quantitative yield.

Compound 16. 2,3,4,6-Hepta-*O*-acetyl-α-lactosyl bromide (5 g, 7.1 mmol) and methyl 6-hydroxyhexanoate (1.3 g, 8.9 mmol) were added to a previously flame-dried flask containing 7 g MS 4 Å and 30 mL anhydrous $CHCl_3$. The resulting suspension was stirred for half an hour. HgO (1.5 g) and $HgBr_2$ (257 mg) were added to the suspension. The mixture was stirred in the dark at ambient temperature for 48 h. The resulting mixture was passed through a Celite packed glass funnel, and washed with saturated $NaHCO_3$ and water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed (hexane:EtOAc, 1:1) to afford product **16** (4.0 g, 74%). 16: ^1H NMR ($CDCl_3$) δ 5.32 (d, J= 2.5 Hz, 1H), 5.17 (t, J=9.0 Hz, 1H), 5.08 (dd, J=8.0, 10.5, 1H), 4.93 (dd, J=3.5, 10.5, 1H), 4.85 (dd, J=8.0, 10.5, 1H), 4.47–4.44 (m, 2H), 4.42 (d, J=8.0 Hz, 1H), 4.11–4.04 (m, 3H), 3.87–3.80 (m, 2H), 3.77 (t, J= 9.5 Hz, 1H), 3.64 (s, 3H), 3.59–3.55 (m, 1H), 3.46–3.41 (m, 1H), 2.27 (t, J=7.5 Hz, 2H), 2.13, 2.10, 2.04, 2.021, 2.020, 2.01, 1.94 (7s, 7 acetyl groups), 1.64–1.50 (m, 4H), 1.38–1.28 (m, 2H). ^{13}C δ174.7, 171.1, 171.0, 170.8, 170.7, 170.5, 170.3, 169.8, 101.7, 101.2, 77.0, 73.5, 73.3, 72.4, 71.7, 71.3, 70.5, 69.8, 67.3, 62.7, 61.5, 34.6, 29.7, 26.0, 25.2, 21.54, 21.49, 21.4(b), 21.3(b), 21.2(b). HRFABMS calcd for $C_{33}H_{48}O_{20}Na^+$ 787.2637, found 787.2618.

Compound 17. To a solution of **16** (3 g) in anhydrous methanol (200 mL) at 0°C was added NaOMe until pH 10. The solution was stirred for 3 h. Dowex cation exchange resin (H form) was added to adjust the pH to 6–7 and filtered. The filtrate was evaporated to give a residue which was dissolved in MeOH:H_2O (1:1). To the above solution was added LiOH until pH 11. The solution was stirred for 1 h. Dowex cation exchange resin (H form) was used to neutralize the above solution which was concentrated to afford a syrup **17** in quantitative yield. **17**: ^1H NMR (D_2O) δ 4.45 (d, J=8.0 Hz, 1H), 4.41 (d, J=7.6 Hz, 1H), 3.96–3.49 (m, 13 H), 3.26 (t, J=8.0 Hz, 1H), 2.22 (t, J=7.2 Hz, 2H), 1.64–1.53 (m, 4H), 1.38–1.32 (m, 2H) ^{13}C δ183.3, 103.5, 102.6, 79.0, 76.0, 75.4, 75.1, 73.5, 73.1, 71.6, 71.1, 69.2, 61.6, 60.7, 37.1, 29.1, 25.8, 25.5. HRFABMS calcd for $C_{18}H_{32}O_{13}Na^+$ 479.1741, found 479.1762.

Compound 18. To a solution of **17** (612 mg) and *p*-aminophenyl-α-D-mannopyranoside (400 mg) in DMF (10 mL) at 0°C was added DPPA (328 μL) and TEA (400 μL). The solution was stirred vigorously overnight. The solution was concentrated in vacuo, followed by gel filtration through Bio-Gel P-2 column eluted by water. The fractions containing the glycoconjugate were collected and lyophilized to afford **18** 628 mg (66% yield). Selected ^1H NMR (D_2O) δ5.39 (s, 1H, anomeric proton of mannose), 4.26 (d, J=7.5 Hz, 2H, two anomeric protons of lactose). **18**: Selected ^{13}C (103.4 (C-1 of glucose), 102.5 (C-1 of galactose), 99.0 (C-1 of mannose). HRFABMS calcd for $C_{30}H_{47}NO_{18}Na^+$ 732.2691, found 732.2665.

Compound 19. Acceptor glycoconjugate **18** (250 mg), UDP-Gal (256 mg), 0.1% BSA and rα1-3GalT (15 unit) were incubated in a solution of Tris–HCl buffer (50 mM, pH 7) containing Mn^{2+} (10 mM) for 48 h at ambient temperature. The mixture was directly applied to Bio-Gel P-2 with water as the eluent. The fractions containing the product were collected and lyophilized to afford the white powder **19** 187 mg (61% yield). **19**: Selected ^1H NMR (D_2O) δ 5.52 (s, 1H, anomeric proton of mannose), 5.09, (d, J=3.2 Hz, 1H, anomeric proton of α-galactose), 4.45 (d, J=7.6 Hz, 1H, anomeric proton), 4.40 (d, J=8.0 Hz, 1H, anomeric proton). Selected ^{13}C δ 103.9 (C-1 of glucose), 103.1 (C-1 of β-galactose), 99.5 (C-1 of mannose), 96.5 (C-1 of α-galactose). HRFABMS calcd for $C_{36}H_{57}NO_{23}Na^+$ 894.3219, found 894.3223.

Agglutination assay. *E. coli* K12 HB101 was grown overnight at 37°C in static LB media, washed, and suspended with PBS to a final concentration of 1×10^8 cells/mL. Yeast (*S. cerevisiae*, wild type) was incubated on YPD media at 25°C for 48 h, washed, and suspended with PBS to a final dilution of 4×10^7 cells/mL. Agglutination assays were carried out on a 9-well spot

plate. Aliquots of 90 μL of a solution of the test compound were first agitated with 30 μL bacterial suspension for 30 s. After that, 30 μL yeast suspension was added, and allowed to develop with agitation for at least 3 min. Agglutination was observed under a magnifying glass.

Purification of anti-Gal IgG from human serum. Polyclonal anti-Gal antibodies were isolated from the human serum according to a modified protocol as reported previously.[32] Male AB type serum was heat-inactivated in a water bath at 56°C for 30 min. The α-Gal Sepharose affinity column was washed with 75 mL PBS buffer. The heat-inactivated serum (25 mL) was run over the column three times, and the flow-through collected. The column was washed with 6 mL PBS 14 times until an OD_{280nm} reading less than 0.01 of the flow-through was reached. The anti-Gal antibodies was eluted from the column with 5 mL of 100 mM glycine·HCl buffer (pH 2.8) 10 times until sufficiently low OD_{280nm} readings were obtained. The fractions were collected, neutralized immediately with 0.5 M sodium bicarbonate, and determined by ELISA assays.

ELISA inhibition assay. Mouse laminin (10 μg/mL in carbonate buffer) as solid-phase antigen was added to a 96-well ELISA (Immulon 4) microtiter plate, and incubated overnight at 4°C. Test compound (0.1 or 1 mM) was incubated with 55 μL of a PBS solution of human polyclonal anti-Gal IgG for 1 h at 37°C with slow agitation. The human anti-Gal IgG concentration used was adjusted to cause 50% of maximal binding to solid-phase antigen. ELISA plate was blocked with 1% BSA in PBS, and incubated 45 min at room temperature. To appropriate wells on ELISA plate was added 50 μL of the test compound/Ab solution. The solution in each well was incubated for 1.5 h at room temperature. The wells were subsequently washed with PBS/0.05% Tween and incubated with goat anti-human IgG-peroxidase conjugate for 1 h at room temperature. After additional washing with PBS/0.05%Tween, the peroxidase reaction was performed with 100 μL of a 3,3′,5,5′-tetramethylbenzidine (TMB)/H_2O_2 (9/1) solution, and the UV absorbance was measured at 655 nm.

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