Synthesis of trisaccharide-coated magnetic nanoparticles for antibody removal

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ABSTRACT

An efficient strategy for the synthesis of blood group A trisaccharide antigen has been developed. Magnetic nanoparticles having Fe₃O₄-Silica core-shell structure were prepared and functionalized with the prepared blood group A trisaccharide antigen derivative, and its excellent removal ability toward anti-A antibody was explored.

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1. Introduction

The ABO blood group system was established based on the expression of carbohydrate antigens on the red blood cell surface of human erythrocyte. Later on, Morgan and coworkers revealed that blood group trisaccharides are determinant fragments of this system with the distinctive structure features as shown in Fig. 1.^{1–3}

It has long been of interests to the biological and medical researches as these antigens are among the most important clinical considerations for both transfusion and transplantation.⁴ In particular, transplantation of solid organs across the ABO blood group barrier is known to cause hyperacute organ rejection, where pre-existing host antibodies cause rapid humoral-mediated graft rejection.⁵ Hyperacute rejection associated with ABO-incompatible organ transplantation could be efficiently suppressed by removal of anti-A and anti-B blood group antibodies increasing the possibility of long-term graft survival.⁶ So far, several methods have been developed to remove anti-ABO antibodies, such as plasmapheresis

and/or immune-adsorption widely employed in ABO-incompatible organ transplantation or bone marrow transplantation.⁷

Mathematical modeling⁸ revealed that the capacity and efficacy of antibody depletion may be strongly depended on the antigen numbers attached to the removable binding materials or devices. Based on our experiences in glyco-nanoparticle synthesis,⁹ we believe that the high surface/volume ratio of nanoparticles offers more contact surface area for attaching carbohydrates and therefore capturing antibodies. Further more, Fe₃O₄ nanoparticles are biologically safe materials applied in bio-separation,¹⁰ biomedical engineering¹¹ and drug delivery process,¹² as well as a unique removing process under added outside magnetic conditions. This removal process would avoid significant effect of flow rate observed in other techniques.^{5,13,14} Herein, we report a synthesis of glyco-magnetic nanoparticles (glyco-MNPs)-based system to remove up to 93% of the target antibody from the medium.

2. Results and discussion

Our target was aiming to the specific removal of anti-A antibody because of its greater clinical significance in ABO-incompatible organ transplantation.¹³ Thus, the corresponding antigen of blood group A trisaccharide GalNAcα1→3(Fucα1→2)Gal was required.

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Fig. 1. The structures of blood group trisaccharides A, B and O.

Chemically, the synthesis of this antigen was more challenging due to the characteristic α -glycosyl bond between residues GalNAc and Gal, although number of methods toward the synthesis of the blood group trisaccharides have been investigated.^{15–18} We have reported a simple one-step procedure for the synthesis of GalNAc α -glycoside products with the promotion of anhydrous FeCl_3 in CH_2Cl_2 (DCM) in good yields.¹⁹ However, in the preparation of this antigen A, an extremely low yield (about 5%) of the desired product was obtained under such reaction conditions. Further exploration revealed that this method is not satisfactory for the glycosidic bond formation between sugar moieties. We thus designed a strategy for the synthesis of blood group A trisaccharide using 2-azido β -D-galactose building block as glycosyl donor, and functionalized the magnetic Fe_3O_4 nanoparticles with this trisaccharide antigen to investigate the related biological studies.

Accordingly, $\text{BF}_3 \cdot \text{Et}_2\text{O}$ catalyzed condensation²⁰ of per-*O*-acetylated β -D-galactose (**1**) and 4-chloro-1-butanol in anhydrous DCM generated stereo-specifically β -D-galactopyranoside **2**, which was subjected to deacetylation with NaOMe in MeOH to afford 4-chlorobutyl β -D-galactopyranoside (**3**) in 63% isolated overall yield. Benzylidenation of **3** with α,α -dimethoxytoluene in acetonitrile under the catalytic amount of camphorsulfonic acid ($\rightarrow$ **4**), followed by regioselective masking of 3-OH with pivaloyl chloride²¹ in pyridine obtained **5** in 82% yield for two steps. Glycosylation of **5** with ethyl 2,3,4-tri-*O*-benzyl-1-thio- β -L-fucopyranoside (**6**) in the presence of *N*-iodosuccinimide (NIS) and catalytic

amount of triflic acid in anhydrous DCM gave predominantly α -disaccharide **7** showing unique doublet at 5.30 ppm with coupling constant of $J = 3.2$ Hz on its ^1H NMR spectrum. Removal of pivaloyl group from compound **7** using NaOMe in MeOH afforded **8** quantitatively, which was further glycosylated with 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl- β -D-galactopyranosyl trichloroacetimidate (**9**)²² in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in anhydrous DCM, to give the desired trisaccharide derivative **10** in 63% yield. The characteristic doublet at 5.45 ppm with coupling constant of $J = 3.6$ Hz clearly indicated α configuration in this newly formed glycosidic bond of **10**. Simultaneous reduction of azido group to amine and cleavage of benzyl groups on fucosyl residue were carried out smoothly via hydrogenation of **10** with $\text{Pd}(\text{OH})_2$ on charcoal under H_2 atmosphere (4.3 Mpa) to afford **11**, which was subsequently acetylated with Ac_2O /DMAP in pyridine to give **12** in 89% overall yield. To facilitate the designed click reaction in glyco-nanoparticle formation, chloride in **12** was transformed into azido group with NaN_3 in the presence of NaI in DMF at 80°C to obtain **13**, which was subjected to global deacetylation with NaOMe in MeOH accomplished the key trisaccharide antigen **14** in an isolated yield of 86% (see Scheme 1).

The assembly of blood group A trisaccharide-functionalized MNPs started from the preparation of superparamagnetic Fe_3O_4 nanoparticles through hydrolysis and reduction²³ of FeCl_3 in the presence of sodium acrylate in a combination of ethylene glycol (EG) and diethylene glycol (DEG) at 200°C (Scheme 2). Treatment of Fe_3O_4 magnetic nanoparticles with tetraethylorthosilicate (TEOS) and NH_4OH obtained the silica layer coated Fe_3O_4 particles.²⁴ The shell thickness could be controlled by the applied TEOS concentration and the reaction time. The resulting silica-coated MNPs were then modified by dipropargyl derivative **15**, which was prepared from the reaction of 3-(triethoxysilyl)propan-1-amine and propargyl bromide in the presence of CaH_2 in DCM,²⁵ to graft silica shell surface with alkynyl group (alkyne-MNPs) in NH_4OH -MeOH co-solvents. Click reaction of alkyne MNPs with trisaccharide antigen derivative **14** in the presence of sodium ascorbate²⁶ and CuSO_4 in THF: H_2O (v/v, 1/1) afforded blood group A trisaccharide-functionalized MNPs (glyco-MNPs).

Scheme 1. Synthesis of blood group A trisaccharide antigen **14**.

Scheme 2. Synthesis of blood group A trisaccharide-functionalized glyco-MNPs.

Fig. 2. TEM of (a,b) MNPs; (c,d) silica-coated MNPs; (e,f) alkyne-MNPs; (g,h) glyco-MNPs.

Transmission electron microscopy (TEM) images of the prepared Fe_3O_4 MNPs (Fig. 2a and b) indicated that the particles were properly dispersed with an approximate average size of 100 nm, while the silica-coated MNPs (Fig. 2c and d) gave an average diameter of 120 nm with well defined core-shell structures. The average particle diameters of alkyne-MNPs (Fig. 2e and f) and glyco-MNPs (Fig. 2g and h) showed no obvious differences comparing to that of silica-coated MNPs under our TEM conditions. Dynamic light scattering (DLS) analysis of alkyne-MNPs and glyco-MNPs in water gave the corresponding hydrodynamic sizes at 251 and 274 nm, respectively.

The FTIR spectrum of blood group A-functionalized MNPs (Fig. 3b), in comparing with the spectra of alkyne-MNPs (Fig. 3a) and blood group A antigen (Fig. 3c), possessed not only the similar characteristic peaks from alkyne-MNPs at 588, 1084, 1411, 1558 cm^{-1} , but also contained peaks at 1714 and 1629 cm^{-1} corresponding to the trisaccharide antigen structure. The typical asymmetric stretching of azido group at 2100 cm^{-1} in Fig. 3c disappeared after click triazole formation, as shown in Fig. 3b, further proved the condensation.

Thermogravimetric analysis (TGA, Fig. 4) of alkyne-MNPs and

glyco-MNPs indicated that the weight losses were 22.1% for alkyne-

Fig. 3. FTIR spectra of a) alkyne-MNPs; b) glyco-MNPs; c) blood group A antigen.

Fig. 4. TGA spectra of a) alkyne MNPs; b) glyco-MNPs.

MNPs and 30.4% for antigen-functionalized MNPs. Based on this calculation, the quantity for immobilized antigens on MNPs was approximately 0.08 mg/mg (carbohydrate antigens/MNPs). These experimental data supported that the blood group A antigen has been incorporated successfully onto the MNPs surface.

To determine the antibody clearance capacity of our antigen-coated glyco-MNPs, we decided to use commercially available monoclonal antibody for our preliminary studies to circumvent the complexities related to the whole blood due to the presence of non-specific proteins. Thus, the interaction between various concentrations of glyco-MNPs with anti-A antibody was investigated. After incubation of glyco-MNPs with anti-A antibody, an outside magnetic field was applied to the binding system through a handheld magnet inducing aggregation of glyco-MNPs on the side of the vial. The residual antibody of supernatants was then recorded with a modified BCA method.²⁷ The starting anti-A concentration was settled at 65 $\mu\text{g/mL}$ for each reaction, while the glyco-MNPs at 10, 20, 30, 40, 50, and 60 $\mu\text{g/mL}$, respectively. From three independent parallel binding experiments, we observed that the maximum clearance quantity of anti-A was about 93%, as shown in Fig. 5. The non-specific binding ratio between anti-A antibody and alkyne-MNPs/glyco-MNPs was determined from an independent control experiment. Approximately 30% of the overall binding capacity contributed from alkyne-MNPs adsorption, instead of antibody-antigen interaction under various concentrations. Based on the above information, the specific antibody-antigen binding ratio was calculated as around 7/9.

Fig. 5. Left: Clearance of anti-A with glyco-MNPs at anti-A concentration of 65 $\mu\text{g/mL}$; Right: Binding ratios between anti-A and a) glyco-MNPs; b) alkyne-MNPs.

3. Conclusion

We have disclosed a new strategy for the preparation of blood group antigen-functionalized magnetic nanoparticles, where the magnetic cores of the nanoparticles possessing a silica-layer coating with blood group A trisaccharide residues outside. By using non-neighboring participation group, 2-azido group, on the C2-position of glycosyl donor, we successfully constructed the specific α -glycosidic bond in between sugar residues of GalNAc and Gal. The blood group A trisaccharide-functionalized glyco-MNPs core-shell structure was confirmed by FTIR, TGA and TEM. The excellent binding abilities of our glyco-MNPs to anti-A antibody extended the potential application of nanoparticles²⁸ and suggested that our technique might be helpful in blood transfusion and organ transplantation. Further studies are currently undergoing in our lab to remove anti-A and anti-B antibodies subsequently through a tandem process with blood group A and B trisaccharides-functionalized glyco-MNPs, respectively.

4. Experimental section

4.1. General methods

All reagents were purchased and used without purification unless otherwise stated. DCM was distilled after CaH_2 treatment. Thin-layer chromatography (TLC) was performed using glass-packed silica gel plates (0.15–0.2 mm thickness). Flash column chromatography was carried out on silica gel (100–200 mesh). Optical rotations were recorded on a digital polarimeter at ambient temperature. NMR spectra were recorded on Bruker spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C NMR). HRMS spectra were taken with ESI (TOF) mode. TGA was recorded on Q600 SDT. FTIR transmission spectra were reported on a thin layer of KBr pallet. TEM images were recorded on Hitachi H-7500 field-emission transmission electron microscope and Hitachi HT-7700 field-emission transmission electron microscope. Measurement of protein was performed with Biotech Synergy 2.

4.1.1. 4-Chlorobutyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranoside (2)

A solution of 4-chloro-1-butanol (30 g, 0.28 mol) in DCM (200 mL) was washed with saturated NaHCO_3 , the DCM solution was then dried over anhydrous Na_2SO_4 . To the above dried solution was added β -D-galactopyranose pentaacetate **1** (90 g, 0.23 mol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (90 mL, 0.72 mol) in anhydrous DCM (220 mL). The reaction mixture was stirred at rt for 2 h, quenched by saturated NaHCO_3 , followed by an extraction with DCM (3 $\times$ 70 mL). The

combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under diminished pressure. Purification of the residue by silica gel column chromatography gave compound **2** (64 g, 63%) as a white foam. $[\alpha]_D^{25}$ -16 (c 1, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 5.37 (s, 1H), 5.18 (t, J = 9.2 Hz, 1H), 5.00 (d, J = 5.2 Hz, 1H), 4.45 (d, J = 7.6 Hz, 1H), 4.19–4.08 (m, 2H), 3.95–3.87 (m, 2H), 3.55–3.48 (m, 3H), 2.14 (s, 3H), 2.05 (d, J = 4.8 Hz, 6H), 1.97 (s, 3H), 1.85–1.78 (m, 2H), 1.75–1.71 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ = 170.57, 170.43, 170.34, 169.56, 101.45, 71.07, 70.81, 69.29, 69.00, 67.20, 61.44, 44.81, 29.28, 26.95, 20.93, 20.85, 20.76. HRMS (ESI) calcd m/z for $\text{C}_{18}\text{H}_{27}\text{ClO}_{10}\text{Na}$ $[(M + \text{Na})^+]$ 461.1185, found m/z 461.1201.

4.1.2. 4-Chlorobutyl 4,6-O-benzylidene- β -D-galactopyranoside (**4**)

To a solution of **2** (55 g, 0.125 mol) in anhydrous methanol (250 mL) was added 1 N NaOMe/MeOH to keep solution pH at 9.5 for 1 h. The reaction mixture was neutralized with Amberlite IR-120 (H^+), and concentrated in *vacuo* to afford solid **3**, which was further dissolved in acetonitrile (150 mL) with subsequent adding of benzaldehyde dimethyl acetal (23.1 mL, 0.15 mol) and Camphorsulfonic acid (4.62 g, 0.02 mol). The reaction mixture was stirred at rt for about 1 h until TLC indicated the reaction completion. Neutralization with Et_3N and evaporation of the solvent under reduced pressure obtained a syrup, which was purified by silica gel column chromatography to give **4** (40.68 g, 90% over two steps) as an amorphous solid. $[\alpha]_D^{25}$ -48 (c 1, CHCl_3); ^1H NMR (400 MHz, MeOD): δ = 7.55–7.53 (m, 2H), 7.35–7.34 (m, 3H), 5.60 (s, 1H), 4.31 (s, 1H), 4.20 (d, J = 11.6 Hz, 2H), 4.14 (d, J = 12.6 Hz, 1H), 3.97–3.92 (m, 1H), 3.64–3.58 (m, 5H), 3.54 (s, 1H), 1.93–1.88 (m, 2H), 1.82–1.76 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ = 137.60, 129.45, 128.44, 126.55, 102.90, 101.63, 75.44, 72.92, 72.03, 69.34, 69.08, 66.88, 45.04, 29.39, 27.09. HRMS (ESI) calcd m/z for $\text{C}_{17}\text{H}_{23}\text{ClO}_6\text{Na}$ $[(M + \text{Na})^+]$ 381.1075, found m/z 381.1067.

4.1.3. 4-Chlorobutyl 3-O-pivaloyl-4,6-O-benzylidene- β -D-galactopyranoside (**5**)

To a solution of **4** (26.35 g, 73.44 mmol) in pyridine (120 mL) was added pivaloyl chloride (10.33 g, 85.66 mmol) dropwise. The reaction mixture was stirred at rt for 1 h and TLC showed the reaction completion. The reaction mixture was evaporated under *vacuo*, and the residue was purified by silica gel column chromatography to give **5** (29.76 g, 91%) as an amorphous solid. $[\alpha]_D^{25}$ -26 (c 1, CHCl_3); ^1H NMR (400 MHz, MeOD): δ = 7.48–7.47 (m, 2H), 7.35–7.34 (m, 3H), 5.56 (s, 1H), 4.82 (dd, J = 12.0, 4.0 Hz, 1H), 4.41–4.37 (m, 2H), 4.22 (d, J = 12.4 Hz, 2H), 4.13 (d, J = 12.4 Hz, 1H), 3.98–3.93 (m, 1H), 3.80 (t, J = 9.0 Hz, 1H), 3.65–3.59 (m, 4H), 1.92–1.87 (m, 2H), 1.82–1.77 (m, 2H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, MeOD): δ = 178.35, 138.38, 128.56, 127.77, 125.94, 103.35, 100.60, 73.95, 73.70, 68.92, 68.79, 68.25, 66.47, 44.42, 38.73, 29.22, 26.85, 26.28. HRMS (ESI) calcd m/z for $\text{C}_{22}\text{H}_{31}\text{ClO}_7\text{Na}$ $[(M + \text{Na})^+]$ 465.1651, found m/z 465.1679.

4.1.4. 4-Chlorobutyl 2,3,4-tri-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3-O-pivaloyl-4,6-O-benzylidene- β -D-galactopyranoside (**7**)

To a solution of **5** (11.35 g, 25.63 mmol) in toluene (120 mL) was added compound **6** (13.34 g, 27.86 mmol), molecular sieves (4 Å, 9.6 g), and NIS (6.90 g, 30.65 mmol) under N_2 atmosphere with ice-water bath. The reaction mixture was stirred at this condition for 15 min, then added TFOH (247 μL , 2.78 mmol) and kept stirring for another hour. At the end of which time, TLC showed the reaction completion. The mixture was neutralized with Et_3N , filtered and the filtrate was evaporated under *vacuo*, and the residue was purified by silica gel column chromatography to give amorphous solid **7** (19.32 g, 88%). $[\alpha]_D^{25}$ -17 (c 1, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 7.49–7.27 (m, 20H), 5.48 (s, 1H), 5.30 (d, J = 3.2 Hz, 1H), 5.08 (dd, J = 9.6, 3.6 Hz, 1H), 4.99 (d, J = 11.6 Hz, 1H), 4.78 (d, J = 10.4 Hz, 2H),

4.68, 4.72 (2 d, J = 12.0 Hz, 2H), 4.61 (d, J = 11.2 Hz, 1H), 4.53 (d, J = 7.6 Hz, 1H), 4.45 (d, J = 2.8 Hz, 1H), 4.37 (q, J = 6.4 Hz, 1H), 4.33–4.24 (m, 2H), 4.11–4.04 (m, 2H), 3.99–3.94 (m, 2H), 3.71 (s, 1H), 3.50–3.45 (m, 4H), 1.81–1.78 (m, 2H), 1.73–1.67 (m, 2H), 1.13 (s, 3H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3): δ = 178.24, 138.99, 138.80, 138.48, 137.76, 128.99, 128.62, 128.53, 128.39, 128.25, 127.78, 127.75, 127.68, 126.22, 101.91, 100.86, 97.00, 79.82, 77.86, 76.29, 75.03, 73.72, 73.22, 72.99, 70.27, 69.19, 68.61, 66.59, 66.33, 44.94, 39.06, 29.57, 27.24, 16.77. HRMS (ESI) calcd m/z for $\text{C}_{44}\text{H}_{59}\text{ClO}_{11}\text{Na}$ $[(M + \text{Na})^+]$ 881.3638, found m/z 881.3633.

4.1.5. 4-Chlorobutyl 2,3,4-tri-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-4,6-O-benzylidene- β -D-galactopyranoside (**8**)

To a solution of **7** (5.0 g, 5.82 mmol) in anhydrous methanol (50 mL) was added NaOMe/MeOH (1 N, 0.6 mL). The reaction mixture was stirred at rt for 1 h, neutralized with Amberlite IR-120 (H^+), and evaporated the filtrate in *vacuo*. The residue was subjected to silica gel column chromatography to give **8** (4.43 g, 99%) as amorphous solid. $[\alpha]_D^{25}$ -54 (c 1, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 7.55–7.53 (m, 2H), 7.40–7.22 (m, 18H), 5.56 (s, 1H), 5.24 (d, J = 3.2 Hz, 1H), 4.97 (d, J = 11.6 Hz, 1H), 4.83–4.74 (m, 4H), 4.66 (d, J = 11.6 Hz, 1H), 4.36 (s, 1H), 4.30 (d, J = 12.4 Hz, 1H), 4.21 (s, 1H), 4.15 (q, J = 6.4 Hz, 1H), 4.07 (d, J = 11.6 Hz, 2H), 3.98–3.91 (m, 2H), 3.85 (d, J = 3.2 Hz, 2H), 3.69 (s, 1H), 3.54–3.46 (m, 3H), 3.42 (s, 1H), 1.85–1.82 (m, 2H), 1.74–1.73 (m, 2H), 1.12 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ = 138.94, 138.79, 137.94, 137.87, 129.27, 128.61, 128.58, 128.57, 128.50, 128.37, 128.34, 128.02, 127.75, 127.69, 127.56, 126.69, 102.01, 101.56, 99.45, 79.80, 77.86, 77.75, 75.72, 74.97, 73.95, 73.70, 73.09, 69.43, 68.43, 67.07, 66.76, 45.12, 29.37, 27.21, 16.91. HRMS (ESI) calcd m/z for $\text{C}_{44}\text{H}_{51}\text{ClO}_{10}\text{Na}$ $[(M + \text{Na})^+]$ 797.3063, found m/z 797.3072.

4.1.6. 4-Chlorobutyl 2-azido-2-deoxy-3,4,6-tri-O-acetyl- α -D-galactopyranosyl-(1 $\rightarrow$ 3)-[2,3,4-tri-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)]-4,6-O-benzylidene- β -D-galactopyranoside (**10**)

To an ice-bath pre-cooled mixture of **8** (2.20 g, 2.84 mmol) and molecular sieves (4 Å, 2.0 g) in anhydrous DCM (25 mL) was added compound **9** (2.80 g, 5.89 mmol) under N_2 atmosphere. After stirring at this condition for 15 min, TMOSTf (80 μL , 0.43 mmol) was added to the mixture stirring for further 2 h. At the end of which time, TLC showed the reaction completion, and the mixture was neutralized with Et_3N , filtered, and the filtrate was evaporated under *vacuo*. The residue was purified by silica gel column chromatography to furnish syrupy **10** (1.94 g, 63%). $[\alpha]_D^{25}$ +57 (c 1, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 7.56–7.24 (m, 20H), 5.55 (s, 1H), 5.45 (d, J = 3.6 Hz, 1H), 5.31 (s, 1H), 5.25 (d, J = 11.2 Hz, 1H), 5.18 (s, 1H), 5.10 (d, J = 12.0 Hz, 1H), 4.94, 4.89 (2 d, J = 12.0 Hz, 2H), 4.79, 4.74 (2 d, J = 12.0 Hz, 2H), 4.62 (d, J = 11.6 Hz, 1H), 4.41–4.32 (m, 4H), 4.25 (d, J = 4.4 Hz, 1H), 4.17–4.08 (m, 3H), 4.00 (d, J = 10.8 Hz, 1H), 3.97–3.92 (m, 1H), 3.87 (dd, J = 9.2, 4.0 Hz, 1H), 3.80 (dd, J = 11.6, 4.0 Hz, 1H), 3.71 (d, J = 1.2 Hz, 1H), 3.59–3.47 (m, 4H), 3.39 (s, 1H), 3.31 (dd, J = 11.6, 3.6 Hz, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 1.96 (s, 3H), 1.84–1.79 (m, 2H), 1.73–1.68 (m, 2H), 1.13 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ = 170.54, 169.92, 169.64, 139.46, 138.94, 138.86, 137.62, 129.00, 128.49, 128.44, 128.33, 128.30, 127.69, 127.59, 127.52, 126.20, 102.13, 100.77, 98.08, 94.21, 80.53, 77.85, 76.14, 74.95, 74.22, 72.73, 72.15, 69.43, 68.91, 68.54, 68.18, 67.66, 66.55, 66.40, 62.87, 57.95, 45.00, 29.53, 27.20, 20.92, 20.81, 16.88. HRMS (ESI) calcd m/z for $\text{C}_{56}\text{H}_{66}\text{ClN}_3\text{O}_{17}\text{Na}$ $[(M + \text{Na})^+]$ 1110.3973, found m/z 1110.3965.

4.1.7. 4-Chlorobutyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl- α -D-galactopyranosyl-(1 $\rightarrow$ 3)-[2,3,4-tri-O-acetyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)]-4,6-di-O-acetyl- β -D-galactopyranoside (**12**)

The solution of **10** (324 mg, 0.30 mmol) in methanol was

hydrogenated at a H₂ pressure of 4.3 MPa in the presence of palladium hydroxide on carbon (20%, 30 mg). TLC monitored the reaction process until the reaction was completed in 30 h. The reaction mixture was filtered and the filtrate was concentrated to a syrup, which was directly dissolved in pyridine (2 mL) containing Ac₂O (2 mL) and 4-dimethylaminopyridine (DMAP, 5 mg). The reaction mixture was allowed to stir overnight, the solvent was evaporated under vacuo, and the residue was subjected to a flush column. Concentration of the eluent obtained **12** (253 mg, 89% for two steps) as colorless solid. [α]_D²⁵ -10 (c 0.4, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 6.24 (d, *J* = 8.4 Hz, 1H), 5.49 (d, *J* = 3.6 Hz, 1H), 5.42 (d, *J* = 1.6 Hz, 1H), 5.37 (d, *J* = 2.4 Hz, 1H), 5.33 (dd, *J* = 10.8, 3.6 Hz, 1H), 5.27 (d, *J* = 2.4 Hz, 1H), 5.23, 5.21 (2 d, *J* = 3.2 Hz, 2H), 4.98 (dd, *J* = 11.6, 3.2 Hz, 1H), 4.52–4.46 (m, 2H), 4.37 (d, *J* = 6.8 Hz, 1H), 4.23 (t, *J* = 5.6 Hz, 1H), 4.14–4.06 (m, 4H), 3.91–3.78 (m, 4H), 3.61–3.56 (m, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.13 (s, 6H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.88–1.86 (m, 2H), 1.85–1.79 (m, 2H), 1.11 (d, *J* = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ = 171.23, 170.71, 170.68, 170.52, 170.44, 170.38, 170.19, 170.13, 170.07, 102.47, 98.18, 96.70, 78.78, 75.25, 71.44, 70.46, 69.62, 68.47, 68.00, 67.61, 67.56, 67.36, 66.90, 65.20, 62.88, 61.45, 48.21, 44.78, 29.20, 27.04, 23.22, 20.94, 20.86, 20.82, 16.08. HRMS (ESI) calcd *m/z* for C₄₀H₅₈ClNO₂₃Na [(M + Na)⁺] 978.2980, found *m/z* 978.2931.

4.1.8. 4-Azidobutyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl- α -D-galactopyranosyl- (1 $\rightarrow$ 3)-[2,3,4-tri-O-acetyl- α -L-fucopyranosyl- (1 $\rightarrow$ 2)]-4,6-di-O-acetyl- β -D-galactopyranoside (13**)**

To a solution of **12** (610 mg, 0.64 mmol) in dry DMF (20 mL) was added NaI (480 mg, 3.20 mmol) and NaN₃ (208 mg, 3.20 mmol). The reaction mixture was stirred at 80 °C overnight, at the end of which time TLC indicated the completion of the reaction. The cooled reaction mixture was diluted with ether (100 mL), washed with water (3 $\times$ 45 mL), and the organic phase was dried over Na₂SO₄, followed by evaporation under diminished pressure. The residue was purified by silica gel column chromatography to generate compound **13** (552 mg, 90%) as a white powder. [α]_D²⁵ -18 (c 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 6.25 (d, *J* = 8.4 Hz, 1H), 5.49 (d, *J* = 3.6 Hz, 1H), 5.43 (s, 1H), 5.36 (s, 1H), 5.32 (d, *J* = 12.0 Hz, 1H), 5.26 (s, 1H), 5.21 (d, *J* = 10.0 Hz, 2H), 4.97 (d, *J* = 11.6 Hz, 1H), 4.52–4.44 (m, 2H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.23 (t, *J* = 5.6 Hz, 1H), 4.14–4.04 (m, 4H), 3.91–3.78 (m, 4H), 3.60–3.56 (m, 1H), 3.34–3.31 (m, 2H), 2.15 (s, 3H), 2.13 (s, 3H), 2.11 (s, 6H), 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.69 (s, 4H), 1.10 (d, *J* = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ = 171.22, 170.72, 170.55, 170.46, 170.41, 170.22, 170.15, 170.09, 102.50, 98.13, 96.70, 78.73, 75.24, 71.44, 70.48, 69.85, 68.46, 68.02, 67.63, 67.58, 67.40, 66.80, 65.21, 62.88, 61.47, 51.24, 48.21, 26.96, 25.72, 23.24, 20.94, 20.87, 20.83, 16.07. HRMS (ESI) calcd *m/z* for C₄₀H₅₈N₄O₂₃Na [(M + Na)⁺] 985.3384, found *m/z* 985.3324.

4.1.9. 4-Azidobutyl 2-acetamido-2-deoxy- α -D-galactopyranosyl- (1 $\rightarrow$ 3)-[α -L-fuco pyranosyl-(1 $\rightarrow$ 2)]- β -D-galactopyranoside (14**)**

To a solution of **13** (667 mg, 0.69 mmol) in anhydrous methanol (10 mL) was added 1 N NaOMe/MeOH until pH reached at 9 to 10. The reaction mixture was stirred at rt for 2 h, then neutralized with Amberlite IR-120 (H⁺). Filtration of the mixture and evaporation of the organic phase obtained a syrupy compound, which was dissolved in DD water and subjected to freeze-drying, finished amorphous solid compound **14** (413 mg, 95%). [α]_D²⁵ +18 (c 0.38, MeOH); ¹H NMR (400 MHz, D₂O): δ = 5.31 (d, *J* = 2.4 Hz, 1H), 5.16 (d, *J* = 2.4 Hz, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.43 (q, *J* = 6.4 Hz, 1H), 4.24–4.20 (m, 3H), 3.98–3.93 (m, 3H), 3.89 (d, *J* = 11.2 Hz, 1H), 3.81–3.69 (m, 9H), 3.64 (d, *J* = 4.8 Hz, 1H), 3.35–3.32 (m, 2H), 2.02 (s, 3H), 1.66–1.62 (m, 4H), 1.21 (d, *J* = 6.4 Hz, 3H); ¹³C NMR (100 Hz,

D₂O): δ = 174.93, 101.52, 98.56, 91.47, 75.98, 75.02, 72.24, 72.02, 71.23, 69.99, 69.84, 68.63, 67.94, 67.87, 66.98, 63.14, 61.47, 61.11, 51.01, 49.62, 26.43, 25.10, 22.07, 15.43. HRMS (ESI) calcd *m/z* for C₂₄H₄₂N₄O₁₅Na [(M + Na)⁺] 649.2539, found *m/z* 649.2540.

4.1.10. Typical procedure for the preparation of Fe₃O₄ magnetic nanoparticles (MNPs)

The solution of ethylene glycol (6 mL) and diethylene glycol (34 mL) was deoxygenated by ultrasonication for 15 min, then sodium acrylate (3 g) and FeCl₃·6H₂O (1.08 g) were added. The mixture was stirred at rt overnight to form a homogeneous yellow solution, which was transferred and sealed to a Teflon-lined stainless-steel autoclave. The autoclave was kept at 205 °C for 12 h, then cooled to rt. The magnetic particles were collected by outside additional magnet and orderly washed with MeOH and water three times and dried in vacuum at 50 °C for 12 h.

4.1.11. Preparation of silica-coated MNPs

The mixture of prepared MNPs (50 mg) in H₂O (6 mL) and MeOH (40 mL) was deoxygenated and homogenized by ultrasonication for 15 min, then ammonium hydroxide (2 mL) was added dropwise. Tetraethylorthosilicate (TEOS, 0.4 mL) was injected into the solution after 5 min, and the reaction bottle was subjected to ultrasonication for 2.5 h. The obtained silica-coated MNPs were washed with MeOH three times with the help of outside magnet and dried in vacuum at 50 °C for 12 h.

4.1.12. Preparation of alkyne-MNPs

The mixture of prepared silica-coated MNPs (25 mg) in H₂O (3 mL) and MeOH (20 mL) was deoxygenated and homogenized through ultrasonication for 30 min, then ammonia solution (1 mL) was added and ultrasonicated for another 10 min. After that, compound **15** was injected and the mixture was kept at ultrasonication conditions for 3 h. The products were collected and washed with MeOH five times with the help of outside magnet, and dried in vacuum at 50 °C for 12 h to afford **16**.

4.1.13. Preparation of antigen-functionalized glyco-MNPs

A suspension of prepared alkyne-MNPs (50 mg) in H₂O (25 mL) and THF (25 mL) was deoxygenated and homogenized through ultrasonication for 15 min, then trisaccharide antigen **14** (250 mg), sodium ascorbate (100 mg) and CuSO₄ (50 mg) were added subsequently. The mixture was ultrasonicated for 48 h at 50 °C, and the solid was collected by outside magnet and washed with MeOH and water repeatedly for five times. Drying the particles under vacuum condition at 50 °C for 12 h accomplished the final product glyco-MNPs **17**.

4.1.14. Typical biological protocol for the cleavage of anti-A with the prepared glyco-MNPs

Anti-A antibody in PBS was purchased and the concentration was calibrated whenever experiment starts. In our study, the antibody concentration was adjusted as 1.0 mg/mL. The prepared glyco-MNPs **17** in reaction vials were scattered into PBS (1 mL) at the amounts of 10, 20, 30, 40, 50, and 60 μ g, respectively, then subjected to ultrasonication for 2 min. To each of the above homogenized PBS solution was added anti-A antibody (65 μ L), respectively, followed by a continuous shaking at 200 rpm at 37 °C for 60 min. The glyco-MNPs were removed using outside magnet, and the concentration of the retained antibody in supernatant was measured by BCA method.²⁷

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tet.2017.04.003>.

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