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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 Fe_3O_4-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.

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 modeling[8] 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, Fe_3O_4 nanoparticles are biologically safe materials applied in bio-separation,[10] biomedical engineering[11] 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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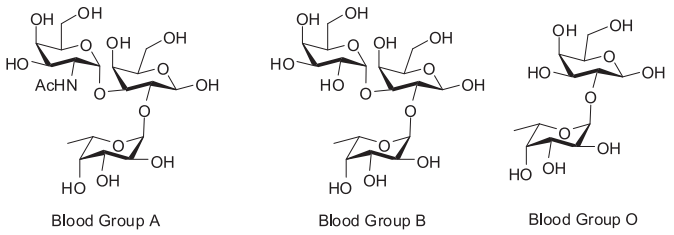
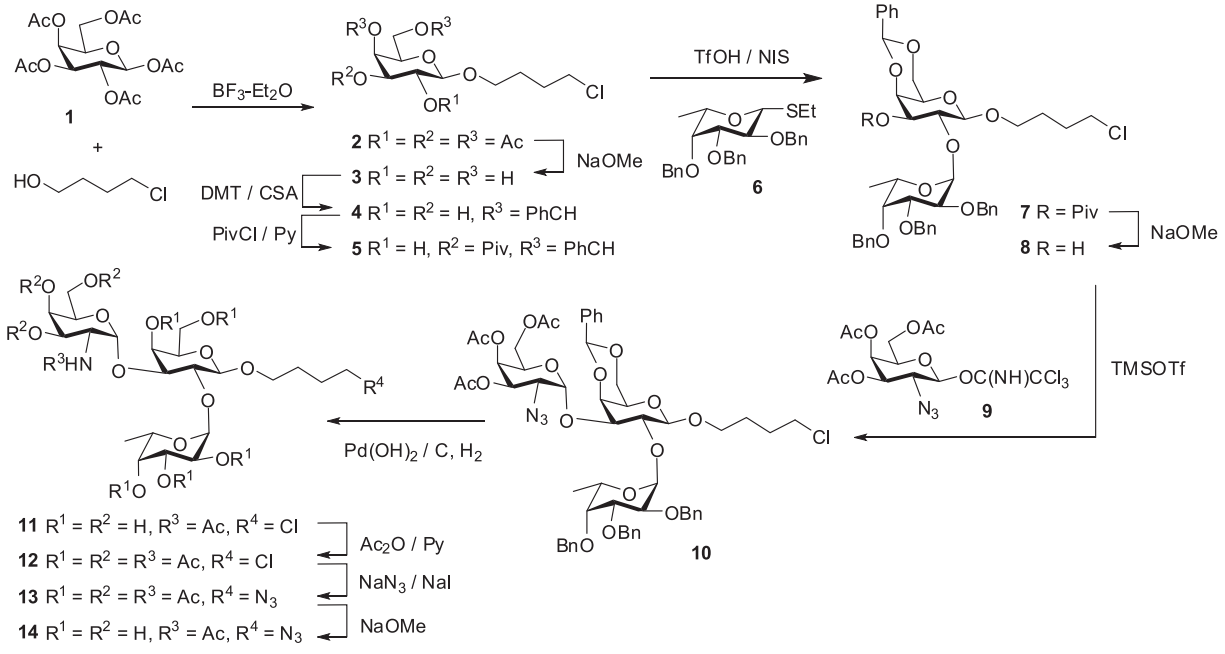


Fig. 1. The structures of blood group trisaccharides A, B and O.

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 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, $BF_3{\cdot}Et_2O$ catalyzed condensation[20] 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 (→ **4**), followed by regioselective masking of 3-OH with pivaloyl chloride[21] 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 ^{1}H 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 $Pd(OH)_2$ 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 reduction[23] 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 ascorbate[26] 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**.