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A Four-Component One-Pot Synthesis of α-Gal Pentasaccharide

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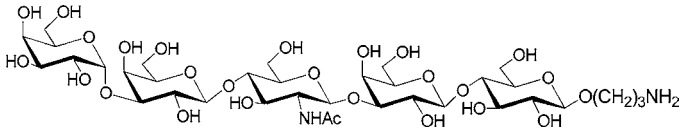
ABSTRACT



A four-component one-pot sequential synthesis of α-Gal pentasaccharide 2 with minimal protecting group manipulations in a very short period of time is described in this paper.

Antibody- and complement-dependent hyperacute rejection (HAR), as the major barrier to successful pig-to-human xenotransplantation, is due to the specific interaction of recipient xenoreactive antibodies with antigens present on the endothelium of the donor organ, followed by activation of the complement cascade.[1] Carbohydrate structure epitopes containing a Galα(1 → 3)Gal terminus (α-Gal epitopes) are established as the main xenoantigens responsible for initiating the hyperacute rejection of pig organs by humans. The pentasaccharide α-Gal epitope Galα1-3Gal*β*1-4GlcNAc*β*1-3Gal*β*1-4Glc*β*-Cer (**1**) (Figure 1) existing in pig vascular endothelium binds specifically with human anti-Gal antibodies. It has been identified that the pentasaccharide residue serves as the binding site for human anti-pig antibodies.[2] To overcome the hyperacute rejection, one of the possible strategies is to use synthetic α-Gal oligosaccharides to antagonize anti-α-Gal antibodies.[3] Such an approach would require access to a substantial amount of α-Gal oligosaccharides and α-Gal analogues.

Several methods were reported for the synthesis of α-Gal epitope pentasaccharide. Boons et al.[4] reported a highly convergent synthesis of the methyl glycoside of the pentasaccharide. The Schmidt group synthesized the pentasaccharide **1** and its methyl glycoside with the trichloroacetimidate methodology.[5] Wang and co-workers described a chemoenzymatic approach to the synthesis of α-Gal epitopes based on the use of recombinant α(1−3)-galactosyltransferase.[6] However, a highly efficient assembly of the

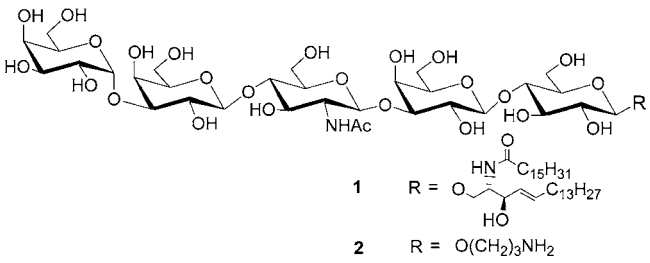


Figure 1. Ceramide pentasaccharide **1** and its derivative **2**.

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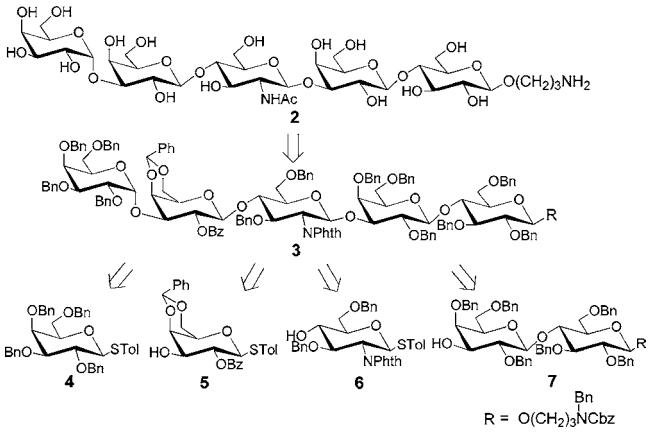
α-Gal pentasaccharide moiety is still in great demand. Herein we present a new method for the rapid synthesis of the pentasaccharide derivative **2** (Figure 1) by a four-component one-pot strategy. The aminopropyl group[7] was incorporated as a side chain for further derivation. The feature of this work lies in the integration of three glycosylation steps into one synthetic operation to furnish the target oligosaccharide in a few hours without the need for protecting group manipulation and intermediate isolation.

The one-pot sequential glycosylation strategy[8] for the assembly of oligosaccharides has achieved success as demonstrated by syntheses of some naturally occurring oligosaccharides such as Le^y,[9] fucosyl GM_1,[10] and Globo H.[11] In all of these examples, a three-component coupling strategy was applied. To enhance the efficiency of the one-pot approach, we tried to explore the possibility of synthesis of the α-Gal pentasaccharide by a four-component one-pot sequential glycosylation method. The retrosynthetic analysis divided the fully protected target pentasaccharide **3** into four building blocks: the galactose building blocks **4** and **5**, the glucosamine building block **6**, and the lactose building block **7** (Scheme 1). According to the reactivity order of thioglycosides that the Wong group reported,[8a,b] building block **4** should have the highest reactivity among the four components, and the reducing end component **7** should have no reactivity because of its *O*-glycosyl linkage, which cannot be activated by promoters of thioglycoside donors. The selection of protecting groups was the main concern in the design of the second and third building blocks, because the presence of a free hydroxyl group and a thiotoluene functionality would make them act as both a glycosyl acceptor and a glycosyl donor. Moreover, their relative reactivities toward glycosylation should fall between **4** and **7**. We finally found that building blocks **5** and **6** were suitable for the successful one-pot synthesis.

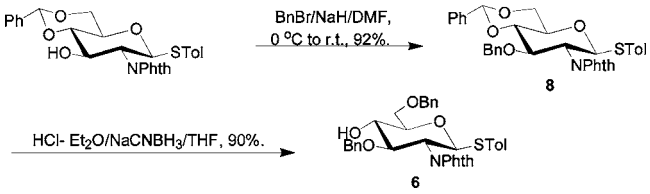
Scheme 1. Retrosynthetic Analysis of the Pentasaccharide Moiety



We chose thioglycosides as glycosyl donors because they are stable enough in most cases and can be activated by a variety of promoters. To perform an efficient one-pot synthesis, we tried several promoter systems, such as dimethyl(thiomethyl)sulfonium triflate (DMTST),[12] *N*-iodosuccinimide and triflic acid (NIS/TfOH),[13] phenylsulfenyl chloride and silver triflate (PhSCl/AgOTf),[14] and 1-benzenesulfinyl piperidine and triflic anhydride (BSP/Tf_2O).[15] Eventually we found that BSP/Tf_2O was the most efficient promoter for the one-pot glycosylation protocol.

Building blocks **4** and **5** were prepared by literature procedures.[8a] The synthesis of building block **6** is shown in Scheme 2. O-Benzylation of *p*-methylphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[8a] gave saccharide **8** in 92% yield. Regioselective reductive ring opening of the 4,6-*O*-benzylidene acetal of **8** produced **6** in 90% yield with the 4-hydroxyl group exposed. The synthesis of building block **7** started from lactose peracetate **9**.[16] Compound **9** was converted to the β-lactoside **10** in 43% yield by coupling with benzyl *N*-(3-hydroxypropyl)-carbamate in the presence of $BF_3 \cdot Et_2O$. Saccharide **10** was deacetylated, treated with dibutyltin oxide, and reacted with allyl bromide to provide regioselectively 3′-*O*-allyl-protected lactoside **11**. The benzylation of the remaining hydroxyls of **11** was performed under the conditions of sodium hydride and benzyl bromide in DMF to give compound **12**. Removal of the allyl protecting group of **12** by palladium(II) chloride yielded building block **7** in 98% yield (Scheme 3).

Scheme 2. Synthesis of Building Block **6**



With all building blocks in hand, the four-component assembly of fully protected pentasaccharide derivative **3** was attempted as outlined in Scheme 4. The one-pot synthetic operation was performed in the presence of the BSP/Tf_2O

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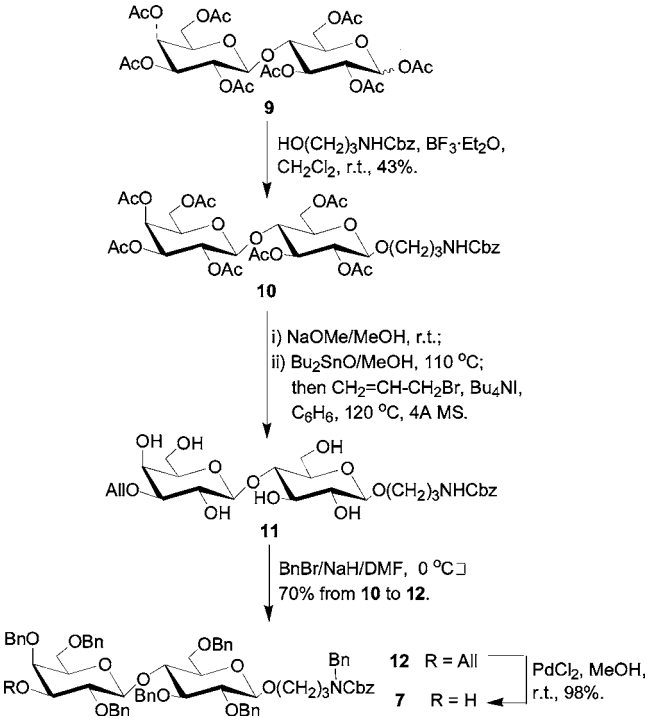
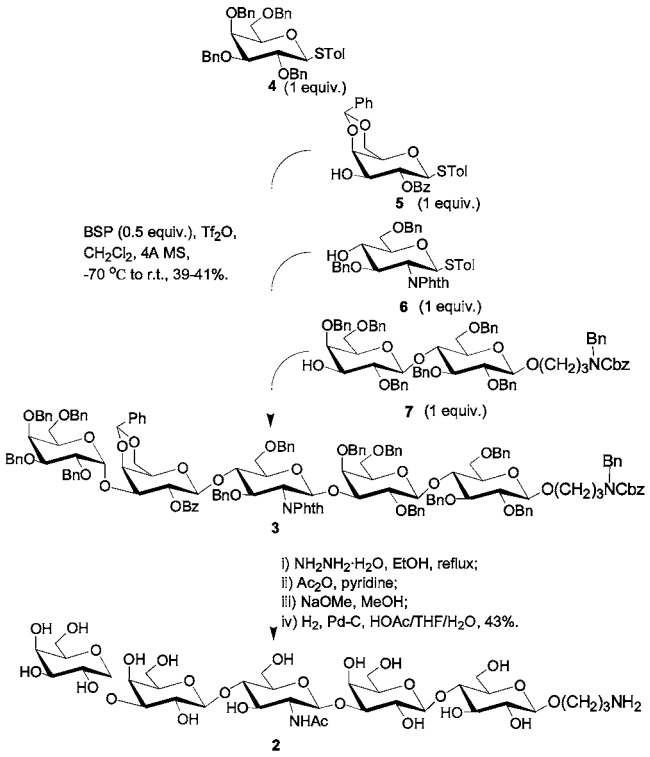
Scheme 3. Synthesis of Lactose Building Block **7**



promoter system.[15] The first glycosylation built up the α-linkage[17] between the donor **4** and the acceptor **5**. In the following two coupling steps, building blocks **6** and **7** were sequentially added to the reaction mixture. The reaction process was monitored by TLC. For the BSP/Tf_2O-promoted reactions, the glycosyl donors must be activated at −70 °C and the reaction temperature is elevated gradually to room temperature. The equivalent of BSP we used ranged from 0.5 to 1.0. Finally, the fully protected pentasaccharide derivative **3** was isolated in 39−41% yield. This finding corresponds to an average yield of 75% per glycosylation step. Global deprotection of **3** was performed in four steps (Scheme 4). The phthalimido functionality was removed in NH_2NH_2·H_2O and EtOH under reflux, and the released amino group was then acetylated with acetic anhydride in pyridine. The benzoyl functionality was cleaved by treatment with NaOMe in methanol. The remaining benzyl, benzylidene, and benzyl carbamate protecting groups were cleaved by catalytic hydrogenolysis over 10% Pd−C. The target pentasaccharide **2** was finally obtained in 43% isolated yield in the form of acetate after purification by C-18 reversed-phase column chromatography. The characterization of **2** and the correct anomeric configuration of each glycosidic linkage were confirmed by its 1D ^1H NMR, ^{13}C NMR and 2D correlated spectroscopy (HSQC, HMBC, TOCSY), and HRMS analysis.[18]

(17) To determine the configuration of the newly formed glycosyl bond, we once isolated the disaccharide and the α-linkage was demonstrated by its proton NMR analysis. Selected ^1H NMR data for one of the two anomeric protons: δ 4.98 (d, J = 3.0 Hz, 1H).

Scheme 4. One-Pot Synthesis of Fully Protected Pentasaccharide **3**[a] and Its Deprotected Product **2**



[a] Key: Compound **5** was added as a solid; **6** and **7** were added in a solution of CH_2Cl_2, respectively.

In conclusion, using a four-component one-pot sequential glycosylation method, we have successfully synthesized the aminopropyl glycoside of the α-Gal pentasaccharide, which plays an important role in the interaction with human anti-Gal antibodies and may be highly desired in the research of xenotransplantation and immunotherapy. A single glycosylation protocol was used, and only easily available common saccharide building blocks were needed for the oligosaccharide assembly. This one-pot strategy should be applicable to the synthesis of other α-Gal derivatives and other complex oligosaccharides, as well as the structure−activity relationship study of biologically important carbohydrates.

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Supporting Information Available: Synthetic procedures and spectral data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Selected NMR data for the five anomeric protons and carbons: ^1H NMR (500 MHz, D_2O) δ 5.14 (d, J = 4.0 Hz, 1H, H-1‴), 4.70 (d, J = 8.5 Hz, 1H, H-1‴), 4.54 (d, J = 8.0 Hz, 1H, H-1′), 4.50 (d, J = 8.0 Hz, 1H, H-1), 4.42 (d, J = 8.0 Hz, 1H, H-1″); ^{13}C NMR (125 MHz, D_2O) δ 103.7 (C-1″), 103.5 (C-1), 103.4 (C-1‴), 102.8 (C-1′), 96.2 (C-1⁗).