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A constraint scaffold enhances affinity of a bivalent *N*-acetylglucosamine ligand against wheat germ agglutinin

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ABSTRACT

Bivalent glycoconjugates have a minimal valence with avidity potential on protein-carbohydrate interactions as well as simplicity of chemical structures enabling simple synthesis with low cost. Understanding the way to maximize the affinities of bivalent glycoconjugates is important for the development of cost-effective tools for therapeutic and diagnostic research. However, there has been little discussion about the effects of constraints imposed from ligand scaffolds on the binding abilities. We synthesized three kinds of biantennary *N*-acetylglucosamine glycosides with different scaffolds using isobutenyl bis(propargyl)ether as a common scaffold precursor. Decoration of the scaffold branches with GlcNAc moieties through copper-catalyzed azide-alkyne cycloaddition and grafting of the alkenyl focal point to another bivalent biotin dendron through thiol-ene and nucleophilic substitution reactions were successfully carried out in an orthogonal manner. The association constants of the ligands against wheat germ agglutinin were determined by a fluorometric titration assay. A bivalent biotin counterpart provided higher affinity than an isobutyl scaffold, whereas an isobutenyl scaffold yielded more enhancement than a bivalent biotin counterpart. The present work suggested that the constraint and steric bulk of ligand scaffolds are possible factors for improving binding properties of glycoconjugates against lectins or proteins.

Lectins are proteins that specifically recognize carbohydrates and they play an important role in various biological events such as cell-cell interaction, cancer metastasis, and infection of bacteria or viruses.[1,2] Interactions of lectins with monomeric carbohydrate moieties are generally weak. When multiple binding sites of lectins interact with multiple copies of carbohydrates, the binding affinities are dramatically enhanced. This avidity is often referred to as the glycoside cluster effect.[3] There is significant variation in the magnitude of enhancement, ranging from 10^0 to 10^6-fold enhancement in affinity compared to the corresponding monovalent ligand.[4] Extensive studies on relationships between valence and affinity have shown that increased affinity with increasing valency appears to be a trend. However, bivalency does not always yield low affinity. For example, enhancements as large as 1000 for bivalent ligands[5] and as low as 0.4 for nonvalent ligands[6] have been reported. Bivalency is a minimal valence with binding enhancement potential by the glycoside cluster effect. Bivalent molecules have simpler chemical structures and lower molecular weights than those of the corresponding higher-valent molecules, which make the synthesis easier with lower cost. In addition, simplicity of bivalent compounds may aid in the understanding of recognition properties. Understanding the way to maximize the affinities of bivalent glycoconjugates is important for the development of cost-effective tools for therapeutic and diagnostic research. Many efforts have been made to control the microstructure of glycoconjugates beside valency. The orientation and spacing between glycans on a scaffold are known to be determined by the length of linkers, topology, and composition. However, there has been little discussion about the possible effect of a constraint imposed from the scaffold on the affinity. Here we report the synthesis of three kinds of biantennary *N*-acetylglucosamine (GlcNAc) glycosides with different scaffolds **1**–**3** (Scheme 1). Wheat germ agglutinin (WGA), a lectin with specificity to GlcNAc[7–9] or *N*-acetylneuraminic acid,[10] was selected as a protein model to gain more insight into the bivalent interaction. The characterization of each ligand by a fluorometric titration assay revealed the contributions of scaffolds to the binding abilities.

At first, we designed and constructed isobutenyl bis(propargyl) ether **6** as a novel scaffold precursor for versatile synthesis of gly-

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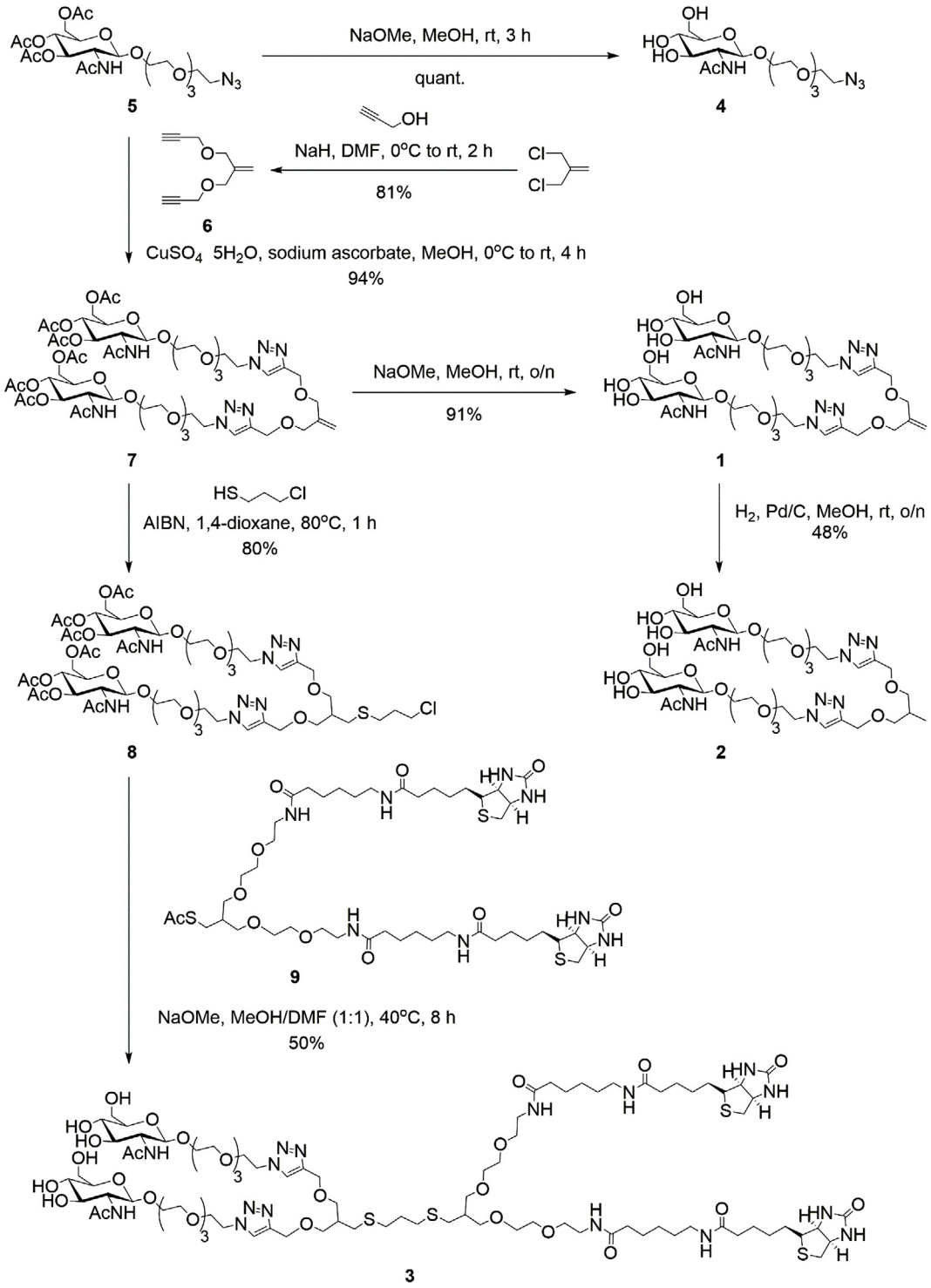

Scheme 1. Synthesis of bivalent GlcNAc glycosides **1**–**3**.

coconjugates. Compound **6** allows the use of a combination of orthogonal reactions. The double alkyne branches may facilitate rapid assembly of epitope carbohydrates by copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.[11] The single isobutenyl focal point may enable further derivatization or grafting to another molecule by thiol-based click reactions. Moreover, the alkenyl group of the branched scaffold would provide constraint to the branches. The synthesis of **6** was accomplished by Williamson etherification reaction as was applied for aliphatic polyether compounds by Fréchet's group.[12–14] Propargyl alcohol was reacted with 3-chloro-2-chloromethyl-1-propene using sodium hydride as a base. We selected THF as a solvent in our first attempt according to the literature, but no product was obtained and the alcohol used was recovered almost quantitatively. Fortunately, the reaction proceeded smoothly by changing the solvent from THF to DMF to afford **6** in 81% yield. With **6** in hand, the triazole-linked bivalent GlcNAc was constructed. The CuAAC reaction between the known monomer **5**[15] and **6** in the presence of sodium