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# Chemical approach for the syntheses of GM4 isomers with sialic acid to non-natural linkage positions on galactose

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ABSTRACT

Cell-surface glycans containing sialic acid are involved in various biological phenomena. However, the syntheses of GM4 derivatives with (2→2) and (2→4) linkages have not been investigated to date. In this study, sialylation of all of the hydroxyl groups on galactose were investigated for the syntheses of GM4 isomers. Regioselective sialylation was achieved via protection of galactosyl acceptors using electron-rich benzyl groups. These synthetic sialylated glycans will prove to be useful tools for studying unidentified carbohydrate-mediated biological roles.



## 1. Introduction

Cell-surface glycans, including N- and O-linked glycans, glycosphingolipids, glycosaminoglycans, glycophospholipid anchors, and lipo–oligo/polysaccharides, participate in a wide range of biological processes.[1,2] Unlike nucleic acids and proteins, oligosaccharides, which often have branched units, are rich in diversity. The combination of three different nucleotides or amino acids can only generate six trimers, while three different hexoses could theoretically generate more than 1000 trisaccharide regioisomers. Thus, by selecting a limited number of combinations from the many theoretical saccharide isomers, organisms construct sugar chain structures with specific functions.

Sialic acids are a family of naturally occurring 2-keto-3-deoxynononic acids that are involved in various functions such as cell–cell interactions, binding for bacterial toxins and viruses, and signal transduction.[3] The structural basis for the diversity of sialic acids arises from substitutions of one or more of the hydroxyl groups of *N*-acetylneuraminic acid (Neu5Ac), Neu5Gc, or KDN with acetyl, methyl, lactyl, phosphate, or sulfate groups.[4] Among the more than 50 derivatives of sialic acid, Neu5Ac is the most common and normally appears at the non-reducing ends of glycoproteins and glycolipids. Because they are generally present at the terminal ends of glycoconjugates located on cell surfaces, sialic acids act either as masks or recognition sites for ligand–receptor interactions in many important biological events.[1,4,5] Therefore, sialic acid-containing glycans have attracted attention in the fields of immunology and medicinal chemistry. In naturally occurring sialosides, Neu5Ac is observed on galactosides linked through the α(2→3) or α(2→6) bonds in glycoproteins and glycolipids.[6] The difference in the linkage to the sialic acid modulates its interaction with receptor proteins.

Influenza A viruses can be classified into subtypes depending on the types of surface glycoprotein present: hemagglutinin (HA) and neuraminidase (NA). There are 16 known HA subtypes, and 9 known NA subtypes of a total of 144 theoretically possible subtypes. Influenza virus infection is initiated by the attachment of the HA to sialic acid-containing ligands such as gangliosides and glycans on glycoproteins, which are located on host cell surfaces. Avian influenza virus HAs bind to sialic acid linked via α(2→3) glycosidic bonds, while human influenza virus HAs bind to α(2→6) receptors. Thus, the difference in the binding position of the sialic acid to the galactose is very important for species recognition. The ability of synthetic chemists to construct desired glycoside bonds has progressed and has contributed to our understanding of the recognition mechanisms for receptor proteins and the development of enzyme inhibitors.[7–10]

Sialylation is one of the most challenging reactions in carbohydrate chemistry and often proceeds with low yield and poor stereoselectivity.[11–13] In particular, the stereoselective synthesis of α-sialosides is complicated because sialic acid does not contain a neighboring C-3 functional group to direct the stereochemical outcome of the glycosylation. Moreover, the electron-withdrawing property of the carboxylic acid group at the anomeric position

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and the lack of a hydroxyl group at C-3 makes sialyl donors prone to undesired 2,3-elimination due to destabilization of the oxocarbenium ion intermediate generated from the sialyl donor.

Consequently, many synthetic sialylation methods have been developed to improve the stereoselectivity and coupling efficiency of the reaction.[11] However, these methodologies are focused on efficient α-sialoside installation with α(2→3) or α(2→6) linkages; thus, the strategies for the synthesis of non-natural sialosides with bonds to the 2- or 4-position of galactose remain undeveloped. Therefore, a synthetic method that enables the introduction of sialic acid groups at the sites of the poor reactive hydroxyl groups in galactose is required. Recently, Mine et al.[14] reported that α2,3-sialyltransferase from *Photobacterium* sp. JT-ISH-224 produced a regio-mistaken sialyl-transferred sialoside which had sialic acid at the C2 hydroxyl group of lactose. This results may indicate that some bacterial sialyltransferases have a potency to synthesize unusual sialosides in vivo. The development of chemical synthesis method for non-natural sialosides enables supply of an adequate amount of the sialoside to explore unknown biological function.

Here, we describe the comprehensive synthesis of sialyl galactoside isomers with both natural and non-natural sialic acid binding positions to galactose (Scheme 1). Access to such a range of gangliosides, including those with non-natural linkages, should lead to the discovery of novel biological functions and new aspects of the enzymatic mechanisms related to gangliosides. In this study, natural sialosides were synthesized to investigate the reactivity of galactosyl acceptors with hydroxyl group protected by acyl or alkyl groups. Based on these results, non-natural gangliosides were then efficiently synthesized by modulating the poor reactive hydroxy groups to more reactive ones.

## 2. Results and discussion

### 2.1. Design of the glycosyl acceptors and sialyl donor

Regioselective glycosylation is generally achieved when the glycosyl acceptor possesses protected hydroxyl groups with only one free hydroxyl group for reaction with the glycosyl donor. The outcomes of sialylations are known to depend primarily on the nature of the protecting groups introduced on the glycosyl donor and acceptor, which differ with respect to their electronic and steric properties. In this study, benzyl and benzoyl groups were selected as the protecting groups for the donor to investigate the reactivity of various glycosyl acceptors with different protecting groups. The allyl group was selected as a temporary protecting group for the aglycones of the glycosyl acceptors. This aglycone can be removed selectively or applied to labeling or immobilization, and so allyl glycoside was used as starting material. However, sialylation to allyl acceptor with thiophenyl sialoside as a donor decreased significantly in the yield under the NIS/TfOH activation method. Therefore, allyl group was hydrogenated using Lindlar catalyst to the corresponding propyl group. The propyl glycosides were used as acceptors to investigate sialylations. A set of selectively protected glycosyl acceptors (**1**–**6**, Fig. 1) prepared from allyl β-D-galactopyranoside **7** was then synthesized as described in Section 2.2–2.5.

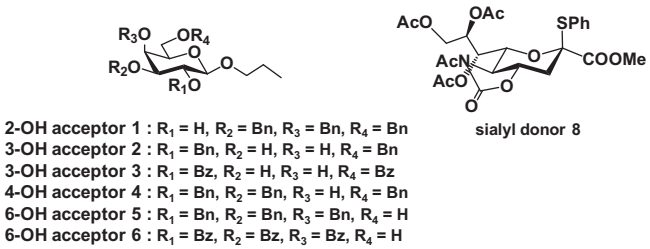



**Figure 1.** Six galactosyl acceptors and sialyl donor.

The nature of the 5-N-protecting group can greatly influence the efficiency and stereoselectivity of the sialylation.[12,13,15] Crich and Li achieved an elegant synthesis of (2→3) and (2→6)-linked sialosides using an *N*-acetyl-5-*N*,4-*O*-oxazolidinone-protected sialyl donor.[16] In addition, it was observed that the sialyl donor without oxazolidinone ring was low reactivity to 6-OH acceptor, whereas oxazolidinone-protected sialyl donor gave higher reactivity to the acceptor. For synthesis of non-natural linkages to axial and sterically hindered hydroxyl groups, the donor should have the high reactivity. Moreover, if β-linked sialosides are produced by sialylation, β-linked sialosides will also be attractive to investigate biological function. Thus, sialyl donor **8** (Fig. 1) bearing the *N*-acetyl-5-*N*,4-*O*-oxazolidinone moiety was employed as a donor in this study.

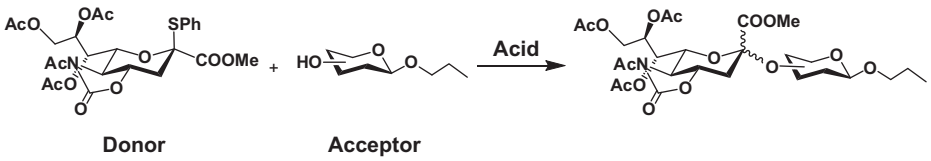
### 2.2. Preparation of the 6-OH acceptor

To obtain the 6-OH glycosyl acceptor, the trityl group was selected as a temporary and orthogonally functional protecting group (Scheme 2).[17] The allyl β-D-galactoside **7** was regioselectively tritylated at the 6-position, and then the remaining hydroxyl groups were protected with benzyl groups to afford **9** (74% yield, in two steps). Removal of the trityl group under acidic conditions and hydrogenolysis with Lindlar's catalyst afforded the 6-OH glycosyl acceptor **5** (77% yield, in two steps). Similarly, the 6-OH glycosyl acceptor **6** was prepared from **7** via benzoylation instead of benzylation.

### 2.3. Preparation of the 3,4-OH acceptor

Regioselective sialylation of the 3,4-diol groups of galactose to synthesize naturally occurring α(2→3) linkages has been successfully achieved.[18–21] In general, glycosyl acceptors for α(2→3) sialylation have 3,4-diol structures designed for regioselective glycosylation at the more reactive C3 hydroxyl group.

To construct the 3,4-diol structure, an isopropylidene acetal group was introduced to the 3- and 4-positions of galactose. Thus, compound **7** was treated with acetone and a catalytic amount of CSA in DMF to afford 3,4-*O*-isopropylidene protected derivative **11** in 64% yield (Scheme 3). The free OH group in **11** was then converted to the benzyl ether **12**, and the isopropylidene acetal group of **12** was selectively removed using 0.5 M aqueous HCl. Finally, the deprotected compound was hydrogenated with Lindlar's catalyst under $H_2$ gas in EtOAc and EtOH to afford the 3,4-OH acceptor **2**




**Scheme 1.** Plan for the synthesis of sialyl galactoside isomers.