Carbohydrates

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One-Pot Synthesis of *N*-Acetyl- and *N*-Glycolylneuraminic Acid Capped Trisaccharides and Evaluation of Their Influenza A(H1N1) Inhibition**

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Abstract: *Human lung epithelial cells natively offer terminal N-acetylneuraminic acid (Neu5Ac) α(2→6)-linked to galactose (Gal) as binding sites for influenza virus hemagglutinin. N-Glycolylneuraminic acid (Neu5Gc) in place of Neu5Ac is known to affect hemagglutinin binding in other species. Not normally generated by humans, Neu5Gc may find its way to human cells from dietary sources. To compare their influence in influenza virus infection, six trisaccharides with Neu5Ac or Neu5Gc α(2→6) linked to Gal and with different reducing end sugar units were prepared using one-pot assembly and divergent transformation. The sugar assembly made use of an N-phthaloyl-protected sialyl imidate for chemoselective activation and α-stereoselective coupling with a thiogalactoside. Assessment of cytopathic effect showed that the Neu5Gc-capped trisaccharides inhibited the viral infection better than their Neu5Ac counterparts.*

Influenza is a persistent global health concern.[1] Apart from the seasonal flu, humans are at times hit by potent viral strains of animal origin. The latest of these include the A(H1N1) flu virus from swine[2] and the avian A(H1N1)[3] and A(H7N9)[4] strains. The attachment of hemagglutinin, a viral envelope glycoprotein, to complementary sialosides on the host cell surface triggers conformational changes, which ultimately result in host cell entry and viral replication.[5] Sialic acids mainly occur at terminal glycan positions linked to galactose (Gal) in either an α(2→3) or α(2→6) manner. Prevalence of one linkage type over the other in critical infection sites limits interspecies transmission.[6] For instance, humans are susceptible to flu viruses that prefer the α(2→6)-linkage, which are dominant in respiratory epithelial cells, and are shielded from avian flu viruses by lung mucins, which are rich in α(2→3)-linkages.[7] Thus, human transmission of avian viruses is relatively rare. Viruses recognizing both linkage types can however, infect swine.[8]

Other glycan features also contribute to hemagglutinin affinity. The sugar unit at the reducing side of Gal can be either glucose (Glc), *N*-acetylglucosamine (GlcNAc), or *N*-acetylgalactosamine. Studies showed the positive influence of GlcNAc in the binding of many human flu viruses.[9] The sialic acid forms common in vertebrates are *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). Many avian flu viruses recognize Neu5Gc-capped glycans[10] and those strains can be transmitted to horses[11] and pigs.[12] Humans, in their evolutionary past, lost the capacity to form Neu5Gc and exclusively generate Neu5Ac for glycan biosynthesis under normal conditions.[13] Hence, pathogens that prefer Neu5Gc cannot readily infect humans,[14] though some human influenza viruses also attach to Neu5Gc-capped glycans.[15] Recent reports suggested that human cells can acquire Neu5Gc from dietary sources, and may increase susceptibility to a wider range of pathogens and diseases.[16]

With much focus on Neu5Ac-containing glycans,[17] chemical strategies aimed at their Neu5Gc counterparts[18] are uncommon and even more uncommon are their biological evaluations.[15] Accordingly, we prepared a set of Neu5Ac- and Neu5Gc-containing trisaccharides having α(2→6)-linkages for evaluation with a common human influenza virus (Scheme 1). The reducing end sugar was also varied. For efficient synthesis, we developed a one-pot method for the assembly of trisaccharide precursors amenable to divergent transformations. The trisaccharide skeletons were generated stereoselectively using the building blocks **1**–**5**. Such a strategy should reduce the effort and waste for the overall synthetic process.

The difficult α sialylation and the poor reactivity of sialyl donors are challenges in the one-pot assembly of glycans with terminal sialic acid units.[19] Various approaches to α sialylation exploit the modulating effect of functionalities at C5 of the sialyl donor.[20] In one such effort, the phthalimido group was found to favor the α isomer as a result of its fixed dipole effect on the oxocarbenium ion.[21] Poor reactivity prevents *p*-tolyl thiosialoside from being used in reactivity-based, one-pot assembly.[19b] To bypass this problem, more-reactive leaving groups, or those that can be selectively activated

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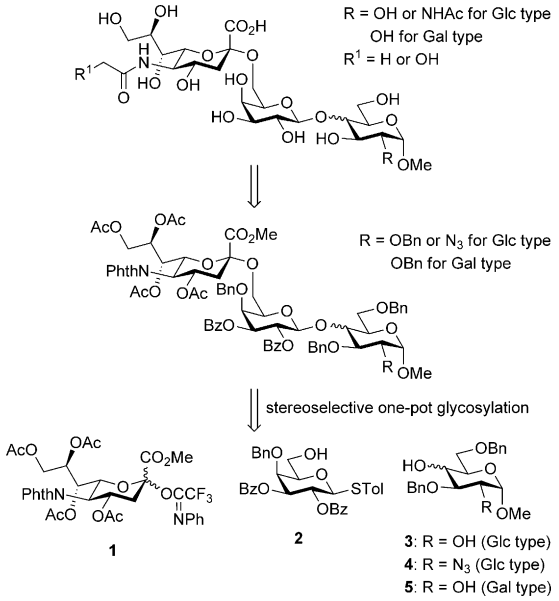
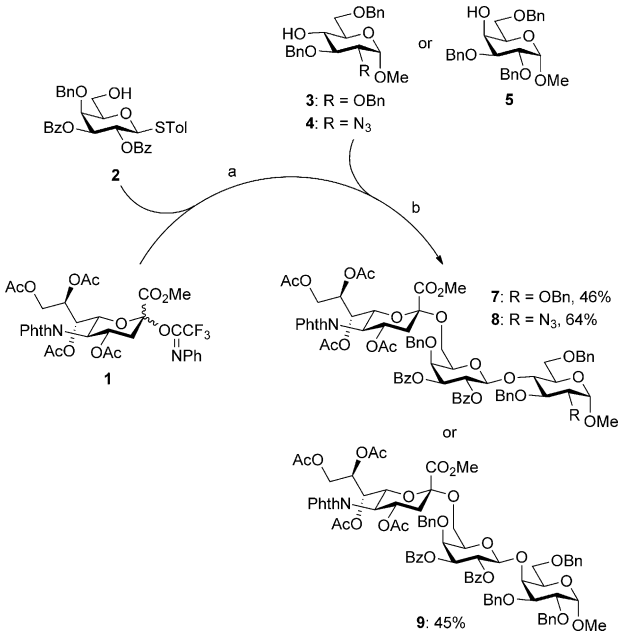


Scheme 1. Retrosynthetic approach to the sialic-acid-capped trisaccharides. Bz = benzoyl, Tol = 4-tolyl.

were employed.[18b,22] Here, we chose the sialyl donor **1**,[21] which carries 5-*N*-phthaloyl protection, to direct α stereoselectivity and the *N*-phenyltrifluoroacetimidate[23] leaving group to enable chemoselective activation using plain trimethylsilyl triflate (TMSOTf). The orthogonal phthaloyl protecting group also permits the installation of either acetyl or glycolyl units during the final transformation.

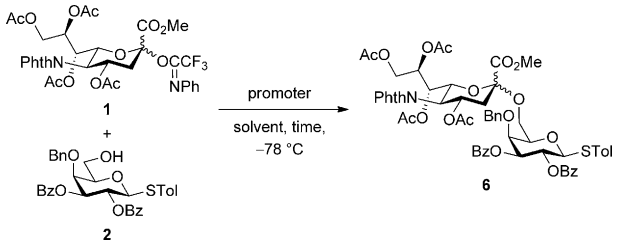
We first examined sialylation conditions best suited for the formation of the disaccharide **6** using the known thiogalactoside **2**[22b] as acceptor (Table 1). Good yield but poor stereoselectivity was observed when **1** was activated with TMSOTf in CH_2Cl_2 at −78 °C (entry 1). Here, the orientation of the newly formed glycosidic bond was confirmed by using the $^3J_{C1,H3ax}$ values for the sialic acid unit.[19a] Changing the solvent from CH_2Cl_2 to EtCN gave full α selectivity, but the yield was markedly reduced, even with $BF_3{\cdot}Et_2O$ as an activator (entries 2 and 3). Successive donor addition improved the yield, the best of which was attained when 1.0 equivalents of **1** was further supplemented with 0.8 equivalents after 30 minutes of reaction (entry 5). These reaction conditions resulted in complete α selectivity with 75% yield for the adduct **6**.

With suitable sialylation conditions in hand, we moved forward on the one-pot assembly (Scheme 2). Thus, 30 minutes after the second addition of **1** at −78 °C, either the acceptor **3**,[24] **4**,[25] or **5**[26] was added together with *N*-iodosuccinimide (NIS) and TMSOTf to activate the thio-



Scheme 2. One-pot assembly of the fully protected sialic-acid-capped trisaccharides. Reagents and conditions: a) 1. TMSOTf, EtCN, −78 °C, 30 min; 2. **1**, −78 °C, 30 min; b) NIS, TMSOTf, −78 °C to −40 °C, 2.5 h.

Table 1: Glycosylation with the sialyl donor **1**.

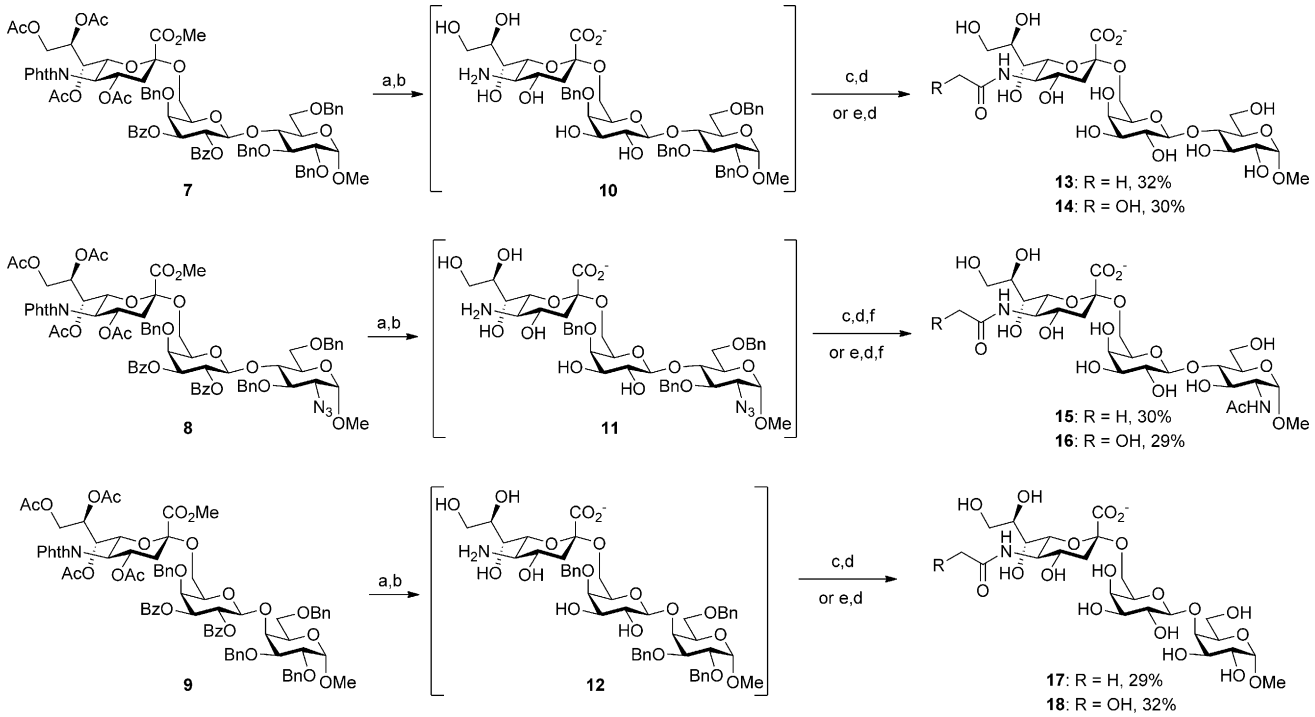


Entry	Donor (equiv)	*t* [h]	Promoter	Solvent	Yield [%][a]	α/β[b]
1	1.2	3.0	TMSOTf	CH_2Cl_2	70	1:1.8
2	1.2	0.5	TMSOTf	EtCN	21	α only
3	1.2	0.5	$BF_3{\cdot}Et_2O$	EtCN	18	α only
4	1.0 + 0.5[c]	0.5 + 0.5[c]	TMSOTf	EtCN	52	α only
5	1.0 + 0.8[c]	0.5 + 0.5[c]	TMSOTf	EtCN	75	α only

[a] Isolated yields.[b] Determined by ^{1}H NMR analysis. [c] Initial use of 1.0 equiv of donor, then additional amount added after 30 min, with quenching 30 min later.

toluene group of the intermediate disaccharide **6α**. Neighboring-group participation of the 2-*O*-benzoyl group in the galactosyl unit and the EtCN solvent effect ensured β glycosylation. This one-pot process delivered the target sialyl-capped trisaccharide backbones **7**, **8**, and **9** in 46, 64, and 45% yields, respectively.

Functional-group transformations toward the final products started with cleavage of the acetate, benzoate, and methyl esters by saponification (Scheme 3). Ethylenediamine treatment then removed the *N*-phthaloyl groups to form the intermediates **10**, **11**, and **12**. With the free amine available on the sialic acid unit, either the acetyl or the glycolyl functionality was introduced. Hydrogenation exposed the hydroxy groups after benzyl removal. It also reduced the azido group in the glucosamine-derived moiety to the amine, which was further selectively acetylated with Ac_2O in MeOH. Accordingly, the trisaccharides **13**–**18** with Neu5Ac and Neu5Gc caps and with Glc, GlcNAc, or Gal reducing end sugar units were acquired in four or five steps in good overall yields.



Scheme 3. Synthesis of Neu5Ac- and Neu5Gc-containing trisaccharides. Reagents and conditions: a) NaOMe, MeOH, RT, 10 min; H_2O, reflux, 18 h; b) $NH_2CH_2CH_2NH_2$, *t*BuOH, reflux, 24 h; c) Ac_2O, MeOH, RT, 5 h; d) H_2, Pd/C, MeOH, RT, 2 d; e) $BnOCH_2COCl$, Et_3N, MeOH, RT, 2 h; f) Ac_2O, MeOH, RT, 0.5 h.

The synthetic sialotrisaccharides were analyzed for their anti-influenza activity through the cytopathic effect (CPE). Mardin–Darby canine kidney (MDCK) cells were used against the common human influenza A/WSN/33(H1N1) virus as a model in this evaluation. MDCK cell surfaces contain α(2→6)-type sialoside linkages.[27] In the absence of multivalent interactions, binding of sialosides to hemagglutinin typically occur in the millimolar range.[28] Thus, MDCK cells were treated with the viral suspension preincubated with 1 mM of the sialotrisaccharides. Among the six compounds tested, only the Neu5Gc-capped trisaccharides **14** and **18** displayed inhibitory activities against the virus (Figure 1 b and c). The cells corresponding to these compounds appear normal and did not display agglutination, which is indicative of successful viral infection and were evident in the controls (Figure 1 a) as well as those cells corresponding to compounds **13**, **15**, **16**, and **17**. These results signify the enhanced affinity of hemagglutinin in the tested human influenza strain with Neu5Gc units compared to Neu5Ac. The Glc or Gal units occupying the reducing end also boosted the interaction. In the case of Neu5Ac-capped trisaccharides as well as compound **16**, the concentration applied is probably just too low to exhibit any inhibitory effect.

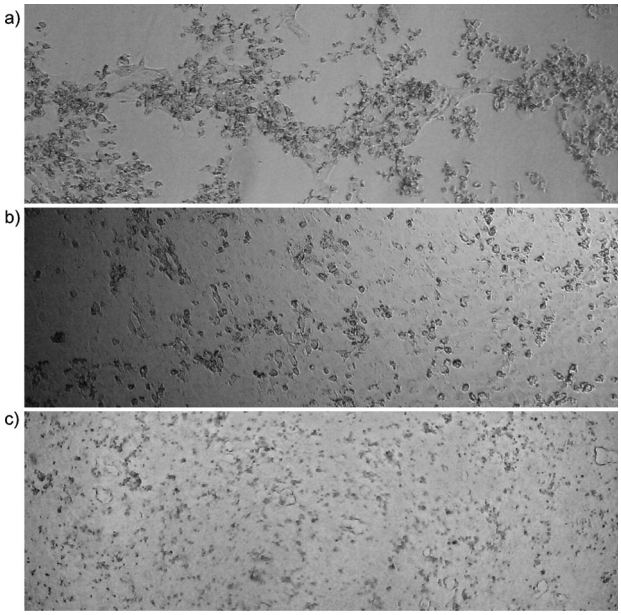


Figure 1. CPE inhibition assay of the synthesized sialotrisaccharides against influenza A/WSN/33 (H1N1) virus. a) Control (cells infected with the virus). b) Cells treated with the virus preincubated with 1 mM of **14**. c) Cells treated with the virus preincubated with 1 mM of **18**.

In summary, we have developed a one-pot and stereoselective glycosylation strategy for the construction of Neu5Ac- and Neu5Gc-capped trisaccharides. The monosaccharide building blocks and the protecting groups used in the reactions were strategically chosen not only to aid in the introduction of the target substituents but also to help control the stereoselectivity of the glycosylation. Neu5Gc-containing trisaccharides with Glc or Gal units at the reducing end position displayed inhibitory activities against a common human A(H1N1) virus. This evaluation shows preliminary evidence of the Neu5Gc sway on human influenza virus

binding. Future studies will focus on the observed enhanced binding ability in greater detail.

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