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Parallel chemoenzymatic synthesis of sialosides containing a C5-diversified sialic acid

Hongzhi Cao, Saddam Muthana, Yanhong Li, Jiansong Cheng, Xi Chen *

Department of Chemistry, University of California-Davis, One Shields Avenue, Davis, CA 95616, United States

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ABSTRACT

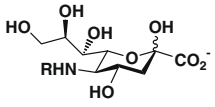
A convenient chemoenzymatic strategy for synthesizing sialosides containing a C5-diversified sialic acid was developed. The α2,3- and α2,6-linked sialosides containing a 5-azido neuraminic acid synthesized by a highly efficient one-pot three-enzyme approach were converted to C5″-amino sialosides, which were used as common intermediates for chemical parallel synthesis to quickly generate a series of sialosides containing various sialic acid forms.

Sialic acids are a family of over 50 naturally occurring acidic monosaccharides with a 9-carbon backbone. They are the common terminal carbohydrate units in glycoproteins and glycolipids on the surface of all types of vertebrate cells.[1,2] Among these, *N*-acetylneuraminic acid (Neu5Ac) **1** and *N*-glycolylneuraminic acid (Neu5Gc) **2** are the two most abundant forms. Other C5-amino derivatives, such as neuraminic acid (Neu) **3**, *N*-acetoxyacetylneuraminic acid (Neu5GcAc) **4**, and *N*-methoxyacetylneuraminic acid (Neu5GcMe) **5** have also been found in nature (Fig. 1).[1,2]

For sialic acid-containing oligosaccharides and glycoconjugates in nature, most sialic acids are either α2,3 or α2,6 linked to galactosides or α2,6 linked to 2-acetamido-2-deoxy-galactosides. In the past decades, accumulating evidence has shown that sialosides mediate or modulate a variety of normal and pathological processes such as cell–cell adhesion, cell recognition, bacterial infection, viral invasion, inflammation, and cancer metastasis.[3–5] The biological significance of sialic acid-containing molecules makes them excellent targets for carbohydrate-based drug discovery and key components of carbohydrate-based vaccines.[6,7]

Despite the advance in chemical glycosylation method development and the recognition of the biological importance and therapeutical potential of sialosides and their derivatives, chemical sialylation remains one of the most challenging glycosylation reactions.[8–12] Recently, it has been shown that Neu5Gc, a non-human natural sialic acid, can be uptaken from food and metabolically incorporated by human cells onto the cell surface. Certain types of human cancer cells can overexpress this exogenous Neu5Gc and induce chronic inflammation.[5,13,14] Sialosides with a non-natural *N*-azidoacetyl group at C5 of neuraminic acid (*N*-azidoacetylneuraminic acid or Neu5NAz) have been shown to be invaluable probes for labeling and studying glycans in living cells, organisms, and animals.[15–17] Sialosides with C5-modified sialic acids have also been used to enhance immune response in carbohydrate-based vaccine studies.[17,18] The scope of the non-natural sialic acid structures that can be metabolically engineered onto cell surface is limited by the restricted substrate specificity of sialoside biosynthetic enzymes.[19,20]

Current chemical synthetic strategies of sialosides focus extensively on synthesizing Neu5Ac-containing structures by using different N-protecting groups (e.g., NAc_2, NHTFA, NHTroc, and NPhth, etc.) at the C5 of Neu5Ac donors to afford improved reactivity and stereoselectivity.[11,21] In principle, after chemical sialylation, the N-protecting groups can be removed and other functional groups can be introduced by coupling the resulted free amino group with different acyl groups to provide naturally and non-naturally existing sialic acid forms. However, these methods



1, R = Acetyl, Neu5Ac;
2, R = Glycolyl, Neu5Gc;
3, R = H, Neu;
4, R = Acetoxyacetyl, Neu5GcAc;
5, R = Methoxyacetyl, Neu5GcMe.

Figure 1. Several naturally occurring sialic acids containing different C-5 groups.

* Corresponding author. Tel.: +1 530 754 6037; fax: +1 530 752 8995.
E-mail address: chen@chem.ucdavis.edu (X. Chen).

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worked well only for Neu5Ac-containing sialosides. Sialosides with other sialic acid forms, such as Neu5Gc, cannot be easily obtained using this approach. This is because that the acyl group used to protect hydroxyl groups can easily migrate to the deprotected amino group at C5 under the common basic or even acidic deprotection conditions.[22] Several research groups have recently developed a number of chemical approaches for the synthesis of Neu5Gc-containing sialosides.[23,24] The *N*-glycolyl-5*N*,4*O*-oxazolidinone protected thiosialoside donor developed by Crich and Wu was successfully employed to an iterative one-pot synthesis of Neu5Gc-containing oligosaccharides in good yields and stereoselectivity.[24] In this approach, the oxazolidinone could not be selectively deprotected in the presence of *N*-glycolyl and the *N*-glycolyl group has to be removed by saponification and subsequently reinstalled (although the *N*-glycolyl group does benefit the stereoselectivity of the glycosylation). Most recently, phosphite sialic acid donors with protected *N*-glycolyl (GcAc and GcBn) developed by Sato and co-workers were used for the direct chemical synthesis of Neu5Gc-containing oligosaccharides.[23] However, the glycosylation only led to moderate yields and stereoselectivities in most cases. The synthesis of sialosides has become the bottleneck process for carrying out their functional studies using the newly developed glycan microarray-based high-throughput format.[25–27]

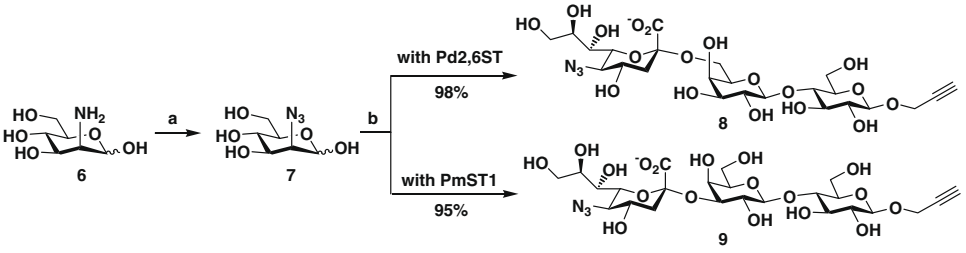
In contrast to chemical sialylation which involves tedious protecting/deprotecting manipulations and purification of desired product from its isomers and elimination byproduct mixtures, enzymatic synthesis offers alternative, high efficient, superior regio-, and stereoselective approaches to access various sialosides.

Chemoenzymatic synthesis of sialosides combines the flexibility of the chemical synthesis and the high efficiency of the enzymatic synthesis. It has been a robust method to prepare complex sialosides.[28,29] For example, enzymatically synthesized sialyloligosaccharide donor building blocks have been used in the chemical synthesis of more complex sialosides and sialylglycoconjugates including sialyl Lewis^x (sLe^x) analogs and GM3 ganglioside.[30–33] We have reported a highly efficient one-pot three-enzyme chemoenzymatic system containing a sialic acid aldolase, a CMP-sialic acid synthetase, and a sialyltransferase for the effective synthesis of sialosides with different sialic acid forms, various sialyl linkages, and diverse underlying glycans.[34–36] In this system, *N*-acetylmannosamine (ManNAc) or mannose derivatives are chemically synthesized and used as sialic acid precursors. These compounds can be converted by a sialic acid aldolase to form different naturally occurring and non-natural sialic acids, which are activated by a CMP-sialic acid synthetase and transferred to suitable acceptors by a sialyltransferase for the formation of diverse sialosides. Based on the activity of the sialyltransferase, either α2,6- or α2,3-linked sialosides can be formed when *Photobacterium damsela* α2,6-sialyltransferase (Pd2,6ST) or *Pasteurella multocida* sialyltransferase 1 (PmST1) is used. A preferred CMP-sialic acid synthetase is *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS) which has flexible substrate specificity.[37] The success of this one-pot three-enzyme approach relies on the substrate promiscuity of all biosynthetic enzymes used. Any substrate modifications that can not be tolerated by any of the enzymes will lead to unsuccessful synthesis. In addition, the pH of the enzymatic reaction mixture needs to be controlled at or lower than 7.5 for synthesizing sialosides containing an *O*-acetyl group to prevent de-O-acetylation. As NmCSS has an optimal pH of 8.5–9.0 and its activity decreases dramatically with the increase or decrease of the pH of the reaction mixture, a large amount of NmCSS and a longer incubation time are usually used for the synthesis of sialosides containing an *O*-acetyl group at pH 7.5. These reaction conditions often lead to lower yields. To overcome these drawbacks, we report herein the improved parallel chemoenzymatic synthesis of sialosides containing C5-derivatized sialic acids. This approach combines the highly efficient one-pot three-enzyme synthesis of common sialoside intermediates (Scheme 1) with additional chemical diversification (Scheme 2) for a quick access of various sialosides containing naturally occurring C5-derivatized sialic acids in preparative scales. This approach can also be easily extended for large-scale synthesis and for the synthesis of sialosides containing non-natural C5-derivatized sialic acids.

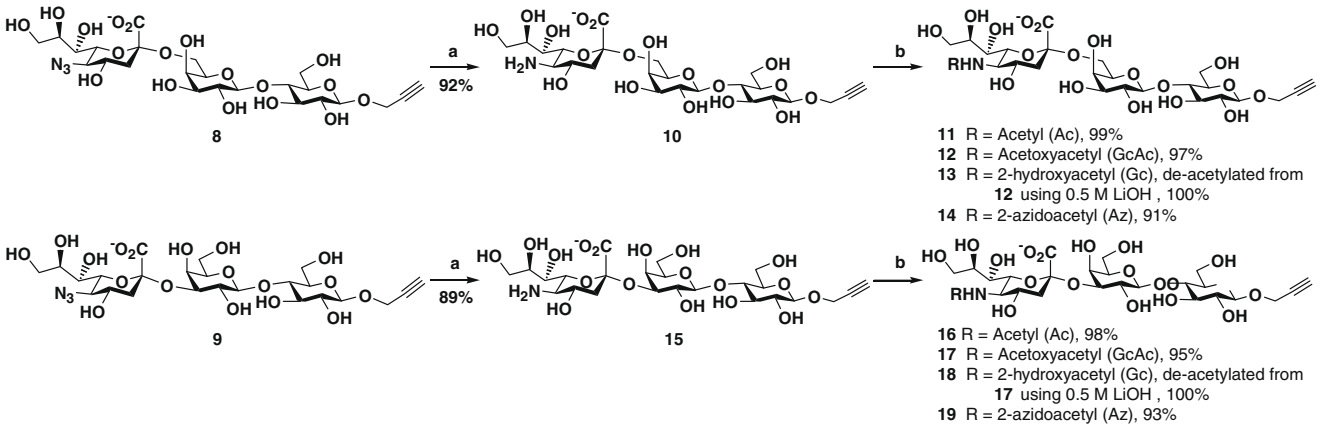
The key of this approach is the enzymatic synthesis of sialosides containing α2,3- and α2,6-linked 5-azidoneuraminic acid (Neu5N$_3$), and their conversion to C5-amino-sialosides as the common intermediates for further chemical diversification.

The common sialoside intermediates with either an α2,6- or α2,3-linked Neu5N$_3$ (**8** or **9**) were conveniently prepared in 200–300 mg amount (see Supplementary data for details) in excellent yields using the one-pot three-enzyme synthetic strategy (Scheme 1)[34–36] from 2-azido-2-deoxy-mannose[38] **7** (ManN$_3$) as a sialic acid precursor and propargyl β-lactoside[39] as a sialyltransferase acceptor. Due to the absence of the *O*-acetyl group in the substrates and the products, the enzymatic reactions were carried out at pH 8.5, the optimal pH for the one-pot three-enzyme sialylation reactions. As shown in Scheme 1, ManN$_3$ was an excellent substrate for the one-pot three-enzyme synthesis of sialosides under the reaction conditions used.

The azido group at the C5 of the sialic acid on sialosides **8** and **9** was reduced to an amino group in the presence of 1,3-dithiopropanol[40,41] and Et$_3$N in a pyridine/water solvent mixture to produce sialosides **10** and **15** in good yields (Scheme 2). With the C5″-amino sialosides **10** and **15** in hand, we next explored the parallel chemical synthesis of various sialosides containing diverse naturally occurring sialic acid forms by introducing different acyl groups to the C5″-amino group. As shown in Scheme 2, coupling **10** and **15** with acetyl chloride in NaHCO$_3$ aqueous solution produced Neu5Ac-containing sialosides **11** and **16**, respectively, in excellent yields. The advantage of the method is highlighted in the synthesis of Neu5GcAc-containing sialosides **12** and **17** using a similar method in exellent yields (97% and 95%, respectively)



Scheme 1. Synthesis of common sialoside intermediates using a one-pot three-enzyme approach. Reagents and conditions: (a) TfN_3, $CuSO_4$, K_2CO_3, CH_2Cl_2, MeOH, 87%; (b) Tris–HCl buffer (100 mM, pH 8.5), $MgCl_2$ (20 mM), CTP, sodium pyruvate, propargyl β-lactoside, *E. coli* sialic acid aldolase, *Neisseria meninigitidis* CMP-sialic acid synthetase (NmCSS). Pd2,6ST, *Photobacterium damsela* α2,6-sialyltransferase; PmST1, *Pasteurella multocida* sialyltransferase 1.



Scheme 2. Parallel chemical diversification at C5 of the sialic acid residue in α2,3- and α2,6-sialosides. Reagents and conditions: (a) 1,3-Dithiopropanol, Et_3N, pyridine, H_2O. (b) For sialosides **11** and **16**, $NaHCO_3$, H_2O, AcCl; for sialosides **12** and **17**, $NaHCO_3$, H_2O, acetoxyacetyl chloride; for sialosides **14** and **19**, NHS-activated 2-azidoacetic ester, DMF, Et_3N.

by treating **10** and **15**, respectively, with acetoxyacetyl chloride in $NaHCO_3$ aqueous solution. Under the reaction conditions, de-O-acetylation was not observed. This was presumably due to a relatively short reaction time (3 h) and the neutralization of the weak basic condition by the HCl byproduct formed. It is worth to mention that it is very challenging to achieve high yields for the direct one-pot three-enzyme synthesis of sialosides **12** and **17** containing a labile *O*-acetyl group on the *N*-glycolyl starting from the corresponding Neu5GcAc precursor. With sialosides **12** and **17** in hand, Neu5Gc-containing sialosides **13** and **18** were readily obtained in quantitative yields by removing the *O*-acetyl group using 0.5 M LiOH aqueous solution. In addition, α2,6- and α2,3-linked sialosides **14** and **19** containing *N*-azidoacetylneuraminic acid (Neu5NAz) were prepared in excellent yields by coupling *N*-hydroxysuccinamide (NHS)-actived 2-azidoacetyl ester[36] with C5″-amino sialosides **10** and **15**, respectively. These Neu5NAz-containing sialosides can be further derivatized to generate a large library of sialosides.

In conclusion, ten α2,6- and α2,3-sialylated trisaccharides (**10**–**19**) containing various naturally occurring C5-diversified sialic acid forms were synthesized in preparative scale from two common azido-containing sialoside intermediates **8** and **9** by a combination of one-pot three-enzyme synthesis and parallel chemical derivatization. The combination of the efficiency of enzyme-catalyzed synthesis of common sialoside intermediates and the flexibility of chemical diversification provides a new approach to quickly access a number of structurally diverse sialosides.

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Supplementary data

Supplementary (experimental procedures and spectroscopic data for sialosides **8**–**19**) data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.078.

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