Enzyme-Catalyzed Synthesis of Oligosaccharides That Contain Functionalized Sialic Acids

Mark D. Chappell and Randall L. Halcomb*

Department of Chemistry and Biochemistry
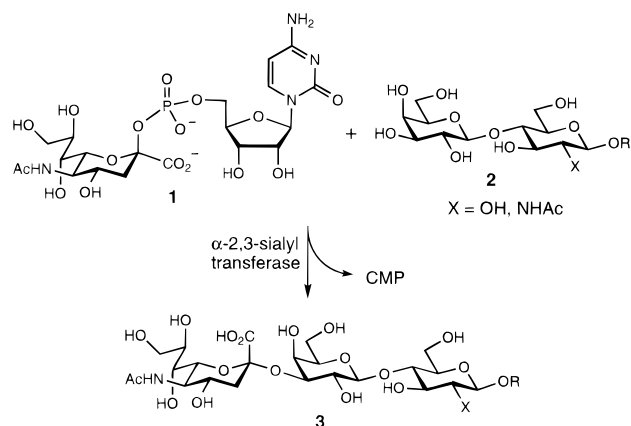*University of Colorado*
Boulder, Colorado 80309-0215

Scheme 1

The explosion of discoveries during the past decade that many cellular processes are mediated by cell-surface carbohydrates and glycoconjugates[1] has renewed the interest of synthetic chemists in this field. Since oligosaccharides and their derivatives are extremely difficult to isolate in homogeneous form from natural sources, the pressure has shifted to organic chemists to develop new and more efficient methods to access this class of compounds. Additionally, application of the structural and functional information that is rapidly being generated to the development of carbohydrate receptor antagonists will require the design and synthesis of novel molecular structures which contain derivatized monosaccharide residues to probe carbohydrate−protein interactions.

The "classical" methods for glycosylation have been extensively reviewed,[2] and many useful and powerful glycosylation methods have been developed in the past decade.[3] An extremely powerful class of methods for oligosaccharide synthesis is based on the use of enzymes to accomplish glycosylations.[4] Enzymatic glycosylations are advantageous over chemical methods in that they can be performed in water, and the need for tedious protection schemes to achieve regioselectivity is usually precluded. Especially powerful enzymes in this regard are glycosyltransferases, which are regiospecific and stereospecific in the reactions they catalyze and which can mediate reactions using complex biomolecules as substrates. A current disadvantage of glycosyltransferase-based technology is the limited ability to incorporate modified sugar residues into oligosaccharides. A major reason that contributes to this drawback is the lack of availability of the requisite sugar−nucleotide glycosyl donors, but some elegant solutions to this problem have recently appeared.[5]

Sialic acids are monosaccharides that are present at the termini of many oligosaccharide chains and are among the most important residues for interactions with receptors.[1b] Therefore, the sialic acid unit is one whose derivatization would be quite desirable in order to probe the importance of the different functional groups of this residue in interactions with various proteins. The method of choice for glycosylation with sialic acid is through catalysis by sialyltransferases.[6] All of the chemical methods for glycosylation with sialic acid are plagued with side reactions, low yields, and poor stereoselectivities.[7] Sialyltransferases have been shown to efficiently transfer the parent sialic acid, *N*-acetylneuraminic acid, onto a variety of lactose- and lactosamine-derived acceptors (Scheme 1).[6] In order to use this technology to incorporate modified sialic acids into complex oligosaccharides, ready access to a number of congeners of cytidine monophospho-*N*-acetylneuraminic acid (CMP-NeuAc, **1**), the sialic acid donor for the enzyme-catalyzed sialylation, is needed. The enzymatic process for producing CMP-NeuAc is not generally amenable to the synthesis of derivatives with modified sialic acids.[8] Furthermore, the enzymes used for this process, particularly CMP-NeuAc synthetase, are not readily available. To overcome these obstacles, a synthetic route to CMP-NeuAc was developed in this lab[9] that promises to be general for the synthesis of virtually any derivative thereof.[9,10] Communicated here is the use of these CMP-NeuAc congeners for the enzymatic synthesis of oligosaccharides which contain derivatized sialic acids.[11]

The CMP-sialic acid donors **1** and **9**−**11** (Scheme 3) were synthesized by a procedure developed in this lab,[9] as illustrated by the synthesis of **1** in Scheme 2. The sialic acid derivative **5** was coupled to the phosphoramidite **6** to give the phosphite **7** (62%). Oxidation to the phosphate and removal of the allyl protecting group afforded compound **8** in 61% yield for two steps. The choice of the allyl protecting group, which could be removed under mild conditions, was found to be essential to the success of the route. Finally, deacetylation followed by hydrolysis of the methyl ester gave CMP-NeuAc **1** (81% for two steps). The derivatives **9**−**11** were also prepared in good

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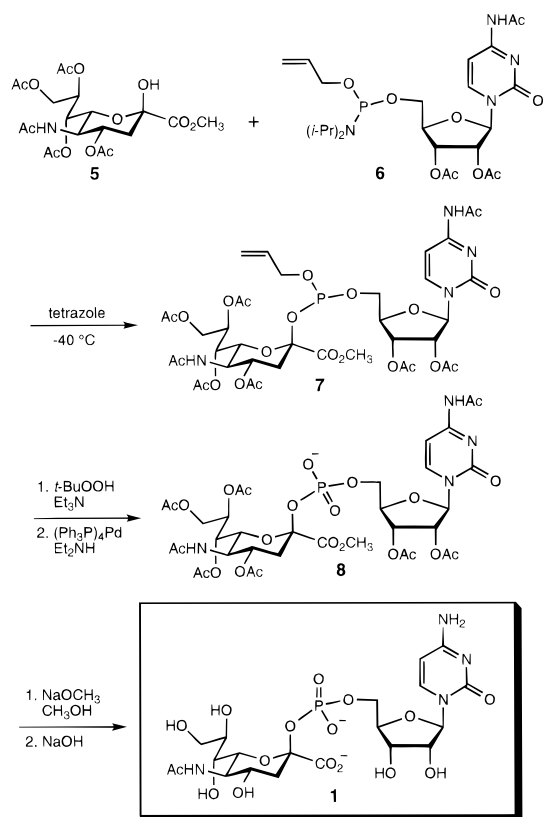
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Scheme 2

Scheme 3

that the nitrogen protecting group can be removed under conditions that are innocuous to the glycosidic bonds. Hydrogenation of **16** would afford a trisaccharide containing a free amine in the sialic acid. Gangliosides containing the latter have been shown to interact with growth factors in certain cell types.[16] Furthermore, this amine would open the possibility of selectively derivatizing C-5 after the glycosylation event with groups that are not compatible with the conditions used in the preparation of other CMP-sialic acids.

In summary, an investigation of the donor substrate specificity of α-2,3-sialyltransferase was conducted, and the utility of the enzyme for the synthesis of oligosaccharides that contain derivatized sialic acids was demonstrated. This method holds promise for synthesizing a variety of sialylated oligosaccharides designed to probe sialic acid recognition in biological systems. Since the sialyltransferase-catalyzed reaction can be performed *in vivo*, this technology along with that developed by others[5,11] has the potential to harness the power of organic synthesis to modify the antigenic properties of intact cells. A detailed comparison of the kinetics of glycosylations using these and other CMP-sialic acid conjugates will be reported in a full account of this work. Additionally, the incorporation of sialyltransferases into strategies for the construction of combinatorial libraries of oligosaccharides has not escaped our attention, and progress in this arena will be reported in due course.

overall yield according to this protocol.[9] Each of these compounds was found to be a substrate for α-2,3-sialyltransferase. In a typical glycosylation procedure, treatment of each CMP-sialic acid donor (ca. 5−10 mg) with the allyl β-lactoside **12** (1.5 equiv), α-2,3-sialyltransferase (25−50 mU), and alkaline phosphatase (1 U) at pH 7.5 (HEPES buffer, 200 mM) for 3−5 days gave the corresponding sialyl lactose in good yield after purification by size exclusion chromatography on BioGel P2.[12]

Following this general protocol, the sialyl lactose derivatives **14−16** were synthesized from compounds **9−11**, respectively, in good overall yields of isolated products (Scheme 3). The parent system **13**[13] was also prepared from CMP-NeuAc **1** for reference and comparison purposes. A variety of modifications at C-5 of the sialic acid component were tolerated by the sialyltransferase. The synthesis of the trisaccharide **14** from **9**[14] demonstrated that an amide at C-5 is not a necessary feature for the substrate. Additionally, the trisaccharide **15** containing a hydroxyacetamide within the sialic acid was also constructed from compound **10**.[15] The hydroxyacetamide is a feature found within numerous gangliosides.[15] Finally, the sialic acid component of **11**, whose nitrogen functionality at C-5 was engaged in a benzyloxy carbamate, was transferred by the sialyltransferase to afford trisaccharide **16**. The latter is interesting in
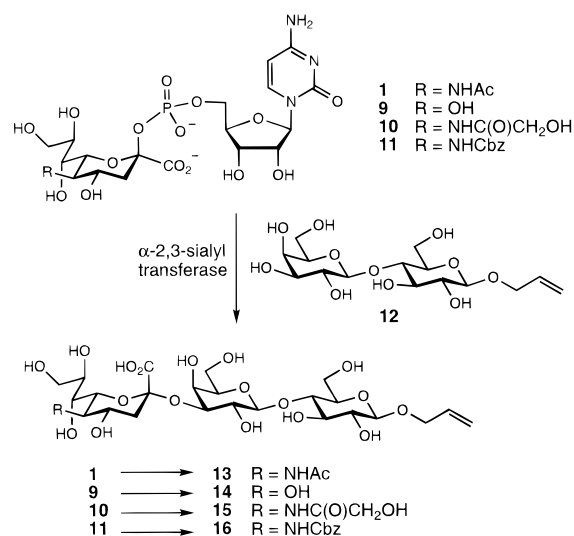
(12) The structure of each product was secured by 1D and 2D ^{1}H and ^{13}C NMR spectroscopies and by high-resolution mass spectrometry. The isolated yields of the trisaccharides were the following: **9**, 71%; **10**, 88%; **11**, 31%; **12**, 18%. The lower yields of **11** and **12** are attributed to a loss of activity of the transferase over time, since these experiments were performed somewhat later than the those which resulted in the syntheses of **9** and **10**. Quantitation of the relative efficiency with which this enzyme transfers the sialic acids from **1** and **5**−**7** will require a thorough kinetics analysis, which will be the subject of future work in this area.

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