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Chemo-enzymatic synthesis of imidazolium-tagged sialyllactosamine probes

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ABSTRACT

Two novel α-linked sialyltrisaccharide imidazolium-type probes (ITags) based on the structures of biologically relevant 6′-sialyllactosamine and 3′-sialyllactosamine were efficiently and stereoselectively prepared using a chemo-enzymatic approach. The apparent kinetic parameters for the enzyme catalyzed transformations with α-2,3-sialyltransferase (α-2,3-ST) and α-2,6-sialyltransferase (α-2,6-ST) were measured by LC–MS using the ionic probes. This strategy demonstrates the suitability of the ITags to probe glycosyltransferase activity and their versatility in the preparation of sialylated epitopes for glycobiology research.

Sialic acids are a diverse family of sugar units with a nine-carbon backbone that are typically found as terminal residues on oligosaccharide chains of various cell-surface glycoproteins and glycolipids and on secreted proteins in vertebrates.[1,2] These unique molecules mediate a wide range of biological processes, including intercellular adhesion, signaling and microbial attachment.[3] For instance, the α-linked sialyltrisaccharides 3′-sialyllactosamine (Neu5Ac-α2,3-Gal-β1,4-GlcNAc-β-OR, **1**) and 6′-sialyllactosamine (Neu5Ac-α2,6-Galβ1,4-GlcNAc-β-OR, **2**) are ligands for influenza viruses.[3–5]

In order to gain a better understanding of the role and function of these important glycan structures in normal physiology and disease and to evaluate their therapeutic potential, efficient and practical synthetic methods to access structurally defined sialylated glycan probes are needed.[6] However, despite the many advances in the area, the chemical synthesis of sialic acid containing oligosaccharides still represents a significant challenge. Most approaches rely on lengthy and time consuming chemical steps to achieve good yields and stereocontrol, mostly due to the hindered and charged tertiary anomeric center and the lack of a participating group to aid in stereochemical control.[7–11] As an alternative, the use of glycosyltransferases to construct oligosaccharides offers several advantages over chemical approaches, when the corresponding enzymes and required sugar nucleotides are available, since these enzymes are able to form glycosidic bonds with exquisite regio- and stereocontrol by catalyzing the transfer of an activated sugar donor to growing oligosaccharide chains without the need for protecting groups.[12]

Imidazolium based ionic liquids (ILs) have emerged as a popular class of reagents in organic chemistry as new vehicles for the immobilization of reagents due to their unique physical and chemical properties.[13,14] In addition, ILs are also ideal as mass spectrometry (MS) probes for fast reaction analysis because of their greater spectral peak intensities and lower limits of detection.[15] Our group recently reported the synthesis of inexpensive and versatile imidazolium-based probes for fast and sensitive reaction monitoring by MS as an alternative to expensive radioactive or fluorescent labels.[14,16–18] In our work, we demonstrated that the ionic tags (ITags) are compatible with several glycosyltransferases and the ionic probes were employed in the qualitative and quantitative biological characterization of bovine milk β-1,4-galactosyltransferase (β-1,4-GalT)[16] and β-1,3-fucosyltransferase (α-1,3-FucT VI).[18]

Encouraged by the simplicity and practicality of our approach, we decided to expand the use of the new probes to another important class of targets. Herein we report the efficient chemo-enzymatic synthesis of α-2,3 and α-2,6-sialyllactosamine ionic-probes. Both trisaccharides were prepared in mg scale from a common intermediate, ITag-LacNAc (Gal-β1,4-GlcNAc-ITag, **4**). Furthermore, the apparent kinetic parameters for the enzymatic sialylations were measured using the ionic probes and are also reported.

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In nature, sialylglycosides are synthesized by sialyltransferases, which are a group of glycosyltransferases that, independent of their source and acceptor specificity, transfer sialic acid from cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) to acceptor oligosaccharides found in glycoproteins, glycolipids or polysaccharides.[19] It has been estimated that there are more than 20 different mammalian sialyltransferases.[20] Each of these enzymes is classified on the basis of the position of attachment to the acceptor being either α-2,3-, α-2,6- or α-2,8-linked and their specificity for its acceptor substrate.[21,22] For instance, two different sialyltransferases, α-2,3-sialyltransferase (α-2,3-ST) and α-2,6-sialyltransferase (α-2,6-ST) are required to introduce the *N*-acetylneuraminic acid (Neu5Ac) groups at C6′ and C3′ of the LacNAc disaccharide core, respectively (Fig. 1).

In our previous work, we developed a *N*-benzenesulfonyl-type ITag for covalent attachment to glycan substrates.[18] The labels are stable to chemical and enzymatic media, readily accessed from commercial materials and contain both a MS probe and a UV moiety for reaction monitoring. Thus, we envisaged that these types of ionic probes would be suitable for the preparation of the sialylated compounds. To that end, core disaccharide ITag-LacNAc **4** was enzymatically prepared in a 95% yield from the reaction of ITag-GlcNAc **3**[18] with an excess of uridine 5′-diphosphogalactose (UDP-Gal) in the presence of bovine milk β-1,4-GalT as the catalyst (see Scheme 1).

With ITag-LacNAc **4** in hand, we first had to ensure that both sialyltransferases were able to tolerate the presence of the sulfonamide-containing ITag. To our delight, incubation of **4** with α-2,3-ST (50 mU) and CMP-Neu5Ac in 3-morpholinopropane-1-sulfonic acid (MOPS) buffer (50 mM, pH 7.9) at 37 °C afforded the desired trisaccharide product **5** in 96% yield after 7 h (Scheme 2).[23] The structure of **5** was confirmed by LC–MS (M^+ = 966) and the characteristic signals in the ^{1}H-NMR spectrum ($\delta H_{3a''}$ = 2.78 ppm, $J_{3a'',3b''}$ = 12.5, $J_{3a'',4''}$ = 4.7 Hz, $\delta H_{3b''}$ = 1.82 ppm, $J_{3b'',3a''} = J_{3b'',4''}$ = 12.1 Hz) (Fig. 2B). Similarly, α-2,6-ST was also able to catalyze the conversion of **4** into 6′-sialyllactosamine analogue **6** under the same reaction conditions, although the reaction appeared to be slower, and after 14 h, **6** was produced in 65% yield. LC–MS (M^+ = 966) and NMR were used to confirm the structure of

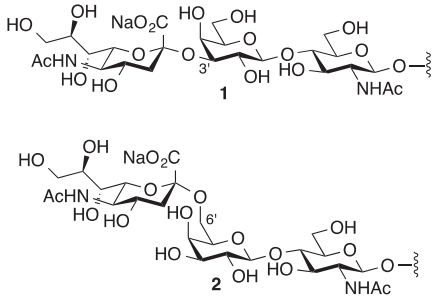
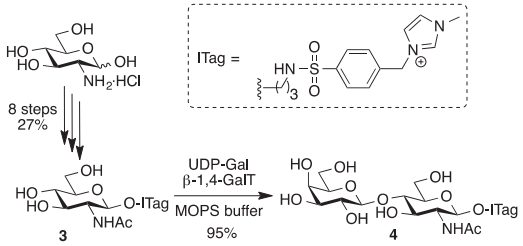
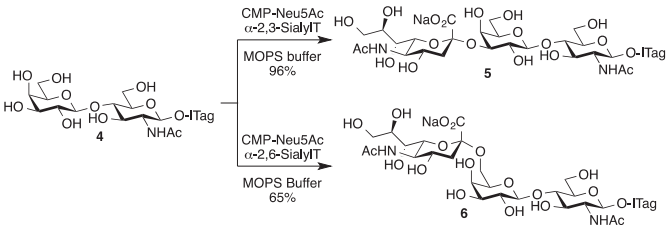


Figure 1. Naturally occurring sialylLacNAc derivatives **1** and **2**.



Scheme 1. Chemo-enzymatic synthesis of core ITag-LacNAc **4**.



Scheme 2. Enzymatic synthesis of ITagged α-2,3/6 sialylated LacNAc **5** and **6**, respectively.

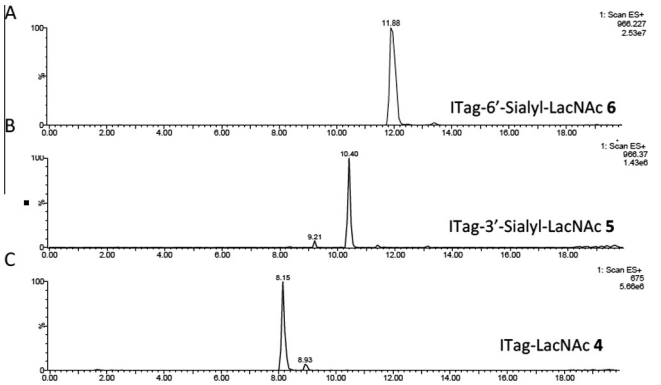


Figure 2. LC–MS traces of ITag-probes. (A) TIC trace for **6** [M^+] 966; (B) TIC trace for **5** [M^+] 966; (C) TIC trace for **4** [M^+] 675.

the trisaccharide ($\delta H_{3a''}$ = 2.69 ppm, $J_{3a'',3b''}$ = 12.4, $J_{3a'',4''}$ = 4.6 Hz, $\delta H_{3b''}$ = 1.72 ppm, $J_{3b'',3a''} = J_{3b'',4''}$ = 12.4 Hz) (Fig. 2A).[24]

Encouraged by our results, we then decided to obtain the apparent kinetic parameters for ITag probe **4** with the two different sialyltransferases using previously reported assays[18,19] and the ITag as the reporting handle. Thus, LC–MS was used to monitor and quantify reaction progress. Assays were performed in duplicate using the appropriate amount of each enzyme. The concentration of the oligosaccharide acceptor was varied around the K_M value, whereas the concentration of CMP-Neu5Ac was kept constant at 0.5 mM and CMP-Neu5Ac consumption was limited to 10–15% to ensure initial rate conditions.[25]

The apparent K_M value for ITag-derivative **4** with α-2,3-ST was determined to be 0.5 ± 0.1 mM with a catalytic efficiency (k_{cat}/K_M) of 21.8 min^{-1} mM^{-1} (Table 1 and Fig. 3A). On the other hand, the apparent K_M value for **4** with α-2,6-ST was measured to be 2.6 ± 0.1 mM with a catalytic efficiency (k_{cat}/K_M) of 2.8 min^{-1} mM^{-1} (Table 1 and Fig. 3B). Although both enzymes are able to turnover ITag-LacNAc **4** efficiently, the catalytic efficiency for the α-2,6-ST was around 8 times lower than that observed for the same substrate with the α-2,3-ST. This is not completely unexpected as different enzymes can react differently to the same chemical modification[29] and perhaps the presence of an imidazolium moiety in close proximity to the anomeric position of the substrate is detrimental for catalytic activity. Indeed, relative rates of transfer for both enzymes with ITag-LacNAc **4** and natural

Table 1
Apparent kinetic parameters for α-2,3- and α-2,6-ST with ITag-LacNAc **4** as substrate

Enzyme	K_M [mM]	V_{max} [μM min^{-1}]	k_{cat} [min^{-1}]	k_{cat}/K_M [min^{-1} mM^{-1}]
α-2,3-ST	0.5 ± 0.1	11.8 ± 0.9	10.9	21.8
α-2,6-ST	2.6 ± 0.1	2.0 ± 0.1	7.2	2.8