Thiosugar Synthesis

Glycosyltransferase-Catalyzed Synthesis of Thiooligosaccharides**

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S-Linked oligomers are metabolically stable mimics of their naturally occurring counterparts, since the rate of hydrolysis of the thioglycosidic bond by glycosylhydrolases is several orders of magnitude slower than that of the corresponding O-glycosides.[1] In addition, the conformational space sampled by thioglycosides is similar to that of O-glycosides. These properties suggest that S-linked carbohydrates may be attractive antigens with extended in vivo activity,[2] and the ability of glycosyltransferases to create such linkages has significant potential.

Considerable attention has been focused on the synthesis of complex oligosaccharides containing S-linked residues, and a variety of chemical approaches are available to construct this linkage.[1,3] Most thiooligosaccharide syntheses take advantage of the reduced basicity and enhanced nucleophilicity of sulfur compared to oxygen, employing a sugar thiol or thiolate anion in a reaction with another carbohydrate bearing an electrophilic leaving group.[4] Other commonly employed methods include glycosylation of thiosugar acceptors with activated glycosyl donors such as trichloroacetimidates,[5] and Michael addition of anomeric thiolates to α,β-unsaturated systems.[6] Recently active-site mutants of glycosylhydrolases, termed thioglycoligases, have been shown to transfer a glycosyl residue to appropriate sugar thiols.[7]

The chemical synthesis of oligosaccharides and their analogues is a laborious pursuit. Glycosyltransferases, which catalyze the formation of glycosidic bonds in nature, are now indispensable tools in synthetic carbohydrate chemistry, both for the production of natural and nonnatural oligosaccharides.[8,9] Since glycosyltransferases establish glycosyl linkages without the need for protecting groups, their use constitutes an obvious advantage over traditional chemical synthesis of

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oligosaccharides. Increasingly, a growing interest in glycobiology and advances in biotechnology and molecular biology are making glycosyltransferases available to the synthetic chemist in multiunit quantities. The ready availability of these enzymes not only facilitates carbohydrate synthesis but also contributes to an increased understanding of these biocatalysts.

Herein we report a new enzymatic method for the synthesis of thiooligosaccharides. In summary, our approach employs glycosyltransferases to catalyze the transfer of a monosaccharide residue from an activated sugar nucleotide donor to a thiol-bearing carbohydrate acceptor. We have examined the ability of two enzymes, a recombinant bovine α1,3-galactosyltransferase (α1,3GalT) and a β1,3-*N*-acetylglucosaminyltransferase (β1,3GlcNAcT) from *Neisseria menigitidis*, to catalyze glycosyl transfer to a 3′-thiolactose acceptor.[10,11] The formation of thioglycoside products is facilitated by addition of a reducing agent such as dithiothreitol (DTT) to the reaction mixture, which serves to maintain a pool of the reactive thiol acceptor for consumption in the reaction.

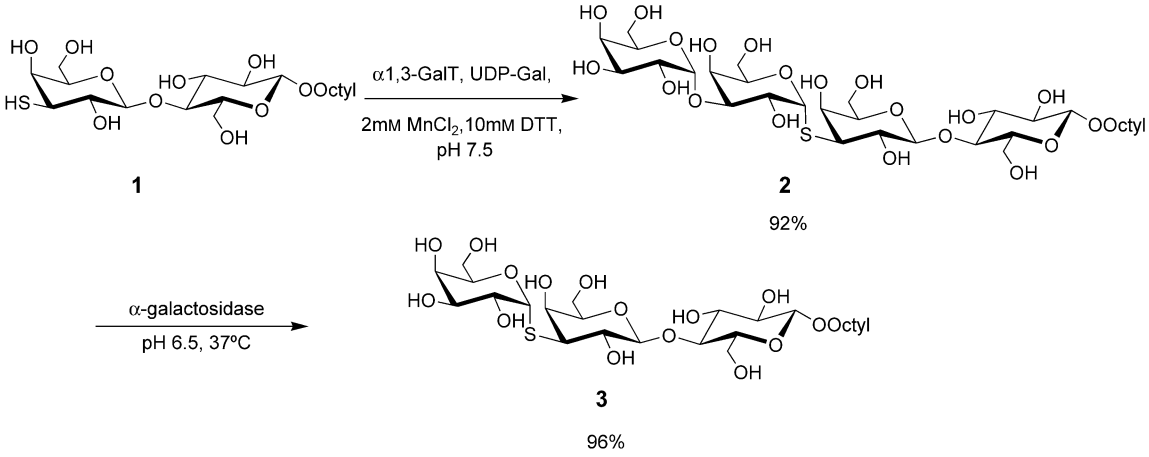
The development of an enzymatic approach to the synthesis of thiooligosaccharides does not obviate the considerable effort required for the construction of thiosugar acceptors. However, this method demonstrates the high yields and stereo- and regioselectivities characteristic of glycosyltransferase-catalyzed oligosaccharide synthesis, renders a final deprotection step unnecessary, and may be more suitable than chemical methods for the formation of thioglycosidic linkages in molecules containing sensitive functionalities. In addition the incorporation of a reducing agent into the reaction mixture, uncommon in traditional chemical approaches to thiooligosaccharide synthesis, prevents the loss of valuable carbohydrate thiols resulting from disulfide formation.

Octyl-β-D-lactose is a substrate for bovine α1,3-GalT, and the product of this reaction is easily purified by solid-phase extraction on C_{18} silica gel. In order to establish whether the analogous S-linked trisaccharide could be synthesized by the same enzyme, octyl β-D-3′-thiolactoside **1** (Scheme 1) was prepared. Beginning with known (2,6-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl β-D-glucopyranose,[12] a linear synthesis was devised affording the thiol acceptor **1** after five steps in 26% overall yield (see the Supporting Information).

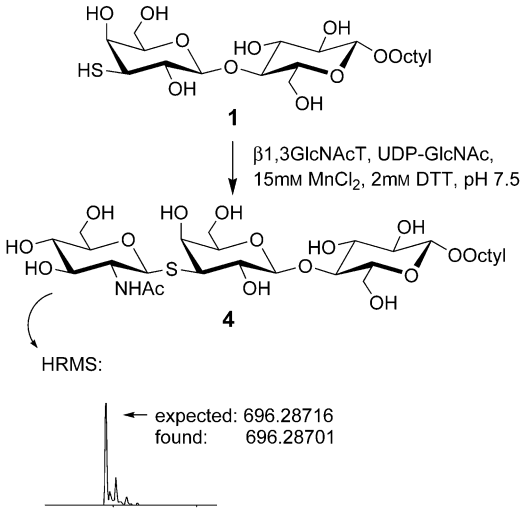
The α1,3-GalT-catalyzed galactosylation of **1** is outlined in Scheme 1. Unexpectedly, incubation of a mixture of the thiol and its disulfide (~3:1) with UDP-Gal and unit quantities of α1,3GalT in the presence of DTT afforded the thio-linked tetrasaccharide **2** in 92% yield after passage through a SepPak C18 cartridge. Thus, following the initial transfer of a galactosyl residue to the thiol acceptor, a second glycosyl transfer occurred. The second reaction is not without precedent, as Wang has reported the galactosylation of an α-linked galactosyl residue by α1,3-GalT, albeit at a rate lower than that of the reaction of the β-linked substrate.[13] Treatment of octyl β-D-lactose under the same conditions yielded the trisaccharide as the major product and a second compound which was identified as the tetrasaccharide by analysis of the high-resolution mass and ^{1}H NMR spectra of the mixture.

Fortunately, the marked difference in stability between O-glycosyl and S-glycosyl bonds could be exploited, and the desired trisaccharide **3**, an analogue of the *Clostridium difficile* toxin A binding ligand, was obtained in near-quantitative yield following treatment with an α-galactosidase from green coffee beans (Scheme 1). This synthetic sidetrack inadvertently reinforced the rationale for the synthesis of thiooligosaccharides.

Encouraged by the successful glycosyl transfer to a sugar thiol acceptor, we hoped to test the generality of our approach. β-Lactosides are known acceptors of a β-1,3-*N*-acetylglucosaminyltransferase from *Neisseria meningitidis*, which has recently been cloned and expressed in *E. coli*.[11] It was thus expected that this enzyme would catalyze the transfer of an *N*-acetylglucosaminyl residue to thiol acceptor **1**, resulting in the formation of the trisaccharide **4** as outlined in Scheme 2. In fact, our initial results confirm that this reaction takes place, and we have established the successful formation of the thioglycoside product by high-resolution mass spectrometry(HRMS). Owing to the significant instability of our enzyme preparation and the slow rate of reaction,



Scheme 1. α1,3-Galactosyltransferase (E.C. 2.4.1.151) catalyzed synthesis of a thiotetrasaccharide **2** and selective hydrolysis of the terminal O-glycosidic linkage by α-galactosidase (E.C. 3.2.1.22) to give thiotrisaccharide **3**.



Scheme 2. β1,3-*N*-acetylglucosaminyl transferase catalyzes glycosyl transfer to thiol **1**. Product formation is confirmed by high-resolution mass spectrometry.

we have not yet generated sufficient product for full characterization by other means.

Standard radiochemical assays,[14] as well as the formation of a tetrasaccharide product (Scheme 1) suggest a substantial decrease in the rate of glycosyl transfer to the 3′-thiolactose acceptor **1** relative to β-octyl lactose. Kinetic analysis showed that the V_{max} for **1** was 0.2 % of that for β-octyl lactose; there was also an effect on K_M which increased from 5 mM to 15 mM. Trisaccharide **3** is a better substrate than **1** with a V_{max} 17 % of the parent lactoside and a K_M of 7 mM, accounting for the preferential formation of tetrasaccharide **2**. In light of the similarity of the K_M values for acceptor **1** and β-octyl lactose, and the substantially diminished rate of transfer to the thiol compound, it seems evident that the nucleophilicity of the thiol is modulated in the enzyme active site.

We have demonstrated here a new method for the synthesis of thiooligosaccharides using glycosyltransferases. Although the observed rate of glycosyl transfer to carbohydrate thiols is diminished relative to unmodified acceptors, the availability of cloned glycosyltransferases makes this a viable method for preparative synthesis. We anticipate that analogous glycosylations of carbohydrate thiols will be observed with a variety of glycosyltransferases. Further, as our understanding of the structure–activity relationships of the active site of these enzymes increases, the development of active site mutants should permit the creation of redesigned glycosyltransferases with improved kinetics for the synthesis of S-linked oligosaccharides.

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