Glycosyltransferase Catalyzed Synthesis of Thiooligosaccharides

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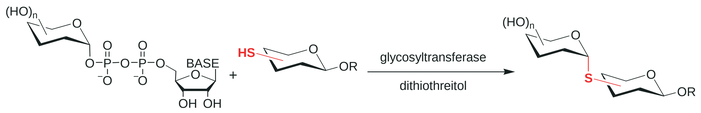
OVERVIEW

The enzymatic formation of sulfur-linked oligosaccharides has been demonstrated by incubation of synthetic carbohydrate thiol acceptors with sugar nucleotide donors and an appropriate mammalian or bacterial glycosyltransferase. These represent the first glycosyltransferase catalyzed syntheses of thiooligosaccharides.



Scheme 1. Enzymatic formation of S-linked oligosaccharides.

BACKGROUND

Oligosaccharide analogues in which the interglycosidic oxygen atom(s) have been replaced by sulfur are known as thiooligosaccharides. These molecules are metabolically stable analogues 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 and the conformational space sampled by *S*-linked glycosides is similar to that of *O*-glycosides. In combination, these properties suggest that *S*-linked carbohydrates may be attractive antigens with extended *in vivo* activity,[1] 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.[2] 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. Recently active site mutants of glycosylhydrolases, termed "thioglycoligases" have been shown to transfer a glycosyl residue to sugar thiols.[3]

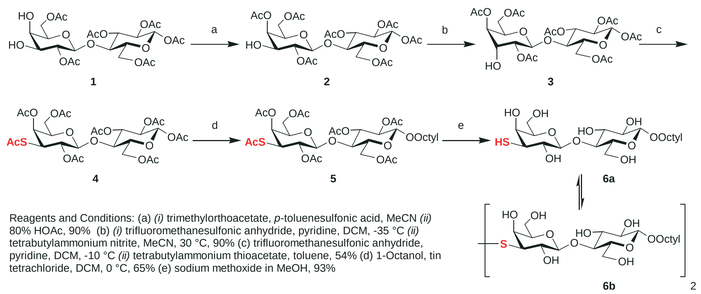
The development of a glycosyltransferase-based approach to the synthesis of thiooligosaccharides does not obviate the considerable effort required for the construction of thiosugar acceptors. However, this method demonstrates the high yields, stereo-, and regio-selectivities characteristic of glycosyltransferase catalyzed reactions, and renders a final deprotection step unnecessary

SURVEYED GLYCOSYLTRANSFERASES

Enzyme	Source	Active?	Relative Rate
α (1,3) Galactosyltransferase[4]	Bovine	Y	0.2%
β (1,3) N-acetylglucosaminyltransferase[5]	*N. Meningitidis* *	Y	0.5%
α (2,3) Sialyltransferase	*C. jejuni*	N	-
ABO(H) blood group glycosyltransferase B (GTB)	Human	N**	-

Table 1. Glycosyltransferases were assayed for their ability to catalyze the synthesis of thiooligosaccharides via a standard radiochemical assay[6]. Rates are given as a % of the rate of transfer to the analogous unmodified acceptor. *β(1,3)-GlcNAcT from another bacterial source was also shown to catalyze this reaction. **The GTB-catalyzed reaction was conducted in the presence of an inhibitor which was not separated from the acceptor.

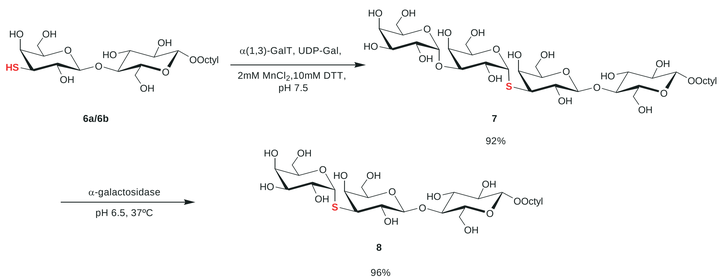
REPRESENTATIVE SYNTHESIS OF A CARBOHYDRATE-THIOL ACCEPTOR



Reagents and Conditions: (a) *(i)* trimethylorthoacetate, *p*-toluenesulfonic acid, MeCN *(ii)* 80% HOAc, 90% (b) *(i)* trifluoromethanesulfonic anhydride, pyridine, DCM, -35 °C *(ii)* tetrabutylammonium nitrite, MeCN, 30 °C, 90% (c) trifluoromethanesulfonic anhydride, pyridine, DCM, -10 °C *(ii)* tetrabutylammonium thioacetate, toluene, 54% (d) 1-Octanol, tin tetrachloride, DCM, 0 °C, 65% (e) sodium methoxide in MeOH, 93%

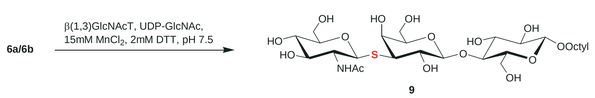
THIOOLIGOSACCHARIDE SYNTHESIS

1. α(1,3)-Galactosyltransferase

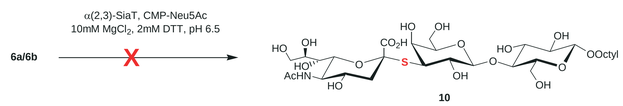


- Surprisingly, two α-linked galactosyl residues were incorporated, yielding tetrasaccharide **7**.
- Owing to the relative stability of the thioglycosidic linkage, the trisaccharide **8** was obtained as the sole product following treatment of **7** with an α-galactosidase.

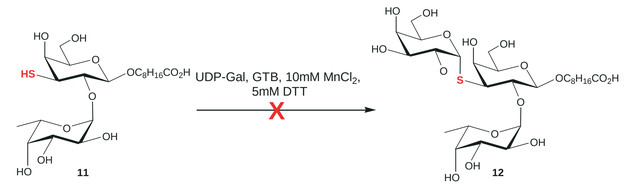
2. β(1,3)-*N*-acetylglucosaminyltransferase



3. α(2,3)-Sialyltransferase



4. ABO(H) blood group glycosyltransferase B



Compound **11**, a sulfur containing analogue of the H-disaccharide, was chemically synthesized in seven steps from known intermediates. A side product formed in the final deprotection step (α-L-Fuc*p*-(1-2)-β-D-(3-deoxy)-Gal*p*-(OR)) was not separated from **11**, and is a known inhibitor of GTB.

KINETICS

Both α(1,3)-GalT and β(1,3)-GlcNAcT catalyze the glycosylation of thiol acceptor **6a**, albeit at <1% of the rate of transfer to β-Octyl lactose. More detailed kinetic parameters have been determined for the reaction of acceptor **6a** catalyzed by α(1,3)-GalT (Table 2). In light of the similarity of the K_M values for **6a** and β-Octyl lactose and the substantially diminished rate of glycosyl transfer observed for the thio-analogue, it seems evident that the nucleophilicity of the thiol is greatly subdued in the active site.

Acceptor	V_{max}	K_M
β-Octyl lactose	1	5 mM
6a	0.002	15 mM
8	0.17	7 mM

Table 2. Kinetic parameters for the glycosylation of **6a** and **8** catalyzed by α(1,3)-GalT

CONCLUSIONS

We have demonstrated a new method for the synthesis of sulfur-linked oligosaccharides using some bacterial and mammalian glycosyltransferases.

The observed rate of glycosyl transfer to thiols is diminished relative to transfer to natural acceptors.

As our understanding of glycosyltransferase activity increases, the development of mutants should permit the creation of redesigned enzymes with improved kinetics for the synthesis of *S*-linked oligosaccharides.

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