

Fidelity and Promiscuity of a Mycobacterial Glycosyltransferase

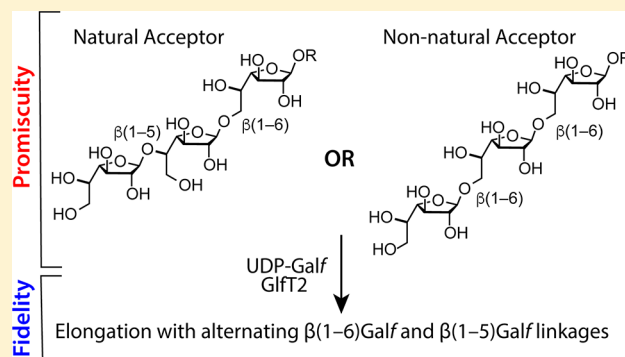
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S Supporting Information

ABSTRACT: Members of the genus *Mycobacterium* cause devastating human diseases, including tuberculosis. *Mycobacterium tuberculosis* can resist some antibiotics because of its durable and impermeable cell envelope. This barrier is assembled from saccharide building blocks not found in mammals, including galactofuranose (Gal_f). Within the cell envelope, Gal_f residues are linked together to afford an essential polysaccharide, termed the galactan. The formation of this polymer is catalyzed by the glycosyltransferase GlfT2, a processive carbohydrate polymerase, which generates a sequence-specific polysaccharide with alternating regioisomeric $\beta(1-5)$ and $\beta(1-6)$ Gal_f linkages. GlfT2 exhibits high fidelity in linkage formation, as it will terminate polymerization rather than deviate from its linkage pattern. These findings suggest that GlfT2 would prefer an acceptor with a canonical alternating $\beta(1-5)$ and $\beta(1-6)$ Gal_f sequence. To test this hypothesis, we devised a synthetic route to assemble oligosaccharides with natural and non-natural sequences. GlfT2 could elongate each of these acceptors, even those with non-natural linkage patterns. These data indicate that the glycosyltransferase is surprisingly promiscuous in its substrate preferences. However, GlfT2 did favor some substrates: it preferentially acted on those in which the lipid-bearing Gal_f residue was connected to the sequence by a $\beta(1-6)$ glycosidic linkage. The finding that the relative positioning of the lipid and the non-reducing end of the acceptor influences substrate selectivity is consistent with a role for the lipid in acceptor binding. The data also suggest that the fidelity of GlfT2 for generating an alternating $\beta(1-5)$ and $\beta(1-6)$ pattern of Gal_f residues arises not from preferential substrate binding but during processive elongation. These observations suggest that inhibiting the action of GlfT2 will afford changes in cell wall structure.



INTRODUCTION

Many glycans essential for microbial pathogenesis, virulence, or viability contain non-mammalian building blocks. Blocking the biosynthesis of these unique glycans could lead to new antibacterial therapies. The galactan of mycobacteria is composed of Gal_f residues, which are not found in mammalian glycans (Figure 1). The galactan is essential for mycobacterial viability, yet no enzymes that assemble the galactan are targeted by current antibiotics. Understanding the catalytic properties of galactan biosynthetic enzymes could lead to new strategies to target mycobacteria.

A key carbohydrate polymerase involved in galactan biosynthesis is GlfT2 (Figure 2). While most glycosyltransferases catalyze the formation of one type of glycosyl bond, some can generate multiple linkage types to yield oligosaccharides or polysaccharides of controlled sequence.^{1–4} Little is known, however, regarding the mechanism or substrate specificity of these multifunctional enzymes. GlfT2 is one such glycosyltransferase. In *Mycobacterium tuberculosis*, GlfT2 (EC 2.4.1.288), which is encoded by the *glfT2* (or *Rv3808c*) gene, is essential.⁵ GlfT2 promotes the formation of linear polysaccharides consisting of about 30–40 Gal_f residues that are connected by alternating $\beta(1-5)$ and $\beta(1-6)$ linkages.⁶ The product polymer, the galactan, extends from the peptidoglycan

and displays pendant arabinofuranose sequences that are linked ultimately to mycolic acids.

The natural glycosyl acceptor for GlfT2 is the tetrasaccharide sequence D-Gal_f- $\beta(1-5)$ -D-Gal_f- $\beta(1-4)$ -L-Rha- $\alpha(1-3)$ -D-GlcNAc linked by a pyrophosphate to a polyprenol lipid (compound 1).^{7–9} This compound is generated through the action of the GlfT1. The ability of GlfT2 to generate polymers depends on the nature of the lipid substituent,^{10,11} but the glycosyltransferase can readily process simple di-Gal_f motifs with lipophilic anomeric substituents.^{6,7,11–13} As a result, synthetic substrates have been used to probe the mechanism and products of GlfT2. GlfT2-catalyzed elongation results in polysaccharides similar in length to those isolated from mycobacteria.^{10,14} During elongation, GlfT2 catalyzes the formation of multiple Gal_f linkages before product release; it is a processive polymerase.^{10,14} Chain-terminating nucleotide UDP-Gal derivatives have been used to reveal that GlfT2 faithfully generates alternating $\beta(1-5)$ and $\beta(1-6)$ Gal_f linkages.^{15,16} These studies revealed GlfT2's fidelity for assembly of products with alternating regioisomeric linkages. The structure of GlfT2, determined by X-ray crystallography,

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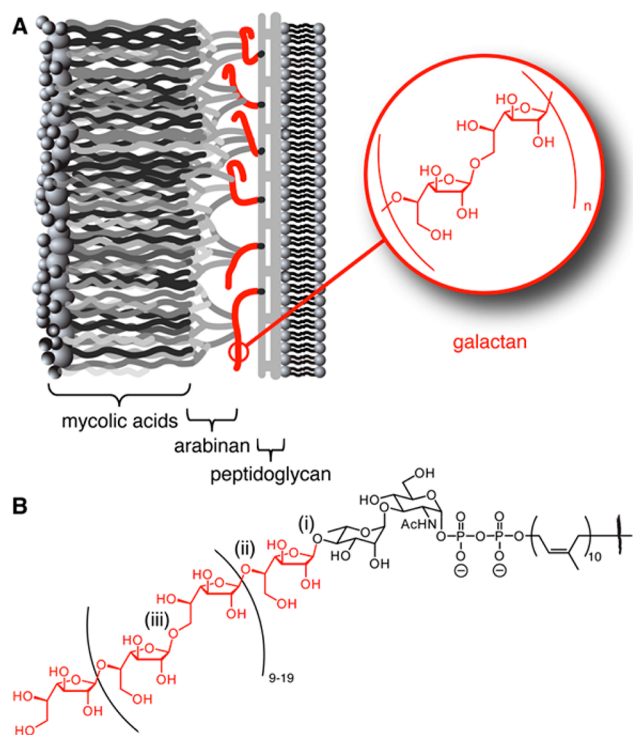


Figure 1. Mycobacterial cell envelope containing an essential polymer composed of Galf residues. (A) Schematic of the mycobacterial cell envelope showing that the galactan (red) links the mycolic acids to the peptidoglycan. (B) Mycobacteria generate the lipid-linked carbohydrate polymer, which then is covalently linked to the peptidoglycan.

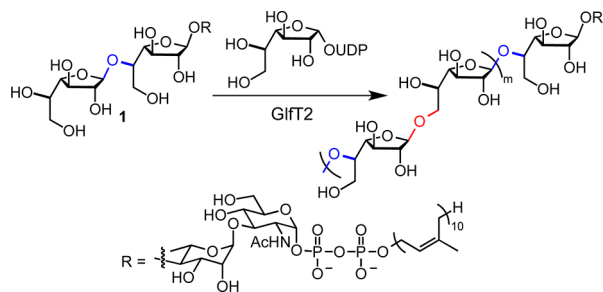


Figure 2. GltT2 mediates galactan formation by catalyzing the addition of 30–40 Galf residues to oligosaccharide-lipid precursor 1. GltT2 is a bifunctional glycosyltransferase that can generate alternating Galf- $\beta(1-5)$ -Galf and Galf- $\beta(1-6)$ -Galf linkages.

indicates that it can form a tetramer, with each monomer unit possessing a single active site.¹⁷ Site-directed mutagenesis studies support a model in which one active site facilitates formation of both glycosidic linkages.¹⁴ Sequence fidelity of the enzyme could be manifested by selectivity in substrate binding, in the process of elongation, or in both. To explore these possibilities, we sought to synthesize and test substrates whose sequences vary.

Our oligosaccharide targets were predicated on findings that recombinant GltT2 can extend acceptor mimics, such as 2, to afford polymers with lengths similar to those isolated from mycobacteria (Figure 3).¹¹ To test the role of sequence in elongation, we focused on synthesizing trisaccharides 4 and 5 with naturally alternating linkages, trisaccharides 6 and 7 with non-natural consecutive linkages, and tetrasaccharide 8 with natural alternating linkages (Figure 3). The comparison of natural substrates (i.e., 4 and 5) to their non-natural (i.e., 6 and

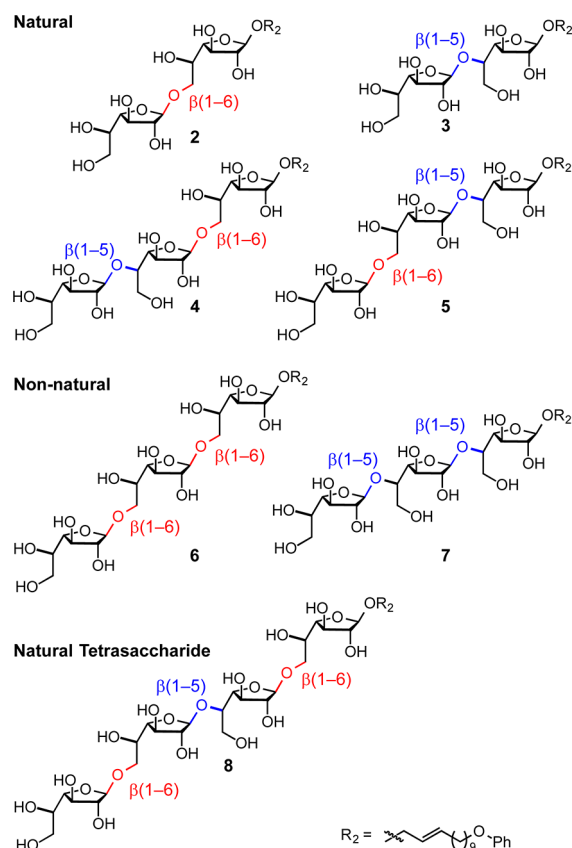


Figure 3. Oligosaccharides synthesized to probe GltT2 specificity. Trisaccharides 4 and 5 and tetrasaccharide 8 have natural alternating glycosidic linkages, while trisaccharides 6 and 7 have non-natural consecutive glycosidic linkages.

7) counterparts would test whether GltT2 can tolerate a “mistake” in the initial acceptor. In this way, we could assess the effect of different structural elements of the acceptor (internal and terminal glycosidic linkage) on the efficiency of polymerization by GltT2.

We developed a divergent approach to produce oligogalactofuranoside acceptors with different sequences. The product oligosaccharides were assessed as substrates for GltT2 by analyzing the efficiency of their elongation and the lengths of the product polysaccharides. Unexpectedly, substrates with perfectly alternating sequence were not preferred over those with “errors”. It was the nature of the first glycosidic linkage following the lipid that had the largest influence on GltT2-promoted glycosylation. The data indicate that the preferential formation of alternating $\beta(1-6)$ -Galf and $\beta(1-5)$ -Galf residues arises not from substrate binding but during catalytic processive polymerization.

Retrosynthesis. The retrosynthesis of tetrasaccharide 8 illustrates our general strategy to assemble oligogalactofuranosides that vary in sequence (see Figure 4). To maximize convergence, the tetrasaccharide was divided into disaccharide units 9 and 10. These building blocks could arise from monosaccharide precursors 11 and 12. Thioethylglycoside 12 was selected as a key intermediate for its ability to function either as an acceptor or as a donor. Specifically, the thioethyl anomeric group is sufficiently stable to allow a glycosylation reaction with the more reactive donor 11.^{18,19} The disaccharide 9 that would result from the first glycosylation reaction could

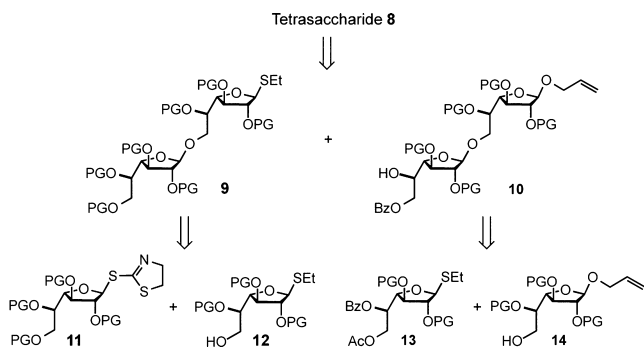


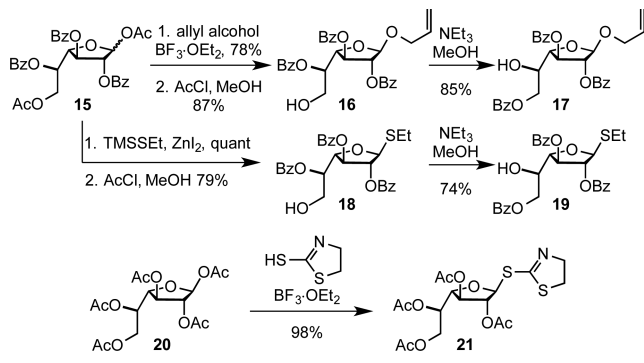
Figure 4. Retrosynthetic analysis of tetrasaccharide acceptor **8**. The precursor for thioglycosides **11** and **12** can be activated for glycosylation under different conditions, and a benzoyl group migration strategy can be employed to access acceptors with either a C5 or a C6 hydroxyl group poised for glycosylation.

be induced to undergo a subsequent reaction to build oligosaccharides.

Another feature of our strategy is that it uses intermediates that can be converted into acceptors with either a free O5 or O6 hydroxyl group. Specifically, the acetyl group from a compound such as **13** could be removed to yield an acceptor with a C6 hydroxyl group (e.g., compound **12**). Compounds with free C5 hydroxyl groups (e.g., **10**) were envisioned to arise from benzoyl group migration from the C5 to the C6 hydroxyl group. Thus, oligosaccharide substrates could be assembled with either alternating or consecutive regioisomeric glycosidic linkages using only a small set of building blocks.

Route to Oligosaccharide Acceptors. The aforementioned retrosynthetic strategy was applied to produce the target monosaccharide building blocks (Scheme 1). Both allyl

Scheme 1. Synthesis of Monosaccharide Building Blocks



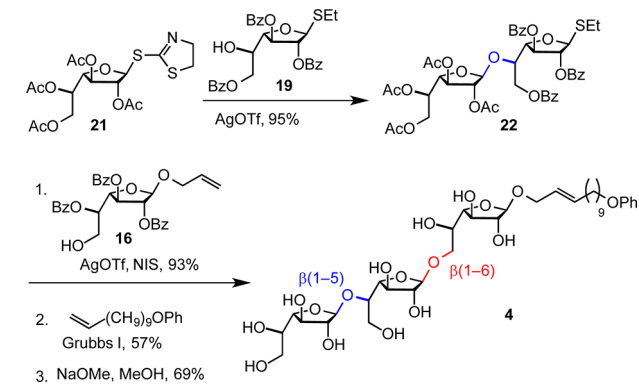
glycoside nucleophile **16** with its C6 hydroxyl group free and **17** with its C5 hydroxyl group free were generated from acetoxy glycoside **15**.^{11,20} Compound **15** was converted into the protected allyl glycoside. Acid-mediated acetate removal from the C6 hydroxyl group provided **16**, which is poised to form a 1,6-linkage. To obtain glycosidic acceptor **17** with a free C5 hydroxyl group, we sought to promote the migration of the benzoyl group from O5 position to O6 position. The desired transformation occurred readily when **16** was treated with triethylamine in methanol to afford **17** in 85% yield.

Similarly, compound **15** was efficiently converted to the S-ethyl thioglycosides **18** and **19**. As anticipated, the S-ethyl anomeric group of **15** survives the acidic conditions for acetate removal. Compounds **18** and **19** can be used first as glycosyl

acceptors, while the thioethyl group remains inert. The product of these reactions can function subsequently as an electrophile in glycosylation. By exploiting acetate group removal from O6 positions under mildly acidic conditions and the triethylamine-catalyzed benzoyl group migration, we could access each of the requisite monosaccharide building blocks.

We next examined the utility of these intermediates for the production of trisaccharide and tetrasaccharide substrates for GlfT2. The synthetic route to trisaccharide **4** is representative (Scheme 2). Glycosylation of **21** with acceptor **19** provided

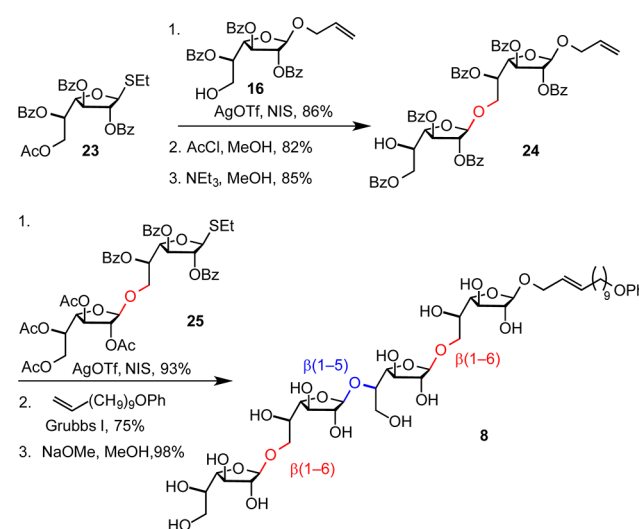
Scheme 2. Synthesis of Trisaccharide 4



disaccharide **22**. Only the thiazolyl glycoside was activated with silver triflate, while the thioethyl functionality of acceptor **19** was undisturbed.^{18,19} When the disaccharide **22** was exposed to catalytic silver triflate and *N*-iodosuccinimide (NIS), it underwent coupling to **16** to generate a trisaccharide with the natural alternating $\beta(1-6)$ -Gal $\beta(1-5)$ -Gal linkage pattern. We employed a cross-metathesis reaction to append the 11-phenoxy-1-undecene moiety. Ester group removal provided the target trisaccharide acceptor **4**. In an analogous manner, trisaccharides **5–7** were synthesized (see Supporting Information).

Our route for trisaccharide assembly exploits common building blocks, yet it is divergent. It could be used to construct longer oligomers, as illustrated by the production of tetrasaccharide **8** (Scheme 3). Compounds **16** and **23** were

Scheme 3. Synthesis of Tetrasaccharide 8



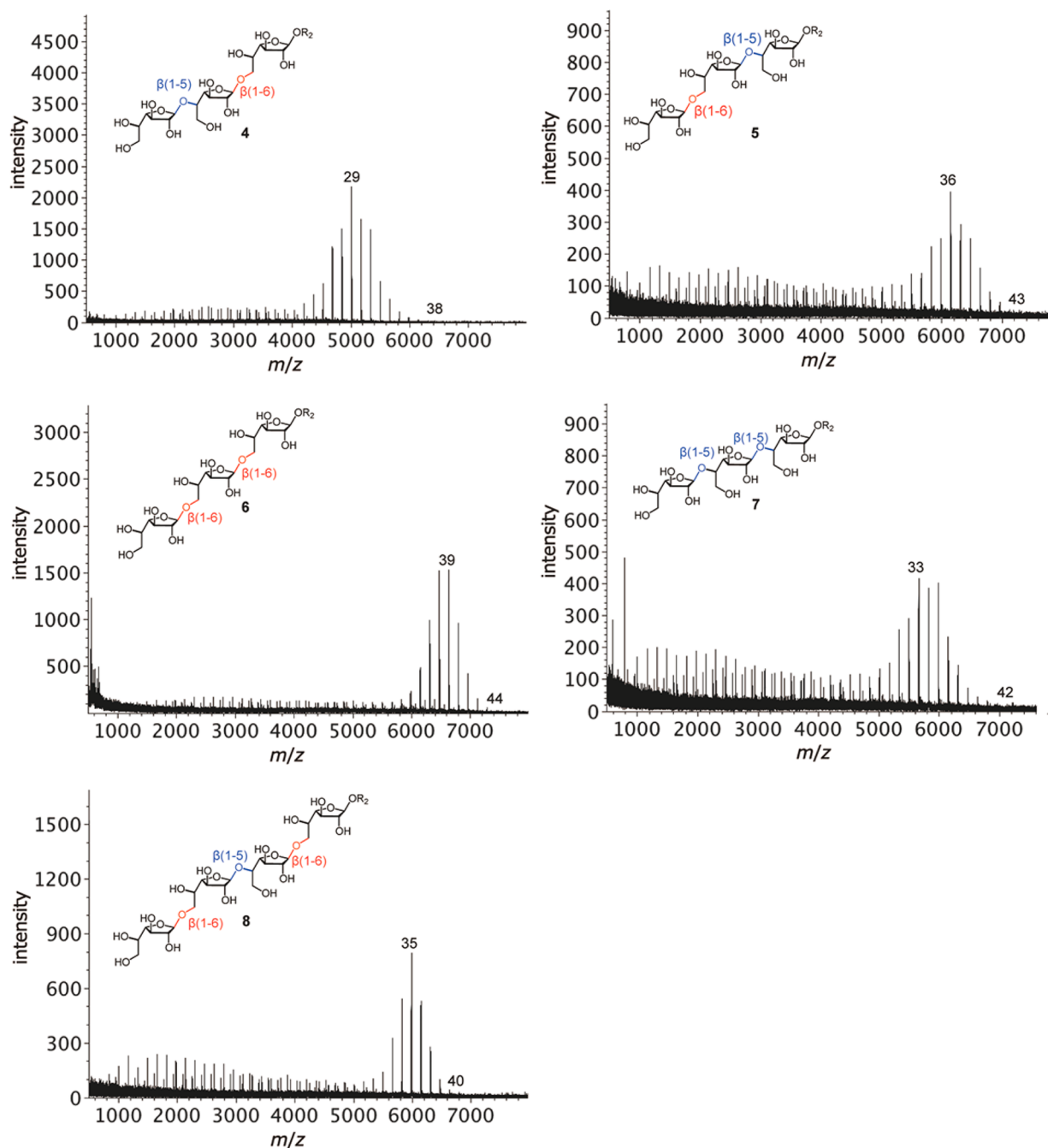


Figure 5. MALDI-TOF mass spectra of GlfT2-catalyzed polymerization reactions with trisaccharide acceptors 4–7 and tetrasaccharide acceptor 8. Synthetic oligosaccharides were incubated at room temperature with GlfT2 in the presence of UDP-Galf. After 20 h, the resulting mixtures were analyzed by MALDI-TOF mass spectrometry. Peaks that correspond to m/z values of $[M+Na]^+$ are labeled with the value of n , which is the number of GalF residues in the product.

exposed to silver triflate to afford a disaccharide bearing a C6 acetate. Acetate removal and benzoyl group migration afforded glycosyl acceptor 24, which is poised for an O5 glycosylation reaction. Glycosylation with disaccharide 25 generated a tetrasaccharide that was transformed into target tetrasaccharide 8. We previously used a chemoenzymatic route to generate this compound, but it afforded only small quantities (1–5 mg) of the tetrasaccharide.¹⁰ The synthetic route described herein is robust and scalable. Its use of orthogonally activatable thioglycosides and application of the mild benzoyl group migration strategy to synthesize both O5 and O6 glycosylation acceptors enabled production of the tetrasaccharide glycolipid 8

in quantities sufficient to characterize GlfT2 elongation (*vide infra*).

Oligosaccharide Elongation Catalyzed by GlfT2. We evaluated trisaccharides 4–7 and tetrasaccharide 8 in GlfT2-catalyzed polymerization reactions. To test their utility as substrates, recombinant GlfT2 from *M. tuberculosis* was mixed with the synthetic oligosaccharides and UDP-Galf.¹¹ After 20 h at room temperature, the crude enzymatic reaction mixtures were analyzed by MALDI-TOF mass spectrometry. The results were unexpected: All of the substrates were elongated, including non-natural trisaccharides 6 and 7 (Figure 5). The polysaccharide products that result have as many GalF residues

as found in the natural galactan (38–44 Galf residues).⁶ Given that GlfT2 processes all sequences, the data highlight the enzyme's promiscuity.

Kinetic Analyses of GlfT2 with Oligosaccharide Acceptors. The intensity of peaks in the mass spectra suggests that some substrates (e.g., 4) are elongated more efficiently than others (e.g., 5). Surprisingly, however, the favorable substrates were not those that had “natural” alternating sequences. To quantify the activity of GlfT2 with different substrates, we used a continuous coupled assay to monitor the rates of GlfT2-catalyzed glycosylation of disaccharides 2 and 3, trisaccharides 4–7, and tetrasaccharide 8. Because GlfT2 produces UDP upon the addition of each Galf residue to an acceptor, turnover can be assessed by linking UDP production to NADH oxidation.^{11,21–23} In agreement with previous results,¹¹ GlfT2 exhibited a kinetic lag phase with disaccharide acceptors 2 and 3 (Figure 6). Though trisaccharide 7 exhibited

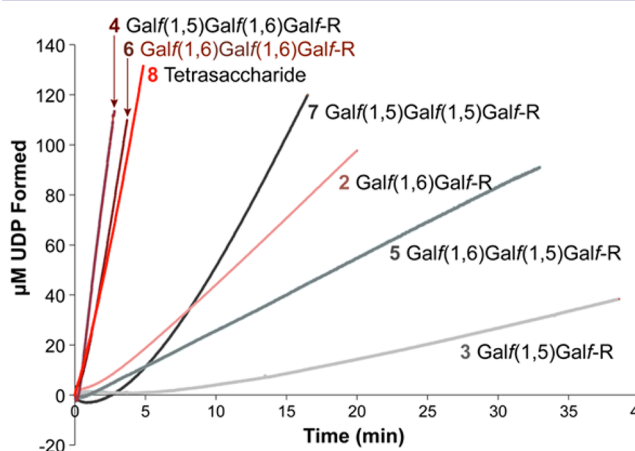


Figure 6. Kinetics of GlfT2-catalyzed polymerization with oligosaccharide acceptors 2–8. GlfT2 turnover was monitored by coupling UDP production to NADH oxidation in a continuous assay.

a minor lag phase, none was observed with trisaccharides 4–6 nor with tetrasaccharide 8. As the products of GlfT1 are tetrasaccharides and pentasaccharides,⁹ endogenous intermediates should be rapidly processed by GlfT2.

We monitored the initial rate of UDP production at different acceptor substrate concentrations to determine apparent K_m and k_{cat} values for GlfT2 (Table 1). Acceptors bearing anomeric lipids with longer chains bound more tightly to the enzyme and afforded higher catalyst turnover number than those with an octyl glycoside at the reducing end.¹³ These results are consistent with the influence of the anomeric lipid substituent on acceptor elongation by GlfT2.

The specific saccharide sequence of each compound influenced its ability to serve as an acceptor. Surprisingly, the

differences were not based on whether the acceptor had a natural or non-natural sequence. For example, a large difference in the kinetic parameters for trisaccharides 4 and 5 was observed, but both have the natural alternating linkages. Specifically, trisaccharide 4, with a $\beta(1-6)$ glycosidic linkage at the reducing end (i.e., near the lipid), had a more favorable apparent K_m value ($13 \pm 1 \mu\text{M}$) and higher catalyst turnover ($k_{cat} = 3.7 \pm 0.1 \text{ s}^{-1}$) than the trisaccharide 5, with a $\beta(1-5)$ glycosidic linkage at the reducing end (apparent $K_m = 190 \pm 86 \mu\text{M}$ and $k_{cat} = 0.72 \pm 0.15 \text{ s}^{-1}$).

Comparison between 4 and 7 or between 5 and 6 further emphasizes the influence of the glycosidic linkage closest to the lipid (reducing end). Both acceptors 4 and 7 terminate with a $\beta(1-5)$ linkage, but 4, with a reducing end $\beta(1-6)$ linkage, exhibited 2-fold smaller apparent K_m and 4-fold higher k_{cat} than did acceptor 7, with a $\beta(1-5)$ linkage at the reducing end. Similarly, acceptor 6, with the internal $\beta(1-6)$ linkage, exhibited 5-fold more favorable apparent K_m and 7-fold higher k_{cat} than did acceptor 5, with the internal $\beta(1-5)$ linkage. These results indicate that the relative positioning of the acceptor lipid substituent impacts substrate binding to GlfT2 and subsequent catalytic turnover. A comparison of the k_{cat}/K_m values for all of the oligosaccharide substrates indicates that the acceptors with a $\beta(1-6)$ near the reducing end (4, 6, 8) are superior substrates to those with a corresponding $\beta(1-5)$ linkage (5, 7). Thus, the positioning or orientation of the saccharide relative to the anomeric lipid substituent is more important than whether the sequence alternates.

DISCUSSION

Data from our group and others indicate that GlfT2 has high fidelity for generating alternating $\beta(1-5)$ and $\beta(1-6)$ linkages.^{15,16} The alternating linkages could be required for subsequent steps in cell envelope biosynthesis or the stability of the cell envelope itself. We therefore examined whether GlfT2 can process acceptors with “mistakes” in the acceptor sequence. To address this issue, we designed oligogalactofuranose glycolipids 4–8 with different saccharide sequences (Figure 3). Synthetic trisaccharide acceptor substrates for GlfT2 have been studied previously, but the reported products of GlfT2-catalyzed reactions did not approach the length of the native galactan.¹³ Moreover, previous investigations focused on substrates with natural alternating glycosidic linkages.¹³ We therefore developed a divergent approach to synthesize GlfT2 substrates that vary in sequence.

Access to the oligogalactofuranose glycolipids was attained by using orthogonally activatable glycosyl donors and a facile method to quickly afford acceptors with different sequences of $\beta(1-6)$ and $\beta(1-5)$ Galf linkages. One key to the strategy was its reliance on *S*-thiazolanyl and *S*-ethyl glycosides as glycosyl donors with either high or moderate reactivity.^{18,19} The stability

Table 1. Apparent K_m and k_{cat} for Oligosaccharides 2 and 4–8 Determined by the Continuous Assay (Figure 6)

acceptor		K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\times 10^3 \text{ M}^{-1}\cdot\text{s}^{-1}$)
Gal f- $\beta(1-6)$ -Gal f ^a	2	66 ± 2	1.0 ± 0.03	15 ± 1
Gal f- $\beta(1-5)$ -Gal f- $\beta(1-6)$ -Gal f	4	13 ± 2	3.7 ± 0.1	280 ± 20
Gal f- $\beta(1-6)$ -Gal f- $\beta(1-5)$ -Gal f	5	190 ± 86	0.72 ± 0.15	3.8 ± 1.9
Gal f- $\beta(1-6)$ -Gal f- $\beta(1-6)$ -Gal f	6	34 ± 16	5.3 ± 1.3	160 ± 80
Gal f- $\beta(1-5)$ -Gal f- $\beta(1-5)$ -Gal f	7	29 ± 1	0.87 ± 0.01	30 ± 1
tetrasaccharide	8	13 ± 1	2.9 ± 0.1	220 ± 20

^aData for compound 2 were obtained from ref 11.

of thioglycosides is an advantage compared to more acid sensitive, reactive intermediates, such as trichloroacetimidate donors.^{20,24} The second important feature of our strategy was the migration of the benzoyl group to the least hindered O6 position.

Acyl group migration is often viewed as a problem in glycan synthesis, but our route exploits it. A previous synthetic approach to galactofuranose derivatives employed a selective protecting group strategy to afford an acceptor nucleophile with a free C5 hydroxyl group. The key intermediate was synthesized by non-selective removal of the primary acetyl group from the C6 position, which resulted in a concomitant benzoyl group migration in low to moderate yield.^{20,24} We found that a base-catalyzed benzoyl group migration provided the means to distinguish between adjacent hydroxyl groups in different steric environments. The orthogonal glycosyl donors and the acyl group migration are the two central strategic features that made it possible to rapidly access acceptors with either natural or non-natural sequences to evaluate with GlfT2. Because the synthetic route outlined relies on a small number of reactions that can be repeated iteratively, we anticipate that it could be used to access longer oligosaccharides with any desired combination of glycosidic linkage pattern.

It is striking that each substrate tested, trisaccharides 4–7 and tetrasaccharide 8, was elongated by GlfT2. The length of each product carbohydrate polymer was similar to that of the endogenous mycobacterial galactan (30–40 *Galf* residues).⁶ Acceptor 4 afforded a slightly shorter galactan than the other three trisaccharide substrates. The observed truncated oligosaccharide could result from efficient consumption of the donor, which would limit the available quantity of UDP-*Galf* as the polymerization proceeds.⁶ We previously demonstrated that the presence and features of the lipid substituent of the initiating acceptor substrates influenced the product carbohydrate polymer length, and we proposed a tethering mechanism for length control by a template-independent polymerase.¹¹ The findings shown herein that all the oligosaccharide acceptor substrates were elongated to the length of the galactan regardless of the different carbohydrate motifs lend additional support to the model. Our results also indicate that GlfT2 exhibits fidelity in the sequence generated during elongation, yet the carbohydrate polymerase is far more promiscuous in processing substrates of different sequences.

Previous studies demonstrated that GlfT2 can elongate disaccharide acceptors with an arabinose residue at the non-reducing end¹³ or even a glycolipid bearing a single *Galf* residue.²⁰ We now show that trisaccharides with non-natural glycosidic linkages are elongated by GlfT2. While this finding was unexpected, in mycobacterial cells, the only available *Galf* glycolipid initiators are those generated from the upstream galactofuranosyltransferase GlfT1.^{7,8,25} This enzyme exhibits high specificity for either the native acceptor or structurally similar analogues. Thus, the high specificity of GlfT1 should ensure that GlfT2 can access the “correct” initial acceptor. The generation of polysaccharides with alternating linkages by GlfT2 appears to occur during processive elongation of the substrate. Specifically, as GlfT2 acts on the substrate, the growing polymer remains bound to the enzyme.¹⁰ In this way, the orientation of the nucleophilic non-reducing end of the growing polymer could be poised to generate the alternating linkages in the enzyme active site.^{14,17}

We found previously that GlfT2 can act on both $\beta(1-6)$ - and $\beta(1-5)$ -linked disaccharide acceptors 2 and 3 to form

products with similar degrees of polymerization.²⁰ There is, however, a significant difference in their initial reaction rate: $\beta(1-6)$ -linked disaccharide 2 has a reaction rate 4-fold faster ($5.7 \pm 3.9 \mu\text{M}/\text{min}$) than that observed for $\beta(1-5)$ -linked disaccharide 3 ($1.3 \pm 0.4 \mu\text{M}/\text{min}$, Figure 6). The data indicate that an acceptor with a $\beta(1-6)$ linkage near the reducing end is a superior substrate for GlfT2 compared to the corresponding $\beta(1-5)$ -linked acceptor. These results indicate a $\beta(1-6)$ near the reducing end promotes both acceptor binding and turnover. These observations are consistent with the saturation transfer difference NMR studies, which indicate that a reducing end *Galf* residue in trisaccharide acceptors makes more intimate contacts with GlfT2 than a non-reducing end *Galf* residue.²⁶ The data suggest that the relative positioning of the lipid and the glycan is critical for elongation.

We previously found that GlfT2 exhibited a kinetic lag phase with a disaccharide acceptor, while no such lag occurred with a chemoenzymatically synthesized tetrasaccharide.¹⁰ The data suggested that the lag phase arose from the lower affinity of the disaccharide acceptor for GlfT2. These findings led us to propose that GlfT2 has subsites, and oligosaccharide acceptors that fill the subsites would not exhibit a kinetic lag phase. This substrate subsite model also has been proposed for other carbohydrate polymerases.^{27–33} It was, however, unknown how many carbohydrate residues are necessary to fully occupy the subsites of GlfT2. Our results indicate that neither trisaccharides 4–6 nor tetrasaccharide 8 gives rise to a kinetic lag phase. Kinetic analyses of trisaccharide 4 and tetrasaccharide 8 revealed that both substrates have similar apparent K_m values, and additional *Galf* residues did not lead to large rate gains during the course of GlfT2-catalyzed glycosylation. This observation is interesting because the natural acceptor substrate for GlfT2 is a glycolipid composed of a tetrasaccharide or pentasaccharide generated by GlfT1. Thus, natural acceptor would fill the enzyme subsites, so that GlfT2 could efficiently elongate the initial acceptor.

We postulate that rapid processive elongation of endogenous acceptors by GlfT2 is inextricably correlated with high fidelity in formation of the alternating $\beta(1-5)$ and $\beta(1-6)$ regioisomeric linkages. Our finding that GlfT2 can process a variety of *Galf*-containing acceptors of varying sequence indicates that substrate binding is not responsible for sequence selectivity. It is in the processive formation of multiple glycosidic linkages that leads to carbohydrate polymers of defined sequence.

CONCLUSION

A robust and divergent synthetic approach has been developed to assemble lipid-modified oligogalactofuranose glycans. Two key features, orthogonally activatable thioglycosides and a benzoyl group migration strategy, enabled the production of both alternating (natural) and non-alternating (non-natural) regioisomeric oligosaccharide acceptors for the mycobacterial galactofuranosyltransferase GlfT2. All of the oligosaccharide acceptors were efficiently processed. These polymerization results revealed the sequence promiscuity of GlfT2 for its initial acceptor. Moreover, the results reveal that the nature of the glycosidic linkage closest to the reducing end of the acceptor makes a larger contribution to the efficiency of the catalysis by GlfT2. These observations provide further support for the importance of the lipid for catalysis. They also suggest that inhibitors that promote GlfT2 release of substrate could afford either shortened galactan or galactan in which deviations from

the alternating $\beta(1-5)$ and $\beta(1-6)$ linkages occur. The possibility that such defects in the cell envelope would be detrimental provides impetus to seek GlfT2 inhibitors.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04481.

Routes employed for the syntheses of trisaccharides 5–7, experimental protocols for the preparation of all new compounds, and spectral data for all new compounds, as well as experimental procedures for protein production and enzyme assays (PDF)

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Notes

The authors declare no competing financial interest.

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