Galactofuranose-based Compounds as Potential Inhibitors of Glf*T*1

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ABSTRACT

The enzyme Galactofuranosyltransferase 1 (Glf*T*1), initiates the elongation of the galactan chain of the mycolyl-arabinogalactan peptidoglycan (mAGP) complex of the mycobacterial cell wall. mAGP is essential for the microorganism's viability and its galactan chain backbone is comprised of alternating 5- and 6- linked β-D-galactofuranose (Gal*f*) units that are not found in humans and, thus, enzymes (e.g. Glf*T*1) involved in its biosynthesis might serve as putative therapeutic targets in diseases such as tuberculosis. Several potential inhibitors resembling the polysaccharide glycan were synthesized and tested against Glf*T*1.

INTRODUCTION

The mycobacterial cell wall is an important component of the organism and serves as a permeability barrier to the passage of antibiotics. Our focus is in the biosynthesis of mycolyl-AG complex, the species responsible for the microorganism's highly impermeable nature. The absence of D-Gal*f* residues in human beings suggests that inhibitors of the galactofuranosyltransferase 1 (Glf*T*1), the first galactofuranosyl transferase involved in the assembly of the galactan chain may be ideal drug candidates.

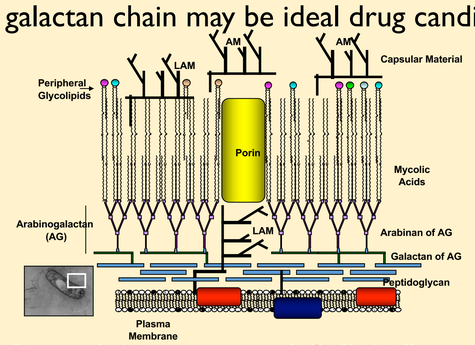


Figure 1. Mycobacterial Cell Wall

The research study aims to synthesize designed compounds resembling the polysaccharide glycans present in the mycobacterial cell wall. These analogues are modified substrates that are predisposed to bind to Glf*T*1.

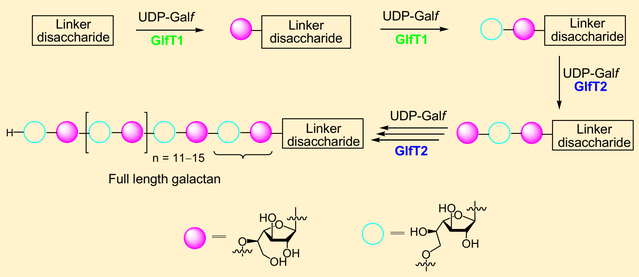
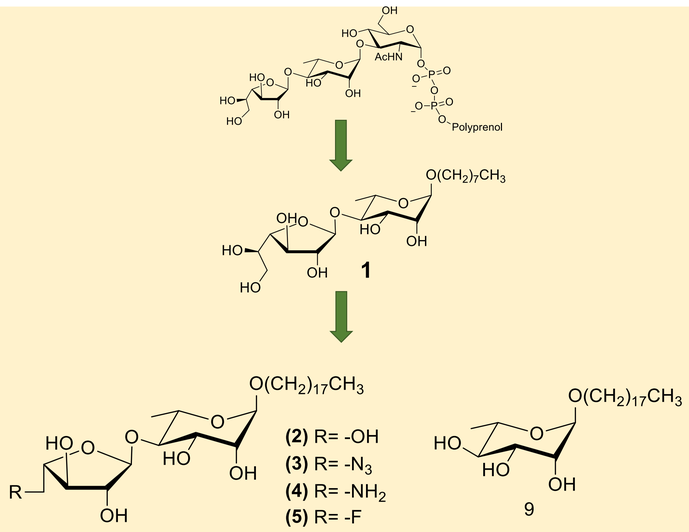


Figure 2. Galactofuranosyltransferase system pathway

DESIGN OF PROBE AND INHIBITORS FOR GlfT1



PROBING GlfT1 SPECIFICITY

MALDI-TOF MS Analaysis of Enzymatic Reactions Mixtures*

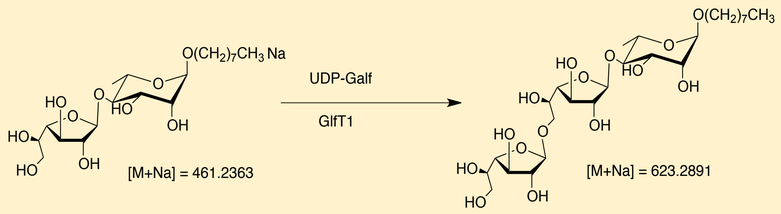
Potential substrate incubated with GlfT1 and UDP-Galf

The mixture was purified using a C-18 Sepak Cartridge, yielding mixtures of oligosaccharides.

Mixture was analyzed using a MALDI-TOF MS

*This assay was done by our collaborators in the University of Alberta, Dr. Zheng and Dr. Lowary.

PROPOSED PROBE IS RECOGNIZED BY GlfT1



K_m = 1754 μM
K_{cat} = 3.25 min^{-1}
K_{cat}/K_m = 1.85 x 10^{-3} (μM-min)$^{-1}$

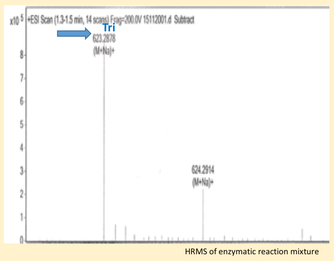
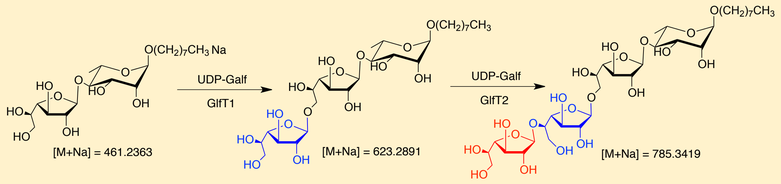


Figure 3. Representative MALDI-TOF mass spectrum obtained from reaction mixture of Probe 1, UDP-Gal*f* and Glf*T*1.



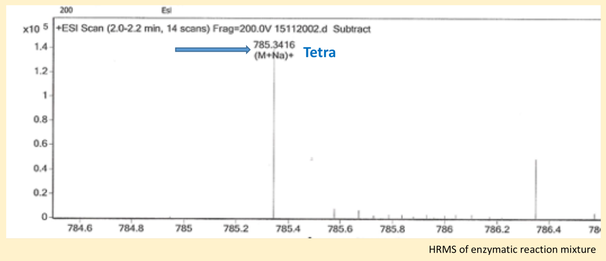
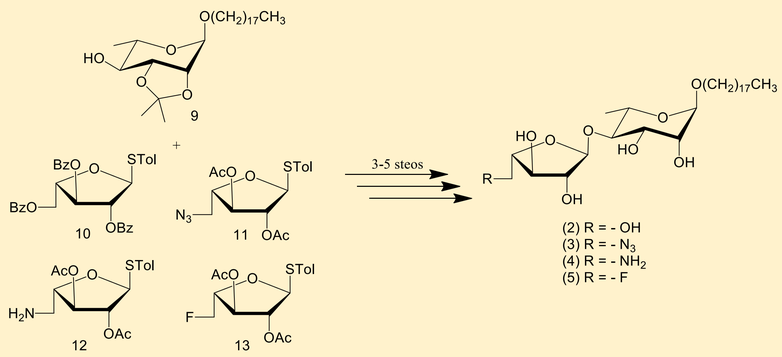
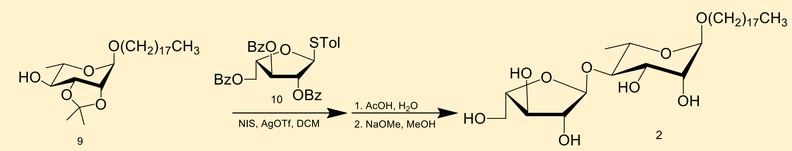


Figure 4. Representative MALDI-TOF mass spectrum obtained from reaction mixture of Probe 1, UDP-Gal*f*, Glf*T*1 and Glf*T*2.

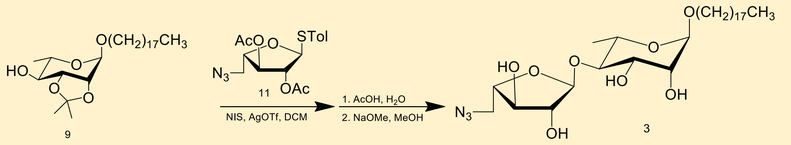
SYNTHETIC ROUTE



Scheme 1. Synthetic strategy to prepare substrate based inhibitors.



Scheme 2. Synthesis of Inhibitor 2.



Scheme 3. Synthesis of Inhibitor 3.

SUMMARY

- Probe 1 was synthesized to probe the specificity of Glf*T*1.
- Kinetic analysis and MS analysis of the reaction products of probe 1 confirmed that it is a substrate of Glf*T*1.
- Results showed that the absence of the pyrophosphate group and GlcNAc moiety can be utilized by Glf*T*1.
- Substrate-inhibitors were designed based on the data acquired.
- Target compounds 2 and 3 were successfully synthesized.
- Synthesized compounds will be tested against Glf*T*1.
- The remaining target compounds (4&5) will be synthesized and tested as inhibitors of Glf*T*1.

ACKNOWLEDGMENT



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