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Note

Mechanism-based candidate inhibitors of uridine diphosphate galactopyranose mutase (UGM) †

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ABSTRACT

Uridine diphosphate-galactopyranose mutase (UGM), an enzyme found in many eukaryotic and prokaryotic human pathogens, catalyzes the interconversion of UDP-galactopyranose (UDP-Gal*p*) and UDP-galactofuranose (UDP-Gal*f*), the latter being used as the biosynthetic precursor of the galactofuranose polymer portion of the mycobacterium cell wall. We report here the synthesis of a sulfonium and selenonium ion with an appended polyhydroxylated side chain. These compounds were designed as transition state mimics of the UGM-catalyzed reaction, where the head groups carrying a permanent positive charge were designed to mimic both the shape and positive charge of the proposed galactopyranosyl cation-like transition state. An HPLC-based UGM inhibition assay indicated that the compounds inhibited about 25% of UGM activity at 500 μM concentration.

Tuberculosis (TB) is one of the world's deadliest diseases. In 2013, about nine million people around the world developed TB and 1.5 million died from this disease.[1] This disease is caused by the bacterium *Mycobacterium tuberculosis*. Traditional methods used to combat this infection are losing effectiveness owing to the appearance of multi-drug-resistant strains. In addition, vaccines against TB are only partially effective.[1] Mycobacterial diseases are difficult to treat with drugs, which is due in part to the particularly impermeable nature of the mycobacterium cell wall.[2,3] The unique cell wall of *M. tuberculosis*, which is impenetrable to many antibiotics, is essential to the viability of the organism.[2,4] A critical component of the cell wall is D-galactan, a polysaccharide chain consisting of D-galactofuranose (Gal*f*) residues. Since Gal*f* residues are not found in mammalian systems, inhibition of the biosynthesis of this polymer constitutes a very attractive and accessible target for new anti-TB drugs without deleterious side effects.[2,4] The biosynthesis of these polymers involves two specific enzymes: UDP-galactopyranose mutase (UGM), which catalyzes the interconversion of UDP-galactopyranose (UDP-Gal*p*) and UDP-galactofuranose (UDP-Gal*f*) (Fig. 1), and UDP-galactofuranosyl transferase (UDP-Gal*f* transferase), which catalyzes the transfer of Gal*f* from UDP-Gal*f* onto a growing oligosaccharide chain.[2,5,6] Because of the pivotal role of UGM in mycobacterial cell-wall biosynthesis, there is a growing interest in the development of UGM inhibitors as potential antimicrobial agents.[4,7] Several UGM inhibitor candidates with micromolar inhibition activities, which can be regarded as substrate-like[8–14] or non-substrate-like,[15–17] have been developed recently.

The mechanism of the reaction catalyzed by UGM has been the subject of much discussion.[6,18–21] Several fluorinated analogues of UDP-Gal*f* and UDP-Gal*p* bearing a fluorine atom at the C-2, C-3 or C-6 position of the galactose ring have been reported as mechanistic probes to study the involvement of oxacarbenium-ion TS or intermediate in the UGM-catalyzed reactions.[22,23] Fluorine substitution has a negative impact on the reaction rate of the UGM-catalyzed reaction and the effect is dependent on the position of the fluorine atom with respect to the anomeric carbon (C1), suggesting a reaction mechanism that possesses some oxacarbenium-ion character. Significant rate reduction was observed for the 2-F analogues compared to the 3-F and 6-F analogues, consistent with greater destabilization of such oxacarbenium-ion TS or intermediates when the electron-withdrawing fluorine atom is closer to the reaction centre (anomeric carbon).[22,23] Recently, Sun et al.[24] have shown that the UGM-catalyzed reaction proceeds through an S_N2-type mechanism in a study using a variety of FAD analogues (Scheme 1). Even with a S_N2-type mechanism, if the bond cleavage is more advanced (leaving group, UDP, departs early) than the bond making process (nucleophilic attack by the FAD cofactor)

† This manuscript is dedicated, with respect, to the memory of Professor Derek Horton.

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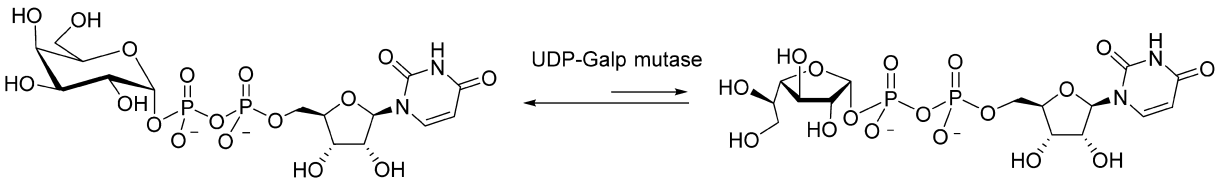
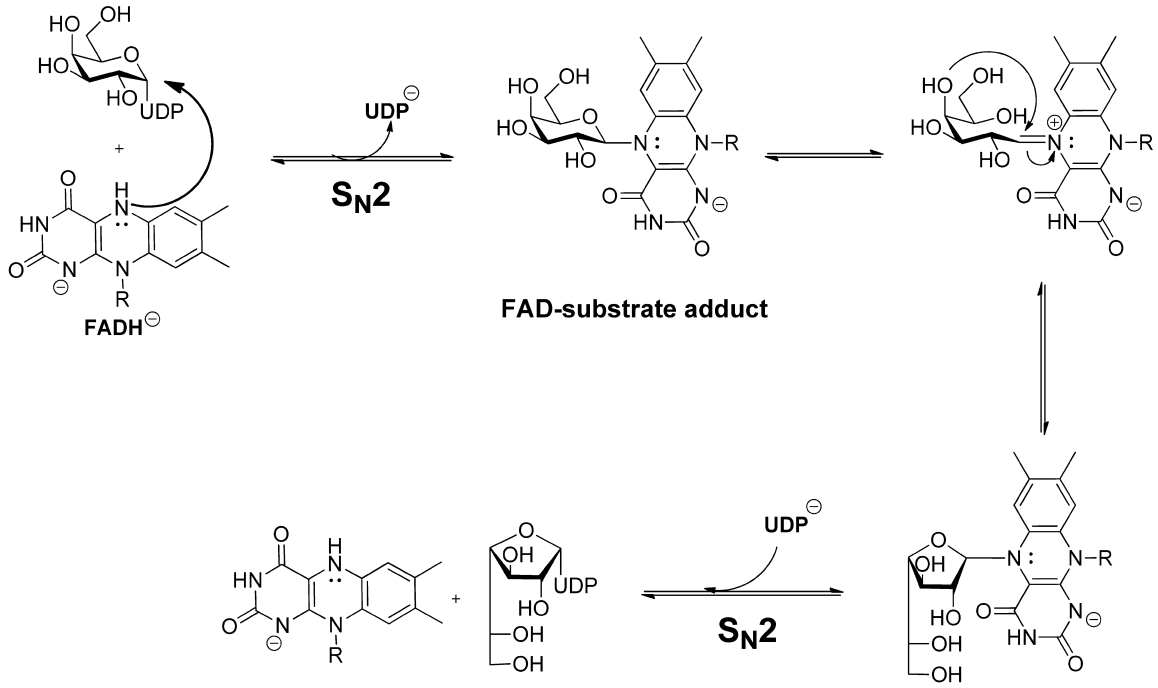


Fig. 1. UGM-catalyzed interconversion of UDP-Gal*p* and UDP-Gal*f*.



Scheme 1. Proposed mechanism for the UGM-catalyzed reaction.

as shown in Fig 2, then the transition state will have more oxacarbenium-ion character. In such a scenario, it should be possible to inhibit the activity of UGM using stable compounds that closely resemble the proposed oxacarbenium ion-like transition state. The situation is analogous to glycosidase-catalyzed hydrolysis reactions which can proceed by S_N2-type mechanisms but which can nevertheless be inhibited by oxacarbenium-ion mimics. Here, we report the synthesis of two compounds, **1** and **2**, as candidate transition-state analogues (Fig. 3) of the UGM-catalyzed reaction.

Several iminosugar-based UGM inhibitors have been reported in the literature as mimics of the proposed oxacarbenium ion-like transition state.[25–28] In general, iminosugars are believed to carry a positive charge at physiological pH and hence are postulated to bind in the active site of the enzyme by mimicry of the charge state of the oxacarbenium ion-like transition state formed during the enzyme-catalyzed reaction.[29] Several α and β-1-*C*-substituted 1,4-dideoxy-1,4-imino-D-galctitol analogues have been synthesized and tested against UGM as galactofuranoside mimics.[27,28] However, most of them showed only modest inhibition of UGM (less than 50% inhibition at 2.5 mM–25 mM). Even modifying one of these inhibitors by attaching the uridine monophosphate (UMP) fragment via an α-linkage to the 1,4-dideoxy-1,4-imino-D-galctitol moiety using a

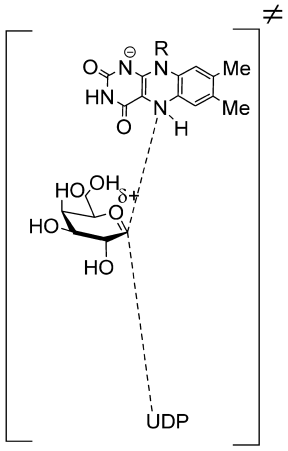


Fig. 2. Proposed transition state for the UGM-catalyzed reaction.

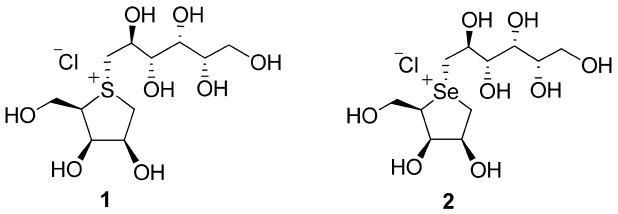


Fig. 3. Novel transition-state analogues for the UGM-catalyzed reaction.