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Potent Ligands for Prokaryotic UDP-Galactopyranose Mutase That Exploit an Enzyme Subsite

Emily C. Dykhuizen† and Laura L. Kiessling*†,‡
Departments of Chemistry and Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin, 53706
kiessling@chem.wisc.edu

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ABSTRACT

UDP-Galactopyranose mutase (UGM or Glf), which catalyzes the interconversion of UDP-galactopyranose and UDP-galactofuranose, is implicated in the viability and virulence of multiple pathogenic microorganisms. Here we report the synthesis of high-affinity ligands for UGM homologues from Klebsiella pneumoniae and Mycobacterium tuberculosis. The potency of these compounds stems from their ability to access both the substrate binding pocket and an adjacent site.

Galactofuranose (Galf) residues are present in many pathogenic microorganisms.1 Perhaps the most notorious of these is Mycobacterium tuberculosis, which depends upon Galf residues as essential cell wall components.2 The biogenesis of Galf-containing glycoconjugates requires the precursor uridine 5′-diphosphogalactofuranose (UDP-Galf). This building block is produced from UDP-galactopyranose (UDP-Galp) by the action of UDP-galactopyranose mutase (UGM) (Figure 1). The gene encoding UGM (glf) is essential for mycobacterial viability,3 indicating that Galf-containing glycoconjugates are necessary components of the mycobacterial cell wall. Moreover, Galf residues are absent from mammalian glycoconjugates, and UGM inhibitors can block mycobacterial cell growth.4 These observations underpin the appeal of UGM as a therapeutic target.

Most inhibitors of UGM mimic the natural ligand, UDP-Gal. Simple sugar derivatives, including Galp or Galf analogs, can serve as inhibitors, but their activities are

Figure 1. The reaction catalyzed by UGM.

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Inhibitors that incorporate the uridine portion of the substrate bind substantially better, with affinities that approximate that of UDP-Galp ($K_d \approx 50 \mu M$).\(^5\) Recently, we and others have identified several non-substrate-based molecules as ligands.\(^4\)–\(^10\) These compounds bind much more tightly ($K_d = 2–3 \mu M$). Moreover, we showed that UGM inhibitors can block mycobacterial growth. Insight into the factors that lead to effective UGM ligands could guide the development of yet more potent inhibitors.

Our efforts to generate high-affinity UGM ligands were informed by our previous design of a fluorescent UGM ligand.\(^1\) We concluded that the UDP moiety of the substrate contributes the majority of the binding energy,\(^9\) and subsequent studies provide additional support.\(^6,11\) Accordingly, we tethered a fluorophore to UDP through the diphosphoryl linkage of derivatives in which the linker was varied systematically. We tethered the fluorophore to the nucleotide portion of the substrate bind substantially better, with affinities that approximate that of UDP-Galp ($K_d \approx 50 \mu M$).\(^5\) Recently, we and others have identified several non-substrate-based molecules as ligands.\(^4\)–\(^10\) These compounds bind much more tightly ($K_d = 2–3 \mu M$). Moreover, we showed that UGM inhibitors can block mycobacterial growth. Insight into the factors that lead to effective UGM ligands could guide the development of yet more potent inhibitors.

Table 1. Dissociation Constants for the Complexes of UGM and UDP-Fluorescein Conjugates 3a–3e (Scheme 1)

<table>
<thead>
<tr>
<th>compound</th>
<th>$K_{d}$ (µM)</th>
<th>$K_{d,m}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>3a</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>3b</td>
<td>1.9</td>
<td>2.5</td>
</tr>
<tr>
<td>3c</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>3d</td>
<td>0.045</td>
<td>0.054</td>
</tr>
<tr>
<td>3e</td>
<td>0.070</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Figure 2. Relative affinity of conjugates 3a–3e for UGM. The affinities are shown relative to that of UDP (1). An increase is observed as the alkyl linker separating the UDP and fluorescein moieties is extended.

Scheme 1. Synthesis of UDP-Fluorescein Conjugates

Figure 3. Relative affinity of conjugates 3a–3e for UGM. The affinities are shown relative to that of UDP (1). An increase is observed as the alkyl linker separating the UDP and fluorescein moieties is extended.

of derivatives 3a–3e for either UGMkleb or UGMmyco vary with linker length. Compound 3a, with the short two methylene linker, binds poorly ($K_d > 30 \mu M$); the next compound in the series, 3b, is slightly more potent (5- to 10-fold) than UDP. Conjugate 3c, which was used in our initial fluorescence polarization-based screen, binds about 100-fold better. The compound with the eight-methylene linker, 3e, binds about 100-fold better than UDP. The compound with the eight-methylene linker, 3e, binds about 100-fold better than UDP.
linker, 3d, had the highest affinity: it is 300-fold more potent than UDP. The separation afforded by the eight-methylene linker seems to be optimal for binding. The affinity of 3e (ten-methylene linker) for UGM is similar to that of 3d. Given the influence of linker length on affinity, we determined UGM inhibition constants (IC₅₀ values) for the ligand series. A similar trend was observed for 3b–3d, with 3d displaying the greatest inhibitory potency against UGMₘｙｃｏ and 3b the least (see Supporting Information). The dependence of binding affinity on linker length suggests that there is a subsite that the fluorophore occupies and that ligands that can exploit this subsite are highly potent.

Because the activities of compounds 3a–3e are influenced dramatically by the linker, we explored its importance. First, we tested the contribution of the linker alone. Uridine diphosphate derivative 2d, with the eight-methylene linker but no fluorophore, has an affinity for UGM that is comparable to that of UDP. These data indicate that the linker has little influence on binding in the absence of the fluorophore. To test whether the chemical composition of the linker contributes to affinity, we synthesized conjugates in which the UDP and fluorescein groups were tethered using oligo(ethylene glycol) linkers. The amino alcohols derived from tri(ethylene glycol) and tetra(ethylene glycol) were converted to UDP-fluorescein conjugates 4a and 4b using the route outlined in Scheme 1 (Figure 3). For UGMₘｙｃｏ, compound 4a, with an eight-atom linker, bound with affinity 20-fold greater than that of UDP. Still, its affinity was more than 10-fold lower than that of the conjugate with the eight-methylene linker (compound 3d). A similar trend was observed with UGMₖｌｅｂ. The affinity difference for the oligo(ethylene glycol) and the corresponding alkyl conjugate could arise from altered conformational preferences. Specifically, the conformation of an oligo(ethylene glycol) linker will be influenced by the gauche effect. Nevertheless, conjugate 4b, with a longer oligo(ethylene glycol) linker, did not exhibit improved binding to either UGMₖｌｅｂ or UGMₘｙｃｏ. Thus, the affinity differences cannot be attributed solely to alterations in conformational preferences. We hypothesize that the alkyl linker gives rise to more favorable binding because it can pack against the fluorophore. Whatever the mechanism, the data indicate that the linker plays a role in the binding of UDP-fluorescein compounds to UGM.

To investigate whether binding of the fluorophore alone could be detected, we synthesized fluorescein derivative 5 (Figure 4). Interestingly, no binding to UGMₘ𝑦ｃｏ or UGMₖ𝑙ｅｂ was observed, even in the presence of high concentrations of UDP. This result indicates that the UDP moiety and the fluorophore must be linked.

Because the fluorescein group is an extended aromatic system, we investigated whether the attachment of other aryl substituents to UDP also would afford potent UGM ligands. Specifically, we appended UDP-octanolamine 2d to naphthyl isothiocyanate to form conjugate 6 (Figure 5). The affinity of compound 6 for UGM was 25-fold greater than that of UDP. These results indicate that the naphthyl group can occupy the subsite we identified.

Our findings led us to test whether we could exploit the putative subsite by elaborating UGM ligands that are not
direct substrate mimics. We recently showed that 2-aminothiazoles function as competitive inhibitors of UGM.9 We postulated that appending a fluorophore to this core could afford an extremely potent UGM ligand. For modification, we selected 2-aminothiazole7 with an affinity of 15 µM4 as a starting point. On the basis of our model for inhibitor binding,4 we reasoned that a fluorescein group could be introduced by converting the pentyl group into a tether.

As a precursor to an aminothiazole with the desired attributes, we synthesized compound 9, which bears an eight-methylene linker terminating in an azide (Scheme 2), and converted it into fluorescein derivative 10. The synthesis of aminothiazole 10 began with a Friedel–Crafts acylation of 8-phenyl-1-octanol to produce the acetophenone.17 The alcohol was converted to the azide through the mesylate followed by α-bromination with CuBr₂ to provide 8.18 The α-bromoketone 8 was cyclized with the thiourea derived from 4-chlorophenylalanine to generate 2-aminothiazole 9.4 The azide was reduced with catalytic hydrogenation to afford the amine, which was modified with fluorescein isothiocyanate (FITC). The resulting fluorescein conjugate 10 has a Kᵣ of 0.38 µM for UGMkleb and 0.30 µM for UGMmyco. Thus, substituted aminothiazole 10 binds 40- to 50-fold more tightly than does thiazole 7. The IC₅₀ value of 3.5 µM for 10 with UGMmyco (Supporting Information) indicates it is one of the most potent inhibitors described to date.4

In summary, we have identified a subsite present on the UGM from K. pneumoniae and M. tuberculosis. This subsite can be exploited to afford potent UGM inhibitors. Indeed, the compounds described herein are the most effective UGM ligands reported; their dissociation constants approach 50 nM. We anticipate that our findings will facilitate the design and optimization of potent, cell permeable UGM inhibitors for use as antimycobacterial agents and probes of the role of UGM in a wide range of pathogenic microorganisms.

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Supporting Information Available: Detailed experimental procedures, including binding curves, and compound synthesis. This information is available free of charge via the Internet at http://pubs.acs.org.