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Design and synthesis of substrate-mimic inhibitors of mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis*

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Abstract—The Staudinger reaction between a polymer-supported triphenylphosphine reagent and pseudo-disaccharide azides is successfully applied to synthesize a variety of substrate-mimic mycothiol analogs. Screening of this new group of analogs against the mycobacterial detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) yielded several modest inhibitors (IC_{50} values around 50 μM) and provided additional structure–activity relationships for future optimization of inhibitors of MCA and its homologs.

Mycothiol (MSH, **1**, Fig. 1) is a low molecular weight thiol[1–3] found exclusively in actinomycetes,[4] a group that includes the pathogen *Mycobacterium tuberculosis*. Mycothiol functions analogously to glutathione in Gram-negative bacteria and eukaryotes and plays an important role as a detoxifying agent by covalently binding to toxins, electrophiles, and drugs to form mycothiol-*S*-conjugates (MSE).[5] This conjugate subsequently is cleaved at the amide bond by a detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) to yield a mercapturic acid conjugate that is ultimately exported from the cell.[5] Mycothiol also is involved in maintaining a redox equilibrium within the cell that is critical for a reducing cellular environment.[4]

The absence of MSH in eukaryotes and Gram-negative bacteria, along with the compelling evidence for increased sensitivity of MSH-deficient mutants toward electrophiles, free radicals, and antibiotics[6], suggest that enzymes involved in MSH biosynthesis and MSH-dependent detoxification (namely MCA and biosynthetic enzymes *MshA–MshD*) are potential targets for new classes of antibiotics. Moreover, because inhibitors of these enzymes may lead to new classes of compounds that target with some specificity to mycobacteria, they are excellent choices for rational drug design and screening of small molecule inhibitors.

Previously, we and others have reported the total synthesis of MSH and mycothiol bimane (Fig. 1, MSmB)[7,8] which are key for performing structural studies of substrates and inhibitors.[9] In addition, we have identified natural products and natural product-like synthetic inhibitors that have shown inhibitory activities against MCA.[10–13] Here, as part of our ongoing effort to discover new generations of anti-mycobacterial agents, we report synthesis and evaluation of novel substrate-mimic inhibitors of MCA built upon a quinic acid-derived scaffold.

Our synthetic strategy to produce MSH-inspired substrate-mimic analogs centered on incorporation of a quinic acid template to replace the inositol ring of natural MSH. This strategy is attractive for a number of reasons: incorporation of quinic acid circumvents the otherwise laborious and low-yielding transformations to obtain optically pure *myo*-D-inositol-containing analogs;[14,15] quinic acid is commerically available as a single isomer; it can be coupled to a glycosyl donor with high efficiency to produce a variety of analogs; and the presence of a relatively unreactive tertiary hydroxyl group as a site of attachment for linkers makes the synthetic scheme amenable to parallel synthesis.

Retrosynthetic analysis (Scheme 1) of quinic acid-containing analogs led us to envision the use of protected acceptor **3**, readily derived from quinic acid; and glycosyl donor **5** that could be obtained from D-mannopyra-

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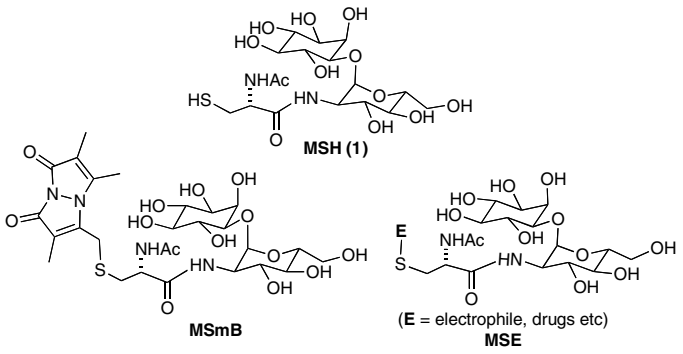
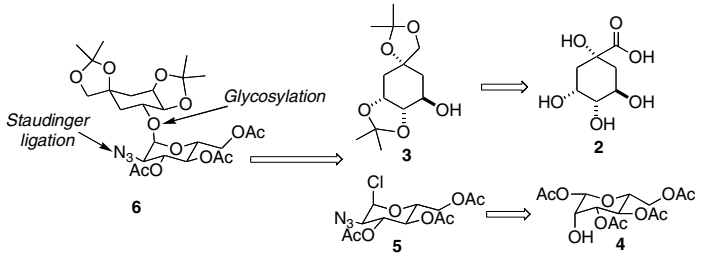


Figure 1. Mycothiol (**1**, MSH), MCA substrate mycothiol bimane (MSmB), and mycothiol-*S*-conjugate (MSE).

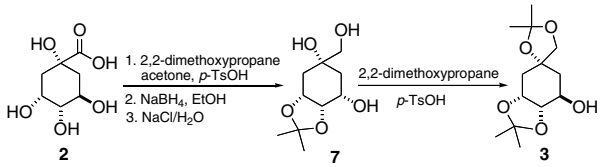


Scheme 1. Retrosynthetic analysis.

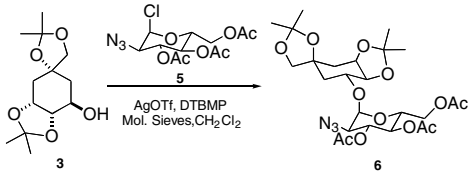
nose derivative **4**. Glycosylation of **3** with **5** would then furnish the core structure **6** for subsequent Staudinger amidation to produce diverse mycothiol analogs.

The forward synthesis of target analogs proceeded with preparation of glycosylation counterparts **3** and **5**. Quinic acid was transformed to protected acceptor **3** in four steps according to published procedures (Scheme 2).[16,17] Treatment of acid **2** with 2,2-dimethoxypropane under acidic conditions gave the protected lactone that upon reduction with $NaBH_4$ gave triol **7**. Subsequent protection of the vicinal diol of **7** provided compound **3** in 65% overall yield.

Glycosyl donor 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride (**5**) was obtained starting from commercially available 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (**4**) (Scheme 3). Following published protocols,[7,18] compound **4** was converted to its corresponding triflate by treatment with Tf_2O in Py/CH_2Cl_2, and the triflate was reacted with sodium azide to give **8**



Scheme 2. Preparation of acceptor **3**.

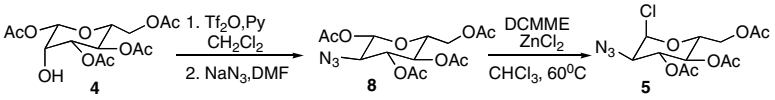


Scheme 4. Glycosylation reaction.

with inversion of configuration at C-2 (Scheme 3). Treatment of **8** with α,α-dichloromethyl methyl ether (DCMME) and $ZnCl_2$ in refluxing chloroform gave glycosyl chloride **5** in good yield (Scheme 4).

The reaction between acceptor **3** and donor **5** was effected by activation with silver triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane to give pseudo-disaccharide **6** with high α selectivity (5:1 α/β ratio) and 71% yield.[19]

Inspired by recent reports describing the use of a polymer-supported triphenylphosphine reagent for derivatization of azides, we explored a similar method to derivatize **6** efficiently.[20–22] Thus, azido pseudo-disaccharide **6** was treated with polymer-supported triphenylphosphine reagent followed by in situ trapping of the iminophosphorane intermediate with 14 varied acid chlorides (Scheme 5).[23] This sequence produced the



Scheme 3. Synthesis of donor **5**.