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Intramolecular α-Glucosaminidation: Synthesis of Mycothiol

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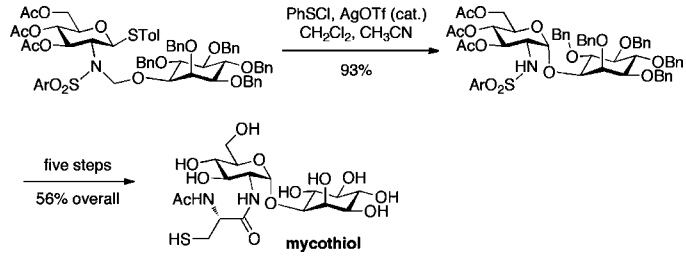
ABSTRACT



A protected cyclitol aglycon was tethered to an (*N*-arylsulfonyl)glucosamine donor by a methylene linker; the exclusively α-selective intramolecular glycosyation reaction was then initiated by electrophilic activation of the thioglycoside donor portion. Further transformations of the glycosylation product to give the *M. tuberculosis* detoxifier mycothiol and its oxidized congener, the disulfide mycothione, are detailed.

Two million people die annually from tuberculosis (TB), and an estimated 8 million develop the disease each year. Because *Mycobacterium tuberculosis*, the TB causative agent, has increasingly developed resistance to many treatments, most of which are largely ineffective against the dormant state, a better understanding of the organism's defense mechanisms is critical to battling infections.[1,2] Mycothiol (**1**, Figure 1)[3] is the low molecular weight thiol used by mycobacteria as their main line of defense against foreign electrophilic agents such as radicals, oxidants, and drugs;[4,5] glutathione plays an analogous role in eukaryotes, which lack **1** altogether. Disruption of mycothiol metabolic pathways[6,7] is fatal to *M. tuberculosis*, so this is a promising avenue for the development of new treatments.[8] The efficient synthesis of **1** and its congener disulfide, mycothione **2**, as well as simpler analogues, could contribute significantly to these efforts.[9−12]

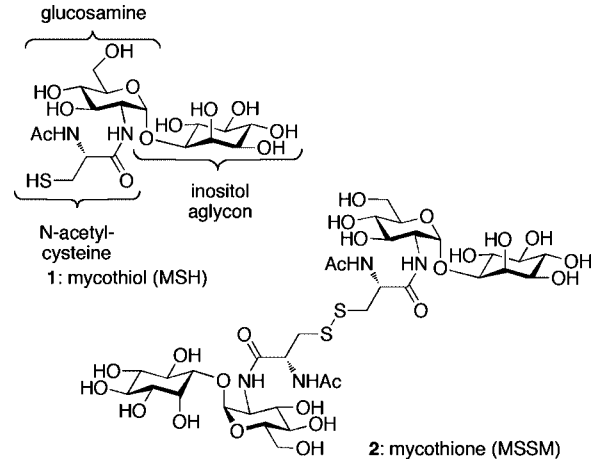


Figure 1. Structures of mycothiol and its disulfide.

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(2) Xie, Z.; Siddiqi, N.; Rubin, E. J. *Antimicrob. Agents Chemother.* **2005**, *49*, 4778–4780.

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Forging the (α) *cis*-1′,2′ glycosidic linkage of **1** is the principal synthetic challenge.[13] Where complex glycosyl donors and acceptors are required, an *intramolecular* glycosylation process[14] (Figure 2) offers several advantages:

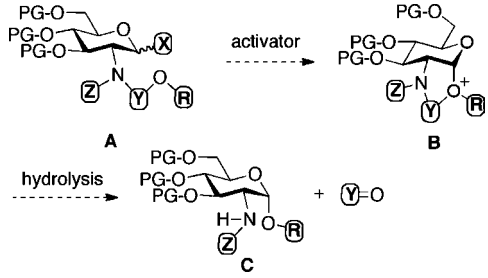
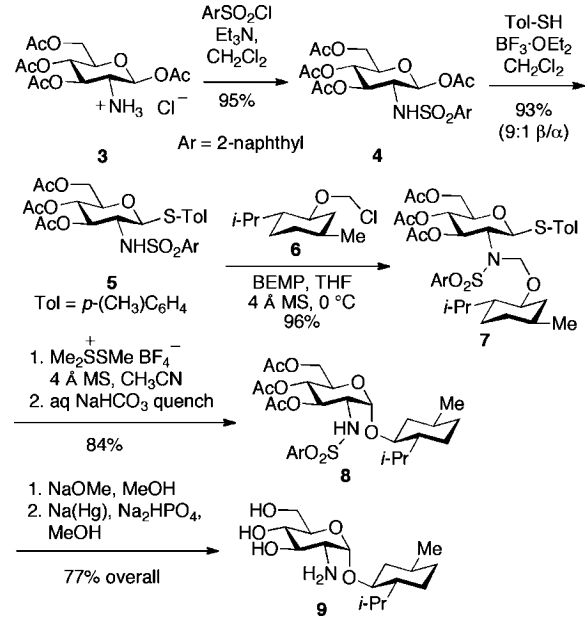


Figure 2. Intramolecular glucosamidation featuring a tether **Y**, an N-protecting group **Z**, and an activatable leaving group **X**.

the donor−acceptor stoichiometry is 1:1, the tether enforces *syn* delivery of the aglycon (O**R**), and the bond formation can potentially be accomplished quite efficiently and to the near exclusion of nonproductive donor fates such as elimination or hydrolysis. While no intramolecular α-glucosaminidation has yet been demonstrated,[15] careful selection of a short and temporary tether **Y**, an easily removable amine protecting group **Z**, and a leaving group **X** that can be activated under mild conditions ought to direct the desired glycosidic bond formation. We are pleased to report the realization of these objectives, and additionally, the efficient transformation of the glycosylation product to **1** and **2**.

1,3,4,6-Tetra-*O*-acetyl-*β*-D-glucosamine[16] (**3**, Scheme 1) was converted[17] to the 2-naphthalenesulfonamide **4** and then

Scheme 1. Model Intramolecular α-Glucosaminidation



further[18] to the *p*-tolyl thioglycoside **5**. Menthol was selected as a model aglycon; coupling of **5** with (+)-menthyl chloroformate (**6**) was particularly efficient in the presence of the phosphazene base 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP).[19] The intramolecular glycosylation of **7** was carried out by activating the thioglycoside with dimethyl(methylthio)sulfonium tetrafluoroborate.[20] Upon quench, the temporary methylene tether was lost, presumably as formaldehyde, and the (exclusively α) glucosaminide **8** was isolated in good yield. Acetate methanolysis followed by reductive removal[21] of the 2-naphthalenesulfonyl protecting group provided the amino triol **9**.

An analogous approach to the synthesis of **1** requires a protected cyclitol aglycon component, such as the penta-*O*-benzylinositol **10** (Scheme 2). Preparation of **10** followed

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(13) Recent glucosaminidation methods: Li, Y.; Yang, Y.; Yu, B. *Tetrahedron Lett.* **2008**, *49*, 3604–3608. Mensah, E. A.; Nguyen, H. M. *J. Am. Chem. Soc.* **2009**, *131*, 8778–8780. Yang, Y.; Li, Y.; Yu, B. *J. Am. Chem. Soc.* **2009**, *131*, 12076–12077.

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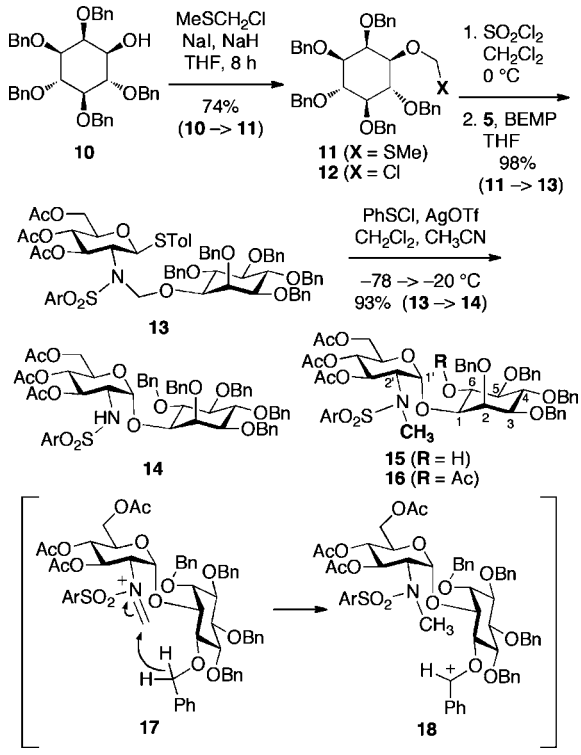
Scheme 2. Mycothiol Core and a 1 → 9 Hydride Shift



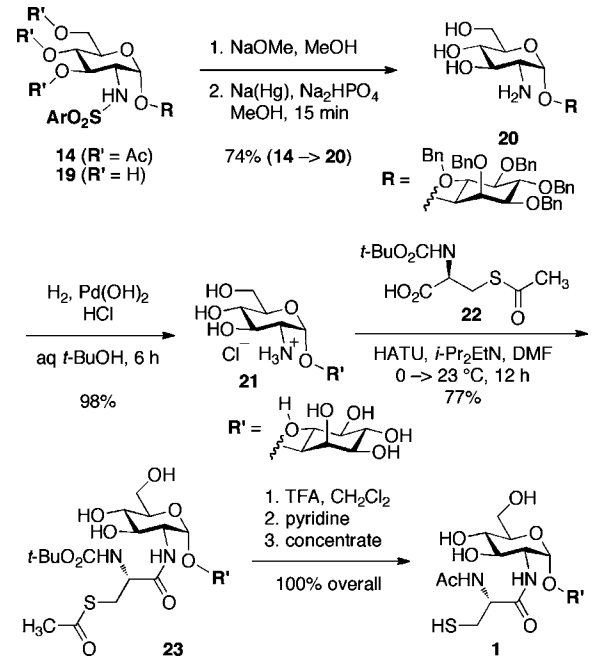
the known route,[22] which includes a resolution based on purification of its (+)-menthol mixed carbonate. Crystallographic analysis (see Supporting Information) confirmed the absolute configuration of the resolved material. The required chloromethyl derivative **12** was generated in situ[23] from methylthiomethyl ether **11**.[24] BEMP-mediated coupling with **5** was again successful and produced the glycosylation substrate **13**.

Use of dimethyl(methylthio)sulfonium tetrafluoroborate as before to trigger the intramolecular glycosylation led not to the desired glucosaminide **14**, but instead to the *N*-methylsulfonamide carbinol **15** (96%) in which one benzyl ether has been selectively cleaved and the methylene linker has been reduced. The structure of **15** was secured by NMR analysis of its acetate derivative **16**. In particular, the methine adjacent to -OH (H-6, $\delta = 4.09$, td, $J = 9.5$, 3.0 Hz) in **15** can be identified by COSY analysis and moves downfield ($\delta = 5.75$, t, $J = 10$ Hz) upon acetylation. An unusually facile 1 → 9 hydride shift[25] within the intermediate iminium ion **17**, followed by hydrolysis of the oxonium species **18**, accounts for this result.

The glycosylation reaction conditions were adjusted to include a good nucleophile (chloride ion) capable of intercepting **17** and blocking the hydride shift, particularly at lower temperatures. Although treatment of **13** with 1.5 equiv each of phenylsulfenyl choride and silver(I) trifluoromethanesulfonate[26] below −20 °C gave **15** only (hydride shift is rapid even at low temperature), the use of PhSeCl with *catalytic* AgOTf gave the desired glycoside **14** in high yield after the quench, with barely a trace of **15**.

Deprotection of the acetates and the 2-naphthylsulfonamide as before led smoothly to the amino triol **20** (Scheme 3).

Scheme 3. Completion of the Synthesis of Mycothiol



The selective reductive cleavage of the *N*-sulfonyl protecting group in the presence of five *O*-benzyl ethers is notable. Hydrogenolysis of these benzyls gave the amino octa-ol as its hydrochloride salt **21**. The amino group, upon liberation by base treatment, was coupled with *N*-Boc-*S*-acetylcysteine **22**,[11] and the resulting amide was deprotected on nitrogen by treatment with trifluoroacetic acid. Neutralization of the resulting ammonium trifluoroacetate salt induced S → N vicinal migration of acetyl, and upon simple concentration the natural product mycothiol (**1**) was obtained essentially quantitatively. When stored under an argon atmosphere, the mercaptan **1** is stable for weeks.

Analysis of **1** by ^{1}H, ^{13}C NMR, and exact mass methods and comparison of the values with those in the literature[9] (see Supporting Information) leave no doubt about its identity. Oxidative conversion of mycothiol to its disulfide mycothione (**2**, Scheme 4) was also quantitative, and similar analysis of **2** confirmed its identity as well. Finally, the amide

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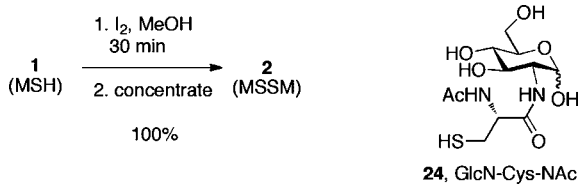
Scheme 4. Conversion of Mycothiol to Mycothione



24, GlcN-Cys-NAc

coupling method in Scheme 3 was applied to D-glucosamine (72% overall yield), resulting in the simplified mycothiol glycon, GlcN-Cys-NAc (**24**). Derivatives of **24** may prove useful in the development of inhibitors of mycothiol processing enzymes.

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Supporting Information Available: Experimental details and spectroscopic characterization for **1**, **2**, and all new compounds, and CIF file for the menthol carbonate of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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