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First Total Synthesis of Mycothiol and Mycothiol Disulfide

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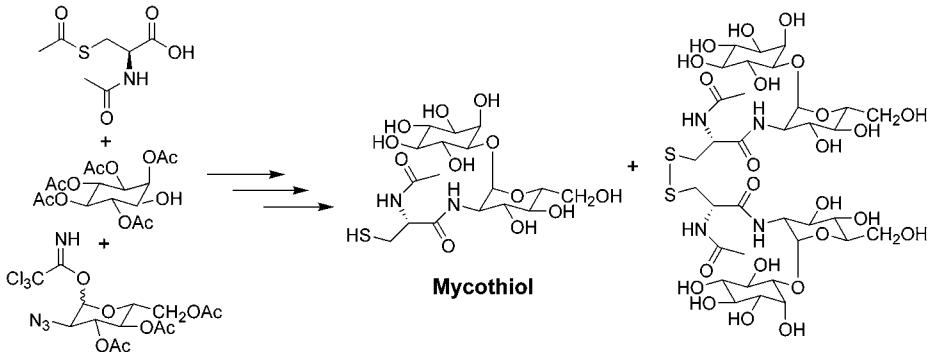
ABSTRACT



The first total synthesis of mycothiol and mycothiol disulfide was achieved by linking D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol, *O*-(3,4,6-tri-*O*-acetyl)-2-azido-2-deoxy-α,*β*-D-glucopyranosyl) trichloroacetimidate, and *N*,*S*-diacetyl-L-cysteine and deprotecting peracetylated mycothiol. The first full spectral characterization is reported for underivatized mycothiol. The structure of mycothiol was confirmed by spectral analysis of the known bimane derivative.

Mycothiol[1] is the major low molecular weight thiol found in most actinomycetes including mycobacteria and streptomycetes.[2] MSH has functional similarities to glutathione, the major thiol found in eukaryotes. MSH appears to maintain a reducing intracellular environment and serves as an antioxidant in MSH-producing organisms.[3]

The biosynthesis and metabolism of MSH have received considerable recent attention as a means of identifying potential new targets for antitubercular drug development.[2] The genes encoding for MSH biosynthetically relevant enzymes including glycosyltransferase (*mshA*),[4] GlcNAc-Ins deacetylase (*mshB*),[5] ATP-dependent Cys:GlcN-Ins ligase (*mshC*),[6] and acetyltransferase (*mshD*)[7] have been identified in *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. Three MSH-dependent enzymes have been identified so far. These include MSH-dependent formaldehyde dehydrogenase, which catalyzes dissimilatory conversion of formaldehyde to formic acid, from *Amycolatopsis methanolica*.[8] MSH disulfide reductase with high homology to glutathione reductase was identified from the *M. tuberculosis* genome.[9] MSH S-conjugate amidase purified from *M. smegmatis* plays a significant role in the detoxification of

(1) Mycothiol: 1-D-*myo*-inosityl 2-deoxy-2-(*N*-acetamido-L-cysteinamido)-α-D-glucopyranoside, MSH, or AcCys-GlcN-Ins.

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alkylating agents and antibiotics.[10] This enzyme cleaves amide bonds of MSH thiol conjugates formed by reactions between electrophiles with the free sulfhydryl group of MSH. Subsequently, cysteine S-conjugates are excreted from the cells leaving behind GlcN-Ins that is recycled for MSH biosynthesis.

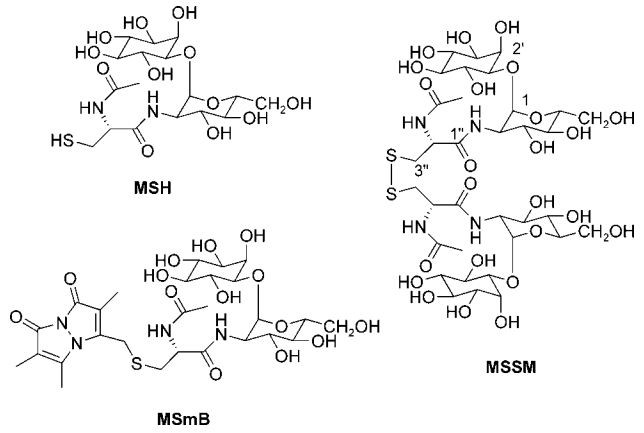


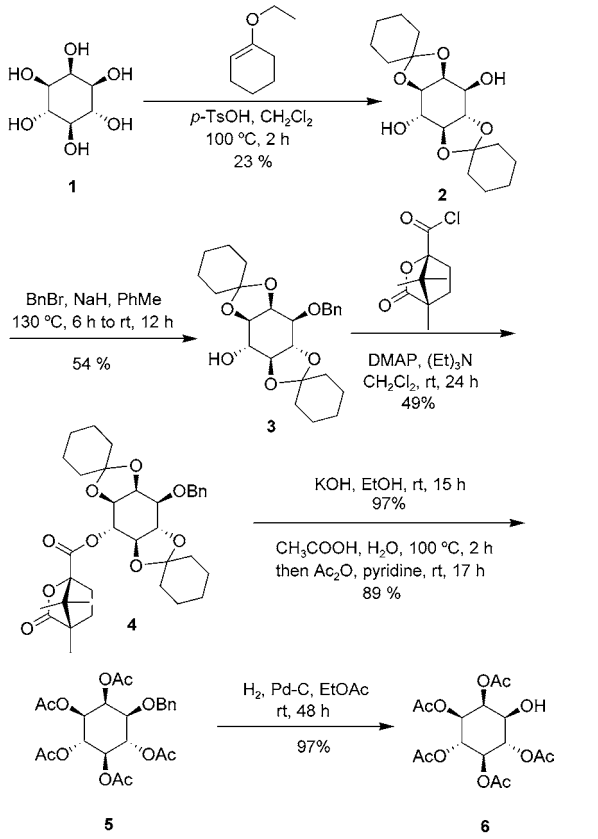
Figure 1. Mycothiol (MSH), the mycothiol disulfide (MSSM), and mycothiol bimane (MSmB).

MSH has been isolated as the disulfide (MSSM) or as the bimane derivative (MSmB) from *Streptomyces* sp. and *Mycobacterium Bovis*.[11−13] We also quantified and isolated MSH as the bimane derivative from *Nocardia* NRRL 5646 and confirmed the structure by NMR and mass spectrometry.[14] To date, efforts to isolate or synthesize free MSH have been elusive. Previous attempts to synthesize MSH and its analogues[15−18] used extensive protection and deprotection strategies. Moreover, α-glycosylation of D-*myo*-inositol and linkage of *N*-acetylcysteine to the resulting inositol−glucosamine pseudodisaccharide and deprotection to MSH have been problematic. Herein, we report the first total synthesis of MSH and MSSM including full spectral characterization of underivatized MSH.

Our approach to the GlcN-Ins component of MSH entailed coupling of the 1-OH of D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol **6** with *O*-(3,4,6-tri-*O*-acetyl)-2-azido-2-deoxy-α,*β*-D-glucopyranosyl) trichloroacetimidate **7**.[19]

Synthesis of **6** was pursued from D/L-*myo*-inositol via literature procedures with minor modifications (Scheme 1).[16,20,21]

Scheme 1. Synthesis of D-2,3,4,5,6-Penta-*O*-acetyl-*myo*-inositol



Treatment of **1** with 1-ethoxycyclohexene gave mixed, *myo*-inositol biscyclohexene ketals. The desired racemic 1,2:4,5-dicyclohexylidene-*myo*-inositol **2** was obtained by crystallization. Benzylation of **2** gave (±)-3-benzyl-1,2:4,5-dicyclohexylidiene-*myo*-inositol **3**. Benzylated D/L isomers of *myo*-inositol were separated as their diasteroisomeric camphanate esters. Confusion of identities of these isomers appears in earlier literature.[20,21] The identities of each isomer were unambiguously established as reported later.[22,23]

The more polar L-isomer was obtained directly by crystallization, whereas **4**, the less polar isomer, was chromatographically separated from mother liquors. Basic hydrolysis of the camphanate ester and ketal cleavage with acetic acid

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(14) Unpublished data: we have quantified and isolated MSH as the bimane derivative form *Nocardia* sp. NRRL 5646.
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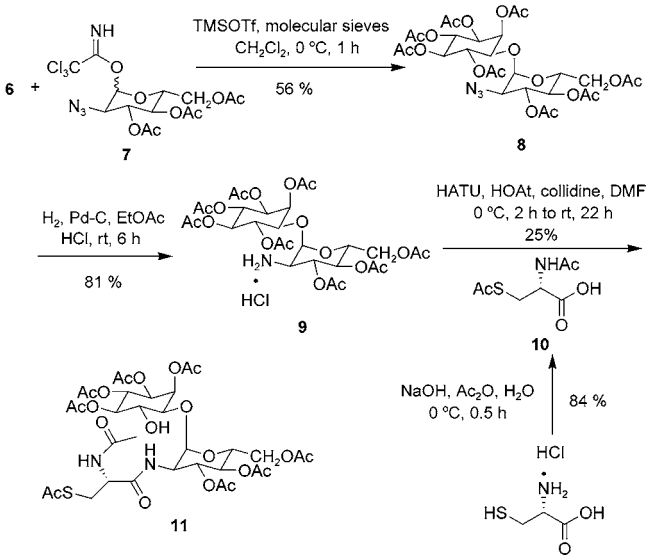
followed by acetylation gave D-1-benzyl-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol **5**. Debenzylation of **5** afforded D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol **6**.

Glycosidic linkage of **6** to **7** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a promoter gave an excellent 9:1 ratio of α and *β* anomers in 56% and 9% yields, respectively (Scheme 2). Reported synthesis of protected

Scheme 2. Conjugation of Protected D-*myo*-Inositol, D-Glucosamine, and L-Cysteine

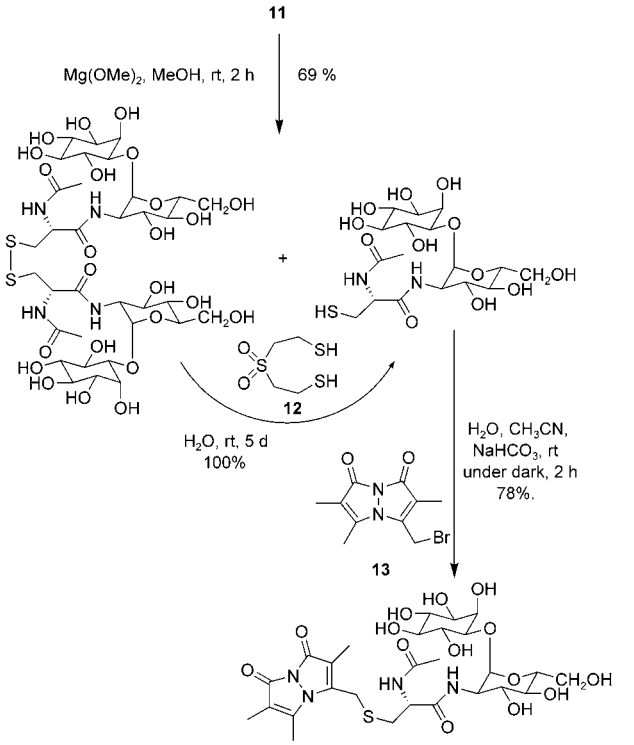


GlcN-Ins using 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenyl-amino)-α-D-glucopyranosyl bromide or 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride in the presence of silver triflate with different glycosylation acceptors gave lower yields of the desired α-anomer and less α,*β* anomeric selectivity compared with use of **7** as a glycosylation donor.[16,17] The reduction of **8** with Pd/C in the presence of HCl under atmospheric pressure gave protected amine hydrochloride **9** in 81% yield.

L-Cysteine hydrochloride was treated with Ac_2O to obtain *N*,*S*-diacetyl-L-cysteine **10**.[24] Reaction of **9** and **10** using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) with 1-hydroxy-7-azabenzotriazole (HOAt) and collidine gave protected MSH **11** (Scheme 2).[25] Carbodiimides such as EDC and DCC were used for amidation of **9** with **10**. However, they caused extensive epimerization at position 2″ of *N*-acetyl-L-cysteine with lower yields.

Although $Mg(OMe)_2$ has not been used to selectively deacylate esters and thioesters while leaving amides intact,[26] this reagent was used in MeOH to deprotect **11** giving MSH and MSSM in 40% and 29% yields, respectively (Scheme 3).

Scheme 3. Synthesis of MSH, MSSM, and MSmB



MSSM was quantitatively reduced to MSH by treatment with bis(2-mercaptoethyl)sulfone (BMS) **12** in water for 5 days (Scheme 3).[27] Advantages of BMS in disulfide reductions versus use of Zn/acid, $NaBH_4$, or triphenylphosphine/HCl include its facile use in water at room temperature without acid and the ready removal of unreacted and oxidized BMS from reactions by EtOAc extraction. The structure of MSH was confirmed directly (MS, ^1H, ^{13}C, 2D NMR, and CD) and by matching spectral characteristics of the MSmB with the same compound isolated from *Nocardia* sp. and also those in the literature.[14,17,28] The major differences in NMR spectra between MSH and MSSM are observed in the ^1H-coupling constant (*J*) and ^{13}C-chemical shift values of the 2″ and 3″ positions of *N*-acetyl-L-cysteine (Supporting Information).

In conclusion, we have achieved the first total synthesis of MSH and MSSM by the linkage of the three moieties D-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol, *O*-(3,4,6-tri-*O*-acetyl)-2-azido-2-deoxy-α,*β*-D-glucopyranosyl) trichloroacetimidate, and *N*,*S*-diacetyl-L-cysteine and the subsequent deprotection of peracetylated MSH. Furthermore, the first full spectral characterization is reported for underivatized MSH and its structure was confirmed by making the known MSH deriva-

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(28) All spectral data (^1H, ^{13}C NMR MS and CD) for synthetic MSmB were identical with natural MSmB from *Nocardia* NRRL 5646 purified by our laboratory.[14] Reported specific rotations of MSmB[17] were concentration-dependent and variable ($[\alpha]^{20}_D = +10$ (*c* = 0.5 in H_2O), $[\alpha]^{20}_D = -12$ (*c* = 0.1 in H_2O)). Our synthetic and natural MSmB both had $[\alpha]^{25}_D = +26$ (*c* = 0.1 in H_2O).

tive, MSmB. We also have shown that MSSM can be conveniently reduced to MSH with BMS. The synthetic MSH and MSSM could help in better understanding of the roles of MSH in organisms producing it.

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Supporting Information Available: Experimental procedures and spectroscopic data of the key intermediates including MSH, MSSM, and synthetic MSmB. This material is available free of charge via the Internet at http://pubs.acs.org.

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