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Desymmetrization of 2,4,5,6-Tetra-*O*-benzyl-D-*myo*-inositol for the Synthesis of Mycothiol

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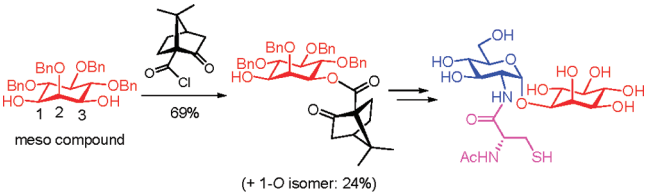
ABSTRACT



An efficient chemical synthesis of mycothiol involving the regioselective ketopinyl desymmetrization of 2,4,5,6-tetrabenzylated D-*myo*-inositol as the key step is described. Together with a highly α-stereoselective D-glucosaminylation, the whole procedure was accomplished in eight steps with an overall yield of 40%.

Low molecular weight thiols safeguard various organisms against toxic oxidants and offer a reducing environment necessary for effective cellular operation.[1] Mycothiol is the principal low molecular weight thiol in actinobacteria,[2] a diverse group of Gram-positive, high-G+C microorganisms that include notable genera as *Mycobacterium* and *Streptomyces*. Similar to glutathione, its functional analog in eukaryotes and Gram-negative bacteria, the sulfhydryl group of mycothiol is acquired from cysteine of which the carboxyl and amino groups are blocked to avoid autoxidation.[3] In mycothiol (**1**), the cysteinyl moiety is *N*-acetylated and is attached as an amide to D-glucosamine (GlcN), which, in turn, is α1′→1-linked to D-*myo*-inositol (Ins) (Figure 1). Mycothiol disulfide (**2**) is produced upon reaction with oxidants but is rapidly recycled back by mycothiol disulfide reductase to **1** preserving a high thiol/disulfide ratio within the confines of the cell.[4] The sulfhydryl group could also attack electrophilic xenobiotics leading to an *S*-conjugated mycothiol (exemplified by the bimane derivative **3**), which is further cleaved at the D-glucosaminyl amide bond by mycothiol-*S*-conjugate amidase to form the cysteine-bound toxin that is excreted out of the cell and the GlcN-Ins pseudodisaccharide that is reused as a substrate for mycothiol biosynthesis.[5] Mycobacterial strains depleted of mycothiol have shown a dramatic increase in susceptibility to oxidative stress and some antitubercular agents.[2a,6] As tuberculosis necessitates extensive treatment coupled with its lethal combination with HIV infection and the emergence of multidrug-resistant strains of its causative agent, *M. tuberculosis*, exploration of mycothiol as an avenue for rational drug design may be critical in fighting the disease.

Mycothiol-based studies would benefit from the increased availability of this compound that is currently

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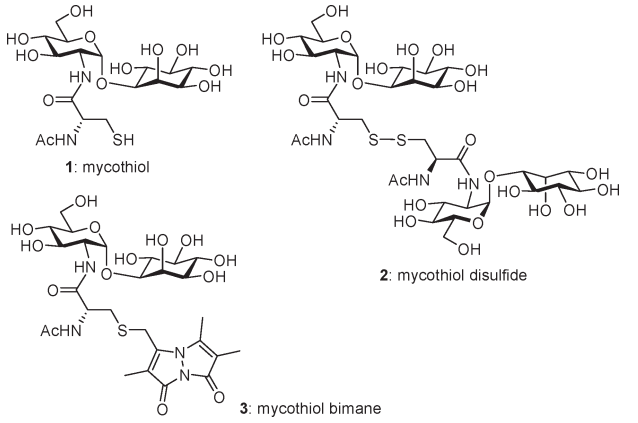
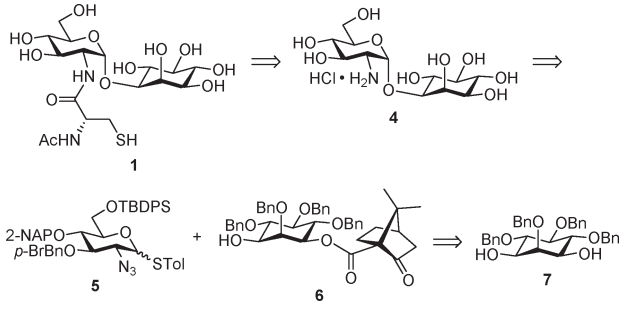


Figure 1. Structures of mycothiol (**1**), mycothiol disulfide (**2**), and mycothiol bimane (**3**).

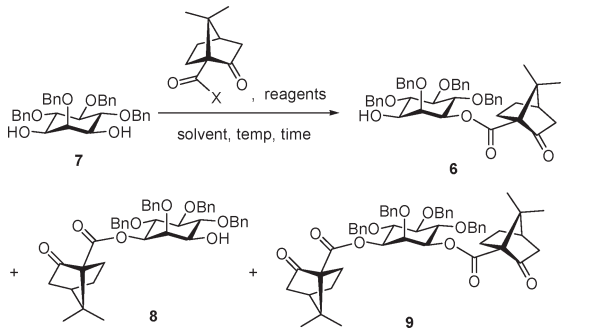
acquired routinely in ≤1.5 mg for every liter of the *M. smegmatis* cell culture.[7] Chemical synthesis,[8] on the other hand, is hampered by difficulties in the regioselective protection and desymmetrization of *myo*-inositol, the α-stereoselective glycosidic bond formation, and the epimerization-prone cysteine introduction. Given our experience with regioselective inositol desymmetrization[9] as well as the α-D-glucosamine formation for heparan sulfate synthesis,[10] we decided to apply these related approaches in mycothiol preparation. Scheme 1 illustrates our retrosynthetic plan. As a direct precursor of **1**, we envisioned that the pseudodisaccharide **4** could be generated from the thioglycoside **5** and the 3-ketopinate **6**. The protecting group combination of the D-glucosamine-derived **5** is

Scheme 1. Retrosynthetic Analysis of Mycothiol (**1**)[a]



[a] 2-NAP: 2-naphthylmethyl, *p*-BrBn: *p*-bromobenzyl, Tol: toluenyl, TBDPS: *tert*-butyldiphenylsilyl.

Table 1. Desymmetrization of the Meso 1,3-Diol **7**



						yield (%)		
entry	X[a]	reagents	solvent	temp (°C)	time (h)	**6**	**8**	**9**
1	ketopinate	–	pyr.	4	4	35	19	10[b]
2	OH	EDC, DMAP	THF	4	4	27	23	7[c]
3	OH	EDC, DMAP	CH_2Cl_2	4	4	32	12	8[d]
4	Cl	Et_3N	CH_2Cl_2	4	4	63	22	0
5	Cl	Et_3N, DMAP	CH_2Cl_2	4	4	35	15	28[e]
6	Cl	Et_3N, DMAP	CH_2Cl_2	−40	24	65	26	0
7	Cl	–	pyr.	4	4	66	21	0
8	Cl	DMAP	pyr.	−40	24	69	24	0
9	Cl	DIPEA	CH_2Cl_2	4	4	60	32	0
10	Cl	DIPEA, DMAP	CH_2Cl_2	−40	24	63	31	0
11	Cl	Et_3N, DMAP	CH_3CN	4	4	30	27	11
12	Cl	Et_3N, DMAP	CH_3CN	−40	24	26	17	0

[a] 1.1 equiv of the (1*S*)-ketopinyl sources were used. [b] 30% of **7** was recovered. [c] 38% of **7** was recovered. [d] 44% of **7** was recovered. [e] 13% of **7** was recovered. EDC: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.

instrumental in assuring exclusive α-linkage in our preparation of heparan sulfate derivatives.[10b] Conversely, the 3-ketopinate **6** would be achievable by desymmetrization of the meso 1,3-diol **7**, which could be generated from the commercially available *myo*-inositol 1,3,5-orthoformate (Kishi's triol) in four known steps.[11] (1*S*)-Ketopinate was chosen from other chiral derivatizing agents (e.g., camphanic acid, *N*-tosyl-L-proline, and Mosher's acid) because its monoesters with **7** could be effectively separated by silica gel column chromatography.

We first examined various conditions for the desymmetrization of the 1,3-diol **7** (Table 1). The favorable formation of the 3-*O*-ketopinyl **6** over its 1-O counterpart (compound **8**) was apparent from the use of (1*S*)-ketopinic

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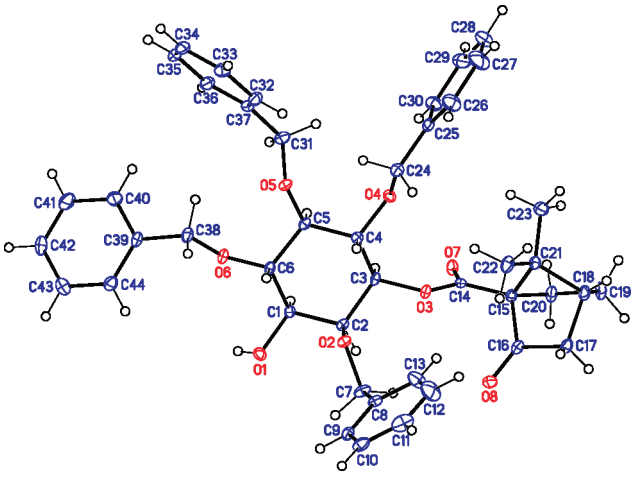


Figure 2. ORTEP drawing of compound **6**.

anhydride, (1*S*)-ketopinic acid, or (1*S*)-ketopinyl chloride as the acyl group source. The anhydride (entry 1) and the acid (entries 2 and 3), however, both gave low yields and modest regioselectivities for the desired compound **6** after 4 h of reaction at 4 °C. The regioselectivity improved remarkably upon the use of (1*S*)-ketopinyl chloride at 4 °C in the presence of pyridine as solvent (entry 7) or CH_2Cl_2 as solvent and either triethylamine (entry 4) or diisopropylethylamine (DIPEA) (entry 9) as base. The addition of catalytic DMAP reduced the regioselectivity of the 3-*O*-esterification at 4 °C (entry 5) but was effective in a slow reaction (entry 6, −40 °C, 24 h). Use of CH_3CN as solvent did not give good results. Overall, the best yield (69%, entry 8) and regioselectivity [3.1/1 (**6**/**8**), entry 7] leading to compound **6** were observed when pyridine was used as both the base and solvent. The absolute structure of **6** was confirmed by X-ray crystallographic analysis (Figure 2).

With compound **6** in hand, we proceeded to generate the pseudodisaccharide backbone of mycothiol (Scheme 2). Condensation of the alcohol **6** with the D-glucosaminyl donor **5** employing NIS and TfOH as promoters led exclusively to the α-glycoside **10** ($^3J_{1',2'}$ = 3.6 Hz) in 84% yield. This outcome affirms our recent report[10b] that the protecting group combination in **5** enables full α-stereoselectivity in glycosylation. Further NaOH-mediated cleavage of the ketopinyl group in **10** provided the 3-alcohol **11** in 95% yield.

We demonstrated regioselective D-mannosylation of a meso *myo*-inositol-derived 4,6-diol in our preparation of phosphatidylinositol dimannosides.[9a,b] In the present case, a direct glycosylation of diol **7** with donor **5** leading to compound **11** would shorten the synthetic process. The plausibility of this approach has been evaluated, and the results are outlined in Table 2. Unfortunately, the glycosylation revealed a higher preference for the 3-O position of the inositol moiety similar to the ketopinyl ester formation. The regioselective formation of the isomer **12** is more pronounced at low temperature (−60 °C) when

Scheme 2. Synthesis of the Pseudodisaccharide **11**

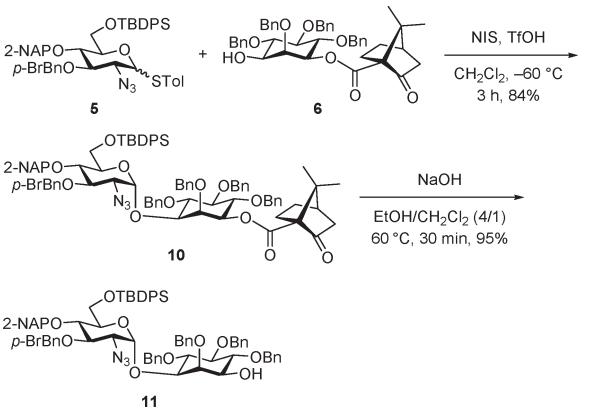
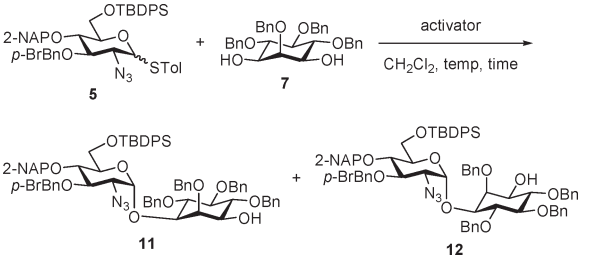


Table 2. Coupling of the D-Glucosamine-Derived Thioglycoside **5** with the Meso 1,3-Diol **7**



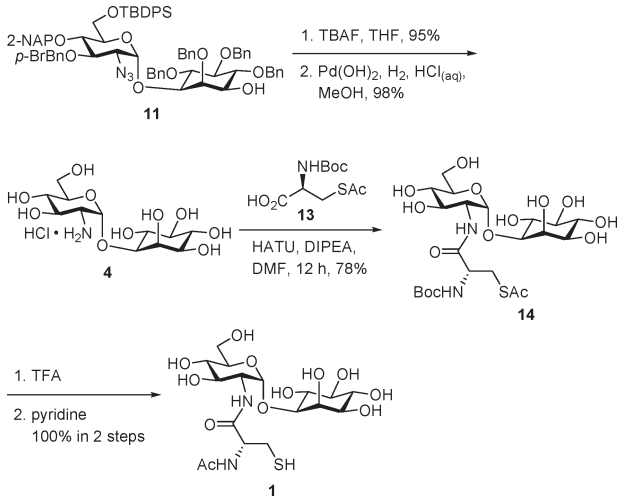
				yield (%)	
entry[a]	activator	temp (°C)	time (h)	**11**	**12**
1	NIS, TfOH	−60	3	30	58
2	NIS, TfOH	−60 → −20	3	41	49
3	NIS, TMSOTf	−60	3	35	57
4	NIS, AgOTf	−60	6	33	37[b]
5	BF_3•Et_2O	−78 → −20	6	29	34[c]

[a] Using 1.5 equiv of donor **5**. [b] 22% of **7** was recovered. [c] 20% of **7** was recovered.

activated by NIS in tandem with TfOH (entry 1) or TMSOTf (entry 3). As expected, the regioselectivity disappeared when the temperature was raised gradually to −20 °C (entry 2) providing the best yield of 41% for the desired **11**. Other activators, such as NIS/AgOTf (entry 4) and BF_3•Et_2O (entry 5), were also used, but no improvements were observed.

Scheme 3 displays the remaining steps toward the synthesis of mycothiol. Cleavage of the silyl group in the 3-alcohol **11** was carried out using TBAF (95%). Subsequent global hydrogenolysis with concomitant azido reduction [$Pd(OH)_2$, H_2] generated the pseudodisaccharide **4** in 98% yield. Coupling of compound **4** with the cysteine derivative **13** using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,

Scheme 3. Synthesis of Mycothiol (**1**)



N′-tetramethyluronium hexafluorophosphate (HATU) and DIPEA[8e] supplied the amide **14** (78%). Trifluoroacetic acid mediated deprotection of the amine group followed by pyridine treatment triggering S→N acetyl migration furnished the target compound **1** in a quantitative yield over two steps. The ^{1}H NMR spectrum of **1** conforms with previous reports.[8c,e]

In conclusion, we have developed an efficient protocol for the chemical synthesis of mycothiol. Our strategy relied on the regioselective ketopinyl desymmetrization of the meso inositol-derived 1,3-diol **7** and the fully α-stereoselective glycosylation with the D-glucosaminyl donor **5**. Starting from compound **7**, the procedure involved eight steps in an overall yield of 40%. Application of this method could ease the acquisition of mycothiol and mycothiol-related compounds for biological studies.

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Supporting Information Available. Experimental procedures, NMR (^{1}H and ^{13}C) spectra and crystallographic data for compound **6**. This material is available free of charge via the Internet at http://pubs.acs.org.