

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

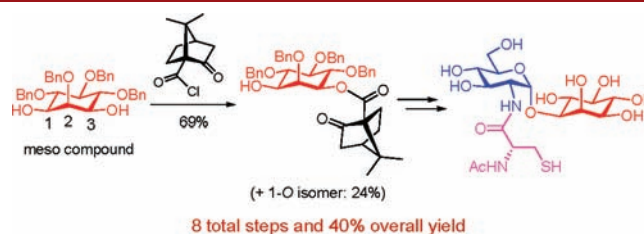
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Received August 16, 2011

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 α 1'→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 bimeane 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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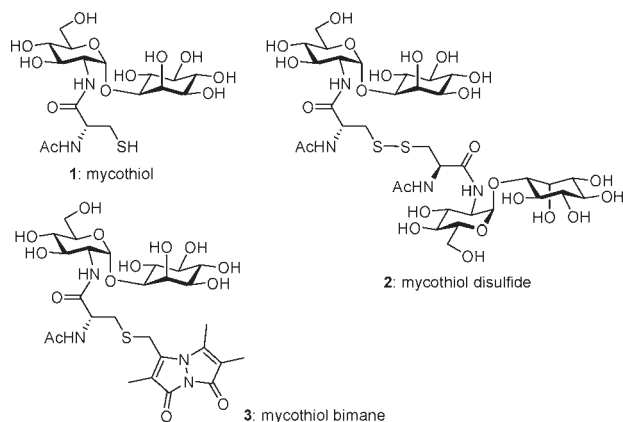
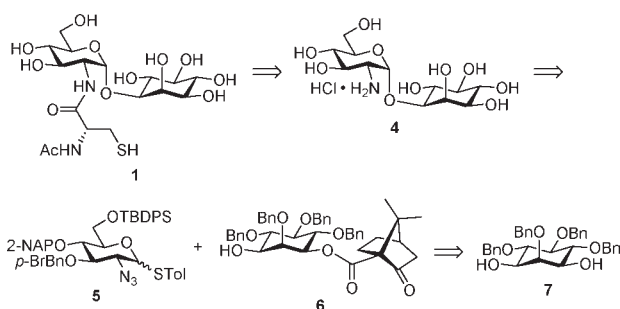


Figure 1. Structures of mycothiol (**1**), mycothiol disulfide (**2**), and mycothiol bimane (**3**).

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 desymmetrization⁹ 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

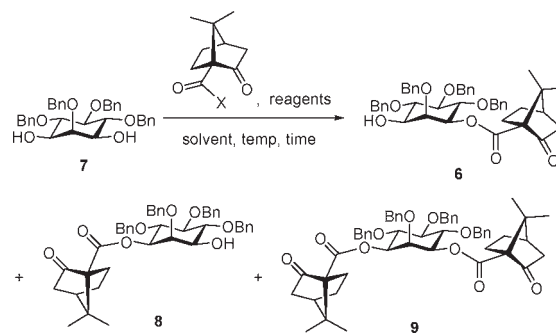
Scheme 1. Retrosynthetic Analysis of Mycothiol (**1**)^a



^a2-NAP: 2-naphthylmethyl, *p*-BrBn: *p*-bromobenzyl, Tol: toluenyl, TBDPS: *tert*-butyldiphenylsilyl.

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



entry	X ^a	reagents	solvent	temp time		yield (%)		
				(°C)	(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 ₂ Cl ₂	4	4	32	12	8 ^d
4	Cl	Et ₃ N	CH ₂ Cl ₂	4	4	63	22	0
5	Cl	Et ₃ N, DMAP	CH ₂ Cl ₂	4	4	35	15	28 ^e
6	Cl	Et ₃ N, DMAP	CH ₂ Cl 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 ₂ Cl ₂	4	4	60	32	0
10	Cl	DIPEA, DMAP	CH ₂ Cl ₂	–40	24	63	31	0
11	Cl	Et ₃ N, DMAP	CH ₃ CN	4	4	30	27	11
12	Cl	Et ₃ N, DMAP	CH ₃ CN	–40	24	26	17	0

^a1.1 equiv of the (1*S*)-ketopinyl sources were used. ^b30% of **7** was recovered. ^c38% of **7** was recovered. ^d44% of **7** was recovered. ^e13% 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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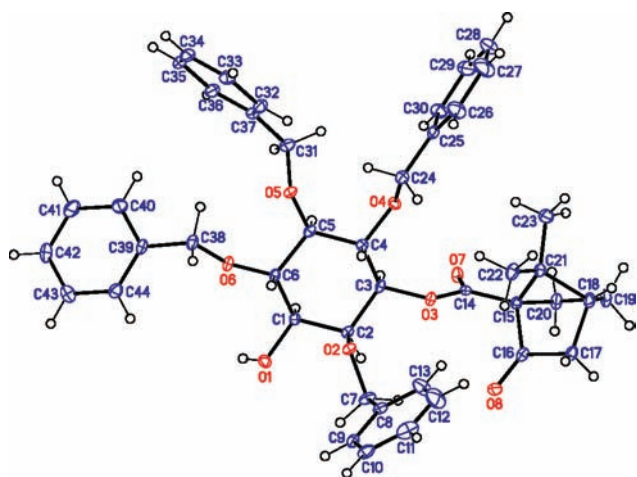


Figure 2. ORTEP drawing of compound 6.

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 CH₂Cl₂ 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 CH₃CN 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**

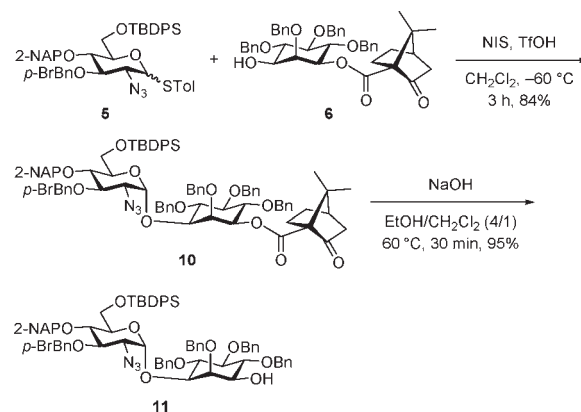
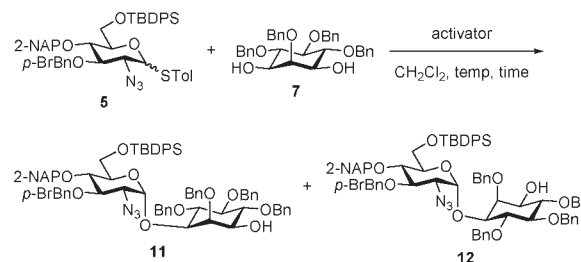


Table 2. Coupling of the *D*-Glucosamine-Derived Thioglycoside **5** with the Meso 1,3-Diol **7**

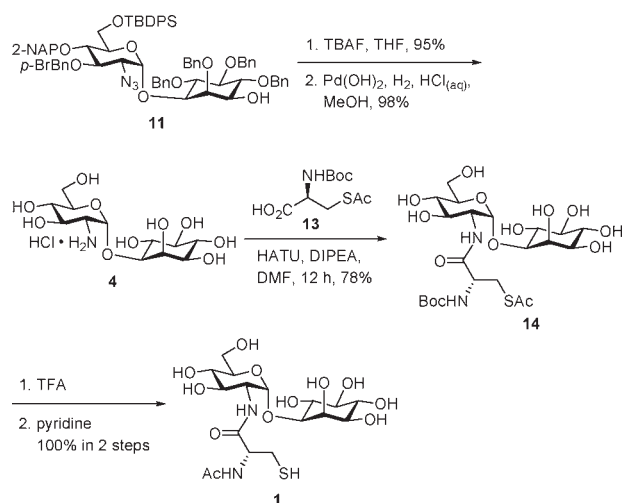


entry ^a	activator	temp (°C)	time (h)	yield (%)	
				11	12
1	NIS, TfOH	–60	3	30	58
2	NIS, TfOH	–60 → –20	3	41	49
3	NIS, TMSOTf	–60	3	35	57
4	NIS, AgOTf	–60	6	33	37 ^b
5	BF ₃ •Et ₂ O	–78 → –20	6	29	34 ^c

^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 BF₃•Et₂O (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, H₂] 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^{8c} 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 ¹H 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 ketopyl desymmetrization of the meso inositol-derived 1,3-diol **7** and the fully α -stereoselective glycosylation with the D-glucosaminy 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.

Acknowledgment. This work was supported by the National Science Council (NSC 97-2113-M-001-033-MY3, NSC 98-2119-M-001-008-MY2) and Academia Sinica.

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