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Synthesis of 1-D- and 1-L-*myo*-Inosityl 2-*N*-Acetamido-2-deoxy-α-D-glucopyranoside Establishes Substrate Specificity of the *Mycobacterium tuberculosis* Enzyme AcGI Deacetylase

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Abstract—Mycothiol (MSH, 1-D-*myo*-inosityl 2-(*N*-acetyl-L-cysteinyl)amido-2-deoxy-α-D-glucopyranoside) is the principal low molecular weight thiol in actinomycetes. The enzyme 1-D-*myo*-inosityl 2-*N*-acetamido-2-deoxy-α-D-glucopyranoside deacetylase (AcGI deacetylase) is involved in the biosynthesis of MSH and forms the free amine 1-D-*myo*-inosityl 2-amino-2-deoxy-α-D-glucopyranoside, which is used in the third of four steps of MSH biosynthesis. Here, we report the synthesis of two isomers of AcGI, which contain either 1-L-*myo*-inositol or 1-D-*myo*-inositol. These synthetic products were used to investigate substrate specificity of the *Mycobacterium tuberculosis* enzyme AcGI deacetylase.

Introduction

Mycothiol[1–3] [MSH, 1-D-*myo*-inosityl 2-(*N*-acetyl-L-cysteinyl)amido-2-deoxy-α-D-glucopyranoside] is the principal low molecular weight thiol produced only by actinomycetes. Analogous to glutathione, the major low molecular weight thiol in eukaryotes and Gram-negative bacteria, MSH plays a central role in detoxification and maintaining a reducing intracellular environment.[4] Because some of the enzymes involved in MSH biosynthesis and MSH-dependent detoxification are novel and limited to actinomycetes, which include pathogenic groups such as mycobacteria, interest in MSH biochemistry continues to rise.

In mycobacteria, biosynthesis of MSH is accomplished in four steps[5,6] by the protein products of genes termed *mshA-D* (illustrated in Scheme 1 and reviewed in ref 7). These include formation of the pseudodisaccharide 1-D-*myo*-inosityl 2-*N*-acetamido-2-deoxy-α-D-glucopyranoside (**1a**, AcGI) by the putative glycosyl transferase *mshA*, followed by deacetylation of AcGI by the enzyme 1-D-*myo*-inosityl 2-*N*-acetamido-2-deoxy-α-D-glucopyranoside deacetylase (*mshB* or AcGI deacetylase),[8] to form the free amine 1-D-*myo*-inosityl 2-amino-2-deoxy-α-D-glucopyranoside (GI).[9] Transfer of cysteine to GI by the cysteine transferase *mshC*[10] and then acetate to Cys-GI by the acetyl transferase *mshD*[11] completes MSH biosynthesis. Three of the four enzymes involved in MSH biosynthesis have been described[9–11] and include *mshB–D* leaving only *mshA* to be identified. In addition, the detoxification enzyme mycothiol-*S*-conjugate amidase (MCA)[12] and the mycothiol-disulfide reducing enzyme mycothione reductase[13] were the first mycothiol-related enzymes to be identified and characterized. Recently, we reported a total synthesis of the bimane derivative of mycothiol, mycothiol bimane (MSmB), which established the absolute stereochemistry of its three subunits as L-cysteine, D-glucosamine and 1-D-*myo*-inositol; and demonstrated specificity of recombinant *Mycobacterium tuberculosis* MCA for MSH possessing that stereochemistry.[14] In this paper, we describe the syntheses of two diastereomers of AcGI containing either 1-D-*myo*-inositol or 1-L-*myo*-inositol,

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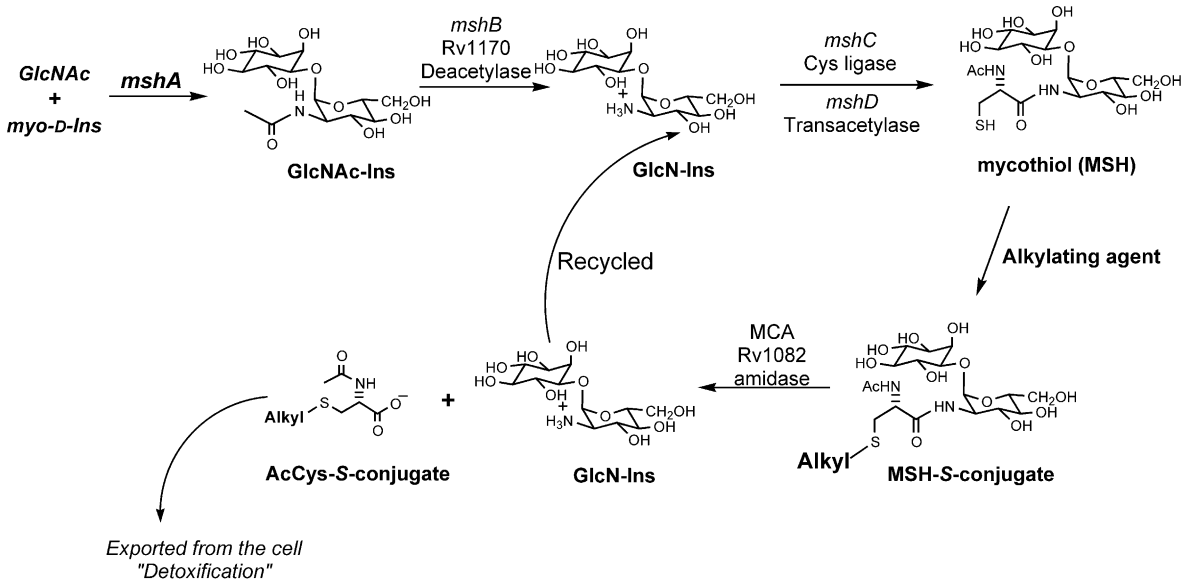
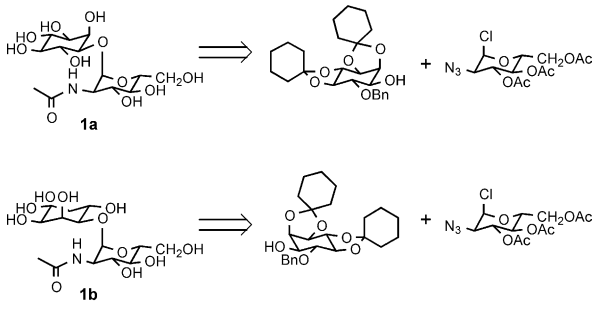


Scheme 1. Mycothiol biosynthetic scheme. With the exception of the first step carried out by the putative glycosyl transferase mshA (denoted in bold), the enzymes involved in each step of MSH biosynthesis and MSH-facilitated detoxification have been described. The *M. tuberculosis* gene products of Rv1170 and Rv1082 are the deacetylase mshB and the amidase MCA, respectively.[8]

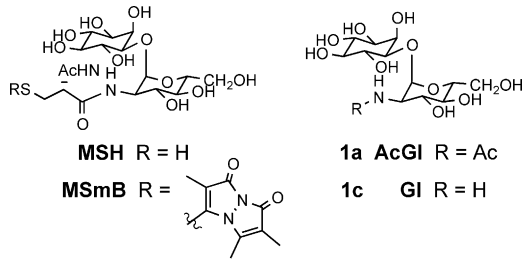
and demonstrate specificity of recombinant *M. tuberculosis* AcGI deacetylase (*mshB*) for the 1-D-*myo*-inositol-containing isomer, consistent with the absolute stereochemistry of MSH.

Chemistry

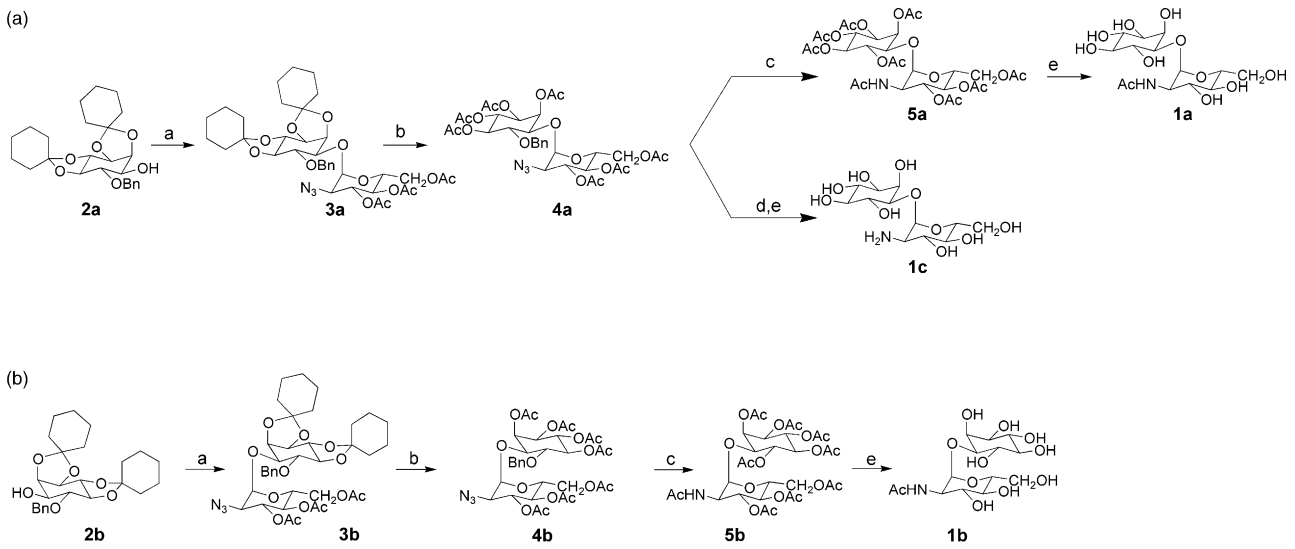
D-GlcNAc-α-(1→1)-*myo*-D-Ins (**1a**) and D-GlcNAc-α-(1→1)-*myo*-L-Ins (**1b**) were synthesized using the same strategy that we used previously for the synthesis of MSmB[14] (Scheme 2) which employs glycosylation of a protected inositol acceptor with an alpha-directing glucopyranosyl donor. Thus, 6-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-1-D-*myo*-inositol (**2a**) and 6-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-1-L-*myo*-inositol (**2b**) were prepared by following the general scheme used in the synthesis of galactinol.[15] Briefly, preparation of the protected L-isomer was accomplished by acylation of 2,3:4,5-di-*O*-cyclohexylidene-1-L-*myo*-inositol with (+)-menthol chloroformate, followed by benzylation of the remaining free hydroxyl at C-6.[15] Removal of the menthyl carbonate was achieved by reduction with lithium aluminum hydride to give the protected 1-L-*myo*-inositol acceptor **2b** in 96% yield. Preparation of the protected 1-D-*myo*-inositol acceptor **2a** was carried out as described previously.[14]



Scheme 2.



As shown in Scheme 3, glycosylation of **2a** and **2b** with 3,4,5-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride[16] in the presence of silver triflate and 2,6-di-*t*-butyl-4-methylpyridine gave a 2:1 ratio of α/β anomers in an approximate yield of 70% for both reactions. The desired α-anomers **3a** and **3b** from each glycosylation reaction (as determined by J_{H1-H2} values of 4 Hz for the α-anomers vs 10 Hz for the β-anomers) were isolated by column chromatography in 44 and 58% yields, respectively. Glycosylation with the 1-L-*myo*-inositol derivative **2b** gave a relatively higher yield of the α-anomer when compared to the 1-D-*myo*-inositol derivative **2a**, and required only two equivalents of 3,4,5-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride for the reaction to go to completion, as shown by TLC. Complete glycosylation of the 1-D-*myo*-inositol acceptor, on the other hand, required 3.8 equivalents of the azide. Deprotection of the cyclohexylidine groups (CSA, ethylene glycol), followed by acetylation (Ac_2O, pyridine) gave compound **4a** in 80% yield over the two steps, and **4b** in 76% yield. Debenzylation and concomitant reduction of the azide with palladium on carbon in the presence of dilute hydrochloric acid gave amine hydrochlorides that were subsequently acetylated to give amides **5a** and **5b** in respective yields of 70 and 51% over two steps. Deacetylation was achieved with



Scheme 3. Reagents (yields are given in the text): (a) 3,4,5-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride, silver (II) trifluoromethane sulfonate, 2,6-diisopropyl-4-methyl pyridine, CH_2Cl_2; (b) (i) ethylene glycol, (+)-camphor sulfonic acid, CH_3CN; (ii) Ac_2O, pyridine, ~80% over two steps; (c) (i) Pd/C, H_2, EtOAc; (ii) Ac_2O, pyridine; (d) Pd/C, H_2, EtOAc; (e) $Mg(MeO)_2$, MeOH.

$Mg(OMe)_2$,[17] and chromatography gave **1a** and **1b** in 63 and 66% yields, respectively.

Several milligrams of the free amine of **1a**, namely D-Gln-α-(1→1)-*myo*-D-Ins (**1c**), were also prepared for use as a standard in the enzyme assays. Thus, following hydrogenolysis of intermediate **4a** to give the intermediate amino alcohol heptaacetate (not shown in Scheme 3a), the reduced product was deprotected with $Mg(OMe)_2$ to give **1c** in approximately 50% yield following purification over silica gel.

Enzyme Activity

Recombinant AcGI deacetylase from *M. tuberculosis* was cloned and expressed in the form of a staphylococcal protein G–AcGI deacetylase fusion protein[18] (protG–AcGI, see Experimental). Because AcGI deacetylase can cleave the cysteinyl–glucosamine amide bond of mycothiol-*S*-conjugates to yield *N*-acetyl-Cys-*S*-conjugates and D-Gln-α-(1→1)-*myo*-Ins (**1c**),[9] initial characterization of protG–AcGI was accomplished using the substrate mycothiol bimane (MSmB) and fluorescence-detected HPLC methods that detect the extent of enzymatic cleavage of MSmB, as described previously.[12] Incubation of 30 or 100 μM MSmB solutions in the presence of 3.5 μg protG–AcGI for 20 min led to 59 and 26% cleavage, respectively, demonstrating activities consistent with those reported earlier for AcGI deacetylase from *M. tuberculosis*.[9]

To establish substrate specificity of AcGI deacetylase from *M. tuberculosis*, solutions of **1a** and **1b** (30 and 100 μM) were incubated in the presence of 3.7 μg recombinant AcGI deacetylase. Quantitated solutions of **1c** [D-Gln-α-(1→1)-*myo*-D-Ins] were used as standards. Following derivatization of the standard solutions and the reaction mixtures with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, the extent of deacetylation was measured by fluorescence-detected HPLC. Deacetylation of the 30 and 100 μM solutions of **1a** proceeded to 72 and 60%, respectively. In contrast, no detectable cleavage of the 1-L-inositol-containing isomer **1b** was observed under the same conditions. Deacetylation of **1b** was further attempted using 20 μg AcGI deacetylase; however, even in the presence of relatively high enzyme concentrations, no cleavage products were detected, thereby establishing the 1-D-*myo*-inositol-containing isomer as the natural substrate of AcGI deacetylase. These results are consistent with a separate study showing that D-Gln-α-(1→1)-*myo*-L-Ins was not a substrate for MSH biosynthesis by crude extracts of *Mycobacterium smegmatis*.[19]

Further characterization of the recombinant enzyme was carried out using time-course experiments to measure specific activities of the enzyme and to determine a range

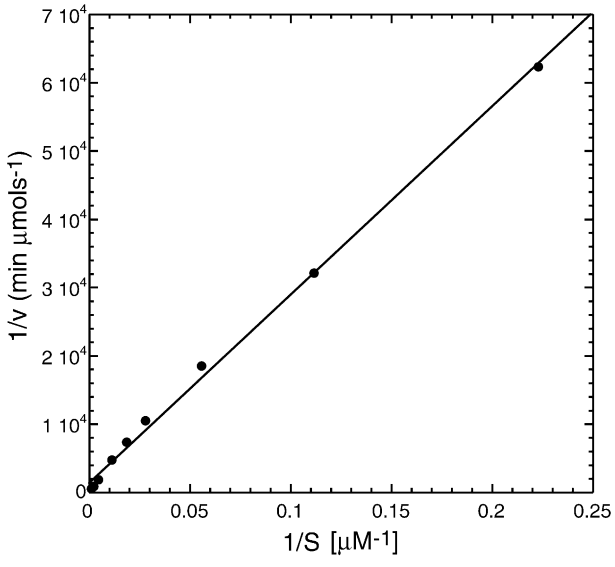


Figure 1.

of protein concentrations cleaving with linear velocities. Once established, experiments using multiple substrate concentrations (ranging from 5 to 1000 μM) in the presence of 3.5 μM protein were carried out. A double reciprocal plot of the data (shown in Fig. 1) yielded values for V_{max} and K_m of 0.68 nmol min^{-1} and 186 μM, respectively.[20] The specific activity under these conditions is 238 nmol min^{-1} mg^{-1} protein, in good agreement with previously reported values.[9]

Conclusions

We have described the synthesis of 1-D- and 1-L-*myo*-inosityl 2-*N*-acetamido-2-deoxy-α-D-glucopyranoside, and established specificity for the 1-D-inositol-containing isomer of the mycothiol biosynthetic enzyme AcGI deacetylase. In addition, we have shown that a recombinant ProtG–AcGI deacetylase fusion protein deacetylates D-GlcNAc-α-(1→1)-*myo*-D-Ins and cleaves MSmB at the scissile amide bond with specific activities comparable to those reported for AcGI deacetylase subcloned from *M. tuberculsosis* H37Rv genomic DNA.[9] Earlier we described the structures and activities of natural product and synthetic inhibitors of mycothiol-*S*-conjugate amidase.[21–23] The results of this work pave the way for future studies aimed toward the design and synthesis of substrate-based inhibitors of AcGI deacetylase and its homologue mycothiol-*S*-conjugate amidase.

Experimental

General

Optical rotations were measured on a Perkin–Elmer 341 polarimeter; IR spectra were recorded on a BioRad FTS-45 FT-IR spectrophotometer as films on either a NaCl disk or a ZnSe crystal. Low- and high-resolution FAB mass spectra were obtained on a Jeol-SX102 instrument. ^1H, ^{13}C and COSY spectra were recorded on Varian Mercury 300, Varian Gemini 300, or Bruker DRX800 spectrometers. HSQC and HSQCLR spectra were recorded on a Bruker DMX500 spectrometer. NMR spectra of synthetic intermediates were run in $CDCl_3$ or CD_3OD and referenced to residual solvent signals at δ_H 7.25 and δ_C 77.05, and δ_H 3.30 and δ_C 49.15, respectively. Primed numbers for ^1H and ^{13}C chemical shift assignments correspond to the inositol ring. Reverse-phase (C18) HPLC (RP-HPLC) was carried out using an Agilent HPLC instrument and a Waters Spherisorb™ ODS column.

Subcloning and protein expression

DNA encoding the enzyme AcGI deacetylase (Rv1170) from *M. tuberculosis* (H37Rv) was amplified by polymerase chain reaction using total genomic DNA as template and primers with the sequences 5′-CAT ATGTCTGAGACGCCGCGGCTG-3′ and 5′-GCT GTTGGATCCTACGTGCCGGACGCGGTGAA - 3′ (Lofstrand Labs), which contain Bam H1 and Xho I restriction sites, respectively. Following purification (Promega), PCR products were cut and gel purified before ligation into the vector GEV2[18] linearized with the same enzymes. Clones containing the correct sequence (ABI Prism) were transformed into competent BL21 (DE3) cells (Novagen) and selected on LB-ampicillin plates (1% agar, 100 μg/mL ampicillin). Recombinant protG-AcGI deacetylase was produced as follows: 500 mL of cells were grown with shaking at 220 rpm at 37 °C for ~6 h in LB medium with 100 μg/mL ampicillin, induced with 1 mM IPTG at an OD^{600} of 0.8, at which time they were moved to a 25 °C incubator and shaken overnight. (Growth at a reduced temperature following induction is required to obtain soluble protein; induction at 37 °C results in nearly all of the recombinant protein being located in inclusion bodies.) The following morning, cells were harvested by centrifugation (10,000*g*, 10 min), lysed with BugBuster™ (Novagen) for 20 min at rt, and the soluble fraction was obtained after centrifugation at 18,000*g*, 30 min, 4 °C. (From this point on, all steps took place at 4 °C.) This supernatant was concentrated to 2 mL using a 20 mL 10,000 MWCO VIVASPIN filter (Millipore) and applied directly to a Superdex75 26/60 gel filtration column (Amersham Pharmacia Biotech) which had been equilibrated with 50 mM Tris, pH 7.4 containing 50 mM NaCl, 1 mM DTT and 100 μM $ZnSO_4$ (Buffer A). The column was eluted with 2 column volumes of Buffer A and 8 mL fractions were collected. Active fractions of greater than 80% purity (SDS gel electrophoresis) were combined, concentrated, and purified by anion exchange chromatography using a 1 mL ResourceQ column (Amersham Pharmacia Biotech) eluting with a linear gradient of 0–0.5 M NaCl in 50 mM Tris, pH 7.4 before a final purification/desalting over an analytical Superdex75 16/60 column (Amersham Pharmacia Biotech) eluting with Buffer A. Active fractions of greater than 95% purity were combined and concentrated to yield 1 mL of a 3.73 mg/mL solution that was stored at 4 °C and used for all assays. FAB-MS: *m*/*z* 38,292.05 (average mass 38,296.67).

Enzyme assays

Assays were carried out in 50 mM Tris (pH 7.4) at 32 °C for 20 min using recombinant protG–AcGI deacetylase and synthetic samples (30 μM of 100 μM) of MSmB, D-GlcNAc-α-(1→1)-*myo*-D-Ins (**1a**), D-GlcNAc-α-(1→1)-*myo*-L-Ins (**1b**), and D-Gln-α-(1→1)-*myo*-D-Ins (**1c**). The extent of cleavage of MSmB was measured directly by fluorescence detected RP-HPLC as described previously.[12] The extent of deacetylation of D-GlcNAc-α-(1→1)-*myo*-D-Ins (**1a**) was determined by fluorescence detected RP-HPLC eluting with a linear gradient of 0–0% MeOH in 5 min, 0–18% MeOH in 10 min, and 18–90% MeOH in 1 min, followed by a 4 min 90% MeOH wash. The fluorescent amino-derivatives, prepared with excess 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate in 100 mM sodium borate, pH 8.0, eluted with a retention time of 12.35 min, and the acetylated, underivatized compounds **1a** and **1b** both eluted at 5.6 min. The injection artifact elutes at 1.15 min under these conditions.

D-*myo*-Inositol 2-*N*-acetamido-2-deoxy-α-D-glucopyranoside (1a). To a solution of **5a** (36 mg, 0.05 mmol) in MeOH (200 μL) was added $Mg(OMe)_2$ (300 μL, 0.974 M). The reaction was monitored by TLC at 30-min intervals and quenched at 2 h with 0.5% TFA. Removal of the solvent in vacuo followed by silica gel chromatography eluting with a gradient from 1:9 to 2:3 MeOH/$CHCl_3$ yielded compound **1a** (12 mg, 63%). $[\alpha]_D^{20}$ +60° (*c* 0.1, MeOH). ^1H NMR (CD_3OD, 300 MHz) δ_H 5.06 (d, J_{HH} = 4 Hz, H1), 4.16 (dd, J_{HH} = 2 Hz, H2′), 3.92 (dd, J_{HH} = 4, 10 Hz, H2), 3.86 (m, H6a), 3.84 (m, H5), 3.78 (dd, J_{HH} = 10, 10 Hz, H6′), 3.69 (dd, J_{HH} = 9, 11 Hz, H3), 3.64 (dd, J_{HH} = 7, 12 Hz, H6b), 3.58 (dd, J_{HH} = 10, 10 Hz, H4′), 3.41 (dd, J_{HH} = 2, 9 Hz, H1′), 3.34 (dd, J_{HH} = 3, 10 Hz, H3′), 3.31 (m, H4), 3.15 (dd, J_{HH} = 10, 10 Hz, H5′), 1.98 (s, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ_C 173.9 ($CH_3\underline{C}$=O), 103.7 (C1), 84.6 (C1′), 79.6 (C6′), 77.1 (C4′), 76.4 (C2′), 76.3 (C5′), 76.2 (C3′), 76.1 (C3), 75.7 (C4), 65.8 (C6), 62.9 (C5′), 56.8 (C2), 23.0 ($\underline{C}H_3C$=O). IR (NaCl, film) 3350 (br), 1684 (C=O), 1204, 1141, 1039 cm^{-1}. MS *m*/*z* 384.1497 MH^+ ($C_{14}H_{26}NO_{11}$ calcd 384.1506).

D-*myo*-Inosityl 2-amino-2-deoxy-α-D-glucopyranoside (1c). (i) To a suspension of palladium on carbon (5%, 12 mg) in EtOAc (0.5 mL) and HCl (30 μL of 1 M solution) was added a solution of compound **4a** (20 mg, 0.033 mmol) in EtOAc (0.5 mL). The mixture was evacuated, flushed with hydrogen and stirred at rt under an atmosphere of hydrogen for 3 h. The reaction mixture was filtered and concentrated to give the crude amine hydrochloride (12 mg). (ii) To a solution of the crude amine in MeOH (200 μL) was added $Mg(OMe)_2$ (200 μL, 0.974 M). The reaction was monitored by TLC and quenched with 0.5% TFA after 2 h. The solvent was removed in vacuo followed by silica gel chromatography, eluting with a gradient from 1:9 to 2:3 MeOH/$CHCl_3$, to yield **1c** (6 mg, 53% over two steps). ^1H NMR (CD_3OD, 500 MHz) δ_H 5.06 (d, J_{HH} = 4 Hz, H1), 4.16 (dd, J_{HH} = 2 Hz, H2′), 3.92 (dd, J_{HH} = 4, 10 Hz, H2), 3.86 (m, H6a), 3.84 (m, H5), 3.78 (dd, J_{HH} = 10,10 Hz, H6′), 3.69 (dd, J_{HH} = 9, 11 Hz, H3), 3.64 (dd, J_{HH} = 7, 12 Hz, H6b), 3.58 (dd, J_{HH} = 10, 10 Hz, H4′), 3.41 (dd, J_{HH} = 2, 9 Hz, H1′), 3.34 (dd, J_{HH} = 3, 10 Hz, H3′), 3.31 (m, H4), 3.15 (dd, J_{HH} = 10,10 Hz, H5′). FAB-MS *m*/*z* 342.11 MH^+ ($C_{12}H_{24}NO_{10}$ calcd 342.14).

Compound 5a. (i) To a suspension of palladium on carbon (5%, 26 mg) in EtOAc (1 mL) and HCl (60 μL of 1 M solution) was added compound **4a** (46 mg, 0.05 mmol) in EtOAc (1 mL). The mixture was evacuated, flushed with hydrogen and stirred at rt under an atmosphere of hydrogen for 3 h. The reaction was filtered and concentrated to give the crude amine hydrochloride (30 mg). (ii) To a solution of the crude amine (30 mg) in pyridine (1 mL) was added acetic anhydride (500 μL). The mixture was stirred at rt for 13 h. Removal of the volatiles in vacuo followed by silica gel chromatography yielded compound **5a** (24 mg, 70% over two steps). $[\alpha]_D^{20}$ +50° (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 5.57 (m, H2′), 5.49 (dd, J_{HH} = 10,10 Hz, H6′), 5.47 (dd, J_{HH} = 10,10 Hz, H4′), 5.10 (dd, J_{HH} = 10, 10 Hz, H5′), 5.05 (dd, J_{HH} = 10, 10 Hz, H4), 5.00 (dd, J_{HH} = 3,11 Hz, H3′), 5.00 (m, H3), 4.95 (d, J_{HH} = 4 Hz, H1), 4.28 (m, H2), 4.20 (dd, J_{HH} = 5, 13 Hz, H6a), 4.08 (m, H6b), 4.04 (m, H5), 3.99 (dd, J_{HH} = 3, 10 Hz, H1′), 2.29 (CH_3C=O), 2.10 (CH_3C=O), 2.02 (CH_3C=O), 2.00 (CH_3C=O), 1.99 (CH_3C=O), 1.95 (CH_3C=O), 1.98 (CH_3C=O), 1.97 (CH_3C=O), 1.92 (CH_3C=O). ^{13}C NMR (CD_3OD, 75 MHz) δ_C 171.3 ($CH_3\underline{C}$=O), 170.8 ($CH_3\underline{C}$=O), 170.3 ($CH_3\underline{C}$=O), 170.2 ($CH_3\underline{C}$=O), 170.1 ($CH_3\underline{C}$=O), 169.7 ($CH_3\underline{C}$=O), 169.6 ($CH_3\underline{C}$=O), 169.5 ($CH_3\underline{C}$=O), 169.3 (CH_3C=O), 98.9 (C1), 72.9 (C1′), 71.6 (C6′), 70.8 (C5′), 70.4 (C3′), 69.4 (C5), 69.1 (C2′ or C3′), 69.0 (C2′ or C3′), 68.7 (C3), 67.5 (C4), 61.9 (C6), 51.5 (C2), 22.9 ($\underline{C}H_3C$=O), 20.8 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.6 ($\underline{C}H_3C$=O), 20.5 ($\underline{C}H_3C$=O), 20.4 ($\underline{C}H_3C$=O), 20.4 ($\underline{C}H_3C$=O). IR (NaCl, film) 1761 (C=O), 1756 (C=O), 1751 (C=O), 1740 (C=O), 1734 (C=O), 1369, 1226, 1039 cm^{-1}. MS *m*/*z* MH^+ 720.2336 ($C_{30}H_{42}NO_{19}$ calcd 720.2351).

Compound 1b. To a solution of **5b** (12 mg, 0.017 mmol) in MeOH (200 μL) was added $Mg(OMe)_2$ (300 μL, 974 mM solution). The reaction was monitored by TLC at 30 min, 1 and 2 h and quenched at 2 h with 0.5% TFA. Removal of the solvent in vacuo followed by silica gel chromatography with a gradient from 1:9 to 2:3 methanol/chloroform yielded compound **1b** (4.3 mg, 66%). $[\alpha]_D^{20}$ +89° (*c* 0.1, MeOH). ^1H NMR (CD_3OD, 300 MHz) ^1H 4.91 (d, J_{HH} = 4 Hz, H1), 4.04 (dd, J_{HH} = 3, 3 Hz, H2′), 3.95 (dd, J_{HH} = 4, 11 Hz, H2), 3.81 (dd, J_{HH} = 2, 12 Hz, H6a), 3.74 (m, H3′), 3.72 (m, H3), 3.69 (m, H5), 3.68 (m, H6b), 3.64 (dd, J_{HH} = 10, 10 Hz, H5′), 3.49 (dd, J_{HH} = 3, 10 Hz, H1′), 3.33 (m, H4), 3.30 (m, H4′), 3.18 (dd, J_{HH} = 9, 9 Hz, H6′), 2.03 (s, $\underline{C}H_3C$=O). ^1H NMR (D_2O, 800 MHz) ^1H 5.04 (br s, H1), 3.96, 9.94, 3.83, 3.84, 3.80, 3.71, 3.66, 3.59, 3.53, 3.48, 3.31, 2.05 (s, CH_3). ^{13}C NMR (D_2O, 75 MHz) δ_C 176.1 ($CH_3\underline{C}$=O), 95.7 (C1), 77.1 (C2′), 75.8, 73.7, 73.6, 72.7, 72.6, 72.4, 71.4, 69.9, 62.0 (C6), 55.2 (C2), 23.5 ($\underline{C}H_3C$=O). IR (NaCl, film) 3337 (br), 2921, 1651 (C=O), 1558, 1542, 1378, 1107, 1039 cm^{-1}. MS *m*/*z* 384.1501 MH^+ ($C_{14}H_{26}NO_{11}$ calcd 384.1506).

Compound 3b. To a solution of **2b** (75 mg, 0.17 mmol), 2,6-diisopropyl-4-methyl pyridine (0.85 mmol, 175 mg), and silver triflate (45 mg, 0.17 mmol) was added 1-chloro-2-azo-3,4,6-acetyl glucosamine (800 μL of a 185 mM soln, 0.15 mmol) dropwise. After 30 min, an aliquot of silver triflate (45 mg, 0.17 mmol) was added, followed by dropwise addition of a second aliquot of 1-chloro-2-azo-3,4,6-acetyl glucosamine (800 μL of a 1 mmol/5.4 mL soln, 0.15 mmol) 30 min later. A third aliquot of silver triflate was then added (45 mg, 0.17 mmol) 30 min later. The reaction was quenched by diluting with EtOAc and washing with aq satd Na_2SO_4, brine, and then dried ($NaSO_4$) and concentrated in vacuo. Purification using flash silica gel chromatography (petroleum ether → 5:1 petroleum ether/EtOAc) gave the α-glycosylation product **3b** (45 mg, 58%). $[\alpha]_D^{20}$ +116° (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 7.27–7.38 (m, ArH), 5.52 (dd, J_{HH} = 9, 10 Hz, H3), 5.27 (d, J_{HH} = 4 Hz, H1), 5.04 (dd, J_{HH} = 10, 10, H4), 4.90 (d, J_{HH} = 11 Hz, $PhCH_2$), 4.71 (d, J_{HH} = 11 Hz, $PhCH_2$),

4.52 (dd, $J_{HH}=5$, 5 Hz, H2′), 4.26 (dd, $J_{HH}=5$, 8 Hz, H3′), 4.19 (m, H5), 4.02 (m, H1′), 4.00 (m, H6a), 3.96 (dd, $J_{HH}=3$, 9 Hz, H6′), 3.92 (m, H6b), 3.84 (dd, $J_{HH}=9$, 10 Hz, H4′), 3.40 (dd, $J_{HH}=9$, 10 Hz, H5′), 3.24 (dd, $J_{HH}=4$, 10 Hz, H2), 2.09 (s, $CH_3C=O$), 2.04, (s, $CH_3C=O$), 2.02 (s, $CH_3C=O$), 1.39–1.80 (m, cyclohexylidene). ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 170.6 ($CH_3\underline{C}=O$), 169.9 ($CH_3\underline{C}=O$), 169.6 ($CH_3\underline{C}=O$), 137.9 (Bn), 128.5 (Bn), 128.3 (Bn), 127.8 (Bn), 113.0 ($O-\underline{C}-O$), 111.2 (s, $O-\underline{C}-O$), 95.3 (C1), 78.5 (C6′), 78.5 (C4′), 78.2 (C5′), 75.8 (C3′), 74.8 (C1′), 72.8 (C2′), 72.8 $\underline{C}H_2Ph$, 70.0 (C3), 68.2 (C4), 67.8 (C5), 61.3 (C6), 60.5 (C2), 37.5, 36.5, 36.4, 34.5, 25.0, 25.0, 23.9, 23.8, 23.7, 20.7 ($2\underline{C}H_3C=O$), 20.6 ($\underline{C}H_3C=O$). IR (NaCl, film) 2937, 2861, 2110 (N_3), 1754 (C=O), 1367, 1227, 1034 cm^{-1}. MS m/z 744.3328 MH^+ ($C_{37}H_{50}N_3O_{13}$ calcd 744.3344).

Compound 4b. (i) To a solution of camphor sulfonic acid (16 mg, 0.07 mmol) and ethylene glycol (34 mg, 0.55 mmol) in CH_3CN (1 mL) was added compound **3b** (85 mg, 0.11 mmol) in CH_3CN (2 mL). The mixture was stirred at rt for 14 h, and quenched by addition of triethylamine and concentrated in vacuo. A solution of the residue in CH_3CN was passed through a layer of silica gel eluting with EtOAc and concentrated to give a crude sample of 55 mg, which was used directly in the following step. (ii) The above material (55 mg) was treated with acetic anhydride (0.5 mL) and pyridine (1 mL), and stirred at rt for 16 h. Flash silica chromatography, eluting with a gradient from petroleum ether through 3:2 and 2:3 petroleum ether/EtOAc, yielded compound **4b** (63 mg, 76% over two steps), $[\alpha]_D^{20}$ +81° (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 7.22–7.31 (m, ArH), 5.70 (dd, $J_{HH}=2$, 2 Hz, H2′), 5.43 (dd, $J_{HH}=10$, 10 Hz, H4′), 5.40 (d, $J_{HH}=9$, 10 Hz, H3), 5.16 (dd, $J_{HH}=10$, 10 Hz, H5′), 5.12 (d, $J_{HH}=3$ Hz, H1), 4.95 (m, H4), 4.93 (m, H3′), 4.81 (d, $J_{HH}=11$ Hz, $PhCH_2$), 4.71 (d, $J_{HH}=11$ Hz, $PhCH_2$), 4.05 (m, H5), 4.00 (dd, $J_{HH}=9$, 10 Hz, H6′), 3.92 (m, H1′), 3.89 (m, H6a/H6b), 3.29 (dd, $J_{HH}=4$, 10 Hz, H2), 2.21 (s, $CH_3C=O$), 2.07 (s, $CH_3C=O$), 2.04 (s, $CH_3C=O$), 2.00 (s, $CH_3C=O$), 1.99 (s, $CH_3C=O$), 1.95 (s, $CH_3C=O$), 1.88 (s, $CH_3C=O$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 170.5 ($CH_3\underline{C}=O$), 170.1 ($CH_3\underline{C}=O$), 170.0 ($CH_3\underline{C}=O$), 169.9 ($CH_3\underline{C}=O$), 169.7 ($CH_3\underline{C}=O$), 169.5 ($CH_3\underline{C}=O$), 137.3 (Bn), 128.5 (Bn), 127.9 (Bn), 127.6 (Bn), 93.8 (C1), 77.6 (C6′), 76.1 ($\underline{C}H_2Ph$), 72.6 (C5′), 72.4 (C1′), 70.0 (C3 or C4′), 69.6 (C3 or C4′), 68.9 (C4 or C3′), 67.9 (C4 or C3′), 67.7 (C5), 65.1 (C2′), 61.2 (C6), 60.5 (C2), 20.8 ($\underline{C}H_3C=O$), 20.7 ($\underline{C}H_3C=O$), 20.7 ($\underline{C}H_3C=O$), 20.7 ($\underline{C}H_3C=O$), 20.7 ($\underline{C}H_3C=O$), 20.6 ($\underline{C}H_3C=O$). IR (NaCl, film) 2945, 2112 (N_3), 1753 (C=O), 1368, 1226, 1036 cm^{-1}. MS m/z 884.1492 MCs^+ ($C_{33}H_{41}N_3O_{17}Cs$ calcd 884.1490).

Compound 5b. (i) To a suspension of palladium on carbon (5%, 30 mg) in EtOAc (1 mL) was added a solution of compound **4b** (40 mg, 0.05 mmol) in EtOAc (1 mL). The reaction vessel was evacuated, flushed with hydrogen and the mixture was stirred at rt under hydrogen for 5 h. The mixture was filtered and concentrated. The crude amine thus obtained was used directly in the next reaction. (ii) To a solution of the above amine in pyridine (1 mL) was added acetic anhydride (500 μL). The reaction was stirred at rt for 13 h. Removal of the volatiles in vacuo followed by silica gel chromatography yielded compound **5b** (19.6 mg, 51% over two steps), $[\alpha]_D^{20}$ +60° (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 6.08 (br d, $J_{HH}=9$ Hz, NH), 5.65 (dd, $J_{HH}=3$, 3 Hz, H2′), 5.50 (dd, $J_{HH}=10$, 10 Hz, H4′), 5.46 (dd, $J_{HH}=10$, 10 Hz, H6′), 5.12 (dd, $J_{HH}=10$, 10 Hz, H5′), 5.10 (dd, $J_{HH}=10$, 10 Hz, H4), 4.98 (m, H3), 4.95 (m, H3′), 4.93 (d, $J_{HH}=3$ Hz, H1), 4.32 (m, H2), 4.16 (dd, $J_{HH}=2$, 12 Hz, H6a), 4.11 (dd, $J_{HH}=4$, 12 Hz, H6b), 3.91 (dd, $J_{HH}=3$, 10 Hz, H1′), 3.87 (m, H5), 2.26 (s, $CH_3C=O$), 2.13 (s, $CH_3C=O$), 2.09 (s, $CH_3C=O$), 2.03 (s, $CH_3C=O$), 2.02 (s, CH_3 C=O), 2.01 (s, $CH_3C=O$), 2.00 (s, $CH_3C=O$), 2.00 (s, $CH_3C=O$), 1.95 (s, $CH_3C=O$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 171.1 ($CH_3\underline{C}=O$), 170.7 ($CH_3\underline{C}=O$), 170.3 ($CH_3\underline{C}=O$), 170.2 ($CH_3\underline{C}=O$), 169.8 ($CH_3\underline{C}=O$), 169.7 ($CH_3\underline{C}=O$), 169.7 ($CH_3\underline{C}=O$), 169.6 ($CH_3\underline{C}=O$), 169.1 ($CH_3\underline{C}=O$), 97.1 (C1), 73.8 (C1′), 70.9 (C5′), 70.5 (C6′), 70.2 (C3), 69.2 (C4′), 68.9 (C5), 68.7 (C3′), 67.8 (C4), 67.1 (C2′), 61.5 (C6), 51.5 (C2), 22.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3). IR (NaCl, film) 2951, 1754 (C=O), 1744 (C=O), 1689, 1369, 1224, 1042 cm^{-1}. MS m/z 720.2354 MH^+ ($C_{30}H_{42}NO_{19}$ calcd 720.2351).

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References and Notes

1. Sakuda, S.; Zhou, Z.; Yamada, Y. *Biosci. Biotech. Biochem.* **1994**, *58*, 1347.
2. Spies, H. S.; Steenkamp, D. J. *Eur. J. Biochem.* **1994**, *224*, 203.
3. Newton, G. L.; Bewley, C. A.; Dwyer, T. J.; Horn, R.; Aharonowitz, Y.; Cohen, G.; Davies, J.; Faulkner, D. J.; Fahey, R. C. *Eur. J. Biochem.* **1995**, *230*, 821.
4. Reviewed in Fahey, R. C. *Ann. Rev. Microbiol.* **2001**, *55*, 333.
5. Bornemann, C.; Jardine, M. A.; Spies, H. S. C.; Steenkamp, D. J. *Biochem. J.* **1997**, *325*, 623.
6. Anderberg, S.; Newton, G. L.; Fahey, R. C. *J. Biol. Chem.* **1998**, *273*, 30391.
7. Newton, G. L.; Fahey, R. C. *Arch. Microbiol.* **2002**, *178*, 388.
8. Owing to the shared sequence homology with mycothiol-*S*-conjugate amidase (MCA), AcGI deacetylase was first identified by subcloning the gene Rv1170 from genomic DNA of *M. tuberculsosis* H37Rv.[9] Thus, AcGI deacetylase is also referred to as Rv1170 and *mshB*. Likewise, MCA has been referred to as Rv1082 as annotated in the *M. tuberculosis* genome sequence published by: Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.;

Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, III, C. R.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davis, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M.-A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G . *Nature* **1998**, *393*, 537.
9. Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *J. Bacteriol.* **2000**, *182*, 6958.
10. Sareen, D.; Steffek, M.; Newton, G. L.; Fahey, R. C. *Biochemistry* **2002**, *41*, 6885.
11. Koledin, T.; Newton, G. L.; Fahey, R. C. *Arch. Microbiol.* **2002**, *178*, 331.
12. Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, *39*, 10739.
13. Patel, M. P.; Blanchard, J. S. *Biochemistry* **1999**, *38*, 11827.
14. Nicholas, G. M.; Kováč, P.; Bewley, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 3492.
15. Mayer, T. G.; Schmidt, R. R. *Liebigs Ann./Recueil.* **1997**, 859.
16. Pavliak, V.; Kováč, P. *Carbohydr. Res.* **1991**, *210*, 333.
17. Xu, Y. C.; Bizuneh, A.; Walker, C. *J. Org. Chem.* **1996**, *61*, 9086.
18. Huth, J. R.; Bewley, C. A.; Jackson, B. M.; Hinnebusch, A. G.; Clore, G. M.; Gronenborn, A. M. *Prot. Sci.* **1997**, *6*, 2359.
19. Jardine, M. A.; Spies, H. S.; Nkambule, C. M.; Gammon, D. W.; Steenkamp, D. J. *Bioorg. Med. Chem.* **2002**, *10*, 875. Note that in this paper the stereochemistry of 1-D-*myo*-inositol is depicted as 1-L, and 1-L-*myo*-inositol is depicted as 1-D.
20. A direct linear plot of the same data yielded similar results with V_{max} and K_m values of 0.57 nmol min^{-1} and 155 μM, respectively.
21. Nicholas, G. M.; Newton, G. L.; Fahey, R. C.; Bewley, C. A. *Org. Lett.* **2001**, *3*, 1543.
22. Nicholas, G. M.; Eckman, L. L.; Ray, S.; Hughes, R.; Pfefferkorn, J.; Barluenga, S.; Nicolaou, K. C.; Bewley, C. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2487.
23. Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 601.