ORGANIC LETTERS

2002
Vol. 4, No. 24
4337−4339

Shortcut to Mycothiol Analogues

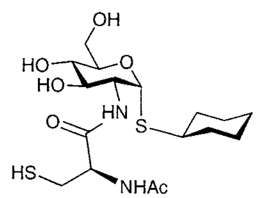
Spencer Knapp,* Silvia Gonzalez, David S. Myers, Lisa L. Eckman,† and Carole A. Bewley†

Department of Chemistry and Chemical Biology, Rutgers The State University of New Jersey, 610 Taylor Rd., Piscataway, New Jersey 08854-8087, and Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892-0820

knapp@rutchem.rutgers.edu

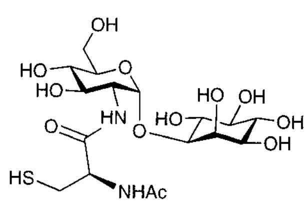
Received September 27, 2002

ABSTRACT

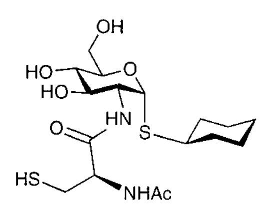


The synthesis of a simplified thioglycosidic analogue (2) of mycothiol (1) is described. Evaluation of 2 against mycothiol S-conjugate amidase from *Mycobacterium tuberculosis* reveals good specific activity (7500 nmol min^{-1} mg-protein^{-1}, vs 14 200 for 1), indicating that 2 can serve as a starting point for antitubercular drug design.

Mycothiol[1] (**1**) is the major low molecular weight thiol found in actinomycetes, including *Mycobacterium tuberculosis*.[2−5] It is thought to protect these organisms against oxidative stress[6−10] and function in the removal of exogenous electrophilic agents.[11−15] The biosynthesis of **1** (Scheme 1) proceeds by way of 1-*O*(2′-acetamido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol **3**, first by deacetylation to give **4**,[16] and then acylation with L-cysteine under the influence of a ligase[17,18] to provide **5**. A transacetylase[17,19] converts **5** to **1**. Two further pathways involving **1** have been elucidated (Scheme 1). A reductase[6−8] regenerates **1** from the corresponding disulfide, mycothione **(6**), thus maintaining the reducing intracellular environment. Upon reaction of **1** with electrophiles E$^+$, the resulting conjugate **7** is cleaved by mycothiol S-conjugate amidase[14,15] into **4** and an *N*-acetylcysteine adduct **8** that is exported from the cell.



1: MSH



2

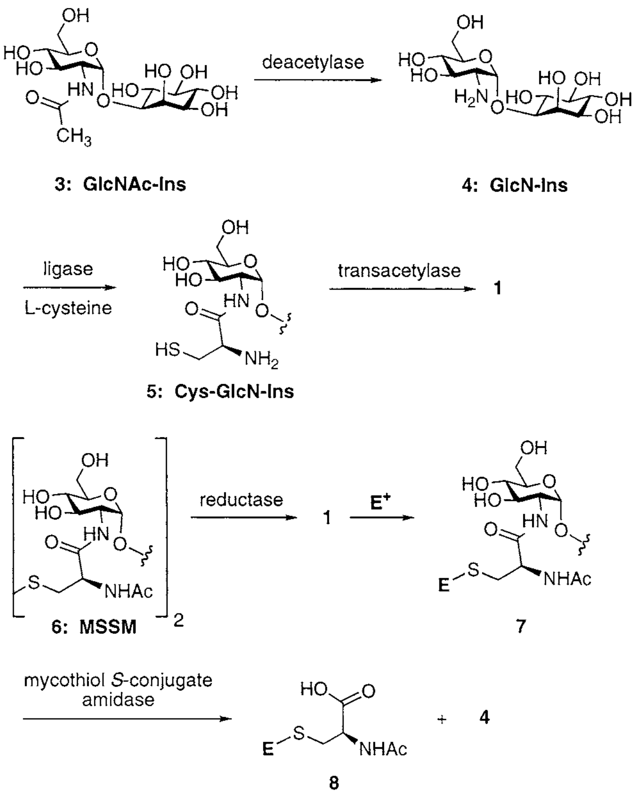
Drug-resistant tuberculosis now threatens a large portion of earth's population,[20] and the development of new treatments for tuberculosis infection has become a national[21] and international[22] priority. The disruption of enzymatic pathways of mycothiol biosynthesis and/or mycothiol-based detoxifi-

† National Institutes of Health.

(1) Systematic name: 1-*O*(2′[*N*-acetyl-L-cysteinyl]amido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol.
(2) Sakuda, S.; Zhou, Z.-Y.; Yamada, Y. *Biosci. Biotech. Biochem.* **1994**, *58*, 1347−1348.
(3) Spies, H. S. C.; Steenkamp, D. J. *Eur. J. Biochem.* **1994**, *224*, 203−213.
(4) Newton, G. L.; Arnold, K.; Price, M. S.; Sherrill, C.; delCardayre, S. B.; Aharonowitz, Y.; Cohen, G.; Davies, J.; Fahey, R. C.; Davis, C. *J. Bacteriol.* **1996**, *178*, 1990−1995.
(5) Fahey, R. C. *Annu. Rev. Microbiol.* **2001**, *55*, 333−356.
(6) Blanchard, J. S.; Patel, M. P. *J. Am. Chem. Soc.* **1998**, *120*, 11538−11539.
(7) Patel, M. P.; Blanchard, J. S. *Biochemistry* **1999**, *38*, 11827−11833.
(8) Patel, M. P.; Blanchard, J. S. *Biochemistry* **2001**, *40*, 5119−5126.
(9) 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*, 821−825.
(10) Newton, G. L.; Unson, M.; Anderberg, S.; Aguilera, J. A.; Oh, N. N.; delCardayre, S.; Davies, J.; Av-Gay, Y.; Fahey, R. C. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 239−244.
(11) Norin, A.; van Ophem, P. W.; Piersma, S. R.; Persson, B.; Duine, J. A.; Jornvall, H. *Eur. J. Biochem.* **1997**, *248*, 282−289.
(12) Misset-Smits, M.; van Ophem, P. W.; Sakuda, S.; Duine, J. A. *FEBS Lett.* **1997**, *409*, 221−222.

10.1021/ol0269796 CCC: $22.00 © 2002 American Chemical Society
Published on Web 11/02/2002

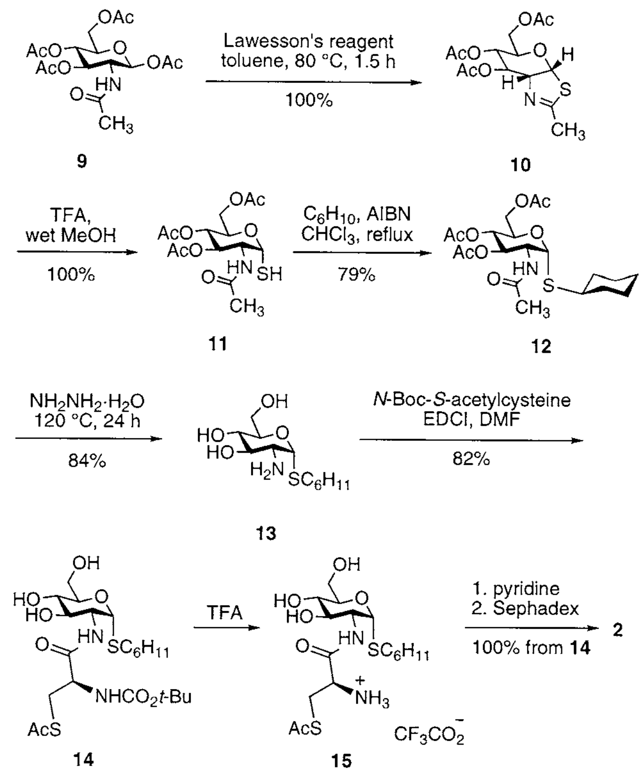
Scheme 1. Enzyme-Mediated Mycothiol Pathways



cation could leave *M. tuberculosis* vulnerable to drugs, oxygen, and other stress factors. The enzymes shown in Scheme 1 accept substrates or produce products that are N-acylated 1-*O*(2′-amino-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositols. For this reason, compounds based on the GlcN−Ins substructure that additionally bear groups on N that resemble those of the respective transition states are potential inhibitors for any one, or more than one, of these enzymes. In previous synthetic studies on **1** and related compounds,[6,15,17,23] the preparation of a protected D-*myo*-inositol glycosylation acceptor has required several steps and a resolution, and both the inositol α-glycosylation and N-acylation steps have been problematic. Some of these synthetic difficulties could be dodged if a stripped-down version of the inositol ring could be used as a component of inhibitors. We recently found that a variety of α-GlcNAc thioconjugates can be prepared stereoselectively and in good yield by *S*-derivatization of an α-GlcNAc mercaptan.[24,25] Thioglycosides are generally more resistant to degradation by glycosidases than *O*-glycosides,[26−28] so this approach to inhibitor design combines several possible advantages.

Commercially available 2-acetamido-2-deoxy-*β*-D-glucopyranose tetraacetate (**9**, Scheme 2) was treated with Lawesson's reagent as described previously,[24,26] and the resulting thiazoline **10** was then hydrolyzed to the acetamido mercaptan **11**.[24] Reaction of **11** with cyclohexene under conditions for free radical addition of anomeric mercaptans to alkenes [chloroform as a cosolvent, azobis(isobutyronitrile) as a radical initiator][25] afforded the cyclohexyl thioglycoside **12** with no trace of the corresponding *β*-isomer. Hydrazinolysis[29] of the four acetyls provided aminotriol **13**, and then coupling with *S*-acetyl-*N*-Boc-L-cysteine[30] gave **14** in good

Scheme 2. Synthesis of Simplified Mycothiol Analogue **2**

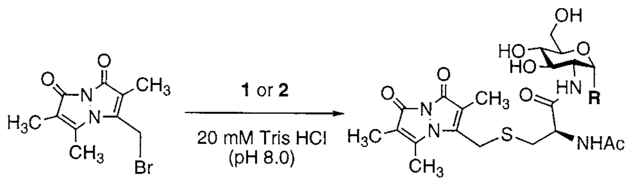


(13) Duine, J. A. *Biofactors* **1999**, *10*, 201−206.
(14) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, *39*, 10739−10746.
(15) Nicholas, G. M.; Kovác, P.; Bewley, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 3492−3493.
(16) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *J. Bacteriol.* **2000**, *182*, 6958−6963.
(17) Bornemann, C.; Jardine, M. A.; Spies, H. S. C.; Steenkamp, D. J. *Biochem. J.* **1997**, *325*, 623−629.
(18) Sareen, D.; Steffek, M.; Newton, G. L.; Fahey, R. C. *Biochemistry* **2002**, *41*, 6885−6890.
(19) Vetting, M. W.; Hegde, S. S.; Javid-Majd, F.; Blanchard, J. S.; Roderick, S. L. *Nature Struct. Biol.* **2002**, *9*, 653−658.
(20) Bloom, B. R.; Murray, C. J. L. *Science* **1992**, *257*, 1055−1064.
(21) For example, see updated web information at: http://www.niaid.nih.gov/publications/tb.htm.
(22) For example, see updated web information at: http://www.who.int/health-topics/tb.htm.
(23) Jardine, M. A.; Spies, H. S. C.; Nkambule, C. M.; Gammon, D. W.; Steenkamp, D. J. *Bioorg. Med. Chem.* **2002**, *10*, 875−881.
(24) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2001**, *66*, 3636−3638.
(25) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2002**, *67*, 2995−2999.
(26) Knapp, S.; Vocadlo, D.; Gao, Z.; Kirk, B.; Lou, J.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118*, 6804−6805.
(27) Bousquet, E.; Spadaro, A.; Pappalardo, M. S.; Bernardini, R.; Romeo, R.; Panza, L.; Ronisvalle, G. *J. Carbohydr. Chem.* **2000**, *19*, 527−541.
(28) Cohen, S. B.; Halcomb, R. L. *J. Org. Chem.* **2000**, *65*, 6145−6152.
(29) Fujinaga, M.; Matsushima, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 185−190.
(30) *S*-Acetyl-*N*-Boc-L-cysteine was prepared from commercially available *N*,*N*′-bis(Boc)-L-cystine by zinc reduction and then in situ S-acetylation. Zahn, H.; Hammerström, K. *Chem. Ber.* **1969**, *102*, 1048−1052.

yield and high isomeric purity. The *N*-Boc protecting group was removed by treatment of **14** with neat trifluoroacetic acid, leading to ammonium salt **15**, and then basification with pyridine in the same pot gave the simplified mycothiol analogue **2** as the result of a spontaneous and convenient intramolecular S-to-N acetyl migration.[31]

Evaluation of **2** as a substrate for *M. tuberculosis* mycothiol S-conjugate amidase[14,15] was carried out by prefatory S-alkylation with bromobimane (**16**, Scheme 3) under mildly basic conditions.[15] The resulting bimane derivative **18** was subjected to cleavage by the amidase in parallel with mycothiol-bimane **17**, while monitoring formation of the cysteine-*S*-bimane product (see **8**) by fluorescence-detected HPLC assay.[15] Specific activities for **18** and **17** are 7500 and 14 200 nmol min^{-1} mg-protein^{-1}, respectively, establishing **18** as a good substrate for this amidase. Neither the inositol hydroxyls nor the glycosidic linking atom (O vs S) plays a major role in enzyme binding. An earlier study[6] had indicated that the inositol ring is not required for reduction of disulfides (see **6**) by the *M. tuberculosis* mycothione reductase. The accumulated information thus suggests that **2**, which dispenses with the inositol hydroxyls and the linking oxygen atom, can serve as a suitable foundation upon which to base inhibitor design.

Scheme 3. Preparation of Bimane Derivatives



16

17: **R** = O-$C_6H_{11}O_5$ (from **1**)
18: **R** = S-C_6H_{11} (from **2**)

Acknowledgment. We thank Merck & Co. and Wyeth-Ayerst Research for financial support and Dr. Gregory J. Morriello and Dr. George A. Doss for assistance with NMR spectroscopy. S.G. was a Johnson & Johnson and Henry Rutgers Undergraduate Research Fellow, and D.S.M. was a Rutgers University Graduate Fellow. The work at NIH was supported in part by the Intramural AIDS Targeted Antiviral Program of the Office of the Director, National Institutes of Health.

Supporting Information Available: Experimental details and spectral characterization for all new compounds and description of the enzyme assay. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0269796

(31) Meyer zu Reckendorf, W.; Bonner, W. A. *J. Org. Chem.* **1961**, *26*, 4596−4599.