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Intramolecular α -Glucosaminidation: Synthesis of Mycothiol

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Abstract

A protected cyclitol aglycon was tethered to an (*N*-arylsulfonyl)glucosamine donor by a methylene linker; the exclusively α -selective intramolecular glycosylation reaction was then initiated by electrophilic activation of the thioglycoside donor portion. Further transformations of the glycosylation product to give the *M. tuberculosis* detoxifier mycothiol and its oxidized congener, the disulfide mycothione, are detailed.

Two million people die annually from tuberculosis (TB), and an estimated 8 million develop the disease each year. Because *Mycobacterium tuberculosis*, the TB causative agent, has increasingly developed resistance to many treatments, and most of them are largely ineffective against the dormant state, a better understanding of the organism's defense mechanisms is critical to battling infections.^{1,2} Mycothiol (**1**; Figure 1)³ is the low molecular weight thiol used by mycobacteria as their main line of defense against foreign electrophilic agents such as radicals, oxidants, and drugs;^{4,5} glutathione plays an analogous role in eukaryotes, which lack **1** altogether. Disruption of mycothiol metabolic pathways^{6,7} is fatal to *M. tuberculosis*, so this is a promising avenue for the development of new treatments.⁸ The efficient synthesis of **1** and its congener disulfide, mycothione **2**, as well as simpler analogues, could contribute significantly to these efforts.^{9,10,11,12}

Forging the (α) *cis*-1',2' glycosidic linkage of **1** is the principal synthetic challenge.¹³ Where complex glycosyl donors and acceptors are required, an *intramolecular* glycosylation process¹⁴ (Figure 2) offers several advantages: the donor-acceptor stoichiometry is 1:1, the tether enforces *syn* delivery of the aglycon (**OR**), and the bond formation can potentially be accomplished quite efficiently and to the near exclusion of non-productive donor fates such as elimination or hydrolysis. While no intramolecular α -glucosaminidation has yet been demonstrated,¹⁵ careful selection of a short and temporary tether **Y**, an easily removable amine protecting group **Z**, and a leaving group **X** that can be activated under mild conditions ought to direct the desired glycosidic bond formation. We are pleased to report the realization of these objectives, and additionally, the efficient transformation of the glycosylation product to **1** and **2**.

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Supporting Information Available: Experimental details and spectroscopic characterization for **1**, **2**, and all new compounds, and the CIF for the menthol carbonate of **10**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

1,3,4,6-Tetra-*O*-acetyl- β -D-glucosamine¹⁶ (**3**, Scheme 1) was converted¹⁷ to the 2-naphthalenesulfonamide **4**, and then further¹⁸ to the *p*-tolyl thioglycoside **5**. Menthol was selected as a model aglycon; coupling of **5** with (+)-menthyl chloroformate (**6**) was particularly efficient in the presence of the phosphazene base 2-*t*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP).¹⁹ The intramolecular glycosylation of **7** was carried out by activating the thioglycoside with dimethyl(methylthio)sulfonium tetrafluoroborate.²⁰ Upon quench, the temporary methylene tether was lost, presumably as formaldehyde, and the (exclusively *alpha*) glucosaminide **8** was isolated in good yield. Acetate methanolysis followed by reductive removal²¹ of the 2-naphthalenesulfonyl protecting group provided the amino triol **9**.

An analogous approach to the synthesis of **1** requires a protected cyclitol aglycon component, such as the penta-*O*-benzylinositol **10** (Scheme 2). Preparation of **10** followed the known route,²² which includes a resolution based on purification of its (+)-menthol mixed carbonate. Crystallographic analysis (See Supporting Information) confirmed the absolute configuration of the resolved material. The required chloromethyl derivative **12** was generated in situ²³ from methylthiomethyl ether **11**.²⁴ BEMP mediated coupling with **5** was again successful, and produced the glycosylation substrate **13**.

Use of dimethyl(methylthio)sulfonium tetrafluoroborate as before to trigger the intramolecular glycosylation led not to the desired glucosaminide **14**, but instead to an N-methylsulfonamide carbinol **15** (96%) in which one benzyl ether has been selectively cleaved and the methylene linker has been reduced. The structure of **15** was secured by NMR analysis of its acetate derivative **16**. In particular, the methine adjacent to -OH (H-6, δ = 4.09, td, J = 9.5, 3.0 Hz) in **15** can be identified by COSY analysis, and moves downfield (δ = 5.75, t, J = 10 Hz) upon acetylation. An unusually facile 1,9 hydride shift²⁵ within the intermediate iminium ion **17**, followed by hydrolysis of the oxonium species **18**, accounts for this result.

The glycosylation reaction conditions were adjusted to include a good nucleophile (chloride ion) capable of intercepting **17** and blocking the hydride shift, particularly at lower temperatures. Although treatment of **13** with 1.5 equiv each of phenylsulfenyl chloride and silver(I) trifluoromethanesulfonate²⁶ below -20 °C gave **15** only (hydride shift is rapid even at low temperature), the use of PhSeCl with *catalytic* AgOTf gave the desired glycoside **14** in high yield after the quench, with barely a trace of **15**.

Deprotection of the acetates and the 2-naphthylsulfonamide as before led smoothly to the amino triol **20** (Scheme 3). The selective reductive cleavage of the *N*-sulfonyl protecting group in the presence of five *O*-benzyl ethers is notable. Hydrogenolysis of these benzyls gave the amino octa-ol as its hydrochloride salt **21**. The amino group, upon liberation by base treatment, was coupled with *N*-Boc-*S*-acetylcysteine **22**,¹¹ and the resulting amide was deprotected on nitrogen by treatment with trifluoroacetic acid. Neutralization of the resulting ammonium trifluoroacetate salt induced S $\rightarrow$ N vicinal migration of acetyl, and upon simple concentration the natural product mycothiol (**1**) was obtained essentially quantitatively. When stored under an argon atmosphere, the mercaptan **1** is stable for weeks.

Analysis of **1** by ¹H, ¹³C NMR, and exact mass methods, and comparison of the values with those in the literature⁹ (see Supporting Information), leaves no doubt about its identity. Oxidative conversion of mycothiol to its disulfide mycothione (**2**, Scheme 4) was also quantitative, and similar analysis of **2** confirmed its identity as well. Finally, the amide coupling method in Scheme 3 was applied to D-glucosamine (72% overall yield), resulting in the simplified mycothiol glycon, GlcN-Cys-NAc (**24**). Derivatives of **24** may prove useful in the development of inhibitors of mycothiol processing enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Barry CE III, Duncan K. *Drug Discov. Today* 2004;1:491–496.
2. Xie Z, Siddiqi N, Rubin EJ. *Antimicrob. Agents Chemother* 2005;49:4778–4780. [PubMed: 16251329]
3. Isolation: Newton GL, Fahey RC, Cohen G, Aharonowitz Y. *J. Bacteriol* 1993;175:27342742. Sakuda S, Zhou Z-Y, Yamada Y. *Biosci. Biotechnol. Biochem* 1994;58:1347–1348. [PubMed: 7765257] Spies HSC, Steenkamp D. *J. Eur. Biochem* 1994;224:203–213.
4. Newton GL, Arnold K, Prince MS, Sherrill C, delCardayré SB, Aharonowitz Y, Cohen G, Davies J, Fahey RC, Davis C. *J. Bacteriol* 1996;178:1990–1995. [PubMed: 8606174]
5. Recent reviews: Jothivasan VK, Hamilton CJ. *Nat. Prod. Rep* 2008;25:1091–1117. [PubMed: 19030604] Newton GL, Buchmeier N, Fahey RC. *Microbiol. Mol. Biol. Rev* 2008;72:471–494. [PubMed: 18772286]
6. Mycothiol biosynthesis: Bornemann C, Jardine MA, Spies HSC, Steenkamp DJ. *Biochem. J* 1997;325:623–629. [PubMed: 9271081] Newton GL, Koledin T, Gorovitz B, Rawat M, Fahey RC, Av-Gay Y. *J. Bacteriol* 2003;185:3476–3479. [PubMed: 12754249] Newton GL, Ta P, Bzymek KP, Fahey RC. *J. Biol. Chem* 2006;281:33910–33920. [PubMed: 16940050] Newton GL, Av-Gay Y, Fahey RC. *J. Bacteriol* 2000;182:6958–6963. [PubMed: 11092856] Koledin T, Newton GL, Fahey RC. *Arch. Microbiol* 2002;178:331–337. [PubMed: 12375100] Sareen D, Steffak M, Newton GL, Fahey RC. *Biochemistry* 2002;41:6885–6890. [PubMed: 12033919]
7. Mycothiol mediated detoxification: Newton GL, Av-Gay Y, Fahey RC. *Biochemistry* 2000;39:10739–10746. [PubMed: 10978158] Park J-H, Roe J-H. *Mol. Microbiol* 2008;68:861–870. [PubMed: 18430082] Steffak M, Newton GL, Av-Gay Y, Fahey RC. *Biochemistry* 2003;42:12067–12076. [PubMed: 14556638]
8. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. *Nat. Rev. Drug Discovery* 2007;6:29–40.
9. MSH synthesis: Lee S, Rosazza JPN. *Org. Lett* 2004;6:365–368. [PubMed: 14748594]
10. Bimane conjugate of MSH: Jardine MA, Spies HSC, Nkambule CM, Gammon DW, Steenkamp DJJ. *Bioorg. Med. Chem* 2002;10:875–881. [PubMed: 11836093] Nichols GM, Kovac P, Bewley CA. *J. Am. Chem. Soc* 2002;124:3492–3493. [PubMed: 11929223]
11. Knapp S, Gonzalez S, Myers DS, Eckman LL, Bewley CA. *Org. Lett* 2002;4:4337–4339. [PubMed: 12443092]
12. MSH analogues: Gammon DW, Hunter R, Steenkamp DJ, Mudzunga TT. *Bioorg. Med. Chem. Lett* 2003;13:2045–2049. [PubMed: 12781192] Knapp S, Amorelli B, Darout E, Ventocilla CC, Goldman LM, Huhn RA, Minnihan EC. *J. Carbohydr. Chem* 2005;24:103–130. Metaferia BB, Ray S, Smith JA, Bewley CA. *Bioorg. Med. Chem. Lett* 2007;17:444–447. [PubMed: 17084627] Metaferia BB, Fetterolf BJ, Shazad-ul-Hussan S, Moravec M, Smith JA, Ray S, Gutierrez-Lugo M-T, Bewley CA. *J. Med. Chem* 2007;50:6326–6336. [PubMed: 18020307] Stewart MJG, Jothivasan VK, Rowan AS, Wagg J, Hamilton CJ. *Org. Biomol. Chem* 2008;6:385–390. [PubMed: 18175009]
13. Recent glucosamidation methods: Li Y, Yang Y, Yu B. *Tetrahedron Lett* 2008;49:3604–3608. Mensah EA, Nguyen HM. *J. Am. Chem. Soc* 2009;131:8778–8780. [PubMed: 19496537] Yang Y, Li Y, Yu B. *J. Am. Chem. Soc* 2009;131:12076–12077. [PubMed: 19663423]
14. Intramolecular glycosylation: Barresi F, Hindsgaul O. *J. Am. Chem. Soc* 1991;113:9376–9377. Stork G, Kim G. *J. Am. Chem. Soc* 1992;114:1087–1088. Bols M. *J. Chem. Soc., Chem. Commun* 1992:913–914. Ito Y, Ogawa T. *Angew. Chem. Int. Ed. Engl* 1994;33:1765–1767. Fairbanks AJ. Synlett 2003:1945–1958. Cumpstey I. *Carbohydr. Res* 2008;343:1553–1573. [PubMed: 18533138]

15. Portions of this work have appeared: Ajayi, K. Ph.D. Dissertation. Rutgers University; Piscataway, NJ: 2010. Lapo RC, Knapp S. Abstracts of Papers of the American Chemical Society 2003;225:U260. Meeting Abstract 56-CARB.
16. Bergmann M, Zervas L. Chem. Ber 1931;64:973–980.
17. Nyasse B, Grehn L, Maia HLS, Monteiro LS, Ragnarsson U. J. Org. Chem 1999;64:7135–7139.
18. Pozsgay V, Jennings HJ. Tetrahedron Lett 1987;28:1375–1376.
19. Schwesinger R, Willaredt J, Schlemper H, Keller M, Schmitt D, Fritz H. Chem. Ber 1994;127:2435–2454.
20. Trost BM, Murayama E. J. Am. Chem. Soc 1981;103:6529–6530.
21. Chavez F, Sherry AD. J. Org. Chem 1989;54:2990–2992.
22. Massy DJR, Wyss P. Helv. Chim. Acta 1990;73:1037–1057. Baker GR, Billington DC, Gani D. Tetrahedron 1991;47:3895–3908. Koto S, Hirooka M, Yoshida T, Takenaka K, Asai C, Nagamitsu T, Sakuma H, Sakurai M, Masuzawa S, Komiya M, Sato T, Zen S, Yago K, Tomonaga F. Bull. Chem. Soc. Jpn 2000;73:2521–2529.
23. Jones JH, Thomas DW, Wood ME. Synth. Commun 1986;16:1607–1610.
24. Corey EJ, Bock MG. Tetrahedron Lett 1975;16:3269–3270.
25. A 1,5 iminium induced hydride shift: Wolfling J, Frank E, Schneider G, Tietze LF. Angew. Chem. Int. Ed. Engl 1999;38:200–201. Wolfling J, Frank E, Schneider G, Tietze LF. Eur. J. Org. Chem 2004:90–100.
26. Martichonok V, Whitesides GM. J. Org. Chem 1996;61:1702–1706. [PubMed: 11667039]

Figure 1.
Structures of mycothiol and its disulfide

Figure 2.
An intramolecular glucosamidation featuring a tether **Y**, an N-protecting group **Z**, and an activatable leaving group **X**.

Scheme 1.
 A Model Intramolecular α -Glucosaminidation

Scheme 2.
The Mycothiol Core and a 1→9 Hydride Shift

Scheme 3.
Completion of the Synthesis of Mycothiol

Scheme 4.
Conversion of Mycothiol to Mycothione