Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 444–447
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of substrate-mimic inhibitors of mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis*

Belhu B. Metaferia, Satyajit Ray, Jeremy A. Smith and Carole A. Bewley*

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Received 22 August 2006; revised 3 October 2006; accepted 12 October 2006
Available online 17 October 2006

Abstract—The Staudinger reaction between a polymer-supported triphenylphosphine reagent and pseudo-disaccharide azides is successfully applied to synthesize a variety of substrate-mimic mycothiol analogs. Screening of this new group of analogs against the mycobacterial detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) yielded several modest inhibitors (IC_{50} values around 50 μM) and provided additional structure–activity relationships for future optimization of inhibitors of MCA and its homologs.

Mycothiol (MSH, **1**, Fig. 1) is a low molecular weight thiol[1–3] found exclusively in actinomycetes,[4] a group that includes the pathogen *Mycobacterium tuberculosis*. Mycothiol functions analogously to glutathione in Gram-negative bacteria and eukaryotes and plays an important role as a detoxifying agent by covalently binding to toxins, electrophiles, and drugs to form mycothiol-*S*-conjugates (MSE).[5] This conjugate subsequently is cleaved at the amide bond by a detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) to yield a mercapturic acid conjugate that is ultimately exported from the cell.[5] Mycothiol also is involved in maintaining a redox equilibrium within the cell that is critical for a reducing cellular environment.[4]

The absence of MSH in eukaryotes and Gram-negative bacteria, along with the compelling evidence for increased sensitivity of MSH-deficient mutants toward electrophiles, free radicals, and antibiotics[6], suggest that enzymes involved in MSH biosynthesis and MSH-dependent detoxification (namely MCA and biosynthetic enzymes *MshA–MshD*) are potential targets for new classes of antibiotics. Moreover, because inhibitors of these enzymes may lead to new classes of compounds that target with some specificity to mycobacteria, they are excellent choices for rational drug design and screening of small molecule inhibitors.

Previously, we and others have reported the total synthesis of MSH and mycothiol bimane (Fig. 1, MSmB)[7,8] which are key for performing structural studies of substrates and inhibitors.[9] In addition, we have identified natural products and natural product-like synthetic inhibitors that have shown inhibitory activities against MCA.[10–13] Here, as part of our ongoing effort to discover new generations of anti-mycobacterial agents, we report synthesis and evaluation of novel substrate-mimic inhibitors of MCA built upon a quinic acid-derived scaffold.

Our synthetic strategy to produce MSH-inspired substrate-mimic analogs centered on incorporation of a quinic acid template to replace the inositol ring of natural MSH. This strategy is attractive for a number of reasons: incorporation of quinic acid circumvents the otherwise laborious and low-yielding transformations to obtain optically pure *myo*-D-inositol-containing analogs;[14,15] quinic acid is commerically available as a single isomer; it can be coupled to a glycosyl donor with high efficiency to produce a variety of analogs; and the presence of a relatively unreactive tertiary hydroxyl group as a site of attachment for linkers makes the synthetic scheme amenable to parallel synthesis.

Retrosynthetic analysis (Scheme 1) of quinic acid-containing analogs led us to envision the use of protected acceptor **3**, readily derived from quinic acid; and glycosyl donor **5** that could be obtained from D-mannopyra-

Keywords: Quinic acid; Mycothiol bimane; Myo-D-inositol; Polymer-supported triphenyl phosphine; Pseudodisaccharide; Mycobacteria.
* Corresponding author. Tel.: +1 301 594 5187; e-mail: caroleb@mail.nih.gov

0960-894X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2006.10.031

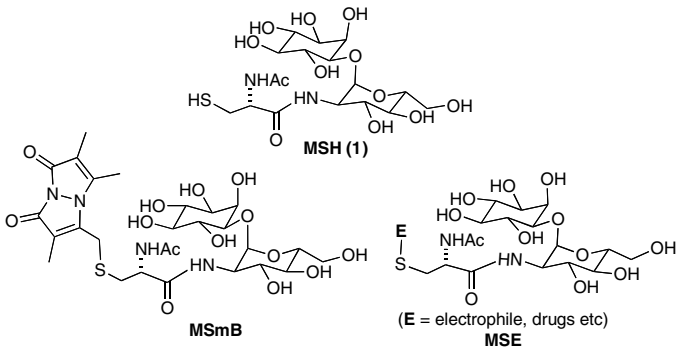
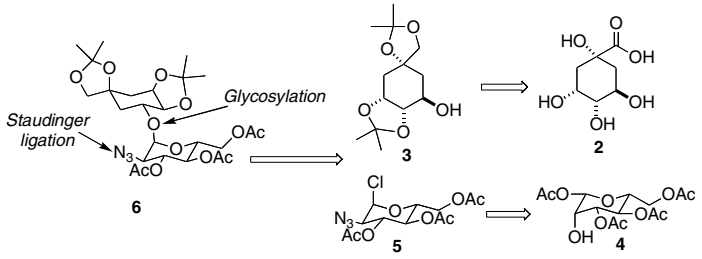


Figure 1. Mycothiol (**1**, MSH), MCA substrate mycothiol bimane (MSmB), and mycothiol-*S*-conjugate (MSE).

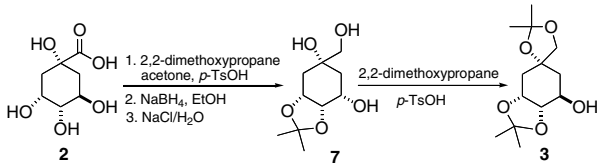


Scheme 1. Retrosynthetic analysis.

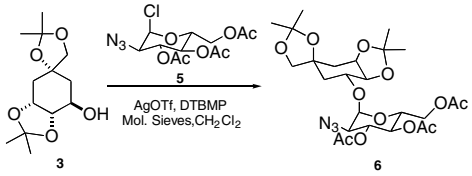
nose derivative **4**. Glycosylation of **3** with **5** would then furnish the core structure **6** for subsequent Staudinger amidation to produce diverse mycothiol analogs.

The forward synthesis of target analogs proceeded with preparation of glycosylation counterparts **3** and **5**. Quinic acid was transformed to protected acceptor **3** in four steps according to published procedures (Scheme 2).[16,17] Treatment of acid **2** with 2,2-dimethoxypropane under acidic conditions gave the protected lactone that upon reduction with $NaBH_4$ gave triol **7**. Subsequent protection of the vicinal diol of **7** provided compound **3** in 65% overall yield.

Glycosyl donor 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride (**5**) was obtained starting from commercially available 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (**4**) (Scheme 3). Following published protocols,[7,18] compound **4** was converted to its corresponding triflate by treatment with Tf_2O in Py/CH_2Cl_2, and the triflate was reacted with sodium azide to give **8**



Scheme 2. Preparation of acceptor **3**.

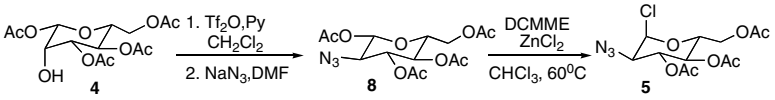


Scheme 4. Glycosylation reaction.

with inversion of configuration at C-2 (Scheme 3). Treatment of **8** with α,α-dichloromethyl methyl ether (DCMME) and $ZnCl_2$ in refluxing chloroform gave glycosyl chloride **5** in good yield (Scheme 4).

The reaction between acceptor **3** and donor **5** was effected by activation with silver triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane to give pseudo-disaccharide **6** with high α selectivity (5:1 α/β ratio) and 71% yield.[19]

Inspired by recent reports describing the use of a polymer-supported triphenylphosphine reagent for derivatization of azides, we explored a similar method to derivatize **6** efficiently.[20–22] Thus, azido pseudo-disaccharide **6** was treated with polymer-supported triphenylphosphine reagent followed by in situ trapping of the iminophosphorane intermediate with 14 varied acid chlorides (Scheme 5).[23] This sequence produced the

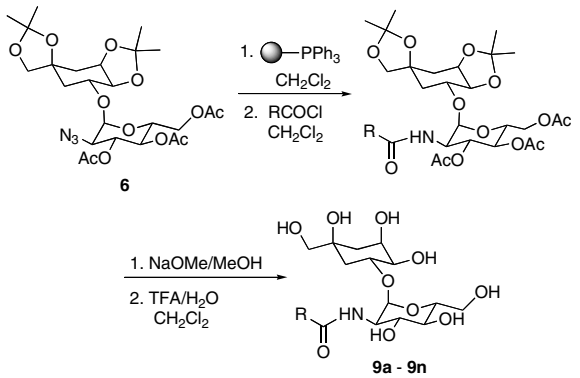


Scheme 3. Synthesis of donor **5**.

desired amide analogs **9a**–**n** cleanly and with good yields ranging from approximately 40 to 80% (Table 1). Among the acid chlorides used for derivatization, reaction with *p*-nitrobenzoyl chloride (to give **9f**) and *p*-trifluoromethyl benzoyl chloride (to give **9g**) gave the highest overall yields (~80%), while the reaction with *p*-(*N*,*N*-dimethyl amino) benzoyl chloride (to give **9e**) gave the lowest yield (~40%) for its amide product. These differences likely arise from the respective electron withdrawing versus electron donating character of the para substituents of the acid chlorides used to generate **9f/g** versus **9e**, and their respective activating or deactivating effect on the carbonyl center that undergoes attack by the iminophosphorane intermediate. The final stages of the synthesis were accomplished by global deprotection to give the desired mycothiol analogs **9a** through **9n**.

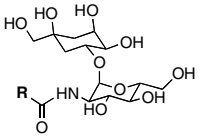
Compounds **9a**–**n** were tested for inhibition of recombinant MCA using a fluorescence detected HPLC assay employing mycothiol bimane (MSmB, Fig. 1) or its cyclohexyl thioglycoside analog[13] as substrate as described previously.[11] Compounds were tested at two concentrations, namely 100 μM and 50 μM, and results from screening at 50 μM are summarized in Table 1. Compounds **9d** and **9e** were found to be the most active against MCA inhibiting the enzyme by approximately 45% at 50 μM to indicate that derivatization with nitrogen-containing moieties such as pyridinyl or para-amino phenyl groups contributes favorably to binding of this scaffold to MCA.

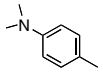
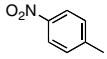
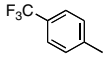
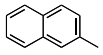
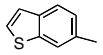
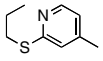
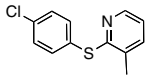
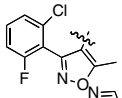
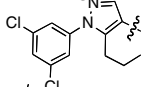
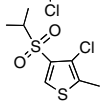
Recent reports describing high density mutagenesis or systematic gene silencing in *M. tuberculosis* have provided further evidence that MSH is essential for growth and survival of this pathogen.[24,25] Discovery and synthesis of small molecule inhibitors and probes of MSH biosynthesis and detoxification will thus continue to be of great importance in relation to the need for new antitubercular. Previously, Knapp and co-workers demonstrated that the cyclitol present in natural MSH can be replaced by a cyclohexyl unit to obtain mycothiol conjugates and analogs that can function, respectively, as substrates[14] or inhibitors.[15] On the other hand, through synthesis of MSH precursors D-GlcN-*myo*-D-Ins and D-GlcN-*myo*-L-Ins we showed previously that the stereochemistry of the hydroxyl groups on the cyclitol are critical as *myo*-L-Ins-containing substrates were tolerated by the biosynthetic or detoxification enzymes.[26]



Scheme 5. Staudinger amidation employing polymer-supported triphenylphosphine reagent.

Table 1. Yields of analogs **9a**–**9n** and inhibition of mycothiol-*S*-conjugate amidase



Analog	R	% inhibition of MCA[a]	% yield[b]
9a		15	65
9b		13	56
9c		13	62
9d		46	71
9e		44	39
9f		15	78
9g		16	79
9h		13	60
9i		NI	58
9j		15	52
9k		10	50
9l		NI	56
9m		26	49
9n		11	67

[a] Values are means of two experiments with final inhibitor concentration of 50 μM; standard deviations were on average ±8%; (NI = less than 10% inhibition).

[b] % overall yields for Scheme 5.

The work described here, which incorporates a new moiety in place of *myo*-D-Ins, namely a quinic acid-derived ring system, provides further insight into the specificity of MSH-associated enzymes and their substrates. In summary, starting from quinic acid and a suitable azido glycosyl chloride intermediate, we have generated with efficiency a small but diverse library of new MCA inhibitors by employing a Staudinger reaction using polymer-supported triphenylphosphine. The discovery that two of these compounds inhibit the *M. tuberculosis* enzyme MCA with IC_{50} values around 50 μM provides a starting point for future elaboration of these scaffolds, which may yield more potent MCA inhibitors and provide additional structure–activity relationships.

Acknowledgments

We thank Drs. John Lloyd for HRMS data and Son Lam for contributory discussions. This work was supported in part by the Intramural Research Program, NIH (NIDDK), and the AIDS Targetted Antiviral Program of the Office of the Director (C.A.B.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.031

References and notes

1. Sakuda, S.; Zhou, Z.; Yamada, Y. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1347.
2. Spies, H. S. C.; Steenkamp, D. J. *Eur. J. Biochem.* **1994**, *224*, 203.
3. Newton, G. L.; Bewley, C. A.; Dwyer, T. J.; Horn, R.; Aharonowitz, Y.; Faulkner, D. J.; Fahey, R. C. *Eur. J. Biochem.* **1995**, *230*, 821.
4. Fahey, R. C. *Annu. Rev. Microbiol.* **2001**, *55*, 333.
5. Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, *39*, 10739.
6. Rawat, M.; Newton, G. L.; Ko, M.; Martinez, G. J.; Fahey, R. C.; Av-Gay, Y. *Antimicrob. Agents Chemother* **2002**, *46*, 3348.
7. Nicholas, G. M.; Kovác, P.; Bewley, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 3492.
8. Lee, S.; Rosazza, J. P. N. *Org. Lett.* **2004**, *6*, 365.
9. Mahadevan, J.; Nicholas, G. M.; Bewley, C. A. *J. Org. Chem.* **2003**, *68*, 3380.
10. Nicholas, G. M.; Eckman, L.; Ray, S.; Hughes, R. O.; Pfefferkorn, J. A.; Barluenga, S.; Nicolaou, K. C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2487.
11. Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 601.
12. Fetterolf, B.; Bewley, C. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3785.
13. Nicholas, G. M.; Bewley, C. A. *Curr. Med. Chem. Anti-Infective Agents* **2004**, *3*, 221.
14. Knapp, S.; Gonzalez, S.; Myers, D. S.; Eckman, L.; Bewley, C. A. *Org. Lett.* **2002**, *4*, 4337.
15. Knapp, S.; Amorelli, B.; Darout, E.; Ventocilla, C. C.; Goldman, L. M.; Huhn, R. A.; Minnihan, E. C. *J. Carbohydr. Chem.* **2005**, *24*, 103.
16. Matsuo, K.; Matsumoto, T.; Nishiwaki, K. *Heterocycles* **1998**, *48*, 1213.
17. Phoon, C. W.; Abell, C. *J. Comb. Chem.* **1999**, *1*, 485.
18. Pavliak, V.; Kovác, P. *Carbohydr. Res.* **1991**, *210*, 333.
19. *General procedure for glycosylation*: In a flame-dried two-necked round bottomed flask under nitrogen, **5** (1.70 g, 4.88 mmol) and **3** (630 mg, 2.44 mmol) were dissolved in CH_2Cl_2 (12 mL) and cooled to −40 °C. To this solution, 2,6-di -*tert*-butyl-4-methylpyridine (1.5 g, 7.32 mmol) and 1 g of freshly dried and powdered 4 Å molecular sieves were added and stirred. Silver triflate (ACROSS Organics) (1.25 g, 4.88 mmol) in four portions was added over a 2-h period in the dark and the reaction was carriedout for 12 h. After filtering through a Celite® pad, the reaction mixture was diluted with ethyl acetate; washed with 10% aqueous sodium thiosulfate, water, and brine; dried over anhydrous sodium sulfate and evaporated to dryness to give **6** (71% yield). Compound (**6**): R_f = 0.23 (40% EtOAc/hexanes); $[\alpha]_D^{22}$ +50.7° (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, *δ*)1.35 (s, 3H), 1.38 (s, 3H), 1.39 (s, 3H), 1.40 (s, 3H), 1.89 (dd, *J* = 4.8, 5.1 Hz, 2H), 2.03 (s, 3H), 2.08 (s, 6H), 2.18 (m, 2H), 3.32 (dd, *J* = 3.6 Hz, 1H), 3.78 (q, *J* = 8.5, 9.0 Hz, 2H), 4.03 (m, 3H), 4.17 (m, 1H), 4.31 (m, 2H), 5.03 (t, *J* = 9.3, 9.9 Hz, 1H), 5.44 (t, *J* = 10.2, 10.5 Hz, 1H), 5.49 (d, *J* = 3.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 25.99, 27.08, 27.91, 28.31, 35.62, 38.59, 61.06, 62.14, 68.12, 68.81, 70.47, 73.61, 74.23, 74.54, 78.79, 79.24, 97.10, 109.29, 110.19, 169.86, 170.22, 170.80; HRMS (ES+) *m/z*: $[M+H]^+$ calcd for $C_{25}H_{38}N_3O_{12}$: 572.2455; found 572.2480, *Δ* = 4.3 ppm.
20. Root, Y. Y.; Bailor, M. S.; Norris, P. *Synth. Commun.* **2004**, *34*, 2499.
21. He, Y.; Hinklin, R. J.; Chang, J.; Kiessling, L. L. *Org. Lett.* **2004**, *6*, 4479.
22. Lindsley, C. W.; Zhao, Z.; Newton, R. C.; Leister, W. H.; Strauss, K. A. *Tetrahedron Lett.* **2002**, *43*, 4467.
23. *General procedure for amidation*: To an ice cooled solution of **6** (20 mg, 0.035 mmol) in dichloromethane (1 mL) was added a polymer-supported triphenylphosphine (2.04 mmol/g loading, 26 mg). The mixture was gently stirred for approximately 5 min at room temperature after which acid chloride (0.07 mmol) was added and the mixture stirred until no additional nitrogen gas was liberated. The mixture was gently refluxed at 40 °C for 12 h, cooled to room temperature, filtered by gravity, and washed with dichloromethane (2× 3 mL). To the filtrate was added acid chloride scavenger MP-Trisamine (~3 mmol/g loading, 58.3 mg, 0.175 mmol) and the mixture was gently stirred for 2 h at room temperature. The mixture was again filtered (gravity), washed with dichloromethane (3× 5 mL), and concentrated in vacuo. The resulting crude products were purified by preparative TLC to give the respective protected amides which were subsequently treated with 0.5 M NaOMe in MeOH followed by TFA/CH_2Cl_2/H_2O (9:1:1) to give the final deprotected analogs **9a**–**n**. Spectral data for each of the final products and for six of the 14 protected amides are available online as Supporting Information.
24. Sassetti, C. M.; Rubin, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12989.
25. Sareen, D.; Newton, G. L.; Fahey, R. C.; Buchmeier, N. *J. Bacteriol.* **2003**, *185*, 6736.
26. Nicholas, G. M.; Eckman, L. L.; Kovac, P.; Otero-Quintero, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 2641.