Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3785–3788
Bioorganic & Medicinal Chemistry Letters

Synthesis of a bromotyrosine-derived natural product inhibitor of mycothiol-*S*-conjugate amidase ☆

Brandon Fetterolf and Carole A. Bewley*

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0820, USA

Received 26 March 2004; revised 27 April 2004; accepted 28 April 2004
Available online 28 May 2004

Abstract—Recently we described the structures of two new bromotyrosine-derived alkaloids that inhibit the detoxification enzyme mycothiol-*S*-conjugate amidase (MCA) from *Mycobacterium tuberculosis*. Here we describe a concise total synthesis of bromotyrosine oxime **1**. The six-step synthesis of **1** utilized a trifluoromethyloxazole intermediate, whose hydrolysis product underwent alkylation and coupling to agmatine to give the inhibitor in ~40% overall yield. Oxime **1** inhibited MCA and its homolog AcGI deacetylase with IC_{50} values of 30 and 150 μM, respectively.

Mycothiol (MSH) is the major low molecular weight thiol limited to actinomycetes, which include mycobacteria. Analogous to glutathione in Gram negative bacteria and eukaryotes, MSH plays a major role in detoxification and maintenance of a reductive intracellular environment.[1] Owing to the continuing need for new classes of antibiotics effective against *Mycobacterium tuberculosis*,[2] studies of enzymes involved in mycothiol biosynthesis and mycothiol-dependent detoxification, as well as those of inhibitors to these enzymes, are rapidly increasing in number. Recently we described a series of marine natural product inhibitors of MCA, some of which are lethal to *M. smegmatis*.[3,4] Of those structures studied, kinetics experiments established that the bromotyrosine-derived series of alkaloids, such as **1**, are among the most active inhibitors of the group and are also competitive inhibitors of the *M. tuberculosis* detoxification enzyme MCA. Kinetics, NMR and modeling experiments suggest that this class of compounds presumably acts by chelation of a metal cation in the active site via the oxime moiety.[4,5] Subsequent structure–activity relationship studies using a synthetic library of compounds[6] inspired by the natural product disulfide psammaplin A[7] (**5**) yielded additional information concerning structural features that contribute to, or abrogate, inhibitory activity toward MCA.[8] Together, these studies established that phenolic oximes exclusive of disulfides or mixed disulfides present in the synthetic library represented the best inhibitors of MCA reported to date. Moreover, inhibitors lacking the disulfides may be more desirable in terms of stability and specificity. Motivated by these findings, we report a concise total synthesis of inhibitor **1**.

Many marine natural products that appear to be biogenetically derived from a bromotyrosine precursor have been described previously (several examples are shown in Fig. 1). Owing to the variety of interesting biological activities exhibited by members of this class of compounds, a number of syntheses have also been completed. Wasserman et al. employed a cyano ylid coupling for chain elongation, followed by formation of an oxime to obtain the natural products verongamine **3**, hemibastadin **2**, and aerothionin **4**.[9] Hoshino et al. synthesized the bromotyrosine disulfide psammaplin A **5** and its mixed disulfide derivatives by opening an oxazole to generate an alpha keto acid intermediate followed by formation of the oxime.[10] During the course of our synthetic studies, Kende et al. completed the first total synthesis of **1** from two complementary schemes starting with 4-hydroxyphenylpyruvic acid, and dibromination with NBS was carried out prior to their

Keywords: Mycobacteria; Marine natural products; Deacetylase; Enzyme.
☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.04.095
* Corresponding author. Tel.: +1-301-594-5187; fax: +1-301-402-0008; e-mail: cb194k@nih.gov

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.095

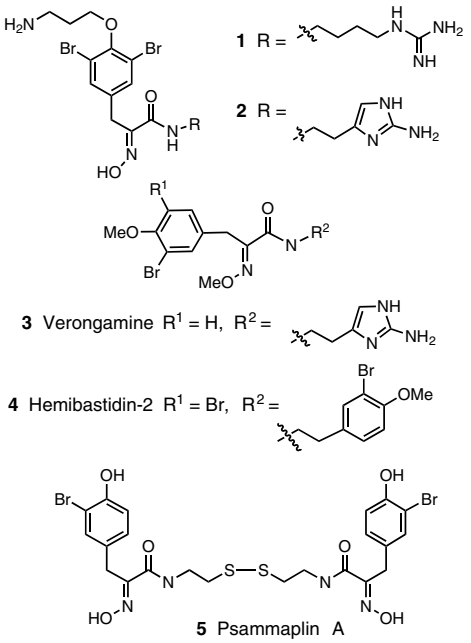
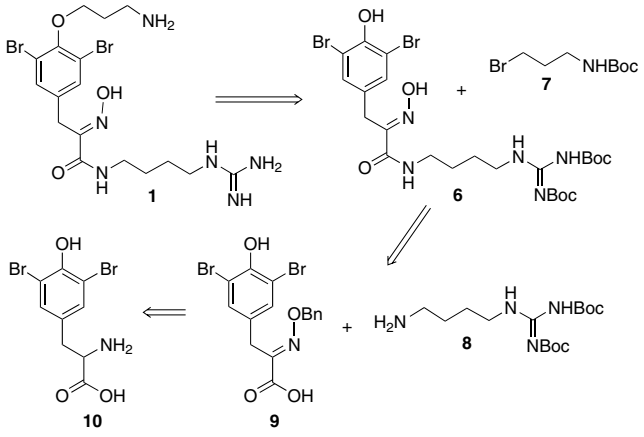


Figure 1. Examples of marine natural product oximes derived from bromotyrosine.

coupling reactions.[11] For the synthesis of **1**, we felt Hoshino's approach to the synthesis of psammaplin A would provide a general route to the oxime, starting with commercially available 3,5-dibromotyrosine. A retrosynthetic analysis (Scheme 1) indicated that **1** could be obtained by *O*-alkylation to connect phenyl alcohol **6** and bromide **7**, and compound **6** could be obtained by coupling protected agmatine **8** with alpha-oximino acid **9**.

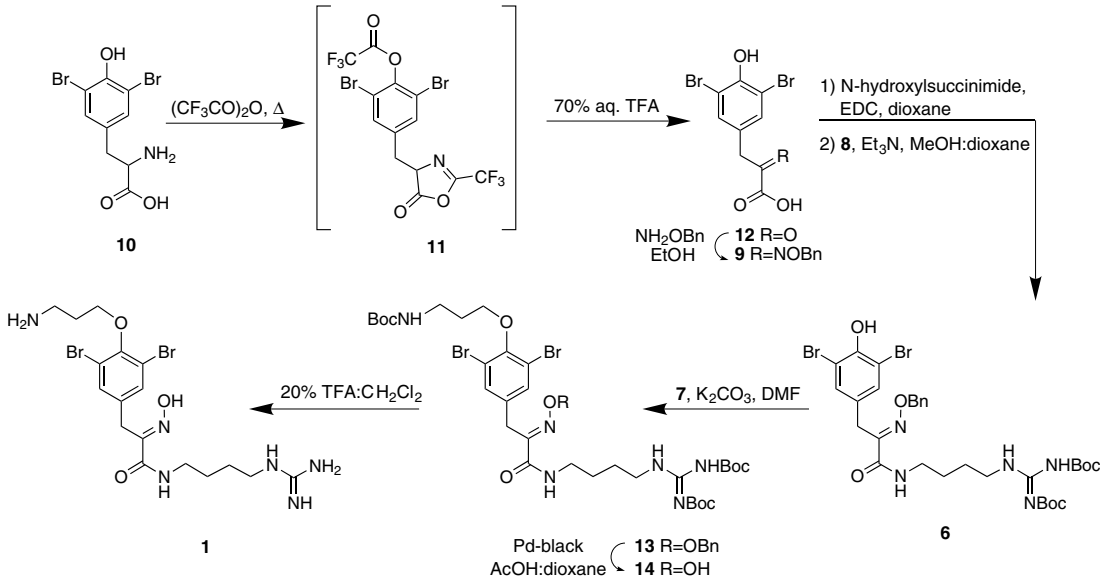
As illustrated in Scheme 2, commercially available 3,5-dibromotyrosine **10** was treated with trifluoroacetic anhydride (TFAA) at 80 °C for 18 h to generate the



Scheme 1. Retrosynthetic analysis.

unstable trifluoromethyloxazolone intermediate **11**.[12] Oxazole **11** was dissolved in 70% aq trifluoroacetic acid (TFA) and allowed to stand overnight, precipitating the alpha keto acid **12** as a white solid in 91% yield over the two steps. Treatment of ketone **12** with O-benzyl hydroxylamine in ethanol provided the alpha protected oxime **9** in 87% yield. For coupling *N*-Boc-protected agmatine[13] **8** to intermediate **9**,[14] several different coupling reagents were tested in an attempt to improve yields. These included the carbonyl activators (1,1′)-carbonyldiimidazole (CDI),[15] dicyclohexylcarbodiimide (DCC), and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDCI). Of the three, we found that treating **9** with EDCI and *N*-hydroxylsuccinimide gave slightly higher yields (63%) of intermediate **6** compared to yields of 53% and 56% for CDI and DCC, respectively.

In the final steps of the synthesis, alkylation of **6** with bromide **7** under basic conditions provided **13** in 86% yield.[16] Initial attempts at hydrogenation of **13** using



Scheme 2. Reactions and conditions.

palladium on carbon resulted in deprotection of the benzyl group and undesirably, reduction of the oxime to an amine as the major product. We next attempted hydrogenation of **13** using palladium black in equal amounts of acetic acid and 1,4-dioxane,[17] which provided **14** in 89% yield with a minimal amount of amine formation.[18] Cleavage of the three *N*-*tert*-butyl carbamate protecting groups using 20% TFA in dichloromethane completed the synthesis of **1** in 38% overall yield starting from the 3,5-dibromotyrosine.[19]

Spectroscopic data for synthetic **1** were recorded and compared to those of natural **1**. Consistent with data from the natural product, HR-FABMS showed an isotopic cluster of 1:2:1 at m/z 521, 523, 525 in the FABMS, and HR-FABMS showed a molecular ion at 523.0336 (calcd 523.0667); and proton and carbon spectra (provided in Supporting Information) were identical to those of the natural product. Last, synthetic **1** was tested for inhibitory activity against recombinant *M. tuberculosis* MCA and the biosynthetic enzyme AcGI deacetylase in an identical manner as described previously.[4,20] As an internal reference in the MCA assays, synthetic **1** was tested in parallel to the natural products psammaplysin[21] and oceanapaside.[22] Multiple independent assays carried out in duplicate confirmed levels of MCA inhibition for synthetic **1** and the natural products equivalent to those reported previously, and yielded an IC_{50} value of approximately 150 μM for inhibition of AcGI deacetylase. In summary, a short and highly efficient synthesis of natural product **1** has been accomplished, and with minor modifications, can be envisaged to supply a plethora of different synthetic derivatives. Related synthetic and biological studies are underway.

Acknowledgements

We thank Lisa Eckman for preparation of recombinant mycothiol-*S*-conjugate amidase, Noel Whittaker, and Victor Livengood for MS data and Belhu Metaferia for helpful discussions. This work was supported in part by the Intramural AIDS Targeted Antiviral Program of the Office of the Director (to C.A.B.).

References and notes

1. Reviewed by Newton, G. L.; Fahey, R. C. *Arch. Microbiol.* **2002**, *178*, 388.
2. (a) Dye, C.; Williams, B. G.; Espinal, M. A.; Raviglione, M. C. *Science* **2002**, *295*, 2042; (b) Pozniak, A. *Ann. N.Y. Acad. Sci.* **2001**, *953*, 192; (c) Grange, J. M.; Zumla, A. *Lancet* **1999**, *353*, 996.
3. Nicholas, G. M.; Newton, G. M.; Fahey, R. C.; Bewley, C. A. *Org. Lett.* **2001**, *3*, 1543.
4. Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 601.
5. Mahadevan, J.; Nicholas, G. M.; Bewley, C. A. *J. Org. Chem.* **2003**, *68*, 3380.
6. Nicolaou, K. C.; Hughes, R.; Pfefferkorn, J. A.; Barluenga, S.; Roecker, A. J. *Chem. Eur. J.* **2001**, *7*, 4280.
7. (a) Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 3229; (b) Arabshahi, L.; Schmitz, F. *J. Org. Chem.* **1987**, *52*, 3584.
8. Nicholas, G. M.; Eckman, L. L.; Ray, S.; Hughes, R. O.; Pfefferkorn, J. A.; Barluenga, S.; Nicolaou, K. C.; Bewley, C. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2487.
9. Wasserman, H. H.; Wang, J. *J. Org. Chem.* **1998**, *63*, 5581, and references cited therein.
10. Hoshino, O.; Maruakata, M.; Yamada, K. *Bioorg. Med. Chem. Lett.* **1992**, *12*, 1561.
11. Kende, A. S.; Lan, J.; Fan, J. *Tetrahedron Lett.* **2004**, *45*, 133.
12. Wilson, M. L.; Coscia, C. J. *J. Org. Chem.* **1979**, *44*, 301.
13. Agmatine **8** was synthesized as described by: Exposito, A.; Fernandez-Suarez, M.; Iglesias, T.; Munoz, L.; Riguera, R. *J. Org. Chem.* **2001**, *66*, 4206.
14. Experimental procedure for coupling product **6**: to a solution of acid **9** (0.45 mmol) in 4.5 mL of 1,4-dioxane at room temperature was added *N*-hydroxylsuccinimide (0.86 mmol) followed by addition of EDCI (0.77 mmol). The reaction was stirred for 2 h at which time all starting material had been consumed. The yellow solution was concentrated in vacuo and the crude yellow oil diluted with EtOAc (50 mL). The mixture was washed with satd aq $NaHCO_3$ (2×25 mL), 1 N HCl (2×25 mL), and brine (25 mL). The clear organic layer was dried over Na_2SO_4 and concentrated to a white solid. The crude succinimide ester was dissolved in 1,4-dioxane (4.5 mL) and to this solution was added a second solution containing amine **8** (0.9 mmol) and triethylamine (0.9 mmol) in anhydrous MeOH (4.5 mL). The yellow solution was stirred for 18 h at ambient temperature and concentrated in vacuo. The crude foam was dissolved in EtOAc and purified by flash chromatography (35:65:1 EtOAc–hexane–Et_3N) to yield amide **6**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 18H), 1.59 (m, 4H), 3.33 (m, 2H), 3.44 (br d, $J = 5.1$ Hz, 2H), 3.80 (s, 2H), 5.21 (s, 2H), 6.76 (t, $J = 6.3$ Hz, 1H), 7.30–7.42 (m, 7H), 8.36 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 14.3, 21.1, 26.7, 26.9, 28.2, 28.4, 28.6, 39.2, 40.6, 60.5, 77.7, 79.5, 83.3, 109.9, 126.4, 128.5, 128.8, 130.7, 133.2, 136.4, 148.3, 151.7, 153.4, 156.2, 162.3, 163.5. FTIR (film): ν 2977, 1717, 1615 cm^{-1}. FABMS (pos) 888.2 (M+Cs).
15. Paul, R.; Anderson, G. W. *J. Am. Chem. Soc.* **1960**, *82*, 4596.
16. Spectroscopic data for compound **13**: white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (s, 9H), 1.50 (s, 18H), 1.6 (m, 4H), 1.97–2.07 (m, 2H), 3.34 (m, 2H), 3.42–3.46 (m, 4H), 3.82 (s, 2H), 4.01 (t, $J = 6$ Hz, 2H), 4.96 (br s, 1H), 5.22 (s, 2H), 6.77 (t, $J = 6.3$ Hz, 1H), 7.29–7.43 (m, 7H), 8.34 (br s, 1H), 11.50 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.7, 27.0, 28.2, 28.4, 28.5, 28.6, 28.8, 29.8, 30.1, 38.2, 39.2, 40.5, 71.3, 77.7, 79.4, 83.3, 118.0, 128.4, 128.5, 128.8, 133.7, 135.0, 136.4, 151.3, 151.6, 153.4, 156.2, 156.3, 162.2, 163.6. FTIR ν 1715, 1637, 1131 cm^{-1}. FABMS (pos) 1045.1 (M+Cs).
17. Masatoshi, M.; Tamura, M.; Hoshino, O. *J. Org. Chem.* **1997**, *62*, 4428.
18. Spectroscopic data for compound **14**: clear oil/foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H), 1.49 (s, 18H), 1.56 (m, 4H), 2.00 (m, 2H), 3.27–3.33 (m, 2H), 3.40 (m, 4H), 3.85 (s, 2H), 3.89 (t, $J = 5.1$ Hz, 1H), 3.99 (t, $J = 5.7$ Hz, 2H), 4.99 (br s, 1H), 6.77 (t, $J = 6.3$ Hz, 1H), 7.48 (s, 2H), 11.47 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.4, 26.7, 28.1, 28.2, 28.4, 28.6, 30.1, 38.3, 39.1, 40.97, 66.1, 71.3, 79.5, 80.4, 83.9, 118.0, 133.7, 135.7, 151.2, 151.4, 153.3, 156.1, 156.5, 163.3. FTIR (film) ν 2977, 1717, 1615, 1132 cm^{-1}. FABMS (pos) 823.1 (M+H).

19. Spectroscopic data for synthetic **1**: white solid. ^{1}H NMR (CD_3OD, 300 MHz) δ 1.56 (m, 4H), 2.12–2.21 (m, 3H), 3.16 (m, 2H), 3.24–3.30 (m, 4H), 3.83 (s, 2H), 4.07 (t, $J = 5.7$ Hz, 2H), 7.49 (s, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ 24.3, 27.3, 27.8, 29.0, 29.1, 39.0, 39.8, 42.2, 71.7, 118.7, 134.7, 138.0, 152.2, 152.4, 165.7. FTIR (film) 3333, 2972, 2930, 1715, 1637, 1131 cm^{-1}. FABMS (pos) 522.23 (M+H).
20. Nicholas, G. M.; Eckman, L. E.; Kovac, P.; Otero-Quintero, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 2641.
21. Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 2916.
22. Nicholas, G. M.; Hong, T. W.; Molinski, T. F.; Lerch, M. L.; Cancilla, M. T.; Lebrilla, C. B. *J. Nat. Prod.* **1999**, *62*, 1678.