ORGANIC LETTERS

2007
Vol. 9, No. 9
1679−1681

Synthesis and Stereochemical Assignment of Brasilibactin A

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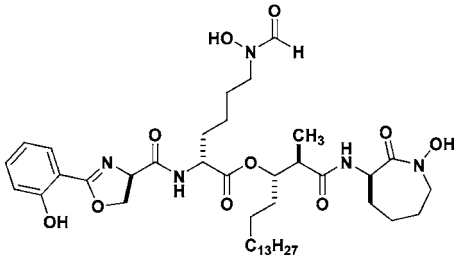
Received February 10, 2007

ABSTRACT



Brasilibactin A, a naturally occurring siderophore related to the mycobactins, has been synthesized in six steps. Use of asymmetric titanium-mediated aldol reactions allowed the preparation of both diastereomers from a common synthetic intermediate, thus allowing the relative stereochemistry of the natural product to be assigned. Brasilibactin A exhibits no inhibition of histone deacetylases (HDACs) in spite of the *N*-formyl-*N*-hydroxy lysine moiety that is expected to affect the activity of these metal-dependent lysine-modifying enzymes.

Natural and synthetic compounds that mediate epigenetic events, including histone lysine acetylation and methylation, have emerged as promising new leads for controlling cancer.[1] After the initial disclosure of histone deacetylase (HDAC) inhibition by trapoxin B and trichostatin (Figure 1),[2] many studies demonstrating control of HDACs with small molecules have emerged.[3] Suberoylanilide hydroxamic acid (SAHA) is the first HDAC inhibitor approved for the treatment of cancer.[4] Control of histone methylation by small molecules is still limited. Inhibitors of metal-dependent[5] (jumonji domain-containing, JHMD/JMJD) and metal-independent[6] (BHC110/LSD1) histone demethylases have recently been reported. Furthermore, there is evidence that HDAC inhibitors also inhibit histone demethylation.[7] Control of both of these epigenetic events is important for further understanding the genetic events that lead to cancer.[8]

We have undertaken the synthesis of brasilibactin A to study its effects on histone modification. Brasilibactin A contains a modified lysine residue for which analogues have been described as inhibitors of HDACs.[9] Although the

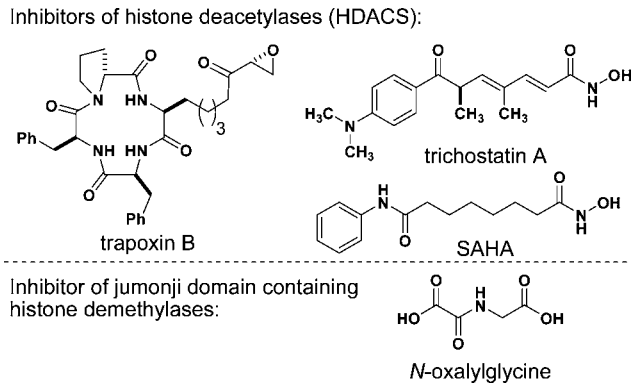


Figure 1. Inhibitors of metal-dependent enzymes that modify lysine residues of histones.

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10.1021/ol070355o CCC: $37.00
Published on Web 03/31/2007

retrohydroxamic acid has been calculated to be less effective at binding zinc,[10] the fact that one class of HDMs (JHDM) and potentially some HDACS employ iron in their active sites suggests that retrohydroxamic acids might still offer unique potencies and/or selectivities. At the outset of our studies, the potential for mycobactin-like structures to affect histone modification had not been explored.

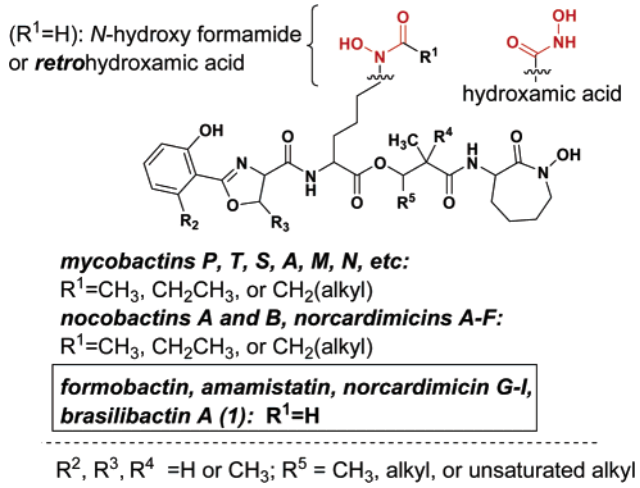


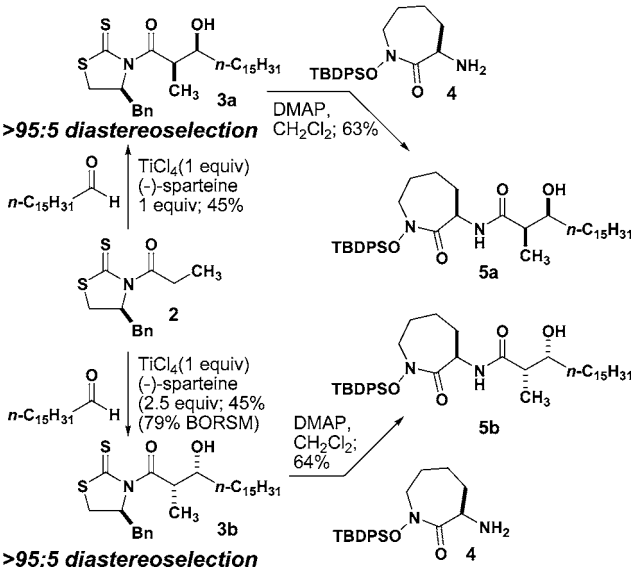
Figure 2. Summary of mycobactins and related siderophore structures.

Brasilibactin A (**1**, Figure 2) is a siderophore recently isolated from *Nocardia brasiliensis* IFM 0995 that exhibits potent cytotoxicity against murine leukemia L1210 (25 nM) and human epidermoid carcinoma KB cells (50 nM).[11] Although this compound exhibits a structural similarity to many mycobactins in the backbone region, it is one of only six compounds in this series reported with an *N*-hydroxy formamide group.[12] Most mycobactins contain long-chain acyl groups at this position. We recognized that this *N*-hydroxyformamide, or "retrohydroxamate", singled out these compounds as potential inhibitors of metal-dependent histone-modifying enzymes such as HDACs and JHDMs.

The absolute stereochemistry of brasilibactin A was previously assigned by total hydrolysis and amino acid analysis, but the absolute stereochemistry of the β-alkoxy acid residue has not been assigned. Analysis of the coupling constants suggested a syn relationship. On the basis of the need to prepare both enantiomers of this segment with either of the two absolute configurations, we chose to employ the titanium-mediated aldol reaction reported by Crimmins,[13] in which a single isomer of the thiazolidinethione auxiliary can be used to form either of the two possible *syn*-aldol products. In addition, this auxiliary allows for the direct cleavage of the auxiliary by amines to form amides.

Aldol reaction of thiazolidinethione **2** with palmitaldehyde proceeded with high diastereoselection (Scheme 1). Use of

Scheme 1. Synthesis of β-Hydroxy Amide Segments from Common Propionyl Thiazolidinethione Auxiliary **2**



1.0 equiv of base provided syn product **3a**, whereas addition of 2.5 equiv of base provided the complementary isomer **3b**. Each isomer was subsequently reacted with amine **4** to provide amides **5a** and **5b**. Matched/mismatched reactivity was observed in this reaction, as a slower reaction was observed in the reaction of **5b**.

The hydroxyl groups of amides **5a** and **5b** were next acylated with protected *N*-Cbz-*N*′-hydroxyleucine (**6**).[14] These reactions exhibited differential rates in their formations of diastereomeric products **7a** and **7b**. Amide **7a** was formed in 60% yield, whereas the reaction to form **7b** was slower and lower yielding (Scheme 2).

Diastereomers **7a** and **7b** were converted to isomers of brasilibactin A (Scheme 3). Hydrogenation to remove the Cbz group and amide coupling to attach the D-serine-derived

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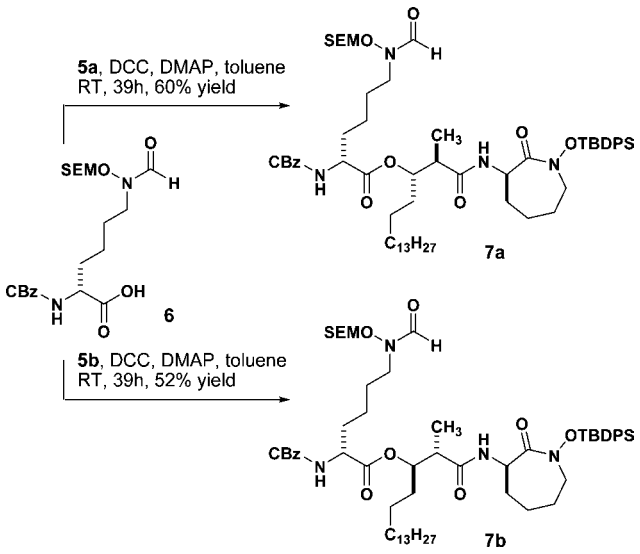
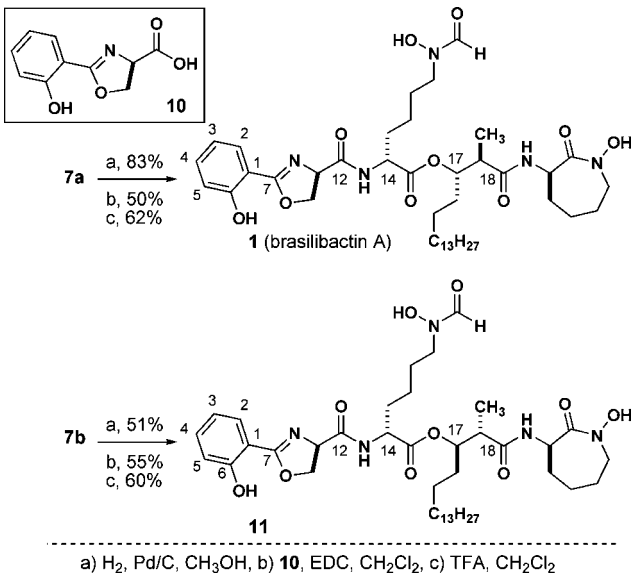
Scheme 2. Attachment of an *N*-Formyl-*N*-hydroxy Lysine Subunit to **5a** and **5b**



Scheme 3. Synthesis of Brasilibactin A (**1**) and 17,18-Bis-*epi*-brasilibactin A (**11**)



2-aryloxazoline[15] proceeded in good yield. Although removal of protecting groups proceeded without incident, isolation of pure, iron-free material proved challenging. Deferration by the method of Snow[16] was successful at removing the orange color associated with the iron complex, but we observed partial decomposition of the product. Deferration using EDTA[17] was also unsuccessful. Ultimately, an aqueous wash with HCl (3.0 M aq) followed by HPLC purification provided pure samples of each diastereomer. Comparison of the ^{1}H NMR spectra with the published signals for brasilibactin A demonstrated that the 17-(*R*),18-(*S*) configuration was consistent with the natural material.[18]

A pure sample of synthetic brasilibactin A was employed in two assays for HDAC inhibition. HeLa cells were treated with brasilibactin A (0.005−5.0 μM) followed by antibodies which reveal nuclear and cytosolic hyperacetylation, which is indicative of HDAC inhibition.[19] No inhibition was observed when compared to SAHA, an unselective HDAC inhibitor. To determine if the inactivity resulted from the fact that brasilibactin was not entering the cells, a second assay using cellular extracts was also conducted. Treatment of cell lysate with brasilibactin A (0.25−1.0 μM) revealed no hyperacetylation of cellular proteins. These results suggest that the cytotoxic activity observed for brasilibactin A, and presumably amamistatin and other *N*-formyl mycobactins, does not emanate from HDAC inhibition. On the basis of these results, our future studies will focus on other cellular processes, including histone methylation.

We have completed an efficient synthesis of brasilibactin A in a total of six steps (longest linear sequence) from known precursors.[20] The use of the Crimmins aldol technology facilitated the straightforward synthesis of both enantiomers of the central β-hydroxy acid residue and allowed the assignment of the configuration of these stereogenic centers in the natural product. The potential for this compound to modulate epigenetic events is under investigation.

Acknowledgment. This research was supported by the NIGMS under the aegis of the Center for Methodology and Library Development (CMLD). J.T.S. thanks Amgen Pharmaceuticals for financial support. The authors thank Prof. Stuart Schreiber (Broad Institute, Harvard University, and the Howard Hughes Medical Institute), Dr. James E. Bradner (Dana Farber Cancer Institute, Broad Institute), and Dr. Stephen J. Haggerty (Broad Institute) for insightful discussions. Prof. Marvin J. Miller and Ms. Leslie Patterson (University of Notre Dame) are acknowledged for helpful advice regarding siderophore deferration. Ms. Jessica Gross (Harvard Medical School, Clardy Research group) is acknowledged for assistance with HPLC purification of the final product. Mr. Edward F. Greenberg (Broad Institute) is acknowledged for performing HDAC assays. The project has been funded in part with federal funds from the NCI's Initiative for Chemical Genetics, NIH, under Contract No. N01-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Supporting Information Available: Experimental procedures and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL070355O

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(20) Compound **4** is prepared in four steps from protected lysine, making the longest linear sequence ten steps from commercially available materials.