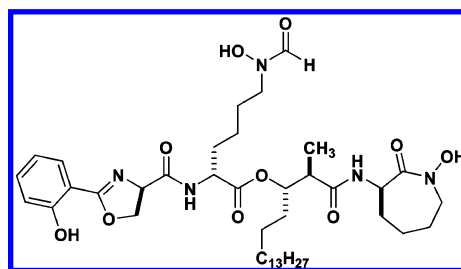


Synthesis and Stereochemical  
Assignment of Brasilibactin AJudith M. Mitchell<sup>†</sup> and Jared T. Shaw<sup>\*,‡</sup>Broad Institute of Harvard and MIT Chemical Biology Program, 7 Cambridge Center,  
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## ABSTRACT



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.<sup>1</sup> After the initial disclosure of histone deacetylase (HDAC) inhibition by trapoxin B and trichostatin (Figure 1),<sup>2</sup> many studies demonstrating control of HDACs with small molecules have emerged.<sup>3</sup> Suberoylanilide hydroxamic acid (SAHA) is the first HDAC inhibitor approved for the treatment of cancer.<sup>4</sup> Control of histone methylation by small molecules is still limited. Inhibitors of metal-dependent<sup>5</sup> (jumonji domain-containing, JHMD/JMJD) and metal-independent<sup>6</sup> (BHC110/LSD1) histone demethylases have recently been reported. Furthermore, there is evidence that HDAC inhibitors also inhibit histone demethylation.<sup>7</sup> Control

of both of these epigenetic events is important for further understanding the genetic events that lead to cancer.<sup>8</sup>

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.<sup>9</sup> Although the

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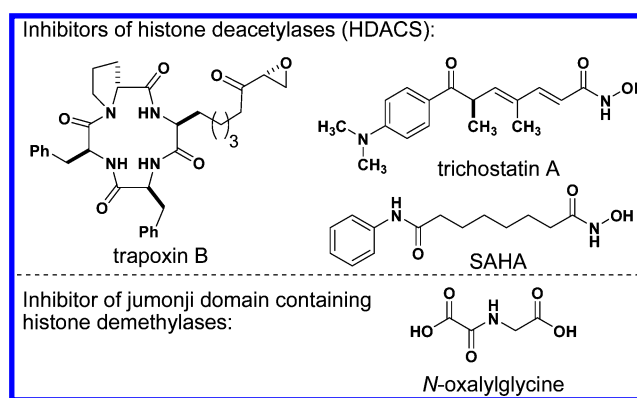
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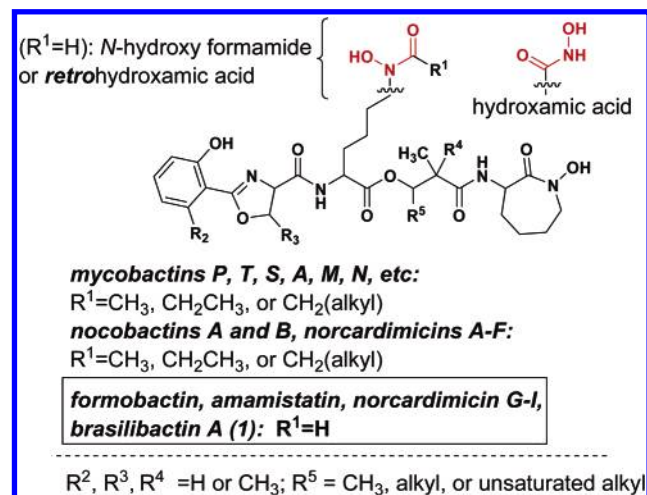
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**Figure 1.** Inhibitors of metal-dependent enzymes that modify lysine residues of histones.

retrohydroxamic acid has been calculated to be less effective at binding zinc,<sup>10</sup> 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).<sup>11</sup> 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.<sup>12</sup> 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.

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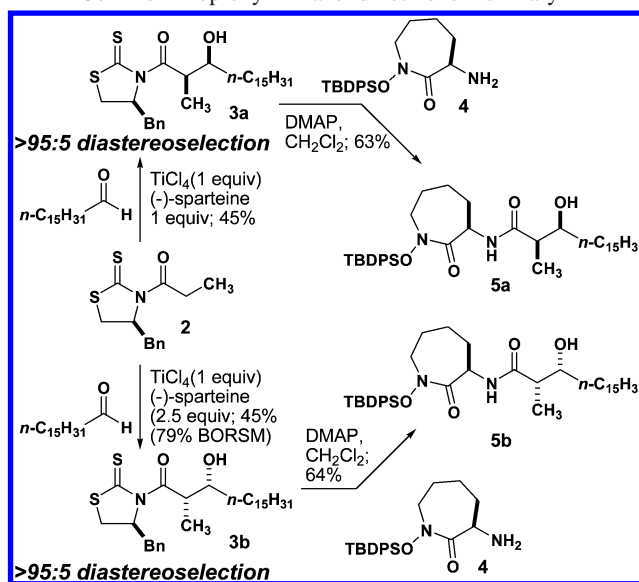
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The absolute stereochemistry of brasilibactin A was previously assigned by total hydrolysis and amino acid analysis, but the absolute stereochemistry of the  $\beta$ -alkoxy acid residue has not been assigned. Analysis of the coupling constants suggested 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,<sup>13</sup> 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  $\beta$ -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**.

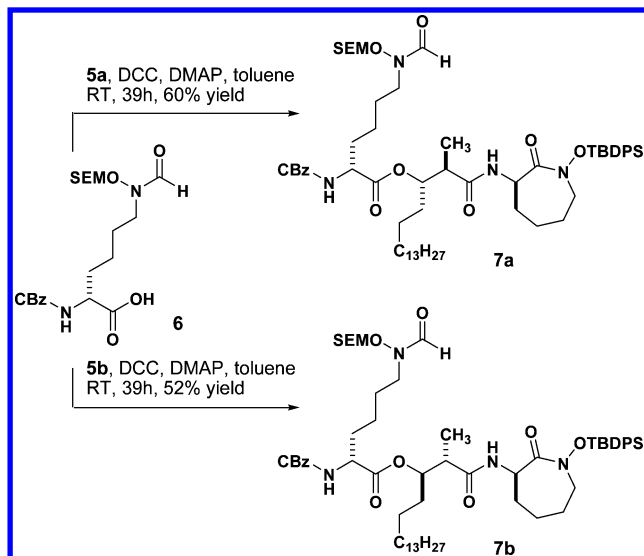
The hydroxyl groups of amides **5a** and **5b** were next acylated with protected *N*-Cbz-*N'*-hydroxyleucine (**6**).<sup>14</sup> These reactions exhibited differential rates in their formations of diastereomeric products **7a** and **7b**. Amide **7a** was formed in 60% yield, whereas the reaction 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**



2-aryloxazoline<sup>15</sup> 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<sup>16</sup> was successful at removing the orange color associated with the iron complex, but we observed partial decomposition of the product. Deferration using EDTA<sup>17</sup> 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 <sup>1</sup>H NMR spectra with the published signals for brasilibactin A demonstrated that the 17-(*R*),18-(*S*) configuration was consistent with the natural material.<sup>18</sup>

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  $\mu$ M) followed by antibodies which reveal nuclear and cytosolic hyperacetylation, which is indicative of HDAC inhibition.<sup>19</sup> 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  $\mu$ M) 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.

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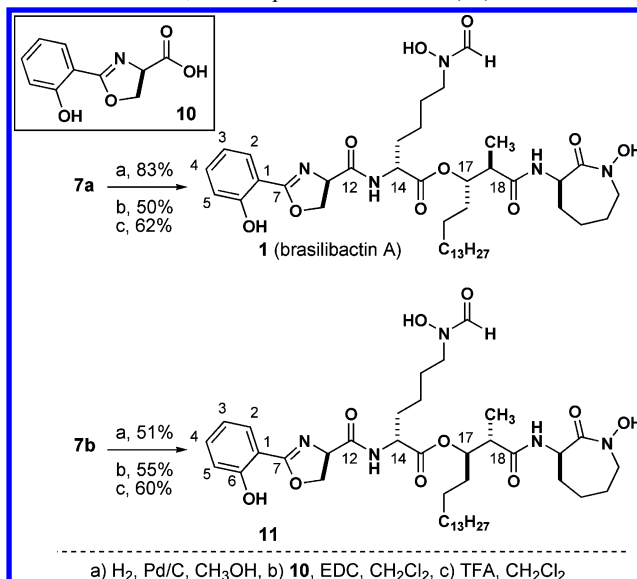
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(20) Compound **4** is prepared in four steps from protected lysine, making the longest linear sequence ten steps from commercially available materials.

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



We have completed an efficient synthesis of brasilibactin A in a total of six steps (longest linear sequence) from known precursors.<sup>20</sup> The use of the Crimmins aldol technology facilitated the straightforward synthesis of both enantiomers of the central  $\beta$ -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.

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**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>.

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