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Total Synthesis and Stereochemical Assignment of Burkholdac B, a Depsipeptide HDAC Inhibitor

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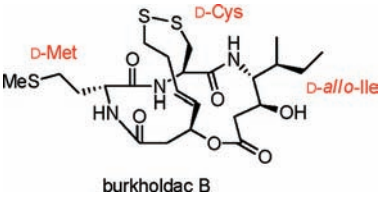
ABSTRACT



Three diastereomers of burkholdac B were prepared by total synthesis, enabling the full stereochemical assignment of the natural product. It is proposed that burkholdac B is identical to thailandepsin A independently isolated by Cheng from the same strain of *Burkholderia thailandensis*. Burkholdac B is the most potent among depsipeptide histone deacetylase inhibitors in growth inhibition of the MCF7 breast cancer cell line with an IC_{50} of 60 pM.

Lysine acetylation is a reversible protein post-translational modification found in both prokaryotes and eukaryotes.[1] Acetylation changes the side chain's size and charge and orchestrates protein−protein and protein−DNA interactions within the nucleus and other cellular compartments. The conversion of *N*-acetyl lysine back to lysine in proteins is catalyzed by histone deacetylases (HDACs). There are 18 HDACs in the human genome, 11 of which are zinc-dependent while the 7 sirtuins are NAD^+-dependent.[2]

Inhibitors of the zinc-dependent HDACs are potential therapeutic agents for a variety of diseases.[3] Natural products are often a rich source of biologically active compounds,[4] and the HDACs are a case in point. Natural product HDAC inhibitors such as trichostatin and apicidin are widely used biological tools, while the depsipeptide FK228 isolated[5] from *Chromobacterium violaceum* No. 968 (Figure 1) is a clinically approved anticancer drug.

FK228 is a prodrug that undergoes intracellular disulfide bond reduction to release the free thiol that acts as a zinc-binding warhead at the HDAC active site. Subsequent to the discovery of FK228, other depsipeptide HDAC inhibitors with an identical warhead were identified. The spiruchostatins isolated[6] from *Pseudomonas* sp. have a statine unit incorporated in the peptide backbone, while largazole[7] from the marine cyanobacterium *Symploca* sp. is an ester prodrug rather than a disulfide. The structural complexity and potent biological activity of these

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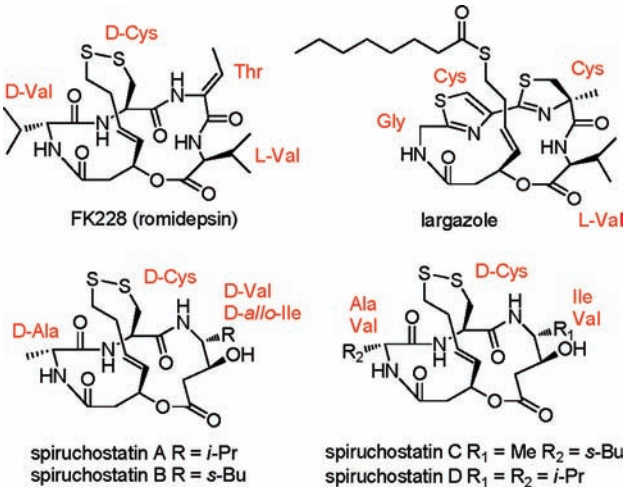

Figure 1. Structures of depsipeptide HDAC inhibitors with amino acid side chains indicated in red.

depsipeptides has led to intensive efforts directed at total and analogue synthesis by ourselves[8] and others.[9−12]

Recently, the Cheng group characterized the FK228 gene cluster.[13] The biosynthesis involves a modular "assembly line" hybrid of nonribosomal peptide synthase and polyketide synthase. By genome mining for homologous open reading frames, Cheng predicted *Burkholderia thailandensis* E264 to be a depsipeptide producer and discovered thailandepsins A (**1**) and B (**2**) as disclosed in a patent (Figure 2).[14] Intriguingly, one amide bond is replaced by a

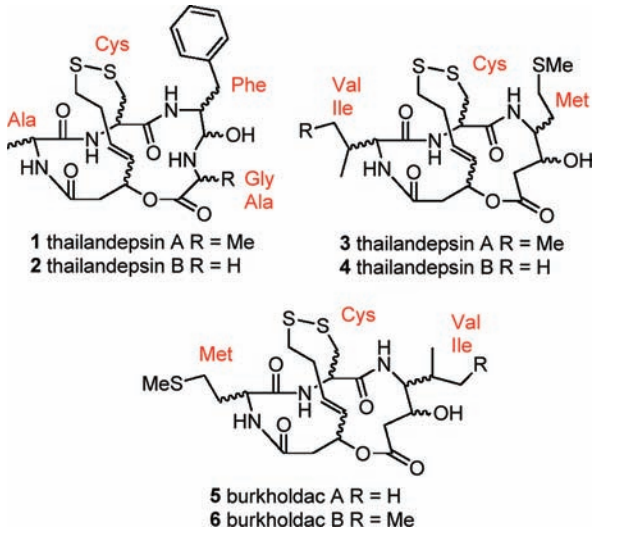

Figure 2. Cheng's original (**1**, **2**) and revised (**3**, **4**) structures of thailandepsins and Brady's burkholdacs (**5**, **6**).

hemiaminal—a rare but not unprecedented motif in cyclic peptides.[15] We explored a thailandepsin A synthesis via cyclization of an aldehyde amine with the disulfide bridge in place but found this to be a complex reaction yielding multiple products. Since the patent provided no characterization data apart from low-resolution MS that did not match the proposed structures, we set aside further work on these compounds.

Cheng later revised the thailandepsin structures to more plausible spiruchostatin congeners **3** and **4** (University of Wisconsin—Madison Research Foundation presentation, 2010). While no stereochemical assignment was made, we postulated that the statine was the syn diastereomer and all

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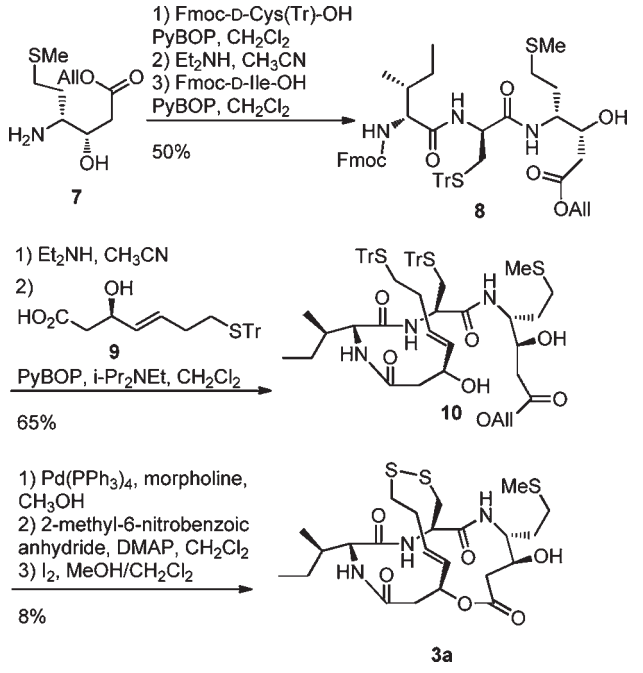
amino acids in the D-series by analogy to the spiruchostatins (Figure 1). A remaining ambiguity is the isoleucine stereochemistry. Epimerization of the α-chiral center of L-Ile gives D-*allo*-Ile, whereas epimerization of α- and *β*-chiral centers gives D-Ile. As both are found in natural products,[16] either diastereomer is possible and this can be resolved only by synthesis.

Scheme 1. Total Synthesis of Thailandepsin A **3a**

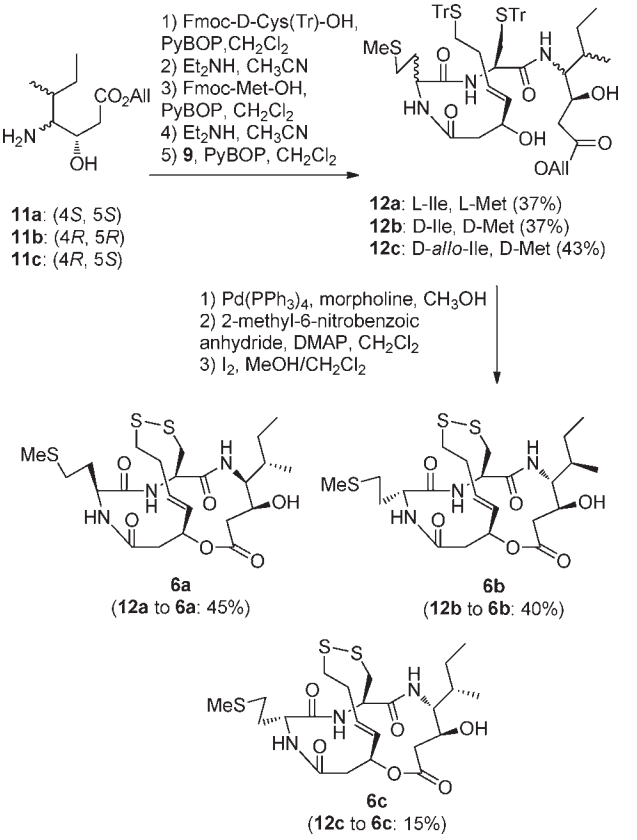


We first prepared the thailandepsin A diastereomer **3a** containing D-Ile (Scheme 1). The statine **7** was obtained by Claisen condensation of the pentafluorophenyl ester of Boc-D-Met and allyl acetate followed by ketone reduction and Boc deprotection.[17] Successive PyBOP-mediated couplings with Fmoc-D-Cys and Fmoc-D-Ile gave the tripeptide **8**. Amide bond formation with *β*-hydroxy acid **9**, obtained by our previously described asymmetric aldol reaction[8a] with the Fujita–Nagao auxiliary,[18] provided linear *seco*-hydroxy ester **10**. Since thailandepsins are sterically unencumbered next to the ester bond (unlike FK228), macrolactonization[8c] with the Shiina reagent[19] was successful and disulfide bond formation completed the synthesis of **3a**. As we were unable to obtain a sample of thailandepsin A or spectral data, the identity of the natural product remained unresolved.

Meanwhile, the Brady group reported the isolation of burkholdacs A (**5**) and B (**6**) from *Burkholderia thailandensis* E264 by overexpression of transcription factors to drive secondary metabolite production.[20] The burkholdacs are isomeric to Cheng's revised thailandepsins, and it is possible that they are unique natural products. However, the isolation of two distinct sets of depsipeptides from the same bacterial strain seems unlikely. We believe Cheng's thailandepsins are identical to Brady's burkholdacs and the latter became our new goal for synthesis.

The connectivity in the Brady structures was secured by 2D NMR. Although Brady did not assign stereochemistry, he suggested the amino acids present in burkholdac B to be L-Ile, D-Cys, and L-Met as the biosynthesis gene cluster contains only one epimerase domain. Our own hypothesis based on spiruchostatin homology would predict all amino acids to be of D-stereochemistry. We targeted three stereoisomers of burkholdac B **6** for total synthesis: (1) the Brady proposal **6a** with L-Ile, D-Cys, and L-Met; (2) the diastereomer **6b** with D-Ile, D-Cys, and D-Met; (3) the diastereomer **6c** with D-*allo*-Ile, D-Cys, and D-Met.

Scheme 2. Total Synthesis of the Three Diastereomers of Brady's Burkholdac B



Following the route described for statine **7**, the statines **11a–c** containing L-Ile, D-Ile, and D-*allo*-Ile side chains were individually prepared from the corresponding isoleucine diastereomer. These were then carried forward to the linear hydroxy esters **12a–c**, which upon macrolactonization and disulfide bridging furnished depsipeptides **6a–c** (Scheme 2). Although the efficiency of macrocyclization

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was variable, the yields are reported for a single experiment and unoptimized.

The NMR spectra of **6a**–**c** show clear and significant differences particularly in the resonances for the isoleucine residue (Supporting Information). Diastereomer **6c** containing D-*allo*-Ile matches Brady's ^{1}H and ^{13}C NMR data and can be definitively assigned as burkholdac B and is likely to be identical to Cheng's thailandepsin A.

Table 1. IC_{50} Values (nM) of Depsipeptides in the Fluor-de-Lys HDAC Enzyme Assay with HeLa Cell Extracts and Growth Inhibition of the MCF7 Breast Cancer Cell Line

compound	HDAC (nM)[a]	MCF7 (nM)[a]
FK228	24 ± 4	0.8 ± 0.2
spiruchostatin A	5.3 ± 3.3	5.7 ± 0.7
largazole	0.04 ± 0.3	5 ± 1
3a	8.3 ± 2.4	10.5 ± 1.8
6a	3312 ± 2472	410 ± 82
6b	3.1	0.25 ± 0.05
6c (burkholdac B)	5.0 ± 3.0	0.06 ± 0.04

[a] The free thiol was generated for HDAC assays, while cell assays were performed with the disulfide prodrug.

The "thailandepsin A" depsipeptide **3a** and burkholdac B diastereomers **6a**–**c** were evaluated in HDAC enzyme and cell line growth inhibition assays (Table 1). In both assays, the diastereomer **6a** with L-amino acids is less potent than the natural products. Cheng's transposed burkholdac **3a** and the diastereomers **6b** and **6c** are all nanomolar HDAC inhibitors with similar activity to FK228 and spiruchostatin A. In the MCF7 growth inhibition assay, the subnanomolar activity of **6b**,**c** is particularly noteworthy. This may be a reflection of improved class I HDAC isoform selectivity as reported by Brady or increased bioavailability or involve other factors. Whatever the reasons, burkholdac B **6c** is by far the most potent of the depsipeptide natural products in this cell assay. With an IC_{50} of 60 pM, burkholdac B is an exciting lead for further investigation.

Structure elucidation was initially a major driver for natural product total synthesis. With the sophistication of current characterization methods, the structures of natural products are usually unambiguous when the synthetic endeavor commences. The thailandepsin/burkholdac case is an exception with issues in both the atom connectivity and stereochemistry.

Several rounds of total synthesis were necessary before the absolute structure of burkholdac B was confirmed. Nevertheless, the additional isomers would not have been otherwise made and have shed useful insights into the SAR of depsipeptide HDAC inhibitors.

For the burkholdacs, the presence of a single epimerase domain in the gene cluster does not lead to a natural product with a single D-amino acid. The same lack of congruence is seen in the related FK228 gene cluster, where the natural product has two D-amino acids. In these depsipeptides, the single epimerase may be acting in trans fashion to invert the other amino acids or the acylation domains may be accepting D-amino acids.

From the activity point of view, our results with **6a** show that introducing multiple L-amino acids into the depsipeptide skeleton is disadvantageous. Cheng's revised structure **3** for thailandepsin A is similar in activity to other natural products in this class, while burkholdac B **6c** is outstanding as an inhibitor of cell growth. Burkholdac B has a lipophilic Met residue, while other depsipeptide HDAC inhibitors contain smaller Gly, Ala, or Val amino acids at the corresponding position. This change appears to be important for the significant increase in activity and isoform selectivity and suggests that further unnatural analogues can be designed to optimize these features.

Note Added in Proof. Cheng has published the isolation of thailandepsins (*J. Nat. Prod.* **2011**, *74*, 2031–2038) and confirmed that thailandepsin A and burkholdac B are identical. Klausmeyer and coworkers at the NCI have isolated the methionine sulfoxide of burkholdac B as an additional natural product (*J. Nat. Prod.* **2011**, *74*, 2039–2044).

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