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Total Synthesis of Spiruchostatin A, a Potent Histone Deacetylase Inhibitor

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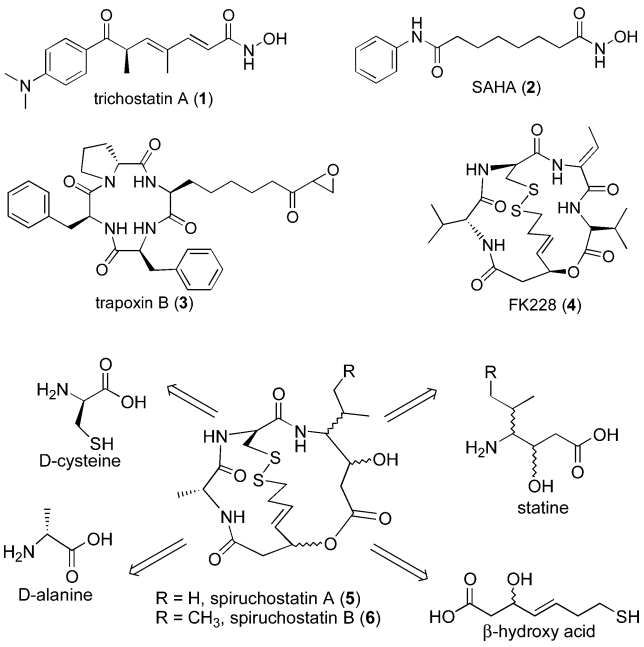
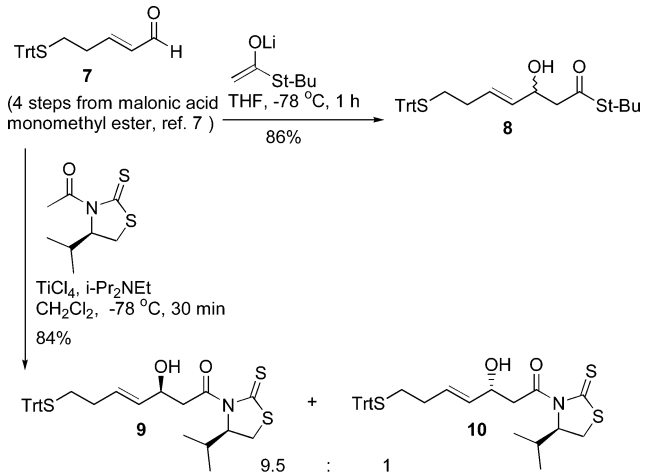
Histone deacetylases (HDACs) are zinc metalloenzymes that catalyze the hydrolysis of acetylated lysine residues. In histones, this returns lysines to their normal protonated state and is a global mechanism of eukaryotic transcriptional control, resulting in tight packaging of DNA in the nucleosome. Additionally, reversible lysine acetylation is an important regulatory process for non-histone proteins. For these reasons, the modulation of HDACs is receiving[1] intense scrutiny. Typical HDAC inhibitors are substrate mimics with a zinc-binding site such as a hydroxamic acid in the natural product trichostatin A[2] (**1**) and the synthetic drug candidate SAHA[3] (**2**), while trapoxin B[4] (**3**) exemplifies a family of cyclic tetrapeptides with an epoxyketone. The natural product depsipeptide FR-901228[5] (**4**, FK228, in phase II clinical trials for cancer) is structurally unique, acting[6] as a prodrug that undergoes disulfide reduction within the cell to release a zinc-binding thiol. The difficulty of modifying FK228 has severely hampered the discovery of any structure−activity relationships. A de novo approach is thus advocated, but Simon's elegant total synthesis[7] is the only example in this area.



In 2001, the structures of spiruchostatin A and B (**5**, **6**), isolated from a *Pseudomonas* extract, were disclosed.[8a] Although the biological activity reported was TGF-β-induced gene expression, the similarity to FK228 suggested underlying HDAC inhibition. Given the successful progression of FK228 to clinical trials, spiruchostatin A is an attractive synthetic target with two additional hurdles. First, the stereochemistry of the β-hydroxy acid and statine was not established. We reasoned that the former was (*S*), as in FK228. The choice of statine diastereomer was less obvious, but was fortunately assigned[8b] as (3*S*,4*R*) during the course of our work. The second challenge is synthetic manipulation of the statine while avoiding β-elimination, protecting group migration, and intramolecular cyclization.

We envisioned preparation of the β-hydroxy acid by Simon's procedure. An achiral aldol uneventfully converted aldehyde **7** (Scheme 1) to *rac*-**8**, but neither the Carreira conditions[9] used by Simon nor the alternative Keck methodology[10] for enantioselective acetate aldols satisfactorily yielded enantiopure **8**. Instead, reaction of **7** with the *N*-acetylthiazolidinethione Nagao auxiliary[11] under Vilarrasa's $TiCl_4$ conditions[12] was highly diastereoselective, yielding the readily separable **9** and **10**. The stereochemical assignment was based on analogy to the literature and confirmed by hydrolysis of **9** to the known β-hydroxy acid prepared by Simon. Since completion of our work, Wentworth and Janda have reported[13] a chloroacetyloxazolidinone Evans aldol and subsequent dechlorination to prepare the same β-hydroxy acid in their total synthesis of FR-901375.

Scheme 1



The statine was obtained by Claisen condensation of Boc-D-valine pentafluorophenyl ester with methyl acetate,[14] followed by diastereoselective reduction of **12** and switching the ester functionality (Scheme 2). The choice of statine protecting groups was highly crucial to our ultimate success. Advanced intermediates with methyl or *tert*-butyl esters could not be unraveled to the free acid under various conditions, while the trichloroethyl ester is cleavable at neutral pH. Meanwhile, the *N*-Boc group in **14** can be removed by acid to provide a protonated amine, preventing undesirable intramolecular cyclization to the γ-lactam. In situ neturalization and condensation with activated D-cysteine yielded dipeptide **15**. Protection of the alcohol and amino acid homologation furnished protected tripeptide **17**.

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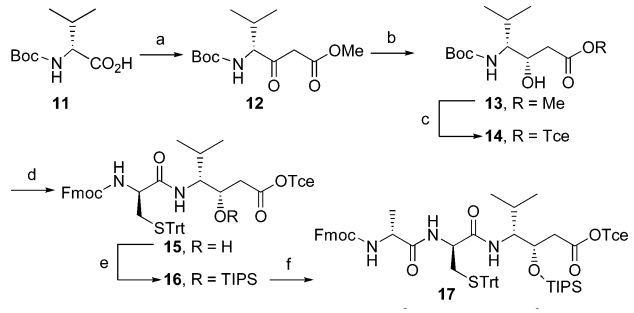
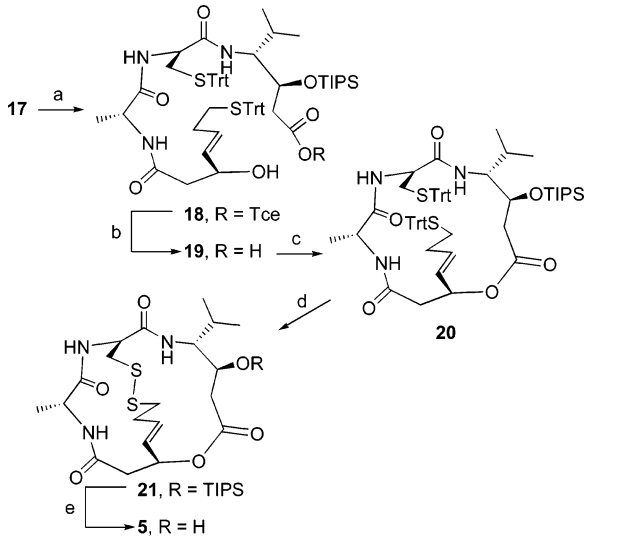
Scheme 2 [a]



[a] Reagents and conditions: (a) (i) PfpOH, EDAC·HCl, DMAP, CH_2Cl_2, 0 °C, 30 min, 20 °C, 4 h; (ii) $LiCH_2CO_2CH_3$, THF, −78 °C, 45 min (66%); (b) KBH_4, MeOH, −78 to 0 °C, 50 min (70%); (c) (i) LiOH, 4:1 THF/H_2O, 0 °C, 1 h; (ii) TceOH, DCC, DMAP, CH_2Cl_2, 0 °C to room temperature, 18 h (95%); (d) (i) TFA, CH_2Cl_2, room temperature, 3 h; (ii) Fmoc-STrt-D-Cys, PyBOP, i-Pr_2NEt, CH_3CN, 20 °C, 20 min (74%); (e) TIPSOTf, 2,6-lutidine, CH_2Cl_2, room temperature, 3 h (93%); (f) (i) 5% Et_2NH/CH_3CN, 20 °C, 3 h; (ii) Fmoc D-Ala, PyBOP, i-Pr_2NEt, CH_3CN, 20 °C, 1 h (82%).

Scheme 3 [a]



[a] Reagents and conditions: (a) (i) 5% Et_2NH/CH_3CN, 20 °C, 5 h; (ii) **9**, DMAP, CH_2Cl_2, 0 °C, then 20 °C, 7 h (84%); (b) Zn, NH_4OAc/THF, 20 °C, 5 h (71%); (c) (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, CH_3CN/THF, 0 °C, then 20 °C, 1 h; (ii) DMAP, toluene, 50 °C, 4 h (53%); (d) I_2, 10% MeOH/CH_2Cl_2, 20 min (84%); (e) HCl, EtOAc, −30 to 0 °C, 3 h (77%).

We anticipated that **9** was sufficiently activated[15] to directly function as an acylating agent. In the event, Fmoc deprotection of **17** and coupling of the crude amine with **9** proceeded smoothly to afford **18** (Scheme 3). Reductive removal of the trichloroethyl ester under buffered conditions unmasked the pivotal *seco*-acid **19**. In Simon's FK228 synthesis, classical macrocyclization by acid activation was unsuccessful, and alcohol activation under carefully controlled Mitsunobu conditions was used instead. In our case, Yamaguchi macrolactonization[16] of **19** to **20** occurred in good yield. Disulfide bond formation[17] and silyl ether removal completed the total synthesis of spiruchostatin A, $[\alpha]_D$ −61.1 (*c* 0.14, MeOH) [lit.[8a] $[\alpha]_D$ −63.6 (*c* 0.14, MeOH)]. The minor aldol product **10** was similarly processed to provide *epi*-spiruchostatin A with (*R*) stereochemistry at the β-hydroxy acid fragment.

Spiruchostatin A caused the accumulation of acetylated histone-H4 and activated the $p21^{waf1}$ promoter in intact breast cancer cells, presumably by a prodrug mechanism similar to that of FK228. Spiruchostatin A inhibited the growth of breast cancer cells with an IC_{50} of approximately 10 nM, compared to 100 nM for the HDAC inhibitor trichostatin A. *epi*-Spiruchostatin A was essentially inactive at 10 μM, highlighting the importance of (*S*) stereochemistry in the β-hydroxy acid for favorable interactions with residues around the rim of HDAC active sites. This observation parallels[2b] trichostatin A, where the unnatural enantiomer is a significantly less active HDAC inhibitor.

Our synthesis unambiguously confirms the complete structure of spiruchostatin A. A noteworthy feature is the dual role of the Nagao auxiliary as a chiral auxiliary for accomplishing acetate aldols *and* as an acylating agent. FK228, and more recently spiruchostatin A, have reached clinical trials as anticancer agents, although they are unlikely to be optimized by Nature for potency or selectivity against human HDACs. Our route paves the way for the preparation and testing of unnatural analogues.

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Supporting Information Available: Chemical and biological experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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