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Total Synthesis of Azumamide A and Azumamide E, Evaluation as Histone Deacetylase Inhibitors, and Design of a More Potent Analogue

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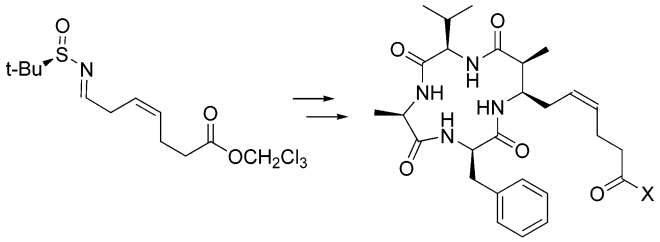
ABSTRACT



The unprecedented diastereoselective Mannich reaction of a *Z*-allylsulfoximine was a key step in the total synthesis of the marine natural products azumamide A and E, and an unnatural analogue. Their relative potency as histone deacetylase inhibitors was evaluated and found to correlate with predicted zinc-binding affinity.

In eukaryotes, DNA is tightly packaged with histone and non-histone proteins into the higher order structure of chromatin. Protein posttranslational modifications including acetylation, methylation, phosphorylation, and ubiquitinylation constitute a "histone code"[1] that ultimately regulates gene transcription by modulating the unwinding of DNA and recruitment of binding partners. Among the chromatin modifying enzymes, zinc metallohydrolase class I/II histone deacetylases (HDACs)[2] have attracted the most attention as anticancer targets. The two most advanced inhibitors in development are Merck's synthetic Vorinostat (Figure 1, SAHA, suberoylanilide hydroxamic acid), recently receiving FDA approval, and the natural product FK228 (depsipeptide) in Phase II clinical trials. These exemplify the classic pharmacophore for HDAC inhibitors: a "warhead" binding the active site zinc, linked by a spacer mimicking the substrate's acetyl-lysine side chain, and a "cap" protruding

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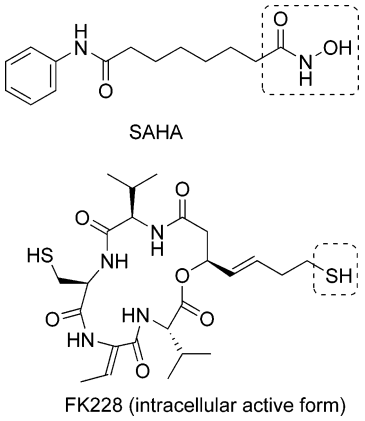


Figure 1. SAHA and FK228, with "warhead" highlighted.

beyond the substrate binding channel and contacting the enzyme's "rim". While FK228 has a weaker thiol warhead compared to a hydroxamic acid, the additional binding interactions from the macrocyclic cap result in an overall potency superior to SAHA, and confer selectivity as the homology betweeen HDAC isoforms is divergent in the rim region.

Given our interest[3] in natural product HDAC inhibitors, the disclosure[4] of the azumamides (Figure 2) was noteworthy

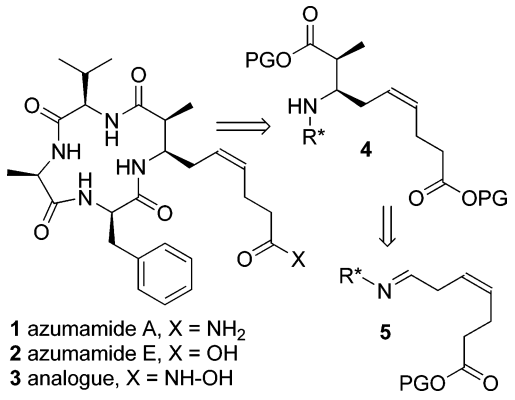


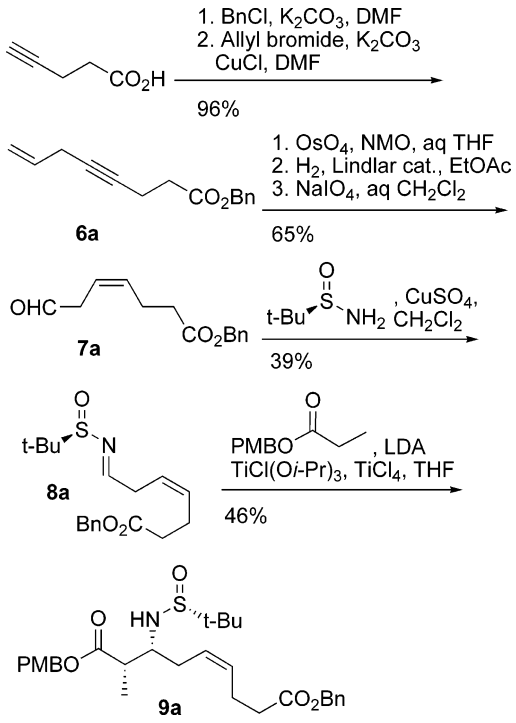
Figure 2. Azumamides and retrosynthesis of the β-amino acid.

for several reasons: (1) Azumamide A contains a carboxamide warhead, hitherto a rare motif among HDAC inhibitors, nevertheless, its reported potency was similar to azumamide E, despite the carboxylic acid being expected to be a stronger metal binder; (2) a structural similarity to FK228, with the depsipeptide replaced by a peptide backbone; and (3) A cyclic tetrapeptide scaffold, with all amino acids in the D-configuration, which would result in unfavorable steric congestion,[5] relieved in the azumamides by expansion of one α-amino acid to a β-amino acid.

Retrosynthetically, macrolactamization of a linear precursor was anticipated to be achievable although likely to be challenging and dependent upon the position of cyclization. This reduced the problem to an efficient preparation of the unnatural β-amino acid **4**. A total synthesis of azumamides A and E has recently appeared[6] where this moiety was assembled by a multistep operation from 1,3-propanediol. Our independent and contemporaneous effort envisioned a Mannich reaction, using Ellman's *tert*-butylsulfinyl auxiliary[7] as R*. While the Mannich reaction is precedented[8] with simpler examples, the choice of β,γ-unsaturated imine **5** appeared audacious due to potential for bond isomerization or migration to conjugated enamine or α,β-unsaturated isomers. Indeed, the SciFinder database showed no examples of α-methylene-β,γ-unsaturated sulfinylimines.

In practice, benzyl 4-pentynoate was subjected to Martín's four-step sequence[9] for conversion of an acetylene to a β,γ-*cis*-unsaturated aldehyde (Scheme 1). The fragile aldehyde

Scheme 1. Synthesis of the β-Amino Acid



7a was successfully converted to sulfinylimine **8a** by $CuSO_4$-mediated dehydration. The ensuing Mannich reaction with a propionate ester enolate proceeded with high diastereoselectivity to afford β-amino acid **9a**. Although the last two reactions were of modest yield due to the poor stability of

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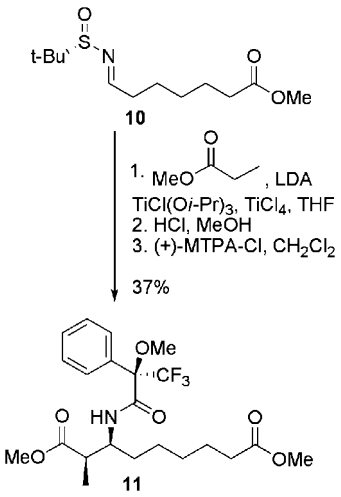
Scheme 2. Synthesis of the Azumamide Degradation Fragment



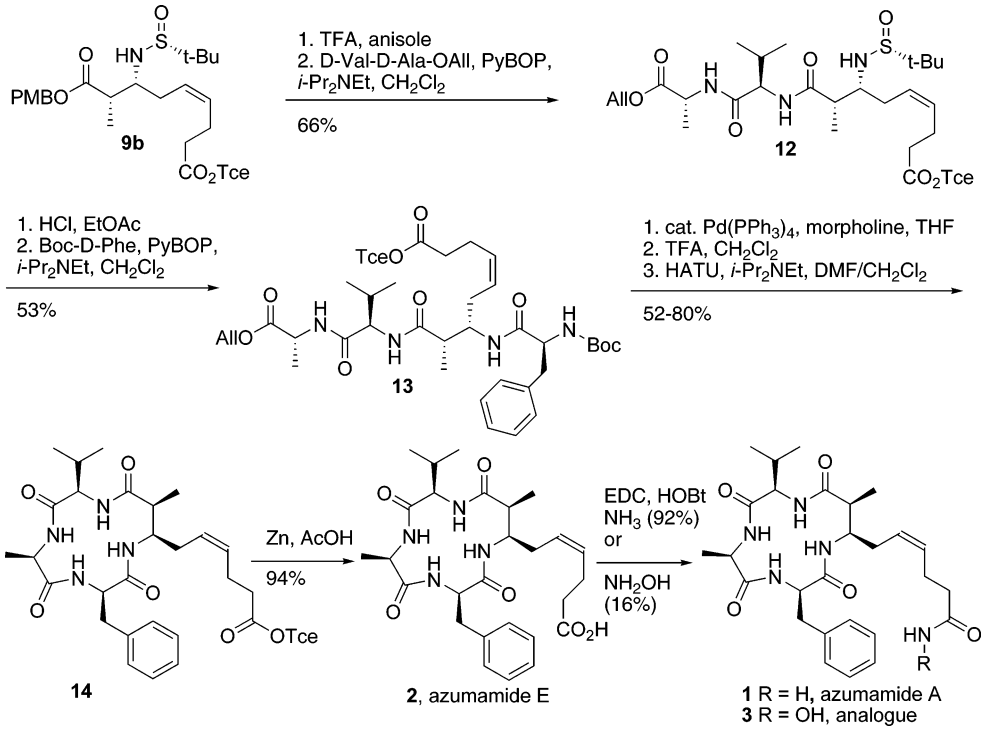
7a and **8a**, they provided gratifying support for the overall strategic disconnection and its potential to deliver the β-amino acid in a concise manner.

Before proceeding further, it was important to establish the nature of the β-amino acid within the azumamides. In the original isolation, this was determined by degradation and comparison with simpler model compounds. To resolve any ambiguity regarding the relative and absolute configuration, we prepared imine **10** (Scheme 2) containing the enantiomeric sulfinylimine to **8a**. With this stable saturated imine, the Mannich reaction occurred in high yield, but in poorer diastereoselectivity providing a 5:1 ratio of products. Removal of the auxiliary and derivatization with Mosher's acid chloride (MTPA-Cl) led to (2*R*,3*S*) diastereomer **11**. Comparison of HPLC and NMR data with that for the MTPA amide obtained from the natural product enabled conslusive assignment of the latter as the (2*S*,3*R*) diastereomer.

With the structure of the natural products secured, we returned to the total synthesis. The β-amino acid **9a** was successfully progressed to azumamide A, but alkaline hydrolysis of the benzyl ester protecting group to provide azumamide E was sluggish and accompanied by epimerization of the β-amino acid. We then devised a second-generation route using the trichloroethyl ester as a protecting group cleavable under neutral conditions. Starting from the trichloroethyl ester analogue **6b**, the chemistry in Scheme 1 proceeded uneventfully with the formation of Ellman imine **8b** and its Mannich reaction both proceeding in an improved >50% yield.

Following selective deprotection of the *p*-methoxybenzyl ester, **9b** was coupled with D-Val-D-Ala-OAll dipeptide to give **12** (Scheme 3). The *tert*-butylsulfinyl group having admirably served its dual purpose as chiral auxiliary and protecting group, it was now cleaved and the amine homologated to linear tetrapeptide **13**. Following deprotection at the N- and C-termini, HATU-mediated macrolactamization furnished the azumamide scaffold **14** in 55−85% yield in individual experiments. In comparison, this was a difficult step requiring significant experimentation in the previous[6] total synthesis. We believe this underscores the importance of the position of macrocylization, which was between the

Scheme 3. Total Synthesis of Azumamide A, Azumamide E, and Hydroxamic Acid Analogue



Phe residue and the β-amino acid, unlike our Phe to Ala cyclization.

The trichloroethyl ester in **14** was reductively removed without side reactions, and occurred nearly quantitatively to afford azumamide E (**2**). Small discrepancies were observed between our ^{1}H NMR data and that of the naturally isolated material. The same phenomenon was previously[6] noted, and attributed to pH sensitivity arising from residual TFA used in the HPLC purification of the natural product. Carbodiimide-mediated reaction of azumamide E (**2**) with ammonia then completed the total synthesis of azumamide A (**1**).

Azumamide A and azumamide E inhibited total HDACs from HeLa cell extracts with IC_{50}s of 5800 $\pm$ 1200 and 110 $\pm$ 33 nM, respectively. Thus, in our hands, the carboxylic acid warhead is much more potent than the carboxamide. This is consistent with expected zinc-binding affinity and we believe the original[4] report might have been compromised by impurities.

The biological activity of the azumamides is significantly weaker than FK228 (IC_{50} 15 $\pm$ 9 nM in the same assay). To determine if this was due to the difference in warhead or the shift from a depsipeptide to peptide scaffold, we converted azumamide E (**2**) to the unnatural hydroxamic acid **3**. This analogue had an IC_{50} of 7.0 $\pm$ 2.5 nM, similar to FK228. This suggests that any deficiencies in switching from depsipeptide to peptide backbone can be overcome by increasing the affinity of the zinc-binding warhead. Interestingly, when a hydroxamic acid was substituted[10] for the epoxyketone warhead in chlamydocin, another cyclopeptide HDAC inhibitor, an increase in potency was not observed.

In summary, we have achieved a total synthesis of azumamides A and E. The stereoselective Mannich reaction of an unusual β,γ-unsaturated sulfinylimine is likely the most challenging transformation accomplished to date with the Ellman auxiliary, and enabled a concise route with only 15 steps in the longest linear sequence. Within the series **1**−**3**, we show that HDAC inhibitory activity is in the order carboxamide < carboxylic acid < hydroxamic acid, as predicted by the relative metal binding of these warheads. We anticipate that variation of the azumamide scaffold will yield additional synthetic HDAC inhibitors of interest.

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

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