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Total Synthesis and Full Histone Deacetylase Inhibitory Profiling of Azumamides A–E as Well as β2-epi-Azumamide E and β3-epi-Azumamide E

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ABSTRACT: Cyclic tetrapeptide and depsipeptide natural products have proven useful as biological probes and drug candidates due to their potent activities as histone deacetylase (HDAC) inhibitors. Here, we present the syntheses of a class of cyclic tetrapeptide HDAC inhibitors, the azumamides, by a concise route in which the key step in preparation of the noncanonical disubstituted β-amino acid building block was an Ellman-type Mannich reaction. By tweaking the reaction conditions during this transformation, we gained access to the natural products as well as two epimeric homologues. Thus, the first total syntheses of azumamides B–D corroborated the originally assigned structures, and the synthetic efforts enabled the first full profiling of HDAC inhibitory properties of the entire selection of azumamides A–E. This revealed unexpected differences in the relative potencies within the class and showed that azumamides C and E are both potent inhibitors of HDAC10 and HDAC11.

INTRODUCTION

Macrocyclic peptides have played important roles in the field of epigenetics due to their potent activities as inhibitors of histone deacetylase (HDAC) enzymes. One of the two HDAC-targeting drugs (1 and 3) that are approved by the U.S. Food and Drug Administration (FDA) for clinical treatment of cutaneous T-cell lymphoma is the macrocyclic natural product romidepsin (3). Furthermore, a cyclic tetrapeptide, trapoxin, played an instrumental role in the first isolation of a mammalian HDAC enzyme. Thus, this class of inhibitors holds promise as tool compounds as well as potential drug candidates targeting HDACs.

Though clearly bearing an overall resemblance to the classical cyclic tetrapeptide HDAC inhibitors [including, for example, apicidin (4)], the azumamides (5–9) are structurally unique in that their extended Zn2+-coordinating amino acid (shown in yellow in Figure 1) is a disubstituted β-amino acid. Furthermore, we found the azumamides interesting due to the relatively strong potencies reported for azumamide E against class I HDACs in spite of its weak Zn2+-coordinating carboxylic acid functionality. Previously, azumamide A and azumamide E have been prepared by multistep chemical syntheses, but only azumamide E was tested against recombinant HDAC isoforms 1–9. Furthermore, in vitro profiling with recombinant HDACs has witnessed important new developments since the publication of those results. We therefore found it relevant to explore the properties of these macrocycles in more detail by preparing the complete selection of natural products (5–9), and profiling their activities against the full panel of recombinant human Zn2+-dependent HDAC enzymes, HDAC1–11.

As total syntheses of azumamides B–D had not been reported previously, this work would also allow unequivocal validation of the proposed structures.

For syntheses of the azumamides, we envisaged two significant challenges: first, efficient stereoselective synthesis...
of the substituted β-amino acid, and second, the macro-
cyclization step, which is known to be difficult for small cyclic
peptides in general and furthermore proved challenging in
previously reported syntheses of azumamide analogues.

RESULTS AND DISCUSSION

Building Block Synthesis. For our synthesis of the β-amino
acid building block, we chose a diastereoselective Ellman-type
Mannich reaction to set the stereochemistry, as also previously
reported by Ganesan and co-workers. However, to avoid having
this important transformation at a late stage in our synthetic route,
we decided to optimize this reaction between a propionate ester
and a simple imine as shown in eq 1.

This should give an intermediate with the correct stereo-
chemistry (2S,3R), which could be readily elaborated to give
the desired β-amino acid by robust organic synthetic trans-
formations (vide infra). Mannich reactions between ester
enolates and chiral sulfinylimines have been studied exten-
sively, and using previously reported conditions as our
starting point we conducted an optimization study as outlined
in Table 1. The tert-butyl ester showed superior selectivity
(entry 5) compared to the less bulky methyl, ethyl, allyl, and
PMB esters (entries 1–4), and furthermore, the methyl ester
did not proceed to completion in our hands. Somewhat
surprisingly, however, the major diastereoisomer in entry 5
proved to have (2S,3S) configuration as determined by X-ray
crystallography upon desilylation (Figure 2).

This indicates that the pathway leading to our major isomer
did not proceed through the six-membered Zimmerman–
Traxler-type transition state, which has been proposed to be
responsible for the diastereoselectivity with similar sub-
strates. By using HMPA as an additive instead of a Lewis
acid, this reaction has previously been shown to proceed through
different a transition state, and indeed we saw the
same product distribution when using HMPA and TiCl(OCR)3
as additives with our substrates (entries 5 and 6). This indicates
that the six-membered transition state, where coordination of
titanium is crucial, is highly unlikely to play a significant role in
the formation of our major isomer. This is not in agreement
with the diastereoselectivities observed with the substrates reported
by Ganesan and co-workers. Thus, to address whether the steric
bulk of the triisopropylsilyl ether was responsible for interrupting
the six-membered transition state, we performed the reaction with
different means of protecting the alcohol (entries 7–9). No
significant effect was observed, however, indicating instead that the
steric bulk of the tert-butyl ester caused the predominance of a
different transition state when using our substrates. This is also in
agreement with the original study by Tang and Ellman where the
level of selectivity decreased for 2,3-disubstituted β-amino acids
when the bulk of the ester increased from methyl to tert-butyl.

Because we were interested in taking advantage of solid-
phase synthesis methods to prepare the linear tetrapeptide
azumamide precursors with a minimum of chromatographic
purification steps, we were keen on keeping the acid-labile tert-
butyl ester protecting group, which would allow easy protecting
group manipulation to give an Fmoc-protected β-amino acid
building block. Hence, instead of substituting this protecting
group, we decided to optimize the Mannich reaction conditions
to deliver the desired stereochemistry. First, we changed the
stereochemistry of the sulfinylimine to the R-enantiomer, which
effectually furnished the enantiomer of entries 5–9 (2R,3R) as
the major isomer (entry 10). We then hypothesized that the
configuration of the 2-position would be sensitive to the E/Z
configuration of the enolate. Using Ireland’s conditions for
forming the enolate in the presence of HMPA, we achieved
>80% Z-isomer, which gratifyingly afforded the (2S,3R)
product as major isomer (entry 11). Under the developed
conditions, we prepared compound 12, which was further
elaborated to give Fmoc-protected β-amino acid 16 in 15% overall
yield with just four column chromatographic purification
steps from compound 10 (Scheme 1).

Table 1. Optimization of Stereochemical Outcome of the Mannich Reaction Shown in Equation 1

<table>
<thead>
<tr>
<th>entry</th>
<th>auxiliary</th>
<th>R1</th>
<th>R2</th>
<th>additive</th>
<th>enolate</th>
<th>dr</th>
<th>major isomer</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>Me</td>
<td>OSi(Pr)3</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>47:39:10:4</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>Et</td>
<td>OSi(Pr)3</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>49:29:11:11</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>allyl</td>
<td>OSi(Pr)3</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>46:34:10:10</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>PMB</td>
<td>OSi(Pr)3</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>46:33:11:10</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>Bu</td>
<td>OSi(Pr)3</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>60:26:8:6</td>
<td>(2S,3S)3</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>Bu</td>
<td>OSi(Pr)3</td>
<td>HMPA</td>
<td>E</td>
<td>71:15:14:0</td>
<td>(2S,3S)3</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>Bu</td>
<td>OEt</td>
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<td>E</td>
<td>70:18:12:0</td>
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<tr>
<td>8</td>
<td>R</td>
<td>Bu</td>
<td>OPMB</td>
<td>TiCl(OCR)3</td>
<td>E</td>
<td>77:13:10:0</td>
<td>(2S,3S)3</td>
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<tr>
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<td>75:21:4:0</td>
<td>(2S,3S)3</td>
</tr>
<tr>
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<td>Bu</td>
<td>OSi(Pr)3</td>
<td>HMPA</td>
<td>E</td>
<td>77:18:5:0</td>
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<td>Bu</td>
<td>OSi(Pr)3</td>
<td>HMPA</td>
<td>Z</td>
<td>64:25:8:2</td>
<td>(2S,3S)3</td>
</tr>
</tbody>
</table>

Footnotes: 1Major configuration of the enolate as determined by NMR and by trapping with BuMe2SiCl. 2Diastereomeric ratio determined by 1H NMR. 3ND = not determined. 4Determined by X-ray crystallography on its desilylated homologue. 5Determined spectroscopically by comparison with its enantiomer from entries 5 and 9. 6HMPA (5.4 equiv) was added prior to the substrate to obtain the (Z)-enolate (>80%). 7Determined by comparison of spectroscopic data of the fully elaborated Boc-protected β-amino acid with previously reported data.
peptide by syringe pump to a solution of Hünig’s base and HATU, as described by Ganesan and co-workers, was tested. Judging from LC–MS analyses of the reaction mixtures, we could not observe any significant differences between the cyclization yields obtained with the different methods. Although all the couplings proceeded satisfactorily, with full conversion of linear peptides and minor amounts of the corresponding dimers as the only observed byproducts, the resulting overall isolated yields were relatively low (10%). We ascribe this to difficulties during purification of the macrocyclic products by preparative reversed-phase HPLC caused by poor water solubility, as we were able to recover more material by purifying the macrocycles by column chromatography. Unfortunately, however, this did not provide the final compounds in satisfyingly high purity for the bioassays, and thus the final compounds were all subjected to preparative reversed-phase HPLC purification although this resulted in a loss of material. Carbodiimide-mediated amidation of the side chain was attempted for conversion of 7 to 6 and 23 to 8, but the reaction was slow and gave varying yields (6 vs 8, Scheme 2). Instead, HATU-mediated coupling was attempted for conversion of 9 to 5, and this proved faster and gave an acceptable yield (5). Spectral data of all the natural products 5–9 were in excellent agreement with those originally reported for the azumamides isolated from natural sources, thus corroborating the original structural assignment (Figures S2–S6 in Supporting Information). Finally, the two epimeric β-amino acid building blocks were applied in analogous syntheses of β-epi-azumamide E (26) and β-epi-azumamide E (29) as shown in Scheme 3.

**HDAC Screening.** As an initial test of the HDAC inhibitory potency of all seven compounds, we first screened against the full panel of recombinant human HDACs at two compound concentrations (50 μM and 5 μM). Protocols for HDAC1–9 were adapted from Bradner et al., using the fluorogenic Ac-LeuGlyLys(Ac)-AMC substrate for HDAC1–3 and 6 while using the Ac-LeuGlyLys(tfa)-AMC substrate for HDAC4, 5, and 7–9. For HDAC10 we used the tetrapeptide Ac-ArgThr-Lys(Lys)(Ac)-AMC, which was recently reported to perform well with this enzyme. Finally, for HDAC11, we used Ac-LeuGlyLys(Ac)-AMC as substrate.

The site-specifically epimerized compounds exhibited no activity as previously reported for an analogue having both stereocenters inverted. It was not surprising that 26 was inactive, but it is noteworthy that the subtle change of inverting the stereochemistry of a single methyl group in 29 had such a detrimental effect across the entire selection of enzymes (Figure 3). Furthermore, none of the compounds 5–9 were able to inhibit class IIa HDAC activity against a trifluoroacetylated substrate (Figure 3).

**Inhibitor Kₐ Values.** Next, we performed dose–response experiments for all compound–HDAC combinations that gave above 50% inhibition in the initial assay (Figure S7 and Table S2 in Supporting Information). The obtained IC₅₀ values were converted to Kₐ values by use of the Cheng–Prusoff equation \( K_a = IC_{50}/(1 + [S]/K_m) \) with the assumption of a standard fast-on–fast-off mechanism of inhibition. Reported Kₐ values were applied for the calculations except HDAC10, where we determined the Kₐ for the used substrate to be 1.5 ± 0.2 μM (Figure 4).

Low potencies were recorded against HDACs 6 and 8, which is in accordance with previous data for azumamide E (Table 2), however, compounds 7 and 9 were both potent inhibitors of HDACs 10 and 11. Although they are classified together...
(class IIb), HDACs 6 and 10 clearly interact very differently with these inhibitors. Generally, we found the compounds with a carboxylic acid Zn\(^{2+}\)-binding group (7 and 9) to be more potent than the
carboxamides (5, 6, and 8), which is in contrast to the originally reported HDAC inhibition data obtained for the natural products against an HDAC-containing cell extract.5 However, the data presented herein agree with subsequent work from Ganesan and co-workers15 on azumamide A (5) and azumamide E (9). We thus show that this applies to all the azumamides, which also confirms that a carboxylate Zn2+–binding group renders HDAC inhibitors significantly more potent than a corresponding carboxamide, as would be expected from literature precendents.19,26,27 Furthermore, compound 7 was more potent than 9 against HDACs 1−3, 6, 10, and 11, which is also in contrast to the original evaluation that found azuE (9) more potent than azuC (7) against crude enzymes from K562 cell extract.5 The tyrosine-containing compound (7) exhibited ∼2-fold higher potency against HDACs 1, 3, 6, 10, and 11, whereas the phenylalanine-containing azumamide E (9) was only more potent against HDAC8, albeit at micromolar Ki values.

Finally, the inhibition of HDAC11 by azumamides C (7) and E (9) is, to the best of our knowledge, the first demonstration of potent cyclic peptide inhibitors of this isozyme.33 Notably, these binding affinities were achieved without the presence of a strong Zn2+ chelator, such as hydroxamic acid.

● CONCLUSIONS

In summary, we report total syntheses of all five azumamides, including for the first time azumamides B−D, which corroborate the originally proposed structures. Our synthetic route furthermore enabled preparation of site-specifically edited analogues for exploration of structure−activity relationships (SAR).34−36 The HDAC profi ling results show that the β-amino acid residue, present in all the azumamides, is sensitive to even slight modifications. In addition, the original HDAC testing using cell extract indicated that azumamide E was the most
potent of the series, but the comprehensive profiling presented herein shows that azumamide C is in fact 2-fold more potent than azumamide E against the majority of the isozymes. By taking advantage of the modular methodologies described in this article and building on the gained SAR information, we are currently investigating collections of azumamide analogues in search of more potent and selective ligands based on this promising scaffold.

**EXPERIMENTAL SECTION**

**General.** All chemicals and solvents were analytical-grade and were used without further purification. Vacuum liquid chromatography (VLC) was performed on silica gel (60 Å, particle size 0.015–0.040 mm). UPLC–MS analyses were performed on a Phenomenex Kinetex column (1.7 μm, 50 × 2.10 mm) by use of a Waters Acquity ultra-high-performance liquid chromatography system. A gradient with eluent I (0.1% HCOOH in water) and eluent II (0.1% HCOOH in acetonitrile) rising linearly from 0% to 95% IV during t = 0.00–5.20 min was applied at a flow rate of 1 mL/min (gradient A) or during t = 0.00–5.20 min (gradient B). Analytical HPLC was performed on a Phenomenex Luna column [150 mm × 4.6 mm, C18 (5 μm)] by use of an Agilent 1100 LC system equipped with a UV detector. Gradient C, with eluent I (0.1% TFA in water) and eluent IV (0.1% TFA in acetonitrile) rising linearly from 0% to 95% IV during t = 2–20 min, was applied at a flow rate of 1 mL/min. Preparative reversed-phase HPLC was performed on a Phenomenex Luna column [250 mm × 20 mm, C18 (5 μm, 100 Å)] by use of an Agilent 1260 LC system equipped with a diode-array UV detector and an evaporative light scattering detector (ELSD). A gradient, with eluent V (95:5:0.1, water–MeCN–TFA) and eluent VI (0.1% TFA in acetonitrile) rising linearly from 0% to 95% IV during t = 5–45 min, was applied at a flow rate of 20 mL/min. All tested compounds were purified to homogeneity and shown by both analytical HPLC (gradient C) and LC–MS (gradient A) to be >95% pure. One- and two-dimensional NMR spectra were recorded on a Varian Mercury 300 instrument or a Varian INOVA 500 MHz instrument. All spectra were recorded at 298 K. Correlation spectroscopy (COSY) spectra were recorded with a relaxation delay of 1.5 s before each scan, a spectral width of 6k × 6k, and eight FIDs and 1k × 12k data points collected. Heteronuclear single quantum coherence (HSQC) spectra were recorded with a relaxation delay of 1.5 s before each scan, a spectral width of 6k × 25k, and 16 FIDs and 1k × 12k data points collected. Heteronuclear two-bond correlation (H2BC) spectra were recorded with a relaxation delay of 1.5 s before each scan, a spectral width of 6k × 35k, and 32 FIDs and 1k × 25k data points collected. Chemical shifts are reported in parts per million (ppm) relative to deuterated solvent peaks as internal standards (δ(D, DMSO-d6 2.50 ppm; δ(C, CDCl3 77.16 ppm)). Coupling constants (J) are given in hertz (Hz).

**Multiplicities of 1H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.**

**General Procedure for Mannich Reactions.** A solution of LDA (2.1 equiv) was added dropwise to a solution of the ester (2.0 equiv) in dry THF at −78 °C. After the mixture was stirred for 30 min, Ti(O-iPr)4·Cl (4.2 equiv) in dry THF was added dropwise. The orange solution was stirred for 30 min and the imine (1.0 equiv) in dry THF was added dropwise. The mixture was stirred for 3 h until thin-layer chromatography (TLC) showed full conversion of the imine. The mixture was quenched with saturated aqueous NH4Cl and allowed to reach room temperature. Water was added and the mixture was decanted into a separatory funnel. EtOAc–water (1:1) was added to the remaining Ti precipitate, and the mixture was stirred vigorously for 5 min before being added to the separatory funnel. The aqueous phase was extracted with EtOAc and the combined organic phases were washed again with water, dried (MgSO4), filtered, and concentrated in vacuo.
Azumamide C, (Z)-6-(2R,5R,8R,11R,12S)-8-(4-Hydroxybenzyl)-2-isopropyl-5,12-dimethyl-3,6,9,13-tetra-azaazacyclotridecan-11-yl)hex-4-enolic Acid (7). LiOH (49 mg, 2.0 mmol, 33 equiv) in water (5.0 mL) was added to the crude cyclic peptide 18 (61 mg) in THF (5.0 mL). The solution was stirred for 16 h and concentrated in vacuo. The resulting residue was dissolved in THF−H2O (1: 1, 10 mL) by adding a few drops of TFA, and then purified by preparative HPLC afforded azumamide C (7) (2.2 mg, 9% overall) as a white solid. \[\text{[a]_D} +53^\circ (c = 0.1, MeOH)\]. \(^1H\) NMR (500 MHz, CD3OD) \(\delta 8.10 (d, J = 7.7 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.84 (br s, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.28−7.16 (m, SH), 5.48 (m, 1H), 5.37 (m, 1H), 4.28 (pentet, J = 7.5 Hz, 1H), 4.16 (m, 1H), 4.08 (m, 1H), 3.59 (m, 1H), 3.25 (dd, J = 13.6, 10.4 Hz, 1H), 3.11 (dd, J = 13.6, 6.1 Hz, 1H), 2.72 (m, 1H), 2.68 (m, 1H), 2.39 (d, J = 1.7 Hz, 6H), 2.39 (m, 6H), 1.28 (d, J = 7.1 Hz, 3H), 1.27 (d, J = 7.4 Hz, 3H), 0.94 (m, 6H). HRMS (ESI-TOF) m/z calc for C27H42N4O6H2+: 651.2869; found 651.2869 [M + H]+. HPLC gradient C, \(t_R = 12.53 \text{ min (95%)}\).

**Assay Materials.** HDAC1 (purity >45% by SDS−PAGE according to the supplier), HDAC4 (purity >90% by SDS−PAGE according to the supplier), and HDAC7 (purity >90% by SDS−PAGE according to the supplier) were purchased from Millipore (Temecula, CA). HDAC2 used for dose−response experiments (full length, purity >94% by SDS−PAGE according to the supplier), HDAC5 (full length, purity ≥4% by SDS−PAGE according to the supplier), and HDAC8 used for dose−response experiments (purity >90% by SDS−PAGE according to the supplier) were purchased from BPS Bioscience (San Diego, CA). HDAC2 used for initial screening experiments (full length, purity 50% by SDS−PAGE according to the supplier), HDAC3−“NCoR1” complex (purity 90% by SDS−PAGE according to the supplier; fusion protein of GST-tagged HDAC3 with the deacetylase activation domain (DAD) of NCoR1 (nuclear receptor corepressor)), HDAC6 (purity >90% by SDS−PAGE according to the supplier), HDAC8 for initial screening experiments (purity >50% by SDS−PAGE according to the supplier), HDAC10 (purity >50% by SDS−PAGE according to the supplier), and HDAC11 (purity >50% by SDS−PAGE according to the supplier) were purchased from Enzo Life Sciences (Postfach, Switzerland). HDAC9 (full length, purity 12% by SDS−PAGE according to the supplier) was purchased from Abnova (Taipei, Taiwan). The HDAC assay buffer consisted of 50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, and bovine serum albumin (0.5 mg/mL). Trypsin [10,000 units/mg, from bovine pancreas, treated with L-(tosylamido-2-phenyl)ethyl chloromethyl ketone (TPCK)] was from Sigma Aldrich (Steinheim, Germany). All peptides were purified to homogeneity (>95% purity by HPLC360nm via reverse-phase preparative HPLC), and the white fluffy materials obtained by lyophilization were kept at −20 °C. For assaying, peptides were reconstituted in DMSO to give 5−10 mM stock solutions, the accurate concentrations of which were determined by co-injection on HPLC with a standard of known concentration.

**In Vitro Histone Deacetylase Inhibition Assays.** For inhibition of recombinant human HDACs, dose−response experiments with internal controls were performed in black low-binding Nunc 96-well microtiter plates. Dilution series (3-fold dilution, 10 concentrations) were prepared in HDAC assay buffer from 5−10 mM DMSO stock solutions. The appropriate dilution of inhibitor (10 μL of Sx the desired final concentration) was added to each well followed by HDAC assay buffer (25 μL) containing substrate [Ac-Leu-Gly-Lys(Ac)-AMC, 40 μM for HDAC1−3 and 80 μM for HDAC6 and 11; Ac-Leu-Gly-Lys(TfA)-AMC, 40 μM for HDAC4, 240 μM for HDAC5, 80 μM for HDAC7, 400 μM for HDAC8, and 160 μM for HDAC9; Ac-Arg-His-Lys(Ac)-Lys(Ac)-AMC, 100 μM for HDAC10]. Finally, a solution of the appropriate HDAC (15 μL) was added and the plate was incubated at 37 °C for 30 min [HDAC1, 150 ng/well; HDAC2, 100 ng/well; HDAC3, 10 ng/well; HDAC4, 2 ng/well; HDAC5, 40 ng/well; HDAC6, 60 ng/well; HDAC7, 2 ng/well; HDAC8, 5 ng/well; HDAC9, 40 ng/well; HDAC10, 500 ng/well; HDAC11, 500 ng/well]. Then trypsin (50 μL, 0.4 mg/mL) was added and the assay development was allowed to proceed for 15−30 min at room temperature, before the plate was read on a Perkin-Elmer Enspire plate reader with excitation at 360 nm and detecting emission at 460 nm. Each assay was performed in duplicate. The data were analyzed by nonlinear regression with GraphPad Prism to afford IC50 values from the dose−response experiments, and K\textsubscript{i} values were determined from the Cheng−Prusoff equation [\(K_i = IC_{50}(1 + [S]/K_c)\)] with the assumption of a standard fast-on−fast-off mechanism of inhibition.
**ASSOCIATED CONTENT**

**Supporting Information**
Two tables showing cyclization experiments performed on a simplified model peptide and IC₅₀ values from dose–response experiments; seven figures showing comparison of ¹H and ¹³C chemical shifts for S18 with previously reported values, ¹H NMR data comparisons for azumamides A–E, and dose–response curves for determination of IC₅₀ values for “active” inhibitors; three schemes illustrating synthesis of β³-εi building block (S6) and β⁻εi building block (S11); additional text with full experimental details and compound characterization data; and ¹H and ¹³C NMR spectra. A CIF file for the X-ray crystal structures is available (CCDC 933151). This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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**ABBREVIATIONS USED**

AMC, 7-amino-4-methylcoumarin; Boc, tert-butoxycarbonyl; DAD, deacetylase activation domain; DIC, N,N′-disopropylcarbodiimide; DMF, N,N′-dimethylformamide; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; FID, free induction decay; Fmoc, fluorenylmethyloxycarbonyl; H3, histone 3 protein; H4, histone 4 protein; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N′,N″-tetramethyluronium hexafluorophosphate; HDAC, histone deacetylase; HMPA, hexamethylphosphoramide; HOBt, hydroxybenzo-triazole; HPLC, high-performance liquid chromatography; KHMD, potassium hexamethyldisilazide; LDA, lithium diisopropylamide; MS, mass spectrometry; NCoR, nuclear receptor corepressor; NMR, nuclear magnetic resonance; PMB, p-methoxybenzyl; rt, room temperature; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TOF, time-of-flight; tᵣₑ, retention time; UPLC, ultra-high-performance liquid chromatography.

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