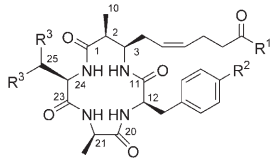
ment of new anticancer agents.[2] Recently, Fusetani and coworkers isolated azumamides A–E (**1**–**5**; Table 1) from the marine sponge *Mycale izuensis*;[3] they represent five unusual cyclic peptides that show potent inhibitory activity on histone deacetylase (HDAC) enzymes (IC_{50}: 0.045–1.3 μM).

Table 1: The azumamides A–E (**1**–**5**).



azumamides A–E

Azumamide	R^1	R^2	R^3
A (**1**)	NH_2	H	Me
B (**2**)	NH_2	OH	Me
C (**3**)	OH	OH	Me
D (**4**)	NH_2	H	H
E (**5**)	OH	H	Me

Natural Product Synthesis

DOI: 10.1002/ange.200602033

Total Synthesis of Azumamides A and E**

Irene Izzo, Nakia Maulucci, Giuseppe Bifulco, and Francesco De Riccardis**

In memory of Luigi Gomez-Paloma and Guido Sodano

In the last years, marine invertebrates have yielded some of the most interesting and biologically important secondary metabolites.[1] Among these fascinating structures, cyclic peptides demonstrate impressive antiproliferative properties and considerable promise as lead structures for the development of new anticancer agents.[2] Recently, Fusetani and coworkers isolated azumamides A–E (**1**–**5**; Table 1) from the marine sponge *Mycale izuensis*;[3] they represent five unusual cyclic peptides that show potent inhibitory activity on histone deacetylase (HDAC) enzymes (IC_{50}: 0.045–1.3 μM).

Histone acetylation plays a fundamental role in gene expression, interfering with transcription-based processes and the regulation of the cell cycle.[4] The modulation of histone-modifying enzymes is recognized as a critical factor for the epigenetic control of oncogene transcription and the activation of tumor repressors.[4b] These combined effects can force cancer cells toward differentiation and growth arrest, rendering HDAC inhibitors as unique leads for the development of specific, noncytotoxic antitumor antibiotics.

Azumamides belong to the rare class of HDAC inhibitors typified by a cyclic tetrapeptide core.[4b] Structurally, they include four nonribosomal amino acid residues, three of which are α-amino acids belonging to the D series (D-Phe, D-Tyr, D-Ala, D-Val), while the fourth one is a unique β-amino acid assigned as (*Z*)-(2*S*,3*R*)-3-amino-2-methyl-5-nonenedioic acid, 9-amide (Amnaa) in azumamides A (**1**), B (**2**), and D (**4**), and as (*Z*)-(2*S*,3*R*)-3-amino-2-methyl-5-nonendioic acid (Amnda) in azumamides C (**3**) and E (**5**).[3]

The configurations at C2 and C3 of the Amnaa residue in azumamide A (**1**) were determined through derivatization with (+)-MTPA-Cl of the free α-methyl-β-amino acid, once isolated and after minor modifications.[3] Comparison of the ^{1}H NMR spectrum of the MTPA amide with those recorded for the stereochemically defined 3-amino-2-methylhexanoic acid models indicated a 2*S*,3*R* pattern. The same configurations were proposed for the remaining azumamides (**2**–**5**) on the basis of their similar chemical shifts and coupling constant values observed in their NMR spectra.[3]

The novel molecular features and the interesting bioactivity of azumamides prompted us to embark on an integrated project consisting of 1) the elaboration of a potentially stereodivergent route for the synthesis of the cyclopeptide architecture of the azumamides and 2) the search of the solution structure for azumamide E (**5**). The first objective served to unequivocally establish the stereochemistry of the Amnaa and Amnda residues, while the second goal was a prerequisite for the inhibitor–enzyme docking studies.

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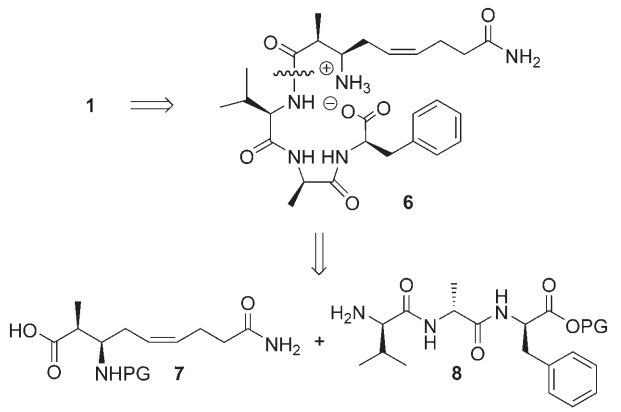
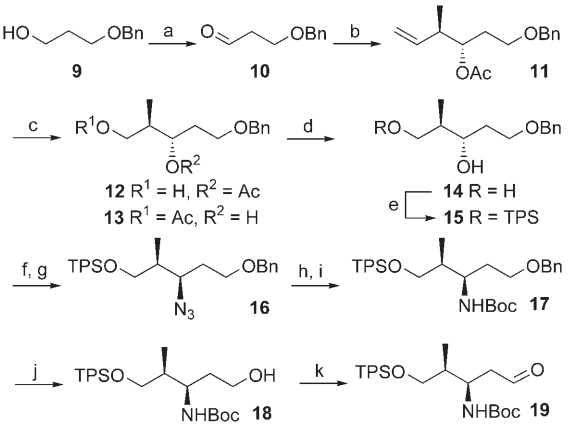
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Initially, we investigated the possible synthesis of azumamide A (**1**). Our convergent approach is shown in Scheme 1 and featured the β-amino acid **7** and the *all*-D tripeptide **8** as key synthons. The synthesis of the α-methyl-β-amino-ω-



Scheme 1. Retrosynthetic plan for azumamide A. PG = protecting group.

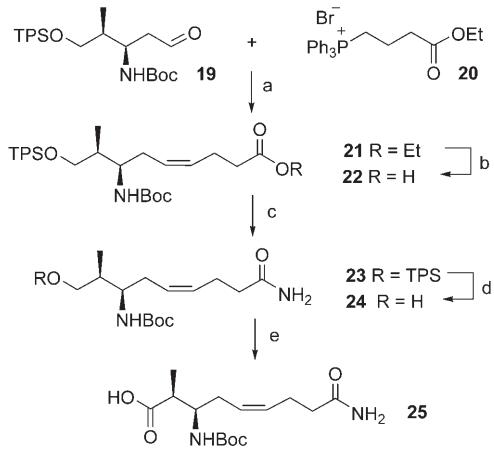
carbamoyl acid **7** relied on the stereochemically flexible Brown's crotylboration reaction[5] and started from the commercially available 3-benzyloxypropanol (**9**, Scheme 2).[6] The homoallylic acetate[7] **11** was obtained through a highly diastereo- and enantioselective (d.r. > 99%; 98% *ee*[8]) crotylation performed with the chiral reagent (+)-(*E*)-crotyl-Ipc_2-borane on the readily available 3-benzyloxypropanal (**10**). The subsequent reductive ozonolysis[9] furnished variable ratios of the regioisomeric acetates **12** and **13**. K_2CO_3-mediated hydrolysis of the mixture of acetates



Scheme 2. Reagents and conditions: a) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, 98%; b) i) (+)-Ipc_2BOMe, (*E*)-2-butene, *t*BuOK, *n*BuLi, $BF_3{\cdot}Et_2O$, THF, −78°C; ii) Ac_2O, py, CH_2Cl_2, 80%; c) O_3, CH_2Cl_2, then PPh_3; $NaBH_4$, EtOH; d) K_2CO_3, MeOH, 61% over three steps; e) TPSCl, DMAP, py, CH_2Cl_2, 90%; f) MsCl, Et_3N, THF; g) NaN_3, DMF, 60°C, 89% over two steps; h) H_2, Pt_2O, EtOAc; i) Boc_2O, Et_3N, CH_2Cl_2, 92% over two steps; j) H_2, Pd/C, EtOH; k) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, 93% over two steps. Bn = benzyl; DMSO = dimethyl sulfoxide; Ipc = isopinocampheyl; py = pyridine; TPS = *tert*-butyldiphenylsilyl; DMAP = 4-dimethylaminopyridine; Ms = mesyl; DMF = *N*,*N*-dimethylformamide; Boc = *tert*-butyloxycarbonyl.

and regioselective silylation of the diol **14** produced the requested (2*R*,3*S*)-1-[(*tert*-butyldiphenylsilyl)oxy]-5-benzyloxy-2-methyl-3-pentanol (**15**).

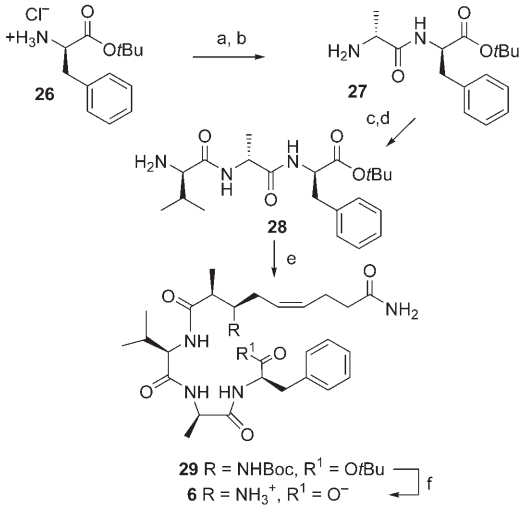
Three-step stereospecific amination at C3 and Boc-protection furnished the orthogonally triprotected aminodiol **17** in good overall yield. Pd-mediated hydrogenation and sequential Swern oxidation afforded the (3*R*,4*S*)-3-[(*tert*-butyloxycarbonyl)amino]-5-[(*tert*-butyldiphenylsilyl)oxy]-4-methylpentanal (**19**) in 93% yield over two steps. With the aldehyde in hand, we were ready to complete the synthesis of the key intermediate (*Z*)-(2*S*,3*R*)-3-[(*tert*-butyloxycarbonyl)amino]-2-methyl-5-nonenedioic acid, 9-amide (**25**, Scheme 3).



Scheme 3. Reagents and conditions: a) KHMDS, THF, −78°C→RT, 76%; b) LiOH, H_2O/THF, 70°C, 91%; c) DPPA, NH_4Cl, Et_3N, DMF, 79%; d) HF/py, py, 60%; e) TEMPO, phospate buffer, $NaClO_2$, NaClO, CH_3CN, 37%. KHMDS = potassium 1,1,1,3,3,3-hexamethyldisilazane; DPPA = diphenylphosphorylazide; TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical.

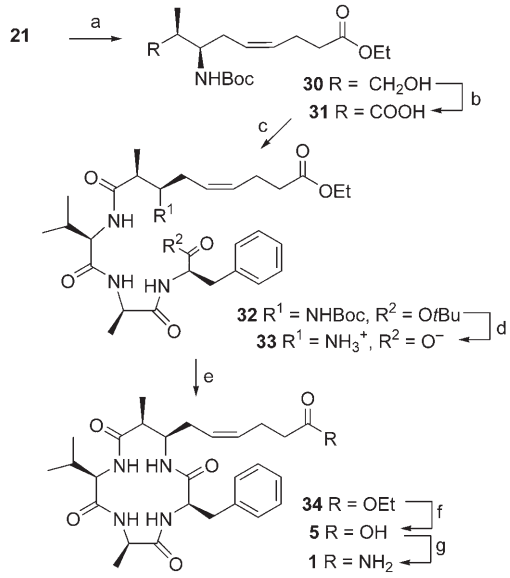
We thus performed a highly stereoselective Wittig olefination[10] by reacting the diprotected aldehyde **19** with the ylide formed from the phosphonium salt **20**.[11] The *Z*-alkene **21** was obtained in good yield and with no detectable accompanying *E* isomer (^{1}H NMR spectroscopy). Lithium hydroxide mediated ester hydrolysis and concurrent DPPA[12] carboxyl group activation gave, in the presence of ammonium chloride, the amide **23** in 72% yield over two steps. Finally, HF-induced desilylation and a disappointingly low-yielding oxidation of the primary alcohol to the carboxylic acid[13] afforded the desired N-protected acid **25** in a modest yield of 22% for two steps.

With fragment **25** in hand, we were ready for the synthesis of the linear tetrapeptide **6**. Its preparation relied on solution-phase peptide synthesis (Scheme 4) and gave key zwitterion **6**, which was ready for the macrolactamization reaction required to produce azumamide A (**1**). Although the linear zwitterion **6** contained the Amnaa residue, which was expected to facilitate the ring closure,[14,15] the macrolactamization reaction performed with the most commonly used reagents for the cyclization of homodetic peptides (DPPA,[12] FDPP,[16] and EDC/HOBt;[15] see Supporting Information)[17] did not give



Scheme 4. Reagents and conditions: a) *N*-Fmoc-D-Ala, EDC, HOBt, DIPEA, CH_2Cl_2; b) Et_2NH, CH_3CN, 93% over two steps; c) *N*-Fmoc-D-Val, EDC, HOBt, DIPEA, CH_2Cl_2; d) Et_2NH, CH_3CN, 89% over two steps; e) **25**, EDC, HOBt, DIPEA, CH_2Cl_2, 61%; f) TFA/CH_2Cl_2 (1:1), quant. Fmoc = 9-fluorenylmethoxycarbonyl; EDC = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide; HOBt = 1-hydroxybenzotriazole; DIPEA = diisopropylethylamine; TFA = trifluoroacetic acid.

the expected azumamide A (**1**). Considering that the number of variables associated with the cyclization step implies a "test it and see" approach, we decided to steer away from the synthetically bothersome carbamoyl function present in the β-amino acid residue, postponing its introduction to a later stage of the synthesis. Thus, we chose to transform the ethyl ester **21**, an intermediate in the synthesis of **25**, into the corresponding (*Z*)-(2*S*,3*R*)-3-[(*tert*-butyloxycarbonyl)amino]-2-methyl-5-nonenedioic acid, 9-ethyl ester (**31**, Scheme 5), a



Scheme 5. Reagents and conditions: a) HF/py, py, quant.; b) TEMPO, phospate buffer, $NaClO_2$, NaClO, CH_3CN, 75%; c) **28**, EDC, HOBt, DIPEA, CH_2Cl_2, 86%; d) TFA/CH_2Cl_2 (1:1), quant.; e) FDPP, DIPEA, DMF, 3 days, 37%; f) LiOH, H_2O/THF, 0°C, 75%; g) DPPA, NH_4Cl, Et_3N, DMF, 54%; FDPP = pentafluorophenyl diphenylphosphinate.

protected form of the Amnda residue, and proceed toward the elaboration of azumamide E (**5**).

As expected, better results were obtained both in the desilylation and in the oxidation[13] steps in the absence of carboxamide. We isolated the protected β-amino acid **31** in a satisfying overall yield of 75%. The C9,N-diprotected Amnda (**31**) was then coupled with the *all*-D tripeptide **28** to give the linear tetrapeptide **32**. Treatment of **32** with a mixture of trifluoroacetic acid/dichloromethane (1:1) led to quantitative deprotection of its carboxylate and amino groups to give **33**. This time, we were delighted to find that the FDPP-mediated macroclactamization reaction performed on **33** produced the C9-protected cyclotetrapeptide **34** in 37% yield.[18] The ethyl ester present in **34** was easily hydrolyzed in the presence of LiOH, and the target azumamide E (**5**) was thus isolated in 75% yield.

Azumamide A (**1**) was subsequently obtained in 54% yield through DPPA-induced amidation of azumamide E (**5**) in the presence of ammonium chloride and a non-nucleophilic base. The analytical data for **1** and **5** ($[\alpha]_D$ values, electrospray mass spectrometry and ^1H and ^{13}C NMR spectroscopy data) measured on the synthetic samples were almost identical to those reported by Fusetani and co-workers[3] for the natural products.[19] A conformational analysis aimed to obtain the experimental structure for azumamide E (**5**) was carried out by means of restrained molecular mechanics (MM) and molecular dynamics (MD) calculations (10 ns, see Supporting Information). The solution-state structure determined from NMR spectroscopy (Figure 1) was obtained by using 17

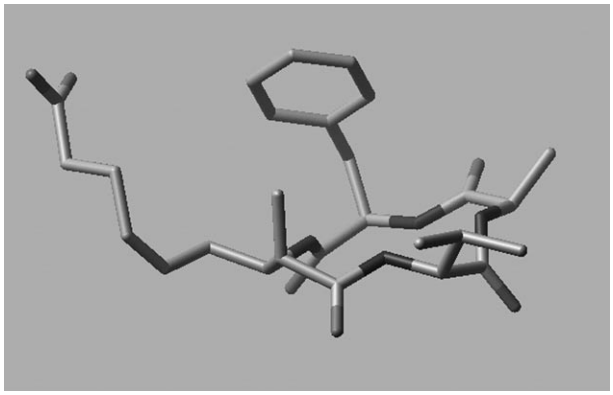


Figure 1. NMR solution-state conformation of azumamide E (**5**) obtained by restrained MD calculations using ROESY data (t_{mix} = 100 ms, 600 MHz, [D_6]DMSO, 300 K). O mid-gray, N dark gray, C pale gray; H atoms omitted.

spatial restraints previously collected in the ROESY spectra ([D_6]DMSO, 600 MHz, t_{mix}: 50, 100, and 150 ms); a careful analysis of its backbone did not reveal the presence of any identifiable turns or defined secondary structure. The presentation of this structure may be considered as an introduction to structural studies of drug–receptor interactions that will follow in due course.

In summary we have developed a successful protocol for the solution-phase synthesis of azumamides A (**1**) and E (**5**),

establishing unequivocally the configurations of the azumamides, and presented a three-dimensional NMR solution-state structure of azumamide E (**5**). Modification of the core structure of the azumamides and evaluation of their effects on the three HDAC enzymes is currently underway.

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Keywords: inhibitors · macrolactamization · natural products · peptides · total synthesis

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[19] The small variations in the values in the NMR spectra observed for synthetic azumamide E (**5**) were attributed to its the sensitivity to the apparent pH value of the CD_3OD solutions (see Supporting Information).

