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Largamides A−H, Unusual Cyclic Peptides from the Marine Cyanobacterium *Oscillatoria* sp.

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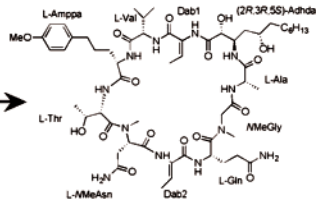
Oscillatoria sp.

8 peptides
3 structural classes



Largamide H

Seven new depsipeptides, termed largamides A−G (**1**−**7**), and one new cyclic peptide, largamide H (**8**), have been isolated from the marine cyanobacterium *Oscillatoria* sp. Their structures were determined by NMR and ESI-MS techniques. The absolute configurations were assigned using LC-MS, chiral HPLC, and combined analysis of homonuclear and heteronuclear $^{2,3}J$ couplings, along with ROE data. Largamides, isolated from a single homogeneous cyanobacterial collection, represent three different structural classes of peptides. Largamides A−C (**1**−**3**) are characterized by the unusual occurrence of a senecioic acid unit, while largamides B (**2**) and C (**3**) possess in addition the rare 2-amino-5-(4′-hydroxyphenyl)pentanoic acid (Ahppa) and the novel 2-amino-6-(4′-hydroxyphenyl)hexanoic acid (Ahpha), respectively. Largamides D−G (**4**−**7**) are the first 3-amino-6-hydroxy-2-piperidone acid (Ahp)-containing depsipeptides reported with the rare Ahppa unit. Largamide H (**8**) is a unique cyclic peptide displaying a new 2,5-dihydroxylated β-amino acid moiety, a methoxylated derivative of Ahppa, and two residues of the nonstandard 2,3-dehydro-2-aminobutanoic acid (Dab). Largamides D−G (**4**−**7**) inhibited chymotrypsin with IC_{50} values ranging between 4 and 25 μM.

Introduction

Marine cyanobacteria are prolific producers of bioactive natural products, many of which originate from mixed polyketide−nonribosomal peptide biosynthetic pathways.[1] Moreover, it has been shown that cyanobacterial metabolites exhibit a broad spectrum of biological activities, including anticancer, antibiotic, antifungal, antiviral, and proteinase-inhibiting activities, all of which are attractive targets of biomedical research.[2−5] In our continuing search for bioactive secondary metabolites from marine organisms, the methanol extract of the marine cyanobacterium *Oscillatoria* sp. was studied. This investigation led to the isolation of seven new depsipeptides, termed largamides A−G (**1**−**7**), and one new cyclic peptide, largamide H (**8**), along with the known glyceryl galactosides (2*S*)-1,2-di-*O*-palmitoyl-3-*O*-β-D-galactopyranosylglycerol[6] and (2*S*)-1-*O*-palmitoyl-3-*O*-β-D-galactopyranosylglycerol.[7] Their structures were elucidated by extensive spectroscopic methods, including 1D (^{1}H and ^{13}C) and 2D NMR experiments (DQF-COSY, HOHAHA, HMBC, HSQC, and ROESY) as well as ESI-MS analysis. The absolute configurations were determined by LC-MS (advanced Marfey's method), chiral HPLC, and combined analysis of homonuclear (H−H) and heteronuclear (C−H) $^{2,3}J$ couplings, and ROE data.

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Results and Discussion

Oscillatoria sp. was collected by hand using scuba from the Florida Keys in September, 2003. The biomass was lyophilized and extracted sequentially with petroleum ether, $CHCl_3$, and MeOH. Chromatography of the MeOH extract over Sephadex LH-20 followed by silica gel MPLC and reversed-phase HPLC yielded compounds **1**−**8**.

The molecular formula of compound **1** was determined to be $C_{41}H_{59}N_7O_{12}$ by HR-ESI-MS (*m*/*z* 842.4319 $[M + H]^+$, calcd for $C_{41}H_{60}N_7O_{12}$, 842.4300) and NMR spectral data (see Table 1). The ^{1}H NMR spectrum exhibited signals characteristic of a peptide containing numerous aliphatic and oxygenated residues, including an exchangeable NH resonance at δ 7.69 (1H, d, J = 9.7 Hz), six α-proton signals at δ 4.72 (1H, dd, J = 9.6, 6.0 Hz), 4.69 (1H, m), 4.62 (1H, d, J = 3.0 Hz), 4.52 (1H, dd, J = 9.6, 4.1 Hz), 4.22 (1H, d, J = 7.2 Hz), and 4.21 (1H, q, J = 7.0 Hz), and five signals corresponding to six methyl doublets at δ 1.42 (3H, d, J = 7.0 Hz), 1.15 (3H, d, J = 6.2 Hz), 0.92 (6H, d, J = 6.2 Hz), 0.80 (3H, d, J = 6.7 Hz), and 0.76 (3H, d, J = 6.7 Hz). Additionally, a downfield pair of doublet of doublets at δ 7.10 (2H, d, J = 8.2, 1.2 Hz) and 6.69 (2H, d, J = 8.2, 1.2 Hz), characteristic of a *para*-substituted phenyl ring, was observed. A detailed analysis of the 2D NMR data obtained from HSQC, HMBC, HOHAHA, and DQF-COSY experiments established the presence of alanine, leucine, tyrosine, threonine, valine, and glutamic acid residues (see Tables 1 and S1).The presence of the nonstandard β-amino acid 2,3-dehydro-2-aminobutanoic acid (Dab) and of the senecioic acid (Sen) residues was deduced on the basis of the HSQC, DQF-COSY, and HMBC spectra (see Tables 1 and S1). Dab was identified by HMBC correlations from proton signals for the propylidene group at δ 6.70 (H-3_{Dab}) and 1.83 (Me-4_{Dab}) to carbon resonances at δ 165.5 (C-1_{Dab}), 130.1 (C-2_{Dab}), and 131.9 (C-3_{Dab}). HMBC correlations from the protons of the two olefinic methyl groups at δ 1.77 (Me-4_{Sen}) and 1.84 (Me-5_{Sen}) to the carbon resonances at δ 132.1 (C-2_{Sen}) and 131.9 (C-3_{Sen}), and from the olefinic proton at δ 6.40 (H-2_{Sen}) to the carbon resonance at 171.5 (C-1_{Sen}), indicated the presence of Sen.

The downfield chemical shift of the threonine α-oxymethine proton at δ 5.24 suggested an ester linkage at this position, which was confirmed by an HMBC correlation between the α-proton of threonine and the carbonyl carbon of leucine (δ 171.8). Moreover, HMBC correlations between α-protons to carbonyl carbons of adjacent amino acid residues allowed us to establish the connectivity of all amino acids except Dab. The connection of Dab to alanine and glutamic acid was established through long-range correlations between the methyl signal at δ 1.83 (Me-4_{Dab}) and the carbonyl resonance at δ 176.7 (C-1_{Ala}), and between the proton at δ 4.52 (H-1_{Glu}) and the carbonyl resonance at δ 165.5 (C-1_{Dab}). A strong HMBC correlation from the proton at δ 4.22 (H-2_{Val}) to the carbonyl resonance at δ 171.5 (C-1_{Sen}) clearly established the connectivity of the Sen unit to the depsipeptide moiety. Additional evidence supporting this sequence was obtained from ESI-MS/MS experiments. Investigation of the ESI-MS/MS spectrum of the major ion peak at *m*/*z* 841 displayed ions at *m*/*z* 660 $[M + H - Sen - Val]^+$, *m*/*z* 642 $[M + H - Sen - Val - H_2O]^+$, and *m*/*z* 480 $[M + H - Sen - Val - H_2O - Tyr]^+$, corroborating the NMR results.

The absolute configurations of L-Val, L-Thr, L-Ala, L-Leu, D-Glu, and D-Tyr residues were assigned by chromatographic comparison of the acid hydrolysate of **1** (5 N HCl, 90 °C, 16 h) with appropriate amino acid standards by LC-MS after derivatizing with L/D-FDLA (1-fluoro-2,4-dinitrophenyl-5-L/D-leucinamide).[8] Retentions times are listed in Table 2.

The molecular formula of **2** was unequivocally established to be $C_{46}H_{61}N_7O_{13}$ by HR-ESI-MS (*m*/*z* 920.4362 $[M + H]^+$, calcd for $C_{46}H_{62}N_7O_{13}$, 920.4406) and NMR data (see Table 1). Its ^{1}H NMR spectrum in comparison to that of **1** showed just a few differences. In particular, it displayed another downfield pair of doublets of doublets at δ 7.00 (1H, d, J = 8.2, 1.8 Hz) and 6.68 (1H, d, J = 8.2, 1.8 Hz), indicating the occurrence of an additional *para*-substituted aromatic ring. It was also evident that the doublet signal at δ 0.92 (6H, d, J = 6.2 Hz) ascribable to the methyl groups of the leucine residue in **1** was absent in **2**. Furthermore, a sequential spin system comprising an α-methine signal (δ_H 4.63, δ_C 52.1) and three sequential methylene signals (δ_H 1.91, 1.62, δ_C 30.9; δ_H 1.57, 1.62, δ_C 28.1; and δ_H 2.55, 2.50, δ_C 35.1) was established on the basis of 2D HOHAHA, DQF-COSY, and HSQC correlations.

Key HMBC correlations between the α-proton at δ 4.63 (H-2_{Ahppa}) and the carbon resonances at δ 171.8 (C-1_{Ahppa}), 30.9 (C-3_{Ahppa}), and 28.1 (C-4_{Ahppa}), between the methylene protons at δ 2.55 (H-$5a_{Ahppa}$) and 2.50 (H-$5b_{Ahppa}$), and the carbon resonances at δ 28.1 (C-4_{Ahppa}), 30.9 (C-3_{Ahppa}), 134.2 (C-$1'_{Ahppa}$), and 130.1 (C-$2'_{Ahppa}$), and between the aromatic proton at δ 7.00 (H-$2'_{Ahppa}$) and the carbon resonance at δ 35.1 (C-5_{Ahppa}) indicated the presence of the rare 2-amino-5-(4′-hydroxyphenyl)pentanoic acid (Ahppa). To our knowledge, this is only the second reported occurrence of Ahppa, the first being in the naturally occurring peptide AM-toxin III, which was isolated from the terrestrial fungus *Alternaria mali*.[9]

Finally, a detailed analysis of the 2D NMR data (2D-HOHAHA, DQF-COSY, HSQC, HMBC) of **2** indicated that it differed from **1** only by the presence of the Ahppa instead of the leucine residue. As in **1**, the hydrolysate of **2** was derivatized and analyzed by LC-MS according to the advanced Marfey's method.[8] In this way, the absolute configuration of the each amino acid residue in **2** was established as L-Val, L-Thr, L-Ala, L-Ahppa, D-Glu, and D-Tyr. The absolute configuration of Ahppa was also determined as L by the advanced's Marfey method in the following manner. The L/D-FDLA bis derivatives of Ahppa (FDLA reacts with both the amino and hydroxyphenyl groups) were detected at retention times of 42.7 and 46.3 min, while the L-FDLA bis derivative was detected at 42.7 min on the reconstructed ion chromatogram for *m*/*z* 796 $[M - H]^-$ (see Table 2). As in the case of tyrosine the L-FDLA bis derivative of Ahppa elutes prior to that of the D-LFDLA.[8,10]

The HR-ESI-MS of compound **3** displayed a major molecular ion at *m*/*z* 934.4604 $[M + H]^+$ ($C_{47}H_{63}N_7O_{13}$, calcd for $C_{47}H_{64}N_7O_{13}$, 934.4562), which was 14 mass units higher than that of **2**. The NMR data (^{1}H, ^{13}C, DQF-COSY, 2D HOHAHA, HSQC, HMBC) of **3** closely resembled that of **2** except for the replacement of resonances belonging to Ahppa for the novel 2-amino-6-(4′-hydroxyphenyl)hexanoic acid (Ahpha). In particular, 2D HOHAHA, DQF-COSY, and HSQC correlations established a sequential spin system comprising an α-methine

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TABLE 1. NMR Spectroscopic Data for Compounds 1 and 4 (600 MHz, CD_3OD)

	1				4		
	δ_C	δ_H (J in Hz)	HMBC[a]		δ_C	δ_H (J in Hz)	HMBC[a]
		Leu				Val 1	
1	171.8			1	174.8		
2	50.7	4.69, m	1, 3, 4, 1_{Glu}	2	58.9	4.49, d (7.4)	1, 3, 5, $1_{MeBrTyr}$
3	40.3	1.73, 1.57, m	1, 2, 4, 5, 6	3	31.8	2.15, m	1, 2, 3, 5
4	25.2	1.60, m	2, 5, 6	4	18.9	0.93, d (6.90)	2, 3, 5
5	21.7	0.92, d (6.2)	3, 4, 6	5	19.8	0.992, d (6.90)	2, 3, 4
6	23.7	0.92, d (6.2)	3, 4, 5			*N*MeBrTyr	
NH		7.69, d (9.7)	1, 2, 1_{Glu}	1	171.4		
		Glu		2	62.6	5.06, dd (11.5, 3.1)	1, 3, 4, 1_{Thr1}
1	172.7			3	33.8	3.40, dd (14.6, 3.1)	2, 4, 5
2	53.7	4.52, dd (9.6, 4.1)	1, 3, 1_{Dab}			2.80, m	1, 2, 4, 5
3	27.1	2.56, 219, m	2, 4, 5	4	130.4		
4	31.4	2.57, 2.50, m	2, 3, 5	5	134.8	7.34, d (2.0)	3, 6, 7, 9
5	176.4			6	110.8		
		Dab		7	154.4		
1	165.5			8	117.6	6.87, d (8.2)	5, 6, 7, 9
2	130.1			9	130.7	7.17, dd (8.2, 2.0)	3, 5, 6,
3	131.9	6.70, q (7.2)	1, 4	NMe	31.2	2.87, s	2, 1_{Thr1}
4	12.4	1.83, d (7.2)	1, 2, 3, 1_{Ala}			Thr1	
		Ala		1	173.0		
1	176.7			2	55.9	4.57, d (6.8)	1, 4, 1_{Ahp}, 6_{Ahp}
2	51.3	4.21, q (7.0)	1, 3, 1_{Thr}	3	66.6	3.74, m	2, 4
3	16.5	1.42, d (7.0)	1, 2	4	19.6	0.57, d (6.2)	2, 3
		Thr				Ahp	
1	170.7			2	171.3		
2	56.6	4.62, d (3.0)	1, 3,1_{Tyr}	3	50.7	4.64, dd (12.4, 6.4)	1, 3, 1_{Leu}
3	73.8	5.24, dq (3.0)	4, 1_{Leu}	4	22.0	2.82, 1.88, m	2, 4, 5
4	16.0	1.15, d (6.2)	2, 3	5	30.6	2.04, 1.87, m	2, 3, 5
		Tyr		6	76.9	5.33 br s	1, 3, 2_{Thr1}
1	173.9					Leu	
2	56.6	4.72, dd (9.6, 6.0)	1, 3, 4, 1_{Val}	1	174.4		
3	38.2	3.09, dd (14.0, 6.0)	1, 2, 4, 5	2	52.9	4.56, dd (8.5, 3.3)	1, 3, 4 1_{Thr2}
		2.83, dd (14.0, 9.6)	1, 2, 4, 5	3	40.2	1.98, ddd (12.6, 12.6, 3.3)	1, 2, 4, 5, 6
4	128.5		3, 6, 7			1.55, m	1, 2, 4, 5, 6
5,9	131.1	7.10, d (8.2, 1.2)	4, 7	4	25.8	1.65, m	5, 6
6,8	116.1	6.69, d (8.2, 1.2)		5	20.0	0.87, d (6.8)	3, 4, 6
7	157.1			6	23.7	0.97, d (6.7)	3, 4, 5
		Val				Thr2	
1	173.3			1	170.9		
2	60.0	4.22, d (7.2)	1, 3, 4, 1_{Sen}	2	56.3	4.78, d (1.2)	1, 3, 4, 1_{Val2}
3	31.9	1.97, m	2, 4, 5	3	73.4	5.59, qd (6.5, 1.2)	1, 4, 1_{Val1}
4	18.5	0.80, d (6.7)	2, 3, 5	4	18.1	1.36, d (6.5)	2, 3
5	19.6	0.76, d (6.7)	2, 3, 4			Val2	
		Sen		1	173.9		
1	171.5			2	59.7	4.39, d (7.4)	1, 3, 4, 5, 1_{Ala}
2	132.1	6.40, dq (6.7, 1.3)	1	3	31.9	2.17, m	1, 2, 4, 5
3	131.9			4	18.5	0.98, d (6.9)	2, 3, 5
4	12.8	1.84, br s	1, 2, 3, 4	5	19.8	0.984, d (6.9)	2, 3, 4
5	13.8	1.77, d (6.7)	1, 2, 3, 5			Ala	
				1	174.4		
				2	49.9	4.45, m	1, 3, 1_{Ahppa}
				3	17.6	1.38, d (7.2)	1, 2
						Ahppa	
				1	173.5		
				2	53.9	4.46, m	1, 3, 4, $1_{Glic.Ac.}$
				3	32.6	1.91, 1.73, m	1, 4, 5
				4	28.8	1.70, 1.66 m	2, 3, 5
				5	35.2	2.57, t (7.3)	3, 4, 1′, 2′, 6′
				1′	133.6		
				2′, 6′	130.2	7.02, dd (8.2, 1.8)	1′, 3′, 4′, 5′
				3′, 5′	115.8	6.70, dd (8.2, 1.8)	1′, 2′, 4′, 6′
				4′	156.2		
						glyceric acid	
				1	174.9		
				2	73.9	4.13, t (3.8)	1, 3
				3	65.2	3.79, d (3.8)	1, 2

[a] Proton showing HMBC correlation to indicated carbon.

signal (δ_H 4.59, δ_C 52.0) and four sequential methylene signals (δ_H 1.93, 1.63, δ_C 31.3; δ_H 1.36 (2H), δ_C 25.8; δ_H 1.59, 1.53, δ_C 32.6; and δ_H 2.50, δ_C 35.6).

Last, key HMBC correlations between the methylene protons at δ 2.50 (H-6_{Ahpha}) and the aromatic carbon resonances at δ 134.2 (C-$1'_{Ahpha}$) and 129.9 (C-$2'$/C-$6'_{Ahpha}$), and be-

CHART 1

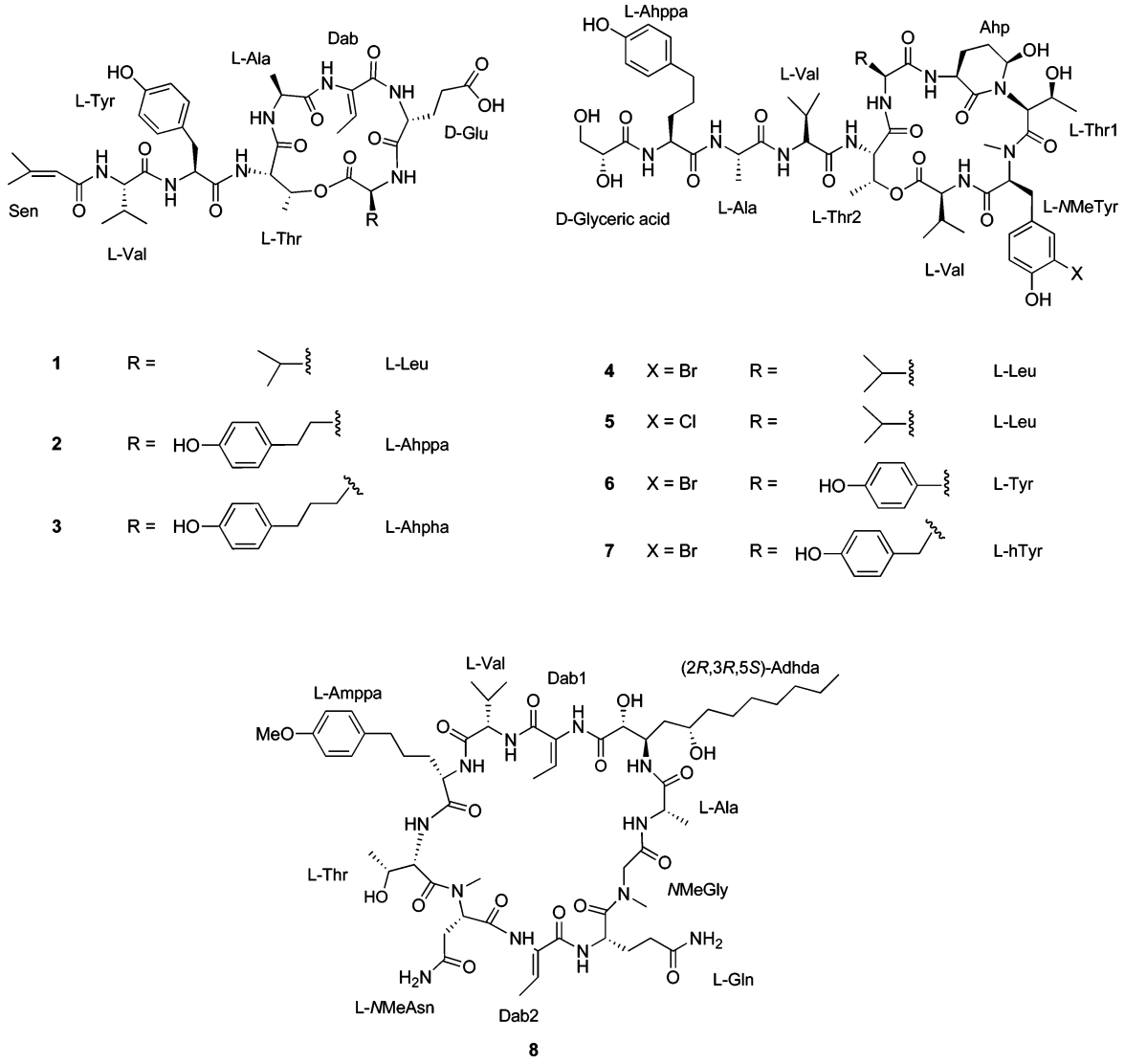

TABLE 2. Retention Times (t_R, min) of L-FDLA and L/D-FDLA-Derivatized Amino Acids from the Hydrolyses of Compounds 1−8 Detected by ESI-LC-MS (Negative Mode)

	1−3		4−7		8		*m*/*z*
	L-FDLA	L/D-FDLA	L-FDLA	L/D-FDLA	L-FDLA	L/D-FDLA	$[M - H]^-$
Val	27.4	27.4, 34.0	27.7	27.7, 34.3	27.7	27.7, 34.0	410
Ala	24.4	24.4, 27.9	24.7	24.7, 28.2	24.3	24.3, 27.1	382
Thr	19.6	19.6, 24.2	19.7	19.7, 24.3	19.6	19.5, 23.3	412
Tyr[a]	44.1	40.8, 44.1	40.8	40.8, 44.1			768
Leu	30.6	30.6, 38.1	30.5	30.6, 38.1			424
Glu	22.8	21.8, 22.8	21.8	21.8, 22.8			440
Ahpha[a]	44.0	43.9, 48.8					810
Ahppa[a]	42.7	42.7, 46.3	42.7	42.8, 46.4			796
*N*MeBrTyr			41.0	41.1, 42.5			860
Amppa					36.7	36.7, 40.7	516
Adhda					41.3	32.5, 41.3	540
Gln[b]					21.2	21.5, 22.8	440
*N*MeAsn[c]					20.3	19.0, 20.3	440

[a] Analyzed as bis derivative. [b] Analyzed as Glu. [c] Analyzed as *N*MeAsp.

tween the α-proton at δ 4.59 ($H\text{-}2_{Ahpha}$) and carbon resonances at δ 171.8 ($C\text{-}1_{Ahpha}$) and 172.7 ($C\text{-}1_{Glu}$) allowed us to establish the occurrence and linkage sites of Ahpha. Once again, the hydrolysate of **3** was derivatized and analyzed by LC-MS according to the advanced Marfey's method.[8] The absolute configuration of each amino acid in **3** was established as L-Val, L-Thr, L-Ala, L-Ahpha, D-Glu, and D-Tyr. The L configuration of Ahpha was established by advanced Marfey's method in a similar fashion as for Ahppa (see Table 2).

The relative stereochemistry for the Dab residues in compounds **1**−**3** was deduced from ROESY spectra recorded in DMSO-d_6 (see Table S2 for ^{1}H and ^{13}C NMR data of **2** in DMSO-d_6). In particular, compound **3** showed a ROE correlation between the Dab NH (δ 10.0, br s) and the Dab methyl group (δ 1.71, d, $J = 6.7$ Hz), indicating a *Z* geometry for the double bond.

The molecular formula of **4** was determined as $C_{56}H_{82}BrN_9O_{17}$ on the basis of the HR-ESI-MS (*m*/*z* 1232.5117 $[M + H]^+$, calcd for $C_{56}H_{83}BrN_9O_{17}$, 1232.5090) and NMR spectral data (see Table 1). The isotopic distribution of the ions $[M + H]^+$, 1:1, at *m*/*z* 1232/1234 clearly indicated the presence of bromine. The ^{1}H NMR spectrum of **4** displayed one methylamide signal (δ 2.87, 3H, s) and aromatic signals ascribable to two spin systems, with one comprising two doublet of doublets characteristic of a 1,4-disubstituted ring (δ 7.02, 2H, $J = 8.2$, 1.8 Hz, δ 6.70, 2H, $J = 8.2$, 1.8 Hz) and the other comprising two doublets at δ 7.34 (1H, $J = 2.0$ Hz) and 6.87 (1H, $J = 8.2$ Hz), and one doublet of doublets (δ 7.17, 1H, $J = 8.2$, 2.0 Hz) characteristic of a 1,3,4-trisubstituted ring. The HSQC spectrum of **4** clearly showed that the nine proton signals at δ 4.30−5.20 were correlated to nine carbons at δ 52.0−63.0, suggesting the occurrence of nine amino acid residues (see Table 1). The analysis of the ^{13}C NMR spectrum on the basis of the HSQC correlations revealed the presence of one primary oxygenated carbon (δ 65.2) and four secondary oxygenated carbons (δ 66.6, 73.4, 74.0, and 76.9). This evidence in combination with the DQF-COSY, 2D-HOHAHA, and HMBC correlations allowed us to establish the presence of alanine, leucine, glyceric acid, *N*-methyl-6-bromotyrosine (*N*MeBrTyr), two valines, and two threonine residues (one of them acylated). The occurrence of the novel Ahppa residue in **4** was established in the same manner as described for **2**. The structure of 3-amino-6-hydroxy-2-piperidone (Ahp) was deduced as follows. A sequential spin system starting from an α-methine group (δ_H 4.64, δ_C 50.7), two sequential methylene groups (δ_H 2.82, 1.88, δ_C 22.0; and δ_H 2.04, 1.87, δ_C 30.6), and one oxymethine group (δ_H 5.33, δ_C 76.9) was obtained from 2D HOHAHA, COSY, HSQC, and HMBC correlations. Long-range correlation peaks observed in the HMBC spectrum between the proton at δ 5.33 (H-6_{Ahp}) and the carbon resonance at δ 55.9 (C-2_{Thr1}) and between the proton at δ 4.57 (H-2_{Thr1}) and the carbon resonance at δ 171.3 (C-1_{Ahp}) indicated that Ahp was connected to the nonacylated threonine (Thr1) to form this hemiaminal-containing residue.

Direct connectivity information of each amino acid residue was obtained from the HMBC spectrum. Long-range correlations between α-protons to carbonyl carbons of adjacent amino acids allowed us to establish the following sequence for **4**: Val1-*N*MeBrTyr-Thr1-Ahp-Leu-Thr2-Val2-Ala-Ahppa-glyceric acid, with the hydroxyl group of Thr2 forming an ester bond with the carboxyl group of Val1. These results are consistent with the observed chemical shifts for the β and γ protons of Thr1, both of which are shifted upfield (δ 3.74 vs ∼5.40 and 0.57 vs ∼1.30, respectively) due to the inner ring current effect of the aromatic ring from *N*MeBrTyr.[11] Absolute configurations determined by application of the advanced Marfey's method to the hydrolysate of **4** revealed that all the amino acid residues possessed L configurations (see Table 2). CrO_3 oxidation of **4** followed by acid hydrolysis and advanced Marfey's analysis led to L-Glu generated from the Ahp unit (see Table 2).[12] Thus, the stereochemistry of C-2_{Ahp} was *S*. In addition, the small $J_{H,H}$ value displayed by H-6_{Ahp} (br s) along with a strong ROE correlation between H-3_{Thr1} and H-6_{Ahp} allowed us to establish the absolute configuration of C-6_{Ahp} as *R*. Chiral HPLC following acid hydrolysis of **4** established the D configuration of the glyceric acid unit.

A strong ROE correlation between the α-protons of *N*MeBrTyr (δ 5.06) and of Thr1 (δ 4.57) revealed a *cis*-amide bond between these two amino acids as has been reported for Ahp-containing cyclic depsipeptides.[11,13]

The HR-ESI-MS of compound **5** displayed an isotopic pattern of the major ion peak $[M + Na]^+$, 3:1, at *m*/*z* 1210.5396/1212.5497, suggesting a molecular formula of $C_{56}H_{82}ClN_9O_{17}$ (calcd for $C_{56}H_{82}ClN_9O_{17}Na$, 1210.5415). The ^{1}H and ^{13}C NMR spectra of **5** were almost identical to those of **4**, with the exception of some signals corresponding to the aromatic ring of the tyrosine residue. In particular, the signals of H-5_{Tyr} (δ 7.19) and C-6_{Tyr} (δ 121.0) were significantly shifted to upfield and downfield values, respectively, when compared to **4** (see Table 1). On the basis of this evidence, **5** was identified as a C-6_{Tyr} chloro analogue of **4**.

Compounds **6** and **7** displayed in the ESI-MS spectrum an isotopic distribution of the ions $[M + H]^+$, 1:1, at *m*/*z* 1282/1284 and *m*/*z* 1318/1320, respectively, indicating the presence of bromine. Their major ion peaks in the HR-ESI-MS spectrum were consistent with a molecular formula of $C_{59}H_{80}N_9O_{18}Br$ (*m*/*z* 1282.4880 $[M + H]^+$, calcd for $C_{59}H_{81}N_9O_{18}Br$, 1282.4883) for compound **6** and $C_{60}H_{82}N_9O_{18}Br$ (*m*/*z* 1318.4918 $[M + Na]^+$, calcd for $C_{60}H_{82}N_9O_{18}BrNa$, 1318.4859) for compound **7**. A detailed analysis of their NMR data (^{1}H, ^{13}C, HSQC, HMBC, DQF-COSY, 2D-HOHAHA, ROESY) clearly indicated that the amino acid sequences of **6** and **7** are the same as that of **4**, except for the substitution in **6** of tyrosine and in **7** of homotyrosine for a leucine residue in **4**. Once again, LC-MS analysis of the L/D-FDLA-derivatized hydrolysates of **6** and **7** allowed us to establish the L configuration for Tyr and hTyr, respectively (see Table 2).

The HR-ESI-MS of **8** showed a major ion peak at *m*/*z* 1199.6708 $[M + H]^+$ corresponding to a molecular formula of $C_{57}H_{90}N_{12}O_{16}$ (calcd for $C_{57}H_{91}N_{12}O_{16}$, 1199.6676). A detailed analysis of the 2D NMR data obtained from HSQC, HMBC, HOHAHA, and DQF-COSY experiments established the presence of valine, threonine, *N*-methylasparagine, glutamine, *N*-methylglycine, and alanine residues along with a methoxy derivative of Ahppa, 2-amino-5-(4′-methoxyphenyl)pentanoic acid (Amppa) (see Table 3). Two Dab units were assigned from the COSY and HMBC data, which provided correlations from the vinyl methyl signals at δ 1.63 (3H, d, $J = 7.4$ Hz, Me-4_{Dab1}) and 1.85 (3H, d, $J = 7.2$ Hz, Me-4_{Dab2}) to their respective carbonyl signals at δ 171.2 (C-1_{Dab1}) and 165.0 (C-1_{Dab2}), and respective olefinic carbon signals at δ 131.1 (C-2_{Dab1}) and 121.2 (C-3_{Dab1}), and 131.0 (C-2_{Dab2}) and 135.3 (C-3_{Dab2}). The methyl signals at δ 1.63 and 1.85 were coupled to their respective vinyl proton signals at 6.07 (1H, q, $J = 7.4$ Hz, H-3_{Dab1}) and 6.78 (1H, q, $J = 7.2$ Hz, H-3_{Dab2}). The presence of the β-amino acid 3-amino-2,5-dihydroxydodecanoic acid (Adhda) was suggested from HSQC, 2D HOHAHA, and DQF-COSY data. In particular,

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TABLE 3. NMR Spectroscopic Data for Compound 8 (600 MHz, CD_3OD)

	8		
	δ_C	δ_H (*J* in Hz)	HMBC[a]
		Dab1	
1	171.2		
2	131.1		
3	121.2	6.07, q (7.4)	1, 4
4	13.1	1.63, d (7.4)	1, 2, 3, 1_{Adhda}
		Val	
1	173.9		
2	63.6	3.76, d (5.8)	1, 3, 4, 5, 1_{Dab1}
3	30.1	2.27, m	1, 2, 4, 5
4	18.5	1.088, d (6.9)	2, 3, 5
5	19.5	1.093, d (6.9)	2, 3, 4
		Amppa	
1	174.9		
2	56.1	4.27, dd (11.3, 4.5)	1, 3, 4, 1_{Val1}
3	31.8	2.08, 2.03 m	1, 2, 4, 5
4	30.0	1.83, 1.69 m	2, 3, 5, 6
5	35.4	2.65, 2.59 m	3, 4, 1′, 2′
1′	135.1		
2′, 6′	129.9	7.09, dd (8.2, 1.2)	5, 1′, 3′, 5′
3′, 5′	114.5	6.82, dd (8.2, 1.2)	1′, 2′, 4′, 6′
4′	159		
OMe	55.4	3.77, s	4′
		Thr	
1	174.0		
2	54.4	4.69, br s	1, 3, 4, 1_{Ahmpa}
3	67.4	4.36, br q (6.6)	1, 4
4	18.3	0.98 d (6.6)	2, 3
		*N*MeAsn	
1	171.2		
2	63.7	4.41, dd (7.7, 5.7)	1, 3, 4, *N*Me, 1_{Thr}
3	35.1	3.19, dd (15.4, 5.7)	1, 2, 4
	2.76	2.76, dd (15.4, 7.7)	1, 2, 4
4	175.7		
*N*Me	39.2	3.20, s	2, 1_{Thr}
		Dab2	
1	165.0		
2	131.0		
3	135.3	6.78, q (7.2)	1, 2, 4
4	13.3	1.85, d (7.2)	1, 2, 3, 1_{NMeAsn}
		Gln	
1	174.0		
2	49.3	5.21, dd (9.8, 3.3)	1, 3, 4, 1_{Dab2}
3	30.1	2.07, 1.90, m	1, 2, 4, 5
4	31.3	2.38, 2.28, m	2, 3, 5
5	178.1		
		*N*MeGly	
1	171.1		
2	51.0	4.72, br d (16.6)	1
		3.48, br d (16.6)	1
*N*Me	36.3	3.15, s	2, 1_{Gln}
		Ala	
1	175.5		
2	49.3	4.64, q (6.9)	1, 3, 1_{NMeGly}
3	14.8	1.34, d (6.9)	1, 2,
		Adhda	
1	173.1		
2	75.9	3.99, d (5.7)	1, 3, 4
3	49.6	4.54, ddd (12.4, 5.7, 2.0)	1, 2, 4, 5, 1_{Ala}
4	39.3	1.58, ddd (14.1, 12.4, 9.3)	2, 3, 4, 5
		1.356, m	2, 5
5	68.4	3.52 m	3, 6, 7
6	38.8	1.356, 1.31 m	5, 7
7	26.6	1.353, 1.24, m	6
8	30.2	1.22, m	7, 9
9	30.7	1.20, m	8, 10
10	32.8	1.228, m	9, 11, 12
11	23.6	1.28, 1.26, m	10, 12
12	14.1	0.89, t (7.0)	10, 11

[a] Proton showing HMBC correlation to indicated carbon.

sequential spin correlations starting with the α-oxymethine proton signal (δ 3.99, d, $J = 5.7$ Hz), β-CH signal (δ 4.54, ddd, $J = 12.4, 5.7, 2.0$ Hz), γ-CH_2 (δ 1.35, m; δ 1.58, ddd, $J = 14.1, 12.4, 2.0$ Hz), and δ-oxymethine signal (δ 3.52, m) were observed. HMBC, 2D HOHAHA, and DQF-COSY experiments were used to extend this spin system to include an additional seven carbons (six methylenes and one methyl).

HMBC correlations between α-protons to carbonyl carbons of adjacent amino acid residues allowed us to establish two fragments: Val-Amppa-Thr-*N*MeAsn and Gln-*N*MeGly-Ala-Adhda. Unambiguous long-range correlations from the methyl signals at δ 1.63 (Me-4_{Dab1}) and 1.85 (Me-4_{Dab2}) to the carbon resonances at δ 173.1 (C-1_{Adhda}) and 171.2 (C-1_{NMeAsn}), respectively, and from the α-proton signals at δ 3.76 (H-1_{Val}) and 5.21 (H-1_{Gln}) to the carbonyl carbon resonances at δ 171.2 (C-1_{Dab1}) and 165.0 (C-1_{Dab2}), respectively, allowed us to establish the connectivity of Dab1 and 2 to these fragments, thus completing the sequence of residues in **8**.

Further evidence supporting this sequence was obtained from the ESI-MS/MS spectrum. The daughter ion spectrum of the major ion at *m/z* 1200 $[M + H]^+$ displayed fragment ions at *m/z* 989 $[M + H - Dab - NMeAsn]^+$, *m/z* 969 $[M + H - NMeAsn - Thr]^+$, *m/z* 860 $[M + H - Dab - NMeAsn]^+$, and *m/z* 829 $[M + H - Dab - Val - Amppa]^+$. This fragmentation pattern was in complete agreement with the NMR results.

Several techniques were used to determine the stereochemistry of **8**. The geometry of the double bond in the Dab residues was deduced from a ROESY spectrum recorded in $CDCl_3$ (see Supporting Information for ^1H and ^{13}C NMR data of **8** in $CDCl_3$). A strong ROE correlation between the NH (8.70, br s) and olefinic proton of Dab1 (6.13, q, $J = 7.3$ Hz) indicated the *E* configuration for the double bond in Dab1, while a strong ROE correlation between the NH (7.61, br s) and methyl group of Dab2 (1.76, d, $J = 7.2$ Hz) demonstrated the *Z* configuration for the olefin in Dab2. The absolute configurations of L-Val, L-Thr, L-Ala, L-*N*MeAsn, and L-Gln residues were assigned by chromatographic comparison of the acid hydrolysate of **1** with appropriate amino acid standards by LC-MS after derivatizing with L/D-FDLA. The L configuration of Amppa was established by advanced Marfey's method in similar fashion as in Ahppa (see Table 2).

The absolute configuration of C-3 of Adhda was also established by this method. The L/D-FDLA derivatives of Adhda were detected at retention times of 32.5 and 41.3 min, while the L-FDLA derivative was detected at 41.3 min on the reconstructed ion chromatogram for *m/z* 540 $[M - H]^-$ (see Table 2). In this case, the L-FDLA derivative of the 3*S*-Adhda should elute before the 3*R*-Adhda derivative since the FDLA derivative bearing a *cis*-type arrangement is expected to be more hydrophobic than a derivative bearing a *trans*-type arrangement.[10,14] Thus, the absolute configuration at C-3 of Adhda was determined to be *R*.

Knowing the absolute configuration at C-3_{Adhda}, the configuration at C-2_{Adhda} and C-5_{Adhda} was assigned using the reported *J*-based configuration analysis method developed by Murata and Tachibana.[15] This method considers that, in flexible systems, the conformation of adjacent asymmetric centers can be represented by six staggered rotamers (Figure 1), and their

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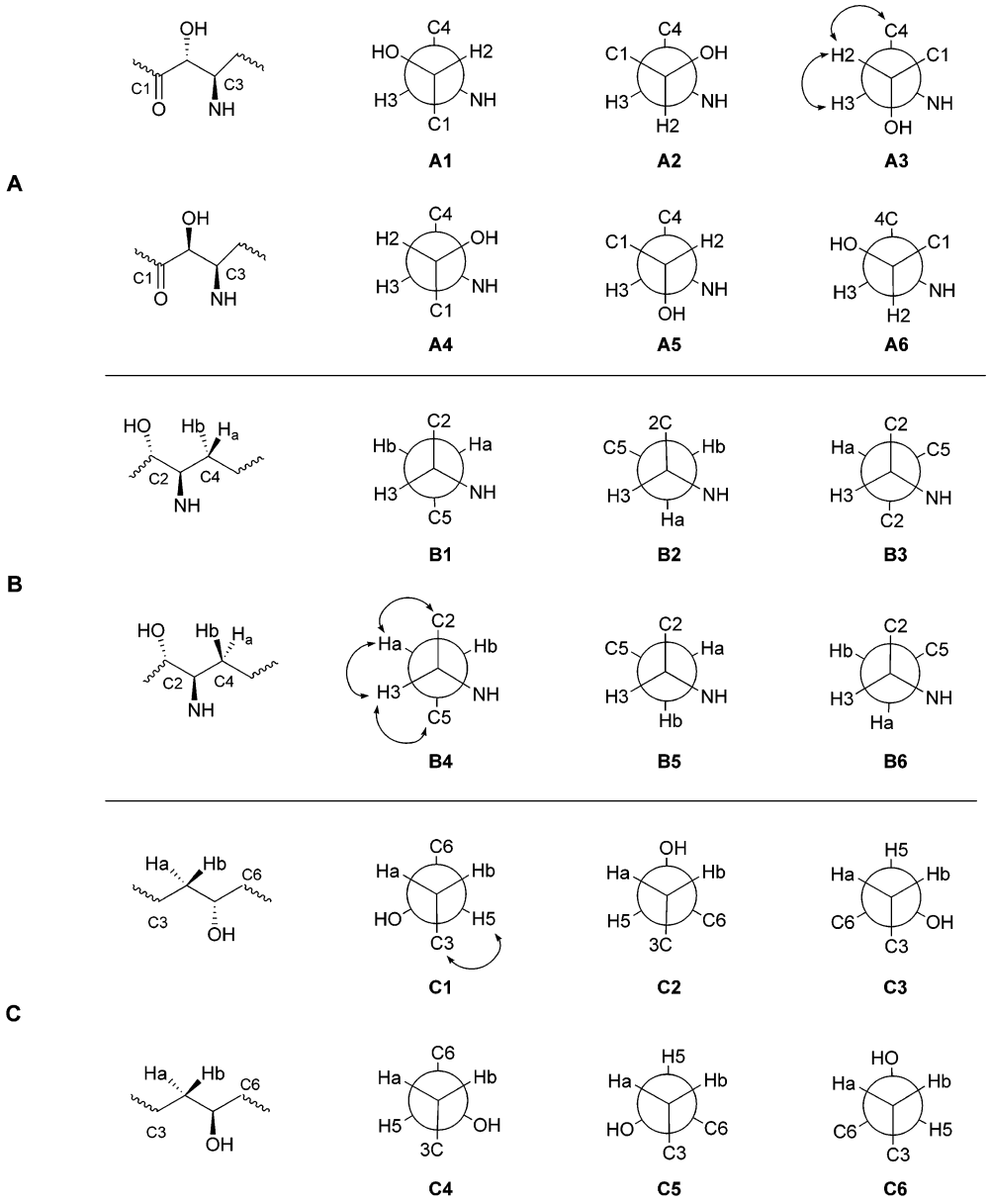

A3
$^{3}J_{H3\text{-}H2}$ = 5.7 Hz
$^{2}J_{H3\text{-}C2}$ = -5.7 Hz
$^{2}J_{H2\text{-}C3}$ = -1.9 Hz

B4
$^{3}J_{H3\text{-}H4a}$ = 2.0 Hz
$^{3}J_{H3\text{-}H4b}$ = 12.4 Hz
$^{2}J_{H4a\text{-}C3}$ = -1.5 Hz
$^{2}J_{H4b\text{-}C3}$ = -7.3 Hz

C1
$^{3}J_{H4a\text{-}H5}$ = 9.3 Hz
$^{2}J_{H4a\text{-}C5}$ = -5.2 Hz
$^{2}J_{H4b\text{-}C5}$ = -1.2 Hz

FIGURE 1. Newman projections showing all possible staggered rotamers in *threo* and *erythro* conformations viewed down bonds (A) C2−C3, (B) C3−C4, and (C) C4−C5, for the amino acid residue Adhda in compound **8**. $^{3}J_{H-H}$ and $^{2}J_{C-H}$ values that led to the assignment of the rotamers **A3**, **B4**, and **C1** are displayed. ROE correlations are also shown as double-sided arrows.

relative configuration can be deduced using $^{3}J_{H-H}$ and $^{2,3}J_{C-H}$ coupling constants in combination with NOE and ROE data. Homonuclear coupling data were obtained from the ^{1}H NMR, PS-COSY, and E.COSY[15] spectra, while heteronuclear coupling constants were accurately measured from a PFG-HETLOC spectrum.[17]

In the PFG-HETLOC spectrum, a large heteronuclear coupling of $^{2}J_{H3-C2} = -5.7$ Hz suggested a gauche configuration between H-3$_{Adhda}$ (δ 4.54) and the hydroxyl group of C-2$_{Adhda}$, while a small coupling of $^{2}J_{H2-C3} = -1.9$ Hz indicated an anti orientation between H-2$_{Adhda}$ (δ 3.99) and NH$_{Adhda}$. Thus, the only model that is consistent with all of these data is **A3** (Figure

1A). Strong ROE correlations between H-2$_{Adhda}$ and H-3$_{Adhda}$ and H-2$_{Adhda}$ and H-4a$_{Adhda}$ (δ 1.58), along with a homonuclear coupling constant of 5.7 Hz between H-2 $_{Adhda}$ and H-3 $_{Adhda}$, further support this result. Thus a 2*S*,3*R* configuration was established for Ahdha.

To establish the configuration of C-5$_{Adhda}$, we first made stereospecific assignments of the methylene protons at C-4$_{Adhda}$. A small $^3J_{H-H}$ of 2.0 Hz between H-3$_{Adhda}$ and H-4a$_{Adhda}$ indicated a gauche conformation between these protons, while a large $^3J_{H-H}$ of 12.4 Hz between H-3$_{Adhda}$ and H-4b$_{Adhda}$ (δ 1.356), indicated an anti conformation between H-3 and H-4b. Together these values rule out models **B1**, **B3**, **B5**, and **B6** (Figure 1B). Measurement of $^2J_{C-H}$ coupling constants of −1.5 and −7.3 Hz between H-4a$_{Adhda}$ and C-3 and between H-4b$_{Adhda}$ and C-3, respectively, further excluded model **B2** and indicated the correct conformation to be that depicted in **B4**. Strong ROE correlations between H-4a$_{Adhda}$ and H-2$_{Adhda}$, H-3$_{Adhda}$ and H-4a$_{Adhda}$, and H-3$_{Adhda}$ and H-5$_{Adhda}$ (δ 3.52) are fully consistent with this result. A large homonuclear coupling constant of 9.3 Hz between H-4a$_{Adhda}$ and H-5$_{Adhda}$ indicated an anti relationship between these two protons, ruling out models **C2**, **C3**, **C4**, and **C5** (Figure 1C). A large heteronuclear coupling of $^2J_{H4a-C5} = -5.2$ Hz, a small heteronuclear coupling of $^2J_{H4b-C5} = -1.2$ Hz, and a strong ROE correlation between H-5$_{Adhda}$ and H-3$_{Adhda}$ supported the configuration displayed by model **C1**. On the basis of these results, the configuration of C-5$_{Adhda}$ was determined to be *S*, giving the absolute configuration of Adhda as 2*S*,5*S*-dihydroxy-3*R*-aminododecanoic acid.

Ahp-containing cyclic depsipeptides have been reported to inhibit serine proteases,[5] where activities toward specific enzymes are related in part to the amino acid residue attached to the amino group of Ahp.[18] In particular, when this residue is hydrophobic the Ahp-containing cyclic depsipeptide strongly inhibits chymotrypsin but not trypsin.[18] As largamides D−G (**4**−**7**) each contain an Ahp residue, compounds **4**−**7** were tested for inhibition of chymotrypsin and trypsin. IC$_{50}$ values for this group of peptides varied significantly. Largamides D and E, which contain the sequence Ahp-Leu-Thr2, inhibited chymotrypsin with an IC$_{50}$ value of 10 μM, while largamides F and G, which contain the respective sequences Ahp-Tyr-Thr2 and Ahp-hTyr-Thr2, inhibited chymotrypsin activity with IC$_{50}$ values of 4.0 and 25.0 μM, respectively. None of the peptides inhibited trypsin. Neither largamides C (**3**) nor H (**8**) was found to be cytotoxic toward HCT116 cancer cell lines at concentrations as high as 250 μM.

Conclusions

In this study, we have isolated eight new cyclic and depsipeptides from a collection of *Oscillatoria* sp. Largamides A−H can be divided into three different classes according to their structural features. Largamides A−C (**1**−**3**) are characterized by the presence of a senecioic acid unit (Sen) that prior to this study has only been observed in terrestrial plant-derived secondary metabolites. In addition, largamides B and C contain the rare amino acid 2-amino-5-(4′-hydroxyphenyl)pentanoic acid (Ahppa) and the novel amino acid 2-amino-6-(4′-hydroxyphenyl)hexanoic acid (Ahpha), respectively. Largamides D−G (**4**−**7**) represent a new variant of 3-amino-6-hydroxy-2-piperidone acid (Ahp)-containing cyclic depsipeptides that comprise a 19-membered ring constructed from six amino acids. In contrast to other Ahp-containing peptides, where dipeptides or glyceric acid-capped dipeptides comprise the linear sequence linked to Thr2, the linear sequence within largamides D−G contains three amino acids, including the unusual Ahppa unit, along with the N-terminal glyceric acid. Largamides D−G also show the rare presence of a threonine residue located between the *N*-methyltyrosine and the Ahp units. Last, largamide H (**8**) represents the third class of cyclic peptide within this collection and is constructed of 10 amino acids. Largamide H is characterized by the presence of a methoxy derivative of Ahppa and a new β-amino acid 3-amino-2,5-dihydroxydodecanoic acid (Adhda), along with two residues of the nonstandard 2,3-dehydro-2-aminobutanoic acid (Dab).

Two interesting previous reports have suggested a defensive role for other Ahp-containing peptides, namely, cyanopeptolins 954 and 963A, on the basis of their protease inhibitory activity.[19,20] Owing to the presence of Ahp residues in largamides D−G, we tested these four depsipeptides for inhibition of chymotrypsin and trypsin proteases and found them to inhibit chymotrypsin, but not trypsin, with low micromolar IC$_{50}$ values (4−25 μM) that vary according to the residue located between Ahp and Thr2. In particular, potency decreased in the order of Tyr > Leu > hTyr. Representative peptides **3** and **8** corresponding to largamides C and H were also tested for cytotoxicity toward HCT-116 as a number of depsipeptides, such as the kahalalides,[21] have been reported to show activity toward this cell line. However, we observed no inhibitory activity for these depsipeptides. In closing, identification of eight new cyclic peptides of three distinct structural classes from a single homogeneous collection of *Oscillatoria* sp. provides another example of the remarkable biosynthetic capabilities of cyanobacteria.

Experimental Section

Biological Material. The marine cyanobacterium *Oscillatoria* sp. (Oscillatoriaceae) was collected in the Florida Keys off the coast of Key Largo (Florida, USA) at a depth of 25−30 feet in September 2003 and frozen in dry ice. Samples were stored at −50 °C until freeze-drying prior to extraction. Upon light microscopic analysis, taxonomic identification was made according to Ripka.[22] A voucher specimen (No. FL31-01) is available upon request.

Extraction and Isolation. The freeze-dried organism (63.4 g) was extracted at room temperature using solvents of increasing polarity, namely, petroleum ether (0.20 g), chloroform (0.12 g), and methanol (15.20 g). The MeOH extract was partitioned with *n*-BuOH−H_2O (1:1) to afford a dried *n*-BuOH extract (1.50 g). The *n*-BuOH extract was fractionated initially on a Sephadex LH-20 column (100 × 2.5 cm) using MeOH as mobile phase, and 22 fractions were obtained. Fraction 6 (73.4 mg) was further

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purified by reverse-phase HPLC (Jupiter Proteo C12, 250 × 10 mm, 4μ, DAD at 280 nm) eluting with a linear gradient of 55−70% MeOH in 0.05% TFA for 35 min to afford compounds **1** (2.5 mg, t_R = 26.8 min), **2** (1.4 mg, t_R = 22.2 min), and **3** (1.6 mg, t_R = 29.4 min). Fraction 8 (120.0 mg) was separated similarly eluting with a linear gradient of 60−90% MeOH in 0.05% TFA for 25 min to afford compounds **4** (7.3 mg, t_R = 15.3 min), **5** (1.2 mg, t_R = 14.6 min), and **8** (5.1 mg, t_R = 29.7 min). Fraction 10 (51.0 mg) was separated by MPLC on Si gel with a gradient of hexane/chloroform/methanol (from 3:1 to 0:100 and from 100:0 to 7:3, stepwise) as mobile phase to afford 37 fractions monitored by TLC. Fractions 22−26 (10.7 mg) were separated by reverse-phase HPLC (Jupiter Proteo C12, 250 × 10 mm, 4μ, DAD at 280 nm), using MeOH−H_2O containing 0.05% TFA as mobile phase (2.5 mL/min) eluting with MeOH (55−70%, 35 min) to yield compounds **6** (1.0 mg, t_R = 18.0 min) and **7** (1.2 mg, t_R = 19.5 min).

Largamide A (**1**): colorless amorphous powder; $[\alpha]^{20}_D$ −63.0° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (4.39), 278 (3.25) nm; ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m*/*z* 842.4319 $[M + H]^+$ (calcd for $C_{41}H_{60}N_7O_{12}$, 842.4300).

Largamide B (**2**): colorless amorphous powder; $[\alpha]^{20}_D$ −71.5° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.53), 221 (sh) (4.29), 278 (3.31) nm; ^{1}H and ^{13}C NMR data for Glu, Dab, Ala, Thr, Tyr, Val, and Sen are identical to those reported for **1** in Table 1; ^{1}H NMR (CD_3OD, 600 MHz) Ahppa δ 4.63 (1H, m, H-2), 1.91 (1H, m, H-3a), 1.62 (1H, m, H-3b), 1.62 (1H, m, H-4a), 1.57 (1H, m, H-4b), 2.55 (1H, m, H-5a), 2.50 (1H, m, H-5b), 7.00 (2H, dd, *J* = 8.2, 1.8 Hz, H-2′/H-6′), 6.68 (2H, dd, *J* = 8.2, 1.8 Hz, H-3′/H-5′), 7.73 (1H, d, *J* = 9.6, NH); ^{13}C NMR (CD_3OD, 150 MHz) Ahppa δ 171.8 (C-1), 52.1 (C-2), 30.9 (C-3), 28.1 (C-4), 35.1 (C-5), 134.2 (C-1′), 130.1 (C-2′/C-6′), 115.8 (C-3′/C-5′), 156.0 (C-4′); HR-ESI-MS *m*/*z* 920.4362 $[M + H]^+$ (calcd for $C_{46}H_{62}N_7O_{13}$, 920.4406).

Largamide C (**3**): colorless amorphous powder; $[\alpha]^{20}_D$ −60.4° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.51), 221 (sh) (4.29), 279 (3.29) nm; ^{1}H and ^{13}C NMR data for Glu, Dab, Ala, Thr, Tyr, Val, and Sen are identical those reported for **1** in Table 1; ^{1}H NMR (CD_3OD, 600 MHz) Ahpha δ 4.59 (1H, m, H-2), 1.93 (1H, m, H-3a), 1.63 (1H, m, H-3b), 1.36 (2H, m, H-4), 1.59 (1H, m, H-5a), 1.53 (1H, m, H-5b), 2.50 (2H, dd, *J* = 8.1, 1.2 Hz, H-6), 7.00 (2H, dd, *J* = 8.2, 1.8 Hz, H-8/H-12), 6.68 (2H, dd, *J* = 8.2, 1.8 Hz, H-9/H-11), 7.73 (1H, d, *J* = 9.6, NH); ^{13}C NMR (CD_3OD, 150 MHz) Ahpha δ 171.8 (C-1), 52.0 (C-2), 31.3 (C-3), 25.8 (C-4), 32.6 (C-5), 35.6 (C-6), 134.2 (C-1′), 129.9 (C-2′/C-6′), 115.8 (C-3′/C-5′), 156.0 (C-4′); HR-ESI-MS *m*/*z* 934.4604 $[M + H]^+$ (calcd for $C_{47}H_{64}N_7O_{13}$, 934.4562).

Largamide D (**4**): colorless amorphous powder; $[\alpha]^{20}_D$ −43.5° (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.26), 281 (3.39) nm; ^{1}H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m*/*z* 1232.5117 $[M + H]^+$ (calcd for $C_{56}H_{83}BrN_9O_{17}$, 1232.5090).

Largamide E (**5**): colorless amorphous powder; $[\alpha]^{20}_D$ −42.7° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.09), 280 (2.85) nm; ^{1}H and ^{13}C NMR data for Val1, Thr1, Ahp, Leu, Thr2, Val2, Ala, Ahppa, and glyceric acid are identical to those of **4** in Table 1; ^{1}H NMR (CD_3OD, 600 MHz) *N*MeClTyr δ 5.06 (1H, dd *J* = 11.5, 3.1 Hz, H-2), 3.40 (1H, dd, *J* = 14.6, 3.1 Hz, H-3a), 2.80 (1H, m, H-3b), 7.19 (1H, d, *J* = 2.0 Hz, H-5), 6.80 (1H, d, *J* = 8.4 Hz, H-8), 7.12 (1H, dd, *J* = 8.4, 2.0 Hz, H-9), 2.87 (3H, s, N-Me); ^{13}C NMR (CD_3OD, 150 MHz) *N*MeClTyr δ 171.4 (C-1), 62.6 (C-2), 33.8 (C-3), 129.7 (C-4), 131.8 (C-5), 121.0 (C-6), 152.9 (C-7), 117.8 (C-8), 130 (C-9); HR-ESI-MS *m*/*z* 1210.5396 $[M + Na]^+$ (calcd for $C_{56}H_{82}ClN_9O_{17}Na$, 1210.5415).

Largamide F (**6**): colorless amorphous powder; $[\alpha]^{20}_D$ −55.0° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.59), 280 (3.28) nm; ^{1}H and ^{13}C NMR data for Val1, *N*MeBrTyr Thr1, Ahp, Thr2, Val2, Ala, Ahppa, and glyceric acid are identical to those of **4** in Table 1; ^{1}H NMR (CD_3OD, 600 MHz) Tyr δ 4.71 (1H, dd, *J* = 11.6, 3.3 Hz, H-2), 3.51 (1H, dd, *J* = 14.7, 3.3 Hz, H-3a), 2.62 (1H, dd, *J* = 14.7, 11.6 Hz, H-3b), 7.03 (2H, d, *J* = 8.2, 1.2 Hz, H-5/H-9), 6.67 (2H, d, *J* = 8.2, 1.2 Hz, H-6/H-8); ^{13}C NMR (CD_3OD, 150 MHz) Tyr δ 173.5 (C-1), 56.5 (C-2), 36.4 (C-3), 129.5 (C-4), 130.5 (C-5/C-9), 116.1 (C-6/C-8), 156.8 (C-7); HR-ESI-MS *m*/*z* 1282.4880 $[M + H]^+$ (calcd for $C_{59}H_{81}N_9O_{18}Br$, 1282.4883).

Largamide G (**7**): colorless amorphous powder; $[\alpha]^{20}_D$ −70.0° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.67), 280 (3.42) nm; ^{1}H and ^{13}C NMR data for Val1, *N*MeBrTyr Thr1, Ahp, Thr2, Val2, Ala, Ahppa, and glyceric acid are identical to those of **4** in Table 1; ^{1}H NMR (CD_3OD, 600 MHz) hTyr δ 4.35 (1H, dd, *J* = 10.9, 2.8 Hz, H-2), 2.43 (1H, m, H-3a), 1.87 (1H, m, H-3b), 2.68 (1H, m, H-4a), 2.51 (1H, m, H-4b), 6.99 (2H, d, *J* = 8.2, 1.2 Hz, H-6/H-10), 6.70 (2H, d, *J* = 8.2, 1.2 Hz, H-7/H-9); ^{13}C NMR (CD_3OD, 150 MHz) Tyr δ 174.0 (C-1), 58.8 (C-2), 33.3 (C-3), 31.7 (C-4), 132.4 (C-5), 130.3 (C-6/C-10), 115.7 (C-7/C-9), 156.4 (C-8); HR-ESI-MS *m*/*z* 1318.4918 $[M + Na]^+$ (calcd for $C_{60}H_{82}N_9O_{18}BrNa$, 1318.4859).

Largamide H (**8**): colorless amorphous powder; $[\alpha]^{20}_D$ −80.6° (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (4.14), 278 (3.21), 285 (3.12) nm; ^{1}H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m*/*z* 1199.6708 $[M + H]^+$ (calcd for $C_{57}H_{91}N_{12}O_{16}$, 1199.6676).

LC/MS Analysis of L/D-FDLA Derivatives.[8,10] Approximately 0.3 mg of compounds **1**−**8** was separately hydrolyzed with 5 N HCl (LabChem Inc., traceable to NIST) (0.8 mL) in an Ace high-pressure tube for 16 h at 90 °C. The hydrolysates were evaporated to dryness and dissolved in H_2O (100 μL). To a 50 μL aliquot of each were added 1 N $NaHCO_3$ (20 μL) and 1% 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA or D-FDLA solution in acetone, 100 μL), and the mixtures were heated to 40 °C for 40 min. The solutions were cooled to room temperature, neutralized with 2 N HCl (20 μL), and evaporated to dryness. The residues were dissolved in CH_3CN and analyzed by LC-MS. The analysis of the L- and L/D-FDLA (mixture of D- and L-FDLA) derivatives was performed on a Phenomenex Jupiter Proteo C12 column (4 μm, 150 × 4.6 mm). Aqueous CH_3CN containing 0.01 M TFA was used as a mobile phase eluting with a linear gradient of 25−70% CH_3CN in 45 min at a flow rate of 0.5 mL/min. A mass spectrometer detector was used for detection in ESI (negative) mode. The fragmentor and capillary voltage were kept at 70 and 1000 V, respectively, and the ion source at 350 °C. A mass range of *m*/*z* 100−1000 was scanned in 0.1 min. Retention times (t_R, min) of the FDLA-derivatized amino acids are summarized in Table 3. Retention times of the FDLA-derivatized authentic standards are L-Val 27.5, D-Val 34.0 *m*/*z* 410 $[M - H]^-$; L-Thr 19.6, D-Thr 24.2 *m*/*z* 412 $[M - H]^-$; L-Ala 24.4, D-Val 27.9 *m*/*z* 382 $[M - H]^-$; L-Glu 21.8, D-Glu 22.8 *m*/*z* 440 $[M - H]^-$; L-Tyr 40.8, D-Tyr 44.1.0 *m*/*z* 768 $[M - H]^-$; L-Leu 30.3, D-Leu 38.1 *m*/*z* 424 $[M - H]^-$; L-*N*MeAsp 20.3, D-*N*MeAsp 19.0 *m*/*z* 440 $[M - H]^-$.

Absolute Stereochemistry of Glyceric Acid. An aliquot (0.1−0.5 mg) of compounds **4**−**7** was hydrolyzed with 5 N HCl

(0.8 mL) in an Ace high-pressure tube for 16 h at 90 °C. The hydrolysates were concentrated to dryness and subjected to chiral HPLC analysis (Phenomenex Chirex Phase 3126 (D) (150 × 4.6 mm) HPLC column; flow rate 0.5 mL/min; detection at 254 nm; solvent 2 mM CuSO4:MeCN 90:10). The retention times of glyceric acid were compared to authentic standards whose retention times were 7.22 min for L-glyceric acid and 9.37 min for D-Glyceric acid.

Chymotrypsin Inhibition Assay. Chymotrypsin inhibition assays were performed according to the manufacturer's instructions. Briefly, α-chymotrypsin type II (Sigma C-4129) was dissolved in 1 mM HCl to prepare a 1 mg/mL solution, and a 2 mM solution of *N*-benzoyl-L-tyrosine ethyl ester in MeOH−H_2O (1:1) was used as substrate. Peptides were dissolved in MeOH−H_2O (1:1); 100 μL of buffer solution (50 mM Tris-HC1/100 mM NaCl/1 mM $CaCl_2$, adjusted to pH 7.8 with HCl), 100 μL of substrate solution, and a 10 μL sample solution were mixed in a microcentrifuge tube followed by addition of 10 μL of enzyme. The change in absorbance at 256 nm was measured immediately and at 30 s intervals for 5 min at 25 °C.

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Supporting Information Available: General experimental procedures; ^1H and ^{13}C NMR data for compounds **3** and **8** in DMSO-d_6 and $CDCl_3$, respectively; ^1H NMR, HSQC, HMBC, 2D HOHAHA, DQF-COSY, and ROESY spectra for compounds **1**−**8** (CD_3OD); ^1H NMR, HSQC, HMBC, and ROESY spectra for compound **2** (DMSO-d_6); ^1H NMR, HSQC, HMBC, and ROESY spectra for compound **8** ($CDCl_3$); and HETLOC spectrum for compound **8** (CD_3OD). This material is available free of charge via the Internet at http://pubs.acs.org.

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