Ulongamides A−F, New β-Amino Acid-Containing Cyclodepsipeptides from Palauan Collections of the Marine Cyanobacterium *Lyngbya* sp.

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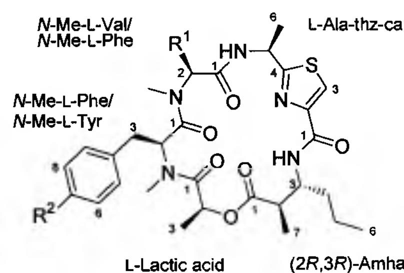
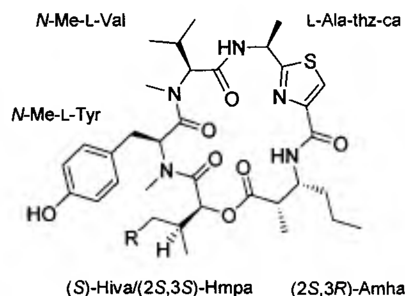
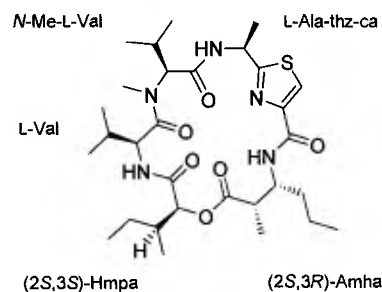
Six new β-amino acid-containing cyclic depsipeptides, termed ulongamides A−F (**1**−**6**), have been isolated from collections of apratoxin-producing cyanobacteria from Palau. Their planar structures have been determined by NMR spectroscopic techniques. The absolute stereochemistry of the hydroxy acid and all the α-amino acid-derived units was ascertained to be *S* by chiral HPLC analysis of degradation products. The stereochemistry of the β-amino acid moiety, 3-amino-2-methylhexanoic acid, was established by advanced Marfey analysis of the acid hydrolyzates and found to be 2*R*,3*R* in compounds **1**−**3** but 2*S*,3*R* in compounds **4**−**6**. All compounds except **6**, which lacks an aromatic amino acid moiety, were weakly cytotoxic against KB cells.

Marine cyanobacteria are well known for their production of bioactive peptides and depsipeptides.[1] Typically, these metabolites can be cyclic, contain modified amino acid units and polyketide portions, or be any combination thereof. These features confer a resistance to enzymatic degradation and contribute to the variety of biological activities that have been reported for many cyanobacterial metabolites. An example is the potent cytotoxin apratoxin A, first isolated from a Guamanian *Lyngbya* sp. and more recently from a Palauan collection of this organism.[2] Apratoxin A-producing cyanobacteria collected in April 1999 and April 2000 at various Palauan dive sites have also afforded six new β-amino acid-containing metabolites, ulongamides A−F (**1**−**6**), whose structure elucidation is described in this report. β-Amino acid units have previously been found in numerous marine cyanobacterial metabolites including the structurally related obyanamide[3] or majusculamide C.[4] They have also appeared in compounds such as the cryptophycins from terrestrial cyanobacteria.[5]







Results and Discussion

Lipophilic extracts of *Lyngbya* sp. NIH309 were subjected to solvent partitioning, normal-phase or reversed-phase chromatography, and then reversed-phase HPLC (see Experimental Section), yielding compounds **1**−**6** as colorless amorphous solids.

Ulongamide A (**1**) possesses a molecular formula of $C_{32}H_{45}N_5O_6S$, as suggested by HRFABMS and NMR data. The ^{1}H NMR spectrum indicated the peptide nature of **1** by displaying signals due to amide protons (δ_H 8.17 and 8.95) and singlets for *N*-methyl protons (δ_H 2.99 and 3.20) indicative of tertiary amide functionalities. ^{1}H−^{1}H COSY and 1D TOCSY analysis revealed the presence of six independent spin systems. ^{13}C NMR, HSQC, and HMBC analysis (Table 1) permitted elaboration of partial structures. Taking the two *N*-methyl singlets and the singlet in the aromatic region (δ_H 8.04) into account, an alanine-derived thiazole carboxylic acid (Ala-thz-ca), *N*-methylvaline, *N*-methylphenylalanine, lactic acid, and a β-amino

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Table 1. NMR Spectral Data for Ulongamide A (**1**) at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$

unit	C/H no.	δ_H (J in Hz)	δ_C[a]	^{1}H−^{1}H COSY	HMBC[b,c]
Amha	1		171.9, s		H-2, H_3-7, H-2 (E)
(A)	2	2.68, qd (6.9, 2.8)	45.2, d	H-3, H_3-7	H_3-7
	3	4.31, m	51.0, d	H-2, H_2-4	H_3-7, H-4a
	4a	1.43, m	35.7, t	H-4b, H_2-5	H_3-6
	4b	1.54, m		H-4a, H_2-5	
	5	1.43, m	19.6, t	H_2-4, H_3-6	H_2-4, H_3-6
	6	0.97, t (7.0)	14.0, q	H_2-5	H-4b
	7	1.22, d (6.9)	14.6, q	H-2	H-2
	NH	8.95, d (10.8)		H-3	
Ala-thz-ca	1		160.8, s		NH (A), H-3
(B)	2		148.9, s		H-3
	3	8.04, s	123.0, d		
	4		169.5, s		H-3, H-5, H_3-6, NH
	5	5.33, quint (6.6)	47.6, d	H_3-6	H_3-6
	6	1.45, d (6.6)	24.5, q	H-5	H-5
	NH	8.17, d (6.6)		H-5	
N-Me-Val	1		167.9, s		H-5 (B), NH (B), H-2
(C)	2	4.50, d (10.8)	66.2, d	H-3	H-3, H_3-4, H_3-5, *N*-CH_3
	3	2.33, m	27.2, d	H_3-4, H_3-5	H-2, H_3-4, H_3-5
	4	0.59, d (7.0)	18.3, q	H-3	H-2, H-3, H_3-5
	5	0.84, d (6.6)	19.3, q	H-3	H_3-4
	N-CH_3	2.99, s	29.0, q		H-2
N-Me-Phe	1		170.3, s		H-2 (C), *N*-CH_3 (C), H-2, H_2-3
(D)	2	6.08, dd (9.4, 5.5)	50.7, d	H_2-3	H_2-3, *N*-CH_3
	3a	3.14, dd (−15.2, 5.5)	35.3, t	H-2, H-3b	H-2, H-5/9
	3b	3.26, dd (−15.2, 9.4)		H-2, H-3a	
	4		136.1, s		H-2, H_2-3, H-6/8
	5/9	7.16, d (7.2)	128.6, d	H-6/8	H_2-3, H-7
	6/8	7.29, m	128.8, d	H-5/9, H-7	H-5/9, H-8/6
	7	7.25, m	127.0, d	H-6/8	H-5/9
	N-CH_3	3.20, s	30.0, q		H-2
lactic acid	1		172.8, s		H-2 (D), *N*-CH_3 (D), H-2, H_3-3
(E)	2	5.15, q (6.6)	66.9, d	H_3-3	H_3-3
	3	1.33, d (6.6)	16.1, q	H-2	H-2

[a] Multiplicity deduced from the HSQC spectrum. [b] Protons showing long-range correlation with indicated carbon. [c] Correlations refer to protons within the same unit if not indicated otherwise.

acid residue, 3-amino-2-methylhexanoic acid (Amha), were identified. Interpretation of HMBC data established their sequence leading to the cyclic planar structure depicted for **1**. The IR spectrum of **1** supported the proposed depsipeptide structure, displaying absorptions at 1635 and 1729 cm^{-1}, which are characteristic of amide and ester functionalities, respectively.

Similar analysis was carried out for the other, closely related metabolites **2**−**6**, and the sequence was verified in each case by HMBC and/or ROESY analysis. Ulongamide B (**2**) exhibited an almost identical ^{1}H NMR spectrum to **1**. However, the signal multiplicity in the aromatic region indicated *para*-substitution of the phenyl ring (Table 2). Further NMR analysis ascertained that the *N*-Me-Phe residue had been replaced by an *N*-Me-Tyr unit, which accounted for the 16 amu difference for **2** when compared to **1** and thus for the molecular formula of $C_{32}H_{45}N_5O_7S$ derived from HRFABMS analysis. The only difference between ulongamide C (**3**) and compound **2** that was discerned by NMR (Table 2) was the presence of two aromatic amino acids, i.e., the switch of the *N*-Me-Val unit to an *N*-Me-Phe residue. This satisfied the four extra degrees of unsaturation required by the HRFABMS molecular formula of $C_{36}H_{45}N_5O_7S$. Ulongamides D (**4**) and E (**5**) also contained the *N*-Me-Tyr moiety, but the hydroxy acid units differed. 2-Hydroxyisovaleric acid (Hiva) in **4** and 2-hydroxy-3-methylpentanoic acid (Hmpa) in **5** had replaced the lactic acid moiety (Table 3). HRFABMS analysis confirmed the molecular formulas for **4** and **5** to be $C_{34}H_{49}N_5O_7S$ and $C_{35}H_{51}N_5O_7S$, respectively. Ulongamide F (**6**) contained no aromatic amino acid, as was evident from NMR data (Table 3). In the position of the *N*-methylated aromatic amino acid in **1**−**5**, a nonmethylated valine residue was found in **6**, which accounted for the additional exchangeable proton at δ_H 6.21. Compound **6** contained the Hmpa unit as in **5**. The gross structure conformed to the molecular formula requirement of $C_{30}H_{49}N_5O_6S$ according to HRFABMS analysis.

Compounds **1**−**6** were subjected to ozonolysis followed by acid hydrolysis. All product mixtures were analyzed by chiral HPLC, and comparison with retention times of authentic standards demonstrated that the configuration of all stereocenters of the liberated α-amino acids and hydroxy acids was *S* in each case. The stereochemistry of the Amha residue was determined by the advanced Marfey's method.[6] For this analysis two diastereomeric Amha isomers (2*R*,3*R* and 2*S*,3*R*) were synthesized, derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA), and analyzed by reversed-phase HPLC. The retention times of the corresponding enantiomers (2*S*,3*S* and 2*R*,3*S*) were inferred after derivatization of each Amha diastereomer with DL-FDLA.[6] Analogous derivatization of the acid hydrolyzates of compounds **1**−**6** followed by HPLC analysis and comparison with the standard derivatives enabled us to deduce the last missing piece of stereochemical information. Interestingly, ulongamides A−C (**1**−**3**) contain the Amha residue in 2*R*,3*R* configuration, while the Amha unit in ulongamides D−F (**4**−**6**) has 2*S*,3*R* configuration. A stereochemical difference for compounds **1**−**3** versus compounds **4**−**6** was already suspected from the NMR analysis since the chemical shifts for this unit were substantially different for both sets of compounds (e.g., $\Delta\delta_{C-2} \approx 2.5$ ppm, $\Delta\delta_{C-4} \approx 6$ ppm, $\Delta\delta_{C-7} \approx 1$ ppm), even though the vicinal coupling of H-2 and H-3 protons deviated only slightly (Tables 1−3). It appears that the configuration at C-2 of the Amha residue correlates with the nature of the adjacent

Table 2. NMR Spectral Data for Ulongamides B (**2**) and C (**3**) at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$

		ulongamide B (**2**)		ulongamide C (**3**)	
unit	C/H no.	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
Amha	1		172.1, s		171.9, s
	2	2.69, qd (7.0, 2.7)	45.4, d	2.67, qd (7.0, 2.9)	45.2, d
	3	4.31, m	51.0, d	4.33, m	50.9, d
	4	1.44, m, 1.55, m	35.6, t	1.47, m, 1.58, m	36.0, t
	5	1.45, m	19.6, t	1.50, m	19.8, t
	6	0.96, t (7.1)	14.0, q	1.06, t (6.9)	14.1, q
	7	1.23, d (7.0)	14.7, q	1.20, d (7.0)	14.5, q
	NH	8.97, d (10.7)		8.87, d (10.8)	
Ala-thz-ca	1		160.8, s		161.0, s
	2		148.9, s		148.8, s
	3	8.06, s	123.0, d	8.05, s	123.1, d
	4		169.5, s		169.6, s
	5	5.32, quint (6.8)	47.6, d	5.41, dq (7.4, 6.8)	47.2, d
	6	1.45, d (6.8)	24.5, q	1.44, d (6.8)	24.6, q
	NH	8.15, d (6.8)		8.06, d (7.4)	
N-Me-Val[b]/	1		167.9, s		167.7, s
N-Me-Phe[c]	2	4.49, d (10.7)	66.2, d	5.30, dd (9.9, 5.1)	61.6, d
	3	2.32, m	27.3, d	2.98, dd (−14.6, 5.1)	35.2, t
				3.00, dd (−14.6, 9.9)	
	4	0.59, d (6.8)	18.3, q		136.9, s
	5/9	0.84, d (6.4)	19.3, q	7.14, m	129.1, d
	6/8			7.16, m	129.0, d
	7			7.06, m	127.1, d
	N-CH_3	2.98, s	29.0, q	3.07, s	28.9, q
N-Me-Tyr	1		170.3, s		170.6, s
	2	5.99, dd (9.1, 5.8)	51.0, d	5.55, dd (10.9, 5.0)	49.9, d
	3	3.08, dd (−15.0, 5.8)	34.5, t	1.44, dd (−15.6, 5.0)	32.7, t
		3.17, dd (−15.0, 9.1)		2.78, dd (−15.6, 10.9)	
	4		128.1, s		127.8, s
	5/9	7.02, d (8.5)	129.8, d	6.76, d (8.5)	129.2, d
	6/8	6.76, d (8.5)	115.7, d	6.70, d (8.5)	115.5, d
	7		154.6, s		154.4, s
	OH	5.23, br s		not observed	
	N-CH_3	3.20, s	30.0, q	2.99, s	29.8, q
lactic acid	1		172.8, s		173.0, s
	2	5.17, q (6.8)	67.0, d	5.08, q (6.8)	66.7, d
	3	1.33, d (6.8)	16.1, q	1.29, d (6.8)	16.1, q

[a] Multiplicity deduced from the HSQC spectrum. [b] Refers to compound **2**. [c] Refers to compound **3**.

hydroxy acid: ulongamides containing a lactic acid moiety (**1**−**3**) possess a 2*R* configuration, while ulongamides with Hiva or Hmpa residues (**4**−**6**) possess a 2*S* configuration.

Compounds **1**−**6** were tested in cytotoxicity assays against KB and LoVo cell lines. Ulongamides A−E (**1**−**5**) displayed weak in vitro cytotoxicity with IC_{50} values of ca. 1 μM and ca. 5 μM against KB and LoVo cells, respectively,[7] comparable with activity found for the structurally related obyanamide, isolated from the marine cyanobacterium *L. confervoides*.[3] Ulongamide F (**6**), however, was inactive at <10 μM against both cell lines, suggesting that the lack of an aromatic amino acid or the *N*-methyl group adjacent to the hydroxy acid is detrimental to activity.

The trivial names for compounds **1**−**6** have been derived from Ulong Channel, the collection site of the cyanobacterium that afforded the majority of these new metabolites. A 3-amino-2-methylhexanoic acid (Amha) residue as in **1**−**6** was also encountered in the cyanobacterial metabolites malevamide B[8] and lyngbyastatin 3[9] as well as in a constituent of the cephalaspidean mollusk *Philinopsis speciosa*, kulokekahilide-1.[10] In comparison to obyanamide, which bears a 3-aminopentanoic acid moiety,[3] compounds **1**−**6** contain a different β-amino acid unit. Remarkably, ulongamide A (**1**) differs from obyanamide otherwise merely in the residue sequence, with the *N*-Me-Val and the *N*-Me-Phe residues being switched. The great structural variety of the ulongamides is intriguing and might suggest that enzymes with relaxed substrate specificity are involved in their biosynthesis[11] or that slight genetic divergence among individual patches of this cyanobacterium might be responsible for the diversity. In conclusion, the finding of the ulongamides **1**−**6** certainly demonstrates once again the biosynthetic versatility of cyanobacteria.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz, respectively, using residual solvent signals as internal references. HSQC experiments were optimized for $^1J_{CH} = 140$ Hz, and the HMBC experiments for $^nJ_{CH} = 7$ Hz. HRFABMS were recorded in the positive mode using a VG-ZAB spectrometer. The synthesis of authentic (2*R*,3*R*)-Amha and (2*S*,3*R*)-Amha will be described elsewhere.[9]

Biological Material. *Lyngbya* sp. NIH309 was collected on April 7, 1999, at Short Dropoff, Palau. The isolation of apratoxins A and C from this collection has already been reported.[2b] Minute cyanobacterial collections of other populations of this cyanobacterium were also carried out on April 5 at Lighthouse Channel, on April 7 at Ngerkuul Pass, and on April 8, 1999, at Ngederrak Lagoon and Big Dropoff, Palau. These were combined before extraction and are referred to as "mixed collection" in the Experimental Section. On April 18, 2000, a population of this organism was collected at Ulong Channel, Palau. Specimens are deposited at the University of Hawaii, Department of Chemistry, and the University of Guam Marine Laboratory.

Extraction and Isolation. Extraction and fractionation of NIH309 from Short Dropoff, collected in 1999, has previously been described.[2b] Compounds **3**−**5** eluted from Si gel with 6% *i*-PrOH in CH_2Cl_2 along with apratoxins A and C. Semipreparative reversed-phase HPLC (Ultracarb, 5 ODS 30, 250 × 10 mm, 3.0 mL/min; UV detection at 220 nm) using an

Table 3. NMR Spectral Data for Ulongamides D–F (**4**–**6**) at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$

		ulongamide D (**4**)		ulongamide E (**5**)		ulongamide F (**6**)	
unit	C/H no.	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)	δ_C[a]
Amha	1		173.4, s		173.4, s		173.6, s
	2	2.94, qd (7.0, 3.9)	42.7, d	2.94, qd (7.2, 3.9)	42.5, d	2.97, qd (7.0, 3.8)	42.9, d
	3	4.17, m	51.4, d	4.14, m	51.5, d	4.22, m	51.2, d
	4	1.04, m, 1.65, m	29.7, t	1.03, m, 1.65, m	29.7, t	1.18, m, 1.65, m	29.6, t
	5	1.34, m, 1.49, m	19.6, t	1.34, m, 1.49, m	19.5, t	1.35, m, 1.48, m	19.5, t
	6	0.94, t (7.4)	14.1, q	0.93, t (7.4)	14.0, q	0.92, t (7.3)	13.9, q
	7	1.19, d (7.0)	13.7, q	1.17, d (7.2)	13.5, q	1.22, d (7.0)	13.7, q
	NH	9.08, d (10.1)		9.08, d (10.1)		9.23, d (10.1)	
Ala-thz-ca	1		160.4, s		160.4, s		160.5, s
	2		148.5, s		148.5, s		148.5, s
	3	8.06, s	123.2, d	8.06, s	123.2, d	8.06, s	123.2, d
	4		169.5, s		169.5, s		169.2, s
	5	5.29, qd (6.8, 6.1)	48.1, d	5.29, qd (6.7, 6.0)	48.2, d	5.26, qd (6.8, 5.8)	48.4, d
	6	1.46, d (6.8)	23.5, q	1.49, d (6.7)	23.4, q	1.47, d (6.8)	23.5, q
	NH	8.41, d (6.1)		8.50, d (6.0)		8.21, d (5.8)	
N-Me-Val	1		167.8, s		167.8, s		167.9, s
	2	4.60, d (10.8)	66.6, d	4.61, d (10.8)	66.6, d	4.45, d (10.7)	66.2, d
	3	2.40, m	27.1, d	2.39, m	27.1, d	2.48, m	27.4, d
	4	0.75, d (6.8)	18.8, q	0.72, d (6.9)	18.7, q	0.94, d (6.9)	19.5, q
	5	0.91, d (6.5)	19.5, q	0.90, d (6.3)	19.5, q	0.96, d (6.5)	19.5, q
	N-CH_3	2.95, s	28.9, q	2.94, s	28.9, q	2.96, s	29.1, q
N-Me-Tyr[b,c]/ Val[d]	1		171.0, s		170.9, s		171.8, s
	2	6.07, dd (10.7, 4.7)	50.4, d	6.07, dd (10.5, 4.6)	50.6, d	4.73, t (9.3)	53.7, d
	3	3.00, dd (−15.7, 4.7), 3.29, dd (−15.7, 10.7)	33.9, t	3.01, dd (−15.8, 4.6), 3.29, dd (−15.8, 10.5)	33.8, t	2.20, m	31.6, d
	4		128.0, s		128.0, s	0.95, d (6.8)	20.2, q
	5/9	7.00, d (8.4)	129.3, d	7.00, d (8.5)	129.3, d	1.08, d (6.6)	18.6, q
	6/8	6.78, d (8.4)	115.8, d	6.78, d (8.5)	115.8, d		
	7		154.6, s		154.7, s		
	OH	5.08, br		5.37, br s			
	N-CH_3	3.20, s	30.4, q	3.21, s	30.5, q		
	NH					6.21, d (9.3)	
Hiva[b]/ Hmpa[c,d]	1		172.2, s		172.5, s		171.5, s
	2	4.94, d (5.6)	74.9, d	4.92, d (7.2)	74.5, d	4.61, d (7.6)	77.6, d
	3	1.95, m	29.8, d	1.64, m	36.5, d	1.60, m	36.4, d
	4	0.67, d (6.8)	17.8, q	0.91, m, 1.31, m	24.3, t	1.01, m, 1.37, m	24.8, t
	5	0.83, d (7.0)	18.6, q	0.58, t (7.4)	10.9, q	0.66, t (7.4)	10.8, q
	6			0.76, d (7.0)	14.3, q	0.75, d (6.9)	14.9, q

[a] Multiplicity deduced from the HSQC spectrum. [b] Refers to compound **4**. [c] Refers to compound **5**. [d] Refers to compound **6**.

isocratic system of 65% aqueous MeCN afforded **3** (0.5 mg, t_R 9.0 min), **4** (0.3 mg, t_R 13.9 min), and **5** (0.7 mg, t_R 16.8 min).

The freeze-dried "mixed collection" from 1999 (~40 g) was extracted with CH_2Cl_2–MeOH (2:1). The resulting lipophilic extract (3.07 g) was partitioned between hexanes and 80% aqueous MeOH. The latter phase was concentrated to dryness and partitioned between *n*-BuOH and H_2O. The concentrated *n*-BuOH portion was applied to an ODS-coated silica column (YMC-ODS-A) and subjected to flash chromatography with 20%, 40%, 50%, 60%, 70%, 80%, and 90% aqueous MeCN, followed by MeCN, MeOH, and CH_2Cl_2. The fraction eluting with 70% aqueous MeCN (30.2 mg) was then subjected to semipreparative reversed-phase HPLC as above, affording **5** (1.2 mg, t_R 16.8 min) and **1** (1.1 mg, t_R 26.0 min).

The freeze-dried collection from Ulong Channel, collected in 2000 (~50 g), was extracted and partitioned as above. The *n*-BuOH phase was concentrated and applied to a Si gel column, and elution was initiated with CH_2Cl_2 followed by mixtures containing progressively increasing amounts of *i*-PrOH before washing with MeOH. The 5% *i*-PrOH in CH_2Cl_2 fraction was concentrated and subjected to semipreparative reversed-phase HPLC as above to afford **6** (0.4 mg, t_R 24.5 min). Similarly, the mixture eluting in the subsequent fraction during the Si gel chromatography step (6% *i*-PrOH in CH_2Cl_2) afforded **2** (0.5 mg, t_R 8.0 min), **3** (0.8 mg, t_R 9.0 min), **4** (0.3 mg, t_R 13.9 min), and **5** (0.6 mg, t_R 16.8 min) by reversed-phase HPLC as above.

Ulongamide A (1): colorless, amorphous solid; $[\alpha]^{25}_D$ +12° (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.25), 230 (3.88) nm; IR (film) ν_{max} 3327, 2963, 2927, 2855, 1729, 1635 (br), 1553, 1523, 1494, 1464, 1271, 1212, 1079, 1044 cm^{-1}; ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, and HMBC data, see Table 1; HRFABMS *m/z* [M + H]$^+$ 628.3195 (calcd for $C_{32}H_{46}N_5O_6S$, 628.3169).

Ulongamide B (2): colorless, amorphous solid; $[\alpha]^{25}_D$ +10° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.24), 230 (3.87) nm; IR (film) ν_{max} 3331, 2967, 2919, 2860, 1737, 1631 (br), 1549, 1519, 1455, 1414, 1273, 1214, 1085, 1045 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* [M + H]$^+$ 644.3132 (calcd for $C_{32}H_{46}N_5O_7S$, 644.3118).

Ulongamide C (3): colorless, amorphous solid; $[\alpha]^{25}_D$ +9° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.54), 230 (4.12) nm; IR (film) ν_{max} 3335, 2955, 2931, 2849, 1731, 1631 (br), 1554, 1519, 1455, 1273, 1214, 1091, 1044 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* [M + H]$^+$ 692.3121 (calcd for $C_{36}H_{46}N_5O_7S$, 692.3118).

Ulongamide D (4): colorless, amorphous solid; $[\alpha]^{25}_D$ +22° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.24), 230 (3.89) nm; IR (film) ν_{max} 3307, 2966, 2919, 2837, 1737, 1635 (br), 1555, 1519, 1461, 1261, 1185, 1096 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* [M + H]$^+$ 672.3423 (calcd for $C_{34}H_{50}N_5O_7S$, 672.3431).

Ulongamide E (5): colorless, amorphous solid; $[\alpha]^{25}_D$ +20° (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.24), 230 (3.89) nm; IR (film) ν_{max} 3310, 2967, 2919, 2849, 1731, 1643, 1555, 1514, 1455, 1267, 1179, 1096 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* [M + H]$^+$ 686.3616 (calcd for $C_{35}H_{52}N_5O_7S$, 686.3587).

Ulongamide F (6): colorless, amorphous solid; $[\alpha]^{25}_D$ +25° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.85), 230 (3.50) nm; IR (film) ν_{max} 3311, 2967, 2919, 2849, 1737, 1643 (br), 1561, 1519, 1467, 1179 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table

3; HRFABMS *m/z* $[M + H]^+$ 608.3456 (calcd for $C_{30}H_{50}N_5O_6S$, 608.3482).

Ozonolysis, Acid Hydrolysis, and Chiral HPLC Analysis. Compounds **1**–**6** (0.1 mg each) were each dissolved in 3 mL of CH_2Cl_2 and ozonized at −78 °C. The solvent was evaporated, and the products were suspended in 6 N HCl (0.5 mL) and heated at 110 °C for 12 h. The hydrolyzates were concentrated to dryness and subjected to chiral HPLC analysis [column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$–MeCN (95:5 or 85:15); flow rate, 1.0 mL/min; detection at 254 nm]. Using 2 mM $CuSO_4$–MeCN (95:5), the retention times (t_R, min) of authentic standards were as follows: L-Ala (6.7), D-Ala (8.0), *N*-Me-L-Val (12.2), *N*-Me-D-Val (17.0), L-Val (17.2), D-Val (23.0), L-lactic acid (19.8), and D-lactic acid (33.0). Most other standards were detected using 2 mM $CuSO_4$–MeCN (85:15): *N*-Me-L-Tyr (16.1), *N*-Me-D-Tyr (18.5), *N*-Me-L-Phe (33.6), *N*-Me-D-Phe (37.2), (*S*)-Hiva (38.2), and (*R*)-Hiva (62.0). The retention times of the α-amino acids and hydroxy acids in the acid hydrolyzates, under the respective conditions, were for **1**: 6.7, 12.2, and 19.8 min (95:5), and 33.6 min (85:15), indicating the presence of L-Ala, *N*-Me-L-Val, L-lactic acid, and *N*-Me-L-Phe; for **2**: 6.7, 12.2, and 19.8 min (95:5), and 16.1 min (85:15), indicating the presence of L-Ala, *N*-Me-L-Val, L-lactic acid, and *N*-Me-L-Tyr; for **3**: 6.7 and 19.8 min (95:5), 16.1 and 33.6 min (85:15), indicating the presence of L-Ala, L-lactic acid, *N*-Me-L-Tyr, and *N*-Me-L-Phe; for **4**: 6.7 and 12.2 min (95:5), 16.1 and 38.2 min (85:15), indicating the presence of L-Ala, *N*-Me-L-Val, *N*-Me-L-Tyr, and (*S*)-Hiva; for **5**: 6.7 and 12.2 (95:5), and 16.1 min (85:15), indicating the presence of L-Ala, *N*-Me-L-Val, and *N*-Me-L-Tyr; and for **6**: 6.7, 12.2, and 17.2 min (95:5), indicating the presence of L-Ala, *N*-Me-L-Val, and L-Val. Hmpa could not be detected under these conditions. The stereochemistry of Hmpa in **5** and **6** was determined using a different chiral column for the HPLC analysis [CHIRALPAK MA(+) (4.6 × 50 mm), Daicel Chemical Industries, Ltd.; solvent, 2 mM $CuSO_4$–MeCN (85:15); flow rate, 1.0 mL/min; detection at 254 nm]. Authentic standards of this hydroxy acid eluted as follows (t_R, min): (2*R*,3*S*)-Hmpa (16.5), (2*R*,3*R*)-Hmpa (19.1), (2*S*,3*R*)-Hmpa (25.0), and (2*S*,3*S*)-Hmpa (30.0). The HPLC profiles of the hydrolyzates of **5** and **6** both showed a peak at 30.0 min, corresponding to (2*S*,3*S*)-Hmpa.

Acid Hydrolysis and Advanced Marfey Analysis.[6] Samples (0.2 mg) of each compound **1**–**6** were subjected to acid hydrolysis at 110 °C for 12 h. The hydrolyzates were dried, reconstituted in H_2O (100 μL), and each divided into two equal portions. To one portion (50 μL) were added 1 M $NaHCO_3$ (50 μL) and a 1% (v/v) solution of 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA). After heating at 80 °C for 3 min, the reaction mixtures were cooled, acidified with 2 N HCl (100 μL), and diluted with MeCN (250 μL). Aliquots were subjected to reversed-phase HPLC analysis [Bondclone 10 C18 (300 × 7.80 mm), 10 μm, Phenomenex; flow rate, 3.0 mL/min; PDA detection from 210 to 540 nm] using a linear gradient of MeCN in 0.1% (v/v) aqueous TFA (30–70% MeCN over 50 min). The retention times of L-FDLA derivatives of synthetic (2*R*,3*R*)-Amha and (2*S*,3*R*)-Amha were 28.2 and 29.6 min, respectively, and the retention times of L-FDLA derivatives of (2*S*,3*S*)-Amha and (2*R*,3*S*)-Amha were 23.8 and 23.1 min, respectively. The latter two values were inferred from the retention times of their enantiomers, obtained by derivatization of (2*R*,3*R*)-Amha and (2*S*,3*R*)-Amha with DL-FDLA, respectively. The retention time and UV/vis spectrum of L-FDLA-derivatized (2*R*,3*R*)-Amha (28.2 min) matched with those of one amino acid derivative in the hydrolyzates of **1**–**3**, while the retention time and UV/vis spectrum of L-FDLA-derivatized (2*S*,3*R*)-Amha (29.6 min) were identical to those of one amino acid derivative in hydrolyzates of **4**–**6**. The derivatives of the other amino acids did not interfere with the analysis, and PDA detection allowed us to distinguish easily *N*-methyl amino acid derivatives from derivatives of nonmethylated ones.[6] To further confirm the result, the second portions of the hydrolyzates of compounds **1**–**6** were derivatized as described above, but using DL-FDLA. As expected, additional peaks arising from non-methylated amino acid derivatives (based on UV/vis spectra) were detected at 23.8 for compounds **1**–**3** and at 23.1 for compounds **4**–**6**, corresponding to retention times of L-FDLA derivatives of (2*S*,3*S*)-Amha and (2*R*,3*S*)-Amha, respectively, being the enantiomers of the Amha isomers in these compounds.

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