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Pompanopeptins A and B, new cyclic peptides from the marine cyanobacterium *Lyngbya confervoides*

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Abstract

A new 3-amino-6-hydroxy-2-piperidone (Ahp) containing peptolide, pompanopeptin A (**1**), and a novel *N*-methyl-2-amino-6-(4′-hydroxyphenyl)hexanoic acid (*N*-Me-Ahpha) containing cyclic pentapeptide connected with a sixth amino acid residue via a rare ureido linkage, pompanopeptin B (**2**), were isolated from the marine cyanobacterium *Lyngbya confervoides* collected from the southeastern coast of Florida. Their planar structures were determined by a combination of NMR spectroscopic analysis and mass spectrometry. The absolute configurations were established using advanced Marfey's method and chiral HPLC analysis of the chemical degradation products. Compound **1** selectively inhibited trypsin over elastase and chymotrypsin, with an IC_{50} value of 2.4 μM; selectivity is conferred by an arginine residue in the cyclic core.

Keywords: Natural product; Cyanobacteria; *Lyngbya confervoides*; Trypsin inhibitor

1. Introduction

Marine cyanobacteria have emerged as an exceptionally rich source of structurally distinct and biologically active metabolites, which include cyclic peptides and depsipeptides.[1] The present study is part of our exploration to discover novel and biologically active metabolites from marine cyanobacteria from coastal Florida. Recent investigation of the marine cyanobacterium *Lyngbya confervoides* collected in Florida led to the isolation of new analogues of dolastatin 13 with elastase and chymotrypsin inhibitory activities, and lyngbyastatins 4–6.[2,3] Re-investigation of a large collection of this cyanobacterium has now resulted in the isolation of a new trypsin inhibitor (**1**), which has structural resemblance to freshwater cyanobacterial isolates, micropeptins from *Microcystis* sp.,[4,5] all of which contain a 3-amino-6-hydroxy-2-piperidone (Ahp) moiety. The same extract yielded another new cyclic peptide (**2**) with a rare ureido unit, which has structural resemblance to several marine derived compounds, mozamide,[6] brunsvicamides,[7] and metabolites from freshwater cyanobacteria, anabaenopeptins.[8] Compound **2** is structurally distinct from other natural products due to the occurrence of a novel *N*-methyl-2-amino-6-(4′-hydroxyphenyl)hexanoic acid (*N*-Me-Ahpha) unit. Here we report the isolation and structure elucidation of **1** and **2**, which we named as pompanopeptins A (**1**) and B (**2**) due to the proximity of the collection sites to Pompano Beach, FL. Compound **1** was evaluated for its biological activity against several serine endopeptidases and demonstrated selective in vitro trypsin inhibition when compared to elastase and chymotrypsin inhibitory activities. Trypsin is a proteolytic enzyme that catalyzes the cleavage of peptide bonds on the carboxyl side of either arginine or lysine. The imbalance of trypsin activation within the pancreatic acinar cells presumably leads to the development of acute pancreatitis.[9] Additionally, an increase in trypsin activity has been associated with conditions like inflammation and angiogenesis.[10]

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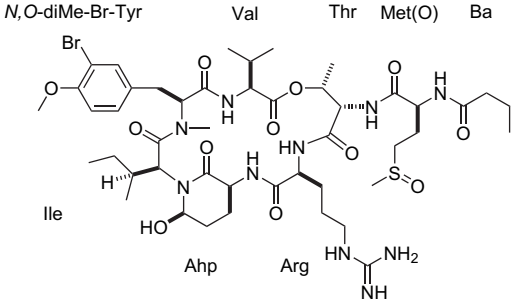

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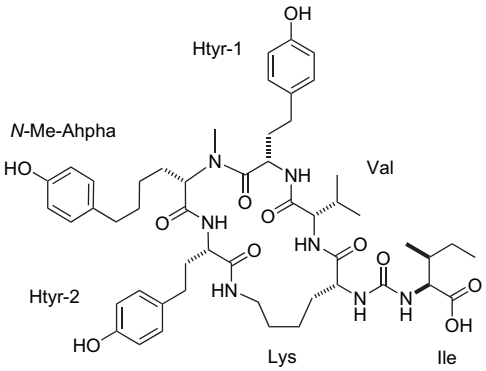

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2. Results and discussion

L. confervoides was collected during the summer of 2005 near Pompano Beach and Ft. Lauderdale, Florida. The freeze-dried sample was lyophilized and extracted with EtOAc–MeOH (1:1). The combined fractions were concentrated in vacuo, defatted with hexanes and partitioned between *n*-BuOH and H_2O. Fractionation of the *n*-BuOH soluble material using HP-20 resin followed by several purification steps by using reversed-phase HPLC resulted in the isolation of pompanopeptins A (**1**) and B (**2**).

Pompanopeptin A (**1**) was isolated as a colorless, amorphous solid with a molecular formula of $C_{46}H_{73}BrN_{10}O_{12}S$ deduced on the basis of HRAPCI/ESIMS [*m/z* $(M+H)^+$ 1069.4400; calcd for $C_{46}H_{74}{}^{79}BrN_{10}O_{12}S$, 1069.4392]. The isotopic distribution of $[M+H]^+$ ions in the ratio of 1:1 at *m/z* 1069 and 1071 revealed the presence of one bromine atom in the molecule. The structure of **1** was deduced through ^1H and ^{13}C NMR in combination with HSQC, HMBC, COSY, TOCSY, and ROESY experiments. NMR analysis in DMSO-d_6 established the presence of valine (Val), *N*,*N*-disubstituted-isoleucine (Ile), threonine (Thr), arginine (Arg), butanoic acid (Ba), and three modified amino acids (Table 1). In agreement with literature values, one unusual spin system was attributed to an Ahp unit.[2,3] The second modified unit consisted of a methine (δ_H 4.57) and two methylenes (δ_H 2.03/1.92 and δ_H 2.79/2.69) based on COSY and TOCSY analysis, and the corresponding methylene carbons both showed HMBC correlations to a methyl singlet (Table 1). This data is in agreement with a methionine sulfoxide [Met(O)] residue as in somamide A[11] and symplostatin 2.[12] The doubling of signals in the ratio of 1:1 for the Met(O) unit in **1** suggested the occurrence of a mixture of *R* and *S* sulfoxide diastereomers as reported earlier[11,12] (Table 1). The sulfoxide is presumably an artifact formed by oxidation of the methionine-containing natural product during isolation.[12] The last unit was an aromatic amino acid; the ^1H NMR spectrum (Table 1) showed signals characteristic of a 1,3,4-trisubstituted aryl ring system [δ_H 7.40, 1H, d (1.5); δ_H 7.01, 1H, d (8.5); δ_H 7.18, 1H, dd (8.5, 1.5)]. ^{13}C NMR and HMBC data revealed that a Br substituent

Table 1
NMR spectral data for pompanopeptin A (**1**) in DMSO-d_6 (500 MHz)

Unit	C/H no.	δ_H (*J* in Hz)	δ_C, mult[a]	COSY	HMBC[b]	ROESY[c]
Val	1		172.3, qC			
	2	4.70, dd (9.0, 5.5)	56.1, CH	H-3, NH	1, 4, 5, 1 (*N*,*O*-diMe-Br-Tyr)	H-3, NH
	3	2.05, m	30.7, CH_2	H-2, H_3-4, H_3-5	4, 5	H-2, H_3-4, H_3-5
	4	0.86, d (7.0)	19.3, CH_3	H-3	2, 3, 5	H-3, H_3-5
	5	0.74, d (7.0)	17.5, CH_3	H-3	2, 3, 4	H-3, H_3-4, H-3 (Thr)
	NH	7.63, d (9.0)		H-2		H-2, H-2 (*N*,*O*-diMe-Br-Tyr)
N,*O*-diMe-Br-Tyr	1		169.3, qC			
	2	5.05, dd (11.5, 2.5)	60.4, CH	H-3a, H-3b		H-3a, H-3b, *N*-Me, NH (Val), H-2 (Ile)
	3a	3.21, dd (−13.5, 11.5)	32.9, CH_2	H-2, H-3b		H-2, H-3b, H-5, H-9
	3b	2.78, dd (−13.5, 2.5)		H-2, H-3[a]		H-2, H-3a, H-5, H-9
	4		131.2, qC			
	5	7.40, d (1.5)	133.5, CH		4, 6, 7, 9	H-3a, H-3b, H-9
	6		111.1, qC			
	7		154.5, qC			
	8	7.01, d (8.5)	112.9, CH	H-9	4, 6, 7, 9	7-OMe
	9	7.18, dd (8.5, 1.5)	130.1, CH	H-8	5, 8	H-3a, H-3b, H-5
	N-Me	2.73, s	30.2, CH_3		1, 2, 1 (Ile)	H-2, 6-OH (Ahp)
	7-OMe	3.76, s	56.3, CH_3		7	H-8
Ile	1		169.6, qC			

(*continued on next page*)

Table 1 (*continued*)

Unit	C/H no.	δ_H (*J* in Hz)	δ_C, mult[a]	COSY	HMBC[b]	ROESY[c]
	2	4.37, d (10.5)	54.1, CH	H-3	1, 3, 6, 6 (Ahp)	H-3, H-2 (*N*,*O*-diMe-Br-Tyr), H-6 (Ahp)
	3	1.79, m	32.9, CH	H-2, H-4a, H-4b, H_3-6		H-2, H-6
	4a	1.09, m	23.7, CH_2	H-3, H-4b/H_3-5		H-6
	4b	0.63, m		H-3, H-4a, H_3-5	3, 4	H-6
	5	0.61, t (6.6)	10.2, CH_3	H-4a, H-4b	3, 4	H-6
	6	−0.16, d (6.0)	13.6, CH_3	H-3	2, 3, 4	H-3, H-4a, H-4b, H_3-5
Ahp	2		169.1, qC			
	3	4.44, m	48.9, CH	H-4a, H-4b, NH		H-4b, H-5b, NH
	4a	2.57, m	21.7, CH_2	H-3, H-4b, H-5a/5b		H-4b, 6-OH, NH
	4b	1.74, m		H-3, H-4a, H-5a/5b		H-3, H-4a
	5a	1.76, m	29.6, CH_2	H-4a, H-4b, H-6, 6-OH		H-5b, H-6, 6-OH
	5b	1.73, m		H-4a, H-4b, H-6, 6-OH		H-3, H-5a, H-6, 6-OH
	6	4.92, br s	73.9, CH	H-5a/5b, 6-OH		H-4a, H-5a/H-5b, 6-OH, H-2 (Ile)
	6-OH	6.13, br s		H-5a/5b, H-6		H-5a/H-5b, H-6, *N*-Me (*N*,*O*-diMe-Br-Tyr)
	NH	7.35, d (8.5)		H-3	2, 1 (Arg)	H-3, H-4a, 2-NH (Arg)
Arg	1		170.0, qC			
	2	4.29, br	51.7, CH	H-3a, H-3b, 2-NH		H-3b/H_2-4, 2-NH
	3a	2.01, m	26.3, CH_2	H-2, H-3b, H_2-4		H_2-4
	3b	1.42, m		H-2, H-3a, H_2-4		H-2, H_2-4
	4	1.44, m (2H)	25.0, CH_2	H-3a, H-3b, H_2-5		H-2, H-3a/3b, H_2-5
	5	3.07, m (2H)	40.1, CH_2	H_2-4, 5-NH		H_2-4, 5-NH
	2-NH	8.50, d (9.5)		H-2		H-2, NH (Ahp), H-2 (Thr)
	5-NH	7.49, t (5.5)		H_2-5		H_2-5, 6-NH_2
	6-NH_2	7.66, br[d]				5-NH
	6-NH	[e]				
	C=N		156.6, qC			
Thr	1		169.2, qC			
	2	4.59, br	55.2, CH	NH	1, 3, 1 (Met (O))	H-3, NH, 2-NH (Arg)
	3	5.52, br q (6.0)	71.8, CH	H_3-4	4, 1 (Val)	H-2, H_3-4, H_3-5 (Val)
	4	1.22, d (6.0)	17.7, CH_3	H-3	2, 3	H-3
	NH	8.06, d (9.0)		H-2	1 (Met (O))	H-2
Met(O)	1		171.8, qC			
	2	4.57, br	51.2, CH	H-3a, H-3b, NH		H-3a/3b, NH
	3a	2.03, m	25.0, CH_2	H-2, H-3b, H-4a, H-4b		H-2, H-4a/4b
	3b	1.92, m		H-2, H-3a, H-4a, H-4b		H-2, H-4a/4b
	4a	2.79, m	48.9, 48.8, [f] CH_2	H-3a, H-3b, H-4b		H-3a/3b, *S*-Me
	4b	2.69, m		H-3a, H-3b, H-4a		H-3a/3b, *S*-Me
	S-Me	2.55, 2.54,[f] s	37.9, 38.0,[f] CH_3		3, 4	H-4a/4b
	NH	8.09, d (7.5)		H-2	1 (Ba)	H-2, H_2-2 (Ba)
Ba	1		172.3. qC			
	2	2.13, t (7.0) (2H)	37.0, CH_2	H_2-3	1, 3, 4	H_2-3, H_3-4, NH (Met (O))
	3	1.52, hex (7.0) (2H)	18.6, CH_2	H_2-2, H_3-4	1, 2, 4	
	4	0.86, t (7.0)	13.7, CH_3	H_2-3	1, 2, 3	

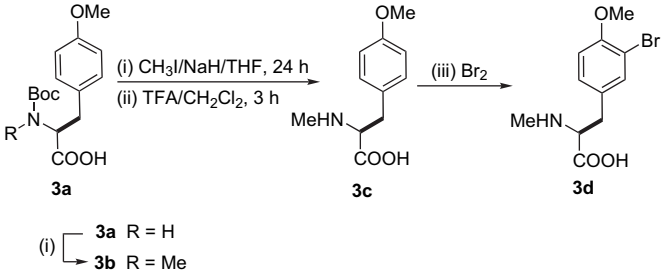
[a] Deduced from ^{13}C NMR (125 MHz), HSQC, and HMBC.
[b] Protons showing long-range correlation with indicated carbon.
[c] Refers to protons within the same unit unless indicated otherwise.
[d] Tentative assignment. No correlation observed in 2D NMR spectra.
[e] Not observed.
[f] Indicates diastereomers at chiral *S**.

was located in the *meta* position ($\delta_{C\text{-}6}$ 111.1) and that the *para* carbon was oxygenated ($\delta_{C\text{-}7}$ 154.5). O-Methylation of the aromatic ring accounted for the low-field chemical shift of one methyl singlet (δ_H 3.76) and the observed HMBC to the carbon resonating at 154.5. Further COSY and HMBC analysis verified that the moiety is tyrosine-derived and that the nitrogen was also methylated (Table 1). These chemical shifts were consistent with a *N*,*O*-diMe-3′-Br-Tyr unit data reported for several largamides having a similar moiety.[13]

A strong ROESY cross-peak was observed between α-protons of *N*,*O*-diMe-Br-tyrosine and isoleucine, suggesting a *cis*-amide bond. The ^1H NMR spectrum of **1** also displayed a significantly shielded methyl doublet (H_3-6, Ile, δ_H −0.16) thereby revealing the spatial proximity of Ile to the shielding region of the aromatic portion of the *N*,*O*-diMe-Br-Tyr unit. The HMBC analysis supported by ROESY correlations enabled us to sequence two partial units as Val-(*N*,*O*-diMe-Br-Tyr)-Ile-Ahp-Arg and Thr-Met(O)-Ba. The connectivity between Thr

and Arg was ascertained based on ROESY cross-peaks between the α-proton of Thr and the NH of Arg (δ_H 4.59, Thr H-2 and δ_H 8.50, Arg NH), characteristic for a *trans*-amide linkage. The ester linkage between Thr and Val was inferred by the HMBC correlation from the deshielded β-proton of Thr (δ_H 5.52) to the quaternary carbonyl (δ_C 172.3) of Val, thus concluding that **1** is a cyclic depsipeptide. The proposed depsipeptide structure shown for **1** accounts for the molecular formula and is also consistent with the IR spectrum, exhibiting bands at 1736 and 1656 cm^{-1} rationalized by carbonyl stretch vibrations of ester and amide functionalities, respectively. The IR spectrum also supports the presence of a methionine sulfoxide moiety by displaying bands at 1056 and 1022 cm^{-1}, a region characteristic for sulfoxide stretch vibrations.

The absolute configuration of C-3 (Ahp) in **1** was deduced as *S* by chiral HPLC analysis, after detecting a peak for the liberated L-glutamic acid (Glu) upon oxidation with CrO_3 oxidation[2,3] and subsequent acid hydrolysis. Further analysis of the ROESY correlations and $^1H-^1H$ coupling constant data (Table 1) and comparison with related Ahp-containing metabolites suggested that the relative configuration of the two chiral centers is the same and thus the absolute configuration of the Ahp unit is 3*S*,6*R*.[2,3] Absolute configurations of all other amino acid units in **1** except *N*,*O*-diMe-Br-Tyr were established as L by chiral HPLC analysis of the acid hydrolyzate. To deduce the configuration of the *N*,*O*-diMe-Br-Tyr residue, an authentic standard of the L-isomer was synthesized from Boc-OMe-L-Tyr followed by a series of reaction steps involving N-methylation,[14] N-deprotection, and bromination (Scheme 1).[15] The amino acid standard and the hydrolyzate of **1** were subsequently derivatized with L-FDLA and DL-FDLA for advanced Marfey's analysis with the aid of LC–MS, revealing L-configuration of this residue as well.[16,17]



Scheme 1. Synthesis of standard *N*,*O*-diMe-3′-Br-L-Tyr (**3d**).

Pompanopeptin B (**2**) was isolated as a colorless, amorphous solid with a molecular formula of $C_{51}H_{71}N_7O_{11}$ deduced on the basis of HRAPCI/ESIMS [*m*/*z* $(M+H)^+$ 958.5319; calcd for $C_{51}H_{72}N_7O_{11}$, 958.5290]. ^1H NMR analysis of **2** in DMF-d_7 suggested the presence of three *para*-substituted phenolic residues, which together with six deshielded NH protons and one *N*-Me singlet were indicative of a peptide framework with three aromatic amino acid units. A detailed analysis of the structure deduced through 1D (^1H, ^{13}C NMR, and APT) and 2D (HMQC, HMBC, COSY, TOCSY, and ROESY) experiments revealed the presence of six amino acid residues: isoleucine (Ile), valine (Val), lysine (Lys), two homotyrosine (Htyr) units, and a novel *N*-Me-2-amino-6-(4′-hydroxyphenyl)hexanoic acid (*N*-Me-Ahpha). The occurrence of this unusual amino acid *N*-Me-Ahpha was hitherto unknown, whereas its demethylated form has been encountered once before, in largamide C.[13] HMBC analysis established the linear sequence of five amino acids as (*N*-Me-Ahpha)-Htyr^2-Lys-Val-Htyr^1, all of which were linked by *trans*-amide bonds based on ROESY correlations between the signals for α-protons and NH protons in adjacent residues. ROESY cross-peaks between the signals for H-2 of *N*-Me-Ahpha and H-2 of Htyr-1 established the *cis*-amide linkage between these two residues and consequently the cyclic pentapeptide core in **2** as cyclo[Val-Htyr^1-(*N*-Me-Ahpha)-Htyr^2-Lys]. The remaining NMR data were in accordance with the literature reports for previously known compounds having similar cyclic cores connected to the side chain through a rare ureido carbonyl linkage.[6–8] In compound **2** an HMBC correlation observed from both the protons at δ_H 4.18 (Lys H-2) and δ_H 4.28 (Ile H-2) to an unassigned sp^2 carbon signal (δ_C 158.6) suggested that NH (Ile) was joined to the 2-NH (Lys) through an ureido moiety. The connection between Lys and Ile was further ascertained by ROESY correlations between 2-NH (Lys, δ_H 6.79) and H-2 (Ile, δ_H 4.28), H-2 (Lys, δ_H 4.18) and NH (Ile, δ_H 6.30), and 2-NH (Lys, δ_H 6.79) and NH (Ile, δ_H 6.30). To account for the molecular formula requirements and the broad singlet in the ^1H NMR spectrum of **2** at δ_H 12.8, the side chain terminated with an Ile carboxylic acid functionality. The NMR assignments reported for the related anabaenopeptins[8] are consistent with the NMR assignments given in Table 2 for **2**.

Chiral HPLC analysis of the acid hydrolyzate of **2** revealed that both Htyr units and Val have L configurations. Additionally, according to chiral HPLC analysis, ozonolysis of **2** with oxidative workup followed by acid hydrolysis generated *N*-Me-(*S*)-2-aminopimelic acid and L-Glu in the hydrolyzate, confirming the presence of *N*-Me-L-Ahpha and L-Htyr, respectively, in **2**. *N*-Me-2-aminopimelic acid standards for the analysis were synthesized by selective N-methylation[14] as discussed in Section 3.5.1 using Boc-(±)-2-aminopimelic acid as starting material (Scheme 2). The configuration of Lys remained uncertain due to its early elution profile during chiral HPLC; hence the hydrolyzate was further subjected to Marfey's analysis, thereby confirming the presence of a D-isomer. However, as reported for related compounds,[6,7,18] the terminal amino acid (Ile) could not be detected under normal acid hydrolysis conditions. Hydrazinolysis[19] of **2**, for the characterization of carboxyl-terminal amino acids, liberated solely Ile residue, which was identified as L-Ile by chiral HPLC analysis.

The cyclic core of pompanopeptin A (**1**) bears similarities to micropeptins 478-A and B[4] where Ile was replaced by Val and *N*-Me-3′-Cl-Tyr by *N*,*O*-diMe-3′-Br-Tyr. Other closely related cyclodepsipeptides, such as micropeptins 90,[20] SD999,[5] and E1992,[21] containing an Arg unit in the cyclic core have been reported to inhibit trypsin with IC_{50} values of 2.0–4.2 μg/mL. Based on the co-crystal structure of A90720A[22] with trypsin, the Arg residue confers selectivity for trypsin over other proteases. Expectedly, pompanopeptin

Table 2
NMR spectral data for pompanopeptin B (**2**) in DMF-d_7 (500 MHz)

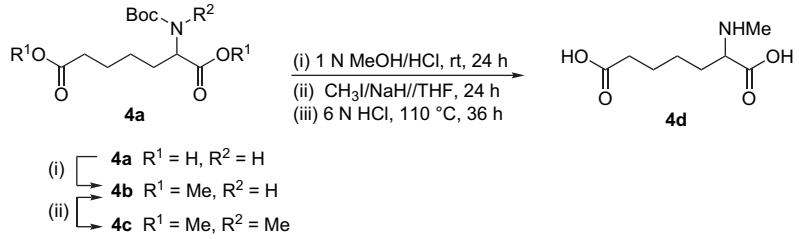
Unit	C/H no.	δ_H (J in Hz)	δ_C, mult[a]	COSY	HMBC[b]
Val	1		174.2, qC		
	2	4.03, dd (8.0, 6.5)	59.8, CH	H-3, NH	1, 3, 4, 5
	3	1.94, m	30.9, CH	H-2, H_3-4, H_3-5	1, 2, 4, 5
	4	1.02, d (6.8)	19.82, CH_3	H-3	2, 3, 5
	5	0.94, d (6.8)	19.79, CH_3	H-3	2, 3, 4
	NH	7.22, d (6.5)		H-2	2, 3, 1 (Lys)
Htyr-1	1		173.3, qC		
	2	4.90, ddd (10, 5.1, 3.2)	50.0, CH	H-3a, H-3b, NH	3
	3a	2.05, m	34.6, CH_2	H-2, H-3b, H-4a, H-4b	4
	3b	1.83, m		H-2, H-3a, H-4a, H-4b	
	4a	2.82, m	32.0, CH_2	H-3a, H-3b, H-4b	
	4b	2.59, m		H-3a, H-3b, H-4a	
	5		132.3, qC		
	6/10	7.05, d (8.5)	130.2, CH	H-7/9	4
	7/9	6.79, d (8.5)	116.1, CH	H-6/10	
	8		156.9, qC		
	OH	9.37, s			7/9
	NH	9.03, d (5.1)		H-2	2, 3, 1 (Val)
N-Me-Ahpha	1		170.9, qC		
	2	4.78, dd (8.5, 5.5)	60.9, CH	H-3a, H-3b	1, 3, 4, *N*-Me, 1 (Htyr-1)
	3a	1.91, m	28.7, CH_2	H-2, H-3b, H-4a, H-4b	2, 4
	3b	1.73, m		H-2, H-3a, H-4a, H-4b	2, 4
	4a	1.62, m	26.8, CH_2	H-3a, H-3b, H-4b	2, 3, 5, 6
	4b	1.26, m		H-3a, H-3b, H-4a	
	5	1.61, m (2H)	32.6, CH_2	H-4b, H_2-6	4
	6	2.50, m (2H)	35.3, CH_2	H_2-5	4
	7		133.4, qC		
	8/12	7.01, d (8.5)	130.0, CH	H-9/11	9/11
	9/11	6.755, d (8.5)	115.9, CH	H-8/12	
	10		156.8, qC		
	OH	9.33, s			
	N-Me	2.72, s	29.0, CH_3		2, 1 (Htyr-1)
Htyr-2	1		172.5, qC		
	2	4.39, ddd (11.5, 8.9, 3.0)	54.1, CH	H-3a, H-3b, NH	1
	3a	2.18, m	35.4, CH_2	H-2, H-3b, H-4a, H-4b	2, 3
	3b	1.91, m		H-2, H-3a, H-4a, H-4b	2, 3
	4a	2.45, m	32.5, CH_2	H-3a, H-3b, H-4b	2, 3, 5, 6/9
	4b	2.35, m		H-3a, H-3b, H-4a	
	5		132.9, qC		
	6/10	6.97, d (8.5)	130.2, CH	H-7/9	4
	7/9	6.751, d (8.5)	115.9, CH	H-6/10	
	8		156.8, qC		
	OH	9.31, s			7/9
	NH	8.94, d (8.9)		H-2	2, 3, 1 (*N*-Me-Ahpha)
Lys	1		173.9, qC		
	2	4.18, ddd (8.3, 6.0, 4.5)	55.9, CH	H-3a, H-3b, 2-NH	1, 3, 4, 1 (ureido)
	3a	1.76, m	32.9, CH_2	H-2, H-3b, H-4a	
	3b	1.68, m		H-2, H-3a, H-4a	2
	4a	1.23, m	21.5, CH_2	H-3a, H-3b, H-4b/H_2-5	6
	4b	1.39, m		H-3a, H-3b, H-4a	
	5a	1.41, m	29.5, CH_2	H-4a, H-4b/5b, H-6a, H-6b	
	5b	1.36, m		H-4a, H-4b, H-6a, H-6b	
	6a	3.66, m	39.1, CH_2	H_2-5, H-6b, 6-NH	
	6b	2.72, m		H_2-5, H-6a, 6-NH	
	2-NH	6.79, d (8.3)		H-2	1, 2, 1 (ureido)
	6-NH	7.46, dd (8.2, 2.1)		H-6a, H-6b	6, 1 (Htyr-2)
Ureido	C=O		158.6, qC		
Ile	1		174.7, qC		
	2	4.28, dd (9.0, 5.0)	58.1, CH	H-3, NH	1, 3, 4, 6, 1 (ureido)
	3	1.83, m	38.5, CH	H-2, H-4a, H-4b, H_3-6	1, 2, 5, 6
	4a	1.46, m	25.8, CH_2	H-3, H-4b, H_3-5	2, 3, 5, 6
	4b	1.18, m		H-3, H-4a, H_3-5	2, 3, 5, 6
	5	0.88, t (7.4)	12.1, CH_3	H-4a, H-4b	3, 4, 6

(*continued on next page*)

Table 2 (*continued*)

Unit	C/H no.	δ_H (*J* in Hz)	δ_C, mult[a]	COSY	HMBC[b]
	6	0.91, d (6.8)	16.3, CH_3	H-3	2
	NH	6.30, d (9.0)		H-2	2, 1 (ureido)
	COOH	12.8, br s			

[a] Deduced from ^{13}C NMR (150 MHz), HMQC, and HMBC.
[b] Protons showing long-range correlation with indicated carbon.



Scheme 2. Synthesis of *N*-Me-(±)-2-aminopimelic acid (**4d**).

A (**1**) also exhibited a comparable trypsin inhibitory activity with an IC_{50} value of 2.4±0.4 μg/mL. Pompanopeptin B (**2**) is structurally related to carboxypeptidase-A inhibitors anabaenopeptins I and J isolated from the cyanobacterium *Aphanizomenon flos-aquae*[8] in which L-Leu/L-Phe and *N*-Me-L-Ala residues were replaced by L-Htyr and *N*-Me-L-Ahpha, respectively. Further biological evaluation will be carried out in due course.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. UV spectra were recorded on a SpectraMax M5 (Molecular Devices). IR spectra were obtained on a Bruker Vector 22 instrument. ^1H, ^{13}C, and 2D NMR spectra for **1** were recorded in DMSO-d_6 (δ_H 2.49, δ_C 39.5 for residual solvent signals) on a Varian Unity Inova 500 spectrometer, operating at 500 MHz (^1H) or 125 MHz (^{13}C). ^1H NMR and 2D NMR data for **2** were acquired on a Bruker Avance 500 spectrometer and ^{13}C NMR and APT spectra acquired on a Bruker Avance 600 MHz spectrometer operating at 150 MHz, using DMF-d_7 as solvent (δ_H 8.02, δ_C 162.9 for residual solvent signals). ^1H and ^{13}C NMR spectra for synthetic compounds were recorded on a Varian Mercury 400 MHz spectrometer, operating at 400 and 100 MHz, respectively, using D_2O (δ_H 4.80 for residual solvent signal, δ_C 67.0 for spiked in 1,4-dioxane) or $CDCl_3$ (δ_H 7.26, δ_C 77.0 for residual solvent signals). HMQC/HSQC experiments were optimized for $^1J_{CH}$=145 Hz, and HMBC experiments were optimized for $^nJ_{CH}$=7 Hz. HRMS data were obtained using an Agilent LC–TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector. HPLC–MS analysis was performed using Agilent 1100 HPLC system equipped with Thermo Finnigan LCQ Advantage ion trap mass spectrometer via an ESI interface. Boc-(±)-2-aminopimelic acid standard was procured from AnaSpec Inc. (San Jose, CA).

3.2. Biological material

Samples of *L. confervoides* were recollected during a persistent cyanobacterial bloom, which occurred near Fort Lauderdale, Florida, USA (26°01.1414′N, 80°05.9973′W; 26°15.134′N, 80°03.908′W)[23] at a depth of 15 m in August 2005. A voucher specimen is retained at the Smithsonian Marine Station.

3.3. Extraction and isolation

The freeze-dried organism was extracted with EtOAc–MeOH (1:1) to afford a crude extract, which was then defatted with hexanes and partitioned between *n*-BuOH and H_2O. The combined *n*-BuOH extract (6.3 g) was applied on a diaion HP-20 resin and subsequently fractionated with water and increasing concentrations of MeOH, and then with MeCN. The fraction eluting with 75% aqueous MeOH to 100% MeOH (864 mg) was subjected to reversed-phase preparative HPLC (Phenomenex LUNA-C18 10 μ, 100×21.20 mm, 10.0 mL/min; UV detection at 220 and 240 nm) using a MeOH–H_2O linear gradient (30–100% over 40 min and then 100% MeOH for 10 min). A fraction eluting between t_R 30 and 32 min was collected and subjected to repeated semipreparative reversed-phase HPLC (YMC-Pack ODS-AQ, 250×10 mm, 2.0 mL/min; UV detection at 220 and 240 nm) using MeOH in 0.05% aqueous TFA (60–90% in 25 min, 90–100% in 10 min), to give **1** (t_R 32.0 min, 5.3 mg). A fraction that eluted at t_R 16.0–23.0 min from the preparative HPLC was separated by repeated semipreparative HPLC using the same gradient as described above to yield **2** (t_R 21.0 min, 1.8 mg).

3.3.1. Pompanopeptin A (*1*)

Colorless, amorphous solid; $[\alpha]_D^{20}$ −42.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.06), 280 (sh) (3.11) nm; IR (film) ν_{max} 3357, 2966, 1736, 1656, 1535, 1203, 1136, 1056, 1022 cm^{-1}; for ^1H NMR, ^{13}C NMR, COSY, HMBC,

and ROESY data, see Table 1; HRAPCI/ESIMS *m/z* $[M+H]^+$ 1069.4400, 1071.4400, ion cluster (1:1) (calcd for $C_{46}H_{74}{}^{79}BrN_{10}O_{12}S$, 1069.4392; $C_{46}H_{74}{}^{81}BrN_{10}O_{12}S$, 1071.4371).

3.3.2. Pompanopeptin B (**2**)

Colorless, amorphous solid; $[\alpha]_D^{20}$ −24.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.07), 280 (sh) (3.37) nm; IR (film) ν_{max} 3331, 2935, 1720–1630, 1549, 1515, 1451, 1239 cm^{-1}; for ^1H NMR, ^{13}C NMR, COSY, HMBC, and ROESY data, see Table 2; HRAPCI/ESIMS *m/z* $[M+H]^+$ 958.5319 (calcd for $C_{51}H_{72}N_7O_{11}$, 958.5290).

3.4. Determination of absolute configuration of amino acids in **1**

3.4.1. Synthesis of standard *N,O*-diMe-3′-Br-L-Tyr (**3d**)

Boc-OMe-L-Tyr (**3a**) (300 mg, 1.02 mmol, 1 equiv) was dissolved in THF (3.06 mL) and the mixture was cooled to 0 °C. To this solution were added CH_3I (8.13 mmol, 8.0 equiv) and dry NaH (73.1 mg, 3.05 mmol, 3 equiv). The solution was stirred at room temperature under N_2 for 24 h. The reaction was quenched by addition of H_2O, acidified, and partitioned with EtOAc and H_2O. The organic layer was neutralized with saturated aqueous $NaHCO_3$ and sequentially washed with H_2O, 5% $Na_2S_2O_3$, and H_2O, and then dried, filtered, and concentrated to afford Boc-*N,O*-diMe-L-Tyr (**3b**)[14] as a pale yellow oil (280 mg, 88% yield) {HRAPCI/ESIMS *m/z* $[M+Na]^+$ 332.1470 (calcd for $C_{16}H_{23}NO_5Na$, 332.1474)}.

Boc deprotection was achieved by treating **3b** with TFA in CH_2Cl_2 and stirring for 3 h at room temperature. The reaction product **3c** was then applied on a Dowex 50WX4-50 ion exchange resin and eluted with 4 N NH_4OH and dried. Compound **3c** was obtained as a colorless, amorphous solid (190 mg, 100% yield); $[\alpha]_D^{20}$ +7.5 (*c* 0.30, H_2O); IR (film) ν_{max} 2961, 2873, 2360, 2341, 1735, 1671, 1613, 1514, 1466, 1442, 1250, 1181, 1143, 1031, 835 cm^{-1}; ^1H NMR (400 MHz, D_2O/pH=2) δ 7.17 (d, 2H, *J*=8.8 Hz), 6.92 (d, 2H, *J*=8.8 Hz), 4.17 (t, 1H, *J*=6.2 Hz), 3.75 (s, 3H), 3.24 (dd, 1H, *J*=14.9, 6.2 Hz), 3.17 (dd, 1H, *J*=14.9, 6.2 Hz), 2.68 (s, 3H); ^{13}C NMR (100 MHz, D_2O/pH=2) δ 171.1, 159.0, 131.1, 126.2, 115.0, 62.6, 55.8, 34.4, 32.3; HRAPCI/ESIMS *m/z* $[M+H]^+$ 210.1128 (calcd for $C_{11}H_{16}NO_3$, 210.1130).

Compound **3c** was then subjected to bromination.[15] Briefly, Br_2 (6.5 μL) was added dropwise to an ice-cooled solution of **3c** (25 mg, 0.12 mmol) in 98% formic acid (150 μL) with vigorous stirring. After stirring for 6 h, the colorless paste was dissolved in 3 N HCl (150 μL), boiled for 1 h, and concentrated to dryness in vacuo. The residue was dissolved in boiling water, filtered, and neutralized (pH=7) with 6 N NH_4OH to obtain **3d** as a colorless amorphous powder (27 mg, 75% yield). $[\alpha]_D^{20}$ −10.4 (*c* 0.08, H_2O); IR (film) ν_{max} 3133, 3038, 1736, 1604, 1499, 1403, 1282, 1258, 1150, 1054, 1018 cm^{-1}; ^1H NMR (400 MHz, D_2O/pH=2) δ 7.47 (d, 1H, *J*=2.0 Hz), 7.22 (dd, 1H, *J*=8.4, 2.0 Hz), 7.03 (d, 1H, *J*=8.4 Hz), 4.11 (t, 1H, *J*=6.0 Hz), 3.85 (s, 3H), 3.23 (dd, 1H, *J*=14.6, 6.0 Hz), 3.16 (dd, 1H, *J*=14.6, 6.0 Hz), 2.70 (s, 3H); ^{13}C NMR (100 MHz, D_2O/pH=2) δ 171.4, 155.3, 134.2, 130.4, 128.0, 113.5, 111.4, 62.9, 56.7, 34.2, 32.3; APCI/ESIMS *m/z* 288/290 (1:1, $[M+H]^+$ ion cluster), HRAPCI/ESIMS *m/z* $[M+H]^+$ 288.0232 (calcd for $C_{11}H_{15}{}^{79}BrNO_3$, 288.0235).

3.4.2. Acid hydrolysis and chiral HPLC analysis of **1**

A sample of compound **1** (0.2 mg) was dissolved in 6 N HCl (0.6 mL) and heated at 110 °C for 24 h. The hydrolyzate was concentrated to dryness, re-suspended in H_2O (100 μL), filtered, and subjected to chiral HPLC analysis (Phenomenex Chirex 3126 *N,S*-dioctyl-(D)-penicillamine, 250×4.60 mm, 5 μm; solvent, 2 mM $CuSO_4$ or 2 mM $CuSO_4$–MeCN (95:5); flow rate, 1.0 mL/min; UV detection 254 nm). The absolute configurations of the amino acid units in **1** (t_R, min) were established as L-Arg (7.5), L-Met(O) (13.5, 14.5) (solvent 2 mM $CuSO_4$); L-Thr (7.8), L-Val (19.2), L-Ile (43.5) (solvent 95:5) by comparison of the retention times t_R (min) with those of standard amino acids. The retention times t_R (min) for the other standard amino acid isomers were 11.4 (D-Arg), 16.5, 18.5 (D-Met(O)) (solvent 2 mM $CuSO_4$); 8.4 (D-Thr), 9.8 (L-*allo*-Thr), 10.2 (D-*allo*-Thr), 25.8 (D-Val), 37.5 (L-*allo*-Ile), 47.0 (D-*allo*-Ile), 57.5 (D-Ile) (solvent 95:5).

3.4.3. Oxidation and chiral HPLC analysis of **1**

Compound **1** (0.5 mg) was dissolved in glacial acetic acid (0.5 mL), followed by the addition of CrO_3 (2 mg) and stirred at room temperature for 2 h. The resulting product was applied onto a C_{18} SPE cartridge eluting with H_2O, dried, and subjected to acid hydrolysis and chiral HPLC analysis using 2 mM $CuSO_4$–MeCN (95:5). A peak detected in the hydrolyzate at t_R 65.0 min corresponded to the generated L-Glu, whereas standard D-Glu eluted at t_R 70.2 min under identical condition.

3.4.4. Determination of absolute configuration of *N,O*-diMe-3′-Br-Tyr in **1** by advanced Marfey's analysis

Aliquots of hydrolyzate of **1** and *N,O*-diMe-3′-Br-L-Tyr standard (**3d**) were each treated with 1 M $NaHCO_3$ (10 μL) and a 1% solution of either L-FDLA or DL-FDLA (1-fluoro-2,4-dinitrophenyl-5-leucinamide) in acetone and heated at 80 °C for 3 min. The solutions were cooled, neutralized with 2 N HCl (5 μL), dried and dissolved in H_2O–MeCN (1:1), and analyzed by LC–MS (Phenomenex Synergi 4u Hydro-RP 80A, 2×150 mm, 4 μm; flow rate, 0.15 mL/min; UV detection 340 nm) using a step gradient of aqueous MeCN containing 0.1% HCOOH and increasing MeCN content (10–50% for 15 min, 50–70% for 55 min, then 70–95% for 60 min). LC–MS analysis of hydrolyzate of **1** of L-FDLA derivatives detected the mono adduct of *N,O*-diMe-Br-L-Tyr (t_R 28.5 min, $[M+H]^+$ *m/z* 568/570) and also the di-L-FDLA derivative (t_R 53.0 min, $[M+H]^+$ *m/z* 862/864) arising because of partial O-demethylation under acid hydrolysis conditions. In contrast, mono and/or di-FDLA derivatives of the corresponding D-isomer were not detected, which would have been eluted at t_R 29.4 and 55.1 min, respectively. These retention times were inferred from derivatization of acid-treated

(6 N HCl, 110 °C, 24 h) *N*,*O*-diMe-Br-L-Tyr with D-FDLA, yielding a chromatographic standard for the L-FDLA-derivatized D-amino acid on a nonchiral column because of the enantiomeric relationship.

3.5. Determination of absolute configuration of amino acids in **2**

3.5.1. Synthesis of N-Me-(±)-2-aminopimelic acid (**4d**)

Boc-(±)-2-aminopimelic acid (**4a**) (140 mg, 0.51 mmol) was dissolved in 1 N methanolic HCl (1.25 mL) and stirred for 24 h at room temperature. The resulting mixture after evaporation was basified (pH=8) with a saturated aqueous solution of $NaHCO_3$, extracted with EtOAc, filtered, and dried to give crude Boc-(±)-2-aminopimelic acid dimethylester (**4b**) (105 mg), a portion of which (55 mg) was methylated using CH_3I and NaH as described above for *N*,*O*-diMe-Br-Tyr to yield Boc-*N*-Me-(±)-2-aminopimelic acid dimethylester (**4c**) (26 mg, 42% overall yield). IR (film) ν_{max} 2953, 1741, 1696, 1437, 1392, 1367, 1328, 1157, 1014, 870, 774 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 1:1 conformers) δ 4.72 (dd, 0.5H, J=10.8, 4.8 Hz), 4.36 (dd, 0.5H, J=10.6, 4.8 Hz), 3.68 (s, 3H), 3.64 (s, 3H), 2.80 (s, 1.5H), 2.74 (s, 1.5H), 2.30 (t, 2H, J=6.8 Hz), 1.96–1.86 (m, 1H), 1.80–1.58 (m, 3H), 1.44 (s, 4.5H), 1.41 (s, 4.5H), 1.33–1.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 172.4 (172.2*), 156.3 (155.5*), 80.3 (80.0*), 59.0 (57.4*), 52.01 (51.98*), 51.5, 33.8, 31.1 (30.4*), 28.8 (28.5*), 28.3 (×3), 25.5, 24.4 (* indicates doubling due to conformers); HRAPCI/ESIMS *m/z* $[M+Na]^+$ 340.1742 (calcd for $C_{15}H_{27}NO_6Na$, 340.1736).

The product **4c** (10 mg) was subjected to acid hydrolysis (1 mL, 6 N HCl, 110 °C, 36 h) to yield *N*-Me-(±)-2-aminopimelic acid (**4d**) as a colorless amorphous solid after concentration to dryness (5.5 mg, 92% yield). IR (film) ν_{max} 3385, 2957, 1722, 1631, 1464, 1410, 1231, 1024, 983 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.81 (dd, 1H, J=8.0, 6.0 Hz), 2.71 (s, 3H), 2.39 (t, 2H, J=7.4 Hz), 2.00–1.88 (m, 2H), 1.68–1.58 (m, 2H), 1.48–1.30 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 182.6, 179.0, 62.3, 33.7, 32.0, 28.9, 24.2, 23.8; HRAPCI/ESIMS *m/z* $[M+H]^+$ 190.1078 (calcd for $C_8H_{16}NO_4$, 190.1079).

3.5.2. Acid hydrolysis and chiral HPLC analysis of **2**

A sample of compound **2** (0.1 mg) was dissolved in 6 N HCl (0.6 mL) and heated at 110 °C for 24 h. The hydrolyzate was treated in a similar manner and analyzed by chiral HPLC as described in Section 3.4.2. The retention time (t_R, min) of the amino acids detected in the hydrolyzate of **2** matched with those of L-Val (11.5) and L-Htyr (43.5), but not with those of D-Val (13.8) and D-Htyr (63.5) (solvent 90:10).

3.5.3. Ozonolysis of pompanopeptin B (**2**)

Compound **2** (0.2 mg) was dissolved in 3 mL CH_2Cl_2–MeOH (1:1) and subjected to ozonolysis by bubbling ozone through the solution for 30 min at room temperature. The solvent was evaporated, and the product was suspended in H_2O_2–HCOOH (1:1), stirred, and heated for 1 h at 80 °C. After evaporation of the solvent, the ozonolysis product was subjected to acid hydrolysis as described above. The resultant mixture was dissolved in H_2O and analyzed by chiral HPLC as described in Section 3.4.2. Since L-Glu (t_R 65 min) and *N*-Me-(*S*)-2-aminopimelic acid (t_R 82 min) were detected in the hydrolyzate, they must have been derived from the parent amino acids L-Htyr and *N*-Me-L-Ahpha, respectively. The corresponding enantiomers were not present in the hydrolyzate since no peak matched the retention times of standards (solvent 95:5): D-Glu (t_R 70.2 min) and *N*-Me-(*R*)-2-aminopimelic acid (t_R 106 min).[24] Additionally, L-Val (19.2) was detected but not D-Val (25.8).

3.5.4. Hydrazinolysis of pompanopeptin B (**2**)

A solution of **2** (0.1 mg) in anhydrous hydrazine (0.5 mL) was heated at 125 °C for 18 h. After cooling, the excess hydrazine was evaporated in vacuo. Analysis of the hydrazinolysis product of **2** by chiral HPLC as above (solvent mixture 95:5) detected only L-Ile (43.5). The retention times for the other Ile isomers were mentioned earlier under Section 3.4.2.

3.5.5. Absolute configuration of Lys in **2** by Marfey's analysis

A portion of the hydrolyzed ozonolysis product from **2** was derivatized with L-FDLA as described earlier. The reaction mixture was dissolved in H_2O–MeCN (1:1) and subjected to reversed-phase HPLC (Alltech Altima HP C18 HL 54, 250×4.6 mm; flow rate, 1 mL/min; PDA detection from 200–500 nm) using a linear gradient of MeCN in 0.1% aqueous TFA (30–70% MeCN over 50 min). The retention times (t_R, min) of the derivatized amino acids in the hydrolyzate of **2** matched with those of D-Lys (42.8), L-Val (23.7), and L-Glu (16.2) and not with that of L-Lys (40.7), D-Val (32.6), and D-Glu (17.6).

3.6. Protease inhibition assays

Compound **1** was tested for serine protease-inhibitory activity. Specifically, a dilution series of **1** was incubated with trypsin (porcine pancreas-Sigma T0303), α-chymotrypsin (bovine pancreas-Sigma C4129), or porcine pancreatic elastase (Elastase-high purity; EPC, EC134) in the presence of chromogenic substrates (*N*-α-benzoyl-DL-arginine 4-nitroanilide hydrochloride for trypsin, *N*-succinyl-Gly-Gly-Phe-*p*-nitroanilide for chymotrypsin, *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide for elastase). Activity was determined as described previously.[3]

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24. The elution order of (*R*)- and (*S*)-isomer of *N*-Me-2-aminopimelic acid standards in HPLC (Section 3.5.3) was confirmed after preparing *N*-Me-(*S*)-2-aminoadipic acid and *N*-Me-(*R*)-2-aminoadipic acid separately followed by chiral HPLC analysis. (*S*)-Isomers eluted first each time under our conditions.