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The Wewakpeptins, Cyclic Depsipeptides from a Papua New Guinea Collection of the Marine Cyanobacterium *Lyngbya semiplena*

Bingnan Han,[†] Doug Goeger,[†] Claudia S. Maier,[‡] and William H. Gerwick*,[†]

College of Pharmacy and Department of Chemistry, Oregon State University, Corvallis, Oregon 97331

bill.gerwick@oregonstate.edu

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wewakpeptin A (**1**)

Four new depsipeptides have been isolated from the marine cyanobacterium *Lyngbya semiplena* collected from Papua New Guinea. The amino and hydroxy acid partial structures of wewakpeptins A−D (**1**−**4**) were elucidated through extensive spectroscopic techniques, including HR-FABMS, 1D ^{1}H and ^{13}C NMR, as well as 2D COSY, HSQC, HSQC-TOCSY, and HMBC spectra. The sequence of the residues was determined through a combination of multifaceted approaches including ESI-MS/MS, HMBC, ROESY, and a modified 1D HMBC experiment. The absolute stereochemistry of each residue was determined by chiral HPLC and chiral GC−MS methods. The wewakpeptins represent an unusual arrangement of amino and hydroxy acid subunits relative to known cyanobacterial peptides and possess a bis-ester, a 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) or 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa) residue, and a diprolyl group reminiscent of dolastatin 15. Wewakpeptin A and B were the most cytotoxic among these four depsipeptides with an LC_{50} of approximately 0.4 μM to both the NCI-H460 human lung tumor and the neuro-2a mouse neuroblastoma cell lines.

Cyanobacteria are an ancient and diverse group of microorganisms which occupy a broad range of habitats from marine to terrestrial. Marine representatives, especially those belonging to the genus *Lyngbya*, are a prolific source of secondary metabolites with pharmaceutical potential. A particularly prevalent structural theme among these is a rich elaboration of cyclic peptides using a diversity of standard as well as modified amino acids.[1] An additional and emerging theme is the production of acyl amides, as revealed by our recent isolation of a series of new anandamide-like fatty acid amides, semiplenamides A−G, from the marine cyanobacterium *Lyngbya semiplena* Gomont, collected in Papua New Guinea.[2] These latter metabolites displayed modest potency in displacing radiolabeled anandamide from the cannabinoid receptor (CB1). In the course of this latter effort, we found the most polar fraction from this organic extract contained a series of four new cyclic depsipeptides, wewakpeptins A−D (**1**−**4**). Herein, we report the isolation and structure elucidation of this new series of *L. semiplena* peptides as well as their cytotoxicity to tumor cells. Wewakpeptins A and B were the most potent among these four depsipeptides, with an LC_{50} of approximately 0.4 μM to both the NCI-H460 human lung tumor and the neuro-2a mouse neuroblastoma cell lines.

Results and Discussion

Collections of a shallow water (1−3 m) strain of *L. semiplena* were made in Wewak Bay, Papua New Guinea. The algae were extracted with CH_2Cl_2/MeOH (2:1) and fractioned by silica gel vacuum liquid chromatography. Preliminary bioassay of the MeOH-eluted fraction showed toxicity in the brine shrimp model (LD_{50} ~1 ppm). Guided

* To whom correspondence should be addressed. Tel: (541) 737-5801. Fax: (541) 737-3999.
† College of Pharmacy.
‡ Department of Chemistry.

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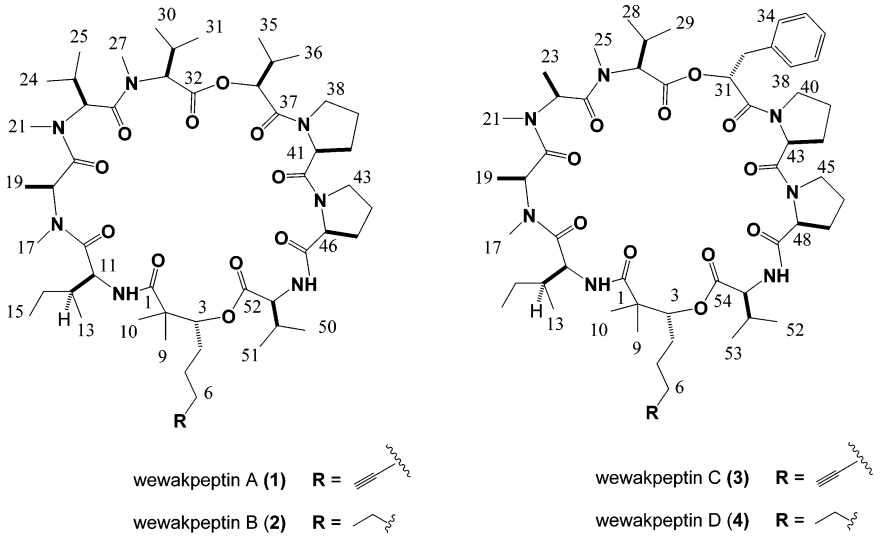
CHART 1



by this assay, this fraction was further chromatographed over a Mega Bond RP_{18} solid-phase extraction (SPE) cartridge and then via reversed-phase HPLC to afford four new depsipeptides, wewakpeptins A−D (**1**−**4**, Chart 1).

The molecular formula of wewakpeptin A (**1**) was determined as $C_{52}H_{85}N_7O_{11}$ on the basis of HR-FABMS and NMR spectral analysis (Table 1). From the proton and carbon NMR data, nine carbonyls accounted for 9 of the 14 degrees of unsaturation implied by the molecular formula. The IR spectrum revealed that **1** contained both amide (1644 cm^{-1}) and ester bonds (1739 cm^{-1}), indicating that it was in fact a peptolide. Its peptidic nature was further supported by the presence of two amide NH signals (δ 8.11, 6.91) and three *N*-methylamide signals (δ 3.02, 2.98, and 2.75) in the ^1H NMR spectrum. Additionally, the ^{13}C NMR spectrum showed two distinctive carbon signals at δ 83.6 and 69.3, consistent with a terminal acetylenic functionality. As previously observed, the carbon at δ 69.3 exhibited no HSQC correlations but showed a $^1J_{CH}$ coupling of 249 Hz to a methine proton at δ 1.93 in the HMBC spectrum.[3,4] This proton also exhibited a $^2J_{CH}$ HMBC correlation to the quaternary carbon at δ 83.6, confirming the presence of an acetylene. The above functionalities accounted for 11 degrees of unsaturation, indicating that wewakpeptin A (**1**) had three rings.

Two oxygenated sp^3 carbons were indicated by signals at δ 79.4 and 77.4 in the ^{13}C NMR spectrum, suggesting the presence of two hydroxy acids in addition to several amino acids. Further NMR analysis revealed a 2-hydroxyisovaleric acid (Hiva) and seven α-amino acids, including two *N*-methylvalines (MeVal), two prolines (Pro), one isoleucine (ILe), one valine (Val), and one *N*-methylalanine (MeAla) (Table 1). Additionally, a 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) was evident on the basis of COSY and HMBC correlations. Geminal dimethyl protons (H_3-9 and H_3-10) showed HMBC correlations to a carbonyl carbon at 174.2 ppm (C-1), a quaternary carbon at 46.4 ppm (C-2), and an oxymethine carbon at 79.4 ppm (C-3). Further, COSY allowed connection of protons H-3 to H-6, the latter of which showed long-range coupling to H-8 (J = 2.6 Hz). In the HMBC spectrum, the quaternary carbon of the terminal acetylene at δ 83.6 ppm (C-7) showed a two-bond correlation with H-8 (δ 1.93) and H-6 (δ 2.16, Table 1).

Determination of the sequence and connection of amino acid residues and other units (Hiva, Dhoya) in **1** was achieved primarily by long-range $^{13}C-^1H$ correlation experiments (HMBC) with different mixing times and a ROESY experiment. Despite several proton and carbon resonances in **1** having close or overlapping chemical shifts, two fragments accounting for all seven amino acid and two hydroxy acid units could be constructed from these data (MeVal-MeVal-MeAla-Ile-Dhoya; Hiva-Pro-Pro-Val). However, neither the Dhoya nor the Hiva moiety showed the connectivity necessary to complete the sequence. Fortunately, a modified decoupled HMBC pulse sequence (1D HMBC) was effectively employed and showed the necessary correlations.[6,7] By irradiation of C-52 (δ 171.0), selective couplings were seen to protons at δ 8.11(NH), 4.59 (H-48), and 4.94 (H-3). Similarly couplings to protons at δ 4.40 (H-28) and 4.96 (H-33) were observed when C-32 (δ 169.75) was irradiated, thus completing the sequence of residues in wewakpeptin A (**1**).

Further evidence supporting this sequence was developed from $(MS)^n$ experiments.[8,9] Collisionally induced ESI-MS/MS of the *m/z* 985 $[C_{52}H_{86}N_7O_{11} + H]^+$ gave ions at *m/z* 899 (Ile-Dhoya-Val-Pro-Pro-Hiva-MeVal-MeVal + $H)^+$, *m/z* 787 (Ile-Dhoya-Val-Pro-Pro-Hiva-MeVal + $H)^+$, *m/z* 673 (Ile-Dhoya-Val-Pro-Pro-Hiva + $H)^+$, *m/z* 573 (Ile-Dhoya-Val-Pro-Pro + $H)^+$, *m/z* 460 (Dhoya-Val-Pro-Pro + $H)^+$, and *m/z* 294 (Val-Pro-Pro + $H)^+$ (Figure 1). These assignments corroborate the NMR results and illustrate

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TABLE 1. NMR Data for Wewakpeptin A (1) and B (2) in $CDCl_3$

		wewakpeptin A (**1**)			wewakpeptin B (**2**)	
unit	position	δ_C	δ_H (J in Hz)	HMBC[a]	δ_C	δ_H[b]
Dhoya	1	174.2			174.4	
	2	46.4			46.4	
	3	79.4	4.94, dd (10.0, 2.0)	2, 4, 9, 10, 52	80.2	4.92
	4	29.8	1.90, 1.38	3, 5	31.0	1.72, 1.28
	5	24.7	1.40, 1.36	4, 6	26.0	1.29, 1.15
	6	18.2	2.16, m	5, 7, 8	31.7	1.26
	7	83.6			22.9	1.28
	8	69.3	1.93, brt (2.6)	7	14.4	0.85[c]
	9	26.2	1.28, s	1, 2, 10	26.2	1.28
	10	23.1	1.26, s	1, 2, 9	23.2	1.26
Ile	NH		6.91, d (8.2)	1, 11		6.89
	11	54.9	5.01, dd (8.2, 2.0)	12, 13, 16	54.8	5.02
	12	39.5	1.71, m	11	39.6	1.71
	13	17.6	1.04	11, 12, 14	17.6	1.04
	14	22.9	1.01, 1.36	15	22.9	1.36, 1.01
	15	12.0	0.88, t (7.0)	12, 14	12.1	0.86
	16	172.3			172.3	
N-Me-Ala	17	30.6	3.02, s	16, 18	30.6	3.02
	18	49.2	5.86, m	16, 17, 19, 20, 21	49.3	5.83
	19	14.9	1.17, d (6.6)	18, 20	14.9	1.19
	20	170.6			170.6	
N-Me-Val-1	21	30.3	2.98, s	20, 22	30.3	2.97
	22	59.4	5.12, d (10.6)	20, 21, 23, 25, 26	59.5	5.12
	23	27.6	2.46, m	24, 25	27.5	2.46
	24	18.4	0.81, d (6.8)	22, 23, 25,	18.4	0.81
	25	20.6	0.95, d (6.8)	22, 23, 24	20.6	0.94
	26	170.4			170.4	
N-Me-Val-2	27	30.2	2.75, s	26, 28, 32	30.2	2.75
	28	65.2	4.40, d (10.6)	26, 27, 29, 32	65.2	4.39
	29	28.0	2.36, m	30, 31	28.0	2.36
	30	19.5	0.98, d (7.1)	28, 29, 31	19.5	0.98
	31	20.6	1.24, d (7.1)	28, 29, 30	20.6	1.25
	32	169.7			169.7	
Hiv	33	77.4	4.96, d (5.0)	32, 34, 35, 36, 37	77.6	4.95
	34	29.5	2.26, m	33	29.5	2.26
	35	20.1	1.07, d (7.0)	33, 34,	20.1	1.07
	36	17.6	1.04, d (7.0)	33, 34, 37	17.6	1.04
	37	166.8			166.9	
Pro-1	38	47.3	3.72, 3.45, q (8.4)	39, 41	47.3	3.77, 3.45
	39	25.6	2.26, 1.92	38, 40, 41	25.5	2.26, 1.92
	40	28.9	2.10, 2.01	38, 39, 41, 42	28.8	2.11, 2.04
	41	58.4	4.64, dd (8.0, 3.7)	38, 39, 40, 42, 43	58.4	4.65
	42	169.9			169.9	
Pro-2	43	47.4	3.82, 3.60, q (8.2)	44, 45, 46	47.4	3.80, 3.62
	44	25.0	2.23, 1.94	43, 45, 46, 47	24.9	2.25, 1.94
	45	29.4	1.89, 1.37	43, 44, 46, 47	29.4	1.85, 1.34
	46	59.7	4.54, dd (7.2, 3.6)	44, 45, 47	59.7	4.57
	47	172.7			172.7	
Val	NH		8.11, d (9.7)	46, 47, 48		8.08
	48	59.0	4.59, dd (9.7, 4.6)	47, 49, 50, 51,52	58.7	4.64
	49	31.5	2.27, m	48, 51, 52	31.8	2.26
	50	19.8	1.07, d (6.8)	48, 49, 51	19.8	1.08
	51	19.1	1.09, d (6.8)	48, 49, 50	19.0	1.09
	52	171.0			171.1	

[a] Proton showing HMBC correlation to indicated carbon. [b] Coupling constants for wewakpeptin B (**2**) are identical to those given for A (**1**) except where noted. [c] Resonance H_3-8 was a triplet with $^3J_{HCCH} = 7.2$ Hz.

the power of $(MS)^n$ experiments in assigning cyclic peptide structures.

The absolute configuration of **1** was established by analysis of degradation products. A small sample was hydrolyzed with 6 N HCl to its constituent amino and hydroxy acid units. These were analyzed by chiral HPLC as well as chiral GC−MS and compared with the retention times of authentic standards. All of the amino acids as well as the Hiva unit were shown to possess the L-configuration. While the Dhoya unit was not stable to acid hydrolysis,[10] its stereochemistry was determined by comparing the retention time of authentic 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa) with that obtained through hydrogenation of **1** followed by acid hydrolysis and chiral GC−MS. The hydrogenated Dhoya product from **1** possessed the same retention time as synthetic *R*-Dhoaa. Proline amide bonds are known to have cis/trans geometry, which correlates with the difference of the proline β and γ ^{13}C NMR values ($\Delta\delta_{\beta\gamma}$).[5] In compound **1**, the small values of $\Delta\delta_{\beta\gamma}$ (3.3 and 4.5 ppm) seen for Pro-1 and Pro-

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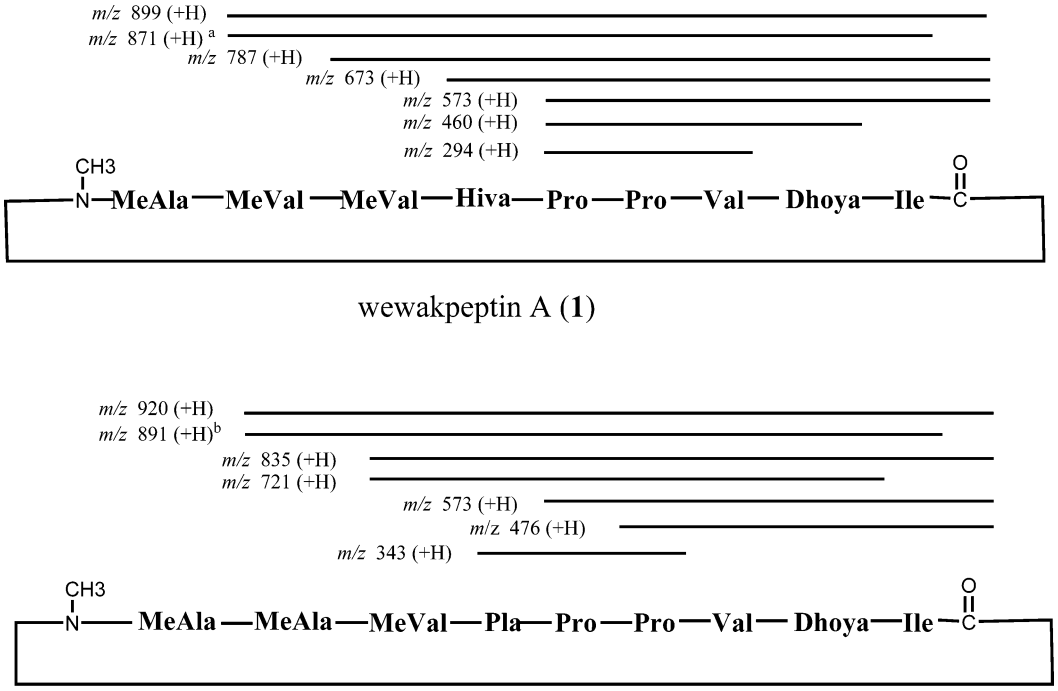

FIGURE 1. Key fragments from collisionally induced ESI-MS/MS experiments with (a) wewakpeptin A (**1**) and (b) wewakpeptin C (**3**). Key: Dhoya = 2,2-dimethyl-3-hydroxy-7-octynoic acid; Hiva = 2-hydroxyisovaleric acid; Pla = 3-phenyllactic acid. [a,b]Fragment ions (= a-type fragment), formed by loss of CO from the respective acylium ions, *m/z* 899 and *m/z* 920 (= b-type fragment).

2, respectively, is indicative of a trans geometry for both proline amide bonds in **1**, a finding that was supported by ROESY interactions observed between H_2-43/H-41 and H_2-38/H-33.

Wewakpeptin B (**2**) was isolated by RP-HPLC from the crude fraction containing wewakpeptin A (**1**). Its high structural homology to **1** was evident by nearly identical ^{1}H and ^{13}C NMR chemical shifts (Table 1). However, it displayed an obvious difference in the Dhoya unit. The acetylenic carbons were absent from the ^{13}C NMR spectrum of **2** and were replaced by two additional high-field carbons at δ 22.9 and 14.4, suggesting that **2** was likely the tetrahydro equivalent of **1**. Indeed, comparison of ^{1}H NMR spectra and FABMS after reducing the triple bond in **1** over Pd−C confirmed these assignments (Table 1). Hydrolysis and stereoanalysis of **2** were not undertaken due to its limited quantity; however, because of the comparable spectroscopic properties of **1** and **2**, we propose they are of the same enantiomeric series.

High-resolution FABMS analysis of wewakpeptin C (**3**) revealed an $[M + H]^+$ ion (*m/z* 1004.6053) consistent with a molecular formula of $C_{54}H_{82}N_7O_{11}$, thus requiring 18 degrees of unsaturation. The IR spectrum of **3** gave characteristic absorption bands for esters and amides at 1741 and 1650 cm^{-1}, and the peptidic nature of **3** was again indicated by two exchangeable *N*H proton resonances at δ 8.12 and 7.00 and three distinct *N*CH_3 proton singlets at δ 3.00, 2.95, and 2.66. Of the 54 carbon resonances in its ^{13}C NMR spectrum, nine amide/ester carbonyls in the δ 165−180 range as well as six characteristic low-field aromatic carbon resonances were observed. Two sp carbons resonating at δ 83.6 and 69.6 suggested a terminal acetylenic functionality in **3**, as in wewakpeptin A (**1**). Oxygenated sp^3 carbons were suspected because of signals at δ 79.4 and 77.4 in the ^{13}C NMR spectrum, suggesting the presence of two hydroxy acids. Detailed analysis of ^{1}H and ^{13}C NMR, COSY, HSQC, HSQC-TOCSY, and HMBC NMR experiments ($CDCl_3$) showed that the structure of **3** was closely related to **1**, with seven α-amino acid residues including two methylalanines (MeAla), two prolines (Pro), one isoleucine (ILe), one valine (Val), and one methylvaline (MeVal) (Table 2). By comparative NMR analysis, one of the nonamino acid moieties in **3** was the hydroxy acid Dhoya. The other hydroxy acid, with proton resonances at δ 7.26−7.33 (H-34−H-38), δ 3.21 and 2.99 (H_2-32), and δ 5.37 (H-31), was similar to those of phenylalanine; however, the chemical shift of the α carbon in this residue (C-31, 74.0 ppm) was typical for an oxymethine, thus indicating that this residue was 3-phenyllactic acid (Pla). Pla is a substructure that has been observed in several other marine metabolites, including the molluscan metabolite kulolide[11] and the cyanobacterial metabolite symplostatin 3[12] (Table 2).

The sequencing of residues in **3** was achieved primarily by long-range ^{13}C-$^{-}$H correlation experiments (HMBC) with different mixing times as well as a ROESY experiment. The modified decoupled HMBC pulse sequence (1D HMBC) was also employed as in the structure elucidation of **1** and indicated two fragments (MeVal-MeAla-MeAla-Ile-Dhoya; Pla-Pro-Pro-Val) which accounted for all seven amino acid and the two hydroxy acid units. Collisionally induced ESI-MS/MS of the *m/z* 1005 $[C_{54}H_{82}N_7O_{11} + H]^+$

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TABLE 2. NMR Spectral Data for Wewakpeptin C (3) and D (4) in $CDCl_3$

		wewakpeptin C (**3**)			wewakpeptin D (**4**)	
unit	position	δ_C	δ_H (J in Hz)	HMBC[a]	δ_C	δ_H[b]
Dhoya	1	174.4			174.5	
	2	46.4			46.3	
	3	79.3	4.91, dd (7.0, 2.0)	2, 4, 9, 10, 54	80.2	4.88
	4	29.9	1.37, 1.87	3, 5	31.0	1.73, 1.29
	5	24.7	1.38, 1.33	4, 6	25.9	1.27, 1.14
	6	18.3	2.12, m	5, 7, 8	31.8	1.26
	7	83.6			22.9	1.30
	8	69.6	1.89, brt (2.5)	7	14.5	0.85[c]
	9	26.11	1.24, s	1, 2, 10	26.2	1.27
	10	23.11	1.22, s	1, 2, 9	23.2	1.24
Ile	NH		7.00, d (8.2)	10		6.95
	11	54.7	4.98, dd (8.0, 2.2)	12, 13, 16	54.7	4.98
	12	39.4	1.66, m		39.4	1.68
	13	17.3	0.99	11, 12, 14	17.3	1.00
	14	23.2	1.30, 1.01	15	23.1	1.30, 0.99
	15	12.3	0.84	12, 14	12.3	0.86
	16	172.1			172.1	
N-Me-Ala-1	17	30.5	3.00, s	16, 18	30.5	3.00
	18	49.0	5.85, m	16, 17, 19, 20, 21	49.0	5.82
	19	14.6	1.25	18, 20	14.5	1.30
	20	170.1			170.1	
N-Me-Ala-2	21	30.2	2.95, s	20, 22	30.3	2.94
	22	49.7	5.55, m	20, 21, 23, 24	49.8	5.54
	23	15.5	1.18, d (6.7)	22, 24	15.5	1.21
	24	171.3			171.2	
N-Me-Val	25	30.6	2.66, s	24, 26	30.7	2.65
	26	64.9	4.19, d (10.0)	24, 25, 27, 30	64.9	4.17
	27	28.7	2.23, m	28, 29	28.8	2.22
	28	19.8	0.83	26, 27, 29	19.8	0.85
	29	20.3	1.01	26, 27, 28	20.4	1.03
	30	169.7			169.7	
Pla	31	74.0	5.37, t (7.0)	30, 32, 33, 34,39	74.0	5.34
	32	37.8	3.21, 2.99, dd (14.4, 3.7)	31, 33, 34, 39	37.8	3.21, 2.98
	33	136.1			136.1	
	34	129.9	7.26	33, 35, 36,	129.9	7.26
	35	129.0	7.33	32, 34, 36, 37, 38	129.0	7.33
	36	127.6	7.28	35, 37, 38	127.6	7.28
	37	129.0	7.33	34, 36,	129.0	7.33
	38	129.9	7.26	36, 37	129.9	7.26
	39	166.6			166.6	
Pro-1	40	47.2	3.58, 3.12, q (7.7)	41,	47.3	3.62, 3.10
	41	25.7	1.95, 2.27	40, 42, 43	25.6	1.96, 2.29
	42	28.9	1.96, 2.24	40, 43, 44	28.9	1.96, 2.24
	43	58.4	4.48	40, 41, 42, 44, 45	58.4	4.49
	44	170.0			170.0	
Pro-2	45	47.6	3.85, 3.59, q (8.0)	46, 47	47.6	3.63, 3.83
	46	25.1	1.71, 2.09	45, 47	25.1	1.66, 1.96
	47	29.6	1.89, 1.37	45, 46, 48	29.5	1.87, 1.35
	48	59.8	4.54	44, 45, 46, 47, 49	59.8	4.57
	49	172.6			172.5	
Val	NH		8.12, d (9.8)	49		8.10
	50	59.2	4.51, dd (9.8, 4.5)	49, 51, 52, 53, 54	59.0	4.56
	51	31.7	2.20, m	50, 52, 53	31.7	2.22
	52	19.1	1.02, d (6.8)	50, 51, 53	19.2	1.04
	53	19.1	1.04, d (6.8)	50, 51, 52	19.0	1.06
	54	170.8			170.9	

[a] Proton showing HMBC correlation to indicated carbon. [b] Coupling constants for wewakpeptin D (**4**) are identical to those given for C (**3**) except where noted. [c] Resonance H_3-8 was a triplet with $^3J_{HCCH} = 7.2$ Hz.

gave ions at *m/z* 920 (Ile-Dhoya-Val-Pro-Pro-Pla-MeVal-MeAla + H)$^+$, *m/z* 835 (Ile-Dhoya-Val-Pro-Pro-Pla-MeVal + H)$^+$, *m/z* 721 (Dhoya-Val-Pro-Pro-Pla-MeVal + H)$^+$, *m/z* 573 (Ile-Dhoya-Val-Pro-Pro + H)$^+$, *m/z* 476 (Ile-Dhoya-Val-Pro + H)$^+$, and *m/z* 343 (Pro-Pro-Pla + H)$^+$ (Figure 1) whose assignment corroborated the NMR results and completed the linkages between the structural units. Chiral HPLC and GC−MS analysis of the acid hydrolysate showed the presence of L-MeAla, L-MeVal, L-Pro, L-Val, L-Ile, and D-Pla. The configuration of Dhoya was determined as *R* using the method as described above for **1**. Curiously, the Pla residue in **3** has the opposite stereochemistry compared with the Hiva residue of **1**.

HR-FABMS of wewakpeptin D (**4**) indicated that the molecular weight was four mass units higher than that of wewakpeptin C (**3**), and NMR data indicated that it was closely related to **3**. However, it also displayed obvious differences in the Dhoya unit in that it lacked the triplet at δ 1.89 for H-6 and the acetylenic carbons at δ 83.6 and 69.3. Chemical reduction (Pd−C, H_2) of the triple bond in **3** produced **4**, confirming these assignments (Table 2). Stereoanalysis of **4** was not undertaken due to

its limited quantity; however, due to the highly comparable spectroscopic properties of **4** and **3**, we again propose that they also are of the same enantiomeric series.

The wewakpeptins were tested for cytotoxicity to NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma cells. Intriguingly, wewakpeptins A and B were approximately 10-fold more toxic than C and D to these cell lines. The LC_{50} for wewakpeptin A was 0.49 and 0.65 μM for neuro-2a and H460 cells, respectively, and 0.20 and 0.43 μM, respectively, for wewakpeptin B. The LC_{50} for wewakpeptin C was 10.7 and 5.9 μM for neuro-2a and H460 cells, respectively, and 1.9 and 3.5 μM, respectively, for wewakpeptin D. These cyclic peptides most likely derive from a nonribosomal polypeptide synthetase (NRPS) pathway,[1] and thus, the structural variation of the wewakpeptins is intriguing and might suggest that adenylation domains with relaxed substrate specificity are involved in their biosynthesis. Hydrolysis of the two ester bonds in the wewakpeptins yields two linear peptides in each case, and these are structurally related to the dolastatins (e.g.,dolastatin 15); thus, it is conceivable that the wewakpeptins undergo these reactions in nature to release more potent fragments.

Experimental Section

Collection. The marine cyanobacterium *Lyngbya semiplena* (voucher specimen available from WHG as collection no. PNG12-7Dec99-3) was collected from shallow waters (1−3 m) in Wewak Bay, Papua New Guinea, on December 7, 1999. Taxonomy was assigned by microscopic comparison with the description given by Desikachary.[13] The material was stored in 2-propanol at −20 °C until extraction.

Extraction and Isolation. Approximately 138 g (dry wt) of the alga was extracted repeatedly with CH_2Cl_2/MeOH (2:1) to produce 3.05 g of crude organic extract. The extract (3.0 g) was fractionated by silica gel vacuum liquid chromatography using a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% MeOH. The fraction eluting with 100% MeOH was found to be active at 1 ppm in the brine shrimp toxicity assay. This fraction was further chromatographed on Mega Bond RP_{18} solid-phase extraction (SPE) cartridges using a stepwise gradient solvent system of decreasing polarity starting from 80% MeOH in H_2O to 100% MeOH. The most active fractions after SPE (85% toxicity at 1 ppm to brine shrimp) were then purified by HPLC [Phenomenex Sphereclone 5 μm ODS (250 × 10 mm), 9:1 MeOH/H_2O, detection at 211 nm] giving compounds **1** (5.0 mg), **2** (0.7 mg), **3** (2.0 mg), and **4** (0.7 mg).

Wewakpeptin A (1): colorless amorphous solid; $[\alpha]^{26}_D$ −45 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} 216 nm (log ϵ 4.6); IR (neat) 3327, 2926, 2875, 1739, 1644, 1454, 1243, 979 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR FABMS *m/z* $[M + H]^+$ 984.6262 (calcd for $C_{52}H_{86}N_7O_{11}$, 984.6302).

Wewakpeptin B (2): colorless amorphous solid; $[\alpha]^{26}_D$ −53 (*c* 0.47, $CHCl_3$); UV (MeOH) λ_{max} 215 nm (log ϵ 4.6); IR (neat) 3316, 2928, 2873, 1739, 1650, 1461, 1242, 976 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR FABMS *m/z* $[M + H]^+$ 988.6843 (calcd for $C_{52}H_{90}N_7O_{11}$, 988.6701).

Wewakpeptin C (3): colorless amorphous solid; $[\alpha]^{26}_D$ −56 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} 220 nm (log ϵ 4.5); IR (neat) 3340, 2932, 2875, 1741, 1650, 1446, 1242, 980 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR FABMS *m/z* $[M + H]^+$ 1004.6053 (calcd for $C_{54}H_{82}N_7O_{11}$, 1004.6072).

Wewakpeptin D (4): colorless amorphous solid; $[\alpha]^{26}_D$ −65 (*c* 0.60, $CHCl_3$); UV (MeOH) λ_{max} 221 nm (log ϵ 4.5); IR (neat) 3343, 2924, 2851, 1741, 1654, 1457, 1244, 989 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR FABMS *m/z* $[M + H]^+$ 1008.6276 (calcd for $C_{54}H_{86}N_7O_{11}$, 1008.6380).

Absolute Stereochemistry of 1. Wewakpeptin A (**1**, 500 μg) was hydrolyzed in 6 N HCl at 105 °C for 16 h, dried under a stream of N_2, and further dried under vacuum. The residue was reconstituted with 300 μL of H_2O prior to chiral HPLC analysis [Phenomenex Chirex 3126 (D), 4.6 × 250 mm; UV 254 nm detector], mobile phase I: 100% 2 mM $CuSO_4$ in H_2O, flow rate 0.7 mL/min; [column, Phenomenex chirex 3126 (D), 4.6 × 50 mm; UV 254 nm detector], mobile phase II: 2 mM $CuSO_4$ in MeCN/H_2O (15:85), flow rate 0.8 mL/min. Mobile phase I elution times (t_R, min) of authentic standards: L-MeAla (16.7), D-MeAla (17.2), L-MeVal (24.0), D-MeVal (39.0), L-Pro (28.4), D-Pro (63.4), L-Val (38.5), D-Val (68.9). Mobile phase II elution times (t_R, min) of authentic standards: L-Hiv (9.2), D-Hiv (14.5). The hydrolysate was chromatographed alone and co-injected with standards to confirm assignments of L-MeAla, L-MeVal (2 equiv), L-Pro (2 equiv), l-Val, and L-Hiv. The presence of L-Ile was confirmed by chiral GC−MS using established methods.[14] Determination of the configuration of the Dhoya unit was established through hydrogenation (10% Pd−C, H_2), acid hydrolysis (6 N HCl, 105 °C for 14 h), and chiral GC-MS analysis of the 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa) residue as direct hydrolysis of wewakpeptin A did not yield a sufficient amount of 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) for analysis.

For chiral GC-MS analysis of methyl-Dhoaa, portions of each standard *R*- and *S*-Dhoaa were separately diluted in 50 μL of MeOH and treated with diazomethane for 10 min. Excess diazomethane and solvent were removed with a stream of N_2, and the residues were resuspended in CH_2Cl_2. Capillary GC-MS analysis was conducted using a Chirasil-Val column (Altech, 25 m × 0.25 mm) using the following conditions: column temperature held at 40 °C to 100 °C at a rate of 3 °C/min, then from 100 °C to 150 °C at a rate of 15 °C/min. The retention time of the methylated Dhoaa residue derived from hydrogenation and hydrolysis of **1** and **3**, followed by methylation (CH_2N_2) matched that of the methylated *R*-Dhoaa standard (34.6 min) but not the methylated *S*-Dhoaa standard (35.2 min).

Absolute Stereochemistry of 3. The wewakpeptin C (**3**, 500 μg) hydrosylate (6 N HCl, 105 °C, 16 h) was worked up and analyzed as described above for the wewakpeptin A hydrolysate. The following residues coeluted with wewakpeptin C hydrosylate peaks; mobile phase I: L-MeAla (16.3 min), L-MeVal (25.9 min), 2 equiv of L-Pro (28.3 min), L-Val (38.2 min); Mobile phase II: d-Pla (66.0 min). The presence of L-Ile and *R*- Dhoya were confirmed by chiral GC−MS as described above for the wewakpeptin A.

Biological Activity. Brine shrimp (*Artemia salina*) toxicity was measured as previously described.[15] After a 24 h hatching period, aliquots of a 10 mg/mL stock solution of compounds A−D were added to test wells containing 5 mL of artificial seawater and brine shrimp to achieve a range of final concentrations from 0.1 to 100 ppm. After 24 h the live and dead shrimp were tallied.

Cytotoxicity was measured in NCI-H460 human lung tumor cells and neuro-2a mouse neurablastoma cells using the method of Alley et al.[16] with cell viability being determined

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by MTT reduction.[17] Cells were seeded in 96-well plates at 6000 cells/well in 180 μL of medium. Twenty-four hours later, the test chemical was dissolved in DMSO and diluted into medium without fetal bovine serum and then added at 20 μg/well. DMSO was less than 0.5% of the final concentration. After 48 h, the medium was removed and cell viability determined.

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Supporting Information Available: General experimental procedures, NMR spectra (^{1}H NMR, ^{13}C NMR, and 2D NMR spectra in $CDCl_3$) for wewakpeptins A−D (**1**−**4**), complete MS spectra for wewakpeptins A (**1**) and C (**3**), and cytotoxicity assays of wewakpeptins A−D with Neuro-2a and H460 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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