Cytotoxic Halogenated Macrolides and Modified Peptides from the Apratoxin-Producing Marine Cyanobacterium *Lyngbya bouillonii* from Guam

Susan Matthew,[†,‡] Lilibeth A. Salvador,[†,‡] Peter J. Schupp,[§] Valerie J. Paul,[⊥] and Hendrik Luesch*[,†]

Department of Medicinal Chemistry, University of Florida, 1600 SW Archer Road, Gainesville, Florida 32610, University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923, and Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949

Received June 17, 2010

Collections of the marine cyanobacterium *Lyngbya bouillonii* from shallow patch reefs in Apra Harbor, Guam, afforded three hitherto undescribed analogues of the glycosidic macrolide lyngbyaloside, namely, 2-*epi*-lyngbyaloside (**1**) and the regioisomeric 18*E*- and 18*Z*-lyngbyalosides C (**2** and **3**). Concurrently we discovered two new analogues of the cytoskeletal actin-disrupting lyngbyabellins, 27-deoxylyngbyabellin A (**4**) and lyngbyabellin J (**5**), a novel macrolide of the laingolide family, laingolide B (**6**), and a linear modified peptide, lyngbyapeptin D (**7**), along with known lyngbyabellins A and B, lyngbyapeptin A, and lyngbyaloside. The structures of **1**−**7** were elucidated by a combination of NMR spectroscopic and mass spectrometric analysis. Compounds **1**−**6** were either brominated (**1**−**3**) or chlorinated (**4**−**6**), consistent with halogenation being a hallmark of many marine natural products. All extracts derived from these *L. bouillonii* collections were highly cytotoxic due to the presence of apratoxin A or apratoxin C. Compounds **1**−**5** showed weak to moderate cytotoxicity to HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells.

Marine cyanobacteria have been attracting increasing attention for probe and drug discovery due to the high incidence of structurally novel bioactive secondary metabolites that complement those known from terrestrial sources. These natural products are predominantly modified peptides and depsipeptides, polyketides, and peptide−polyketide hybrids, many of which are cyclic and oftentimes halogenated.[1] One intriguing characteristic of marine cyanobacteria is that a single organism commonly produces several distinct classes of natural products so that up to 10% of the genome may be dedicated to secondary metabolism. One example of such a "superproducer" is *Lyngbya bouillonii*.[2,3] Various collections carried out over the past two decades have consistently yielded apratoxin A,[4] lyngbyabellin A,[5] lyngbyapeptin A,[6] and, with variable reproducibility, also lyngbyastatin 2,[7,8] lyngbyabellin B,[6] apratoxin B,[9] and apramides A−G.[10] Recently we found that the secondary metabolite content of *L. bouillonii* may be depth-dependent, because at the same site (Finger's Reef, Guam) but at greater depth (14 m instead of 2 m) apratoxin E rather than apratoxin A was the major apratoxin produced by a morphologically identical cyanobacterium.[8] The latter, however, lacked the usually coexisting snapping shrimp *Alpheus frontalis* that is known to use *L. bouillonii* as food and tubular shelter.[11−13]

Previous investigations of *L. bouillonii* from Finger's Reef in Apra Harbor were guided by cancer cell viability assays, leading to the isolation of the major cytotoxins. We have now meticulously isolated other extract components that previously had escaped isolation efforts due to low abundance, lower activity, and/or complexity of the metabolite profile. A dereplication strategy was utilized with the aid of LC-MS for initial characterization of unknowns and identification of previously encountered compounds. A sensitive 1 mm triple-resonance high-temperature superconducting (HTS) cryogenic probe was used to elucidate structures by NMR.[14] To investigate the chemical diversity of *L. bouillonii* within Apra Harbor and to potentially identify new natural apratoxins to complement our structure−activity relationship and mechanistic studies,[8,15] we examined another site (Western Shoals) that was a habitat for an apparently similar cyanobacterium with largely identical chemistry, yet it additionally yielded apratoxin C, previously isolated from a Palauan variety of this organism.[9] Here we describe the structure elucidation and initial biological evaluation of seven new compounds (**1**−**7**) stemming from these collections, all of which are related to known metabolites from *L. bouillonii* (Figure 1). Six out of the seven compounds are halogenated, a hallmark of many marine-derived metabolites. All extracts contained apratoxin A as the major cytotoxic constituent (Figure 1).

Results and Discussion

Investigations of two *L. bouillonii* collections from Finger's Reef and one collection from Western Shoals (both sites located in Apra Harbor, Guam) led to the isolation of lyngbyaloside, originally identified from a *L. bouillonii* strain from Papua New Guinea,[16] and three novel bromine-containing lyngbyaloside analogues, 2-*epi*-lyngbyaloside (**1**), 18*E*-lyngbyaloside C (**2**), and 18*Z*-lyngbyaloside C (**3**) (Figure 1). On the contrary, there was no evidence for the presence of the structurally related macrolides lyngbouilloside[17] and lyngbyaloside B[18] produced by *L. bouillonii* varieties from Papua New Guinea and Palau, respectively. In addition to lyngbyabellins A and B,[5,6,19] we also discovered two new members of this structural class of *L. bouillonii* metabolites, 27-deoxylyngbyabellin A (**4**) and lyngbyabellin J (**5**), both bearing two chlorine atoms characteristic of the lyngbyabellins (Figure 1). Laingolide B (**6**) is a chlorinated analogue of the Papua New Guinea *L. bouillonii* isolates laingolide[20] and laingolide A[21] (Figure 1), neither of which has been identified yet in Guamanian or Palauan cyanobacteria. The linear modified peptide lyngbyapeptin D (**7**) was found to be coproduced with lyngbyapeptin A,[6,22] albeit in minute quantities relative to the parent compound, and readily decomposed to compound **7a** (Figure 1). The isolation was achieved by silica gel column chromatography of extracts followed by one or more rounds of reversed-phase HPLC.

The HRESI/APCIMS spectrum of **1** displayed two $[M + Na]^+$ peaks at *m*/*z* 683.2410 and 685.2382 in equal intensity, suggesting the presence of one bromine atom. Initial 2D NMR analysis of **1** in $CDCl_3$ at 600 MHz (COSY, TOCSY, HSQC, HMBC) in combination with HRESI/APCIMS analysis revealed that **1** has the same planar structure as the co-isolated tricyclic glycoside macrolide lyngbyaloside (Figure 1). The ^1H and ^{13}C NMR chemical shift data for **1** were mostly identical to those for lyngbyaloside (Table 1).[16] Significant differences were observed for H-4a and H-4b. NOESY

* To whom correspondence should be addressed. Tel: (352) 273-7738. Fax: (352) 273-7741. E-mail: luesch@cop.ufl.edu.
† University of Florida.
‡ Contributed equally.
§ University of Guam Marine Laboratory.
⊥ Smithsonian Marine Station.

10.1021/np1004032
Published on Web 08/12/2010

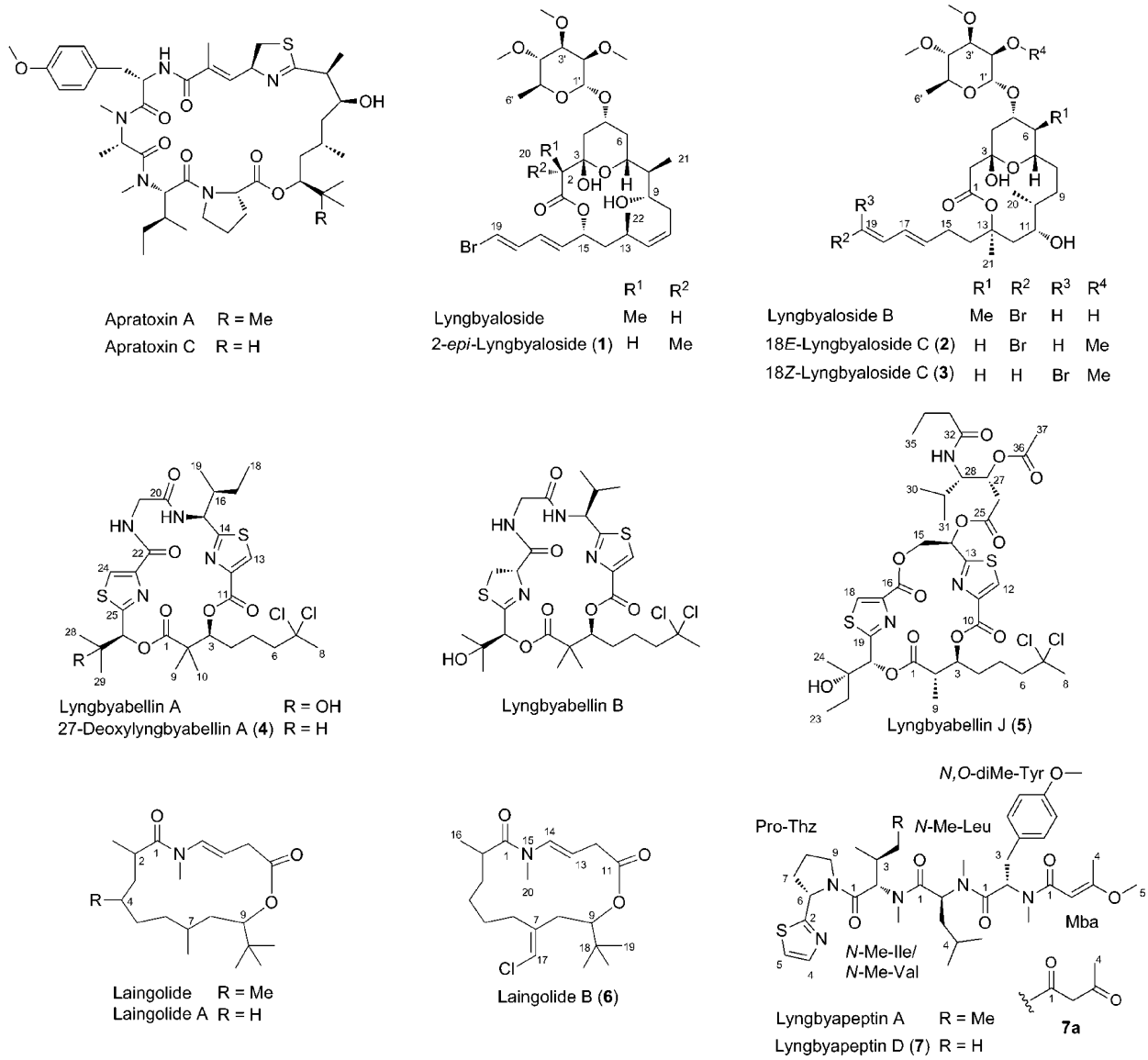

Figure 1. Natural products isolated from shallow-water *L. bouillonii* found in Apra Harbor (Guam) and closely related analogues found in Palau (lyngbyaloside B) or Papua New Guinea (laingolide and laingolide A).

correlations between H_3-20/H-4b and H-2/H-4a suggested that **1** has the opposite configuration at C-2 compared with lyngbyaloside, with CH_3-20 occupying an axial position (Figure 2). The van der Waals interaction between H-4b and CH_3-20 causes deshielding of the axial methylene proton H-4b relative to the corresponding proton in lyngbyaloside ($\Delta\delta$ +0.5 ppm). This configuration allows a shielding 1,3-interaction between H-2 and H-4a, causing an upfield shift of H-4a relative to the H-4a resonance in lyngbyaloside ($\Delta\delta$ −0.24 ppm) (Figure 2). Thus, the chemical shifts of the diastereotopic methylene protons H-4a and H-4b are very similar ($\Delta\delta$ 0.33 ppm). Because the configuration of the other stereocenters in lyngbyaloside (which on the basis of NMR data were the same as for compound **1**) had not been assigned yet,[16] we attempted to use proton−proton coupling constants and NOESY data to propose a stereostructure for both molecules. The chemical shifts for H-6a and H-6b differed substantially ($\Delta\delta$ 1.18 ppm), suggesting that CH_3-21 is equatorial and H-8 axial, by analogy with the analysis above for the configuration of C-2: a shielding 1,3-diaxial interaction exists between H-6b and H-8, leading to a relative upfield shift of the H-6b resonance, while the van der Waals interaction of H-6a with CH_3-21 causes the H-6b signal to appear more downfield (Figure 2). The splitting pattern and $^3J_{H,H}$ coupling constants (dt 10.9, 2.9 Hz) of H-9 together with the absence of a NOESY correlation with H-7 suggested that H-9 occupies a pseudoequatorial position (Figure 2). A *cis* geometry of the double bond at C-11 and C-12 was evident from the $^3J_{H,H}$ coupling constant of 10.6 Hz. NOESY correlations between H-12/H_3-22, H-13/H-15, and H-14b/H-15 implied a pseudoequatorial orientation for CH_3-22 and axial position for H-15 (Figure 2). Therefore, we propose a 2*R**,3*S**,5*R**,7*R**,8*S**,9*S**,13*R**,15*R** configuration for compound **1** and consequently a 2*S**,3*S**,5*R**,7*R**,8*S**,9*S**,13*R**,15*R** configuration for lyngbyaloside (Figure 1). For other lyngbyalosides, the glycoside portion could be related to the aglycone on the basis of NOE or ROESY correlations,[16,18] some of which were also observed here for **1**. The structure depicted for **1** is the more likely enantiomer since L-rhamnose was also found in related metabolites, aurisides A and B.[23] Furthermore, the specific rotations for all lyngbyaloside-like molecules have at least the same sign.

The HRESI/APCIMS spectrum of **2** showed a $[M + Na]^+$ peak at *m/z* 671.2399 and an isotope peak of equal intensity at *m/z* 673.2381, suggesting the presence of one bromine atom. The molecular formula of **2** was deduced as $C_{30}H_{49}BrO_{10}$, with 6 degrees of unsaturation. Detailed ^{1}H and 2D NMR analysis in $CDCl_3$ (Table 2) revealed that **2** is closely related to lyngbyaloside B, with the same molecular formula on the basis of HRESI/APCIMS. HSQC, HMBC, COSY, and TOCSY spectra suggested the presence of an ester moiety (δ_C 172.3), a hemiketal (δ_C 96.6), a hexapyranoside

Table 1. NMR Data for 2-*epi*-Lyngbyaloside (**1**) in $CDCl_3$ (600 MHz)

	δ_C[a]	δ_H (J in Hz)	HMBC	NOESY
1	174.2, C			
2	49.0, CH	2.63, q (7.0)	1, 3, 20	3-OH, H-4a
3	98.2, C			
3-OH		5.15, s		H-2, H-7
4a	39.8, CH_2	1.96, m	5, 6	H-2,H-5
4b		1.63, m	5	H_3-20
5	69.4, CH	4.21, m	1′	H-4a, H-6a, H-7, H-1′
6a	36.0, CH_2	2.20, m		H-5, H_3-21, H-1′
6b		1.00, m		
7	70.6, CH	3.73, td (10.6, 1.6)		3-OH, H-5
8	44.1, CH	1.86, m		H-9
9	72.3, CH	3.93, dt (10.9, 2.9)		H-8, H-10a, H_3-21
10a	27.8, CH_2	2.20, m		H-9
10b		1.90, m		H-11
11	123.4, CH	5.45, dt (10.6, 7.9)	9	H-10b
12	139.3, CH	5.37, t (10.6)	10, 22	H_3-22
13	28.3, CH	2.48, m		H-15
14a	43.3, CH_2	1.66, ddd (−14.1, 6.3, 3.3)	13	H-16
14b		1.45, brdd (−14.1, 11.0)		H-15
15	73.1, CH	5.54, brdd (11.0, 6.3)		H-13, H-14b
16	132.3, CH	5.73, dd (15.3, 6.3)	15, 18	H-14a
17	128.7, CH	6.15, dd (15.3, 10.9)		H-18
18	136.4, CH	6.69, dd (13.5, 10.9)	19	H-17
19	110.0, CH	6.39, d (13.5)	17	
20	11.2, CH_3	1.25, d (7.1)	2, 3	H-4b
21	10.3, CH_3	0.90, d (7.0)		H-6a, H-9
22	19.3, CH_3	1.05, d (6.6)	12	H-12
1′	94.7, CH	5.02, d (1.0)	3′, 5′	H-5, H-6a, 2′-*O*-Me
2′	77.6, CH	3.52, dd (3.1, 1.0)	2′-*O*-Me	
2′-*O*-Me	59.2, CH_3	3.50, s	2′	H-1′
3′	81.0, CH	3.46, dd (9.4, 3.1)	3′-*O*-Me′	
3′-*O*-Me	57.8, CH_3	3.46, s	3′	
4′	82.1, CH	3.12, brt (9.4)	3′, 5′, 6′, 4′-*O*-Me	
4′-*O*-Me	60.9, CH_3	3.55, s	4′	
5′	68.0, CH	3.59, dq (9.4, 6.2)		
6′	17.6, CH_3	1.29, d (6.2)	4′, 5′	

[a] Deduced from HSQC and HMBC experiments.

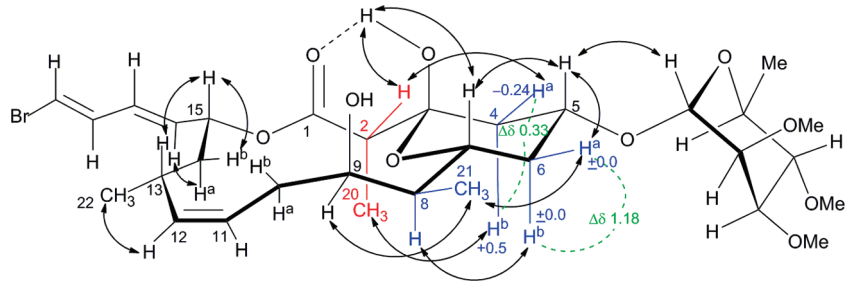

Figure 2. Proposed stereostructure and selected key NOESY correlations for 2-*epi*-lyngbyaloside (**1**). Configurational difference from lyngbyaloside is indicated in red. Other groups and protons with critical stereoelectronical effects discussed in the text are shown in blue. Δδ values for H-4a/b and H-6a/b relative to the corresponding resonances in lyngbyaloside are shown in blue, and Δδ values between diastereotopic protons in **1** are depicted in green.

group (δ_C/δ_H 94.6/4.97, 77.7/3.46, 80.9/3.42, 82.1/3.08, 67.9/3.53), and a terminally brominated conjugated hexadiene side chain (δ_C/δ_H 137.7/66.3, 127.7/5.99, 137.7/6.63, 106.3/6.17 for C/H-16 to 19). In contrast to lyngbyaloside B,[18] **2** lacks a methyl group at C-6 of the aglycone but is O-methylated at C-2′ of the glycoside; thus both compounds are constitutional isomers (Figure 1). $^3J_{H,H}$ coupling constants and key NOESY correlations between 3-OH/H-7, H-5/H-1′, H-11/H-14a, and H-11/H-14b suggested that the configuration of the macrolide portion of **2** is likely the same as in lyngbyaloside B. The $^3J_{H,H}$ coupling constants for H-18/H-19 (13.9 Hz), H-17/H-18 (10.6 Hz), and H-16/H-17 (14.6 Hz) and NOESY correlations between H-15/H-16, H-16/H-18, and H-17/H-19 suggested an *E*,*E* configuration of the conjugated diene in **2**.

An identical molecular formula of $C_{30}H_{49}BrO_{10}$ and isotope pattern at *m*/*z* 671.2410 and 673.2379 $[M + Na]^+$ were obtained for **3**. Interpretation of NMR spectra (Table 2) revealed that the planar structures of **3** and **2** are identical. NOESY data and $^3J_{H,H}$ coupling constants, however, indicated that the conjugated diene side chain of **3** has a different configuration from that of **2**. The $^3J_{H,H}$ coupling constants for H-18/H-19 (7.0 Hz), H-17/H-18 (10.2 Hz), and H-16/H17 (15.4 Hz) indicated an *E*,*Z* configuration of the diene side chain (Figure 1). This was further confirmed by NOESY correlations between H-18/H-19 and H-16/H-18. Thus, compound **3** was a regioisomer of compound **2**.

Compound **4** had a molecular formula of $C_{29}H_{40}Cl_2N_4O_6S_2$ based on HRESI/APCIMS with $[M + H]^+$ isotopic peaks at *m*/*z* 675/677/679 in the ratio of 5:4:1, indicating the presence of two chlorine atoms in the molecule. 1D and 2D NMR data suggested a close relationship of **4** with lyngbyabellins (Table 3, Figure 1).[5,6,19,24−26] COSY analysis combined with characteristic HMBC correlations of the methyl protons at δ 2.05 (H_3-8) to the *gem*-dichloro carbon at δ 90.0 (C-7) and the methylene carbon at δ 49.3 (C-6) established the aliphatic

Table 2. NMR Data for Lyngbyalosides C (**2** and **3**) in $CDCl_3$, (600 MHz)

	18*E*-lyngbyaloside C (**2**)				18*Z*-lyngbyaloside C (**3**)	
	δ_C[a]	δ_H (*J* in Hz)	HMBC	NOESY	δ_C[a]	δ_H (*J* in Hz)
1	172.3, C				172.3, C	
2a	46.9, CH_2	2.49, d (−12.1)	3	H-4b	47.0, CH_2	2.53, d (−12.1)
2b		2.38, d (−12.1)		3-OH, H-4a		2.41, d (−12.1)
3	96.6, C				96.6, C	
3-OH		4.57, brs	2, 4	H-2b, H-7		4.56, s
4a	41.5, CH_2	2.09, m	4, 6	H-2b, H-5	41.4, CH_2	2.15, m
4b		1.28, m	2	H-2a		1.33, m
5	69.2, CH	4.10, m	1′	H-4a, H-6a, H-7, H-1′	69.1, CH	4.10, m
6a	37.7, CH_2	1.89, m	4, 7	H-5	37.9, CH_2	1.92, m
6b		1.15, m				1.17, m
7	69.8, CH	3.79, t (10.1)	9	3-OH, H-5	69.9, CH	3.79, t (10.1)
8a	31.4, CH_2	1.70, m	11	H-11	31.5, CH_2	1.70, m
8b		1.45, m				1.46, m
9a	32.4, CH_2	1.45, m	10, 12	H_3-20	32.3, CH_2	1.46, m
9b		1.32, m				1.38, m
10	37.0, CH	1.48, m	11, 12	H-11	37.0, CH	1.55, m
11	65.5, CH	4.26, m	13	H-8a, H-10a, H-12a, H-14a, H_3-20	65.7, CH	4.30, m
12a	44.1, CH_2	2.77, d (−15.6)	13, 14	H-11, H_3-20, H_3-21	44.2, CH_2	2.80, d (−15.4)
12b		1.44, dd (−15.6, 5.3)	10, 11			1.47, dd (−15.4, 6.0)
13	86.2, C				86.3, C	
14a	38.6, CH_2	1.97, m	13, 16	H-11	38.6, CH_2	2.03, m
14b		1.63, m				1.67, m
15a	26.7, CH_2	2.17, m	16, 17	H-16, H-17	27.0, CH_2	2.28, m
15b		2.18, m				2.26, m
16	135.9, CH	5.76, dt (14.6, 5.9)	14, 15, 18	H-15, H-18	138.8, CH	5.96, dt (15.4, 7.0)
17	127.7, CH	5.99, dd (14.6, 10.6)	15	H-15, H-19	126.2, CH	6.44, dd (15.4, 10.2)
18	137.7, CH	6.63, dd (13.9, 10.6)	16, 19	H-16	132.6, CH	6.60, dd (10.2, 7.0)
19	106.3, CH	6.17, d (13.9)	17, 18	H-17	105.6, CH	6.04, d (7.0)
20	13.5, CH_3	0.80, d (7.0)	11	H-9a/9b, H-11, H-12a	13.3, CH_3	0.81, d (6.7)
21	23.3, CH_3	1.50, s	10, 11	H-12a	23.4, CH_3	1.50, s
1′	94.6, CH	4.97, brs	2′, 3′, 5′	H-5, H-5′	94.5, CH	4.97, d (1.0)
2′	77.7, CH	3.46, dd (3.0, 1.0)	3′, 2′-*O*-Me	H-3′, 3′-*O*-Me	77.8, CH	3.46, dd (2.8, 1.0)
2′-*O*-Me	58.9, CH_3	3.49, s			58.9, CH_3	3.50, s
3′	80.9, CH	3.42, dd (9.0, 3.0)		H-2′, H-5′	81.0, CH	3.44, dd (9.4, 2.8)
3′-*O*-Me	57.6, CH_3	3.48, s		H-2′	57.5, CH_3	3.49, s
4′	82.1, CH	3.08, dd (10.0, 9.0)	3′, 4′-*O*-Me	4′-*O*-Me, H-5′, H_3-6′	82.2, CH	3.10, brt (9.4)
4′-*O*-Me	60.8, CH_3	3.52, s		H-4′	60.7, CH_3	3.54, s
5′	67.9, CH	3.53, dq (10.0, 6.2)	4′	H-1′, H-3′, H-4′, H_3-6′	68.0, CH	3.56, dq (9.4, 6.0)
6′	17.8, CH_3	1.25, d (6.2)	4′, 5′	H-4′, H-5′	17.6, CH_3	1.26, d (6.0)

[a] Deduced from HSQC and HMBC experiments.

chain as 7,7-dichloro-3-acyloxy-2,2-dimethyloctanoate.[5,6,19] Further 2D NMR analysis revealed that the molecule also has two 2-alkylthiazole-4-carboxylic acid units (C-11 to C-14 and C-22 to C-25), a glycine moiety, and an isoleucine-derived unit as in lyngbyabellin A, but an α-hydroxyisovaleric instead of the α,β-dihydroxyisovaleric acid derived moiety in lyngbyabellin A (Table 3, Figure 1). The molecular formula of **4** ($C_{29}H_{40}Cl_2N_4O_6S_2$) indicated the loss of one oxygen atom compared to lyngbyabellin A, consistent with the lack of the hydroxy group at C-27, as evident from our NMR analysis. Otherwise NMR data were virtually identical to those for lyngbyabellin A, indicating that the relative configuration of both compounds was the same. Therefore compound **4** had to be the deoxy derivative of lyngbyabellin A and was named 27-deoxylyngbyabellin A (**4**). The specific rotation of **4** matched reported values for natural[5] and synthetic[27,28] lyngbyabellin A, suggesting that it had the exact absolute configuration as lyngbyabellin A.

The LRESIMS spectrum of **5** showed a characteristic isotope pattern for a dichlorinated compound at *m/z* 886/888/890 (5:4:1). HRESI/APCIMS ($[M + Na]^+$ *m/z* 886.2198) implied a molecular formula of $C_{37}H_{51}Cl_2N_3O_{12}S_2$. NMR analysis (Table 4) suggested the presence of a *gem*-dichloro moiety (δ_C 90.0 for C-7), and HMBC correlations ascertained that this moiety is part of a 7,7-dichloro-3-acyloxy-2-methyloctanoate residue, found in certain lyngbyabellin-type compounds.[24−26,29] HMBC correlations of a highly shielded methyl group (CH_3-23: δ_C 7.88, δ_H 0.92) allowed for the assignment of the α,β-dihydroxy-β-methylpentanoic acid (Dhmpa, C-19 to C-24) unit in **5**. The presence of two disubstituted thiazole rings was determined from HMBC correlations of two methine singlets at δ_H 8.24 (H-18) and 8.12 (H-12) to nonprotonated heteroaromatic carbons C-17/19 (δ_C 145.9/165.2) and C-11/13 (δ_C 146.3/165.8), respectively. The cyclic core structure of **5** (Figure 1) was constructed on the basis of HMBC correlations between H-20/C-1, H-15/C-16, and H-14/C-13 (Table 4). The planar cyclic core of **5** is identical to that of lyngbyabellin C;[24] however, acylation of the hydroxy group at C-14 was evident from the low-field signal for H-14 (δ_H 6.45) and HMBC correlation of H-14 to C-25 (Table 4). HMBC and COSY analysis revealed a bis-acylated γ-amino-β-hydroxy acid putatively derived from condensation of valine and acetate (Table 4). Although no HMBC correlations were observed between C-32 and H-28 or 28-NH, the chemical shift of C-32 (δ_C 173.4) was indicative of an amide functionality. HMBC and COSY correlations suggested that C-32 was derived from a butyric acid moiety. Similarly, the hydroxy group was found to be acetylated. The deduced planar structure of **5** (Figure 1) is closely related to lyngbyabellins C−I[24−26] and hence given the trivial name lyngbyabellin J. The absolute configuration of **5** was established using enantioselective analysis after ozonolysis in combination with acid or base hydrolysis. Peaks corresponding to (2*R*,3*S*)-Dhmpa and D-glyceric acid were observed in the base hydrolysate. The configuration of the γ-amino-β-hydroxy acid unit at C-27 and C-28 was established by comparing the coupling constants of the α-methylene signal (H-26a/b), in accordance with the recently proposed NMR-based technique.[30] H-26a showed a small coupling ($^3J_{H,H}$ = 3.9 Hz) to H-27, while H-26b gave a large coupling ($^3J_{H,H}$ = 7.0 Hz), suggesting a *syn* arrangement at C-27 and C-28. The absolute configuration at C-28 was established by acid hydrolysis

Table 3. NMR Data for 27-Deoxylyngbyabellin A (**4**) in $CDCl_3$ (600 MHz)

C/H no.	δ_C[a]	δ_H (*J* in Hz)	COSY	HMBC
1	173.1, C			
2	46.5, C			
3	78.2, CH	5.30, dd (10.3, 1.3)	H-4a, H-4b	
4a	29.5, CH_2	1.71, m	H-3, H-4b, H_2-5	
4b		1.40, m	H-3, H-4a, H_2-5	
5	22.3, CH_2	1.60, m	H-4, H-6a, H-6b	3, 4, 6
6a	49.3, CH_2	2.22, m	H_2-5, H-6b	5, 6, 7
6b		2.01, m	H_2-5, H-6a	
7	90.0, C			
8	37.1, CH_3	2.05, s		7
9	24.1, CH_3	1.28, s		1, 2, 3
10	20.2, CH_3	1.34, s		1, 2, 3
11	160.5, C			
12	146.9, C			
13	127.5, CH	8.06, s		12, 14
14	168.3, C			
15	55.1, CH	5.23, dd (7.7, 7.5)	15-NH, H-16	16, 17, 19
15-NH		7.26, d (10.4)	H-15	
16	40.1, CH	1.96, m	H-15, H-17a, H-17b H_3-19	15, 17, 19
17a	25.4, CH_2	1.50, m	H-16, H-17b, H_3-18	16, 18
17b		1.13, m	H-16, H-17a, H_3-18	
18	11.2, CH_3	0.92, t (7.3)	H-17	17
19	14.9, CH_3	0.77, d (6.8)	H-16	16
20	168.3, C			
21a	42.9, CH_2	4.61, dd (−16.9, 8.8)	21-NH	20, 22
21b		3.75, dd (−16.9, 4.8)		
21-NH		7.97, br	H-21	
22	160.6, C			
23	148.7, C			
24	124.7, CH	8.20, s		23, 25
25	168.5, C			
26	76.7, CH	5.85, d (9.9)	H-27	1, 25, 27, 28, 29
27	32.9, CH	2.34, m	H-26, H_3-28, H_3-29	26, 28, 29
28	18.7, CH_3	0.95, d (6.6)	H-27	26, 27
29	18.6, CH_3	1.10, d (6.6)	H-27	26, 27

[a] Deduced from HSQC and HMBC experiments.

(leading to partial dehydration of the liberated hydroxy acid), subsequent ozonolysis, oxidative workup, and analysis of the liberated valine unit. Enantioselective HPLC-MS established the presence of L-Val in the hydrolysate. Comparison of the coupling constant between H-2 and H-3 (8.4 Hz) with data for lyngbyabellin E (9.5 Hz) and hectochlorin (7.4 Hz) suggested that **5** should have the same relative configuration at C-2 and C-3 and likely the same absolute configuration, as all lyngbyabellins isolated to date have a 3*S* configuration. These data support the absolute configuration of lyngbyabellin J (**5**) as 2*S*,3*S*,14*R*,20*R*,21*S*,27*R*,28*S*.

Compound **6** was deduced to have a molecular formula of $C_{20}H_{32}ClNO_3$ on the basis of NMR and HRESI/APCIMS (*m*/*z* 370.2147 for $[M + H]^+$). The presence of one chlorine atom was consistent with the $[M + H]^+$ isotope ion cluster at *m*/*z* 370/372 in a 3:1 ratio. The final structure assignment of **6** was achieved through 1D and 2D NMR experiments including COSY, HSQC, HMBC, and NOESY (Table 5). This compound possessed a 15-membered macrocyclic lactone with enamide and *tert*-butyl functionalities observed for the two laingolides reported from the same species collected at Laing Island in Papua New Guinea (Figure 1).[20,21] Compound **6**, hence termed laingolide B, was more similar to laingolide A[21] because of the lack of a methyl group at C-4; however, the methyl group at C-7 in laingolide A was replaced by an exocyclic vinyl chloride, a group commonly found in cyanobacterial compounds such as in many ubiquitously occurring malyngamides.[31−35] The ^{13}C NMR chemical shifts were characteristic for an exomethylene group (δ_{C7} 138.0, δ_{C17} 112.8), yet the signal for H-17 integrated only for one proton due to the chlorine substitution. HMBC correlations from protons at H_2-8 (δ 2.32/2.36) to C-6, C-7, and C-17 as well as from the olefinic proton singlet at H-17 (δ 5.80) to C-6 (δ 30.0), C-7 (δ 138.0), and C-8 (δ 33.3) allowed unambiguous placement of this functionality (Table 5). The *E* geometry of the vinyl chloride in **6** was deduced on the basis of NOESY cross-peaks from H-17 to H-8b and H-9. Laingolide B (**6**) underwent degradation over time similar to its reported analogues, thus hampering the determination of the absolute configuration at its two asymmetric centers.

Table 4. NMR Data for Lyngbyabellin J (**5**) in $CDCl_3$ (600 MHz)

C/H no.	δ_C[a]	δ_H (*J* in Hz)	COSY	HMBC
1	173.9, C			
2	43.0, CH	3.00, dq (8.4, 6.8)	H_3-9	1, 3
3	74.4, CH	5.25, m		
4a	31.3, CH_2	1.81, m		5
4b		1.75, m		5
5	21.0, CH_2	1.69, m	H-6a, H-6b	4, 6
6a	49.1, CH_2	2.20, m	H-5, H-6b	
6b		2.10, m	H-5, H-6a	
7	90.7, C			
8	37.3, CH_3	2.09, s		6, 7
9	14.4, CH_3	1.22, d (6.8)	H-2	1, 3
10	[b]			
11	146.3, C			
12	128.5, CH	8.12, s		11, 13
13	165.8, C			
14	70.4, CH	6.45, t (6.3)	H-15a, H-15b	13, 25
15a	64.4, CH	4.95, dd (−11.3, 5.2)	H-14, H-15b	14, 16
15b		4.50, dd (−11.3, 6.8)	H-14, H-15a	14, 16
16	160.3, C			
17	145.9, C			
18	130.0, CH	8.24, s		17, 19
19	165.2, CH			
20	74.4, CH	5.70, s		1
21	74.0, C			
22	31.0, CH	1.64, m	H-23	
23	7.9, CH_3	0.92, t (6.2)	H-22	21
24	21.5, CH	1.07, s		21
25	169.4, C			
26a	36.6, CH_2	2.79, dd (−16.1, 3.9)	H-26b, H-27	25, 27
26b		2.70, dd (−16.1, 7.0)	H-26a, H-27	25, 27
27	69.6, CH	5.22, m	H-26a, H-26b, H-28	25, 26
28	55.1, CH	4.15, m	H-27, 28-NH	
28-NH		5.65, d (10.1)	H-28	
29	28.0, CH	1.85, m	H_3-30, H_3-31	28
30	20.0, CH_3	0.93, d (7.5)	H-29	29
31	16.5, CH_3	0.87, d (7.4)	H-29	29
32	173.4, C			
33	38.9, CH_2	2.12, t (7.4)	H-34	32
34	19.2, CH_2	1.60, m	H_2-33, H_2-35	
35	13.8, CH_3	0.87, t (7.9)	H_2-34	34
36	170.7, C			
37	21.0, CH_3	2.04, s		36

[a] Deduced from HSQC and HMBC experiments. [b] Not observed.

The LRESIMS spectrum of lyngbyapeptin D (**7**) showed a pseudomolecular ion peak for $[M + Na]^+$ at *m*/*z* 706.5, consistent with the molecular formula $C_{36}H_{53}N_5O_6S$ postulated on the basis of NMR data (Table 6). The ^1H NMR spectrum of **7** showed two *O*-Me singlets for methyl groups deshielded by anisotropy from unsaturated systems (δ_H 3.49 and 3.74) and three *N*-Me singlets for secondary amides (δ_H 2.95, 2.85, and 2.66) and suggested the presence of five C-Me groups, one of which was deshielded by an adjacent π system (δ_H 2.19) and attached to a nonprotonated carbon (singlet). 2D NMR analysis inclusive of COSY, HSQC, and HMBC (Table 6) suggested that compound **7** was comprised of three

Table 5. NMR Data for Laingolide B (**6**) in $CDCl_3$ (600 MHz)

C/H no.[a]	δ_C[b]	δ_H (*J* in Hz)	COSY	HMBC
1	175.8, C			
2	35.0, CH	3.02, ddq (10.8, 10.6, 6.5)	H-3a/3b, H_3-16	1, 3, 4, 16
3a	35.7, CH_2	1.63, m	H-2, H-3b, H-4a/4b	1, 2, 4, 5, 16
3b		1.44, m	H-2, H-3a, H-4a/4b	
4a	26.0, CH_2	1.40, m	H-3a/3b, H-4b, H-5	3, 5, 6
4b		1.18, m	H-3a/3b, H-4a	
5a/5b	26.0, CH_2	1.40, 2H, m	H-4a/4b, H-6a/6b	
6a	30.0, CH_2	2.46, m	H-5, H-6b, H-17	4, 5, 7, 8, 17
6b		1.90, m	H-5, H-6a, H-17	
7	138.0, C			
8a	33.3, CH_2	2.32, m	H-8b, H-9, H-17	6, 7, 9, 17, 18
8b		2.26, m	H-8a, H-9, H-17	
9	77.6, CH	4.96, dd (9.6, 1.8)	H-8a/8b	7, 9, 10, 11, 19
11	171.7, C			
12a	37.2, CH_2	3.09, ddd (−11.4, 5.6, 1.5)	H-12b, H-13, H-14	11, 13, 14
12b		2.94, dd (−11.4, 10.8)	H-12a, H-13, H-14	
13	103.6, CH	5.10, ddd (13.8, 10.8, 5.6)	H-12a/12b, H-14	11, 12, 14
14	133.1, CH	6.97, d (13.8)	H-12a/12b, H-13	1, 12, 13, 20
16	17.7, CH_3	1.16, d (6.5)	H-2	1, 2, 3
17	112.8, CH	5.80, s	H-6a/6b, H-8a/8b	6, 7, 8
18	34.3, C			
19	(25.8 × 3), CH_3	(0.93, s) × 3		9, 18
20	30.3, CH_3	3.10, s		1, 13

[a] Nomenclature for laingolide B adapted from laingolide and laingolide A.[20,21] [b] Deduced from HSQC and HMBC experiments.

Table 6. NMR Data for Lyngbyapeptin D (**7**) in $CDCl_3$ (600 MHz)

unit	C/H no.	δ_C[a]	δ_H (*J* in Hz)	COSY	HMBC
Mba	1	166.8, C			
	2	89.8, CH	5.12, s	H_3-4	
	3	[b]			
	4	19.0, CH_3	2.19, s	H-2	
	O-Me	51.0, CH_3	3.49, s		
N,*O*-diMe-Tyr	1	169.0, C			
	2	54.4, CH	5.75, dd (9.5, 6.2)	H-3a, H-3b	1, 3
	3a	34.6, CH_2	3.25, dd (−13.3, 9.5)	H-2, H-3b	2, 4, 5/9
	3b		2.71, dd (−13.3, 6.2)	H-2, H-3a	
	4	129.8, C			
	5/9	130.7, CH	7.17, d (8.2)	H-6/8	6/8, 7
	6/8	114.0, CH	6.76, d (8.2)	H-5/9	5/9, 7
	7	158.6, C			
	O-Me	54.5, CH_3	3.74, s		7
	N-Me	31.1, CH_3	2.95, s		2, 1 (Mba)
N-Me-Leu	1	171.1, C			
	2	50.7, CH	5.48, m	H-3a, H-3b	1, 3
	3a	37.9, CH_2	1.65, m	H-2, H-3b, H-4	2, 4
	3b		1.50, m	H-2, H-3a	
	4	24.5, CH	1.35, m	H-3a/3b, H_3-5, H_3-6	5, 6
	5	23.3, CH_3	0.93, d (6.5)	H-4	3, 4, 6
	6	22.1, CH_3	0.93, d (6.5)	H-4	3, 4, 5
	N-Me	30.0, CH_3	2.85, s		2, 1 (*N*,*O*-diMe-Tyr)
N-Me-Val	1	169.6, C			
	2	59.9, CH	4.88, d (11.5)	H-3	1, 3, 1 (*N*-Me Leu)
	3	27.6, CH	2.08, m	H-2, H_3-4, H_3-5	2
	4	18.7, CH_3	0.57, d (6.5)	H-3	2, 3, 5
	5	19.1, CH_3	0.88, d (6.5)	H-3	2, 3, 4
	N-Me	30.3, CH_3	2.66, s		2, 1 (*N*-Me-Leu)
Pro-thz	2	171.4, C			
	4	141.1, CH	7.69, d (2.5)	H-5	
	5	119.0, CH	7.24, d (2.5)	H-4	
	6	58.3, CH	5.45, m	H-7a, H-7b	2
	7a	31.8, CH_2	2.31, m	H-6, H-7b, H-8a, H-8b	
	7b		2.24, m	H-6, H-7a	
	8a	24.3, CH_2	2.13, m	H-7a, H-8b, H-9a, H-9b	
	8b		1.99, m	H-7a, H-8a, H-9a, H-9b	
	9a	47.5, CH_2	3.88, m	H-8a, H-8b, H-9b	
	9b		3.77, m	H-8a, H-8b, H-9a	

[a] Deduced from HSQC and HMBC experiments. [b] Not observed.

methylated proteinogenic amino acid residues, namely, *N*,*O*-diMe-tyrosine, *N*-Me-leucine, and *N*-Me-valine, and a proline-derived pyrrolidine substituted with a thiazole ring (Pro-thz). In addition **7** showed evidence for the presence of a 3-methoxy-2-butenoic acid (Mba) moiety. HMBC analysis together with NOESY experiments enabled the sequencing of all units as depicted for **7** (Figure 1), and the NOESY cross-peak between the *O*-Me and olefinic (H-2) signal of the Mba unit indicated an *E* configuration of the double bond. Thus compound **7** is a close analogue of lyngbyapeptin A where an *N*-Me-Ile was replaced with *N*-Me-Val (Figure 1).[6,22]

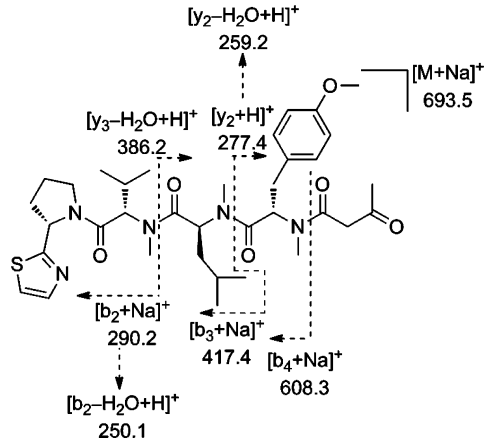

Figure 3. ESIMS/MS of the lyngbyapeptin D degradation product **7a**.

Table 7. Cytotoxic Activity (IC_{50}, μM) of Lyngbyalosides and Lyngbyabellins from Apra Harbor (Guam) against Two Cancer Cell Lines

structural class	compound	HT29	HeLa
lyngbyalosides	lyngbyaloside	37	35
	2-*epi*-lyngbyaloside (**1**)	38	33
	18*E*-lyngbyaloside C (**2**)	13	9.3
	18*Z*-lyngbyaloside C (**3**)	>100	53
lyngbyabellins	lyngbyabellin A	0.047	0.022
	27-deoxylyngbyabellin A (**4**)	0.012	0.0073
	lyngbyabellin B	1.1	0.71
	lyngbyabellin J (**5**)	0.054	0.041

Compound **7** suffered decomposition to **7a** by O-demethylation and subsequent tautomerization of the enol to the corresponding ketone (Figure 1). This was verified by HRESI/APCIMS analysis of the decomposition product (**7a**), which showed a pseudomolecular ion peak at *m/z* 692.3450 for $[M + Na]^+$, in agreement with the molecular formula $C_{35}H_{51}N_5O_6S$. The structure and amino acid sequence was further confirmed by ESIMS/MS of **7a** (Figure 3). The absolute configuration of **7a** (and thus indirectly of **7**) was established using enantioselective HPLC-MS analysis following ozonolysis, oxidative workup, and acid hydrolysis. Peaks corresponding to *N*-Me-L-Val, *N*-Me-L-Asp (from degradation of *N,O*-diMe-L-Tyr), *N*-Me-L-Leu, and L-Pro were detected, indicating the all-*S* configuration as in lyngbyapeptins A–C.[6,22,24]

Lyngbyalosides[17,18] and lyngbyabellins[5,6,19,26] have been reported to exhibit weak to moderate cytotoxicity toward cancer cells, respectively. Consequently we tested these compounds for antiproliferative activity against two cancer cell lines (Table 7). As expected, lyngbyalosides were only weakly active. Lyngbyaloside and its 2-epimer **1** had almost identical IC_{50} values of 33–38 μM against HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells, indicating that the configuration at C-2 is not critical for cytotoxicity. Compound **2** was approximately 3-fold more active and the most potent of the lyngbyalosides tested, while the C-18 regioisomer **3** was the least active and approximately 5-fold less potent against HeLa cells compared with **2** (Table 7). Minor toxicity was noted against HT29 cells at the highest concentration tested (100 μM). However, it was insufficient to provide an IC_{50} value, suggesting that the orientation of the bromine atom is a considerable determinant for cytotoxicity.

The lyngbyabellins are known to disrupt the actin cytoskeleton[5,26] and exhibited mostly nanomolar cytotoxicity in our assays. Lyngbyabellin B was the least active of this structural class, with IC_{50} values near 1 μM (Table 7). Lyngbyabellin A, its deoxy analogue **4**, and lyngbyabellin J (**5**) possessed similar activity, with **4** being slightly more potent (Table 7). The comparable activity for lyngbyabellin A and deoxy analogue **4** is consistent with data obtained by Gerwick and co-workers, who previously probed the effect of hydroxylation at that position in other lyngbyabellins and found no major differences.[26] The configuration of the hydroxy acid-derived unit esterified to the 7,7-dichloro-3-acyloxy-2-methyloctanoic acid residue (here Dhmpa) did not have a profound effect on the activity, as previously observed.[25] Furthermore, the cytotoxicity of cyclic and acyclic lyngbyabellins appears to be similar.[25,26] As mentioned above, laingolide B (**6**) and lyngbyapeptin D (**7**) decomposed during our characterization studies, preventing us from testing any bioactivity of these compounds.

In summary, *L. bouillonii* from Apra Harbor, Guam, continues to be a source of novel secondary metabolites, particularly halogenated compounds. In addition to the previously reported chemical diversity of structures from this cyanobacterium, the various compounds described here document the tremendous biosynthetic potential of cyanobacteria to produce small "focused" libraries of structural analogues, which may be exploited for drug discovery.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a SpectraMax M5 (Molecular Devices). ^{1}H and 2D NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II 600 MHz spectrometer equipped with a 1 mm triple-resonance high-temperature superconducting (HTS) cryogenic probe[14] using residual solvent signals (δ_H 7.26; δ_C 77.0 ppm $CDCl_3$) as internal standards. HSQC and HMBC experiments were optimized for $^1J_{CH}$ = 145 and $^nJ_{CH}$ = 7 Hz, respectively. HRMS data were obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector. Enantioselective LC-MS data were obtained on a 3200 Q TRAP (Applied Biosystems) connected to a Shimadzu LC system. ESIMS/MS data were obtained on a 3200 Q TRAP by direct infusion using a syringe driver.

Biological Material. The cyanobacterium *Lyngbya bouillonii* from Apra Harbor, Guam, was collected by snorkeling or scuba in shallow waters at Finger's Reef and Western Shoals. At both sites the cyanobacterium was encountered with the shrimp *Alpheus frontalis*. Finger's Reef collections were carried out in May 2005 (VP) and in May 2007 (1–4 m depth, SL-1). The VP collection is a re-collection of VP417 (16S rRNA gene sequence has been deposited under GenBank accession nos. AY049750 and AY049751),[9] and a voucher specimen is located at the Smithsonian Marine Station. A voucher sample of SL-1 has been given the accession number GH0011398 in the GUAM herbarium. A morphologically identical cyanobacterium containing the shrimp was collected from Western Shoals in January 2007 (1–3 m depth, WSL-1). A specimen of the WSL-1 collection is deposited in the GUAM herbarium (accession number GH0011397).

Dereplication Strategy. Cyanobacterial samples were fractionated as described below. Fractions from Si gel chromatography were analyzed by HPLC-PDA-MS and ^{1}H NMR and compared with authentic standards of all compounds previously isolated from *L. bouillonii* from Apra Harbor, Guam.[4–10,25] Novel chlorinated and brominated compounds were rapidly identified on the basis of their characteristic isotope clusters of pseudomolecular ions, and the close relationship to known lyngbyalosides and lyngbyabellins was established by ^{1}H NMR analysis.

Extraction and Isolation. *L. bouillonii* collected in 2005 from Finger's Reef (VP) was extracted with CH_2Cl_2 and MeOH (2:1) and the extract (10 g) chromatographed on silica gel with CH_2Cl_2 containing increasing concentrations of *i*-PrOH to afford 16 fractions. Fraction 5 (2% *i*-PrOH in CH_2Cl_2; 100 mg) and fractions 6 and 7 (5% *i*-PrOH in CH_2Cl_2; 71 and 70 mg) were individually subjected to semipreparative HPLC (Phenomenex Phenyl-hexyl, 250 × 10 mm, 5 μ, 2.0 mL/min; PDA detection) using a MeOH–H_2O linear gradient (90–100% MeOH for 30 min and 100% MeOH for 10 min). Fractions were pooled on the basis of retention times, ^{1}H NMR analysis, and low-resolution MS measurements to afford mixtures of lyngbyabellins A and B, lyngbyapeptin A, apratoxin A, and mixtures of lyngbyalosides (t_R 14–16 min; ~4 mg). Lyngbyalosides were purified by reversed-phase semipreparative HPLC (Phenomenex Synergi Hydro-RP, 250 × 10 mm, 5 μm, 2.0 mL/min; PDA detection) using the same program as described above to afford 2-*epi*-lyngbyaloside (**1**) (t_R 14.3 min, 0.5 mg), 18*E*-

lyngbyaloside C (**2**) (t_R 15.5 min, 2.5 mg), 18*Z*-lyngbyaloside C (**3**) (t_R 14.7 min, 0.2 mg), and lyngbyaloside (t_R 16.0 min, 0.2 mg).

The *L. bouillonii* sample collected from Finger's Reef in 2007 (SL-1) was freeze-dried and extracted with EtOAc−MeOH (1:1) to yield 16.1 g of crude material. The extract was subjected to flash chromatography on silica gel, eluting with CH_2Cl_2 followed by increasing concentrations of *i*-PrOH in CH_2Cl_2 and finally with MeOH. The fractions that eluted with 4% *i*-PrOH (305 mg), 6% *i*-PrOH (58 mg), and 8% *i*-PrOH (24.8 mg) were subjected to semipreparative reversed-phase HPLC (Phenomenex Ultracarb, ODS 250 × 10 mm, 5 μm, 3.0 mL/min; PDA detection; isocratic 80% MeCN(aq) for 30 min; 80−100% MeCN for 30−40 min; and 100% MeCN for 40−60 min) to afford several impure fractions (t_R 3−8 min, 13 mg; t_R 9−12 min, 7.4 mg; t_R 13−17 min, 3 mg; t_R 17 min, 6.4 mg; t_R 20−21 min, 4.6 mg) along with a lyngbyapeptin A-enriched fraction (t_R 12.0 min; 20.6 mg), mixtures of lyngbyalosides (t_R 16−17.0 min; 6.4 mg), laingolide B (**6**) (t_R 21.0 min; 3.5 mg), and apratoxin A (t_R 22.0 min; 1.0 mg).

The collection of *L. bouillonii* from Western Shoals (WSL-1) was freeze-dried and then extracted with EtOAc−MeOH (1:1) to yield 5.5 g of organic extract, which was subjected to flash chromatography on silica gel as described above. The fractions eluted with 4% *i*-PrOH (209 mg), 6% *i*-PrOH (50 mg), and 8% *i*-PrOH (15.7 mg) were further separated by semipreparative reversed-phase HPLC as described above for SL-1 to yield several impure fractions (t_R 3−11 min; 18.6 mg) along with semipure lyngbyapeptin A (t_R 12.5 min; 13.8 mg), lyngbyalosides mixtures (t_R 16−17.0 min; 6.2 mg), laingolide B (**6**) (t_R 21.0 min; 4.0 mg), and apratoxin A (t_R 22.0 min; 1.1 mg). Another fraction that eluted with 4% *i*-PrOH from silica gel (50 mg) also yielded minor amounts of apratoxin C (t_R 19.0 min; 0.5 mg) in addition to the above-mentioned compounds.

The final purification of the semipure compounds from either WSL-1 or SL-1 collection was achieved by semipreparative reversed-phase HPLC (Phenomenex Phenyl-hexyl, 250 × 10 mm, 5 μm, 2.0 mL/min; PDA detection) using a MeOH−H_2O linear gradient (90−100% MeOH for 30 min and 100% MeOH for 10 min), yielding laingolide B (**6**) (t_R 14.9 min; 3.5 mg), lyngbyapeptin D (**7**) (t_R 15.5 min; 0.2 mg), 27-deoxylyngbyabellin A (**4**) (t_R 18.0 min; 0.3 mg), lyngbyaloside C mixtures (t_R 14.5 min; ~1 mg), semipure lyngbyabellin J (t_R 12.3 min; 1 mg), lyngbyabellin A (t_R 13.3 min; 2 mg), lyngbyabellin B (t_R 14.7 min; 1 mg), and lyngbyapeptin A (t_R 16.5 min; 8 mg). In addition to the above-mentioned compounds, repurification of the semipure fractions obtained from the SL-1 collection (t_R 9−12 min, 7.4 mg, and t_R 17 min, 6.4 mg) also yielded lyngbyapeptin D (**7**) (t_R 15.5 min; 0.2 mg) and 27-deoxylyngbyabellin A (**4**), (t_R 18.0 min; 0.3 mg), respectively. The lyngbyabellin J-containing mixture was further purified using semipreparative reversed-phase HPLC (Phenomenex Synergi Hydro-RP, 250 × 10 mm, 5 μm, 2.0 mL/min; PDA detection) using a MeCN−H_2O linear gradient (70−100% MeOH for 30 min and 100% MeOH for 10 min) to yield lyngbyabellin J (**5**) (t_R 13.3 min; 0.4 mg).

2-*epi*-Lyngbyaloside (1): colorless, amorphous solid; $[\alpha]^{20}_D$ −24 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.40), 240 (3.57) nm; ^{1}H NMR, ^{13}C NMR, HMBC, and NOESY data, see Table 1; HRESI/APCIMS *m/z* $[M + Na]^+$ 683.2410 (calcd for $C_{31}H_{49}{}^{79}BrO_{10}Na$, 683.2401), 685.2382 (calcd for $C_{31}H_{49}{}^{81}BrO_{10}Na$, 685.2389) (100:100 $[M + Na]^+$ ion cluster).

18*E*-Lyngbyaloside C (2): colorless, amorphous solid; $[\alpha]^{20}_D$ −13 (*c* 0.13, MeOH); UV (MeOH); λ_{max} (log ε) 220 (3.15), 240 (3.23) nm. ^{1}H NMR, ^{13}C NMR, HMBC, and NOESY data, see Table 2; HRESI/APCIMS *m/z* $[M + Na]^+$ 671.2399 (calcd for $C_{30}H_{49}{}^{79}BrO_{10}Na$, 671.2401), 673.2381 (calcd for $C_{30}H_{49}{}^{81}BrO_{10}Na$, 673.2387) (100:100 $[M + Na]^+$ ion cluster).

18*Z*-Lyngbyaloside C (3): colorless, amorphous solid; $[\alpha]^{20}_D$ −21 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.13), 240 (3.28) nm; ^{1}H and ^{13}C NMR data, see Table 2; HRESI/APCIMS *m/z* $[M + Na]^+$ 671.2410 (calcd for $C_{30}H_{49}{}^{79}BrO_{10}Na$, 671.2401), 673.2381 (calcd for $C_{30}H_{49}{}^{81}BrO_{10}Na$, 673.2379) (100:100 $[M + Na]^+$ ion cluster).

27-Deoxylyngbyabellin A (4): colorless, amorphous solid; $[\alpha]^{20}_D$ −75 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.11), 230 (4.00), 280 (3.04) nm; ^{1}H NMR, ^{13}C NMR, COSY, and HMBC data, see Table 3; HRESI/APCIMS *m/z* $[M + H]^+$ 675.1843 (calcd for $C_{29}H_{41}{}^{35}Cl_2N_4O_6S_2$, 675.1845), *m/z* 675/677/679 (100:80:23 $[M + H]^+$ ion cluster).

Lyngbyabellin J (5): colorless, amorphous solid; $[\alpha]^{20}_D$ +25 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.59), 236 (3.72) nm; ^{1}H NMR, ^{13}C NMR, COSY, and HMBC data, see Table 4; HRESI/APCIMS *m/z* $[M + Na]^+$ 886.2198 (calcd for $C_{37}H_{51}{}^{35}Cl_2N_3O_{12}S_2Na$, 886.2183), *m/z* 886/888/890 (100:80:23 $[M + Na]^+$ ion cluster).

Laingolide B (6): colorless, amorphous solid; $[\alpha]^{20}_D$ +170 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.13) nm; ^{1}H NMR, ^{13}C NMR, COSY, and HMBC data, see Table 5; HRESI/APCIMS *m/z* $[M + H]^+$ 370.2147 (calcd for $C_{20}H_{33}{}^{35}ClNO_3$, 370.2147), *m/z* 370/372 (100:33 $[M + H]^+$ ion cluster).

Lyngbyapeptin D (7): colorless, amorphous solid; $[\alpha]^{20}_D$ −60 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.59), 250 (4.42) nm; ^{1}H NMR, ^{13}C NMR, COSY, and HMBC data, see Table 6; LRESIMS *m/z* $[M + Na]^+$ 706.5 (calcd for $C_{36}H_{53}N_5O_6SNa$, 706.3614).

Compound 7a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, J = 3.0 Hz, 1H), 7.27 (d, J = 2.8 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 8.9 Hz, 1H), 5.75 (dd, J = 9.2, 5.9 Hz, 1H), 5.48 (m, 2H), 4.89 (d, J = 11.9 Hz, 1H), 3.75−3.94 (m, 2H), 3.74 (s, 1H), 3.53 (d, J = 7.3 Hz, 1H), 3.23 (dd, J = 13.2, 9.7 Hz, 1H), 3.07 (m, 1H), 2.95 (s, 3H), 2.86 (s, 3H), 2.80 (s, 1H), 2.76 (d, J = 5.5 Hz, 1H), 2.68 (m, 1H), 2.66 (s, 3H), 2.21−2.29 (m, 3H), 2.19 (s, 3H), 2.05−2.18 (m, 2H), 1.90−2.03 (m, 3H), 1.25 (s, 1H), 0.86−0.98 (m, 10H), 0.55 (d, J = 6.47 Hz, 3H); HRESI/APCIMS *m/z* $[M + Na]^+$ 692.3450 (calcd for $C_{35}H_{51}N_5O_6SNa$, 692.3458).

Absolute Configuration of 5. A sample of **5** (200 μg) was dissolved in CH_2Cl_2 and ozonized at −78 °C for 30 min. The ozonized sample was divided into two portions, and the solvent was evaporated. One portion of the ozonized sample was subjected to base hydrolysis using 1 N KOH(aq) and MeOH (1:1) at 80 °C for 3 h. This was analyzed by enantioselective HPLC (CHIRALPAK MA (+) (4.6 × 50 mm), 0.5 mL/min, detection at 254 nm). The retention times for standard D-glyceric acid and L-glyceric acid were 8.6 and 7.4 min, respectively, using 0.5 mM $CuSO_4$. The base hydrolysate was determined to contain D-glyceric acid (8.6 min). Another portion of the base hydrolysate was analyzed using enantioselective HPLC-MS for 2,3-dihydroxy-3-methylpentanoic acid (Dhmpa) [column, Chirobiotic TAG (4.6 × 250 mm), Supelco; solvent, MeOH-10 mM NH_4OAc (60:40, pH 5.58); flow rate, 0.5 mL/min; detection by ESIMS in negative ion mode (MRM scan)]. (2*R*,3*S*)-Dhmpa eluted at 6.3 min. The retention times (t_R, min; MRM ion pair) of the authentic standards were as follows: (2*S*,3*R*)-Dhmpa (4.2; 147→57), (2*S*,3*S*)-Dhmpa (4.9), (2*R*,3*R*)-Dhmpa (5.6), (2*R*,3*S*)-Dhmpa (6.3). MS parameters were as follows: Dhmpa: DP −25.0, EP −4.0, CE −25.0, CXP −4.0, CEP −8.0; CUR 40, CAD medium, IS −4500, TEM 750, GS1 65, GS2 65. The absolute configuration of the Dhmpa unit was further confirmed by enantioselective HPLC (CHIRALPAK MA (+) (4.6 × 50 mm), 0.5 mL/min, detection at 254 nm) using a mobile phase of 2 mM $CuSO_4$−CH_3CN (95:5). The retention times (min) of Dhmpa standards were (2*R*,3*R*)-Dhmpa (19.0), (2*R*,3*S*)-Dhmpa (29.0), (2*S*,3*R*)-Dhmpa (46.0), and (2*S*,3*S*)-Dhmpa (47.0). The base hydrolysate was found to contain (2*R*,3*S*)-Dhmpa (29.0).

Another portion of the ozonized sample was hydrolyzed using 6 N HCl at 110 °C for 20 h. The hydrolysate was dried under N_2 and the residue dissolved in MeOH and ozonized at −78 °C for 30 min. After evaporation of the solvent under N_2, the residue was dissolved in H_2O_2−HCOOH (1:2) and left to stir overnight at room temperature before refluxing at 100 °C for 1 h. The solvent was removed, and the sample was analyzed by enantioselective HPLC-MS [column, Chirobiotic TAG (4.6 × 250 mm), Supelco; solvent, MeOH−10 mM NH_4OAc (40:60, pH 5.30); flow rate, 0.5 mL/min; detection by ESIMS in positive ion mode (MRM scan)]. L-Val eluted at 8.20 min. The retention times (t_R, min; MRM ion pair) of the authentic amino acids were as follows: L-Val (8.20; 118→72), D-Val (15.1). MS parameters were as follows: Val: DP 5.7, EP 9.0, CE 40.0, CXP 8.0, CEP 10.0; CUR 40, CAD high, IS 4500, TEM 750, GS1 65, GS2 65.

Acid Hydrolysis of 7a and Enantioselective Amino Acid Analysis by LC-MS. A sample of **7a** (50 μg) was dissolved in CH_2Cl_2 and subjected to ozonolysis at room temperature for 30 min. The solvent was evaporated, and the residue was treated with 0.6 mL of H_2O_2−HCOOH (1:2) at 70 °C for 20 min. The resulting oxidation product was concentrated to dryness and subsequently hydrolyzed with 0.6 mL of 6 N HCl at 110 °C for 20 h. The hydrolyzed product was dried, reconstituted in 100 μL of H_2O, and analyzed by enantioselective HPLC-MS [column, Chirobiotic TAG (4.6 × 250 mm), Supelco; solvent, MeOH−10 mM NH_4OAc (40:60, pH 5.23); flow rate, 0.5 mL/min; detection by ESIMS in positive ion mode (MRM scan)]. *N*-Me-L-Val, *N*-Me-L-Leu, and L-Pro eluted at t_R 14.1, 16.6, and 19.0 min,

respectively. The retention times (t_R, min; MRM ion pair) of the authentic amino acids were as follows: *N*-Me-L-Val (14.1; 132→86), *N*-Me-D-Val (47.9), *N*-Me-L-Leu (16.6; 146→100), *N*-Me-D-Leu (117.2), L-Pro (19.0; 116→70), D-Pro (61.5). Compound-dependent parameters were as follows: *N*-Me-Val: DP 29.4, EP 4.2, CE 17.4, CXP 2.7; *N*-Me-Leu: DP 32.4, EP 4.0, CE 17.1, CXP 2.49; Pro: DP 35.0, EP 7.7, CE 22.7, CXP 5.0. Source gas parameters used were as follows: CUR 30, CAD high, IS 4500, TEM 450, GS1 40, GS2 40. *N*-Me-Asp derived from *N*-Me-Tyr was detected using the negative ion mode with the same LC conditions (t_R 5.6 min in the hydrolysate). The retention times (t_R, min; MRM ion pair) of the authentic standards were as follows: *N*-Me-L-Asp (5.6; 146→102), *N*-Me-D-Asp (13.2). The MS parameters used were as follows: DP −24.4, EP −2.0, CE −17.3, CXP −13.4, CUR 30, CAD high, IS −4500, TEM 450, GS1 40, GS2 40.

Cell Viability Assay. HT29 colorectal adenocarcinoma and HeLa cervical carcinoma cells were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone) under a humidified atmosphere with 5% CO_2 at 37 °C. HeLa cells (3000/well) and HT29 cells (12 500/well) were seeded in 96-well plates. Upon 24 h incubation, cells were treated with varying concentrations of **1**−**5**. The cells were incubated for an additional 48 h, and cell viability was measured using MTT reagent according to the manufacturer's instructions (Promega). Data shown are from duplicate experiments.

Acknowledgment. This research was supported by the National Institutes of Health (NIGMS grant P41GM086210 to H.L. and V.J.P.) and the James & Esther King Biomedical Research Program (Grant No. 06-NIR07 to H.L.). P.J.S. acknowledges NIH MBRS SCORE grant S06-GM-44796 for support. The authors gratefully acknowledge NSF for funding through the External User Program of the National High Magnetic Field Laboratory (NHMFL), which supported some of the NMR studies at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute of the University of Florida (UF). The 600 MHz, 1 mm triple-resonance HTS cryogenic probe was developed through collaboration between UF, NHMFL, and Bruker Biospin. We thank P. Williams for providing standards of 2,3-dihydroxy-3-methylpentanoic acid. This is contribution #660 of the University of Guam Marine Laboratory and contribution #829 from the Smithsonian Marine Station at Fort Pierce.

Supporting Information Available: NMR spectra for compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tan, L. T. *Phytochemistry* **2007**, *68*, 954–979.
(2) Hoffmann, L.; Demoulin, V. *Belg. J. Bot.* **1991**, *124*, 82–88.
(3) Luesch, H. The search for new anticancer drugs from marine cyanobacteria and investigations on the biosynthesis of cyanobacterial metabolites. Ph.D. Thesis, University of Hawaii, Manoa, HI, 2002.
(4) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418–5423.
(5) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L. *J. Nat. Prod.* **2000**, *63*, 611–615.
(6) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 1437–1439.
(7) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 1702–1706.
(8) Matthew, S.; Schupp, P. J.; Luesch, H. *J. Nat. Prod.* **2008**, *71*, 1113–1116.
(9) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Bioorg. Med. Chem.* **2002**, *10*, 1973–1978.
(10) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 1106–1112.
(11) Banner, D. M.; Banner, A. H. *Rec. Aust. Mus. Suppl.* **1982**, *34*, 359–362.
(12) Cruz-Rivera, E.; Paul, V. J. *Proceedings of 9th International Coral Reef Symposium*, Bali, Indonesia, October 23−27, 2000; International Society for Reef Studies: Melbourne, Florida.
(13) Cruz-Rivera, E.; Paul, V. J. *Coral Reefs* **2006**, *25*, 617–627.
(14) Brey, W.; Edison, A. S.; Nast, R. E.; Rocca, J. R.; Saha, S.; Withers, R. S. *J. Magn. Reson.* **2006**, *179*, 290–293.
(15) Liu, Y.; Law, B. K.; Luesch, H. *Mol. Pharmacol.* **2009**, *76*, 91–104.
(16) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *J. Nat. Prod.* **1997**, *60*, 1057–1059.
(17) Tan, L. T.; Márquez, B. L.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 925–928.
(18) Luesch, H.; Yoshida, W. Y.; Harrigan, G. G.; Doom, J. P.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 1945–1948.
(19) Milligan, K. E.; Marquez, B. L.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 1440–1443.
(20) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *Tetrahedron Lett.* **1996**, *37*, 7519–7520.
(21) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *J. Nat. Prod.* **1999**, *62*, 934–936.
(22) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Castillo, G.; Demoulin, V. *Tetrahedron Lett.* **1999**, *40*, 695–696.
(23) Sone, H.; Kigoshi, H.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 8956–8960.
(24) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Tetrahedron* **2002**, *58*, 7959–7966.
(25) Williams, P. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 595–598.
(26) Han, B.; McPhail, K. L.; Gross, H.; Goeger, D. E.; Mooberry, S. L.; Gerwick, W. H. *Tetrahedron* **2005**, *61*, 11723–11729.
(27) Yokokawa, F.; Sameshima, H.; Katagiri, D.; Aoyama, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 9445–9458.
(28) Pang, H.; Xu, Z.; Chen, Z.; Ye, T. *Lett. Org. Chem.* **2005**, *2*, 699–702.
(29) Marquez, B. L.; Watts, K. S.; Yokochi, A.; Roberts, M. A.; Verdier-Pinard, P.; Jimenez, J. I.; Hamel, E.; Scheuer, P. J.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 866–871.
(30) Preciado, A.; Williams, P. G. *J. Org. Chem.* **2008**, *73*, 9228–9234.
(31) Cardellina, J. H.; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240–242.
(32) Milligan, K. E.; Márquez, B.; Williamson, R. T.; Davies-Coleman, M.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 965–968.
(33) Appleton, D. R.; Sewell, M. A.; Berrid, M. V.; Copp, B. R. *J. Nat. Prod.* **2002**, *65*, 630–631.
(34) Nogle, L. M.; Gerwick, W. H. *J. Nat. Prod.* **2003**, *66*, 217–220.
(35) Kwan, J. C.; Teplitski, M.; Gunasekera, S. P.; Paul, V. J.; Luesch, H. *J. Nat. Prod.* **2010**, *73*, 463–466.

NP1004032