

Antimalarial Peptides from Marine Cyanobacteria: Isolation and Structural Elucidation of Gallinamide A

Roger G. Linington,^{*,†,‡,§,||} Benjamin R. Clark,[†] Erin E. Trimble,[‡] Alejandro Almanza,[‡] Luis-David Ureña,[‡] Dennis E. Kyle,^{‡,§} and William H. Gerwick^{†,⊥}

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, INDICASAT, Clayton, Edificio 175, PO Box 7250, Panamá 5, Panamá, Department of Global Health, College of Public Health, University of South Florida, 3720 Spectrum Boulevard, Suite 304, Tampa, Florida 33612, and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, California 92093

Received June 13, 2008

As part of a continuing program to identify novel treatments for neglected parasitic diseases, the Panama International Cooperative Biodiversity Group (ICBG) program has been investigating the antimalarial potential of secondary metabolites from Panamanian marine cyanobacteria. From over 60 strains of cyanobacteria evaluated in our biological screens, the organic extract of a *Schizothrix* species from a tropical reef near Piedras Gallinas (Caribbean coast of Panama) showed potent initial antimalarial activity against the W2 chloroquine-resistant strain of *Plasmodium falciparum*. Bioassay-guided fractionation followed by 2D NMR analysis afforded the planar structure of a new and highly functionalized linear peptide, gallinamide A. Subsequent degradation and derivatization methods were used to determine the absolute configuration at most stereogenic centers in this unusual new metabolite.

Antimalarial therapy continues to suffer from a lack of international attention in comparison to diseases of developed countries such as cancer and heart disease. The for-profit nature of most commercial drug discovery programs precludes malaria as a viable therapeutic target because the majority of the victims of malaria live in countries with very low per capita incomes.¹ By contrast, academic research efforts are driven by different motivations and can thus investigate and hopefully contribute important lead molecules for treatment of this disease.² In line with this concept the Panama ICBG program is exploring the natural products diversity of plants, endophytic fungi, and marine cyanobacteria in the search for new antimalarial drug leads using an assay against *Plasmodium falciparum*.^{3,4}

While many of the current frontline antimalarial drugs are originally of plant origin (e.g., quinine, artemisinin), there has been relatively little investigation of the contributions that marine natural products may play in the discovery of lead compounds against malaria.^{5–11} Given the level of success that has been achieved from terrestrial natural products and the tremendous untapped biosynthetic resource that the marine environment represents, we hypothesize that antimalarial drug discovery from marine organisms has the potential to produce potent and selective lead compounds. As an extension of this hypothesis, we are more broadly evaluating cyanobacterial extracts against a panel of tropical parasites, including malaria, leishmaniasis, Chagas disease, and dengue fever, as well as for cytotoxicity to cancer cells. Initial hits are prioritized by their profile of activities across this panel of screens prior to further chemical and biological investigation.

Results and Discussion

During a field expedition to the Portobelo Marine Park on the Caribbean coast of Panama, a small quantity of a red-tipped *Schizothrix* sp. was collected by hand using scuba from a shallow

reef near Piedras Gallinas. The organic extract from this material (556 mg) was subjected to our standard prefractionation protocol.⁴ These fractions were screened against a panel of tropical parasites including the W2 chloroquine-resistant strain of *Plasmodium falciparum*. The fraction eluting with 2:8 hexanes/EtOAc showed activity against the malaria parasite (IC₅₀ = 1 μg/mL) and was thus evaluated by HPLC-MS and subsequently fractionated by HPLC to give 3.4 mg of compound **1** as a clear glass, which was given the common name gallinamide A. Pure gallinamide A was evaluated in the *P. falciparum* assay and shown to possess moderate antimalarial activity (IC₅₀ = 8.4 μM).

The HRESITOFMS spectrum of gallinamide A (**1**) gave an [M + H]⁺ ion at *m/z* 593.3907 that was consistent with the pseudo-molecular formula C₃₁H₅₃N₄O₇ (calcd for C₃₁H₅₃N₄O₇, 593.3909). ¹H and ¹³C NMR analysis showed that it was peptidic in nature; however, there were several resonances that were not attributable to the common ribosomally encoded amino acids, implying that **1** possessed a highly functionalized and interesting structure. The presence of a sharp singlet proton resonance (δ_H 2.25, δ_C 41.7) that integrated for six protons and showed self-correlation in the HMBC spectrum strongly suggested that **1** possessed an *N,N*-dimethyl terminal residue, a motif with significant precedence in cyanobacterial natural products chemistry.¹² Interpretation of the 2D NMR data confirmed this assignment and identified the presence of an *N,N*-dimethylisoleucine residue (subunit **a**, Figure 1). Standard 2D NMR analyses using a combination of COSY, HSQC, HMBC, and TOCSY experiments identified the presence of an isopropionic acid moiety (subunit **b**, Figure 1). Consideration of the multiplicity edited HSQC experiment revealed two broad doublets not attached to carbon that integrated for one proton each (δ_H 6.70, 6.77). By COSY, the resonance at δ_H 6.77 could be attributed to the amide NH proton of a leucine residue (subunit **c**, Figure 1). The second exchangeable resonance at δ_H 6.70 gave a COSY correlation to a multiplet at δ_H 4.55, which in turn showed COSY correlations to a methyl doublet at δ_H 1.22 and a doublet of doublets at δ_H 6.88. Finally, this spin system was completed by a COSY correlation from δ_H 6.88 to a sharp doublet at δ_H 7.33. Interpretation of these connectivities in conjunction with HMBC information allowed assignment of this subunit as 4-(*S*)-amino-2-(*E*)-pentenoic acid (Apa), as depicted in subunit **d** of Figure 1. The *E* geometry for the olefin in subunit **d** was determined by coupling constant analysis

* To whom correspondence should be addressed. Tel: (831) 459-3014. Fax: (831) 459-2935. E-mail: rogerl@chemistry.ucsc.edu.

[†] Scripps Institution of Oceanography.

[‡] INDICASAT, Panamá.

[§] University of South Florida.

[⊥] Skaggs School of Pharmacy and Pharmaceutical Sciences.

^{||} Current address: Department of Chemistry and Biochemistry, University of California, Santa Cruz.

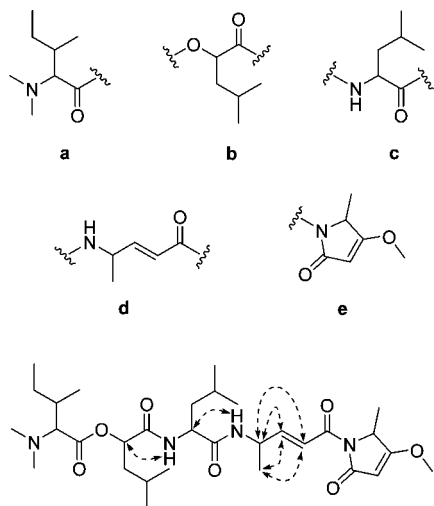
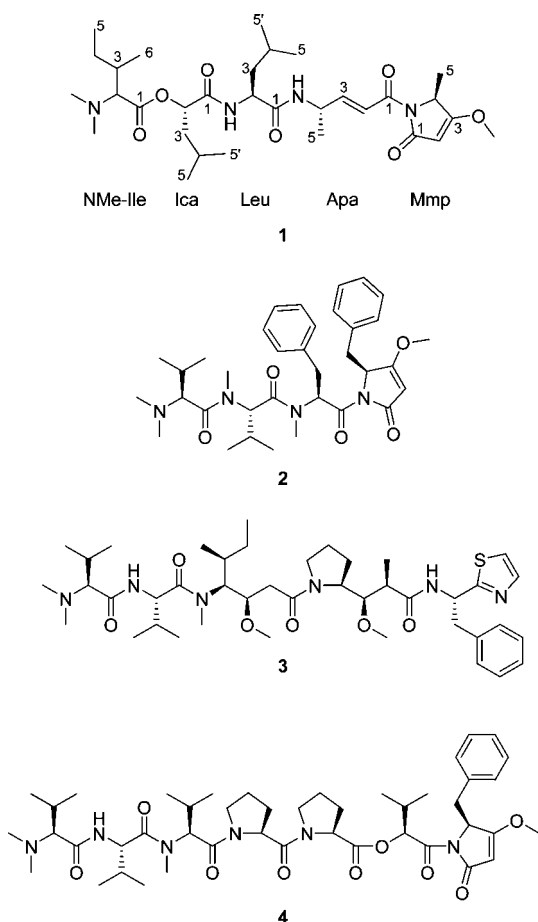


Figure 1. Subunits a–e and selected ROESY correlations for **1**.

of the olefinic protons at δ_{H} 6.88 and 7.33 ($^3J_{\text{HH}} = 15.7$ Hz). This was confirmed by ROESY correlations from Apa-5 to both olefinic protons (Apa-2 and Apa-3), which is only possible for the *E* configuration (for the *Z* isomer, the Apa-2 to Apa-5 distance is greater than 5 Å for all possible conformations; see Supporting Information).



Consideration of the molecular formula by subtracting the atoms comprising subunits a–d identified the remaining subunit (e) to be of $\text{C}_6\text{H}_8\text{NO}_2$ composition. The presence of a quaternary carbonyl carbon at δ 170.8, a methine carbon at δ 93.7, and a quaternary carbon at δ 182.1 was strongly indicative of a trisubstituted β -oxygenated α,β -unsaturated amide-type carbonyl group. HMBC

correlations from a methyl doublet at δ 1.42 (Mmp-5) and a methine quartet at δ 4.56 (Mmp-4) to the quaternary carbon at δ 182.1 suggested the presence of a modified alanine residue, and an additional HMBC correlation from a methyl singlet at δ 3.85 to the quaternary carbon at δ 182.1 provided evidence for the presence of a methoxy group attached to this latter quaternary center. Consideration of the remaining atoms of this subunit required the presence of a nitrogen atom, which could only be installed between carbons Mpp-1 and Mpp-4 to form a methylmethoxy-pyrrolinone moiety (Mmp) as the C-terminal residue of gallinamide A (**1**).

Subunits a–e were connected using a combination of HMBC correlations from the α -protons and amide NH resonances to the amide carbonyls and ROESY correlations between α -protons and amide NH resonances to give the assembled planar structure of **1** as depicted in Figure 1.

Stereoanalysis of **1** was accomplished using two complementary approaches. Determination of the configuration for subunits c, d, and e was achieved by oxidative ozonolysis followed by acid hydrolysis and Marfey's analysis, which showed the exclusive presence of L-alanine and L-leucine. The configuration of subunit b was determined by sequential basic and acidic hydrolyses followed by chiral HPLC and comparison with commercially available standards of *R*- and *S*-isocaproic acid. Co-injections showed **1** to contain exclusively *S*-isocaproic acid. Due to lack of material, a similar analytical approach was not successful in determining the configuration of subunit a despite exploring numerous conditions; however, based on the precedent that all marine natural products containing an *N,N*-dimethyl terminal amino acid residue possess the L configuration at this center^{13–21} (e.g., belamide A (**2**)) and that all but one of these have been shown to derive from cyanobacteria, it is likely that the configuration at this position in **1** is also L.

Gallinamide A (**1**) was tested for its antimalarial activity against the W2 chloroquine-resistant strain of the malaria parasite. Compound **1** showed moderate *in vitro* activity against *Plasmodium falciparum* ($\text{IC}_{50} = 8.4 \mu\text{M}$), cytotoxicity to mammalian Vero cells ($\text{TC}_{50} = 10.4 \mu\text{M}$), and activity against *Leishmania donovani* ($\text{IC}_{50} = 9.3 \mu\text{M}$). Compound **1** was inactive up to the highest tested concentrations against *Trypanosoma cruzi* ($16.9 \mu\text{M}$) (for positive controls and activities for all bioassays see the Supporting Information).

Marine cyanobacteria are proving a valuable source of antimalarial lead compounds of diverse structure types, including alkylated phenols, alkaloids, and cyclic⁴ and linear peptides.³ Of these, gallinamide A most closely resembles the structures of the linear peptides dolastatin 10 (**3**) and 15 (**4**), which have been shown to exhibit both antimalarial and mammalian cell antiproliferative effects.²² Intriguingly, despite showing moderate cytotoxicity to Vero cells ($\text{TC}_{50} = 10.4 \mu\text{M}$) gallinamide A shows no *in vitro* cytotoxicity toward NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma cell lines up to the highest tested concentrations ($16.9 \mu\text{M}$). This is in stark contrast to dolastatin 15 (**4**), which exhibits both nanomolar antimalarial activity and a TC_{50} against the NCI-H460 human lung tumor cell line of 2.4 ± 1.2 nM. In addition we have recently found symprostatin 1,²³ a close analogue to dolastatin 10, to be active in our Panama-based antimalarial assays (data not shown). Thus, it appears that linear peptides with terminating *N,N*-dimethylvaline or *N,N*-dimethylisoleucine groups are a class of therapeutic agents that exert antiparasitic as well as antiproliferative activities. In the case of gallinamide A, activity against both *P. falciparum* and human host cells is significantly lower in potency than either dolastatin 15 or symprostatin 1. One possible rationalization of this observation is that these compounds work by fundamentally different modes of action; however the number of antimalarial lead compounds isolated by our program from the marine environment that possess dolastatin-like structural motifs suggests that this class of compounds share a degree of

commonality in their interactions with *P. falciparum*. As previously reported by Fennell et al.,²² dolastatins 10 and 15 and synthetic analogues exhibited varying relative levels of activity against mammalian cells and *P. falciparum*; however the study failed to identify any compounds with greater potency against *P. falciparum* than host cells.²² By contrast, gallinamide A is a reasonably effective antimalarial whose relative potency against parasites (8–10 μM) versus mammalian cells (generally >17 μM) suggests that this structural framework could be an attractive foundation for further SAR investigations.

The isolation of gallinamide A (**1**) represents an important addition to the current understanding of cyanobacterial secondary metabolite chemistry.^{12,24–26} Compound **1** contains the unusual 4-(*S*)-amino-2-(*E*)-pentenoic acid subunit that has little precedent in nature^{27–29} and has never been previously isolated from the marine environment. The presence of a methyl-methoxyproline moiety at the *C*-terminus is also relatively unusual, with only a handful of natural products containing this subunit having been reported.^{30–32} It is also intriguing to recognize a tandem repeat of ketide-extended alanine residues (residues 4 and 5) in gallinamide A, the first of which is reduced and dehydrated from its intermediate β -carbonyl, and the second is enolized, *O*-methylated, and then cyclized to the pyrrolinone system. It may be that a gene duplication event was involved in the creation of these tandem NRPS-PKS modules. The inclusion of structural motifs from several cyanobacterial natural products in a single compound makes gallinamide an interesting discovery from a biosynthetic perspective whose origins would be intriguing to investigate.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Rudolf Research Analytical Autopol II polarimeter. UV spectra were acquired on a Shimadzu UV2401-PC spectrophotometer. IR spectra were obtained on a Thermo Electron Nicolet IR100 spectrophotometer. NMR spectra were acquired on a JEOL Eclipse 400 MHz spectrometer and referenced to residual solvent proton and carbon signals (δ_{H} 1.94, δ_{C} 118.2 for CD_3CN). Low-resolution APCI mass spectra were acquired on a JEOL LC-mate mass spectrometer (INDI-CASAT). Accurate mass ESI mass spectra were acquired on an Agilent ESI-TOF mass spectrometer (The Scripps Research Institute). HPLC purifications were performed on an Agilent 1100 series HPLC system employing a G1312A binary gradient pump, a G1322A degasser, and a G1314A variable wavelength detector tuned to 210 nm with a Phenomenex Jupiter C₁₈ (4.6 \times 250 mm) RP-HPLC column. All solvents were HPLC grade and were used without further purification.

Collection. The cyanobacterium *Schizothrix* sp. (44.3 g dry wt) was collected by hand using scuba from a depth of 12–15 m near Piedras Gallinas (09°33.799' N 79°41.642' W) in the Portobelo National Marine Park, Colon Province, on the North coast of Panama. The cyanobacterium was strained through a mesh bag to remove excess seawater, frozen on site, and stored at –4 °C until workup. The taxonomy was identified by comparison with characteristics described by Geitler.³³ A voucher was deposited at the Smithsonian Tropical Research Institute, Panama (voucher number PAP-04-OCT-05-2).

Extraction and Isolation. Freshly thawed material was extracted exhaustively with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (2:1, 6 \times 500 mL), and the combined organic extracts were partitioned against H_2O (300 mL) and concentrated to dryness *in vacuo* to give 556 mg of a dark brown gum. This material was subjected to flash Si gel CC (Aldrich, Si gel 60, 230–400 mesh, 40 \times 180 mm) eluting with 100% hexanes (300 mL); 9:1 hexanes/EtOAc (300 mL); 8:2 hexanes/EtOAc (300 mL); 6:4 hexanes/EtOAc (300 mL); 4:6 hexanes/EtOAc (300 mL); 2:8 hexanes/EtOAc (300 mL); 100% EtOAc (300 mL); 3:1 EtOAc/MeOH (300 mL); and 100% MeOH (300 mL). The fraction eluting with 2:8 hexanes/EtOAc showed strong antimalarial activity (1 $\mu\text{g}/\text{mL}$) and so was passed through a C₁₈ SPE cartridge coupled to a 0.22 μm nylon filter eluting with 100% MeOH. The eluent was concentrated *in vacuo*, and the resulting brown gum subjected to C₁₈ RP-HPLC (Phenomenex Jupiter C₁₈ 4.6 \times 250 mm RP-HPLC column, 5 μm , 67% MeOH/33% H_2O , 210 nm, 1 mL/min, t_{R} 32.6 min) to give **1** as a colorless glass (3.4 mg, 0.6% of crude extract).

Table 1. NMR Data for Gallinamide A (**1**) (CD_3CN)

residue	position	δ_{H}^a	mult. (Hz)	δ_{C}^b
<i>N,N</i> -diMe-Ile	1			171.8, qC
	2	2.91	d, 10.6	72.9, CH
	3	1.80	m	34.1, CH
	4	1.12, 1.65	m	25.6, CH ₂
	5	0.87	d, 7.7	10.6, CH ₃
	6	0.82	d, 7.0	16.2, CH ₃
Ica	NMe	2.25	s	41.7, CH ₃
	1			171.1, qC
	2	4.99	m	73.2, CH
	3	1.52, 1.73	m	41.6, CH ₂
	4	1.66	m	23.4, CH
	5	0.92 ^c		25.3 ^c , CH ₃
Leu	5'	0.94 ^c		25.6 ^c , CH ₃
	1			172.1, qC
	2	4.33	dd, 8.0, 14.6	52.3, CH
	3	1.56, 1.60	m	42.0, CH ₂
	4	1.65	m	25.5, CH
	5	0.92	d, 7.0	21.8, CH ₃
Apa	5'	0.92	d, 7.0	21.9, CH ₃
	NH	6.77	d, 8.1	
	1			164.8, qC
	2	7.33	dd, 1.8, 15.7	123.0, CH
	3	6.88	dd, 5.1, 15.7	149.4, CH
	4	4.55	m	46.9, CH
Mmp	5	1.22	d, 7.0	20.1, CH ₃
	NH	6.70	d, 7.7	
	1			170.8, qC
	2	5.09	s	93.7, CH
	3			182.1, qC
	4	4.56	m	56.4, CH
	5	1.42	d, 6.6	17.3, CH ₃
	OMe	3.85	s	59.6, CH ₃

^a Recorded at 400 MHz. ^b Recorded at 100 MHz. ^c Resonances interchangeable.

Gallinamide A (1): colorless glass; $[\alpha]_{\text{D}}^{25} -22.5$ (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.95), 247 (3.92) nm; IR (CH_2Cl_2) ν_{max} 3282, 2960, 1731, 1653, 1622 cm^{-1} ; for ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z $[\text{M} + \text{H}]^+$ 593.3907 (calcd for C₃₁H₅₃N₄O₇, 593.3909).

Ozonolysis of 1. A stream of ozone gas (4% O₃ in O₂, 0.0625 L/min) was bubbled through a solution of **1** (0.1 mg in 200 μL CH_2Cl_2) at room temperature for 5 min. The solvent was removed under N₂ and dried *in vacuo* for 1 h. Subsequent hydrolysis and Marfey's analysis of the corresponding ozonate was performed as described below.

Marfey's Analysis of 1. The ozonate of **1** (0.1 mg, 0.2 μmol) was treated with 6 N HCl in a sealed vial at 120 °C for 18 h. The solution was concentrated to dryness *in vacuo* and treated with a solution of 1-fluoro-2,4-dinitrophenyl-5-L-valine-amide (FDVA) (0.25 mg, 0.8 μmol) in acetone (50 μL) and a solution of 0.1 M NaHCO₃ (100 μL) in a sealed vial at 90 °C for 5 min. The reaction mixture was neutralized with 2 N HCl (50 μL) and diluted with CH_3CN (100 μL). The resulting solution was analyzed by RP-HPLC employing a HP LiChrospher 100 RP-18 (5 μm , 4 \times 125 mm) column and a gradient elution profile of 15% $\text{CH}_3\text{CN}/85\%$ H_2O (acidified with 0.05% HCOOH) to 50% $\text{CH}_3\text{CN}/50\%$ H_2O (acidified with 0.05% HCOOH) over 45 min at a flow of 0.8 mL/min, monitoring at 340 nm. Comparison with commercially available amino acid standards derivatized using identical methodology established **1** as containing exclusively L-alanine (17.8 min) and L-leucine (25.2 min). Retention times in minutes for the derivatized amino acid standards were as follows: L-alanine 17.8; D-alanine 21.6; L-leucine 25.2; D-leucine 31.3.

Chiral HPLC Analysis of 1. An authentic sample of **1** (0.1 mg, 0.2 μmol) was treated with 1 M NaOH/MeOH (1:1) for 18 h at 25 °C, concentrated to dryness *in vacuo*, and then treated with 6 N HCl in a sealed vial at 120 °C for 18 h. The resulting hydrolysate was analyzed by chiral HPLC employing a Phenomenex Chirex-D column (4.6 \times 50 mm) eluting with 85% 2 mM CuSO₄/15% MeCN at a flow of 0.8 mL/min, monitoring at 228 nm. Comparison with commercially available standards established **1** as containing exclusively *S*-isocaproic acid (6.4 min). The identity of *S*-isocaproic acid in **1** was confirmed by co-injection. Retention times in minutes for commercially available standards were as follows: *S*-isocaproic acid 6.4; *R*-isocaproic acid 8.1.

Acknowledgment. We thank the Government of Panama for support of the International Cooperative Biodiversity Group (ICBG) program, J. Wingerd for NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma bioassay data, I. Torres and A. O’Dea for assistance with the collection of the cyanobacterium, A. O’Dea for source organism photographs, and D. Newman for assistance with the NCI database. Financial support was provided by the Fogarty International Center’s International Cooperative Biodiversity Groups (ICBG) program in Panama (ICBG TW006634), the National Center on Minority Health and Health Disparities CREO program (5T37MD001480-13), and the SENACYT Estimulo program (EST06-O-022).

Supporting Information Available: ¹H and ¹³C NMR spectra of **1**, interpretation of ROESY correlations for the Apa subunit, complete biological screening data, and a color photograph of the field-collected *Schizothrix* sp. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Nwaka, S.; Ridley, R. G. *Nat. Rev. Drug Discovery* **2003**, *2*, 919–928.
- (2) Gelb, M. H. *Curr. Opin. Chem. Biol.* **2007**, *11*, 440–445.
- (3) McPhail, K. L.; Correa, J.; Linington, R. G.; González, J.; Ortega-Barría, E.; Capson, T. L.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 984–988.
- (4) Linington, R. G.; González, J.; Ureña, L.-D.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 397–401.
- (5) Giddens, A. C.; Nielsen, L.; Boshoff, H. I.; Tasdemir, D.; Perozzo, R.; Kaiser, M.; Wang, F.; Sacchetti, J. C.; Copp, B. R. *Tetrahedron* **2008**, *64*, 1242–1249.
- (6) Fattorusso, E.; Tagliatalata-Scafati, O. *Stud. Nat. Prod. Chem.* **2005**, *32*, 169–207.
- (7) Wright, A. D.; Lang-Unnasch, N. *Planta Med.* **2005**, *71*, 964–966.
- (8) Laurent, D.; Pietra, F. *Mar. Biotechnol.* **2006**, *8*, 433–447.
- (9) Hamann, M. T. *Curr. Pharm. Des.* **2007**, *13*, 653–660.
- (10) Laurent, D.; Jullian, V.; Parenty, A.; Knibiehler, M.; Dorin, D.; Schmitt, S.; Lozach, O.; Lebouvier, N.; Frostin, M.; Alby, F.; Maurel, S.; Doerig, C.; Meijer, L.; Sauvain, M. *Bioorg. Med. Chem.* **2006**, *14*, 4477–4482.
- (11) Wang, G. Y.; Tang, W. P.; Bidigare, R. R. In *Natural Products as Therapeutic Drugs and Preventive Medicines*; Zhang, L., Demain, A. L., Eds.; Humana Press: New Jersey, 2005; pp 191–221.
- (12) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 2001; Vol. 57, pp 75–184.
- (13) Pettit, G. R.; Singh, S. B.; Hogan, F.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463–5465.
- (14) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005–6006.
- (15) Sone, H.; Nemoto, T.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8445–8448.
- (16) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874–1880.
- (17) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod.* **2001**, *64*, 907–910.
- (18) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod.* **2002**, *65*, 16–20.
- (19) Horgen, F. D.; Kazmierski, E. B.; Westenburg, H. E.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2002**, *65*, 487–491.
- (20) Mugishima, T.; Tsuda, M.; Kasai, Y.; Ishiyama, H.; Fukushi, E.; Kawabata, J.; Watanabe, M.; Akao, K.; Kobayashi, J. *J. Org. Chem.* **2005**, *70*, 9430–9435.
- (21) Simmons, T. L.; McPhail, K. L.; Ortega-Barria, E.; Mooberry, S. L.; Gerwick, W. H. *Tetrahedron Lett.* **2006**, *47*, 3387–3390.
- (22) Fennell, B. J.; Carolan, S.; Pettit, G. R.; Bell, A. J. *Antimicrob. Chemother.* **2003**, *51*, 833–841.
- (23) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1075–1077.
- (24) Tidgewell, K.; Clark, B. R.; Gerwick, W. H. In *Comprehensive Natural Products Chemistry*; Pergamon Press, 2009 (in press); Vol. 8.
- (25) Tan, L. T. *Phytochemistry* **2007**, *68*, 954–979.
- (26) Van Wagoner, R. M.; Drummond, A. K.; Wright, J. L. C. *Adv. Appl. Microbiol.* **2007**, *61*, 89–217.
- (27) Oka, M.; Nishiyama, Y.; Ohta, S.; Kamei, H.; Konishi, M.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1988**, *41*, 1331–1337.
- (28) Oka, M.; Ohkuma, H.; Kamei, H.; Konishi, M.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1988**, *41*, 1906–1909.
- (29) Terui, Y.; Nishikawa, J.; Hino, H.; Kato, T.; Shoji, J. *J. Antibiot.* **1990**, *43*, 788–795.
- (30) Reichenbach, H.; Hoefle, G.; Gerth, K. (Gesellschaft fuer Biotechnologische Forschungsb.H. (GBF), Germany). Application: DE, 1998.
- (31) Paik, S.; Carmeli, S.; Cullingham, J.; Moore, R. E.; Patterson, G. M. L.; Tius, M. A. *J. Am. Chem. Soc.* **1994**, *116*, 8116–8125.
- (32) Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336–6343.
- (33) Geitler, L. *Cyanophyceae*; Koeltz Scientific Books: Koenigstein, Germany, 1932.

NP8003529