Symplocamide A, a Potent Cytotoxin and Chymotrypsin Inhibitor from the Marine Cyanobacterium *Symploca* sp.

Roger G. Linington,[†‡▽] Daniel J. Edwards,[§⊥▽] Cynthia F. Shuman,[†] Kerry L. McPhail,[§] Teatulohi Matainaho,[∥] and William H. Gerwick*[,†§]

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California 92093, Instituto de Investigaciones Científicas Avanzados y Servicios de Alta Tecnología, INDICASAT, Clayton, Edificio 175, P.O. Box 7250, Panamá 5, Panamá, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, and Discipline of Pharmacology, School of Medicine and Health Sciences, University of Papua New Guinea, National Capital District, Papua New Guinea

Received June 12, 2007

Investigation of a *Symploca* sp. from Papua New Guinea has led to the isolation of symplocamide A (**1**), a potent cancer cell cytotoxin, which also inhibits serine proteases with a 200-fold greater inhibition of chymotrypsin over trypsin. The complete stereostructure of symplocamide A was determined by detailed NMR and MS analysis as well as chiral HPLC analysis of the component amino acid residues. The presence of several unusual structural features in symplocamide A provides new insights into the pharmacophore model for protease selectivity in this drug class and may underlie the potent cytotoxicity of this compound to H-460 lung cancer cells (IC_{50} = 40 nM) as well as neuro-2a neuroblastoma cells (IC_{50} = 29 nM).

Marine cyanobacteria are a continuing source of many novel natural product structures, some of which possess highly potent biological properties.[1] Most of these metabolites are partially composed of amino acids, and these are often integrated with polyketide sections to make a wide range of nitrogen-rich lipids. These lipopeptide backbones are often modified by oxidation, methylation, or various halogenations, including those catalyzed by the recently described radical halogenases.[2,3] There is a growing body of literature that supports the hypothesis that the presence of halogen atoms in natural products enhances their biological potency.[4,5] Hence, we have been interested in isolating new types of halogenated lipopeptides from marine cyanobacteria with the goals of further defining the extent of their structural diversity and biological properties.

Proteases are implicated in the pathogenesis of many human diseases, including cancer and Alzheimer's, and thus the therapeutic modulation of proteolytic activity offers an attractive potential treatment modality. Indeed, it has been estimated that of the approximately 400 human proteases known in 2001, 14% were under pharmaceutical investigation.[6] In addition, proteases from infectious pathogens such as HIV, HCV, and dengue virus are crucial drug targets for the improvement of human health. However, with this multitude of different proteases and many potential therapeutic applications, discovery of agents with selectivity for specific proteases is crucial to the development of truly useful pharmaceuticals in this class.

While freshwater cyanobacteria are a rich source of structurally diverse protease inhibitors,[7] their marine relatives represent an under-explored resource of this enzyme-inhibitor class. Hence, we have initiated a program to survey marine cyanobacterial extracts, fractions, and newly isolated pure compounds for interesting profiles of protease inhibition. This line of research has been productive and led to the identification of the depsipeptide symplocamide A (**1**), which shows strong serine protease inhibitory activity. In addition, symplocamide A shows a high level of cytotoxicity to cancer cells in vitro (H-460 lung cancer cells, IC_{50} = 40 nM; neuro-2a neuroblastoma cells, IC_{50} = 29 nM). These biological properties combined with its unusual structure, including *N,O*-dimethylbromotyrosine and citrulline residues, make symplocamide A a valuable and insightful addition to the 3-amino-6-hydroxypiperidone (Ahp) class of depsipeptides.

Results and Discussion

The marine cyanobacterium *Symploca* sp. was collected by hand using scuba from a depth of 25 m from Sunday Island in Papua New Guinea. Prefractionation of the crude extract by normal-phase vacuum liquid chromatography (NP-VLC) gave nine subfractions, the most polar of which (100% MeOH) was subjected to gradient reversed-phase solid-phase extraction (RP-SPE) followed by final HPLC purification to afford **1** as a pale yellow solid (3.4 mg).

Symplocamide A (**1**) gave an $[M + H]^+$ pseudomolecular ion at *m/z* 1051.4457 by HRESITOFMS, which was consistent with the molecular formula $C_{46}H_{72}{}^{79}BrN_{10}O_{13}$. ^1H and ^{13}C NMR data suggested that **1** was of peptidic nature due to the presence of five exchangeable doublets between δ_H 7.33 and 8.51, consistent with amide NH protons, and eight methine protons between δ_H 4.24 and 5.05 consistent with the presence of α-protons. Standard NMR analysis using a combination of ^1H, ^{13}C, gCOSY, gHSQC, and gHMBC experiments quickly established the presence of four standard amino acids plus one short chain fatty acid (subunits **a**−**e**, Figure 2). In addition to these straightforward assignments three unusual fragments were also identified (**f**−**h**, Figure 2).

Consideration of the COSY data starting from the amide proton at δ_H 7.33 indicated a contiguous spin system incorporating the NH, a methine, and two methylenes and terminating in an oxygen-bearing methine (δ_H 4.91) as depicted in subunit **f**. HMBC correlations from two methine protons at δ_H 4.44 and 4.91 to the same carbonyl carbon at δ_C 168.8 indicated that this substructure was cyclized and confirmed the presence of a 3-amino-6-hydroxypiperidone (Ahp) functionality, a well-known structural component of cyanobacterial metabolites.

In an analogous fashion, consideration of the COSY and HSQC correlations for subunit **g** starting with the amide proton at δ_H 8.51 indicated the presence of a linear amino acid terminating in a

* To whom correspondence should be addressed. Tel: (858) 534-0578. Fax: (858) 534-0529. E-mail: wgerwick@ucsd.edu.
† University of California San Diego.
‡ INDICASAT, Panamá.
▽ These authors contributed equally to the work.
§ Oregon State University.
∥ University of Papua New Guinea.
⊥ Present address: Department of Chemistry, Physical Science Building, California State University Chico, Chico, CA 95929-0210.

10.1021/np070280x CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 12/29/2007

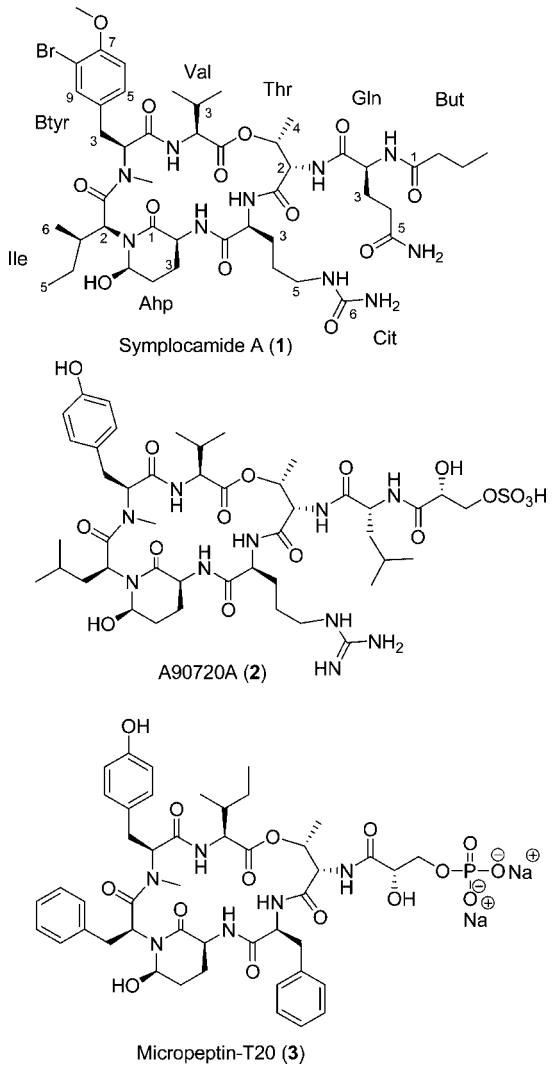

Figure 1. Structure of symplocamide A (**1**) and related compounds.

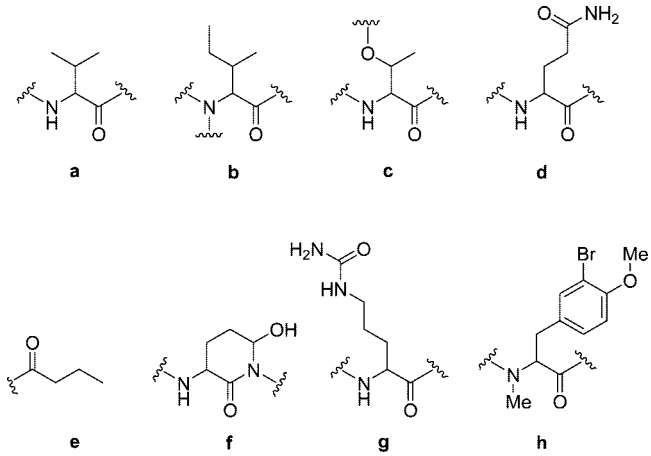

Figure 2. Subunits for **1** determined by NMR analysis.

nitrogenous functional group. The NMR signals of this subunit bore strong resemblance to those for arginine; however, the presence of a carbonyl carbon at δ_C 158.7 in conjunction with the absence of one NH signal from the terminal guanidino group identified subunit **g** as a citrulline residue.[8]

Finally, the presence of three nonequivalent aromatic methine signals (δ_H 7.02, d, δ_C 113.0; δ_H 7.19, dd, δ_C 130.1; δ_H 7.39, d, δ_C 133.6) as well as the presence of a further three quaternary aromatic carbons (δ_C 111.0, 131.3, 154.5), all of which showed a variety of homo- and heteronuclear coupling interactions with one another (Table 1), strongly suggested the presence of a tyrosine moiety possessing an additional aromatic substituent. HMBC correlations between an *N*Me singlet at δ_H 2.73 and the α-carbon at δ_C 60.4 and between an *O*Me singlet at δ_H 3.76 and a quaternary carbon at δ_C 154.5 indicated that the tyrosine residue was present as its *N*,*O*-dimethyl derivative. Given that all ^{1}H and ^{13}C NMR signals were now accounted for in subunits **a**−**h** and that the HREIMS molecular formula included one bromine atom, this final subunit was therefore assigned as *N,O*-dimethyl-3-bromotyrosine. The structures of these subunits were further verified by 1D-TOCSY experiments, which confirmed the presence of the predicted contiguous spin systems in all cases where isolated proton signals were available for selective irradiation (see Supporting Information).

Subunits **a**−**h** were assembled into one linear fragment as shown in Figure 3 by extensive consideration of HMBC and ROESY correlations in both DMSO-d_6 and MeOH-d_4. The number of double-bond equivalents calculated from the molecular formula called for the presence of one additional ring, suggesting that **1** was a cyclic peptide. However, the α-protons for both Gln and Ile occurred at the same chemical shift (δ_H 4.37) and gave rise to two possible sites for the cyclization of **1** (Figure 3). Fortunately, an HMBC signal from Ahp-5 (δ_H 4.91) to Ile-2 (δ_C 54.2) provided evidence for the direct connectivity of these two subunits, thus proving that the linear subunit was cyclized, as indicated by the solid arrows in Figure 3, and completing the planar structure of symplocamide A (**1**).

Stereoanalysis of the standard amino acids in **1** was accomplished by acid hydrolysis followed by Marfey's analysis and comparison with commercially available standards, and identified the presence of L-valine, L-isoleucine, L-glutamine (analyzed as the corresponding glutamic acid), L-threonine, and L-citrulline (analyzed as the corresponding ornithine). In order to determine the stereochemistry of the Ahp moiety, **1** was subjected to oxidation using PCC/CH_2Cl_2 followed by acid hydrolysis and Marfey's analysis as outlined above. The exclusive presence of L-glutamic acid in the hydrolysate showed that the Ahp moiety also possessed the L-configuration.

Using the advanced Marfey's method with HPLC-MS analysis[9] we were able to demonstrate that the novel amino acid *N,O*-dimethylbromotyrosine also possessed an L-configuration. Direct Marfey's analysis using LC-MS identified an HPLC peak of *m*/*z* 568/570 with a retention time of 64.8 min. Subsequent hydrolysis and racemization of the constituent amino acids for **1** using literature procedures[9] provided a mixture of both D and L isomers, which were also analyzed using an identical LC-MS protocol. In this instance two peaks of *m*/*z* 568/570 were observed at 64.8 and 68.0 min. Given that L enantiomers consistently elute before D enantiomers in situations where the Marfey's derivatives adopt specific conformations (as indicated by a strong λ_{max} at 340 nm),[9] we were able to confidently assign the configuration of the *N,O*-dimethylbromotyrosine moiety as L.

The last remaining undefined stereocenter was that of the alcohol-bearing carbon at position 5 on the Ahp residue. ROESY correlations between δ_H 4.44 (Ahp-2) and δ_H 2.57 (Ahp-3′), and between δ_H 2.57 (Ahp-3′) and δ_H 6.14 (Ahp-OH), showed that the α-proton (Ahp-2) and the alcohol (Ahp-OH) must reside on the same face of the six-membered ring, thus establishing the *R* absolute configuration, consistent with the analysis and configuration of other Ahp-containing peptides.[10,11]

On the basis of the structural similarity of symplocamide A (**1**) to protease inhibitors discovered from freshwater and marine cyanobacteria, as well as some terrestrial sources,[12] it was proposed that **1** may inhibit serine proteases. Structural studies of the related compounds A90720A (**2**)[13] and scyptolin A,[14] bound to trypsin and elastase, respectively, show that the residue immediately

Table 1. NMR Data for Symplocamide A (**1**) Acquired in DMSO-d_6

position	δ_H (J in Hz)[a]	δ_C, mult.[b]	HMBC
Val 1		172.3, qC	
2	4.67, m	56.2, CH	Val-1, Val-3, Val-4, Val-5
3	2.04, m	30.7, CH	Val-2, Val-4, Val-5
4	0.86, d (7.0)	19.3, CH_3	Val-2, Val-3, Val-5
5	0.74, d (7.0)	17.6, CH_3	Val-2, Val-3, Val-4
NH	7.65, d (10.2)		Btyr-1
Btyr 1		169.0, qC	
2	5.05, dd (11.1, 2.7)	60.4, CH	Btyr-1, Btyr-3, Btyr-NMe
3	2.77, dd (11.1, 14.8)	32.8, CH_2	Btyr-4, Btyr-5
3′	3.21 dd (14.8, 2.7)	32.8, CH_2	Btyr-4, Btyr-5
4		131.3, qC	
5	7.19, dd (1.8, 8.4)	130.1, CHx	Btyr-3, Btyr-6, Btyr-7, Btyr-9
6	7.02, d (8.4)	113.0, CH	Btyr-5, Btyr-7, Btyr-8
7		154.5, qC	
8		111.0, qC	
9	7.39, d (1.8)	133.6, CH	Btyr-3, Btyr-4, Btyr-7, Btyr-8
OMe	3.76, s	56.3, CH_3	Btyr-7
NMe	2.73, s	30.2, CH_3	Ile-1, Btyr-2
Ile 1		169.7, qC	
2	4.37, m	54.2, CH	Ahp-5, Ile-3, Ile-6
3	1.79, m	33.0, CH	
4	1.09, m	23.7, CH_2	Ahp-5, Ile-2
5	0.62, m	10.3, CH_3	Ile-3, Ile-4
6	−0.16, d (6.7)	13.8, CH_3	Ile-2, Ile-3, Ile-4
Ahp 1		168.8, qC	
2	4.44, m	48.9, CH	Ahp-1, Ahp-3
3	1.74/2.57, m	21.8, CH_2	
4	1.74, m	29.7, CH_2	
5	4.91, bs	74.0, CH	Ahp-1, Ile-2
OH	6.14, bs		
NH	7.33, d (9.3)		Cit-1
Cit 1		170.2, qC	
2	4.24, ddd (4.0, 9.4, 9.4)	52.4, CH	
3	1.42/1.95, m/ m	27.6, CH_2	
4	1.34, m	26.6, CH_2	Cit-2, Cit-5
5	2.92, dt (6.0, 6.3)	38.9, CH_2	Cit-3, Cit-4, Cit-6
NH′	5.99, bt (6.0)		
6		158.7, qC	
NH2	5.37, bs		
NH	8.51, d (7.9)		Thr-1, Cit-2
Thr 1		169.0, qC	
2	4.63, m	54.8, CH	Gln-1, Thr-1, Thr-3
3	5.50, q (6.3)	71.9, CH	Val-1, Thr-1, Thr-4
4	1.20, d (6.7)	17.7, CH_3	Thr-2, Thr-3
NH	7.84, d (9.3)		Gln-1
Gln 1		172.5, qC	
2	4.37, m	52.0, CH	But-1, Gln-1, Gln-3
3	1.72/1.89, m/ m	27.3, CH_2	Gln-1, Gln-2, Gln-4, Gln-5
4	2.13, m	31.5, CH_2	Gln-2, Gln-3, Gln-5
5		173.9, qC	
NH2	6.75/7.29, bs/ bs		Gln-4, Gln-5
NH	8.05, d (8.0)		But-1, Gln-2, Gln-3
But 1		172.3, qC	
2	2.11, t (7.0)	37.1, CH_2	But-1, But-3, But-4
3	1.52, m	18.7, CH_2	But-1, But-2, But-4
4	0.86, t (7.0)	13.6, CH_3	But-2, But-3

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz.

following the Ahp unit should bind in the S1 specificity pocket (according to the terminology of Schechter and Berger[15]), as shown in Figure 4. Due to the presence of the bulky and slightly basic citrulline moiety in this position for **1**, and the preference of trypsin for basic residues (Arg and Lys) and chymotrypsin for large hydrophobic residues (Tyr, Phe, Trp) at this position, it was unclear whether **1** would inhibit trypsin or chymotrypsin; hence, this was experimentally tested. Symplocamide A (**1**) inhibited trypsin with an IC_{50} value of 80.2 ± 0.7 μM and chymotrypsin with an IC_{50} value of 0.38 ± 0.08 μM, a greater than 200-fold difference in IC_{50} values.

Of the more than 100 compounds in this structural class, about half have been reported to inhibit trypsin or chymotrypsin (Table 2). In general, a basic residue (L-Arg or L-Lys) at the P1 position in the inhibitor is necessary for trypsin inhibition at concentrations under 10 μM. The influence of the residue at this position on chymotrypsin inhibition is less clear for this class of compounds. While a number of active compounds possess large hydrophobic residues (Tyr, Phe) at this position, smaller hydrophobic and neutral side chains (Leu, Abu, HcAla, Gln) are also tolerated. The most potent chymotrypsin inhibitor in this class, micropeptin-T20 (**3**), was the only example with L-Phe at this position.[16] Symplocamide A (**1**) is one of the most potent chymotrypsin inhibitors from this class of Ahp-containing depsipeptides (Table 2). The citrulline moiety at the proposed P1 position of **1** has not previously been reported within this class of compounds and may contribute to the specificity for chymotrypsin over trypsin. However, the specificity and potency of these serine protease inhibitors are also expected to be influenced by interactions with more distal regions of the proteases. Thus, the potency of **1** for chymotrypsin may also be

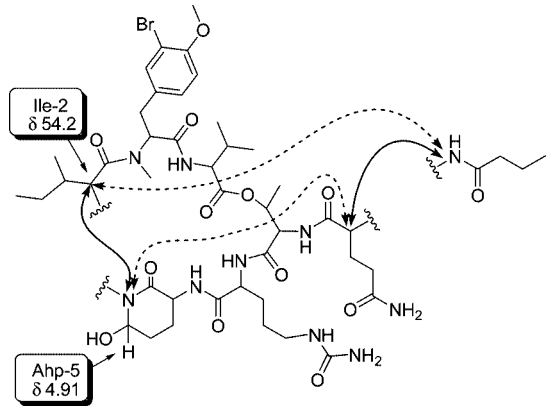

Figure 3. Two possible bond connections (solid versus dashed arrows) for the construction of **1**. The correct formulation is depicted with the solid arrows (determined by HMBC between Ahp-5 and Ile-2; see text and Table 1).

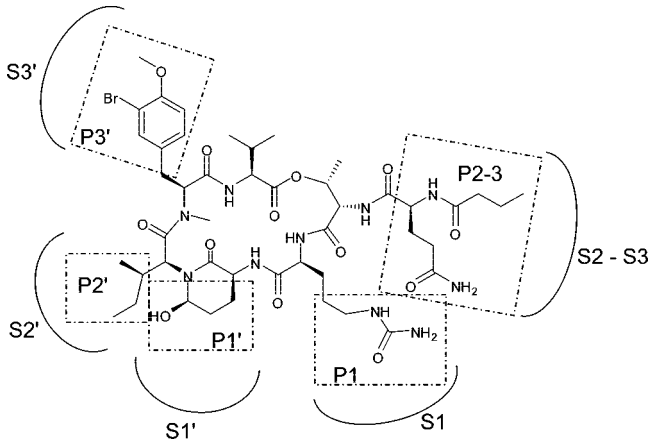

Figure 4. Proposed binding sites of **1** in the active site of serine proteases.

influenced by the *N,O*-dimethylbromotyrosine moiety at residue P3′ as well as the gln-butanoyl side chain of the compound. The overall specificity of this class of protease inhibitors toward serine proteases other than trypsin and chymotrypsin is not reflected in this limited data set. For example, many of the compounds that inhibit trypsin have also been found to inhibit thrombin or plasmin, and many that inhibit chymotrypsin also inhibit elastase. Should more symplocamide be obtained through total synthesis or reisolation, it would be of great value to explore further the inhibitory activity of **1** toward a wider range of proteases.

The biological activity of symplocamide A (**1**) was evaluated in several other assays. Compound **1** showed cytotoxicity to NCI-H460 nonsmall lung cancer cells (IC_{50} = 40 nM) and neuro-2A mouse neuroblastoma cells (IC_{50} = 29 nM). Additionally, **1** was screened against three tropical parasites with the following results: malaria (W2 *Plasmodium falciparum*, IC_{50} = 0.95 μM), Chagas disease (*Trypanasoma cruzi*, IC_{50} > 9.5 μM), and leishmaniasis (*Leishmania donovani*, IC_{50} > 9.5 μM).

Symplocamide A (**1**) represents an important addition to the Ahp-containing class of depsipeptides. The presence of a citrulline residue has not previously been reported for this class of compounds, and the *N,O*-diMe-Br-Tyr moiety is an unusual amino acid residue rarely found in nature.[35] Symplocamide A displays an unusual biological profile, with a high selectivity index for chymotrypsin over trypsin (>200-fold). There is an ever-growing recognition of the importance of proteases in cancer,[36] and protease inhibitors are emerging as an exciting new class of anticancer agents. It is tempting to speculate that there may be a connection between the potent chymotrypsin inhibition of **1** and the observed cytotoxicity to H-460 cancer cells. As proteosomal degradation in cells is known to include a chymotrypsin-like activity, and because proteosome inhibition has shown promise for the treatment of some cancers, it would be of interest to investigate the potential of symplocamide A more generally as a proteosome inhibitor.[37] The Ahp-containing protease inhibitors have the potential to play an important role as lead compounds for rational drug design.[7] The addition of symplocamide A (**1**) to this group provides further information about the structural features required for activity of this class of compounds and highlights the value of natural products as tools for developing a greater understanding of small-molecule−protein interactions.

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. NMR spectra were acquired on Bruker Avance 400 and 600 MHz spectrometers and referenced to residual solvent ^1H and ^{13}C signals (δ_H 2.49, δ_C 39.5 for DMSO-d_6; δ_H 3.30, δ_C 49.5 for MeOH-d_4). Low-resolution APCI mass spectra were acquired on a JEOL LC-mate mass spectrometer. Accurate mass measurements were acquired on an Agilent ESI-TOF mass spectrometer. HPLC-MS studies for Marfey's analysis were performed on an Agilent 1100 series HPLC system employing a G1312A binary gradient pump, a G1322A degasser, a G1314A variable-wavelength detector tuned to 340 nm coupled to a JEOL LC-mate mass spectrometer, and a Phenomenex Jupiter C_{18} (4.6 × 250 mm) RP-HPLC column. All solvents were HPLC grade and were used without further purification. HPLC isolation was performed using a Waters 515 binary pump, a Waters 996 PDA detector, and a Phenomenex Synergi Hydro RP-HPLC column (10 × 250 mm).

Enzymes and substrates were obtained from Sigma-Aldrich. The protease activity and inhibition assays were modified from the Sigma protocol to accommodate reaction volumes of 100–110 μL. Assays were run on a Cary50 UV spectrophotometer (Varian).

Isolation Procedure. The marine cyanobacterium *Symploca* sp. (voucher specimen available from WHG as collection number PNG-12/15/03-5) was collected from a depth of 25 m from Sunday Island in Papua New Guinea (S 09°10.171′, E 150°50.667′). The sample was stored in 1:1 H_2O/EtOH and frozen at −20 °C until workup. This material was extracted exhaustively with 2:1 CH_2Cl_2/MeOH and concentrated to dryness in vacuo to give a dark green gum. VLC of the extract used a stepwise gradient (100% hexanes; 9:1 hexanes/EtOAc; 8:2 hexanes/EtOAc; 6:4 hexanes/EtOAc; 4:6 hexanes/EtOAc; 2:8 hexanes/EtOAc; 100% EtOAc; 3:1 EtOAc/MeOH; and 100% MeOH). The 100% MeOH fraction was further purified by RP_{18} SPE (stepwise gradient from 1:1 H_2O/MeOH to 100% MeOH) followed by HPLC purification to give symplocamide A (**1**) as a pale yellow glass (3.4 mg, 0.003% dry weight).

PCC Oxidation. To a stirred solution of **1** (0.1 mg) in CH_2Cl_2 (100 μL) was added pyridinium chlorochromate (2 mg), and the resulting orange solution was stirred at room temperature for 16 h. The reaction mixture was quenched with water (200 μL) and the organic phase removed. The aqueous phase was washed with CH_2Cl_2 (2 × 500 μL), and the combined organics were concentrated to dryness under a stream of N_2 gas prior to Marfey's analysis as outlined below.

Marfey's Analysis General Procedure. Hydrolysates and commercially available standards were separately derivatized by treatment with a solution of N_α-(2,4-dinitro-5-fluorophenyl)-L-valinamide (FDVA) (0.25 mg, 0.8 μmol) in acetone (50 μL) and a solution of 0.1 M $NaHCO_3$ (100 μL) in sealed vials at 90 °C for 5 min. The reaction mixtures were acidified with 2 N HCl (50 μL) and diluted with CH_3CN (100 μL). The resulting solutions were analyzed by RP-HPLC employing a Phenomenex Jupiter C_{18} column (4.6 × 250 mm) and a gradient elution profile of 15:85 CH_3CN/H_2O (acidified with 0.02% HOAc) to 1:1 CH_3CN/H_2O (acidified with 0.02% HOAc) over 70 min at a flow of 0.5 mL/min, monitoring at 340 nm.

Racemization of Symplocamide A (1) Hydrolysate. Symplocamide A (**1**, 0.1 mg, 0.1 μmol) was stirred with 6 N HCl in a sealed vial at 110 °C for 18 h and the solvent removed in vacuo. The resulting solid was dissolved in H_2O (200 μL), and triethylamine (80 μL) and acetic anhydride (80 μL) were added sequentially. The reaction mixture

Table 2. Structure–Activity Relationships for All Ahp-Containing Depsipeptides with Inhibitory Activity against Trypsin or Chymotrypsin[a]

	protease inhibition (μM)		primary structure								
compound	trypsin	chymotrypsin	residue 1	residue 2	residue 3	AHP	residue 5	Thr	residue 7	residue 8	side chain
micropeptin T-20[16]	nt	0.0025	L-Ile	N-Me-L-Tyr	L-Phe	L-Ahp	L-Phe	L-Thr	D-Gac-Phos		
cyanopeptolin 954[17]	ni	0.04	L-Val	NMe-Cl-L-Tyr	L-Phe	Ahp	L-Leu	L-Thr	L-Gln		Ac
nostopeptin BN920[12]	ni	0.11	L-Val	NMe-L-Tyr	L-Phe	Ahp	L-Leu	L-Thr	L-Gln		Ac
lyngbyastatin 4[18]	ni	0.30	L-Val	NMe-Tyr	Phe	L-Ahp	Abu	L-Thr	L-Htyr	L-Ala	D-Gac-sulfate
symplocamide A	**80.2**	**0.38**	**L-Val**	**NMe, OMe- L-Br-Tyr**	**L-Ile**	**Ahp**	**L-Cit**	**L-Thr**	**L-Gln**		**But**
micropeptin 88A[19]	ni	0.40	L-Ile	NMe-L-Phe	L-Val	Ahp	HcAla	L-Thr	L-Glu		
planktopeptin BL1125[1]	ni	0.80	L-Ile	NMe, OMe-L-Tyr	L-Thr	Ahp	L-Leu	L-Thr	L-Gln	L-Htyr	L-Gac
cyanopeptolin 963A[20]	ni	0.90	L-Val	NMe- L-Phe	L-Leu	Ahp	L-Tyr	L-Thr	L-Asp		Hex
micropeptin 103[21]	ni	0.96	L-Val	NMe-L-Trp	L-Phe	Ahp	L-Gln	L-Thr	L-Thr	Gly	Hex
micropeptin 88Y[22]	ni	1.30	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Tyr	L-Thr	L-Glu	L-Tyr	Ac
nostopeptin A[23]	ni	1.47	L-Ile	NMe-L-Tyr	L-Ile	Ahp	L-Leu	Hmp	L-Gln		But
nostopeptin B[23]	ni	1.73	L-Ile	NMe-L-Tyr	L-Ile	Ahp	L-Leu	Hmp	L-Gln		Ac
oscillapeptin B[24]	nt	1.78	L-allo-Ile	NMe-OMe-L-Tyr	L-Ile	Ahp	L-7-Me-Htyr	L-Thr	L-Htyr		D-Mgs
oscillapeptin D[24]	nt	1.95	L-allo-Ile	NMe-L-Phe	L-Ile	Ahp	HcAla	L-Thr	D-Htyr		D-Mgs
oscillapeptin A[25]	ni	1.89	L-allo-Ile	NMe-OMe-L-Tyr	L-Ile	Ahp	L-Htyr	L-Thr	L-Htyr		D-Mgs
planktopeptin BL1061[1]	ni	2.10	L-Ile	NMe-OMe-L-Tyr	L-Thr	Ahp	L-Leu	L-Thr	L-Gln	L-Leu	L-Gac
micropeptin SD979[26]	ni	2.40	L-Val	NMe-L-Tyr	L-Ile	Ahp	L-Tyr	L-Thr	L-Asp		Hex
oscillapeptin E[24]	nt	2.64	L-allo-Ile	NMe-L-Phe	L-Ile	Ahp	L-Htyr	L-Thr	D-Htyr		D-Mgs
oscillapeptin C[24]	nt	2.82	L-allo-Ile	NMe-L-Phe	L-Ile	OMe-Ahp	HcAla	L-Thr	D-Htyr		D-Mga
micropeptin T1[12]	ni	2.85	L-Val	NMe-L-Trp	L-Phe	Ahp	L-Tyr	L-Thr	L-Glu		Hex
micropeptin 88F[19]	ni	3.02	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Tyr	L-Thr	OMe-L-Glu	L-Phe	But
micropeptin SD1002[26]	ni	3.13	L-Val	NMe-L-Trp	L-Ile	Ahp	L-Tyr	L-Thr	L-Asp		Hex
nostopeptin J[27]	ni	3.2	L-Ile	NMe-OMe-L-Tyr	L-Leu	OMe-Ahp	L-Leu	Hmp	L-Gln		Ac
nostopeptin I[27]	ni	3.5	L-Ile	NMe-OMe-L-Tyr	L-Leu	Ahp	L-Leu	Hmp	L-Gln		Ac
nostopeptin E[27]	ni	3.6	L-Ile	NMe-L-Tyr	L-Leu	Ahp	L-Leu	Hmp	L-Gln		Ac
nostopeptin F[27]	ni	3.8	L-Ile	NMe-L-Tyr	L-Leu	OMe-Ahp	L-Leu	Hmp	L-Gln		Ac
largamide F[28]	ni	4.00	L-Val	L-NMe-Br-Tyr	L-Thr	L-Ahp	L-Tyr	L-Thr	L-Val	L-Ala	L-Ahppa, D-Gac
micropeptin SF909[29]	ni	4.40	L-Ile	L-NMe-Tyr	L-Leu	Ahp	L-Gln	L-Thr	L-Hpla		
micropeptin 88C[19]	ni	4.50	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Tyr	L-Thr	L-Glu	L-Phe	But
micropeptin 88E[19]	ni	4.90	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Leu	L-Thr	L-Glu	L-Phe	But
micropeptin 88D[19]	ni	8.96	L-Ile	NMe-L-Phe	L-Val	Ahp	HcAla	L-Thr	L-Glu	L-Phe	But
largamide D[28]	ni	10.00	L-Val	L-NMe-Br-Tyr	L-Thr	L-Ahp	L-Leu	L-Thr	L-Val	L-Ala	L-Ahppa, D-Gac
largamide E[28]	ni	10.00	L-Val	L-NMe-Cl-Tyr	L-Thr	L-Ahp	L-Leu	L-Thr	L-Val	L-Ala	L-Ahppa, D-Gac
planktopeptin BL843[1]	ni	ni	L-Ile	NMe-OMe-L-Tyr	L-Thr	Ahp	L-Leu	L-Thr	L-Gln		L-Glu-γ-lactam
micropeptin 88N[22]	ni	ni	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Tyr	L-Thr	L-Glu	L-Leu	But
largamide G[28]	ni	ni	L-Val	L-NMe-Br-Tyr	L-Thr	L-Ahp	L-Htyr	L-Thr	L-Val	L-Ala	L-Ahppa, D-Gac
nostopeptin C[27]	ni	ni	L-Ile	NMe-L-Tyr	L-Ile	Ahp	L-Leu	Hmp	L-Gln		Ac
nostopeptin D[27]	ni	ni	L-Ile	NMe-L-Tyr	L-Ile	Ahp	L-Leu	Hmp	L-Gln		Pr
A90720A[30]	0.0096	nt	L-Val	L-NMe-Tyr	L-Leu	Ahp	L-Arg	L-Thr	D-Leu		L-Gac-sulfate
micropeptin A[31]	0.07	ni	L-Val	NMe-L-Tyr	L-Leu	Ahp	L-Arg	L-Thr	L-Glu		Oct
micropeptin T2[12]	0.10	ni	L-Val	NMe-L-Trp	L-Phe	Ahp	L-Lys	L-Thr	L-Glu		Hex
oscillapeptin F[24]	0.18	ni	L-allo-Ile	NMe-L-Phe	L-Ile	Ahp	L-Lys	L-Thr	D-Htyr		D-Mgs
micropeptin SF995[29]	0.20	ni	L-Val	L-NMe-Trp	L-Ile	Ahp	L-Arg	L-Thr	L-Asp		Hex
cyanopeptolin S[32]	0.24	nt	L-Ile	NMe-L-Phe	L-Ile	Ahp	L-Arg	L-Thr			Gac-sulfate
micropeptin B[31]	0.26	ni	L-Val	NMe-L-Tyr	L-Leu	Ahp	L-Arg	L-Thr	L-Glu		Hex
micropeptin 90[33]	2.08	ni	Val	NMe-Tyr	L-Phe	Ahp	Arg	L-Thr			Gac-sulfate
micropeptin EI992[34]	3.83	ni	L-Ile	NMe-L-Tyr	L-Phe	Ahp	L-Arg	L-Thr	L-Asp		But
micropeptin SD999[26]	4.00	ni	L-Val	NMe-L-Kyn	L-Ile	Ahp	L-Arg	L-Thr	L-Asp		Hex
micropeptin E1964[34]	4.36	ni	L-Ile	NMe-L-Tyr	L-Phe	Ahp	L-Arg	L-Thr	L-Asp		Ac
micropeptin SD944[26]	8.47	ni	L-Val	NMe-L-Tyr	L-Ile	Ahp	L-Lys	L-Thr	L-Asp		Hex
micropeptin 88B[19]	ni	ni	L-Ile	NMe-L-Phe	L-Val	Ahp	L-Glu	L-Thr	L-Glu	L-Phe	But

[a] ni = no inhibition at 10 μM or IC_{50} > 10 μM. nt = not tested. Abbreviations used in this table: Ahp: 3-amino-6-hydroxy-2-piperidone; Gac-phos: glyceric acid phosphate; Gac-sulfate: glyceric acid sulfate; NMe-Cl-Tyr: *N*-methyl-3-chlorotyrosine; NMe-OMe-Br-Tyr: *N*-methyl-*O*-methyl-3-bromotyrosine; Ac: acetate; Abu: 2-amino-2-butenoic acid; Htyr: homotyrosine; Cit: citrulline; But: butanoic acid; HcAla: (4-hydroxy-2-cyclohexenyl)alanine; Hex: hexanoic acid; Oct: octanoic acid; *N*-Me-Kyn: *N*-methylkynurenine; Hmp: 3-hydroxy-4-methylproline; Mgs: 2-*O*-methylglyceric acid-3-*O*-sulfate; Mga: 2-*O*-methylglyceric acid; Ahppa: 2-amino-5-(4′-hydroxyphenyl)pentanoic acid; Hpla: p-hydroxyphenyllactic acid; Pr: propionic acid.

was stirred in a sealed vial at 60 °C for 1 h, and the solvents were removed in vacuo. This *N*-acetylated product material was hydrolyzed by stirring with 6 N HCl in a sealed vial at 110 °C for 18 h and the solvent removed in vacuo. The resulting product was analyzed by Marfey's analysis as outlined above.

Symplocamide A (1): colorless glass; $[\alpha]^{23}_{D}$ −43.2 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 221 (sh) (6.70), 280 (0.67) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 1051.4457 (calcd for $C_{46}H_{72}{}^{79}BrN_{10}O_{13}$, 1051.4463).

Trypsin Assay. Solutions of 0.5 mM *N*-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) and 0.05 mg/mL trypsin were prepared fresh daily in 67 mM NaH_2PO_4 buffer, pH 7.6, at 25 °C or on ice, respectively. A 1:2 dilution series of symplocamide A (**1**) from 1.0×10^{-2} to 1.5×10^{-4} M was prepared in 100% EtOH, yielding final concentrations of 4.5×10^{-4} to 6.8×10^{-6} M in a 110 μL reaction volume. The substrate BAEE (100 μL) and 5 μL of symplocamide A (**1**) or EtOH blank were mixed in the cuvette and incubated at 25 °C until a stable temperature was reached. Trypsin (5 μL) was then added and rapidly mixed, and the production of *N*-benzoyl-L-arginine from BAEE was followed at 253 nm for 5 min. The 0.5 to 2 min interval was used for subsequent analysis. In the final reaction volume of 110 μL, the reagent concentrations and conditions were 0.0023 mg/mL trypsin (2.75 BAEE U), 0.45 mM BAEE, 4.5% (v/v) MeOH, 64 mM NaH_2PO_4, pH 7.6 at 25 °C. The final BAEE U for uninhibited trypsin was calculated on the basis of a BAEE unit definition of the amount of trypsin needed to produce a change in absorbance at 253 nm of 0.001/min at pH 7.6 at 25 °C in a reaction volume of 3.2 mL.

Chymotrypsin Assay. A 1.18 mM *N*-benzoyl-L-tyrosine ethyl ester (BTEE) solution was prepared fresh daily in 60% (v/v) MeOH, and a 0.05 mg/mL chymotrypsin solution was prepared in cold 1 mM HCl. Dilutions of symplocamide A (**1**) from 1.25×10^{-3} to 1.5×10^{-7} M were prepared in 100% MeOH, yielding final concentrations of 6.25×10^{-5} to 7.5×10^{-9} M in a 100 μL reaction volume. Substrate (90 μL BTEE) and inhibitor (5 μL symplocamide A (**1**)) or MeOH blank were mixed and incubated at 25 °C in the spectrophotometer until a stable temperature was reached. Chymotrypsin (5 μL) was then added and rapidly mixed. The production of *N*-benzoyl-L-tyrosine from BTEE was followed at 256 nm for 5 min and the 0.5 to 1.5 min interval was used for subsequent analysis. In the final assay volume of 100 μL, reagent concentrations and conditions were 0.0025 mg/mL chymotrypsin (0.0086 U), 0.53 mM BTEE, 32% MeOH (v/v), 53 mM $CaCl_2$, 0.5 mM HCL, 38 mM TrisHCl, pH 7.8 at 25 °C.

Antiparasitic Assays. Antiparasitic assays were performed using standard assay protocols as described in the isolation of venturamides A and B.[38]

Acknowledgment. We thank the government of Papua New Guinea for permission to collect the *Symploca* cyanobacterium and the captain (C. De Wit) and crew of the *MV Golden Dawn* for scuba diving support. We also thank the Biochemistry and Chemistry NMR Facilities at OSU for 600 MHz NMR spectrometer time, and J. Morré and the EIHS Center at OSU for mass spectrometric data acquisition (NIEHS grant P30 ES00210). We thank the B. Moore lab at SIO for use of their UV spectrophotometer and the Theodorakis lab in the Department of Chemistry at UCSD for the use of their polarimeter. We acknowledge NIH grants CA100851 and NS053398 for support for this work at SIO and OSU and the Fogarty International Center's International Cooperative Biodiversity Groups (ICBG) program for efforts in Panama (ICBG TW006634).

Supporting Information Available: 1D and 2D NMR spectra for symplocamide A (**1**), 1D selective TOCSY NMR data for **1**, HPLC analysis of the FDVA derivatized hydrolysate of **1** and its racemate, IC_{50} data for the protease inhibition assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418–5423. (b) Li, W. I.; Berman, F. W.; Okino, T.; Yokokawa, F.; Shioiri, T.; Gerwick, W. H.; Murray, T. F. *P. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7599–7604. (c) Grach-Pogrebinsky, O.; Sedmak, B.; Carmeli, S. *Tetrahedron* **2003**, *59*, 8329–8336.
(2) Flatt, P. M.; O'Connell, S. J.; McPhail, K. L.; Zeller, G.; Willis, C. L.; Sherman, D. H.; Gerwick, W. H. *J. Nat. Prod.* **2006**, *69*, 938–944.
(3) Galonic, D. P.; Vaillancourt, F. H.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 3900–3901.
(4) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355–357.
(5) Renneberg, B.; Kellner, M.; Laatsch, H. *Liebigs Ann. Chem.* **1993**, *8*, 847–852.
(6) Southan, C. *Drug Discovery Today* **2001**, *6*, 681–688.
(7) Radau, G. *Curr. Enzyme Inhib.* **2005**, *1*, 295–307.
(8) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. *Alkaloids* **2001**, *57*, 75–184.
(9) Harada, K.-I.; Fujii, K.; Mayumi, T.; Hibino, Y.; Suzuki, M.; Ikai, Y.; Oka, H. *Tetrahedron Lett.* **1995**, *36*, 1515–1518.
(10) Ishida, K.; Matsuda, H.; Murakami, M.; Yamaguchi, K. J. *Nat. Prod.* **1997**, *60*, 184–187.
(11) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655–658.
(12) (a) Ploutno, A.; Carmeli, S. *Tetrahedron* **2002**, *58*, 9949–9957. (b) Kodani, S.; Suzuki, S.; Ishida, K.; Murakami, M. *FEMS Microbiol. Lett.* **1999**, *178*, 343–348.
(13) Lee, A. Y.; Smitka, T. A.; Bonjouklian, R.; Clardy, J. *Chem. Biol.* **1994**, *1*, 113–117.
(14) Matern, U.; Oberer, L.; Falchetto, R. A.; Erhard, M.; Konig, W. A.; Herdman, M.; Weckesser, J. *Phytochemistry* **2001**, *58*, 1087–1095.
(15) Abramowitz, N.; Schechter, I; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *29*, 862–867.
(16) Okano, T.; Sano, T.; Kaya, K. *Tetrahedron Lett.* **1999**, *40*, 2379–2382.
(17) von Elert, E.; Oberer, L.; Merkel, P.; Huhn, T.; Blom, J. F. *J. Nat. Prod.* **2005**, *68*, 1324–1327.
(18) Matthew, S.; Ross, C.; Rocca, J. R.; Paul, V. J.; Luesch, H. *J. Nat. Prod.* **2007**, *70*, 124–127.
(19) Ishida, K.; Matsuda, H.; Murakami, M. *Tetrahedron* **1998**, *54*, 5545–5556.
(20) Bister, B.; Keller, S.; Baumann, H. I.; Nicholson, G.; Weist, S.; Jung, G.; Suessmuth, R. D.; Juettner, F. *J. Nat. Prod.* **2004**, *67*, 1755–1757.
(21) Murakami, M.; Kodani, S.; Ishida, K.; Yamaguchi, K. *Tetrahedron Lett.* **1997**, *37*, 3035–3038.
(22) Yamaki, H.; Sitachitta, N.; Sano, T.; Kaya, K. J. *J. Nat. Prod.* **2005**, *68*, 14–18.
(23) Okino, T.; Sun, Q.; Ishida, K.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 158–161.
(24) Itou, Y.; Ishida, K.; Shin, H. J.; Murakami, M. *Tetrahedron* **1999**, *55*, 6871–6882.
(25) Shin, H. J.; Murakami, M.; Matsuda, H.; Ishida, K.; Yamaguchi, K. *Tetrahedron Lett.* **1995**, *36*, 5235–5238.
(26) Reshef, V.; Carmeli, S. *Tetrahedron* **2001**, *57*, 2885–2894.
(27) Sun, Q.; Ishida, K.; Matsuda, H.; Murakami, M. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1998**, *40*, 499–504.
(28) Plaza, A.; Bewley, C. A. *J. Org. Chem.* **2006**, *71*, 6898–6907.
(29) Banker, R.; Carmeli, S. *Tetrahedron* **1999**, *55*, 10835–10844.
(30) Bonjouklian, R.; Smitka, T. A.; Hunt, A. H.; Occolowitz, J. L.; Perun, T. J., Jr.; Doolin, L.; Stevenson, S.; Knauss, L.; Wijayaratne, R.; Szewczyk, S. *Tetrahedron* **1996**, *52*, 395–404.
(31) Okino, T.; Murakami, M.; Haraguchi, R.; Munekata, H.; Matsuda, H.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, *34*, 8131–8134.
(32) Jakobi, C.; Oberer, L.; Quiquerez, C.; Koenig, W. A.; Weckesser, J. *FEMS Microbiol. Lett.* **1995**, *129*, 129–134.
(33) Ishida, K.; Murakami, M.; Matsuda, H.; Yamaguchi, K. *Tetrahedron Lett.* **1995**, *36*, 3535–3538.
(34) Ploutno, A.; Shoshan, M.; Carmeli, S. *J. Nat. Prod.* **2002**, *65*, 973–978.
(35) (a) Montanari, A. M.; Fenical, W.; Lindquist, N.; Lee, A. Y.; Clardy, J. *Tetrahedron* **1996**, *52*, 5371–80. (b) Ciminiello, P.; Fattorusso, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **1995**, *58*, 689–696.
(36) Decock, J.; Paridaens, R.; Cufer, T. *Curr. Opin. Oncol.* **2005**, *17*, 545–550.
(37) Giaccone, G. *Eur. J. Cancer Suppl.* **2004**, *2*, 25–28.
(38) Linington, R. G.; Gonzalez, J.; Ureña, L.-D.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 397–401.

NP070280X