



## FAU Institutional Repository

<http://purl.fcla.edu/fau/fauir>

This paper was submitted by the faculty of [FAU's Harbor Branch Oceanographic Institute](#).

Notice: ©2009 American Chemical Society and American Society of Pharmacognosy. This document is the accepted manuscript version of a published work that appeared in final form in *Journal of Natural Products* after peer review and technical editing by the publisher. To access the final edited and published work see <http://dx.doi.org/10.1021/np900183v>. This article may be cited as: Wright, A. E., Roth, G. P., Hoffman, J. K., Divlianska, D. B., Pechter, D., Sennett, S. H., Guzman, E. A., Linley, P., McCarthy, P. J., Pitts, T. P., Pomponi, S. A., & Reed, J. K. (2009). Isolation, synthesis, and biological activity of Aphrocallistin, an adenine-substituted bromotyramine metabolite from the hexactinellida sponge *Aphrocallistes beatrix*. *Journal of Natural Products*, 72(6), 1178-1183. doi:10.1021/np900183v

## Isolation, Synthesis, and Biological Activity of Aphrocallistin, an Adenine-Substituted Bromotyramine Metabolite from the Hexactinellida Sponge *Aphrocallistes beatrix*

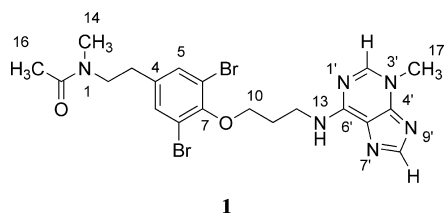
Amy E. Wright,<sup>\*,†</sup> Gregory P. Roth,<sup>\*,‡</sup> Jennifer K. Hoffman,<sup>‡</sup> Daniela B. Divlianska,<sup>‡</sup> Diana Pechter,<sup>†</sup> Susan H. Sennett,<sup>†</sup> Esther A. Guzmán,<sup>†</sup> Patricia Linley,<sup>†</sup> Peter J. McCarthy,<sup>†</sup> Tara P. Pitts,<sup>†</sup> Shirley A. Pomponi,<sup>†</sup> and John K. Reed<sup>†</sup>

Center for Marine Biomedical and Biotechnology Research, Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Ft. Pierce, Florida 34946, and Burnham Institute for Medical Research at Lake Nona, Conrad Prebys Center for Chemical Genomics, 6400 Sanger Road, Orlando, Florida 32827

Received March 23, 2009

A new adenine-substituted bromotyrosine-derived metabolite designated as aphrocallistin (**1**) has been isolated from the deep-water Hexactinellida sponge *Aphrocallistes beatrix*. Its structure was elucidated on the basis of spectral data and confirmed through a convergent, modular total synthetic route that is amenable toward future analogue preparation. Aphrocallistin inhibits the growth of a panel of human tumor cell lines with IC<sub>50</sub> values ranging from 7.5 to >100 μM and has been shown to induce G1 cell cycle arrest in the PANC-1 pancreatic carcinoma cell line. Aphrocallistin has been fully characterized in the NCI cancer cell line panel and has undergone *in vitro* ADME pharmacological profiling.

Hexactinellida sponges occur almost exclusively in deep-water marine habitats at depths greater than 200 m. The skeleton of the hexactinellids is made entirely of silicon oxides, giving them a “glassy” look and thus the common designation “glass sponges”. The lack of sponginess leads to a fairly rigid body with limited resilience or flexibility, making collection of these deep-water sponges using methods such as trawling difficult, as the sponges crush and fragment in the trawl. Although these sponges are fairly common, there is very little published on their chemistry. Thiel and co-workers have described the lipid complement of a series of hexactinellid sponges as part of a chemotaxonomic investigation,<sup>1,2</sup> and ergosta-4,24(28)-dien-3-one has been reported from an Antarctic hexactinellid.<sup>3</sup> As part of our ongoing search for compounds with potential utility against cancer, a crude ethanol extract of the sponge *Aphrocallistes beatrix beatrix* Gray, 1858 (order Hexactinosida, family Aphrocallistidae) showed moderate inhibition of the proliferation of the PANC-1 human pancreatic carcinoma and the DLD-1 human colon carcinoma cell lines. Bioassay-guided fractionation led to the purification of a novel compound that we have designated as aphrocallistin (**1**). This paper describes the isolation, structure elucidation, synthesis, cytotoxic activity, and preliminary pharmacokinetics of aphrocallistin.



### Results and Discussion

A sample of the sponge *A. beatrix beatrix* Gray, 1858 was collected using the Johnson-Sea-Link manned submersible east of Fort Pierce, Florida, on deep-water *Lophelia* coral mounds. The specimen used in this study was stored at –20 °C immediately after collection until further workup. The frozen sponge was extracted exhaustively with EtOH, and after concentration by

distillation under reduced pressure, the extract was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc partition was further separated by vacuum column chromatography on a C-18 stationary phase using a step gradient of CH<sub>3</sub>CN in H<sub>2</sub>O. Active fractions were further purified by medium-pressure liquid chromatography followed by HPLC to yield a fraction that ultimately contained aphrocallistin (**1**) as a light brown oil.

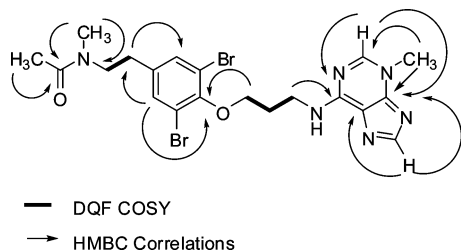
Low-resolution ESIMS analysis suggested the presence of a single compound in the active fraction having a three-ion cluster *m/z* 539/541/543 with typical ion abundance for a dibrominated compound. HRESIMS analysis showed a similar pattern and suggested a formula of C<sub>20</sub>H<sub>24</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub> requiring 11 unsaturation equivalents in the molecule. Inspection of the <sup>1</sup>H and <sup>13</sup>C NMR spectra revealed a series of resonances that appeared doubled and significantly more carbons than would be consistent with the molecular formula suggested by HRESIMS. The presence of two closely related compounds was suggested by a series of repeating resonances in the <sup>1</sup>H and DQF-COSY 2D-NMR spectra that appeared to constitute two 1,2-substituted ethyl groups, two *N*-methyl groups, and two acetyl methyls. Careful integration of the <sup>1</sup>H NMR spectrum allowed for the determination that the active fraction consists of two closely related compounds that were present in a ratio of 3:2. Nominally the integrated <sup>13</sup>C NMR spectrum appeared to have 29 carbon resonances, 15 sp<sup>2</sup> hybridized and 14 sp<sup>3</sup> hybridized. Some of the resonances were obviously doubled, while others were not. Two resonances were extremely broad, suggesting either attachment to nitrogen or the presence of a tautomeric species. As not all resonances in the <sup>13</sup>C NMR spectrum were doubled, initial assignment of the resonances was difficult. Collection of an inverse gated carbon spectrum led to a <sup>13</sup>C spectrum that could be readily integrated and allowed for the assignment of the carbons in the major, minor, or both compounds.

The NMR spectra of **1** exhibited resonances attributable to an *N*-methyl functionality [ $\delta_{\text{H}}$  2.98 (3H, s),  $\delta_{\text{C}}$  36.9 (CH<sub>3</sub>)]; an acetyl functionality [ $\delta_{\text{H}}$  2.02 (3H, s),  $\delta_{\text{C}}$  21.6 (CH<sub>3</sub>);  $\delta_{\text{C}}$  173.4 (qC)]; and an A<sub>2</sub>X<sub>2</sub> spin system [ $\delta_{\text{H}}$  2.74 (2H, t *J* = 7.6 Hz),  $\delta_{\text{C}}$  33.0 (CH<sub>2</sub>);  $\delta_{\text{H}}$  3.50 (2H, t *J* = 7.6 Hz),  $\delta_{\text{C}}$  50.1 (CH<sub>2</sub>)], consistent with an ethyl amine functionality. Correlations in the HMBC spectrum between the *N*-methyl protons and the nitrogen-bearing methylene carbon of the ethyl chain (C-2) and the amide carbonyl carbon (C-15) suggested the presence of an *N*-methyl amide in **1**. Correlations in the HMBC spectrum between H<sub>2</sub>-2 and both C-14 and C-15 and from H<sub>3</sub>-16 to C-15 also support the presence of an *N*-methyl-*N*-acetylmethylamine in **1**. The presence of an aromatic singlet ( $\delta_{\text{H}}$  7.45)

\* To whom correspondence should be addressed. Isolation, tumor biology: Wright. Tel: 772-465-2400, ext 459. Fax: 772-461-2221. E-mail: awright33@fau.edu. Synthesis, pharmacology: Roth. Tel: 407-745-2062. Fax: 407-745-2001. E-mail: groth@burnham.org.

<sup>†</sup> Harbor Branch Oceanographic Institute at Florida Atlantic University.

<sup>‡</sup> Burnham Institute for Medical Research at Lake Nona.



**Figure 1.** Selected  $^1\text{H}$ – $^{13}\text{C}$  HMBC correlations for aphrocallistin (**1**).

attributable to two hydrogen atoms along with carbon resonances ( $\delta_{\text{C}}$  134.4 and 118.9) each integrating for two carbon atoms suggested the presence of a 1,2,3,5-symmetrically substituted aromatic ring in **1**. The  $^1\text{H}$  resonance observed at  $\delta_{\text{H}}$  7.45 (H-5, H-9) had correlations to the ethyl carbon (C-3) as well as to C-4, C-8, and C-7 of the aromatic ring. The C-5 and C-7 substituents of the aromatic ring were assigned as bromine on the basis of comparison of the chemical shifts to related compounds. The chemical shift of C-6 suggested oxygen substitution ( $\delta_{\text{C}}$  152.7). The DQF-COSY spectrum showed the presence of a 3-aminopropan-1-ol group in **1** [ $\delta_{\text{H}}$  4.11 (2H, m),  $\delta_{\text{C}}$  71.9 (CH<sub>2</sub>);  $\delta_{\text{H}}$  2.27 (2H, m),  $\delta_{\text{C}}$  30.5 (CH<sub>2</sub>);  $\delta_{\text{H}}$  4.04 (2H, m),  $\delta_{\text{C}}$  40.4 (CH<sub>2</sub>)]. A correlation observed in the HMBC spectrum from the methylene protons observed at  $\delta_{\text{H}}$  4.11 (H<sub>2</sub>-10) to C-7 of the aromatic ring allowed for attachment of the propyl functionality to C-7 of the dibromophenyl ring through an ether linkage. The chemical shifts of H<sub>2</sub>-12 ( $\delta_{\text{H}}$  4.04) and C-12 ( $\delta_{\text{C}}$  40.4) were consistent with nitrogen substitution at C-12. Atoms 1–13 of **1** constitute a common bromotyramine-derived functionality observed in over 83 marine natural products and has been given the common name moloka'iamine.<sup>4</sup> All data observed for N-1 through C-13 in aphrocallistin are consistent with those reported for moloka'iamine.

The remaining atoms to be incorporated into the structure are C<sub>6</sub>H<sub>5</sub>N<sub>4</sub>. The  $^1\text{H}$ ,  $^{13}\text{C}$ , and edited-g-HSQC NMR spectra showed the presence of three quaternary sp<sup>2</sup>-hybridized carbons ( $\delta_{\text{C}}$  153.5, 149.2, 112.6); two methine sp<sup>2</sup>-hybridized carbons [ $\delta_{\text{H}}$  8.64 (s),  $\delta_{\text{C}}$  149.7 (CH);  $\delta_{\text{H}}$  8.42 (s),  $\delta_{\text{C}}$  145.6 (CH)]; and one methyl group [ $\delta_{\text{H}}$  4.06 (3H, s),  $\delta_{\text{C}}$  36.8 (CH<sub>3</sub>)]. These atoms were connected together on the basis of chemical shift arguments,  $^1J_{\text{C-H}}$  coupling constants, and correlations observed in the  $^1\text{H}$ – $^{13}\text{C}$  (Figure 1) and  $^1\text{H}$ – $^{15}\text{N}$  HMBC spectra, resulting in the assignment of a 3-methyladenine (adenine numbering system) substituent attached to the moloka'iamine moiety through N-13. In the HMBC spectrum, residual signals from the unsuppressed one-bond  $^1J_{\text{C-H}}$  for the two methine resonances could be observed, and both were greater than 200 Hz, suggesting that both carbons bear two nitrogen substituents.<sup>5</sup> Both of the protons showed correlations in the HMBC spectrum to the same quaternary carbon observed at  $\delta_{\text{C}}$  149.2 (C-4'), suggesting that both protons are three bonds away from this carbon. The N-methyl resonance observed at  $\delta_{\text{H}}$  4.06 showed HMBC correlations to C-2' and to C-4', placing it on N-3'. The H-2' proton showed HMBC correlations to a carbon observed at  $\delta_{\text{C}}$  153.5 (C-6'), which in turn had coupling from H<sub>2</sub>-12 linking the adenine functionality to the moloka'iamine functionality through N-13. The proton observed at  $\delta_{\text{H}}$  8.42 (H-8') showed long-range coupling in the HMBC spectrum to the final quaternary carbon observed at  $\delta_{\text{C}}$  112.6 (C-5'), which is consistent with the chemical shift reported for C-5 of an adenine functionality. The  $^1\text{H}$ – $^{15}\text{N}$  HMBC experiment also supports this assignment with correlations observed between the H<sub>3</sub>-17 methyl and a nitrogen observed at  $\delta_{\text{N}}$  148 (N-4'); H-2' and two nitrogens observed at  $\delta_{\text{N}}$  148 (N-4') and  $\delta_{\text{N}}$  226 (N-1'); H<sub>2</sub>-11 and a nitrogen observed at  $\delta_{\text{N}}$  106 (N-13); and H<sub>2</sub>-3, H<sub>3</sub>-14 and H<sub>3</sub>-16, all showing correlations to a nitrogen observed at  $\delta_{\text{N}}$  111. This completes the overall structure of aphrocallistin.

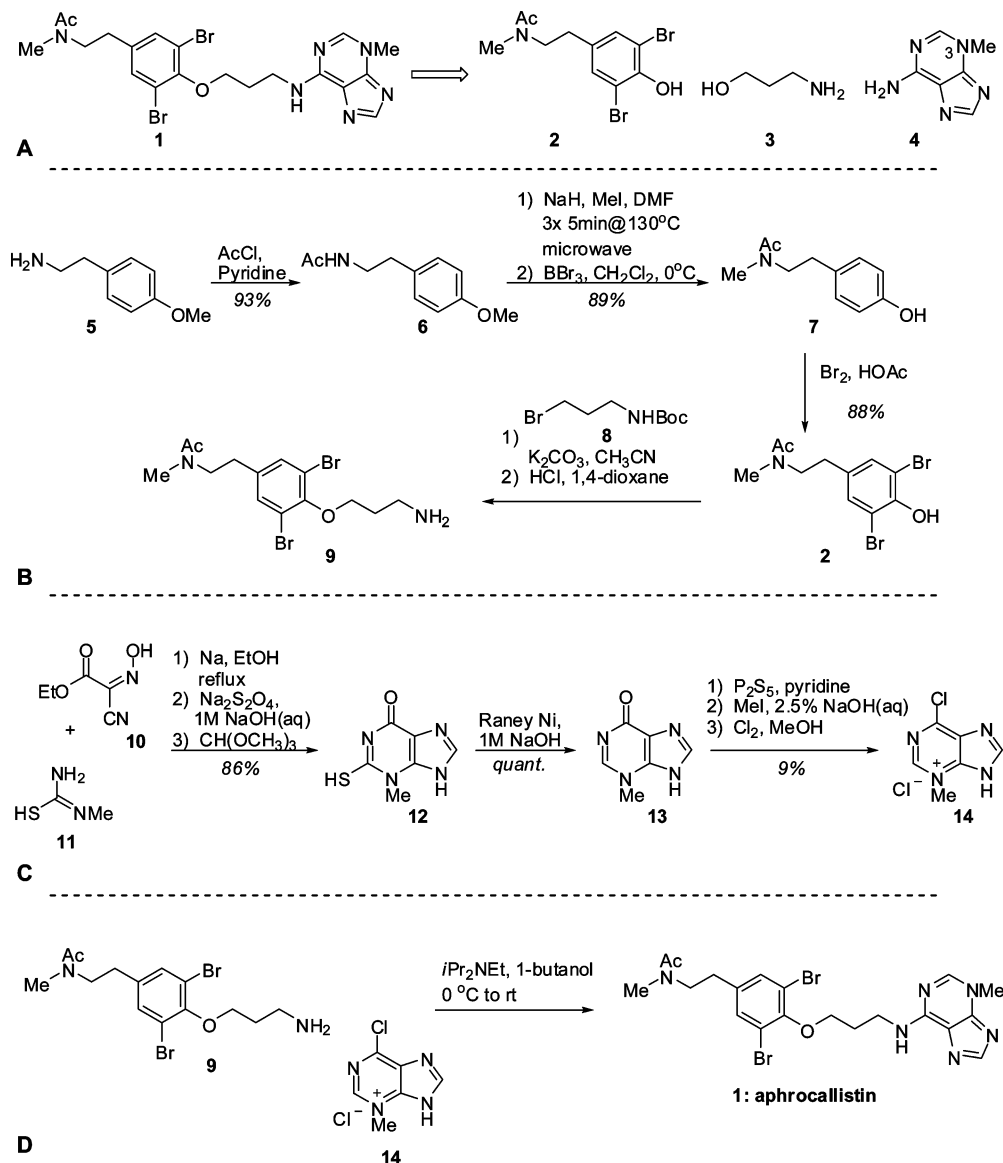
Aphrocallistin has a relatively unusual 3-methyladenine functionality that has not been observed in other marine natural products. It is also the first in the bromotyramine-derived compounds to have both acetyl and methyl substitution at N-1 of the moloka'iamine moiety. It is this functionality that leads to the 3:2 mixture in which the major component is *cis* across the peptide bond and the minor component is *trans* as determined by NOE measurement [the major has a strong NOE between the N-1 CH<sub>3</sub> and the N-acetyl methyl, while the minor has a strong NOE between the N-acetyl methyl and H<sub>2</sub>-2 methylene protons].

In order to confirm the structure and to obtain larger amounts of the compound for biological evaluation, a chemical synthesis of **1** was conducted. Aphrocallistin (**1**) can be regarded as a three-part construct of a tyramine derivative **2** and an adenine moiety **4** connected together by a three-carbon chain presumably stemming from 3-aminopropan-1-ol (**3**), a linker commonly found in many natural products (Figure 2A). Some prominent structural features in the molecule of **1** can be noted. The tyramine nitrogen is present as a tertiary amide, an attribute not common in nature, and the adenine piece is unusually functionalized at position 3 (adenine numbering) with a methyl group.

Aphrocallistin (**1**) can be assembled in a convergent biomimetic manner from tyramine **9** and the purine **14** via *S<sub>N</sub>Ar*-like chemistry. Initial efforts to prepare the tyramine derivative **7** en route to **9** via reductive methylation of tyramine followed by acetylation to form the corresponding tertiary amide were unsuccessful. The two-step approach did not lead to satisfactory results, as a competing Pictet–Spengler-like cyclization gave diminished yields of the desired secondary amine. This early challenge was easily circumvented by reversing the order of synthesis. Installation of the acetate functionality on the *O*-protected compound **5** was achieved following Okuda's method.<sup>6</sup> Improvement of the protocol provided **6** in excellent yield. Treatment of **6** with sodium hydride (NaH) and subsequent addition of iodomethane (MeI) followed by conventional heating (refluxing DMF) did not provide the desired tertiary amide but returned the starting material, an outcome attributed to the high volatility of iodomethane and the extreme reaction conditions, high temperature, and long reaction time, to achieve alkylation. A microwave-assisted protocol (5 min at 130 °C) led to a substantial improvement in the conversion; however the reaction required repetition and resubmission of crude material to the reaction conditions for three cycles in order to obtain a good yield of product. Removal of the phenolic *O*-methyl group in the presence of boron tribromide delivered compound **7** in good yield (Figure 2B). With compound **7** in hand, we were able to prepare the left-hand side of aphrocallistin (compound **2**) via a reaction of **7** with bromine under acidic conditions.<sup>7</sup> Linker **3** was then incorporated using a two-step procedure. Alkylation of the phenolic oxygen of **2** with the alkyl halide **8**, followed by unmasking of the primary amine functionality, set the stage for the final attachment of the purine moiety.

The purine moiety **14** was prepared following literature procedures,<sup>8–10</sup> which were adapted and optimized to achieve good conversion and high yields (Figure 2C). The synthetic route was initiated by preparing pyrimidin-4-one **12** in three steps. Commercially available starting materials **10** and **11** were heated at reflux in anhydrous EtOH in the presence of freshly prepared sodium ethoxide.<sup>8</sup> The resulting cyclic nitroso compound was then reduced with sodium dithionite to the corresponding amino analogue,<sup>8</sup> which was condensed with trimethyl orthoformate<sup>9</sup> to provide **12** in good overall yield. Desulfuration of **12** in the presence of Raney Ni<sup>8,10</sup> led to intermediate **13**. Reaction of the purin-6-one **13** with phosphorus pentasulfide,<sup>10</sup> alkylation on the sulfur residue,<sup>10</sup> and subsequent chloride displacement<sup>11</sup> formed **14**, a key intermediate for the final step of the synthesis of aphrocallistin (**1**).

The convergent synthesis of aphrocallistin (**1**) concluded with a nucleophilic aromatic substitution of purine **14** with the tyramine



**Figure 2.** (A) Retrosynthetic analysis of aphrocallistin (**1**); (B) synthesis of tyramine moiety **9**; (C) synthesis of purine **14**; (D) final step in the synthesis of **1**.

moiety **9** (Figure 2D).<sup>12</sup> Ultimately, 70 mg of **1** was prepared in eight linear steps from commercially available starting materials in good overall yield. Spectral analysis (<sup>1</sup>H NMR and LCMS) confirmed the synthetic material to be identical with the natural product.

Aphrocallistin was evaluated in a panel of human tumor cell lines and gave IC<sub>50</sub> values ranging from 7.5 μM in the DLD-1 colon carcinoma cell line to >100 μM in the extremely resistant AsPc-1 pancreatic carcinoma cell line (Table 2). Cell cycle analysis of aphrocallistin in the Panc-1 pancreatic adenocarcinoma cell line showed significant cell cycle arrest at G1 at doses ≤46.5 μM. Interestingly aphrocallistin shows the greatest inhibition of tumor proliferation for cell lines having a p53 mutation, being about 3 times more potent against Panc-1 cells and about 10 times more potent against DLD-1 than Vero cells (cell line derived from normal kidney cells of an African monkey). These cell lines cannot initiate p21 modulated response and DNA repair mechanisms due to the loss of the p53 pathway. Aphrocallistin has been fully characterized in the NCI cancer cell line panel (see Supporting Information for full data) and showed significant selectivity of growth inhibition in the following cell lines: SR (leukemia), HCT-116 (colon), SK-MEL-5 (melanoma), T-47D and MDA-MB-468 (breast). In a study aimed at detecting whether aphrocallistin binds directly to DNA,

no displacement of ethidium bromide was observed, suggesting that it does not directly bind to DNA. Aphrocallistin was tested for antimicrobial activity against *Candida albicans*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* and did not show significant inhibition under the conditions used for the assay.

The pharmacological properties of aphrocallistin were evaluated in various *in vitro* assays. The solubility of the compound was assessed to be high at three physiologically relevant pH levels. It exhibited moderate permeability as measured by the parallel artificial membrane permeation assay at pH 6.2 and 7.4 and low effective permeability at pH 5.0. Aphrocallistin also showed moderate protein binding and excellent stability (3 h) at both test concentrations in human and mouse plasma. However, it was rapidly metabolized with 96.9% and 98.5% of the compound consumed within 60 min in both human and mouse liver microsomes, respectively. This suggests that aphrocallistin will be subject to significant first pass hepatic metabolism *in vivo*. Data are detailed in the Supporting Information.

## Experimental Section

**General Experimental Procedures.** NMR data used in the structure elucidation were collected on a JEOL ECA-600 spectrometer operating

**Table 1.** NMR Spectroscopic Data (600 MHz, CD<sub>3</sub>OD) for Aphrocallistin (1)

position	$\delta_C$ , mult.	aphrocallistin major rotamer					minor rotamer	
		$\delta_N$	$\delta_H$ (J in Hz)	COSY	HMBC <sup>a</sup>	NOESY	$\delta_C$	$\delta_H$ (J in Hz)
1		111						
2	50.1, CH <sub>2</sub>		3.50, t (7.6)	3	3, 4, 14, 15	3, 5, 9, 14	53.0	3.55, t (6.9)
3	33.0, CH <sub>2</sub>		2.74, t (7.6)	2	1, 2, 4, 5, 9	2, 5, 9	33.9	2.82, t (6.9)
4	140.0, qC						139.4	
5 and 9	134.4, CH		7.45, s		3, 6, 7, 5 or 9	2, 3	134.6	7.47, s
6 and 8	118.9, qC						119.1	
7	152.8, qC						153.1	
10	71.9, CH <sub>2</sub>		4.11, m	11	7, 11, 12	11	71.9	4.11, m
11	30.5, CH <sub>2</sub>		2.27, m	10, 12	10, 12, 13	10, 12	30.5	2.27, m
12	40.4, CH <sub>2</sub>		4.04, m	11	10, 11, 6'	11	40.4	4.04, m
13		106						
1'		227						
2'	149.7, CH		8.64, s		1', 3', 4', 6', 17	17	149.7	8.64, s
3'		149						
4'	149.2 br, qC						149.2	
5'	112.6 br, qC						112.6	
6'	153.5, qC						153.5	
7' <sup>b</sup>								
8'	145.6 br, CH		8.42, s		4', 5'		145.6	8.42, s
9' <sup>b</sup>								
14	36.9, CH <sub>3</sub>		2.98, s		1, 2, 15	3, 16	33.8	2.88, s
15	173.4, qC						173.3	
16	21.6 CH <sub>3</sub>		2.02, s		1, 15	14	20.9	1.84, s
17	36.8, CH <sub>3</sub>		4.06, s		2', 3', 4'	2'	36.8	4.06, s

<sup>a</sup> HMBC correlations optimized for 8 Hz are from proton(s) stated to the listed carbon or nitrogen. <sup>b</sup> not observed

**Table 2.** Cytotoxicity Data for Aphrocallistin (1) in a Panel of Tumor Cell Lines

cell line	IC <sub>50</sub> $\mu$ M $\pm$ SE
A549	15.9 $\pm$ 2.1
AsPC-1	>100
BxPC-3	70.9 $\pm$ 2.2
DLD-1	7.5 $\pm$ 2.0
MiaPaca-2	26.6 $\pm$ 2.0
NCI/ADR-Res	34.3 $\pm$ 2.0
PANC-1	22.8 $\pm$ 2.0
Vero	76.1 $\pm$ 2.0

at 600.17 MHz for <sup>1</sup>H, 150.9 MHz for <sup>13</sup>C, and 60.8 MHz for <sup>15</sup>N (instrument reference set to liquid NH<sub>3</sub>). The edited-g-HSQC was optimized for 140 Hz and the g-HMBC was optimized for 8 Hz. Chemical shifts are referenced to solvent, e.g., CD<sub>3</sub>OD  $\delta_H$  observed at 3.31 ppm and  $\delta_C$  observed at 49.0 ppm. Chemical shifts for <sup>15</sup>N were referenced to liquid NH<sub>3</sub> with long-range  $J_{H,N}$  optimized for 6 Hz. The HRFABMS were measured using a JEOL AccuTOF mass spectrometer. Flow cytometry was measured on a BD FACSCalibur.

All reactions involving air- and moisture-sensitive reagents and solvents were performed under a nitrogen atmosphere using standard chemical techniques. The solvents DMF, CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>CN, and 1,4-dioxane were purchased in Sure/Seal bottles from Sigma-Aldrich and were used "as is"; EtOAc, pyridine, and EtOH were purchased and used fresh from Sigma-Aldrich. All organic reagents were used as purchased. Unless otherwise noted, a typical reaction workup was performed by addition of H<sub>2</sub>O and extraction with EtOAc; the organic phase thus obtained was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Microwave-assisted reactions were performed using a CEM Discover system. All NMR spectra for the synthetic materials were recorded on a Bruker Avance II 400 MHz instrument. <sup>1</sup>H and <sup>13</sup>C chemical shifts are reported in  $\delta$  values in ppm in the corresponding solvent. Analytical thin-layer chromatography was performed on Partisil K6F silica gel 60 Å, 250  $\mu$ m. Reversed-phase purification and LCMS analysis of synthetic materials were completed on a Waters Autopurification system, which consists of a 2767 sample manager, a 2545 binary gradient module, a system fluidics organizer, a 2489 UV/vis detector, and a 3100 mass detector, all controlled with MassLynx software. A Sunfire Analytical C18 5  $\mu$ m column (4.6  $\times$  50 mm) and a Sunfire Preparative OBD 5  $\mu$ m column (19  $\times$  50 mm) were used for analytical and preparative LCMS, respectively. All solvents used for chromatography on the synthetic materials were Fisher Chemical HPLC grade, and the water was Millipore Milli-Q PP filtered.

**Biological Material.** A sample of a sponge identified as *Aphrocallistes beatrix beatrix* (Gray) [phylum Porifera, class Hexactinellida, order Hexactinosida, family Aphrocallistidae] was collected by manned submersible at a depth of 725.4 m approximately 40 nautical miles east of Fort Pierce on deep-water *Lophelia* coral mounds (latitude 27°39.430' N, longitude 79°34.967' W). The sponge is vasiform with the appearance of folded plates and fingers. It is crispy in texture and friable. It was collected growing in association with yellow zoanthids. A reference sample preserved in ethanol has been deposited in the Harbor Branch Oceanographic Museum (catalog number 002:00022, DBMR number 20-V-04-1-005) and is available for taxonomic evaluation by those skilled in the art.

**Isolation of Aphrocallistin.** The frozen sponge (106 g) was diced and extracted exhaustively with EtOH (Pharmco 100%). The combined EtOH extracts were concentrated to dryness, and the residue was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc partition was concentrated to dryness to yield 0.493 g of an oily residue. The residue from the EtOAc partition was chromatographed by vacuum flash chromatography on a custom prepared RP-18 stationary phase using a step gradient of H<sub>2</sub>O–CH<sub>3</sub>CN–IPA as eluent. Column size was 150 mL. The eluent series is as follows: fraction 1, 100 mL of H<sub>2</sub>O–CH<sub>3</sub>CN (80:20 v/v); fraction 2, 100 mL of H<sub>2</sub>O–CH<sub>3</sub>CN (60:40 v/v); fraction 3, 100 mL of H<sub>2</sub>O–CH<sub>3</sub>CN (40:60 v/v); fraction 4, 100 mL of H<sub>2</sub>O–CH<sub>3</sub>CN (20:80 v/v); fraction 5, 100 mL of CH<sub>3</sub>CN; fraction 6, 100 mL of H<sub>2</sub>O–CH<sub>3</sub>CN–TFA (20:80:0.1 v/v); fraction 7, 100% CH<sub>3</sub>CN; fraction 8, 100% IPA. Fractions 6 and 7 (84.4 mg) were combined and further purified by medium-pressure liquid chromatography on a C-18 reversed-phase stationary phase using a Combiflash Companion (Isco) with the following gradient program [solvent A: H<sub>2</sub>O–CH<sub>3</sub>CN–TFA, 95:5:0.1 v/v; solvent B: CH<sub>3</sub>CN containing 0.1% TFA;  $t = 0$  min, A:B (100:0);  $t = 1$  min, A:B (100:0);  $t = 11$  min, A:B (70:30);  $t = 17$  min, A:B (0:100); flow = 15 mL/min; detected by UV absorption observed at 230 nm] to yield 40 mg of aphrocallistin.

**Aphrocallistin (1):** light brown oil; UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 285 (4.0); <sup>1</sup>H NMR (600.2 MHz, CD<sub>3</sub>OD, see Table 1); <sup>13</sup>C NMR data (160.9 MHz, CD<sub>3</sub>OD see Table 1); HRESIMS  $m/z$  539.042786 [M + H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>25</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub> 539.040571).

**Preparation of *N*-(4-Hydroxyphenethyl)-*N*-methylacetamide (7).** Acetamide **6** (550.0 mg, 2.85 mmol) was placed in a microwave tube and dissolved in DMF (14 mL). Sodium hydride (60% suspension in oil, 230.0 mg, 5.75 mmol, 2.0 equiv) was added at room temperature, and the reaction mixture was stirred for 15 min until the bubbling ceased. Iodomethane (888  $\mu$ L, 14.23 mmol, 5.0 equiv) was added, and the vessel was sealed and placed in the microwave reactor. The reaction was heated for 5 min at 130 °C with stirring. After the heating was

completed the vessel was uncapped and another portion of iodomethane was added to it followed by heating. This process was repeated once to ensure full consumption of the starting amide. The reaction was worked up following the standard protocol to afford 590 mg (100%) of the desired tertiary amide as an off-white solid. The compound was used without further purification. *N*-(4-Methoxyphenethyl)-*N*-methylacetamide:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.13 (d,  $J = 8.6$  Hz, 2H, minor), 7.06 (d,  $J = 8.6$  Hz, 2H, major), 6.85 (d,  $J = 8.6$  Hz, 2H, major), 6.83 (d,  $J = 8.6$  Hz, 2H, minor), 3.788 (s, 3H, major), 3.786 (s, 3H, minor), 3.54 (dd,  $J = 7.6, 7.6$  Hz, 2H, minor), 3.46 (t,  $J = 7.1$  Hz, 2H, major), 2.93 (s, 3H, minor), 2.89 (s, 3H, major), 2.79 (t,  $J = 7.1$  Hz, 2H, major), 2.78 (dd,  $J = 7.6, 7.6$  Hz, 2H, minor), 2.06 (s, 3H, major), 1.85 (s, 3H, minor);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.7 (minor), 170.5 (major), 158.5 (major), 158.2 (minor), 131.3 (major), 130.3 (minor), 129.83 (two carbons, minor), 129.81 (two carbons, major), 114.2 (two carbons, major), 114.0 (two carbons, minor), 55.35 (major), 55.32 (minor), 52.9 (major), 50.0 (minor), 37.0 (minor), 33.9 (major), 33.5 (minor), 32.9 (major), 22.1 (minor), 21.1 (major).

To a solution of *N*-(4-Methoxyphenethyl)-*N*-methylacetamide (1.05 g, 5.07 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL) at 0 °C was added dropwise  $\text{BBR}_3$  (1 M, 25.33 mL, 25.33 mmol, 5.0 equiv). The reaction mixture was slowly brought to room temperature, stirred for 2 h, and then was quenched with  $\text{H}_2\text{O}$ . The aqueous phase was extracted with EtOAc once. The combined organic layers were extracted with 1 M NaOH. The aqueous layer was neutralized with 1 M HCl and extracted with EtOAc, dried ( $\text{MgSO}_4$ ), and concentrated *in vacuo* to afford 870 mg (89%) of the unprotected phenol **7** as an off-white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.10 (br s, 1H), 7.03 (d,  $J = 8.5$  Hz, 2H, minor), 6.94 (d,  $J = 8.5$  Hz, 2H, major), 6.740 (d,  $J = 8.5$  Hz, 2H, major), 6.736 (d,  $J = 8.5$  Hz, 2H, minor), 3.59 (dd,  $J = 7.3, 7.3$  Hz, 2H, minor), 3.48 (dd,  $J = 6.3, 6.3$  Hz, 2H, major), 2.97 (s, 3H, major), 2.91 (s, 3H, minor), 2.755 (dd,  $J = 6.3, 6.3$  Hz, 2H, major), 2.752 (dd,  $J = 7.3, 7.3$  Hz, 2H, minor), 2.03 (s, 3H, minor), 1.65 (s, 3H, major);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  171.9 (major), 171.1 (minor), 155.9 (major), 155.1 (minor), 129.84 (major, two carbons), 129.75 (minor, two carbons; minor, one carbon), 128.9 (major), 115.8 (major, two carbons), 115.4 (minor, two carbons), 52.9 (major), 49.3 (minor), 36.4 (minor), 33.5 (major), 33.5 (major), 32.7 (minor), 21.7 (minor), 20.7 (major).

**Preparation of *N*-(3,5-Dibromo-4-hydroxyphenethyl)-*N*-methylacetamide (**2**).** Phenol **7** (510.0 mg, 2.64 mmol) was dissolved in glacial acetic acid (5.30 mL) and then treated with  $\text{Br}_2$  (340  $\mu\text{L}$ , 6.62 mmol, 2.5 equiv) dropwise at room temperature. The reaction went to completion within 3 h, as monitored by TLC, and was quenched with 10% aqueous  $\text{Na}_2\text{S}_2\text{O}_3$ . The product **2** was extracted with EtOAc, dried ( $\text{MgSO}_4$ ), and concentrated *in vacuo* to provide 820 mg (88%) of tan-colored material, which was used without further purification:  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.37 (s, 2H, minor), 7.36 (s, 2H, major), 3.54 (t,  $J = 7.0$  Hz, 2H, minor), 3.50 (dd,  $J = 7.7, 7.7$  Hz, 2H, major), 2.98 (s, 3H, major), 2.90 (s, 3H, minor), 2.78 (t,  $J = 7.0$  Hz, minor), 2.71 (dd,  $J = 7.5, 7.5$  Hz, 2H, major), 2.05 (s, 3H, major), 1.87 (s, 3H, minor);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  173.3, 151.1 (major), 150.8 (minor), 134.6 (major), 134.1 (minor), 133.9 (two carbons, minor), 133.7 (two carbons, major), 112.3 (two carbons, minor), 112.2 (two carbons, major), 53.3 (minor), 50.5 (major), 37.2 (major), 33.8 (minor), 33.7 (minor), 32.8 (major), 21.7 (major), 21.0 (minor).

***N*-(4-(3-Aminopropoxy)-3,5-dibromophenethyl)-*N*-methylacetamide (**9**).** Phenol **2** (1.54 g, 4.39 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (22 mL) and treated with  $\text{K}_2\text{CO}_3$  (910 mg, 6.58 mmol, 1.5 equiv) at room temperature. The resulting suspension was heated to 50 °C, and alkyl bromide **8** (1.04 g, 4.37 mmol, 1 equiv) in  $\text{CH}_3\text{CN}$  (22 mL) was added to it. The reaction was allowed to proceed overnight, then cooled to room temperature and worked up following the standard protocol to give 1.78 g (80%) of the desired alkylated phenol as an off-white solid. In addition, the aqueous phase was neutralized with 1 M HCl and further extracted with EtOAc (3  $\times$  50 mL), dried ( $\text{MgSO}_4$ ), and concentrated *in vacuo* to yield 120 mg of the unreacted starting material (8% recovery). The product was used without further purification:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.36 (s, 2H, major), 7.32 (s, 2H, minor), 4.04 (t,  $J = 5.8$  Hz, 2H, minor), 4.03 (t,  $J = 5.8$  Hz, 2H, major), 3.53 - 3.42 (m, 4H), 2.94 (s, 3H, minor), 2.93 (s, 3H, major), 2.77 (t,  $J = 7.7$  Hz, 2H, minor), 2.75 (dd,  $J = 7.7, 7.7$  Hz, 2H, major), 2.08 (s, 3H, major), 2.04 (m, 2H), 1.99 (s, 3H, minor), 1.44 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.7 (major), 170.4 (minor), 156.2, 152.1 (minor), 151.6 (major), 138.1 (major), 137.0 (minor), 133.1 (two carbons, major), 132.9

(two carbons, minor), 118.6 (two carbons, minor), 118.3 (two carbons, major), 79.3, 71.4, 52.2 (minor), 49.5 (major), 38.2, 37.1 (major), 33.8 (minor), 33.6 (minor), 32.6 (major), 30.2, 28.6 (three carbons), 22.1 (major), 21.3 (minor).

***tert*-Butyl-3-(2,6-dibromo-4-(2-(*N*-methylacetamido)ethyl)phenoxy)-propylcarbamate** (1.78 g, 3.50 mmol) was dissolved in 1,4-dioxane (40 mL), and the resulting solution was placed in an ice bath. Concentrated HCl (500  $\mu\text{L}$ , 37%) was added dropwise, and the bath was removed. The reaction was completed in 1.5 h, as revealed by TLC. The pH of the solution was adjusted to 7 by adding 1 M NaOH and extracted with EtOAc (3  $\times$  100 mL), dried ( $\text{MgSO}_4$ ), and concentrated *in vacuo* to yield 1.37 g (96%) of **9**:  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.40 (br s, 2H), 7.63 (s, 2H, major), 7.53 (s, 2H, minor), 4.95 (br s, 1H), 3.98 (t,  $J = 5.9$  Hz, 2H), 3.44 (m, 2H), 3.01 (t,  $J = 7.4$  Hz, 2H), 2.93 (s, 3H, minor), 2.786 (s, 3H, major), 2.785 (m, 2H, minor), 2.69 (dd,  $J = 7.5, 7.5$  Hz, 2H, major), 2.05 (m, 2H), 1.94 (s, 3H, minor), 1.86 (s, 3H, major);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  169.5 (minor), 169.3 (major), 150.6 (major), 150.4 (minor), 139.0 (minor), 138.6 (major), 133.3 (two carbons, major), 132.9 (two carbons, minor), 117.3 (two carbons, major), 117.2 (two carbons, minor), 70.59 (major), 70.53 (minor), 51.0 (minor), 47.7 (major), 36.5, 35.7 (minor), 32.6 (major), 32.3 (minor), 31.4 (major), 28.4, 21.6 (minor), 20.9 (major).

**Preparation of 3-Methyl-3H-purin-6(9H)-one (**12**).** Sodium metal (1.47 g, 64.0 mmol, 2 equiv) was dissolved in absolute EtOH (60.0 mL) to prepare 1.0 M sodium ethoxide in EtOH. Compound **10** (4.55 g, 32.0 mmol) was added to the solution at room temperature and stirred until all material dissolved. A solution of thiourea **11** (2.88 g, 32.0 mmol) in 42 mL of 2-methoxyethanol was combined with the reaction mixture, and the resulting mix was refluxed for 5.5 h. After cooling to 0 °C, 1.0 M HCl was added dropwise until a pH of 2 was obtained. A blue-gray solid formed, which was filtered, washed with a small portion of ice-cold  $\text{H}_2\text{O}$ , and dried in an oven overnight to yield 5.10 g (86%) of 6-amino-2-mercapto-1-methyl-5-nitrosopyrimidin-4(1H)-one. The compound was used without purification.

6-Amino-2-mercapto-1-methyl-5-nitrosopyrimidin-4(1H)-one (5.10 g, 27.4 mmol) was dissolved in 100 mL of 1.0 M NaOH, and sodium dithionite (17.65 g, 101.4 mmol, 3.7 equiv) was added. After 1.5 h at room temperature, the solids were filtered off and dried in an oven to obtain 4.7 g of 5,6-diamino-2-mercapto-1-methylpyrimidin-4(1H)-one in quantitative yield. These steps were repeated to provide sufficient material for the last stage of the synthesis of **12**.

5,6-Diamino-2-mercapto-1-methylpyrimidin-4(1H)-one (7.74 g, 44.95 mmol) was dissolved in DMF (10 mL) and trimethyl orthoformate (73.76 mL, 674.18 mmol, 15.0 equiv). The mixture was heated at reflux for 5 days. After full conversion of the starting material was achieved (LC-MS analysis) the reaction mixture was concentrated and the residual material was recrystallized from water to obtain 8.19 g (100%) of compound **12**:  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  13.84 (br s, 1H), 12.47 (br s, 1H), 8.17 (s, 1H), 3.78 (s, 3H).

**3-Methyl-3H-purin-6(9H)-one (**13**).** Raney nickel (16.0 g) was washed three times with 2-methoxyethanol and then suspended in minimal  $\text{H}_2\text{O}$ . A solution of purinone **12** (8.20 g, 45.0 mmol) in 1.0 M NaOH (55.0 mL) was added to the Raney nickel slurry. The reaction mixture was heated to 80 °C for 3 h until all starting material was consumed as determined by TLC. The room-temperature mixture was filtered through a pad of Celite, which was washed with  $\text{H}_2\text{O}$  and 2-methoxyethanol. The filtrate was concentrated *in vacuo*. A total of 6.76 g of **13** was obtained and was used without purification:  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.86 (s, 1H), 7.32 (s, 1H), 3.66 (s, 3H).

**6-Chloro-3-methyl-9H-purin-3-um chloride (**14**).** To purinone **13** (100 mg, 666  $\mu\text{mol}$ ) was added pyridine (5.45 mL) and phosphorus pentasulfide (454.5 mg, 2.04 mmol, 3.1 equiv), and the reaction was refluxed for 4 h and then concentrated *in vacuo*. To the resulting brown solid was added boiling water. A suspension formed, which was allowed to cool to room temperature. The solids were filtered off and dried in an oven overnight to deliver 56 mg (51%) of the corresponding purine-6(9H)-thione:  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  13.65 (br s, 1H), 8.42 (s, 1H), 8.36 (s, 1H), 3.86 (s, 3H).

3-Methyl-3H-purine-6(9H)-thione (5.34 g, 32.13 mmol) was combined with 2.5% aqueous NaOH (66.74 mL, 1.3 equiv) and iodomethane (4.00 mL, 64.26 mmol, 2.0 equiv) at room temperature. The resulting mixture was stirred for 2 h at room temperature, during which time a white solid precipitated. The precipitate was filtered off and dried to deliver 3.56 g (61%) of the methylthio product:  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.84 (s, 1H), 8.15 (s, 1H), 4.11 (s, 3H), 2.74 (s, 3H).

Chlorine gas was bubbled through ice-cold anhydrous MeOH (2.5 mL). To this saturated solution was slowly added 3-methyl-6-(methylthio)-3*H*-purine (215.0 mg, 1.19  $\mu$ mol) in MeOH (1.5 mL), after which Cl<sub>2</sub> was bubbled through the solution for an additional 10 min. The resultant precipitate was filtered, washed with cold benzene, and dried *in vacuo* to afford a light yellow solid salt, **14** (70 mg, 29%).

**Preparation of Aphrocallistin (1).** A solution of amine **9** (139 mg, 341  $\mu$ mol) in 1-butanol (5 mL) and Hünig's base (130  $\mu$ L, 751  $\mu$ mol) was cooled to 0 °C. To this solution was added salt **14** (70 mg, 341  $\mu$ mol), and the mixture was stirred at 0 °C for 1 h, after which the solution was warmed to room temperature and stirred for 16 h. The reaction was quenched with brine, the pH was adjusted to 9 with saturated Na<sub>2</sub>CO<sub>3</sub> solution, and the reaction was extracted with EtOAc (3  $\times$  20 mL), dried (MgSO<sub>4</sub>), and concentrated *in vacuo*.

**Salt Formation.** Crude **1** was dissolved into anhydrous methanol (5 mL), and TFA (100  $\mu$ L) was added. The solution was stirred for 30 min at room temperature. The reaction was concentrated *in vacuo*, redissolved in MeOH (5 mL), and concentrated *in vacuo* (2 $\times$ ). The resulting yellow oil was purified via reversed-phase HPLC to obtain 70 mg of **1**·TFA (31% unoptimized yield). Spectral analysis (<sup>1</sup>H NMR and LCMS, see Supporting Information) confirmed the synthetic material to be identical with the natural product.

**Cytotoxicity Assays.** Aphrocallistin was analyzed as to its effects on proliferation of A549 human lung adenocarcinoma, AsPC-1 human pancreatic adenocarcinoma, PANC-1 human pancreatic carcinoma, BxPC-3 pancreatic adenocarcinoma, MIA PaCa2 human pancreatic carcinoma, DLD-1 human colorectal adenocarcinoma, NCI-ADR-RES (formerly MCF-7/ADR) human ovarian carcinoma, and the Vero monkey kidney cell lines. The A549 (ATCC# CCL-185), PANC-1 (ATCC# CRL-1469), DLD-1 (ATCC# CCL-221), AsPC-1 (ATCC# CRL-1682), BxPC-3 (ATCC# CRL-1687), Mia-Paca2 (ATCC# CRL-1420), and Vero (ATCC# CCL-81) cell lines were obtained from the American Type Culture Collection (Rockville, MD). The NCI/ADR-RES cell line was obtained from the NCI Cancer Cell Repository. Assays were run using protocols described previously.<sup>13</sup> All samples were assayed a minimum of three times to derive the final IC<sub>50</sub> value.

**Cell Cycle Analysis.** PANC-1 human pancreatic carcinoma cells were used as targets to observe the effects of aphrocallistin on the cell cycle. Cell cycle analysis was performed as follows: PANC-1 cells were incubated in tissue culture media (TCM = Roswell Park Memorial Institute (RPMI) medium 1640 supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 60 mg/mL L-glutamine, 18 mM HEPES, 0.05 mg/mL gentamicin, 100  $\mu$ g/mL sodium pyruvate, 2.5 mg/mL glucose, and 10% fetal bovine serum) at 37 °C in 5% CO<sub>2</sub> in air in the presence or absence of varying concentrations of aphrocallistin, methanol (vehicle control), or paclitaxel (positive control) for 24 h. At the end of this incubation, cells were harvested, fixed in ethanol, and stained with 0.02 mg/mL of propidium iodide together with 0.1 mg/mL of RNase A. Stained preparations were analyzed on a BD FACSCanto. A total of 10 000 events were collected per sample. Raw histogram data were further analyzed using the DNA analysis software ModFit (Verity, Topsham, ME).

**Antimicrobial Testing.** The antimicrobial activity of **1** was determined through the use of broth dilution assays using the following strains identified by their ATCC catalog number: *Candida albicans* 44506; methicillin-resistant *Staphylococcus aureus* 700787; and *Pseudomonas aeruginosa* 27853. Assays were performed as

standard microtiter assays in a total volume of 50  $\mu$ L at a seeding density of 10<sup>3</sup>/mL. *C. albicans* was grown in Sabouraud dextrose broth. The bacteria were grown in cation-supplemented Mueller-Hinton broth. Assays were performed at 37 °C. The level of growth was determined after 24 h with the minimum inhibitory concentration being defined as the lowest concentration of the compound that completely inhibited growth of the test strain. **1** gave MICs of 250  $\mu$ g/mL against *C. albicans* and methicillin-resistant *S. aureus* and >500  $\mu$ g/mL against *P. aeruginosa*.

**Acknowledgment.** The work described in this paper was funded in part by NIH grant number CA-93455 and the State of Florida Fish and Wildlife Research Institute Drug Discovery from Florida's Oceans contract. We acknowledge a grant from HRSA, which funded in part the purchase of the JEOL NMR and FACSCanto flow cytometer used in this work. We thank C. Cody at JEOL for high-resolution mass spectral data; Dr. H. M. Reiswig of the Department of Biology, University of Victoria, Victoria, British Columbia, Canada, for the sponge identification; and the Burnham Exploratory Pharmacology group (M. Vicchiarelli and A. Mangravita-Novo) for performing the *in vitro* ADME profiling assays. This is Harbor Branch Oceanographic Institute contribution number 1733.

**Supporting Information Available:** Copies of the <sup>1</sup>H and <sup>13</sup>C NMR spectra along with selected 2D NMR spectra (g-DQF-COSY, edited-g-HSQC, g-HMBC, g-NOESY) for the natural product; the <sup>1</sup>H NMR spectrum and LC-MS analysis of synthetic aphrocallistin; details of preparation of compound **6**; and details of the pharmacological testing are available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) Thiel, V.; Blumenberg, M.; Hefter, J.; Pape, T.; Pomponi, S.; Reed, J.; Reiter, J.; Wörheide, G.; Michaelis, W. *Naturwissenschaften* **2002**, *89*, 60–66.
- (2) Blumenberg, M.; Thiel, V.; Pape, T.; Michaelis, W. *Naturwissenschaften* **2002**, *89*, 415–419.
- (3) Guella, G.; Mancini, I.; Pietra, F. *Comp. Biochem. Phys. B* **1988**, *90B*, 113–15.
- (4) Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 6565–6569.
- (5) Killday, K. B.; Yarwood, D.; Sills, M. A.; Murphy, P. T.; Hooper, J. N. A.; Wright, A. E. *J. Nat. Prod.* **2001**, *64*, 525–526.
- (6) Okuda, K.; Kotake, Y.; Ohta, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2853–2855.
- (7) Georgiades, S. N.; Clardy, J. *Org. Lett.* **2006**, *8*, 4251–4254.
- (8) Woo, P. W. K.; Kostlan, C. R.; Sircar, J. C.; Dong, M. K.; Gilbertsen, R. B. *J. Med. Chem.* **1992**, *35*, 1451–1457.
- (9) (a) Nagamatsu, T.; Ukai, M.; Yoneda, F.; Brown, D. J. *Chem. Pharm. Bull.* **1985**, *33*, 3113–3121. (b) Richter, E.; Loeffler, J. E.; Taylor, E. C. *J. Am. Chem. Soc.* **1960**, *82*, 3144–3146.
- (10) Bergmann, F.; Levin, G.; Kalmus, A.; Kwietny-Govrin, H. *J. Org. Chem.* **1961**, *26*, 1504–1508.
- (11) Bergmann, F.; Nieman, Z.; Kleiner, M. *J. Chem. Soc.* **1966**, *10*, 15.
- (12) Huang, L.-K.; Cherg, Y.-C.; Cheng, Y.-R.; Jang, J.-P.; Chao, Y.-L.; Cherg, Y.-J. *Tetrahedron* **2007**, *63*, 5323–5327.
- (13) Guzmán, E. A.; Johnson, J. D.; Meyer, C. I.; Carrier, M. K.; Pitts, T. P.; Gunasekera, S. P.; Wright, A. E. *Anticancer Drugs* **2009**, *20*, 149–155.

NP900183V