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Caldoramide, a Modified Pentapeptide from the Marine Cyanobacterium *Caldora penicillata*

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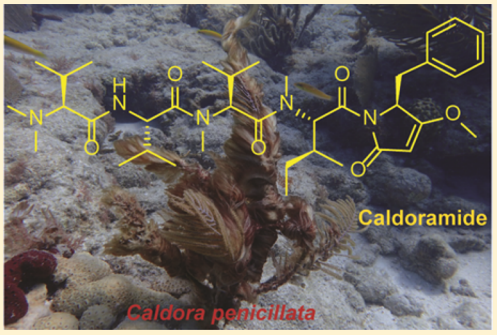
Supporting Information

ABSTRACT: The isolation, structure determination, and biological activities of a new linear pentapeptide, caldoramide (**5**), from the marine cyanobacterium *Caldora penicillata* from Florida are described. Caldoramide (**5**) has structural similarities to belamide A (**4**), dolastatin 10 (**1**), and dolastatin 15 (**2**). We profiled caldoramide against parental HCT116 colorectal cancer cells and isogenic cells lacking oncogenic KRAS or hypoxia-inducible factors 1α (HIF-1α) and 2α (HIF-2α). Caldoramide (**5**) showed differential cytotoxicity for cells containing both oncogenic KRAS and HIF over the corresponding knockout cells. LCMS dereplication indicated the presence of caldoramide (**5**) in a subset of *C. penicillata* samples.



Organisms from the largely unexplored marine environment have proven to be a rich source of secondary metabolites that are potential therapeutics. Of these, the benthic marine cyanobacteria have been reported to be a source of compounds rich in diversity and biological activity.[1−3] Cyanobacteria inhabit diverse environments, which could explain the chemical diversity of the secondary metabolites that have been isolated from these marine organisms. Among the compounds that showed interesting biological activities were the small peptides and depsipeptides collectively named dolastatins, which were originally isolated from the sea hare *Dolabella auricularia*.[4] The most potent among the series of dolastatins, dolastatin 10 (**1**), has been subsequently isolated from the cyanobacterium *Symploca* sp. (now known as *Caldora penicillata*) in a yield several magnitudes higher than from the sea hare.[5,6] Specifically, dolastatins 10 (**1**) and 15 (**2**) have displayed extraordinary cytotoxicity to cancer cells.[7,8] Their interesting biological activities have triggered synthetic efforts, resulting in several drug leads that advanced to clinical trials.[9] Inspired by the structure of dolastatin 10, a related cytotoxin served as the payload for the antibody−drug conjugate brentuximab vedotin (Adcetris), approved for the treatment of relapsed Hodgkin lymphoma and anaplastic large-cell lymphoma.[10] Largazole (**3**), a histone deacetylase (HDAC) inhibitor, has also been isolated from the cyanobacterium *Symploca* (now *C. penicillata*). Furthermore, a closely related structure to the dolastatins, belamide A (**4**), was reported as the major metabolite of the Panamanian cyanobacterium *Symploca* sp. and showed moderate cytotoxicity to the HCT116 colorectal cancer cell line.[11]

Here we report caldoramide (**5**), a new, closely related structural analogue of belamide A and dolastatin 15 (**2**), from the cyanobacterium *Caldora penicillata*. The taxonomy of this species has recently been clarified.[12] We have also detected via our dereplication method[13] that various geographically dispersed collections of *C. penicillata* consistently yielded known compounds such as dolastatin 10 (**1**) and/or the related homologue symplostatin 1 (**6**); these metabolites can serve as chemotaxonomic markers for this taxon.[12,13] In addition to caldoramide (**5**), largazole[14,15] and dolastatin 10 (**1**)[15] were isolated from this sample. Cytotoxicity profiling of caldoramide using three isogenic colorectal cancer cell lines[16,17] revealed preferential targeting of oncogenic KRAS and hypoxia-inducible factor (HIF) pathways.

The wet sample of *C. penicillata*, a re-collection from the Florida Keys, was stored frozen and freeze-dried prior to solvent extraction. The freeze-dried material was extracted first with EtOAc−MeOH (1:1) and then with EtOH−H_2O (1:1) to give the nonpolar and polar extracts, respectively. The two extracts were further partitioned to give EtOAc-soluble, *n*-BuOH-soluble, and H_2O-soluble fractions. The EtOAc-soluble fraction was subjected to further purification via repeated chromatography to give a new compound, caldoramide (**5**), as well as the known cytotoxic compounds largazole (**3**) and dolastatin 10 (**1**).

Caldoramide (**5**) was obtained as an optically active white powder. HRESI/TOFMS supported the molecular formula of

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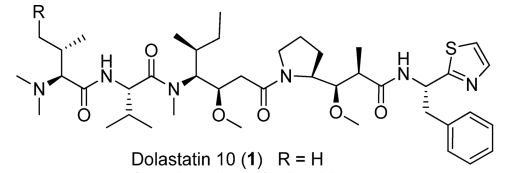

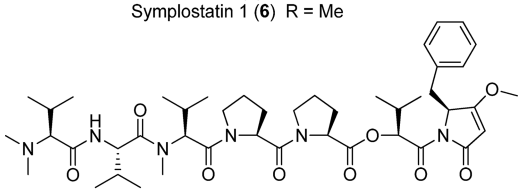

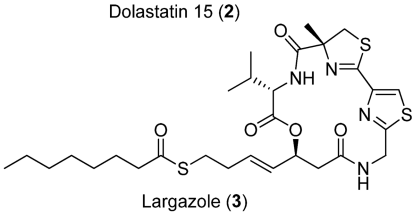

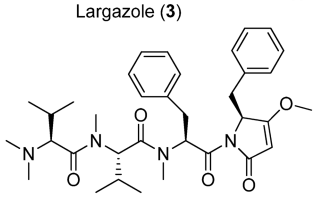

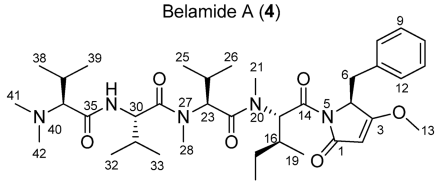

$C_{37}H_{60}N_5O_6$. Following the interpretation of DQF COSY, edited HSQC, and HMBC experiments (Table 1), the ^1H and ^{13}C NMR signals were assignable to one *O*Me group, *N*,*N*-dimethylvaline (C-35 to C-42), valine (C-29 to N-34), *N*-Me-valine (C-22 to C-28), *N*-Me-isoleucine (C-14 to C-21), a putative phenylalanine (C-4 to C-12), and a conjugated enol ether (C-1 to C-3). The remarkable chemical shifts recorded for a methine carbon (C-2, δ_C 94.8; δ_H 4.80) adjacent to a nonprotonated carbon (C-3, δ_C 178.1) suggested oxygenation at the latter position. Further, HMBC correlations between H-2 (δ_H 4.80) and C-1 (δ_C 169.1) and C-4 (δ_C 59.7); H-4 (δ_H 4.83) and C-2 (δ_C 94.8); and H_2-6 (δ_H 3.30, 3.08) and C-3 (δ_C 178.1) were observed, and these data established a substituted pyrrolinone ring (C-1 to C-5) similar to that reported for belamide A (**4**).[11] The HMBC correlation connected the *O*Me (δ_H 3.72) to the C-3 (δ_C 178.1) position in the pyrrolinone ring. Additional HMBC correlations from the H_2-6 methylene protons (δ_H 3.30, 3.08) to C-3 (δ_C 178.1), C-7 (δ_C 134.7), and C-8/12 (δ_C 129.6) connected the benzyl moiety to the C-4 position of the pyrrolinone ring. These data supported a 3-methoxy-4-benzylpyrrolinone moiety present at the C-terminus, and therefore, it was apparent that the *N*,*N*-dimethylvaline residue formed the N-terminus. Sequencing of the remaining three amino acid residues was accomplished by analyzing the HMBC and NOESY data (Table 1). NOESY correlations of H-34 (N−H, δ_H 6.92) to H-36 (δ_H 2.44) and H_3-41/42 (*N*,*N*-dimethyl, δ_H 2.23) of the *N*,*N*-dimethylvaline and to H-30 (δ_H 4.81) and H-31 (δ_H 1.96) of the valine moiety indicated the connection between these two amino acids. HMBC correlations of NH-34 to C-30 (δ_C 53.6) and C-35 (δ_C 171.8) and H-36 to C-35 confirmed the connection between the *N*,*N*-dimethylvaline and valine moieties. HMBC correlations between H_3-28 (*N*-Me, δ_H 3.10) and C-23 (δ_C 58.3) and C-29 (δ_C 172.8) and NOESY correlations shown between H_3-28 and H-23 (δ_H 5.27) and H_3-28 and H-30 (δ_H 4.81) of valine linked the valine and *N*-Me-valine residues. The NOESY correlations observed between H_3-21 (δ_H 3.21) and H-15 (δ_H 6.45), H-16 (δ_H 2.09), and H-23 (δ_H 5.27) and HMBC correlations between H_3-21 and C-15 (δ_C 58.7) and C-22 (δ_C 171.2) connected the *N*-Me-valine to the *N*-Me-isoleucine, which in turn is connected to the remaining C-terminal unit to satisfy the molecular formula. These data established the planar structure of caldoramide (**5**) as *N*,*N*-diMe-Val-Val-*N*-Me-Val-*N*-Me-Ile-3-*O*-Me-4-benzylpyrrolinone.

The absolute configuration was determined by enantioselective HPLC analysis. Determination of the configuration at C-4 required ozonolysis of **5** prior to hydrolysis with 6 N HCl. The ozonolysis[18] prior to hydrolysis yielded free phenylalanine in the hydrolysate.[11] The retention times of the components of the hydrolysate were compared to the amino acid standards and indicated the L-configuration for all amino acids in caldoramide (**5**).

Differential cytotoxicity profiling of isogenic knockouts of cancer genes can be used to determine target pathway specificity. Oncogenic KRAS and activated HIF pathways have been linked to angiogenesis, cell growth, survival, and metastasis.[16,17,19] To test if caldoramide's mechanism of action involves these pathways, we used the parental HCT116 colorectal cancer cell line that contains one oncogenic KRAS allele and one wild-type KRAS allele, HCT116 cells without the oncogenic KRAS allele (HCT $116^{WT\ KRAS}$), and HCT116 cells depleted of HIF-1α and HIF-2α ($HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$).[16] In a recently reported gene expression profiling of isogenic HIF and KRAS knockout cell lines, we showed that there is a significant overlap between global gene expression affected by oncogenic KRAS with genes affected by both HIF-1α and HIF-2α.[17] This signature overlap indicates that HIF transcriptional activity is regulated by oncogenic KRAS. Caldoramide (**5**) showed preferential antiproliferative activity against cells containing oncogenic KRAS or HIF transcription factors, viz., parental HCT 116 over $HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$ and over $HCT116^{WT\ KRAS}$ (Figure 1). The potency of caldoramide in $HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$ was slightly decreased (IC_{50} shifts from 3.9 μM to 5.2 μM) and maximum efficacy reduced to 67% compared with parental HCT116 cells. The IC_{50} shift in $HCT116^{WT\ KRAS}$ was more pronounced (3.9 μM to 8.6 μM), while efficacy was unaffected.

The cytotoxicity profiles of both dolastatin 10 (**1**) and largazole (**3**) have been previously determined using the same cell lines. Knockout cells were much less susceptible to dolastatin 10 with >50-fold shifts in IC_{50} values compared with parental cells with concomitant reduced efficacy (Table 2).[17] The HDAC inhibitor largazole also showed differential cytotoxicity for parental HCT116 over $HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$, but this difference in cytotoxicity is only seen with $HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$ and not in the cells lacking oncogenic KRAS (Table 2).[17] Caldoramide (**5**), although structurally related, is less potent than dolastatin 10[17] across the three cell lines investigated. Thus, all three compounds have varying activity profiles against the HCT116 isogenic cell lines that were tested, suggesting that the dose−

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Table 1. NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Caldoramide (5)

unit	C/H no.	δ_C mult.	δ_H (J in Hz)	COSY[a]	HMBC[b]	NOESY[c]
Bn-*O*-Me-pyrrolinone	1	169.1, C				
	2	94.8, CH	4.80, s		1, 4	13
	3	178.1, C				
	4	59.7, CH	4.83, dd (4.8, 3.4)	6	3, 7	6
	N-5					
	6	35.6, CH_2	3.30, dd (13.7, 4.8)	4	3, 4, 7, 8/12	4, 8/12
			3.08, dd (13.7, 3.4)	4	3, 4, 7, 8/12	4, 8/12
	7	134.7, C				
	8/12	129.6, CH	7.00, dd (7.5, 1.3)	9/11	6, 10	4, 11
	9/11	128.3, CH	7.20, m	8/12, 10	7	
	10	127.0, CH	7.14, m	9/11	8/12	
	13	58.4, CH_3	3.72, s		3	2, 8/12
N-Me-Ile	14	172.2, C				
	15	58.7, CH	6.45, d (10.9)	16	14, 17, 19, 21	17, 19
	16	34.3, CH	2.09, m	15, 17, 19	15, 17, 18	17, 18, 19
	17	25.1, CH_2	1.26, m	16, 18	15, 16, 18, 19	15, 16, 18
			1.10, m	16, 18	15, 16, 18, 19	15, 16, 18
	18	10.6, CH_3	0.84, t (7.5)	17	16, 17	16, 17
	19	14.5, CH_3	0.89, d (6.8)	16	15, 16, 17	15, 16
	N-20					
	21	31.8, CH_3	3.21, s		15, 22	15, 16, 23
N-Me-Val	22	171.2, C				
	23	58.3, CH	5.27, d (10.3)	24	22, 24, 25, 28	21, 25, 26, 28
	24	27.2, CH	2.38, m	23, 25, 26	22, 23, 25	23, 25, 26, 28
	25	19.6, CH_3	0.89, d (6.9)	24	23, 24, 26	23, 24, 28
	26	18.3, CH_3	0.75, d (6.9)	24	23, 24, 25	23, 24, 28,
	N-27					
	28	30.5, CH_3	3.10, s		23, 29	23, 24, 30, 31
Val	29	172.8, C				
	30	53.6, CH	4.81, dd (8.9, 3.4)	31, 34	29, 31, 32, 33	28, 31, 32, 34
	31	31.0, CH	1.96, m	30, 32, 33	29, 30, 32, 33	30, 32, 33, 34
	32	17.8, CH_3	0.93, d (6.9)	31	30, 31, 33	30, 31, 34
	33	19.9, CH_3	0.98, d (6.9)	31	30, 31, 32	30, 31
	NH-34		6.92, d (8.9)	30	30, 35	30, 31, 36, 41/42
N,*N*-diMe-Val	35	171.8, C				
	36	76.6, CH	2.44, d (6.2)	37	35, 38, 39, 41	34, 37, 38, 41/42
	37	27.7, CH	2.08, m	36, 38, 39	35, 36, 38, 39	36, 38, 39, 41/42
	38	20.2, CH_3	0.99, d (6.9)	37	36, 37, 39	36, 37, 41/42
	39	17.7, CH_3	0.92, d (6.9)	37	36, 37, 38	36, 37, 41/42
	40-N					
	41	43.0, CH_3	2.23, s		36	34, 36, 38, 39
	42	43.0, CH_3	2.23, s		36	34, 36, 38, 39

[a]COSY correlations are from proton(s) stated to the indicated proton(s). [b]HMBC correlations, optimized for $^{2/3}J_{CH}$ = 8 Hz, are from proton(s) stated to the indicated carbon. [c]NOESY correlations are from proton(s) stated to the indicated proton(s).

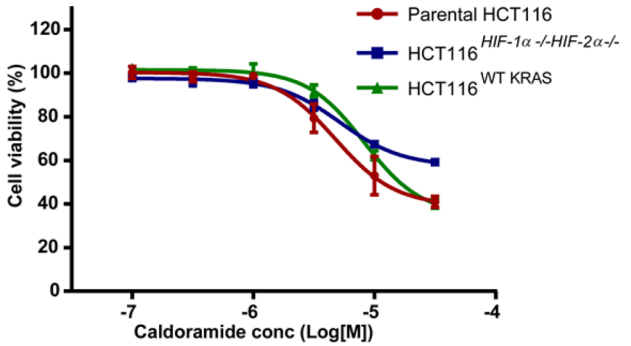

Figure 1. Effects of caldoramide (**5**) on cell viability of isogenic HCT116 cell lines.

response analyses of these cell lines might be used as a platform to group antiproliferative compounds by mechanism.

Extracts, both nonpolar and polar, for the different collections of cyanobacterial samples were eluted with MeOH through C18 solid phase extraction columns to remove interference from the polar compounds for HPLC-MS analysis. Detection of known compounds has provided a guide into prioritization of extracts at the early stage for further purification. The sensitive, reliable, and fast HPLC tandem mass spectrometry fragmentation (HPLC-MS/MS) was used to screen several extracts that were collected from various sites for the presence of known compounds. We monitored the parent ion and the most stable fragment ion to detect the presence of each compound. Using this technique, we have simultaneously scanned for the presence of dolastatin 10 (**1**),

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Table 2. Comparison of Cytotoxicity Profiles of the Three Coproduced Metabolites against Isogenic HCT116 Cells

	IC_{50}			efficacy relative to parental HCT116, %		
compound	parental	*HIF-1α−/−2α−/−*	oncogenic KRAS ko ($HCT116^{WT\ KRAS}$)	parental	*HIF-1α−/−2α−/−*	oncogenic KRAS ko ($HCT116^{WT\ KRAS}$)
caldoramide (**5**)	3.9 μM	5.2 μM	8.6 μM	100	67	100
dolastatin 10 (**1**)[a]	0.48 nM	65 nM	24 nM	100	74	83
largazole (**3**)[a]	6.41 nM	18 nM	3.7 nM	100	70	67

[a]Values taken from ref 17.

symplostatin 1 (**6**), largazole (**3**), and caldoramide (**5**) in an array of extracts and partitions from various collections of cyanobacterial samples (Figure 2). Most samples contained the

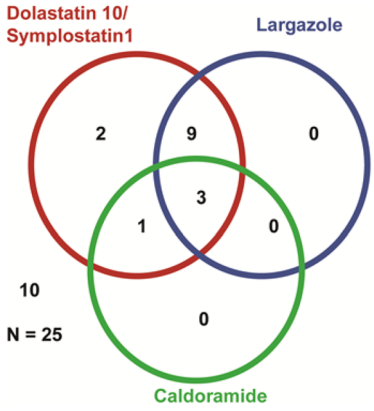

Figure 2. Distribution of the three structural classes of compounds isolated from marine cyanobacteria from Florida.

metabolites dolastatin 10 (**1**) and/or the structurally related symplostatin 1 (**6**), as well as largazole (**3**). Furthermore, we found that in the extracts tested, caldoramide (**5**) did not elute alone, but has been consistently coproduced with dolastatin 10 (**1**) or symplostatin 1 (**6**). This characteristic has been shown by largazole (**3**), which was reported to be coproduced with the same metabolites.[13] Lastly, from the extracts tested, caldoramide (**5**) was detected in four samples, all of which belong to *C. penicillata*. This suggests that the presence of caldoramide (**5**) is genus or species specific.

We have described the isolation of a modified peptide, caldoramide (**5**), from the marine cyanobacterium *C. penicillata*. Our tandem MS/MS method has revealed that this compound, like largazole, is coproduced with dolastatin 10 (**1**) and/or its homologue symplostatin 1 (**6**). While only moderately cytotoxic, similar to belamide A (**4**), we have shown that caldoramide (**5**) is able to inhibit the growth of cells that have both oncogenic KRAS and HIF-1α and HIF-2α transcription factors (parental HCT 116) to a greater extent than cells lacking those genes ($HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$ and $HCT116^{WT\ KRAS}$). A related yet not identical selectivity profile has been determined for dolastatin 10 (**1**) and largazole (**3**).[17] Consequently, all three coproduced compounds derived from the same extract appear to act as indirect HIF inhibitors and exert different profiles, suggesting complementary mechanisms.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were recorded on a Rudolph Research Analytical Autopol III automatic polarimeter. UV spectrophotometric data was acquired on a Shimadzu PharmaSpec UV−visible spectrophotometer. NMR data were collected on a JEOL ECA-600 spectrometer operating at 600.17 MHz for ^1H and 150.9 MHz for ^{13}C. The edited-HSQC experiment was optimized for J_{CH} = 140 Hz, and the HMBC spectrum was optimized for $^{2/3}J_{CH}$ = 8 Hz. ^1H NMR chemical shifts (referenced to residual $CHCl_3$ observed at δ_H 7.25) were assigned using a combination of data from 2D DQF COSY and multiplicity-edited HSQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to $CDCl_3$ observed at δ_C 77.0) were assigned on the basis of multiplicity-edited HSQC experiments. The HRMS data were obtained using an Agilent 6210 LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector at the Mass Spectrometer Facility at the University of California, Riverside, California. Silica gel 60 (EMD Chemicals, Inc. 230−400 mesh) was used for column chromatography. All solvents used were of HPLC grade (Fisher Scientific).

Collection, Extraction, and Isolation. The sample of cyanobacterial assemblage of *Caldora penicillata* for this study was collected from Big Pine Ledges (24.55302 N × 81.38028 W), a reef site near Big Pine Key, Florida, on March 30, 2015. This sample was a re-collection of FK 12-18, collected at the same location and well characterized by both microscopic and molecular methods in our recent paper that described this species,[12] and was consistent with the prior collection morphologically and chemically.[12,13] A voucher specimen of the re-collection (FK 15-1) is maintained at the Smithsonian Marine Station, Fort Pierce, FL. The freeze-dried material (96.6 g) was first extracted with EtOAc−MeOH (1:1) followed by H_2O−EtOH (1:1). The combined lipophilic and polar extract was partitioned between EtOAc and H_2O, and the aqueous portion subsequently partitioned between *n*-BuOH and H_2O. Concentration of these extracts furnished 0.775 g (0.8%) of an EtOAc-soluble fraction and 2.275 g (2.3%) of a BuOH-soluble fraction. The EtOAc-soluble fraction (0.760 g) was purified on a column of SiO_2 (25 g) using a step gradient system of hexanes−30% EtOAc, EtOAc, EtOAc−10% MeOH, and MeOH to give four subfractions (1a−1d). Subfraction 1b (0.106 g), eluting with EtOAc, was further chromatographed on a column of C_{18} silica (7 g) using a step gradient system of MeOH−10% H_2O, MeOH, and EtOAc to give three subfractions. The first subfraction (0.068 g), eluting with MeOH−10% H_2O, was rechromatographed on a Si column (6 g) using a step gradient system of hexanes−30% EtOAc, hexanes−60% EtOAc, EtOAc, and MeOH to give another four subfractions. The last two subfractions were combined (0.053 g) and pushed through a SiO_2 Sep-Pak with EtOAc followed by MeOH. Fraction 1 (0.019 g), eluting with EtOAc, was further purified by reversed-phase HPLC (semi-preparative 250 × 10 mm, 5 μm, RP-18, flow 3.0 mL/min) using MeOH−8.5% H_2O to give the known compound largazole (**3**) (t_R = 8.8 min, 2.0 mg yield, 0.002% dry wt) and caldoramide (**5**) (t_R = 9.4 min, 5.9 mg yield, 0.006% dry wt). Subfraction 1d (0.194 g), eluting with MeOH, was separated on a column of C_{18} (12 g) using a step gradient system of MeOH−H_2O followed by MeOH to give four subfractions. Subfraction 2 (6.5 mg), eluting with MeOH−10% H_2O, was further purified by reversed-phase HPLC (semipreparative 250 × 10 mm, 5 μm, RP-18, flow 3.0 mL/min) using MeOH−23% H_2O to give the known compound dolastatin 10 (**1**) (1.3 mg yield, t_R = 24 min, 0.001% dry wt). The two known compounds largazole[14] and dolastatin 10[15] were identified by LCMS data, and their structures were confirmed by comparing ^1H NMR spectra with the ^1H NMR spectra published in the literature.

*Caldoramide (**5**):* colorless, amorphous powder; $[\alpha]^{25}_D$ +11.1 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.19) nm; ^1H and ^{13}C NMR data, Table 1; HRESI/TOFMS *m/z* 670.4527 $[M + H]^+$ (calcd for $C_{37}H_{60}N_5O_6$, 670.4538).

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Ozonolysis, Acid Hydrolysis, and Enantioselective HPLC Analysis. Compound **5** (1.0 mg) was dissolved in 1.5 mL of CH_2Cl_2 and ozonized at −78 °C. The solvent was evaporated, and the product was suspended in 6 N HCl (0.5 mL) and heated at 110 °C for 12 h in a sealed tube. The hydrolysate was concentrated to dryness. The residue was reconstituted in 0.2 mL of H_2O and analyzed by enantioselective HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) penicillamine, 4.6 × 250 mm, 5 μm; solvent 2.0 mM $CuSO_4$ and mixtures of 2.0 mM $CuSO_4$−CH_3CN (95:5 or 92:8); detection at 254 nm]. Using 2.0 mM $CuSO_4$ with a flow rate of 0.8 mL/min, the retention times (t_R min) for authentic standards were *N*,*N*-diMe-L-Val (11.5) and *N*,*N*-diMe-D-Val (14.4), and with a flow rate of 1.0 mL/min, the retention times (t_R min) for authentic standards were *N*-Me-L-Val (19.5) and *N*-Me-D-Val (31.6). Using 2.0 mM $CuSO_4$−CH_3CN (95:5) with a flow rate of 1.0 mL/min, the retention times (t_R min) for authentic standards were L-Val (14.4) and D-Val (19.4) and with a flow rate of 0.8 mL *N*-Me-L-*allo*-Ile (25.3), *N*-Me-D-*allo*-Ile (37.1), and *N*-Me-L-Ile (29.1). *N*-Me-D-Ile was not available for this analysis. *N*-Me-D-Ile and *N*-Me-D-*allo*-Ile are reported to have close retention times.[18] No peak appeared close to the retention time of 37 min in the hydrolysate. Using 2.0 mM $CuSO_4$−CH_3CN (92:8) with a flow rate of 1.0 mL/min, the retention times (t_R min) for authentic standards were L-Phe (80.3) and D-Phe (84.5). The absolute configurations of the amino acid moieties in the hydrolysate were confirmed by comparing the retention times in their respective chromatography traces as *N*,*N*-diMe-L-Val (11.5), *N*-Me-L-Val (19.5), L-Val (14.4), *N*-Me-L-Ile (29.1), and L-Phe (80.3).

HPLC-MS Profiling. Cyanobacteria samples that were previously fractionated using 100% MeOH through a C18 solid phase extraction column were reconstituted in MeOH to 1 mg/mL concentration. Samples for analysis were diluted to 10 000 ng/mL in MeCN from the methanolic stock solutions. For each sample, 10 μL was injected and analysis was done using the following conditions: (1) HPLC: 0.1% HCOOH in MeCN−0.1% HCOOH in H_2O [20−100% MeCN for 25 min followed by 100% MeCN for 5 min, flow rate 0.25 mL/min, on a Hypersil GOLD aQ C18 polar end-capped LC column]; (2) MS and MS/MS: Thermo Fisher LCQ-Deca system with ESIMS in positive ion mode and CID 35%. Detection of largazole (**3**), symplostatin 1 (**6**), and dolastatin 10 (**1**) using MRM was previously reported.[13]

Cell Viability Assay. Human colorectal cancer cell lines HCT116, $HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$, and $HCT\ 116^{WT\ KRAS}$ were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and maintained in 5% CO_2 at 37 °C. The cells were seeded in a 96-well plate (10 000 cells/well) and treated with different concentrations of **5** after 24 h. The cell viability was measured 48 h following treatment with MTT dye using the manufacturer's protocol (Promega).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00203.

^1H, ^{13}C, COSY, HMBC, 2D NOESY, and HSQC NMR spectra of caldoramide (**5**) in $CDCl_3$ (PDF)

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Notes

The authors declare no competing financial interest.

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