Selected Papers

Kurahamide, a Cyclic Depsipeptide Analog of Dolastatin 13 from a Marine Cyanobacterial Assemblage of *Lyngbya* sp.

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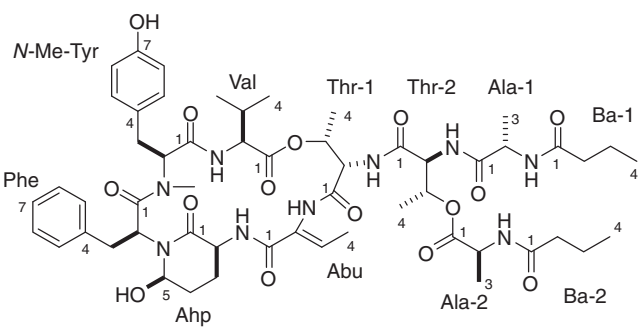
Kurahamide, a new dolastatin 13 analog, was isolated from a marine cyanobacterial assemblage, consisting mostly of *Lyngbya* sp. Its gross structure was elucidated by spectroscopic analysis, and the stereochemistries were assigned based on a chiral HPLC analysis of hydrolysis products. Kurahamide strongly inhibited elastase and chymotrypsin in vitro. In addition, kurahamide moderately inhibited the growth of human cancer cells, including HeLa and HL60 cells.

Marine microorganisms are considered to be good sources of new biologically active substances. Marine cyanobacteria, in particular, produce a variety of important secondary metabolites.[1] Several of these compounds, such as dolastatin 10[2] and biselyngbyaside,[3] have received attention due to their remarkable physiological activities.[4] In our continuing search for new bioactive substances from marine cyanobacteria,[5] we investigated the constituents of a marine cyanobacterial assemblage, consisting mostly of *Lyngbya* sp., and isolated a cyclodepsipeptide, kurahamide (**1**, Figure 1). The cyclic moiety of **1** is common to dolastatin 13[6] and its analogs. Several dolastatin 13 analogs have been isolated from marine cyanobacteria, including lyngbyastatins 4–10,[7] somamides A and B,[8] symplostatin 2,[9] and molassamide,[10] all of which differ with respect to the pendant side chain.

Results and Discussion

The marine cyanobacterial samples[11] were collected at Kuraha, Okinawa, and were extracted with methanol. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with reversed-phase column chromatography (ODS silica gel, MeOH–H_2O) and reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, MeOH–H_2O; Cosmosil Cholester, MeCN–H_2O) to give kurahamide (**1**) (3.7 mg) as a colorless oil.



kurahamide (**1**)

Figure 1. Structure of kurahamide (**1**).

The molecular formula of kurahamide (**1**) was found to be $C_{55}H_{77}N_9O_{15}$ by ESIMS (*m*/*z* 1126.5458, calcd for $C_{55}H_{77}N_9O_{15}Na$ $[M + Na]^+$ 1126.5437). The NMR data for **1** are summarized in Table 1. The ^{1}H NMR data suggested that **1** is a depsipeptide, with several exchangeable protons (δ_H ca. 6.5–7.5) and α-protons (δ_H ca. 4–5). Two low-field methine protons connected to the corresponding low-field shifted carbon signals (δ_H/δ_C 5.13/73.6, 5.19/71.7), as shown in the HMQC spectrum (Table 1), indicating methines adjacent to an ester linkage. Further analysis of the ^{1}H NMR, ^{13}C NMR, COSY, HMQC, and HMBC spectra recorded in CD_3CN revealed the presence of valine, *N*-methyltyrosine, phenylalanine, two threonines and two alanines. In Thr-1, there was no visible coupling between H-2 and H-3, as in other compounds with the same cyclic core.[7,9,10] HMBC and NOESY (Table 1) correlations established the partial structure of Thr-1. Additionally, the presence of residues derived from 3-amino-6-hydroxy-2-piperidone (Ahp), 2-amino-2-butenoic acid (Abu), and butanoic acid (Ba) were also established based on the detailed analysis of the COSY and HMBC spectra.

The sequence of each residue for **1** was determined on the basis of HMBC data and supported by NOESY data (Table 1). Two HMBC correlations from H-2 of Val (δ 4.08) and NH (δ 7.48) to C-1 of *N*-Me-Tyr (δ 172.4) connected these two residues. A strong HMBC correlation between the *N*-Me of *N*-Me-Tyr (δ 2.58) and C-1 of Phe (δ 172.9) connected these two units. Additionally, an HMBC correlation between H-2 of Phe (δ 4.63) and C-5 of Ahp (δ 76.2) revealed the connectivity between these two residues. Similarly, an HMBC correlation from NH of Ahp (δ 6.84) to C-1 of Abu (δ 165.1) and NOESY correlations observed at NH of Abu (δ 7.49)/H-2 of Thr-1 (δ

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Table 1. NMR Data for Kurahamide (**1**) in CD_3CN

Unit	Position	δ_C[a)]	δ_H[b)] (*J* in Hz)	COSY	HMBC	NOESY
Val	1	175.4				
	2	59.9	4.08, m	3, NH	1, 3, 4, 5, 1 (*N*-Me-Tyr)	3, 4, 5, NH
	3	31.8	1.85, m	2, 4, 5	2, 4, 5	2, 4, 5, NH
	4	20.07[c)]	0.69, d (7.0)	3	2, 3, 5	2, 3, *N*-Me (*N*-Me-Tyr)
	5	19.3	0.62, d (7.0)	3	2, 3, 4	2, 3, NH, *N*-Me (*N*-Me-Tyr)
	NH		7.48, br	2	2, 1 (*N*-Me-Tyr)	2, 3, 5, 2 (*N*-Me-Tyr), *N*-Me (*N*-Me-Tyr)
N-Me-Tyr	1	172.4				
	2	63.2	4.74, dd (11.5, 2.8)	3a, 3b	1, 3, 4, *N*-Me, 1 (Phe)	3a, 2 (Phe), 5/9, NH (Val)
	3a	34.4	3.10, dd (14.6, 2.8)	2, 3b	2, 4, 5/9	2, 3b, 5/9
	3b		2.51, dd (14.6, 11.5)	2, 3a	2, 4, 5/9	3a, 5/9
	4	130.2				
	5/9	132.1	6.84, d (8.6)	6/8	3, 4, 9/5, 6/8, 7	2, 3a, 3b, 6/8
	6/8	117.0	6.61, d (8.6)	5/9	4, 8/6, 7	5/9
	7	157.4				
	N-Me	32.1	2.58, s		2, 1 (Phe)	2 (Val), 4 (Val), 5 (Val), NH (Ahp), NH (Val)
Phe	1	172.9				
	2	52.7	4.63, dd (11.7, 4.4)	3a, 3b	1, 3, 1 (Ahp), 5 (Ahp)	3b, 5/9, 2 (*N*-Me-Tyr), NH (Ahp)
	3a	36.4	2.68, dd (14.7, 11.7)	2, 3b	2, 4, 5/9	3b, 5/9, 5 (Ahp)
	3b		1.78, m	2, 3a		2, 3a, 5/9
	4	138.5				
	5/9	131.0	6.64, dd (8.2, 1.7)	6/8, 7	3, 7	2, 3a, 3b, 5 (Ahp), 6/8, 4b (Ahp)
	6/8	129.4	7.00, m	5/9, 7	4, 8/6	5/9
	7	127.8	6.95, m	5/9, 6/8	5/9	
Ahp	1	170.78[d)]				
	2	50.1	3.64, ddd (2.4, 6.7, 12.7)	3a, 3b, NH	1	3b, NH
	3a	22.9	2.08, m	2, 3b, 4a, 4b		3b, OH, NH
	3b		1.44, m	3a, 4a, 4b	1, 5	2, 3a, 4a
	4a	30.8	1.57, m	3a, 3b, 4b, 5	2	3b, 5, OH
	4b		1.49, m	3a, 3b, 4a, 5	2	5, 5/9 (Phe)
	5	76.2	4.99, br s	4a, 4b, OH		4a, 4b, OH, 3a (Phe), 5/9 (Phe)
	OH		4.06, m	5		3a, 4a, 5, NH (Abu)
	NH		6.84, d (6.7)	2	1 (Abu)	2, 3a, 2 (Phe), NH (Abu), *N*-Me (*N*-Me-Tyr)
Abu	1	165.1				
	2	129.4				
	3	136.9	5.66, qd (7.1, 0.8)	4, NH	1, 2	4
	4	14.9	1.79, dd (7.1, 0.7)	3, NH	1, 2, 3	3
	NH		7.49, br s	3, 4	1, 2, 3, 1 (Thr-1)	NH (Ahp), 2 (Thr-1), 3 (Thr-1), NH (Thr-1), OH (Ahp)
Thr-1	1	170.81[d)]				
	2	57.5	4.27, d (9.4)	NH	1, 3, 4	3, 4, NH, NH (Abu)
	3	73.6	5.13, q (7.3)	4	1, 4, 1 (Val)	2, 4, NH (Abu)
	4	20.10[c)]	1.05, d (7.3)	3	2, 3	2, 3, NH
	NH		7.11, d (9.4)	2	2, 3, 1 (Thr-2)	2, 4, 2 (Thr-2), 3 (Thr-2), NH (Abu)
Thr-2	1	171.3				
	2	58.2	4.37, dd (8.0, 4.6)	3, NH	1, 3, 4, 1 (Ala-1)	3, 4, NH, NH (Thr-1)
	3	71.7	5.19, dq (4.6, 6.4)	2, 4	4, 1 (Ala-2)	2, 4, NH, NH (Thr-1)
	4	17.9	1.02, d (6.4)	3		2, 3, NH
	NH		7.04, d (8.0)	2	2, 3, 1 (Ala-1)	2, 3, 4, 3 (Ala-1), 2 (Ala-1)
Ala-1	1	174.8				
	2	50.5	4.17, dq (7.3, 7.0)	3, NH	1, 3	3, NH, NH (Thr-2)
	3	18.1	1.09, d (7.0)	2	1, 2	2, NH, NH (Thr-2)
	NH		6.71, d (7.3)	2	1, 2	2, 3, 2 (Ba)
Ala-2	1	173.8				
	2	49.8	4.07, m	3, NH	1, 3	3, NH
	3	18.1	1.08, d (7.5)	2	1, 2	2, NH
	NH		6.59, m	2	2, 3, 1 (Ba-2)	2, 3, 2 (Ba)

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Unit	Position	δ_C[a)]	δ_H[b)] (J in Hz)	COSY	HMBC	NOESY
Ba-1	1	174.4				
	2	39.01[e)]	1.91, m	3	1, 3, 4	3, NH (Ala)
	3	20.26[f)]	1.35, tq (7.7, 7.7)	2, 4	1, 2, 4	2, 4
	4	14.47[g)]	0.68, t (7.7)	3	2, 3	3
Ba-2	1	174.4				
	2	38.77[e)]	1.91, m	3	1, 3, 4	3, NH (Ala)
	3	20.36[f)]	1.35, tq (7.7, 7.7)	2, 4	1, 2, 4	2, 4
	4	14.55[g)]	0.67, t (7.7)	3	2, 3	3

a) Measured at 100 MHz. b) Measured at 400 MHz. c), d), e), f), g) These carbon signals are interchangeable.

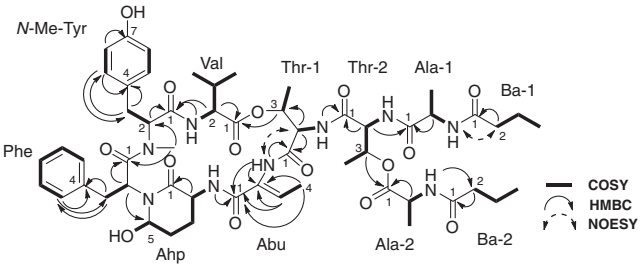

Figure 2. Structure of kurahamide (**1**), based on 2D NMR analyses.

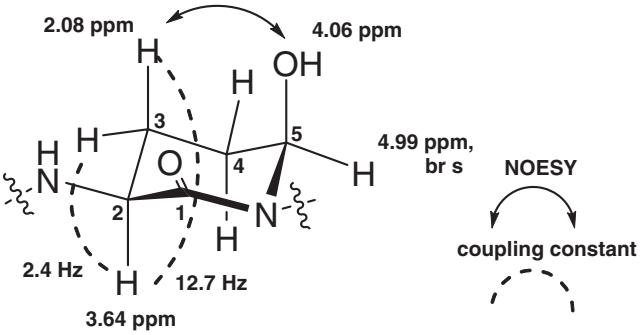

Figure 3. Selected NOESY correlations and coupling constants showing the relative stereochemistry of the Ahp residue in kurahamide (**1**).

4.27) and NH of Abu (δ 7.49)/H-3 of Thr-1 (δ 5.13) expanded the sequence to Val–*N*-Me–Tyr–Phe–Ahp–Abu–Thr-1. Additionally, HMBC correlations from NH of Thr-1 (δ 7.11) to C-1 of Thr-2 (δ 171.3), from H-2 of Thr-2 (δ 4.37) to C-1 of Ala-1 (δ 174.8), and from H-3 of Thr-2 (δ 5.19) to C-1 of Ala-2 (δ 173.8) revealed the linear sequence for all nine amino acids. Furthermore, an HMBC correlation between H-3 of Thr-1 (δ 5.13) and C-1 of Val (δ 175.4) indicated the presence of an ester bond between these amino acids. The low-field chemical shift of H-3 of Thr-1 also supported the presence of an ester bond at this position and thereby established the cyclic partial structure of **1**. Finally, a NOESY correlation between NH of Ala-1 (δ 6.71) and H-2 of Ba-1 (δ 1.91) and an HMBC correlation between NH of Ala-2 (δ 6.59) and C-1 of Ba-2 (δ 174.4) allowed us to determine the location of two butanoic acid moieties, thereby completing the gross structure of kurahamide, as shown in Figure 2.

The absolute configurations of amino acids except for the Ahp moiety were determined by Marfey's analysis[13] and a chiral HPLC analysis of the acid hydrolysates of **1**. These analyses revealed that all of the amino acids had L-configurations. With regard to the Ahp moiety, the absolute configuration was determined by chiral HPLC analysis of the acid hydrolysates of a PCC oxidation product. Oxidation followed by acid hydrolysis liberated glutamic acid, the configuration of which was assigned to be L. In the Ahp ring system, the magnitude of the coupling constant ($^3J_{H2–H3a} = 12.7$ Hz, determined by a decoupling experiment) suggested that H-2 and H-3a are located in axial positions. Additionally, a NOESY correlation between H-3a (δ 2.08) and 5-OH (δ 4.06), and the observation of two small coupling constants for H-5 of Ahp (δ 4.99, br s) revealed that 5-OH is also located in an axial position of the piperidone ring. Thus, the absolute configuration of the Ahp unit was determined to be 2*S*, 5*R* (Figure 3). A four-bond HMBC correlation between H-4 (δ 1.79) and C-1 (δ 165.1) of Abu indicated a "w" configuration for bonds between these atoms and, therefore, a *Z* geometry for the double bond.[10,14] The absolute stereochemistry of kurahamide was determined as shown in **1**.

Table 2. IC_{50} Values of Kurahamide for Serine Protease Activities In Vitro

Compounds	IC_{50}/μM		
	Elastase	Chymotrypsin	Trypsin
Kurahamide (**1**)	0.10	9.0	>100
PMSF	860	200	1100

Because analogs of dolastatin 13 were reported to exhibit serine protease-inhibitory activity,[7a,7b,10] **1** was evaluated for this activity. The elastase, chymotrypsin, and trypsin activities were tested in vitro. As shown in Table 2, **1** showed strong inhibitory activity against elastase and chymotrypsin, with IC_{50} values of 0.10 and 9.0 μM, respectively. The inhibition activity of **1** was much stronger than that of phenylmethylsulfonyl fluoride (PMSF) which was used as a positive control. Meanwhile, there was no apparent inhibition of trypsin at 100 μM, the highest concentration tested. This is a similar selectivity profile to that previously reported for other dolastatin 13 analogs. According to a previous paper, the adjacent residue on the *N*-terminal side of Ahp unit should bind in the S1 specificity pocket of trypsin,[15] and a basic residue (L-Arg or L-Lys) at this position in the inhibitor is necessary for trypsin inhibition at concentrations under 10 μM.[16] In the case of kurahamide (**1**), the present residue is Abu, and this fact can explain why **1** is not an efficient trypsin inhibitor. Meanwhile, the inhibitory activities of **1** against elastase and chymotrypsin can come from the hydrophobicity of the Abu unit.[16,17] To evaluate the growth-

inhibitory activities against cancer cells by **1**, MTT assays with HeLa cells and HL60 cells were used. The cells were treated in 96-well plates with various concentrations of the compounds for 72 h. The data from these assays revealed that **1** inhibited the growth of both HeLa cells and HL60 cells, with IC_{50} values of 16 and 2.5 μM, respectively.

In conclusion, kurahamide (**1**), a novel analog of dolastatin 13, was isolated from a marine cyanobacterial assemblage, consisting mostly of *Lyngbya* sp. The structure of **1** was established by spectroscopic and chiral HPLC analysis of acid hydrolysates. Kurahamide (**1**) exhibited strong protease-inhibitory activity against elastase and chymotrypsin, with IC_{50} values of 0.10 and 9.0 μM, respectively. Compound **1** was also shown to inhibit the growth of HeLa cells and HL60 cells. Several dolastatin 13 analogs have been reported as protease inhibitors, and their modes of action have recently been reported.[17]

Experimental

General Experimental Procedures. Chemicals and solvents were the best grade available and were used as received from commercial sources. All NMR spectral data were recorded on a JEOL JNM-ECX400 spectrometer for ^{1}H (400 MHz) and ^{13}C (100 MHz). ^{1}H NMR chemical shifts (referenced to residual CHD_2CN observed at δ 1.93) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to CD_3CN observed at δ 118.2) were assigned based on HMBC and HMQC experiments. ESI mass spectra were obtained on an LCT premier EX spectrometer (Waters). Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO RT/IR-4200 instrument.

Collection, Extraction, and Isolation. The marine cyanobacterial samples were collected at Kuraha, Okinawa Prefecture, Japan, at a depth of 0–1 m in March 2013. The collected cyanobacteria (2.3 kg) were extracted with methanol (4 L) for one week. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between ethyl acetate (3 × 0.4 L) and water (0.4 L). The material obtained from the organic layer was partitioned between 90% aqueous methanol (0.4 L) and hexane (3 × 0.4 L). The aqueous methanol fraction (1.3 g) was first separated by column chromatography on ODS (13 g) eluted with 40% methanol, 60% methanol, 80% methanol, and methanol. The fraction (313 mg) eluted with 60% methanol was subjected to HPLC [Cosmosil 5C_{18}-MS-II (ϕ20 × 250 mm); flow rate 5 mL min^{-1}; detection, UV 215 nm; solvent 65% MeOH] in six batches to give a fraction that contained kurahamide (7.5 mg, t_R = 33.0 min). This fraction was further separated by HPLC [Cosmosil Cholester (ϕ20 × 250 mm); flow rate 5 mL min^{-1}; detection, UV 215 nm; solvent 40% MeCN] to give kurahamide (**1**) (3.7 mg, t_R = 40.0 min).

Kurahamide (1): colorless oil; $[\alpha]_D^{28}$ −28.4 (*c* 0.2, CH_3OH); IR (film): λ_{max} 3288, 2965, 1735, 1653, 1636, 1534, 1446, 1382, 1202 cm^{-1}; ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and NOESY data, see Table 1; HRESIMS *m/z* 1126.5458 $[M + Na]^+$ (calcd for $C_{55}H_{77}N_9O_{15}Na$, 1126.5437).

Acid Hydrolysis, Chiral HPLC Analysis, and Marfey's Analysis. Kurahamide (**1**) (0.5 mg) was treated with 9 M HCl (100 μL) for 23 h at 110 °C. The hydrolyzed product was evaporated to dryness and could be separated into each component, except for a mixture of Ala and Thr [Conditions for HPLC separation: column, Cosmosil 5C_{18}-PAQ (ϕ20 × 250 mm); flow rate, 5.0 mL min^{-1}; detection at 215 nm; solvent H_2O. Retention times (min) of components: Ala and Thr (t_R = 10.5 min), Val (t_R = 12.6 min), *N*-Me-Tyr (t_R = 31.2 min), Phe (t_R = 42.4 min)].

Each fraction was dissolved in H_2O (50 μL) and analyzed by chiral HPLC, and the retention times were compared to those of authentic standards [DAICEL CHIRALPAK (MA+) (ϕ4.6 × 50 mm); flow rate, 1.0 mL min^{-1}; detection at 254 nm; solvent 2.0 mM $CuSO_4$, 2.0 mM $CuSO_4$–MeOH (95:5) and 2.0 mM $CuSO_4$–MeCN (90:10)]. With 2.0 mM $CuSO_4$, the retention times (t_R min) for authentic standards were D-Val (3.8) and L-Val (6.1). With 2.0 mM $CuSO_4$–MeOH (95:5), the retention times for authentic standards were *N*-Me-D-Tyr (10.8) and *N*-Me-L-Tyr (13.2). With 2.0 mM $CuSO_4$–MeCN (90:10), the retention times for authentic standards were D-Phe (5.9) and L-Phe (8.6). The retention times (min) (and the respective HPLC conditions) of the amino acids in the hydrolysate were 6.1 (100:0), 13.2 (95:5), and 8.6 (90:10), indicating the presence of L-Val, *N*-Me-L-Tyr, and L-Phe in the hydrolysate.

The Ala- and Thr-containing fraction was dissolved in H_2O (100 μL). A 1.0% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (Marfey's reagent) solution in acetone (200 μL) and 50 μL of 1 M $NaHCO_3$ were added, and the mixture was heated at 80 °C for 3 min. The solution was cooled to room temperature, neutralized with 1 M HCl, and evaporated to dryness. The residue was resuspended in 50 μL of MeCN–H_2O (1:1), and the solution was analyzed by reversed-phase HPLC. [Cosmosil 5C_{18}-AR-II (ϕ4.6 × 250 mm); flow rate 1.0 mL min^{-1}; detection, UV 340 nm; solvent 0.02 M NaOAc–MeOH (45:55)]. The retention times (t_R min) of the derivatized amino acids in the hydrolysate matched those of L-Thr (6.4) and L-Ala (9.1), but not L-*allo*-Thr (6.6), D-*allo*-Thr (11.1), D-Thr (18.2) or D-Ala (23.6).

Oxidation, Acid Hydrolysis and Chiral HPLC Analysis. Kurahamide (**1**) (0.5 mg) was dissolved in CH_2Cl_2 (1.0 mL), and PCC (2.0 mg) was added. The reaction mixture was allowed to stand for 12 h at room temperature, after which it was washed with H_2O. The CH_2Cl_2 portion was dried under N_2. The PCC-oxidized kurahamide was heated with 9 M HCl (100 μL) for 23 h at 110 °C. The hydrolyzed product was evaporated to dryness and could be separated to give a Glu-containing fraction [Conditions for HPLC separation: column, Cosmosil 5C_{18}-PAQ (ϕ20 × 250 mm); flow rate, 5.0 mL min^{-1}; detection at 215 nm; solvent TFA–H_2O (0.1:99.9). Retention time (t_R min): Glu (10.9)]. The Glu-containing-fraction was dissolved in H_2O (50 μL) and analyzed by chiral HPLC, and the retention time was compared to those of authentic standards [DAICEL CHIRALPAK (MA+) (ϕ4.6 × 50 mm); flow rate, 1.0 mL min^{-1}; detection at 254 nm; solvent 2.0 mM $CuSO_4$]. The retention times (t_R min) for authentic standards were D-Glu (11.3) and L-Glu (16.4). The retention time (min) of Glu in the hydrolysate was 16.4, indicating the presence of L-Glu in the hydrolysate.

Gene Sequencing. A cyanobacterial filament was isolated under a microscope. Genomic DNA was extracted using the DNeasy Plant Mini kit (Qiagen) following the manufacturer's specifications. The 16S rRNA genes were PCR-amplified from isolated DNA using the primer set 16S 27F, a universal primer,

and 23S 30R, a cyanobacterial-specific primer. The PCR reaction contained DNA derived from a cyanobacterial filament, 12.5 μL of GoTaq (Promega), 1.0 μL of each primer (10 pM), and 10.5 μL of H_2O for a total volume of 25 μL. The PCR reaction was performed as follows: initial denaturation for 10 min at 95 °C, amplification by 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C, and final elongation for 7 min at 72 °C. PCR products were analyzed on agarose gel (1%) in TAE buffer and visualized by ethidium bromide staining. The obtained DNA was sequenced with 16S 27F, 16S 1494R, and 23S 30R primers. The 16S rRNA gene sequence is available in the DDBJ/EMBL/GenBank databases under accession number AB857842.

Protease Inhibition Assays. Serine protease-inhibitory activities were determined as previously reported with slight modifications.[7a] Elastase inhibitory activity was assessed using high-purity porcine pancreatic elastase (Sigma, E0258). The assay buffer used was 50 mM Tris-HCl (pH 8.0). Assay buffer (73.4 μL), elastase solution (75 μg mL^{-1} in assay buffer, 5 μL), and various concentrations of **1** (6.6 μL, dissolved in MeOH) were preincubated for 15 min at room temperature in a 96-well plate. After this time, 15 μL of substrate solution was added (2 mM *N*-succinyl-Ala–Ala–Ala-*p*-nitroanilide in assay buffer) to each well, and the reaction was followed by measuring the absorbance at 405 nm for 30 min. Inhibitory activities against chymotrypsin and trypsin were determined using α-chymotrypsin from bovine pancreas (Sigma, C4129) and trypsin from porcine pancreas (Sigma T0303), with 2 mM *N*-succinyl-Gly–Gly–Phe-*p*-nitroanilide as a substrate solution for chymotrypsin and 2 mM *N*α-benzoyl-L-arginine 4-nitroanilide hydrochloride for trypsin. The assay buffer was 50 mM Tris-HCl, 100 mM NaCl, and 1 mM $CaCl_2$ (pH 7.8). Assay buffer (40 μL), enzyme solution (5 μL, 1 mg mL^{-1} in assay buffer), and various concentrations of **1** (5 μL, dissolved in MeOH) were preincubated for 10 min at 37 °C, before 25 μL of substrate solution was added. The reaction was followed by measuring the absorbance at 405 nm for 30 min. PMSF (Nacalai tesque, Japan) was used as a positive control. Enzyme activity in each well was calculated based on the slope of the reaction curve compared to that of the solvent control.

Cell Growth Analysis. HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM (Nissui, Japan) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg mL^{-1} streptomycin, 0.25 μg mL^{-1} amphotericin, 300 μg mL^{-1} L-glutamine, and 2.25 mg mL^{-1} $NaHCO_3$. HL60 cells were cultured at 37 °C with 5% CO_2 in RPMI (Nissui, Japan) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg mL^{-1} streptomycin, 0.25 μg mL^{-1} amphotericin, 300 μg mL^{-1} L-glutamine, and 2.25 mg mL^{-1} $NaHCO_3$. HeLa cells were seeded at 2×10^4 cells/well in 96-well plates (Iwaki, Japan) and cultured overnight. HL60 cells were seeded at 1×10^5 cells/well in 96-well plates. Various concentrations of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by the MTT assay.

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Supporting Information

^{1}H NMR, ^{13}C NMR, NOESY, HMQC, and HMBC spectra in CD_3CN for kurahamide (**1**). HPLC chromatograms for determination of absolute configurations. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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11 Most of the cyanobacterium was morphologically classified into the genus *Lyngbya* because it was composed of short cells with a thick sheath. From the phylogenetic tree inferred from 959 bp of 16S rRNA gene sequences (see Supporting Information, S12) it was revealed that the present cyanobacterium (Maeda 130904A, accession no. AB857842) forms a clade with *Oscillatoria miniata* NAC8-50 (GU724208), and is closely related to *Trichodesmium* spp.[12] The identification described above was carried out using the same cyanobacterium collected at the same location in September 2013, and we confirmed that its extract contained kurahamide (**1**). Despite our efforts, other minor cyanobacteria in the assemblage were not identified by 16S rRNA gene analysis. A detailed classification will be reported later.

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