Isolation, Structure Determination, and Biological Activity of Dolastatin 12 and Lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix calcicola* Cyanobacterial Assemblages

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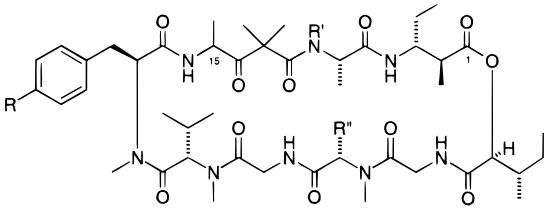
Lyngbyastatin 1 (**1a**), a new cytotoxic analogue of dolastatins 12 (**2a**) and 11 (**4**), was isolated as an inseparable mixture with its C-15 epimer (**1b**) from extracts of a *Lyngbya majuscula/Schizothrix calcicola* assemblage and a *L. majuscula* strain collected near Guam. Dolastatin 12 (**2a**) was also encountered as an inseparable mixture with its C-15 epimer (**2b**) in *L. majuscula/S. calcicola* assemblages. At least one of the compounds in each mixture appeared to exist in solution as a mixture of slowly interconverting conformers resulting in broadened signals in ^{1}H NMR spectra. Structure elucidation therefore relied principally on mass spectroscopy and chemical degradation studies. Both **1ab** and **2ab** proved toxic with only marginal or no antitumor activity when tested against colon adenocarcinoma #38 or mammary adenocarcinoma #16/C. Both **1ab** and **2ab** were shown to be potent disrupters of cellular microfilament networks.

Terrestrial and marine blue-green algae have proven to be an extremely valuable source of novel bioactive agents such as the cryptophycins[1] and curacins.[2] Cryptophycin-52 is planned for human clinical trials as an anticancer drug soon. As part of an ongoing program to search for new anticancer compounds from cyanophytes, we have found that extracts of a *Lyngbya majuscula* strain and two separate *L. majuscula/Schizothrix calcicola* assemblages, collected near Guam, exhibit marginally selective solid tumor toxicity in the Corbett soft agar disk diffusion assay modeled after the one commonly used in antifungal and antibacterial testing.[3] Bioassay-guided chromatographic fractionation resulted in the isolation of two separate active complexes, **1ab** and **2ab**.



	R	R'	R"	
1ab	OCH_3	CH_3	$(CH_3)_2CHCH_2$-	epimeric at C-15
2 ab	H	CH_3	$(CH_3)_2CHCH_2$-	epimeric at C-15
3	OCH_3	H	$CH_3CH_2(CH_3)CH$-	S at C-15
4	OCH_3	H	$(CH_3)_2CHCH_2$-	S at C-15

Results and Discussion

Although electrospray mass spectroscopy suggested the presence of only one component in each complex, extensive signal doubling and broadening in the ^{1}H and ^{13}C NMR spectra determined in a variety of solvents indicated that both complexes either contained at least two isomeric species or existed as a mixture of slowly interconverting conformers in solution. Variable-temperature experiments, which included heating each complex to 100 °C in DMSO-d_6 and in DMF-d_6, offered minimal improvement, and this complicated any attempt to use NMR spectroscopy as a significant tool in structure elucidation. It was evident from these spectral data, however, that **1ab** and **2ab** contained N-methylated depsipeptides and were closely related to each other. The ^{1}H NMR spectra of these complexes recorded in CD_3CN at 70 °C differed mainly in that **1ab** showed a methoxy signal at δ 3.78, which was absent in the spectrum of **2ab**, and a pair of doublets at δ 6.86 (J = 8.5 Hz) and δ 7.18 (J = 8.5 Hz) as opposed to a 5H multiplet centered at δ 7.24 for **2ab**. This suggested that **1ab** was a methoxy analogue of **2ab**. This was further supported by HRFABMS, which indicated a 30 mass unit difference between these two; the components in **1ab** had a molecular formula of $C_{51}H_{82}N_8O_{12}$, whereas the components in **2ab** had a molecular formula of $C_{50}H_{80}N_8O_{11}$. The molecular formulas and the cyanobacterial origin of these components suggested that they were N-methylated analogues of majusculamide C (**3**) or even of dolastatins 11 (**4**) and 12 (**2a**). Majusculamide C (**3**) is a known *L. majuscula* metabolite,[4] whereas dolastatins 11 (**4**) and 12 (**2a**) are constituents of the sea hare, *Dolabella auricularia*,[5] which is known to be a generalist algal herbivore. Supporting evidence for this proposal was obtained from amino acid analysis, which demonstrated that glycine was the only typical amino acid in **1ab** and **2ab**. This meant that if **1ab** was, for example, an N-methylated analogue of majusculamide C (**3**) or dolastatin 11 (**4**), then the site of N-methylation was the alanine residue.

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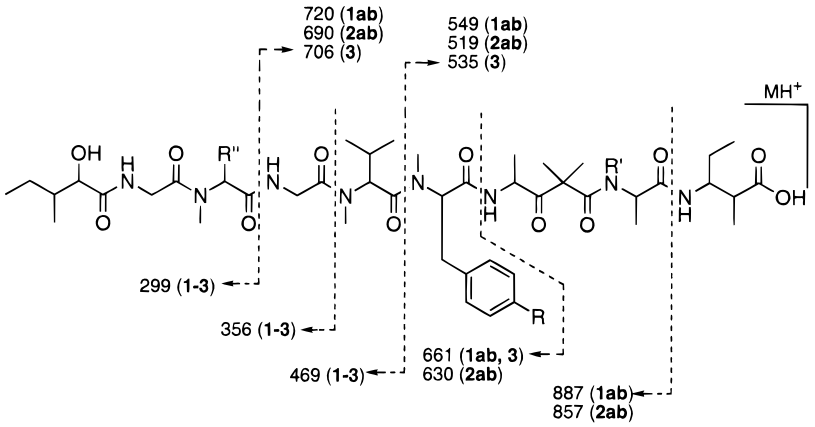
Scheme 1. FABMS Spectral Fragmentation Pattern of Base Hydrolysates of **1ab**, **2ab**, and Majusculamide C (**3**)[a]



	R	R'	R"
1ab	OCH_3	CH_3	$(CH_3)_2CHCH_2$-
2ab	H	CH_3	$(CH_3)_2CHCH_2$-
3	OCH_3	H	$CH_3CH_2(CH_3)CH$-

[a] Numbers in parentheses indicate to which hydrolysate a particular mass fragment refers. With the exception of the peak at *m/z* 356 all mass fragments were also observed in the corresponding CIDMS spectra.

Complexes **1ab**, **2ab**, and majusculamide C (**3**) were hydrolyzed in CH_3OH/0.5M NaOH (1:1). The ^1H NMR spectra of the hydrolysis products of **1ab** and **2ab** exhibited the same extensive broadening of signals observed in the NMR spectra of the parent compounds. Significantly, the hydrolysis product of **3** exhibited doubling of signals and an increased complexity in the ^1H NMR spectrum attributable to epimerization of the acid/base sensitive 4-amino-2,2-dimethyl-3-oxopentanoic acid (Ibu) residue. Diagnostic peaks included four methyl doublets (CD_3CN, 300 MHz) at δ 0.21 (J = 6.6 Hz), 0.28 (J = 6.6 Hz), 0.62 (J = 6.6 Hz), and 0.75 (J = 6.6 Hz). In majusculamide C (**3**), only two methyl doublets in this region at δ 0.20 (J = 6.1 Hz) and 0.62 (J = 6.8 Hz), corresponding to the *N*-methylvaline methyl groups, were recorded.

The base hydrolysis products were subjected to both HRFABMS and MS/MS. The fragmentation patterns shown in Scheme 1 supported the proposal that **1ab** and **2ab** were almost identical to majusculamide C (**3**) and dolastatins 11 (**4**) and 12 (**2a**).

Marfey analysis[6] of the acid hydrolysates of **1ab**, **2ab**, and majusculamide C (**3**) confirmed that **1ab** and **2ab** differed from majusculamide C (**3**) in possessing an *N*-methyl-L-alanine rather than an L-alanine residue. Moreover, **1ab** and **2ab** contained an *N*-methyl-L-leucine rather than an *N*-methyl-L-isoleucine residue, indicating a closer structural relationship to dolastatin 11 (**4**) and dolastatin 12 (**2a**) than to majusculamide C (**3**). These were the only differences, however, and even after comparison with the NMR spectra of dolastatin 12 (**2a**),[5] they did not immediately indicate the causes of the extensive broadening and doubling of signals in the NMR spectra of **1ab** and **2ab**. Chromatographic peaks corresponding to racemic 2-amino-4-methyl-3-pentanone derived from the Ibu residue in the Marfey profile offered the prospect that **1ab** and **2ab** each existed as a pair of C-15 epimers. However, proof of this possibility is made problematic by the acid/base sensitivity of the Ibu residue. Racemic mixtures of 2-amino-4-methyl-3-pentanone are obtained in the acid hydrolysis of majusculamide C (**3**) where the chirality at C-15 has been assigned as S on the basis of synthetic endeavors.[7] This stereochemistry also exists in dolastatins 11 (**4**) and 12 (**2a**).[7]

Epimerization of majusculamide C (**3**) could be effected with 5% TFA in CH_3CN on overnight standing at room temperature. This resulted in doubling and broadening of signals in its ^1H NMR spectrum. Diagnostic peaks included three distinct methoxy signals (CD_3CN, 300 MHz) at δ 3.78, 3.81, and 3.82 in an approximate 2:1:1 ratio. No changes were observed in the ^1H NMR spectra of **1ab** and **2ab** on similar acid treatment, indicating that epimerization at C-15 had already occurred.

Because of the complexity of the NMR spectra of **1ab** and **2ab**, however, it was still necessary to rule out all other isomeric possibilities. Samples of **1ab** and **2ab** were fully hydrolyzed in 6 N HCl and individual components isolated by semipreparative C_{18} reversed-phase HPLC. As expected from the Marfey analysis, ^1H NMR analysis of the amino acids thus obtained supported the presence only of the amino acids indicated in the structures given. The isolated hydroxy acid was determined to be L-isoleucic acid by CD and ^1H NMR spectral comparison with a synthetic standard. No leucic acid was detected. This left only epimerization at C-15 as a source of the extensive broadening and doubling of peaks in the NMR spectra of **1ab** and **2ab**. We have assigned the 15*S* epimer **1a** the trivial name lyngbyastatin 1 and the 15*R* epimer **1b** as epilyngbyastatin 1. The C-15 epimer of dolastatin 12 (**2a**) was similarly assigned as epidolastatin 12 (**2b**).

Complexes **1ab** and **2ab** had MICs of 0.1 and <0.05 μg/mL against KB (a human nasopharyngeal carcinoma cell line), respectively, and of 0.5 and 0.08 μg/mL against LoVo (a human colon adenocarcinoma cell line). The original cyanobacterial extracts and purified **1ab** and **2ab** showed moderate solid tumor selectivity in vitro when tested against the following cell types: a murine leukemia (L1210), two murine solid tumor (colon adenocarcinoma 38 and mammary 17/Adr), a human solid tumor (colon H116), and a normal cell (hematopoietic CFU-GM) (Table 1). In this assay, those agents which demonstrate a 250 or greater zone differential against one or more solid tumor cell lines and either the leukemia and/or normal cell (CFU-GM) are considered solid tumor selective.[3] Those that show a zone differential of between 150 and 250 units, as shown here, are considered to be moderately solid tumor selective.

Table 1. Evaluation of **1Ab**, **2Ab**, and **3** in the Corbett Solid Tumor Bioassay

compd/-extract	μg/disk	L1210	colon 38	mammary 17/Adr	colon H-116	CFU-GM
VP337L	35	800	900	430		−600
VP216L	26	350	580	260		−700
VP229L	115	410	610	350	550	400
1ab	2	450	670	650	580	570
2ab	2	420	630	270	480	500
3	12	500	730		710	450

Complex **1ab** at concentrations of 2 and 0.2 μg/mL caused the complete loss of filamentous (F)-actin coincident with dramatic changes in cell morphology when tested against A-10 cells, smooth muscle cells with an easily visualized cytoskeleton (Figure 1). The normally fibroblastic cells became neuron-like with small central areas and multiple long processes extending from the central regions. The effects were specific for microfilaments as there was no evidence of microtubule loss at these concentrations. Binuclear cells were present, consistent with inhibition of the actin-dependent process of cytokinesis. Evidence of apoptosis and the breakdown of nuclei into apoptotic bodies was prevalent at the 2 μg/mL concentration, although some cells treated at 0.2 μg/mL had fragmented nuclei. Similar results were obtained with complex **2ab**. Altered cellular morphology accompanied total disruption of the microfilament network at concentrations of 2 and 0.2 μg/mL. Complex **2ab** at concentrations of 0.01−2 μg/mL caused the inhibition of cytokinesis and the formation of many binuclear cells without microtubule loss. Cells containing apoptotic nuclei were present following treatment with 2 μg/mL. Cytochalasin B caused only partial disruption of the microfilament network when tested at 2 μg/mL.

Other than differences in potency, the toxicological profiles of **1ab** and **2ab** were similar when tested against mice. Both were poorly tolerated by intravenous (IV), subcutaneous, and intraperitoneal routes of administration. The trials were carried out by the IV route and resulted in sickness, stupor, lethargy, scruffiness, and a body temperature drop. Necrosy of drug deaths (from efficacy and toxicity trials) revealed an inflamed, bloody, upper GI tract with vascular damage to the pancreas and hemorrhage in the lungs. The spleen size was near normal, indicating that general marrow toxicity was not the usual cause of death. At maximum tolerated dose levels, there was no antitumor activity against either colon adenocarcinoma #38 or mammary adenocarcinoma #16/C (Tables 2 and 3). These tumors (combined) are able to detect almost all clinically useful agents with the exception of bleomycin, BCNU, and amethopterin.

It is evident that many biologically active compounds originally isolated from marine macroorganisms have a cyanobacterial or other microbial origin. Secondary metabolites of *L. majuscula*, for example, have previously been isolated from the sea hare *Stylocheilus longicauda*.[8] A significance of the results presented herein is that it represents the first time that analogues of dolastatin 11 (**4**) and 12 (**2a**) have been isolated from cyanobacteria and implies that other dolastatins may prove to be of cyanobacterial origin.

Experimental Section

General Experimental Methods. ^{1}H NMR analyses were conducted at 400 MHz on a Varian 400 spectrometer with residual nondeuterated solvent as an internal reference. ^{1}H NMR analyses on products of acid hydrolysates were carried out at 300 MHz on a GE 300 model. Electrospray mass spectra were run on a MAT 900 spectrometer. HRFABMS were performed on a VG ZAB2SE spectrometer, and MS/MS analyses used He as the collision gas. All MS experiments were run in the positive mode. CD spectra were obtained on a JASCO Model J-600 spectrometer.

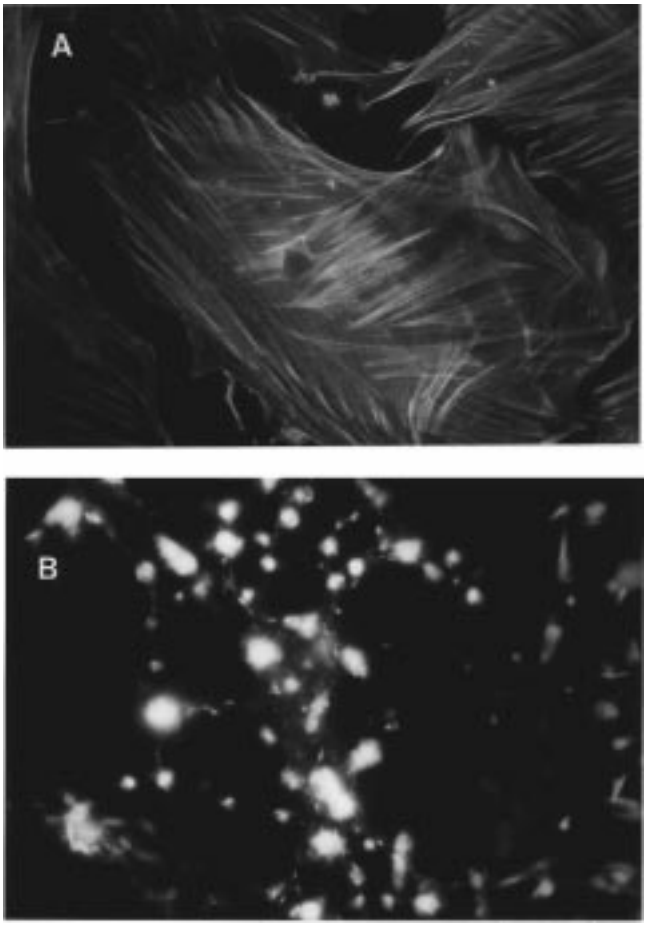


Figure 1. Complex **1ab**-induced microfilament loss. Cellular microfilaments composed of filamentous actin were visualized in A-10 cells using TRITC-labeled phalloidin. (A) Control cells treated with vehicle. Here, the extensive microfilament network and stress fiber bundles of actin filaments are clearly seen. (B) The cells were treated with complex **1ab** (0.2 μg/mL), which caused the complete loss of the cellular microfilament network.

Isolation. Cyanobacterial assemblage VP337 was a mixture of *L. majuscula*/*S. calcicola* collected at Tumon Bay, Guam, Sep 6, 1995. The freeze-dried collection was extracted with CH_2Cl_2/*i*-PrOH (1:1) to afford a lipophilic extract VP337L (11.64 g). Extraction was then continued with 70% aqueous CH_3OH to yield VP377A (∼1.0 g).

VP337L was partitioned between hexanes and 80% aqueous CH_3OH. The 80% aqueous CH_3OH fraction was dried and partitioned between *n*-BuOH and H_2O. The bioactive *n*-BuOH fraction was subjected to reversed-phase C_{18} chromatography (YMC-Gel ODS-A, I-230/70 mesh) eluting with 10% aqueous CH_3CN followed by H_2O mixtures containing progressively increasing amounts of CH_3CN. The 60 and 80% fractions were then subjected to semipreparative reversed-phase HPLC (Econosil C_{18}, 10 μM, 1.0 × 25.0 cm, 2 mL/min; detection at 220 nm) using a CH_3CN−0.1% HCOOH linear gradient (20−100% over 20 min and then 100% CH_3CN for 20 min). Complexes **1ab** (150.0 mg) and **2ab** (166.6 mg) were collected at t_R (min) 24.2 and 24.8, respectively.

VP 337A was partitioned as above. The bioactive *n*-BuOH fraction was subjected to chromatography on Sephadex LH-20 (Sigma), eluting initially with hexanes/CH_2Cl_2 (2:3), followed by CH_2Cl_2, CH_2Cl_2:$(CH_3)_2CO$ (1:1), $(CH_3)_2CO$, and finally CH_3OH. The hexanes:CH_2Cl_2 (2:3) fraction was then chro-

Table 2. Evaluation of **1Ab** and **2Ab** against Early Stage Colon Adenocarcinoma #38 in BDF Female Mice[a]

treatment	drug route	schedule	total dose (mg/kg)	% body wt loss at Nadir	drug deaths	median tumor burden in mg on day 13 (range)	*T/C* %	antitumor activity
no treatment (five mice)						839 (384−1232)		
complex **1ab**	IV	d 3,4,7−10	15	−8	3/4	toxic	toxic	toxic
	IV	QD 3−11	13	−3	0/4	832 (526−1363)	93	none
complex **2ab**	IV	QD 3−11	10.3	−15	2/4	368 (172−564)	41	marginal (toxic dose)
	IV	QD 3−11	7.5	−8	2/4	822 (732−911)	92	none (toxic dose)

[a] Tumors were trocared bilatrerally as 30−60 mg fragments on day 0. Treatment started on day 3.

Table 3. Evaluation of **1Ab** and **2Ab** against Early Stage Mammary Adenocarcinoma #16/C in C_3H Female Mice[a]

treatment	drug route	schedule	total dose (mg/kg)	% body wt loss at Nadir	drug deaths	median tumor burden in mg on day 13 (range)	*T/C* %	antitumor activity
no treatment (five mice)				−2		1273 (700−2393)		
complex **1ab**	IV	d 1,2	4.8	toxic	5/5	toxic	toxic	toxic
	IV	QD 1-9	11.7	−4	0/5	776 (663−1380)	61	none
complex **2ab**	IV	d 1,2	3.2	−13	2/5 (toxic)	862 (641−863)	68	none (toxic dose)
	IV	QD 1-8	6.4	−3	0/5	1100 (478−1613)	86	none

[a] Tumors were trocared bilatrerally as 30−60 mg fragments on day 0. Treatment started on day 3. Lower dosages for both **1ab** and **2ab** were also inactive, data not shown.

matographed on C_{18} eluting initially with 65% aqueous CH_3CN followed by H_2O mixtures containing progressively increasing amounts of CH_3CN. The earliest eluting 65% aqueous fraction was subjected to semipreparative HPLC as above to yield **1ab** (10.8 mg) and **2ab** (10.0 mg).

Cyanobacterium VP216 was a *L. majuscula* strain obtained from Guam Piti Bomb Hole, Feb 6, 1995. The freeze-dried organism was extracted with CH_2Cl_2 and the lipophilic extract evaporated to yield 1.14 g of a gum. This material was fractionated on a Varian Mega Bond Elut Si column, eluting initially with EtOAc and then with EtOAc mixtures containing progressively increasing amounts of MeOH. The active fraction was eluted with 20% MeOH in EtOAc. This fraction was chromatographed on a second Varian Mega Bond Elut Si column, eluting with CH_2Cl_2 mixtures containing progressively increasing amounts of MeOH. The active fraction eluted at 2% MeOH in CH_2Cl_2. The latter fraction was subjected to semipreparative HPLC on silica gel (Econosil Silica,10 μM, 1.0 × 25.0 cm, 3 mL/min; RI detection) and eluted initially with 80% EtOAc in hexanes. Complex **1ab** (43.3 mg) eluted as a long tailing peak when the solvent was changed to 100% EtOAc. A sample of **1ab** (17.9 mg) was then further purified by reversed-phase semipreparative HPLC as above eluting as a sharp peak at t_R 24.2 min.

Cyanobacterial collection VP229 was a *L. majuscula/S. calcicola* (9:1) assemblage collected at Jeff's Pirate Cove, Guam, Feb 27, 1995. Extraction of the freeze-dried material with CH_2Cl_2 afforded 5.92 g of extract. This extract was subjected to chromatography on Sephadex LH-20 (Sigma) eluting with MeOH/CH_2Cl_2. The bioactive fraction was further fractionated on Varian Mega Bond Elut Si eluting with CH_2Cl_2 mixtures containing progressively increasing amounts of MeOH. The active fraction eluted at 4% CH_3OH in CH_2Cl_2. This fraction was subjected to semipreparative HPLC on silica gel eluting with 4% CH_3OH in CH_2Cl_2, yielding 24.9 mg of complex **2ab**. This was then further purified by reversed-phase semipreparative HPLC as above to give a further 10.0 mg of **2ab**.

Attempts to separate the epimers of **1ab** and **2ab** and acid-treated majusculamide C (**3**) on analytical HPLC columns (Alltech, 0.46 × 25.0 cm) were unsuccessful. Stationary phases included C_{18} (Econosil, 10 μM), C_4 (Hypersil, 5 μM), phenyl (Hypersil, 5 μM), and CN (Econosil, 5 μM). Elution conditions included an aqueous CH_3CN linear gradient (20−100% over 20 min followed by 100% CH_3CN for 20 min) and isocratic conditions employing aqueous CH_3CN mixtures ranging from 50 to 80% CH_3CN. The flow rate in all cases was 1 mL/min. Detection was at 220 nm.

Absolute Configuration of Amino Acids. Complexes **1ab** (1.0 mg), **2ab** (1.0 mg), and majusculamide C (**3**) (1.0 mg) were each suspended in 6 N HCl (1 mL) and incubated at 108 °C for 18 h. They were then concentrated to dryness. The residues were each dissolved in H_2O (50 μL), and to the resulting mixtures were added a 1% (w/v) solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey reagent) in acetone (100 μL) and 1 M $NaHCO_3$ (20 μL). After being heated at 37 °C for 1 h, the reaction was cooled, acidified with 2 N HCl (20 μL), and evaporated to dryness. The resulting products were then resuspended in DMSO/H_2O (1:1) (2 mL), and aliquots were subjected to reversed-phase HPLC analysis (Econosil (Alltech) C_{18}, 10 μM, 0.46 × 25.0 cm, 1 mL/min: detection at 340 nm) using a CH_3CN−50 mM NH_4OAc linear gradient (10−50% over 60 min). Elution times, t_R (min), of amino acids were as follows: L-Ala (19.0), Gly (19.2), *N*-Me-L-Ala (20.4), *N*-Me-L-Val (29.0), *N*,*O*-diMe-L-Tyr (30.8), (3*S*,2*R*)-3-amino-2-methylpentanoic acid (MAP, 30.8), *N*-Me-L-Phe (31.8), *N*-Me-L-Leu (31.8), and *N*-Me-L-Ile (32.4). Two peaks at t_R 59.5 and 60.1 min in the profiles of all three hydrolysates were attributed to racemic 2-amino-4-methyl-3-pentanone. Unreacted Marfey reagent eluted at t_R 40.0 min. The elution times of all amino acids except MAP were confirmed by comparison and co-injection with authentic standards. The retention time of MAP was confirmed after its isolation from an acid hydrolysate (see below). Repeating this Marfey experiment with a different elution profile consisting of a CH_3CN−50 mM NH_4OAc linear gradient (10−20% over 20 min) followed by an isocratic profile at 20% CH_3CN−50 mM NH_4OAc for 90 min afforded the same conclusions as above.

Base Hydrolysis of 1ab, 2ab, and Majusculamide C (3). Complexes **1ab** (2.0 mg), **2ab** (2.0 mg), and majusculamide C (**3**) (2.0 mg) were each suspended in 2 mL of a CH_3OH/0.5 M NaOH (1:1) solution and allowed to stand for 12 h at room temperature. The reaction mixtures were each dried under air to remove CH_3OH and then acidified to litmus by the addition of HCl. The acidified reaction mixtures were then extracted with EtOAc, and the organic layer was dried under N_2 to give the base hydrolysis products. Hydrolysate of **1ab:** FABMS *m/z* 1017 (30), 887 (8), 720 (5), 661 (32), 631 (12), 549 (9), 469 (100), 356 (5), 299 (38); CIDMS on *m/z* 1018 *m/z* 886 (100), 719 (33), 659 (35), 548 (45), 469 (36); HRFABMS *m/z* 1017.6214 (calcd for $C_{51}H_{84}N_8O_{13}$ + H, 1017.6236). Hydrolysate of **2ab:** FABMS *m/z* 987 (16), 857 (8), 690 (8), 690 (7), 630 (33), 519 (7), 469 (89), 356 (7), 299 (43), 244 (8), 185 (11); CIDMS on *m/z* 988 *m/z* 958 (57), 856 (100), 689 (34), 630 (29), 520 (33), 469 (40), 299 (9); HRFABMS *m/z* 987.6188 (calcd for $C_{50}H_{82}N_8O_{12}$ + H, 987.6131). Hydrolysate of **3**: FABMS *m/z* 1003 (37), 706 (24), 661 (43), 560 (7), 535 (15), 469 (100), 356 (5) 299 (66); CIDMS on *m/z* 1004 *m/z* 985 (33), 705 (100), 659 (45), 535 (35), 468 (42); HRFABMS *m/z* 1003.6100 (calcd for $C_{50}H_{82}N_8O_{13}$ + H, 1003.6080).

Isolation and Identification of Amino and Hydroxy Acids. Complex **1ab** (65 mg) was suspended in 6 N HCl (5 mL) and incubated at 108 °C for 18 h. The reaction mixture was evaporated to dryness and then subjected to semipreparative reversed-phase HPLC (Econosil C_{18}, 10 μM, 1.0 × 25.0 cm, 2 mL/min; detection at 220 nm) eluting with a CH_3CN−0.1% HCOOH linear gradient (0−100% over 60 min after a 5 min period at 0% CH_3CN). This afforded as follows: Gly (8.0 mg) at 5.0−6.0 min [^{1}H NMR (D_2O) δ 3.52 (s, H-2)], *N*-Me-L-Ala (6.8 mg) at 6.0−8.8 min [^{1}H NMR (D_2O) δ 1.38 (3H, d, J = 7.3 Hz, H-3), 2.58 (3H, s, NCH_3), 3.69 (1H, q, J = 7.3 Hz, H-2)], *N*-Me-L-Val (3.5 mg) at 8.8−10.1 min [^{1}H NMR (D_2O) δ 0.87 (3H, d, J = 7.4 Hz, H-4), 0.90 (3H, d, J = 7.4 Hz, H-4′), 2.08 (1H, m, H-3), 2.57 (3H, s, NCH_3), 3.29 (1H, d, J = 4.6 Hz, H-2)], *N*-Me-L-Val and MAP (4.0 mg) at 10.1−13.5 min [^{1}H NMR (D_2O) δ 0.85 (3H, t, J = 7.3 Hz, H-5), 1.03 (3H, d, J = 7.3 Hz, 2-CH_3), 1.50 (1H, dq, J = 7.3, 14.6 Hz, H-4), 1.55 (1H, dq, J = 7.3, 14.6 Hz, H-4), 2.51 (1H, dq, J = 5.9, 7.1 Hz, H-2), 3.21 (1H, br q, J = 6.5 Hz, H-3)], MAP (3.1 mg) at 13.5−17.4 min, fraction A collected between 17.4 and 20.0 min, *N*,*O*-diMe-L-Tyr (6.0 mg) at 22.0−25.0 min [^{1}H NMR (D_2O and TFA-*d*) δ 2.26 (3H, s, NCH_3), 2.80 (2H, m, H-3), 3.32 (3H, s, OCH_3), 3.75 (1H, t, J = 5.8 Hz, H-2), 6.48 (2H, d, J = 8.3 Hz, H-5/9), 6.74 (2H, d, J = 8.3 Hz, H-6/8)] and L-isoleucic acid (4.0 mg) at 28.5−30.0 min [CD (0.01 N HCl) $[\theta]_{209}$ +2930; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 7.2 Hz, H-5), 1.01 (3H, d, J = 6.6 Hz, 3-CH_3), 1.30 (1H, m, H-4), 1.40 (1H, m, H-4), 1.96 (1H, br s, H-3), 4.17 (br s, H-2)]. An authentic standard of L-leucic acid, when injected, coeluted with L-isoleucic acid but was not encountered in the hydrolysate mixture as determined by ^{1}H NMR. Fraction A was resubjected to semipreparative reversed-phase HPLC eluting isocratically with 0.1% HCOOH at the same flow rate as before. This afforded 3.6 mg of a mixture of 2-amino-4-methyl-3-pentanone collected at 23.0−31.2 min [^{1}H NMR (D_2O and TFA-*d*) δ 0.78 (3H, d, J = 7.3 Hz, H-5), 0.84 (3H, d, J = 7.3 Hz, H-5′), 1.09 (3H, d, J = 7.3 Hz, H-1), 1.42 (1H, sept, J = 7.3 Hz, H-4) 4.10 (1H, q, J = 7.3 Hz, H-2)] and *N*-Me-L-Leu [^{1}H NMR (D_2O and TFA-*d*) δ 0.71 (6H, d, J = 6.5 Hz, H-5), 1.45−1.55 (3H, m, H-3, H-4), 2.48 (3H, s, NCH_3) 3.70 (1H, t, J = 6.3 Hz, H-2)] and *N*-Me-L-Leu (4.7 mg) at 31.2−42.0 min.

Complex **2ab** (35.7 mg) was also subjected to total hydrolysis followed by HPLC fractionation of the hydrolysate as described above. This afforded *N*-Me-L-Phe (3.7 mg) at t_R 22.1 min [^{1}H NMR (D_2O and TFA-*d*) δ 2.50 (3H, s, NCH_3), 3.06 (1H, dd, J = 6.2, 14.9, H-3), 3.10 (1H, dd, J = 6.2, 14.9, H-3), 4.05 (1H, t, J = 6.2, H-2), 7.05 (2H, br d, J = 7.8, H-5/9), 7.17 (3H, m, H-6/7/8)] and L-isoleucic acid (2.4 mg).

Lyngbyastatin 1 and epilyngbyastatin 1 (1ab): clear glassy oil; ESIMS *m*/*z* $[M + H]^+$ 1000.00 (11.2), $[M + Na]^+$ 1022.0 (100); ^{1}H NMR ($CDCl_3$) δ 0.34 (br s, *N*-Me-Val-CH_3), 0.70 (br s, *N*-Me-Val CH_3), 0.85−0.95 (4 × CH_3), 1.19 (d, J = 7.1 Hz, 2-CH_3, 15-CH_3), 1.32 (s, 11-CH_3), 2.74−3.09 (4 × NCH_3), 3.76 (br s, OCH_3), 6.83 (br d, J = 8.0 Hz, *N*,*O*-diMe-Tyr H-6/8), 7.14 (br d, J = 8.5 Hz, N,O-diMe-Tyr H-5/9); HRFABMS *m*/*z* 999.6248 (calcd for $C_{51}H_{82}N_8O_{12}$ +H, 999.6130) (100).

Dolastatin 12 and epidolastatin 12 (2ab): clear glassy oil; ESIMS *m*/*z* $[M + H]^+$ 970.0 (100), $[M + Na]^+$ 991.9 (54); ^{1}H NMR ($CDCl_3$) δ 0.34 (br s, *N*-MeVal-CH_3), 0.70 (br s, *N*-MeVal CH_3), 0.85−0.95 (4 × CH_3), 1.19 (d, J = 7.1 Hz, 2-CH_3, 15-CH_3), 1.32 (s, 11-CH_3), 2.74−3.09 (4 × NCH_3), 7.25 (5H, m, Ar-*H*); HRFABMS *m*/*z* 969.6069 (calcd for $C_{50}H_{80}N_8O_{11}$ + H, 969.6025) (100).

Selective Cytotoxicity Assay and in Vivo Trials. The solid tumor selective assay was performed as described previously.[3] Fractionation of the cyanobacterial extracts active in this assay was guided by monitoring toxicity toward KB and LoVo cell lines. Isolated **1ab** and **2ab** were then tested in the Corbett assay as described in the text. In vivo trials were carried out as described previously.[9]

Microfilament Disrupting Activity. Complexes **1ab** and **2ab** were tested for microfilament-disrupting activity using rhodamine−phalloidin. A-10 cells were grown on glass coverslips in Basal Medium Eagle (BME) containing 10% fetal calf serum. The cells were incubated with the test compounds for 24 h and then fixed with 3% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 2 min, and chemically reduced with sodium borohydride (1 mg/mL in PBS) three times for 5 min each. Following a 45 min incubation with 100nM TRITC-phalloidin in phosphate buffered saline, the coverslips were washed, stained with 4,6-diamidino-2-phenylindole (DAPI), mounted on microscope slides, and examined using a Zeiss Axioplan fluorescence microscope.

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