Lyngbyastatins 5–7, Potent Elastase Inhibitors from Floridian Marine Cyanobacteria, *Lyngbya* spp.

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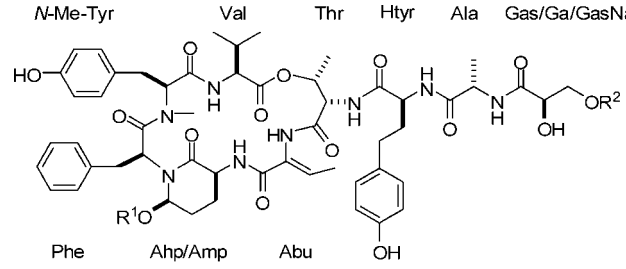
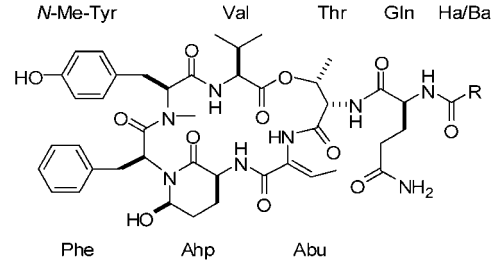
Three new analogues of dolastatin 13, termed lyngbyastatins 5–7 (**1**–**3**), were isolated from two different collections of marine cyanobacteria, *Lyngbya* spp., from South Florida. Their planar structures were deduced by a combination of NMR techniques, and the absolute configurations were established by modified Marfey's analysis of the acid hydrolyzates. The related cyclodepsipeptide somamide B (**4**), previously reported from a Fijian cyanobacterium, has also been found in one of the extracts, and its absolute stereochemistry was unambiguously assigned for the first time. Compounds **1**–**4** were found to selectively inhibit elastase over several other serine proteases, with IC_{50} values for porcine pancreatic elastase ranging from 3 to 10 nM.

Marine cyanobacteria are a rich source of structurally intriguing bioactive compounds[1] and also appear to be the true source of many compounds isolated from sea hares, including dolastatins.[2] We recently reported the isolation of a new analogue of dolastatin 13[3] with protease inhibitory activity, lyngbyastatin 4, from the marine cyanobacterium *Lyngbya confervoides* collected off the South Florida Atlantic coast.[4] Re-collections of this cyanobacterium now afforded two lyngbyastatin 4 analogues, lyngbyastatins 5 (**1**) and 6 (**2**). Chemical investigation of a collection of *Lyngbya* sp. from Summerland Key in the Florida Keys yielded one more dolastatin 13 analogue, lyngbyastatin 7 (**3**), along with the already reported compound somamide B (**4**), originally derived from an assemblage of *Lyngbya majuscula* and *Schizothrix* sp. encountered in the Pacific Ocean.[5] We report the isolation, structure determination, and preliminary biological evaluation of compounds **1**–**4**, particularly for elastase-inhibitory activity. Elastase overactivity is involved in tissue destruction and inflammation characteristic of various diseases, such as chronic obstructive pulmonary disease, hereditary emphysema, cystic fibrosis, adult respiratory distress syndrome, and ischemic-reperfusion injury.[6] It is also believed to contribute to cutaneous wrinkling.[7] Consequently, enzyme inhibition has been recognized as a valid therapeutic approach for various indications, and drug discovery efforts have resulted in several small molecules that have entered clinical trials.[8]



Lyngbyastatin 4 $R^1 = H$ $R^2 = SO_3H$
Lyngbyastatin 5 (**1**) $R^1 = H$ $R^2 = H$
Lyngbyastatin 6 (**2**) $R_1 = CH_3$ $R^2 = SO_3Na$



Lyngbyastatin 7 (**3**) $R = (CH_2)_4CH_3$
Somamide B (**4**) $R = (CH_2)_2CH_3$

Results and Discussion

The freeze-dried sample of the lyngbyastatin 4-producing *L. confervoides* from reef habitats near Fort Lauderdale, Florida, was extracted with organic solvents, and the extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was fractionated over HP-20 resin, and fractions were tested for serine protease inhibitory activities. The active fractions were further chromatographed and subsequently purified by reversed-phase HPLC to afford lyngbyastatins 5 (**1**) and 6 (**2**) in submilligram amounts, along with the major metabolite lyngbyastatin 4.[4] Due to these limited amounts, the structure determination of **1** and **2** required the use of an ultrasensitive 1 mm triple-resonance high-temperature superconducting (HTS) cryogenic probe.[9]

Lyngbyastatin 5 (**1**) was isolated as a colorless, amorphous solid. NMR data combined with a $[M + Na]^+$ peak at *m/z* 1079.4711 in the HRESI/APCIMS of **1** suggested a molecular formula of $C_{53}H_{68}N_8O_{15}$. Analysis of the ^1H NMR, COSY, TOCSY, ROESY, HSQC, and HMBC spectra recorded in DMSO-d_6 revealed the presence of alanine, valine, threonine, phenylalanine, *N*-methyltyrosine, glyceric acid (Ga), homotyrosine (Htyr), 2-amino-2-butenoic acid (Abu), and 3-amino-6-hydroxy-2-piperidone (Ahp) (Table 1). ^1H and ^{13}C NMR spectral data of **1** closely matched the data reported for lyngbyastatin 4.[4] Despite the lack of many HMBC correlations, sequencing of all amino acid units in **1** was facilitated by ROESY correlations (Table 1) that had also been observed for lyngbyastatin 4 (Figure S1, Supporting Information), supporting the linear sequence of Val-*N*-Me-Tyr-Phe-Ahp-Abu-Thr-Htyr-Ala-Ga. The cyclized structure for **1** was readily proposed due to the low-field chemical shift of the Thr H-3 (δ_H 5.51). Chemical shift differences from NMR data for lyngbyastatin 4 were only apparent for the glyceric acid unit. Compared to H-3 signals in the Ga sulfate

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Table 1. NMR Spectral Data for Lyngbyastatins 5 (**1**) and 6 (**2**) in DMSO-d_6 (600 MHz)[a]

	lyngbyastatin 5 (**1**)						lyngbyastatin 6 (**2**)			
unit	C/H no.	δ_H (J in Hz)	δ_C, mult.[b]	COSY	HMBC[c,d]	ROESY[d]	δ_H (J in Hz)	δ_C, mult.[b]	COSY	ROESY[d]
Val	1		[e]					[e]		
	2	4.65, br	56.6, CH	H-3, NH		H-3, H_3-4, H_3-5, NH, *N*-Me (*N*-Me-Tyr)	4.71, br	56.5, CH	H-3, NH	H-3, H_3-4, H_3-5, NH, *N*-Me (*N*-Me-Tyr)
	3	2.07, m	31.1, CH	H-2, H_3-4, H_3-5		H-2, H_3-4, H_3-5, NH	2.08, m	31.6, CH	H-2, H_3-4, H_3-5	H-2, H_3-4, H_3-5, NH
	4	0.85, d (6.6)	19.7, CH_3	H-3	2, 3, 5	H-2, H-3, H_3-5, 6-OH (Ahp), H_3-4 (Thr)	0.90, d (5.4)	19.7, CH_3	H-3	H-2, H-3, H_3-5, NH, *N*-Me (*N*-Me-Tyr)
	5	0.74, d (6.6)	17.8, CH_3	H-3	2, 3, 4	H-2, H-3, H_3-4, NH, 6-OH (Ahp), H_3-4 (Thr)	0.79, d (5.4)	17.6, CH_3	H-3	H-2, H-3, H_3-4, NH
	NH	7.51, br		H-2		H-2, H-3, H_3-4, H_3-5, H-2 (*N*-Me-Tyr), *N*-Me (*N*-Me-Tyr), H-2 (Phe), 6-OH (Ahp)	6.72, br d (8.4)		H-2	H-2, H-3, H_3-4, H_3-5, H-2 (*N*-Me-Tyr), *N*-Me (*N*-Me-Tyr), H-2 (Phe), 6′-*O*-Me (Amp)
N-Me-Tyr	1		[e]					[e]		
	2	4.86, d (11.4)	61.2, CH	H-3a, H-3b		H-3a, H-3b, H-5/9, *N*-Me, NH (Val), H-2 (Phe), H-3b (Phe)	4.96, d (11.4)	61.4, CH	H-3a, H-3b	H-3a, H-3b, H-5/9, *N*-Me, NH (Val), H-2 (Phe), H-3a (Phe), H-3b (Phe), H-5/9 (Phe)
	3a	3.09, d (–12.6)	33.1, CH_2	H-2, H-3b		H-2, H-3b, H-5/9	3.09, d (–12.5)	32.9, CH_2	H-2, H-3b	H-2, H-3b, H-5/9, *N*-Me
	3b	2.69, dd (–12.6, 11.4)		H-2, H-3a		H-2, H-3a, H-5/9	2.72, dd (–12.5, 11.4)		H-2, H-3a	H-2, H-3a, H-5/9, *N*-Me
	4		127.5, qC					[e]		
	5/9	6.98, d (7.8)	130.8, CH	H-6/8	3, 4, 7, 9/5	H-2, H-3a, H-3b, H-6/8, H-2 (Phe)	6.98, d (7.0)	130.9, CH	H-6/8	H-2, H-3a, H-3b, H-6/8, *N*-Me
	6/8	6.76, d (7.8)	115.7, CH	H-5/9	4, 7, 8/6	H-5/9, 7-OH	6.77, d (7.0)	115.8, CH	H-5/9	H-5/9, 7-OH
	7		156.5, qC					[e]		
	7-OH	9.36, s				H-6/8	9.39, s			H-6/8, H-5/9 (Phe)
	N-Me	2.74, s	30.8, CH_3		2, 1 (Phe)	H-2, H-2 (Val), NH (Val), 6-OH (Ahp)	2.77, s	30.5, CH_3		H-2, H-3a, H-3b, H-2 (Val), H_3-4 (Val), H_3-5 (Val), NH (Val), 6′-*O*-Me (Amp)
Phe	1		170.8, qC					[e]		
	2	4.72, dd (10.2, 3.0)	50.7, CH	H-3a, H-3b		H-3a, H-3b, H-5/9, H-2 (*N*-Me-Tyr), H-5/9 (*N*-MeTyr), NH (Val), H-6 (Ahp), 6-OH (Ahp)	4.78, dd (10.5, 1.2)	50.4, CH	H-3a, H-3b	H-3a, H-3b, H-5/9, H-2 (*N*-Me-Tyr), NH (Val)
	3a	2.85, dd (–12.6, 10.2)	35.5, CH_2	H-2, H-3b		H-2, H-5/9, H-6 (Ahp), 6-OH (Ahp)	2.95, dd (–12.6, 10.5)	35.2, CH_2	H-2, H-3b	H-2, H-3b, H-5/9, H-6 (Amp), H-2 (*N*-Me-Tyr)
	3b	1.83, dd (–12.6, 3.0)		H-2, H-3a		H-2, H-5/9, H-2 (*N*-Me-Tyr), H-6 (Ahp), 6-OH (Ahp)	1.72, dd (–12.6, 1.2)		H-2, H-3a	H-2, H-3a, H-5/9, H-6 (Amp), H-2 (*N*-Me-Tyr)
	4		136.9, qC					[e]		
	5/9	6.81, d (7.2)	129.7, CH	H-6/8	3, 7, 9/5	H-2, H-3a, H-3b, H-6/8, H-7, H-6 (Ahp)	6.85, d (6.6)	129.7, CH	H-6/8	H-2, H-3a, H-3b, H-6/8, H-2 (*N*-Me-Tyr), 7-OH (*N*-Me-Tyr), H-5a (Amp), H-5b (Amp), H-6 (Amp)
	6/8	7.18, m	128.3, CH	H-5/9, H-7	4, 8/6	H-5/9, H-7	7.18, m	128.3, CH	H-5/9, H-7	H-5/9, H-7
	7	7.14, m	126.7, CH	H-5/9	5/9	H-5/9, H-6/8	7.16, m	126.8, CH	H-6/8	H-6/8

Table 1. Continued

	lyngbyastatin 5 (**1**)						lyngbyastatin 6 (**2**)			
unit	C/H no.	δ_H (J in Hz)	δ_C, mult.[b]	COSY	HMBC[c,d]	ROESY[d]	δ_H (J in Hz)	δ_C, mult.[b]	COSY	ROESY[d]
Ahp[f]/Amp[g]	2		[e]					[e]		
	3	3.79, m	48.6, CH	H-4a, H-4b, NH		H-4b, H-5a, NH	3.76, m	48.7, CH	H-4a, H-4b, NH	H-4b, H-5a, NH
	4a	2.40, m	22.2, CH_2	H-3, H-4b, H-5a		H-4b, 6-OH, NH	2.23, m	22.4, CH_2	H-3, H-4b, H-5a, H-5b	H-4b, NH, 6′-*O*-Me
	4b	1.55, m		H-3, H-4a, H-5a		H-3, H-4a	1.57, m		H-3, H-4a, H-5a, H-5b	H-3, H-4a
	5a	1.72, m	29.7, CH_2	H-4a, H-4b, H-5b, H-6		H-3, H-5b, H-6, 6-OH	2.03, m	23.9, CH_2	H-4a, H-4b, H-5b, H-6	H-3, H-5b, H-6, 6′-*O*-Me, H-5/9 (Phe)
	5b	1.56, m		H-4a, H-4b, H-5a, H-6		H-5a, H-6, 6-OH	1.39, m		H-4a, H-4b, H-5a, H-6	H-5a, H-6, 6′-*O*-Me, H-5/9 (Phe)
	6	5.06, s	74.1, CH	H-5a, H-5b, H-6-OH		H-5a, H-5b, 6-OH, H-2 (Phe), H-3a (Phe), H-3b (Phe), H-5/9 (Phe)	4.60, s	83.0, CH	H-5a, H-5b	H-3, H-5a, H-5b, 6′-*O*-Me, H-3a (Phe), H-3b (Phe), H-5/9 (Phe)
	6-OH[f] 6′-*O*-Me[g]	6.08, s		H-6[f]		H-4a, H-5a, H-5b, H-6, H-2 (Phe), H-3a (Phe), H-3b (Phe), *N*-Me (*N*-Me-Tyr), H_3-4 (Val), H_3-5 (Val), NH (Val)	3.08, s	55.8, CH_3		H-4a, H-5a, H-5b, H-6, *N*-Me (*N*-Me-Tyr), H_3-4 (Val), H_3-5 (Val), NH (Val)
	NH	7.20, d (7.2)		H-3		H-3, H-4a, H-4b, NH (Abu)	7.04, br		H-3	H-3, H-4a, NH (Abu)
Abu	1		163.0, qC					[e]		
	2		130.2, qC					[e]		
	3	6.51, q (7.2)	132.6, CH	H_3-4	1	H_3-4	6.51, q (6.2)	132.5, CH	H_3-4	H_3-4
	4	1.46, d (7.2)	13.5, CH_3	H-3	2, 3	H-3, NH, H-6/10 (Htyr)	1.46, d (6.2)	14.1, CH_3	H-3	H-3, NH, H-2 (Thr), H-6/10 (Htyr)
	NH[h]	9.23, br				H_3-4, H-2 (Thr), H-3 (Thr), NH (Ahp)	9.25, br			H_3-4, H-2 (Thr), H-3 (Thr), NH (Amp)
Thr	1		[e]					[e]		
	2	4.64, br	56.6, CH	NH		H_3-4, NH, NH (Abu)	4.63, br	56.1, CH	NH	H-3, H_3-4, NH, NH (Abu)
	3	5.51, br	72.0, CH	H_3-4		H_3-4, NH (Abu)	5.55, br	72.5, CH	H_3-4	H-2, H_3-4, NH, NH (Abu)
	4	1.22, d (6.0)	18.3, CH_3	H-3	3	H-2, H-3, H_3-4 (Val), H_3-5 (Val)	1.23, d (6.0)	18.8, CH_3	H-3	H-2, H-3
	NH	7.93, br		H-2		H-2, H-2 (Htyr)	7.98, br			H-2, H_3-4, H-2 (Htyr), H-3a (Htyr), H-3b (Htyr), NH (Htyr)
Htyr	1		[e]							
	2	4.46, dd (12.0, 6.6)	52.8, CH	H-3a, H-3b, NH		H-3a, H-3b, H_2-4, H-6/10, NH, NH (Thr)	4.47, m	52.8, CH	H-3a, H-3b, NH	H-3a, H-3b, H_2-4, H-6/10, NH, NH (Thr)
	3a	1.80, m	30.9, CH_2	H-2, H-3b, H_2-4		H-2, H-3b, H_2-4, H-6/10, NH	1.81, m	30.9, CH_2	H-2, H-3b, H_2-4	H-2, H-3b, H_2-4, H-6/10, NH, NH (Thr)
	3b	1.91, m		H-2, H-3a, H_2-4		H-2, H-3a, H_2-4, H-6/10, NH	1.89, m		H-2, H-3a, H_2-4	H-2, H-3a, H_2-4, H-6/10, NH, NH (Thr)
	4	2.46, m (2H)	30.8, CH_2	H-3a, H-3b		H-2, H-3a, H-3b, H-6/10, NH	2.46, m (2H)	30.8, CH_2	H-3a, H-3b	H-2, H-3a, H-3b, H-6/10, NH
	5		132.0, qC					[e]		
	6/10	6.94, d (8.1)	129.5, CH	H-7/9	4, 10/6, 8	H-2, H-3a, H-3b, H_2-4, H-7/9, H_3-4 (Abu)	6.94, d (7.2)	129.6, CH	H-7/9	H-2, H-3a, H-3b, H_2-4, H-7/9, H_3-4 (Abu)
	7/9	6.64, d (8.1)	115.5, CH	H-6/10	5, 9/7, 8	H-6/10, 8-OH	6.65, d (7.2)	115.5, CH	H-6/10	H-6/10, 8-OH
	8		155.8, qC							
	8-OH	9.14, s				H-7/9	9.15, s			H-7/9
	NH	8.20, br		H-2		H-2, H-3a, H-3b, H_2-4, H-2 (Ala), H_3-3 (Ala), NH (Ala)	8.23, br		H-2	H-2, H-3a, H-3b, H_2-4, NH (Thr), H-2 (Ala), H_3-3 (Ala), NH (Ala)

Table 1. Continued

unit	lyngbyastatin 5 (**1**) C/H no.	δ_H (J in Hz)	δ_C, mult.[b]	COSY	HMBC[c,d]	ROESY[d]	lyngbyastatin 6 (**2**) δ_H (J in Hz)	δ_C, mult.[b]	COSY	ROESY[d]
Ala	1		172.4, qC					[e]		
	2	4.37, m	48.2, CH	H_3-3, NH		H_3-3, NH, NH (Htyr)	4.37, m	47.5, CH	H_3-3, NH	H_3-3, NH, NH (Htyr)
	3	1.28, d (7.2)	18.9, CH_3	H-2	1, 2	H-2, NH, NH (Htyr), H-3a (Ga)	1.25, d (7.2)	18.8, CH_3	H-2	H-2, NH, NH (Htyr)
	NH	7.82, d (7.2)		H-2		H-2, H_3-3, H-2 (Ga), 2-OH (Ga), H-3a (Ga), H-3b (Ga), NH (Htyr)	7.86, d (7.2)		H-2	H-2, H_3-3, H-2 (GasNa), 2-OH (GasNa), H-3a (GasNa), NH (Htyr)
Ga[f]/GasNa[g]	1		[e]					[e]		
	2	3.93, m	73.1, CH	2-OH, H-3a, H-3b		2-OH, H-3a, H-3b, NH (Ala)	4.10, br s	71.5, CH	2-OH, H-3a, H-3b	2-OH, H-3a, H-3b, NH (Ala)
	2-OH	5.69, d (5.4)		H-2		H-2, H-3a, NH (Ala)	5.93, br s		H-2	H-2, H-3a, H-3b, NH (Ala)
	3a	3.60, m	64.4, CH_2	H-2, H-3b		H-2, 2-OH, H-3b, H_3-3 (Ala), NH (Ala)	4.02, d (−10)	68.9, CH_2	H-2, H-3b	H-2, 2-OH, H-3b, NH (Ala)
	3b	3.50, m		H-2, H-3a		H-2, 2-OH, H-3a, NH (Ala)	3.74, m		H-2, H-3a	H-2, 2-OH, H-3a

[a] 1 mm HTS cryoprobe. [b] Deduced from HSQC and/or HMBC spectra. [c] Protons showing HMBC correlations to the indicated carbon. [d] Refers to nuclei within the same unit unless indicated otherwise. [e] Could not be detected due to lack of HMBC correlation. [f] Refers to lyngbyastatin 5 (**1**). [g] Refers to lyngbyastatin 6 (**2**). [h] Proton showed weak TOCSY correlations to H-3 and H_3-4 of the Abu unit.

(Gas) unit of lyngbyastatin 4,[4] signals for H-3a and H-3b (δ_H 3.60 and 3.50) in **1** were shifted upfield ($\Delta\delta$ = 0.41 and 0.23, respectively). This discrepancy suggested that the Ga unit is not sulfated in **1**, even though no additional OH signal was observed, presumably due to broadening. This conclusion is consistent with the molecular formula requirements derived from HRMS analysis indicating that compound **1** lacks $-SO_3$ compared to lyngbyastatin 4. Thus all atoms were accounted for by the proposed structure for **1**. To determine if compound **1** was an isolation artifact arising from desulfation of lyngbyastatin 4 during HPLC purification, lyngbyastatin 4 was exposed to TFA to mimic isolation conditions. Repeated HPLC analysis (elution with 0.05% aqueous TFA in 75% MeOH followed by solvent removal under N_2) yielded a single peak corresponding to lyngbyastatin 4, suggesting that lyngbyastatin 5 (**1**) is indeed a natural product.

The same extract afforded lyngbyastatin 6 (**2**) as a colorless solid. The molecular formula of **2** was deduced as $C_{54}H_{69}N_8O_{18}SNa$ by HRESI/APCIMS ($[M + Na]^+$ at m/z 1195.4257) and NMR spectral data (Table 1), suggesting a Na salt. NMR analysis revealed that **2** was a close analogue of compound **1**, with the same amino acid and hydroxy acid composition. The ^1H NMR, COSY, TOCSY, ROESY, and HSQC spectral data were almost identical to those of lyngbyastatin 4,[4] with the exception of the lack of the 6-OH signal of the Ahp unit and an extra signal corresponding to an *O*-methyl group (δ_H 3.08 s, δ_C 55.8), accounting for the additional carbon in **2** according to HRMS. Furthermore, the signal for H-6 of this residue (δ_H 4.60) was shifted upfield by 0.46 ppm and the corresponding C-6 (δ_C 83.0) was shifted downfield by 8.9 ppm compared to **1** (Table 1). These NMR data are in agreement with a 3-amino-6-methoxy-2-piperidone (Amp) unit in which the *O*-Me group has a shielding effect on H-6 and a deshielding effect on C-6, consistent with data for the Amp-containing compound oscillapeptin C.[10] Due to insufficient HMBC correlations owing to scarcity of sample, sequencing of all the amino acid units of **2** was achieved only with the aid of ROESY (Table 1). Again, ROESY data ascertained the sequence Val-*N*-Me-Tyr-Phe-Amp-Abu-Thr-Htyr-Ala-Ga (Figure S1, Supporting Information), and the low-field chemical shift of Thr H-3 (δ_H 5.55) allowed us to propose a cyclic depsipeptide structure for **2** rather than a linear peptide. MS data combined with NMR data gave substantial evidence for the presence of the glyceric acid 3′-*O*-sodium sulfate (GasNa) in the side chain.

A sample of the marine cyanobacterium *Lyngbya* sp. was collected from a mangrove channel at Summerland Key in the Florida Keys and extracted with organic solvents. Fractionation by solvent partition and successive chromatographic steps using silica, C_{18} cartridges, and finally reversed-phase HPLC afforded lyngbyastatin 7 (**3**) along with somamide B (**4**).[5]

Compound **3** was isolated as a colorless, amorphous solid. NMR data combined with a $[M + Na]^+$ peak at m/z 969.4710 in the HRESI/APCIMS of **3** suggested a molecular formula of $C_{48}H_{66}N_8O_{12}$. Analysis of ^1H NMR, ^{13}C NMR, HMQC, COSY, TOCSY, and HMBC spectra revealed the presence of valine, threonine, phenylalanine, *N*-methyltyrosine, glutamine, hexanoic acid (Ha), Abu, and Ahp moieties (Table 2). HMBC and ROESY analysis (Table 2) and comparison of ^1H and ^{13}C NMR data for **1** and **3** revealed that the cyclic core structure for these compounds is identical. Furthermore, ROESY correlations between Thr NH (δ_H 7.87) and Gln H-2 (δ_H 4.39) and from Gln 2-NH (δ_H 8.07) to Ha H_2-2 (δ_H 2.13) are consistent with the proposed structure shown for **3**. Compound **3** is most closely related to the previously reported cyanobacterial metabolite somamide B (**4**), which differs from **3** only by the presence of a terminal butanoic acid (Ba) residue in the side chain instead of the hexanoic acid (Ha) residue in **3**. In fact, our further chemical investigation of the lyngbyastatin 7-containing extract also yielded somamide B (**4**); however, whether

Table 2. NMR Spectral Data for Lyngbyastatin 7 (**3**) in DMSO-d_6

unit	C/H no.	δ_H (J in Hz)[a]	δ_C, mult.[b]	COSY[a]	HMBC[a,c,d]	ROESY[a,d]
Val	1		173.9, qC			
	2	4.71, br	56.1, CH	H-3, NH	1 (*N*-Me-Tyr)	H-3, H_3-4, H_3-5, NH
	3	2.08, m	30.9, CH	H_3-4, H_3-5	1, 2, 4, 5	H-2, H_3-4, H_3-5, NH
	4	0.86, d (6.8)	19.3, CH_3	H-3	2, 3, 5	H-2, H-3, H_3-5, NH, *N*-Me (*N*-Me-Tyr), H_3-4 (Thr)
	5	0.74, d (6.8)	17.5, CH_3	H-3	2, 3, 4	H-2, H-3, H_3-4, NH, *N*-Me (*N*-Me-Tyr)
	NH	7.47, br d (8.5)		H-2		H-2, H-3, H_3-4, H_3-5, *N*-Me (*N*-Me-Tyr), H-2 (*N*-Me-Tyr), 6-OH (Ahp)
N-Me-Tyr	1		169.4, qC			
	2	4.88, d (11.7)	60.8, CH	H-3a, H-3b	1, 3, 4	H-3a, H-3b, *N*-Me, H-5/9, H-2 (Phe), H-3b (Phe), H-5/9 (Phe), NH (Val)
	3a	3.07, d (–13.5)	32.8, CH_2	H-2, H-3b	2, 5/9	H-2, H-3b, H-5/9, H-2 (Phe)
	3b	2.69, dd (–13.5, 11.7)		H-2, H-3a	1, 2, 5/9	H-2, H-3a, H-5/9
	4		127.8, qC			
	5/9	6.97, d (8.4)	130.5, CH	H-5/9	4, 5, 7, 8	H-2, H-3a, H-3b, H-6/8, *N*-Me, H-2 (Phe)
	6/8	6.75, d (8.4)	115.3, CH	H-6/8	4, 7, 9	H-5/9, 7-OH, H-5/9 (Phe)
	7		156.2, qC			
	7-OH	9.37, s			7, 5/9	H-6/8
	N-Me	2.74, s	30.4, CH_3		2, 1 (Phe)	H-2, H-5/9, H_3-4 (Val), H_3-5 (Val), NH (Val)
Phe	1		170.5, qC			
	2	4.73, dd (11.5, 4.4)	50.3, CH	H-3a, H-3b	1, 3, 2 (Ahp)	H-3a, H-3b, H-5/9, H-2 (*N*-Me-Tyr), H-3a (*N*-Me-Tyr), H-5/9 (*N*-Me-Tyr), H-3 (Ahp), H-6 (Ahp)
	3a	2.86, dd (–13.7, 11.5)	35.3, CH_2	H-2, H-3b	2, 4, 5/9	H-2, H-3b, H-5/9, H-6 (Ahp), 6-OH (Ahp)
	3b	1.81, dd (–13.7, 4.4)		H-2, H-3a	2, 4, 5/9	H-2, H-3a, H-6 (Ahp), H-2 (*N*-Me-Tyr)
	4		136.7, qC			
	5/9	6.83, d (6.9)	129.4, CH	H-6/8	3, 5/9, 7	H-2, H-3a, H-6/8, H-2 (*N*-Me-Tyr), H-6/8 (*N*-Me-Tyr), H-3 (Ahp)
	6/8	7.18, m	127.5, CH	H-5/9	4, 6/8	H-5/9, H-7
	7	7.14, m	126.3, CH	H-6/8	5/9, 6/8	H-6/8
Ahp	2		168.9, qC			
	3	3.79, ddd (11, 9, 6)	48.2, CH	H-4a, H-4b, NH	2, 4	H-4b, H-5a, NH, H-2 (Phe), H-5/9 (Phe), H-3 (Abu)
	4a	2.40, m	21.9, CH_2	H-4b, H-5a, H-5b, H-3		H-4b, 6-OH, NH
	4b	1.56, m		H-4a, H-5a, H-3		H-3, H-4a
	5a	1.72, m	29.3, CH_2	H-4a, H-4b, H-5b, H-6		H-3, H-5b, H-6
	5b	1.55, m		H-5a, H-6, H-4a		H-5a, H-6, 6-OH
	6	5.07, s	73.8, CH	6-OH, H-5a, H-5b		H-5a, H-5b, 6-OH, H-2 (Phe), H-3a (Phe), H-3b (Phe)
	6-OH	6.09, s		H-6		H-4a, H-5a, H-5b, H-6, NH (Val), H-3a (Phe)
	NH	7.17, d (9)				H-3, H-4a, H-4b, NH (Abu)
Abu	1		162.8, qC			
	2		130.0, qC			
	3	6.51, q (6.9)	131.8, CH	H_3-4	1, 2, 4	H_3-4, H-3 (Ahp)
	4	1.48, d (6.9)	13.1, CH_3	H-3	2, 3	H-3, NH, H-2 (Thr)
	NH	9.17, br s				H_3-4, H-2 (Thr), NH (Ahp)
Thr	1		173.0,[e] qC			
	2	4.53, br	55.7, CH			H-3, H_3-4, H_3-4 (Abu), NH (Abu)
	3	5.47, br	71.8, CH	H_3-4		H_3-4, H-2
	4	1.21, d (6.5)	18.1, CH_3	H-3	2, 3	H-2, H-3, H_3-4, H_3-4 (Val), H-2 (Gln), NH
	NH	7.87, br		H-2		H-2 (Gln)
Gln	1		172.7, qC			
	2	4.39, ddd (8, 8, 6)	52.2, CH		1, 4	H-3a, H-3b, H_2-4, H_3-4 (Thr), NH (Thr), H_2-3 (Ha)
	3a	1.91, m	26.9, CH_2	H-3b, H-2, H_2-4		H-2 , H-3b, H_2-4
	3b	1.71, m		H-3a, H-2, H_2-4	1, 2, 4, 5	H-2, H-3a, H_2-4, 2-NH, H_2-2 (Ha)
	4	2.12, m (2H)	31.5, CH_2	H-3a, H-3b	5	H-2, H-3b, 2-NH, 5-NHa
	5		173.8, qC			
	2-NH	8.07, br s		H-2		H-3b, H_2-4, H_2-2 (Ha)
	5-NHa	7.22, br s			5	H_2-4, H_2-2 (Ha)
	5-NHb	6.72, br s			4	
Ha	1		172.5, qC			
	2	2.13, m (2H)	35.1, CH_2	H_2-3	1, 3	H_2-3, H_2-4/5, H-3b (Gln), 2-NH (Gln), 5-NHa (Gln)
	3	1.49, m (2H)	24.9, CH_2	H_2-2, H_2-4, H_2-5	2, 4, 5	H_3-6, H_2-2, H_2-4/5, 2-NH (Gln), H-2 (Gln), H_2-4 (Gln)
	4	1.27, m (2H)	30.9, CH_2	H_2-3	3	H_2-2, H_2-3, H_2-5, H_3-6
	5	1.27, m (2H)	21.9, CH_2	H_3-6	4, 6	H_2-2, H_2-3, H_2-4, H_3-6
	6	0.84, t (7.0)	13.9, CH_3	H_2-5	4, 5	H_2-3, H_2-4/5

[a] Recorded at 500 MHz. [b] Recorded at 150 MHz. [c] Protons showing HMBC correlations to the indicated carbon. [d] Refers to nuclei within the same unit unless indicated otherwise. [e] No HMBC correlation observed. Carbon assigned to Thr unit based on remaining unassigned signal in the ^{13}C NMR.

or not the absolute configurations for our and the published compound are identical was still unknown up to this point.

ROESY cross-peaks between the Abu methyl group and the Abu NH in compounds **1**–**4** unequivocally established the *Z* geometry of the Abu group. The absolute configuration of the amino acid residues in compounds **1**–**4** determined by modified Marfey's analysis[11] suggested that all the amino acids are in the L-form. The absolute configuration at C-3 of each Ahp residue was determined after CrO_3 oxidation and acid hydrolysis. This reaction sequence liberated L-glutamic acid, which permitted us to establish the configuration of the Ahp residues as 3*S*. We had found earlier for lyngbyastatin 4 that oxidation prior to hydrolysis increases the yield of phenylalanine;[4] this procedure again enabled us to clearly assign the 2*S* configuration to each Phe residue in **1**–**4**. Proton–proton coupling constants and ROESY correlations within the Ahp residues of **1**–**4** (Tables 1 and 2) suggested that the relative configuration and conformation of the Ahp moieties are identical to the one in symplostatin 2,[12] somamide A,[5] and lyngbyastatin 4[4] (3*S*,6*R*). For our compound **4** the ^{13}C NMR and ^{1}H NMR chemical shifts are equivalent to those reported for somamide B,[5] suggesting that their relative configurations are identical and thus that these compounds are not diastereomers. Although we were unable to reliably detect an optical rotation for **4**, the fact that lyngbyastatin 7 (**3**) and our compound **4** had the same absolute configuration based on Marfey's analysis and that optical rotation data for lyngbyastatin 7 (**3**) matched closely the data reported for somamide B[5] indicated that compound **4** is indeed somamide B itself, not an enantiomer.

The inhibitory activity of compounds **1**–**4** was determined against purified serine proteases, elastase, chymotrypsin, and trypsin and compared side-by-side with the activity of lyngbyastatin 4 at substrate concentrations near the K_m values for each enzyme to allow for better assessment of selectivity. Porcine pancreatic elastase inhibitory activities displayed by compounds **1**–**4** were similar without statistically significant difference, with IC_{50} values of 3.2 ± 2.0 nM (**1**), 3.3 ± 0.8 nM (**2**), 8.3 ± 5.4 nM (**3**), and 9.5 ± 5.2 nM (**4**), which were in the same range as for lyngbyastatin 4 (13.9 ± 3.1 nM). Compared with elastase activity, chymotrypsin activity was less compromised upon enzyme incubation with compounds **1**–**4**, IC_{50} values being 2.8 ± 0.3 μM (**1**), 2.5 ± 0.8 μM (**2**), 2.5 ± 0.2 μM (**3**), and 4.2 ± 0.5 μM (**4**). For comparison, lyngbyastatin 4 inhibited chymotrypsin with an IC_{50} of 4.3 ± 0.8 μM under identical conditions. Expectedly, trypsin activity was unaffected by treatment with compounds **1**–**4** (up to 30 μM tested), which is consistent with our previous findings for lyngbyastatin 4.[4]

There have been numerous publications describing the isolation of related Ahp-containing protease inhibitors from cyanobacteria, which are assumed to be enzyme substrate mimics.[10,13,14] In agreement with this assumption, compounds **1**–**4** inhibited elastase in a competitive manner obliging Michaelis–Menten kinetics (data not shown). Since the residue between Ahp and Thr units presumably determines the specificity toward certain serine proteases,[14–17] the Abu moiety appears to strongly contribute to the observed selectivity for elastase (S1 subsite = recognition pocket) so that the cyclic core structure for **1**–**4** represents a potent inhibitor prototype. In the co-crystal structure of the Abu-containing bicyclic inhibitor FR901277 bound to porcine pancreatic elastase,[16] it has been previously observed that the ethylidene moiety of Abu is stabilized by CH/π interaction.[18] Such an enzyme–inhibitor interaction may also exist for the monocyclic inhibitors **1**–**4**. Furthermore, for related Ahp-containing protease inhibitors, the carbonyl group of the residue that occupies the S1 enzyme subsite displays hydrogen bonding with NH of Ser-195 of porcine pancreatic elastase. However, the carbonyl moiety of the Abu unit of FR901277 did not form this hydrogen bond.[16] This fact may be attributed to a rigid and coplanar conformation of the backbone atoms due to the carbon–carbon double bond,[16] potentially affecting elastase-inhibitory activity.

The side chain in related inhibitors has been postulated to provide additional interaction points for hydrogen bonding with the enzyme.[10] The Thr unit that forms the ester bond to yield the cyclodepsipeptide core occupies the S2 subsite of the protease. The two consecutive residues located *N*-terminal to this Thr residue are important determinants for efficient elastase-inhibitor complexes based on the co-crystal structures for FR901277 and scyptolin A with the enzyme (S3 and S4 subsites).[16,17] However, comparable bioassay data for cyclodepsipeptides **1**–**4** indicate that the corresponding compositional difference in the side chain between **1** and **2** (Htyr-Ala) versus **3** and **4** (Gln-Ha/Ba) is overall less influential on the elastase-inhibitory activity. In agreement, scyptolin A,[17] planktopeptin BL1125, and planktopeptin BL1061,[19] all of which contain Leu instead of the Abu unit, display similar activities (IC_{50}'s 40–160 nM), although the side chains differ for each compound. Some marginal selectivity for elastase and chymotrypsin was observed among the two planktopeptins.[19] However, planktopeptin BL843 contains only one residue (Glu-γ-lactam) *N*-terminal to the Thr-Ahp sequence (thus has no residue to occupy the S4 enzyme subsite) and exhibits one order of magnitude lower protease-inhibitory activity.[19] This indicates the requirement of at least two units at these positions for strong activity.

Remarkably, the fact that the protease-inhibitory activity is retained in the *O*-methylated (Amp) derivative, lyngbyastatin 6 (**2**), demonstrates that the hydroxyl proton in the Ahp unit is not critical for the inhibition of elastase or chymotrypsin. Inhibitor–enzyme co-crystal structures obtained for related Ahp-containing cyclodepsipeptides also revealed that the OH group of Ahp does not take part in any hydrogen bond formation with the enzyme, but the hydroxyl oxygen atom forms intra- and intermolecular hydrogen bonds with NH of L-Val and a water molecule, respectively.[15–17] Thus, its role as a hydrogen acceptor and its conformation appear unaltered by *O*-methylation, in agreement with virtually identical NMR data in DMSO-d_6 for lyngbyastatins 4 and 5 (**1**) versus 6 (**2**). This is in contrast to activity data reported for the Amp-containing compound oscillapeptin C, which supposedly does not inhibit elastase because of its *O*-Me group (but still inhibits chymotrypsin).[10] The remarkable selectivity of all of the dolastatin 13 analogues **1**–**4** toward inhibiting elastase will be explored further.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on SpectraMax M5 (Molecular Devices). ^{1}H, ^{13}C, and 2D NMR spectra were recorded in DMSO-d_6 either on a Bruker Avance 500 MHz or 600 MHz or on a Bruker Avance II 600 MHz spectrometer equipped with a 1 mm triple-resonance high-temperature superconducting cryogenic probe using residual solvent signals (δ_H 2.49, δ_C 39.5) as internal standards. HMQC and HSQC experiments were optimized for $^{1}J_{CH} = 145$ Hz, and HMBC experiments were optimized for $^{n}J_{C,H} = 7$ Hz. HRMS data were obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector.

Extraction and Isolation. Samples of *Lyngbya confervoides*[20] were collected off the coast of Fort Lauderdale, Florida (26°05.9902′ N, 80°05.0184′ W) at a depth of 15 m in August 2005. A voucher specimen is retained at the Smithsonian Marine Station, and the 16S rDNA sequence is published.[20] The freeze-dried cyanobacterium was extracted with EtOAc–MeOH (1:1) to afford a crude lipophilic extract, which was then partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract (6.3 g) was applied to a diaion HP-20 polymeric resin and subsequently fractionated with H_2O and increasing concentrations of MeOH, and then with MeCN. The fraction eluting with 75% aqueous MeOH (175 mg) was subjected to preparative RP HPLC (LUNA-C18,10 μ, 100 × 21.20 mm, 10.0 mL/min; UV detection at 220 and 240 nm) using a MeOH–H_2O linear gradient (30–100% over 40 min and then 100% MeOH for 10 min). Fractions eluting between t_R 12 and 20 min were then repeatedly subjected to semipreparative reversed-phase HPLC (YMC-Pack ODS-AQ, 250 ×10 mm, 2.0 mL/min; UV detection at 220 and 240 nm) using a linear gradient of 0.05% aqueous TFA in

MeOH (60–90% for 25 min, then 90–100% for 10 min, and finally 100% MeOH for 10 min) to afford lyngbyastatin 5 (**1**), t_R 13.7 min (0.47 mg), and lyngbyastatin 6 (**2**), t_R 15.0 min (0.17 mg), along with known lyngbyastatin 4, t_R 12.2 min (9.6 mg), as the most potent elastase inhibitors in the sample.

Lyngbya sp. was collected from a mangrove channel, Kemp Channel, at the northern end of Summerland Key, Florida Keys (24°39.730′ N, 81° 27.791′ W) in May 2006. We suspect it is a *L. majuscula* of gray-black color, yet it appears thinner than generally described (cell width: 17.3 μm; sheath: 0.9 μm; length: 3.8 μm). A voucher specimen is retained at the Smithsonian Marine Station. The freeze-dried sample was extracted with CH_2Cl_2–MeOH (1:1). The resulting lipophilic extract (24.1 g) was partitioned between hexanes and 20% aqueous MeOH, the methanolic phase was evaporated to dryness, and the residue was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was concentrated and subjected to chromatography over Si gel using CH_2Cl_2 and increasing gradients of *i*-PrOH. Consecutive fractions that eluted with 50 and 75% *i*-PrOH were individually applied to C_{18} SPE cartridges, and elution was initiated with H_2O followed by aqueous solutions containing 25, 50, 75, and 100% MeOH. Both times, the fractions eluting with 75% aqueous MeOH were then purified by semipreparative RP HPLC (YMC-Pack ODS-AQ, 250 × 10 mm, 2.0 mL/min; UV detection at 220 and 254 nm) using a MeOH–H_2O linear gradient (50–100% for 60 min and then 100% MeOH for 10 min). The fraction that had eluted with 50% *i*-PrOH from Si gel yielded compound **3**, t_R 35.2 min (7.4 mg), while the 75% *i*-PrOH fraction furnished additional amounts of **3** (3.1 mg) and somamide B (**4**), t_R 26.2 min (1.2 mg). Both compounds accounted for most of the elastase-inhibitory activity of the extract.

Lyngbyastatin 5 (1): colorless, amorphous powder;[21] UV (MeOH) λ_{max} (log ε) 210 (4.57), 280 (sh) (3.79) nm; ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and ROESY data, see Table 1; HRESI/APCIMS *m/z* [M + Na]$^+$ 1079.4711 (calcd for $C_{53}H_{68}N_8O_{15}Na$ 1079.4702).

Lyngbyastatin 6 (2): colorless, amorphous powder;[21] UV (MeOH) λ_{max} (log ε) 210 (4.48), 280 (sh) (3.65) nm; ^{1}H NMR, ^{13}C NMR, COSY, and ROESY data, see Table 1; HRESI/APCIMS *m/z* [M + Na]$^+$ 1195.4257 (calcd for $C_{54}H_{69}N_8O_{18}SNa_2$ 1195.4246).

Lyngbyastatin 7 (3): colorless, amorphous powder; $[\alpha]^{20}_D$ –7.4 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.80), 280 (sh) (3.12); IR (film) ν_{max} 3373 (br), 2961, 1733, 1645 (br), 1539, 1446, 1203, 1026 cm^{-1}; ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and ROESY data, see Table 2; HRESI/APCIMS *m/z* [M + Na]$^+$ 969.4710 (calcd for $C_{48}H_{66}N_8O_{12}Na$ 969.4698).

Somamide B (4): colorless, amorphous powder,[21] UV (MeOH) λ_{max} (log ε) 230 (3.74), 280 (sh) (3.10) nm; NMR data, see ref 5; HRESI/APCIMS *m/z* [M + Na]$^+$ 941.4407 (calcd for $C_{46}H_{62}N_8O_{12}Na$ 941.4385).

Amino Acid Analysis by Modified Marfey's Method.[11] Samples (~50 μg each) of compounds **1**–**4** were subjected to acid hydrolysis (6 N HCl) at 110 °C for 24 h. The hydrolyzates were evaporated to dryness, dissolved in H_2O (100 μL), and divided into two equal portions. To one portion were added 1 M $NaHCO_3$ (50 μL) and a 1% v/v solution of 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA) in acetone, and the mixture was heated at 80 °C for 3 min. The reaction mixture was then cooled, acidified with 2 N HCl (100 μL), dried, and dissolved in H_2O–MeCN (1:1). Aliquots were subjected to RP HPLC (Alltech Alltima HP C18 HL 5 μm, 250 × 4.6 mm, UV detection at 340 nm) using a linear gradient of MeCN in 0.1% (v/v) aqueous TFA (30–70% MeCN over 50 min). The retention times (t_R, min) of the derivatized amino acids in the corresponding hydrolyzates of compounds **1**–**4** matched with those of L-Thr (13.8), L-Val (23.6), L-Phe (28.5), and *N*-Me-L-Tyr (40.6). HPLC profiles derived from compounds **1** and **2** additionally revealed peaks for derivatives of L-Ala (19.8) and L-Htyr (44.8), while the profiles of compounds **3** and **4** additionally gave peaks for L-Glu (16.2). Here glutamic acid must have derived from glutamine present in **3** and **4**. For comparison, the L-FDLA derivatives of the other standard amino acids not detected in the hydrolyzates had the following retention times (t_R in min): L-*allo*-Thr (14.8), D-*allo*-Thr (16.9), D-Thr (19.1), D-Val (32.5), D-Phe (35.5), *N*-Me-D-Tyr (42.6), D-Ala (22.3), D-Htyr (48.4), and D-Glu (17.6).

Portions of the hydrolyzates derived from compounds **1** and **2** were also subjected to chiral HPLC analysis (Phenomenex Chirex phase 3126 *N,S*-dioctyl-(D)-penicillamine, 4.60 × 250 mm, 5 μm; solvents, 2 mM $CuSO_4$–MeCN (85:15); flow rate 1.0 mL/min; detection at 254 nm), which allowed detection of D-glyceric acid (t_R 13.8 min) but not L-glyceric acid (t_R of standard, 10.6 min).

CrO_3 oxidations of **1**–**4** followed by acid hydrolysis were carried out as described.[4] The resulting hydrolyzates were derivatized with L-FDLA and aliquots subjected to reversed-phase HPLC as above. When compared to the Marfey profiles without prior oxidation, the HPLC profiles for derivatives resulting from compounds **1** and **2** showed one new peak for L-Glu (t_R 16.2 min) and one peak with increased intensity for L-Phe (t_R 28.5 min). For compounds **3** and **4**, both peaks were already present in the original profile; however, they appeared to be larger after oxidation, while the corresponding D-amino acid derivatives were not detected.

Protease Inhibition Assays. The test samples for **1**–**4** were prepared in DMSO by (log/2)-fold dilutions ranging from 1 mM to 100 pM. All assays were performed in triplicate. Phenylmethylsulfonyl fluoride (PMSF) was used as a positive control in the enzyme assays.

To test the inhibition of porcine pancreatic elastase (Elastase-high purity; EPC, EC134), 75 μg/mL solution of elastase was prepared using Tris-HCl (pH 8.0). The K_m for elastase was determined to be 1.5 mM for *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide, a concentration that was used subsequently for the inhibitor dose–response experiments. After pre-incubation of 165 μL of Tris-HCl (pH 8.0), 10 μL of elastase solution, and 10 μL of test samples in DMSO (5% final concentration) in a microtiter plate at 30 °C for 20 min, 15 μL of substrate solution (1.5 mM final concentration) was added to the mixture. The increase in absorbance was measured for 30 min at intervals of 5 min at 405 nm. Competitive binding was determined by plotting enzyme activity against substrate concentrations in the presence of different inhibitor concentrations (Lineweaver–Burk plot).

Inhibitory activity against α-chymotrypsin (bovine pancreas; Sigma, C4129) was determined as follows. A 1 mg/mL solution of chymotrypsin was prepared in assay buffer (50 mM Tris-HCl/100 mM NaCl/1 mM $CaCl_2$, pH 7.8). After preincubation of 80 μL of assay buffer solution, 10 μL of enzyme solution, and 10 μL of test solution in DMSO in a microtiter plate at 37 °C for 10 min, 50 μL of substrate solution (*N*-succinyl-Gly-Gly-Phe-*p*-nitroanilide, 0.75 mM final concentration corresponding to K_m) was added to the mixture. The increase in absorbance was measured for 30 min at intervals of 5 min at 405 nm.

Inhibitory activity against trypsin was assayed as described above for chymotrypsin, using trypsin from porcine pancreas (Sigma, T0303) and *N*α-benzoyl-DL-arginine-4-nitroanilide hydrochloride as the substrate solution.

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Note Added after ASAP Publication: HPLC analysis was done with 0.05% aqueous TFA, not 0.5%. This appears correctly in the version posted on October 9, 2007.

Supporting Information Available: Figure S1 depicting key ROESY correlations in compounds **1** and **2** in comparison with lyngbyastatin 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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