Antimalarial Linear Lipopeptides from a Panamanian Strain of the Marine Cyanobacterium *Lyngbya majuscula*

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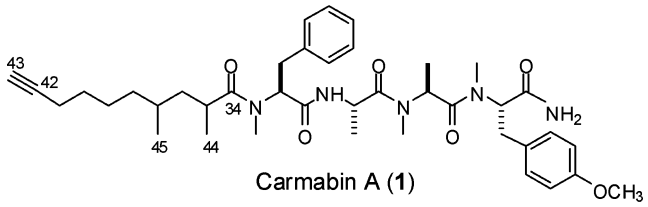
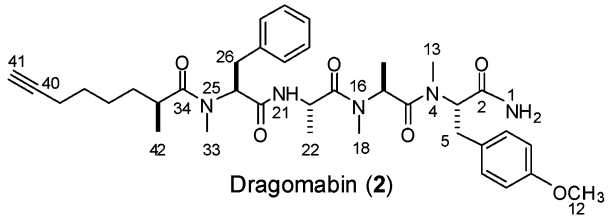
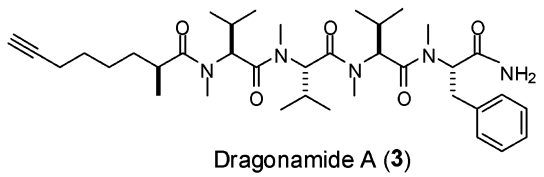
As part of the Panama International Cooperative Biodiversity Groups (ICBG) project, two new (**2**, **4**) and two known (**1**, **3**) linear alkynoic lipopeptides have been isolated from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscula*. Carmabin A (**1**), dragomabin (**2**), and dragonamide A (**3**) showed good antimalarial activity (IC_{50} 4.3, 6.0, and 7.7 μM, respectively), whereas the nonaromatic analogue, dragonamide B (**4**), was inactive. The planar structures of all four compounds were determined by NMR spectroscopy in combination with mass spectrometry, and their stereoconfigurations were established by chiral HPLC and by comparison of their optical rotations and NMR data with literature values.

Artemisinin combination treatments (ACTs) for falciparum malaria are currently the only first-line antimalarial drugs amenable to widespread use against all chloroquine-resistant malaria parasites.[1] However, their effective distribution to combat malaria in economically disadvantaged regions could require an annual global subsidy of $300−500 million.[2] Furthermore, in the event of successful widespread use of the artemisinins, the development of resistance to these drugs before effective replacements or alternatives are at hand is a cause for profound concern. Therefore, the development of new classes of antimalarial drugs remains an enormous challenge and is a focus of many collaborative research efforts, including the International Cooperative Biodiversity Groups project in Panama, which investigates Panamanian terrestrial plants, endophytes, and marine organisms as sources of tropical disease treatments.[3] As part of this program, we have been investigating marine cyanobacteria as a source of antimalarial agents and found that the organic extracts of a red Panamanian strain of the marine cyanobacterium *Lyngbya majuscula* were active against chloroquine-resistant *Plasmodium falciparum*. To the best of our knowledge, there are only three reports of marine cyanobacterial metabolites isolated with antimalarial activity. Most recently, members of our group isolated antimalarial venturamides A and B (IC_{50} 8.2 and 5.6 μM, respectively) from a Panamanian *Oscillatoria* sp.[4] These modified hexapeptides showed good differential toxicity to parasite versus mammalian host cells. An inseparable mixture of phenolic hierridins A and B from *Phormidium ectocarpi* inhibited chloroquine-resistant *P. falciparum* with an IC_{50} of 5.2 μg/mL,[5] and the indolophenanthridine alkaloids calothrixins A and B showed nanomolar potency (IC_{50} 58 and 180 nM, respectively) against the same strain.[6] Interestingly, the anticancer microtubule inhibitors dolastatins 10 and 15,[7] which have been shown to be of cyanobacterial origin,[8] were tested and shown to be potent antimalarials (IC_{50} 100 pM and 200 nM, respectively),[9] consistent with the proposal that parasite tubulin is a valid target for antiprotozoal agents.

Herein, we report the antimalarial bioassay-guided isolation of two new lipopeptides, dragomabin (**2**) and dragonamide B (**4**), and the related known metabolites carmabin A (**1**) and dragonamide (**3**, designated here as dragonamide A), from four separate Panamanian collections of *Lyngbya majuscula* Gomont (Oscillatoriaceae). Compounds **1**, **2**, and **3** were responsible for the antimalarial activity of the crude extract. Jamaicamides A and B,[10] previously isolated in our laboratories, were also tested for antimalarial activity given their somewhat related chemical structures to these new *L. majuscula* metabolites. Linear metabolites derived from a putative mixed polyketide synthase and nonribosomal peptide synthetase biogenesis represent a familiar motif in







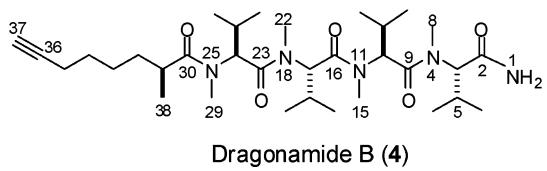


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Table 1. ^{13}C and ^{1}H NMR Data in $CDCl_3$ for Carmabin A (**1**)[a] and Dragomabin (**2**)[b]

		carmabin A (**1**)		dragomabin (**2**)	
unit[c]	position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
NH_2	1		5.54/5.40, br s		5.44, br s
N,*O*-diMeTyr	2	171.7/171.4, qC		171.7/171.5, qC	
	3	62.3/57.9, CH	4.73, dd (11, 2)/5.32, ob	62.3/57.9, CH	4.74, ob/5.32, ob
	4	N		N	
	5	33.5/32.5, CH_2	3.19/3.16, ob	33.3/32.4, CH_2	3.17, m
	6	129.6, qC		129.6, qC	
	7	130.4/129.8, CH	7.10/7.05, d (8)	130.4/129.8, CH	7.10/7.06, d (8)
	8	114.4/114.1, CH	6.83/6.80, d (8)	114.4/114.1, CH	6.83/6.80, d (8)
	9	158.7/158.6, qC		158.7/158.6, qC	
	10	114.4/114.1, CH	6.83/6.80, d (8)	114.4/114.1, CH	6.83/6.80, d (8)
	11	130.4/129.8, CH	7.10/7.05, d (8)	130.4/129.8, CH	7.10/7.06, d (8)
	12	55.3, CH_3	3.77, s	55.4, CH_3	3.77, s
	13	31.0/29.0, CH_3	2.76/2.90, s	31.1/29.1, CH_3	2.76/2.90, s
N-MeAla	14	172.98/172.4, qC		172.9/172.4, qC	
	15	50.2/48.8, CH	5.35/4.81, ob	50.2/48.8, CH	5.35/4.81, ob
	16	N		N	
	17	13.9/14.0, CH_3	1.18/0.48, d (7)	13.9/14.0, CH_3	1.18/0.51, d (7)
	18	29.2, CH_3	2.24, s	29.2, CH_3	2.28, s
Ala	19	171.3, qC		171.3, qC	
	20	45.7/45.4, CH	4.78/4.58, p (7)	45.7/45.5, CH	4.78/4.60, p (7)
	21	NH	6.94, t (7)	NH	6.90, ob
	22	18.1/17.7, CH_3	1.08/1.20, d (7)	18.2/17.8, CH_3	1.08/1.20, ob
N-MePhe	23	169.5, qC		169.5, qC	
	24	56.7/56.3, CH	5.52, ob	57.0/56.5, CH	5.50, m
	25	N		N	
	26	33.7, CH_2	3.28/3.25, ob	33.6, CH_2	3.28/3.25, m
	27	137.0/136.9, qC		137.1/137.0, qC	
	28	128.86/128.80, CH	7.18, ob	128.85/128.80, CH	7.18, d (ob)
	29	126.60/126.58, CH	7.17, ob	126.6, CH	7.17, ob
	30	128.4, CH	7.24, m	128.5, CH	7.24, m
	31	126.60/126.58, CH	7.17, ob	126.6, CH	7.17, ob
	32	128.86/128.80, CH	7.18, d (ob)	128.85/128.80, CH	7.18, d (ob)
	33	30.8, CH_3	2.88, s	30.8, CH_3	2.89, s
Moya/Mdya	34	178.3/178.1, qC		177.8/177.6, qC	
	35	33.60/33.55, CH	2.60, sextet (6)	35.9, CH	2.53, sextet (6)
	36	40.8/40.7, CH_2	1.10/1.13, m	33.33/33.25, CH_2	1.46, m
					1.20, m
	37	30.2, CH	1.12, m	26.3, CH2	1.00, m
	38	36.4/36.3, CH_2	0.93, m	28.40/28.36, CH_2	1.34, m
	39	25.7, CH_2	1.21, m	18.2/17.8, CH_2	2.06, m
			1.29 m		
	40	28.7, CH_2	1.42 m	84.49/84.45, qC	
	41	18.3, CH_2	2.15, t (6.6)	68.24/68.18, CH	1.93, br s
	42	84.6, qC		17.4, CH_3	1.04, d (7)
	43	68.2, CH	1.95, br s		
	44	17.0/17.1, CH_3	1.02/1.07, d (6.8)		
	45	19.45/19.50, CH_3	0.68, d (6.1)		

[a] Measured at 100 MHz (^{13}C) and 600 MHz (^{1}H), ratio of conformers = 3:2. [b] Measured at 75 MHz (^{13}C) and 400 (^{1}H) MHz, ratio of conformers = 3:2. [c] Moya = 2-methyloct-7-ynoic acid; Mdya = 2,4-dimethyldec-9-ynoic acid. ob = obscured.

other cyanobacterial natural products, such as the microcolins,[11] apramides,[12] and majusculamides A, B,[13] and D.[14]

Results and Discussion

The organic extracts of two collections of *L. majuscula* from different sites in Bocas del Toro, Panama (2002 and 2003), showed significant activity against chloroquine-resistant *P. falciparum* (IC_{50} = 6 and 26 μg/mL). Crude fractionation by normal-phase vacuum-liquid chromatography (NP-VLC) of the most active collection from Isla Bastimentos (Bocas del Toro, 2002) produced two relatively polar fractions (100% EtOAc and 25% MeOH−EtOAc) with good antimalarial activity (<2 and 1 μg/mL, respectively). Solid-phase extraction (SPE) and reversed-phase HPLC of the 25% MeOH−EtOAc NP-VLC fraction yielded carmabin A (**1**) and dragomabin (**2**) as the active components.

Structure elucidation of linear lipopeptides such as compounds **1** and **2** is complicated due to the presence of *N*-methyl amide functionalities, which results in several geometrical isomers exisiting in solution, as noted in the original isolation and structure elucidation work on carmabin A (**1**).[15] Ready identification of carmabin A in our extracts was further hampered because the original structure work used only DMSO-d_6 as the NMR solvent, while the work detailed below was derived from samples dissolved in $CDCl_3$. Nevertheless, the following deduction of the molecular formula and several partial structures from NMR analysis identified compound **1** as carmabin A. Carmabin A (**1**) analyzed for $C_{40}H_{57}N_5O_6$ by a combination of HRFABMS (obsd $[M - NH_2]^+$ at *m*/*z* 687.4174 for $C_{40}H_{55}N_4O_6$) and NMR analysis. ^{1}H NMR data for compound **1** revealed the presence of two major *N*-methyl peptide conformers (approximate ratio = 3:2) with signals for three *N*-methyl substituents (δ 2.77−3.03), an *O*-methyl substituent (δ 3.78), four α-protons (δ 4.79−5.36), four overlapped high-field methyl doublets, and aromatic protons consistent with both *para*-disubstituted and monosubstituted phenyl moieties. Spin systems delineating tyrosine, phenylalanine, and two alanine residues were confirmed from COSY data for carmabin A (**1**), and the tetrapeptide segment of **1** could be specifically assigned as *N*,*O*-dimethyltyrosyl-*N*-(methylalanyl)alanyl-*N*-methylphenylalanine (Table 1) from HSQC and HMBC data. The LRFABMS fragmentation pattern for compound **1** was consistent with a sequential loss of *N*,*O*-

dimethyltyrosine (191), *N*-methylalanine (85.5), and alanine (71) from the $[M - NH_2]^+$ peak at *m/z* 688 and confirmed the assignment of **1** as carmabin A.[15] The identity of carmabin A in this extract assisted structure elucidation of the new compounds, dragomabin (**2**) and dragonamide B (**4**), as described below.

Dragomabin (**2**) also gave a prominent $[M - NH_2]^+$ peak by HRFABMS for $C_{37}H_{49}N_4O_6$ (*m/z* 645.3677), which is 42 mass units (C_3H_6) less than the major ion for carmabin A (**1**), consistent with the loss of a propyl unit in **2** relative to **1**. Furthermore, the ^{1}H NMR spectrum for **2** lacked the distinctive upfield methyl doublet at δ 0.68 that was present in the spectrum for **1**. This signal was assigned to the 4-methyl group of the 2,4-dimethyldec-9-ynoyl unit in **1**. The identical tetrapeptide segments in dragomabin (**2**) and carmabin A (**1**), the lack of the second methyl group, and the reduced molecular weight of **2** compared to **1** implied the presence of the 2-methyloct-7-ynoic acid in dragomabin. Indeed, close examination of a 2D HSQC-TOCSY experiment for dragomabin delineated a $-CH(CH_3)CH_2(CH_2)_3-$ spin system consistent with this proposal. HMBC correlations positioned the aliphatic methyl and methine adjacent to the C-34 carbonyl carbon (δ 177.8/177.6). The distal methylene group of this partial structure was placed adjacent to the terminal acetylene by an HMBC correlation from the methylene protons (H_2-39, δ 2.06) to the quaternary acetylene carbon (δ 84.45/84.49), thus confirming the nature and location of the aliphatic group in **2**. Hence, dragomabin (**2**) was defined as the 2-methyloct-7-ynoyl amide of the same tetrapeptide unit present in carmabin A (**1**).

SPE and reversed-phase HPLC of the second antimalarial NP-VLC fraction (100% EtOAc) yielded one major component. The ^{1}H and ^{13}C NMR data again indicated a lipopeptide metabolite, with signals suggestive of phenylalanine and valine residues, as well as a fatty acid unit (Supporting Information, Table S10). Comparison of these data with literature values, as well as characteristic FAB mass spectrometric fragments at *m/z* 638 ($[M - NH_2]^+$), 476 (638 − *N*-MePhe), 363 (476 − *N*-MeVal), 250 (476 − 2 × *N*-MeVal), and 137 (476 − 3 × *N*-MeVal), identified the compound as dragonamide (**3**).[16]

A parallel investigation was undertaken of a small, biologically inactive 2003 Isla Bastimentos collection of red *L. majuscula*, which was collected separately due to its "fuzzy" appearance. Upon microscopic investigation, this "fuzzy" facade resulted from a thick coating of epiphytic chain diatoms on the cyanobacterial filaments. ^{1}H NMR profiling of the 100% EtOAc NP-VLC fraction of the crude extract once again revealed *N*-methyl amide singlets, upfield methyl doublets, and midfield multiplets indicative of peptide metabolites. Therefore, SPE and reversed-phase HPLC were carried out to afford pure compound **4**, which exhibited ^{1}H NMR signals for four *N*-methyl amide substituents, nine overlapped methyl doublets coupled to ^{1}H multiplets, and no aromatic signals. Examination of the ^{13}C NMR and multiplicity-edited HSQC spectra for this metabolite revealed quaternary and methine ^{13}C NMR signals at δ 84.2 and 68.4, respectively, consistent with a terminal acetylene as present in compounds **1**−**3**. Together with five carbonyl signals, this accounted for the seven degrees of unsaturation inherent in the formula of $C_{33}H_{59}N_5O_5$ calculated from HRESIMS data ($[M + Na]^+$, *m/z* 628.4420) for **4**. Moreover, these data suggested a similar tetrapeptide-coupled fatty acid motif as observed in compounds **1**−**3**. Isopropyl spin systems of four separate valine residues (Table 2) were delineated by COSY and multiplicity-edited HSQC correlations, and the order of these residues was assigned by careful analysis of the HMBC data. The presence of a 2-methyloct-7-ynoic acid residue attached to this tetravaline partial structure was evident from COSY data and chemical shift comparisons with dragonamide (**3**). On the basis of this otherwise close similarity to dragonamide (**3**), we have designated this nonaromatic analogue as dragonamide B (**4**), and therefore reassign dragonamide (**3**) as dragonamide A in the present report.

Table 2. ^{13}C and ^{1}H NMR Data in $CDCl_3$ for Dragonamide B (**4**)[a]

unit	position	δ_C, mult.	δ_H (*J* in Hz)
NH_2	1		6.02, s
N-Me Val-1	2	172.0, qC	
	3	62.0, CH	4.57, d (11)
	4	N	
	5	25.4, CH	2.29, m
	6	17.8, CH_3	0.74, d (ob)[d]
	7	19.60, CH_3	0.98, d (6)
	8	30.6, CH_3	3.05, s
N-Me Val-2	9	171.4, qC	
	10	58.0, CH	5.21, d (ob)
	11	N	
	12	27.21[b], CH	2.35, m
	13	19.47[c], CH_3	0.85, d (7)
	14	18.0, CH_3	0.82, d (7)
	15	30.38, CH_3	3.028, s
N-Me Val-3	16	170.4, qC	
	17	58.1, CH	5.18, d (11)
	18	N	
	19	27.4, CH	2.35, m
	20	17.71, CH_3	0.76, d (ob)
	21	19.47[c], CH_3	0.87, d (6)
	22	30.45, CH_3	3.033, s
N-Me Val-4	23	171.0, qC	
	24	57.8, CH	5.24, d (11)
	25	N	
	26	27.17[b], CH	2.35, m
	27	19.55[c], CH_3	0.89, d (7)
	28	18.0, CH_3	0.74, d (ob)
	29	30.2, CH_3	2.99, s
Moya	30	176.9, qC	
	31	36.2, CH	2.72, m
	32	33.6, CH_2	1.35, m
			1.70, m
	33	26.8, CH_2	1.34, m
	34	28.4, CH_2	1.49, m
	35	18.3, CH_2	2.16, 2.15, dd (7)
	36	84.2, qC	
	37	68.4, CH	1.92, t (2)
	38	17.67, CH_3	1.12, d (7)

[a] Measured at 75 MHz (^{13}C) and 300 MHz (^{1}H). [b,c] Chemical shift assignments to atom number are interchangeable. [d] ob = obscured signal.

Stereoconfiguration of the amino acid residues in lipopeptides **1**−**4** was assigned by chiral HPLC comparison of their acid hydrolysates with D and L amino acid standards. All standards could be obtained commercially except for *N*,*O*-dimethyl-D-tyrosine, which was synthesized by methylation of Cbz-protected D-tyrosine following a literature procedure.[17] Carmabin A (**1**) and dragomabin (**2**) contained *N*-methyl-L-phenylalanine, *N*-methyl-L-alanine, L-alanine, and *N*,*O*-dimethyl-L-tyrosine, while dragonamides A (**3**) and B (**4**) contained *N*-methyl-L-phenylalanine and *N*-methyl-L-valine residues, and all *N*-methyl-L-valine residues, respectively. Furthermore, closely similar NMR data for the 2-methyloct-7-ynoic acid unit in dragonamides A and B (see Supporting Information and Table 2, respectively) suggested the same relative stereoconfiguration at the fatty acyl α position in these two compounds and, due to the common source for all four compounds, led us to propose the same stereoconfiguration for dragomabin (**2**). In the original literature report of dragonamide A (**3**),[16] this fatty acyl α stereocenter (C-35) was assigned an *R* configuration on the basis of the optical rotation for the 2-methyloctanoic acid liberated following hydrogenation and acid hydrolysis of dragonamide A. This was assigned by comparison to previous literature values for 2-methylalkanoic acids (although no optical rotation values were provided in ref 14). Subsequently, the total synthesis of dragonamide A led to reassignment of the natural product as 35*S* on the basis of comparisons of its NMR and optical rotation data with both synthetic enantiomers (35*R* and 35*S*).[18] NMR and optical rotation data for the dragonamide A that we have isolated closely match those reported for both the natural product[16] and the 35*S* synthetic

product, but differ significantly from the 35*R* synthetic product.[18] Therefore, we conclude that dragonamide A (**3**), dragonamide B (**4**), and dragomabin (**2**) all contain 2*S*-methyloct-7-ynoic acid. Interestingly, this is the opposite configuration of that proposed for the 2-methyloct-7-ynoic acid unit in apramides A and D based on calculation and analysis of the molar optical rotations of these two compounds.[19]

To the best of our knowledge, carmabin A (**1**) is the only reported compound containing a 2,4-dimethyldec-9-ynoic acid moiety, the absolute stereochemistry of which has not been assigned. However, the related acyl group, 2,4-dimethyloctanoic acid, is present in the potent immunosuppressant natural products microcolins A and B[11] and the closely related metabolites majusculamide D and its deoxy homologue,[14] all of which were also isolated from *L. majuscula* strains. The four 2,4-dimethyloctanoic acid stereoisomers were synthesized from hexanoyl chloride in seven steps as part of the total asymmetric synthesis of microcolin A (36*R*, 38*R*) and its three 2,4-dimethylacyl analogues.[20] We considered three approaches to the assignment of the absolute configuration of carmabin A (**1**): (1) reduction, hydrolysis, and isolation of the resulting free 2,4-dimethyldecanoic acid from **1**, followed by characterization of the free acid by optical rotation and NMR chemical shift comparison with synthetic 2,4-dimethyloctanoic acid stereoisomers; (2) detection, isolation, and characterization of free 2,4-dimethyldecanoic acid as a co-metabolite from nonpolar fractions of the *L. majuscula* extract, which would suggest the same stereoconfiguration in **1**; (3) direct comparison of NMR chemical shifts for the acid unit in tetrahydrocarmabin A with those for the four microcolin diastereomers. Unfortunately, we isolated an insufficient amount of material to perform the degradative sequence suggested in the first approach. We were unable to detect free 2,4-dimethyldecanoic acid in the crude lipid extract, thus precluding the second approach. Finally, approach 3 was discounted because the chemical shifts for the acid unit of reduced **1** differed significantly from those for the microcolins, presumably due to the adjacent phenylalanine residue in carmabin A (**1**) as opposed to the leucine residue in the microcolins. In the ROESY spectrum for **1**, methine H-35 (δ2.60) showed strong correlations to CH_3-45 (δ0.68) as well as to *N*-methyl CH_3-33 (δ2.88). This implies a configuration of 35*S*, 37*R* or 35*R*, 37*S* for **1** (Figure S9, Supporting Information) although insufficient spectroscopic resolution precluded observation of any complementary correlation between H-37 (δ 1.12) and CH_3-44 (δ 1.02/1.07). Thus, the configurations of the two asymmetric methyl groups at C-35 and C-37 remain unassigned in carmabin A (**1**).

These three collections of red *L. majuscula* from different sites in the Bocas del Toro region of Panama (approximately 20 square miles) were profiled by LC-MS together with a collection of a morphologically similar *L. majuscula* from Cacique, near Portobelo (approximately 180 miles directly east of Bocas del Toro). Lipopeptides **1**−**4** were all present in both the Bastimentos and Bocas del Drago collections, while the Crawl Cay collection yielded dragomabin (**2**) and dragonamide A (**3**). Therefore, all *L. majuscula* collections from Bocas del Toro appear to biosynthesize both the dragomabin/carmabin series as well as the dragonamides. Interestingly, only carmabin A (**1**) and dragomabin (**2**) were detected in the Cacique collection (i.e., dragonamides containing valyl residues attached to the acyl group are not present in this population).

Pure compounds **1**−**3** were tested against the W2 chloroquine-resistant malaria strain, both as they were isolated and subsequently side-by-side, thus providing a good indication of their relative levels of activity in this assay. While initial testing gave IC_{50} values of 1.4, 21.0, and 10.7 μM for carmabin A (**1**), dragomabin (**2**), and dragonamide A (**3**), respectively, same plate evaluation gave IC_{50} values of 4.3, 6.0, and 7.7 μM, therefore indicating no significant difference between their levels of antimalarial activity. However, carmabin A was more cytotoxic to Vero cells (IC_{50} 9.8 μM) than dragomabin (IC_{50} 182.3 μM) or dragonamide A (IC_{50} 67.8 μM), and thus, dragomabin possesses the best differential toxicity between parasite and mammalian cells. It appears that the presence of three extra carbons in the aliphatic chain leads to the increase in cytotoxicity of carmabin A (**1**) over that of dragomabin (**2**). Dragonamide B (**4**) was isolated and tested by itself at a later date, and its lack of activity suggests that an aromatic amino acid at the carboxy terminus is necessary for antimalarial activity in this compound series. Interestingly, the nonaromatic ring-containing alkynoic lipopeptide jamaicamide B,[10] isolated from *L. majuscula* collected from Hector's Bay, Jamaica, showed weak antimalarial activity (IC_{50} 18.4 μM) and a similar level of cytotoxicity (IC_{50} 16.2 μM) to Vero cells. However, the terminal bromoacetylene homologue jamaicamide A[10] was inactive in this assay (IC_{50} >50 μM).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1010 polarimeter. IR data were obtained on a Nicolet 510 FTIR spectrophotometer. NMR spectra were recorded on Bruker Avance DRX 300 MHz, Bruker Avance DPX 400 MHz, and Bruker Avance DRX 600 MHz spectrometers operating at ^{1}H frequencies of 300.13, 400.13, and 600.01 MHz, respectively, and carbon frequencies of 75.47, 100.61, and 150.90 MHz, respectively, with the solvent used as an internal standard ($CDCl_3$ at δ_C 77.23, δ_H 7.26). Mass spectra were recorded on Kratos MS50TC and Waters Micromass LCT mass spectrometers. HPLC separations were performed using either Waters 515 HPLC pumps, a Rheodyne 7725i injector, and a Waters 996 photodiode array detector or Shimadzu LC-6AD HPLC pumps, a Rheodyne 7125 injector, and an SPD-10A dual-wavelength UV detector. NP-VLC was performed with Merck Si gel G for TLC.

Collection, Extraction, and Isolation. *Lyngbya majuscula* was collected in 2002 and 2003 by hand while snorkeling (2−4 ft) at Isla Bastimentos (two separate collections), Bocas del Drago and Crawl Cay in Bocas del Toro, Panama. The four samples were stored at −20 °C in 70% EtOH until workup. Voucher specimens are available from K.L.M. (collection numbers PAB-09/13/02-1, PAB-05/25/03-1, PAB-05/27/03-3, and PAB-05/27/03-2). The first Bastimentos collection (56.5 g dry wt) was extracted five times with CH_2Cl_2−MeOH (2:1) to give a crude organic extract (3.6 g). A portion of the extract (3.5 g) was fractionated on Si gel by NP-VLC to give nine fractions using a stepwise gradient of hexanes−EtOAc and EtOAc−MeOH. Fraction 8 (25% MeOH−EtOAc) was further chromatographed to produce five subfractions on a Waters RP-18 SPE cartridge (2 g; MeOH−H_2O, 1:1, 7:3, 9:1, 100% MeOH, and CH_2Cl_2). The subfraction eluting in 7:3 MeOH−H_2O was subjected to RP-HPLC (Phenomenex RP-18, 5 μm, 10 × 250 mm; 3:1 MeOH−H_2O, 3 mL/min) to yield dragomabin (**2**, 2.2 mg, 0.06% of extract). RP-SPE (2 g) of NP-VLC fraction 7 (100% EtOAc) to give five subfractions (MeOH−H_2O, 6:4, 8:2, 9:1, 100% MeOH, and CH_2Cl_2) was followed by RP-HPLC (Phenomenex RP-18, 5 μm, 10 × 250 mm; 8:2 MeOH−H_2O, 3 mL/min) of the 8:2 MeOH−H_2O subfraction to yield dragonamide A (**3**, 6.0 mg, 0.17% of extract). Following the same extraction, NP-VLC, and RP-SPE protocols, the Bocas del Drago collection yielded 2.2 g of crude extract, of which NP-VLC fraction 8 (25% MeOH−EtOAc) yielded 5.5 mg of carmabin A (**1**, 0.25% of extract) after repeated RP-HPLC (YMC-ODS RP-18, 10 × 250 mm; 65:35 MeCN−H_2O, 77:23 MeCN−H_2O, 2 mL/min).

Approximately 8.7 g (dry wt) of a diatom-covered red *L. majuscula* collected from Isla Bastimentos was repeatedly extracted with CH_2Cl_2−MeOH (2:1) to afford 1.5 g of extract. RP-SPE of a polar EtOAc NP-VLC fraction (100% EtOAc) produced a fraction eluting with 100% MeOH that possessed interesting, peptide-like ^{1}H NMR signals. This fraction was purified by RP-HPLC (Phenomenex Synergi Hydro RP-18, 4.6 × 250 mm, 1:1 MeOH−H_2O to 100% MeOH gradient over 50 min, 0.8 mL/min) to afford pure dragonamide B (**4**, 2.3 mg, 0.15% of extract).

Carmabin A (1): white, amorphous solid; $[\alpha]^{25}_D$ −137 (*c* 0.440, $CHCl_3$); IR ν_{max} (film) 3395, 2931, 2859, 1635, 1523, 1466, 1412, 1253, 1088 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; FABMS (3-nba, positive) *m*/*z* (%) 726 (5, $[M + Na]^+$), 705 (17, $[M + H]^+$), 687 (80, $[M - NH_2]^+$), 496 (22), 411 (5), 340 (33); HRFABMS (3-nba, positive) obsd $[M - NH_2]^+$ *m*/*z* 687.4174 (calcd for $C_{40}H_{55}N_4O_6$, 687.4121).

Dragomabin (2): white, amorphous solid; $[\alpha]^{25}_{D}$ −106 (*c* 0.500, $CHCl_3$); IR ν_{max} (film) 3308, 2931, 2859, 1640, 1519, 1471, 1413, 1379, 1253, 1084 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 1; ESIMS (positive) *m*/*z* (%) 684 (20, $[M + Na]^+$), 661 (14, M^+), 645 (100), 454 (28); HRESIMS obsd $[M - NH_2]^+$ *m*/*z* 645.3677 (calcd for $C_{37}H_{49}N_4O_6$, 645.3652).

Dragonamide A (3): pale yellow oil; $[\alpha]^{25}_{D}$ −244 (*c* 0.250, $CHCl_3$); IR ν_{max} (film) 2963, 2931, 2873, 1694, 1633, 1467, 1401, 1257, 1093 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table S10 (Supporting Information); FABMS (3-nba, positive) *m*/*z* (%) 638 (100), 476 (20), 363 (35), 250 (80).

Dragonamide B (4): pale yellow oil; $[\alpha]^{25}_{D}$ −289 (*c* 0.700, $CHCl_3$); IR ν_{max} (film) 2965, 2941, 2883, 1693, 1645, 1466, 1408, 1263, 1093 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 2; ESIMS (positive) *m*/*z* (%) 628 (88, $[M + Na]^+$), 589 (7), 476 (29), 363 (78), 250 (100); HRESIMS obsd $[M + Na]^+$ *m*/*z* 628.4420 (calcd for $C_{33}H_{59}N_5O_5Na$, 628.4414).

Absolute Configurations of the Peptide Portions of Compounds 1−4. Each of compounds **1**−**4** (0.2 mg) was hydrolyzed separately in 6 N HCl (microwave, 48 s) and then dried in vacuo. The residue of each was reconstituted in 200 μL of EtOH for chiral HPLC analysis using two different column/mobile phase combinations: (1) column Phenomenex Chirex phase 3126 (D) (4.6 × 250 mm), mobile phase 2 mM $CuSO_4$, flow rate 0.8 mL/min, detection 254 nm; retention times for authentic standards (t_R min) L-Ala (10.6), D-Ala (16.7), *N*-Me-L-Ala (14.8), *N*-Me-D-Ala (15.5), *N*-Me-L-Val (24.4), *N*-Me-D-Val (40.4); hydrolysates of **1**−**4** showed peaks at 10.6 min (L-Ala), 14.8 min (*N*-Me-L-Ala), and 24.4 min (*N*-Me-L-Val); (2) column Chirobiotic T (4.6 × 150 mm), mobile phase 50% aqueous EtOH, flow rate 1 mL/min, detection 254 nm; retention times for authentic standards (t_R min) *N*-Me-L-Phe (9.1), *N*-Me-D-Phe (13.0), *N*,*O*-diMe-L-Tyr (9.4), *N*,*O*-diMe-D-Tyr (14.0); hydrolysates of **1** and **2** showed peaks at 9.1 and 9.4 min consistent with *N*-Me-L-Phe and *N*,*O*-diMe-L-Tyr, respectively.

Malaria Assay. The antiplasmodial activity was evaluated in a chloroquine-resistant strain (Indochina W2) of *Plasmodium falciparum* using a fluorometric method based on the detection of parasite DNA with the fluorochrome PicoGreen.[3] A modified Trager and Jensen method[21] was used to maintain the parasites in vitro.

Cytotoxicity Assay. Vero cells adhering to 96-well plates were used to evaluate the toxicity of the compounds purified from *L. majuscula* on the basis of reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma).[22] After treatment with the test compound and 4 h incubation at 37 °C, cell viability was evaluated in an ELISA reader at 570 nm.

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Supporting Information Available: ^1H and ^{13}C NMR spectra in $CDCl_3$ for carmabin A (**1**), dragomabin (**2**), dragonamide A (**3**), and dragonamide B (**4**). Figure showing ROESY correlations for the 2,4-dimethyldec-9-ynoyl unit of carmabin A (**1**). Table of NMR data for dragonamide A (**3**) in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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