Apramides A−G, Novel Lipopeptides from the Marine Cyanobacterium *Lyngbya majuscula*

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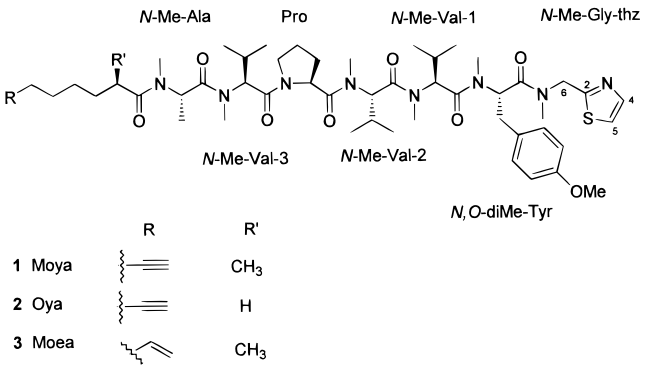
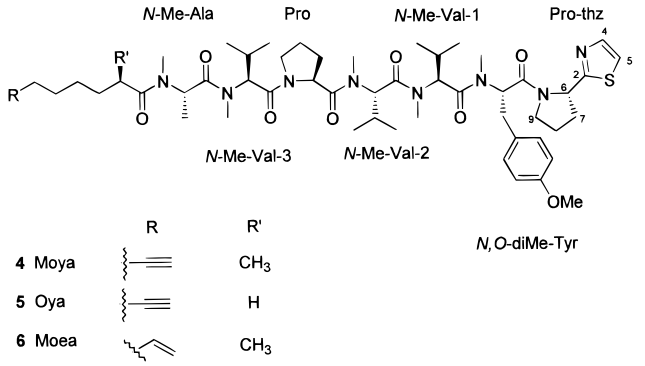
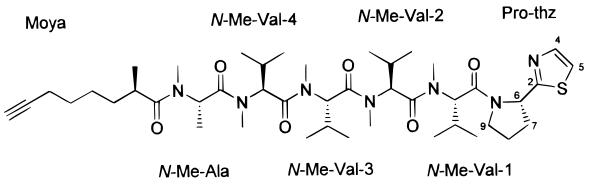
Six new metabolites have been isolated from a lyngbyastatin 2-producing strain of the marine cyanobacterium *Lyngbya majuscula* collected at Apra Harbor, Guam, and their structures elucidated. These linear lipopeptides have been assigned the trivial names apramides A−F (**1**−**6**). From a more recent collection of this cyanobacterium, a structurally related compound, apramide G (**7**), has been found instead of apramides A−F (**1**−**6**). Structure elucidation of the lipopeptides **1**−**7** is based on spectroscopic techniques and chiral chromatography of hydrolysis products. The apramides appear as NMR-spectroscopically distinguishable conformers in solution, and this has been ascribed to the presence of a thiazole-containing modified amino acid unit.

Cyanobacteria produce numerous and structurally diverse secondary metabolites, mainly of a peptide nature, having antitumor, antifungal, enzyme-inhibiting, and other remarkable bioactivities.[1] Noteworthy is the extraordinarily high number of cytotoxic compounds that are frequently encountered in cyanobacterial extracts. We[2] and others[3] have therefore focused our attention on the cyanobacteria as potential providers of new anticancer drugs. Recently, efforts in our laboratory have led to the discovery of several new, significantly active cytotoxins from marine cyanobacteria.[4] For example, chemical investigations of cytotoxic extracts from various collections of a reef-inhabiting strain of *Lyngbya majuscula* Harvey ex Gomont, found at Apra Harbor, Guam, have afforded the peptolides lyngbyastatin 2 and norlyngbyastatin 2.[4c] In this paper we report the isolation and identification of a series of linear lipopeptides, apramides A−G (**1**−**7**), from some of these extracts. The apramides are structurally related to the carmabins[5] and microcolins[6] from other strains of *L. majuscula*.







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Results and Discussion

Apramides A−F (**1**−**6**), along with the cytotoxins lyngbyastatin 2 and norlyngbyastatin 2, were isolated from the extract of a collection made in November 1993. The most abundant of these metabolites was apramide A (**1**), which possessed a molecular formula of $C_{52}H_{80}N_8O_8S$ as determined by HRFABMS. ^{1}H and ^{13}C NMR spectra indicated the presence of a minor conformer. A conformational ratio of 3:1 was seen in all of the solvents used (CD_2Cl_2, $CDCl_3$, C_6D_6). NMR analysis, however, was not obstructed and was carried out for the major conformer. Singlets at δ 2.40, 2.61, 2.75, 2.80, 3.00, and 3.17 in the ^{1}H NMR spectrum recorded in C_6D_6 suggested the presence of six *N*-methylated amino acids. In C_6D_6 all of the *N*-Me resonances were clearly distinguished, and this facilitated the sequencing of partial structures by HMBC and NOESY or ROESY experiments. Other striking features in the ^{1}H NMR spectrum were two doublets at δ 6.56 and 7.45 (J = 3.2 Hz), which indicated the presence of a thiazole ring, and a triplet at δ 1.77 (J ca. 2.4 Hz) due to an acetylenic proton. COSY and HMBC analysis detected the presence of a 2-methyl-7-octynoic acid moiety (Moya), six amino acid residues (*N*-

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Table 1. NMR Spectral Data for the Major Conformer of Compound **1** in C_6D_6

unit, C/H no.		δ_H (J in Hz)[a]	δ_C[b,c]	$^1H-^1H$ COSY	HMBC[d,e]
N-Me-Gly-thz	2		166.3, s		H-5, H-6
(A)	4	7.45, d (3.2)	142.5, d	H-5	
	5	6.56, d (3.2)	119.9, d	H-4	
	6	4.30, d (15.0), 4.92, d (15.0)	49.3, t		N-CH_3
	N-CH_3	2.75, s	35.0, q		H-6
N,*O*-diMe-Tyr	1		169.7, s		H-2, H-6 (A), N-CH_3 (A)
(B)	2	5.88, dd (9.3, 6.2)	54.0, d	H-3	N-CH_3
	3	3.01, dd (14.2, 9.3), 3.13, dd (14.2, 6.2)	34.7, t	H-2	H-2
	4		129.3, s		H-3, H-6/8
	5/9	7.09, d (8.4)	131.0, d	H-6/8	H-3, H-9/5
	6/8	6.82, d (8.4)	114.1, d	H-5/9	H-8/6
	7		159.2, s		H-5/9, H-6/8, O-CH_3
	O-CH_3	3.57, s	54.9, q		
	N-CH_3	3.00, s	30.4, q		H-2
N-Me-Val-1	1		169.2, s		H-2, N-CH_3 (B)
(C)	2	5.22, d (10.5)	58.7, d	H-3	H-4, H-4b, N-CH_3
	3	2.36, m	27.0, d	H-2, H-4a, H-4b	H-2, H-4a, H-4b
	4a	0.61, d (6.6)	17.6, q	H-3	H-4b
	4b	0.78, d (6.2)	19.9, q	H-3	H-2, H-4a
	N-CH_3	2.40, s	29.2, q		H-2
N-Me-Val-2	1		170.0, s		H-2, H-2 (C), N-CH_3 (C)
(D)	2	5.13, d (10.7)	58.9, d	H-3	H-4a, H-4b, N-CH_3
	3	2.48, m	27.3, d	H-2, H-4a, H-4b	H-2, H-4a, H-4b
	4a	1.14, d (6.6)	18.7, q	H-3	H-4b
	4b	0.94, d (6.2)	20.3, q	H-3	H-4a
	N-CH_3	2.80, s	29.9, q		H-2
Pro	1		172.6, s		N-CH_3 (D)
(E)	2	4.51, dd (7.8, 4.4)	56.8, d	H-3	
	3	1.32, m, 1.43, m	28.9, t	H-2, H-4	H-2
	4	1.41, m, 1.70, m	25.0, t	H-3, H-5	H-2
	5	3.68, m, 4.17, m	47.6, t	H-4	
N-Me-Val-3	1		169.0, s		H-2
(F)	2	5.26, d (11.1)	59.9, d	H-3	H-4a, H-4b, N-CH_3
	3	2.36, m	28.3, d	H-2, H-4a, H-4b	H-2, H-4a, H-4b
	4a	0.81, d (6.6)	19.2, q	H-3	H-2
	4b	1.14, d (6.6)	19.2, q	H-3	H-4a
	N-CH_3	3.17, s	30.3, q		H-2
N-Me-Ala	1		172.6, s		H-2, H-3, H-2 (F), N-CH_3 (F)
(G)	2	5.72, q (6.6)	49.1, d	H-3	H-3, N-CH_3
	3	1.22, d (6.6)	14.9, q	H-2	H-2
	N-CH_3	2.61, s	29.9, q		H-2
Moya	1		175.4, s		2-CH_3, H-2 (G), N-CH_3 (G)
(H)	2	2.28, m	36.0, d	2-CH_3, H-3	2-CH_3
	2-CH_3	0.97, d (6.8)	18.1, q	H-2	
	3	1.18, m, 1.73, m	33.8, t	H-2	2-CH_3
	4	1.22, m, 1.25, m	26.9, t		H-6
	5	1.31, m	28.8, t	H-6	H-6
	6	1.94, m	18.5, t	H-5, H-8	
	7		84.3, s		H-6
	8	1.77, t (~2.4)	68.9, d	H-6	H-6

[a] Recorded at 400 MHz. [b] Recorded at 100 MHz. [c] Multiplicity deduced by DEPT spectroscopy. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations refer to protons within the same unit if not indicated otherwise.

Me-Ala, Pro, *N*,*O*-diMe-Tyr, 3 × *N*-Me-Val) and a C-terminally modified amino acid unit (*N*-Me-Gly-thz) (Table 1). Due to overlapping carbonyl carbon resonances at δ 172.6 (Table 1) and because Pro protons did not show any correlation to carbonyl carbons, complete sequencing of the individual spin systems by HMBC analysis was unsuccessful. The partial structures that could be assembled were the following: the sequences Moya-(*N*-Me-Ala), (*N*-Me-Gly-thz)−(*N*,*O*-diMe-Tyr)−(*N*-Me-Val-1)−(*N*-Me-Val-2), and the unlinked amino acid units *N*-Me-Val-3 and Pro, the latter lacking connectivity to its carbonyl carbon. A NOESY experiment, however, established the sequential relationship of these partial structures (Figure 1) and permitted the assignment of the gross structure shown for **1**. The structure deduced from NMR analysis is consistent with the molecular formula established by HRFABMS. The FABMS fragmentation pattern of **1** confirmed the sequence derived from the NMR experiments (Figure 2).

The ^1H NMR spectrum of apramide B (**2**) lacked the signal corresponding to the methyl substituent in the

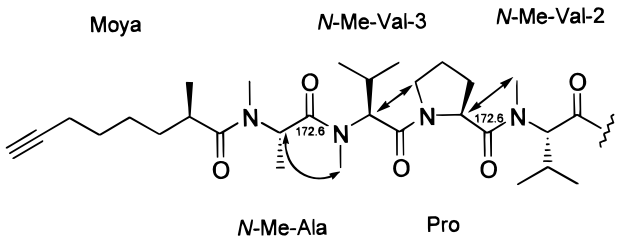

Figure 1. Selected NOESY correlations for compound **1**.

2-position of the lipid chain, but otherwise appeared virtually unchanged (Table 2). The HRFABMS indicated a molecular formula of $C_{51}H_{78}N_8O_8S$, one methylene mass unit smaller than for **1**. In addition to NMR analysis, FABMS fragmentation supported the presence of a 7-octynoic acid unit (Oya) in lieu of the Moya moiety and confirmed the sequence, inasmuch as all fragments were 14 mass units smaller than the ones outlined in Figure 2 for apramide A (**1**).

Table 2. NMR Spectral Data for Compounds **2** and **3** (Major Conformers) in C_6D_6

		2		**3**	
unit, C/H no.		δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]
N-Me-Gly-thz	2		166.3, s		166.3, s
(A)	4	7.45, d (3.2)	142.5, d	7.45, d (3.3)	142.5, d
	5	6.56, d (3.2)	119.9, d	6.56, d (3.3)	119.9, d
	6	4.30, d (15.0), 4.92, d (15.0)	49.3, t	4.30, d (15.1), 4.92, d (15.1)	49.3, t
	N-CH_3	2.75, s	35.0, q	2.75, s	35.0, q
N,*O*-diMe-Tyr	1		169.7, s		169.7, s
(B)	2	5.87, dd (9.4, 6.1)	54.0, d	5.88, dd (9.4, 6.4)	54.0, d
	3	3.01, dd (14.1, 9.4), 3.12, dd (14.1, 6.1)	34.7, t	3.02, dd (14.2, 9.4), 3.11, dd (14.2, 6.4)	34.7, t
	4		129.2, s		129.3, s
	5/9	7.09, d (8.6)	131.0, d	7.09, d (8.5)	131.0, d
	6/8	6.82, d (8.6)	114.1, d	6.83, d (8.5)	114.2, d
	7		159.2, s		159.2, s
	O-CH_3	3.56, s	54.9, q	3.57, s	54.9, q
	N-CH_3	3.00, s	30.4, q	3.00, s	30.4, q
N-Me-Val-1	1		169.2, s		169.2, s
(C)	2	5.21, d (10.6)	58.7, d	5.22, d (10.7)	58.7, d
	3	2.37, m	27.0, d	2.37, m	27.0, d
	4a	0.61, d (7.0)	17.6, q	0.61, d (6.8)	17.6, q
	4b	0.78, d (6.6)	19.9, q	0.78, d (6.4)	19.9, q
	N-CH_3	2.40, s	29.2, q	2.40, s	29.2, q
N-Me-Val-2	1		170.0, s		170.0, s
(D)	2	5.12, d (10.6)	58.9, d	5.12, d (10.7)	58.9, d
	3	2.47, m	27.3, d	2.49, m	27.3, d
	4a	1.14, d (~6.8)	18.7, q	1.15, d (~6.6)	18.7, q
	4b	0.93, d (6.3)	20.2, q	0.94, d (6.4)	20.3, q
	N-CH_3	2.80, s	30.0, q	2.80, s	29.9, q
Pro	1		172.55, s		172.57, s
(E)	2	4.52, dd (8.1, 4.6)	56.8, d	4.51, dd (8.1, 4.7)	56.8, d
	3	1.36 m, 1.47, m	28.9, t	1.32, m, 1.44, m	28.9, t
	4	1.43, m, 1.70, m	25.0, t	1.41, m, 1.70, m	25.0, t
	5	3.67, m, 4.15, m	47.6, t	3.67, m, 4.17, m	47.6, t
N-Me-Val-3	1		169.0, s		169.0, s
(F)	2	5.27, d (11.0)	59.8, d	5.27, d (11.2)	59.9, d
	3	2.40, m	28.3, d	2.39, m	28.3, d
	4a	0.83, d (6.6)	19.3, q	0.82, d (6.8)	19.2, q
	4b	1.14, d (~6.8)	19.2, q	1.15, d (~6.6)	19.2, q
	N-CH_3	3.17, s	30.4, q	3.18, s	30.3, q
N-Me-Ala	1		172.63, s		172.59, s
(G)	2	5.67, q (7.0)	49.0, d	5.73, q (6.8)	49.1, d
	3	1.20, d (7.0)	14.8, q	1.22, d (6.8)	14.9, q
	N-CH_3	2.52, s	30.0, q	2.63, s	29.9, q
Oya[c]/Moea[d]	1		171.7, s		175.5, s
(H)	2	1.85, m, 1.87, m	33.3, t	2.32, d	36.1, d
	2-CH_3			1.01, d (6.8)	18.1, q
	3	1.56, m	24.7, t	1.28, m, 1.83, m	34.2, t
	4	1.26, m	28.65, t	1.17, m, 1.26, m	27.4, t
	5	1.34, m	28.57, t	1.30, m	29.3, t
	6	1.95, td (6.8, 2.7)	18.5, t	1.94, m	34.0, t
	7		84.3, s	5.75, ddt (17.1, 10.3, 6.8)	139.0, d
	8	1.78, t (2.7)	68.9, d	4.98, br dd (10.3, ~2), 5.02, br dd (17.1, ~2)	114.6, t

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Refers to compound **2**. [d] Refers to compound **3**.

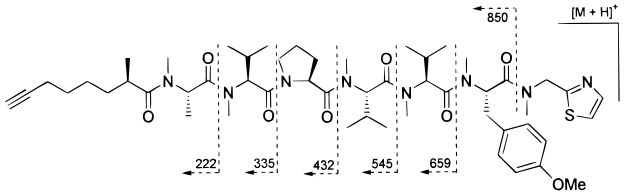

Figure 2. FABMS fragmentation pattern of apramide A (**1**).

The molecular weight of apramide C (**3**) showed a positive deviation of two mass units from that of apramide A (**1**) based on the HRFABMS. The only obvious differences in their ^{1}H NMR spectra were the missing acetylenic resonance for **1** and the appearance of three olefinic proton signals (δ 4.98, 5.02, and 5.75) for **3** (Table 2). In the ^{13}C NMR spectrum the signals at δ 84.3 and 68.9 for C-7 and C-8 for the Moya unit in **1** were shifted to δ 139.0 and 114.6 in **3**, consistent with the partial reduction of the terminal triple bond to a double bond. Therefore, **3** contains a 2-methyl-7-octenoic acid moiety (Moea) instead of the Moya unit in **1**. Furthermore, FABMS fragments were two mass units bigger than the ones shown in Figure 2 for compound **1**, upholding this conclusion.

Apramides D−F (**4**−**6**) seemed to be closely related to compounds **1**−**3** in comparing their ^{1}H and ^{13}C NMR spectra. The two easily recognizable doublets at δ 4.30 and 4.92 in the ^{1}H NMR spectra of **1**−**3**, due to the methylene protons of the *N*-Me-Gly-thz unit, were not present in the spectra of compounds **4**−**6**. Instead, another set of signals typical for a proline residue appeared. Furthermore, the NMR spectra for **4**−**6** were simplified by the fact that the conformational ratio was displaced to approximately 20:1, thus facilitating the NMR analysis. Compounds **4**−**6** differ from **1**−**3**, respectively, only by bearing a Pro-thz unit instead of the *N*-Me-Gly-thz residue, which must have caused this drastic impact on the conformational behavior. HRFABMS data as well as FABMS fragmentation patterns confirm the NMR result. All significant fragment ions

Table 3. NMR Spectral Data for Compounds **4**, **5**, and **6** in C_6D_6

		4		**5**		**6**	
unit, C/H no.		δ_H (*J* in Hz)[a]	δ_C[b]	δ_H (*J* in Hz)[a]	δ_C[b]	δ_H (*J* in Hz)[a]	δ_C[b]
Pro-thz	2		171.9, s		172.0, s		171.9, s
(A)	4	7.50, d (3.2)	142.4, d	7.51, d (3.2)	142.4, d	7.50, d (3.2)	142.4, d
	5	6.60, d (3.2)	118.9, d	6.59, d (3.2)	118.8, d	6.60, d (3.2)	118.8, d
	6	5.52, dd (8.1, 1.9)	58.5, d	5.52, dd (8.3, 2.1)	58.5, d	5.52, dd (8.1, 2.1)	58.5, d
	7	1.54, m, 2.16, m	31.1, t	1.55, m, 2.16, m	31.1, t	1.54, m, 2.16, m	31.1, t
	8	1.37, m, 1.80, m	24.6, t	1.36, m, 1.80, m	24.6, t	1.36, m, 1.80, m	24.6, t
	9	3.41, ~td (~9.7, 3.2), 3.55, ~td (~9.7, 7.2)	47.0, t	3.41, ~td (~9.5, 3.2), 3.56, ~td (~9.5, 7.1)	47.0, t	3.41, ~td (~9.3, 3.2), 3.56, ~td (~9.3, 7.1)	47.0, t
N,*O*-diMe-Tyr	1		169.3, s		169.3, s		169.3, s
(B)	2	5.90, dd (10.5, 5.7)	55.4, d	5.91, dd (10.5, 5.6)	55.4, d	5.90, dd (10.5, 5.6)	55.4, d
	3	2.99, dd (14.8, 10.5), 3.07, dd (14.8, 5.7)	34.5, t	2.98, dd (14.7, 10.5), 3.07, dd (14.7, 5.6)	34.5, t	2.98, dd (14.7, 10.5), 3.07, dd (14.7, 5.6)	34.5, t
	4		129.2, s		129.2, s		129.2, s
	5/9	7.00, d (8.5)	130.5, d	7.00, d (8.5)	130.5, d	7.00, d (8.5)	130.5, d
	6/8	6.81, d (8.5)	114.2, d	6.81, d (8.5)	114.2, d	6.81, d (8.5)	114.2, d
	7		159.2, s		159.2, s		159.2, s
	O-CH_3	3.59, s	54.9, q	3.59, s	54.9, q	3.59, s	54.9, q
	N-CH_3	3.13, s	30.9, q	3.14, s	30.9, q	3.14, s	30.9, q
N-Me-Val-1	1		170.2, s		170.2, s		170.2, s
(C)	2	5.32, d (10.5)	58.8, d	5.32, d (10.6)	58.8, d	5.32, d (10.6)	58.8, d
	3	2.44, m	27.2, d	2.44, m	27.2, d	2.44, m	27.2, d
	4a	0.65, d (6.8)	17.7, q	0.65, d (6.9)	17.7, q	0.65, d (6.9)	17.7, q
	4b	0.92, d (7.0)	20.15, q	0.92, d (6.4)	20.15, q	0.92, d (7.1)	20.15, q
	N-CH_3	2.46, s	29.3, q	2.47, s	29.4, q	2.47, s	29.3, q
N-Me-Val-2	1		169.9, s		170.0, s		170.0, s
(D)	2	5.11, d (10.7)	58.9, d	5.11, d (10.6)	58.9, d	5.11, d (10.6)	58.9, d
	3	2.46, m	27.3, d	2.46, m	27.3, d	2.46, m	27.3, d
	4a	1.14, d (~6.6)	18.8, q	1.15, d (~6.4)	18.8, q	1.15, d (~6.7)	18.8, q
	4b	0.91, d (6.8)	20.23, q	0.90, d (6.4)	20.22, q	0.91, d (6.7)	20.23, q
	N-CH_3	2.80, s	30.0, q	2.81, s	30.0, q	2.81, s	30.0, q
Pro	1		172.6, s		172.57, s		172.6, s
(E)	2	4.52, dd (8.0, 4.8)	56.8, d	4.52, dd (7.8, 4.6)	56.8, d	4.52, dd (8.1, 4.8)	56.8, d
	3	1.34, m, 1.46, m	28.9, t	1.36, m, 1.47, m	28.9, t	1.38, m, 1.47, m	28.9, t
	4	1.44, m, 1.70, m	25.0, t	1.44, m, 1.70, m	25.0, t	1.41, m, 1.70, m	25.0, t
	5	3.68, dt (9.7, 7.3), 4.17, dt (9.7, 6.1)	47.6, t	3.68, dt (9.7, 7.3), 4.16, dt (9.7, 6.5)	47.6, t	3.68, dt (9.7, 7.3), 4.17, dt (9.7, 6.6)	47.6, t
N-Me-Val-3	1		169.0, s		169.0, s		169.0, s
(F)	2	5.27, d (11.2)	59.9, d	5.28, d (11.3)	59.8, d	5.27, d (11.0)	59.9, d
	3	2.38, m	28.3, d	2.40, m	28.3, d	2.39, m	28.3, d
	4a	0.82, d (6.6)	19.2, q	0.84, d (6.7)	19.3, q	0.82, d (6.7)	19.2, q
	4b	1.15, d (~6.6)	19.2, q	1.15, d (~6.4)	19.2, q	1.15, d (~6.7)	19.2, q
	N-CH_3	3.17, s	30.3, q	3.18, s	30.4, q	3.18, s	30.3, q
N-Me-Ala	1		172.6, s		172.63, s		172.6, s
(G)	2	5.71, q (6.8)	49.1, d	5.67, q (6.8)	49.0, d	5.73, q (6.7)	49.1, d
	3	1.22, d (6.8)	14.9, q	1.20, d (6.8)	14.8, q	1.22, d (6.7)	14.9, q
	N-CH_3	2.61, s	29.9, q	2.52, s	30.0, q	2.63, s	29.9, q
Moya[c]/Oya[d]/Moea[e]	1		175.4, s		171.7, s		175.5, s
(H)	2	2.27, m	36.0, d	1.85, m, 1.87, m	33.3, t	2.32, m	36.1, d
	2-CH_3	0.98, d (6.6)	18.1, q	-	-	1.01, d (6.9)	18.1, q
	3	1.18, m, 1.75, m	33.8, t	1.57, m	24.7, t	1.27, m, 1.84, m	34.2, t
	4	1.22, m, 1.26, m	26.9, t	1.26, m	28.65, t	1.17, m, 1.26, m	27.4, t
	5	1,31, m	28.8, t	1.34, m	28.57, t	1.30, m	29.3, t
	6	1.94, m	18.5, t	1.95, td (6.8, 2.7)	18.5, t	1.96, br q (6.9)	34.0, t
	7		84.3, s		84.3, s	5.75, ddt (17.1, 10.2, 6.9)	139.0, d
	8	1.78, t (2.6)	68.9, d	1.78, t (2.7)	68.9, d	4.98, br dd (10.2, ~2), 5.03, br dd (17.1, ~2)	114.6, t

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Refers to compound **4**. [d] Refers to compound **5**. [e] Refers to compound **6**.

appeared to be the same for **4**−**6** as for **1**−**3**, respectively, except that the $[M + H]^+$ peaks differed.

Acid hydrolysis of **1** followed by chiral HPLC established that the configurations of all the amino acids were L. NMR data (Tables 1 and 2) indicated that the relative stereochemistry in compounds **2** and **3** is the same as in compound **1**, and the same sign of optical rotation indicated identical absolute stereochemistry. Analogous analysis for **4** showed the L stereochemistry of the amino acid units in **4**−**6**. The *S* configuration of the Pro-thz unit was confirmed by chiral HPLC after an ozonolysis-acid hydrolysis sequence. An increase was observed in the intensity of the peak corresponding to L-Pro compared to the HPLC profile of the nonozonized sample.

The only missing stereochemical information was the configuration at C-2 in the Moya and Moea units of **1**, **3**, **4**, and **6**, which can be inferred from data for optical rotations. Because no or only weak interactions with the other stereocenters can be assumed due to their distant (nonadjacent) location, we would expect approximately additive contributions of appropriate fragments to the total molar rotation angle.[7] Comparison of the molar optical rotations within the same series of compounds suggests a negative contribution of the additional stereocenter in the methylated compounds (Table 4). Even though the impact of the added stereocenter should be small, our experimental values for the methylated compounds were consistently more negative, indicating at least a qualitative trend. We

Table 4. Observed Specific and Molar Rotations for Compounds **1**−**6** (in $CHCl_3$)

compound	$[\alpha]^{22}_D$	$[M]^{22}_D$
1	−206°	−2010°
2	−189°	−1820°
3	−206°	−2020°
4	−234°	−2350°
5	−213°	−2110°
6	−231°	−2320°

predict the stereochemistry to be 2*R*, because it is known for a closely related model compound that the 2*S* epimer gives a more positive rotation in $CHCl_3$ than the corresponding epimer with *R* configuration in the lipid chain.[8]

A collection of the same cyanobacterium at the same location almost four years later did not afford any of the previously isolated apramides A−F (**1**−**6**). Instead, a closely related metabolite was found: apramide G (**7**), with molecular formula $C_{44}H_{73}N_7O_6S$ based on HRFABMS. It contained the Moya and the Pro-thz unit like apramide D (**4**) (Table 5). Most obvious in the NMR spectra was the lack of the *N*,*O*-diMe-Tyr unit. Closer examination of the spectra also disclosed the absence of the Pro unit. A fourth *N*-Me-Val unit was encountered instead. Sequencing could be achieved by NMR (HMBC and ROESY, see Table 5) and also from the FABMS fragmentation pattern (Figure 3), leading to the gross structure depicted for **7**.

All of the amino acids present in **7** were found to possess L configuration, and also the Pro-thz was L-Pro derived, determined again by ozonolysis and/or acid hydrolysis followed by chiral HPLC of the hydrolyzate. The configuration of C-2 in the Moya unit is expected to be the same as in the other apramides that contain a methyl group in this position. Apramides A−F (**1**−**7**) are noncytotoxic.[9] Further testing with **1** revealed no antibacterial, antifungal, or protease-inhibiting activity (trypsin, chymotrypsin, papain, thrombin, elastase), but indicated that **1** enhanced elastase activity.

The trivial names of the new lipopeptides **1**−**7** have been assigned after the collection site of the cyanobacterium producing these metabolites. Structurally similar C_8 units as found in the apramides have been identified as building blocks of some other natural products isolated from marine mollusks, for example, the kulolides,[10] kulomo'opunalides,[10b] and dolastatin 17,[11] which are probably also cyanobacterial metabolites and of dietary origin. Carmabin A, a lipopeptide containing a 2,4-dimethyl-9-decynoic acid residue, and yanucamides A and B, bearing a 2,2-dimethyl-3-hydroxy-

Table 5. NMR Spectral Data for Compound **7** in C_6D_6

unit, C/H no.		δ_H (*J* in Hz)[a]	δ_C[b]	HMBC[c,d]	ROESY[d]
Pro-thz	2		172.0, s	H-5, H-6	
(A)	4	7.48, d (3.3)	142.3, d		
	5	6.55, d (3.3)	118.8, d		
	6	5.49, dd (8.0, 2.7)	58.6, d		
	7	1.65, m, 2.20, m	31.4, t		
	8	1.38, m, 1.76, m	24.5, t		
	9	3.53, ddd (11.4, 7.8, 4.3), 3.79 dt (10.2, 7.8)	47.3, t		H-2 (B)
N-Me-Val-1	1		168.9, s	H-2	
(B)	2	5.27, d (10.9)	60.0, d	H-4a, H-4b, N-CH_3	H-9 (A), H-4a, H-4b
	3	2.44, m	27.7, d	H-2, H-4a, H-4b	
	4a	0.744, d (6.7)	18.2, q	H-4b	H-2
	4b	0.99, d (6.5)	19.6, q	H-2, H-4a	H-2
	N-CH_3	3.23, s	30.6, q	H-2	H-2 (C)
N-Me-Val-2	1		170.7, s	N-CH_3 (B), H-2 (B), H-2	
(C)	2	5.399, d (10.7)	58.5, d	H-4a, H-4b, N-CH_3	N-CH_3 (B), H-4a, H-4b
	3	2.47, m	27.6, d	H-2, H-4a, H-4b	
	4a	0.736, d (6.7)	17.8, q	H-2, H-4b	H-2
	4b	0.85, d (6.5)	19.6, q	H-2, H-4a	H-2
	N-CH_3	3.18, s	30.5, q	H-2	H-2 (D)
N-Me-Val-3		1	170.5, s	N-CH_3 (C), H-2	
(D)	2	5.396, d (10.9)	58.6, d	H-4a, H-4b, N-CH_3	N-CH_3 (C), H-4a, H-4b
	3	2.47, m	27.5, d	H-2, H-4a, H-4b	
	4a	0.72, d (6.7)	17.8, q	H-4b	H-2
	4b	0.86, d (6.5)	19.6, q	H-4a	H-2
	N-CH_3	3.16, s	30.3, q	H-2	H-2 (E)
N-Me-Val-4	1		170.6, s	N-CH_3 (D), H-2	
(E)	2	5.37, d (10.7)	58.7, d	H-4a, H-4b, N-CH_3	N-CH_3 (D), H-4a, H-4b
	3	2.48, m	27.3, d	H-2, H-4a, H-4b	
	4a	0.87, d (6.5)	18.3, q	H-2, H-4b	H-2
	4b	0.90, d (6.3)	19.8, q	H-4a	H-2
	N-CH_3	3.05, s	29.9, q	H-2	H-2 (F)
N-Me-Ala	1		172.9, s	H-2 (E), N-CH_3 (E), H-2, H-3	
(F)	2	5.67, q (7.0)	48.8, d	H-3, N-CH_3	N-CH_3 (E)
	3	1.18, d (7.0)	15.0, q	H-2	
	N-CH_3	2.72, s	30.2, q	H-2	H-2 (G)
Moya	1		176.0, s	H-2 (F), N-CH_3 (F), 2-CH_3	
(G)	2	2.33, m	36.2, d	2-CH_3	N-CH_3 (F)
	2-CH_3	1.02, d (6.7)	17.8, q		
	3	1.23, m, 1.77, m	33.8, t	2-CH_3	
	4	1.25, m, 1.30, m	26.9, t		
	5	1.33, m	28.8, t	H-6	
	6	1.96, td (6.8, 2.7)	18.5, t		
	7		84.3, s		
	8	1.78, t (2.7)	68.9, d		

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Protons showing long-range correlation with indicated carbon. [d] Correlations refer to protons within the same unit if not indicated otherwise.

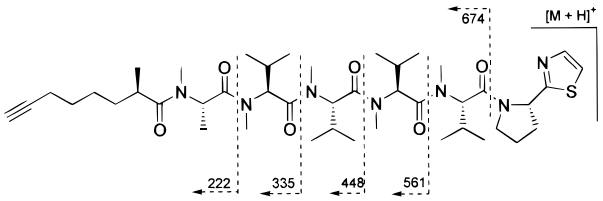

Figure 3. FABMS fragmentation pattern of apramide G (**7**).

7-octynoic acid unit, have been isolated directly from marine cyanobacteria.[5,12]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded in C_6D_6 at 400/500 MHz and 100/125 MHz, respectively, using residual solvent signals as internal references. The HMQC experiments were optimized for $^1J_{CH}$ = 140 Hz, and the HMBC experiments for $^nJ_{CH}$ = 7 Hz. HRFABMS were recorded in the positive mode.

Biological Material. Cyanobacterium VP503 was a *L. majuscula* strain collected at Finger's Reef, Apra Harbor, Guam, in November 1993, and yielded apramides A−F (**1**−**6**). Several re-collections at the same site (VP417) were carried out. The first of them was made on August 4, 1997, and afforded apramide G (**7**). The isolation of the cytotoxins lyngbyastatin 2 and norlyngbyastatin 2 from both collections has already been reported.[4c] A specimen of the cyanobacterium (VP417) preserved in formalin has been deposited at the University of Hawaii.

Extraction and Isolation. Fractionation of the lipophilic extracts of VP503 and of the initial collection of VP417 has previously been described.[4c] Apramides A−F (**1**−**6**) and apramide G (**7**) were found in the cytotoxic fractions after Si gel chromatography along with lyngbyastatin 2 and norlyngbyastatin 2. Subjection of the cytotoxic fraction from VP503 to semipreparative reversed-phase HPLC (Ultracarb, 5 ODS 30, 250 × 10 mm, 2 mL/min; UV detection at 220 nm) using an isocratic system of 80% aqueous MeCN allowed separation of the apramides from the cytotoxins and afforded **2** (0.7 mg, t_R 17.1 min), **5** (1.0 mg, t_R 18.2 min), **1** (6.7 mg, t_R 21.5 min), **4** (2.0 mg, t_R 22.9 min), **3** (1.5 mg, t_R 39.9 min), and **6** (1.1 mg, t_R 43.0 min). The lyngbyastatin 2-containing fraction of VP417 was purified similarly (flow rate, 3 mL/min) and yielded **7** (0.6 mg, t_R 26.0 min).

Apramide A (1): colorless, amorphous solid; $[\alpha]^{22}_D$ −206° (*c* 2.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.74), 230 (sh) (4.29) nm; IR (film) ν_{max} 2961, 2930, 2870, 1637 (br), 1513, 1470, 1402, 1250, 1095 cm^{-1}; ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, and HMBC data, see Table 1; FABMS *m*/*z* 978 [M + H]$^+$ (6), 850 (4), 659 (9), 545 (11), 432 (4), 335 (100), 222 (49); HRFABMS *m*/*z* [M + H]$^+$ 977.5906 (calcd for $C_{52}H_{81}N_8O_8S$, 977.5898).

Apramide B (2): colorless, amorphous solid; $[\alpha]^{22}_D$ −189° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.74), 230 (sh) (4.27) nm; IR (film) ν_{max} 2963, 2926, 2852, 1638 (br), 1514, 1470, 1402, 1250, 1097 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; FABMS *m*/*z* 963 [M + H]$^+$ (8), 835 (8), 644 (14), 531 (12), 321 (100), 208 (40); HRFABMS *m*/*z* [M + H]$^+$ 963.5744 (calcd for $C_{51}H_{79}N_8O_8S$, 963.5742).

Apramide C (3): colorless, amorphous solid; $[\alpha]^{22}_D$ −206° (*c* 0.56, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.74), 230 (sh) (4.30) nm; IR (film) ν_{max} 2964, 2929, 2872, 1638 (br), 1514, 1470, 1403, 1251, 1096 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; FABMS *m*/*z* 979 [M + H]$^+$ (7), 851 (4), 660 (8), 547 (12), 434 (5), 337 (100), 224 (49); HRFABMS *m*/*z* [M + H]$^+$ 979.6009 (calcd for $C_{52}H_{83}N_8O_8S$, 979.6055).

Apramide D (4): colorless, amorphous solid; $[\alpha]^{22}_D$ −234° (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.75), 230 (sh) (4.30) nm; IR (film) ν_{max} 2960, 2928, 2871, 1634 (br), 1514, 1470, 1402, 1250, 1096 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 1004 [M + H]$^+$ (6), 850 (6), 659 (15), 545 (12), 335 (100), 222 (45); HRFABMS *m*/*z* [M + H]$^+$ 1003.6067 (calcd for $C_{54}H_{83}N_8O_8S$, 1003.6055).

Apramide E (5): colorless, amorphous solid; $[\alpha]^{22}_D$ −213° (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.75), 230 (sh) (4.29) nm; IR (film) ν_{max} 2960, 2928, 2870, 1637 (br), 1513, 1458, 1401, 1250, 1099 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 989 [M + H]$^+$ (9), 835 (7), 644 (23), 531 (20), 321 (100), 208 (49); HRFABMS *m*/*z* [M + H]$^+$ 989.5936 (calcd for $C_{53}H_{81}N_8O_8S$, 989.5898).

Apramide F (6): colorless, amorphous solid; $[\alpha]^{22}_D$ −231° (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.75), 230 (sh) (4.28) nm; IR (film) ν_{max} 2961, 2928, 2870, 1638 (br), 1514, 1470, 1402, 1250, 1097 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 1006 [M + H]$^+$ (6), 852 (5), 660 (14), 547 (14), 434 (4), 337 (100), 224 (49); HRFABMS *m*/*z* [M + H]$^+$ 1005.6207 (calcd for $C_{54}H_{85}N_8O_8S$, 1005.6211).

Apramide G (7): colorless, amorphous solid; $[\alpha]^{22}_D$ −160° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.44), 240 (sh) (3.77) nm; IR (film) ν_{max} 2960, 2921, 2850, 1625 (br), 1261, 1099 cm^{-1}; ^{1}H NMR, ^{13}C NMR, HMBC, and ROESY data, see Table 5; FABMS *m*/*z* 829 [M + H]$^+$ (37), 674 (30), 561 (57), 448 (60), 335 (86), 222 (100); HRFABMS *m*/*z* [M + H]$^+$ 828.5385 (calcd for $C_{44}H_{74}N_7O_6S$, 828.5421).

Absolute Stereochemistry of the Amino Acid Derived Units. Compound **1** (0.6 mg) was dissolved in 0.5 mL 6 N HCl and the solution heated at 110 °C for 24 h. The product mixture was dried and analyzed by chiral HPLC, comparing the retention times with those of authentic standards [column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$−MeCN (95:5), except for *N*,*O*-diMe-Tyr, $CuSO_4$−MeCN (90:10); flow rate, 0.8 mL/min; detection at 254 nm]. The retention times (t_R, min) of the authentic amino acids were *N*-Me-L-Ala (8.4), *N*-Me-D-Ala (8.8), L-Pro (11.5), D-Pro (22.2), *N*-Me-L-Val (12.7), *N*-Me-D-Val (17.2), *N*,*O*-diMe-L-Tyr (81.5), and *N*,*O*-diMe-D-Tyr (87.5). The retention times of the amino-acid components in the acid hydrolyzate were 8.4, 11.5, 12.7, and 81.5 min, indicating the presence of *N*-Me-L-Ala, L-Pro, *N*-Me-L-Val, and *N*,*O*-diMe-L-Tyr, respectively, and the absence of D-amino acids. An additional small peak at 5.6 min was attributed to *N*-Me-Gly due to partial hydrolysis of the *N*-Me-Gly-thz unit. Another peak at 26.2 min (solvent mixture, 90:10) was assigned to *N*-Me-L-Tyr, formed due to *O*-demethylation under the hydrolysis conditions. It also appeared when the standard *N*,*O*-diMe-L-Tyr was treated similarly with 6 N HCl.

Compounds **4** and **7** (0.2 mg each) were treated and analyzed in a similar manner as **1**. The acid hydrolyzate of **4** showed the same HPLC profile as the acid hydrolyzate of **1**, but lacked the *N*-Me-Gly peak at 5.6 min. The HPLC profile of the acid hydrolyzate of **7** exhibited two major peaks at 8.4 and 12.7 min for *N*-Me-L-Ala and *N*-Me-L-Val, respectively, and a small peak at 11.5 min for L-Pro, the latter being attributed to partial hydrolysis of the Pro-thz unit. To clearly establish the stereochemistry of the Pro-thz unit, samples of **4** and **7** (0.2 mg each) were dissolved in 3 mL CH_2Cl_2 and subjected to ozonolysis at −78 °C. The solvent was evaporated, the residues were hydrolyzed as described above, and the dried hydrolyzates were analyzed by chiral HPLC using the same conditions as before. The HPLC profiles differed from the previous ones by an increase in intensity of only the L-Pro peak (t_R 11.5 min).

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