The Guineamides, Novel Cyclic Depsipeptides from a Papua New Guinea Collection of the Marine Cyanobacterium *Lyngbya majuscula*

Lik Tong Tan,[†] Namthip Sitachitta,[‡] and William H. Gerwick*,[†]

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, and Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330

Received October 18, 2002

The guineamides (**1**−**6**) are novel cyclic depsipeptides isolated and characterized from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya majuscula*. The planar structures of these new natural products were established using an extensive array of 1D and 2D NMR experiments, including HSQC, TOCSY, and HMBC. Absolute stereochemistry was determined using a combination of chemical manipulations as well as Marfey's method. These metabolites all contain *β*-amino or *β*-hydroxy carboxylic acid residues, an increasingly common feature in marine cyanobacterial metabolites. The identification of 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha) in guineamides E (**5**) and F (**6**) represents the first report of such a residue in a natural product. In addition, characterization of the unique *β*-amino acid 2-methyl-3-aminopentanoic acid (Mapa) in guineamide A (**1**), which has also been reported as a component of several marine molluscan metabolites, especially from those of *Dolabella auricularia*, further supports the diet-derived nature of such compounds as isolated from marine invertebrates. Guineamides B (**2**) and C (**3**) possess moderate cytotoxicity to a mouse neuroblastoma cell line with IC_{50} values of 15 and 16 μM, respectively.

Marine cyanobacteria, especially members belonging to the genus *Lyngbya*, are a prolific source of secondary metabolites with pharmaceutical potential. For example, the pantropical species *Lyngbya majuscula* Gomont has yielded no less than 150 nitrogen-containing compounds, most belonging to the lipopeptide structural class.[1] These lipopeptides occur as either linear or cyclic forms with a variety of significant associated bioactivities,[1,2] including antiproliferative (e.g., curacin A),[3] cytotoxicity (e.g., apratoxin),[4] neurotoxicity (e.g., kalkitoxin),[5] and antifungal activity (e.g., lyngbyabellin B).[6] Some of the metabolic signatures of cyanobacterial lipopeptides include a high degree of *N*-methylated α-amino acids, α-hydroxy acids, *β*-amino acids, and *β*-hydroxy acids. These features are also present in a number of molluscan metabolites, most notably the dolastatins reported from *Dolabella auricularia*, leading to speculations that these substances are sequestered from its cyanobacterial diet.[7] Indeed, four of the dolastatins (3, 10, 12, and 16), as well as 12 close analogues of these molecules, have been identified to date from marine cyanobacteria,[8] including the potent antitumor agent dolastatin 10, which is currently in phase II clinical evaluation.[9]

In a continuing search for novel and biologically active natural products, we have made extensive cyanobacterial collections from various tropical and subtropical locations. A preliminary screening of these cyanobacterial extracts for antifungal and cytotoxic properties led to the identification of an organic extract of *Lyngbya majuscula*, Gomont (PNSM-4/Sep/98-01) collected from Papua New Guinea, as possessing significant activity. Correspondingly, this extract was fractionated by normal-phase Si VLC to yield two fractions enriched with diverse peptides based on ^{1}H NMR analyses. Further bioassay-guided fractionation, using a combination of Sep Pak RP and HPLC on RP-8 and phenylhexyl columns, led to the isolation of six novel cyclic depsipeptides (**1**−**6**). All six possessed a number of *N*-methylated amino acids and α-hydroxy, *β*-amino, and *β*-hydroxy acids and were given the trivial names guineamides A (**1**, 10.0 mg), B (**2**, 1.7 mg), C (**3**, 2.0 mg), D (**4**, 9.5 mg), E (**5**, 1.8 mg), and F (**6**, 3.7 mg). Described herein are the results of their structure determination, including stereochemistry, as well as some of the biological activities of these metabolites.

Results and Discussion

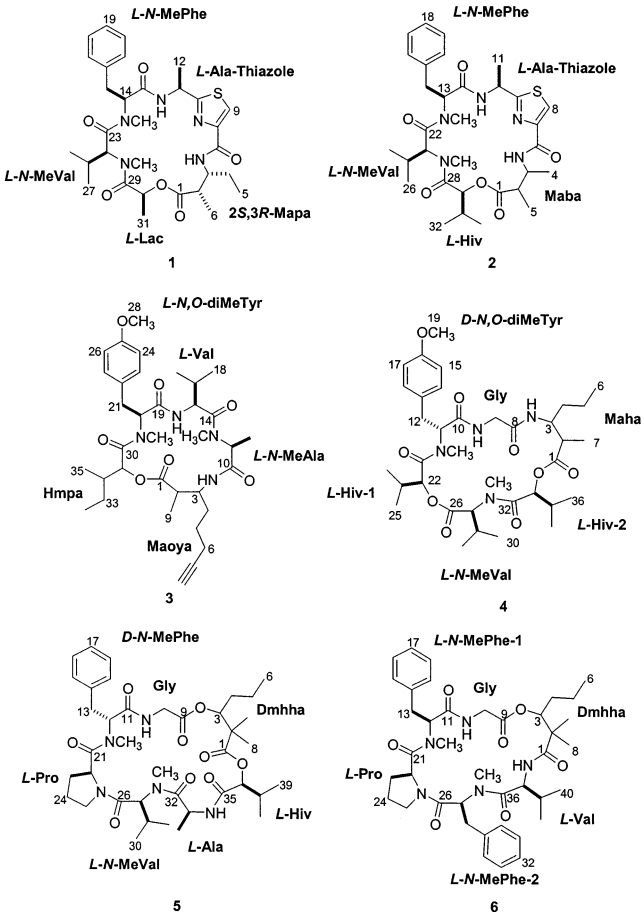
High-resolution FABMS of the major metabolite, guineamide A (**1**), gave an $[M + H]^+$ peak at 614.3022 for a molecular formula of $C_{31}H_{44}N_5O_6S$, requiring 13 degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 1732 and 1669 cm^{-1}, indicating the presence of both ester and amide functionalities, respectively. The ^{13}C NMR spectrum of **1** exhibited the presence of five ester/amide carbonyls and a monosubstituted phenyl ring [*δ* 136.8 (s), 128.8 (d, 2C), 128.2 (d, 2C), and 126.5 (d)] (Table 1), accounting for nine of the 13 degrees of unsaturation.

Interpretation of 1D and 2D NMR spectral data of **1** allowed construction of five partial structures assigned as *N*-methylphenylalanine (*N*-MePhe), *N*-methylvaline (*N*-MeVal), 2-methyl-3-aminopentanoic acid (Mapa), lactic acid (Lac), and an alanine-thiazole unit. A disubstituted thiazole ring, with one substituent being a conjugated amide carbonyl (C-7, *δ*159.9), was formulated on the basis of the characteristic ^{13}C NMR chemical shifts at *δ* 170.0 (C-10), 147.5 (C-8), and 124.0 (C-9) as well as HMBC correlations detected from H-9 (*δ* 8.21s) to C-8 and C-10. With only a single degree of unsaturation remaining, guineamide A was deduced to be of overall monocyclic structure.

The partial structures of **1** were connected from HMBC data. The connection between *N*-MePhe-Ala-thiazole units was achieved through correlations from the amide proton at *δ* 7.76 (*N*H−Ala) to C-10 and C-13. A heteronuclear coupling from the amide proton at *δ* 9.01 (*N*H−Mapa) to C-7 linked the Mapa unit to the carbonyl of the thiazole unit. The coupling between H-14 and C-23 connected the

* To whom correspondence should be addressed. Tel: (541) 737 5801. Fax: (541) 737 3999. E-mail: Bill.Gerwick@orst.edu.
[†] Oregon State University.
[‡] Chulalongkorn University.

10.1021/np020492o CCC: $25.00
Published on Web 05/16/2003



N-MeVal to the *N*-MePhe. Three-bond couplings from H-24 to C-29 and from H-30 to C-1 placed the Lac unit between the *N*-MeVal and Mapa residues, completing the planar structure of guineamide A (**1**).

The absolute configurations of the *N*-MePhe, Ala-thiazole, and *N*-MeVal residues in guineamide A (**1**) were determined by first treating **1** with O_3 to destroy the aromaticity of the thiazole ring, a structural feature that would potentially facilitate racemization of C-11 during acid hydrolysis.[10] Acid hydrolysis of the ozonide product of **1** was followed by Marfey's analysis[11] and revealed that all residues had L-stereochemistry. The L-configuration of the analyzed Ala corresponds to the *S* configuration at C-11 in **1**. The absolute configuration of the Lac unit was deduced as L by comparisons with both L- and D-lactic acid standards using chiral HPLC.

The absolute stereochemistry of the Mapa unit was determined by a combination of synthetic manipulations and the use of both L- and D-Marfey's reagents.[12] Four distinct peaks, equating to the four stereoisomers of Mapa, were observed from HPLC analyses. These peaks were attributable to L-Marfey-2*S*,3*R*-Mapa (**8**, 40.7 min), D-Marfey-2*S*,3*R*-Mapa (**9**, 35.9 min, identical t_R to the enantiomer, L-Marfey-2*R*,3*S*-Mapa), L-Marfey-2*R*,3*R*-Mapa [39.3 min, 2*R*,3*R*-Mapa was obtained from epimerization of 2*S*,3*R*-Mapa (**7**)], and D-Marfey-2*R*,3*R*-Mapa (36.4 min; identical t_R to the enantiomer, L-Marfey-2*S*,3*S*-Mapa). From careful analysis and co-injections with the Marfey-derivatized natural Mapa unit (40.7 min) from guineamide A (**1**), the absolute configuration was determined to be 2*S*,3*R* (Figure 1). The 2*S*,3*R*-Mapa unit has also been reported in other marine metabolites, such as majusculamide C[7a] and dolastatins 11 and 12.[13] Indeed, a recent report described a new cytotoxic guineamide A analogue, obyanamide, isolated from the marine cyanobacterium *Lyngbya confervoides*, and it differs from **1** by having 3-aminopentanoic acid instead of 2-methyl-3-aminopentanoic acid.[14]

Table 1. NMR Spectral Data for Guineamide A (**1**) at 600 MHz (^{1}H) and 100 MHz (^{13}C) in DMSO-d_6

position	δ_H	mult	*J* (Hz)	δ_C	HMBC
2(*S*)-Methyl-3(*R*)-aminopentanoic acid (2*S*,3*R*-Mapa)					
1				171.2	
2	2.72	m		44.4	C-3
3	4.08	m		52.3	
4a	1.52	m		25.5	C-3, C-5
4b	1.60	m			C-3, C-5
5	0.94	t	7.3	10.9	C-3, C-4
6	1.11	d	6.9	14.5	C-1, C-3
*N*H	9.01	d	10.6		C-7
L-Ala-thiazole					
7				159.9	
8				147.5	
9	8.21	s		124.0	C-7, C-8, C-10
10				170.0	
11	5.11	m		47.4	C-10, C-12
12	1.37	d	6.7	23.6	C-10, C-11
*N*H	7.76	d	6.3		C-10, C-13
L-*N*-MePhe					
13				167.6	
14	5.39	t	7.5	60.0	C-13, C-16, C-22, C-23
15a	2.83	dd	14.0, 7.0	37.1	C-13, C-14, C-16, C-17/21
15b	3.19	dd	14.0, 7.1		C-13, C-14, C-16, C-17/21
16				136.8	
17/21	7.17	t	7.0	128.8	C-16, C-18/20
18/20	7.14	t	7.0	128.2	C-17/21, C-19
19	7.07	t	7.0	126.5	C-17/21
22 (*N*-CH_3)	2.99	s		28.6	C-14
L-*N*-MeVal					
23				168.7	
24	4.89	d	10.5	57.0	C-23, C-25, C-26, C-27, C-28, C-29st
25	2.15	m		26.4	C-24, C-26, C-27
26	0.27	d	6.5	17.9	C-25
27	0.77	d	6.5	18.0	C-25
28 (*N*-CH_3)	3.00	s		29.7	C-24
L-Lactic acid (L-Lac)					
29				172.5	
30	5.28	q	6.8	67.5	C-1, C-31
31	1.29	d	6.8	15.5	C-29, C-30

A molecular formula of $C_{32}H_{45}N_5O_6S$ (13° unsaturation) was determined for guineamide B (**2**) by HR FABMS. The peptide nature of **2** was suggested by initial inspection of the ^{1}H NMR spectrum, which showed two D_2O exchangeable protons at δ 8.85 and 8.07 and two *N*-Me singlets at δ 3.07 and 3.22, attributable to secondary and tertiary amide functionalities, respectively. In addition, the ^{1}H NMR spectrum (in $CDCl_3$) of **2** was similar to that of guineamide A (**1**), indicating that **2** possessed similar amino acid residues.

Examination of the 2D NMR data for **2** revealed the presence of *N*-MePhe, *N*-MeVal, a thiazole-alanine unit, 2-hydroxyisovaleric acid (Hiv), and 2-methyl-3-aminobutanoic acid (Maba). The sequential relationship of these residues was established from HMBC, leading to the planar structure shown (**2**). For example, HMBC correlations were observed from *N*H-Maba (δ 8.85) to C-6; H-8 to C-6; H-10 and *N*H-Ala-thiazole (δ 8.07) to C-12; H-13 and H-21 to C-22; and H-23 and H-27 to C-28, revealing the sequence Maba/Ala-thiazole/*N*-MePhe/*N*-MeVal/Hiv. The final ring closure between Hiv and Maba was revealed from an HMBC correlation of the α-H (H-29) of the Hiv unit to the carbonyl carbon (C-1) of the Maba unit. While metabolites

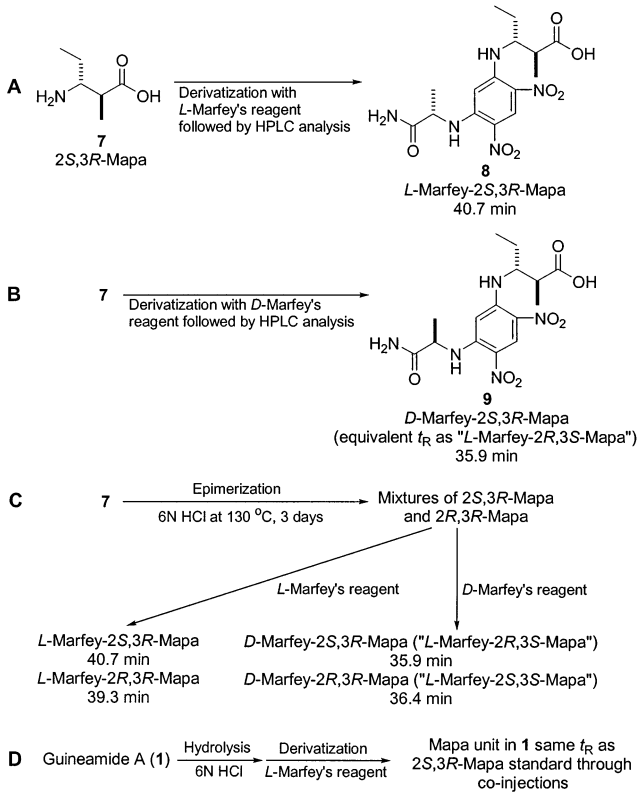


Figure 1. Absolute stereochemical analysis of the 2*S*,3*R*-Mapa unit in guineamide A (**1**).

1 and **2** share a common Ala-thiazole/*N*-MePhe/*N*-MeVal sequence, **2** deviates from guineamide A (**1**) in having an Hiv and Maba unit instead of a Lac and Mapa unit.

Absolute configurations of the α-amino acids and the Hiv unit in **2** were determined by Marfey's analysis and chiral GC−MS, respectively. As in guineamide A, guineamide B (**2**) was first subjected to ozonolysis to prevent epimerization at the α position of the Ala-thiazole unit during acid hydrolysis. The aqueous layer of the acid hydrolysate of **2** was divided into two portions, and one-half was subjected to Marfey's analysis to yield L-Ala, L-*N*-MePhe, and L-*N*-MeVal. The other half was subjected to chiral GC−MS analysis, leading to an assignment of L-Hiv in **2**.

Pure guineamide C (**3**) analyzed for a molecular formula of $C_{35}H_{53}N_4O_7$ by high-resolution FABMS. The IR spectrum of **3** indicated the presence of ester (1712 cm^{-1}) and amide (1652 cm^{-1}) functionalities. By ^{13}C NMR, **3** possessed five such ester/amide carbonyl signals along with resonances at δ 135.0, 129.3, 114.0, and 159.0, characteristic of a *para*-disubstituted phenyl ring. Collectively, this accounted for nine of the 12 degrees of unsaturation inherent to the molecular formula of **3**.

Interpretation of 1D and 2D NMR spectral data of **3** generated five partial structures, including 2-hydroxy-3-methylpentanoic acid (Hmpa), *N*,*O*-dimethyltyrosine (*N*,*O*-diMeTyr), Val, and *N*-MeAla. An additional β-amino acid unit was deduced from COSY data. A correlation observed from H-6 to H-8 in the TOCSY spectrum, together with the HMBC correlations from H-6 to C-7 and C-8, defined this unit as 2-methyl-3-amino-oct-7-ynoic acid (Maoya). The presence of a terminal acetylene functionality in **3** accounted for two of the three remaining degrees of unsaturation. Hence, guineamide C (**3**) also possessed a single macrocyclic ring.

The connectivity between these partial structures was accomplished using HMBC data. A three-bond correlation from the α-proton (H-11) of *N*-MeAla to C-14 of Val linked these two residues. A two-bond heteronuclear coupling from an *N*H (δ 7.09; amide proton of Val) to C-19 connected *N*,*O*-diMeTyr to Val. The Hmpa unit was placed adjacent to the *N*,*O*-diMeTyr on the basis of HMBC correlations from H-20 and H_3-29 to C-30. Finally, a two-bond correlation from the amide proton of the Maoya unit (δ 5.43) to C-10 of *N*-MeVal and from H-31 of the Hmpa unit to C-1 of the Maoya unit completed the cyclic structure assignment of guineamide C (**3**). The absolute configurations of these α-amino acids in **3** (*N*,*O*-diMeTyr, Val, and *N*-MeAla) were determined as L using Marfey's analysis.

The $C_{36}H_{56}N_4O_9$ (11 degrees of unsaturation) molecular formula of guineamide D (**4**) was determined by HR-FABMS. IR data again indicated the presence of ester/amide carbonyl functionalities (1731 and 1663 cm^{-1}). ^1H NMR spectra of guineamide D (**4**) in different solvents ($CDCl_3$, CD_3OD, and C_6D_6) were complicated due to the presence of *N*-methyl amide groups, which gave rise to several different conformers in solution. Fortunately, a single conformer predominated in DMSO-d_6, and all NMR experiments were subsequently measured in this solvent. Three distinct methyl singlets were observed in the ^1H NMR spectrum, attributable to an *O*Me (δ 3.67) and two *N*Me (δ 3.07 and 2.88) groups. The presence of two hydroxy acid units in **4** was inferred from two carbon resonances at δ 74.7 and 75.0. A *para*-disubstituted phenyl ring was deduced from two low-field aromatic doublet proton signals at δ 7.14 (2H) and 6.79 (2H). In addition, six ester/amide carbonyl signals in the 165−175 ppm range were detected in the ^{13}C NMR spectrum, accounting for 10 degrees of unsaturation. Thus, the remaining degree of unsaturation was once again due to the macrocyclic nature of the molecule.

Six substructures in guineamide D (**4**) were generated from detailed analyses of 1D and 2D NMR spectral data. These included two Hiv (2-hydroxyisovaleric acid) units (Hiv-1 and Hiv-2), *N*,*O*-diMeTyr, *N*-MeVal, and Gly, and the unique β-amino acid unit, 2-methyl-3-aminohexanoic acid (Maha). The structure of the latter residue in **4** was deduced mainly from HMBC and TOCSY data. Two spin systems were deduced from TOCSY [H_3-7/H-2/H-3 and *N*H-(δ 7.00)/H-3/H_2-4], while HMBC correlations from H_3-7 to C-1 and C-3, H_2-4 to C-3, C-5, and C-6, and H_2-5 to C-3 and C-4 confirmed the structure of this β-amino acid. This Maha residue has been reported only in one other cyanobacterial metabolite, malevamide B, recently isolated from *Symploca laete-viridis*.[15]

The sequential connectivities of the above substructures were identified from HMBC correlations. These correlations included H-2, H-7, and H-33 to C-1, *N*H-Maha (δ 7.00) and H-9 to C-8, *N*H-Gly (δ 8.55), H-9, and H-11 to C-10, H-11, H-20, and H-22 to C-21, and H-22, H-27, and H-28 to C-26, giving rise to the sequence Hiv-2/Maha/Gly/*N*,*O*-diMeTyr/Hiv-1/*N*-MeVal. Finally, the linkage between *N*-MeVal and Hiv-2 was also provided from HMBC data, which showed correlations between the *N*-CH_3 of *N*-MeVal and the α-H of Hiv-2 to the carbonyl carbon (C-32) of Hiv-2.

The absolute configurations of the α-hydroxy and α-amino acids in guineamide D (**4**) were determined by Marfey's analysis and chiral GC−MS. Derivatization of the acid hydrolysate of **4** with Marfey's reagent, followed by HPLC analyses with amino acid standards, yielded L-*N*-MeVal and D-*N*,*O*-diMeTyr. From chiral GC−MS analysis, only L-Hiv was observed from the acid hydrolysate of guineamide D (**4**).

High-resolution FABMS analysis of guineamide E (**5**) revealed an $[M + H]^+$ ion consistent with a molecular formula of $C_{39}H_{59}N_5O_9$, thus requiring 13 double-bond equivalents. The IR spectrum of guineamide E (**5**) gave characteristic absorption bands for esters and amides at 1742 and 1666 cm^{-1}, and the peptide nature of **5** was indicated by two exchangeable *N*H proton resonances at δ 8.94 and 6.66 and two distinct *N*CH_3 proton singlets at δ 3.27 and 2.87. All 39 carbon atoms were observed in the ^{13}C NMR data of guineamide E (**5**) and included characteristic low-field aromatic carbon resonances, suggesting a monosubstituted phenyl group and seven amide/ester carbonyls in the 165−180 ppm range. Hence, compound **5** possessed two additional rings. Two oxygenated sp^3 carbons resonating at δ 78.2 and 77.9 were also detected in the ^{13}C NMR spectrum.

From 1D and 2D NMR data (HMBC and TOCSY) seven substructures could be assembled for guineamide E (**5**). These included five α-amino acids (*N*-MePhe, Pro, *N*-MeVal, Ala, and Gly) and two hydroxy acids [2-hydroxyisovaleric acid (Hiv) and 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha)]. The structure of the latter hydroxy acid, Dmhha, was deduced from HMBC and TOCSY data and is a new β-hydroxy acid unit to be reported in a natural product. The proton spin system from H-3 to H-6 in the Dmhha unit was revealed by TOCSY, and connection of this to the remainder of the residue was achieved by HMBC correlations: H_3-7 and H_3-8 to C-1, C-2, and C-3; H-3 to C-1, C-2, C-4, and C-5; H-4 to C-3, C-5, and C-6; and H-6 to C-4 and C-5.

The sequence of the seven residues in guineamide E (**5**) was established largely from HMBC data. Sequential HMBC correlations were observed from H-22, H-20, and H-12 to C-21; H-12, H-10, and *N*H-Gly (δ 8.94) to C-11; H-10 and H-3 to C-9; H-3, H-7, H-8, and H-36 to C-1; H-36 and *N*H-Ala (δ 6.66) to C-35; and H-31 and H-27 to C-32. This gave rise to the Pro/*N*-MePhe/Gly/Dmhha/Hiv/Ala/*N*-MeVal sequence. No HMBC correlations were detected between the *N*-MeVal and Pro; however, as this was the only remaining possible site for a connection and was required to satisfy the inherent degrees of unsaturation, this assignment could be made with confidence. The *cis* conformation of the Pro amide bond in **5** was determined by a $\Delta\delta_{\beta\gamma}$ (differential value of ^{13}C chemical shifts of C_β and C_γ in Pro) value of 9.6 ppm.[16] Absolute stereochemistry of the α-amino/hydroxy acid units in guineamide E (**5**) were determined by either Marfey's analysis or chiral GC−MS analysis, which indicated the presence of L-Pro, L-*N*-MeVal, L-Ala, D-*N*-MePhe, and L-Hiv.

An $[M + H]^+$ peak observed by HRFABMS for guineamide F (**6**) indicated a molecular formula of $C_{40}H_{55}N_5O_7$, accounting for 16 degrees of unsaturation. The peptide nature of **6** was clear from two exchangeable *N*H protons, two *N*Me groups, and six ester/amide carbonyls. Similar to guineamide E (**5**), four singlet methyl signals (δ 3.02, 3.44, 1.23, and 1.20) were present in the ^1H NMR spectral data. Twelve olefinic carbon signals were present in the 128−132 ppm range, with four peaks possessing two degenerate carbon signals each. Taken together with 10 low-field aromatic proton signals (6.8−7.4 ppm) in the ^1H NMR spectrum, two monosubstituted phenyl groups were deduced in **6**. In contrast to guineamide A (**5**), only one oxygenated sp^3 carbon signal was observed (δ 77.6).

Careful analyses of TOCSY and HMBC data revealed that guineamide F had one residue less than guineamide E (**5**). These included two *N*-MePhe residues, one each of Pro, Val, Gly, and the unique residue 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha). The structure of the Dmhha residue in guineamide F (**6**) was determined in a similar way as in **5** using data from both HMBC and TOCSY experiments.

The planar structure of guineamide F (**6**) was determined from key proton correlations observed by HMBC. These included H-23, H-20, and H-12 to C-21; H-12, H-13, H-10, and *N*H-Gly (δ 8.84) to C-11; H-10 and H-3 to C-9; H-3, H-7, H-8, H-37, and *N*H-Val (δ 5.86) to C-1; and H-27 and H-35 to C-36, and gave rise to the sequence Pro/*N*-MePhe-1/Gly/Dmhha/Val/*N*-MePhe-2, which is identical to the Pro/*N*-MePhe-1/Gly/Dmhha section in guineamide E (**5**). Although no HMBC correlations were observed for the final *N*-MePhe-2-Pro connection, it is the only possible linkage and is again required to account for the 16 degrees of unsaturation. As in guineamide E, the Pro amide bond in **6** was deduced to be *cis* from a $\Delta\delta_{\beta\gamma}$ value of 8.0 ppm. From $^1H-^1H$ COSY, the shielded proton at δ −0.03 was assigned to one of the methylene protons on the β-carbon of the Pro unit, possibly arising from through-space shielding effects from the nearby aromatic amino acids. Marfey's analysis was used to determine the absolute stereochemistry of the α-amino acids in guineamide F (**6**) as L-Pro, L-*N*-MePhe, and L-Val.

A cytotoxicity bioassay using neuro-2a mouse neuroblastoma cells was used to evaluate the properties of these compounds. Guineamide A (**1**) had no activity at 10 μg/mL, while guineamides C (**3**) and B (**2**) had only moderate activities with IC_{50} values of 16 and 15 μM, respectively.

The present study further demonstrates the robustness of marine cyanobacteria to produce diverse cyclic lipopeptide metabolites. From a biosynthetic perspective, the guineamides (**1**−**6**) are interesting because of the presence of unusual β-amino and β-hydroxy acid residues. These residues are becoming structural signatures in marine cyanobacterial cyclic peptides, and at least 65% of the macrocyclic peptides reported to date from marine cyanobacteria contain these moieties.[1] Of the β-amino acid residues present in the guineamides, three (Mapa in **1**, Maba in **2**, and Maoya in **3**) are also present as constituents in molluscan metabolites (e.g., dolastatins D, 11, and 12 and onchidin A).[13,17] Of interest is the Mapa residue where the stereochemistry (2*S*,3*R*) is conserved in both cyanobacterial and molluscan metabolites. These observations support the hypothesis that the secondary metabolites isolated from sea-hares are diet derived, especially those from *Philinopsis speciosa* and *Dolabella auricularia*.[7,18] There is also a preponderance of *N*-methylated amino acids such as *N*-MeVal and *N*-MePhe in the guineamides. An additional and highly intriguing feature of this report is the finding of both L- and D-*N*,*O*-dimethyltryrosine in compounds **3** and **4**, respectively. Previous reports of this distinctive residue in marine cyanobacterial metabolites have predominately described the L-stereoisomer.[1] Overall, these unique structural features of the guineamides are hallmarks of cyanobacterial metabolism, products of hybrid polyketide synthase and nonribosomal peptide synthetase biosynthetic pathways.

Experimental Section

General Experimental Procedures. Optical rotations were measured on either a Perkin-Elmer 141 polarimeter or a Perkin-Elmer 243 polarimeter. UV and IR spectra were recorded on a Beckman DU 640B and a Nicolet 510 spectrophotometer, respectively. 1D and 2D NMR experiments were measured on either a Bruker AM 400 MHz NMR spectrometer or a Bruker DRX600 spectrometer with the solvents $CDCl_3$ (^1H NMR δ 7.24, ^{13}C NMR δ 77.0) or DMSO-d_6 (^1H NMR δ

Table 2. NMR Spectral Data for Guineamide B (**2**) at 600 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$

position	δ_H	mult	J (Hz)	δ_C	HMBC
2-Methyl-3-aminobutanoic acid (Maba)					
1				172.5	
2	2.66	dt	6.7, 2.4	46.9	C-1, C-3, C-5
3	4.52	m		47.0	C-1
4	1.24	d	7.3	20.0	C-1
5	1.25	d	7.2	14.6	C-1
*N*H	8.85	d	10.4		C-3, C-6
L-Ala-thiazole					
6				160.7	
7				148.8	
8	7.95	s		123.2	C-6, C-7, C-9
9				169.5	
10	5.03	m		48.1	C-9, C-11, C-12
11	1.37	d	6.8	23.8	C-9
*N*H	8.07	d	6.1		C-9, C-10, C-12
L-*N*-MePhe					
12				167.7	
13	5.41	dd	10.4, 4.0	61.1	C-12, C-14, C-15, C-21, C-22
14a	2.72	dd	13.4, 3.7	37.5	C-12, C-13, C-15, C-16/20
14b	3.45	m			C-12, C-13, C-15, C-16/20
15				136.5	
16/20	7.17	brd	7.4	129.3	C-14, C-18
17/19	7.12	brt	7.5	128.7	C-15
18	7.02	t	7.2	127.0	C-16/20
21 (*N*-CH_3)	3.07	s		29.1	C-13, C-22
L-*N*-MeVal					
22				170.6	
23	5.27	d	10.6	57.8	C-22, C-24, C-25, C-27, C-28
24	2.38	m		27.7	C-23, C-26
25	0.94	d	6.5	19.1	C-23, C-24, C-26
26	0.82	d	6.5	19.6	C-23, C-24, C-25
27 (*N*-CH_3)	3.22	s		30.5	C-23, C-28
2(*S*)-Hydroxyisovaleric acid (L-Hiv)					
28				172.2	
29	4.97	d	5.7	75.4	C-1, C-28, C-30, C-32
30	2.09	m		30.2	C-29, C-32
31	0.88	d	6.9	18.8	C-29, C-30, C-32
32	0.82	d	6.5	18.4	C-29, C-30, C-31

2.50, ^{13}C NMR δ 39.5) used as internal standard. Chemical shifts are reported in ppm, and coupling constants (J) are reported in Hz. FAB mass spectra were recorded on a Kratos MS50TC mass spectrometer. GC−MS data were obtained on a Hewlett-Packard 5890 Series II GC connected to a Hewlett-Packard 5971 mass spectrometer. HPLC was performed with a Waters Millipore Model 590 and detected with a Waters Millipore Lambda-Max Model 480 LC spectrophotometer. All Marfey-derivatized products were analyzed using dual Waters 515 HPLC pumps and a Waters 996 photodiode array detector.

Algal Collection. The marine cyanobacterium *Lyngbya majuscula* Gomont (Oscillatoriaceae) (voucher specimen available as collection number PNSM-4/Sep/98-01 from W.H.G.) was collected by hand from reefs of Seamount (S 3°33.237′ E 143°39.845′, approximately 2 miles north of Wewak), Papua New Guinea, at a depth of 54 ft using scuba. Upon collection, the microalga was stored in ethanol at reduced temperature until workup.

Extraction and Isolation. Approximately 0.5 L wet volume of preserved alga was extracted with CH_2Cl_2−MeOH (2:1) three times to give ca. 3.0 g of crude organic extract. The organic extract was subjected to Si gel VLC using a stepped gradient elution from 100% hexanes to 50% EtOAc in MeOH, giving seven distinct fractions. Fraction 5 was purified further by Mega-Bond Sep-Pak RP-18 and HPLC on Phenomenex 10 C_8 90A (250 × 4.6 mm), 34% H_2O in MeOH, to yield guineamides A (**1**, 10.0 mg), B (**2**, 1.7 mg), and C (**3**, 2.0 mg). The second subfraction obtained from Mega-Bond Sep-Pak RP-18 was also subjected to further purification using HPLC on a Phenomenex Luna 5 μm phenyl-hexyl (250 × 4.6 mm), 28% H_2O in MeOH, to give guineamides E (**5**, 1.8 mg) and F (**6**, 3.7 mg). Fraction 6 obtained from Si VLC of the organic extract was purified by Mega-Bond Sep-Pak RP-18 and HPLC using a Phenomenex Luna 5 μm phenyl-hexyl column (250 × 4.6 mm), 23% H_2O in MeOH, yielding guineamide D (**4**, 9.5 mg).

Table 3. NMR Spectral Data for Guineamide C (**3**) at 600 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$

position	δ_H	mult	J (Hz)	δ_C	HMBC
2-Methyl-3-amino-oct-7-ynoic acid (Maoya)					
1				174.2	
2	2.80	m		44.9	C-4, C-9
3	4.18	m		51.0	
4a	1.53	m		26.6	C-5, C-6
4b	1.89	m			C-5, C-6
5a	1.60	m		25.5	C-3, C-4, C-6, C-7
5b	1.80	m			C-3, C-4, C-6, C-7
6	2.20	m		17.8	C-5, C-7, C-8
7				84.0	
8	1.92	t	2.4	68.6	
9	1.20	d	7.2	13.8	C-1, C-2, C-3
*N*H	5.43	d	9.0		C-10
L-*N*-MeAla					
10				168.2	
11	3.43	q	6.8	61.2	C-10, C-12, C-13, C-14
12	1.48	d	6.8	12.4	C-10
13 (*N*-CH_3)	3.10	s		37.7	C-11
L-Val					
14				167.3	
15	4.48	dd	9.6, 9.6	55.1	C-16, C-17, C-18
16	2.28	m		29.6	
17	0.97	d	6.9	18.4	C-15
18	0.96	d	6.9	20.1	C-15
*N*H	7.09	d	9.6		C-19
L-*N*,*O*-diMeTyr					
19				167.2	
20	5.62	dd	13.0, 4.5	58.7	C-19, C-29, C-30
21a	2.82	dd	15.5, 13.0	32.9	C-20, C-23/27, C-24/26
21b	3.80	dd	15.5, 4.5		C-20, C-23/27, C-24/26
22				135.0	
23/27	7.11	d	8.5	129.3	C-25
24/26	6.81	d	8.5	114.0	C-25
25				159.0	
28	3.76	s		55.1	C-25
29 (*N*-CH_3)	3.01	s		31.3	C-30
2-Hydroxy-3-methylpentanoic acid (Hmpa)					
30				171.8	
31	4.52	d	8.3	75.9	C-1, C-30
32	1.52	m		36.4	
33a	0.55	m		23.3	C-31, C-32, C-34
33b	0.58	m			C-31, C-32, C-34
34	0.60	t	7.0	11.2	C-32, C-33
35	0.85	d	6.6	14.8	C-31, C-32, C-33

Guineamide A (1): colorless oil; $[\alpha]^{26}_D$ −8° (c 0.10, $CHCl_3$); UV (MeOH) λ_{max} 214 nm (ϵ 14 400); IR (neat) 3309, 2964, 2930, 1732, 1669, 1620, 1551, 1519, 1496, 789 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6), see Table 1; LRFABMS m/z 614 (100), 522 (6), 437 (7), 329 (5), 293 (5); HRFABMS m/z $[M + H]^+$ 614.3023 (calcd for $C_{31}H_{44}N_5O_6S$, 614.3012).

Guineamide B (2): white amorphous solid; $[\alpha]^{26}_D$ −5° (c 0.17, $CHCl_3$); UV (EtOH) λ_{max} 213 nm (ϵ 24 900); IR (neat) 3308, 2967, 2934, 1730, 1628, 1549, 1520, 1255, 1084, 752 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; LRFABMS m/z 628 (48), 244 (14), 196 (100), 134 (51), 86 (61); HRFABMS m/z $[M + H]^+$ 628.3161 (calcd for $C_{32}H_{46}N_5O_6S$, 628.3169).

Guineamide C (3): colorless oil; $[\alpha]^{26}_D$ −76° (c 0.10, MeOH); UV (MeOH) λ_{max} 222 nm (ϵ 16 400); IR (neat) 3361, 2961, 2930,

Table 4. NMR Spectral Data for Guineamide D (**4**) at 600 MHz (^{1}H) and 100 MHz (^{13}C) in DMSO-d_6

position	δ_H	mult	*J* (Hz)	δ_C	HMBC
2-Methyl-3-aminohexanoic acid (Maha)					
1				174.7	
2	2.67	m		41.2	C-1, C-7
3	3.79	m		50.8	
4ab	1.25	m		36.7	C-3, C-4, C-5, C-6
5ab	1.24	m		19.5	C-3, C-4
6	0.85	m		14.6	C-4, C-5
7	1.08	d	7.0	15.9	C-1, C-2, C-3
*N*H	7.00	d	9.1		C-8
Gly					
8				169.3	
9a	3.30	m		43.7	C-8, C-10
9b	3.93	dd	16.8, 6.3		C-8, C-10
*N*H	8.55	m			C-9, C-10
D-*N*,*O*-DiMeTyr					
10				172.4	
11	5.40	brt	8.1	56.6	C-10, C-12, C-13, C-20, C-21
12ab	2.89	m		35.1	C-10, C-11, C-13
13				129.8	
14/18	7.14	d	8.1	130.6	C-12, C-14/16, C-15/17, C-16
15/17	6.79	d	8.1	114.5	C-13, C-16
16				158.8	
19 (*O*-CH_3)	3.67	s		55.9	
20 (*N*-CH_3)	3.07	s		31.3	C-11, C-21
2(*S*)-Hydroxyisovaleric acid (L-Hiv-1)					
21				169.0	
22	5.20	brd	2.7	75.0	C-21, C-23, C-24, C-25, C-26
23	1.45	m		29.8	C-24, C-25
24	0.67	d	6.8	19.3	C-22, C-23, C-25
25	0.43	d	6.5	16.9	C-22, C-23, C-24
L-*N*-MeVal					
26				168.6	
27	3.79	m		66.7	C-26, C-28, C-31, C-32
28	2.25	m		27.9	C-26, C-27, C-29
29	0.94	m		19.5	C-27, C-28, C-30
30	0.85	m		19.2	C-27, C-28, C-29
31 (*N*-CH_3)	2.88	s		28.8	C-26, C-27
2(*S*)-Hydroxyisovaleric acid (L-Hiv-2)					
32				169.6	
33	5.03	brd	2.8	74.7	C-1, C-32, C-34, C-35, C-36
34	2.04	m		29.9	C-35, C-36
35	0.85	m		17.1	C-33, C-34, C-36
36	0.94	m		20.2	C-33, C-34, C-35

2872, 1712, 1652, 1513, 1458, 782 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; LR-FABMS *m*/*z* 641 (100), 625 (6), 376 (5), 345 (5), 288 (21); HRFABMS *m*/*z* $[M + H]^+$ 641.3941 (calcd for $C_{35}H_{53}N_4O_7$, 641.3914).

Guineamide D (4): white amorphous solid; $[\alpha]^{26}_D$ +55° (*c* 0.95, $CHCl_3$); UV (EtOH) λ_{max} 210 nm (ϵ 21 200); IR (neat) 2965, 2935, 1731, 1663, 1514, 1248, 1182 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6), see Table 4; LRFABMS *m*/*z* 689 (100), 274 (46), 164 (38), 121 (19), 86 (24); HRFABMS *m*/*z* $[M + H]^+$ 689.4130 (calcd for $C_{36}H_{57}N_4O_9$, 689.4126).

Guineamide E (5): white amorphous solid; $[\alpha]^{26}_D$ −2.7° (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} 215 nm (ϵ 38 300); IR (neat) 3259, 2966, 2937, 1742, 1666, 1631, 1203, 753 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 5; LRFABMS *m*/*z* 742 (100), 267 (10), 217 (10), 134 (25), 107 (24); HRFABMS *m*/*z* $[M + H]^+$ 742.4383 (calcd for $C_{39}H_{60}N_5O_9$, 742.4391).

Guineamide F (6): white amorphous solid; $[\alpha]^{26}_D$ −49° (*c* 0.37, $CHCl_3$); UV (EtOH) λ_{max} 215 nm (ϵ 28 700); IR (neat) 3301, 2962, 1745, 1661, 1640, 1524, 1198, 752 cm^{-1}; ^{1}H NMR

Table 5. NMR Spectral Data for Guineamide E (**5**) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$

position	δ_H	mult	*J* (Hz)	δ_C	HMBC
2,2-Dimethyl-3-hydroxyhexanoic acid (Dmhha)					
1				175.2	
2				48.2	
3	5.54	dd	7.1, 5.3	77.9	C-1, C-2, C-4, C-5, C-7, C-8, C-9
4ab	1.56	m		31.9	C-3, C-5, C-6
5ab	1.40	m		20.0	
6	0.96	t	7.2	14.6	C-4, C-5
7	1.29	s		25.0	C-1, C-2, C-3, C-8
8	1.28	s		18.3	C-1, C-2, C-3, C-7
Gly					
9				172.5	
10a	4.51	dd	18.0, 8.2	41.2	C-9, C-11
10b	3.62	dd	18.0, 4.1		C-9, C-11
*N*H	8.94	brdd	8.0, 4.0		C-11
D-*N*-MePhe					
11				171.5	
12	5.79	dd	12.1, 4.8	57.5	C-11, C-13, C-14, C-20, C-21
13a	2.86	m		34.7	C-11, C-12, C-14, C-15/19
13b	3.55	dd	15.0, 4.8		C-11, C-12, C-14, C-15/19
14				137.5	
15/19	7.19	m		129.7	
16/18	7.21	m		128.8	
17	7.17	m		127.0	
20 (*N*-CH_3)	2.87	s		31.4	C-11, C-12
L-Pro					
21				171.6	
22	4.61	m		58.8	C-21, C-23, C-24, C-25
23a	0.89	m		31.3	C-24
23b	1.84	m			C-21, C-22, C-24
24a	0.90	m		21.7	
24b	1.40	m			
25a	3.35	m		46.8	C-23, C-24
25b	3.40	m			
L-*N*-MeVal					
26				168.8	
27	4.74	d	10.9	58.1	C-26, C-28, C-29, C-30, C-31, C-32
28	2.23	m		28.3	C-26, C-27, C-29, C-30
29	0.81	d	6.7	18.7	C-27, C-28, C-30
30	0.79	d	6.4	19.4	C-27, C-28, C-29
31 (*N*-CH_3)	3.27	s		30.8	C-27, C-32
L-Ala					
32				175.2	
33	4.63	m		46.6	C-32, C-34
34	1.51	d	7.2	15.8	C-32, C-33
*N*H	6.66	brd	5.1		C-33, C-34, C-35
2(*S*)-Hydroxyisovaleric acid (L-Hiv)					
35				169.6	
36	5.46	d	2.2	78.2	C-1, C-35, C-37, C-38, C-39
37	2.35	m		32.7	C-38, C-39
38	0.89	d	5.5	16.6	C-36, C-37, C-39
39	0.88	d	5.6	19.2	C-36, C-37, C-38

(400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 6; LRFABMS *m*/*z* 718 (100), 196 (12), 134 (83), 107 (11), 97 (14); HRFABMS *m*/*z* $[M + H]^+$ 718.4197 (calcd for $C_{40}H_{56}N_5O_7$, 718.4180).

Ozonolysis of Guineamides A (1) and B (2). A stream of O_3 was bubbled into 1 mL of CH_2Cl_2 containing either **1** or **2** (200 μg) at 25 °C for about 10 min. Solvent was removed under a stream of N_2, and the resulting residue was subjected to acid hydrolysis and derivatization as described below.

Stereochemical Determination of the α-Amino Acids in Guineamides A (1) and B (2). Hydrolysis of the ozonolyzed guineamide A (**1**) or B (**2**) was achieved in 1 mL of 6 N HCl placed a sealed ampule at 120 °C for 20 h. Traces of HCl were removed in vacuo. The resulting hydrolysate was resus-

Table 6. NMR Spectral Data for Guineamide F (**6**) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$

position	δ_H	mult	J (Hz)	δ_C	HMBC
2,2-Dimethyl-3-hydroxyhexanoic acid (Dmhha)					
1				176.1	
2				47.1	
3	5.27	brt	6.0	77.6	C-1, C-2, C-4, C-5, C-7, C-8, C-9
4ab	1.55	m		32.6	C-2, C-3, C-5, C-6
5ab	1.31	m		20.0	
6	0.95	t	6.1	14.4	C-4, C-5
7	1.23	s		18.0	C-1, C-2, C-3, C-8
8	1.20	s		26.2	C-1, C-2, C-3, C-7
Gly					
9				171.0	
10a	3.20	m		41.5	C-9, C-11
10b	4.69	dd	16.9, 9.6		C-9, C-11
*N*H	8.84	d	9.4		C-11
L-*N*-MePhe-1					
11				169.2	
12	3.89	dd	10.2, 3.1	64.2	C-11, C-13, C-14, C-20, C-21
13a	2.87	dd	13.7, 10.2	34.5	C-11, C-12, C-14, C15/19
13b	3.68	dd	14.5, 3.1		C-11, C-12, C-14, C15/19
14				138.5	
15/19	7.10	m		129.7	C-13, C-17
16/18	7.23	m		127.2	
17	7.23	m		127.4	
20 (*N*-CH_3)	3.02	s		31.4	C-12, C-21
L-Pro					
21				171.5	
22	3.41	m		57.8	C-23, C-24, C-25
23a	0.74	m		30.2	C-21, C-24, C-25
23b	−0.03	m			C-21, C-24, C-25
24ab	1.27	m		22.2	
25a	3.20	m		46.5	
25b	3.39	m			
L-*N*-MePhe-2					
26				169.1	
27	5.14	dd	10.2, 4.9	54.2	C-26, C-28, C-29, C-35, C-36
28a	2.78	dd	12.6, 4.9	37.9	C-26, C-27, C-29, C-30/34
28b	3.12	dd	12.6, 10.3		C-26, C-27, C-29, C-30/34
29				137.2	
30/34	7.00	m		129.3	C-28, C-30/34, C-32
31/33	7.23	m		129.2	C-29
32	7.19	m		127.5	
35 (*N*-CH_3)	3.44	s		31.7	C-27, C-36
L-Val					
36				173.4	
37	4.53	brt	7.4	55.2	C-1, C-36, C-38, C-39, C-40
38	1.93	m		31.0	C-36, C-37, C-39, C-40
39	0.95	m		18.5	C-37, C-38, C-40
40	0.95	m		19.3	C-37, C-38, C-39
*N*H	5.86	d	7.9		C-1

pended in 100 μL of H_2O. A 0.1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide solution in acetone (L-Marfey's reagent, 20 μL) and 1 N $NaHCO_3$ (10 μL) were added to a portion of each hydrolysate, and the mixtures were heated at 40 °C for 1 h. The solutions were cooled to room temperature, neutralized with 2 N HCl (5 μL), and evaporated to dryness. The residues were resuspended in H_2O (50 μL) and analyzed by reversed-phase HPLC on a Waters NOVAPAK C_{18} (150 × 3.9 mm column) with a linear gradient of triethylammonium phosphate (50 mM, pH 3.0)−MeCN, 90:10 to 60:40 in 60 min at 1 mL/min (UV detection at λ 340 nm). The analyses established the presence of L-*N*-MePhe (36.79 min; D-*N*-MePhe, 37.01 min), L-*N*-MeVal (25.67 min; D-*N*-MeVal, 28.01 min), and L-Ala (11.21 min; D-Ala, 14.80 min).

Stereochemical Determination of the Lactic Acid in Guineamide A (1). A portion of the hydrolysate of **1** (50 μL) was subjected to chiral HPLC analysis [Chirex (D)-penicillamine 50 × 4.6 mm, in 100% 2 mM $CuSO_4H_2O$] to detect L-Lac (6.1 min; D-Lac, 6.5 min).

Epimerization of 2(*S*)-Methyl-3(*R*)-Aminopentanoic Acid (2*S*,3*R*-Mapa, 7). 2*S*,3*R*-Mapa (**7**, 1.0 mg) was dissolved in 1 mL of 6 N HCl and transferred into an Ace high-pressure tube. The sample was heated at 130 °C for 3 days, after which excess HCl was removed under a stream of N_2 and redissolved in 1 mL of H_2O. The aqueous solution was divided into two equal portions and derivatized with either L- or D-Marfey's reagent using the procedure outlined above.

Marfey's Analysis of 2(*S*)-Methyl-3(*R*)-Aminopentanoic acid (Mapa). A sample of 2*S*,3*R*-Mapa (**7**, 0.5 mg) was derivatized with L-Marfey's reagent and analyzed on HPLC using a RP-18 column to give a retention time of 40.7 min. Derivatization of the same sample (0.5 mg) with D-Marfey's reagent gave a peak with a retention time of 35.9 min. C-2 epimerized Mapa (see above) was similarly derivatized with either L- or D-Marfey's reagent and analyzed by HPLC. Four peaks were observed, attributable to L-Marfey-2*S*,3*R*-Mapa (40.7 min), L-Marfey-2*R*,3*R*-Mapa (39.3 min), D-Marfey-2*S*,3*R*-Mapa (35.9 min), and D-Marfey-2*R*,3*R*-Mapa (36.4 min). By single injection, as well as by co-injection with the above standards, the Mapa unit derivatized with L-Marfey from guineamide A (**1**) was found to be identical with L-Marfey-2*S*,3*R*-Mapa (40.7 min).

Stereochemical Determination of the 2-Hydroxyisovaleric Acid in Guineamide B (2). The aqueous layer of the acid hydrolysate of **2** (50 μL) was dried under a stream of N_2, diluted in 0.4 mL of diethyl ether, and treated with diazomethane for 10 min. Excess CH_2N_2 and solvent were removed with a stream of N_2, and the residue was resuspended in CH_2Cl_2. Capillary GC−MS analysis of the methyl ester derivative was carried out on a Chiralsil-Val column (Alltech, 25 m × 0.25 mm) using the following conditions: initial column temperature held at 40 °C for 10 min after injection of the sample, then increased from 40 °C to 100 °C at a rate of 3 °C/min, then from 100 °C to 150 °C at a rate of 15 °C/min. The retention time found for the guineamide B-derived Hiv was recorded at 8.30 min. Standards of D- and L-Hiv were also converted to the corresponding methyl ester derivatives by the same procedure and analyzed under the same conditions to give D-Hiv at 9.10 min and L-Hiv at 8.30 min.

Stereochemical Determination of the α-Amino Acids in Guineamide C (3). Compound **3** (0.2 mg) was treated with 1 mL of 6 N HCl and the suspension heated at 110 °C for 18 h. Excess HCl was removed in vacuo and the hydrolysate suspended in 100 μL of H_2O. This was divided into two equal portions and subjected to Marfey's analysis and chiral GC−MS as described above for guineamide B (**2**). Standard L-*N*,*O*-diMeTyr was derivatized with D-Marfey's reagent to yield the chromatographic equivalent of D-*N*,*O*-diMeTyr. This was used as the D-*N*,*O*-diMeTyr equivalent. The following amino acids were observed in guineamide C (**3**) from Marfey's analysis: L-MeAla (9.23 min; D-Ala, 11.76 min), L-Val (28.22 min; D-Val, 35.53 min), and L-*N*,*O*-diMeTyr (37.26 min; D-*N*,*O*-diMeTyr, 38.33 min).

Stereochemical Determination of the α-Amino Acids and Hiv in Guineamide D (4). Stereochemistry of the amino acids present in guineamide D (**4**) were analyzed by Marfey's method following peptide hydrolysis and gave the following amino acids: L-*N*-MeVal (36.43 min; D-*N*-MeVal, 40.12 min) and D-*N*,*O*-diMeTyr (38.33 min; L-*N*,*O*-diMeTyr, 37.26 min). From the chiral GC−MS analysis, only L-Hiv was observed from the acid hydrolysate of **4**.

Stereochemical Determination of the α-Amino Acids in Guineamides E (5) and F (6). The absolute stereochemistries of amino acid residues in guineamide E (**5**, 0.1 mg) were determined using the same method outlined above and gave the following from Marfey's analysis: L-Pro (22.33 min; D-Pro, 25.79 min), L-*N*-MeVal (36.45 min; D-*N*-MeVal, 40.12 min), L-Ala (20.63 min; D-Ala, 25.98 min), and D-*N*-MePhe (39.32

min; L-*N*-MePhe, 38.38 min). The Hiv unit in **5** was established as L by chiral GC−MS analysis. The same methods were used for determining the absolute stereochemistry of amino acids in guineamide F (**6**). Marfey's analysis gave L-Pro (22.32 min; D-Pro, 25.78 min), L-Val (28.22 min; D-Val, 35.53 min), and L-*N*-MePhe (38.38 min; D-*N*-MePhe, 39.32 min).

Acknowledgment. We gratefully acknowledge B. Arbogast (Chemistry, OSU) for mass spectral data, G. Zeller (Pharmacy, OSU) for cytotoxicity data, T. Williamson (Wyeth-Ayerst Research, Pearl River, NY) for recording 2D NMR spectra for compounds **1** and **3**, R. Bates (University of Arizona) for the gift of the (2*S*,3*R*)-2-methyl-3-aminopentanoic acid, the Government of Papua New Guinea for permission to make these collections, and C. DeWitt (Golden Dawn Enterprises) for assistance with technical aspects of the scuba operation. Financial support for this work came from the National Institutes of Health (GM 63554 and CA52955).

Supporting Information Available: 1D, 2D NMR and mass spectral data of **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *The Alkaloids. Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: San Diego, 2001; Vol. 57, pp 75−184.
(2) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347−9377.
(3) Gerwick, W. H.; Proteau, P. J.; Nagle, D. G.; Hamel, E.; Blokhin, A.; Slate, D. L. *J. Org. Chem*. **1994**, *59*, 1243−1245.
(4) (a) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418−5423. (b) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Bioorg. Med. Chem*. **2002**, *10*, 1973−1978.
(5) (a) Wu, M.; Okino, T.; Nogle, L. M.; Marquez, B. L.; Williamson, R. T.; Sitachitta, N.; Berman, F. W.; Murray, T. F.; McGough, K.; Jacobs, R.; Colsen, K.; Asano, T.; Yokokawa, F.; Shioiri, T.; Gerwick, W. H. *J. Am. Chem. Soc.* **2000**, *122*, 12041−12042. (b) Berman, F. W.; Gerwick, W. H.; Murray, T. F. *Toxicon* **1999**, *37*, 1645−1648.
(6) Milligan, K. E.; Marquez, B. L.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod*. **2000**, *63*, 1440−1443.
(7) Poncet, J. *Curr. Pharm. Des.* **1999**, *5*, 139−162.
(8) (a) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. W. P.; Todd, J. S. *J. Org. Chem*. **1984**, *49*, 236−242. (b) Mynderse, J. S.; Hunt, A. H.; Moore, R. E. *J. Nat. Prod*. **1988**, *51*, 1299−1301. (c) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod*. **1998**, *61*, 1075−1077. (d) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod*. **1998**, *61*, 1221−1225. (e) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod*. **1999**, *62*, 655−658. (f) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod*. **1999**, *62*, 1702−1706. (g) Mitchell, S. S.; Faulkner, D. J.; Rubins, K.; Bushman, F. D. *J. Nat. Prod*. **2000**, *63*, 279−282. (h) Nogle, L. M.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod*. **2001**, *64*, 716−719. (i) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod*. **2001**, *64*, 907−910. (j) Nogle, L. M.; Gerwick, W. H. *J. Nat. Prod*. **2002**, *65*, 21−24. (k) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod*. **2002**, *65*, 16−20. (l) Horgen, F. D.; Kazmierski, E. B.; Westenburg, H. E.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod*. **2002**, *65*, 487−491.
(9) Margolin, K.; Longmate, J.; Synold, T. W.; Gandara, D. R.; Weber, J.; Gonzalez, R.; Johansen, M. J.; Newman, R. Baratta, T.; Doroshow, J. H. *Invest. New Drugs* **2001**, *19*, 335−340.
(10) McDonald, L. A.; Ireland, C. M. *J. Nat. Prod*. **1992**, *55*, 376−379.
(11) Marfey, P. *Carlsberg Res. Commun*. **1984**, *49*, 591−596.
(12) Harada, K.; Fujii, K.; Hayashi, K.; Suzuki, M.; Ikai, Y.; Oka, H. *Tetrahedron Lett.* **1996**, *37*, 3001−3004.
(13) Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R. J.; Schmidt, M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553−558.
(14) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod*. **2002**, *65*, 29−31.
(15) Horgen, F. D.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod*. **2000**, *63*, 461−467.
(16) Siemion, I. Z.; Wieland, T.; Pook, K. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702−703.
(17) (a) Sone, H.; Nemoto, T.; Ishiwata, H.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8449−8452. (b) Rodriguez, J.; Fernandez, R.; Quinoa, E.; Riguera, R.; Debitus, C.; Bouchet, P. *Tetrahedron Lett.* **1994**, *35*, 9239−9242.
(18) (a) Reese, M. T.; Gulavita, N. K.; Nakao, Y.; Hamann, M. T.; Yoshida, W. Y.; Coval, S. J.; Scheuer, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 11081−11084. (b) Nakao, Y.; Yoshida, W. Y.; Szabo, C. M.; Baker, B. J.; Scheuer, P. J. *J. Org. Chem*. **1998**, *63*, 3272−3280. (c) Kimura, J.; Takada, Y.; Inayoshi, T.; Nakao, Y.; Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem*. **2002**, *67*, 1760−1767.

NP020492O