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Isolation and Total Synthesis of Mabuniamide, a Lipopeptide from an *Okeania* sp. Marine Cyanobacterium

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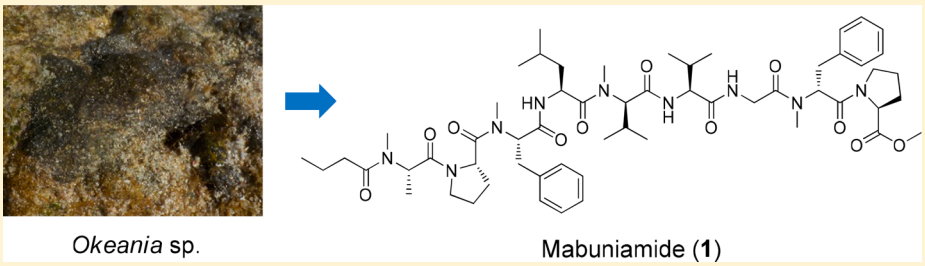
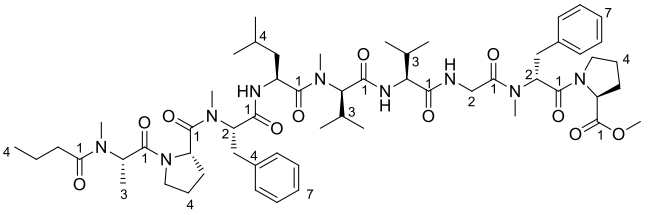
Supporting Information



ABSTRACT: The bioassay-guided fractionation of an *Okeania* sp. marine cyanobacterium collected in Okinawa led to the isolation of the lipopeptide mabuniamide (**1**). The gross structure of **1** was determined by spectroscopic analyses, and its absolute configuration was determined using Marfey's analysis of the acid hydrolysate of **1**. The absolute configuration of **1** was confirmed by total synthesis. Mabuniamide (**1**) stimulated glucose uptake in cultured rat L6 myotubes. In addition, mabuniamide (**1**) and its stereoisomer (**2**) exhibited moderate antimalarial activity.

Marine organisms are well known to be a rich source of bioactive substances.[1,2] Marine cyanobacteria also are good producers of secondary metabolites possessing various bioactivities with structural diversity.[3] In the past 10 years, numerous marine cyanobacteria-derived peptides have been discovered.[4] Most of the linear peptides isolated from marine cyanobacteria exhibit biological activities, such as antitumor, antimicrobial, antimalarial, and enzyme inhibition properties.[4,5] For example, bisebromoamide, isolated from a *Lyngbya* sp., showed potent cytotoxicity against HeLa S_3 cells.[6] Almiramides B and C, isolated from a *Lyngbya majuscula*, showed strong *in vitro* antiparasitic activity against *Leishmania donovani*.[7] Meanwhile, some peptides that show bioactivity related to metabolic disease such as type 2 diabetes and osteoporosis have been isolated from marine cyanobacteria. For example, largazole, isolated from a *Symploca* sp., exhibited *in vitro* and *in vivo* osteogenic activity.[8,9] Ypaoamide B, isolated from an *Okeania* sp., stimulated glucose uptake in cultured L6 myotubes by activation of AMP-activated protein kinase (AMPK).[10]

In our search for novel bioactive substances, we recently isolated mabuniamide (**1**), which stimulated glucose uptake in cultured L6 myotubes and showed antimalarial activity. Here, we report the isolation, structure elucidation, synthesis, and biological activity of **1**.



Mabuniamide (**1**)

RESULTS AND DISCUSSION

An *Okeania* sp. marine cyanobacterium (392.1 g, wet weight) was collected from the coast of Odo, Okinawa, Japan, and extracted with MeOH. The extract was subjected to fractionation guided by glucose uptake activity in L6 myotubes with solvent partitioning, ODS silica gel column chromatog-

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Table 1. NMR Data for Mabuniamide (1) in $CDCl_3$

unit	no.	δ_C,[a] type	δ_H mult (J in Hz)[b]	unit	no.	δ_C,[a] type	δ_H mult (J in Hz)[b]
Pro1	1	172.5, C		Leu	1	175.0, C	
	2	59.0, CH	4.34, dd (8.6, 5.6)		2	49.4, CH	4.69, m
	3a	28.9, CH_2	2.09, m		3a	39.7, CH_2	1.81, m
	3b		1.81, m		3b		1.36, m
	4a	25.1, CH_2	1.86, m		4	24.8, CH	1.71, m
	4b		1.72, m		5	23.5, CH_3	0.90, d (6.6)
	5	46.8, CH_2	3.23, m		6	21.3, CH_3	0.87, d (6.6)
	O-Me	51.3, CH_3	3.68, s		NH		8.78, d (6.3)
N-Me-Phe1	1	168.0, C		*N*-Me-Phe2	1	170.16,[c] C	
	2	56.4, CH	5.52, dd (8.7, 6.1)		2	62.9, CH	4.95, dd (11.8, 2.8)
	3a	35.2, CH_2	3.27, m		3a	33.8, CH_2	3.22, dd (14.2, 2.8)
	3b		2.73, dd (13.4, 6.1)		3b		2.87, m
	4	137.3, C			4	138.1, C	
	05/9	129.5, CH	7.11, m		05/9	129.6, CH	7.20, m
	06-/8	128.4, CH	7.21. m		06/8	129.0, CH	7.25, m
	7	127.0, CH	7.19, m		7	126.7, CH	7.16, m
	N-Me	29.8, CH_3	2.86, s		*N*-Me	29.3, CH_3	2.82, s
Gly	1	168.2, C		Pro2	1	173.3, C	
	2a	41.4, CH_2	4.16, dd (17.5, 5.3)		2	55.2, CH	4.33, dd (7.9, 5.2)
	2b		3.91, dd (17.5, 3.4)		3	28.3, CH_2	0.76, m
	NH		6.93, brt (5.3, 3.4)		4a	25.4, CH_2	1.94, m
Val	1	171.2, C			4b		1.54, m
	2	59.1, CH	4.24, dd (8.4, 7.0)		5a	47.6, CH_2	3.53, m
	3	30.2, CH	2.20, m		5b		3.47, m
	4	19.6, CH_3	0.93, m	*N*-Me-Ala	1	170.1, C	
	5	18.4, CH_3	0.93, m		2	49.6, CH	5.39, q (6.9)
	NH		6.86, m		3	14.3, CH_3	1.18, d (6.9)
N-Me-Val	1	170.19[c], C			*N*-Me	30.6, CH_3	2.91, s
	2	63.2, CH	4.64, m	Ba	1	173.2, C	
	3	26.0, CH	2.36, m		2	35.7, CH_2	2.26, t (7.5)
	4	20.4, CH_3	1.01, d (6.4)		3	18.4, CH_2	1.63, m
	5	18.7, CH_3	0.83, d (6.8)		4	14.0, CH_3	0.93, t (7.1)
	N-Me	31.5, CH_3	3.10, s				0.90, d (6.6)

[a]Measured at 125 MHz. [b]Recorded at 500 MHz. [c]These signals are interchangeable.

raphy (MeOH–H_2O), and reversed-phase HPLC to give mabuniamide (**1**).

Mabuniamide (**1**) was obtained as a colorless oil, and its molecular formula was determined to be $C_{58}H_{87}N_9O_{11}$ by HRESIMS. The NMR data for **1** are summarized in Table 1. The ^1H NMR spectra suggested the presence of three NH groups (δ_H 8.78, 6.93, 6.86), four *N*-methylamide groups (δ_H 3.10, 2.91, 2.86, 2.82), and one methyl ester moiety (δ_H 3.68). In addition, the ^1H and ^{13}C NMR spectra revealed the presence of several α-protons ($\sim\delta_H$ 4.24–5.52) and several carbonyl carbons ($\sim\delta_C$ 168.0–175.0). Thus, the ^1H and ^{13}C NMR spectra suggested that **1** was a peptidic compound (Table 1). The analyses of COSY, HSQC, and HMBC spectra recorded in $CDCl_3$ revealed the presence of two prolines (Pro1 and Pro2), two *N*-methyl-phenylalanines (*N*-Me-Phe1 and *N*-Me-Phe2), glycine (Gly), valine (Val), *N*-methyl-valine (*N*-Me-Val), leucine (Leu), *N*-methyl-alanine (*N*-Me-Ala), and butanoic acid (Ba).

The sequence for **1** was determined by HMBC and NOESY analyses. The HMBC signals H_2-5 of Pro1 (δ_H 3.23)/C-1 of *N*-Me-Phe1 (δ_C 168.0), *N*-Me of *N*-Me-Phe1 (δ_H 2.86)/C-1 of Gly (δ_C 168.2), NH of Gly (δ_H 6.93)/C-1 of Val (δ_C 171.2), NH of Val (δ_H 6.86)/C1 of *N*-Me-Val (δ_C 170.19), *N*-Me of *N*-Me-Val (δ_H 3.10)/C-1 of Leu (δ_C 175.0), NH of Leu (δ_H 8.78)/C-1 of *N*-Me-Phe2 (δ_H 170.16), *N*-Me of *N*-Me-Phe2 (δ_H 2.82)/C-1 of Pro2 (δ_C 173.3), and *N*-Me of *N*-Me-Ala (δ_H 2.91)/C-1 of Ba (δ_C 173.2) and the NOESY correlation H_2-5 of Pro2 (δ_H 3.53 and 3.47)/H-2 of *N*-Me-Ala (δ_H 5.39) established the linear sequence Pro1-*N*-Me-Phe1-Gly-Val-*N*-Me-Val-Leu-*N*-Me-Phe2-Pro2-*N*-Me-Ala-Ba. Thus, the gross structure of **1** was determined and is shown in Figure 1a. The sequence of residues in **1** was further confirmed by analysis of ESIMS/MS fragmentation patterns shown in Figure 1b. Thus, the gross structure of **1** was determined and is shown in Figure 1a.

The configurations of the Pro1, Val, *N*-Me-Val, Leu, Pro2, and *N*-Me-Ala units in **1** were L, L, D, L, L, and L, respectively, as determined by hydrolysis of **1** following Marfey's method.[11] The analysis described above revealed that **1** contained *N*-Me-L-Phe and *N*-Me-D-Phe. To distinguish them from one another, the partial acid hydrolysate of **1** was subjected to reversed-phase HPLC-guided fractionation. Treatment of **1** with 4 M HCl for 30 min at 100 °C afforded the partial acid hydrolysate of **1**.[12,13] The ESIMS analysis of each fraction allowed us to identify fragments of *N*-Me-Phe-Gly-Val (fragment A) and Leu-*N*-Me-Phe-Pro (fragment B). Complete acid hydrolysis of their fragments and reversed-phase HPLC-guided fractionation generated the *N*-Me-Phe from each fragment, respectively. Marfey's analysis of each *N*-Me-Phe revealed that the absolute configuration of the *N*-Me-Phe adjacent to Leu and Pro was L

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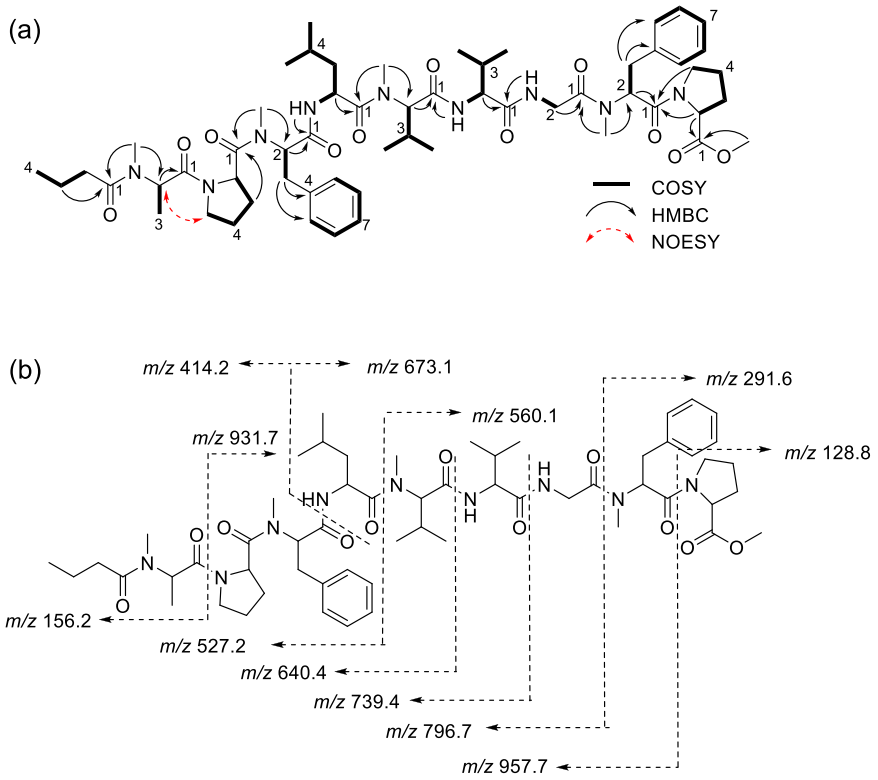
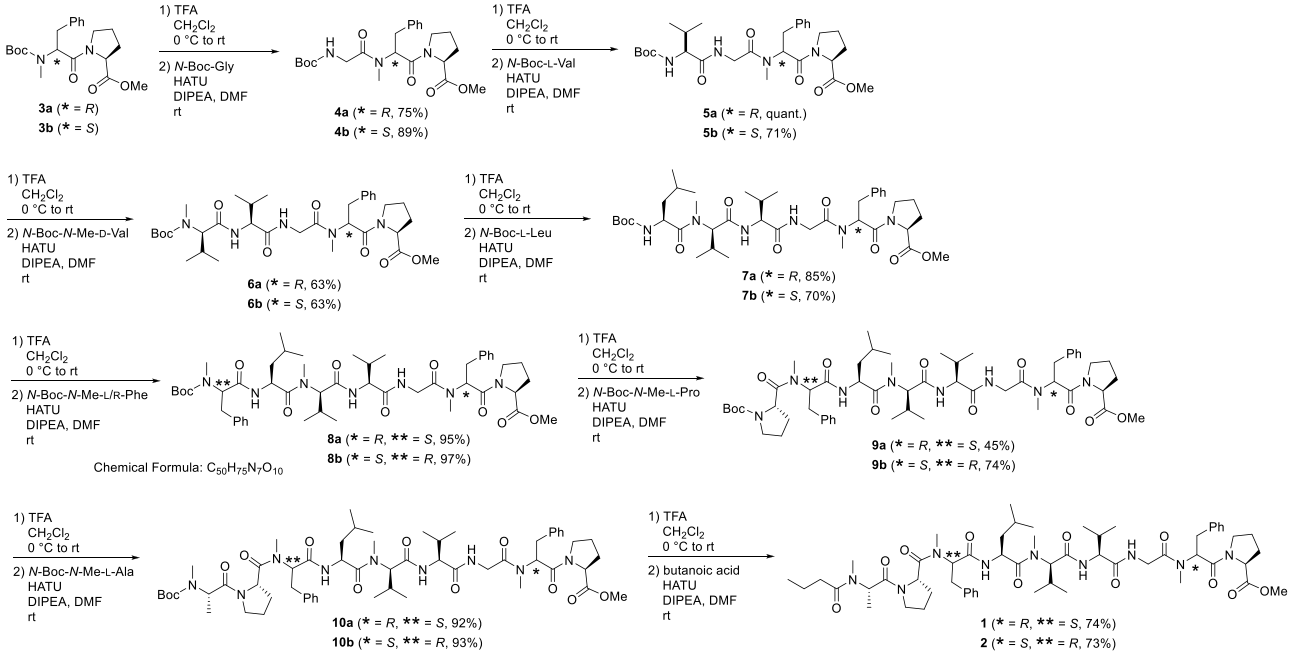


Figure 1. (a) Key 2D NMR correlations for mabuniamide (**1**). (b) ESIMS/MS fragmentation patterns of mabuniamide (**1**).

Scheme 1. Total Synthesis of Mabuniamide (1) and its Stereoisomer 2



and that the other was D. This completed the structural assignments of mabuniamide (**1**).

To confirm the absolute configuration of **1**, we synthesized mabuniamide (**1**) and its stereoisomer **2**, whose configurations of the two *N*-Me-Phe residues were exchanged (Scheme 1). Known dipeptide **3a**[14] was condensed with the following amino acids, Gly, L-Val, and *N*-Me-D-Val, one by one, affording pentapeptide **6a**. We further extended the sequence of **6a** by the condensation of the following residues, L-Leu, *N*-Me-L-Phe, and *N*-Me-L-Pro, sequentially. The addition of L-alanine to the resulting octapeptide **9a** followed by the condensation of butanoic acid gave mabuniamide (**1**, 12% through 16 steps). Its stereoisomer **2**, which possessed the inverted configurations regarding the two *N*-Me-Phe residues, was synthesized from known dipeptide **3b**[15] in a similar manner, as shown in Scheme 1 (14% through 16 steps). The spectral data of natural mabuniamide were consistent with those of synthetic **1**, but

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not **2**. Therefore, we confirmed the correctness of our structure determination.

Mabuniamide (**1**) is structurally similar to malevamide A[16] with the replacement of several amino acid moieties and a 2-methylhexanoic acid residue (Mha). The proton and carbon chemical shifts of **1** also were very similar to those of malevamide A. However, the configurations of two *N*-Me-Phe residues and the Mha moiety in malevamide A have not been determined. Therefore, the similar spectroscopic data of **1** may suggest the absolute configuration of malevamide A.

The biological activity of mabuniamide (**1**) was evaluated using a glucose uptake assay in cultured rat L6 myotubes.[7,17] Mabuniamide (**1**) showed no cytotoxicity at 10−40 μM (Figure 2a) and stimulated glucose uptake in a dose-dependent

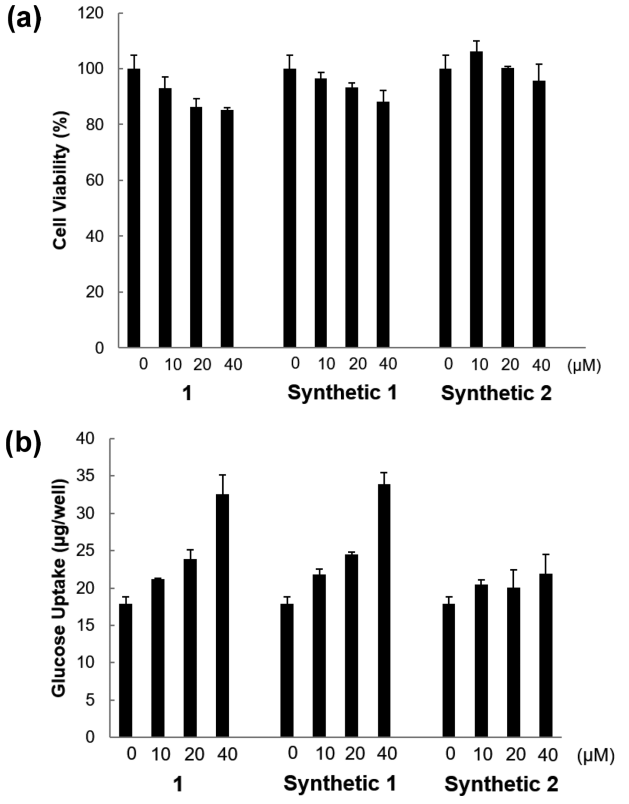

Figure 2. Effects of natural **1**, synthetic **1**, and its stereoisomer **2** on cell viability and glucose uptake in cultured L6 myotubes. (a) Cells were treated with the indicated concentrations of compounds. After incubation for 22 h, cell viability was determined based on an MTT assay. Values are the mean ± SD of quadruplicate determinations. (b) Cells were preincubated in Krebs-Henseleit-HEPES buffer (KHH buffer) without glucose for 2 h. They were then incubated in KHH buffer containing 11 mM glucose with the indicated concentrations of compounds for 22 h. Glucose uptake was measured using a Glucose CII test kit. Values are the mean ± SD of quadruplicate determinations.

and insulin-independent manner (Figure 2b). Synthetic **1** also stimulated glucose uptake in a dose-dependent and insulin-independent manner. The effect of synthetic **1** was slightly stronger than that of **1** (Figure 2b). In addition, we assessed the antimalarial activities of **1** and **2** against the asexual erythrocytic stage of the *Plasmodium falciparum* 3D7 clone, which is a standard reference that is sensitive to most antimalarials. As a result, mabuniamide (**1**) and its stereoisomer **2** exhibited antiplasmodial activity with IC_{50} values of 1.4 ± 0.2 and 2.8 ± 0.1 μM, respectively. However, the IC_{50} value of chloroquine as a positive control, 7.6 ± 0.5 nM, was much lower than those of mabuniamide (**1**) and its analogue (**2**).

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotation was measured on a JASCO P-1010 polarimeter or DIP-1000. UV spectra were measured on a JASCO V-660 UV visible spectrometer. IR spectra were measured on a JASCO FT/IR-6100 or RT/IR-4200 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 (500 MHz) or JEOL JNM-ECX400 (400 MHz). Chemical shifts were reported relative to the residual solvent signals ($CDCl_3$: δ_H 7.26, δ_C 77.2). ESIMS data were obtained using a Waters Quattro micro API mass spectrometer; HRESIMS was performed on a Waters Micromass Q-TOF or LC Premier EX spectrometer. HPLC was carried out with a JASCO PU-2080 Plus Intelligent HPLC pump and a JASCO UV-2075 Plus Intelligent UV/vis detector. The absorbance of the assay mixture was determined using a BioTek ELx 800 absorbance microplate reader. Chemicals and solvents were the best grade available and used as received from commercial sources.

Identification of the Marine Cyanobacterium. A cyanobacterial filament collected in May 2018 was isolated under a microscope and crushed with freezing and thawing. The 16S rDNA genes were PCR-amplified from isolated DNA using the primer set CYA106F, a cyanobacterial-specific primer, and 16S1541R, a universal primer. The PCR reaction contained DNA derived from a cyanobacterial filament, 0.5 μL of KOD-Multi & Epi- (Toyobo), 1.0 μL of each primer (50 pM, respectively), 12.5 μL of 2× PCR buffer for KOD-Multi & Epi-, and H_2O for a total volume of 25 μL. The PCR reaction was performed as follows: initial denaturation for 2 min at 94 °C, amplification by 40 cycles of 10 s at 98 °C, 10 s at 58 °C, 1 min at 68 °C, and final elongation for 7 min at 68 °C. PCR products were analyzed on agarose gel (1%) in Tris-borate-EDTA buffer and visualized by ethidium bromide staining. The obtained DNA was sequenced with CYA106F and 16S1541R primers. This sequence is available in the DDBJ/EMBL/GenBank databases under the accession number LC466966. The nucleotide sequence of the 16S rRNA gene obtained in this study was used for phylogenetic analysis with the sequences of related cyanobacterial 16S rRNA genes.[18] All sequences were aligned by SINA Web service (version 1.2.11)[19] with default settings. The poorly aligned positions and divergent regions were removed by Gblocks Server (version 0.91b),[20] implementing the options for a less stringent selection, including the 'Allow smaller final blocks', 'Allow gap positions within the final blocks', and 'Allow less strict flanking positions' options. The obtained 1190 nucleotide positions have been used for phylogenetic analysis. JModeltest (version 2.1.7)[21] with default settings was used to select the best model of DNA substitution for the Maximum Likelihood (ML) analysis and Bayesian analysis according to the Akaike information criterion (AIC). The ML analysis was conducted by PhyML (version 20131016),[22] using the GTR+I+G model with a gamma shape parameter of 0.4830, a proportion of invariant sites of 0.5300, and nucleotide frequencies of F(A) = 0.2465, F(C) = 0.2272, F(G) = 0.3151, F(T) = 0.2112. Bootstrap resampling was performed on 1000 replicates. The ML tree was visualized with Njplot (version 2.3).[23] The Bayesian analysis was conducted by MrBayes (version 3.2.5)[24] using the GTR+I+G model. The Markov chain Monte Carlo process was set at 2 chains, and 1 000 000 generations were conducted. Sampling frequency was assigned at every 500 generations. After analysis, the first 100 000 trees were deleted as burn-in, and the consensus tree was constructed. The Bayesian tree was visualized with FigTree (version 1.4.0, http://tree.bio.ed.ac.uk/software/figtree). As a result, the cyanobacterium formed a clade with *Okeania*. Therefore, the cyanobacterium was classified into the genus *Okeania*.

Extraction and Isolation. The *Okeania* sp. cyanobacterium (392.1 g, wet weight) was collected at Odo, Okinawa, Japan (26°09′ N, 127°71′ E), in May 2018. The cyanobacterium was extracted with

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MeOH (1.5 L) at room temperature for 4 days. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between H_2O (0.2 L) and EtOAc (0.2 L × 3). The material obtained from the organic layer was further partitioned between 90% aqueous MeOH (0.1 L) and *n*-hexane (0.1 L × 3). The aqueous 90% MeOH fraction (0.25 g) was separated by column chromatography on ODS (4.0 g) using 40% aqueous MeOH, 60% aqueous MeOH, 80% aqueous MeOH, and MeOH. The fraction (47.2 mg) that eluted with 80% aqueous MeOH was subjected to reversed-phase HPLC [Cosmosil 5C_{18}-AR-II (20 × 250 mm), 80% MeOH at 5.0 mL/min, UV detection at 215 nm] to yield mabuniamide (**1**, 32.0 mg, t_R = 40.1 min). The purity of **1** was determined as >95% by HPLC analysis.

*Mabuniamide (**1**):* colorless oil; $[\alpha]^{26}_D$ −4.86 (*c* 1.00, $CHCl_3$), UV (MeOH) λ_{max} (log ε) 200, 278 nm (4.93); IR (neat) 3285, 2960, 1747, 1642, 754 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, Table 1; HRESIMS *m*/*z* 1086.6572 $[M + H]^+$ (calcd for $C_{58}H_{88}N_9O_{11}$, 1086.6603).

Total Hydrolysis and Derivatization with L-FDAA. Mabuniamide (**1**, 1.8 mg) was treated with 6 M HCl (500 μL) for 18 h at 110 °C. The hydrolyzed product was evaporated to dryness and subjected to HPLC [Cosmosil HILIC (4.6 × 250 mm), MeCN/10 mM $AcONH_4$ = 85:15 at 1.0 mL/min, UV detection 215 nm] to yield Pro, *N*-Me-Phe, Val, *N*-Me-Val, Leu, and *N*-Me-Ala. Each amino acid was mixed with a 0.1% solution of N^{α}-(6-fluoro-2,4-dinitrophenyl)-L-alaninamide (L-FDAA, Marfey's reagent, 100 μL) in acetone and 0.5 M $NaHCO_3$ (300 μL) followed by heating at 40 °C for 90 min. After cooling to room temperature (rt), the reaction mixture was neutralized with 2 M HCl (75 μL) and diluted with MeOH (1.0 mL). The solution was subjected to reversed-phase HPLC [Cosmosil 5C_{18}-AR-II (4.6 × 250 mm), MeOH/20 mM AcONa = 60:40 (solvent A), 55:45 (solvent B), 50:50 (solvent C), or 45:65 (solvent D) at 1.0 mL/min, UV detection at 340 nm]. The L-DAA derivatives of standard amino acids were prepared by the same procedure. The retention times (min) of the derivatives of authentic standards were as follows: L-Leu (6.8) and D-Leu (14.6) in solvent A, *N*-Me-L-Phe (8.3), *N*-Me-D-Phe (10.3), *N*-Me-L-Val (8.2), *N*-Me-D-Val (15.2), *N*-Me-L-Ala (6.8), and *N*-Me-D-Ala (9.3) in solvent B, L-Val (7.6) and D-Val (21.5) in solvent C, L-Pro (6.4) and D-Pro (10.5) in solvent D. The retention time and ESIMS product ion (*m*/*z* $[M + Na]^+$) of the L-DAA derivative of Leu from hydrolysate was 6.8 min (406.1) in solvent A. The retention times and ESIMS product ions (*m*/*z* $[M + Na]^+$) of the L-DAA derivatives of *N*-Me-Phe from hydrolysate were 8.3 min (454.1) and 10.3 min (454.0) in solvent B, respectively. The retention times and ESIMS product ions (*m*/*z* $[M + Na]^+$) of the L-DAA derivatives of *N*-Me-Val and *N*-Me-Ala from hydrolysate were 15.2 min (406.1) and 6.8 min (378.2) in solvent B, respectively. The retention time and ESIMS product ion (*m*/*z* $[M + Na]^+$) of the L-DAA derivative of Val from hydrolysate was 7.6 min (392.1) in solvent C. The retention time and ESIMS product ion (*m*/*z* $[M + Na]^+$) of the L-DAA derivative of Pro from hydrolysate was 6.4 min (390.2) in solvent D.

Partial Hydrolysis and Derivatization with L-FDAA. Mabuniamide (**1**, 1.0 mg) was treated with 4 M HCl (600 μL) for 30 min at 100 °C. The hydrolysate was evaporated to dryness and subjected to HPLC [Cosmosil 5C_{18}-AR-II (4.6 × 250 mm), 45% MeOH containing 0.1% trifluoroacetic acid (TFA) at 1.0 mL/min, UV detection 215 nm]. Eight fractions were analyzed by ESIMS and assigned peptide sequences. After the assignments of each peptide sequence, two fractions were identified to contain *N*-Me-Phe-Gly-Val (fragment A) and Leu-*N*-Me-Phe-Pro (fragment B). Fragment A and fragment B were treated with 6 M HCl (600 μL) for 12 h at 110 °C. The hydrolysates were dried and mixed with a 0.1% solution of L-FDAA (100 μL) in acetone and 0.5 M $NaHCO_3$ (200 μL) followed by heating at 40 °C for 90 min. After cooling to rt, the reaction mixture was neutralized with 2 M HCl (50 μL) and diluted with MeOH (1.0 mL). The solution was subjected to reversed-phase HPLC [Cosmosil 5C_{18}-AR-II (4.6 × 250 mm), MeOH/20 mM AcONa = 55:45 (solvent B) at 1.0 mL/min, UV detection at 340 nm]. The retention times (min) of the authentic standards were as follows: *N*-Me-L-Phe (8.3) and *N*-Me-D-Phe (10.3) in solvent B. The retention time and ESIMS product ion (*m*/*z* $[M + Na]^+$) of the L-DAA derivative of *N*-Me-Phe from the hydrolysate of fragment B was 8.3 min (454.1) in solvent B. The retention times and ESIMS product ion (*m*/*z* $[M + Na]^+$) of the L-DAA derivative of *N*-Me-Phe from the hydrolysate of fragment A was 10.3 min (454.0) in solvent B.

Total Synthesis Procedures. *Methyl N-((tert-Butoxycarbonyl)-glycyl)-N-methyl-D-phenylalanyl-L-prolinate (**4a**).* To a stirred solution of known dipeptide **3a** (395 mg, 1.01 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **3a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **3a**, *N*-Boc-glycine (212 mg, 1.21 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (462 mg, 1.22 mmol) in dimethylformamide (DMF, 2 mL) was added *N*,*N*-diisopropylethylamine (DIPEA) (0.85 mL) at room temperature. After stirring for 3 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (hexane−EtOAc, 1:1 v/v) to give compound **4a** as a colorless oil (339 mg, 0.76 mmol, 75%): $[\alpha]^{24}_D$ +63 (*c* 1.0, $CHCl_3$); IR (neat) 3325, 2965, 1744, 1640, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30−7.18 (m, 5H), 5.58 (dd, *J* = 8.4, 7.2 Hz, 1H), 5.36 (brs, 1H, NH), 4.42 (dd, *J* = 8.8, 5.2 Hz, 1H), 4.01 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.77 (dd, *J* = 17.6, 3.6 Hz, 1H), 3.72 (s, 3H), 3.44−3.33 (m, 2H), 3.28 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.96 (s, 3H), 2.83 (dd, *J* = 14.0, 7.2, 1H), 2.17 (m, 1H), 1.98−1.79 (m, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 168.6, 168.1, 155.8, 137.2, 129.6, 128.5, 126.8, 79.8, 59.1, 56.4, 52.4, 47.0, 42.4, 35.1, 29.7, 29.0, 28.5, 25.2; HRMS (ESI-TOF) *m*/*z* 448.2434 $[M + H]^+$ (calcd for $C_{23}H_{34}N_3O_6$ 448.2448).

*Methyl N-(tert-Butoxycarbonyl)-L-valylglycyl-N-methyl-D-phenylalanyl-L-prolinate (**5a**).* To a stirred solution of tripeptide **4a** (255 mg, 0.57 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **4a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **4a**, *N*-Boc-L-Val (149 mg, 0.69 mmol), and HATU (261 mg, 0.69 mmol) in DMF (0.9 mL) was added DIPEA (0.5 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 100:1 v/v) to give compound **5a** as a colorless oil (310 mg, 0.57 mmol, quant.). The ratio of major and minor rotamers is 10:1; $[\alpha]^{24}_D$ +46 (*c* 1.0, $CHCl_3$); IR (neat) 3325, 2967, 1747, 1638, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.30−7.18 (m, 5H), 6.79 (brs, 1H, NH), 5.57 (dd, *J* = 7.6, 7.6 Hz, 1H), 5.03 (brs, 1H), 4.42 (dd, *J* = 8.8, 5.2 Hz, 1H), 4.09 (dd, *J* = 18.0, 4.0 Hz, 1H), 4.03 (m, 1H), 3.91 (dd, *J* = 18.0, 3.6 Hz, 1H), 3.72 (s, 3H), 3.42−3.33 (m, 2H), 3.27 (dd, *J* = 14.0, 8.0 Hz, 1H), 2.99 (s, 3H), 2.83 (dd, *J* = 14.0, 7.2, 1H), 2.24−2.11 (m, 2H), 2.00−1.79 (m, 3H), 1.44 (s, 9H), 0.95 (d, *J* = 7.2 Hz, 3H), 0.87 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 172.6, 171.6, 168.1, 168.0, 155.9, 137.1, 129.5, 128.5, 126.8, 80.0, 59.8, 59.1, 56.4, 52.4, 46.9, 41.3, 35.1, 31.2, 29.8, 28.9, 28.4, 25.2, 19.4, 17.5; HRMS (ESI-TOF) *m*/*z* 547.3124 $[M + H]^+$ (calcd for $C_{28}H_{43}N_4O_7$ 547.3132).

*Methyl N-N-(tert-Butoxycarbonyl)-N-methyl-D-valyl-L-valylglycyl-N-methyl-D-phenylalanyl-L-prolinate (**6a**).* To a stirred solution of tetrapeptide **5a** (261 mg, 0.48 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **5a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **5a**, *N*-Boc-*N*-Me-D-Val

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(133 mg, 0.58 mmol), and HATU (218 mg, 0.57 mmol) in DMF (0.7 mL) was added DIPEA (0.41 mL) at rt. After stirring for 3 h, the mixture was diluted with EtOAc (10 mL), washed with 10% aqueous citric acid (8 mL), saturated aqueous sodium hydrogen carbonate (8 mL), and brine (8 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 140:1 v/v) to give compound **6a** as a colorless oil (195 mg, 0.30 mmol, 63%): $[\alpha]^{24}_D$ +80 (*c* 1.0, $CHCl_3$); IR (neat) 3319, 2965, 1748, 1638, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30−7.18 (m, 5H), 7.03 (brs, 1H, NH), 6.79 (brs, 1H, NH), 5.57 (dd, *J* = 7.2, 7.2 Hz, 1H), 4.41 (dd, *J* = 8.8, 5.6 Hz, 1H), 4.30 (m, 1H), 4.06 (brd, *J* = 15.2 Hz, 1H), 4.03 (m, 1H), 3.90 (brd, *J* = 15.2 Hz, 1H), 3.72 (s, 3H), 3.42−3.33 (m, 2H), 3.28 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.98 (s, 3H), 2.82 (m, 1H), 2.81 (s, 3H), 2.32 (m, 1H), 2.24−2.11 (m, 2H), 2.00−1.79 (m, 3H), 1.47 (s, 9H), 0.96 (d, *J* = 7.2 Hz, 3H), 0.94 (d, *J* = 8.0 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 171.3, 171.0, 168.0, 157.3, 137.1, 129.6, 128.5, 126.8, 80.5, 65.7, 59.1, 58.4, 56.3, 52.4, 46.9, 41.3, 35.1, 31.1, 30.6, 29.8, 29.0, 28.5, 26.3, 25.2, 20.0, 19.6, 18.8, 17.7; HRMS (ESI-TOF) *m*/*z* 660.3979 $[M + H]^+$ (calcd for $C_{34}H_{54}N_5O_8$ 660.3972).

*Methyl N,N-((tert-Butoxycarbonyl)-L-leucyl)-N-methyl-D-valyl-L-valylglycyl-N-methyl-D-phenylalanyl-L-prolinate (**7a**).* To a stirred solution of pentapeptide **6a** (169 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (0.8 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **6a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **6a**, *N*-Boc-L-Leu·H_2O (77 mg, 0.31 mmol), and HATU (178 mg, 0.47 mmol) in DMF (0.35 mL) was added DIPEA (0.22 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 120:1 v/v) to give compound **7a** as a colorless oil (172 mg, 0.22 mmol, 85%): $[\alpha]^{24}_D$ +58 (*c* 1.0, $CHCl_3$); IR (neat) 3320, 2962, 1747, 1639, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25−7.11 (m, 5H), 6.82 (m, 2H, NH), 5.54 (m, 2H), 4.63 (dd, *J* = 8.4, 6.8 Hz, 1H), 4.48 (d, *J* = 11.6 Hz, 1H), 4.41−4.33 (m, 2H), 4.23 (dd, *J* = 17.6, 5.2 Hz, 1H), 3.81 (dd, *J* = 17.6, 3.2 Hz, 1H), 3.72 (s, 3H), 3.35−3.27 (m, 2H), 3.06 (s, 3H), 3.05 (m, 1H), 2.98 (s, 3H), 2.79 (dd, *J* = 14.0, 6.4 Hz, 1H), 2.38−2.12 (m, 4H), 2.00−1.79 (m, 3H), 1.51−1.41 (m, 2H), 1.40 (s, 9H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 172.6, 170.9, 170.2, 168.11, 168.06, 155.8, 137.2, 129.6, 128.5, 126.8, 79.6, 63.8, 59.1, 58.3, 56.5, 52.4, 49.8, 47.0, 42.3, 41.3, 35.2, 31.0, 30.5, 29.9, 29.0, 28.5, 25.8, 25.2, 24.9, 23.3, 22.1, 20.0, 19.6, 18.8, 17.6; HRMS (ESI-TOF) *m*/*z* 773.4828 $[M + H]^+$ (calcd for $C_{40}H_{65}N_6O_9$ 773.4813).

*Methyl N,N,N-(tert-Butoxycarbonyl)-N-methyl-L-phenylalanyl-L-leucyl-N-methyl-D-valyl-L-valylglycyl-N-methyl-D-phenylalanyl-L-prolinate (**8a**).* To a stirred solution of hexapeptide **7a** (152 mg, 0.20 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (0.8 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **7a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **7a**, *N*-Boc-*N*-Me-L-Phe (73 mg, 0.26 mmol), and HATU (100 mg, 0.26 mmol) in DMF (0.35 mL) was added DIPEA (0.2 mL) at rt. After stirring for 6 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 120:1 v/v) to give compound **8a** as a colorless oil (173 mg, 0.19 mmol, 95%). The ratio of major and minor rotamers is 1:0.7; $[\alpha]^{24}_D$ +56 (*c* 1.0, $CHCl_3$); IR (neat) 3303, 2963, 1748, 1632, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.25−7.11 (m, 10H), 6.99−6.78 (m, 2H, NH), 6.74 (brt, *J* = 3.7 Hz, 1H, NH), 5.56 (dd, *J* = 8.4, 6.4 Hz, 1H), 5.00−4.78 (m, 2H), 4.56 (m, 1H), 4.38 (dd, *J* = 8.8, 5.6 Hz, 1H), 4.29−4.07 (m, 2H), 3.84 (dd, *J* = 18.0, 3.6 Hz, 1H), 3.70 (s, 3H), 3.40−3.20 (m, 3H), 3.07−2.87 (m, 9H), 2.80 (dd, *J* = 14.0, 6.8 Hz, 1H), 2.72 (s, 3H), 2.34 (m, 1H), 2.22−2.07 (m, 2H), 1.94−1.74 (m, 3H), 1.54 (m, 1H), 1.35 (s, 9H), 1.01−0.87 (m, 15H), 0.83 (d, *J* = 6.8 Hz, 3H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 173.9, 172.5, 170.8, 170.5, 170.3, 169.8, 168.0, 156.1, 137.7, 137.2, 129.6, 129.0, 128.4, 126.7, 80.8, 63.2, 59.0, 58.5, 56.3, 52.3, 48.2, 46.8, 42.1, 41.7, 41.3, 35.1, 34.1, 30.8, 30.7, 29.7, 28.9, 28.3, 25.8, 25.1, 24.9, 23.2, 22.1, 19.9, 19.5, 18.6, 17.9, 17.7; HRMS (ESI-TOF) *m*/*z* 934.5637 $[M + H]^+$ (calcd for $C_{50}H_{76}N_7O_{10}$ 934.5654).

*tert-Butyl (S)-2-(((2R,8S,11R,14S,17S)-2-Benzyl-14-isobutyl-8,11-diisopropyl-1-((S)-2-(methoxycarbonyl)pyrrolidin-1-yl)-3,12-dimethyl-1,4,7,10,13,16-hexaoxo-18-phenyl-3,6,9,12,15-pentaazaoctadecan-17-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (**9a**).* To a stirred solution of heptapeptide **8a** (148 mg, 0.16 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (0.8 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **8a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **8a**, *N*-Boc-L-Pro (41 mg, 0.19 mmol), and HATU (72 mg, 0.19 mmol) in DMF (0.25 mL) was added DIPEA (0.14 mL) at rt. After stirring for 5 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 100:1 v/v) to give compound **9a** as a colorless oil (75 mg, 72 μmol, 45%): $[\alpha]^{24}_D$ +48 (*c* 1.0, $CHCl_3$); IR (neat) 3304, 2962, 1748, 1650, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 6.8 Hz, 1H, NH), 7.25−7.07 (m, 10H), 6.94−6.76 (m, 2H, NH), 5.54 (dd, *J* = 9.2, 6.4 Hz, 1H), 4.99 (d, *J* = 11.2 Hz, 1H), 4.76 (m, 1H), 4.65 (m, 1H), 4.37 (dd, *J* = 8.4, 5.2 Hz, 1H), 4.25−4.14 (m, 3H), 3.92 (dd, *J* = 17.6, 3.6 Hz, 1H), 3.71 (m, 1H), 3.69 (s, 3H), 3.41−3.19 (m, 6H), 3.11 (s, 3H), 2.94 (m, 1H), 2.89 (s, 3H), 2.86 (s, 3H), 2.75 (dd, *J* = 14.0, 6.0 Hz, 1H), 2.39 (m, 1H), 2.21 (m, 1H), 2.11 (m, 1H), 1.93−1.78 (m, 4H), 1.48 (m, 1H), 1.42 (s, 9H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.98−0.82 (m, 15H), 0.74 (m, 1H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 174.2, 172.6, 171.3, 170.3, 168.2, 168.1, 154.7, 138.1, 137.3, 129.6, 129.0, 128.5, 127.0, 126.7, 80.2, 62.6, 59.0, 56.4, 54.3, 52.3, 49.3, 47.3, 46.9, 41.4, 39.8, 35.2, 34.0, 30.2, 29.8, 29.4, 28.9, 28.6, 25.9, 25.1, 24.9, 24.8, 23.2, 21.5, 20.3, 19.6, 18.7, 18.4; HRMS (ESI-TOF) *m*/*z* 1031.6198 $[M + H]^+$ (calcd for $C_{55}H_{83}N_8O_{11}$ 1031.6181).

*Methyl N,N,N,N-(tert-Butoxycarbonyl)-N-methyl-L-alanyl-L-prolyl-N-methyl-L-phenylalanyl-L-leucyl-N-methyl-D-valyl-L-valylglycyl-N-methyl-D-phenylalanyl-L-prolinate (**10a**).* To a stirred solution of octapeptide **9a** (106 mg, 0.10 mmol) in CH_2Cl_2 (1.2 mL) was added TFA (0.6 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **9a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **9a**, *N*-Boc-*N*-Me-L-Ala (35 mg, 0.17 mmol), and HATU (60 mg, 0.16 mmol) in DMF (0.15 mL) was added DIPEA (0.11 mL) at rt. After stirring for 3 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 100:1 v/v) to give compound **10a** as a colorless oil (103 mg, 92 μmol, 92%): $[\alpha]^{24}_D$ +11 (*c* 1.0, $CHCl_3$); IR (neat) 3340, 2964, 1747, 1648, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (m, 1H, NH), 7.25−7.06 (m, 10H), 6.94−6.84 (m, 2H, NH), 5.51 (dd, *J* = 8.8, 6.4 Hz, 1H), 5.00−4.93 (m, 2H), 4.75−4.60 (m, 2H), 4.44−4.33 (m, 2H), 4.24 (dd, *J* = 8.0, 7.2 Hz, 1H), 4.17 (dd, *J* = 17.6, 5.2 Hz, 1H), 3.91 (dd, *J* = 17.6, 3.2 Hz, 1H), 3.68 (s, 3H), 3.54 (m, 1H), 3.42 (m, 1H), 3.33−3.20 (m, 4H), 3.09 (s, 3H), 3.05 (m, 1H), 2.93

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(m, 1H), 2.86 (s, 3H), 2.82 (s, 3H), 2.72 (s, 3H), 2.70 (m, 1H), 2.37 (m, 1H), 2.20 (m, 1H), 2.09 (m, 1H), 1.97 (m, 1H), 1.90−1.67 (m, 5H), 1.57 (m, 1H), 1.42 (s, 9H), 1.34 (m, 1H), 1.17 (d, J = 6.8 Hz, 3H), 1.01 (d, J = 6.4 Hz, 3H), 0.96−0.81 (m, 15H), 0.68 (m, 1H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 173.4, 172.5, 171.3, 170.2, 170.1, 168.1, 168.0, 155.6, 138.1, 137.3, 129.6, 129.5, 129.0, 128.4, 127.0, 126.7, 80.2, 62.9, 59.0, 56.4, 55.2, 53.5, 52.3, 51.4, 49.5, 47.4, 46.8, 41.4, 39.5, 35.1, 33.8, 30.2, 29.8, 29.6, 29.3, 28.9, 28.5, 25.9, 25.4, 25.1, 24.8, 23.4, 21.3, 20.4, 19.6, 18.7, 18.4, 14.3; HRMS (ESI-TOF) m/z 1116.6685 $[M + H]^+$ (calcd for $C_{59}H_{90}N_9O_{12}$ 1116.6709).

*Mabuniamide (**1**).* To a stirred solution of nonapeptide **10a** (69 mg, 62 μmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.4 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **10a** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **10a**, butanoic acid (10 μL, 110 μmol), and HATU (35 mg, 93 μmol) in DMF (1.1 mL) was added DIPEA (0.1 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 70:1 v/v) to give mabuniamide (**1**) as a colorless oil (50 mg, 46 μmol, 74%); $[\alpha]^{26}_D$ −3.18 (c 0.41, $CHCl_3$); IR (neat) 3338, 2964, 1745, 1647, 753 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) see Table 1; HRMS (ESI-TOF) m/z 1086.6576 $[M + H]^+$ (calcd for $C_{58}H_{88}N_9O_{11}$ 1086.6603).

*Methyl N-((tert-Butoxycarbonyl)glycyl)-N-methyl-L-phenylalanyl-L-prolinate (**4b**).* To a stirred solution of known dipeptide **3b** (925 mg, 2.37 mmol) in CH_2Cl_2 (5 mL) was added TFA (2.5 mL) at 0 °C. After stirring at rt for 2 h, the mixture was diluted with benzene (6 mL) and concentrated. The trifluoroacetate salt of deprotected amine **3b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **3b**, *N*-Boc-glycine (498 mg, 2.84 mmol), and HATU (1.08 g, 2.84 mmol) in DMF (5 mL) was added DIPEA (2 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (30 mL), washed with 10% aqueous citric acid (25 mL), saturated aqueous sodium hydrogen carbonate (25 mL), and brine (25 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (hexane−EtOAc, 1:1 v/v) to give compound **4b** as a colorless oil (950 mg, 2.12 mmol, 89%). The ratio of major and minor rotamers is 5:1; $[\alpha]^{24}_D$ −33 (c 1.0, $CHCl_3$); IR (neat) 3325, 2975, 1744, 1647, 752 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.33−7.19 (m, 5H), 5.66 (dd, J = 8.1, 7.6 Hz, 1H), 5.33 (brs, 1H, NH), 4.42 (dd, J = 8.8, 4.0 Hz, 1H), 3.93 (dd, J = 17.6, 4.4 Hz, 1H), 3.74 (m, 1H), 3.71 (s, 3H), 3.60 (m, 1H), 3.41 (m, 1H), 3.25 (dd, J = 14.8, 7.6 Hz, 1H), 3.00 (m, 1H), 2.99 (s, 3H), 2.15 (m, 1H), 2.05−1.89 (m, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 172.3, 169.1, 168.0, 155.7, 136.5, 129.0, 128.5, 126.8, 79.6, 59.1, 55.0, 52.2, 47.0, 42.4, 35.0, 29.8, 29.0, 28.3, 24.8; HRMS (ESI-TOF) m/z 448.2430 $[M + H]^+$ (calcd for $C_{23}H_{34}N_3O_6$ 448.2448).

*Methyl N-(tert-Butoxycarbonyl)-L-valylglycyl-N-methyl-L-phenylalanyl-L-prolinate (**5b**).* To a stirred solution of tripeptide **4b** (715 mg, 1.60 mmol) in CH_2Cl_2 (4 mL) was added TFA (2 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (6 mL) and concentrated. The trifluoroacetate salt of deprotected amine **4b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **4b**, *N*-Boc-L-Val (417 mg, 1.92 mmol), and HATU (730 mg, 1.92 mmol) in DMF (2.2 mL) was added DIPEA (1.1 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (30 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (25 mL), and brine (25 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 140:1 v/v) to give compound **5b** as a colorless oil (616 mg, 1.13 mmol, 71%). The ratio of major and minor rotamers is 5:1; $[\alpha]^{24}_D$ −87 (c 1.0, $CHCl_3$); IR (neat) 3326, 2970, 1745, 1640, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.28−7.16 (m, 5H), 6.79 (brt, J = 4.0 Hz, 1H, NH), 5.63 (dd, J = 8.8, 7.6 Hz, 1H), 5.10 (brd, J = 8.4 Hz, 1H), 4.41 (dd, J = 8.0, 4.0 Hz, 1H), 4.01 (dd, J = 18.4, 4.0 Hz, 1H), 3.96 (m, 1H), 3.82 (dd, J = 18.4, 4.0 Hz, 1H), 3.69 (s, 3H), 3.60 (m, 1H), 3.40 (m, 1H), 3.24 (dd, J = 14.4, 6.8 Hz, 1H), 2.99 (s, 3H), 2.97 (m, 1H), 2.15 (m, 1H), 2.02−1.85 (m, 4H), 1.41 (s, 9H), 0.90 (d, J = 6.8 Hz, 3H), 0.86 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 172.4, 171.6, 169.0, 168.5, 155.8, 136.5, 129.0, 128.6, 126.9, 79.8, 59.7, 59.1, 55.1, 52.3, 47.0, 41.4, 35.0, 31.2, 30.0, 29.1, 28.4, 24.9, 19.3, 17.7; HRMS (ESI-TOF) m/z 547.3115 $[M + H]^+$ (calcd for $C_{28}H_{43}N_4O_7$ 547.3132).

*Methyl N,N-(tert-Butoxycarbonyl)-N-methyl-D-valyl-L-valylglycyl-N-methyl-L-phenylalanyl-L-prolinate (**6b**).* To a stirred solution of tetrapeptide **5b** (518 mg, 0.95 mmol) in CH_2Cl_2 (3.5 mL) was added TFA (1.8 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (5 mL) and concentrated. The trifluoroacetate salt of deprotected amine **5b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **5b**, *N*-Boc-*N*-Me-D-Val (263 mg, 1.14 mmol), and HATU (432 mg, 1.14 mmol) in DMF (1.8 mL) was added DIPEA (0.66 mL) at rt. After stirring for 4 h, the mixture was diluted with EtOAc (20 mL), washed with 10% aqueous citric acid (15 mL), saturated aqueous sodium hydrogen carbonate (15 mL), and brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 140:1 v/v) to give compound **6b** as a colorless oil (397 mg, 0.60 mmol, 63%). The ratio of major and minor rotamers is 5:1; $[\alpha]^{24}_D$ +9.0 (c 1.0, $CHCl_3$); IR (neat) 3316, 2965, 1746, 1640, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.28−7.16 (m, 5H), 6.98 (brd, J = 8.4 Hz, 1H, NH), 6.73 (brs, 1H, NH), 5.64 (dd, J = 7.6, 7.6 Hz, 1H), 4.41 (dd, J = 9.2, 4.0 Hz, 1H), 4.24 (m, 1H), 4.00 (m, 1H), 3.78 (m, 1H), 3.69 (s, 3H), 3.65−3.50 (m, 2H), 3.40 (m, 1H), 3.23 (dd, J = 14.4, 7.6 Hz, 1H), 2.98 (s, 3H), 2.97 (m, 1H), 2.77 (s, 3H), 2.27 (m, 1H), 2.15 (m, 1H), 2.06−1.89 (m, 4H), 1.44 (s, 9H), 0.95−0.84 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 172.4, 171.2, 170.9, 169.1, 168.5, 157.3, 136.5, 129.1, 128.6, 126.9, 80.5, 65.5, 59.2, 58.4, 55.1, 52.3, 47.1, 41.5, 35.1, 31.0, 30.7, 30.0, 29.1, 28.4, 26.2, 24.9, 20.0, 19.5, 18.8, 17.8; HRMS (ESI-TOF) m/z 660.3977 $[M + H]^+$ (calcd for $C_{34}H_{54}N_5O_8$ 660.3972).

*Methyl N,N-((tert-Butoxycarbonyl)-L-leucyl)-N-methyl-D-valyl-L-valylglycyl-N-methyl-L-phenylalanyl-L-prolinate (**7b**).* To a stirred solution of pentapeptide **6b** (312 mg, 0.47 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) at 0 °C. After stirring at rt for 1.5 h, the mixture was diluted with benzene (5 mL) and concentrated. The trifluoroacetate salt of deprotected amine **6b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **6b**, *N*-Boc-L-Leu·H_2O (142 mg, 0.57 mmol), and HATU (225 mg, 0.59 mmol) in DMF (1.2 mL) was added DIPEA (0.48 mL) at rt. After stirring for 1.5 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$−MeOH, 140:1 v/v) to give compound **7b** as a colorless oil (257 mg, 0.33 mmol, 70%). The ratio of major and minor rotamers is 10:2:1; $[\alpha]^{24}_D$ +7.7 (c 1.0, $CHCl_3$); IR (neat) 3316, 2962, 1745, 1638, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.31−7.13 (m, 5H), 6.75 (m, 1H, NH), 5.66 (dd, J = 8.0, 8.0 Hz, 1H), 5.47 (d, J = 8.4 Hz, 1H), 4.63 (m, 1H), 4.49 (d, J = 11.6 Hz, 1H), 4.42 (dd, J = 8.8, 4.0 Hz, 1H), 4.29 (dd, J = 8.4, 5.2 Hz, 1H), 3.92 (d, J = 4.0 Hz, 1H), 3.70 (s, 3H), 3.66−3.53 (m, 2H), 3.41 (m, 1H), 3.26 (dd, J = 14.4, 7.6 Hz, 1H), 3.01 (s, 3H), 2.98 (s, 3H), 2.97 (m, 1H), 2.33 (m, 1H), 2.22−2.11 (m, 2H), 2.03−1.89 (m, 3H), 1.51−1.43 (m, 2H), 1.39 (s, 9H), 1.01−0.82 (m, 18H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 174.5, 172.4, 170.7, 170.1, 168.9, 168.5, 155.8, 136.6, 129.2, 128.6, 126.9, 79.6, 63.7, 59.2, 58.4, 55.1, 52.3, 49.7, 47.1, 42.3,

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41.5, 35.2, 30.9, 30.7, 30.0, 29.8, 29.1, 28.5, 25.8, 24.9, 23.3, 22.1, 20.0, 19.5, 18.7, 17.8; HRMS (ESI-TOF) m/z 773.4837 $[M + H]^+$ (calcd for $C_{40}H_{65}N_6O_9$ 773.4813).

*Methyl N,N,N-(tert-Butoxycarbonyl)-N-methyl-D-phenylalanyl-L-leucyl-N-methyl-D-valyl-L-valylglycyl-N-methyl-L-phenylalanyl-L-prolinate (**8b**).* To a stirred solution of hexapeptide **7b** (228 mg, 0.29 mmol) in CH_2Cl_2 (1.2 mL) was added TFA (0.6 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **7b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **7b**, *N*-Boc-*N*-Me-D-Phe (99 mg, 0.35 mmol), and HATU (134 mg, 0.35 mmol) in DMF (0.5 mL) was added DIPEA (0.25 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 100:1 v/v) to give compound **8b** as a colorless oil (262 mg, 0.28 mmol, 97%). The ratio of major and minor rotamers is 1:0.75; $[\alpha]^{24}_D$ +43 (c 1.0, $CHCl_3$); IR (neat) 3310, 2963, 1746, 1638, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.25–7.14 (m, 10H), 6.95–6.60 (m, 3H, NH), 5.64 (dd, J = 8.0, 8.0 Hz, 1H), 5.05 (dd, J = 10.0, 5.6 Hz, 1H), 4.89 (m, 1H), 4.57 (d, J = 10.8 Hz, 1H), 4.40 (m, 1H), 4.19 (m, 1H), 4.01–3.75 (m, 2H), 3.69 (s, 3H), 3.57 (m, 1H), 3.42–3.27 (m, 2H), 3.23 (dd, J = 14.8, 7.2 Hz, 1H), 3.07–2.93 (m, 4H), 2.92 (s, 3H), 2.82 (m, 1H), 2.75 (s, 3H), 2.31 (m, 1H), 2.20–2.05 (m, 2H), 1.60 (m, 1H), 1.54–1.38 (m, 2H), 1.32 (s, 9H), 1.00–0.87 (m, 15H), 0.81 (d, J = 6.8 Hz, 3H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 173.9, 172.5, 170.8, 170.5, 170.3, 169.8, 168.0, 156.1, 137.7, 137.2, 129.6, 129.0, 128.4, 126.7, 80.2, 63.2, 59.6, 59.0, 58.5, 58.3, 56.3, 52.3, 48.2, 46.8, 42.1, 41.7, 41.3, 35.1, 34.1, 31.4, 30.8, 30.7, 29.7, 28.9, 28.3, 25.8, 25.1, 24.9, 23.3, 22.1, 19.9, 19.5, 18.6, 17.9; HRMS (ESI-TOF) m/z 934.5698 $[M + H]^+$ (calcd for $C_{50}H_{76}N_7O_{10}$ 934.5654).

*tert-Butyl (S)-2-(((2S,8S,11R,14S,17R)-2-Benzyl-14-isobutyl-8,11-diisopropyl-1-((S)-2-(methoxycarbonyl)pyrrolidin-1-yl)-3,12-dimethyl-1,4,7,10,13,16-hexaoxo-18-phenyl-3,6,9,12,15-pentaazaoctadecan-17-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (**9b**).* To a stirred solution of heptapeptide **8b** (213 mg, 0.23 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.5 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **8b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **8b**, *N*-Boc-L-Pro (59 mg, 0.27 mmol), and HATU (104 mg, 0.27 mmol) in DMF (0.4 mL) was added DIPEA (0.2 mL) at rt. After stirring for 3 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 140:1 v/v) to give compound **9b** as a colorless oil (180 mg, 0.17 mmol, 74%). The ratio of major and minor rotamers is 5:1; $[\alpha]^{24}_D$ +36 (c 1.0, $CHCl_3$); IR (neat) 3339, 2964, 1744, 1654, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.25–7.12 (m, 10H), 6.89 (m, 2H, NH), 5.72 (dd, J = 12.4, 5.2 Hz, 1H), 5.63 (dd, J = 8.0, 8.0 Hz, 1H), 4.79 (m, 1H), 4.69 (d, J = 11.2 Hz, 1H), 4.44–4.37 (m, 2H), 4.19 (dd, J = 8.4, 6.8 Hz, 1H), 3.95 (dd, J = 8.0, 3.6 Hz, 1H), 3.67 (s, 3H), 3.57 (m, 1H), 3.45–3.35 (m, 3H), 3.35–3.27 (m, 2H), 3.21 (dd, J = 14.4, 7.2 Hz, 1H), 3.01 (s, 3H), 2.99 (s, 3H), 2.96–2.82 (m, 3H), 2.92 (s, 3H), 2.30 (m, 1H), 2.18–2.04 (m, 2H), 1.95–1.86 (m, 3H), 1.77–1.63 (m, 5H), 1.47 (m, 1H), 1.40 (s, 9H), 1.11 (m, 1H), 0.99 (d, J = 6.4 Hz, 3H), 0.95–0.89 (m, 12H), 0.80 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 174.8, 173.6, 172.5, 171.1, 170.0, 168.8, 168.5, 154.8, 137.6, 136.7, 129.2, 129.0, 128.5, 128.3, 126.8, 126.5, 79.7, 62.6, 59.2, 59.1, 56.4, 55.9, 55.1, 54.3, 52.3, 49.0, 47.1, 47.0, 41.6, 40.6, 35.1, 33.6, 31.2, 30.5, 29.9, 29.2, 28.9, 28.6, 25.8, 24.9, 24.7, 24.5, 23.3, 22.0, 20.3, 19.6, 18.7, 18.5; HRMS (ESI-TOF) m/z 1053.5984 $[M + Na]^+$ (calcd for $C_{55}H_{82}N_8O_{11}Na$ 1053.6001).

*Methyl N,N,N,N-(tert-Butoxycarbonyl)-N-methyl-L-alanyl-L-prolyl-N-methyl-D-phenylalanyl-L-leucyl-N-methyl-D-valyl-L-valylglycyl-N-methyl-L-phenylalanyl-L-prolinate (**10b**).* To a stirred solution of octapeptide **9b** (159 mg, 0.15 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.4 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (4 mL) and concentrated. The trifluoroacetate salt of deprotected amine **9b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **9b**, *N*-Boc-L-Ala (38 mg, 0.19 mmol), and HATU (70 mg, 0.18 mmol) in DMF (0.25 mL) was added DIPEA (0.13 mL) at rt. After stirring for 3 h, the mixture was diluted with EtOAc (15 mL), washed with 10% aqueous citric acid (10 mL), saturated aqueous sodium hydrogen carbonate (10 mL), and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 100:1 v/v) to give compound **10b** as a colorless oil (160 mg, 0.14 mmol, 93%). The ratio of major and minor rotamers is 1:0.15; $[\alpha]^{24}_D$ +28 (c 1.0, $CHCl_3$); IR (neat) 3340, 2964, 1744, 1638, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major rotamer δ 7.24–7.12 (m, 10H), 6.95 (brd, J = 8.8 Hz, 1H, NH), 6.90 (brt, J = 4.2 Hz, 1H, NH), 5.67 (dd, J = 12.0, 4.8 Hz, 1H), 5.62 (dd, J = 7.6, 7.6 Hz, 1H), 4.97 (brdd, J = 12.6, 6.3 Hz, 1H), 4.75 (m, 1H), 4.69 (d, J = 10.8 Hz, 1H), 4.53 (m, 1H), 4.39 (dd, J = 8.8, 4.0 Hz, 1H), 4.19 (dd, J = 8.4, 7.2 Hz, 1H), 3.96 (dd, J = 9.2, 4.0 Hz, 1H), 3.67 (s, 3H), 3.60–3.51 (m, 3H), 3.44–3.32 (m, 2H), 3.21 (dd, J = 14.0, 7.2 Hz, 1H), 3.05 (m, 1H), 3.03 (s, 3H), 3.02 (s, 3H), 2.95–2.82 (m, 3H), 2.90 (s, 3H), 2.73 (brs, 3H), 2.30 (m, 1H), 2.18–2.07 (m, 2H), 1.98–1.85 (m, 4H), 1.80–1.68 (m, 4H), 1.45 (m, 1H), 1.43 (s, 9H), 1.20 (d, J = 6.8 Hz, 3H), 1.16 (m, 1H), 0.99 (d, J = 6.4 Hz, 3H), 0.95–0.90 (m, 12H), 0.81 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major rotamer δ 175.0, 173.0, 172.5, 171.2, 171.0, 170.7, 170.0, 168.8, 168.5, 155.7, 137.6, 136.7, 129.2, 128.9, 128.5, 128.3, 126.8, 126.6, 80.1, 62.6, 59.21, 59.15, 56.5, 55.1, 53.7, 52.3, 51.4, 49.2, 47.2, 47.1, 41.6, 40.2, 35.1, 35.6, 31.3, 30.5, 29.9, 29.1, 28.5, 28.0, 25.8, 25.3, 24.9, 24.8, 23.4, 21.8, 20.3, 19.6, 18.7, 18.5, 14.3; HRMS (ESI-TOF) m/z 1116.6721 $[M + H]^+$ (calcd for $C_{59}H_{90}N_9O_{12}$ 1116.6709).

*Iso-Mabuniamide (**2**).* To a stirred solution of nonapeptide **10b** (115 mg, 0.10 mmol) in CH_2Cl_2 (0.6 mL) was added TFA (0.3 mL) at 0 °C. After stirring at rt for 1 h, the mixture was diluted with benzene (3 mL) and concentrated. The trifluoroacetate salt of deprotected amine **10b** was afforded and used in the next reaction without purification. To a stirred solution of the trifluoroacetate salt of deprotected amine **10b**, butanoic acid (15 μL, 170 μmol), and HATU (47 mg, 0.12 mmol) in DMF (0.15 mL) was added DIPEA (0.09 mL) at rt. After stirring for 2 h, the mixture was diluted with EtOAc (8 mL), washed with 10% aqueous citric acid (6 mL), saturated aqueous sodium hydrogen carbonate (6 mL), and brine (6 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 60:1 v/v) to give iso-mabuniamide (**2**) as a colorless oil (79 mg, 73 μmol, 73%): $[\alpha]^{24}_D$ +18 (c 1.0, $CHCl_3$); IR (neat) 3338, 2963, 1745, 1651, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.24–7.12 (m, 10H), 6.94–6.79 (m, 2H, NH), 5.69 (dd, J = 11.6, 4.8 Hz, 1H), 5.64 (dd, J = 7.6, 7.6 Hz, 1H), 5.43 (q, J = 7.2 Hz, 1H), 4.79 (m, 1H), 4.71 (d, J = 10.8 Hz, 1H), 4.49 (dd, J = 9.6, 9.6 Hz, 1H), 4.40 (dd, J = 8.0, 4.0 Hz, 1H), 4.20 (dd, J = 8.0, 8.0 Hz, 1H), 3.96 (m, 1H), 3.68 (s, 3H), 3.62–3.49 (m, 4H), 3.41–3.34 (m, 2H), 3.22 (dd, J = 14.0, 7.2 Hz, 1H), 3.04 (s, 3H), 3.03 (s, 3H), 2.93 (s, 3H), 2.91 (s, 3H), 2.91–2.76 (m, 3H), 2.31 (m, 1H), 2.28 (t, J = 7.6 Hz, 2H), 2.14 (m, 2H), 1.98–1.87 (m, 4H), 1.82–1.71 (m, 4H), 1.47 (m, 1H), 1.24 (m, 1H), 1.22 (d, J = 7.2 Hz, 3H), 1.01 (d, J = 6.4 Hz, 3H), 0.97–0.90 (m, 15H), 0.83 (d, J = 6.8 Hz, 3H); some signals overlapped with a water signal; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 173.0, 172.9, 172.4, 171.0, 170.4, 169.9, 168.7, 168.4, 137.6, 136.7, 129.1, 128.8, 128.4, 128.3, 126.7, 126.5, 62.5, 59.1, 59.0, 56.4, 56.2, 55.0, 52.3, 49.5, 49.0, 47.4, 47.0, 41.6, 40.3, 35.6, 35.1, 33.5, 31.3, 30.9, 30.6, 30.5, 29.8, 29.1, 28.0, 25.7, 25.3, 24.9, 24.7, 23.4, 21.8, 20.3, 19.5, 18.6, 18.44, 18.36,

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14.3, 14.0; HRMS (ESI-TOF) m/z 1086.6626 $[M + H]^+$ (calcd for $C_{58}H_{88}N_9O_{11}$ 1086.6603).

Culturing of L6 Myoblasts. L6 myoblasts were purchased from the Japanese Collection of Research Bioresources. L6 myotube cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and 2.0% penicillin/streptomycin in a humidified 5% CO_2 incubator at 37 °C. To differentiate myotubes, L6 myoblasts were seeded at 5.0×10^3 cells/well in 96-well plates and cultured to 90% confluency for 2 days. Then the cells were cultured to form myotubes in DMEM containing 2% FBS for 1 week. The medium was renewed every 2 days.

Determination of Glucose Uptake. L6 myotubes were incubated in filter-sterilized Krebs-Henseleit-HEPES buffer (1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 119 mM NaCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, pH 7.4) containing 0.1% bovine serum albumin, 10 mM HEPES, and 2 mM sodium pyruvate (KHH buffer) for 2 h. The myotubes were then cultured for 22 h in KHH buffer containing 11 mM glucose without or with mabuniamide (**1**, 10−40 μM). The differences in the glucose concentrations in the KHH buffer before and after culture were determined by a commercial assay kit (Glucose CII-Test Wako), and the absorbance at 490 nm was measured using a microplate reader (Model ELx 800; Bio Tek, Japan). The amounts of glucose consumed were calculated from the differences in glucose concentrations between before and after culture.

Cell Viability. After the glucose uptake assay, an MTT solution (10 μL, 5 mg/mL in H_2O) was added to each well, and the plate was incubated at 37 °C with 5% CO_2 for 4 h. All remaining supernatant was then removed, and DMSO (50 μL) was added to each well to dissolve the resultant formazan crystals. Absorbance was measured by using a microplate reader at the wavelength of 540 nm.

Growth-Inhibitory Assay against Malarial Parasites. The *P. falciparum* 3D7 line was obtained from the Malaria Research and Reference Reagent Resource Center (MR4). The *P. falciparum* axenic culture[25] and the drug sensitivity assay[26] were described previously. In brief, parasites were cultivated in RPMI-1640 medium containing 5% heat-inactivated human serum and 0.25% Albumax II (Invitrogen), 200 mM hypoxanthine (Sigma), 10 μg/mL gentamicin (Invitrogen), and human RBC (type O) at 2% hematocrit. One hundred microliter cultures at 0.3% parasitemia containing different concentrations of drugs were prepared in 96-well plates. After 72 h of cultivation in an incubator containing 5% O_2, 5% CO_2, and 90% N_2 at 37 °C, parasite growth was monitored by measurement of the absorbance at 650 nm using a DTX880 multimode detector (Beckman Coulter) in the lactate dehydrogenase assay as previously described.[26]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00749.

Phylogenetic tree of cyanobacteria and NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Gerwick, W. H.; Moore, B. S. *Chem. Biol.* **2012**, *19*, 85−98.
(2) Jimenez, C. *ACS Med. Chem. Lett.* **2018**, *9*, 959−961.
(3) Shah, S. A. A.; Akhter, N.; Auckloo, B. N.; Khan, I.; Lu, Y.; Wang, K.; Wu, B.; Guo, Y. W. *Mar. Drugs* **2017**, *15*, 354.
(4) Mi, Y.; Zhang, J.; He, S.; Yan, X. *Mar. Drugs* **2017**, *15*, 132.
(5) Liu, L.; Rein, K. S. *Mar. Drugs* **2010**, *8*, 1817−1837.
(6) Teruya, T.; Sasaki, H.; Fukazawa, H.; Suenaga, K. *Org. Lett.* **2009**, *11*, 5062−5065.
(7) Sanchez, L. M.; Lopez, D.; Vesely, B. A.; Togna, G. D.; Gerwick, W. H.; Kyle, D. E.; Linington, R. G. *J. Med. Chem.* **2010**, *53*, 4187−4197.
(8) Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806−1807.
(9) Lee, S. U.; Kwak, H. B.; Pi, S. H.; You, H. K.; Byeon, S. R.; Ying, Y.; Luesh, H.; Hong, J.; Kim, S. H. *ACS Med. Chem. Lett.* **2011**, *2*, 248−251.
(10) Sueyoshi, K.; Yamada, M.; Yamano, A.; Ozaki, K.; Sumimoto, S.; Iwasaki, A.; Suenaga, K.; Teruya, T. *J. Nat. Prod.* **2018**, *81*, 1103−1107.
(11) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(12) Takada, K.; Ninomiya, A.; Naruse, M.; Sun, Y.; Miyazaki, M.; Nogi, Y.; Okada, S.; Matsunaga, S. *J. Org. Chem.* **2013**, *78*, 6746−6750.
(13) Jang, J. P.; Hwang, G. J.; Kwon, M. C.; Ryoo, I. J.; Jang, M.; Takahashi, S.; Ko, S. K.; Osada, H.; Jang, J. H.; Ahn, J. S. *J. Nat. Prod.* **2018**, *81*, 806−810.
(14) Takayanagi, A.; Iwasaki, A.; Suenaga, K. *Tetrahedron Lett.* **2015**, *56*, 4947−4949.
(15) Ye, B.; Jiang, P.; Zhang, T.; Ding, Y.; Sun, Y.; Hao, X.; Li, L.; Wang, L.; Chen, Y. *J. Org. Chem.* **2018**, *83*, 6741−6747.
(16) Horgen, F. D.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 461−467.
(17) Sueyoshi, K.; Yamano, A.; Ozaki, K.; Sumimoto, S.; Iwasaki, A.; Suenaga, K.; Teruya, T. *Mar. Drugs* **2017**, *15*, 367.
(18) Engene, N.; Paul, V. J.; Byrum, T.; Gerwick, W. H.; Thor, A.; Ellisman, M. H. *J. Phycol.* **2013**, *49*, 1095−1106.
(19) Pruesse, E.; Peplies, J.; Glöckner, F. O. *Bioinformatics* **2012**, *28*, 1823−1829.
(20) (a) Talavera, G.; Castresana, J. *Syst. Biol.* **2007**, *56*, 564−577. (b) Castresana, J. *Mol. Biol. Evol.* **2000**, *17*, 540−552.
(21) (a) Darriba, D.; Taboada, G. L.; Doallo, R.; Posada, D. *Nat. Methods* **2012**, *9*, 772−772. (b) Guindon, S.; Gascuel, O. *Syst. Biol.* **2003**, *52*, 696−704.
(22) Guindon, S.; Gascuel, O. *Syst. Biol.* **2003**, *52*, 696−704.
(23) Perrière, G.; Gouy, M. *Biochimie* **1996**, *78*, 364−369.
(24) Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D. L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M. A.; Huelsenbeck, J. P. *Syst. Biol.* **2012**, *61*, 539−542.
(25) Alexandre, J. S. F.; Yahata, K.; Kawai, S.; Torii, M.; Kaneko, O. *Parasitol. Int.* **2011**, *60*, 313−320.
(26) Gamo, F. J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J. L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305−310.

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