Trungapeptins A−C, Cyclodepsipeptides from the Marine Cyanobacterium *Lyngbya majuscula*

Sutaporn Bunyajetpong,[†] Wesley Y. Yoshida,[‡] Namthip Sitachitta,*[,†,‡] and Kunimitsu Kaya[§]

Department of Chemistry, Chulalongkorn University, Phayathai, Bangkok 10330, Thailand, Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, and Department of Environmental Sciences, Graduate School of Environmental Studies, Tohoku University, Aoba 20, Sendai 980-8579, Japan

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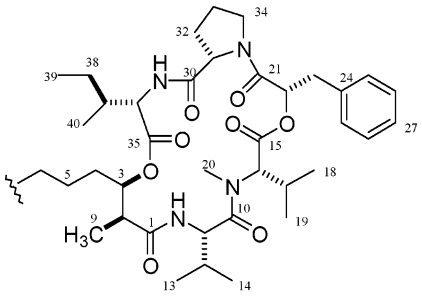
Trungapeptins A−C (**1**−**3**) were isolated from the marine cyanobacterium *Lyngbya majuscula* collected from Trung Province, Thailand. Their gross structures were elucidated by interpretation of spectroscopic data. The absolute configurations of the amino acids and phenyllactic acid were determined by Marfey's and chiral HPLC analyses, respectively. The relative stereochemistry of 3-hydroxy-2-methyl-7-octynoic acid (Hmoya) of trungapeptin A was elucidated by application of the *J*-based configuration analysis, and its absolute stereochemistry was established to be 2*S*, 3*R* by Mosher's method. The structures of compounds **1**−**3** are closely related to the antanapeptins, a series of depsipeptides isolated from a Madagascan collection of *L. majuscula*.

Marine cyanobacteria, especially *Lyngbya majuscula*, have proven to be an exceptional source of novel potential pharmaceuticals such as antillatoxin,[1] apratoxins,[2,3] and kalkitoxin.[4] Interestingly, secondary metabolites isolated from marine cyanobacteria are predominantly lipopeptides.[5] Carmabin A,[6] georgamide,[7] pitipeptolide A,[8] yanucamides,[9] and antanapeptins[10] represent a group of unique cyanobacterial lipopeptides that possess a lipid residue with terminal acetylene. Such features are also found in molluskan metabolites such as the onchidins,[11] the kulolides,[12] and the kulomo'opunalides.[12b] These structural similarities indicated that the natural products found in the molluskan extracts are likely to be of dietary origin. Herein, we wish to report the isolation and structure elucidation of three new depsipeptides, trungapeptins A−C (**1**−**3**), from a sample of *L. majuscula* collected from Trung Province, Thailand. The structures of compounds **1**−**3** contain the unique 3-hydroxy-2-methyl-7-octynoic acid (Hmoya), 3-hydroxy-2-methyl-7-octenoic acid (Hmoea), and 3-hydroxy-2-methyl-7-octanoic acid (Hmoaa) residues, respectively.



Results and Discussion

A collection of *L. majuscula* was obtained from Ratchamonkol Beach, Trung Province, Thailand, in April 2002. The organic extract of the alga, which exhibited brine shrimp toxicity and ichthyotoxicity, was initially separated by silica gel liquid chromatography. Bioassay-guided fractionation by Sephadex LH-20, RP-Sep-Pak, and RP-HPLC afforded colorless oils of compounds **1** (31.6 mg), **2** (4.6 mg), and **3** (4.4 mg).

High-resolution FABMS of trungapeptin (**1**) gave an $[M + H]^+$ peak at *m*/*z* 723.4329, corresponding to a molecular composition of $C_{40}H_{58}N_4O_8$, which required 14 degrees of unsaturation. The IR spectrum of **1** displayed strong absorption bands at 1734 and 1654 cm^{-1}, indicating the presence of ester and amide functionalities, respectively. The ^{13}C NMR and DEPT spectra of **1** exhibited six ester/amide carbonyls and a monosubstituted phenyl ring (Table 1), accounting for 10 of the 14 degrees of unsaturation. The ^1H NMR spectrum of **1** showed two NH signals at δ 5.91 and 7.84 as well as an N-Me singlet at δ 3.00, characteristic of amino acids containing secondary and tertiary amide functionalities, respectively. A series of 1D TOCSY[13] experiments followed by a HSQC experiment led to the identification of the amino acids valine (Val), *N*-methylvaline (*N*-Me-Val), proline (Pro), and isoleucine (Ileu). A fifth residue exhibited ^1H NMR resonances at δ 7.26−7.32 (H-25 to H-29), 3.17 (H-23a), 3.34 (H-23b), and 5.07 (H-22), which were characteristic of those reported for phenylalanine. However, the HSQC spectrum showed that H-22 was attached to an oxymethine carbon (δ 74.8) and thus more consistent with a 3-phenyllactic acid moeity. The final residue containing C-1 through C-9 was constructed on the basis of the 1D and 2D NMR spectroscopic data. Signals in the ^{13}C NMR spectrum at δ 69.2 (C-8) and 83.7 (C-7) and a ^1H NMR resonance at δ 1.99 (H-8, t, J = 2.6 Hz) were diagnostic for a terminal alkyne functionality. HMBC correlations from H-9 to C-1 and C-3 yielded 3-hydroxy-2-methyl-7-octynoic acid (Hmoya). Since Hmoya and Pro accounted for three of four remaining degrees of unsaturation, compound **1** must be a monocyclic depsipeptide. Correlations observed between NH-1/C-1, H_3-20/C-10, H-22/C-15, H-34b/C-21, and NH-2/C-30 led to the sequence Hmoa/Val/N-MeVal/Pla/Pro/Ileu. The HMBC correlation observed between H-3/C-35 completed the planar structure of trungapeptin A (**1**).

Acid hydrolysis and derivatization of **1** with Marfey's reagent[14] followed by HPLC analysis revealed the L-configuration of valine, *N*-Me-valine, proline, and *allo*-isoleucine residues. The absolute stereochemistry of L-3-phenyllactic acid was determined by chiral HPLC analysis. *J*-based configuration analysis[15] was employed to determine the relative stereochemistry of C-2 and C-3 of the Hmoya unit. The $^{2-3}J_{CH}$ values were measured by either a sensitivity- and gradient-enhanced hetero (ω) half-filtered TOCSY (HETLOC)[16] or a 2D gradient-selected HSQMBC experiment.[17] The measured homonuclear coupling constant between H-2 and H-3 was small

* Corresponding author. Phone: 808-956-9878. Fax: 808-956-5908. E-mail: namthip@hawaii.edu.
† Chulalongkorn University.
‡ University of Hawaii at Manoa.
§ Tohoku University.

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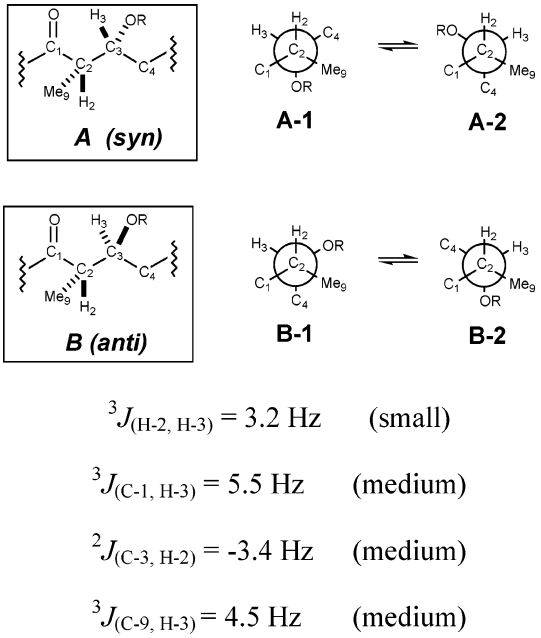
Table 1. NMR Data for Trungapeptin A (**1**) in $CDCl_3$

unit	C/H no.	δ_H (mult., *J* in Hz)	δ_C (mult.)	HMBC[a,b]
Hmoa	1		172.7 (qC)	
	2	2.45 (qd, 6.9, 3.2)	43.2 (CH)	C: 1, 3, 9
	3	4.98 (dt, 10.1, 3.2)	74.3 (CH)	C: 1, 2, 5, 35
	4a	1.85 (m)	28.3 (CH_2)	
	4b	1.99 (m)		
	5a	1.41 (m)	24.2 (CH_2)	C: 7
	5b	1.52 (m)		
	6	2.22 (td, 6.8, 2.6)	17.7 (CH_2)	C: 4, 5, 7, 8
	7		83.7 (qC)	
	8	1.99 (t, 2.6)	69.2 (CH)	C: 5 ,6
	9	1.17 (d, 6.9)	12.4 (CH_3)	C: 1, 2, 3
Val	10		173.5 (qC)	
	11	4.61 (t, 9.7)	53.6 (CH)	C: 1 ,10, 12, 13
	12	2.05 (m)	31.5 (CH)	C: 10, 11
	13	0.95 (d, 6.7)	18.5 (CH_3)	C: 11, 12, 14
	14	0.92 (d, 6.6)	19.6 (CH_3)	C: 11, 12, 13
	NH-1	5.97 (d, 9.7)		C: 1 ,11
N-Me Val	15		171.6 (qC)	
	16	4.42 (d, 7.7)	65.4 (Ch)	C: 10, 15, 18, 19, 20
	17	2.41 (m)	29.3 (CH)	C: 15
	18	1.06 (d, 6.8)	19.9 (CH_3)	C: 16, 17
	19	1.37 (d, 6.9)	21.1 (CH_3)	C: 16, 17
	20	3.00 (s)	30.4 (CH_3)	C: 10, 16
Pla	21		169.0 (qC)	
	22	5.07 (dd, 10.5, 5.6)	74.8 (CH)	C: 15,[c] 23
	23a	3.17 (dd, 12.9, 10.5)	38.1 (CH_2)	C: 21, 22, 24, 25/29
	23b	3.34 (dd, 12.9, 5.6)		C: 21, 22, 24, 25/29
	24		134.0 (qC)	
	25/29	7.26 (m)	129.7 (CH)	C: 23, 27
	26/28	7.32 (m)	129.1 (CH)	C: 24, 25/29
	27	7.30 (m)	127.8 (CH)	
Pro	30		170.15 (qC)	
	31	3.31 (d, 7.6)	60.9 (CH)	C: 30, 34
	32a	0.95 (m)	30.5 (CH_2)	C: 30
	32b	2.13 (br dd, 12.6, 6.4)		C: 30, 33, 34
	33a	1.45 (m)	21.7 (CH_2)	
	33b	1.70 (m)		
	34a	3.38 (m)	46.4 (CH_2)	C: 33
	34b	3.49 (m)		C: 21,[c] 33
Ileu	35		170.01 (qC)	
	36	4.17 (t, 8.3)	57.6 (CH)	C: 30, 35, 38, 40
	37	1.85 (m)	34.5 (CH)	
	38a	1.15 (m)	25.2 (CH_2)	C: 37, 39, 40
	38b	1.37 (m)		C: 36, 37, 39
	39	0.81 (t, 7.6)	10.4 (CH_3)	C: 36, 37, 38
	40	0.83 (d, 6.6)	15.7 (CH_3)	C: 36, 37, 38
	NH-2	7.84 (d, 8.3)		C: 30

[a] Carbons showing long-range correlation with indicated protons. [b] If not indicated otherwise, correlations were observed after optimization for $^nJ_{CH} = 7$ Hz. [c] Correlation observed after optimization for $^nJ_{CH} = 4$ Hz.

($^3J_{H2-H3} = 3.2$ Hz), and all of the observed heteronuclear coupling constants for these two protons were medium strength (Figure 1). Unfortunately, the data corresponded to a mixture of rotamers for C-2 and C-3 (A1/A2 and B1/B2 as shown in Figure 1), and the *syn* and *anti* configurations for these carbons were indistinguishable. Fortuitously, *anti*- and *syn*-3-hydroxy-2-methyl alkanoates generally exhibit $^3J_{H-2,H-3}$ values of 7 and 4 Hz, respectively.[18,19] Compound **1** was subjected to methanolysis, resulting in the isolation of fragment **4** (Figure 2), the structure of which was confirmed by ^{1}H NMR and 1D TOCSY spectral data. The observed $^3J_{H-2,H-3}$ value of 3.0 Hz was consistent with the *syn* stereochemistry for C-2 and C-3. Subsequent treatment of **4** with Mosher's reagent showed unambiguously that C-3 had the *R* configuration (for $\Delta\delta$ values, see Figure 2),[20] and consequently the absolute configuration for



$$^3J_{(H\text{-}2,\ H\text{-}3)} = 3.2\ \text{Hz} \quad \text{(small)}$$

$$^3J_{(C\text{-}1,\ H\text{-}3)} = 5.5\ \text{Hz} \quad \text{(medium)}$$

$$^2J_{(C\text{-}3,\ H\text{-}2)} = -3.4\ \text{Hz} \quad \text{(medium)}$$

$$^3J_{(C\text{-}9,\ H\text{-}3)} = 4.5\ \text{Hz} \quad \text{(medium)}$$

Figure 1. The measured $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values of the Hmoya portion (C-1 to C-9) of trungapeptin A (**1**). The designated magnitude of the coupling constants are based on ref 15. The *syn* (A-1/A-2) rotamer and *anti* (B-1/B-2) rotamer could not be distinguished solely by *J*-based configuration analysis.

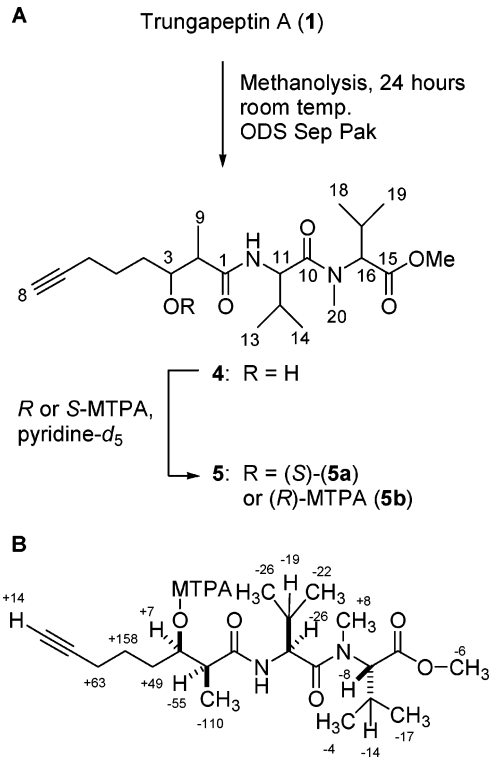


Figure 2. (A) Preparation of **5a** and **5b**. (B) $\Delta\delta$ ($\delta_S - \delta_R$) values ($\times 10^{-3}$ ppm).

C-2 and C-3 was 2*S*, 3*R*. This stereochemistry is identical to that of the Hmoya unit of the kulomo'opunalides,[12b] but is diastereomeric to that of the Hmoya unit in onchidin B (2*R*, 3*R*).[11b]

The HRFABMS of trungapeptins B (**2**) and C (**3**) established corresponding molecular formulas of $C_{40}H_{60}N_4O_8$ and $C_{40}H_{62}N_4O_8$, respectively. The ^{1}H and ^{13}C NMR chemical shifts (Table 2) of compounds **2** and **3** were nearly identical to those for **1**. A closer examination of the data revealed differences in chemical shifts for only the Hmoya unit. The acetylenic signal at δ 1.99 for H-8 in

Table 2. ^{1}H and ^{13}C NMR Data for Trungapeptins B (**2**) and C (**3**) in $CDCl_3$

	trungpeptin B (**2**)		trungapeptin C (**3**)	
position	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)
1	172.8 (qC)		172.9 (qC)	
2	43.2 (CH)	2.43 (m)	43.1 (CH)	2.43 (m)
3	74.7 (CH)	4.96 (m)	74.7 (CH)	4.94 (dt, 9.6, 3.5)
4	29.6 (CH_2)	2.39 (m) 1.86 (m)	29.7 (CH_2)	2.39 (m) 1.86 (m)
5	24.9 (CH_2)	1.28 (m) 1.41 (m)	25.7 (CH_2)	1.28 (m) 1.41 (m)
6	33.2 (CH_2)	2.06 (m)	31.4 (CH_2)	2.06 (m)
7	138.1 (CH)	5.79 (m)	22.5 (CH_2)	1.28 (m)
	115.1 (CH_2)	4.99 (br dd, 10.1, 1.8)	14.0 (CH_3)	0.87 (t, 7.2)
8	5.05 (br dd, 15.2 ,1.8)9			
12.1 (CH_3)	1.14 (d, 6.9)	12.0 (CH_3)	1.14 (d, 7.1)	
10	173.6 (qC)		173.6 (qC)	
11	53.7 (CH)	4.60 (t, 9.6)	53.7 (CH)	4.60 (t, 9.5)
12	31.5 (CH)	2.03 (m)	31.5 (CH)	2.03 (m)
13	18.5 (CH_3)	0.95 (d, 6.7)	18.6 (CH_3)	0.95 (d, 6.6)
14	19.7 (CH_3)	0.93 (d, 6.7)	19.7 (CH_3)	0.92 (d, 6.4)
NH-1		5.96 (d, 9.6)		6.11 (d, 9.5)
15	171.5 (qC)		171.5 (qC)	
16	65.3 (CH)	4.41 (d, 9.6)	65.3 (CH)	4.42 (d, 9.6)
17	29.3 (CH)	2.39 (m)	29.3 (CH)	2.39 (m)
18	20.0 (CH_3)	1.36 (d, 6.4)	20.0 (CH_3)	1.36 (d, 6.7)
19	21.1 (CH_3)	1.06 (d, 6.5)	21.1 (CH_3)	1.06 (d, 6.7)
20	30.4 (CH_3)	3.00 (s)	30.4 (CH_3)	3.00 (s)
21	168.9 (qC)		168.9 (qC)	
22	74.9 (CH)	5.10 (dd, 10.4, 5.6)	75.1 (CH)	5.11 (dd, 10.5, 5.5)
23	38.1 (CH_2)	3.16 (dd, 12.6, 10.4) 3.33 (dd, 12.6, 5.6)	38.1 (CH_2)	3.16 (dd, 12.8, 10.5) 3.33 (dd, 12.8, 5.5)
24	134.0 (qC)		134.0 (qC)	
25/29	129.7 (CH)	7.24 (m)	129.7 (CH)	7.25 (m)
26/28	129.1 (CH)	7.31 (m)	129.0 (CH)	7.31 (m)
27	127.7 (CH)	7.26 (m)	127.7 (CH)	7.29 (m)
30	169.9 (qC)		169.9 (qC)	
31	60.9 (CH)	3.33 (m)	61.0 (CH)	3.37 (m)
32	30.5 (CH_2)	2.13 (dd, 12.4, 5.8) 0.90 (dd, 12.4, 6.7)	30.5 (CH_2)	2.15 (dd, 12.5, 6.1) 0.90 (m)
33	21.7 (CH_2)	1.47 (m) 1.68 (m)	21.7 (CH_2)	1.47 (m) 1.68 (m)
34	46.4 (CH)	3.36 (m) 3.46 (m)	46.3 (CH)	3.36 (m) 3.46 (m)
35	170.0 (qC)		170.0 (qC)	
36	57.4 (CH)	4.21 (t, 8.4)	57.3 (CH)	4.21 (t, 8.8)
37	34.6 (CH)	1.86 (m)	34.6 (CH)	1.84 (dt, 10.2, 3.5)
38	25.2 (CH_2)	1.16 (m) 1.37 (m)	25.1 (CH_2)	1.16 (m) 1.37 (m)
39	10.5 (CH_3)	0.81 (t, 7.4)	10.5 (CH_3)	0.81 (t, 7.3)
40	15.7 (CH_3)	0.84 (d, 6.7)	15.6 (CH_3)	0.83 (d, 6.7)
NH-2		7.85 (d, 8.4)		7.85 (d, 8.8)

the ^{1}H NMR spectrum of **1** was replaced with signals in the olefinic region at δ 5.79, 4.99, and 5.05 in **2** and by high-field methylene signals at δ 1.28 and a methyl signal at δ 0.87 in **3**. In addition, the ^{13}C NMR spectra of **2** and **3** were missing the acetylenic resonances at δ 69.2 and 83.7 present in the spectrum for **1**. These were replaced by two additional sp^2 carbon signals at δ 115.1 and 138.1 and high-field carbon resonances at δ 22.5 and 14.0 in the ^{13}C NMR spectra of **2** and **3**, respectively. These observations implied that the terminal triple bond of Hmoya in **1** had been reduced to a double bond in **2** and a single bond in **3**. Detailed analysis of the 1D and 2D NMR data for **2** and **3** confirmed that these molecules possessed gross structures nearly identical with **1**, differing only in the presence of the octenoic and octanoic acid moieties, respectively. Since the ^{1}H and ^{13}C NMR data as well as the optical rotations for compounds **2** and **3** were virtually identical with those of **1**, it is likely that the molecules possessed identical absolute configurations, and thus the stereochemical analysis of **2** and **3** was not performed.

Trungapeptin A (**1**) showed mild brine shrimp toxicity at 10 ppm and mild ichthyotoxicity at 6.25 ppm but did not exhibit cytotoxicity against KB and LoVo cells at 10 μg/mL. Due to a limited supply of compounds **2** and **3**, their biological activities were not evaluated.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Perkin-Elmer model 341 polarimeter. UV and IR spectra were measured on an HP4853 UV−visible spectrophotometer and an Impact 410 Nicolet Fourier transform infrared spectrophotometer, respectively. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity Inova 500 instrument operating at 500.115 MHz for ^{1}H NMR and at 125.766 MHz for ^{13}C NMR. ^{1}H and ^{13}C NMR chemical shifts were referenced to the residual solvent peaks: δ_H 7.26 and δ_C 77.0 for $CDCl_3$. The HSQC experiments were optimized for $^1J_{CH}$ = 140 and 250 Hz, and the HMBC experiments for $^nJ_{CH}$ = 7 or 4 Hz. LR-FAB mass spectra were recorded on a VG-70SE mass spectrometer, and HR-FAB mass spectra were performed with a JEOL JMS-700 spectrometer. The isolation of **1** to **3** was performed with a Waters Model 662 pump, a Waters Model 486MS variable-wavelength UV detector, and a Waters Model 600S controller.

Biological Material. The marine cyanobacterium *Lyngbya majuscula* was collected from Ratchamonkol Beach (depth 2−3 ft), Trung Province, Thailand, in April 2002. The sample was immediately preserved in 2-propanol and stored at −40 °C until extraction.

Isolation and Purification. Approximately 1 L wet wt of preserved alga was extracted with CH_2Cl_2/MeOH (2:1) three times to give 2.02 g of crude organic extract and 168.6 g dry wt of extracted algal material. A portion of the organic extract (1.73 g) was subjected to Si gel column chromatography with a stepwise gradient elution from 100% hexanes to 100% EtOAc to 100% MeOH, giving 16 distinct fractions. Fraction 9 was purified by Sephadex LH-20 using CH_2Cl_2/MeOH (1:1) as eluent to provide five major fractions (fraction 9-1 to 9-5). Fraction 9-3 was further separated by C-18 Sep-Pak using stepwise gradient elution from 100% H_2O to 100% MeOH. Five fractions (fraction 9-3-1 to 9-3-5) were collected and concentrated to dryness. Reversed-phase HPLC (Alltech Econosil C18, 5 μm, 4.6 × 250 mm; 75% aqueous MeOH in 110 min at 1.0 mL/min, and monitoring at 220 nm) of fraction 9-3-3 afforded trungapeptin A (31.6 mg, t_R = 28.0 min), trungapeptin B (4.6 mg, t_R = 56.5 min), and trungapeptin C (4.4 mg, t_R = 80.9 min).

Trugapeptin A (1): colorless oil; $[\alpha]_D^{25}$ −53 (*c* 0.37, MeOH); UV (MeOH) λ_{max} 205 nm (ϵ 23 000); IR (neat) 3353, 2970, 2939, 1734, 1654, 1527, 1451, 1249, 1197 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; HRFABMS *m*/*z* $[M + 1]^+$ 723.4329 (calcd for $C_{40}H_{59}N_4O_8$, 723.4325).

Trungapeptin B (2): colorless oil; $[\alpha]_D^{25}$ −58 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 204 nm (ϵ 17 000); IR (neat) 3354, 2960, 2929, 2858, 1736, 1654, 1521, 1454 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 2; HRFABMS *m*/*z* $[M + 1]^+$ 725.4442 (calcd for $C_{40}H_{61}N_4O_8$, 725.4394).

Trungapeptin C (3): colorless oil; $[\alpha]_D^{25}$ −54 (*c* 0.72, MeOH); UV (MeOH) λ_{max} 204 nm (ϵ 13 000); IR (neat) 3344, 2960, 2929, 2852, 1741, 1659, 1526, 1454, 1250, 1193 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 2; HRFABMS *m*/*z* $[M + 1]^+$ 727.4548 (calcd for $C_{40}H_{63}N_4O_8$, 727.4538).

Marfey's Analysis of Amino Acids. Hydrolysis of trungapeptin A (0.1 mg) was achieved in 0.5 mL of 6 N HCl placed in a sealed ampule at 105 °C for 12 h. Trace HCl was removed under a N_2 stream. The resulting hydrolyzate was resuspended in 50 μL of 0.1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA, Marfey's reagent) solution in acetone, and 100 μL of 0.1 N $NaHCO_3$ was added. The reaction mixture was heated at 80 °C for 3 min. The solution was cooled to RT, neutralized with 50 μL of 0.2 N HCl, and diluted with 100 μL of 40% MeCN containing 0.05% TFA. This solution was analyzed by reversed-phase HPLC with two isocratic solvent systems (Alltech Econosil C18; solvent system I: 40% MeCN + 0.05% TFA, solvent system II: 37.5%

MeCN + 0.05% TFA) in 20 min at 1.0 mL/min (UV detection at λ 340 nm). Analyses in solvent I established the residues L-Val (8.31 min; D-Val, 11.54 min), L-*N*-MeVal (11.04 min; D-*N*-MeVal, 13.54 min), and L-Pro (5.65 min; D-Pro, 6.06 min). Analyses in solvent II established the residue L-*allo*-Ile (19.79 min; D-*allo*-31.45 min; L-Ile, 19.97 min; D-Ile, 32.09 min).

Absolute Stereochemistry of the 3-Phenyllactic Acid. Trungapeptin A (**1**, 0.1 mg) was hydrolyzed in 6 N HCl at 105 °C for 12 h. The hydrolyzate was concentrated to dryness and analyzed by chiral HPLC [column, CHIRALPAK MA(+) (4.6 × 50 nm), Daicel Chemical Industries, Ltd, solvent, 2 mM $CuSO_4$/MeCN (85:15); flow rate, 0.5 mL/min; detection at 254 nm]. Phenyllactic acid eluted after $t_R = 39.5$ min, corresponding to the retention time of an authentic standard of L-3-phenyllactic acid and therefore indicating an *S* configuration [t_R of D-3-phenyllactic acid: 28.5 min].

Methanolysis of Trungapeptin A. A solution of trungapeptin A (5 mg) in 5% methanolic potassium hydroxide (0.5 mL) was stirred for 24 h at RT. The reaction mixture was diluted with ether (10 mL), and the organic layer was washed with brine, dried over $MgSO_4$, and dried under N_2. Purification of **4** (1.8 mg) was accomplished by C_{18} Sep Pak [MeOH/H_2O (1:1), MeOH/MeOH (3:2), and MeOH/MeOH (7:3)].

MTPA Esters of 4. The sample of **4** was divided into two equal portions (0.9 mg), and each sample was placed in a 5 mm NMR tube containing 0.75 mL of pyridine-d_5. To one tube was added 6.0 mg of *R*-MTPACl and to the other 6.0 mg of *S*-MTPACl, and the reaction was monitored by ^{1}H NMR spectroscopy until completion. The reactions were quenched with 10 μL of *N*,*N*-dimethylaminopropylamine after 6 h, and the solvent was evaporated under a stream of nitrogen. The corresponding esters (**5a** and **5b**) were purified on Si Sep Pak in 1:1 hexanes/EtOAc.

5a (major conformer): ^{1}H NMR ($CDCl_3$) δ 6.412 (NH), 5.361 (H-3), 4.921 (H-16), 4.829 (H-11), 3.688 (s, MeO), 3.085 (*N*-CH_3), 2.521 (H-2), 2.207 (H-16), 2.164 (H-6), 1.990 (H-11), 1.936 (H-8), 1.750 (H_2-4), 1.513 (H_2-5), 1.093 (H_3-9), 0.998 (H_3-18), 0.933 (H_3-13), 0.804 (H_3-19), 0.825 (H_3-14).

5b (major conformer): ^{1}H NMR ($CDCl_3$) δ 6.384 (NH), 5.354 (H-3), 4.929 (H-16), 4.855 (H-11), 3.694 (MeO), 3.077 (N-CH_3), 2.576 (H-2), 2.221 (H-16), 2.101 (H_2-6), 2.009 (H-11), 1.922 (H-8), 1.701 (H_2-4), 1.355 (H_2-5), 1.808 (H_3-9), 0.959 (H_3-13), 1.002 (H_3-18), 0.821 (H_3-19), 0.847 (H_3-14).

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References and Notes

(1) Orjala, J.; Nagle, D. G.; Hsu, V. L.; Gerwick, W. H. *J. Am. Chem. Soc.* **1995**, *117*, 8281−8282.
(2) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418−5423.
(3) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Bioorg. Med. Chem.* **2002**, *10*, 1973−1978.
(4) 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*, 1440−1443.
(5) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. *Alkaloids Chem. Biol.* **2001**, *57*, 75−184.
(6) Hooper, G. H.; Orjala, J.; Schatzman, R. C.; Gerwick, W. H. *J. Nat. Prod.* **1998**, *61*, 529−533.
(7) Wan, F.; Erickson, K. *J. Nat. Prod.* **2001**, *64*, 143−146.
(8) Luesch, H.; Pangillinan, R.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2001**, *64*, 304−307.
(9) Sitachitta, N.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 197−200.
(10) Nogle, L. M.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 21−24.
(11) (a) Rodríguez, J.; Fernández, R.; Quińoá, E.; Riguera, R.; Debitus, C.; Bouchet, P. *Tetraherdon Lett.* **1994**, *35*, 9239−9242. (b) Fernández, R.; Rodríguez, J.; Quińoá, E.; Rigeura, R.; Muńoz, L.; Fernández-Suárez, M.; Debitus, C. *J. Am. Chem. Soc.* **1996**, *118*, 11635−11643.
(12) (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.
(13) Uhŕin, D.; Barlow, P. N. *J. Magn. Reson.* **1997**, *126*, 248−255.
(14) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(15) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(16) Uhŕin, D.; Batta, G.; Hruby, V. J.; Barlow, P. N.; Kövér, K. E. *J. Magn. Reson.* **1998**, *130*, 155−161.
(17) Williamson, R. T.; Márquez, B. L.; Gerwick, W. H. *Magn. Reson. Chem.* **2000**, *38*, 265−273.
(18) Meyers, A. I.; Yamamoto, Y. *Tetrahedron* **1984**, *40*, 2309−2315.
(19) Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Org. Chem.* **1994**, *59*, 7219−7226.
(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, 4092−4096.

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