Dictyonamides A and B, New Peptides from Marine-Derived Fungus

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Marine-derived fungi have proven to be a good source of structurally novel and biologically active secondary metabolites, which might be useful leads in the development of new pharmaceutical agents.[1] In our continuing search for bioactive compounds from marine-derived fungi,[2] we previously isolated a new anthracycline-derived pentacyclic metabolite, seragakinone A, from the mycelium of a fungus (K063), which was separated from an Okinawan marine red alga. Further investigation on extracts of the mycelium of the same fungus resulted in the isolation of two new peptides, dictyonamides A (**1**) and B (**2**). In this paper, we describe the isolation and structure elucidation of **1** and **2**.

The fungus (K063) was separated from the red alga *Ceratodictyon spongiosum* collected off Seragaki Beach in Okinawa and grown in PYG broth [peptone (1%), yeast extract (0.5%), and glucose (2%) in seawater, pH 7.5] at 28 °C for 14 days. The mycelium (82 g from 10 L of culture) was extracted with $CHCl_3$/MeOH (1:1), and the extracts were partitioned with EtOAc and H_2O. The aqueous layer was extracted with *n*-BuOH, and the *n*-BuOH-soluble portions were separated by a silica gel column ($CHCl_3$/MeOH, 1:1) to afford a mixture of peptides, which was purified by C_{18} HPLC (CH_3CN/H_2O/TFA, 42:58:0.05) to give dictyonamides A (**1**, 0.026% wet weight) and B (**2**, 0.018%) (Chart 1).

Dictyonamide A (**1**) [$[\alpha]^{22}_D$ −169° (*c* 1.0, MeOH)] was obtained as a colorless amorphous solid and showed the pseudomolecular ion peak at *m/z* 1274 $(M + H)^+$ in the FABMS. The molecular formula $C_{63}H_{108}N_{12}O_{15}$ was established by HRFABMS [*m/z* 1273.8090 $(M + H)^+$, Δ −4.5 mmu]. The IR spectrum suggested the presence of hydroxy and/or amino (3429 cm^{-1}) and amide carbonyl (1682 and 1633 cm^{-1}) groups, while the UV absorption at 296 nm implied the presence of aromatic functionality. The ^1H NMR (Table 1) spectrum suggested **1** to be a peptide. Amino acid analysis of the hydrolysate of **1** revealed 1 mol each of threonine (Thr), alanine (Ala), valine (Val), and isoleucine (Ile). Extensive analyses of ^1H and ^{13}C NMR data (Table 1) in CD_3OD including $^1H-^1H$ COSY, TOCSY, HMQC, and HMBC disclosed 1 mol each of *N*-methylthreonine [(NMe)Thr] and *N*-methylglycine [(NMe)Gly] and 5 mol of *N*-methylvaline [(NMe)Val] in addition to the four normal amino acid residues as described above. The remaining ^1H and ^{13}C NMR signals (δ_H 7.14, 7.55, 8.08, and 8.58; δ_C 118.5, 122.0, 124.8, 133.3, 136.1, 142.9, and 172.3) and 2D NMR data (Figure 1) implied the presence of a 2-aminobenzoic acid (Abz) moiety.

The sequence of Ala-Thr in the N-terminus of **1** was deduced from analysis by peptide sequencer. HMBC correlations of *N*-Me (δ_H 3.17) of (NMe)Thr^3 to Thr^2-CO (δ_C 173.4) and *N*-Me (δ_H 3.11) of (NMe)Val^4 to (NMe)-Thr^3-CO (δ_C 173.4) indicated that the N-terminal sequence was Ala^1-Thr^2-(NMe)Thr^3-(NMe)Val^4- (Figure 1). NOESY correlations of NH (δ_H 11.40 in DMSO-d_6) of the Abz^{12} to (NMe)Gly^{11}-α (δ_H 3.98 and 4.22 in DMSO-d_6) and HMBC correlations of *N*-Me (δ_H 3.25) of (NMe)Gly^{11} to (NMe)Val^{10}-CO (δ_C 173.2) and *N*-Me (δ_H 3.09) of (NMe)-Val^{10} to Ile^9-CO (δ_C 175.1) revealed that the C-terminal sequence was -Ile^9-(NMe)Val^{10}-(NMe)Gly^{11}-Abz^{12} (Figure 1). Since the sequence of the remaining amino acid residues, 1 mol of Val and 3 mol of (NMe)Val, was not elucidated by 2D NMR data, the remaining sequence was analyzed on the basis of the FABMS/MS methods (Figures 2 and 3). The collisionally activated dissociation (CAD) spectrum of the pseudomolecular ion [*m/z* 1273.82 $(M + H)^+$] of **1** provided evidences for the amino acid sequence as shown in Figure 2. On the other hand, the negative-ion FABMS/MS [*m/z* 1271.76 $(M - H)^-$] data revealed the whole sequence and the fragmentations of the side chain of the amino acid residues of **1** (Figure 3). LC-ESIMS analyses[3] of the acid hydrolysate of **1** using Marfey's procedure revealed that the absolute configuration of each amino acid residue was all L, although that of (NMe)Thr was not clear due to inseparable peaks of D- and L-forms. Chiral HPLC analyses of the acid hydrolysate of **1** revealed that the absolute configuration of (NMe)Thr was also L. Thus the structure of dictyonamide A (**1**) was concluded to be L-Ala-L-Thr-L-(NMe)-Thr-L-(NMe)Val-L-Val-L-(NMe)Val-L-(NMe)Val-L-(NMe)-Val-L-Ile-L-(NMe)Val-(NMe)Gly-Abz.

The molecular formula of dictyonamide B (**2**) revealed by the HRFABMS [*m/z* 1435.8620 $(M + H)^+$, Δ −4.3 mmu] was $C_{69}H_{118}N_{12}O_{20}$, which was larger than that of **1** by $C_6H_{10}O_5$. The IR spectrum suggested the presence of hydroxy and/or amine (3428 cm^{-1}) and amide carbonyl (1681 and 1633 cm^{-1}) groups, while the UV absorption at 296 nm implied the presence of Abz. Amino acid analysis of the hydrolysate of **2** revealed 1 mol each of Thr, Ala, Val, and Ile. The ^1H and ^{13}C NMR (Table 1) spectra of **2** were similar to those of **1** except for carbon signals (Table 1) at δ_C 62.7, 65.9, 71.5, 78.0, 84.8, and 97.4, indicating the presence of a sugar moiety in **2**. Since an anomeric proton signal was not observed, the sugar moiety was elucidated to be a ketohexose. Comparison of NMR data of the sugar moiety in **2** with those of the *O*-methyl glycosides of known ketohexoses indicated the sugar moiety to be fructose.[4] FABMS/MS fragmentation patterns of **2** other than the sugar moiety were similar to those of **1**, indicating that the sequence of the amino

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10.1021/jo0156767 CCC: $20.00
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Chart 1

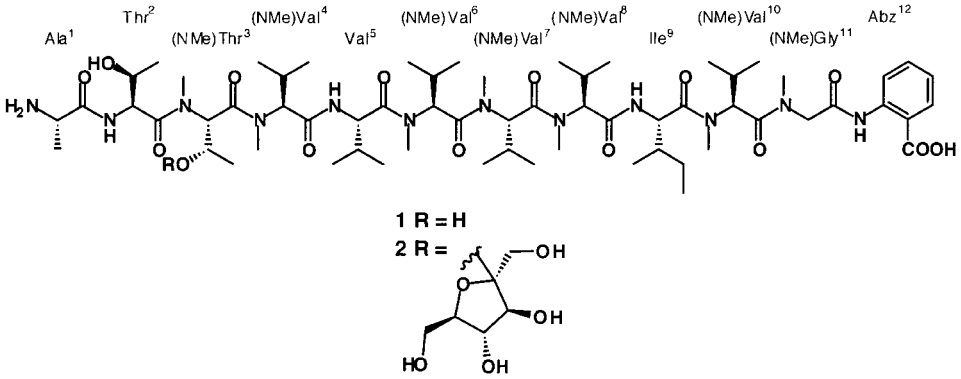

Table 1. ^{1}H and ^{13}C NMR Data of Dictyonamides A (1) and B (2) in CD_3OD

		1				2			
		^{13}C[a]	^{1}H[a]		*J* (Hz)	^{13}C[a]	^{1}H[a]		*J* (Hz)
Ala	NH		n.o.[b]				n.o.		
	α	50.9	4.08	q	6.9	58.7	4.15	q	6.9
	β	18.4	1.49	d	6.9	17.3	1.57	d	6.9
	CO	171.3				172.0			
Thr	NH		8.49				n.o.		
	α	56.9	4.93	d	5.0	56.9	4.96	d	5.0
	β	69.3	4.08	m		69.2	4.11	m	
	γ	21.4	1.20	d	6.2	20.9	1.25	d	6.2
	CO	173.4				173.1			
(NMe)Thr	NMe	32.5	3.17	s		33.1	3.22	s	
	α	61.0	5.27	d	8.1	60.9	5.30	d	8.1
	β	66.9	4.25	m		66.9	4.29	m	
	γ	21.4	1.17	d	6.2	21.0	1.21	d	6.2
	CO	173.4				173.1			
(NMe)Val	NMe	32.0	3.11	s		32.0	3.09	s	
	α	64.4	4.63	m		64.3	4.67	m	
	β	28.6	2.22	m		28.6	2.26	m	
	γ	20.7	0.87	m		20.4	0.94	m	
		19.7	0.77	m		18.4	0.81	m	
	CO	172.1				172.2			
Val	NH		8.03	br			8.15	br	
	α	56.7	4.63	m		56.8	4.67	m	
	β	33.1	2.22	m		32.6	2.07	m	
	γ	19.5	0.91	m		20.7	0.96	m	
		19.5	0.91	m		19.5	0.96	m	
	CO	171.8				172.0			
(NMe)Val	NMe	32.1[c]	3.14[d]	s		32.1[e]	3.12[f]	s	
	α	64.4	4.63	m		64.4	4.67	m	
	β	28.6	2.22	m		28.6	2.26	m	
	γ	20.7	0.87	m		20.5	0.94	m	
		19.3	0.80	m		19.5	0.80	m	
	CO	172.2				172.6			
(NMe)Val	NMe	32.4[c]	3.05[d]	s		32.3[e]	3.14[f]	s	
	α	60.5	5.20	d	10.6	60.3	5.24	d	10.6
	β	29.3	2.33	m		29.2	2.38	m	
	γ	20.7	0.87	m		20.7	0.93	m	
		19.3	0.80	m		19.5	0.81	m	
	CO	172.9				173.0			
(NMe)Val	NMe	33.1[c]	3.11[d]	s		32.5[e]	3.17[f]	s	
	α	60.5	5.35	br		60.5	5.36	br	
	β	29.3	2.33	m		29.3	2.38	m	
	γ	20.7	0.87	m		20.7	0.92	m	
		19.3	0.80	m		20.0	0.84	m	
	CO	173.2				173.0			
Ile	NH		8.11	br			8.15	br	
	α	55.4	4.74	d	8.7	55.6	4.77	d	8.7
	β	38.6	1.77	m		38.4	1.92	m	
	γ-Me	16.5	0.83	m		16.7	0.94	m	
	γ-CH_2	26.3	1.48	m		26.3	1.19	m	
			1.16	m			1.52	m	
	δ	11.8	0.79	m		11.9	0.84	m	
	CO	175.1				174.9			
(NMe)Val	NMe	32.5	3.09	s		32.4	3.15	s	
	α	60.5	5.20	br		60.5	5.24	br	
	β	29.3	2.33	m		29.3	2.38	m	
	γ	20.7	0.87	m		20.7	0.92	m	
		19.3	0.80	m		20.0	0.84	m	
	CO	173.2				172.9			
(NMe)Gly	NMe	38.4	3.25	s		38.4	3.29	s	
	α	54.7	4.18	d	16.2	54.7	4.20	d	16.2
			4.26	d	16.2		4.30	d	16.2
	CO	169.6				169.4			
Abz	NH		n.o.				n.o.		
	1	118.5				120.3			
	2	142.9				142.8			
	3	122.0	8.58	d	8.7	121.8	8.62	d	8.7
	4	136.1	7.55	dd	8.7, 7.5	136.0	7.59	dd	8.7, 7.5
	5	124.8	7.14	dd	7.5, 7.5	124.7	7.19	dd	7.5, 7.5
	6	133.3	8.08	d	7.5	133.2	8.12	d	7.5
	COOH	172.3				172.0			
Fructose	1′					65.9	3.76	d	12.5
							4.06	d	12.5
	2′					97.4			
	3′					71.5	3.92	m	
	4′					78.0	4.00	m	
	5′					84.8	4.09	m	
	6′					62.7	3.70	d	9.3
							3.83	dd	9.3, 3.1

[a] *δ* in ppm. [b] Not observed. [c–f] Signals may be interchangeable.

acid residues in **2** was the same as that of **1** (Figures 4 and 5). Treatment of **2** with 0.001% HCl in MeOH at 110 °C for 2 h afforded the corresponding aglycon, whose spectral data and retention time of HPLC were identical with those of **1**. Detection of the Ala-Thr moiety for **2** by peptide sequencer and the FABMS/MS fragment ions at *m*/*z* 451.0 and 271.2 (positive mode) implied that the fructose was connected to the hydroxy group at (NMe)-Thr[3]. The oximethine carbon resonance of Thr[2] in CD_3-OH was slightly shifted (0.05 ppm) as compared with that in CD_3OD, whereas the oximethine carbon resonance of (NMe)Thr[3] was not shifted, indicating that the fructose was connected to the hydroxy group of (NMe)Thr[3]. Chiral HPLC analyses of the acid hydrolysates of **2** revealed that the absolute configurations of all amino acid residues were L. The absolute stereochemistry of the fructose was determined to be D-configuration by GC analyses using chiral column (Chirasil-Val) of TMS derivative of the methanolysis product of **2**.

Dictyonamides A (**1**) and B (**2**) are new linear dodecapeptides from the mycelium of a marine-derived fungus (K063). The characteristic features of **1** and **2** are the

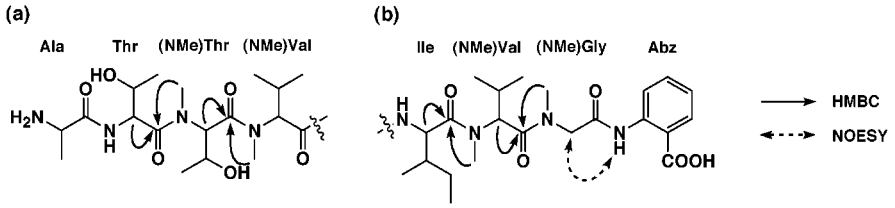

Figure 1. N- and C-terminal sequence (**a** and **b**, respectively) of dictyonamide A (**1**).

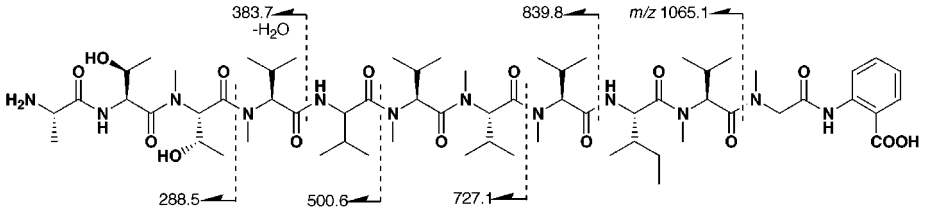

Figure 2. Positive-ion FAB MS/MS fragmentation patterns of dictyonamide A (**1**) (precursor ion: *m*/*z* 1273.82 $[M + H]^+$).

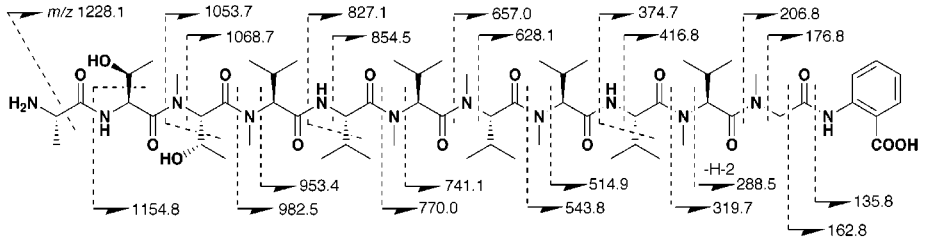

Figure 3. Negative-ion FAB MS/MS fragmentation patterns of dictyonamide A (**1**) (precursor ion: *m*/*z* 1271.76 $[M - H]^-$).

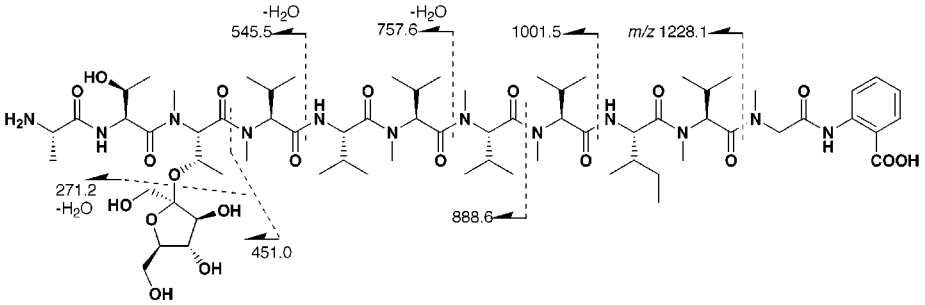

Figure 4. Positive-ion FAB MS/MS fragmentation patterns of dictyonamide B (**2**) (precursor ion: *m*/*z* 1435.85 $[M + H]^+$).

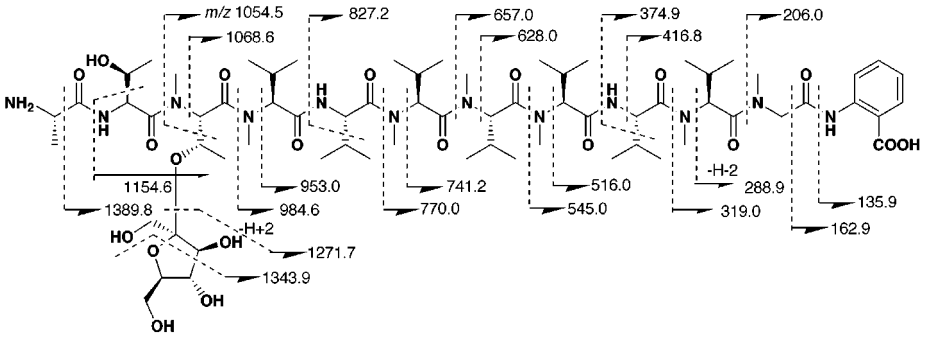

Figure 5. Negative-ion FAB MS/MS fragmentation patterns of dictyonamide B (**2**) (precursor ion: *m*/*z* 1433.85 $[M - H]^-$).

presence of many *N*-methylamino acids and an anthranilic acid (Abz) at the C-terminus. Peptides containing an anthranilic acid such as **1** and **2** are very rare, although some peptides such as actinomycin D from *Streptomyces* sp.,[5] cycloaspeptides from *Aspergillus* sp.,[6] and viridic acid from *Penicillium virdicatum*[7] have been reported. Dictyonamide A (**1**) showed inhibitory activity against cyclin-dependent kinase 4 with IC_{50} value of 16.5

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μg/mL, while compound **2** did not show such activity (IC_{50} > 50 μg/mL).

Experimental Section

General Methods. The 3.35 ppm resonance of residual CH_3OH and 49.8 ppm of CD_3OD were used as internal references for ^1H and ^{13}C NMR spectra, respectively. FAB mass spectra were obtained using glycerol as a matrix.

Fungal Material. K063 strain did not show any taxonomically useful cellular morphology for the fungal identification on the various media tested. K063 strain should belong to ascomycetous fungi by molecular methods for fungal identification using an analysis of nuclear large subunit (26S) ribosomal DNA partial sequence have been introduced.[2] Subcultures of the organism are deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Collection and Cultivation. The fungus (K063) was separated from the marine rhodophyta *Ceratodictyon spongiosum*, which was collected off Seragaki Beach at Okinawa Island. The fungus was grown in the PYG broth [peptone (1%), yeast extract (0.5%), and glucose (2%) in seawater, pH 7.5] at 28 °C for 14 days. The cultured broth (10 L) was filtered.

Extraction and Separation. The mycelium (82 g of wet weight) of the culture was extracted with $CHCl_3$/MeOH (1:1, 500 mL × 2) and evaporated under reduced pressure. The extracts were partitioned between EtOAc (100 mL × 3) and H_2O (100 mL), and the aqueous layer was extracted with *n*-BuOH (100 mL × 3). The *n*-BuOH-soluble portions were separated by a silica gel column ($CHCl_3$/MeOH, 1:1) to afford a crude peptide mixture. The mixture was purified by C_{18} HPLC (JUPITER, Phenomenex, 1.0 × 25 cm, CH_3CN/H_2O/TFA, 42:58:0.05, flow rate: 2.5 mL/min, UV detection at 296 nm) to give dictyonamides A (**1**, 21.8 mg, t_R 29 min) and B (**2**, 14.9 mg, t_R 24 min).

Dictyonamide A (1): colorless amorphous solid; $[\alpha]^{22}_D$ −169° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 208 (ϵ 21 900) and 296 (2600) nm; IR (film) ν_{max} 3429, 1682, and 1633 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 1274 $(M + H)^+$; HRFABMS *m*/*z* 1273.8090 $(M + H)^+$ (calcd for $C_{63}H_{108}N_{12}O_{15}$, 1273.8135).

Dictyonamide B (2): colorless amorphous solid; $[\alpha]^{22}_D$ −132° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 209 (ϵ 19 200) and 296 (1500) nm; IR (film) ν_{max} 3428, 1681, and 1633 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 1436 $(M + H)^+$; HRFABMS *m*/*z* 1435.8620 $(M + H)^+$ (calcd for $C_{69}H_{118}N_{12}O_{20}$, 1435.8663).

Amino Acid Analysis of Acid Hydrolysate of 1 and 2. Each (0.3 mg) of dictyonamides A (**1**) and B (**2**) in 3 N HCl (100 μL) was hydrolyzed at 100 °C for 16 h, and the solution was adjusted at pH 2 with 1 N NaOH(aq) and subjected to automatic amino acid analyzer. A total of 1 mol each of Thr, Ala, Val, and Ile was found for the hydrolysate of **1** and **2**.

Determination of Absolute Stereochemistry of Amino Acid Residues. (a) LC-MS Analysis of FDAA Derivatives. Dictyonamide A (**1**, 0.1 mg) was dissolved in 6 N HCl and heated at 110 °C for 16 h. The solvent was removed in vacuo, and the residue was placed under high vacuum. An aqueous solution of the hydrolysates of **1** in H_2O (50 μL) was treated with saturated $NaHCO_3$(aq) (20 μL) and 1% *N*-(3-fluoro-4,6-dinitrophenyl)-L-alaninamide in acetone (100 μL) at 40 °C for 1 h. The reaction was quenched by the addition of 2 N HCl (20 μL). The reaction mixture was diluted with 210 μL of CH_3CN, and an aliquot was applied to C_{18} HPLC (Develosil ODS HG-5, Nomura Chemical, 2 × 150 mm) using CH_3CN-H_2O containing 0.01 M TFA as the mobile phase under a linear gradient elution mode (CH_3CN, 30−70%, 40 min) at flow rate 0.2 mL/min. Derivatized amino acids were detected by absorption at 340 nm and by ESI (mass range 250−700 Da) and compared with similarly derivatized amino acid standards. Retention times (min) are given in parentheses: L-Thr (4.6), D-Thr (5.7), L-(NMe)Thr (4.6), D-(NMe)Thr (5.7), L-Ala (6.3), D-Ala (7.1), L-Val (10.3), D-Val (16.5), L-(NMe)Val (15.4), D-(NMe)Val (19.2), L-Ile (15.9), and D-Ile (22.6). The retention times (min) of FDAA derivatives of hydrolysates of **1** were as follows; L-Thr (4.6), L-(NMe)Thr (4.6), L-Ala (6.3), L-Val (10.3), L-(NMe)Val (15.4), and L-Ile (15.9).

(b) Chiral HPLC Analysis. Each (0.1 mg) of dictyonamides A (**1**) and B (**2**) was dissolved in 6 N HCl and heated at 110 °C for 16 h. The solvent was removed in vacuo, and the residue was placed under high vacuum. Chiral HPLC analyses were carried out using a SUMICHIRAL OA-5000 [Sumitomo Chemical Industry, 4.6 × 150 mm, flow rate: 1.0 mL/min; UV detection at 254 nm]. Retention times (min) of standard amino acids were as follows: L-Ala (5.6), D-Ala (7.8), L-Val (17.2), D-Val (30.0), L-Thr (6.0), D-Thr (7.1), L-(NMe)Val (9.0), D-(NMe)Val (14.4), L-(NMe)Thr (10.6), D-(NMe)Thr (7.1), L-(NMe)alloThr (16.8), and D-(NMe)alloThr (7.4) [eluent: H_2O containing 1.0 mmol/L of $CuSO_4$]; L-Ile (24.7) and D-Ile (40.2) [eluent: MeOH/H_2O (15:85) containing 1.0 mmol/L of $CuSO_4$]. The retention times (min) of hydrolysates of **1** and **2** were as follows; L-Ala (5.6), L-Val (17.2), L-Thr (6.0), L-(NMe)Val (9.0), L-(NMe)Thr (10.6), and L-Ile (24.7).

Determination of Absolute Stereochemistry of Fructose. Dictyonamide B (**2**, 0.5 mg) was dissolved in 0.001% HCl in MeOH (100 μL) and heated at 110 °C for 2 h. The solvent was removed in vacuo, and the residue was separated by C_{18} HPLC (Develosil ODS HG-5, 0.4 × 25 cm, CH_3CN/H_2O/TFA, 45:55:0.05, flow rate: 1.0 mL/min, detection at 296 nm) to give dictyonamide A (**1**) and a fraction containing sugar. The fraction was dissolved in pyridine (200 μL), and then trimethylsilyl chloride (10 μL) and 1,1,1,3,3,3-hexamethyldisilazane (20 μL) were added. After the reaction mixture was stirred at 40 °C for 10 min, an aliquot of the reaction mixture was applied to GC analysis. Capillary GC analyses were performed using a Chirasil-Val column (Alltech, 0.32 mm × 25 m, carrier gas: He, detection: FID) at 150 °C. The authentic samples of D- and L-fructose were converted into the corresponding TMS/Me derivatives by the same procedure as described above. Peaks (5.6, 5.9, and 6.8 min) of the TMS/Me derivative of methanolysis products of **2** corresponded to those of the TMS/Me derivative of standard D-fructose (5.6, 5.9, and 6.8 min) but not to those of L-fructose (5.5, 6.7, and 7.4 min).

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Supporting Information Available: UV, NMR, and FABMS/MS spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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