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Dictyodendrins A−E, the First Telomerase-Inhibitory Marine Natural Products from the Sponge *Dictyodendrilla verongiformis*[1]

Kaoru Warabi,[†] Shigeki Matsunaga,[†] Rob W. M. van Soest,[‡] and Nobuhiro Fusetani*[,†]

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, and Institute for Systematics and Ecology, University of Amsterdam, 1090 GT Amsterdam, The Netherlands

anobu@mail.ecc.u-tokyo.ac.jp

Received December 3, 2002

Five new alkaloids, dictyodendrins A−E (**1**−**5**), were isolated from the Japanese marine sponge *Dictyodendrilla verongiformis* as telomerase inhibitors. Their structures were elucidated by spectroscopic and chemical methods. Dictyodendrins are tyramine-based pyrrolocarbazole derivatives containing three or four *p*-hydroxybenzene groups. They inhibited telomerase completely at a concentration of 50 μg/mL.

Telomerase is a ribonucleoprotein enzyme that adds repeats of the DNA sequence, TTAGGG, called telomeres, onto the 3′-ends of chromosomes.[2−4] Telomerase activity is found in about 90% of human tumors, but not in normal cells.[5] Thus, inhibitors of telomerase are potential antitumor agents.[6−8] In fact, some synthetic inhibitors based on the function of telomerase have been successful in clinical trials.[9] It should be noted that several natural products have been reported to inhibit telomerase,[10,11] but no telomerase-inhibitory marine natural products are known to date. In our screening for anti-telomerase activity of the extracts of Japanese marine invertebrates, the marine sponge *Dictyodendrilla verongiformis* collected in southern Japan showed significant activity. Bioassay-guided fractionation led to the isolation of dictyodendrins A−E (**1**−**5**, respectively). This paper describes the isolation and structure elucidation of dictyodendrins.

Results and Discussion

Frozen sponge (80 g, wet weight) was successively extracted with MeOH, EtOH, and acetone. The extracts were combined, concentrated, and partitioned between water and $CHCl_3$. The aqueous layer was further extracted with *n*-butanol, and the active *n*-butanol layer was separated by ODS flash chromatography with aqueous methanol followed by gel filtration on Sephadex LH-20 with methanol. The active fractions were finally purified by reverse-phase HPLC to afford dictyodendrins A (**1**, 12.8 mg), B (**2**, 0.9 mg), C (**3**, 4.6 mg), D (**4**, 1.5 mg), and E (**5**, 2.7 mg) along with two known compounds, **6** (21.8 mg) and **7** (1.5 mg), which were isolated as aldose reductase inhibitors from a marine sponge of the same genus.[12]

Dictyodendrin A (**1**) was isolated as a red amorphous solid exhibiting UV−vis absorptions at 328 and 480 nm. Its molecular formula was established as $C_{43}H_{33}N_2O_{11}SNa$ on the basis of HR-FABMS and NMR data. IR bands at 3400 and 1724 cm^{-1} suggested the presence of hydroxyl and ester moieties, respectively. The ^1H NMR spectrum exhibited 13 signals between 5.9 and 7.5 ppm, in addition to signals for a methine, two methylenes, and an *O*-methyl. Some of the signals were extremely broadened, which indicated the presence of a conformational equilibrium. Interpretation of COSY data led to three pairs of mutually coupled 2H-signals ascribable to 1,4-disubstituted benzene rings: ring E, δ 7.40 [H-18 (H-22)] and 6.96 [H-19 (H-21)]; ring F, 6.23 [H-26 (H-30)] and 6.48 [H-27 (H-29)]; and ring H, 7.03 [H-39 (H-43)] and 6.71 [H-40 (H-42)]. There was another set of signals attribut-

* Corresponding author.
[†] Laboratory of Aquatic Natural Products Chemistry and Graduate School of Agricultural and Life Sciences.
[‡] Institute for Systematics and Ecology.

(1) Bioactive Marine Metabolites 117. Part 116: Nakao, Y.; Yoshida, S.; Matsunaga, S.; Fusetani, N. Submitted for publication.
(2) Blackburn, E. H. *Cell* **2002**, *106*, 661−673.
(3) Cech, T. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 34−43.
(4) Maser, R. S.; Depinto, R. A. *Science* **2002**, *297*, 565−569.
(5) Lavelle, F.; Riou, J.-F.; Laoui, A.; Mailliet, P. *Crit. Rev. Oncol. Hematol.* **2000**, *34*, 111−126.
(6) White, L. K.; Wright, W. E.; Shay, J. W. *Trends Biotechnol.* **2001**, *19*, 114−120.
(7) Neidle, S.; Parkinson, G. *Nat. Rev. Drug Discov.* **2002**, *1*, 383−393.
(8) Helder, M. N.; Wisman, G. B. A.; van der Zee, A. G. J. *Cancer Invest.* **2002**, *20*, 82−101.
(9) Gowan, S. M.; Harrison, J. R.; Patteson, L.; Valenti, M.; Read, M. A.; Neidle, S.; Kelland, L. R. *Mol. Pharmacol.* **2002**, *61*, 1154−1162.
(10) Togashi, K.; Kakeya, H.; Morishita, M.; Song, Y. X.; Osada, H. *Oncol. Res.* **1998**, *10*, 449−453.
(11) Shin-ya, K.; Wiezba, K.; Matsuo, K.; Ohtani, T.; Yamada, Y.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Am. Chem. Soc.* **2001**, *123*, 1262−1263.
(12) Sato, A.; Morishita, T.; Shiraki, T.; Yoshioka, S.; Horikoshi, H.; Kuwano, H.; Hanazawa, H.; Hata, T. *J. Org. Chem.* **1993**, *58*, 7632−7634.

10.1021/jo0267910 CCC: $25.00
Published on Web 02/26/2003

TABLE 1. ^{1}H and ^{13}C NMR Data for Dictyodendrin A (1) (CD_3OH)

no.	δ_C	δ_H (mult., J in Hz)	HMBC	no.	δ_C	δ_H (mult., J in Hz)	HMBC
2	132.4			23	48.0	3.73 (2H, br)	
3	119.0			24	36.2	2.19 (2H, t, J = 9.0)	C23, C26, C30
4	119.0			25	130.3		
5	115.4			26 (30)	130.5	6.23 (2H, d, J = 8.3)	C24, C26, C27, C28, C29, C30
6	127.1			27 (29)	115.7	6.48 (2H, d, J = 8.3)	C26, C27, C28, C29, C30
7	122.2	5.94 (1H, d, J = 7.4)	C9, C11	28	156.5		
8	118.7	6.56 (1H, br)	C6, C10	31	130.0		
9	116.7	7.17 (1H, d, J = 8.8)	C10, C11	32	135.0	7.23 (1H, d, J = 8.7)	C3, C31, C36, C34
10	138.6			33	116.0	6.880 (1H, dd, J = 2.3, 8.7)	C34
11	133.9			34	158.3		
12		9.96 (1H, s)	C5, C6, C11, C13	35	116.0	6.895 (1H, dd, J = 2.3 8.4)	C34
13	128.8			36	134.6	7.37 (1H, d, J = 8.4)	C3, C31, C32, C34
14	138.6[a]			37	48.9	5.28 (1H, s)	C2, C3, C31, C44
15	113.3			38	129.5		
16	130.8[a]			39 (43)	130.7	7.03 (2H, d, J = 8.4)	C37, C39, C41, C43
17	128.8			40 (42)	116.2	6.71 (2H, d, J = 8.4)	C38, C41
18 (22)	134.1	7.40 (2H, br)	C15, C18, C20, C22	41	157.5		
19 (21)	116.6	6.96 (2H, br)	C17	44	174.1		
20	158.4			45	48.9	3.52 (3H, s)	C44

[a] Interchangeable.

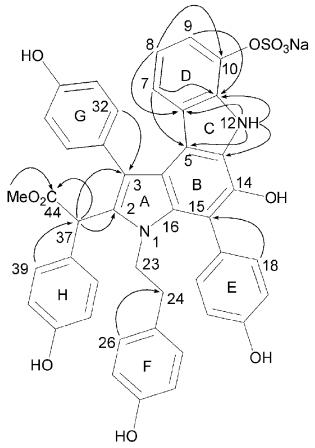
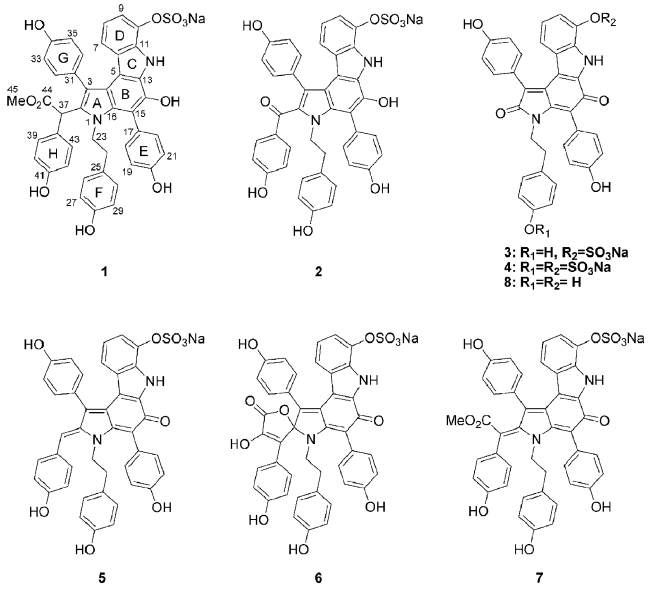


FIGURE 1. Key HMBC correlations in **1**.

able to a 1,4-disubstituted benzene ring [ring G, δ 7.23 (H-32), 6.880 (H-33), 6.895 (H-35), 7.37 (H-36)], in which each proton gave a discrete signal, indicating a restricted rotation for this ring. Carbon chemical shift values of δ 129−130 and 158−159 for the nonprotonated carbons as well as those of protonated carbons in these rings demonstrated that they were all *p*-substituted phenols (Table 1). Interpretation of the HMBC data allowed further expansion of partial structures from these rings (Figure 1). The HMBC correlation between H-18 (H-22) and C-15 indicated that ring E was connected to an sp^2-hybridized carbon atom, while that between H-26 (H-30) and C-24 showed that ring F was linked to a methylene carbon (δ_H 2.19, δ_C 36.2), which was in turn connected to a nitrogenous methylene (C-23: δ_H 3.74, δ_C 48.0).



Ring G was attached to an sp^2 carbon at δ 119.0 on the basis of an HMBC cross-peak between H-32 (H-36) and C-3. HMBC cross-peaks between H-39 (H-43) and C-37, between H-37 and C-44, and between H-37 and C-2 connected ring H to the C-37 methine (δ_H 5.28; δ_C 48.9), which was linked to a methoxycarbonyl functionality (δ_{C-44} 174.1; δ_{C-45} 52.4, δ_{H-45} 3.52) and an sp^2 carbon at δ 132.4 (C-2). Although H-37 exhibited a correlation with one of the two carbons resonating at δ 119, it was not possible to confirm whether the signal was identical with

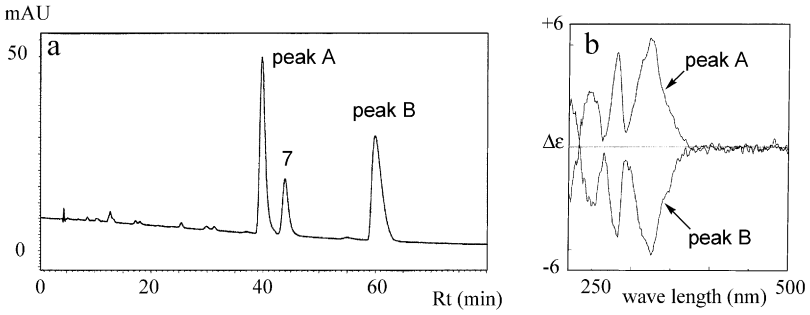


FIGURE 2. (a) Reverse-phase chiral HPLC chromatogram of dictyodendrin A (**1**): column, Chiralpak OJR (4.6 mm × 150 mm); solvent, 22% MeCN−0.3 M $NaClO_4$; flow rate, 0.5 mL/min; detection, UV absorption at 335 nm. (b) CD spectra of peaks A and B.

TABLE 2. ^{13}C NMR Data for 2−5 (CD_3OH)[a]

no.	2	3	4	5
2	134.4[a]	173.2	173.2	158.9[a]
3	126.0	130.4	130.5	143.8
4	118.1	135.2	135.0	ND
5	116.0	114.0	114.0	116.0
6	126.8	126.8	126.8	126.8
7	122.6	121.8	121.9	121.5
8	118.9	122.3	122.4	121.2
9	117.2	118.2	118.2	117.6
10	138.7	140.6	140.7	140.0
11	133.9	133.4	133.5	133.0
13	129.5	nd[b]	133.7	135.6
14	141.8	180.7	180.6	ND
15	112.6	129.4	119.3	114.3
16	136.2[a]	150.3	150.2	148.8[a]
17	127.1	124	124.0	126.9
18 (22)	134.0	133.4	133.8	133.3
19 (21)	116.8	116.0	116.3	116.6
20	158.8	159.3	158.8	157.8
23	46.4	46.1	44.0	49.6
24	37.7	34.9	35.1	34.2
25	130.5	130.0	135.8	130.0
26 (30)	130.5	130.9	130.5	130.9
27 (29)	116.2	116.0	122.3	115.8
28	156.9	157.0	152.8	156.9
31	129.1	123.6	123.7	126.1
32 (36)	134.3	133.4	133.5	133.2
33 (35)	115.6	116.4	116.5	116.4
34	157.9	160.3	160.6	159.6
37	191.8			120.7
38	131.8			127.8
39 (43)	133.2			133.8
40 (42)	115.6			116.9
41	163.2			159.8

[a] Interchangeable within the column. [b] Chemical shift value not determined.

TABLE 3. ^{1}H NMR Data for 2−5 (CD_3OH)

no.	2	3	4	5
7	5.99	6.13	6.15	5.76
8	6.55	6.70	6.72	6.63
9	7.16	7.19	7.20	7.17
12	10.21	11.59	11.6	10.21
18 (22)	7.43	7.22	7.24	7.43
19 (21)	7.00	6.90	6.92	6.96
23	3.94	3.40	3.44	3.47
24	2.46	2.73	2.48	2.26
26 (30)	6.39	6.63	6.76	6.40
27 (29)	6.45	6.55	7.04	6.40
32 (36)	7.04	7.30	7.34	7.14
33 (35)	6.63	6.88	6.90	6.97
37				5.99
39 (43)	7.32			7.21
40 (42)	6.53			6.81

C-3 or not due to a limited resolution of the HMBC data. The three contiguous aromatic protons (H-7−H-9) on ring D were assigned on the basis of the $^3J_{HH}$ values of 8 Hz and large $^3J_{CH}$ and small $^2J_{CH}$ values.

The HMBC cross-peaks (H-7/C-5, C-11; H-8/C-6, C-10; H-9/C-11; 12-NH/C-5, C-6, C-11, C-13) required fusion of ring D with a pyrrole, leading to a 2,3,7-trisubstituted indole. We were not able to specify the substituent at C-10 due to the unfeatured chemical shift of 139 ppm for C-10. Two of the three unassigned carbons resonated at δ 119.0 and 138.6, while the other was buried under other signals. Further structural analysis by NMR data was hampered by the absence of additional HMBC cross-peaks. Fortunately, 20% of dictyodendrin A was converted to the known compound **7**[12] after being kept in CD_3OD at 4 °C in an NMR tube, thus indicating that an oxidative transformation took place. Therefore, it was possible to assign a pyrrolocarbazole unit for the remaining part of **1**, in which C-10 was substituted by a sulfate group.

With the structure of dictyodendrin A (**1**) in hand, we considered the reason for the broad ^{1}H NMR signals. Because broadening of ^{1}H signals was not observed for **7**, it was presumed that a restricted rotation at C-2−C-37 bond caused the broadening; ROESY cross-peaks from H-39 (H-43) to H-24, H-32 (H-36), and H-45 cannot be accounted for by the presence of a single rotamer. Despite the presence of a stereogenic center, the CD spectrum of dictyodendrin A (**1**) exhibited no Cotton effect, indicating its racemic nature. This was proved by chiral reverse-phase HPLC, in which dictyodendrin A (**1**) was separated into two major peaks (Figure 2a), both of which gave ^{1}H NMR spectra identical to those of the starting material, accompanied by a minor peak for **7**. The CD spectra of the two major peaks exhibited mirror images (Figure 2b), confirming their enantiomeric relationship or, in other words, the racemic nature of our initial preparation.

Dictyodendrin B (**2**) was isolated as a yellowish amorphous solid exhibiting UV−vis absorptions at 305 and 397 nm. The molecular formula of $C_{41}H_{29}N_2O_{10}SNa$ was smaller than dictyodendrin A by elements of C_2H_4O. A facile loss of a SO_3 unit in the FABMS was in accordance with the presence of a sulfate group. Analysis of NMR data (Tables 2 and 3) resulted in the identification of an isolated ethylene, four 1,4-disubstituted benzene rings, and a 2,3,7-trisubstituted indole, which were all present in dictyodendrin A. The *O*-methyl and C-37 methine signals found in **1** were missing, while a conjugated ketone carbon signal was observed at δ 191.8. Therefore,

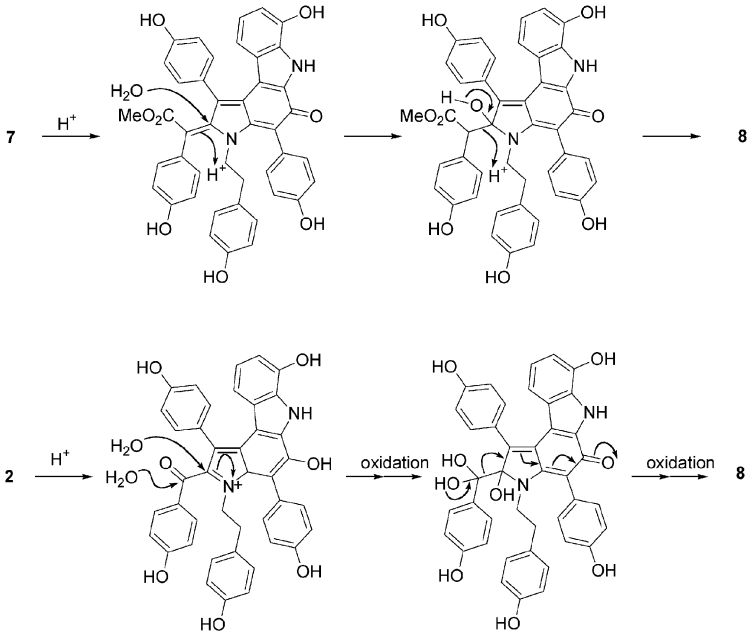


FIGURE 3. Proposed mechanism for the acid-catalyzed formation of **8** from **7** and **2**.

it was likely that dictyodendrin B (**2**) was an oxidative decarboxylation product of **1**, which was evident from the HR-FABMS data. Although it was not possible to corroborate this assumption by interpretation of NMR data due to the lack of HMBC cross-peaks from several carbons, the presence of the common core unit was demonstrated by chemical transformation. Upon treatment with acid, compound **7** underwent a fragmentation reaction to give the pyrrolidinone **8** (Figure 3). Fortunately, dictyodendrin B (**2**) also gave **8** by treatment with acid (Figure 3). The presence of a sulfate ester at C-10 was indicated by comparing the NMR data with those of **1**.

The UV−vis (λ_{max} 302 and 415 nm), IR (ν_{max} 3400 cm^{-1}), and FABMS data indicated that dictyodendrin C (**3**) was closely related to both **1** and **2**; the molecular formula of **3** was smaller than **2** by a C_7H_5O unit. The most significant ^1H NMR spectral feature of **3** was the absence of C-37 and ring H, which was substantiated by the ^{13}C NMR data. The C_9-unit attached at C-2 in **7** was replaced by an oxygen atom in **3**, which was in agreement with HR-FABMS and NMR data (Tables 2 and 3), thus indicating **3** to be the 10-sulfate of **8** as demonstrated by a downfield shift of 0.6 ppm for H-9. This was confirmed by conversion of **3** to **8** by acid hydrolysis.

Dictyodendrin D (**4**) exhibited spectral data almost superimposable on those of dictyodendrin C (**3**), except for the FABMS data, which revealed the molecular ion peak 80 units larger than that of **3**. Serial loss of SO_3 and $NaSO_3$ in the FABMS suggested the presence of two sulfate groups. A downfield shift of 0.5 ppm observed for H-27 (H-29) assigned **4** as the 28-*O*-sulfate of dictyodendrin C. Again, this was confirmed by NMR data (Tables 2 and 3) and acid hydrolysis of dictyodendrin D (**4**) to **8**.

Dictyodendrin E (**5**) was a labile reddish solid showing UV−vis absorptions at 280 and 464 nm. The ^1H NMR spectrum measured in CD_3OH exhibited two sets of signals in a ratio of 4:1. Interpretation of the major set of NMR data demonstrated the presence of four 1,4-disubstituted benzene rings, two mutually coupled methylenes, and a 2,3,7-trisubsituted indole. The 37-methoxycarbonyl group in **7** was missing. Instead, a vinyl proton, which was long-range coupled to C-3 and C-39 (C-43), was observed at δ 5.99 (H-37). The remaining NMR signals (Tables 2 and 3) were superimposable on those of **7**. Therefore, dictyodendrin E (**5**) was the *des*-methoxycarbonyl derivative of **7**. (*Z*)-Geometry of the $\Delta^{2,37}$ olefin was inferred from a ROESY cross-peak between H-37 and H-32 (H-36). Although complete analysis of the minor set of signals was not possible due to signal overlapping, C-37 could be assigned as a methoxymethine on the basis of HMBC data, thereby indicating that the minor set of signals were attributable to the MeOH adduct of the major constituent at C-37. In fact, the intensity of the FABMS ion for the MeOH adduct increased significantly after being kept in MeOH. The presence of the pyrrolocarbazole framework in dictyodendrin E (**5**) was confirmed by its transformation to **8** with acid treatment.

Dictyodendrins A−E (**1**−**5**, respectively) and compounds **6** and **7** showed 100% inhibition of telomerase activity at a concentration of 50 μg/mL. Interestingly, **8** showed no activity at a concentration of 50 μg/mL, indicating the importance of the sulfate group. To our knowledge, dictyodendrins are the first marine natural products that inhibit telomerase.[13] Dictyodendrins are closely related to purpurone from a marine sponge *Iotrochota* sp.[14] and ningalins from an ascidian *Didemnum* sp.[15] Dictyodendrins are also biogenetically related to lamellarins,[16] lukianols,[17] polycitone A,[18] storniamide

(13) Faulkner, D. J. *Nat. Prod. Rep*. **2002**, *19*, 1−48 and previous reviews in this series.

(14) Chan, G. W.; Francis, T.; Threen, D. R.; Offen, P. H.; Pierce, N. J.; Westley, J. W.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem*. **1993**, *58*, 2544−2546.

(15) Kang, H.; Fenical, W. *J. Org. Chem*. **1997**, *62*, 3254−3262.

(16) Ridley, C. P.; Reddy, M. V. R.; Rocha, G.; Bushman, F. D.; Faulkner, D. J. *Bioorg. Med. Chem*. **2002**, *10*, 3285−3290 and references therein.

A,[19] and prunolides[20] obtained from either tunicates or a sponge.

Experimental Section

Animal Material. The sponge was collected off Nagashima Island (32° 12′ N, 130° 11′ E) of the Amakusa Islands in June of 2000 by hand using scuba at depths of 15−20 m and kept frozen until extraction. A voucher specimen (ZMA POR 16722) was deposited at the Institute for Systematics and Ecology (Zoological Museum), University of Amsterdam, Netherlands, and was identified as *Dictyodendrilla verongiformis*.

Telomerase Inhibition Assay.[21,22] A pellet of 2×10^6 cells of telomerase positive HeLa cells was extracted with 200 μL of CHAPS buffer (1 mM $MgCl_2$, 1 mM EGTA, 10 mM Tris-HCl, pH 7.5, 0.1 mM benzamidine, 5 mM β-mercaptoethanol, 0.5% CHAPS, and 10% glycerol). Telomerase activity was measured by the telomeric amplification protocol (TRAP) method with a slight modification.[21,22] A 0.5 μL portion of the test solution was added to a mixture of 8.3 μL of TRAP buffer solution[21] and 0.5 μL of the cell extract, and the mixture was preincubated on ice for 20 min. After addition 0.7 μL of a solution that contained 25 ng of TS primer[21] and 0.5 nmol of dNTP mixture, the reaction mixture was incubated at 30 °C for 40 min and then heated at 80 °C for 10 min in order to inactivate telomerase. After the reaction mixture was cooled to room temperature, additions of 0.15 U of TaKaRa Ex Taq (Takara Bio Inc., Japan) and 20 μL of a solution prepared by mixing 96 μL of TaKaRa Ex Taq buffer, 19.2 μL of dNTP solution (2.5 mM), 2.59 μL of TS primer solution (1 μg/μL), 2.59 μL of ACX primer solution[22] (1 μg/μL), 1.20 μL of NT primer solution[22] (0.5 μg/μL), 12.0 μL of TSNT solution[22] (0.01 amol), and 826.5 μL of water were made; PCR was then conducted for 30 cycles consisting of 3 cycles (94 °C for 30 s, 66 °C for 30 s, and 72 °C for 30 s), 3 cycles (94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s), and 24 cycles (94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s). The PCR products were analyzed by SDS PAGE on a 7%/15% polyacrylamide gel in Tris-borate buffer (2.22 mM Tris, 2.22 mM H_3BO_3, and 0.055 mM $EDTANa_2$) at 150 V for 90 min. The gel was stained with Gel Star and photographed under a UV lamp.

Extraction and Isolation. A 60 g portion of the frozen sponge was extracted with MeOH (0.9 L), and another 20 g portion was successively extracted with MeOH (0.2 L), EtOH (0.2 L), and acetone (0.2 L). The extracts were combined, concentrated, and partitioned between H_2O and $CHCl_3$. The aqueous layer was further extracted with *n*-butanol. The *n*-butanol layer (0.83 g) was chromatographed on ODS with H_2O, MeOH/H_2O (20:80), MeOH/H_2O (50:50), MeOH/H_2O (70:30), and MeOH, in order. The fractions eluted with MeOH/H_2O (20:80) and MeOH/H_2O (50:50) were combined and gel-filtered on Sephadex LH-20 with MeOH. The active fractions were purified by ODS HPLC using a gradient elution with aqueous MeOH containing 0.3 M $NaClO_4$. The active fraction was finally purified by reverse-phase HPLC on a phenylhexyl column using a gradient elution with aqueous MeOH containing 0.3 M $NaClO_4$ or with aqueous MeCN containing 0.3 M $NaClO_4$ to afford dictyodendrin A (**1**, 12.8 mg, 0.0160%), B (**2**, 0.9 mg, 0.001%), C (**3**, 4.6 mg, 0.0058%), D (**4**, 1.5 mg, 0.0019%), and E (**5**, 2.7 mg, 0.0034%) along with **6** (21.8 mg, 0.0273%) and **7** (1.5 mg, 0.0019%).

Dictyodendrin A (1): red amorphous solid; $[\alpha]^{19}_D$ −4.6° (*c* 0.01, 60% MeOH−0.3 M $NaClO_4$); UV (MeOH) λ_{max} 208 (ϵ 55 200), 227 (56 600), 328 (18 500), 480 (3950) nm; IR (film) ν_{max} 3408, 1724, 1614, 1514, 1440, 1226, 1103, 1055, 1006 cm^{-1}; FABMS (positive, glycerol + *p*-TsOH) *m/z* 809 $[M + H]^+$, 787 $[M - Na + 2H]^+$, 707 $[M - NaSO_3 + 2H]^+$; HR-FABMS $[M + Na]^+$ *m/z* 831.1633 ($C_{43}H_{33}N_2O_{11}SNa_2$, calcd 831.1600); for ^1H and ^{13}C NMR data, see Table 1.

Dictyodendrin B (2): yellow amorphous solid; UV (MeOH) λ_{max} 228 (ϵ 34 700), 305 (sh), 397 (9010) nm; IR (film) ν_{max} 3381, 2926, 2847, 1608, 1514, 1442, 1234, 1167, 1105, 1055, 1006 cm^{-1}; FABMS (negative, triethanolamine) *m/z* 741 $[M - H]^-$, 661 $[M - NaSO_3]^-$; HR-FABMS $[M - Na]^-$ *m/z* 741.1553 ($C_{41}H_{29}O_{10}N_2S$, calcd 741.1543); for ^1H and ^{13}C NMR data, see Tables 2 and 3.

Dictyodendrin C (3): greenish-yellow amorphous solid; UV (MeOH) λ_{max} 238 (ϵ 18 300), 277 (10 500), 302 (8600), 415 (6540) nm; IR (film) ν_{max} 3362, 2926, 2854, 1699, 1602, 1512, 1456, 1394, 1267, 1170, 1113, 1057, 1024 cm^{-1}; FABMS (positive, glycerol + *p*-TsOH) *m/z* 659 $[M + H]^+$, 637 $[M - Na + 2H]^+$, 557 $[M - NaSO_3 + 2H]^+$; HR-FABMS $[M + Na]^+$ *m/z* 681.0891 ($C_{34}H_{23}N_2O_9SNa_2$, calcd 681.0920); for ^1H and ^{13}C NMR data, see Tables 2 and 3.

Dictyodendrin D (4): greenish-yellow amorphous solid; UV (MeOH) λ_{max} 243 (ϵ 12 500), 276 (6610), 305 (6230), 353 (5520), 416 (4960) nm; IR (film) ν_{max} 3454, 2926, 2851, 1707, 1602, 1510, 1475, 1439, 1394, 1236, 1170, 1055, 1024 cm^{-1}; FABMS (positive, glycerol + *p*-TsOH) *m/z* 717 $[M - Na + 2H]^+$, 637 $[M - NaSO_3 + 2H]^+$; FABMS (negative, triethanolamine) *m/z* 761 $[M + H]^-$, 739 $[M - Na + 2H]^-$, 659 $[M - NaSO_3 + 2H]^-$, 637 $[M - 2Na - SO_3 + 3H]^-$; HR-FABMS $[M - Na]^-$ *m/z* 737.0512 ($C_{34}H_{22}N_2O_{12}S_2Na$, calcd 737.0512); for ^1H and ^{13}C NMR data, see Tables 2 and 3.

Dictyodendrin E (5): red amorphous solid; UV (MeOH) λ_{max} 230 (ϵ 33 900), 280 (17 700), 464 (20,600) nm; IR (film) ν_{max} 3369, 2928, 2858, 1724, 1664, 1604, 1512, 1467, 1263, 1078 cm^{-1}; FABMS (positive, glycerol + *p*-TsOH) *m/z* 727 $[M - Na + 2H]^+$, 647 $[M - NaSO_3 + 2H]^+$; HR-FABMS $[M - Na + 2H]^+$ *m/z* 727.1739 ($C_{41}H_{31}N_2O_9S$, calcd 727.1750); for ^1H and ^{13}C NMR data, see Tables 2 and 3.

Acid Hydrolysis of 7. A 1.4 mg portion of **7** was dissolved in TFA/MeOH (1:9, 0.5 mL). The solution was heated at 35 °C for 20 min and then at 42 °C for 31 min. After the mixture was cooled, the solvents were removed in a stream of N_2 gas to afford the desulfation product (0.7 mg), exhibiting the $[M + H]^+$ ion at *m/z* 705 in FABMS. A half portion of the product was dissolved in MeOH/1 N HCl (1:20, 1.05 mL), and the mixture was heated at 100 °C for 80 min. After dilution with MeOH, the reaction mixture was cooled to room temperature. The reaction mixture was dried in a stream of N_2 gas. The residue was purified by ODS-HPLC with 56% aqueous MeOH containing 0.05% TFA to furnish **8** (0.2 mg). **8**: greenish-yellow amorphous solid; HR-FABMS $[M + H]^+$ *m/z* 557.1729 ($C_{34}H_{25}N_2O_6$, calcd 557.1712); ^1H NMR (CD_3OD, 600 MHz) δ 7.31 (2H, d, J = 8.5 Hz; H-32 (36)), 7.24 (2H, d, J = 8.5 Hz; H-18 (22)), 6.93 (2H, d, J = 8.5 Hz; H-19 (21)), 6.89 (2H, d, J = 8.5 Hz; H-33 (35)), 6.66 (2H, d, J = 8.5 Hz; H-26 (30)), 6.58 (t, J = 7.3 Hz; H-8), 6.57 (dd, J = 1.9, 7.3 Hz; H-9), 6.56 (2H, d, J = 8.5 Hz; H-27 (29)), 5.82 (dd, J = 1.9, 7.3 Hz; H-7), 3.42 (2H, t, J = 7.8 Hz; H_2-23), 2.40 (2H, t, J = 7.8 Hz; H_2-24).

Acid Hydrolysis of Dictyodendrin B (2). A 100 μg portion of dictyodendrin B (**2**) was dissolved in MeOH/1 N HCl (1:4, 100 μL) and heated at 100 °C for 65 min. After being diluted with MeOH, the reaction mixture was dried in a stream of N_2 gas. The residue was purified by ODS HPLC with 60% aqueous MeOH containing 0.05% TFA to afford **8** (ca. 0.1 mg).

Acid Hydrolysis of Dictyodendrin C (3). A 0.8 mg portion of dictyodendrin C (**3**) was dissolved in TFA/MeOH (1:9, 0.5 mL) and heated at 38 °C for 30 min, and the mixture was processed as described above to yield **8** (0.4 mg).

(17) Yoshida, W. Y.; Lee, K. K.; Carroll, A. R.; Scheuer, P. J. *Helv. Chim. Acta* **1992**, *75*, 1721−1725.
(18) Rudi, A.; Goldberg, I.; Stein, Z.; Frolow, F.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Org. Chem*. **1994**, *59*, 999−1003.
(19) Palermo, J. A.; brasco, M. F. R.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727−2734.
(20) Carroll, A. R.; Healy, P. C.; Quinn, R. J.; Tranter, C. J. *J. Org. Chem*. **1999**, *64*, 2680−2682.
(21) Kim, N.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. *Science* **1994**, *266*, 2011−2015.
(22) Kim, N. W.; Wu, F. *Nucleic Acids Res*. **1997**, *25*, 2595−2597.

Acid Hydrolysis of Dictyodendrin D (4). A 0.3 mg portion of dictyodendrin D (**4**) was dissolved in TFA/MeOH (1:9, 0.5 mL) and heated at 40° for 50 min, and the mixture was processed as described above to afford **8** (0.2 mg).

Acid Hydrolysis of Dictyodendrin E (5). A 0.3 mg portion of dictyodendrin E (**5**) was dissolved in MeOH/1 N HCl (2:9, 330 μL). The solution was heated at 40 °C for 20 min and then at 100 °C for 35 min. The reaction mixture was processed as described above to furnish **8** (ca. 0.1 mg).

Acknowledgment. We thank Prof. Raymond J. Andersen at University of British Columbia for editorial comments. Thanks are also due to the crew of R/V Toyoshio-maru for assistance in collecting the sponge. This work was partly supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

Supporting Information Available: Tables of NMR data for **2**−**5** and one- and two-dimensional NMR spectra for **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0267910