Five New Discodermolide Analogues from the Marine Sponge *Discodermia* Species

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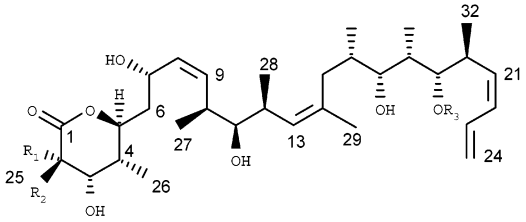
Discodermolide (**1**) and five new discodermolide analogues trivially named 2-*epi*-discodermolide (**2**), 2-*des*-methyldiscodermolide (**3**), 5-hydroxymethyldiscodermolate (**4**), 19-*des*-aminocarbonyldiscodermolide (**5**), and 9(13)-cyclodiscodermolide (**6**) have been isolated from marine sponges belonging to the genus *Discodermia* collected from the Caribbean Sea. The isolation, structure elucidation, and biological activities of **2**−**6** are described. The natural analogues, which were isolated in trace amounts, exhibited significant variation of cytotoxicity against the cultured murine P-388 leukemia and A-549 human adenocarcinoma cells and suggested the importance of the C_7 through C_{17} moiety for potency against cultured tumor cell lines.

In 1990, we reported the isolation and structure determination of the polypropionate-derived polyhydroxy-δ-lactone, (+)-discodermolide (**1**), from the Caribbean marine sponge *Discodermia dissoluta*.[1] Biological studies by our group and others have demonstrated its immunosuppressive and antimitotic activities.[2−4] (+)-Discodermolide has been shown to promote the rapid polymerization of purified tubulin and to hyperstabilize the microtubule complex in cultured cells[5,6] with a mechanism of action similar to that of paclitaxel (Taxol). (+)-Discodermolide also inhibits the *in vitro* growth of several cancer cell lines, including paclitaxel-resistant ovarian and colon cancer cells.[7−9]

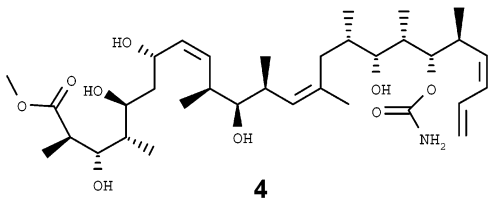
The Schreiber group has synthesized both antipodes of discodermolide, establishing the absolute configuration,[10] and prepared a number of structural variants.[11] Since then, several other groups have synthesized (+)-discodermolide,[12,13] antipode (−)-discodermolide,[14,15] or various fragments of discodermolide using different synthetic approaches.[16−29] In 2001, the Paterson group[30] synthesized (+)-discodermolide and three epimeric discodermolides. Recently, we reported the preparation, structure elucidation, biological activity, and structure−activity relationship of seven new acetylated analogues[31,32] and the previously reported discodermolide-3,7,11,17-tetraacetate[1] of natural (+)-discodermolide. Herein, we report the isolation, structure elucidation, biological activity, and structure−activity relationship of five new naturally occurring discodermolide analogues, **2**−**6**, from sponge samples of the genus *Discodermia*.

Results and Discussion

The sponge samples, *Discodermia* species, were collected in 1993 and 1998 from the Bahamian archipelago and stored at −20 °C until extraction. The EtOH extracts of the thawed sponges were partitioned between EtOAc and H_2O. The EtOAc-soluble fractions were chromatographed over Si gel with CH_2Cl_2/MeOH or EtOAc/MeOH step gradient, and the fractions collected were monitored for the presence of discodermolide analogues using TLC, P-388 assay and NMR techniques. The cytotoxic fractions, which showed ^{1}H NMR spectra similar to that of discodermolide, were further purified by HPLC to yield compounds **2**−**6**.



Compound	R_1	R_2	R_3
1	H	Me	$CONH_2$
2	Me	H	$CONH_2$
3	H	H	$CONH_2$
5	H	Me	H



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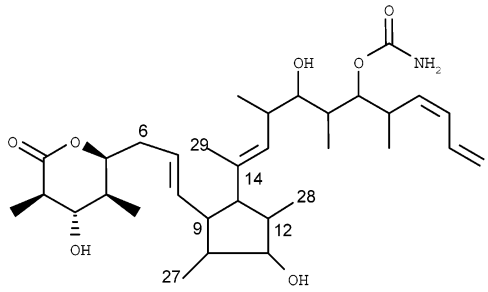

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HRFABMS of 2-*epi*-discodermolide (**2**) supported the molecular formula $C_{33}H_{55}NO_8$ [$(M + H)^+$ *m*/*z* 594.4003, Δ 0.2 mmu], and it is identical to that reported for discodermolide. The ^{1}H and ^{13}C NMR spectra of 2-*epi*-discodermolide were very similar to that of discodermolide and

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Table 1. ^{1}H NMR Data of Discodermolide (**1**) and Natural Analogues **2** and **3**[a]

	discodermolide (**1**)	2-*epi*-discodermolide (**2**)	2-*des*-methyldiscodermolide (**3**)
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
2	2.56 (dq, 4.2, 7.2)	2.48 (dq, 2.3, 7.5)	2.57 (dd, 3.8, 17.6)
2			2.50 (dd, 2.9, 17.6)
3	3.61 (ddd, 4.2, 4.2, 4.6)	3.73 (ddd, 2.3, 3.5, 4.7)	3.95 (dddd, 2.9, 3.8, 3.8, 4.2)
3-OH	3.27 (d, 4.6)	3.32 (d, 4.7)	3.27 (d, 3.8)
4	1.83 (ddq, 2.0, 4.2, 6.9)	1.79 (m)	1.70 (m)
5	4.45 (dt, 2.0, 10.1)	4.48 (dt, 2.0, 10.0)	4.50 (dt, 2.0, 10.7)
6	1.72 (m)	1.72 (m)	1.72 (m)
6	1.47 (ddd, 2.4, 10.8, 13.0)	1.48 (ddd, 2.5, 10.8, 13.0)	1.48 (ddd, 2.3, 10.9, 13.0)
7	4.44 (m)	4.44 (m)	4.46 (m)
7-OH	2.75 (d, 5.3)	2.73 (d, 5.2)	2.74 (d, 5.3)
8	5.38 (ddd, 2.2, 9.1, 10.9)	5.38 (dd, 9.1, 10.9)	5.38 (dd, 8.4, 10−0)
9	5.54 (ddd, 1, 10.1, 10.9)	5.54 (dd, 10.1, 10.9)	5.54 (dd, 10.1, 10.9)
10	2.62 (m)	2.62 (m)	2.62 (m)
11	3.05 (m)	3.05 (m)	3.06 (m)
11-OH	2.64(d, 5.2)	2.64 (d, 5.0)	2.64 (d, 5.3)
12	2.29 (ddq, 5.2, 6.6, 10.0)	2.27 (ddq, 5.2, 6.5, 10.0)	2.27 (ddq, 5.2,6.6, 10.0)
13	4.97 (d, 10.0)	4.95 (d, 10.0)	4.96 (d, 10.0)
15	1.76 (m)	1.76 (m)	1.78 (m)
15	1.63 (dd, 3.6, 12.2)	1.60 (dd, 3.6, 12.2)	1.61 (dd, 3.6, 12.2)
16	1.76 (m)	1.72 (m)	1.71 (m)
17	3.13 (dd, 3.5, 6.2)	3.13 (ddd, 3.5, 6.2, 6.5)	3.13 (ddd, 4.1, 6.2, 6.4)
17-OH	2.59 (d, 6.5)	2.59 (d, 6.5)	2.59 (d, 6.4)
18	1.72 (m)	1.75 (m)	1.72 (m)
19	4.71 (dd, 4.1, 8.0)	4.71 (dd, 4.2, 8.0)	4.72 (dd, 4.2, 8.0)
20	3.07 (m)	3.07 (m)	3.08 (m)
21	5.42 (dd, 10.6, 10.7)	5.42 (dd, 10.6, 10.6)	5.42 (dd, 10.4, 10.9)
22	6.06 (dd, 10.7, 11.0)	6.07 (dd, 10.6, 11.0)	6.07 (dd, 10.9, 11.0)
23	6.68 (ddd, 10.5, 11.0, 16.6)	6.66 (ddd, 10.5, 11.0, 16.6)	6.67 (ddd, 10.1, 11.0, 16.8)
24	5.21 (d, 16.6)	5.24 (d, 16.6)	5.24 (d, 16.8)
24	5.10 (d, 10.1)	5.14 (d, 10.0)	5.14 (d, 10.1)
25	1.18 (d, 7.2)	1.16 (d, 6.7)	-
26	0.97 (d, 6.9)	1.02 (d, 6.2)	1.01 (d, 6.8)
27	1.00 (d, 6.9)	1.00 (d, 5.4)	1.01 (d, 6.8)
28	0.88 (d, 6.6)	0.87 (d, 6.5)	0.88 (d, 6.6)
29	1.57 (s)	1.56 (s)	1.57 (s)
30	0.73 (d, 6.2)	0.73 (d, 6.2)	0.73 (d, 6.2)
31	0.80 (d, 6.5)	0.79 (d, 6.5)	0.80 (d, 6.5)
32	0.93 (d, 6.7)	0.95 (d, 6.7)	0.95 (d, 6.7)
NH_2	5.05 (br s)	5.05 (br s)	5.05 (br s)

[a] All spectra run at 500 MHz in CDCN. Chemical shifts are reported in ppm, and J values in Hz.

indicated a few minor chemical shift and coupling constant differences around the δ-lactone functionality. The NOESY spectrum of discodermolide (**1**) gave correlations of C_{25}−Me/C_3−H, C_{25}−Me/C_4−H, C_2−H/C_3−H, C_{26}−Me/C_3−H, and C_3−H/C_4−H, in agreement with the reported solution structure that has an axial methyl at C_2, an axial hydroxyl at C_3, and an equatorial methyl at the C_4 positions,[33] whereas the NOESY spectrum of 2-*epi*-discodermolide gave correlations of C_{25}−Me/C_3−H, C_2−H/C_4−H, C_{26}−Me/C_3−H, and C_3−H/C_4−H. The strong NOE correlation between C_2−H and C_4−H indicated the one−three diaxial arrangement of these hydrogen atoms. These data confirmed that the C_{25}−Me, which has an axial arrangement in discodermolide, has flipped to an equatorial arrangement in 2-*epi*-discodermolide. Comparison of NMR data in Tables 1 and 3 together with the high-resolution mass spectral data confirmed the structure of 2-*epi*-discodermolide (**2**).

HRFABMS of 2-*des*-methyldiscodermolide (**3**) supported the molecular formula $C_{32}H_{53}NO_8$ [$(M + H)^+$ m/z 580.3853, Δ 0.4 mmu], and it indicated a difference in elements CH_2 (14 mmu) from discodermolide and 2-*epi*-discodermolide. The ^{1}H NMR spectrum of 2-*des*-methyldiscodermolide was very similar to that of discodermolide. The ^{1}H NMR spectrum indicated signals for seven methyl groups instead of the eight methyl groups present in discodermolide. Detailed analysis of the ^{1}H NMR indicated the absence of the signal corresponding to the C_{25} methyl doublet that appears characteristically downfield in discodermolide due to deshielding by the adjacent carbonyl group. The DEPT spectrum showed the replacement of the C_2 methine carbon by a methylene carbon appearing at 40.3 ppm. The COSY spectrum clearly showed the coupling of this new methylene group observed at 2.52 and 2.56 ppm to the C_3 hydroxy methine observed at 3.95 ppm. Comparison of the NMR data in Tables 1 and 3 together with the mass spectral data confirmed the structure of 2-*des*-methyldiscodermolide (**3**).

HRFABMS of 5-hydroxymethyldiscodermolate (**4**) supported the molecular formula $C_{34}H_{59}NO_9$ [$(M + H)^+$ m/z 626.4252, Δ 1.6 mmu], and it indicated a difference in elements CH_4O (32 mmu) from discodermolide. The ^{1}H NMR spectrum of 5-hydroxymethyldiscodermolate as expected was very similar to that of discodermolide. The ^{1}H NMR spectrum showed an additional three-proton singlet for a methoxy group at 3.63 ppm. The C_5 δ-lactone proton, which appeared at 4.46 ppm in discodermolide, indicated an upfield shift to 3.90 ppm, suggesting the presence of a free hydroxyl group at this position. The ^{13}C NMR spectrum showed an upfield shift of 4.6 ppm for C_5 compared to that of discodermolide and an additional signal at 52.2 ppm characteristic for the methyl ester group. The selective INAPT spectrum revealed a three-bond correlation between the methoxy protons and the ester carbonyl at 176.7 ppm. Comparison of the NMR data in Tables 1, 2, and 3 together with mass spectral data established the structure of 5-hydroxymethyldiscodermolate (**4**). The stereochemistry was confirmed by comparison with an authentic sample of **4** prepared by acid-catalyzed methanolysis of discodermolide (**1**).

Table 2. ^{1}H NMR Data of Natural Analogues **4**, **5**, and **6**[a]

	5-hydroxymethyl discodermolate (**4**)[b]	19-*des*-aminocarbonyl discodermolide (**5**)[b]	9(13)-cyclodiscodermolide (**6**)[c]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
2	2.66 (m)	2.56 (dq, 4.2, 7.2)	2.61 (dq, 4.0, 7.3)
3	3.91 (m)	3.63 (ddd, 4.0, 4.2, 4.5)	3.61 (dd, 4.0, 5.0)
3-OH	4.08 (d, 4.9)	3.27 (d, 4.5)	
4	1.64 (ddq, 2.5, 5.0, 6.9)	1.85 (m)	1.96 (ddq, 5.0, 5.4, 7.1)
5	3.90 (m)	4.45 (m)	4.33 (ddd, 5.4, 7.9, 10.0)
5-OH	3.97 (d, 4.2)		
6	1.63 (m)	1.71 (m)	2.25 (ddd, 5.6, 7.9, 14.9)
6	1.45 (ddd, 3.1, 9.3, 12.0)	1.49 (ddd, 2.3, 10.7, 12.4)	2.46 (ddd, 5.6, 10.0, 14.9)
7	4.50 (ddd, 7.5, 9.1, 9.3)	4.45 (m)	5.36 (ddd, 5.6, 5.6, 15.4)
7-OH	3.02 (d, 4.5)	2.76 (d, 5.4)	
8	5.42 (m)	5.35 (dd, 8.7, 11.0)	5.26 (dd, 8.5, 15.4)
9	5.42 (m)	5.49 (dd, 10.5, 11.0)	2.08 (ddd, 8.5, 10.1, 10.1)
10	2.64 (m)	2.62 (m)	1.68 (m)
11	3.07 (m)	3.09 (ddd, 4.8, 5.3, 6.8)	3.61 (m)
11-OH	2.65 (d, 4.8)	2.62 (d, 5.3)	
12	2.36 (ddq, 5.2, 6.8, 9.9)	2.35 (ddq, 5.4, 6.8, 10.0)	1.68 (m)
13	5.03 (d, 9.9)	5.03 (d, 10.0)	1.57 (m)
14			
15	1.79 (m)	1.79 (m)	4.75 (d, 9.9)
15	1.68 (m)	1.73 (m)	
16	1.76 (m)	1.76 (m)	2.47 (m)
17	3.14 (dd, 4.3, 6.2)	3.27 (ddd 4.1, 5.0, 6.2)	3.30 (m)
17-OH	2.59 (d, 6.5)	2.98 (d, 5.0)	
18	1.74 (m)	1.72 (m)	1.82 (d, 3.0, 6.5, 7.0)
19	4.70 (dd, 4.7, 7.5)	3.41 (ddd, 3.3, 4.2, 6.8)	4.81 (m)
19-OH		2.90 (d, 3.3)	
20	3.08 (m)	2.84 (ddq, 6.9, 7.0, 10.0)	3.14 (ddq, 7.2, 9.8, 10.8)
21	5.42 (m)	5.40 (dd, 10.5, 10.5)	5.46 (dd, 10.8, 11.0)
22	6.04 (dd, 10.9, 11.0)	6.06 (dd, 10.5, 11.0)	6.04 (dd, 11.0, 11.0)
23	6.67 (ddd, 10.4, 11.0, 16.8)	6.68 (ddd, 10.5, 11.0, 16.8)	6.68 (ddd, 10.2, 11.0, 16.7)
24	5.23 (d, 16.8)	5.21 (d, 16.8)	5.25 (d, 16.7)
24	5.13 (d, 10.4)	5.10 (d, 10.5)	5.16 (d, 10.2)
25	1.07 (d, 7.1)	1.19 (d, 7.2)	1.24 (d, 7.3)
26	0.76 (d, 7.0)	0.97 (d, 6.9)	0.99 (d, 7.1)
27	0.98 (d, 6.7)	1.00 (d, 6.9)	0.92 (d, 7.1)
28	0.88 (d, 6.7)	0.90 (d, 6.8)	0.93 (d, 7.2)
29	1.59 (s)	1.61 (s)	1.54 (s)
30	0.74 (d, 6.3)	0.76 (d, 6.5)	0.94 (d, 7.0)
31	0.84 (d, 6.7)	0.88 (d, 6.9)	0.78 (d, 7.0)
32	0.95 (d, 6.6)	0.93 (d, 6.9)	0.99 (d, 7.2)
1-OMe	3.63 (s)		
NH_2	5.05 (br s)		

[a] All spectra run at 500 MHz. Chemical shifts are reported in ppm, and J values in Hz. [b] In CD_3CN. [c] In CD_3OD.

HRFABMS of 19-*des*-aminocarbonyldiscodermolide (**5**) supported the molecular formula $C_{32}H_{54}O_7$ [$(M + H)^+$ m/z 551.3937, Δ 1.0 mmu], and it indicated a difference in elements CHNO (43 mmu) from discodermolide. The ^{1}H NMR spectrum of 19-*des*-aminocarbonyldiscodermolide closely resembled that of discodermolide. The ^{1}H NMR spectrum indicated the absence of the characteristic two-proton signal corresponding to the NH_2 group in the aminocarbonyl group. The two aminocarbonyl protons appear as a broad signal at 5.05 ppm in discodermolide (see Table 1). The C_{19} aminocarbonyloxymethine proton that appears at 4.71 ppm in discodermolide showed an upfield shift to 3.41 ppm in **5**, indicating the presence of a typical hydroxymethine proton. The ^{13}C NMR spectrum did not contain a signal corresponding to an aminocarbonyloxy carbon, which appears at 158.4 ppm in discodermolide (see Table 3). Comparison of the NMR data in Tables 1, 2, and 3 together with mass spectral data confirmed the structure of 19-*des*-aminocarbonyldiscodermolide (**5**).

HRFABMS of 9(13)-cyclodiscodermolide (**6**) supported the molecular formula $C_{33}H_{53}NO_7$ [$(M + H)^+$ m/z 576.3966, Δ 6.6 mmu], and it indicated a difference in elements H_2O (18 mmu) from discodermolide. The ^{13}C spectral comparison revealed that the resonances attributed to one oxygenated carbon and one methylene carbon observed for discodermolide have been replaced by two methine carbons in 9(13)-cyclodiscodermolide (see Table 3). These data together with the molecular formula suggested an additional unsaturation equivalent in 9(13)-cyclodiscodermolide accounting for a second ring system in the molecule. The ^{1}H NMR spectral pattern of 9(13)-cyclodiscodermolide (**6**) was similar to that of discodermolide, but the chemical shift values of the proton signals from C_6 through C_{16} were quite different. The ^{1}H NMR spectrum indicated a downfield shift of the two C_6 methylene protons by 0.71 ppm to an allylic position. In the COSY spectrum of **6**, the C_7 olefinic proton at 5.36 ppm indicated couplings to the C_6 allylic methylene protons observed at 2.25 and 2.46 ppm and to the C_8 olefinic proton observed at 5.26 ppm. The C_6 allylic methylene protons were in turn coupled to the C_5 oxymethine proton observed at 4.33 ppm. The *trans* arrangement of C_7 and C_8 olefinic protons was evident from the coupling constant of 15.4 Hz. Similarly, in the COSY spectrum, the C_9 allylic methine proton observed at 2.08 ppm (ddd, J = 8.5, 10.1, 10.1 Hz) showed couplings to the protons at C_8, 5.26 ppm (dd, J = 8.5, 15.4 Hz); C_{10}, 1.68 ppm (m); and allylic C_{13}, 1.57 ppm (m). The C_{10} methine proton was coupled to the C_{27} methyl and to the C_{11} hydroxymethine proton observed at 3.61 ppm, which was in turn coupled to the C_{12} methine proton observed at 1.68 ppm. The C_{12} methine proton, which was coupled to the C_{28} methyl protons observed at 0.93 ppm, indicated additional coupling to the C_{13} allylic proton,

Table 3. ^{13}C NMR Data of Compounds **1**−**6**[a]

position	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]
1	174.7 (s)	174.3 (s)	170.7 (s)	176.7 (s)	174.7 (s)	173.1 (s)
2	44.1 (d)	43.5 (d)	40.3 (t)	43.1 (d)	44.1 (d)	44.7 (d)
3	73.2 (d)	73.1 (d)	68.2 (d)	76.6 (d)	73.2 (d)	73.7 (d)
4	36.4 (d)	39.9 (d)	38.3 (d)	42.0 (d)	36.3 (d)	34.2 (d)
5	77.6 (d)	78.8 (d)	78.1 (d)	73.0 (d)	77.6 (d)	82.3 (d)
6	42.2 (t)	42.9 (t)	42.6 (t)	41.7 (t)	42.2 (t)	36.6 (t)
7	63.5 (d)	63.4 (d)	63.3 (d)	65.6 (d)	63.6 (d)	125.6 (d)
8	133.9 (d)	134.0 (d)	133.9 (d)	134.5 (d)	133.8 (d)	138.3 (d)
9	133.8 (d)	133.8 (d)	133.8 (d)	133.7 (d)	133.7 (d)	54.7 (d)
10	36.4 (d)	36.3 (d)	36.4 (d)	36.5 (d)	36.5 (d)	47.0 (d)
11	79.8 (d)	79.8 (d)	79.8 (d)	79.7 (d)	79.6 (d)	81.1 (d)
12	37.1 (d)	37.2 (d)	37.2 (d)	36.5 (d)	36.9 (d)	44.1 (d)
13	131.2 (d)	131.2 (d)	131.2 (d)	131.2 (d)	131.2 (d)	62.9 (d)
14	133.9 (s)	133.9 (s)	133.9 (s)	133.8 (s)	133.8 (s)	133.0 (s)
15	36.3 (t)	36.2 (t)	36.3 (t)	36.3 (t)	36.3 (t)	130.9 (d)
16	34.3 (d)	34.3 (d)	34.3 (d)	34.3 (d)	34.4 (d)	37.8 (d)
17	76.0 (d)	76.0 (d)	76.0 (d)	76.1 (d)	79.4 (d)	76.1 (d)
18	38.5 (d)	38.5 (d)	38.5 (d)	38.4 (d)	37.5 (d)	40.0 (d)
19	79.3 (d)	79.4 (d)	79.4 (d)	79.1 (d)	79.0 (d)	80.5 (d)
20	34.8 (d)	34.7 (d)	34.8 (d)	34.9 (d)	36.5 (d)	34.9 (d)
21	134.3 (d)	134.2 (d)	134.3 (d)	134.2 (d)	136.5 (d)	134.1 (d)
22	130.4 (d)	130.6 (d)	130.6 (d)	130.4 (d)	130.3 (d)	130.7 (d)
23	133.3 (d)	133.2 (d)	133.3 (d)	133.3 (d)	133.9 (d)	133.5 (d)
24	118.3 (t)	118.6 (t)	118.6 (t)	118.3 (t)	117.9 (t)	118.4 (t)
25	15.8 (q)	13.3 (q)		9.3 (q)	15.8 (q)	16.2 (q)
26	13.1 (q)	14.7 (q)	14.3 (q)	12.9 (q)	13.1 (q)	13.0 (q)
27	19.7 (q)	19.8 (q)	19.8 (q)	19.3 (q)	19.3 (q)	18.9 (q)
28	17.5 (q)	17.6 (q)	17.6 (q)	16.7 (q)	17.1 (q)	18.1 (q)
29	23.3 (q)	23.3 (q)	23.3 (q)	23.4 (q)	23.5 (q)	14.0 (q)
30	15.5 (q)	15.6 (q)	15.5 (q)	15.2 (q)	15.4 (q)	13.3 (q)
31	9.2 (q)	9.1 (q)	9.1 (q)	9.2 (q)	7.2 (q)	9.0 (q)
32	18.2 (q)	18.2 (q)	18.2 (q)	18.2 (q)	18.1 (q)	18.4 (q)
33	158.4 (s)	158.4 (s)	158.4 (s)	158.3 (s)		158.3 (s)
1-OCH_3			52.2 (q)			

[a] All spectra run at 125.7 MHz. Chemical shifts are reported in ppm. [b] In CD_3CN, [c] In CD_3OD.

which in turn did not show coupling to the C_{15} olefinic proton observed at 4.75 ppm. The C_{29} olefinic methyl observed at 1.54 (s) indicated allylic coupling to the C_{15} olefinic proton, which in turn was coupled to the C_{16} allylic methine proton observed at 2.47 ppm. These data together with the absence of the second methylene signal corresponding to the C_{15} methylene in discodermolide established the C_6 through C_{16} substructure for this compound. The remaining coupling patterns (C_2 through C_5 and C_{17} through C_{24}) were similar to that of discodermolide. Comparison of the ^{13}C data with that of discodermolide indicated that one hydroxymethine doublet (C_7) and a methylene triplet (C_{15}) present in discodermolide have been replaced by two methine doublets (C_9 and C_{13}) in **6**. The high-field chemical shift value of 14.0 ppm for the C_{29} olefinic methyl (see Table 3) indicated *trans* stereochemistry for the C_{14} double bond. These data in combination with the high-resolution mass spectral data confirmed the structure of 9(13)-cyclodiscodermolide (**6**). The stereochemistry of the C_9 to C_{20} side chain was not determined due to insufficient material on hand after biological testing.

Biological Activity of Discodermolide (1) and Natural Analogues 2−6. Discodermolide (**1**) and its naturally occurring analogues **2**−**6** were tested for their in vitro cytotoxicity to cultured murine P-388 leukemia and human lung adenocarcinoma A-549 cell lines. These compounds inhibited the in vitro proliferation of the P-388 cell line, with IC_{50} values of **1**, 35; **2**, 134; **3**, 172; **4**, 65.8; **5**, 128; and **6**, 5043 nM[34] and the A-549 cell line, with IC_{50} values of **1**, 13.5; **2**, 67; **3**, 120; **4**, 74; **5**, 74; and **6**, 4487 nM.[34] These activity data indicated that changes in the δ-lactone ring, more specifically at C_1 (opened lactone, **4**) and C_{25} (methyl group, **2**, **3**), have a minor contribution toward the activity. Similarly, any changes at the tail end of the molecule (carbamate group, **5**) indicated no significant decrease in activity. Interestingly, changes in the middle section of the molecule (**6**) indicated a complete loss of activity. These results are consistent with our earlier findings[31,32] that acetylation at C_{11} and more specifically at C_{17} caused a dramatic reduction in activity. These data supported our earlier conclusion[31,32] that the C_7 through C_{17} moieties contribute to the overall cytotoxicity of discodermolide. The complete biological activity profile, including the effects of analogues on tubulin polymerization both within cells and using purified tubulin and the G_2/M blocking activity, will be published elsewhere. The details of P-388 and A-549 assays were described in the preceding publications.[31,32]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP-360 digital polarimeter. IR spectra were obtained on a Midac M-1200 with Galactic GRAMS/386 software. 1D and 2D NMR spectra were measured on a Bruker AMX-500 instrument. The ^1H NMR chemical shifts (referenced to CD_3CN observed at 1.93 ppm or CD_3OD observed at 3.30 ppm) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to solvent) were assigned on the basis of DEPT and HMQC experiments. The HRMS were obtained on a Finnigan MAT95Q mass spectrometer at the Spectroscopic Services Group, University of Florida, Gainesville, FL.

Collection and Taxonomy. The sponge sample *Discodermia* sp. HBOI # 23-XI-98-1-005, family Theonellidea, was collected on November 1998 by manned submersible east of Bell Channel Bouy, Grand Bahama Island, Bahamas (latitude 26°30.662′ N; longitude 78°34.976′ W, depth 147 m). The morphology of the sponge varies from large cup-shaped to irregular cups forming branches. The sponge is tan in color

and rare in this location. A taxonomic reference sample has been deposited in the Harbor Branch Oceanographic Museum (HBOM), catalog number 003:00971. The sponge samples *Discodermia* species HBOI #s 23-XI-98-3-001 and 23-XI-98-3-002 were collected on November 1998 by manned submersible from Lucaya, Grand Bahama Island, Bahamas (latitude 26°30.727′ N; longitude 78°35.026′ W, depth 157 m). The specimen 23-XI-98-3-001 is cream in color and morphology is thin, irregular anastomosing branches. A taxonomic reference sample has been deposited in the HBOM, catalog number 003:00972. The specimen 23-XI-98-3-002 is tan in color and morphology is a cluster of fingers. A taxonomic reference sample has been deposited in the HBOM, catalog number 003:00973. The sponge samples *Discodermia* species HBOI #s 6-VI-93-1-005, -007, -008, -009, -010 were collected on June 6, 1993, by a manned submersible from the Tartar Bank, south of Cat Island, Bahamas (latitude 24°02.104′ N; longitude 75°28.309′ W, depth 183−198 m). The specimens are cup-shaped and white to tan to yellow in color. Taxonomic reference samples have been deposited in the HBOM, catalog numbers 003:00984 to 003:00988.

Extraction and Isolation of Discodermolide (1) and 2-*epi*-Discodermolide (2). The sponge specimen *Discodermia* sp. 23-XI-98-1-005 (1931 g), which was stored at −20 °C, was thawed and extracted with EtOH (3 L). The EtOH extract was concentrated on a water bath under vacuum, and the concentrate was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (4.2 g) was chromatographed over silica gel (200 g) with CH_2Cl_2/MeOH gradient, and fractions were monitored by thin-layer chromatography and ^{1}H NMR for discodermolide and discodermolide analogues. The fraction that showed the presence of discodermolide and discodermolide analogues by ^{1}H NMR on further purification by HPLC (SiO_2, 5 μm, 250 × 10 mm) with 6% MeOH/CH_2Cl_2 gave 2-*epi*-discodermolide (**2**) as a white solid (0.3 mg, yield, 0.00001% of wet weight) and discodermolide (**1**) as a white solid (20 mg, yield, 0.001% of wet weight). The identity of discodermolide was confirmed by TLC, specific rotation, and NMR comparison with an authentic sample.

2-*Epi*-discodermolide (2): $[\alpha]^{21}_D$ +10.7° (*c* 0.1, MeOH); IR (neat/NaCl) ν_{max} 3394, 1720, 1041, 1028 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS (3-nitrobenzyl alcohol) *m/z* 594.4003, Δ 0.2 mmu for $C_{33}H_{56}NO_8$ $(M + H)^+$.

Extraction and Isolation of 2-*Des*-methyldiscodermolide (3) and 5-Hydroxymethyldiscodermolate (4). The frozen sponge specimen *Discodermia* sp. 23-XI-98-3-002 (3570 g) was extracted as above, and the residue was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (7.8 g) was chromatographed over silica gel (200 g) with CH_2Cl_2 followed by EtOAc/MeOH gradient, and the fractions were monitored by thin-layer chromatography and ^{1}H NMR spectra for the presence of discodermolide and discodermolide analogues. The TLC pattern and the ^{1}H NMR spectra of the fractions that eluted with 2−5% MeOH/EtOAc showed the presence of discodermolide analogues in addition to discodermolide. The fraction that eluted with 5% MeOH/EtOAc on further purification by HPLC (SiO_2, 5 μm, 250 × 10 mm) with 7% MeOH/CH_2Cl_2 gave 2-*des*-methyldiscodermolide as a white solid (1.0 mg, yield, 0.00003% of wet weight). Similarly, the fraction that eluted with 2% MeOH/EtOAc on further purification by HPLC (SiO_2, 5 μm, 250 × 10 mm) with 7% MeOH/CH_2Cl_2 followed by HPLC with 4% MeOH/CH_2Cl_2 gave 5-hydroxymethyldiscodermolate (**4**) as a white solid (1.1 mg, yield, 0.00003% of wet weight).

2-*Des*-methyldiscodermolide (3): $[\alpha]^{21}_D$ +10.2° (*c* 0.1, MeOH); IR (neat/NaCl) ν_{max} 3374, 1721, 1710, 1323, 1037 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS (glycerol) *m/z* 580.3853, Δ 0.4 mmu for $C_{32}H_{54}NO_8$ $(M + H)^+$.

5-Hydroxymethyldiscodermolate (4): $[\alpha]^{21}_D$ +14.6° (*c* 0.1, MeOH); IR (neat/NaCl) ν_{max} 3361, 1710, 1393, 1046 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS (glycerol) *m/z* 626.4252, Δ 1.6 mmu for $C_{34}H_{60}NO_9$ $(M + H)^+$.

Preparation of 5-Hydroxymethyldiscodermolate (4). A solution of discodermolide (4.0 mg) in 10% MeOH/CH_2Cl_2 (2.0 mL) and SiO_2 gel (20 mg, EM Science, 230−400 mesh) was stirred for 5 days at 35 °C. The reaction mixture was filtered, and the filtrate was evaporated under a stream of N_2 to give a colorless residue (∼4 mg). The residues was subjected to HPLC on a SiO_2 gel (Lichrosorb 5μ, 250 × 10 mm) column using a mixture of 7% MeOH in CH_2Cl_2 to yield 5-hydroxymethyldiscodermolate (**4**, 0.8 mg) and unreacted discodermolide (**1**, 2.8 mg).

Extraction and Isolation of 19-*Des*-aminocarbonyldiscodermolide (5). The frozen sponge specimen *Discodermia* sp. 23-XI-98-3-001 (2480 g) was extracted as above, and the residue was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (4.4 g) was chromatographed over silica gel (200 g) with MeOH/EtOAc, and fractions were monitored by thin-layer chromatography and ^{1}H NMR spectra for discodermolide and discodermolide analogues. The ^{1}H NMR spectrum of the fraction that eluted with 2% MeOH/EtOAc showed the presence of a discodermolide analogue in addition to discodermolide. This fraction (0.10 g) on further purification by HPLC (SiO_2, 5 μm, 250 × 10 mm) with 6% MeOH/CH_2Cl_2 followed by HPLC with 3% MeOH/CH_2Cl_2 gave 19-*des*-aminocarbonyldiscodermolide (**5**) as a white solid (1.1 mg, yield, 0.00006% of wet weight).

19-Desaminocarbonyldiscodermolide (5): $[\alpha]^{21}_D$ +18.0° (*c* 0.1, MeOH); IR (neat/NaCl) ν_{max} 3393, 1103, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS (glycerol) *m/z* 551.3937, Δ 1.0 mmu for $C_{32}H_{55}NO_7$ $(M + H)^+$.

Extraction and Isolation of 9(13)-Cyclodiscodermolide (6). The frozen sponge specimens *Discodermia* spp. 6-VI-93-1-005, -007, -008, -009, -010 (2500 g) were processed as above, and the residue was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (1.8 g) was chromatographed over silica gel (60 g) with a CH_2Cl_2/MeOH gradient, and the fractions were monitored by thin-layer chromatography and ^{1}H NMR for discodermolide and discodermolide analogues. The fraction that eluted with 5% MeOH/CH_2Cl_2 on further purification by HPLC (SiO_2, 5 μm, 250 × 10 mm) with 5.5% MeOH/CH_2Cl_2 gave 9(13)-cyclodiscodermolide (**6**) as a white solid (1.2 mg, yield, 0.00005% of wet weight).

9-(13)-Cyclodiscodermolide (6): $[\alpha]^{21}_D$ +24.0° (*c* 0.01, MeOH); IR (neat/NaCl) ν_{max} 3393, 1595, 1392, 1047, 976 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS (glycerol) *m/z* 576.3966, Δ 6.6 mmu for $C_{33}H_{54}NO_7$ $(M + H)^+$.

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