Acetylated Analogues of the Microtubule-Stabilizing Agent Discodermolide: Preparation and Biological Activity

Sarath P. Gunasekera,* Ross E. Longley, and Richard A. Isbrucker

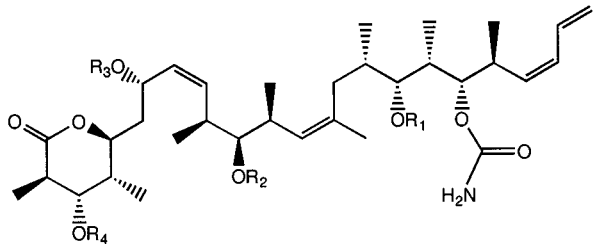
Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, 5600 U.S. 1 North, Fort Pierce, Florida 34946

Received August 30, 2000

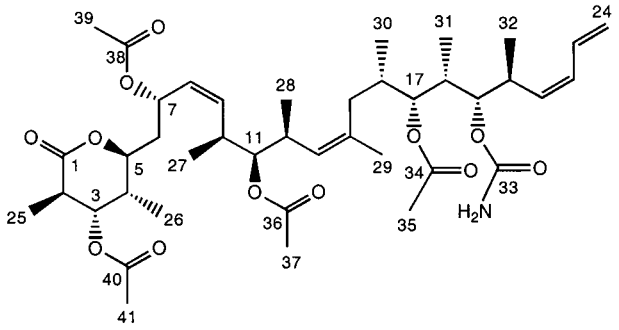
A series of eight discodermolide acetates have been prepared using natural (+)-discodermolide and evaluated for *in vitro* cytotoxicity against the cultured murine P-388 leukemia cells. The acetylated analogues showed a significant variation of cytotoxicity and suggested the importance of C-11 and C-17 hydroxyl groups for potency. The preparation and structure elucidation of the new analogues are described.

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

The interesting biological activity of **1** has stimulated efforts toward its total synthesis due to its low yield (0.001 to 0.002% wet wt) in the marine sponge. The Schreiber group has synthesized both antipodes, establishing the absolute configuration,[10] along with the preparation of 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.[16−28] Herein, we wish to report the preparation, structural elucidation, biological activity, and structure−activity relationship of seven new acetylated analogues and the previously reported discodermolide-3,7,11,17-tetraacetate[1] of natural (+)-discodermolide.



Compound	R_1	R_2	R_3	R_4
1	H	H	H	H
3	Ac	H	Ac	Ac
4	H	Ac	Ac	Ac
5	H	H	Ac	Ac
6	H	Ac	H	Ac
7	Ac	H	H	Ac
8	H	H	H	Ac
9	H	H	Ac	H



Discodermolide-3,7,11,17-tetraacetate (**2**)

Results and Discussion

The starting material, natural (+)-discodermolide, was isolated from the sponge *D. dissoluta* and crystallized following the reported procedure.[1] The acetylated analogues were prepared by treating compound **1** in dry pyridine with acetic anhydride at controlled temperature around 15 °C. The reaction mixture was periodically monitored using TLC to achieve the highest number of reaction products. The solvents were evaporated and the resulting mixture was separated by HPLC to give eight discodermolide analogues, **2**−**9**. The high-resolution mass spectral data of the analogues in combination with the number of acetoxymethyl groups appearing in the respective ^{1}H NMR spectra (Tables 1 and 2) suggested the presence of one tetraacetate (**2**), two triacetates (**3**, **4**), three diacetates (**5**, **6**, **7**), and two monoacetates (**8**, **9**). The number of acetoxymethyl signals and acetoxycarbonyl signals in their respective ^{13}C NMR spectra (Table 3) established the presence of the above four sets of acetylated analogues. The ^{1}H NMR data of the new analogues (**3**−**9**) were compared to those reported for discodermolide and discodermolide tetraacetate,[1] and using the COSY data in combination with the HMQC data, the proton chemical shift values of all analogues were assigned (Tables 1 and 2). The ^{1}H NMR spectra of the analogues indicated a significant downfield shift of the signals corresponding to the acetoxymethines (see Table 4), and these data were used to determine the position of acetylation in each analogue. The ^{1}H NMR spectrum of **2**, when compared with that of discodermolide, showed the presence of four additional acetoxymethyls (δ 2.04, 2.04, 1.97, 1.96) and downfield shifts of H-3 (Δ 1.26 ppm), H-7 (Δ 1.01 ppm), H-11 (Δ 1.49 ppm), and H-17 (Δ 1.56 ppm), and these data

* To whom correspondence should be addressed. Tel: 561-465-2400. Fax: 561-461-2221. E-mail: sgunaseker@hboi.edu.

10.1021/np000423e CCC: $20.00
Published on Web 01/05/2001

Table 1. ^{1}H NMR Data for Compounds **2**−**5** in $CDCl_3$ (chemical shifts ppm from solvent)

position	**2** δ 1H (mult., *J* in Hz)	**3** δ 1H (mult., *J* in Hz)	**4** δ 1H (mult., *J* in Hz)	**5** δ 1H (mult., *J* in Hz)
2	2.66 (dq, 5.8, 7.3)	2.70 (dq, 5.8, 7.4)	2.73 (dq, 5.8, 7.4)	2.72 (dq, 5.8, 7.5)
3	4.83 (dd 4.3, 4.2)	4.88 (dd, 4.2, 4.2)	4.89 (dd, 4.1, 4.1)	4.88 (dd, 4.1, 5.8)
4	2.09 (m)	2.05 (m)	2.08 (m	2.06 (m)
5	4.22 (dt, 1,5, 9.7)	4.32 (dt, 1.5, 9.7)	4.30 (dt, 1.5, 9.7)	4.35 (dt 1.5, 9.7)
6	2.10 (ddd, 8.3, 9.7, 12.6)	2.10 (m)	2.10 (ddd, 8.3, 9.7, 12.6)	2.10 (m)
6	1.64 (ddd, 3.2, 9.7, 12.6)	1.78 (ddd, 3.2, 9.7, 12.6)	1.70 (ddd, 3.2, 9.7, 12.6)	1.81 (ddd, 3.2, 9.5, 14.8)
7	5.61 (dt, 3.2, 8.9)	5.72 (dt 3.2, 8.9)	5.72 (dt, 3.2, 8.9)	5.74 (3.2, 9.0, 14.8)
8	5.21 (dd, 9.0, 10.7)	5.35 (dd, 7.8, 10.3)	5.27 (dd, 9.0, 10.7)	5.37 (dd, 9.0, 10.8)
9	5.42 (dd, 10.7, 10.7)	5.45 (dd, 9.1, 10.3)	5.44 (dd, 10.7, 10.7)	5.45 (dd, 10.2, 10.8)
10	2.79 (ddq, 6.1, 6.9, 10.7	2.72 (ddq, 6.1, 6.5, 10.3)	2.89 (ddq, 6.1, 6.9, 10.7)	2.73 (ddq, 6.1, 6.5, 10.2)
11	4.58 (dd, 6.1, 5.1)	3.12 (dd, 5.2, 6.5)	4.70 (dd, 5.6, 5.6)	3.18 (m)
12	2.44 (ddq, 5.1, 6.6, 9.9)	2.45 (ddq, 5.2, 7.1, 9.7)	2.63 (ddq, 5.1, 6.7, 9.7)	2.60 (ddq, 5.1, 6.7, 9.5)
13	4.89 (d, 9.9)	5.16 (d, 9.7)	4.94 (d, 9.7)	5.20 (d, 9.5)
15	1.80 (dd, 12.5, 11.8)	1.89 (dd, 17.0, 12.1)	1.94 (m)	1.90 (m)
15	1.61 (dd, 12.5, 10.0)	1.69 (dd, 12.1, 1.0)	1.61 (m)	1.90 (m)
16	2.00 (m)	2.07 (m)	1.92 (m)	1.88 (m)
17	4.71 (dd, 5.6, 5.8)	4.79 (dd, 5.9, 5.9)	3.25 (dd, 4.9, 4.9)	3.27 (m)
18	1.91 (ddq, 5.6, 6.1, 6.8)	2.01 (ddq, 5.9, 6.1, 7.2)	1.87 (m)	1.85 (m)
19	4.52 (dd, 6.1, 6.1)	4.61 (dd, 6.1, 6.1)	4.71 (dd, 6.1, 6.1)	4.72 (dd, 4.4, 7.2)
20	3.07 (ddq, 6.1, 6.6, 11.0)	3.12 (ddq, 6.1, 6.9, 10.6)	3.01 (ddq, 6.1, 6.5, 10.4)	2.97 (ddq, 6.2, 6.9, 10.2)
21	5.25 (ddd, 1.1, 11.0, 10.0)	5.32 (dd, 10.6, 11.0)	5.29 (dd, 10.4, 10.4)	2.34 (dd, 10.6, 11.0)
22	5.97 (dd, 11.0, 11.0)	6.02 (dd, 11.0, 11.0)	6.02 (dd, 11.0, 11.0)	6.01 (dd, 11.0, 11.0)
23	6.65 (ddd, 10.2, 11.0, 16.8)	6.69 (ddd, 10.8, 11.0, 16.9)	6.54 (ddd, 9.5, 11.0, 16.8)	5.60 (ddd, 9.6, 11.0, 16.9)
24	5.16 (d, 16.8)	5.20 (d, 16.9)	5.14 (d, 16.8)	5.19 (d, 16.9)
24	5.10 (d, 10.2)	5.14 (d, 10.8)	5.10 (d, 9.5)	5.10 (d, 9.6)
25	1.24 (d, 7.3)	1.29 (d, 7.3)	1.30 (d, 7.4)	1.30 (d, 7.5)
26	0.93 (d, 6.7)	0.98 (d, 6.5)	0.98 (d, 6.5)	0.98 (d, 6.5)
27	0.90 (d, 6.9)	0.98 (6.9)	0.99 (d, 6.1)	0.99 (d, 6.5)
28	0.80 (d, 6.6)	0.91 (d, 7.1)	0.89 (d, 6.7)	0.94 (d, 6.7)
29	1.55 (s)	1.62 (s)	1.60 (s)	1.63 (s)
30	0.63 (d, 6.6)	0.72 (d, 6.6)	0.80 (d, 5.8)	0.82 (d, 5.9)
31	0.84 (d, 6.8)	0.91 (d, 7.2)	1.00 (d, 6.5)	0.98 (d, 6.5)
32	0.90 (d, 6.9)	0.97 (d, 6.9)	0.95 (d, 6.6)	0.99 (d, 6.5)
35	2.04 (s)	2.07 (s)		
37	1.97 (s)		2.01 (s)	
39	1.96 (s)	2.02 (s)	2.00 (s)	2.02 (s)
41	2.04 (s)	2.08 (s)	2.09 (s)	2.08 (s)

confirmed its structure. The two triacetate analogues indicated the presence of three acetoxy methyls in each compound and downfield shifts of H-3 (Δ 1.31 ppm), H-7 (Δ 1.12 ppm), and H-17 (Δ 1.64 ppm) in **3** and downfield shifts of H-3 (Δ 1.32 ppm), H-7 (Δ 1.12 ppm), and H-11 (Δ 1.61 ppm) in **4**. Similarly, the three diacetate analogues showed two acetoxymethyls in each compound and downfield shifts of H-3 (Δ 1.31 ppm) and H-7 (Δ 1.14 ppm) in **5**, H-3 (Δ 1.33 ppm) and H-11 (Δ 1.63 ppm) in **6**, and H-3 (Δ 1.32 ppm) and H-17 (Δ 1.63 ppm) in **7**. The remaining two monoacetates revealed the presence of one acetoxymethyl in each compound and downfield shifts of H-3 (Δ 1.32 ppm) in **8** and H-7 (Δ 1.10 ppm) in **9**. The combination of the above data confirmed the structures of all acetylated analogues.

Biological Activity of Discodermolide Acetates. Discodermolide (**1**) and its acetates **2**−**9** were tested for their *in vitro* cytotoxicity to cultured murine P388 leukemia cells. These compounds inhibited the *in vitro* proliferation of cultured murine leukemia cells with IC_{50} values of **1**, 35; **2**, 837; **3**, >6925; **4**, 166; **5**, 0.74; **6**, 103; **7**, 1149; **8**, 12.6; and **9**, 3.9 nM.[29] Acetylation of analogues on the left-hand side of the molecule, at positions C-3 and C-7, confers a greater cytotoxicity to the discodermolide structure as seen with discodermolide-3-acetate (**8**), discodermolide-7-acetate (**9**), and discodermolide-3,7-diacetate (**5**). Interestingly, analogues with acetyl groups at position C-11, discodermolide-3,11-diacetate (**6**) and discodermolide-3,7,11-triacetate (**4**), showed a reduced cytotoxicity as compared to the parent molecule, whereas compounds that include an acetylation at position C-17 cause a dramatic reduction in the activity of the specific analogues, as seen with discodermolide-3,7,11,17-tetraacetate (**2**), discodermolide-3,7,-17-triacetate (**3**), and discodermolide-3,17-diacetate (**7**). These results are in agreement with Hung *et al.*,[11] who reported a 10-fold reduction in the activity of discodermolide-17-acetate as compared to discodermolide in the [^{3}H]thymidine incorporation assay using MG63 cells. From these data it could be concluded that the C-11 and C-17 hydroxyl groups contribute to the overall cytotoxicity of discodermolide. Current research in this lab has indicated that neither **8** nor **9** is subject to degradation or enzymatic deacetylation under similar culture conditions using a human cancer cell line. The complete biological activity profile, including the effects of analogues on tubulin polymerization both within cells and using purified tubulin as well as cytotoxicity and G_2/M blocking activity, will be published elsewhere.

Experimental Section

General Experiment Procedures. 1D and 2D NMR spectra were measured on a Bruker AMX-500 instrument. The ^{1}H NMR chemical shifts were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts 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.

Preparation, Purification, and Identification of Discodermolide Acetates 2−**9.** Discodermolide (5.0 mg) was dissolved in dry pyridine (2.5 mL) and treated with acetic anhydride (7 μL) in a Reacti-vial. The content was stirred and maintained at 15−18 °C. The reaction mixture was monitored periodically by TLC to yield the most number of analogues, and the reaction was halted by adding a small piece of ice when

Table 2. ^{1}H NMR Data for Compounds **6**−**9** in $CDCl_3$ (chemical shifts ppm from solvent)

position	**6** δ 1H (mult., *J* in Hz)	**7** δ 1H (mult., *J* in Hz)	**8** δ 1H (mult., *J* in Hz)	**9** δ 1H (mult., *J* in Hz)
2	2.75 (dq, 4.5, 7.3)	2.74(dq, 5.8, 7.4)	2.74 (dq, 4.3, 7.4)	2.62 (dq, 5.8, 7.2)
3	4.90 (dd, 4.5, 4.3)	4.89 (dd, 4.2, 4.2)	4.89 (dd, 3.9, 4.1)	3.71 (dd, 5.8, 5.8)
4	2.09 (m)	2.07 (m)	2.05 (m)	1.90 (m)
5	4.57 (dt, 1,5, 10.0)	4.55 (dt, 1.5, 9.7)	4.58 (dt, 2.0, 10.1)	4.40 (dt, 1.5, 10.0)
6	1.80 (m)	1.82 (ddd, 8.3, 9.7, 12.3)	2.10 (m)	2.02 (m)
6	1.64 (ddd, 3.2, 9.7, 12.6)	1.70 (ddd, 3.2, 9.7, 12.3)	1.82 (ddd, 3.2, 9.7, 12.6)	1.80 (ddd, 3.2, 9.6, 12.5)
7	4.75 (m)	4.70 (dt 3.2, 8.9)	4.71 (dt, 3.2, 8.7)	5.70 (dt, 3.2, 8.9)
8	5.39 (dd, 7.8, 10.7)	5.48 (dd, 8.9, 10.7)	5.50 (dd, 7.8, 10.7)	5.36 (dd, 8.9, 10.3)
9	5.42 (dd, 10.7, 10.7)	5.45 (dd, 10.3, 10.7)	5.42 (dd, 10.3, 10.7)	5.46 (dd 10.3, 10.3)
10	2.88 (ddq, 6.4, 6.5, 10.7)	2.74 (m)	2.79 (ddq, 5.7, 6.7, 10.3)	2.75 (ddq, 10.3, 7.0)
11	4.72 (dd, 6.4, 5.5)	3.13 (m)	3.18 (dd, 5.7, 5.7)	3.19 (dd, 7,0. 4.2)
12	2.60 (ddq, 5.5, 6.6, 10.1)	2.40 (ddq, 5.1, 6.1, 9.9)	2.56 (ddq, 5.7, 7.7, 10.2)	2.61 (m)
13	4.94 (d, 10.1)	4.94 (d, 9.9)	5.10 (d,10.2)	5.19 (d, 10.1)
15	1.79 (dd, 12.5, 11.5)	1.91 (m)	1.89 (m)	1.90 (m)
15	1.61 (m)	1.63 (m)	1.69 (m)	1.61 (m)
16	1.89 (m)	2.05 (m)	1.91 (m)	1.90 (m)
17	3.25 (dd, 5.0, 5.5)	4.78 (dd, 5.0, 5.0)	3.27 (ddd, 5.0, 5.9, 5.9)	3.27 (dd, 5.5, 5.5)
18	1.85 (m)	2.00 (m)	1.92. (m)	1.92 (m)
19	4.61 (dd, 6.1, 6.4)	4.61 (dd, 6.1, 6.1)	4.71 (dd, 4.4, 7.2)	4.69 (dd, 7.4, 4.4)
20	3.00 (ddq, 6.4, 6.5, 10.6)	3.13 (m)	2.99 (ddq, 6.5, 7.2, 10.6)	2.98 (ddq, 7.4, 10.9, 6.5)
21	5.25 (dd, 10.6, 11.0)	5.32 (dd, 10.6, 10.6)	5.34 (dd, 10.6, 10.6)	5.36 (dd, 10.9, 10.9)
22	6.01 (dd, 11.0, 11.0)	6.02 (dd, 10.6, 11.0)	6.01 (dd, 10.6, 11.0)	6.01 (dd, 10.9, 11.0)
23	6.61 (ddd, 10.2, 11.0, 16.8)	6.70 (ddd 10.1, 11.0, 16.9)	6.60 (ddd, 10.4, 11.0, 16.9)	6.60 (ddd, 11.0, 16.9, 10.2)
24	5.24 (d, 16.8)	5.14 (d, 16.9)	5.19 (d, 16.9)	5.21 (d, 16.9)
24	5.11 (d, 10.2)	5.06 (d,10.1)	5.15 (d, 10.4)	5.10 (d, 10.3)
25	1.31 (d, 7.3)	1.30 (d, 7.4)	1.30 (d, 7.4)	1.29 (d, 7.2)
26	0.97 (d, 6.7)	0.97 (d, 6.0)	0.98 (d, 6.5)	1.03 (d, 6.8)
27	0.99 (d, 6.5)	1.00 (d, 6.7)	1.00 (d, 6.7)	0.99 (d, 7.0)
28	0.87 (d, 6.6)	0.98 (d, 6.1)	0.92 (d, 7.7)	0.90 (d, 6.8)
29	1.62 (s)	1.63 (s)	1.64 (s)	1.63 (s)
30	0.80 (d, 6.6)	0.71 (d, 6.6)	0.81 (d, 6.2)	0.81 (d, 6.0)
31	0.97 (d, 6.8)	0.91 (d, 6.4)	0.96 (d, 7.2)	0.97 (d, 6.5)
32	0.90 (d, 6.5)	0.95 (d, 6.6)	0.98 (d, 6.5)	0.96 (d, 6.5)
35		2.07 (s)		
37	2.03(s)			
39				2.01 (s)
41	2.08 (s)	2.07 (s)	2.08 (s)	

no discodermolide was visible on TLC. The solvents were evaporated under a stream of nitrogen. The reaction was repeated with another 11 mg of discodermolide. The residues were combined and subjected to HPLC on a SiO_2 gel (5 μm, Phenomenex semi-prep Lichrosorb) column using a mixture of 6% MeOH in CH_2Cl_2 to yield a mixture of less polar acetates, a mixture of polar acetates, and traces of unreacted discodermolide. The less polar acetate mixture was rechromatographed using the same HPLC system with 3% MeOH in CH_2Cl_2 to give pure discodermolide-3,7,11,17-tetraacetate (**2**, 3.5 mg), discodermolide-3,7,17-triacetate (**3**, 1.3 mg), discodermolide-3,7,11-triacetate (**4**, 3.0 mg), discodermolide-3,7-diacetate (**5**, 2.8 mg), discodermolide-3,11-diacetate (**6**, 1.0 mg), and discodermolide-3,17-diacetate (**7**, 0.8 mg). Similarly, the polar fraction upon rechromatography under the same conditions with 5% MeOH in CH_2Cl_2 gave pure discodermolide 3-acetate (**8**, 1.0 mg) and discodermolide 7-acetate (**9**, 0.8 mg).

Discodermolide-3,7,11,17-tetraacetate (2): $[\alpha]^{22}_D$ 20.1° (*c* 0.1, CH_2Cl_2) [lit.[1] $[\alpha]^{25}_D$ 19.2° (*c* 0.3, $CHCl_3$)]; IR (neat/KBr) ν_{max} 1732, 1716, 1372, 1238 cm^{-1}; HRFABMS (thioglycerol) *m/z* 702.4203, Δ 1.4 mmu for $C_{39}H_{60}NO_{10}$ $(M - CH_3COO)^+$.

Discodermolide-3,7,17-triacetate (3): $[\alpha]^{22}_D$ 22.0° (*c* 0.2, CH_2Cl_2); IR (neat/KBr) ν_{max} 3490, 1737, 1716, 1372, 1240 cm^{-1}; HRFABMS (thioglycerol) *m/z* 742.4053, Δ 8.9 mmu for $C_{39}H_{61}NNaO_{11}$ $(M + Na)^+$.

Discodermolide-3,7,11-triacetate (4): $[\alpha]^{22}_D$ 34.4° (*c* 0.3, CH_2Cl_2); IR (neat/KBr) ν_{max} 3497, 1732, 1715, 1375, 1245 cm^{-1}; HRFABMS (thioglycerol) *m/z* 742.4104, Δ 3.9 mmu for $C_{39}H_{61}NNaO_{11}$ $(M + Na)^+$.

Discodermolide-3,7-diacetate (5): $[\alpha]^{22}_D$ 24.6° (*c* 0.5, CH_2Cl_2); IR (neat/KBr) ν_{max} 2470, 1722, 1711, 1371, 1240 cm^{-1}; HRFABMS (thioglycerol) *m/z* 700.4182, Δ 4.5 mmu for $C_{37}H_{59}NNaO_{10}$ $(M + Na)^+$.

Discodermolide-3,11-diacetate (6): $[\alpha]^{22}_D$ 37.2° (*c* 0.1, CH_2Cl_2); IR (neat/KBr) ν_{max} 3465, 1737, 1722, 1374, 1239 cm^{-1}; HRFABMS (3-nitrobenzyl alcohol) *m/z* 678.4186, Δ 3.1 mmu for $C_{37}H_{60}NO_{10}$ $(M + H)^+$.

Discodermolide-3,17-diacetate (7): $[\alpha]^{22}_D$ 25.0° (*c* 0.1, CH_2Cl_2); IR (neat/KBr) ν_{max} 3471, 1724, 1373, 1238 cm^{-1}; HRFABMS (3-nitrobenzyl alcohol) *m/z* 678.4191, Δ 2.6 mmu for $C_{37}H_{60}NO_{10}$ $(M + H)^+$.

Discodermolide-3-acetate (8): $[\alpha]^{22}_D$ 25.4° (*c* 0.5, CH_2Cl_2); IR (neat/KBr) ν_{max} 3433, 1723, 1711, 1372, 1245 cm^{-1}; HRFABMS (3-nitrobenzyl alcohol) *m/z* 636.4112, Δ 0.1 mmu for $C_{35}H_{58}NO_9$ $(M + H)^+$.

Discodermolide-7-acetate (9): $[\alpha]^{22}_D$ 23.4° (*c* 0.2, CH_2Cl_2); IR (neat/KBr) ν_{max} 3430, 1714, 1373, 1244 cm^{-1}; HRFABMS (3-nitrobenzyl alcohol) *m/z* 636.4110, Δ 0.3 mmu for $C_{35}H_{58}NO_9$ $(M + H)^+$.

^{1}H NMR and ^{13}C NMR data see Tables 1−3.

P388 Assay. P388 cells were obtained from Dr. R. Camalier, National Cancer Institute, Bethesda, MD. The cell line was maintained in RPMI-1640 tissue culture medium (TCM) supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 60 mg/mL l-glutamine, 18 mM HEPES, 0.05 mg/mL gentamicin (Life Technologies, Gaithersburg, MD), and 10% Rehatuin fetal bovine serum (Intergen Co., Purchase, NY) and cultured in plastic tissue culture flasks at 37 °C in humidified air containing 5% CO_2. Stock cultures of P388 cells were subcultured 1:20 in fresh TCM every 2 to 3 days. To assess the antiproliferative effects of agents against the P388 cell line, 200 μL cultures (96-well tissue culture plates, Nunc, Denmark) were established at 1×10^5 cells/mL in TCM or TCM containing the test agent at 0.03−5.0 μg/mL. After 48 h exposures, P388 cells were enumerated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) as described in the literature.[30] The results were expressed as percent inhibition compared to the negative (no drug) control. Positive drug controls of varying dilutions of 5-fluorouracil and adriamycin (Sigma Chemical Co., St. Louis, MO) were included

Table 3. ^{13}C NMR Data for **2**−**9** in $CDCl_3$

C	**2** δ C	**3** δ C	**4** δ C	**5** δ C	**6** δ C	**7** δ C	**8** δ C	**9** δ C
1 (s)	171.7	172.0	171.9	172.1	172.5	172.4	173.3	173.3
2 (d)	40.0	40.1	40.1	40.1	40.1	40.2	40.1	43.2
3 (d)	74.5	74.6	74.5	74.6	74.6	74.7	74.7	73.2
4 (d)	33.7	33.4	33.5	33.3	34.0	33.9	33.6	35.6
5 (d)	76.8	76.9	76.7	77.5	77.0	77.4	77.7	77.0
6 (t)	38.7	38.8	38.9	38.9	41.2	41.1	41.0	38.7
7 (d)	66.5	67.2	66.7	67.3	63.4	63.9	63.3	67.6
8 (d)	128.2	128.5	127.8	128.3	132.2	132.8	132.5	128.3
9 (d)	135.1	136.1	135.6	136.6	134.1	134.2	134.2	136.6
10 (d)	35.1	36.4	35.6	36.7	35.0	35.7	36.0	37.3
11 (d)	80.2	79.1	80.1	78.9	80.2	79.1	78.9	79.1
12 (d)	34.1	34.2	33.8	34.7	34.2	34.9	35.1	35.0
13 (d)	128.9	130.5	128.9	130.0	128.8	130.2	129.8	129.9
14 (s)	133.4	132.6	133.7	133.1	133.9	133.0	133.0	133.2
15 (t)	35.6	35.6	36.0	35.8	36.9	35.6	35.7	36.0
16 (d)	31.8	32.0	32.8	33.1	32.8	32.0	33.0	33.2
17 (d)	77.9	78.0	75.7	75.9	75.8	78.0	75.7	75.9
18 (d)	36.4	36.4	37.4	37.3	37.4	36.4	37.2	36.8
19 (d)	77.8	77.7	78.5	78.8	78.5	77.7	78.9	79.0
20 (d)	34.1	34.4	34.7	34.7	34.7	34.2	34.5	34.8
21 (d)	133.0	133.1	133.8	133.7	133.7	133.0	133.4	133.7
22 (d)	130.2	130.2	129.8	129.9	129.9	130.3	129.9	130.0
23 (d)	132.2	132.2	132.2	132.1	132.1	132.2	132.0	132.1
24 (t)	118.2	118.2	117.8	117.9	117.9	118.2	117.9	117.9
25 (q)	15.3	15.6	15.5	15.6	15.6	15.5	15.5	15.6
26 (q)	12.4	12.5	12.5	12.6	12.5	12.5	12.4	12.5
27 (q)	17.5	17.4	17.4	17.5	17.5	18.5	18.0	17.6
28 (q)	16.6	14.8	15.9	14.8	16.3	15.1	15.5	15.0
29 (q)	22.8	23.1	23.1	23.3	23.0	23.1	23.1	23.2
30 (q)	13.6	13.3	13.7	13.8	13.7	13.5	13.8	13.7
31 (q)	9.5	9.5	8.9	8.8	8.9	9.5	8.7	8.9
32 (q)	17.5	17.5	17.4	17.4	18.4	17.5	17.4	17.4
33 (s)	156.7	156.7	156.9	157.0	156.9	156.7	157.6	157.0
34 (s)	170.9	170.9[a]				170.9[a]		
35 (q)	20.8	20.9				20.9		
36 (s)	170.6		170.7[a]		170.8[a]			
37 (q)	20.9		20.9		20.9			
38 (s)	169.8	170.1[a]	169.9[a]	170.1[a]				170.1
39 (s)	20.9	20.9	21.0	20.9				21.3
40 (s)	170.4	170.3[a]	170.3[a]	170.3[a]	170.4[a]	170.5[a]	170.6	
41 (q)	21.2	21.3	21.3	21.3	21.0	21.0	20.7	

[a] Chemical shift value assignments may be interchanged

Table 4. ^1H NMR Data for the Hydroxymethines and Acetoxymethines in Compounds **1** in $CDCl_3$ and 5% CD_3OD and **2**−**9** in $CDCl_3$

position	**1** δ H	**2** δ H	**3** δ H	**4** δ H	**5** δ H	**6** δ H	**7** δ H	**8** δ H	**9** δ H
3-H	3.57	4.83	4.88	4.89	4.88	4.90	4.89	4.89	3.71
7-H	4.60	5.61	5.72	5.72	5.74	4.75	4.70	4.71	5.70
11-H	3.09	4.58	3.12	4.70	3.18	4.72	3.13	3.18	3.19
17-H	3.15	4.71	4.79	3.25	3.27	3.25	4.78	3.27	3.27

to monitor drug sensitivity of the cell line. To quantitate the effects on cell proliferation and resulting IC_{50} values, 75 μL of warm growth media containing 5 mg/mL MTT was added to each well, and cultures were returned to the incubator and left undisturbed for 3 h. Prior to quantitation of reduced formazan, the plates were centrifuged (500*g*, 10 min), culture fluids were removed by aspiration, and 200 μL of acidified 2-propanol (2 mL HCl/L 2-propanol) was added per well. The absorbance of the resulting solutions was measured in a plate reader (Tecan Spectra SLT; TECAN U.S., Research Triangle Park, NC) at 570 nm and a 650 nm reference filter. The absorbance of test wells was divided by the absorbance of drug-free wells, and the concentration of agent that results in 50% of the absorbance of untreated cultures (IC_{50}) is determined by linear regression of logit-transformed data.[31] A linear relationship between tumor cell number and formazan production has been routinely observed over the range of cell densities observed in these experiments.

Acknowledgment. We thank Dr. Shirley A. Pomponi for the collection and identification of the sponge *D. dissoluta*. We also thank Patricia Linley for her assistance with the cytotoxicity assays and Dr. David Powell of the University of Florida for the HRFABMS measurements. This research was partly funded by a grant from the National Institutes of Health/National Cancer Institute CA 74227. This is Harbor Branch Oceanographic Institution Contribution No. 1388.

References and Notes

(1) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem*. **1990**, *55*, 4912−4915 (correction: **1991**, *56*, 1346).
(2) Longley, R. E.; Gunasekera, S. P.; Faherty, D.; McLane, J.; Dumont, F. *Ann. N. Y. Acad. Sci*. **1993**, *696*, 94−107.
(3) Longley, R. E.; Caddigan, D.; Harmody, D.; Gunasekera, M.; Gunasekera, S. P. *Transplantation* **1991**, *52*, 650−656.
(4) Longley, R. E.; Caddigan, D.; Harmody, D.; Gunasekera, M.; Gunasekera, S. P. *Transplantation* **1991**, *52*, 656−661.
(5) ter Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243−250.
(6) Hung, G. T.; Chen, J.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 287−293.
(7) Kowalski, R. J.; Giannakakou, P.; Gunasekera, S. P.; Longley, R. E.; Day, B. W.; Hamel, E. *Mol. Pharm*. **1997**, *52*, 613−622.
(8) Balachandran, R.; ter Haar, E.; Welsh, M. J.; Grant, S. G.; Day, B. W. *Anticancer Drugs* **1998**, *6*, 67−76.
(9) Balachandran, R.; Grant, S. G.; Welsh, M. J.; Day, B. W. *Breast J*. **1998**, *4*, 409−419.
(10) Nerenberg, J. B.; Hung, D. T.; Somers, P. K.; Schreiber, S. L *J. Am. Chem. Soc*. **1993**, *115*, 12621−12622.
(11) Hung, D. T.; Nerenberg, J. B.; Schreiber, S. L *J. Am. Chem. Soc*. **1996**, *118*, 11054−11080.
(12) Peterson, I.; Florence, G. J.; Gerlach, K.; Scott, J. P. *Angew. Chem*. **2000**, in press.
(13) Smith, A. B., III; Qui, Y.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc*. **1995**, *117*, 12011−12012.
(14) Marshall, J. A.; Johns, B. A. *J. Org. Chem*. **1998**, *63*, 7885−7892.
(15) Harried, S. S.; Yang, G.; Strawn, M. A.; Myles, D. C. *J. Org. Chem*. **1997**, *62*, 6098−6099.
(16) Clark, D. L.; Heathcock, C. H. *J. Org. Chem*. **1993**, *58*, 5878−5879.
(17) Paterson, I.; Wren, S. P. *J. Chem. Soc., Chem. Commun*. **1993**, 1790−1792.
(18) Golec, J. M. C.; Jones, S. D. *Tetrahedron Lett*. **1993**, *34*, 8159−8162.
(19) Evans, P. L.; Golec, J. M. C.; Gillespie, R. J. *Tetrahedron Lett*. **1993**, *34*, 8163−8166.
(20) Golec, J. M. C.; Gillespie, R. J. *Tetrahedron Lett*. **1993**, *34*, 8167−8168.
(21) Yang, G.; Myles, D. C. *Tetrahedron Lett*. **1994**, *35*, 1313−1316.
(22) Yang, G.; Myles, D. C. *Tetrahedron Lett*. **1994**, *35*, 2503−2504.
(23) Paterson, I.; Schlapbach, A. *Synlett* **1995**, *5*, 498−500.
(24) Miyazawa, M.; Oonuma, K.; Maruyama, M.; Miyashita, M. *Chem. Lett*. **1997**, 1191−1192.
(25) Marshall, J. A.; Zhi-Hui, L.; Johns, B. A. *J. Org. Chem*. **1998**, *63*, 817−823.
(26) Evans, D. A.; Halstead, D. P.; Allison B. D. *Tetrahedron Lett*. **1999**, *40*, 4461−4462.
(27) Filla, S. A.; Song, J. J.; Chen, L.; Masamune, S. *Tetrahedron Lett*. **1999**, *40*, 5449−5453.
(28) Misske, A. M.; Hoffmann, H. M. R. *Tetrahedron* **1999**, *55*, 4315−4324.
(29) Gunasekera, S. P.; Longley R. E. Serial number 9/412,552, U.S. Patent filed on October 5, 1999.
(30) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res*. **1988**, *48*, 589−601.
(31) Finney, D. J. *Statistical Method in Biological Assay*; Charles Griffin Company: London, 1978; pp 316−348.

NP000423E