Synthetic Analogues of the Microtubule-Stabilizing Agent (+)-Discodermolide: Preparation and Biological Activity

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A series of seven synthetic discodermolide analogues **2**−**8**, which are minor side products generated during the final stages in the synthesis of (+)-discodermolide (**1**), have been purified and evaluated for in vitro cytotoxicity against A549, P388, MFC-7, NCI/ADR, PANC-1, and VERO cell lines. These synthetic analogues showed a significant variation of cytotoxicity and confirmed the importance of the C-7 hydroxy through C-17 hydroxy molecular fragment for potency. Specifically, these analogues suggested the relevance of the C-11 hydroxyl group, the C-13 double bond, and the C-16 (*S*) stereochemistry for the potency of (+)-discodermolide. The preparation, purification, structure elucidation, and biological activity of these new analogues are described.

(+)-Discodermolide (**1**) was first reported in 1990[1] and has been shown to promote the rapid polymerization of purified tubulin and to hyperstabilize the microtubule complex in cultured cells. (+)-Discodermolide inhibits the in vitro growth of several cancer cell lines, including paclitaxel-resistant ovarian and colon cancer cells,[2−4] and synthetic (+)-discodermolide is currently undergoing Phase I clinical trials as a potential antitumor drug. Recently, we reported the structure elucidation, biological activity, and structure−activity relationship studies of 20 semisynthetic discodermolide analogues[5−9] and five natural discodermolide analogues.[10] Herein, we report the preparation, purification, structural elucidation, and biological activity of seven (**2**−**8**) new synthetic analogues of (+)-discodermolide (**1**). The Schreiber group first synthesized both antipodes of discodermolide, establishing the absolute configuration,[11] and prepared a number of structural variants.[12] Since then, several other groups have synthesized (+)-discodermolide,[13,14] antipode (−)-discodermolide,[15,16] or various fragments of discodermolide using different synthetic approaches.[17−30] Recently, the Paterson group[31] synthesized (+)-discodermolide and three (5-*epi*-, 7-*epi*-, and 5,7-bis-*epi*-) new discodermolide analogues. The chemical development group at Novartis has recently completed a large-scale synthesis of (+)-discodermolide.[32−36]

In the final stage of the synthesis of (+)-discodermolide,[36] the three protecting groups in (6*Z*,11*Z*)-(1*S*,2*R*,3*R*,4*S*,8*S*,9*S*,10*S*,13*S*,15*S*,16*S*,17*S*,18*R*)-3,9,17-tris(*tert*-butyldimethylsilanyloxy)-13,15-dihydroxy-18-(methoxymethylcarbamoyl)-2,4,6,8,10,16-hexamethyl-1-((*Z*)-(*S*)-1-methylpenta-2,4-dienyl)nonadeca-6,11-dienyl carbamate (**9**) were removed by a one-step acid-catalyzed procedure. This acid-catalyzed hydrolysis procedure with concomitant lactonization gave crude (+)-discodermolide in 85% purity in about 85% yield. The reaction sequence leading to (+)-discodermolide is indicated in Figure 1. Further purification of this crude discodermolide by column chromatography on reversed-phase Si gel gave pure (+)-discodermolide as a monohydrate after crystallization from CH_3CN/H_2O at pH 4.0.

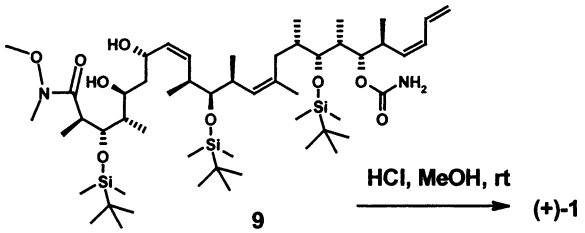

Figure 1. Reaction sequence leading to (+)-discodermolide.

Quite a number of cyclized side products in trace quantities were isolated from the various column washings after further chemical treatment. This paper describes the HPLC purification, structure determination, and the cytotoxic activity of 3-*O*-(*tert*-butyldimethylsilyl)discodermolide (**2**) and six cyclo-discodermolide analogous (**3**−**8**).

Results and Discussion

Structurally, (+)-discodermolide possesses 13 stereogenic centers, a tetrasubstituted *δ*-lactone (C-1 to C-5), four secondary hydroxyl groups (C-3, C-7, C-11, and C-17), a pendant carbamate moiety (C-19), one di- (C-8, *Z*) and one trisubstituted (C-13, *Z*) double bond, and a terminal (*Z*)-diene (C-21 to C-24). Both in the solid state[1] and in solution,[38] (+)-discodermolide adapts a hairpin conformation where the two internal (*Z*)-olefins in the side chain act as conformational locks by minimizing (1,3) strain between their respective substituents in concert with the avoidance of *syn*-pentane interactions. This hairpin confirmation brings the two isolated double bonds closer together in correct geometry for the proton-catalyzed cyclization.

Treatment of the Weinreb amide (**9**) with HCl under carefully controlled conditions leads ultimately to the isolation of (+)-discodermolide.[36] This apparently simple process is in reality very complex. The first reaction to occur is ring closure to produce 3,11,17-tris-*O*-(*tert*-butyldimethylsilyl)discodermolide. This compound oils out of the reaction mixture and takes no further part in the reaction, resulting in a decrease in yield. Careful washing of the vessel walls periodically with methanol is necessary to maintain it in the reaction mixture. However, it can easily

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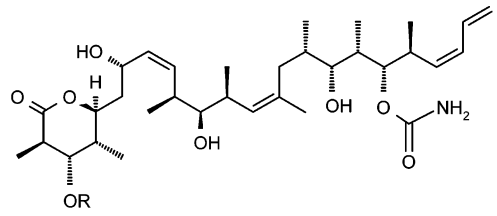
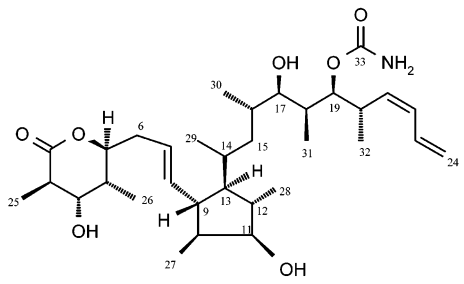
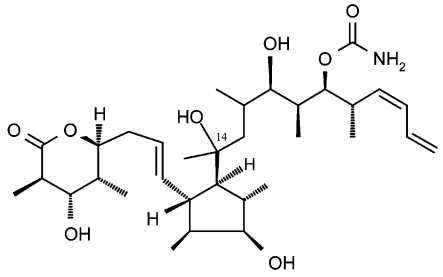


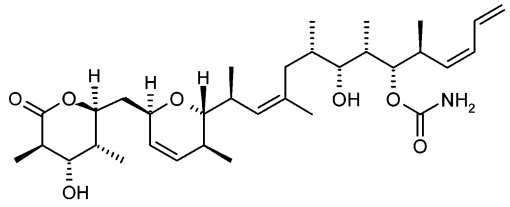
1 R = H
2 R = *t*-Butyldimethylsilyl



3 Δ 14 - 15
4 Δ 14 - (29)



5 14*R**, 16*S*
6 14*S**, 16*S*
7 14*S**, 16*R*



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be recovered by chromatography of the reaction mixture (see Experimental Section).

The actual hydrolysis is very complex. HPLC of the reaction mixture indicates the presence of at least seven major products apart from **1** as well as a number of other minor components. The major products are very likely to be intermediates with all the various combinations of silyl ether cleavage, whereby the last silyl group to cleave is that at the 3-position. If the process is run for a significantly longer time, degradation products of discodermolide can be identified. The (+)-3-*O*-(*tert*-butyldimethylsilyl)-discodermolide (**2**) was isolated by chromatography of the column washings obtained from the reversed-phase column chromatography of the cleavage reaction mixture.

As was indicated above, the 3,11,17-tris-*O*-(*tert*-butyldimethylsilyl)discodermolide oils out of the reaction mixture. For technical reasons the crude mixture must be

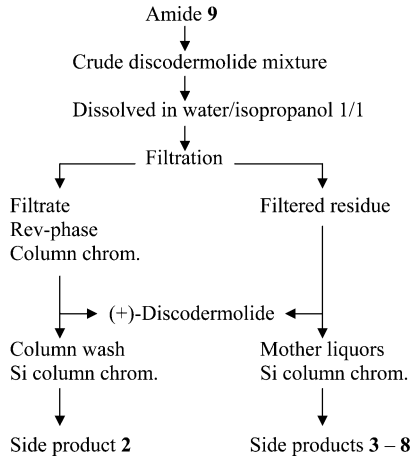

Figure 2. Schematic diagram of the isolation process.

filtered before applying to the reversed-phase column and in the polar medium required for the reversed-phase purification. The 3,11,17-tris-*O*-(*tert*-butyldimethylsilyl)-discodermolide remains on the filter and can easily be reisolated and hydrolyzed separately to give, after crystallization, **1**. Chromatography of the mother liquors of this crystallization allowed the isolation and identification of a series of novel cyclic structures, **3**−**8**, most likely derived from the acid-catalyzed cyclization of (+)-discodermolide (**1**). Indeed it can be demonstrated that treatment of **1** itself with HCl also produces the same structures. Figure 2 outlines the isolation and purification process.

The structures of the cyclized compounds **3**−**8** are supported by the spectral data. All compounds gave correct HRMS values for the molecular mass. The ^{1}H and ^{13}C NMR data of all analogues were compared to those reported for (+)-discodermolide (see Table 1), and the chemical shifts were assigned using $^{1}H-^{1}H$ COSY data. DEPT, HMQC, and in some instances HMBC data were used to assign ^{13}C data of all analogues presented in Table 4. The NOE data were used to determine the stereochemistry of all cyclo-analogues.

HRMS of **2** supported the molecular formula $C_{39}H_{69}NO_8Si$ [$(M + H)^+$, *m*/*z* 708.4863] expected for a mono-*O*-(*tert*-butyldimethylsilyl)discodermolide. The comparison of ^{1}H and ^{13}C NMR spectra of **2** (see Table 1) with that of (+)-discodermolide (**1**) suggested the presence of one *tert*-butyldimethylsilyl ether group in the molecule. The ^{1}H spectrum of **2** was identical to that of **1** except at the C-3 position. The COSY spectrum of **2** indicated the absence of a cross-peak corresponding to the C-3OH group and further revealed a significant downfield shift of δ 0.15 ppm for the signal corresponding to the oxymethine at the C-3 position. The presence of additional signals in the ^{1}H spectrum [(0.086 ppm (3H, s), 0.080 (3H, s), and 0.880 (9H, s)] and in the ^{13}C spectrum [(−4.8 (CH_3), −6.3 (CH_3), 18.5 (qC), and 26.0 (3 × CH_3)] of **2** confirmed the presence of one *tert*-butyldimethyl group in the molecule. Combination of the above data and the ready acid-catalyzed hydrolysis of **2** to **1** confirmed the structure of **2** as 3-*O*-(*tert*-butyldimethylsilyl)discodermolide.

HRMS of (7*E*,9*S*,13*S*,14*E*)-7-deoxy-7,14-dien-9-13-cyclo-discodermolide (**3**) supported the molecular formula $C_{33}H_{53}NO_7$ [$(M + H)^+$ *m*/*z* 576.3895], and it indicated a difference in elements H_2O (18 mmu) from (+)-discodermolide. The ^{13}C NMR spectral comparison of **3** with that of **1** revealed that the resonances attributed to one of the oxygenated carbons and one of the methylene carbons observed for **1** have been replaced by two methine carbons (δ 54.6 and

Table 1. ^{1}H and ^{13}C NMR Data[a] for **1** and **2**

position	δ_H (**1**)	m (*J* Hz)	δ_H (**2**)	m (*J* Hz)	δ_C (**1**)	m	δ_C (**2**)	m
1					174.7	C	174.3	C
2	2.56	dq (4.2, 7.2)	2.56	dq (3.0, 7.3)	44.1	CH	44.9	CH
3	3.61	ddd (4.2, 4.2, 4.6)	3.76	dd (2.4, 3.0)	73.2	CH	75.1	CH
3-OH	3.27	d (4.6)						
4	1.83	ddq (2.0, 4.2, 6.9)	1.88	ddq (2.4, 10.0, 6.9)	36.4	CH	34.9	CH
5	4.45	dt (2.0, 10.1)	4.49	dt (2.0, 10.0)	77.6	CH	78.2	CH
6	1.72	m[b]	1.72	m	42.2	CH_2	36.3	CH_2
6	1.47	ddd (2.4, 10.8, 13.0)	1.48	ddd (2.0, 11.0, 13.0)				
7	4.44	m[b]	4.47	m	63.5	CH	63.4	CH
7-OH	2.75	d (5.3)	2.78	d (5.3)				
8	5.38	ddd (2.2, 9.1, 10.9)	5.38	dd (8.6, 10.9)	133.9	CH	133.9	CH
9	5.54	ddd (1, 10.1, 10.9)	5.53	dd (10.1, 10.9)	133.8	CH	133.6	CH
10	2.62	m[b]	2.62	m[b]	36.4	CH	36.3	CH
11	3.05	m[b]	3.05	m[b]	79.8	CH	79.6	CH
11-OH	2.64	d (5.2)	2.63	d (5.0)				
12	2.29	ddq (5.2, 6.6, 10.0)	2.29	ddq (5.2, 6.5, 10.0)	37.1	CH	36.9	CH
13	4.97	d (10.0)	4.99	d (10.0)	131.2	CH	131.2	CH
14					133.9	C	133.6	C
15	1.76	m[b]	1.75	m	36.3	CH_2	42.3	CH_2
15	1.63	dd (3.6, 12.2)	1.60	dd (3.6, 12.2)				
16	1.76	m[b]	1.72	m[b]	34.3	CH	34.2	CH
17	3.27	dd (3.5, 6.2)	3.14	ddd (3.5, 6.2, 6.5)	76.0	CH	75.9	CH
17-OH	2.59	d (6.5)	2.59	d (6.5)				
18	1.72	m[b]	1.72	m[b]	38.5	CH	38.4	CH
19	4.71	dd (4.1, 8.0)	4.71	dd (4.2, 8.0)	79.3	CH	79.1	CH
20	3.07	m[b]	3.07	m[b]	34.8	CH	34.7	CH
21	5.42	dd (10.6, 10.7)	5.42	dd (10.6, 10.6)	134.3	CH	134.2	CH
22	6.06	dd (10.7, 11.0)	6.07	dd (10.6, 11.0)	130.4	CH	130.5	CH
23	6.68	ddd (10.5, 11.0, 16.6)	6.66	ddd (10.5, 11.0, 16.6)	133.3	CH	133.2	CH
24	5.21	d (16.6)	5.24	d (16.6)	118.3	CH_2	118.5	CH_2
24	5.10	d (10.1)	5.14	d (10.0)				
25	1.18	d 7.2	1.18	d (7.2)	15.8	CH_3	16.5	CH_3
26	0.97	d (6.9)	0.98	d (6.9)	13.1	CH_3	14.3	CH_3
27	1.00]	d (6.9)	1.00	d (6.9)	19.7	CH_3	19.5	CH_3
28	0.88	d (6.6)	0.87	d (6.6)	17.5	CH_3	17.3	CH_3
29	1.57	s	1.56	s	23.3	CH_3	23.3	CH_3
30	0.73	d (6.2)	0.73	d (6.2)	15.5	CH_3	15.4	CH_3
31	0.80	d (6.5)	0.81	d (6.5)	9.2	CH_3	9.2	CH_3
32	0.93	d (6.7)	0.94	d (6.7)	18.2	CH_3	18.1	CH_3
33					158.4	C	158.2	C
34			0.086	s			−4.8	CH_3
34			0.080	s			−6.3	CH_3
35							18.5	C
36			0.88	s			26.0	CH_3
NH_2	5.05	br s	5.05	br s				

[a] All spectra run in CD_3CN. Chemical shifts are reported in ppm, and *J* values in Hz. [b] Overlapped signal.

62.4), and these data together with the molecular formula suggested an additional unsaturation in **3** accounting for a second ring system in the molecule. The ^{1}H NMR spectral pattern of **3** was similar to that of **1**, 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 0.70 and 0.73 ppm to an allylic position. In the COSY spectrum of **3**, the C-7 olefinic proton at 5.32 ppm indicated couplings to the C-6 allylic methylene protons observed at 2.20 and 2.42 ppm and to the C-8 olefinic proton observed at 5.24 ppm. The C-6 allylic methylene protons were in turn coupled to the C-5 oxymethine proton observed at 4.26 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 2.04 ppm (ddd, J = 8.5, 8.8, 8.9 Hz) showed couplings to the protons at C-8, 5.24 ppm (dd, J = 8.5, 15.4 Hz); C-10, 1.64 ppm (m); and allylic C-13, 1.52 ppm (m). The C-10 methine proton was coupled to C-27 at 0.88 ppm and to the C-11 hydroxymethine proton observed at 3.56 ppm, which was in turn coupled to the C-12 methine proton observed at 1.65 ppm. The C-12 methine proton that was coupled to the C-28 methyl protons observed at 0.90 ppm indicated additional coupling to the C_{13} allylic proton, which in turn did not show coupling to the C_{15} olefinic proton observed at 4.73 ppm. The C-14 olefinic methyl observed at 1.51 (br s, C-29) indicated allylic coupling to the C-15 olefinic proton, which in turn was coupled to the C-16 allylic methine proton observed at 2.38 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 NMR data with those of discodermolide indicted that one hydroxymethine (C_7) and a methylene (C_{15}) signal present in discodermolide have been replaced by two methine signals (C_9 and C_{13}) in **3**. The highfield chemical shift value of 14.6 ppm for the C_{29} olefinic methyl (see Table 2) indicated *trans* stereochemistry for the C_{14} double bond. The absolute stereochemistry of the substituted cyclopentane ring was assigned on the basis of NOESY correlations and using the assumption that the absolute stereochemistry of the chiral centers at C_{10}−C_{12} were not changed during the cyclization reaction. The strong NOESY correlations observed (H-9/H-12, H-9/Me-27, and H-9/Me-29) indicated that H-9, Me-

Table 2. ^{1}H NMR Data[a] of Cyclo-analogues **3**−**5**

position	δ_H (**3**)	m (*J* Hz)	δ_H (**4**)	m (*J* Hz)	δ_H (**5**)	m (*J* Hz)
2	2.55	m	2.55	dq (4.2, 7.3)	2.55	dq (4.2, 7.3)
3	3.61	m	3.59	m	3.63	ddd (4.1, 4.2, 5.0)
3-OH	3.29	d (3.8)			3.33	d (4.1)
4	1.93	m	1.93	m	1.94	m
5	4.26	ddd (5.0, 6.4, 10.0)	4.29	ddd (5.0, 6.5, 10.0)	4.32	ddd (5.0, 6.5, 10.0)
6	2.42	m	2.41	ddd (4.3, 5.0, 15.4)	2.47	ddd (4.3, 5.0, 15.4)
6	2.20	m	2.24	ddd (6.5, 6.5, 15.4)	2.26	ddd (6.5, 6.5, 15.4)
7	5.32	ddd (4.7, 6.5, 15.4)	5.31	m	5.34	m
8	5.24	dd (8.5, 15.4)	5.28	dd (8.5, 15.4)	5.44	dd (8.5, 15.4)
9	2.04	ddd (8.5, 8.8, 8.9)	2.01	ddd (8.5, 8.8, 8.9)	2.23	ddd (8.5, 8.8, 8.9)
10	1.64	m	1.64	m	1.70	m
11	3.56	m	3.56	m	3.43	dd (4.1, 6.0)
11-OH	2.39	d (4.8)				
12	1.65	m	1.68	m	1.99	m
13	1.52	m	1.73	m	1.42	dd (3.4, 7.8)
14						
15	4.73	d (10.0)	1.95	m	1.35	dd (7.8, 14.4)
15			1.58	dd (10.7, 12.5)	1.28	dd (3.2, 14.4)
16	2.38	m	1.86	m	1.87	m
17	3.21	ddd (5.6, 5.7, 6.3)	3.16	dd (5.6, 5.7)	3.16	ddd (5.6, 5.7, 6.1)
17-OH	2.60	d (6.3)			2.60	d (6.1)
18	1.77	m	1.78	m	1.75	m
19	4.70	dd (4.0, 9.0)	4.68	m	4.63	dd (4.7, 7.0)
20	3.06	m	3.04	m	3.00	ddq (6.8, 7.0, 10.6)
21	5.40	dd (10.6, 11.0)	5.37	dd (10.6, 11.0)	5.38	dd (10.6, 11.0)
22	6.04	dd (10.9, 11.0)	5.99	dd (10.9, 11.0)	6.03	dd (11.0, 11.0)
23	6.67	ddd (10.0, 11.0, 16.8)	6.64	ddd (10.5, 11.0, 16.8)	6.67	ddd (10.2, 11.0, 16.7)
24	5.23	d (16.8)	5.19	d (16.8)	5.21	d (16.7)
24	5.14	d (10.0)	5.09	d (10.5)	5.10	d (10.2)
25	1.18	d (7.3)	1.18	d (7.3)	1.20	d (7.3)
26	0.94	d (6.5)	0.95	d (6.8)	0.97	d (6.9)
27	0.88	d (6.7)	0.85	d (6.8)	0.87	d (6.7)
28	0.90	d (6.5)	0.93	d (6.5)	0.98	d (7.2)
29	1.51	s	4.78	s	1.11	s
29			4.68	s		
30	0.88	d (6.8)	0.77	d (6.5)	0.90	d (6.8)
31	0.71	d (6.7)	0.88	d (6.7)	0.90	d (6.8)
32	0.93	d (7.0)	0.94	d (6.9)	0.93	d (6.8)
NH_2					5.05	br s

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

27, and Me-29 are arranged on one side of the cyclopentane ring system. Similarly, strong NOE correlations observed (H-10/H-11, H11-/Me-28, and H-13/Me-28) indicated that H-10, H-11, H-13, and Me-28 are arranged on the opposite side of the cyclopentane system. These NOE correlations established the stereochemistry of the cyclopentane ring and that the two side chains are on the opposite sides of the plane of the cyclopentane ring. Since the absolute stereochemistries at C-10, C-11, and C-12 are known from the structure of discodermolide, the two new chiral centers were assignesd 9*S* and 13*S* stereochemistry. These data in combination with the experimental evidence that **3** is an acid-catalyzed cyclized product of (+)-discodermolide confirmed the structure of **3** as (7*E*,9*S*,13*S*,14*E*)-7-deoxy-7,14-dien-9−13-cyclodiscodermolide. A closely related compound having a different specific rotation value has been reported[10] by one of us as a natural product without the definition of the side chain stereochemistry. The related natural product is not available for direct comparison studies. The amounts of **3** isolated here allowed stereochemical assignment of the two side chains attached to the cyclopentane ring.

HRMS of **4** supported the molecular formula $C_{33}H_{53}NO_7$ [$(M + H)^+$ *m/z* 576.3894], indicating that **3** and **4** are isomers. The detailed analysis of the ^{1}H and ^{13}C NMR data suggested that **3** and **4** are double-bond isomers. Further comparison of the ^{1}H and ^{13}C NMR data of **4** with that of **3** indicated the presence of a new *exo*-methylene group [δ_H 4.78 (s), 4.68 (s); δ_C 111.0 (t)] at C-14 instead of the olefinic methine and vinylic methyl group present in **3**. The upfield shift of the H_2-15 to allylic methylene group and three-bond HMBC correlations of the H_2-29 *exo*-methylene protons to C-13 and C-15 established the position of the *exo*-methylene group in the molecule. The NOE correlations in the cyclopropane ring system were identical to that reported for **3**. These data confirmed the structure of **4** as (7*E*,9*S*,13*S*)-7-deoxy-7,14(29)-dien-9−13-cyclodiscodermolide.

HRMS of **5** [*m/z* 616.3821 $(M + Na)^+$] and **6** [*m/z* 594.4000 $(M + H)^+$] calculated to the same molecular formula, $C_{33}H_{55}NO_8$, suggesting that **5** and **6** are isomers, and indicated a difference in elements H_2O (+18 mmu) from **3** and **4**. Comparison of the ^{1}H NMR data (Tables 2 and 3) and ^{13}C NMR data (Table 4) of **5** and **6** with those of **3** and **4** indicated an addition of a molecule of water across the double bond at C-14, C-15 in **3** or at C-14 (C-29) in **4** to give a tertiary hydroxyl group at C-14 in **5** and **6**. The HMBC spectra of **5** and **6** revealed three-bond correlations between the H-29 methyl protons [(H 1.11 (s) in **5**; 1.06 (s) in **6**] and C-13 methine and C-15 methylene carbons. These data established the position of the tertiary hydroxyl group in **5** and **6**. The NOE correlations in the cyclopentane ring system were identical to that reported for **3**. The ^{1}H NMR spectrum of **5** indicated the H-13 methine at 1.42 ppm and the H-15 methylene at 1.35 and 1.28 ppm. Similarly, the ^{1}H NMR spectrum of **6** indicated the H-13 methine at 1.39 ppm and the H-15 methylene at 1.75 and 1.09 ppm. The absolute stereochemistry at the C-14 chiral center was not rigorously established. However, the significant chemical shift differences observed for the

Table 3. ^{1}H NMR Data[a] of Cyclo-analogues **6**−**8**

position	δ_H (**6**)	m (*J* Hz)	δ_H (**7**)	m (*J* Hz)	δ_H (**8**)	m (*J* Hz)
2	2.55	dq (4.2, 7.3)	2.54	dq (3.8, 7.3)	2.55	dq (4.6, 7.3)
3	3.63	dd (4.2, 5.0)	3.61	m	3.64	dd (4.4, 4.2)
3-OH	3.27	br s	3.24	d (4.3)	3.27	s
4	1.94	m	1.94	m	2.08	m
5	4.30	ddd (5.0, 6.5, 10.0)	4.28	ddd (5.0, 6.5, 10.0)	4.39	ddd (5.5, 5.7, 9.4)
6	2.42	ddd (4.3, 5.0, 15.4)	2.43	ddd (4.5, 4.6, 14.8)	1.83	dd (5.8, 6.2)
6	2.26	ddd (6.5, 6.5, 15.4)	2.26	ddd (6.0, 6.0, 14.8)	1.83	dd (5.8, 6.2)
7	5.41	m	5.38	m	4.26	ddd (2.1, 4.2, 6.2)
8	5.44	dd (8.5, 15.4)	5.40	dd (8.5, 15.4)	5.72	ddd (1.7, 1.8, 10.2)
9	2.23	ddd (7.8, 8.9, 8.91)	2.23	ddd (7.8, 8.9, 8.9)	5.67	ddd (1.7, 2.4, 10.2)
10	1.71	m	3.43	dd (4.1, 6.0)	3.09	dd (4.2, 7.0)
11-OH			2.91	d (6.0)		
12	2.04	m	1.93	m	1.68	ddq (6.5, 7.5, 9.7)
13	1.39	dd (2.7, 7.8)	1.53	dd (4.5, 8.9)	5.06	d (9.7)
14						
15	1.75	dd (6.3, 14.4)	1.82	dd (8.4, 13.0)	1.80	m
15	1.09	dd (4.7, 14.4)	1.48	dd (4.0, 13.0)	1.80	m
16	2.10	m	2.40	m	1.79	m
17	3.37	ddd (5.6, 5.7, 6.3)	3.61	m	3.14	dd (5.5, 5.6, 6.2)
17-OH	2.84	d (5.7)			2.59	d (6.2)
18	1.84	m	1.92 m		1.79	m
19	4.65	dd (3.5, 7.9)	4.59	dd (2.5, 9.0)	4.68	dd (5.6, 5.7)
20	3.01	ddq (6.8, 7.0, 10.4)	2.97	ddq (2.5, 10.3, 6.5)	3.04	ddq (5.6, 10.6, 6.8)
21	5.34	dd (10.4, 11.0)	5.29	dd (10.3, 11.0)	5.40	dd (10.6, 11.0)
22	6.00	dd (11.0, 11.0)	5.98	dd (11.0, 11.0)	6.02	dd (10.9, 11.0)
23	6.67	ddd (10.2, 11.0, 16.7)	6.67	ddd (10.2, 11.0, 16.7)	6.66	ddd (10.5, 11.0, 16.8)
24	5.20	d (16.7)	5.19	d (16.7)	5.20	d (16.8)
24	5.10	d (10.2)	5.09	d (10.2)	5.11	d (10.5)
25	1.18	d (7.3)	1.18	d (7.3)	1.21	d (7.3)
26	0.97	d (6.5)	0.97	d (7.3)	0.99	d (7.0)
27	0.88	d (6.7)	0.86	d (6.7)	0.99	d (7.0)
28	0.98	d (7.2)	1.03	d (7.3)	0.92	d (6.5)
29	1.06	s	1.07	s	1.60	s
30	0.84	d (7.0)	0.95	d (7.3)	0.77	d (6.2)
31	0.97	d (6.8)	0.99	d (6.5)	0.87	d (6.8)
32	0.95	d (6.8)	0.94	d (6.5)	0.94	d (6.8)
NH_2			4.97	br s	5.03	br s

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

H-15 methylene protons were used to establish the stereochemistry at the C-14 chiral center in **5** and **6**. In a model it was established that the minimum *syn*-pentane interactions between the C-29Me and C-16 substituents (Me-30 and C-17 carbon chain) occurred when C-16H is positioned between the C-14 substituents [C-14OH and C-14Me (29Me)]. This arrangement with 14*S* stereochemistry could shift one of the C-15 diastereotopic methylene protons downfield due to its proximity to the C-14OH group.[12] The downfield shift of one of the C-15 protons is clearly seen in the ^{1}H NMR of **6** and therefore established the structure of **6** as (7*E*,9*S*,13*S*,14*S**)-7-deoxy-14-hydroxy-7-en-9,13-cyclodiscodermolide and the structure of **5** as (7*E*,9*S*,13*S*,14*R**)-7-deoxy-14-hydroxy-7-en-9,13-cyclodiscodermolide.

HRMS of (7*E*,9*S*,13*S*,14*S**,16*R*)-7-deoxy-14-hydroxy-7-en-9,13-cyclodiscodermolide (**7**) indicated a loss of H_2O (−18 mmu) from the molecular ion and gave the highest mass peak at *m*/*z* 576.3894 $[M + H - H_2O]^+$ (calcd for $C_{33}H_{54}NO_7$, 576.3895). The ^{1}H and ^{13}C NMR spectra closely resembled those of **6** (see Tables 3 and 4), and significant chemical shift differences are observed for H-15 (1.82, 1.48 ppm), H-16 (2.40 ppm), and H-17 (3.61 ppm). The NOE data for the cyclopentane ring system were comparable to those of compounds **3**−**6** and thus established the 9*S* and 13*S* stereochemistry similar to these analogues. The significant chemical shift differences observed for H-16 and H-17 suggested 16*R* stereochemistry for this compound. The 16*R* analogue was formed as a minor product during an aldol reaction in the early stages of the synthesis.[34] It is believed that this minor product was carried throughout the synthesis and was converted to the cyclized product during the acid-catalyzed reaction. These data established the structure of **7** as (7*E*,9*S*,13*S*,14*S**,16*R*)-7-deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide.

HRMS of (7*R*,11*S*)-7,11-oxycyclodiscodermolide (**8**) supported the molecular formula $C_{33}H_{53}NO_7$ $[(M + H)^+$ *m*/*z* 576.3895], and it indicated a difference in elements of H_2O (18 mmu) from discodermolide. The ^{1}H and ^{13}C NMR spectra were very similar to those of (+)-discodermolide (**1**). The ^{1}H NMR spectral comparison of **8** with that of **1** revealed the presence of all signals corresponding to protons except the resonances attributed to 7-OH and 11-OH groups. These data together with the additional unsaturation calculated from the molecular formula suggested a second ring system in the molecule. The presence of six oxymethine protons in the ^{1}H spectrum and six oxygen-bearing carbon signals in the ^{13}C spectrum as in **1** suggested a pyrano ring system in **8**. The ^{1}H NMR spectrum indicated an upfield shift of the two C-6 methylene protons to 1.83 ppm compared to those observed for the other cyclo-compounds **3**−**7** (2.2 to 2.4 ppm) and thus indicated the presence of a nonallylic methylene group at C-6 in **8** as in **1**. In the COSY spectrum of **8**, the C-6 methylene protons observed at 1.83 ppm indicated couplings to the C-7 oxymethine proton at 4.26 ppm, which was in turn coupled to the C-8 olefinic proton observed at 5.72 ppm. This olefinic proton was coupled to the C-9 olefinic proton observed at 5.67 ppm and was in turn coupled to the C-10 methine observed at 2.08 ppm. The C-10 methine proton was coupled to the C-27 methyl at 0.99 ppm and to the C-11 oxymethine proton observed at 3.09 ppm, which was in

Table 4. ^{13}C NMR Data of Cyclo-analogues **3**−**8**[a]

	3		**4**		**5**		**6**		**7**		**8**	
position	δ_C	m	δ_C	m	δ_C	m	δ_C	m	δ_C	m	δ_C	m
1	175.1	C	175.9	C	175.0	C	174.9	C	174.7	C	174.6	C
2	44.3	CH	44.2	CH	44.1	CH	44.5	CH	44.7	CH	43.9	CH
3	73.4	CH	73.1	CH	73.2	CH	73.2	CH	73.2	CH	72.9	CH
4	33.8	CH	33.8	CH	34.5	CH	34.1	CH	34.2	CH	33.8	CH
5	81.2	CH	81.3	CH	81.0	CH	81.0	CH	80.8	CH	79.0	CH
6	36.5	CH_2	36.3	CH_2	36.4	CH_2	36.4	CH_2	36.4	CH_2	39.0	CH_2
7	125.5	CH	125.2	CH	125.0	CH	124.8	CH	124.8	CH	67.3	CH
8	137.9	CH	138.2	CH	141.3	CH	141.2	CH	140.4	CH	128.9	CH
9	54.6	CH	56.3	CH	50.0	CH	50.1	CH	51.5	CH	131.1	CH
10	43.7	CH	43.3	CH	44.7	CH	44.2	CH	44.5	CH	35.5	CH
11	80.6	CH	80.6	CH	81.6	CH	81.6	CH	81.8	CH	80.6	CH
12	47.3	CH	47.0	CH	44.1	CH	43.4	CH	44.2	CH	33.8	CH
13	62.4	CH	58.9	CH	64.1	CH	64.2	CH	63.3	CH	131.3	CH
14	134.6	C	149.5	C	75.5	C	75.3	C	83.9	C	134.4	C
15	130.1	CH	39.5	CH_2	44.7	CH_2	45.9	CH_2	46.8	CH_2	36.2	CH_2
16	37.5	CH	33.6	CH	32.2	CH	31.1	CH	35.4	CH	34.6	CH
17	76.1	CH	76.2	CH	76.8	CH	75.3	CH	80.6	CH	75.7	CH
18	39.4	CH	38.3	CH	38.1	CH	37.7	CH	35.6	CH	38.4	CH
19	79.8	CH	78.7	CH	78.4	CH	78.1	CH	77.7	CH	78.8	CH
20	34.8	CH	35.1	CH	35.4	CH	35.5	CH	35.5	CH	35.0	CH
21	134.4	CH	134.7	CH	135.2	CH	135.6	CH	135.9	CH	134.6	CH
22	130.5	CH	130.3	CH	130.3	CH	130.3	CH	130.3	CH	130.3	CH
23	133.4	CH	133.3	CH	133.4	CH	133.4	CH	133.5	CH	133.3	CH
24	118.3	CH_2	118.2	CH_2	118.2	CH_2	118.2	CH_2	118.2	CH_2	118.2	CH_2
25	16.1	CH_3	15.9	CH_3	16.0	CH_3	16.0	CH_3	16.0	CH_3	15.7	CH_3
26	12.9	CH_3	12.7	CH_3	12.7	CH_3	12.8	CH_3	12.8	CH_3	13.1	CH_3
27	13.3	CH_3	12.8	CH_3	12.2	CH_3	12.2	CH_3	12.2	CH_3	19.9	CH_3
28	18.4	CH_3	17.9	CH_3	21.9	CH_3	22.0	CH_3	22.3	CH_3	17.0	CH_3
29	14.6	CH_3	111.0	CH_2	26.3	CH_3	25.2	CH_3	24.0	CH_3	23.6	CH_3
30	18.7	CH_3	14.2	CH_3	15.9	CH_3	16.4	CH_3	16.4	CH_3	14.8	CH_3
31	8.6	CH_3	9.5	CH_3	9.8	CH_3	10.1	CH_3	11.0	CH_3	9.3	CH_3
32	18.2	CH_3	17.9	CH_3	17.9	CH_3	17.8	CH_3	17.8	CH_3	18.1	CH_3
33	158.6	C	158.9	C	158.0	C	158.3	C	157.8	C	158.2	C

[a] All spectra run in CD_3CN at 125.7 MHz. Chemical shifts are reported in ppm from solvent.

turn coupled to the C-12 methine proton observed at 1.68 ppm. The *cis* arrangement of C-8 and C-9 olefinic protons was evident from the coupling constant of 10.2 Hz. The remaining coupling patterns (C-2 through C-5 and C-13 through C-24) were similar to that of (+)-discodermolide. The three-bond long-range correlations between H-7/C-5, C-9, C-11 and H-11/C-7, C-9, C-13, C-27, C-28 established the presence of a 2,5,6-trisubstituted pyrano-3-ene system in the molecule. The absolute stereochemistry of the substituted pyrano ring was assigned on the basis of NOESY correlations and using the assumption that the absolute stereochemistries of the chiral centers at C-10(*S*) and C-12(*S*) were not changed during the cyclization reaction. The strong NOESY correlations H-6/H-11, H-11/Me-27 indicated that H_2-6, H-11, and Me-27 are arranged on the same side of the pyrano ring system. Since the absolute stereochemistry at C-10(*S*) is known from the structure of (+)-discodermolide, the two new chiral centers were assigned 7*R* and 11*S* stereochemistry. These data in combination with the experimental evidence that **8** is an acid-catalyzed cyclized product of (+)-discodermolide confirmed the structure of **8** as (7*R*,11*S*)-7,11-oxycyclodiscodermolide.

Cytotoxic Activity of (+)-Discodermolide (1) and Analogues 2−8. (+)-Discodermolide (**1**) and its synthetic analogues **2**−**8** were tested for their in vitro cytotoxicity against five tumor cell lines (A549, P388, MFC-7, NCI/ADR, and PANC-1) and one normal cell line (VERO). The results of these assays are given in Table 5. Previously, we have reported that C-11 and C-17 hydroxy groups in discodermolide are essential for strong biological activity against a set of cancer cell lines[5,6] and also showed that the C-10 through C-16 fragment[7−10] is essential for biological activity. Overall, all cyclo-compounds **3**−**8** showed

Table 5. Cytotoxic Activity of **1**−**8**[a]

	IC_{50} in μM					
compound	A549	P388	MCF-7	NCI/ADR	PANC-1	VERO
1	0.015	0.033	0.0024	0.017	0.049	30.0
2	1.99	0.79	0.96	2.8	2.65	0.42
3	6.64	2.13	2.10	>5	6.57	21.9
4	0.10	0.19	0.13	3.40	0.33	0.69
5	0.84	2.42	2.36	>5	3.03	4.3
6	2.64	2.09	1.73	>5	>5	16.6
7	>5	7.65	7.35	>5	>5	11.4
8	1.46	1.00	1.80	4.40	3.13	3.65

[a] A549 (human lung adenocarcinoma cell line); P388 (cultured murine leukemia cell line); MCF-7 (human breast cancer cell); NCI/ADR (adriamycin drug resistant cell line); PANC-1 (human pancreatic cancer cell line); VERO (normal kidney epithelial cell line of African green monkey).

significant loss of activity in all tested cell lines compared to (+)-discodermolide. Compound **7**, which has the 16*R* chirality opposite that of discodermolide, 16(*S*), indicated the least cytotoxicity among all cyclo-analogues. This is in agreement with the earlier finding by Scheiber group[12] that C-16(*S*) stereochemistry is essential for biological activity of (+)-discodermolide. The significant reduction of activity in all five five-membered cyclo-compounds and also in the six-membered cyclo-compound **8** indicated that the hairpin folding observed[1,38] in (+)-discodermolide is essential for its biological activity. Compound **4**, which has an *exo*-methylene group at C-14 (29), showed the highst cytotoxicity among the cyclo-analogues in all assays tested except in the NCI/ADR cancer cell line. However, the activity values of **4** are more than 10-fold less than that of (+)-discodermolide. The VERO (normal kidney epithelial cell line of African green monkey) cell assay measured the

cytotoxicity of the compound against noncancerous cells. The data given in Table 5 showed that (+)-discodermolide has the highest IC_{50} value in the VERO assay and thus indicated that (+)-discodermolide is less toxic than all analogues to normal noncancerous cells. The complete biological activity profile, including the effects of analogues on tubulin polymerization and the G_2/M blocking activity, will be published elsewhere. The details of the cytotoxicity assays were described in the preceding publications.[39,40]

Experimental Section

General Experiment Procedures. UV spectra were measured with a Hitachi U-3010 spectrophotometer. 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 ^{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 measurements were performed on FT/MS APEX III equipped with an electrospray ion source and operated in positive ion mode.

Acid-Catalyzed Deprotection Procedure. A solution of (1*S*,2*R*,3*R*,4*S*,6*Z*,8*S*,9*S*,10*S*,11*Z*,13*S*,15*S*,16*S*,17*S*,18*R*)-3,9,17-tris(*tert*-butyldimethylsilanyloxy)-13,15-dihydroxy-18-(methoxymethylcarbamoyl)-2,4,6,8,10,16-hexamethyl-1-((*Z*)-(*S*)-1-methylpenta-2,4-dienyl)nonadeca-6,11-dienyl carbamate (75 g, 75.17 mmol), prepared according to the method of Kinder[37] *et al*., was dissolved in a total of 27 L of MeOH. This solution was initially treated at room temperature every 15 min with 1.02 kg portions of 3.0 M HCl (total 16 portions, 16.32 kg) followed by 4 portions of 2.03 kg of 3.0 M HCl (total 8.12 kg). After the final portion had been added the reactor wall was washed carefully with 2.4 kg of MeOH. The reaction mixture was stirred at room temperature for 3 h, and a final portion of 8.13 kg of 3.0 M HCl was added. Stirring was continued for a further 2 h at room temperature, and 144 kg of a 5% solution of $NaHCO_3$ was added. The neutralized mixture was then treated with 5.54 kg of pH 6−7 phosphate buffer solution and concentrated in a vacuum at 30 °C until 33 L of distillate was obtained (the pH was monitored during the distillation; if the pH value became too basic, it was readjusted to a value of 6.5 by the addition of 3.0 M HCl). Saturated NaCl (26.6 kg) was then added to the remaining thin suspension followed by EtOAc (16.0 kg). The mixture was stirred for 5 min, whereby the solid redissolved, and 20.7 kg of *tert*-butylmethyl ether was added. The organic phase was separated. The aqueous phase (190 L) was re-extracted with 8 kg of EtOAc, and the phases were allowed to separate. *tert*-Butylmethyl ether (20.7 kg) was added and the two-phase mixture stirred for 5 min. The organic phase was separated and combined with the previous organic extract. The combined organic phases were dried by the addition of 7.75 kg of anhydrous $MgSO_4$ and filtered, and the solid was washed with three portions of 4.4 kg of *tert*-butylmethyl ether. The solvent was then removed by distillation under vacuum at 30 °C. The residue was kept under vacuum (10 mBar) for a further 30 min at 30 °C in order to remove final traces of solvent to give 85% pure (+)-discodermolide as an oily solid (38.0 g, 85%).

Isolation of Side Products. The crude (+)-discodermolide dissolved in 8.81 kg of 2-propanol was treated with water (78.4 kg). This mixture was then applied to a reversed-phase C_{18} column via a filter in order to isolate pure (+)-discodermolide. This process will be described elsewhere. The filtered residue from above was dissolved in 30 L of 2-propanol. Evaporation of the solution gave an oily mixture (13.3 g) containing 3,11,17-*O*-*tris*-(*tert*-butyldimethylsilyl)discodermolide (78.9%), bis-silylated discodermolide (9.8%), 3-*O*-(*tert*-butyldimethylsilyl)-discodermolide (5.7%), and (+)-discodermolide (5.6%). This mixture was dissolved in 90% CH_3CN/water (600 mL) and filtered, cooled to 0 °C, and treated with 37% HCl (36 mL) followed by 60 mL of water. The reaction mixture was stirred at room temperature for 28 h. Water (120 mL) was added, and the mixture concentrated in a vacuum at 23−25 °C. After around 150 mL of distillate had been obtained, a sticky solid began to form. CH_3CN (50 mL) was added and evaporation continued. After a further 350 mL of distillate had been collected a crystal suspension had formed. This suspension was stirred for a further 2 h at room temperature and filtered, and the solid was washed with 4 portions of 50 mL of water. After drying 6.57 g of (+)-discodermolide monohydrate was obtained. The mother liquors were extracted once with 200 mL of heptane. The aqueous phase was re-extracted with 2 portions of 200 mL of EtOAc. The EtOAc phases were combined and evaporated to dryness to give 2.0 g of oil. This oil was chromatographed on a Si gel column (200 g) and eluted with 5% MeOH/CH_2Cl_2 to give the following main fractions: fractions 1 (104 mg), 2 + 3 (77 mg), 4 (75 mg), 5 (308 mg), and 6 (400 mg). Fraction 6 was identified as (+)-discodermolide.

Purification of 3-*O*-(*tert*-Butyldimethylsilyl)discodermolide (2). The Si gel column utilized in the purification of (+)-discodermolide, vide supra, was washed with CH_3CN (100 mL). The washings were then concentrated in a vacuum at 35 °C to give an oily residue (10.7 g). This was dissolved in 1% MeOH/CH_2Cl_2 (5 mL) and chromatographed on a column of Si gel (250 g). Elution with 1% MeOH/CH_2Cl_2 followed by 3% MeOH/CH_2Cl_2 furnished 96% pure 3,11,17-tris-*O*-(*tert*-butyldimethylsilyl)discodermolide (3.79 g), 86% pure 3-*O*-(*tert*-butyldimethylsilyl)discodermolide (2.93 g), (+)-discodermolide (0.35 g), and a mixture of products (1.0 g). The 86% pure 3-*O*-(*tert*-butyldimethylsilyl)discodermolide fraction (5.0 mg) on further purification by HPLC (Si gel, Lichrosorb 5μ, Phenomenex 250 × 10 mm) with 3.5% MeOH in CH_2Cl_2 gave pure **2** (4.1 mg).

3-*O*-(*tert*-Butyldimethylsilyl)discodermolide (2): $[\alpha]^{21}_D$ 26.6° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.32) nm; IR (neat/NaCl) ν_{max} 3410, 2964, 2933, 1713, 1601, 1461, 1381, 1326, 1251, 1035, 970 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS *m/z* 708.4863 [M + H]$^+$ (calcd for $C_{39}H_{70}NO_8Si$, 708.4865).

Purification of (7*E*,9*S*,13*S*,14*E*)-7-Deoxy-7,14-dien-9-13-cyclodiscodermolide (3). The chromatography fraction 5 (50 mg) was subjected to HPLC on a Si gel (Lichrosorb 5μ, Phenomenex 250 × 10 mm) column using a mixture of 8% MeOH in CH_2Cl_2 to yield **3** (30.1 mg).

(7*E*,9*S*,13*S*,14*E*)-7-Deoxy-7,14-dien-9-13-cyclodiscodermolide (3): $[\alpha]^{21}_D$ −18.0° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.27) nm; IR (neat/NaCl) ν_{max} 3374, 2975, 1713, 1607, 1462, 1397, 1320, 1043, 979 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; HRMS *m/z* 576.3895 [M + H]$^+$ (calcd for $C_{33}H_{54}NO_7$, 576.3895).

Purification of (7*E*,9*S*,13*S*)-7-Deoxy-7,14(29)-dien-9-13-cyclodiscodermolide (4), (7*E*,9*S*,13*S*,14*R)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (5), and (7*E*,9*S*,13*S*,14*S**)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (6).** Fraction 4 (20 mg) was subjected to HPLC on a Si gel (Lichrosorb 5μ, Phenomenex 250 × 10 mm) column using a mixture of 6% MeOH in CH_2Cl_2 to yield **4** (10.5 mg), **5** (1.9 mg), and slightly impure **6** (1.4 mg). Further HPLC purification of impure **6** (1.4 mg) using the same solvent system yielded pure **6** (0.9 mg).

(7*E*,9*S*,13*S*)-7-Deoxy-7,14(29)-dien-9-13-cyclodiscodermolide (4): $[\alpha]^{21}_D$ 3.0° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.22) nm; IR (neat/NaCl) ν_{max} 3387, 2933, 1713, 1607, 1464, 1393, 1328, 1238, 980 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; HRMS *m/z* 576.3894 [M + H]$^+$ (calcd for $C_{33}H_{54}NO_7$, 576.3895).

(7*E*,9*S*,13*S*,14*R)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (5):** $[\alpha]^{21}_D$ 32.4° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.23) nm; IR (neat/NaCl) ν_{max} 3395, 2979, 2929, 1713, 1607, 1461, 1381, 1326, 1231, 970 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; HRMS *m/z* 616.3821 [M + Na]$^+$ (calcd for $C_{33}H_{55}NO_8Na$, 616.3821).

(7*E*,9*S*,13*S*,14*S)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (6):** $[\alpha]^{21}_D$ 36.0° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.29) nm; IR (neat/NaCl) ν_{max} 3395, 2969, 2929, 1713, 1601, 1456, 1391, 1326, 1231, 976 cm^{-1}; ^{1}H and ^{13}C NMR

data, see Tables 3 and 4; HRMS *m*/*z* 594.4000 $[M + H]^+$ (calcd for $C_{33}H_{56}NO_8$, 594.4002).

Purification of (7*E*,9*S*,13*S*,14*S,16*R*)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (7) and (7*R*,11*S*)-7-11-Oxycyclodiscodermolide (8).** Fraction 1 (60 mg) was subjected to HPLC on a Si gel (Lichrosorb 5μ, Phenomenex 250 × 10 mm) column using a mixture of 5% MeOH in CH_2Cl_2 to yield **8** (15.5 mg) and impure **7** (1.3 mg), which on further purification with 3.5% MeOH in CH_2Cl_2 gave pure **7** (0.7 mg).

(7*E*,9*S*,13*S*,14*S,16*R*)-7-Deoxy-14-hydroxy-7-en-9-13-cyclodiscodermolide (7):** $[\alpha]^{21}_D$ 18.0° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.15) nm; IR (neat/NaCl) ν_{max} 3395, 2969, 2929, 1713, 1603, 1457, 1374, 1317, 1031, 969 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; HRMS *m*/*z* 576.3894 $[M + H - H_2O]^+$ (calcd for $C_{33}H_{54}NO_7$, 576.3895).

(7*R*,11*S*)-7-11-Oxycyclodiscodermolide (8): $[\alpha]^{21}_D$ 24.2° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.54) nm; IR (neat/NaCl) ν_{max} 3405, 2964, 2934, 1712, 1601, 1456, 1386, 1326, 1226, 1041 905 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRMS *m*/*z* 576.3895 $[M + H]^+$ (calcd for $C_{33}H_{54}NO_7$, 576.3895).

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