New Dimeric Macrolide Glycosides from the Marine Sponge *Myriastra clavosa*

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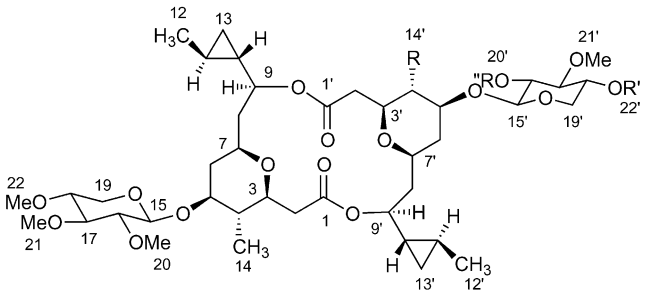
Clavosolides A−D (**1**−**4**), dimeric macrolides incorporating cyclopropyl, tetrahydropyranyl, and glycosidic ring systems, were isolated from the cytotoxic extract of a Philippines collection of the marine sponge *Myriastra clavosa*. The structures of the clavosolides, which occurred as only trace metabolites, were elucidated through extensive NMR spectroscopic analyses. Clavosolides A (**1**) and B (**2**) were recently reported metabolites from *M. clavosa*, while the unsymmetrical dimers clavosolides C (**3**) and D (**4**) had new structures.

The aqueous extract of a Philippines collection of the marine sponge *Myriastra clavosa* (order Astrophorida, family Ancorinidae) produced a distinctive pattern of differential cytotoxicity and antiproliferative effects when it was tested in the NCI's 60-cell antitumor screen.[1] This suggested that the *M. clavosa* extract might contain cytotoxic compounds capable of modulating specific molecular targets;[2] thus detailed chemical studies were initiated. A previous investigation of *M. clavosa* collected in Palau provided clavosines A−C,[3] which are potent cytotoxic compounds related to the calyculins[4] and calyculinamides.[5] The chemical separation of our Philippines sample of *M. clavosa* revealed no detectable clavosines; however, four dimeric macrolides, clavosolides A−D (**1**−**4**), were isolated and characterized. These compounds were obtained only in trace amounts after extensive chromatography of the sponge extract. An 18.7 g portion of the extract was subjected to C_4 vacuum liquid chromatography, gel permeation with Sephadex LH-20, and preparative thin-layer chromatography to afford 0.3 mg of clavosolide A (**1**), 0.2 mg of clavosolide B (**2**), 0.4 mg of clavosolide C (**3**), and 0.2 mg of clavosolide D (**4**). While this present paper was in preparation, the isolation and structural elucidation of clavosolides A (**1**) and B (**2**) were reported by Rao and Faulkner from a different collection of *M. clavosa* that also came from the Philippines.[6] The structures of clavosolides A (**1**) and B (**2**) that we isolated were independently solved by extensive NMR analyses, and they were identical to the two compounds described by Faulkner's group.[6]

Results and Discussion

HRFABMS established the molecular formula of clavosolide C (**3**) as $C_{43}H_{70}O_{16}$, while the presence of alcohol, ester, and ether functionalities were indicated by IR bands at 3446, 1738, and 1077 cm^{-1}, respectively. The structure of **3** was elaborated by detailed analyses of NMR data sets acquired in $CDCl_3$, C_6D_6, and DMSO-d_6, and it was apparent from the high degree of signal doubling and direct overlap that **3** was a nonsymmetrical dimer related to compounds **1** and **2**. Spectral dispersion and resolution



1 R = R' = R'' = CH_3
2 R = R' = CH_3, R'' = H
3 R = R'' = CH_3, R' = H
4 R = H, R' = R'' = CH_3

varied depending upon the solvent, and some of the correlations that were critical for the structural elucidation of **3** were observed only in one or the other of the solvent systems. Because of the limited sample size, ^{13}C chemical shifts of protonated carbons were obtained indirectly from HSQC correlations, while those of the nonprotonated carbons were assigned from HMBC data (Table 1). The ^1H NMR and HSQC data for **3** established the presence of five methoxyl groups and, in conjunction with $^1H-^1H$ COSY[7] and TOCSY spectra, allowed assignment of all of the proton spin systems in **3**. The two sugar moieties were identified as pyranoses through HMBC correlations (C_6D_6) of the anomeric carbons at δ 105.9 (C-15) and 105.7 (C-15′) to H-19b and H-19b′, respectively. Three-bond correlations from the methoxyl hydrogens to the oxymethine pyranose carbons placed methoxyl groups at C-16, C-17, C-18, C-16′, and C-17′. The ^1H and ^{13}C resonances recorded for the sugar moieties in **3** were in excellent agreement with literature values for similar methylated sugars,[8] which supported the assignment of the lone hydroxyl group at C-18′. Careful analysis of the ^1H NMR spectral data for **3** obtained in DMSO-d_6 allowed resolution of all the pyranoside protons. The relative stereochemistry and β-linkage of the two sugars were defined by large (J = 8−9 Hz) diaxial couplings that were measured for the oxymethine protons. All of the oxygenated substituents in the sugars were assigned to be equatorial; thus compound **3** contained two methylated β-xylose residues. The point of attachment of the xylose substituents was established by HMBC correlations from the H-15 and H-15′ anomeric protons to C-5 and C-5′, respectively. In support of this assignment, a series of 1-D NOESY experiments ($CDCl_3$) revealed NOE

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Table 1. NMR Spectral Data for Clavosolide C (**3**) in $CDCl_3$ and C_6D_6

pos.	δ_C ($CDCl_3$)[a]	δ_H mult (*J* in Hz) ($CDCl_3$)[b]	HMBC[c]	δ_C (C_6D_6)[a]	δ_H mult (*J* in Hz) (C_6D_6)[b]	HMBC[c]
1	171.0[d]		2a, 2b, 9′	171.3[d]		2a, 2b, 3, 9′
2	39.1	2.39 dd (6.2, 17.2)		40.6	2.67 dd (5.5, 18.2)	
		2.52 dd (3.3, 17.2)			2.83 dd (4.0, 18.3)	
3	76.8	3.42 m	2a, 14	76.6	3.87 m	2a, 2b, 14
4	42.4	1.35 m	14	44.0	1.43 m	2a, 14
5	83.1	3.22 m	4, 6a, 6b, 14, 15	82.7	3.11 m	4, 6a, 6b, 14, 15
6	40.6	1.35 m		41.5	1.56 ddd (11.5, 11.8, 12.0)	
		2.02 ddd (4.9, 5.2, 12.2)			2.12 ddd (4.7, 5.3, 12.6)	
7	74.8	3.42 m	6a, 9	75.1	3.61 m	3, 6a, 8b
8	41.1	1.66 br d (14.2)		41.7	1.45 br d (13.9)	10
		1.86 ddd (8.4, 9.6, 14.4)			1.95 ddd (9.5, 10.5, 11.2)	
9	76.8	4.40 t (8.8)	8b	77.0	4.70 br t (8.5)	8b, 13a, 13b
10	24.6	0.69 dddd (4.4, 4.7, 8.4, 8.8)	12	24.7	0.60 dddd (4.4, 4.4, 8.1, 8.8)	12, 13b
11	11.8	0.80 m		11.7	0.83 m	12, 13a
12	18.4	0.95 d (5.5)		18.5	0.92 d (5.5)	10, 11, 13a, 13b
13	10.8	0.20 ddd (4.7, 4.8, 8.8)	12	11.1	0.04 ddd (4.7, 4.8, 8.8)	
		0.32 ddd (4.4, 4.8, 8.4)			0.23 m	
14	12.5	0.95 d (5.5)		12.6	1.08 d (6.6)	
15	105.4	4.24 d (7.7)	16	105.9	4.20 d (7.3)	5, 19b
16	83.8	2.94 dd (7.4, 9.1)	17, 20	84.6	3.13 m	18, 20
17	85.1	3.07 m	18, 21	86.3	3.19 m	16, 21
18	79.4	3.22 m	17, 19a, 22	80.2	3.19 m	17, 19b, 22
19	63.2	3.06 m		63.4	3.00 m	
		3.93 dd (4.2, 11.6)			3.84 dd (4.9, 11.4)	
20	60.7	3.56 s, 3H	16	60.5	3.56 s, 3H	16
21	60.6	3.59 s, 3H	17	60.7	3.60 s, 3H	17
22	58.7	3.44 s, 3H	18	58.2	3.15 s, 3H	
1′	171.2[d]		2a′, 2b′, 9	171.1[d]		2a′, 2b′, 3′, 9
2′	39.1	2.39 dd (6.2, 17.2)		40.5	2.63 dd (5.3, 18.2)	
		2.52 dd (3.3, 17.2)			2.80 dd (4.0, 18.1)	
3′	76.8	3.42 m	2a′, 14′	76.6	3.87 m	2a′, 2b′, 14′
4′	42.4	1.35 m	14′	44.0	1.41 m	2a′, 14′
5′	83.1	3.22 m	4′, 6a′, 6b′, 15′	82.7	3.08 m	4′, 6a′, 6b′, 14′, 15′
6′	40.6	1.35 m		41.5	1.52 ddd (11.7, 11.8, 12.0)	
		2.02 ddd (4.9, 5.2, 12.2)			2.11 ddd (4.7, 5.3, 12.6)	
7′	74.8	3.42 m	6a′, 9′	75.1	3.61 m	3′ 6a′, 8b′
8′	41.1	1.66 br d (14.2)		41.7	1.42 br d (13.9)	10′
		1.86 ddd (8.4, 9.6, 14.4)			1.92 ddd (9.5, 10.5, 11.2)	
9′	76.8	4.40 t (8.8)	8b′	77.0	4.70 br t (8.5)	8b′, 13a′, 13b′
10′	24.6	0.69 dddd (4.4, 4.7, 8.4, 8.8)	12′	24.7	0.60 dddd (4.4, 4.7, 8.1, 8.8)	12′, 13b′
11′	11.8	0.80 m		11.7	0.83 m	12′, 13a′
12′	18.4	0.95 d (5.5)		18.5	0.92 d (5.5)	10′, 11′, 13a′, 13b′
13′	10.8	0.20 ddd (4.7, 4.8, 8.8)	12′	11.1	0.04 ddd (4.7, 4.8, 8.8)	
		0.32 ddd (4.4, 4.8, 8.4)			0.23 m	
14′	12.5	0.95 d (5.5)		12.6	1.04 d (6.6)	
15′	105.1	4.34 d (7.7)	16′	105.7	4.22 d (7.0)	5′, 16′, 19b′
16′	83.1	3.01 dd (7.1, 8.7)	15′, 17′, 20′	83.9	3.08 m	17′, 18′, 20′
17′	84.8	3.07 m	16′, 21′	85.7	3.01 t (8.5)	16′, 21′
18′	69.1	3.58 m	17′	69.9	3.52 ddd (5.1, 8.5, 9.9)	16′, 17′, 19a′, 19b′
19′	64.5	3.20 d (10.0, 11.6)		65.1	3.08 m	
		3.92 dd (4.4, 11.1)			3.84 dd (4.9, 11.4)	
20′	60.2	3.55 s, 3H	16′	60.0	3.45 s, 3H	16′
21′	60.6	3.59 s, 3H	17′	60.4	3.46 s, 3H	17′

[a] Assignments made from HSQC correlations. [b] With geminal protons, the smaller δ value is given the "a" designation, the larger δ value is given the "b" designation. [c] Protons that correlated with the listed carbon. [d] Assignments made from HMBC correlations.

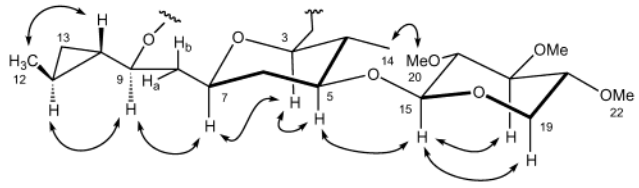

Figure 1. Selected NOE interactions for **3**.

interactions between H-5 and the anomeric proton H-15 and between H-5′ and H-15′. In addition, NOESY correlations were observed from H_3-14 and H_3-14′ to H_3-20 and H_3-20′, respectively (Figure 1).

HMBC correlations between the two carbonyl carbons (δ 171.0 and 171.2, $CDCl_3$) and the two overlapped oxygenated methines at δ 4.40 (H-9 and H-9′, $CDCl_3$) indicated ester links at these positions. A weak HMBC correlation between the δ 3.87 hydrogens (H-3 and H-3′) and the δ 75.1 carbons (C-7 and C-7′, C_6D_6) coupled with strong NOE interactions between the corresponding protons (H-3 and H-3′ with H-7 and H-7′) suggested the presence of two tetrahydropyran (THP) rings that incorporated C-3 through C-7 and C-3′ through C 7′. The H-3 and H-3′ hydrogens also showed NOE correlations to the δ 3.11 (H-5) and 3.08 (H-5′) hydrogens and to the adjacent methyl groups at δ 1.08 (H_3-14) and 1.04 (H_3-14′) (C_6D_6). The methylene protons at δ 1.56 (H-6a) and 1.52 (H-6a′) each appeared as a ddd with three large *J* values of approximately 12 Hz. These data helped define axial orientations for the H-3 (H-3′), H-5 (H-5′), and H-7 (H-7′) oxymethines and placed the methyl groups (H_3-14 and H_3-14′) equatorial on the THP ring (Figure 1).

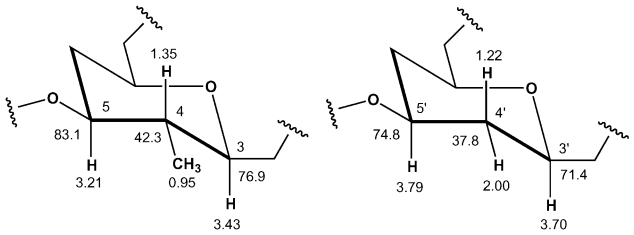

Figure 2. Selected ^{1}H and ^{13}C δ values for the THP rings of **4**.

The *trans* relationship of the cyclopropyl substituents was indicated by a $J_{10,11}$ ($J_{10',11'}$) value of 4.4 Hz.[9] This was confirmed by NOE correlations (Figure 1) between H_3-12 (H_3-12′) and H-10 (H-10′) and between the H-11 (H-11′) cyclopropyl proton and the oxymethine H-9 (H-9′). The latter proton, in turn, displayed a NOE correlation to the axial THP hydrogen H-7 (H-7′), placing H-11, H-9, and H-7 all on the same face of the molecule. In keeping with the conformation suggested by the NOE data and depicted in Figure 1, the large couplings of approximately 9−10 Hz observed for $J_{7,8b}$, $J_{8b,9}$, and $J_{9,10}$ were indicative of large dihedral angles between these pairs of protons. Conversely, $J_{7,8a}$ and $J_{8a,9}$ were close to 0 Hz, indicating a near 90° angle between these protons. Thus, the structure and relative stereochemistry of clavosolide C (**3**) was assigned as shown.

Approximately 0.2 mg of an additional compound, clavosolide D (**4**), was isolated from the 2:1 MeOH−H_2O eluant of the initial C_4 column separation of the aqueous *M. clavosa* extract. While the limited mass of this sample made complete spectral characterization difficult, it was possible to propose a structure for this compound based on its similarity to compounds **1**−**3**. HRFABMS established the molecular formula of clavosolide D (**4**) as $C_{43}H_{70}O_{16}$, rendering it isomeric with **2** and **3**. ^{1}H, COSY, TOCSY, and HSQC NMR data in both $CDCl_3$ and C_6D_6 established that both pyranose moieties in **4** were identical and trimethylated. These data revealed that one of the THP rings in compound **4** lacked a methyl group. Figure 2 illustrates differences in the ^{1}H and ^{13}C resonances ($CDCl_3$) recorded for the two THP rings of **4**. The methyl group previously substituted at C-4′ in **1**−**3** was missing in **4**, and instead two H-4′ methylene protons at δ 1.22 and 2.00 were evident. Loss of the C-14′ methyl group's deshielding effect on the α- (C-4′) and β-carbons (C-3′ and C-5′) resulted in an upfield shift of these resonances. Proton−proton coupling constant analysis revealed that the relative stereochemistry of the demethylated THP ring in **4** was the same as that found in compounds **1**−**3**. In the C_6D_6 ^{1}H NMR spectrum of **4**, H-4a′ appeared as a quartet with a *J* value of approximately 11 Hz. The multiplicity and magnitude of this coupling established H-4a′ as axial with two axial neighbors, H-3′ and H-5′. A 1-D NOESY experiment (C_6D_6) irradiating H-3′ gave correlations to H-4b′, H-5′, and H-7′, which verified that H-3′, H-5′, and H-7′ were all axial.

Clavosolides A−D (**1**−**4**) represent a new family of marine natural products with a number of unusual structural features such as the incorporation of two cyclopropyl groups into a dimeric macrolide structure and bis-glycosylation with highly methylated xylose residues. Glycosylated macrolides have been reported from a relatively limited number of marine sources which include the polycavernosides from the red alga *Polycavernosa tsudai*,[10] the callipeltosides from the sponge *Callipelta* sp.,[11] the aurisides from the sea-hare *Dolabella auricularis*,[12] lyngbyaloside[13] and lyngbouilloside[14] from the cyanobacterium *Lyngbya bouillonii*, and the macrolactins,[15] maduralide,[16] and halichoblelide[17] from marine bacterial isolates. A thorough evaluation of the cytotoxic properties of the clavosolides **1**−**4** was not possible because of the limited mass of these compounds. Rao and Faulkner reported that clavosolides A (**1**) and B (**2**) were noncytotoxic,[6] and when we tested clavosolide C (**3**) for cytotoxicity against 10 different human tumor cell lines, it was inactive at a high test concentration of 10 μg/mL. Continuing efforts are being made to isolate additional quantities of **1**−**4** for more detailed biological evaluations and to identify the compound(s) responsible for the cytotoxic activity observed in the *M. clavosa* extract.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a Beckman DU-640 and a Perkin-Elmer 1600 FTIR spectrometer, respectively. ^{1}H NMR spectra were obtained at 500 MHz and ^{13}C NMR spectra at 125 MHz on a Varian INOVA 500 spectrometer. Chemical shifts were reported in ppm relative to the solvent signals ($CDCl_3$ δ_H 7.24, δ_C 77.0; C_6D_6 δ_H 7.15, δ_C 128.0; DMSO-d_6 δ_H 2.49, δ_C 39.5). Inverse-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH}$ = 140 Hz) and HMBC (optimized for $^nJ_{CH}$ = 5.5 and 8.5 Hz) pulse sequences. Assignments for the ^{13}C resonances were based on HSQC and HMBC correlations. High-resolution mass spectra were acquired on a JEOL SX102 mass spectrometer, and CsI was added to the samples prior to analysis. VLC was carried out with wide-pore C_4 and YMC 60 Å diol-bonded silica stationary phases. TLC was done on EM HPTLC 10 × 10 cm glass-backed plates, 200 μm thickness.

Animal Materials. The sample (290 g, wet weight) of *Myriastra clavosa* Ridley 1884 (Astrophorida, Ancorinidae) was collected around Pamilican Island in the Bohol Sea, Philippines, in 1997. The sponge was frozen shortly after collection and transported frozen to Frederick, MD, where the extracts were prepared. The collection was made by the Coral Reef Research Foundation, and taxonomic identification was made by Michelle Kelly. A voucher specimen (0CDN5139) for this collection is maintained at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen sponge was ground to a coarse powder and percolated overnight with H_2O. The solid sponge material was separated from the H_2O solution by centrifugation, and the clarified liquid was lyophilized to provide 75.3 g of aqueous extract. An 18.7 g portion of the extract was subjected to VLC on a wide-pore (WP) C_4 column in four separate batches employing a sequence of five solvent systems: 100% H_2O, H_2O−MeOH (2:1), H_2O−MeOH (1:2), 100% MeOH, and MeOH−CH_2Cl_2 (1:1). The 100% MeOH fractions (120 mg total) were taken up in 1:1 MeOH−CH_2Cl_2 and filtered, and the filtrate (107 mg) was passed through a Sephadex LH-20 column eluted with MeOH−CH_2Cl_2 (1:1). Pooled fractions from this column provided 60 mg of an oil that was subjected to VLC on a diol-bonded silica column eluted with increasingly polar mixtures of MeOH in $CHCl_3$. The 2% MeOH−$CHCl_3$ fraction (15 mg) was further fractionated by TLC on RP-C18 plates developed with 15% H_2O−MeOH to give 1.8 mg of material, which was then subjected to amino-bonded silica TLC with 10% 2-propanol−hexane and then diol-bonded silica TLC developed with the same solvent mixture (2×) to provide 0.4 mg of **3**.

The H_2O−MeOH (1:2) fractions from the WP-C_4 columns (323 mg total) were processed in the same manner as described above through the diol VLC step to give 5.6 mg of material. Reversed-phase TLC on RP-C18 plates developed with 10% H_2O−MeOH (2×) afforded three fractions: A, (2.0 mg), B (0.4 mg), and C (0.4 mg). Fraction A, after TLC on amino-bonded silica with 3% 2-propanol−hexane (3×), gave 0.3 mg of **1**. Fractions B and C were purified in the same manner as fraction A with four developments, rather than three, to give 0.2 mg each of **2** and **4**.

Table 2. ^{1}H and ^{13}C NMR Spectral Data for Clavosolide D (**4**) in $CDCl_3$

pos.	δ_C [a]	δ_H mult (*J* in Hz)	pos.	δ_C [a]	δ_H mult (*J* in Hz)
1	n.o.[b]		1′	n.o.[b]	
2	38.8	2.39 dd (6.6, 18.0) 2.52 bd (17.6)	2′	38.8	2.32 dd (3.7, 17.8) 2.52 d (18.0)
3	76.9	3.43 m	3′	71.4	3.70 m
4	42.3	1.35 m	4′	37.8	1.22 m 2.00 dt (4.9, 11.6)
5	83.1	3.21 ddd (5.3, 10.3, 10.5)	5′	74.8	3.79 m
6	40.7	1.35 m 2.02 br dt (4.9, 11.6)	6′	39.4	1.24 m 1.93 br d (12.4)
7	75.0	3.42 m	7′	75.0	3.42 m
8	41.0	1.67 br d (14.0) 1.87 m	8′	41.0	1.67 br d (14.0) 1.89 m
9	77.0	4.39 t (8.9)	9′	77.0	4.41 bt (9.2)
10	24.7	0.68 m	10′	24.7	0.68 m
11	11.8	0.81 m	11′	11.8	0.81 m
12	18.4	0.94 d (5.8)	12′	18.4	0.94 d (5.8)
13	10.8	0.20 ddd (4.8, 4.7, 8.4) 0.32 ddd (4.4, 4.8, 8.7)	13′	10.8	0.20 ddd (4.7, 4.8, 8.4) 0.32 ddd (4.4, 4.8, 8.7)
14	12.5	0.95 d (5.8)			
15	105.4	4.24 d (7.6)	15′	102.0	4.28 d (7.5)
16	83.6	2.93 dd (7.6, 9.1)	16′	83.5	2.92 dd (7.5, 9.2)
17	85.3	3.06 m	17′	85.2	3.06 m
18	79.2	3.22 ddd (5.3, 10.3, 10.5)	18′	79.2	3.22 ddd, 5.3, 10.3, 10.5)
19	63.1	3.06 m 3.92[c] dd (5.5, 9.2)	19′	63.1	3.06 m 3.94[c] dd (5.2, 11.6)
20	60.6[e]	3.56[d] s, 3H	20′	60.5[e]	3.53[d] s, 3H
21	60.6[e]	3.59[d] s, 3H	21′	60.6[e]	3.58[d] s, 3H
22	58.7	3.44 s, 3H	22′	58.7	3.44 s, 3H

[a] Assigned from HSQC data. [b] Not observed. [c–e] Values may be interchanged.

Clavosolide C (3): white solid; $[\alpha]_D$ −20° (*c* 0.04, MeOH); IR ν_{max} (neat) 3445, 2924, 2853, 1738, 1455, 1372, 1261, 1157, 1077 cm^{-1}; ^{1}H and ^{13}C NMR in $CDCl_3$ and C_6D_6, see Table 1; ^{1}H NMR (DMSO-d_6) δ 0.19 (m, H-13a and H-13a′), 0.37 (m, H-13b and H-13b′), 0.68 (m, H-11 and H-11′), 0.76 (m, H-10 and H-10′), 0.86 (d, J = 6.6 Hz, H_3-14 and H_3-14′), 0.92 (d, J = 5.8 Hz, H_3-12 and H_3-12′), 1.15 (m, H-6a and H-6a′), 1.20 (m, H-4 and H-4′), 1.58 (br d, J = 13.9 Hz, H-8a and H-8a′), 1.73 (dt, J = 14.3, 8.1 Hz, H-8b and H-8b′), 1.95 (m, H-6b and 6b′), 2.22 (dd, J = 17.2, 6.6 Hz, H-2a and H-2a′), 2.56 (dd, J = 17.2, 3.3 Hz, H-2b and H-2b′), 2.74 (dd, J = 9.4, 8.1 Hz, H-16), 2.78 (dd, J = 8.8, 8.1 Hz, H-16′), 2.92 (dd, J = 9.2, 8.8 Hz, H-17), 3.03 (dd, J = 10.3, 9.5 Hz, H-17′ and H-19a′), 3.05 (dd, J = 10.9, 10.3 Hz, H-19a), 3.12 (m, H-18), 3.42 (s), 3.43 (s), 3.45 (s), 3.47 (s) 3.61 (dd, J = 10.6, 5.8 Hz, H-19b′), 3.87 (dd, J = 12.1, 5.8 Hz, H-19b), 4.24 (m, H-9 and H-9′), 4.29 (d, J = 8.1 Hz, H-15), 4.31 (d, J = 7.4 Hz, H-15′); H-3, H-3′, H-5, H-5′, H-7, H-7′, and H-18′ were obscured by the residual H_2O signal; COSY, see ref 7; HRFABMS (M + Cs)$^+$ *m*/*z* 975.3756, calcd for $C_{43}H_{70}O_{16}Cs$, 975.3718.

Clavosolide D (4): oil; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2; ^{1}H NMR (C_6D_6) δ 0.04 (m, H-13a and H-13a′), 0.22 (ddd, J = 4.4, 4.6, 8.7 Hz, H-13b and H-13b′), 0.60 (m, H-10 and H-10′), 0.86 (m, H-11 and H-11′), 0.92 (d, J = 5.4 Hz, H_3-12 and H_3-12′), 1.07 (d J = 6.6 Hz, H-14), 1.21 (q, J = 11.3 Hz, H-4a′), 1.35 (m, H-8a′), 1.36 (m, H-6a′), 1.41 (m, H-4), 1.46 (br d, J = 15.0 Hz, H-8a), 1.56 (ddd, J = 11.7, 11.8, 12.1 Hz, H-6a), 1.90 (m, H-8b′), 1.92 (m, H-6b′), 1.95 (m, H-8b and H-4b′), 2.15 (br dd, J = 3.7, 12.1 Hz, H-6b), 2.41 (dd, J = 4.8, 17.9 Hz, H-2a′), 2.65 (dd, J = 4.4, 17.8 Hz, H-2a), 2.80 (dd, J = 4.4, 18.3 Hz, H-2b), 2.85 (dd, J = 5.1, 18.3 Hz, H-2b′), 3.00 (m, H-19a and H-19a′), 3.14 (m, H-5, H-16 and H-16′), 3.15 (s, H_3-22), 3.17 (s, H_3-22′), 3.21 (m, H-17, H-17′, H-18 and H-18′), 3.47 (m, H-7′), 3.53 (m, H-7), 3.53 (s, H_3-20′), 3.56 (s, H_3-20), 3.60 (s, H_3-21), 3.63 (s, H_3-21′), 3.66 (m, H-5′), 3.84 (m, H-3, H-19b and 19b′), 4.00 (br ddd, J = 4.8, 5.1, 10.2 Hz, H-3′), 4.22 (d, J = 7.4 Hz, H-15), 4.23 (d, J = 7.3 Hz, H-15′), 4.66 (br t, J = 8.8 Hz, H-9′), 4.69 (br t, J = 9.1 Hz, H-9); HRFABMS (M + Cs)$^+$ *m*/*z* 975.3733, calcd for $C_{43}H_{70}O_{16}Cs$, 975.3718.

Cytotoxicity Assays. DMSO solutions of the crude extract, chromatography fractions, and purified compounds were tested as described previously.[18] Ten different human tumor cell lines, COLO 205 (colon), LOX and MALME-3M (melanoma), K-562 and MOLT-4 (leukemia), A549 and H-460 (non-small cell lung), OVCAR-3 (ovarian), SNB-19 (CNS), and MCF7 (breast), were employed to test purified clavosolide C (**3**) for cytotoxic activity. None of these cell lines revealed either cytotoxic or antiproliferative effects at a high test concentration of 10 μg/mL of **3**.

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