Prenylbicyclogermacrane Diterpenoids from the Formosan Soft Coral *Nephthea pacifica*

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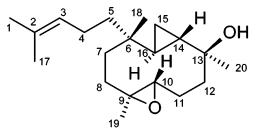
Ten new prenylbicyclogermacrane diterpenoids, pacificins A−J (**1**−**10**), were isolated from the methylene chloride solubles of the Formosan soft coral *Nephthea pacifica.* The structures were elucidated by 1D and 2D NMR spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

Soft corals of the genus *Nephthea* are rich in terpenoids[1−11] and steroids.[12] As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Nephthea pacifica* Kükenthal (family Nephtheidae) was studied as CH_2Cl_2 extracts and showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[13,14] Bioassay-guided fractionation resulted in the isolation of 10 new prenylbicyclogermacrane diterpenoids, pacificins A−J (**1**−**10**).



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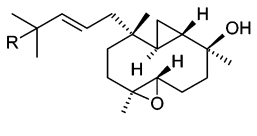

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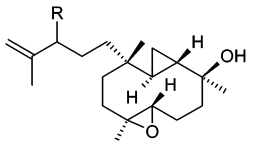
3, R = OOH



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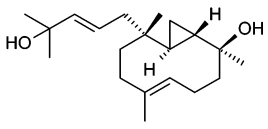

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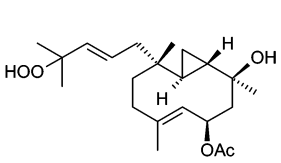

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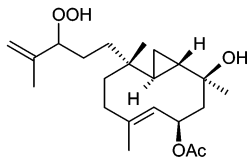

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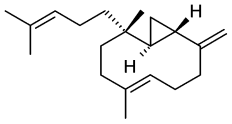

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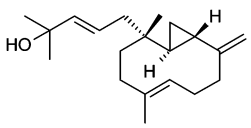

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Results and Discussion

Pacificin A (**1**) was isolated as a colorless amorphous solid. HREIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **1** as $C_{20}H_{34}O_2$, with four degrees of unsaturation. ^{13}C NMR and DEPT spectra of **1** exhibited the presence of four methyls, seven sp^3 methylenes, three sp^3 methines, one sp^2 methine, three sp^3 quaternary carbons, and one sp^2 quaternary carbon. The presence of two sp^2-hybridized carbon atoms in the molecule, as deduced from the ^{13}C and DEPT NMR spectra (Table 2), corresponding to one carbon−carbon double bond as the only double bond, indicated compound **1** to be tricyclic. The presence of a trisubstituted epoxy group in **1** was shown by the NMR data (δ_H 2.81 d; δ_C 61.9 qC, 66.2 CH) (Tables 1 and 2). The NMR data (δ_H 0.20 m, 0.38 m, 0.73 m, 1.10 m; δ_C 3.9 CH_2, 27.1 CH, 28.8 CH) (Tables 1 and 2) pointed to a cyclopropane ring in **1**. The ^1H NMR spectrum also contained signals for five tertiary methyl groups (δ_H 0.46, 0.73, 1.30, 1.58, 1.65). In addition, a signal at δ_H 5.06 was attributed to an olefinic proton and was confirmed by ^{13}C NMR spectroscopy (δ_C 125.0 CH). The presence of an ambiguous carbon bearing an oxygen (δ_C 72.9 qC) was shown in the ^{13}C NMR spectrum. The spectral data of **1** exhibited some similarity to those of a prenylbicyclogermacrane diterpenoid, palmatol, isolated from *Alcyonium palmatum*,[15] except for the differences of chemical shifts in the vicinity of C-9/C-10. Measurement of the $^{13}C-^{13}C$ homonuclear shift correlation 2D spectrum (INADEQUATE) (Supporting Information) of **1** together with COSY, HMQC, and HMBC (Figure 1) experiments established its chemical structure and enabled also the assignment of all resonances in the NMR spectra. The relative stereochemistry of **1** was deduced from a 2D NOESY experiment (Figure 2), which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From these data, pacificin A can be formulated as **1**.

The molecular formula of pacificin B (**2**) proved to be $C_{20}H_{34}O_3$ by HREIMS and ^{13}C NMR data. Detailed comparison of ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of **2** and **1** revealed that **2** differed from **1** in the side chain. COSY correlation between H-3/H-4 and H-4/H-5, HMBC correlations from H-1/H-17 to C-2/C-3 and H-5 to C-3/C-6/C-7, and a $J_{3,4}$ of 15.6 Hz placed an *E* double bond between C-3 and C-4. The relative stereochemistry of **1** was determined by a 2D NOESY experiment, which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From the aforementioned data, pacificin B can be formulated as **2**.

Pacificin C (**3**) had the molecular formula $C_{20}H_{34}O_4$, 16 mass units higher than that of **2**. The ^1H and ^{13}C NMR

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Table 1. ^{1}H NMR Data of **1**–**10**

H	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[a]	**8**[a]	**9**[a]	**10**[a]
1	1.65 s	1.33 s	1.34 s	4.86 s, 4.93 s	5.02 s, 5.04 s	1.23 s	1.35 s	5.01 s, 5.04 s	1.69 s	1.33 s
3	5.06 t (6.9)	5.63 d (15.6)	5.58 d (15.6)	4.00 t (6.9)	4.25 t (6.9)	5.59 d (15.9)	5.55 d (15.6)	4.23 t (6.9)	5.11 t (6.9)	5.61 d (15.6)
4	2.04 m	5.69 m	5.76 m	1.22 m	1.29 m	5.72 m	5.74 m	1.60 m	2.10 m	5.69 m
5	1.15 m, 1.28 m	1.96 m, 2.04 m	1.97 m, 2.07 m	1.21 m	1.11 m	1.86 m, 2.01 m	2.02 m	1.22 m	1.31 m	1.99 m
7	1.50 m, 1.74 m	1.56 m, 1.69 m	1.53 m, 1.68 m	1.49 m, 1.75 m	1.48 m, 1.74 m	1.40 m, 1.76 m	1.51 m, 1.74 m	1.44 m, 1.78 m	1.40 m, 1.91 m	1.42 m, 1.79 m
8	1.26 m, 2.01 m	1.23 m, 2.02 m	1.24 m, 2.05 m	1.27 m, 2.06 m	1.28 m, 2.06 m	1.96 m, 2.29 m	2.33 dt (2.4, 12.6), 2.18 m	2.36 dt (2.4, 12.6), 2.18 m	2.08 m, 2.31 m	2.08 m, 2.32 m
10	2.81 br d (10.2)	2.86 br d (10.5)	2.83 br d (9.9)	2.83 br d (9.9)	2.84 br d (8.4)	5.23 br d (11.1)	5.23 br d (10.8)	5.23 br d (10.5)	5.36 br d (10.5)	5.35 br d (11.1)
11	1.36 m, 1.96 m	1.40 m, 2.06 m	1.42 m, 2.05 m	1.36 m, 2.04 m	1.37 m, 2.05 m	1.99 m, 2.25 m	5.44 dt (4.5, 10.8)	5.44 dt (3.9, 10.5)	2.15 m, 2.34 m	2.14 m, 2.35 m
12	1.94 m	1.96 m	1.97 m	1.97 m	1.96 m	1.80 m	2.17 dd (11.4, 4.2), 2.04 m	2.13 dd (12.3, 3.9), 1.97 m	2.16 m, 2.48 m	2.17 m, 2.46 m
14	1.10 m	1.15 m	1.14 m	1.15 m	1.14 m	0.91 m	1.03 m	1.00 m	0.98 m	0.97 m
15	0.20 m, 0.38 m	0.30 m, 0.44 m	0.28 m, 0.43 m	0.23 m, 0.39 m	0.25 m, 0.43 m	0.23 m, 0.49 m	0.27 m, 0.41 m	0.23 m, 0.37 m	0.60 m, 0.68 m	0.64 m, 0.69 m
16	0.73 m	0.74 m	0.73 m	0.73 m	0.74 m	0.56 m	0.55 m	0.52 m	0.55 m	0.53 m
17	1.58 s	1.33 s	1.34 s	1.74 s	1.74 s	1.23 s	1.35 s	1.74 s	1.62 s	1.33 s
18	0.46 s	0.51 s	0.51 s	0.50 s	0.50 s	0.58 s	0.61 s	0.60 s	0.59 s	0.59 s
19	1.30 s	1.31 s	1.30 s	1.32 s	1.32 s	1.64 s	1.83 s	1.84 s	1.60 s	1.58 s
20	0.73 s	0.77 s	0.76 s	0.76 s	0.77 s	0.68 s	0.79 s	0.79 s	4.19 s, 4.58 s	4.17 s, 4.58 s
OAc							2.02 s	2.02 s		

[a] Recorded in $CDCl_3$ at 300 MHz. [b] Recorded in acetone-d_6 at 300 MHz.

spectral data (Tables 1 and 2) closely resembled those of **2** except that the tertiary hydroxyl attached to C-2 was replaced by a hydroperoxide. HMBC correlations from H-1/H-17 to C-2/C-3 and H-5 to C-3/C-6/C-7 confirmed the position of the hydroperoxide. The relative stereochemistry of **3** was deduced from a 2D NOESY experiment, which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From these data, pacificin C was formulated as **3**.

HREIMS and NMR data revealed pacificin D (**4**) to have a molecular formula of $C_{20}H_{34}O_3$. The ^{1}H and ^{13}C NMR spectral data exhibited the presence of a terminal methylene (δ_H 4.86, 4.93; δ_C 111.8, 147.3) and a secondary hydroxyl (δ_H 4.00; δ_C 76.9). The ^{1}H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) closely resembled those of **1** except for NMR signals due to the side chain. HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the 3-hydroxyisopentenyl side chain. The relative stereochemistry of **4** was establised by a 2D NOESY experiment, which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. Therefore, the structure of pacificin D was established as **4**.

Pacificin E (**5**) was isolated as a colorless resin of molecular formula $C_{20}H_{34}O_4$, as indicated by HRFABMS and NMR spectra. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) were very close to those of **4** except that the tertiary hydroxyl attached to C-3 was replaced by a hydroperoxyl.[16] HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the position of the hydroperoxyl. The relative stereochemistry of **5** was deduced from a 2D NOESY experiment, which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From the above data, pacificin E was thus formulated as **5**.

Pacificin F (**6**) was shown to have the molecular formula of $C_{20}H_{34}O_2$ by HREIMS and NMR spectra. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) exhibited some similarity to those of **2** except that the trisubstituted epoxide was replaced by a *E*-trisubstituted double bond. HMBC correlations (Supporting Information) from H-11 to C-9/C-10/C-12/C-13 confirmed the position of the *E*-trisubstituted double bond. The relative stereochemistry of **6** was determined on the basis of a 2D NOESY experiment (Supporting Information), which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From these data, pacificin F can be formulated as **6**.

The molecular formula of pacificin G (**7**) was obtained from HRFABMS and NMR spectra. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) resembled those of **6** except for NMR signals due to the side chain terminus and an additional acetoxy group on the 10-membered ring. 2D COSY correlation (H-10/H-11) and HMBC correlations from H-11 to C-9/C-10 confirmed the position of the acetoxy group. The side chain was identical to that of **3**. The relative stereochemistry of **7** was deduced from a 2D NOESY experiment (Supporting Information), which indicated that Me-19, Me-20, H-11, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From the aforementioned data, pacificin G can be formulated as **7**.

Pacificin H (**8**) was isolated as a colorless amorphous solid of molecular formula $C_{20}H_{36}O_5$, as established by HRFABMS and NMR spectra. The ^{1}H and ^{13}C NMR

Table 2. ^{13}C NMR Spectral Data of **1**−**10**

	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[a]	**8**[a]	**9**[a]	**10**[a]
1	25.7	30.0	24.5	111.8	114.7	29.8	24.5	114.9	25.8	30.0
2	131.2	70.9	82.2	147.3	143.0	69.4	82.3	143.5	130.9	70.9
3	125.0	141.3	136.7	76.9	90.4	141.7	136.3	90.6	125.5	140.6
4	22.2	122.3	127.4	29.6	24.7	122.3	127.9	24.7	22.4	123.6
5	46.6	48.1	49.1	41.7	41.7	48.6	49.0	40.5	45.9	48.2
6	34.7	35.3	35.7	34.3	34.3	36.0	36.4	35.1	35.9	36.8
7	36.6	36.5	37.1	36.8	36.7	37.2	37.4	36.9	37.7	38.1
8	35.4	35.5	35.4	35.4	35.4	36.0	36.4	35.1	36.4	36.4
9	61.9	62.0	61.9	61.9	62.8	132.2	139.9	140.4	136.1	136.0
10	66.2	66.4	66.3	66.3	66.3	127.2	125.8	125.7	126.1	126.1
11	24.4	24.5	24.3	24.4	24.4	24.7	69.0	69.0	30.4	30.3
12	41.7	41.8	41.7	42.1	42.2	44.4	49.0	49.0	40.4	40.5
13	72.9	73.0	73.0	72.9	72.9	72.0	71.5	71.6	154.0	153.8
14	28.8	28.9	28.9	28.6	28.9	30.5	30.3	30.3	24.9	25.0
15	3.9	3.9	4.3	3.9	3.9	5.8	4.9	4.5	12.9	13.1
16	27.1	27.0	27.8	27.0	26.9	27.5	28.0	27.1	35.5	35.9
17	17.6	30.0	24.5	17.2	17.1	29.8	24.5	17.0	17.6	30.0
18	19.4	19.3	19.5	19.6	19.5	15.5	20.3	19.3	18.4	17.6
19	17.1	17.1	17.0	17.0	17.1	17.9	18.2	17.0	15.8	15.8
20	20.6	20.5	20.6	20.7	20.7	19.9	21.3	21.3	103.2	103.3
OAc							21.4	21.4		
							170.5	170.4		

[a] Recorded in $CDCl_3$ at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments). [b] Recorded in acetone-d_6 at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments).

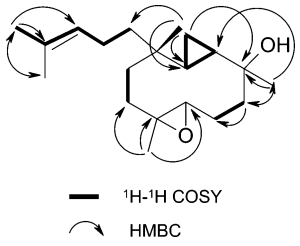

Figure 1. Key COSY and HMBC correlations of **1**.

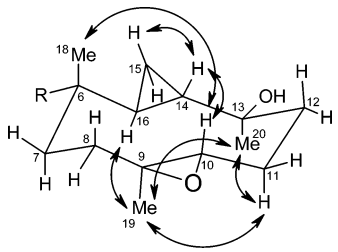

Figure 2. Selected NOESY correlations of **1**.

spectral data (Tables 1 and 2) were quite similar to those of **7** except for NMR signals due to the side chain. HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the 3-hydroperoxyisopentenyl side chain. The relative stereochemistry of **8** was established by a 2D NOESY experiment, which indicated that Me-19, Me-20, H-11, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From these data, pacificin H was formulated as **8**.

The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of pacificin H (**9**) were identical with those of an acetylation byproduct of an isolate from an octocoral *Alcyonium palmatum*.[15] However, after our detailed analysis of the 2D NMR spectra of **9**, the ^1H and ^{13}C NMR chemical shifts at C-11, C-14, and C-16 should be revised as in Table 2. Compound **9** is a new natural product.

Pacificin I (**10**) analyzed for $C_{20}H_{32}O$ by mass spectrometry in combination with interpretation of ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) were analogous to those of **9** except for NMR signals due to the side chain. COSY correlation between H-3/H-4 and H-4/H-5, HMBC correlations from H-1/H-17 to C-2/C-3 and H-5 to C-3/C-6/C-7, and a $J_{3,4}$ of 15.6 Hz placed an *E* double bond between C-3 and C-4. The relative stereochemistry of **9** was determined by a 2D NOESY experiment, which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. Therefore, the structure of pacificin I was established as **10**.

Pacificins C and H exhibited cytotoxicity against P-388 cells with ED_{50}'s of 1.44 and 2.01 μg/mL, respectively. The other isolates were inactive against P-388 and HT-29 cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C, respectively, using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *N. pacifica* was collected at Green Island, off Taiwan, in March 2002, at a depth of 5 m and was stored for 1 week in a freezer until extraction. A voucher specimen, NSUGN-058, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *N. pacifica* were freeze-dried to give 1.10 kg of a solid, which was extracted with CH_2Cl_2 (3.0 L × 3, overnight for each cycle) at room temperature. After removal of solvent in vacuo, the residue (47 g) was chromatographed over Si gel 60 using *n*-hexane−EtOAc and MeOH−EtOAc mixtures as eluting solvents. Elution by *n*-hexane−EtOAc (85:15) afforded fractions containing **9**. Elution by *n*-hexane−EtOAc (65:35) afforded fractions containing **1**, **7**, and **8**. Elution by *n*-hexane−EtOAc (55:45) afforded fractions containing **6** and **10**. Elution by *n*-hexane−EtOAc (35:65) afforded fractions containing **2**−**5**. Compound **1** (360 mg, 7.6%) was further purified by Si gel column chromatography, eluting with *n*-hexane−acetone (4:1). Compounds **2** (3 mg, 0.006%), **3** (3 mg, 0.006%), **4** (5 mg, 0.011%), and **5** (6 mg, 0.013%) were further purified by HPLC (LiChrosorb Si 60, 7 μm, 25 × 250 mm), eluting with *n*-hexane−acetone (3:1). Compound **6** (3 mg, 0.006%) was further

purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), eluting with MeOH−H_2O (90:10). Compounds **7** (4 mg, 0.009%) and **8** (3 mg, 0.006%) were further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), by eluting with MeOH−H_2O (73:27). Compound **9** (20 mg, 0.042%) was further purified by Si gel column chromatography, eluting with *n*-hexane−EtOAc (9:1). Compound **10** (4 mg, 0.009%) was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), eluting with MeOH−H_2O (90:10).

Pacificin A (1): $[\alpha]^{25}_D$ −62° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3450 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 306 $[M]^+$ (9), 288 (12), 270 (32), 81 (100); HREIMS *m/z* 306.2558 (calcd for $C_{20}H_{34}O_2$, 306.2550).

Pacificin B (2): $[\alpha]^{25}_D$ −53° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3520 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 322 $[M]^+$ (3), 304 (8), 81 (100); HREIMS *m/z* 322.2492 (calcd for $C_{20}H_{34}O_3$, 322.2499).

Pacificin C (3): $[\alpha]^{25}_D$ −46° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3480 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 339.2529 (calcd for $C_{20}H_{35}O_4$, 339.2526).

Pacificin D (4): $[\alpha]^{25}_D$ −43° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3490 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 322 $[M]^+$ (3), 304 (6), 216 (12), 81 (100); HREIMS *m/z* 322.2495 (calcd for $C_{20}H_{34}O_3$, 322.2499).

Pacificin E (5): $[\alpha]^{25}_D$ −38° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3510 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 339.2532 (calcd for $C_{20}H_{35}O_4$, 339.2526).

Pacificin F (6): $[\alpha]^{25}_D$ −51° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3460 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 306 $[M]^+$ (4), 288 (6), 271 (12), 215 (8), 189 (70), 95 (100); HREIMS *m/z* 306.2543 (calcd for $C_{20}H_{34}O_2$, 306.2550).

Pacificin G (7): $[\alpha]^{25}_D$ −26° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3480, 1730 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 381.2638 (calcd for $C_{22}H_{37}O_5$, 381.2631).

Pacificin H (8): $[\alpha]^{25}_D$ −18° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3550, 1732 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m/z* 381.2636 (calcd for $C_{22}H_{37}O_5$, 381.2631).

Pacificin I (9): $[\alpha]^{25}_D$ −28° (*c* 0.1, $CHCl_3$); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 272 $[M]^+$ (9), 257 (12), 95 (100).

Pacificin J (10): $[\alpha]^{25}_D$ −22° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3450 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 288 $[M]^+$ (3), 270 (6), 220 (12), 95 (100); HREIMS *m/z* 288.2440 (calcd for $C_{20}H_{32}O$, 288.2445).

Cytotoxicity Testing. P-388 cells were kindly supplied by J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[14] Three concentrations (50, 5, and 0.5 μg/mL) of the tested compounds were used in the cytotoxicity assays.

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Supporting Information Available: ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and $^{13}C-^{13}C$ homonuclear shift correlation 2D spectrum (INADEQUATE) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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