Cytotoxic C_{21} and C_{22} Terpenoid-Derived Metabolites from the Sponge *Ircinia* sp.

Jui-Hsin Su,[*,†,‡,§] Shang-Wei Tseng,[†,‡] Mei-Chin Lu,[†,‡] Li-Lian Liu,[§,⊥] Yalan Chou,[⊥] and Ping-Jyun Sung[†,‡,§,∥,▽]

[†]Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan, Republic of China

[‡]National Museum of Marine Biology & Aquarium, Pingtung 944, Taiwan, Republic of China

[§]Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[⊥]Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[∥]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[▽]Department of Life Science and Institute of Biotechnology, National Dong Hwa University, Hualien 974, Taiwan, Republic of China

S *Supporting Information*

ABSTRACT:
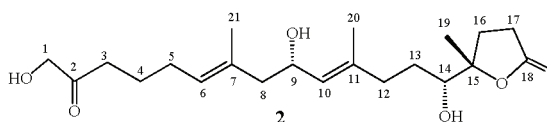
One novel C_{21} terpenoidal natural product, ircinolin A (**2**), two new C_{22} furanoterpene metabolites, 15-acetylirciformonin B (**3**) and 10-acetylirciformonin B (**4**), and two known compounds, irciformonin B (**1**) and irciformonin F (**5**), were isolated from the sponge *Ircinia* sp. The structures of these compounds were elucidated on the basis of their spectroscopic data. Moreover, the absolute configuration of **1** was determined by Mosher's method. Among these metabolites, **2** is the first C_{21} terpenoid-derived metabolite to be reported from this genus. Compounds **1** and **3**–**5** exhibited significant cytotoxic activity against K562, DLD-1, HepG2, and Hep3B cancer cell lines.

Previous chemical investigations on sponges of the genus *Ircinia* have led to the isolation and identification of various terpenoid metabolites.[1−16] Some of these have been found to possess several kinds of biological activities, such as cytotoxic,[13−15] antimicrobial,[12] and antiviral properties.[6,10,16] The current chemical investigation of *Ircinia* sp. led to the discovery of one novel C_{21} terpenoid-related metabolite, ircinolin A (**2**), two new C_{22} furanoterpene metabolites, 15-acetylirciformonin B (**3**) and 10-acetylirciformonin B (**4**), and two known compounds, irciformonin B (**1**)[14] and irciformonin F (**5**).[16] The structures of **1**−**5** were established by detailed spectroscopic analysis, including extensive examination of 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) correlations. The absolute configuration of **1** was determined using a modified Mosher's method and is reported herein for the first time.[17] The cytotoxicity of metabolites **1**−**5** against human chronic myelogenous leukemia (K562), human colon adenocarcinoma (DLD-1), and human liver carcinoma (HepG2 and Hep3B) cell lines was studied in order to discover bioactive compounds.
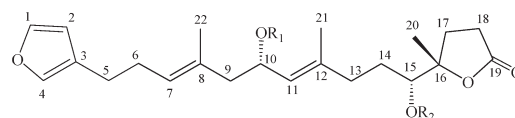
1: R₁ = H, R₂ = H
3: R₁ = H, R₂ = Ac
4: R₁ = Ac, R₂ = H
5: R₁ = Ac, R₂ = Ac
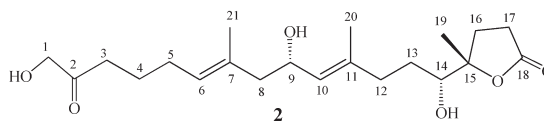
The EtOAc extract of the freeze-dried specimen was fractionated by silica gel column chromatography, and the eluted

1a: R = (S)-MTPA
1b: R = (R)-MTPA

1c: R₁ = (R)-MTPA, R₂ = (S)-MTPA
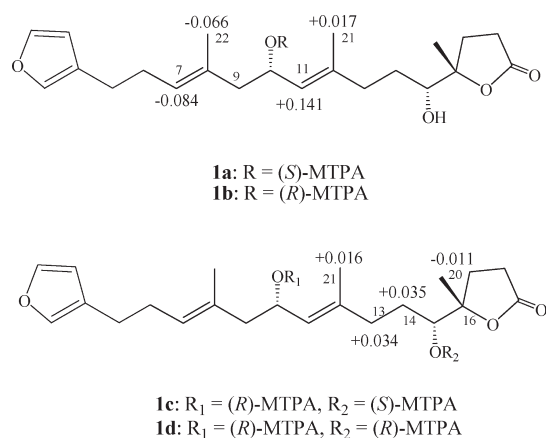**1d**: R₁ = (R)-MTPA, R₂ = (R)-MTPA

Figure 1. Absolute configuration of **1**: ^{1}H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the Mosher's esters of **1a/1b** and bis-Mosher's esters **1c/1d**.

fractions were further separated utilizing normal-phase HPLC to yield metabolites **1**–**5**. The new compounds were given the trivial names ircinolin A (**2**), 15-acetylirciformonin B (**3**), and 10-acetylirciformonin B (**4**). The known compounds were identified as irciformonin B (**1**)[14] and irciformonin F (**5**).[16] Compound **1** was found to be identical to the previously reported metabolite obtained from the sponge *Ircinia formosana*[14] by comparison of the physical (specific rotation) and spectroscopic (^{1}H and ^{13}C NMR) data. In the original report, the authors gave a planar structure for the 10-hydroxy and only a relative configuration at C-15/C-16. Therefore, the presence of two secondary alcohols at C-10 and C-15 of **1** allowed us to determine its absolute configuration by using the modified Mosher's method.[17] The (S)- and (R)-MTPA esters of **1** (**1a** and **1b**, respectively) were prepared using the corresponding R-(−)- and S-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively. Determination of the chemical shift differences ($\delta_S - \delta_R$) for the protons neighboring C-10 led to the assignment of the S configuration at C-10 in **1** (Figure 1). Furthermore, the (S)- and (R)-MTPA esters of **1b** were prepared under the same conditions, resulting in the corresponding bis-MTPA esters **1c** and **1d**. Determination of the chemical shift differences ($\delta_S - \delta_R$) for the protons neighboring C-15 led to the assignment of the R configuration at C-15 in **1** (Figure 1). NOE correlations were observed between H-15 and both H-14β and H₃-19, between H₃-19 and H-17β, and between H-17α and H-14α, indicating that H₃-19 has a β-orientation. The key NOE correlations and calculated distances between those protons fit well with the structure of **1** from C-14 to C-19 that was generated by molecular modeling (Figure 2). On the basis of these results, **1** was found to possess the 10S, 15R, 16S configuration.

Ircinolin A (**2**) was obtained as a colorless oil. The HRESIMS spectrum of **2** exhibited a pseudomolecular ion peak at *m/z* 381.2284 [M − H]⁻, which established a molecular formula of $C_{21}H_{34}O_6$, implying five degrees of unsaturation. IR absorptions were observed at 3413, 1761, and 1723 cm⁻¹, suggesting the presence of hydroxy and carbonyl groups in **1**. The ^{13}C NMR and DEPT spectroscopic data (Table 1), signals of three methyls, nine sp³ methylenes (including one oxymethylene), two sp³ oxymethines, one sp³ quaternary carbon, two sp² methines, and four sp² quaternary carbons (including one ketone and one ester carbonyl), were observed. From the ^{1}H NMR spectrum of **2**, the
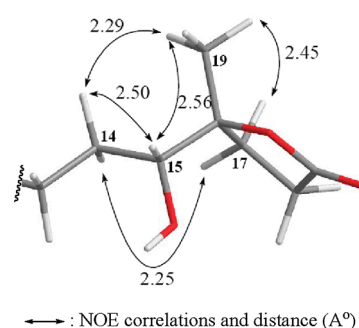

: NOE correlations and distance (Aᵒ)

Figure 2. Selected NOE correlations of C-14–C-19 of **1**.

resonances of two olefinic protons (δ_H 5.22, d, *J* = 8.5 Hz; 5.21, t, *J* = 6.0 Hz), two oxygenated methines (δ_H 4.48, dd, *J* = 8.0, 8.0; 3.62, d, *J* = 9.0 Hz), one oxygen-bearing methylene (δ_H 4.24, 2H, s), and three methyls (δ_H 1.71, s; 1.65, s; 1.35, s) were observed. The planar structure and all of the ^{1}H and ^{13}C chemical shifts of **2** were elucidated by 2D NMR spectroscopic analysis, in particular ^{1}H–^{1}H COSY and HMBC experiments (Figure 3). Thus, **2** was found to possess two double bonds, at C-6/C-7 and C-10/C-11, two hydroxy groups, at C-9 and C-14, and a ketone group at C-2. The *E* configurations of two double bonds (C-6/C-7 and C-10/C-11) in **2** were assigned on the basis of the ^{13}C NMR chemical shifts at C-20 (δ_C 16.5) and C-21 (δ_C 16.2). Furthermore, comparison of the NMR data between **2** and **1** confirmed both compounds have the same partial structure from C-5 to C-21 of **2** and from C-6 to C-22 of **1**. Moreover, the correlations observed in the NOESY spectrum of **2** also showed that the configuration of this metabolite is identical with that of **1**.

15-Acetylirciformonin B (**3**) was isolated as a colorless oil with the molecular formula $C_{24}H_{34}O_6$, which possesses eight degrees of unsaturation, as indicated by HRESIMS (*m/z* 441.2261, [M + Na]⁺) and NMR spectroscopic data (Table 1). By comparison of the NMR data of **3** with those of **1**, it was found that the ^{1}H and ^{13}C NMR data of **3** were very similar to those of **1**, with the difference that **3** contains one more acetyl group relative to **1**. The chemical shift of H-15 in **1** (δ_H 3.62) was shifted downfield (δ_H 5.01) in **3**, suggesting that **3** is the 15-acetyl derivative of **1**. This was further supported by acetylation of **1** and **3** with acetic anhydride in pyridine to yield the same diacetate **5**, giving further support to this conclusion. Thus, compound **3** was established as the 15-acetyl derivative of **1**.

10-Acetylirciformonin B (**4**) was isolated as a colorless oil and showed a [M + Na]⁺ ion peak in the HRESIMS spectrum corresponding to the molecular formula $C_{24}H_{34}O_6$, the same as that of **3**. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **4** with those of **1** revealed **4** to be an acetyl derivative of **1**. It was found that H-10 of **1** resonated upfield (δ_H 4.43) relative to **4** (δ_H 5.60, dd, *J* = 7.6, 7.6 Hz). Thus, the hydroxy group at C-10 of **1** was replaced by an acetoxy group in **4**. Furthermore, acetylation of **4** gave a product that was found to be identical to **5** by comparison of their physical (specific rotation) and spectroscopic (^{1}H and ^{13}C NMR) data. Thus, **4** was determined to be the 10-acetyl derivative of **1**.

It is noteworthy to mention that metabolite **2** is the first C_{21} terpenoid-derived metabolite to be reported from this genus. Metabolites **2**–**5** were isolated together with **1** from the same organism and possess very similar molecular skeletons, and on the basis of a shared biosynthetic pathway, it is proposed that these five compounds have the same absolute configurations

Table 1. NMR Spectroscopic Data for Compounds 2−4

	2		3		4	
C/H	$\delta_C{}^a$ type	$\delta_H{}^b$ (J in Hz)	$\delta_C{}^a$ type	$\delta_H{}^b$ (J in Hz)	$\delta_C{}^c$ type	$\delta_H{}^d$ (J in Hz)
1	68.1, $CH_2{}^e$	4.24, s	142.8, CH	7.35, s	142.6, CH	7.34, s
2	209.9, C		110.9, CH	6.27, s	111.0, CH	6.28, s
3	37.7, CH_2	2.44, m	124.6, C		124.7, C	
4	23.5, CH_2	1.73, m	138.9, CH	7.21, s	138.8, CH	7.21, s
5	27.3, CH_2	2.07, m	24.8, CH_2	2.48, m	24.8, CH_2	2.46, m
6	127.3, CH	5.21, t (6.0)	28.4, CH_2	2.30, m	28.4, CH_2	2.20, m
7	132.9, C		128.2, CH	5.27, t (6.5)	127.4, CH	5.21, t (7.2)
8	48.1, CH_2	2.18, m	132.2, C		131.3, C	
9	65.8, CH	4.48, dd (8.0, 8.0)	48.0, CH_2	2.13, m	45.1, CH_2	2.33, 2.13, m
10	128.0, CH	5.22, d (8.5)	65.5, CH	4.40, dd (7.5, 7.5)	69.8, CH	5.60, dd (7.6, 7.6)
11	137.8, C		128.1, CH	5.15, d (8.5)	124.3, CH	5.15, d (10.4)
12	36.0, CH_2	2.29, 2.12, m	136.6, C		139.6, C	
13	28.6, CH_2	1.45, 1.57, m	35.6, CH_2	2.01, dd (8.0, 8.5)	36.1, CH_2	2.26, 2.10, m
14	75.2, CH	3.62, d (9.0)	27.7, CH_2	1.66, 1.78, m	28.7, CH_2	1.46, 1.59, m
15	88.7, C		76.0, CH	5.01, dd (10.5, 2.5)	75.3, CH	3.60, d (10.4)
16	27.7, CH_2	1.82, 2.42, m	86.2, C		88.6, C	
17	29.3, CH_2	2.63, m	29.8, CH_2	1.90, 2.24, m	27.8, CH_2	1.82, 2.43, m
18	177.0, C		28.7, CH_2	2.61, m	29.3, CH_2	2.61, m
19	22.9, CH_3	1.35, s	176.1, C		177.0, C	
20	16.5, CH_3	1.71, s	22.6, CH_3	1.40, s	22.8, CH_3	1.35, s
21	16.2, CH_3	1.65, s	16.5, CH_3	1.67, s	16.7, CH_3	1.68, s
22			16.2, CH_3	1.64, s	16.4, CH_3	1.62, s
OAc			20.9, CH_3	2.01, s	21.2, CH_3	1.99, s
			170.3, C		170.4, C	

[a] 500 MHz in $CDCl_3$. [b] 125 MHz in $CDCl_3$. [c] 400 MHz in $CDCl_3$. [d] 100 MHz in $CDCl_3$. [e] Numbers of attached protons were deduced by DEPT experiments.
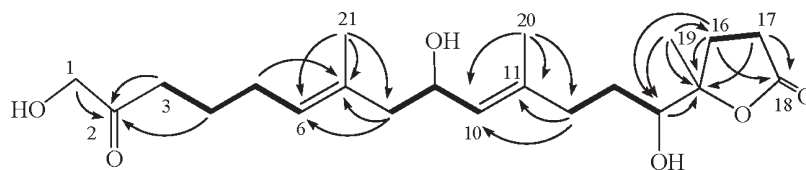
Figure 3. Selected $^1H-^1H$ COSY (−) and HMBC (→) correlations of **2**.

Table 2. Cytotoxicity (IC$_{50}$ μM) of Compounds 1−5[a]

	cell lines			
compound	K562	DLD-1	HepG 2	Hep3B
1	7.8	0.04	1.0	6.4
3	5.4	0.03	0.5	1.1
4	9.6	0.2	2.2	10.6
5	10.2	0.6	1.7	7.0
doxorubicin C	0.14	<0.01	0.04	0.1

[a] Compound **2** was inactive against all four cell lines (IC$_{50}$ > 20 μM).

(C-10, C-15, and C-16 in **1**, **3**−**5** and C-9, C-14, and C-15 in **2**). The cytotoxicities of compounds **1**−**5** against K562, DLD-1, HepG2, and Hep3B cancer cells are shown in Table 2. The results show that compound **3**, the most potent of compounds **1**−**5**, exhibited cytotoxicity against the K562, DLD-1, HepG2,

and Hep3B cancer cell lines with IC$_{50}$'s of 5.4, 0.03, 0.5, and 1.1 μM, respectively. Furthermore, compounds **1**, **4**, and **5** also were found to exhibit significant cytotoxicity toward some of the cell lines (Table 2). It seems that the furan moiety in compounds **1** and **3**−**5** is critical for the cytotoxic activity of the C$_{22}$ furanoterpenoids.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation values were measured with a Jasco P-1010 digital polarimeter. IR spectra were recorded on a Varian Digilab FTS 1000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a Varian Mercury Plus 400 FT-NMR (or Varian Unity INOVA 500 FT-NMR) instrument at 400 MHz (or 500 MHz) for ^1H NMR and 100 MHz (or 125 MHz) for ^{13}C NMR, respectively, in $CDCl_3$. ESIMS data were obtained with a Finnigan LCQ ion-trap mass spectrometer. HRESIMS data were recorded on a LTQ Orbitrap XL mass spectrometer. Gravity

column chromatography was performed on silica gel (230−400 mesh, Merck). TLC was carried out on precoated Kieselgel 60 F254 (0.2 mm, Merck), and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. High-performance liquid chromatography was performed using a system comprised of a Hitachi L-7100 pump and a Rheodyne 7725 injection port. A preparative normal-phase column (250 × 21.2 mm, 5 μm) was used for HPLC.

Animal Material. Sponges of the genus *Ircinia* Nardo, 1833, belonging to the family Irciniidae Gray, 1867, the order Dictyoceratida Minchin, 1900, have a spongin skeleton as their supporting structure.[18] They are largely distributed within 20 m depth in coral reefs. The *Ircinia* sp. (diameter 10 cm, height 5 cm) studied in this paper was collected from Orchid Island, Taiwan, at a depth of 20 m. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan (specimen no. LYSG-2). Morphology: globular and massive. Remarkably elastic, very tough, and extremely hard when dry. Difficult to cut or tear. Its size can reach more than 20 cm in length. The specimen has medium to very coarse sand embedded in the base and sparsely distributed in the endoderm. Cortex black, golden-brown internally. The surface is strongly conulose, conule 1−2 mm in height and 2−4 mm apart on surface. Oscules, 1.5−2 mm in diameter, open at the tips of several lobes (Supporting Information, Figure S23). There is some sand embedded within the cortex, though not forming a crust under the cortex. The mesohyl appears fleshy, and golden-brown fibers are visible within the mesohyl (Figure S24). Skeleton: This species lacks spicules and has densely irregular primary and fine secondary filaments. The primary fibers often incorporate a core of foreign material and can form complex, often massive fascicles (Figures S25 and S26) like other species in the genus *Ircinia*.[19] Secondary fibers are generally uncored. Primary fibers are 40−100 μm in diameter; secondary fibers are 4−16 μm in diameter. Remarks: Although the new species *Ircinia formosana* has previously been reported by Shen et al.,[16] there is no description of the specimen. Thus, the relation between this *Ircinia* sp. and the new species is undetermined.

Extraction and Isolation. The frozen bodies of *Ircinia* sp. (130 g fresh wt) were collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOAc (5 × 1 L). The EtOAc extract (1.3 g) was chromatographed over silica gel by column chromatography and eluted with EtOAc in *n*-hexane (0−100%, stepwise) to yield 12 fractions. Fraction 8 (60.5 mg), eluted with *n*-hexane−EtOAc (5:1), was subjected to normal-phase HPLC (*n*-hexane−EtOAc, 5:1) to obtain compound **5** (9.5 mg). Fraction 9 (133.5 mg), eluting with *n*-hexane−EtOAc (3:1), was further separated by silica gel column chromatography with gradient elution (*n*-hexane−EtOAc, 3:1 to 2:1) to afford five subfractions (A1−A5). Subfraction A2 (30.2 mg) was separated by normal-phase HPLC using *n*-hexane−EtOAc (2:1) to afford **4** (6.2 mg). Subfraction A4 (20.5 mg) was also separated by normal-phase HPLC using *n*-hexane−EtOAc (3:2) to afford **3** (2.5 mg). Fraction 12 (250 mg), eluted with *n*-hexane−EtOAc (1:2), was further separated by silica gel column chromatography with gradient elution (*n*-hexane−EtOAc, 1:1 to 1:5) to yield six subfractions (B1 to B4). Subfraction B2 was separated by normal-phase HPLC using *n*-hexane−EtOAc (1:2) to afford **1** (100.5 mg). Subfraction B3 was also separated by normal-phase HPLC using *n*-hexane−EtOAc (1:3) to afford **2** (3.5 mg).

Irciformonin B (1): colorless oil; $[\alpha]^{24}_D$ +8.2 (*c* 1.50, acetone) [lit. $[\alpha]^{25}_D$ +3.1 (*c* 3, acetone)].[14]

Ircinolin A (2): colorless oil; $[\alpha]^{24}_D$ −6.0 (*c* 0.21, CHCl$_3$); IR (neat) ν_{max} 3413, 2961, 2925, 2855, 1761, 1723, 1382, and 1267 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 1; ESIMS *m/z* 381 [M − H]$^-$; HRESIMS *m/z* 381.2284 [M−H]$^-$ (calcd for $C_{21}H_{33}O_6$, 381.2272).

15-Acetylirciformonin B (3): colorless oil; $[\alpha]^{24}_D$ −7.1 (*c* 0.12, CHCl$_3$); IR (neat) ν_{max} 3459, 2960, 2925, 2855, 1774, 1744, 1376, and 1232 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 1; ESIMS *m/z*

441 [M + Na]$^+$; HRESIMS *m/z* 441.2261 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_6$Na, 441.2248).

10-Acetylirciformonin B (4): colorless oil; $[\alpha]^{24}_D$ −4.1 (*c* 0.17, CHCl$_3$); IR (neat) ν_{max} 3457, 2961, 2926, 2856, 1769, 1732, 1374, and 1260 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 1; ESIMS *m/z* 441 [M + Na]$^+$; HRESIMS *m/z* 441.2261 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_6$Na, 441.2248).

Irciformonin F (5): colorless oil; $[\alpha]^{24}_D$ −3.0 (*c* 0.56, CH$_2$Cl$_2$) [lit. $[\alpha]^{25}_D$ −4.2 (*c* 4.6, CH$_2$Cl$_2$)].[16]

Preparation of (S)- and (R)-MTPA Esters of 1. To a solution of **1** (15 mg) in pyridine (0.5 mL) was added (*R*)-MTPA chloride (25 μL), and the mixture was allowed to stand overnight at room temperature (rt). After dilution with EtOAc, the solution was evaporated in vacuo and the residue purified by normal-phase HPLC using *n*-hexane−EtOAc (3:1) to obtain the (*S*)-MTPA ester **1a** (12.5 mg). The same procedure was used to prepare the (*R*)-MTPA ester, **1b** (14.5 mg from 15 mg of **1**), with (*S*)-MTPA chloride. Selected ^{1}H NMR (CDCl$_3$, 400 MHz) data for **1a**: δ 5.227 (1H, d, *J* = 9.2 Hz, H-11), 5.167 (1H, t, *J* = 6.4 Hz, H-7), 1.788 (3H, s, H$_3$-21), 1.575 (3H, s, H$_3$-22); ESIMS *m/z* 615 [M + Na]$^+$. Selected ^{1}H NMR (CDCl$_3$, 400 MHz) data for **1b**: δ 5.086 (1H, d, *J* = 9.2 Hz, H-11), 5.251 (1H, t, *J* = 6.4 Hz, H-7), 1.771 (3H, s, H$_3$-21), 1.641 (3H, s, H$_3$-22) (see Supporting Information); ESIMS *m/z* 615 [M + Na]$^+$.

Preparation of (S)- and (R)-MTPA Esters of 1b. To a solution of **1b** (6 mg) in pyridine (0.5 mL) was added (*R*)-MTPA chloride (25 μL), and the mixture was allowed to stand overnight at rt. After dilution with EtOAc, the solution was evaporated in vacuo and the residue purified by normal-phase HPLC using *n*-hexane−EtOAc (5:1) to obtain the (*S*)-MTPA ester **1c** (3.2 mg). The same procedure was used to prepare the (*R*)-MTPA ester, **1d** (2.5 mg from 6 mg of **1b**), with (*S*)-MTPA chloride. Selected ^{1}H NMR (CDCl$_3$, 400 MHz) data for **1c**: δ 1.608 (1H, m, H-14a), 1.967 (2H, m, H$_2$-13), δ 1.349 (3H, s, H$_3$-20), 1.728 (3H, s, H$_3$-21); ESIMS *m/z* 831 [M + Na]$^+$. Selected ^{1}H NMR (CDCl$_3$, 400 MHz) data for **1d**: δ 1.573 (1H, m, H-14a), 1.942 (2H, dd, *J* = 8.0, 8.0, H$_2$-13), δ 1.360 (3H, s, H$_3$-20), 1.712 (3H, s, H$_3$-21) (see Supporting Information); ESIMS *m/z* 831 [M + Na]$^+$.

Acetylation of 1. A solution of **1** (5.0 mg) in pyridine (0.2 mL) was mixed with Ac$_2$O (0.1 mL), and the mixture was stirred at rt for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatography over Si gel using *n*-hexane−EtOAc (5:1) to yield the diacetyl derivative **5** (5.5 mg, 90%). The specific rotation $[[\alpha]^{24}_D$ −3.0 (*c* 0.27, CH$_2$Cl$_2$)] was in full agreement with that of the natural product **5**.

Acetylation of 3. A solution of **3** (2.0 mg) in pyridine (0.2 mL) was mixed with Ac$_2$O (0.1 mL), and the mixture was stirred at rt for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatograph over Si gel using *n*-hexane−EtOAc (5:1) to give the diacetyl derivative **5** (2.0 mg, 91%). The specific rotation $[[\alpha]^{24}_D$ −3.5 (*c* 0.1, CH$_2$Cl$_2$)] was in full agreement with that of the natural product **5**.

Acetylation of 4. A solution of **4** (2.0 mg) in pyridine (0.2 mL) was mixed with Ac$_2$O (0.1 mL), and the mixture was stirred at rt for 24 h. After evaporation of excess reagent, the residue was subjected to column chromatography over Si gel using *n*-hexane−EtOAc (5:1) to yield the diacetyl derivative **5** (2.0 mg, 91%). The specific rotation $[[\alpha]^{24}_D$ −3.2 (*c* 0.1, CH$_2$Cl$_2$)] was in full agreement with that of the natural product **5**.

Cytotoxicity Testing. Cytotoxicity assays of compounds **1**−**5** were conducted against K562 (human chronic myelogenous leukemia), DLD-1 (human colon adenocarcinoma), and HepG2 and Hep3B (human liver carcinoma) cell lines and were performed using the tetrazolium-based colorimetric assay.[20,21]

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information.** NMR spectra of compounds **1**−**5**, the MTPA esters of **1a** /**1b**, and bis-MTPA esters **1c**/**1d**

and a photograph of the marine sponge *Ircinia* sp. are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: +886-8-8822370. Fax: +886-8-8825087. E-mail: x2219@ nmmba.gov.tw.

■ ACKNOWLEDGMENT

■ REFERENCES

(1) Cafieri, F.; Fattorusso, E.; Santacroce, C.; Minale, L. *Tetrahedron* **1972**, *28*, 1579–1583.

(2) Faulkner, D. J. *Tetrahedron Lett.* **1973**, *14*, 3821–3822.

(3) Rothberg, I.; Shubiak, P. *Tetrahedron Lett.* **1975**, *10*, 769–772.

(4) González, A. G.; Rodriguez, M. L.; Barrientos, A. S. M. *J. Nat. Prod.* **1983**, *46*, 256–261.

(5) Manes, L. V.; Crews, P.; Ksebati, M. B.; Schmitz, F. J. *J. Nat. Prod.* **1986**, *49*, 787–793.

(6) Barrow, C. J.; Blunt, J. W.; Munro, M. H. G.; Perry, N. B. *J. Nat. Prod.* **1988**, *51*, 1294–1298.

(7) Garcia, M. O.; Rodriguez, A. D. *Tetrahedron* **1990**, *46*, 1119–1124.

(8) De Giulio, A.; De Rosa, S.; Di Vincenzo, G.; Strazzullo, G.; Zavodnik, N. *J. Nat. Prod.* **1990**, *53*, 1503–1507.

(9) Martinez, A.; Duque, C.; Hara, N.; Fujimoto, Y. *Nat. Prod. Lett.* **1995**, *6*, 281–284.

(10) Bifilco, G.; Bruno, I.; Minale, L.; Riccio, R.; Debitus, C; Bourdy, G.; Vassas, A.; Lavayre, J. *J. Nat. Prod.* **1995**, *58*, 1444–1449.

(11) De Rosa, S.; Milone, A.; De Giulio, A.; Crispino, A.; Iodice, C. *Nat. Prod. Lett.* **1996**, *8*, 245–251.

(12) Martinez, A.; Duque, C.; Sato, N.; Fujimoto, Y. *Chem. Pharm. Bull.* **1997**, *45*, 181–184.

(13) Issa, H. H.; Tanaka, J.; Higa, T. *J. Nat. Prod.* **2003**, *66*, 251–254.

(14) Shen, Y.-C.; Lo, K.-L.; Lin, Y.- C.; Khalil, A. T.; Kuo, Y.-H.; Shih, P.-S. *Tetrahedron Lett.* **2006**, *47*, 4007–4010.

(15) Rifai, S.; Fassouane, A.; Pinho, P. M.; Kijjoa, A.; Nazareth, N.; Nascimento, M. S. J.; Herz, W. *Mar. Drugs* **2005**, *3*, 15–21.

(16) Shen, Y.-C.; Shih, P.-S.; Lin, Y.-S.; Lin, Y.-C.; Kuo, Y.- H.; Kuo, Y.-C.; Khalil, A. T. *Helv. Chim. Acta* **2009**, *92*, 2101–2110.

(17) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

(18) Hooper, J. N. A.; van Soest, R. W. M. *Systema Porifera: A Guide to the Classification of Sponges*; Kluwer Academic/Plenum: New York, 2002; p 1810.

(19) Cook, S.; De, C.; Bergquist, P. R. *N. Z. J. Mar. Freshwater Res.* **2009**, *33*, 545–563.

(20) 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.

(21) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.