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Hippolides A−H, Acyclic Manoalide Derivatives from the Marine Sponge *Hippospongia lachne*

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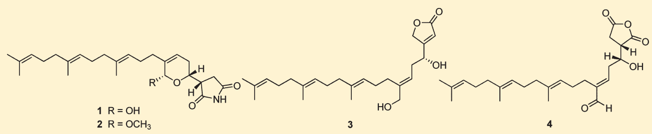
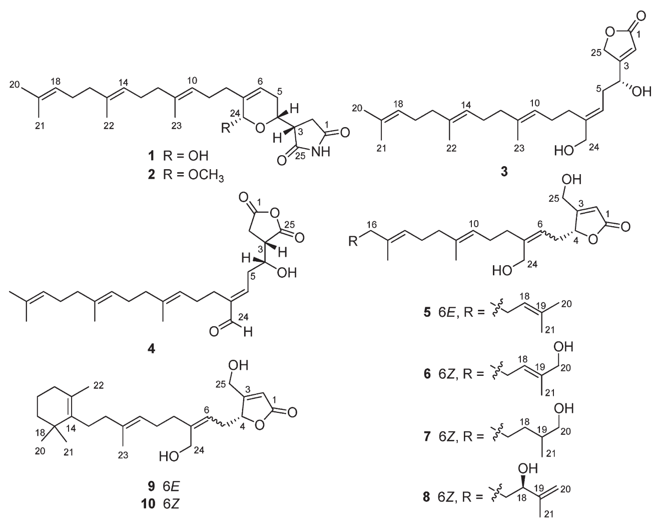
Supporting Information

ABSTRACT: Eight new acyclic manoalide-related sesterterpenes, hippolides A−H (**1**−**8**), together with two known manoalide derivatives, (6*E*)-neomanoalide (**9**) and (6*Z*)-neomanoalide (**10**), were isolated from the South China Sea sponge *Hippospongia lachne*. The absolute configurations of **1**−**8** were established by the modified Mosher's method and CD data. Compound **1** exhibited cytotoxicity against A549, HeLa, and HCT-116 cell lines with IC_{50} values of 5.22 × 10^{-2}, 4.80 × 10^{-2}, and 9.78 μM, respectively. Compound **1** also showed moderate PTP1B inhibitory activitiy with an IC_{50} value of 23.81 μM, and compound **2** showed moderate cytotoxicity against the HCT-116 cell line and PTP1B inhibitory activity with IC_{50} values of 35.13 and 39.67 μM, respectively. In addition, compounds **1** and **5** showed weak anti-inflammatory activity, with IC_{50} values of 61.97 and 40.35 μM for PKCγ and PKCα, respectively.



Manoalide, isolated from the marine sponge *Luffariella variabilis*,[1] is well known for its potent anti-inflammatory activity as a selective inhibitor of phospholipase A_2 (PLA_2).[2] A variety of sesterterpenes related to manoalide have been reported from sponges of the genera *Luffariella*, *Hyrtios*, *Thorecta*, *Thorectandra*, *Fascaplysinopsis*, *Fasciospongia*, *Cacospongia*, *Sarcotragus*, *Acanthodendrilla*, and *Aplysinopsis*.[3,4] Acyclic manoalide derivatives are sesterterpenoids that possess a terminal geranyl group instead of the cyclohexene ring in manoalide.[3] They have been isolated exclusively from three sponges, *Thorectandra excavatus*,[5] *Hyrtios* sp.,[6] and *Fasciospongia cavernosa*.[7] Besides their anti-inflammatory activity, these acyclic manoalide derivatives also showed significant cytotoxicity to cancer lines.[6,7]

Marine sponges of the genus *Hippospongia* (family Spongiidae, order Dictyoceratida) have attracted a great deal of attention, as they contain bioactive sesquiterpenes,[8] sesterterpene sulfates,[9] furanoterpenes,[10] and triterpenoic acid.[11] However, manoalide derivatives have not yet been isolated from this genus. In our search for cytotoxic secondary metabolites from marine invertebrates of the South China Sea, eight new acylic manoalide derivatives, hippolides A−H (**1**−**8**), together with two known manoalide derivatives, (6*E*)-neomanoalide (**9**) and (6*Z*)-neomanoalide (**10**), were isolated from the sponge *Hippospongia lachne*. Herein we report the isolation, structure elucidation, and bioactivity of these compounds.



RESULTS AND DISCUSSION

The EtOH extract of dried *H. lachne* was partitioned between EtOAc and H_2O. The EtOAc-soluble extract was further partitioned to yield petroleum ether- and CH_2Cl_2-soluble fractions.

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Table 1. ^{13}C NMR Data of Compounds 1−8 ($CDCl_3$)

carbon	1[a]	2[b]	3[b]	4[b]	5[a]	6[a]	7[b]	8[b]
1	178.7 qC	178.0 qC	173.7 qC	176.9 qC	172.9 qC	172.4 qC	172.4 qC	172.5 qC
2	30.5 CH_2	30.5 CH_2	115.1 CH	29.5 CH_2	116.1 CH	116.2 CH	116.2 CH	116.1 CH
3	46.0 CH	45.8 CH	171.7 qC	47.4 CH	172.0 qC	171.2 qC	171.2 qC	171.3 qC
4	64.5 CH	64.4 CH	68.2 CH	68.1 CH	81.7 CH	81.6 CH	81.6 CH	81.7 CH
5	28.4 CH_2	28.2 CH_2	34.7 CH_2	34.6 CH_2	30.2 CH_2	30.3 CH_2	30.2 CH_2	30.3 CH_2
6	120.9 CH	120.8 CH	118.8 CH	148.4 CH	117.3 CH	119.9 CH	119.9 CH	119.9 CH
7	137.4 qC	136.5 qC	144.2 qC	145.5 qC	143.5 qC	143.3 qC	143.4 qC	143.2 qC
8	32.6 CH_2	32.5 CH_2	28.4 CH_2	24.4 CH_2	28.5 CH_2	35.7 CH_2	35.7 CH_2	35.6 CH_2
9	26.0 CH_2	25.9 CH_2	26.5 CH_2	26.8 CH_2	26.6 CH_2	26.0 CH_2	26.8 CH_2	26.6 CH_2
10	123.6 CH	123.6 CH	123.1 CH	122.8 CH	123.3 CH	124.5 CH	123.8 CH	123.9 CH
11	135.8 qC	135.7 qC	136.4 qC	136.6 qC	136.2 qC	134.6 qC	135.6 qC	135.5 qC
12	39.7 CH_2	39.7 CH_2	39.7 CH_2	39.7 CH_2	39.7 CH_2	39.2 CH_2	39.6 CH_2	39.5 CH_2
13	26.7 CH_2	26.6 CH_2	26.7 CH_2	26.7 CH_2	26.7 CH_2	26.3 CH_2	26.2 CH_2	26.1 CH_2
14	124.2 CH	124.3 CH	123.9 CH	124.0 CH	124.1 CH	123.8 CH	124.1 CH	124.7 CH
15	135.0 qC	135.0 qC	135.2 qC	135.1 qC	135.1 qC	135.7 qC	135.1 qC	134.8 qC
16	39.7 CH_2	39.6 CH_2	39.7 CH_2	39.7 CH_2	39.7 CH_2	39.5 CH_2	39.8 CH_2	35.7 CH_2
17	26.8 CH_2	26.7 CH_2	26.8 CH_2	26.6 CH_2	26.8 CH_2	26.7 CH_2	25.2 CH_2	33.1 CH_2
18	124.4 CH	124.1 CH	124.3 CH	124.3 CH	124.4 CH	126.2 CH	32.6 CH_2	75.7 CH
19	131.3 qC	131.3 qC	131.4 qC	131.3 qC	131.3 qC	134.6 qC	35.6 CH	147.3 qC
20	25.7 CH_3	25.7 CH_3	25.7 CH_3	25.7 CH_3	25.7 CH_3	69.0 CH_2	68.4 CH_2	111.1 CH_2
21	17.7 CH_3	17.7 CH_3	17.7 CH_3	17.7 CH_3	17.7 CH_3	13.7 CH_3	16.6 CH_3	17.6 CH_3
22	16.1 CH_3	16.0 CH_3	16.0 CH_3	16.0 CH_3	16.1 CH_3	16.0 CH_3	15.9 CH_3	16.0 CH_3
23	16.0 CH_3	16.0 CH_3	16.0 CH_3	16.0 CH_3	16.0 CH_3	16.0 CH_3	15.9 CH_3	16.0 CH_3
24	91.4 CH	98.0 CH	66.2 CH_2	194.7 CH	66.2 CH_2	60.3 CH_2	60.4 CH_2	60.3 CH_2
25	176.9 qC	176.7 qC	71.2 CH_2	179.1 qC	58.6 CH_2	58.7 CH_2	58.7 CH_2	58.6 CH_2
26		55.4 CH_3						

[a] Recorded at 125 MHz. [b] Recorded at 100 MHz.

The CH_2Cl_2-soluble extract (cytotoxic against human hepatocellular carcinoma BEL-7402 and human lung adenocarcinoma SPC-A-1 cancer cell lines with IC_{50} values of 16.7 and 13.3 μg/mL, respectively) was subjected to repeated silica gel column chromatography and semipreparative HPLC to afford eight new compounds (**1**−**8**), along with two known metabolites, (6*E*)-neomanoalide (**9**) and (6*Z*)-neomanoalide (**10**).

Hippolide A (**1**) was obtained as a white, amorphous powder. Its molecular formula was established as $C_{25}H_{37}NO_4$ on the basis of its HRESIMS (*m*/*z* 438.2618, $[M + Na]^+$) and supported by NMR data (Tables 1 and 2). The ^{13}C NMR and DEPT spectra indicated 25 resonances for four methyl, eight methylene, seven methine, and six quaternary carbons (Table 1). The ^{1}H NMR spectrum displayed resonances for four olefinic protons at δ_H 5.63 (1H, br s), 5.12 (1H, t, J = 7.5 Hz), 5.10 (1H, t, J = 7.5 Hz), and 5.09 (1H, t, J = 7.5 Hz), one acetal proton at δ_H 5.19 (1H, s), one oxygenated methine proton at δ_H 4.56 (1H, m), four methyl groups attached to quaternary carbons at δ_H 1.68 (3H, s) and 1.60 (9H, s, overlapped), in addition to one NH proton at δ_H 8.36 (s), and one OH proton at δ_H 3.33 (d, J = 5.0 Hz) (Table 2). Six of the eight degrees of unsaturation of **1** were accounted for by four double bonds and two carbonyl carbons, indicating that the structure included two rings. The strong HMBC correlations of four methyl groups, H_3-20/C-18, C-19, and C-21, H_3-21/C-18, C-19, and C-20, H_3-22/C-14, C-15, and C-16, and H_3-23/C-10, C-11, and C-12, together with the COSY correlations for H-8/H-9/H-10, H-12/H-13/H-14, and H-16/H-17/H-18, indicated a farnesyl group (Figure 1). The COSY correlations of H-4/H-5/H-6, and H-24 (δ_H 5.19, 1H, s) with an exchangeable proton at δ_H 3.33 (1H, d, J = 5.0 Hz, 24-OH), and the HMBC correlations from H-24 to C-4/C-6/C-7 and from H-5 to C-7 indicated the presence of a δ-hydroxy lactol moiety. The presence of a succinimide moiety fulfilled the remaining unsaturation requirements. The COSY correlations of H-2/H-3 and HMBC correlations from H-2 to C-1 and C-25 confirmed the group as depicted. The aforementioned three moieties were connected by HMBC cross-peaks of H-2/C-4 and H-8/C-24. The NOESY correlations for H-6/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 (Figure 2) suggested the 6*Z*, 10*E*, 14*E*-configuration.

The *syn*-relationship between H-4 and H-24 was suggested by the NOE correlations of H-4 (δ_H 4.56)/H-24 (δ_H 5.19), H-4/H-5 (δ_H 2.03), and H-24/H-5 (**1a** in Figure 2). The relative configurations of C-3 and C-4 in **1** were assigned as *S** and *R**, respectively, based on NOESY correlations between H-3 (δ_H 2.99) and H-4, between H-3 and H-5, and between H-5 and H-2a (δ_H 2.88), as shown in the Newman projection of **1b** (Figure 2). The absolute configuration of C-24 was assigned by application of the modified Mosher method.[12] The (*S*)- and (*R*)-MTPA esters of **1** were prepared by treatment with (*R*)- and (*S*)-MTPA chloride, respectively. The $\Delta\delta_{S-R}$ values observed for the protons near the secondary C-24 hydroxy group for the esters indicated the *S*-configuration for the carbinol stereogenic center in **1** (Figure 3). On the basis of its relative configuration, the absolute configuration of **1** was thus determined as 3*S*, 4*R*, 24*S*.

Table 2. ^{1}H NMR Data of Compounds **1**−**8** ($CDCl_3$, *J* in Hz)

position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[a]	**7**[b]	**8**[b]
2	2.88, dd (5.0, 20.0); 2.69, dd (10.0, 20.0)	2.91, dd (3.2, 12.0); 2.71, dd (6.4, 12.0)	5.99, br s	2.90, dd (4.8, 17.2); 2.70, dd (8.6, 17.2)	6.04, br s	6.02, br s	6.02, br s	6.01, br s
3	2.99, m	2.98, ddd (3.2, 5.2, 6.4)		2.94, m				
4	4.56, m	4.47, m	4.66, t (6.2)	4.44, m	5.07, m	5.08, m	5.11, m	5.10, m
5	2.03, m	2.01, m	2.50, t (7.1)	2.56, m	2.73, m; 2.46, m	2.80, ddd (5.0, 7.5, 10.5); 2.55, ddd (5.5, 8.0, 10.5)	2.79, m; 2.57, m	2.81, ddd (5.0, 7.0, 9.9); 2.56, m
6	5.63, br s	5.60, br s	5.21, t (7.3)	6.50, t (7.2)	5.33, t (6.8)	5.37, t (7.5)	5.23, t (7.5)	5.23, t (7.6)
8	2.10, m	2.04, m	2.12, m	2.28, m	2.10, m	2.14, m	2.11, m	2.13, m
9	2.14, m	2.12, m	2.04, m	2.02, m	2.06, m	2.12, m	2.11, m	2.10, m
10	5.12, t (7.5)	5.09, m	5.07, t (6.4)	5.09, m	5.10, m	5.09, m	5.09, m	5.09, m
12	1.97, m	1.98, m	1.97, m	1.96, m	1.98, m	2.02, m	2.00, m	2.01, m
13	2.05, m	2.12, m	2.04, m	2.02, m	2.06, m	2.12, m	2.08, m	2.07, m
14	5.10, t (7.5)	5.09, m	5.09, t (6.4)	5.09, m	5.08, m	5.08, m	5.09, m	5.13, m
16	1.97, m	1.98, m	1.97, m	1.96, m	1.98, m	2.02, m	1.96, m	2.03, m
17	2.05, m	2.05, m	2.04, m	2.02, m	2.06, m	2.12, m	1.39, m	1.65, m
18	5.09, t (7.5)	5.09, m	5.10, t (6.4)	5.09, m	5.08, m	5.38, t (6.3)	1.39, m; 1.08, m	4.04, m
19							1.62, m	
20	1.68, s	1.70, s	1.67, s	1.67, s	1.68, s	3.99, s	3.50, dd (5.2, 10.8); 3.42, dd (6.4, 10.8)	4.93, s; 4.85, s
21	1.60, s	1.59, s	1.59, s	1.59, s	1.60, s	1.66, s	0.92, d (6.7)	1.73, s
22	1.60, s	1.59, s	1.59, s	1.59, s	1.60, s	1.60, s	1.58, s	1.61, s
23	1.60, s	1.59, s	1.59, s	1.57, s	1.60, s	1.60, s	1.58, s	1.58, s
24	5.19, s	4.66, s	4.10, s	9.40, s	4.04, s	4.14, d (12.0); 4.10, d (12.0)	4.16, d (11.6); 4.11, d (11.6)	4.19, d (12.1); 4.09, d (12.1)
25			4.88, br s		4.49, d (17.0); 4.42, d (17.0)	4.54, d (17.0); 4.46, d (17.0)	4.56, d (16.8); 4.46, d (16.8)	4.54, d (17.0); 4.46, d (17.0)
26		3.33, s						
OH	3.33, d (5.0)			8.59, s				
NH	8.36, s	7.95, s						

[a] Recorded at 500 MHz. [b] Recorded at 400 MHz.

Hippolide B (**2**) was obtained as a colorless oil. The molecular formula of $C_{26}H_{39}NO_4$ was deduced from its HRESIMS (*m/z* 452.2778, $[M + Na]^+$) combined with its NMR data (Tables 1 and 2). The ^{1}H and ^{13}C NMR data of **2** were similar to those of hippolide A (**1**), except for the presence of a methoxy instead of a hydroxy group (δ_H 3.33 and δ_C 55.4). Analysis of the 2D NMR data suggested that the C_1−C_{25} portion of **2** possessed the same skeleton as **1**. In addition, the succinimide group was confirmed by the HMBC correlations from NH (δ_H 7.95) to C-2 and C-3, from H-2 (δ_H 2.91 and 2.71) to C-25, and from H-3 (δ_H 2.98) to C-1, and COSY correlations of H-2/H-3 (Figure 1). The HMBC correlation from the oxygenated methyl proton (δ_H 3.33, 3H, s) to C-24 (δ_C 98.0) indicated that the methoxy group was attached to C-24. The ROESY correlations of H-6/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 showed the 6*Z*, 10*E*, 14*E*-configuration (Figure 2).

The *syn*-relationship between H-4 and H-24 was determined by the ROESY correlations of H-4 (δ_H 4.47)/H-24 (δ_H 4.66), H-4/H-5 (δ_H 2.01), and H-5/H-24 and the absence of correlations between H-4 and H_3-26 (δ_H 3.33) (**2a** in Figure 2). The relative configurations of C-3 and C-4 in **2** were determined as *S** and *R**, respectively, by the observation of ROESY correlations of H-3 (δ_H 2.98)/H-4, H-3/H-5, and H-5/H-2a (δ_H 2.91), shown in the Newman projection of **2b** (Figure 2). The CD spectrum of **2** showed a negative Cotton effect near at 199 nm similar to that of **1** (Figure 4). This, in conjunction with the same relative configuration at C-3, C-4, and C-24 of **1** and **2**, defined the absolute configuration of **2** as 3*S*, 4*R*, 24*S*. Compound **2** might be an artifact resulting from etherification of **1** during the isolation procedure with MeOH.

Hippolide C (**3**) was obtained as a colorless oil. The molecular formula of $C_{25}H_{38}O_4$ was determined by HRESIMS (*m/z* 425.2665, $[M + Na]^+$) and ^{13}C NMR data, indicating seven

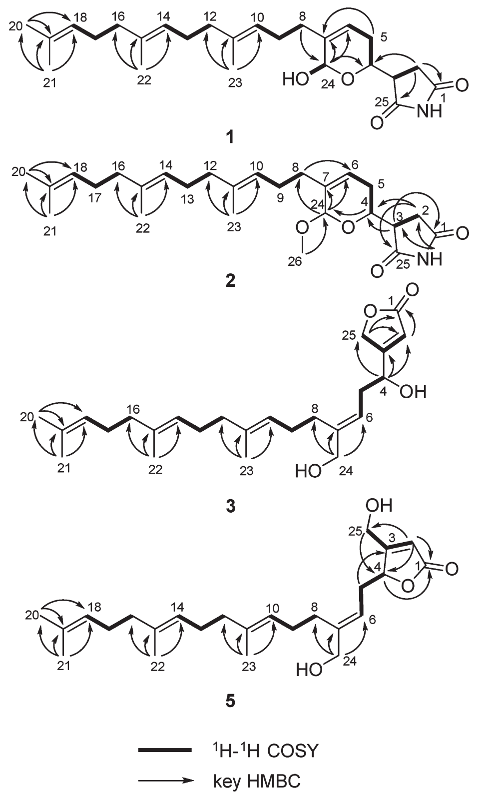

Figure 1. $^1H-^1H$ COSY and HMBC correlations of **1**, **2**, **3**, and **5**.

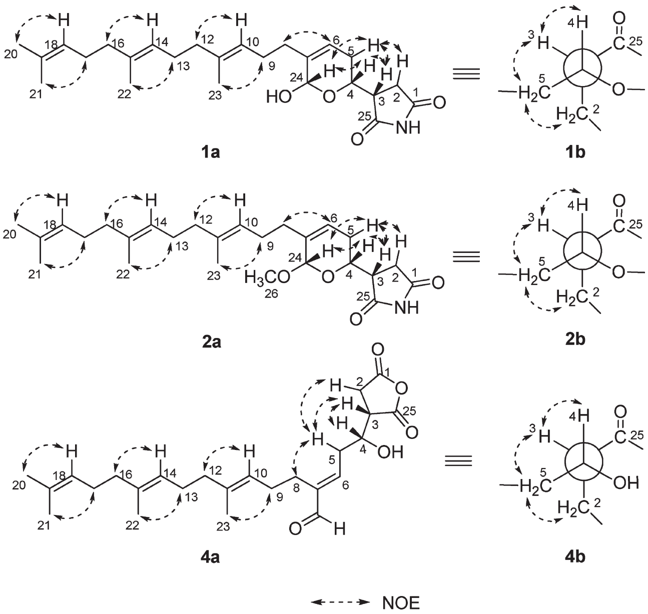

Figure 2. Selected NOE correlations of **1**, **2**, and **4**.

degrees of unsaturation. Analysis of ^1H NMR data (Table 2) in conjunction with the HSQC spectrum revealed the presence of four methyl, nine methylene, two of which were oxygenated, five protonated olefinic, and one oxygenated methine carbon. The ^{13}C NMR spectrum (Table 1) further showed the presence of one carbonyl and five nonprotonated sp^2 carbons. The HMBC and COSY correlations (Figure 1) suggested that **3** possessed an oxygenated geranylgeranyl group (C_5-C_{24} region). A butenolide

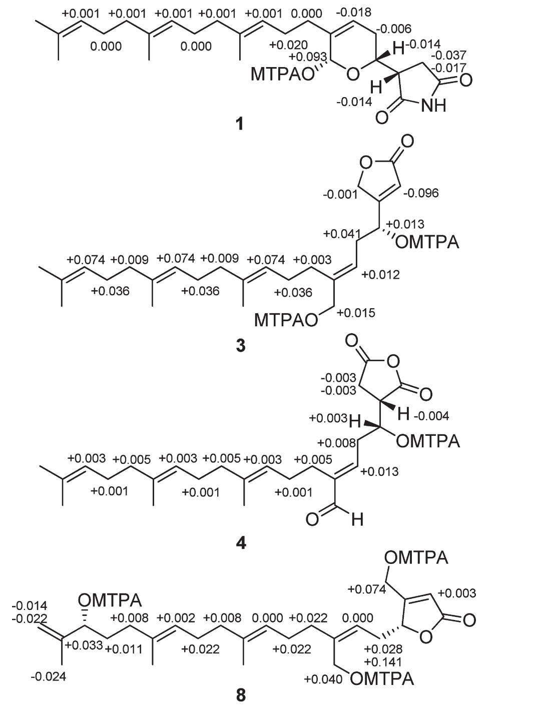

Figure 3. $\Delta\delta_{S-R}$ values (ppm) for the MTPA derivatives of **1**, **3**, **4**, and **8** in $CDCl_3$.

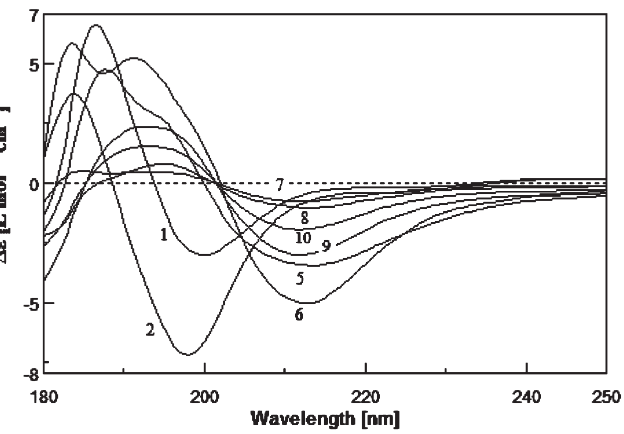

Figure 4. CD curves of compounds **1**, **2**, **5**, **6**, **7**, **8**, **9**, and **10**.

moiety was determined by the COSY correlations of H-2/H-25 and HMBC correlations of H-2/C-1 and H-25/C-1 and C-2. The connection of the geranylgeranyl group to the butenolide moiety at C-4 was supported by the COSY correlations of H-4/H-5/H-6 and the HMBC correlations from H-4 to C-2/C-3/C-25. ROESY correlations of H-6/H_2-24, H-5/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 supported the 6*E*, 10*E*, 14*E*-configuration. The absolute configuration of C-4 of **3** was determined by the modified Mosher's method. The $\Delta\delta_{S-R}$ values observed for the protons near the secondary hydroxy group at C-4 for the (*S*)- and (*R*)-MTPA esters of **3** indicated 4*R* absolute configuration (Figure 3).

Hippolide D (**4**) was obtained as a colorless oil, and its molecular formula $C_{25}H_{36}O_4$ was established by HRESIMS (*m/z* 423.2510, $[M + Na]^+$) and NMR data (Tables 1 and 2). The HMBC and COSY correlations indicated the presence of a geranylgeranyl group, and the HMBC correlations from H-24 (δ_H 9.40) to C-6 (δ_C 148.4, CH), C-7 (δ_C 145.5, qC), and C-8 (δ_C 24.4, CH_2) suggested the presence of a formyl group at C-7. A dihydrofuran-2,5-dione group and the connection with the geranylgeranyl group were confirmed by COSY correlations of H-2/H-3 and H-4/H-5/H-6 and HMBC correlations of H-2/C-1, C-4, and C-25, H-3/C-1, and H-4/C-3 and C-25. The ROESY correlations for H-5/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 suggested the 6*E*, 10*E*, 14*E*-configuration. The relative configurations of C-3 and C-4 were defined by the observation of ROESY correlations of H-4 (δ_H 4.44)/H-3 (δ_H 2.94), H-3/H-5 (δ_H 2.56), and H-5/H-2 (δ_H 2.90 and 2.70) (Newman projection **4b** in Figure 2). According to the $\Delta\delta_{S-R}$ values observed for the protons near the secondary hydroxy group at C-4 for the (*S*)- and (*R*)-MTPA esters of **4**, the absolute configuration at C-4 was determined to be *R* (Figure 3). The absolute configuration of **4** was thus determined as 3*S*, 4*R*.

Hippolide E (**5**) was obtained as a colorless oil. The molecular formula was established as $C_{25}H_{38}O_4$ on the basis of the HRESIMS (*m/z* 425.2671, $[M + Na]^+$) and ^{13}C NMR data. The presence of one carbonyl carbon (δ_C 172.9, C-1) in the ^{13}C NMR spectrum and the HMBC correlations of (H-2 and H-4)/C-1 and H-2/C-4 indicated that the C-1−C-4 fragment formed a butenolide ring (Figure 1). The COSY correlations for H_2-25/H-2 and HMBC correlations from H_2-25 to C-2/C-3/C-4 suggested that a hydroxymethyl group (δ_C 58.6, C-25) was connected to the butenolide ring at C-3. Analysis of the HMBC and COSY correlations revealed the presence of an oxygenated geranylgeranyl group (C_5−C_{24}), the same as in **3**. The connection of the oxygenated geranylgeranyl group to the butenolide ring at C-4 was supported by the COSY correlations of H-4/H-5/H-6 and an HMBC correlation from H-5 to C-3. The NOESY correlations H-6/H_2-24, H-5a (δ_H 2.73)/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 suggested the 6*E*, 10*E*, 14*E*-configuration.

Hippolide F (**6**) was obtained as a colorless oil. The quasimolecular ion peak at *m/z* 441.2619 $[M + Na]^+$ in the HRESIMS and the ^{13}C NMR data were consistent with the molecular formula $C_{25}H_{38}O_5$. Comparison of the ^1H and ^{13}C NMR spectra of **6** with those of **5** showed that they were similar except for an oxygenated methylene group at C-20 (δ_H 3.99 and δ_C 69.0) in **6** replacing the methyl group (δ_H 1.68 and δ_C 25.7) in **5**. The HMBC correlations from H_3-21 to C-18, C-19, and C-20 and from H-20 (δ_H 3.99, 2H, s) to C-18, C-19, and C-21 confirmed the structure as depicted. The NOESY correlations of H-6/H-8, H_2-24/H-5a (δ_H 2.80) and 5b (δ_H 2.55), H-10/H-12, H-9/H_3-23, H-14/H-16, H-13/H_3-22, H-18/H-20, and H-17/H_3-21 suggested the 6*Z*, 10*E*, 14*E*, 18*E*-configuration.

Hippolide G (**7**) was obtained as a colorless oil, and its molecular formula was established as $C_{25}H_{40}O_5$ on the basis of HRESIMS (*m/z* 443.2771, $[M + Na]^+$) and ^{13}C NMR data. The resonances for **7** were similar to those for **6** except for C-18, C-19, and C-21 (δ_C 32.6, 35.6, and 16.6 in **7** compared to δ_C 126.2, 134.6, and 13.7 in **6**, respectively). The COSY correlations of H-16/H-17/H-18 and H-19/H-20/H-21 and HMBC correlations from H-18a (δ_H 1.39) to C-17/C-20/C-21, from H-20 (δ_H 3.50 and 3.42) to C-18/C-19/C-21, and from H_3-21 to C-18/C-19/C-20 suggested that **7** lacked the C-18 double bond as seen in **6**. The NOESY correlations of H-6/H-8, H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 supported the 6*Z*, 10*E*, 14*E*-configuration.

Hippolide H (**8**) had the same formula of $C_{25}H_{38}O_5$ as **6** on the basis of HRESIMS and ^{13}C NMR data. The difference of the chemical shifts of C-18, C-19, and C-20 in compounds **8** and **6** [δ_C 75.7, 147.3, and 111.1 (CH_2) in **8** compared to δ_C 126.2, 134.6, and 69.0 in **6**, respectively] suggested that the C-18 double bond switched to C-19 and the hydroxy group switched to C-18 in **8**. The COSY correlations of H-16/H-17/H-18 and H-20/H-21 and the HMBC correlations of H_3-21/C-18, C-19, and C-20, H-20a (δ_H 4.93)/C-18, C-19, and C-21, H-20b (δ_H 4.85)/C-18 and C-21, and H-18/C-19, C-20, and C-21 confirmed the structure as depicted. The NOESY correlations of H-6/H-8, H_2-24/H-5 (δ_H 2.81 and 2.56), H-10/H-12, H-9/H_3-23, H-14/H-16, and H-13/H_3-22 suggested the 6*Z*, 10*E*, 14*E*-configuration.

The absolute configuration at C-4 of compounds **5**−**10** was determined by applying the CD method. The CD spectra of compounds **5**, **6**, **7**, **8**, and the two known neomanoalides **9** and **10** showed similar negative Cotton effects near 212 nm as those of the 4*R*-configured neomanoalides,[13,14] thus indicating the 4*R*-configuration for compounds **5**−**10** (Figure 4). The 18*R* absolute configuration of **8** was determined by the modified Mosher's method (Figure 3).

The structures of the two known compounds, (6*E*)-neomanoalide (**9**) and (6*Z*)-neomanoalide (**10**),[15] were determined on the basis of HRESIMS and 1D and 2D NMR experiments including HSQC, COSY, HMBC, and NOESY.

The new compounds **1**−**8** were tested in vitro for cytotoxicity against cancer cell lines, PTP1B inhibitory activity, and anti-inflammatory activity. Protein tyrosine phosphatase 1B (PTP1B), one of the protein tyrosine phosphatases (PTPases), is known to be a negative regulator of insulin signal transduction by dephosphorylating the insulin receptor as well as its substrate, insulin receptor substrates.[16] The PTP1B inhibitors are recognized as potential therapeutic agents for the treatment of type II diabetes and obesity.[17] Compound **1** exhibited significant cytotoxicity against A549 human lung epithelial cells, HeLa human cervical carcinoma cells, and HCT-116 human colon cancer cells with IC_{50} values of 5.22×10^{-2}, 4.80×10^{-2}, and 9.78 μM, respectively. Compound **1** also exhibited moderate PTP1B inhibitory activity, with an IC_{50} value of 23.81 μM. Compound **2** showed moderate cytotoxicity against the HCT-116 cell line and PTP1B inhibitory activity, with IC_{50} values of 35.13 and 39.67 μM, respectively. In addition, compounds **1** and **5** showed weak anti-inflammatory activities, with IC_{50} values of 61.97 and 40.35 μM for PKCγ and PKCα, respectively. These results implied an important role for the C-24 acetal group in compounds **1** and **2** for the observed cytotoxic activity and PTP1B inhibitory activity.

■ EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were recorded on a Bruker vector 22 spectrometer with KBr pellets. Optical rotation data were recorded on a Perkin-Elmer model 341 polarimeter with a 1 dm cell. The CD spectra were obtained with a JASCO J-715 spectropolarimeter. The NMR experiments were measured on Bruker AMX-400 MHz and Bruker AMX-500 MHz instruments in $CDCl_3$ with TMS as an internal standard. ESIMS and HRESIMS spectra were recorded on a Waters Q-Tof micro YA019 mass spectrometer. Reversed-phase HPLC was performed on a YMC-Pack Pro C_{18} RS column (250 × 10 mm, 5 μm) using a Waters 600 HPLC instrument with a Waters 996 UV detector. Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia) and YMC ODS-A (50 μm). Vacuum liquid chromatography (VLC) was performed on silica gel (200−300 mesh, Qingdao Ocean Chemical Company, China); the fractions were monitored by

TLC (HSGF 254, Yantai, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in H_2O.

Animal Material. A specimen of *H. lachne* was collected off Yongxing Island and Seven Connected Islets in the South China Sea in June 2007 and was identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China). A voucher sample (No. B-2) was deposited in the Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, China.

Extraction and Purification. The sponge (3.6 kg, wet weight) was extracted with 95% aqueous EtOH, and combined extracts were concentrated under reduced pressure to yield the crude extract. This extract was suspended in H_2O and extracted with EtOAc and *n*-BuOH to afford the EtOAc- and *n*-BuOH-soluble extracts. The EtOAc-soluble extract was partitioned between MeOH/H_2O (9:1) and petroleum ether to yield a brownish-red oil (84 g). The MeOH/H_2O (9:1) phase was diluted 3:2 with H_2O and extracted with CH_2Cl_2 to afford the CH_2Cl_2-soluble extract (110 g). This CH_2Cl_2-soluble extract was subjected to VLC on silica gel using CH_2Cl_2/MeOH (100:1, 50:1, 30:1, 20:1, 10:1, 5:1, and 1:1) as eluent to give nine subfractions (A−I). Subfraction D was subjected to CC on Sephadex LH-20 and ODS and further purified by HPLC (YMC-Pack Pro C_{18} RS, 5 μm, 10 × 250 mm, 2.0 mL/min, UV detection at 210 and 254 nm) eluting with MeOH/H_2O (82:18) to yield pure compounds **1** (72.1 mg), **2** (7.1 mg), and **3** (6.2 mg). Similarly, compounds **4** (32.1 mg), **5** (13.7 mg), **6** (12.5 mg), **7** (3.4 mg), and **8** (5.1 mg), together with the two known compounds **9** (10.1 mg) and **10** (20.1 mg), were purified from subfraction G.

Cytotoxicity Assay. Cytotoxicity and the corresponding IC_{50} values were determined using an MTT assay as described previously.[18] Compounds were solubilized in DMSO with the working concentration of test substances ranging from 1 to 100 μg/mL. Cells were inoculated in 96-well plates. After incubation for 24 h, the cells were treated with various concentrations of test substances for 48 h and then incubated with 1 mg/mL MTT at 37 °C for 4 h, followed by solubilization in DMSO. The formazan dye product was measured by the absorbance at 570 nm on a microplate reader.

PTP1B Inhibitory Assay. PTP1B inhibitory activity was determined using a PTP1B inhibitory assay as described in a previous report.[19] The enzymatic activities of the PTP1B catalytic domain were determined at 30 °C by monitoring the hydrolysis of *p*NPP. Dephosphorylation of *p*NPP generates product *p*NP, which was monitored at an absorbance of 405 nm. In a typical 100 μL assay mixture containing 50 mmol/L 3-[*N*-morpholino]propanesulfonic acid (MOPs), pH 6.5, 2 mmol/L *p*NPP, and 30 nmol/L recombinant PTP1B, activities were continuously monitored and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve.

Anti-inflammatory Activity Assay. Anti-inflammatory activity was determined using the Kinase-Glo Plus assay format.[20] For the Kinase-Glo Plus format test: ATP, substrates, and enzymes were prepared in assay buffers (25 mM HEPES, 10 mM $MgCl_2$, 0.01% Triton X-100, 100 μg/mL BSA, 2.5 mM DTT, pH 7.4). One microliter of compounds, 5 μL of ATP, 5 μL of substrates, and 4 μL of kinase were added to the assay plates, respectively. The assay plates were centrifuged, and the kinase was incubated at 30 °C for 1 h. The kinase reaction was stopped by the addition of kinase-Glo Plus (20 μL/well) and incubated at room temperature (RT) for 20 min. Finally, the plates were read and the luminescence measured.

For ADP-Glo format test: ATP, substrates, and enzymes were prepared in assay buffers (25 mM HEPES, 10 mM $MgCl_2$, 0.01% Triton X-100, 100 μg/mL BSA, 2.5 mM DTT, pH 7.4). One microliter of compounds, 5 μL of ATP, 5 μL of substrates, and 4 μL of AMPK (A2/B1/G1) were added to the assay plates. The plates were centrifuged, and the kinase was incubated at 30 °C for 1 h. The kinase reaction was stopped by the addition of ADP-Glo. After incubation at RT for 40 min, 20 μL/well of kinase detection buffers was transferred to the assay plates, and the assay plates were incubated at RT for 30 min. Finally, the plates were read and the luminescence was measured.

Hippolide A (1): white, amorphous powder; $[\alpha]^{23}_{D}$ +29 (*c* 0.06, MeOH); CD (*c* 1.27 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 186.5 (6.60), 200 (−2.99) nm; IR (KBr) ν_{max} 3289, 2927, 1712, 1355, 1190, 1015, 800 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 438.2618 $[M + Na]^+$ (calcd for $C_{25}H_{37}NO_4Na$, 438.2620).

Hippolide B (2): colorless oil; $[\alpha]^{23}_{D}$ −12 (*c* 0.03, MeOH); CD (*c* 1.08 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 184 (3.74), 198 (−7.17) nm; IR (KBr) ν_{max} 3442, 2926, 1775, 1462, 1377, 1196, 999, 918, 823 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 452.2778 $[M + Na]^+$ (calcd for $C_{26}H_{39}NO_4Na$, 452.2777).

Hippolide C (3): colorless oil; $[\alpha]^{23}_{D}$ +10 (*c* 0.04, MeOH); IR (KBr) ν_{max} 3429, 2926, 1747, 1633, 1462, 1379, 1173, 1059 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 425.2665 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_4Na$, 425.2668).

Hippolide D (4): colorless oil; $[\alpha]^{20}_{D}$ +2 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3425, 2924, 1713, 1622, 1462, 1379, 1188 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 423.2510 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_4Na$, 423.2511).

Hippolide E (5): colorless oil; $[\alpha]^{23}_{D}$ +17 (*c* 0.03, MeOH); CD (*c* 1.08 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 187.5 (4.74), 213.5 (−3.44) nm; IR (KBr) ν_{max} 3435, 2929, 1747, 1454, 1378, 1170, 1063 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 425.2671 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_4Na$, 425.2668).

Hippolide F (6): colorless oil; $[\alpha]^{20}_{D}$ −3.6 (*c* 0.06, MeOH); CD (*c* 5.85 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 183.5 (5.84), 187.5 (4.59), 191.0 (5.22), 212.5 (−5.02) nm; IR (KBr) ν_{max} 3427, 2919, 1743, 1643, 1446, 1146, 1063, 1009, 854 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 441.2619 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_5Na$, 441.2617).

Hippolide G (7): colorless oil; $[\alpha]^{20}_{D}$ +36 (*c* 0.03, MeOH); CD (*c* 2.38 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 185 (0.50), 212 (−0.76) nm; IR (KBr) ν_{max} 3423, 2917, 2850, 1736 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 443.2771 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_5Na$, 443.2773).

Hippolide H (8): colorless oil; $[\alpha]^{20}_{D}$ +15 (*c* 0.02, MeOH); CD (*c* 1.67 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 195.0 (0.81), 213.5 (−1.03) nm; IR (KBr) ν_{max} 3427, 2919, 2851, 1743, 1063 cm^{-1}; ^{13}C NMR data see Table 1; ^1H NMR data see Table 2; HRESIMS *m/z* 441.2620 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_5Na$, 441.2617).

Preparation of MTPA Esters. A previously described modified Mosher's method was used.[12] The (*S*)- and (*R*)-MTPA esters of **1** (1*S*, 1*R*), **3** (3*S*, 3*R*), **4** (4*S*, 4*R*), and **8** (8*S*, 8*R*) were obtained by treatment of **1** (0.6 and 1.3 mg, respectively), **3** (0.7 and 0.5 mg, respectively), **4** (0.9 and 0.7 mg, respectively), and **8** (0.6 and 0.7 mg, respectively) with (*R*)- and (*S*)-MTPA chlorides (10 μL) in dry pyridine (0.5 mL) and stirred at room temperature overnight. The MTPA esters were purified by mini-column chromatography on silica gel (200 mesh, petroleum ether/EtOAc, 1:1, for **1**, **4**, and **8** and CH_2Cl_2/MeOH, 13:1, for **3**).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Physical data (1D and 2D NMR, HRESIMS, IR, etc.) for compounds **1**−**8** and ^1H NMR data of MTPA esters (1*S*, 1*R*, 3*S*, 3*R*, 4*S*, 4*R*, 8*S*, 8*R*). This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) De Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1980**, *21*, 1611–1614.

(2) Reynolds, L. J.; Morgan, B. P.; Hite, G. A.; Mihelich, E. D.; Dennis, E. A. *J. Am. Chem. Soc.* **1988**, *110*, 5172–5177.

(3) Ebada, S. S.; Lin, W. H.; Proksch, P. *Mar. Drugs* **2010**, *8*, 313–346.

(4) Ueoka, R.; Nakao, Y.; Fujii, S.; Van Soest, R. W. M.; Matsunaga, S. *J. Nat. Prod.* **2008**, *71*, 1089–1091.

(5) Cambie, R. C.; Craw, P. A.; Berquist, P. R.; Karuso, P. *J. Nat. Prod.* **1988**, *51*, 331–334.

(6) Bourguet-Kondracki, M. L.; Debitus, C.; Guyot, M. *J. Chem. Res. Synop.* **1996**, 192–193.

(7) De Rosa, S.; De Giulio, A.; Crispino, A.; Iodice, C.; Tommonaro, G. *Nat. Prod. Lett.* **1997**, *10*, 267–274.

(8) (a) Ishibashi, M.; Ohizumi, Y.; Cheng, J. F.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1988**, *53*, 2855–2858. (b) Kobayashi, J.; Murayama, T.; Ohizumi, Y. *J. Nat. Prod.* **1989**, *52*, 1173–1176. (c) Kobayashi, J.; Naitoh, K.; Sasaki, T.; Shigemori, H. *J. Org. Chem.* **1992**, *57*, 5773–5776. (d) Shen, Y. C.; Chen, C. Y.; Kou, Y. H. *J. Nat. Prod.* **2001**, *64*, 801–803. (e) Liu, H. W.; Wang, G. H.; Namikoshi, M.; Kobayashi, H.; Yao, X. S.; Cai, G. P. *Pharm. Biol.* **2006**, *44*, 522–527. (f) Oda, T.; Wang, W. F.; Ukai, K.; Nakazawa, T.; Mochizuki, M. *Mar. Drugs* **2007**, *5*, 151–156. (g) Oda, T.; Wang, W. F.; Fujita, A.; Mochizuki, M.; Ukai, K.; Namikoshi, M. *J. Nat. Med.* **2007**, *61*, 434–437.

(9) (a) Musman, M.; Ohtani, I. I.; Nagaoka, D.; Tanaka, J.; Higa, T. *J. Nat. Prod.* **2001**, *64*, 350–352. (b) Lee, H. S.; Lee, T. H.; Yang, S. H.; Shin, H. J.; Shin, J.; Oh, K. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2483–2486.

(10) (a) Umeyama, A.; Shoji, N.; Arihara, S.; Ohizumi, Y.; Kobayashi, J. *Aust. J. Chem.* **1989**, *42*, 459–462. (b) Kobayashi, J.; Shinonaga, H.; Shigemori, H.; Sasaki, T. *Chem. Pharm. Bull.* **1993**, *41*, 381–382. (c) Rochfort, S. J.; Atkin, D.; Hobbs, L.; Capon, R. J. *J. Nat. Prod.* **1996**, *59*, 1024–1028. (d) Guo, Y. W.; Trivellone, E. *Chin. Chem. Lett.* **2000**, *11*, 327–330. (e) Rifai, S.; Fassouane, A.; Kijjoa, A.; Soest, R. V. *Mar. Drugs* **2004**, *2*, 147–153.

(11) (a) Ohta, S.; Uno, M. *Tetrahedron Lett.* **1996**, *37*, 7765–7766. (b) Craig, K. S.; Williams, D. E.; Hollander, I.; Frommer, E.; Mallon, R.; Collins, K.; Wojciechowicz, D.; Tahir, A.; Soest, R. V.; Andersen, R. J. *Tetrahedron Lett.* **2002**, *43*, 4801–4804.

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

(13) Soriente, A.; Crispino, A.; De Rosa, M.; De Rosa, S.; Scettri, A.; Scognamiglio, G.; Villano, R.; Sodano, G. *Eur. J. Org. Chem.* **2000**, 947–953.

(14) Kobayashi, M.; Okamoto, T.; Hayashi, K.; Yokoyama, N.; Sasaki, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 265–270.

(15) De Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1981**, *22*, 3147–3150.

(16) (a) Saltiel, A. R.; Pessin, J. E. *Trends Cell Biol.* **2002**, *12*, 65–71. (b) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814–3839.

(17) Johnson, T. O.; Eromlieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 696–709.

(18) Zhang, H. J.; Yi, Y. H.; Yang, G. J.; Hu, M. Y.; Cao, G. D.; Yang, F.; Lin, H. W. *J. Nat. Prod.* **2010**, *73*, 650–655.

(19) Yang, X. N.; Li, J. Y.; Shen, Q.; Chen, J. W.; Li, J. *Biochim. Biophys. Acta* **2005**, *1726*, 34–41.

(20) Singh, P.; Harden, B. J.; Lillywhite, B. J.; Broad, P. M. *Assay Drug Dev. Technol.* **2004**, *2*, 161–169.