Breviane Spiroditerpenoids from an Extreme-Tolerant *Penicillium* sp. Isolated from a Deep Sea Sediment Sample

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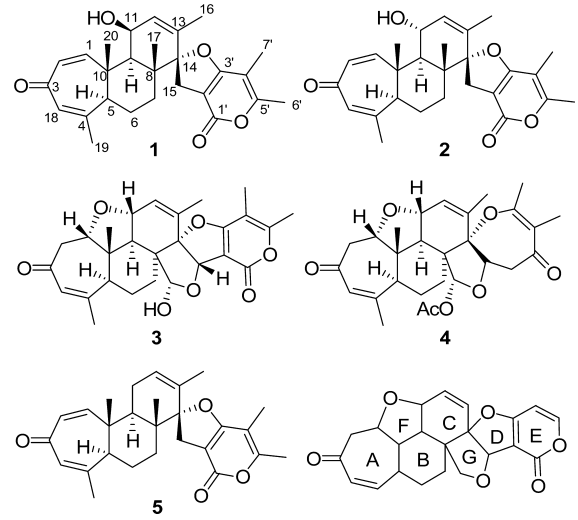
Breviones F−H (**1**−**3**), three new bioactive breviane spiroditerpenoids, have been isolated from the crude extract of an extreme-tolerant *Penicillium* sp. obtained from a deep sea sediment sample that was collected at a depth of 5115 m. The structures of these compounds were elucidated primarily by NMR spectroscopy. The absolute configurations of **1** and **3** were assigned by application of the modified Mosher method, and **3** is further confirmed by X-ray crystallographic analysis of its *S*-MTPA ester. Compound **2** could be the precursor for **3**, which features a previously undescribed skeleton of mixed biogenesis.

Although marine-derived fungi, especially the *Penicillium* spp., are rich sources of chemically diverse natural products with a broad range of biological activities,[1−7] only a limited number of metabolites have been reported from truly deep sea fungi.[8,9] Examples include the anserinones, (+)-formylanserinone B, (−)-epoxyserinone A, and (+)-epoxyserinone B isolated from a deep sea (−1335 m) *Penicillium* sp.;[10] the meleagrins, the roquefortines, and the conidiogenones from a deep water (−5080 m) sediment-derived *Penicillium* sp.;[11] and trisorbicillinone A from a deep sea (−5059 m) fungus *Phialocephala* sp.[12] In a search for new bioactive natural products from the extremophilic/extreme-tolerant fungi,[13,14] a marine-derived *Penicillium* sp. (MCCC 3A00005), isolated from a deep water sediment sample that was collected at a depth of 5115 m in the East Pacific, was grown in a solid-substrate fermentation culture. Its organic solvent extract showed cytotoxic activity against HeLa cells, and an inhibitory effect on HIV-1 replication in C8166 cells. Fractionation of the extract afforded three new breviane spiroditerpenoids, which have been named breviones F−H (**1**−**3**), along with the known compound brevione E (**4**).[15] Details of the isolation, structure elucidation, and biological activities of these metabolites are reported herein.



Results and Discussion

The structure of the known compound brevione E (**4**) was identified by comparison of the NMR and MS data with those reported.[15] It is the first naturally occurring 3(4→18)-*abeo*-1′-norbreviane and was initially isolated from a terrestrial *Penicillium* sp. as an allelopathic agent.

Brevione F (**1**) was assigned the molecular formula $C_{27}H_{32}O_5$ by HRESIMS (*m*/*z* 459.2151 $[M + Na]^+$). Analysis of its NMR spectroscopic data (Table 1) revealed the presence of one exchangeable proton, six methyl groups, three methylene units, three methines, three sp^3 quaternary carbons, five olefins, one carboxyl carbon, and one conjugated ketone carbon. Interpretation of the $^1H-^1H$ COSY NMR data of **1** established the C-1/C-2 olefin unit. HMBC correlations from H_3-20 to C-1, C-5, C-9, and C-10; from H_3-19 to C-4, C-5, and C-18; and from H-1, H-2, and H-18 to C-3 permitted completion of a 3-methylcyclohept-2-enone moiety (A). Further analysis of its 2D NMR data led to the identification of a decalin (B and C), a dihydrofuran (D), and an α-pyrone (E) in **1**. Relevant HMBC cross-peaks indicated that ring A was fused to the decalin moiety at C-5 and C-10, ring D was fused to E at C-2′ and C-3′, and ring C was joined to D at C-14 to form a spirocycle. These assignments established the gross structure of **1** as the 11-OH analogue of **5**, a metabolite isolated from a terrestrial *Penicillium* sp. as an allelopathic agent.[15]

The relative configuration of **1** was assigned by analysis of the NOESY data. NOESY correlations of H-9 with H-5 and H-11 and of H-5 with H-7a indicated that these protons are all on the same face of the ring system, whereas those of H_3-17 with H_2-15 and H_3-20, and H_3-17 with H-7b were used to place them on the opposite face of the ring system, thereby establishing the relative configuration of brevione F as **1**.

The absolute configuration of **1** was determined by application of the modified Mosher method.[16] Treatment of **1** with (*S*)-MTPA Cl and (*R*)-MTPA Cl afforded the (*R*)-MTPA ester (**1a**) and (*S*)-MTPA ester (**1b**), respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **1b** and **1a** was calculated in order to assign the absolute configuration at C-11. Calculations for all of the relevant signals suggested the 11*S* absolute configuration. Therefore, the 5*S*, 8*R*, 9*R*, 10*S*, 11*S*, and

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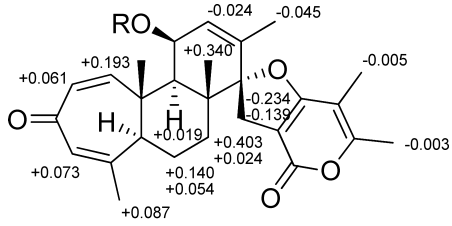
Table 1. NMR Data of Breviones F−H (**1**−**3**) in Acetone-d_6

	brevione F (**1**)		brevione G (**2**)		brevione H (**3**)			
position	δ_C[a], mult.	δ_H[b] (*J* in Hz)	δ_C[c], mult.	δ_H[d] (*J* in Hz)	δ_C[a], mult.	δ_H[b] (*J* in Hz)	HMBC (H → C#)	NOESY[e]
1	153.5, CH	6.87, d (13)	160.0, CH	7.80, d (13)	86.1, CH	3.78, dd (13, 3.0)	20	11, 20
2a	128.1, CH	5.77, d (13)	127.3, CH	5.63, d (13)	49.7, CH_2	3.01, t (13) 2.58, dd (13, 3.0)	1, 3, 10	5, 9
2b							1, 3, 10, 18	
3	193.2, qC		192.8, qC		196.6, qC			
4	154.0, qC		155.3, qC		161.9, qC			
5	49.7, CH	2.90, d (13)	49.5, CH	3.04, d (13)	46.2, CH	3.14, d (13)	4, 10, 20	2a, 7a, 9
6a	22.1, CH_2	1.76, dt (13, 3.5)	22.2, CH_2	1.70, dt (13, 3.5)	24.1, CH_2	1.93, dt (13, 3.5)	4, 8, 10	
6b		1.92, td (13, 3.5)		1.82, td (13, 3.5)		2.12, td (13, 3.5)	5, 7, 10	17, 19, 20
7a	31.6, CH_2	1.48, td (13, 3.5)	31.6, CH_2	1.42, td (13, 3.5)	30.6, CH_2	1.75, td (13, 3.5)	8, 17	5, 9
7b		1.56, dt (13, 3.5)		1.57, dt (13, 3.5)		1.53, dt (13, 3.5)	5, 6, 9	17
8	44.6, qC		43.1, qC		52.3, qC			
9	46.3, CH	1.97, s	47.8, CH	2.21, d (10)	54.4, CH	2.02, d (12)	1, 5, 7, 8, 12, 14, 17, 20	2a, 5, 7a, 18
10	41.6, qC		42.6, qC		46.5, qC			
11	65.0, CH	4.79, s	68.6, CH	4.49, d (10)	74.4, CH	4.65, d (12)	13	1, 20
12	131.5, CH	5.82, s	134.7, CH	5.69, s	131.3, CH	6.05, s	9, 14, 16	16
13	132.7, qC		132.0, qC		135.3, qC			
14	99.6, qC		99.1, qC		103.1, qC			
15a	29.5, CH_2	2.93, d (16)	29.1, CH_2	2.81, d (16)	83.4, CH	5.31, s	13, 14, 1′, 2′, 4′	16
15b	2.99, d (16)		3.01, d (16)					
16	18.5, CH_3	1.73, s	17.6, CH_3	1.67, s	17.7, CH_3	1.61, s	12, 13, 14	12, 15
17	19.2, CH_3	1.32, s	17.2, CH_3	1.14, s	102.6, CH	5.48. s	7, 8, 14, 15	7b, 20
18	131.7, CH	5.96, s	131.4, CH	5.96, s	130.9, CH	5.82, s	2, 5, 19	19
19	23.6, CH_3	1.96, s	23.8, CH_3	1.99, s	23.1, CH_3	1.95, s	4, 5, 18	6b, 18
20	15.7, CH_3	1.52, s	14.4, CH_3	1.35, s	18.8, CH_3	1.18, s	1, 9, 10	1, 11, 17
1′	171.2, qC		171.1, qC		173.0, qC			
2′	99.8, qC		99.8, qC		101.3, qC			
3′	161.2, qC		161.3, qC		164.1, qC			
4′	103.0, qC		103.0, qC		103.1, qC			
5′	161.0, qC		161.0, qC		160.0, qC			
6′	9.5, CH_3	1.85, s	9.6, CH_3	1.90, s	9.3, CH_3	1.96, s	1′, 3′, 4′, 5′	7′
7′	17.1, CH_3	2.16, s	17.1, CH_3	2.17, s	17.4, CH_3	2.23, s	1′, 3′, 4′	6′
OH-11		3.88, brs		4.35, brs				

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] Recorded at 150 MHz. [d] Recorded at 600 MHz. [e] Recorded at 400 MHz.



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

Figure 1. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MTPA esters **1a** and **1b**.

14*S* absolute configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 1.

Brevione G (**2**) was assigned the same molecular formula $C_{27}H_{32}O_5$ as **1** by analysis of its HRESIMS (*m*/*z* 459.2152 [M + Na]$^+$). Although its NMR spectra revealed structural similarity to **1**, the resonances for the decalin moiety (B and C) were significantly different from those in **1**, suggesting that **2** could be a stereoisomer of **1**. Interpretation of the 2D NMR data of **2** established the same planar structure as **1** and, surprisingly, identical relative configuration for all of the stereogenic centers except for C-11 as well. Considering the specific rotation values for **1** ($[\alpha]_D$ +27) and **2** ($[\alpha]_D$ −20), and the fact that they were not enantiomers, the chirality of C-11 in **2** could be reversed compared to that in **1**. In addition, the CD spectra of **1** and **2** were nearly identical, suggesting that **2** could be an 11-epimer of **1**. On the basis of these data, the absolute configuration of **2** was tentatively proposed as 5*S*, 8*R*, 9*R*, 10*S*, 11*R*, and 14*S*.

The elemental composition of **3** was established as $C_{27}H_{30}O_7$ by HRESIMS (*m*/*z* 489.1875 [M + Na]$^+$). The ^{1}H and ^{13}C NMR data for **3** (Table 1) differed significantly from those of **1** and **2**, suggesting a substantial structural change. Upon analysis of the ^{1}H−^{1}H COSY and HMBC data, the same partial structure of rings A−E, like those appearing in **1** and **2**, was established. However, a hydrogenation of the C-1/C-2 olefin and an oxidation of C-15 (δ_C 83.4) were observed. HMBC correlations from H-1 to C-20; from H-2b to C-1, C-3, C-10, and C-18; from H-18 to C-2, C-5, and C-19; and from H_3-19 to C-4, C-5, and C-18 confirmed the presence of a 3-methylcyclohept-2-enone moiety (A) that was fused to the B ring at C-5 and C-10. In turn, correlations from H-17 to C-7, C-8, and C-14 led to the connection of C-17 to C-8. A key HMBC cross-peak of H-17 with C-15 revealed an ether linkage between C-15 and C-17 of a THF moiety (G). Although no HMBC correlation was observed either from H-1 to C-11 or vice versa, considering the chemical shifts for C-1 (δ_C 86.1) and C-11 (δ_C 74.4 in **3** versus 68.6 in **2**), as well as the unsaturation requirement for **3**, these two carbons were attached to the same oxygen to form the second THF ring (F). The chemical shift for C-17 (δ_C 102.6) revealed its hemiacetal nature; thereby the only exchangeable proton in **3** was assigned as OH-17 to complete a heptacyclic planar structure as depicted.

The relative configuration of **3** was also assigned by analysis of the NOESY data (Figure 2). NOESY correlations of H-5 with H-2a, H-7a, and H-9 indicated that these protons are all on the same face of the ring system. Those of H-1 with H-11 and H_3-20 and of H-17 with H-7b and H_3-20 were used to place them on the opposite face of the molecule. The observed NOE effects between H-15 and H_3-16 indicated that H-15 and H-17 were on the same face of the D ring, which was exactly the same as in **4**,[15] suggesting that both **3** and **4** adopted the same relative configuration.

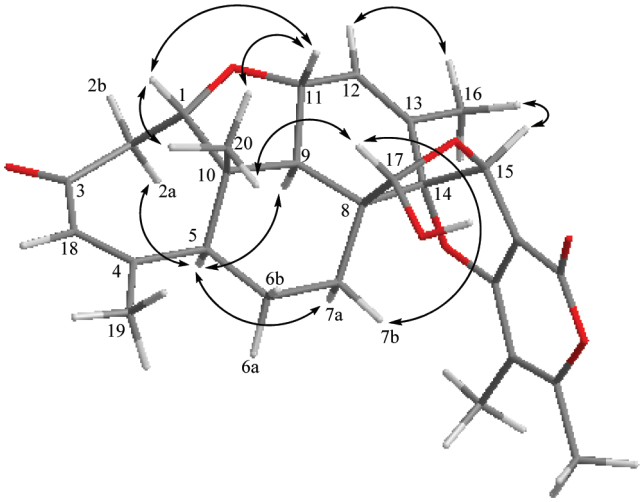
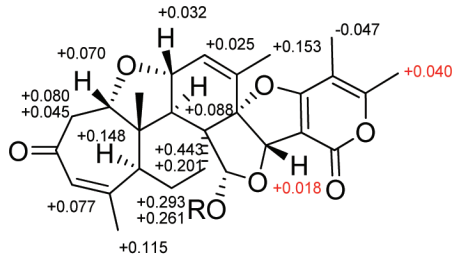


Figure 2. Key NOESY correlations for brevione H (**3**).



3a R = (*R*)-MTPA
3b R = (*S*)-MTPA

Figure 3. $\Delta\delta$ values (in ppm) $= \delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MTPA esters **3a** and **3b**.

The modified Mosher method was again applied to assign the absolute configuration of **3**. Treatment of **3** with (*S*)-MTPA Cl and (*R*)-MTPA Cl afforded the (*R*)-MTPA ester (**3a**) and (*S*)-MTPA ester (**3b**), respectively. The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **3b** and **3a** were calculated in order to assign the absolute configuration at C-17. However, the result from calculations for all of the relevant signals showed anomalous values for H-15 and H_3-6′ (Figure 3). To our knowledge, such deviation was also observed in decaspirone A due to the presence of a dioxynaphthalene moiety that aligned parallel to the aryl ring of the Mosher ester and influenced the anisotropic effect of the reagent.[17] The presence of a similarly aligned, highly conjugated α-pyrone in **3** might exert the same effect and cause the anomalous results for the closest proton H-15 and the δ-methyl protons H_3-6′. Ultimately, the structure of **3** was confirmed by single-crystal X-ray crystallographic analysis of its *S*-MTPA ester, and a perspective ORTEP plot is shown in Figure 4. Therefore, the 1*S*, 5*S*, 8*S*, 9*R*, 10*S*, 11*R*, 14*S*, 15*S*, and 17*S* absolute configuration was proposed for **3**. The opposite specific rotation values for **3** and **4** ($[\alpha]_D$ −36 for **3** versus +46 for **4**) were attributed to the opposite absolute configuration at C-17.

Compounds **1**−**3** were evaluated against HeLa cells and showed inhibitory effects of 25.2%, 44.9%, and 25.3%, respectively, at 10 μg/mL. **1**−**3** were also tested for *in vitro* activity against HIV-1, and **1** displayed an inhibitory effect on HIV-1 replication in C8166 cells, with an EC_{50} value of 14.7 μM (the CC_{50} value is greater than 100 μM; the positive control indinavir sulfate showed an EC_{50} value of 8.71 nM), whereas **2** and **3** did not show noticeable *in vitro* activity (EC_{50} > 50 μM).

Breviones F−H (**1**−**3**) are new members of the family of breviane spiroditerpenoids with mixed biogenesis.[15] Compound **1** is closely related to **5**, but differs in having one more hydroxy group

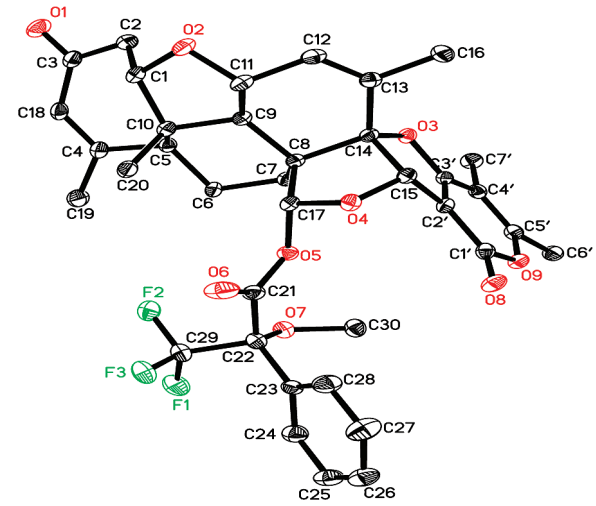


Figure 4. Thermal ellipsoid representation of **3b**. (Note: The numbering of structure **3b** presented here is consistent with the backbone numbering for compound **1**. A different numbering system is used for the structural data deposited with the CCDC.)

at C-11, whereas **2** is the 11-epimer of **1**. They could be derived from the same precursor originated from three units of acetyl-SCoA and geranylgeranyl diphosphate.[15] Even though **3** is structurally related to **4**, it possesses a previously undescribed skeleton. Biogenetically, **3** could be derived from **2** via oxidation of C-15 and C-17, followed by cyclization resulting from the nucleophilic attacks of C-11-OH to C-1 and C-15-OH to C-17 to form the corresponding THFs (F and G).

Although some breviane spiroditerpenoids have been reported from terrestrial fungi, ferns, and marine algae,[15,18−22] compounds **1**−**3** are the first examples of this class to be reported from a deep sea (−5115 m) sediment-derived *Penicillium* sp., an extreme-tolerant fungus that survived such harsh conditions as high pressure and salinity. It is widely accepted that organisms produce metabolites that serve as chemical defenses against their environment.[23] For extremophilic/extreme-tolerant fungi, even though the significance of secondary metabolites in their survival in extremes remains to be further explored, growing evidence indicates that they can be a valuable source of bioactive natural products with diverse structures.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-400, -500, and -600 spectrometers using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.1) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0T and APEXII FT-ICR spectrometers, respectively.

Fungal Material. The *Penicillium* sp. was isolated by one of the authors (D.Y.) from a deep water sediment sample collected at a depth of 5115 m in the East Pacific (145°2′ W, 07°37′ N), in September 2003. The isolate was characterized as an unidentified species of *Penicillium* by one of authors (Z.S.) based on sequence (Genbank accession number EU139854) analysis of the ITS region of the rDNA and assigned the accession number 3A00005 in the Marine Culture Collection Center (MCCC) at the Third Institute of Oceanography, the State Oceanic Administration, Xiamen, People's Republic of China. The fungal strain was cultured on slants of potato dextrose agar (PDA) with artificial seawater (NaCl 23.5 g, $MgCl_2 \cdot 6H_2O$ 10.6 g, $CaCl_2 \cdot 2H_2O$ 1.5 g, KCl 0.66 g, Na_2SO_4 3.9 g, $NaHCO_3$ 0.2 g, H_3BO_3 0.03 g in 1 L of distilled

H_2O) at 25 °C for 7 days. Fermentation was carried out in six 500 mL Fernbach flasks each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Artificial seawater (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was extracted repeatedly with EtOAc (4 × 600 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (3.5 g), which was fractionated by silica gel vacuum liquid chromatography (VLC) using petroleum ether/EtOAc gradient elution. The fractions eluted with 50% (100 mg) and 60% (150 mg) EtOAc were combined and separated again by Sephadex LH-20 column chromatography using 1:1 CH_2Cl_2/CH_3OH as eluent. Purification of the resulting subfractions with different isocratic elutions (**1** and **2**: 60% CH_3OH in H_2O for 60 min; **3** and **4**: 65% CH_3OH in H_2O for 35 min) by semipreparative reversed-phase HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 2 mL/min) afforded breviones F (**1**; 6.0 mg, t_R 44.2 min), G (**2**; 2.5 mg, t_R 36.8 min), H (**3**; 10.0 mg, t_R 17.6 min), and E (**4**; 5.0 mg, t_R 20.0 min).

Brevione E (4): colorless oil; $[\alpha]_D$ +46 (*c* 0.28, $CHCl_3$); ^{1}H NMR, ^{13}C NMR, and ESIMS data were fully consistent with the literature.[15]

Brevione F (1): white powder; $[\alpha]_D$ +27 (*c* 0.15, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 212 (4.02), 243 (3.80), 294 (3.48) nm; IR (neat) ν_{max} 3422 (br), 2947, 1696, 1647, 1621, 1574, 1439, 1276, 1118, 1064, 938 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data (acetone-d_6, 500 MHz) H-1 → C-2, 3, 5, 9, 10; H-2 → C-10, 18; H-5 → C-6, C-10, C-20; H-6a → C-7; H-6b → C-5, 7, 8, 10; H-7a → C-6; H-7b → C-5, 6; H-9 → C-8, 10, 11, 14, 17, 20; H-12 → C-9, 14, 16; H-15a → C-8, 13, 14, 1′, 2′; H-15b → C-8, 13, 14, 1′, 2′; H_3-16 → C-12, 13, 14; H_3-17 → C-7, 8, 9, 14; H-18 → C-2, 5, 19; H_3-19 → C-4, 5, 18; H_3-20 → C-1, 5, 9, 10; H_3-6′ → C-1′, 4′, 5′; H_3-7′ → C-4′, 5′; NOESY correlations (acetone-d_6, 600 MHz) H-1 ↔ H-9, H-11, H_3-20; H-5 ↔ H-7a, H-9, H_3-19; H-6a ↔ H_3-19; H-6b ↔ H_3-17, H_3-19, H_3-20; H-7b ↔ H-15b, H_3-17, H_3-20; H-9 ↔ H-1, H-5, H-11; H-11 ↔ H-1, H-5, H-9; H-12 ↔ H-15a, H_3-16; H-15a ↔ H-12, H_3-16; H-15b ↔ H-7b, H_3-17; H_3-16 ↔ H-12, H-15a; H_3-17 ↔ H-7b, H-15b, H_3-20; H-18 ↔ H_3-19; H_3-19 ↔ H-5, H-6a, H-6b, H-18; H_3-20 ↔ H-1, H-6b, H-7b, H_3-17; H_3-6′ ↔ H_3-7′; HRESIMS *m*/*z* 459.2151 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_5Na$, 459.2142).

Preparation of (*R*)-MTPA Ester (1a) and (*S*)-MTPA Ester (1b). A sample of **1** (1.0 mg, 0.002 mmol) was dissolved in CH_2Cl_2 (2.0 mL) in a 10 mL round-bottomed flask. DMAP (4.5 mg) and (*S*)-MTPA Cl (5.0 μL, 0.026 mmol) were quickly added, the flask was sealed, and all contents were stirred at room temperature for 12 h. The mixture was evaporated to dryness and purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 2 mL/min, 85% CH_3OH in H_2O for 20 min; 2 mL/min) to afford **1a** (1.0 mg, t_R 18.0 min): white powder; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.54 (1H, d, *J* = 13 Hz, H-1), 6.06 (1H, s, H-12), 6.06 (1H, s, H-11), 5.91 (1H, s, H-18), 5.78 (1H, d, *J* = 13 Hz, H-2), 3.09 (2H, s, H_2-15), 3.02 (1H, t, *J* = 13 Hz, H-5), 2.29 (1H, s, H-9), 2.18 (3H, s, H_3-7′), 1.87 (3H, s, H_3-19), 1.87 (3H, s, H_3-6′), 1.83 (3H, s, H_3-16), 1.77 (1H, td, *J* = 13, 3.5 Hz, H-6b), 1.62 (1H, dt, *J* = 13, 3.5 Hz, H-6a), 1.54 (1H, dt, *J* = 13, 3.5 Hz, H-7b), 1.28 (3H, s, H_3-20), 1.13 (1H, td, *J* = 13, 3.5 Hz, H-7a), 0.65 (3H, s, H_3-17).

In a similar fashion, a sample of **1** (1.0 mg, 0.002 mmol), CH_2Cl_2 (2.0 mL), DMAP (4.5 mg), and (*R*)-MTPA Cl (5.0 μL, 0.026 mmol) were allowed to react in a 10 mL round-bottomed flask at room temperature for 12 h, and the reaction mixture was processed as described above for **1a** to afford **1b** (0.8 mg, t_R 17.5 min): white powder; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.73 (1H, d, *J* = 13 Hz, H-1), 6.06 (1H, m, H-11), 6.03 (1H, m, H-12), 5.98 (1H, s, H-18), 5.84 (1H, d, *J* = 13 Hz, H-2), 2.96 (1H, d, *J* = 16 Hz, H-15b), 2.87 (1H, d, *J* = 16 Hz, H-15a), 2.82 (1H, d, *J* = 13 Hz, H-5), 2.31 (1H, s, H-9), 2.17 (3H, s, H_3-7′), 1.96 (3H, s, H_3-19), 1.87 (3H, s, H_3-6′), 1.84 (1H, td, *J* = 13, 3.5 Hz, H-6b), 1.79 (3H, s, H_3-16), 1.77 (1H, dt, *J* = 13, 3.5 Hz, H-6a), 1.57 (1H, dt, *J* = 13, 3.5 Hz, H-7b), 1.53 (1H, td, *J* = 13, 3.5 Hz, H-7a), 1.21 (3H, s, H_3-20), 0.99 (3H, s, H_3-17).

Brevione G (2): white powder; $[\alpha]_D$ −20 (*c* 0.15, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 212 (4.06), 244 (3.94), 294 (3.65) nm; IR (neat) ν_{max} 3402 (br), 3287, 2954, 1701, 1650, 1619, 1576, 1439, 1109, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HMBC data (acetone-d_6, 600 MHz) H-1 → C-2, 3, 5, 9, 10; H-2 → C-10, 18; H-5 → C-10; H-6a → C-7; H-6b → C-5, 7, 8, 10; H-7a → C-6; H-7b → C-5, 6; H-9 → C-8, 10, 11, 14, 17, 20; H-12 → C-9, 14, 16; H-15a → C-8, 13, 14, 1′, 2′; H-15b → C-8, 13, 14, 1′, 2′; H_3-16 → C-12, 13, 14; H_3-17 → C-7, 8, 9, 14; H-18 → C-2, 5, 19; H_3-19 → C-4, 5, 18; H_3-20 → C-1, 5, 9, 10; H_3-6′ → C-1′, 4′, 5′; H_3-7′ → C-4′, 5′; NOESY correlations (acetone-d_6, 600 MHz) H-1 ↔ H-9, H_3-20; H-5 ↔ H-7a, H-9; H-6a ↔ H_3-19; H-6b ↔ H_3-17, H_3-19, H_3-20; H-7a ↔ H-9; H-7b ↔ H-15b, H_3-17, H_3-20; H-9 ↔ H-1, H-5, H-7a; H-11 ↔ H-15a, H_3-17, H_3-20; H-12 ↔ H-15a, H_3-16; H-15b ↔ H-7b, H_3-17; H-15a ↔ H-11, H-12, H_3-16; H_3-16 ↔ H-12, H-15a; H_3-17 ↔ H-6b, H-7b, H-11, H-15b, H_3-20; H-18 ↔ H_3-19; H_3-19 ↔ H-6a, H-6b, H-18; H_3-20 ↔ H-1, H-6b, H-11, H_3-17; H_3-6′ ↔ H_3-7′; HRESIMS *m*/*z* 459.2152 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_5Na$, 459.2142).

Brevione H (3): white powder; $[\alpha]_D$ −36 (*c* 0.1, $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 212 (4.11), 244 (3.98), 293 (3.60) nm; IR (neat) ν_{max} 3430 (br), 2941, 1724, 1645, 1574, 1449, 1385, 1360, 1112, 1032, 989, 968 cm^{-1}; ^{1}H NMR, ^{13}C NMR, HMBC, and NOESY data, see Table 1; HRESIMS *m*/*z* 489.1875 $[M + Na]^+$ (calcd for $C_{27}H_{30}O_7Na$, 489.1884).

Preparation of (*R*)-MTPA Ester (3a) and (*S*)-MTPA Ester (3b). A sample of **3** (2.5 mg, 0.005 mmol) was dissolved in CH_2Cl_2 (4.0 mL) in a 10 mL round-bottomed flask. DMAP (8.5 mg) and (*S*)-MTPA Cl (10.0 μL, 0.052 mmol) were quickly added, the flask was sealed, and all contents were stirred at room temperature for 12 h. The mixture was evaporated to dryness and purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 80% CH_3OH in H_2O for 15 min; 2 mL/min) to afford **3a** (1.2 mg, t_R 12.0 min): colorless platelets; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.71 (1H, s, H-17), 6.16 (1H, s, H-12), 5.75 (1H, s, H-18), 5.47 (1H, s, H-15), 4.35 (1H, d, *J* = 12 Hz, H-11), 3.82 (1H, dd, *J* = 13, 3.0 Hz, H-1), 3.13 (1H, d, *J* = 13 Hz, H-5), 3.03 (1H, t, *J* = 13 Hz, H-2a), 2.55 (1H, dt, *J* = 13, 3.0 Hz, H-2b), 2.29 (3H, s, H_3-7′), 2.02 (1H, d, *J* = 12 Hz, H-9), 1.95 (3H, s, H_3-6′), 1.76 (3H, s, H_3-19), 1.70 (1H, td, *J* = 13, 3.5 Hz, H-6b), 1.65 (1H, dt, *J* = 13, 3.5 Hz, H-6a), 1.64 (3H, s, H_3-16), 1.63 (1H, dt, *J* = 13, 3.5 Hz, H-7b), 1.33 (1H, td, *J* = 13, 3.5 Hz, H-7a), 0.98 (3H, s, H_3-20).

In a similar fashion, a sample of **3** (2.5 mg, 0.005 mmol), CH_2Cl_2 (4.0 mL), DMAP (8.5 mg), and (*R*)-MTPA Cl (5.0 μL, 0.026 mmol) were allowed to react in a 10 mL round-bottomed flask at room temperature for12 h, and the reaction mixture was processed as described above for **3a** to afford **3b** (2.5 mg, t_R 11.8 min): colorless platelets; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.79 (1H, s, H-17), 6.19 (1H, s, H-12), 5.82 (1H, s, H-18), 5.48 (1H, s, H-15), 4.38 (1H, d, *J* = 12 Hz, H-11), 3.89 (1H, dd, *J* = 13, 3.0 Hz, H-1), 3.29 (1H, d, *J* = 13 Hz, H-5), 3.11 (1H, t, *J* = 13 Hz, H-2a), 2.60 (1H, dt, *J* = 13, 3.0 Hz, H-2b), 2.24 (3H, s, H_3-7′), 2.11 (1H, d, *J* = 12 Hz, H-9), 1.99 (3H, s, H_3-6′), 1.97 (1H, td, *J* = 13, 3.5 Hz, H-6b), 1.94 (1H, dt, *J* = 13, 3.5 Hz, H-6a), 1.88 (3H, s, H_3-19), 1.83 (1H, dt, *J* = 13, 3.5 Hz, H-7b), 1.79 (3H, s, H_3-16), 1.77 (1H, td, *J* = 13, 3.5 Hz, H-7a), 1.17 (3H, s, H_3-20).

X-ray Crystallographic Analysis of 3b.[24] Upon crystallization from MeOH/H_2O (10:1) using the vapor diffusion method, colorless crystals were obtained for **3b**, a crystal (0.30 × 0.21 × 0.05 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Bruker SMART 1000 CCD diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 173(2) K. Crystal data: $C_{37}H_{37}F_3O_9$, *M* = 682.67, space group orthorhombic, $P2_12_12_1$; unit cell dimensions *a* = 8.4483(17) Å, *b* = 15.923(3) Å, *c* = 23.947(5) Å, *V* = 3221.5(11) Å^3, *Z* = 4, D_{calcd} = 1.408 mg/m^3, μ = 0.111 mm^{-1}, *F*(000) = 1432. The structure was solved by direct methods using SHELXL-97[25] and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied with the Siemens Area Detector Absorption Program (SADABS).[26] The 40 187 measurements yielded 4139 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0540 and wR_2 = 0.1171 [$I > 2\sigma(I)$].

MTT Assay.[27] In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO or appropriate concentration of test compounds (10 mg/mL as stock solution of a compound in DMSO and serial dilutions). Cells were

treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first, and then the medium was changed to fresh Dulbecco's modified Eagle medium (DMEM). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from the wells, and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well and shaken at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

Anti-HIV Assays. Anti-HIV assays included cytotoxicity and HIV-1 replication inhibition evaluations.[28] Cells (3×10^4/well) were seeded into a 96-well microtiter plate in the absence or presence of various concentrations of test compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2. After a 4-day incubation period, cell viability was measured by the MTT method. The concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined. In parallel with the MTT assay, an HIV-1 replication inhibition assay was determined by p24 antigen capture ELISA. C8166 cells were exposed to HIV-1_{LAI} (MOI = 0.058) at 37 °C for 1.5 h, washed with PBS to remove free viruses, and then seeded into a 96-well microtiter plate at 3×10^4 cells per well in the absence or presence of test compounds (indinavir sulfate was used as positive control). After 4 days, the supernatant was collected and inactivated by 0.5% Triton X-100. The supernatant was diluted three times, added to the plate coating with anti-p24 McAb (provided by Dr. Bin Yan, Wuhan Institute of Virology, Wuhan, People's Republic of China), and incubated at 37 °C for 1 h. After washing five times with PBST, the HRP-labeled anti-p24 antibody (provided by Dr. Bin Yan) was added and incubated at 37 °C for 1 h. The plate was washed five times with PBST, followed by adding OPD reaction mixture. The assay plate was read at 490 nm using a microplate reader within 30 min. The inhibition rate and the EC_{50} based on p24 antigen expression level were calculated.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of breviones F−H (**1**−**3**) and CD spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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