γ-Lactones and *ent*-Eudesmane Sesquiterpenes from the Endophytic Fungus *Eutypella* sp. BCC 13199

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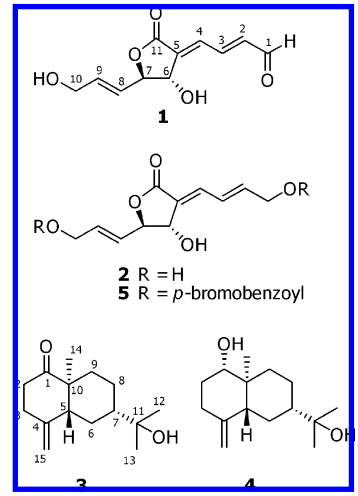
Two new γ-lactones, eutypellins A (**1**) and B (**2**), and two *ent*-eudesmane sesquiterpenes, *ent*-4(15)-eudesmen-11-ol-1-one (**3**) and *ent*-4(15)-eudesmen-1α,11-diol (**4**), together with three known pimarane diterpenes, diaporthein B, scopararane A, and libertellenone C, were isolated from the endophytic fungus *Eutypella* sp. BCC 13199. The structures of these compounds were elucidated by interpretation of spectroscopic data. The absolute configuration of **4** was confirmed by application of the modified Mosher's method. Eutypellin A (**1**) and sesquiterpene **3** exhibited weak cytotoxic activities.

Endophytic fungi have been the source of a wide range of novel bioactive secondary metabolites.[1−3] As part of our research program on the utilization of fungal sources in Thailand, we have investigated the endophyte *Eutypella* sp. BCC 13199, as an extract of this strain showed cytotoxic activity against human small-cell lung cancer cells (NCI-H187) with an IC_{50} value of 7.1 μg/mL. Scale-up fermentation and chemical studies led to the isolation of the new γ-lactones eutypellins A (**1**) and B (**2**), the *ent*-eudesmane sesquiterpenes *ent*-4(15)-eudesmen-11-ol-1-one (**3**) and *ent*-4(15)-eudesmen-1α,11-diol (**4**), and three known pimarane diterpenes, diaporthein B,[4] scopararane A,[5] and libertellenone C.[6] Details of the isolation, structure elucidation, and biological activities of the new compounds are presented here.

Eutypellin A (**1**) was isolated as a yellow, amorphous solid. The molecular formula of **1** was determined to be $C_{11}H_{12}O_5$ by HRESIMS in conjunction with the ^1H and ^{13}C NMR spectroscopic data. The IR spectrum showed broad and intense carbonyl absorption bands at ν_{max} 1749 and 1678 cm^{-1}. The ^1H and ^{13}C NMR, DEPT, and HMQC spectroscopic data revealed that this compound contained an aldehyde (δ_H 9.70, d, $J = 7.8$ Hz; δ_C 194.9), an ester (δ_C 170.7), a quaternary sp^2 carbon (δ_C 137.6), five olefinic methines, two oxymethines (δ_C 71.0 and 85.9), and an oxymethylene (δ_C 60.8) group. The COSY data indicated the connections from C-1 to C-4 and from C-6 to C-10. The *E*-configuration of C-2/C-3 and C-8/C-9 olefinic bonds was evident from the respective *J*-values of 15.3 and 15.5 Hz. The α-alkylidene-γ-lactone moiety was established by the HMBC correlations from H-4 and H-7 to the carbonyl carbon at δ_H 169.3 (C-11), from H-3 and H-6 to C-5, and from H-4 to C-6. An intense NOESY correlation between H-3 and H-6 indicated the 4*E*-configuration; therefore, compound **1** possesses the 2*E*,4*E*,8*E* triene geometry. To address the relative configuration at C-6 and C-7, the γ-lactone conformations for two possible diastereomers, *cis*- and *trans*-H-6/H-7, were examined using molecular model as well as computational energy minimization (MM2 and MOPAC). The *cis*-H-6/H-7 isomer adopted a stable conformation with the dihedral angle of H−C(6)−C(7)−H close to 0°, whereas the *trans*-H-6/H-7 isomer had a dihedral angle within the range 90−120°. Therefore, the latter configuration (*trans*) was more consistent with the observed small *J*-value (2.3 Hz) between H-6 and H-7.



The molecular formula of eutypellin B (**2**) was determined by HRESIMS as $C_{11}H_{14}O_5$. The signals in its ^1H and ^{13}C NMR spectra closely matched those of **1** except for the difference that the formyl group (C-1) in **1** was replaced with a hydroxymethyl (δ_H 4.13, 2H, dd, $J = 4.3$, 1.5 Hz; δ_C 61.4). The location of the hydroxymethyl group (H-1) was confirmed by the COSY correlations, indicating a vicinal $^1H-^1H$ coupling with H-2 and an allylic coupling with H-3 (δ_H 6.70, ddt, $J = 15.2$, 11.6, 1.5 Hz). H-6 resonated as a broad singlet with narrow peak width, which demonstrated the same relative configuration (*trans*-H-6/H-7) as **1**. Detailed interpretation of the NMR spectroscopic data confirmed that eutypellin B (**2**) is the C-1 alcohol derivative of **1**.

To determine the absolute configuration, we examined the application of the modified Mosher's method.[7,8] The reaction of **2** with *p*-bromobenzoyl chloride in pyridine gave the bis-*p*-bromobenzoate **5**. However, the reaction of **5** with (*S*)- or (*R*)-MTPACl in pyridine or DMAP/CH_2Cl_2, in all cases, did not provide the expected 6-*O*-MTPA ester derivative but gave only polymeric products. Attempts at crystallization of **5** and other mono- and bis-acylated derivatives of **2** for X-ray crystallographic analysis were also unsuccessful; therefore, the absolute configuration of the eutypellins remains unassigned.

The molecular formula of sesquiterpene **3** was determined by HRESIMS as $C_{15}H_{26}O_2$. The IR absorption at ν_{max} 1702 cm^{-1} and a ^{13}C NMR resonance at δ_C 215.0 indicated the presence of an aliphatic ketone functionality. Detailed analyses of the NMR

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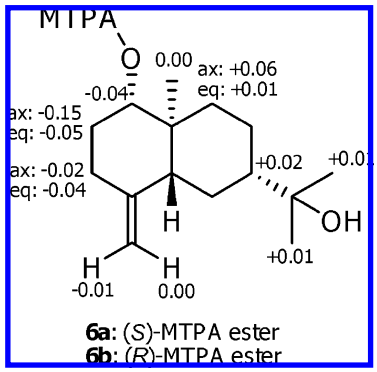

Figure 1. $\Delta\delta$ values [$\delta_S - \delta_R$] of the MTPA esters **6a** and **6b**.

spectroscopic data (^{1}H−^{1}H COSY, HMQC, and HMBC) revealed the eudesmene skeleton with an exomethylene (C-4/C-15), a ketone (C-1), and a tertiary alcohol (C-11) functionality. Compound **4**, $C_{15}H_{26}O_2$ (HRESIMS), was a C-1 alcohol analogue of **3**. The vicinal *J*-values of 11.6 and 4.6 Hz for H-1 (δ_H 3.41, dd, J = 11.6, 4.6 Hz) with H_2-2 indicated an axial orientation of H-1. $LiBH_4$ reduction of **3** gave an exclusive product whose MS and ^{1}H NMR data were identical to those of **4**.[9−12] The observed levorotation for **3** ($[\alpha]^{27}_D$ −87, *c* 0.05, $CHCl_3$) and **4** ($[\alpha]^{28}_D$ −48, *c* 0.725, $CHCl_3$) was opposite of those of the known eudesmane sesquiterpenes. Thus, compound **4** is the enantiomer of 4(15)-eudesmen-1β,11-diol, which was previously isolated from higher plants *Pterocarpus marsupium* ($[\alpha]^{31}_D$ +56.4, *c* 1.5, $CHCl_3$),[9] *Cryptomeria japonica* ($[\alpha]^{30}_D$ +56, *c* 1.5, $CHCl_3$),[10] and *Cymbopogon proximus*.[11] Compound **3** is the enantiomer of the semisynthetic compound previously obtained by Jones' oxidation of 4(15)-eudesmen-1β,11-diol[9] or as one of the acid degradation products ($[\alpha]_D$ +110.0, *c* 0.05, $CHCl_3$) of hinesol.[13] The absolute configuration of **4**, isolated from BCC 13199, was further confirmed by application of the modified Mosher's method.[7,8] The $\Delta\delta$ values [$\delta_S - \delta_R$] of the MTPA esters **6a** and **6b** indicated the 1*S*-configuration (Figure 1).

Compounds **1**−**4** were tested for their cytotoxic activities against three cancer cell lines, NCI-H187, MCF-7 (human breast cancer), and KB (human oral carcinoma), and noncancerous Vero cells (African green monkey kidney fibroblasts) (Table 2). Eutypellin A (**1**) exhibited weak cytotoxicity, whereas its alcohol analogue, **2**, was almost inactive. *ent*-Eudesmanone **3** also showed weak cytotoxicity, but the corresponding alcohol derivative **4** was inactive to all cell lines. Compounds **1**−**4** were also subjected to our antimalarial (*Plasmodium falciparum* K1), antituberculosis (*Mycobacterium tuberculosis* H37Ra), and antifungal (*Candida albicans* and *Magnaporthe grisea*) assays; however, they were all inactive.

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on an Analytikjena SPEKOL 1200 spectrophotometer. FTIR spectra were taken on a Perkin-Elmer 2000 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 2.50/δ_C 40.0 for DMSO-d_6, and δ_H 7.26/δ_C 77.0 for $CDCl_3$). ESITOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. The fungus used in this study was isolated from Earth Ginger *Etlingera littoralis* in Doi Suthep-Pui National Park, Chiang Mai Province, Thailand, and it was deposited in the BIOTEC Culture Collection (BCC) on February 12, 2003, as BCC 13199. This fungus was identified as a *Eutypella* sp. (order Xylariales, family Diatrypaseae) on the basis of the sequence data of the 18S rDNA and ITS genes by one of the authors (N.B.).

Fermentation and Isolation. *Eutypella* sp. BCC 13199 was maintained on potato dextrose agar at 25 °C. The agar was cut into plugs and inoculated into 3 × 1 L Erlenmeyer flasks containing 250 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 3 days on a rotary shaker (200 rpm), 600 mL of the primary culture was transferred into a 10 L bioreactor containing 5.4 L of malt extract broth (MEB; malt extract 6.0 g, yeast extract 1.2 g, maltose 1.8 g, dextrose 6.0 g, per liter), and final fermentation was carried out at 25 °C for 12 days under agitation at 200 rpm and aeration at 0.5 vvm. The cultures were filtered to separate broth (filtrate) and mycelia (residue). Culture broth was extracted with EtOAc (2 × 6 L), and the combined organic phase was concentrated to obtain a brown gum (broth extract; 3.46 g). The MeOH extract from mycelia (191 mg) did not contain any unique compounds. The broth extract was subjected to column chromatography (CC) on silica gel (5.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 100:0) to obtain five pooled fractions: fraction 1 (630 mg), 2 (1.40 g), 3 (732 mg), 4 (534 mg), and 5 (170 mg). Fraction 1 was triturated in MeOH (2 mL) and filtered. The residual solid (40 mg) was subjected to preparative HPLC using a reversed-phase column (LiChroCART 250-10, 10 × 250 mm, 10 μm; mobile phase MeCN/H_2O, 65:35, flow rate 4 mL/min) to furnish **5** (16 mg, t_R 5 min) and **6** (5 mg, t_R 9 min). The filtrate from trituration was chromatographed on a Sephadex LH-20 column in MeOH to obtain four fractions: fraction 1-1 (300 mg), 1-2 (160 mg), 1-3 (10 mg), and 1-4 (56 mg). Fraction 1-1 was separated by preparative HPLC (NovaPak HR C18, 40 × 100 mm, 10 μm; mobile phase MeOH/H_2O, 60:40, flow rate 15 mL/min) to collect fractions for t_R 5 min (fraction 1-1-1, 51 mg) and t_R 9 min (fraction 1-1-2, 200 mg). Fraction 1-1-1 was further purified by CC on silica gel (2.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 100:0) to afford **4** (37 mg). Fraction 1-1-2 was also purified by CC on silica gel to furnish **3** (169 mg). Fractions 1-2 and 1-3 were combined and fractionated by preparative HPLC to obtain diaporthein B (51 mg) and scopararane A (14 mg). Fractions 2 (1.40 g) and 3 (732 mg), from the first silica gel CC, were combined and further fractionated by CC on silica gel. The major fraction (853 mg) was subjected to preparative HPLC (NovaPak HR C18, 40 × 100 mm, 10 μm; mobile phase MeOH/H_2O, 60:40, flow rate 15 mL/min) to obtain **1** (36 mg; t_R 6 min) and a fraction (t_R 8.5 min, 6 mg) mainly composed of libertellenone C. Libertellenone C was further purified by short CC on silica gel (MeOH/CH_2Cl_2) to a colorless solid (3.1 mg). Fractions 4 and 5 were combined and triturated in MeOH (2 mL). The filtrate was concentrated to leave **2** (610 mg).

Eutypellin A (1): brown, amorphous solid; $[\alpha]^{25}_D$ +31 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 272 (4.55), 329 sh (3.79) nm; IR (KBr) ν_{max} 3423, 1749, 1678, 1651, 1193, 1099, 1043, 976 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), Table 1; HRMS (ESI-TOF) *m*/*z* 247.0586 $[M + Na]^+$ (calcd for $C_{11}H_{12}O_5Na$ 247.0582).

Eutypellin B (2): pale brown, amorphous solid; $[\alpha]^{24}_D$ +14 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 269 (4.63) nm; IR (KBr) ν_{max} 3424, 1746, 1649, 1195, 1091, 1046, 977 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), Table 1; HRMS (ESI-TOF) *m*/*z* 249.0734 $[M + Na]^+$ (calcd for $C_{11}H_{14}O_5Na$, 249.0739).

***ent*-4(15)-Eudesmen-11-ol-1-one (3):** colorless solid; $[\alpha]^{27}_D$ −87 (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max} 3343, 2948, 1702 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.01 (1H, br d, J = 1.3 Hz, Ha-15), 4.79 (1H, br d, J = 1.3 Hz, Hb-15), 2.70 (1H, m, Ha-2), 2.61 (1H, m, Ha-3), 2.44 (1H, m, Hb-3), 2.38 (1H, m, Hb-2), 2.14 (1H, br d, J = 11.1 Hz, H-5), 1.83 (2H, m, Ha-6 and Ha-9), 1.77 (1H, m, Ha-8), 1.59 (1H, dt, J = 4.0, 13.7 Hz, Hb-9), 1.37 (1H, m, Hb-6), 1.33 (1H, m, H-7), 1.25 (1H, m, Hb-8), 1.23 (3H, s, H-12), 1.22 (3H, s, H-13), 0.99 (3H, s, H-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.0 (C, C-1), 146.6 (C, C-4), 109.1 (CH_2, C-15), 72.6 (C, C-11), 48.5 (CH, C-7), 48.4 (C, C-10), 47.9 (CH, C-5), 38.1 (CH_2, C-2), 34.6 (CH_2, C-3), 32.1 (CH_2, C-9), 27.6 (CH_3, C-12), 27.0 (CH_3, C-13), 24.6 (CH_2, C-6), 22.0 (CH_2, C-8), 16.6 (CH_3, C-14); HRMS (ESI-TOF) *m*/*z* 259.1671 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

***ent*-4(15)-Eudesmen-1α,11-diol (4):** colorless solid; $[\alpha]^{28}_D$ −48 (*c* 0.725, $CHCl_3$); IR (KBr) ν_{max} 3357, 2936, 1016, 888 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.76 (1H, d, J = 1.3 Hz, Ha-15), 4.51 (1H, d, J = 1.3 Hz, Hb-15), 3.41 (1H, dd, J = 11.6, 4.6 Hz, H-1), 2.30 (1H, ddd, J = 13.6, 4.9, 2.2 Hz, Ha-3), 2.10 (1H, dt, J = 5.2, 13.6 Hz, Hb-3), 1.96 (1H, ddd, J = 12.3, 3.4, 2.8 Hz, Ha-9), 1.81 (1H, m, Ha-2), 1.55 (1H, dq, J = 5.0, 12.2 Hz, Hb-2), 1.70 (1H, m, H-5), 1.68 (2H, m, Ha-6 and Ha-8), 1.34 (1H, tt, J = 12.0, 3.2 Hz, H-7), 1.23 (1H, m, Hb-8), 1.21 (1H, m, Hb-6), 1.20 (6H, s, H-12 and H-13), 1.16

Table 1. NMR Spectroscopic Data (DMSO-d_6) for Eutypellins A (**1**) and B (**2**)

	eutypellin A (**1**)			eutypellin B (**2**)		
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC	δ_C, mult	δ_H, mult. (*J* in Hz)	HMBC
1	194.9, CH	9.70, d (7.8)	2	61.4, CH_2	4.13, dd (4.3, 1.5)	2, 3
2	138.9, CH	6.66, dd (15.3, 7.8)	1, 4	147.8, CH	6.49, dt (15.2, 4.3)	1, 4
3	145.2, CH	7.76, dd (15.3, 11.7)	1, 5	124.0, CH	6.70, ddt (15.2, 11.6, 1.5)	1, 2, 4, 5
4	136.0, CH	7.37, dd (11.7, 1.8)	2, 6, 11	140.2, CH	7.16, dd (11.6, 1.1)	2, 5, 6, 11
5	137.6, qC			127.4, qC		
6	71.0, CH	4.86, m	5	70.8, CH	4.62, br s	4, 5, 8, 11
6-O*H*		6.29, br d (6.4)			not obsd	
7	85.9, CH	4.81, ddd (6.9, 2.3, 0.7)	6, 11	85.8, CH	4.73, m	6, 9, 11
8	125.1, CH	5.71, ddt (15.5, 6.9, 1.8)	6, 7, 9, 10	125.8, CH	5.64, ddt (15.5, 6.6, 1.7)	7, 9, 10
9	135.8, CH	5.92, ddt (15.5, 0.7, 4.2)		134.6, CH	5.83, ddt (15.5, 1.0, 4.4)	10
10	60.8, CH_2	3.98, m	8, 9	60.9, CH_2	3.94, d (4.4)	8, 9
11	169.3, qC			170.6, qC		

Table 2. Cytotoxic Activities of Compounds **1**−**4** (IC_{50}, μM)

compound	NCI-H187	MCF-7	KB	Vero
1	12	84	38	88
2	206	>221	144	>221
3	11	20	32	32
4	>210	>210	>210	>210
doxorubicin[a]	0.25	1.5	0.28	—[b]
ellipticine[a]	3.6	—[b]	2.5	5.5

[a] Reference compounds for the cytotoxicity assays. [b] Not tested.

(1H, m, Hb-9), 0.67 (3H, s, H-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.9 (C, C-4), 106.8 (CH_2, C-15), 79.3 (CH, C-1), 72.8 (C, C-11), 49.0 (CH, C-7), 47.5 (CH, C-5), 40.2 (C, C-10), 37.0 (CH_2, C-9), 34.2 (CH_2, C-3), 31.5 (CH_2, C-2), 27.2 (CH_3, C-12), 27.1 (CH_3, C-13), 24.5 (CH_2, C-6), 22.2 (CH_2, C-8), 10.2 (CH_3, C-14); HRMS (ESI-TOF) *m*/*z* 261.1823 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830).

Preparation of the Bis-*p*-Bromobenzoate Derivative 5. Compound **2** (56 mg) was treated with *p*-bromobenzoyl chloride (170 mg) in pyridine (1.0 mL) at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was subjected to preparative HPLC (MeCN/H_2O, 75:25) to furnish the bis-*p*-bromobenzoate **5** (60 mg) along with a mono-*p*-bromobenzoate derivative (60 mg).

Compound 5: colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (2H, d, J = 8.3 Hz, *p*-bromobenzoyl), 7.90 (2H, d, J = 8.3 Hz, *p*-bromobenzoyl), 7.61 (2H, d, J = 8.3 Hz, *p*-bromobenzoyl), 7.59 (2H, d, J = 8.3 Hz, *p*-bromobenzoyl), 7.31 (1H, d, J = 11.7 Hz, H-4), 6.81 (1H, dd, J = 15.1, 11.7 Hz, H-3), 6.42 (1H, dt, J = 15.1, 5.5 Hz, H-2), 6.05 (1H, dt, J = 15.5, 5.2 Hz, H-9), 5.85 (1H, dd, J = 15.5, 5.3 Hz, H-8), 4.98 (2H, d, J = 5.5 Hz, H-1), 4.87−4.81 (4H, m, H-6, H-7, and H-10), 2.48 (1H, d, J = 6.9 Hz, 6-O*H*); MS (ESITOF) *m*/*z* 612.94, 614.94, and 616.94 $[M + Na]^+$.

$LiBH_4$ Reduction of 3. To a solution of **3** (4.0 mg) in THF (0.3 mL), cooled on an ice−water bath, was added $LiBH_4$ (7 mg), and the mixture was stirred for 1.5 h. Water was added and the mixture extracted with EtOAc. The organic layer was concentrated under reduced pressure to leave a colorless solid, which was purified by a short silica gel column (3.3 mg). The ESIMS and ^{1}H NMR spectroscopic data were identical to those of the isolated compound **4**.

Preparation of the MTPA Esters 6a and 6b. Compound **4** (1.0 mg) was treated with (−)-(*R*)-MTPACl (20 mg) in pyridine (0.2 mL) at room temperature for 14 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was purified by a short silica gel column (MeOH/CH_2Cl_2) to obtain the (*S*)-MTPA ester **5a** (1.1 mg). Similarly, (*R*)-MTPA ester **5b** was prepared from **4** and (+)-(*S*)-MTPACl.

(*S*)-MTPA Ester 6a: colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52−7.39 (5H, m, phenyl), 4.86 (1H, dd, J = 11.7, 4.6 Hz, H-1), 4.80 (1H, br s, Ha-15), 4.68 (1H, br s, 11-O*H*), 4.57 (1H, br s, Hb-3), 3.51 (3H, br s, OCH_3), 2.34 (1H, m, Ha-3), 2.19 (1H, m, Hb-3), 1.98 (1H, m, Ha-2), 1.86 (1H, m, Ha-9), 1.74−1.60 (3H, m, H-5, Ha-6 and Ha-8), 1.60 (1H, m, Hb-2), 1.36 (1H, m, H-7), 1.30−1.22 (2H, m, Hb-6 and Hb-8), 1.22 (1H, m, Hb-9), 1.21 (6H, s, H-12 and H-13), 0.72 (3H, s, H-14); HRMS (ESITOF) *m*/*z* 477.2226 $[M + Na]^+$ (calcd for $C_{25}H_{33}O_4F_3Na$, 477.2223).

(*R*)-MTPA Ester 6b. colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53−7.38 (5H, m, phenyl), 4.90 (1H, dd, J = 11.8, 4.6 Hz, H-1), 4.81 (1H, br s, Ha-15), 4.68 (1H, br s, 11-O*H*), 4.57 (1H, br s, Hb-3), 3.55 (3H, br s, OCH_3), 2.38 (1H, m, Ha-3), 2.20 (1H, m, Hb-3), 2.03 (1H, m, Ha-2), 1.85 (1H, m, Ha-9), 1.75 (1H, m, Hb-2), 1.72 (1H, m, H-5), 1.62−1.55 (2H, m, Ha-6 and Ha-8), 1.33 (1H, m, H-7), 1.28−1.18 (2H, m, Hb-6 and Hb-8), 1.20 (6H, s, H-12 and H-13), 1.16 (1H, m, Hb-9), 0.72 (3H, s, H-14); HRMS (ESITOF) *m*/*z* 477.2202 $[M + Na]^+$ (calcd for $C_{25}H_{33}O_4F_3Na$, 477.2223).

Biological Assays. The cytotoxic activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were performed using the resazurin microplate assay.[14] Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) was evaluated using the green fluorescent protein microplate assay (GFPMA).[15]

Supporting Information Available: NMR spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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