Absolute Stereochemistry of Amphidinolide E

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The absolute configurations at eight chiral centers in amphidinolide E (**1**), a cytotoxic 19-membered macrolide isolated from a marine dinoflagellate *Amphidinium* sp., were determined to be 2*R*, 7*R*, 8*R*, 13*S*, 16*S*, 17*R*, 18*R*, and 19*R* on the basis of detailed analysis of NMR data and by chemical means.

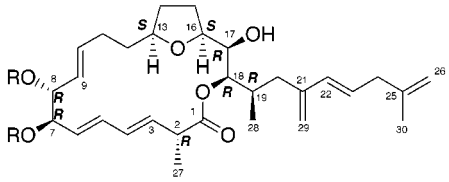
Introduction

Our continuing search for bioactive metabolites from symbiotic marine dinoflagellates of the genus *Amphidinium* has resulted in the isolation of a series of cytotoxic macrolides[1] and two types of polyketides.[2] Amphidinolide E (**1**) is a unique cytotoxic 19-membered macrolide, which has been isolated from the Y-5′ strain of a dinoflagellate *Amphidinium* sp.[3] The gross structure was elucidated by 2D NMR data, whereas the stereochemisty remains unsolved because of the lack of sample. Recently, the relative and absolute configurations at eight chiral centers in **1** were elucidated on the basis of detailed analysis of NMR data and by chemical means because a substantial amount (2 mg) of **1** has been obtained through repeated cultivation. This contribution describes the determination of the relative and absolute configurations of **1** (Chart 1).

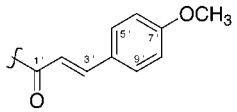
Results and Discussion

The relative stereochemistry of eight chiral centers (C-2, C-7, C-8, C-13, C-16, C-17, C-18, and C-19) in amphidinolide E (**1**) was elucidated by a combination of the *J*-based configuration method[4] and detailed analyses of NOESY data, while the absolute stereochemistry was determined by the following experiments: (a) application of the exciton chirality method[5] for a 1,2-diol at C-7 and C-8, (b) application of a modified Mosher method[6] for a hydroxyl group at C-17 in **1** and for that at C-1 of the C-1−C-7 segment obtained by oxidative degradation, and (c) comparison of NMR data for MTPA esters of the C-8−C-17 segment obtained by oxidative degradation with those of the corresponding synthetic compound.

Chart 1



1 : R = H
2 : R = –C(CH$_3$)$_2$–,
4 : R =



Relative Stereochemistry. The relative stereochemistry of H-13/H-16 on a tetrahydrofuran ring in **1** was elucidated to be syn from the NOESY correlation for H-13/H-16 (Figure 1a). On the other hand, the relative stereochemistry of the 7,8-diol was assigned as threo by NOESY correlations (Figure 1b) and the ^3J(H-7, H-8) value (8.8 Hz) of the 7,8-*O*-isopropylidene derivative (**2**) of **1**, which was obtained by treatment of **1** with 2,2-dimethoxypropane (DMP) and pyridinium *p*-toluenesulfonate (PPTS).

For the C-16−C-17 bond, the ^3J(H-16, H-17) value (7.5 Hz) was typical for an anti relationship (Figure 2a), while the value for ^2J(H-17, C-16) (−6.0 Hz), which was obtained from the hetero half-filtered TOCSY (HETLOC)[7] spectrum of **1**, indicated that H-16 was gauche to 17-OH. Gauche relations between C-15 and C-18 and between C-15 and H-17 were implied by NOESY correlations for H_2-15/H-18 and H_2-15/H-17, thus suggesting that the relative configuration of C-16−C-17 was threo. An erythro relationship for C-18−C-19 was deduced from the

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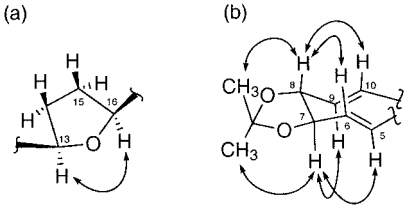


Figure 1. NOESY correlations and relative stereochemistry of (a) a tetrahydrofuran ring in amphidinolide E (**1**) and (b) C-7−C-8 in the 7,8-*O*-isopropylidene derivative (**2**) of **1**. NOESY correlations are illustrated by solid arrows.

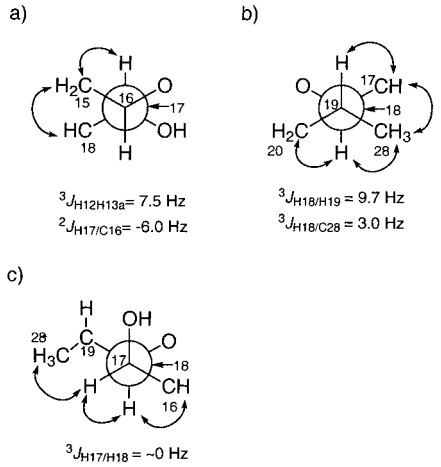
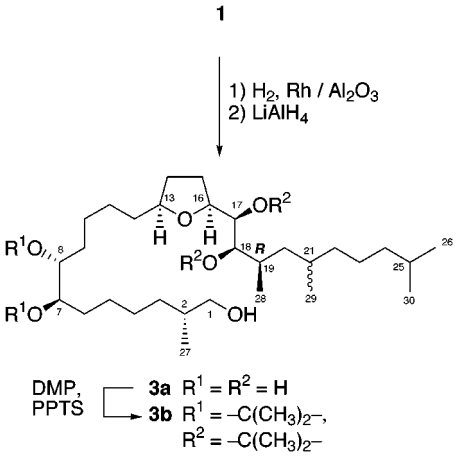


Figure 2. Rotation models for the (a) C-16−C-17, (b) C-18−C-19, and (c) C-17−C-18 bonds of amphidinolide E (**1**). NOESY correlations are illustrated by solid arrows.

Scheme 1



^3J(H-18, H-19) (9.7 Hz) and ^3J(H-18/C-28) (3.0 Hz) values and NOESY correlations, as shown in Figure 2b.

The ^3J(H-17, H-18) (~0 Hz) value and the NOESY correlation for H-17/H-18 were attributed to a gauche relation for H-17 and H-18 (Figure 2c). NOESY correlations for H-16/H-18 and H-17/H_3-28 were suggestive of both gauche relations between C-16 and H-18 and between H-17 and C-19. This threo relation for the C-17−C-18 bond was supported by analysis of NOESY correlations of the linear 7,8,17,18-di-*O*-isopropylidene derivative (**3b**) of **1**, obtained as follows (Scheme 1). Reduction of six olefins with hydrogen gas and the Rh−Al_2O_3 catalyst followed by reduction of the ester carbonyl group at C-1 with $LiAlH_4$ gave a crude pentaalcohol of dodecahydroamphidinolide E (**3a**). Hydrogenation using

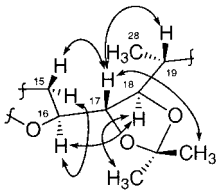


Figure 3. NOESY correlations and relative stereochemistry of C-15−C-19 in the 7,8,17,18-di-*O*-isopropylidene derivative (**3b**) of amphidinolide E (**1**). NOESY correlations are illustrated by solid arrows.

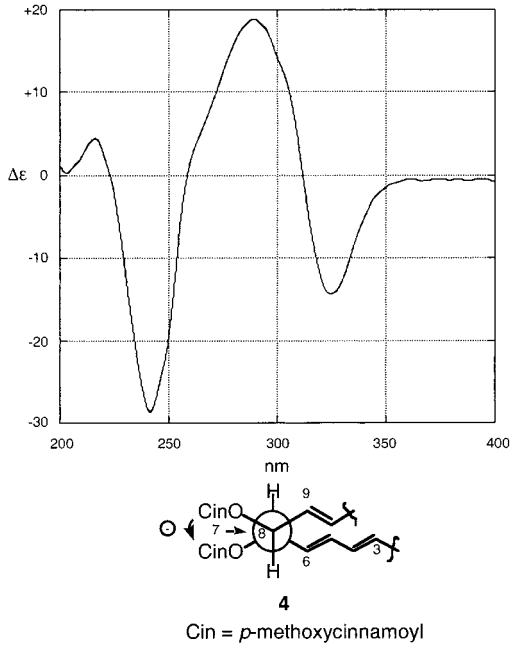


Figure 4. CD spectrum of the 7,8-bis-*O*-*p*-methoxycinnamoyl derivative (**4**) of amphidinolide E (**1**).

Rh−Al_2O_3 in MeOH caused considerable epimerization (>20%) at the C-2 carbon of the carbonyl group, while no epimerization was observed under acidic conditions with 1% acetic acid. The pentaalcohol **3a** was converted into **3b** through acetonidation. NOESY correlations from an acetonide methyl signal (δ_H 1.60, s) to H-18 (δ_H 4.27) and from the other methyl signal (δ_H 1.53, s) to H-17 (δ_H 3.90) indicated that the relative configuration of H-17/H-18 in **3b** was anti (Figure 3). Therefore, a threo relation for the C-17−C-18 bond was established.

Absolute Stereochemistry. For application of the exciton chirality method, the *O*-*p*-methoxycinnamoyl group was chosen as an exciton chromophore of the 7,8-diol in amphidinolide E (**1**) because the UV spectrum of **1** showed a strong absorption at 230 nm (ϵ 26 000). Treatment of **1** with *p*-methoxycinnamoyl chloride afforded the 7,8-bis-*O*-*p*-methoxycinnamate (**4**) and the 7,8,17-tris-*O*-*p*-methoxycinnamate in a ratio of 3:2, respectively. In the ^{1}H NMR spectrum of **4**, the vicinal coupling constant between H-7 and H-8 was 8.8 Hz, suggesting that H-7 and H-8 had an anti relationship to each other. The CD spectrum of **4** disclosed a negative first Cotton effect (λ_{ext} 324 nm, $\Delta\epsilon$ −14.3) and a positive second Cotton effect (λ_{ext} 289 nm, $\Delta\epsilon$ +18.9) (Figure 4), indicating 7*R* and 8*R* configurations.[8]

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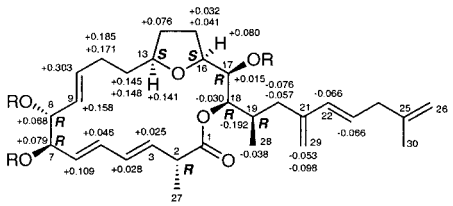
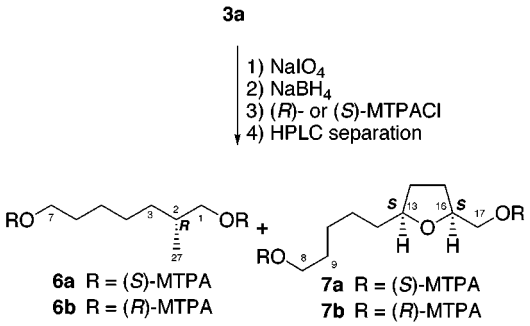


5a R = (*S*)-MTPA
5b R = (*R*)-MTPA

Figure 5. $\Delta\delta$ values ($\Delta\delta$ (in ppm) $= \delta_S - \delta_R$) obtained for the 7,8,17-tris-(*S*)- and (*R*)-MTPA esters (**5a** and **5b**, respectively) of amphidinolide E (**1**).

Scheme 2



6a R = (*S*)-MTPA
6b R = (*R*)-MTPA
7a R = (*S*)-MTPA
7b R = (*R*)-MTPA

A modified Mosher method was used to elucidate the absolute configuration at C-17. Amphidinolide E (**1**) was treated with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) to afford the 7,8,17-tris-(*S*)- and (*R*)-MTPA esters (**5a** and **5b**, respectively).[9] $\Delta\delta$ values ($\delta_S - \delta_R$) obtained from ^{1}H NMR data of **5a** and **5b** are shown in Figure 5. The $\Delta\delta$ values for H-13, H_2-14, H_2-15, and H_2-16 are positive, while negative $\Delta\delta$ values are observed for H-18, H-19, H_2-20, H-22, H-23, H_3-28, and H_2-29, thus indicating a 17*R* configuration. Therefore, the absolute configurations at C-13, C-16, C-17, C-18, and C-19 were assigned as *S*, *S*, *R*, *R*, and *R*, respectively, because the relative stereochemistry among them was elucidated as described above.

To elucidate the absolute configuration at C-2, oxidative degradation of two 1,2-diol units (C-7−C-8 and C-17−C-18) of a reductive product (**3a**) of amphidinolide E (**1**) was performed as follows. The pentaalcohol **3a** was subjected to $NaIO_4$ degradation of the two 1,2-diol units, followed by $NaBH_4$ reduction and esterification with (*R*)- and (*S*)-MTPACl (Scheme 2). HPLC separation of the reaction mixture furnished the 1,7-bis-(*S*)- and (*R*)-MTPA esters of the C-1−C-7 segment (**6a** and **6b**, respectively) and the 8,17-bis-(*S*)- and (*R*)-MTPA esters of the C-8−C-17 segment (**7a** and **7b**, respectively).[10] The absolute configuration at C-2, where a methyl group was located, was elucidated on the basis of chemical shift differences and signal patterns of the two geminal protons[10] at C-1 of **6a** and **b**. The methylene protons at C-1 of **6a** appeared as two separated doublet signals at δ_H 4.12 and 4.17 (chemical shift difference ($\Delta\delta$) 0.05) (Figure 6), while the $\Delta\delta$ value (0.16) of H_2-1 (δ_H 4.03 and 4.19) for **6b** was larger than that for **6a**, indicating that the absolute configuration at C-2 was *R*.

The absolute stereochemistry at C-13 and C-16 of the C-8−C-17 segment was established by comparison of the

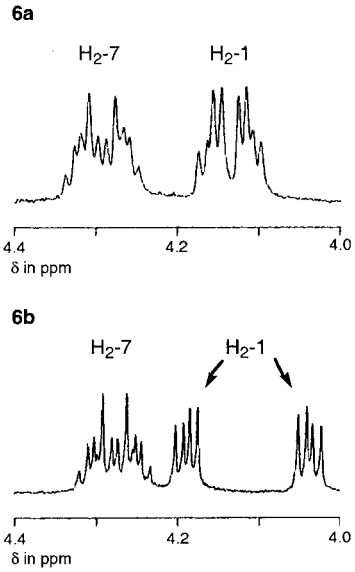
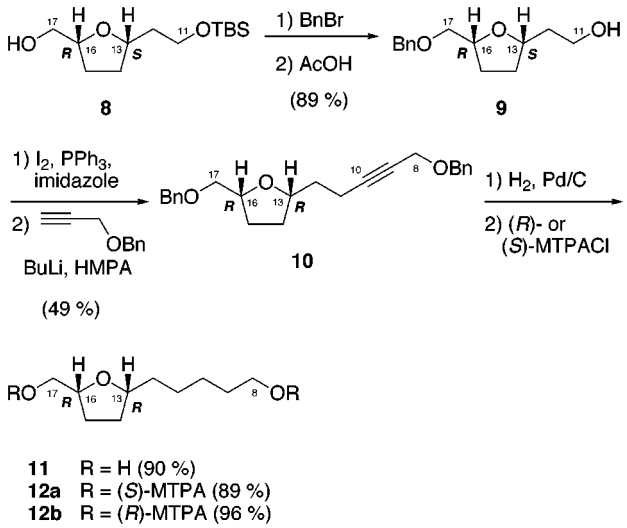


Figure 6. Proton signal patterns of H_2-1 of the 1,7-bis-(*S*)- and (*R*)-MTPA esters (**6a** and **6b**, respectively) of C-1−C-7 in amphidinolide E (**1**).

Scheme 3



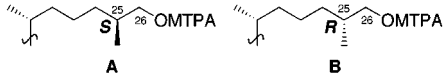
11 R = H (90 %)
12a R = (*S*)-MTPA (89 %)
12b R = (*R*)-MTPA (96 %)

NMR data of **7a** and **b** with that of the corresponding synthetic segment (**12a** and **b**). **12a** and **b** are enantiomers of **7b** and **a**, respectively. The C-8−C-17 segment was synthesized from the (13*S*,16*R*)-alcohol **8**, which was prepared from (*R*)-5-benzyloxymethyl-γ-butyrolactone. **8** was converted into the benzyl ether **9**, which was subjected to iodination followed by a coupling reaction with 3-benzyloxypropyne to afford an acetylide **10** (Scheme 3). Reduction and deprotection of **10** gave a (13*R*,16*R*)-

(9) Esterification of the 7,8-*O*-isopropylidene derivative (**2**) of amphidinolide E (**1**) with MTPACl did not afford the 17-MTPA ester of **2**.

(10) The (*S*)- and (*R*)-MTPA esters of C-18−C-26 segments were not obtained.

(11) Mosher's method has been applied for the determination of the absolute stereochemistry of a methyl group at C-25 of steroids with a primary hydroxyl group at C-26. In the ^{1}H NMR spectra of the (+)-(*R*)-MTPA esters, two methylene-26 protons of the 25-(*S*) isomer (**A**) are much closer ($\Delta\delta$ ca. 0.04) to each other than are those ($\Delta\delta$ ca. 0.14) of the 25-(*R*) isomer (**B**), whereas in the (−)-(*S*)-MTPA esters, the mutual relation is reversed. (a) De Riccardis, F.; Minale, L.; Riccio, R.; Giovannitti, B.; Iorizzi, M.; Debitus, C. *Gazz. Chim. Ital.* **1993**, *123*, 79−86. (b) Finamore, E.; Minale, L.; Riccio, R.; Rinaldo, G.; Zollo, F. *J. Org. Chem.* **1991**, *56*, 1146−1153.



A **B**

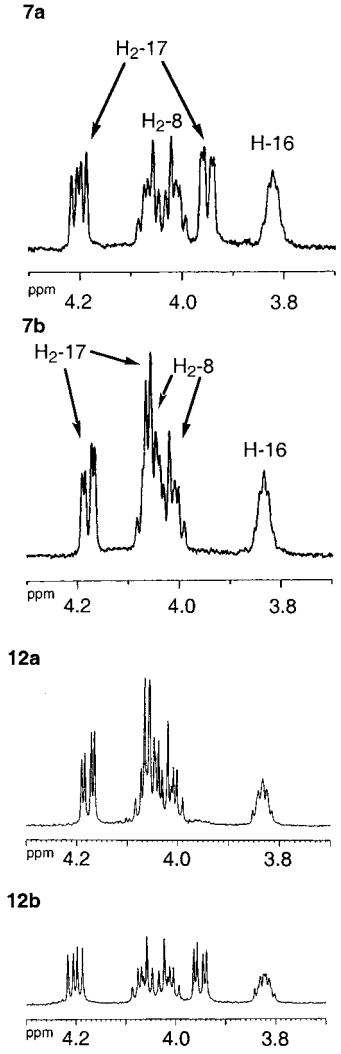


Figure 7. Proton signal patterns of H_2-17 of the 8,17-bis-(*S*)- and (*R*)-MTPA esters (**7a** and **7b**, respectively) of C-8−C-17 in amphidinolide E (**1**) and the bis-(*S*)- and (*R*)-MTPA esters (**12a** and **12b**, respectively) of the synthetic C-8−C-17 segment.

C-8−C-17 segment (**11**), which was converted into the 8,17-bis-(*S*)- and (*R*)-MTPA esters (**12a** and **12b**, respectively). Though the bis-(*S*)- and (*R*)-MTPA esters (**7a** and **7b**) had very similar NMR profiles, significant differences were observed for the signal patterns of H_2-17 (Figure 7). In the ^{1}H NMR spectrum of the bis-(*S*)-MTPA ester (**7a**), the methylene protons at C-17 resonated at δ_H 3.93 (1H, dd, J = 1.3 and 11.5 Hz, H-17) and 4.19 (1H, dd, J = 6.3 and 11.5 Hz, H-17), while in the ^{1}H NMR spectrum of **7b**, those at C-17 were observed as a 2H multiplet signal at δ_H 4.07 (m) and 4.19 (dd, J = 3.8 and 11.3 Hz). The ^{1}H NMR spectrum of the bis-(*S*)-MTPA ester (**7a**) derived from a natural specimen was identical to that of the bis-(*R*)-MTPA ester (**12b**) of the synthetic (13*R*,16*R*)-C-8−C-17 segment, while that of **7b** was identical to that of the synthetic **12a**. Thus, the absolute configurations at C-13 and C-16 were determined to be *S*.

Therefore, the absolute configurations at eight chiral centers in amphidinolide E (**1**) were found to be 2*R*, 7*R*, 8*R*, 13*S*, 16*S*, 17*R*, 18*R*, and 19*R*.

Experimental Section

General Methods. FABMS spectra were recorded in positive mode using *p*-nitrobenzyl alcohol as a matrix. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300 K using 2.5 mm micro cells for $CDCl_3$ (Shigemi Co. Ltd.). HETLOC experiments were obtained with the pulse sequence proposed by Woolborn and Leibfritz,[12] with composite pulses for broadband constant rotations (bandwidth ±0.60).[13] The duration of the trim pulse, the delay in the BIRD pulse, and the constant time for J_{CH} evaluations were 2.5, 300, and 3.57 ms, respectively. The MLEV17 spin-lock period was set to 30 ms for $^{2,3}J_{C,H}$. For 256 t_1 increments, 256 transients with 16 dummy scans were accumulated in 1K data points. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. The measuring time was ca. 48 h. Cultivation conditions and isolation procedures were described previously.[3]

7,8-*O*-Isopropylidene Derivative (2) of Amphidinolide E (1). Amphidinolide E (**1**, 0.4 mg) in acetone (30 μL) was treated with DMP (10 μL) and PPTS (0.24 mg) at room temperature for 10 min. After addition of Et_3N (0.24 μL) and evaporation of the solvent, the residue was subjected to a silica gel column (hexane/EtOAc, 3:1) to afford the 7,8-*O*-isopropylidene derivative (**2**, 0.3 mg) of **1** as a colorless oil: ^{1}H NMR (C_6D_6) δ 1.05 (3H, d, J = 6.6 Hz, H_3-28), 1.05 (1H, m, H-14), 1.21 (3H, d, J = 6.7 Hz, H_3-27), 1.26 (1H, m, H-15), 1.38 (1H, m, H-12), 1.53 (1H, m, H-14), 1.53 (3H, s, CH_3), 1.54 (3H, s, CH_3), 1.59 (1H, m, H-15), 1.69 (3H, s, H_3-30), 1.73 (2H, m, H-11 and H-12), 2.04 (1H, dd, J = 13.3 and 10.9 Hz, H-20), 2.10 (1H, m, H-11), 2.45 (1H, d, J = 2.0 Hz, 17-OH), 2.74 (2H, m, H_2-24), 2.76 (1H, m, H-19), 2.82 (1H, dd, J = 13.3 and 3.3 Hz, H-20), 3.17 (1H, dq, J = 9.0 and 6.7 Hz, H-2), 3.31 (1H, m, H-13), 3.66 (1H, dt, J = 6.4 and 8.6 Hz, H-16), 3.73 (1H, dd, J = 8.6 and 2.0 Hz, H-17), 3.98 (1H, t, J = 8.8 Hz, H-7), 4.09 (1H, t, J = 8.8 Hz, H-8), 4.89 (1H, d, J = 8.2 Hz, H-18), 4.86 (1H, brs, H-26), 4.89 (1H, brs, H-26), 4.97 (1H, brs, H-29), 5.05 (1H, brs, H-29), 5.30 (1H, ddd, J = 15.2, 9.0, and 2.0 Hz, H-9), 5.45 (1H, dd, J = 14.4 and 9.0 Hz, H-3), 5.47 (1H, dd, J = 15.0 and 8.8 Hz, H-6), 5.49 (1H, m, H-10), 5.90 (1H, dd, J = 15.0 and 10.8 Hz, H-5), 5.99 (1H, dt, J = 15.9 and 7.4 Hz, H-23), 6.00 (1H, dd, J = 14.4 and 10.8 Hz, H-4), and 6.14 (1H, d, J = 15.9 Hz, H-22); ESIMS m/z 563 $(M + Na)^+$; HRFAB−MS m/z 541.3542 [calcd for $C_{33}H_{49}O_6$ $(M + H)^+$, 541.3529].

7,8,17,18-Di-*O*-isopropylidene Derivative (3b) of Amphidinolide E (1). Amphidinolide E (**1**, 0.2 mg) in 1% AcOH/MeOH (30 μL) was treated with 5% Rh−Al_2O_3 (0.2 mg) under an H_2 atmosphere at room temperature for 1 h. After filtration and evaporation of the solvent, the residue in THF solution (30 μL) was treated with $LiAlH_4$ (0.5 mg) at room temperature for 1 h. After addition of phosphate buffer (pH 6.85, 100 μL), the mixture was extracted with EtOAc (200 μL × 3). The organic phase was evaporated in vacuo to afford a crude pentaalcohol (**3a**). To a solution of **3a** in acetone (15 μL) were added DMP (7.5 μL) and PPTS (0.12 mg), and the mixture was stirred at room temperature for 1 h. After addition of Et_3N (0.12 μL), the mixture was evaporated, and then the residue was dissolved in $CHCl_3$ (10 μL). After evaporation, the residue was subjected to silica gel column chromatography (hexane/acetone, 6:1) to afford the 7,8,17,18-di-*O*-isopropylidene derivative (**3b**, 0.12 mg) as a colorless oil: ^{1}H NMR (C_6D_6) δ 0.85−1.00 (15H, m, H_3-26, H_3-27, H_3-28, H_3-29, and H_3-30), 1.10−2.20 (32H, m), 1.49 (6H, s, CH_3 × 2), 1.57 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.22 (1H, m, H-1), 3.28 (1H, m, H-1), 3.67 (2H, m, H-7 and H-8), 3.83 (1H, m, H-13), 3.90 (1H, m, H-17), 3.95 (1H, m, H-16), and 4.27 (1H, m, H-18); ESIMS m/z 619 $(M + Na)^+$; HRFAB−MS m/z 597.5056 [calcd for $C_{38}H_{69}O_6$ $(M + H)^+$, 597.5094].

7,8-Bis-*O*-*p*-methoxycinnamoyl Ester (4) of Amphidinolide E (1). To a CH_2Cl_2 solution (15 μL) of amphidinolide E (**1**, 0.1 mg) were added DMAP (30 μg), Et_3N (1 μL), and *p*-methoxycinnanmoyl chloride (0.6 mg) in CH_2Cl_2 (15 μL) at room temperature, and stirring was continued for 3 h. After addition of phosphate buffer (pH 6.85, 50 μL), the reaction

(12) Wollborn, U.; Leibfritz, D. *J. Magn. Reson.* **1992**, *98*, 142−146.
(13) Shaka, A. J.; Pines, A. *J. Magn. Reson.* **1987**, *71*, 495−503.

mixture was extracted with $CHCl_3$ (100 μL × 3), and then the organic layer was evaporated in vacuo. The residue was subjected to C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical Ltd., 10 × 250 mm; eluent CH_3CN/H_2O, 95:5; flow rate, 2.5 mL/min; UV detection at 311 nm) to afford the 7,8-bis-*O*-*p*-methoxycinnamate (**4**, 0.03 mg, t_R 15 min) and 7,8,17-tris-*O*-*p*-methoxycinnamate (0.02 mg, t_R 19 min) of **1**. **4** (a colorless oil): UV (MeOH) λ_{max} 310 (ϵ 60 000) and 228 nm (sh); ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, J = 6.6 Hz, H_3-28), 1.21 (3H, d, J = 6.7 Hz, H_3-27), 1.20−1.30 (2H, m, H-14 and H-15), 1.42−1.52 (2H, m, H-14 and H-12), 1.62 (1H, m, H-15), 1.71 (3H, s, H_3-30), 1.70−1.78 (2H, m, H-20 and H-12), 1.80 (1H, m, H-11), 2.25−2.31 (2H, m, H-11 and H-19), 2.41 (1H, brd, J = 13.7 Hz, H-20), 2.76 (1H, dd, J = 7.2 and 15.4 Hz, H-24), 2.80 (1H, dd, J = 6.2 and 15.4 Hz, H-24), 3.27 (1H, m, H-2), 3.47 (1H, m, H-13), 3.60 (1H, dt, J = 7.5 and 7.0 Hz, H-16), 3.72 (1H, brd, J = 6.6 Hz, H-17), 3.81 (6H, s, MeO × 2), 4.68 (1H, d, J = 9.7 Hz, H-18), 4.71 (1H, brs, H-26), 4.74 (1H, brs, H-26), 4.87 (1H, brs, H-28), 4.98 (1H, brs, H-28), 5.32 (1H, dd, J = 7.9 and 15.4 Hz, H-9), 5.50−5.60 (3H, m, H-7, H-8, and H-6), 5.66−5.78 (2H, m, H-3 and H-23), 5.82 (1H, ddd, J = 5.3, 8.3, and 14.7 Hz, H-10), 6.05 (1H, d, J = 15.6 Hz, H-22), 6.22 (1H, d, J = 15.9 Hz, H-2′), 6.24 (1H, d, J = 15.9 Hz, H-2′), 6.24 (1H, dd, J = 10.8 and 15.1 Hz, H-4), 6.38 (1H, dd, J = 10.8 and 14.4 Hz, H-5), 6.85 (4H, d, J = 8.9 Hz, H_2-6′ and H_2-8′), 7.41 (2H, J = 8.9 Hz, H-5′ and H-9′), 7.42 (2H, J = 8.9 Hz, H-5′ and H-9′), 7.59 (1H, d, J = 15.9 Hz, H-3′), and 7.60 (1H, d, J = 15.9 Hz, H-3′); ESIMS m/z 843 (M + Na)$^+$; HRESIMS m/z 843.4062 [calcd for $C_{50}H_{60}O_{10}Na$ (M + Na)$^+$, 843.4084].

7,8,17-Tris-(*S*)-MTPA Ester (5a) of Amphidinolide E (1). To a CH_2Cl_2 solution (20 μL) of amphidinolide E (**1**, 0.1 mg) were added DMAP (20 μg), triethylamine (1.2 μL), and (*R*)-(−)-MTPACl (0.6 μL) at room temperature, and stirring was continued for 6 h. *N*,*N*-Dimethyl-1,3-propanediamine (0.6 μL) was added, and the reaction mixture was stirred for 10 min. After addition of phosphate buffer (pH 6.85, 50 μL), the reaction mixture was extracted with $CHCl_3$ (100 μL × 3), and then the organic layer was evaporated in vacuo. The residue was subjected to C_{18} HPLC (Develosil ODS-HG-5, 10 × 250 mm; eluent CH_3CN/H_2O, 95:5; flow rate, 2.5 mL/min; UV detection at 210 nm) to afford the 7,8,17-tris-(*S*)-MTPA ester (**5a**, 0.12 mg) of **1**. **5a (**a colorless oil): ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 6.7 Hz, H_3-28), 1.25 (3H, d, J = 6.7 Hz, H_3-27), 1.35 (1H, m, H-14), 1.45 (1H, m, H-15), 1.56 (1H, m, H-12), 1.67 (1H, m, H-15), 1.70 (3H, s, H_3-30), 1.72 (1H, m, H-12), 1.74 (1H, m, H-20), 1.79 (1H, m, H-14), 1.81 (1H, m, H-19), 2.00 (1H, m, H-11), 2.25 (1H, m, H-20), 2.28 (1H, m, H-11), 2.71 (2H, brt, J = 7.1 Hz, H_2-24), 3.24 (1H, m, H-2), 3.31 (1H, m, H-13), 3.34 (3H, s, MeO), 3.39 (3H, s, MeO), 3.56 (3H, s, MeO), 3.64 (1H, dt, J = 6.7 and 9.3 Hz, H-16), 4.67 (1H, brs, H-26), 4.72 (1H, brs, H-29), 4.74 (1H, brs, H-26), 4.78 (1H, d, J = 10.4 Hz, H-18), 4.93 (1H, brs, H-29), 5.21 (1H, dd, J = 7.4 and 15.6 Hz, H-9), 5.38 (1H, d, J = 9.3 Hz, H-17), 5.39 (1H, dd, J = 9.3 and 15.3 Hz, H-6), 5.54 (1H, dt, J = 16.0 and 7.1 Hz, H-23), 5.60 (1H, brt, J = 9.3 Hz, H-7), 5.65 (1H, m, H-8), 5.67 (1H, m, H-3), 5.75 (1H, dt, J = 15.6 and 7.1 Hz, H-10), 5.96 (1H, d, J = 16.0 Hz, H-22), 6.17 (1H, dd, J = 10.4 and 15.3 Hz, H-4), 6.40 (1H, dd, J = 10.8 and 15.3 Hz, H-5), 7.31−7.47 (14H, m, Ph), and 7.64 (1H, d, J = 7.8 Hz, Ph); ESIMS m/z 1171 (M + Na)$^+$; HRFAB MS m/z 1171.4238 [calcd for $C_{60}H_{65}O_{12}F_9Na$ (M + Na)$^+$, 1171.4230].

7,8,17-Tris-(*R*)-MTPA Ester (5b) of Amphidinolide E (1). Amphidinolide E (**1**, 0.1 mg) was treated with (*S*)-(+)-MTPACl (0.6 μL) by the same procedure as described above to afford the bis-(*R*)-MTPA ester (**5b**, 0.15 mg) of **1**. **5b** (colorless oil): ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, d, J = 6.7 Hz, H_3-28), 1.29 (3H, d, J = 6.7 Hz, H_3-27), 1.29 (1H, m, H-14), 1.41 (2H, m, H-12 and H-15), 1.58 (1H, m, H-12), 1.63 (1H, m, H-15), 1.69 (3H, s, H_3-30), 1.71 (1H, m, H-14), 1.80 (1H, dd, J = 10.4 and 13.8 Hz, H-20), 1.82 (1H, m, H-11), 2.00 (1H, m, H-19), 2.09 (1H, m, H-11), 2.33 (1H, dd, J = 3.4 and 13.4 Hz, H-20), 2.72 (2H, m, H_2-24), 3.17 (1H, m, H-13), 3.26 (1H, m, H-2), 3.43 (3H, s, MeO), 3.46 (3H, MeO), 3.46 (1H, m, H-16), 3.53 (3H, s, MeO), 4.67 (1H, brs, H-26), 4.73 (1H, brs, H-26), 4.81 (1H, d, J = 10.4 Hz, H-18), 4.82 (1H, s, H-29), 4.99 (1H, brs, H-29), 5.05 (1H, dd, J = 7.1 and 15.6 Hz, H-9), 5.28 (1H, dd, J = 9.3 and 15.3 Hz, H-6), 5.36 (1H, d, J = 9.3 Hz, H-17), 5.45 (1H, dt, J = 15.6 and 7.1 Hz, H-10), 5.52 (1H, brt, J = 9.3 Hz, H-7), 5.58 (1H, m, H-8), 5.61 (1H, m, H-23), 5.64 (1H, m, H-3), 6.03 (1H, d, J = 15.6 Hz, H-22), 6.15 (1H, dd, J = 10.8 and 15.3 Hz, H-4), 6.35 (1H, dd, J = 10.4 and 15.3 Hz, H-5), 7.31−7.47 (14H, m, Ph), and 7.61 (1H, d, J = 7.8 Hz, Ph); ESIMS m/z 1171 (M + Na)$^+$; HRFAB MS m/z 1171.4207 [calcd for $C_{60}H_{65}O_{12}F_9Na$ (M + Na)$^+$, 1171.4230].

Oxidative Degradation of Amphidinolide E (1). To a solution of **3a**, which was prepared from amphidinolide E (**1**, 0.5 mg) by the same procedure as described above, in THF/1 M phosphate buffer (1:2, 180 μL) was added $NaIO_4$ (1.5 mg), and the mixture was stirred at room temperature for 1 h. After addition of phosphate buffer (pH 6.85, 100 μL), the mixture was extracted with EtOAc (200 μL × 3), and the organic layer was evaporated in vacuo. To a solution of the residue in EtOH (75 μL) was added $NaBH_4$ (0.5 mg), and stirring was continued at 0 °C for 30 min. The mixture was partitioned between EtOAc (200 μL × 3) and phosphate buffer (100 μL). The organic phase was evaporated, and the residue was dissolved in 1% DMAP solution in CH_2Cl_2 (60 μL). To half of the mixture were added Et_3N (4 μL) and (*R*)-(−)-MTPACl (2 μL), and stirring was continued at room temperature for 14 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (2 μL), the solvent was evaporated in vacuo. The residue was purified by C_{18} HPLC (Develosil ODS-HG-5, 10 × 250 mm; eluent CH_3CN/H_2O, 95:5; flow rate, 2.5 mL/min; UV detection at 210 nm) to give **6a** (0.08 mg, t_R 22 min) and **7a** (0.05 mg, t_R 27 min) as colorless oils. **6b** (0.07 mg, t_R 23 min) and **7b** (0.04 mg, t_R 27 min) were prepared by treatment of the other half of the $NaBH_4$ reduction product with (*S*)-(+)-MTPACl, and then HPLC separation was carried out as described above. **6a** (a colorless oil): ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, d, J = 6.7 Hz, H_3-27), 1.12 (1H, m, H-3), 1.17−1.35 (5H, H-3, H_2-4, and H_2-5), 1.66 (2H, brt, J = 6.6 Hz, H_2-6), 1.80 (1H, m, H-2), 3.54 (6H, s, MeO), 4.11 (1H, dd, J = 5.7 and 10.7 Hz, H-1), 4.16 (1H, dd, J = 6.5 and 10.7 Hz, H-1), 4.27 (1H, m, H-7), 4.31(1H, m, H-7), 7.45−7.43 (6H, m, Ph), and 7.46−7.55 (4H, m, Ph); ESIMS m/z 601 (M + Na)$^+$; HRESIMS m/z 601.2003 [calcd for $C_{28}H_{32}O_6F_6Na$ (M + Na)$^+$, 601.2001]. **6b** (a colorless oil): ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.7 Hz, H_3-27), 1.10 (1H, m, H-3), 1.18−1.33 (5H, m, H-3, H_2-4, and H_2-5), 1.65 (2H, brt, J = 6.9 Hz, H_2-6), 1.79 (1H, m, H-2), 3.52 (6H, s, MeO), 4.04 (1H, dd, J = 6.5 and 10.7 Hz, H-3), 4.19 (1H, dd, J = 5.8 and 10.7 Hz, H-3), 4.25 (1H, dt, J = 10.9 and 6.6 Hz, H-7), 4.30 (1H, dt, J = 10.9 and 6.6 Hz, H-7), 7.33−7.40 (6H, m, Ph), and 7.53−7.57 (4H, m, Ph); ESIMS m/z 601 (M + Na)$^+$; HRESIMS m/z 601.1991 [calcd for $C_{28}H_{32}O_6F_6Na$ (M + Na)$^+$, 601.2001]. **7a** (a colorless oil): ^{1}H NMR (C_6D_6) δ 1.05−1.28 (6H, m), 1.30−1.52 (6H, m), 3.46 (3H, s, MeO), 3.52 (1H, m, H-13), 3.52 (3H, s, MeO), 3.81 (1H, m, H-16), 3.93 (1H, dd, J = 1.3 and 11.5 Hz, H-17), 4.01 (1H, m, H-8), 4.05 (1H, m, H-8), 4.19 (1H, dd, J = 6.3 and 11.5 Hz, H-17), 7.05−7.17 (6H, m, Ph), 7.72 (2H, d, J = 7.7 Hz, Ph), and 7.78 (2H, d, J = 7.5 Hz, Ph); ESIMS m/z 643 (M + Na)$^+$; HRESIMS m/z 643.2103 [calcd for $C_{30}H_{34}O_7F_6Na$ (M + Na)$^+$, 643.2107]. **7b** (a colorless oil): ^{1}H NMR (C_6D_6) δ 1.05−1.14 (4H, m), 1.18−1.25 (2H, m), 1.38−1.51 (6H, m), 3.46 (3H, s, MeO), 3.51 (3H, s, MeO), 3.53 (1H, m, H-13), 3.83 (1H, m, H-16), 4.01 (1H, m, H-8), 4.03−4.08 (2H, m, H-8 and H-17), 4.17 (1H, dd, J = 3.8 and 11.3 Hz, H-17), 7.05−7.18 (6H, m, Ph), 7.73 (2H, d, J = 7.6 Hz, Ph), and 7.77 (2H, d, J = 7.8 Hz, Ph); ESIMS m/z 643 (M + Na)$^+$; HRESIMS m/z 643.2120 [calcd for $C_{30}H_{34}O_7F_6Na$ (M + Na)$^+$, 643.2107].

2-[(2′*S*,5′*R*)-5′-Benzyloxymethyltetrahydrofuran-2′-yl]-ethan-1-ol (9). To a suspension of NaH (283 mg, 7.08 mmol, 60% oil dispersion) in DMF (1.6 mL) and THF (2.7 mL) was added a solution of (2*R*,5*S*)-{5-[2′-(*tert*-butyldimethylsilyloxy)-ethyl]tetrahydrofuran-2-yl}methanol (**8**, 1.00 g, 3.84 mmol) in THF (0.7 mL) at 0 °C. After stirring at 0 °C for 30 min and then at room temperature for 2 h, benzyl bromide (0.6 mL, 5.1 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 18 h. After addition of MeOH (0.4 mL) and then H_2O (11 mL), the mixture was extracted with ether (20 mL × 3). The organic phase was dried with $MgSO_4$ and

concentrated to give a crude benzyl ether (1.30 g). To the crude product was added the mixture (60 mL) AcOH/H_2O/THF (1:1:1), and the solution was stirred at room temperature for 6 h. After extraction with $CHCl_3$, the organic phase was dried with $MgSO_4$, evaporated, and purified by silica gel column chromatography (hexane/EtOAc, 9:1) to yield benzyl alcohol **9** (809 mg, 3.43 mmol, 89% by two steps) as a colorless oil: $[\alpha]_D^{22}$ +10.1° (*c* 1.00, $CHCl_3$); IR (neat) $\tilde{\nu}_{max}$ 3439, 3062, 3030, 2939, 2869, 1494, 1453, 1365, and 1078 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55−2.05 (6H, m), 3.48 (2H, m), 3.78 (2H, t, J = 5.6 Hz), 4.05−4.15 (2H, m), 4.55 (1H, d, J = 12.5 Hz), 4.57 (1H, d, J = 12.5 Hz), and 7.25−7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ 27.8 (t), 31.0 (t), 37.5 (t), 61.2 (t), 72.8 (t), 73.3 (t), 78.4 (d), 79.8 (d), 127.6 (d), 127.7 (2C, d), 128.3 (2C, d), and 138.2 (s); FAB MS *m*/*z* 237 $(M + H)^+$; HRFAB−MS *m*/*z* 237.1473 [calcd for $C_{14}H_{21}O_3$ $(M + H)^+$, 237.1491].

(2*R*,5*R*)-2-Benzyloxymethyl-5-(5′-benzyloxypent-3′-ynyl)-tetrahydrofuran (10). To a solution of **9** (200 mg, 847 μmol) in toluene (8 mL) were added imidazole (143 mg, 2.10 mmol), triphenylphosphine (552 mg, 2.10 mmol), and iodine (407 mg, 1.60 mmol), and stirring was continued at room temperature for 30 min. The mixture was washed with saturated aqueous Na_2SO_3 and brine, dried with $MgSO_4$, evaporated, and purified by silica gel column chromatography (hexane/EtOAc, 10:1) to yield an iodide (220 mg, 636 μmol, 75.1%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.52 (1H, m), 1.70 (1H, m), 1.88−2.12 (4H, m), 3.25 (2H, m), 3.47 (2H, m), 3.94 (1H, m), 4.10 (1H, m), 4.56 (1H, d, J = 12.5 Hz), 4.59 (1H, 12.5 Hz), and 7.25−7.39 (5H, m); ^{13}C NMR ($CDCl_3$) δ 24.5 (t), 27.8 (t), 30.3 (t), 39.9 (t), 72.7 (t), 73.1 (t), 78.1 (d), 79.3 (d), 127.3 (d), 127.4 (2C, d), 128.1 (2C, d), and 138.2 (s). To a solution of propyn-2-ynyloxymethylbenzene (187 mg, 1.28 mmol) in THF (3.2 mL) was added a 2.46 M solution of *n*-BuLi in hexane (546 μL, 134 mmol) at −78 °C, and the mixture was then allowed to warm to −20 °C over 2.5 h. To this mixture was added a solution of the iodide (220 mg, 636 μmol) in HMPA (1.12 mL) and THF (1 mL) at −78 °C. The mixture was allowed to warm to room temperature, and stirring was continued for 16 h. The reaction was quenched with the addition of saturated aqueous NH_4Cl (5 mL), and the mixture was extracted with Et_2O (10 mL × 3). The extract was washed with H_2O and brine, dried with $MgSO_4$, evaporated, and purified by silica gel column chromatography (hexane/EtOAc, 10:1 to 8:2) to yield the acetylene compound **10** (150.4 mg, 413 μmol, 65.0%) as a colorless oil: $[\alpha]_D^{22}$ +7.5° (*c* 1.00, $CHCl_3$); IR (neat) $\tilde{\nu}_{max}$ 3062, 3029, 2940, 2857, 2222, 1495, 1452, 1356, and 1073 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (1H, m), 1.73 (2H, m), 1.83 (1H, m), 1.98 (2H, m), 2.39 (2H, m), 3.49 (2H, m), 4.00 (1H, m), 4.11 (1H, m), 4.17 (2H, m), 4.53−4.65 (4H, m), and 7.26−7.43 (10H, m); ^{13}C NMR ($CDCl_3$) δ 15.6 (t), 28.0 (t), 30.5 (t), 34.7 (t), 57.5 (t), 71.1 (t), 72.9 (t), 73.1 (t), 75.8 (s), 77.9 (d), 78.4 (d), 86.6 (s), 127.3 (d), 127.4 (2C, d), 127.5 (d), 127.8 (2C, d), 128.1 (2C, d), 128.2 (2C, d), 137.5 (s), and 138.2 (s); FAB−MS *m*/*z* 365 $(M + H)^+$; HRFAB MS *m*/*z* 365.2097 [calcd for $C_{24}H_{29}O_3$ $(M + H)^+$, 365.2117].

5-[(2′*R*,5′*R*)-5′-Hydroxymethyltetrahydrofuran-2′-yl]-pentan-1-ol (11). To a solution of **10** (100 mg, 275 μmol) in EtOH (2.5 mL) was added 5% Pd/C (100 mg), and stirring was continued under an H_2 atmosphere at room temperature for 16 h. After filtration of insoluble materials followed by evaporation, the residue was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 100:0 to 80:20) to afford **11** (46.5 mg, 247 μmol, 89.8%) as a colorless oil; $[\alpha]_D^{20}$ −10.8° (*c* 1.00, $CHCl_3$); IR (neat) $\tilde{\nu}_{max}$ 3369, 2933, 2859, 1461, and 1044 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26−1.71 (10H, m), 1.81−1.98 (2H, m), 3.45 (1H, dd, J = 5.9 and 11.5 Hz), 3.58 (2H, t, J = 6.5 Hz), 3.63 (1H, dd, J = 3.1 and 11.5 Hz), 3.83 (1H, m), and 3.95 (1H, m); ^{13}C NMR ($CDCl_3$) δ 25.7 (t), 25.9 (t), 27.0 (t), 31.2 (t), 32.5 (t), 35.7 (t), 62.5 (t), 65.2 (t), 79.4 (d), and 80.0 (d); FAB−MS *m*/*z* 189 $(M + H)^+$; HRFAB MS *m*/*z* 189.1487 [calcd for $C_{10}H_{21}O_3$ $(M + H)^+$, 189.1491].

Bis-(*S*)-MTPA Ester (12a) of 11. To a CH_2Cl_2 solution (30 μL) of **11** (0.5 mg, 2.7 nmol) were added DMAP (30 μg), Et_3N (2 μL), and (*R*)-(−)-MTPACl (1 μL) at room temperature, and stirring was continued for 6 h. *N*,*N*-Dimethyl-1,3-propanediamine (1 μL) was added, and the reaction mixture was stirred for 10 min. After addition of phosphate buffer (pH 6.85, 50 μL), the reaction mixture was extracted with $CHCl_3$ (100 μL × 3), and then the organic layer was evaporated in vacuo. The residue was purified by C_{18} HPLC (Develosil ODS-HG-5, 10 × 250 mm; eluent CH_3CN/H_2O, 95:5; flow rate, 2.5 mL/min; UV detection at 210 nm) to give **12a** (1.5 mg, 2.4 nmol, 89%, t_R 27 min) as a colorless oil; ^1H NMR (C_6D_6) δ 1.05−1.14 (4H, m), 1.18−1.25 (2H, m), 1.38−1.41 (4H, m), 1.42−1.51 (2H, m), 3.46 (3H, s, MeO), 3.51 (3H, s, MeO), 3.53 (1H, m, H-13), 3.83 (1H, m, H-18), 4.01 (1H, m, H-8), 4.03−4.08 (2H, m, H-8 and H-17), 4.17 (1H, dd, J = 3.8 and 11.3 Hz, H-17), 7.05−7.18 (6H, m, Ph), 7.73 (2H, d, J = 7.6 Hz, Ph), and 7.77 (2H, d, J = 7.8 Hz, Ph); FAB−MS *m*/*z* 621 $(M + H)^+$; HRFAB MS *m*/*z* 621.2274 [calcd for $C_{30}H_{35}O_7F_6$ $(M + H)^+$, 621.2287].

Bis-(*S*)-MTPA Ester (12b) of 11. 11 (0.5 mg, 2.7 nmol) was treated with (*S*)-(+)-MTPACl (1 μL) by the same procedure as described above to afford the bis-(*R*)-MTPA ester (**12b**, 1.6 mg, 2.6 nmol, 96%) of **11**. **12b** (a colorless oil): ^1H NMR (C_6D_6) δ 1.05−1.28 (6H, m), 1.30−1.40 (4H, m), 1.42−1.52 (2H, m), 3.46 (3H, s, MeO), 3.52 (1H, m, H-13), 3.52 (3H, s, MeO), 3.81 (1H, m, H-16), 3.93 (1H, dd, J = 1.3 and 11.5 Hz, H-17), 4.01 (1H, m, H-8), 4.05 (1H, m, H-8), 4.19 (1H, dd, J = 6.3 and 11.5 Hz, H-17), 7.05−7.17 (6H, m, Ph), 7.72 (2H, d, J = 7.7 Hz, Ph), and 7.78 (2H, d, J = 7.5 Hz, Ph); FAB−MS *m*/*z* 643 $(M + H)^+$; HRFAB MS *m*/*z* 621.2275 [calcd for $C_{30}H_{35}O_7F_6$ $(M + H)^+$, 621.2287].

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Supporting Information Available: ^1H NMR spectra of **1**, **2**, **4**, **5a** and **b**, **6a** and **b**, **7a** and **b**, and **12a** and **b**. This material is available via the Internet at http://pubs.acs.org.

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