Amphidinolide T, Novel 19-Membered Macrolide from Marine Dinoflagellate *Amphidinium* sp.

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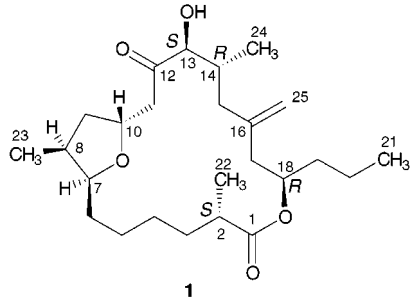
A novel 19-membered macrolide, amphidinolide T (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp., and the structure was elucidated on the basis of spectroscopic data. Relative stereochemistry at C-7, C-8, and C-10 was deduced from the NOESY correlations, while absolute configurations at C-2, C-13, C-14, and C-18 were assigned on the basis of NMR data of the MTPA esters of **1** and those of degradation products of **1**.

Introduction

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel secondary metabolites with unique structures.[1−5] In our continuing search for bioactive metabolites from Okinawan marine organisms,[6−8] we have investigated extracts of laboratory-cultured marine dinoflagellates *Amphidinium* sp., which were symbionts of the Okinawan marine acoel flatworms *Amphiscolops* sp., and isolated a series of cytotoxic macrolides, amphidinolides, with various backbone skeletons.[9] Here we describe the isolation and structure elucidation of a novel 19-membered macrolide, amphidinolide T (**1**), which was isolated from extracts of a strain (Y-56) of the dinoflagellate *Amphidinium* sp.

Results and Discussion

The dinoflagellate *Amphidinium* sp. was separated from a flatworm *Amphiscolops* sp., which was collected off Zanpa, Okinawa, and mass cultured unialgally at 25 °C for 2 weeks in a seawater medium enriched with 1% ES supplement.[10] The harvested algal cells (420 g, wet weight, from 580 L of culture) were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH) and a Sep-Pak C_{18} cartridge (MeOH/H_2O) followed by C_{18} HPLC (CH_3CN/H_2O) to afford amphidinolide T (**1**, 0.005%, wet weight).



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Stereochemistry at C-7, C-8, and C-10 is relative

Amphidinolide T (**1**) had the molecular formula of $C_{25}H_{42}O_5$ as revealed by HRFABMS [*m*/*z* 423.3107 (M + H)$^+$, Δ −0.4 mmu]. IR absorptions at 3490 and 1720 cm^{-1} indicated the presence of hydroxyl(s) and carbonyl group(s), respectively. ^{1}H and ^{13}C NMR data (Table 1) disclosed the presence of a ketone, an ester carbonyl, an *exo*-methylene, seven sp^3 methines, four of which were oxygen-bearing, 10 methylenes, and four methyl groups. Since three out of five unsaturations were accounted for, amphidinolide T (**1**) was inferred to contain two rings. Interpretation of the ^{1}H−^{1}H COSY and TOCSY spectra revealed proton connectivities of the following units: (**a**) from H_3-22 to H_2-11 and H_3-23, (**b**) from 13-OH to H_2-15 and H_3-24, and (**c**) from H_2-17 to H_3-21 (Figure 1). Connections among units **a**−**c** and remaining three carbons (C-1, C-12, and C-16) were assigned on the basis of ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum as follows. HMBC correlations from H_2-11 (δ_H 2.50 and 1.95) and H-13 (δ_H 4.34) to the ketone carbonyl at δ_C 212.0 (C-12) suggested the connectivity between units **a** and **b** through C-12. The connection between units **b** and **c** through an *exo*-methylene at C-16 was deduced from HMBC correlations from one proton (δ_H 5.17) of H_2-25 to C-16 (δ_C 143.5) and C-17 (δ_C 40.1) and

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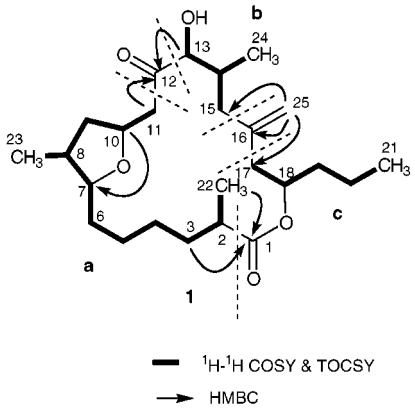

Figure 1. Selected 2D NMR correlations for amphidinolide T (**1**).

Table 1. ^{1}H and ^{13}C NMR Data of Amphidinolide T (1) in C_6D_6.

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position (H)	δ_C	δ_H (m, Hz)	HMBC (H)[c]
1	174.9 s		4b, 22
2	41.7 d	2.49 m	3a, 22
3	35.1 t	1.62 m 1.29 m	2, 5, 22
4	26.8 t	1.55 m 1.18 m	3a, 6
5	26.2 t	1.53 m 1.34 m	4a, 6b
6	29.7 t	1.47 m 1.10 m	4, 5
7	78.6 d	3.69 ddd, 2.6, 4.1, 10.7	6, 9, 10, 23
8	36.5 d	1.73 m	23
9	39.7 t	1.36[a] m	11, 23
10	73.6 d	4.53 m	11
11	45.0 t	2.50 dd, 9.2, 14.2	9
		1.95 dd, 2.7, 14.2	
12	212.0 s		10, 11, 13
13	78.2 d	4.34 d, 5.5	14. 15. 24
13-OH		3.64 d, 5.5	
14	32.0 d	2.47 m	13, 15, 24
15	41.2 t	2.57 dd, 10.3, 13.5	14, 24, 25b
16	143.5 s	2.34 m	15, 17, 25
17	40.1 t	2.33[a] m	25a
18	71.7 d	5.38 m	17, 19
19	35.7 t	1.58 m 1.44 m	20, 21
20	19.1 t	1.35 m 1.32 m	19, 21
21	13.9 q	0.91[b] t, 7.3	20
22	18.0 q	1.12[b] d, 6.9	
23	14.0 q	0.67[b] d, 7.1	7
24	13.7 q	0.97[b] d, 6.6	13, 15a
25	116.1 t	5.17 s 4.96 s	15, 17

[a] 2H. [b] 3H. [c] a and b denote low-field and high-field resonances, respectively, of a geminal pair.

from another proton (δ_H 4.96) of H_2-25 to C-15 (δ_C 41.2) and C-16. The HMBC correlation from H-10 (δ_H 4.53) to C-7 (δ_C 78.6) revealed that an ether linkage was present between C-7 and C-10, thereby constructing a tetrahydrofuran ring. The presence of an ester carbonyl at C-1 was implied by C−H long-range correlations from H_3-22 (δ_H 1.12) and H-3 (δ_H 1.29) to C-1 (δ_C 174.9). The relatively lower-field resonance of H-18 (δ_H 5.38) indicated that C-18 was involved in an ester linkage with C-1. Thus the gross structure of amphidinolide T was elucidated to be **1**.

Relative stereochemistry of three methine protons (H-7, H-8, and H-10) in the tetrahydrofuran ring was suggested to be 7,8-*syn* and 7,10-*anti*, since NOESY correlations were observed for H_2-6/H_3-23, H-7/H-11a, and H-10/H_3-23 (Figure 2). Absolute configuration at C-13 was assigned as *S* on the basis of modified Mosher's

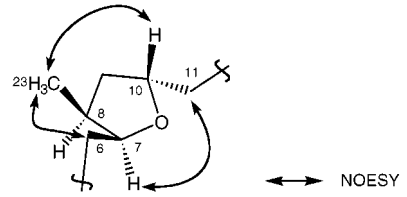


Figure 2. Relative stereochemistry of tetrahydrofuran ring in amphidinolide T (**1**)

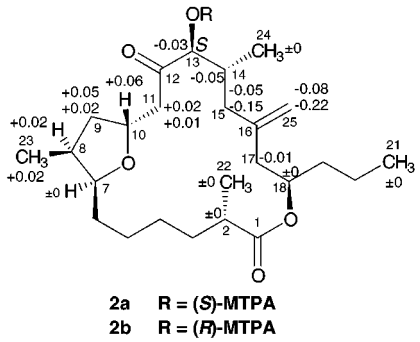
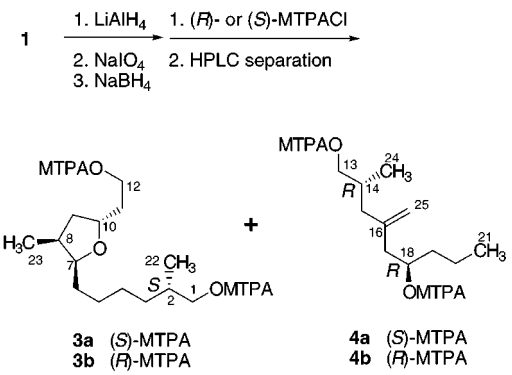


Figure 3. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of amphidinolide T (**1**).

Scheme 1



method[11] using the (*S*)- or (*R*)-MTPA (2-methoxy-2-trifluoromethyl-2-phenylacetic acid) esters (**2a** and **2b**, respectively) of **1** (Figure 3). Absolute configurations at C-2, C-14, and C-18 were investigated by NMR data of degradation products of **1** as follows. Reduction of **1** with $LiAlH_4$, oxidation with $NaIO_4$, reduction with $NaBH_4$, esterification with (*R*)- or (*S*)-MTPACl, and then HPLC separation furnished the bis-(*S*)- or (*R*)-MTPA esters of C-1−C-12 (**3a** and **3b**, respectively) and C-13−C-21 segments (**4a** and **4b**, respectively) (Scheme 1). Absolute stereochemistry of C-18 was assigned as *R* by modified Mosher's method based on ^{1}H NMR data of **4a** and **4b** (Figure 4). Absolute configurations at C-2 and C-14 with a methyl group were elucidated on the basis of chemical shift differences and signal patterns of two geminal protons[12] at C-1 of **3a** and **3b** and C-13 of **4a** and **4b**, respectively. The methylene protons of C-1 for **3a** appeared as separated double doublet signals at δ_H 4.06 and 4.23 (Figure 5), while those for **3b** were observed as overlapped 2H signal at δ_H 4.15, indicating that absolute configuration at C-2 was *S*. On the other hand, the chemical shift differences (Figure 6) of H_2-13 for **4a** ($\Delta\delta$

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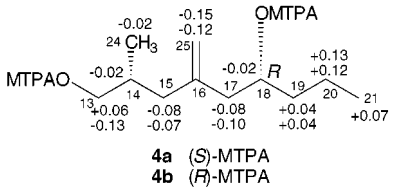

Figure 4. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**4a** and **4b**, respectively) of C-13−C-21 segment.

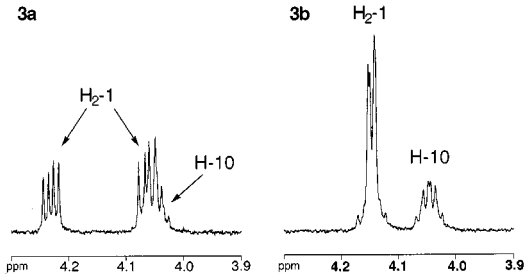

Figure 5. Proton signal patterns of H_2-1 of bis-(*S*)- and bis-(*R*)-MTPA esters (**3a** and **3b**, respectively) of C-1−C-12 segment.

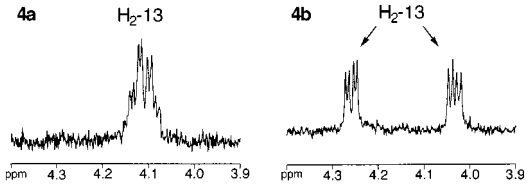

Figure 6. Proton signal patterns of H_2-13 of bis-(*S*)- and bis-(*R*)-MTPA esters (**4a** and **4b**, respectively) of C-13−C-21 segment.

0.03; δ_H 4.09 and 4.12) and **4b** ($\Delta\delta$ 0.23; δ_H 4.03 and 4.26) suggested the 14*R*-configuration. Thus the stereostructure of amphidinolide T was concluded to be **1**.

Amphidinolide T (**1**) is a novel 19-membered macrolide possessing a tetrahydrofuran ring, one *exo*-methylene, three branched methyls, one ketone, and one hydroxyl group. It is known that more than a half of amphidinolides isolated so far have unique structural features such as an odd-numbered macrocyclic lactone ring and/or vicinally located one-carbon branches. Amphidinolide T (**1**) possesses an odd-numbered macrocyclic lactone ring but has no vicinally located one-carbon branches. The C_{21} backbone with four C_1 branches (C-2, C-8, C-14, and C-16) of **1** are different from the carbon frameworks of two other 19-membered macrolides, amphidinolides E[13] and K,[14] which have a C_{26} backbone with four C_1 branches (C-2, C-19, C-21, and C-25) and a C_{22} backbone with five C_1 branches (C-2, C-4, C-6, C-7, and C-14), respectively. Biosynthetic studies of amphidinolides J,[15] G, and H[16] suggest that amphidinolide T (**1**) might be also generated from mixed polyketide chains consisting of successive polyketide chains and unusual carbon units derived from C-2 of acetates. Amphidinolide T (**1**) exhibited cytotoxicity against murine leukemia L1210 cells in vitro with IC_{50} value of 18 μg/mL.

Experimental Section

General Methods. ^{1}H and 2D NMR spectra were recorded on a 600 MHz spectrometer, and ^{13}C NMR spectra were measured on a 500 MHz spectrometer. NMR spectra of all MTPA esters were measured using 5 mm symmetrical thin-wall micro sample tubes for $CDCl_3$ (Shigemi Co. Ltd.). FABMS spectra were recorded using *p*-nitrobenzyl alcohol as a matrix in positive mode. Cytotoxic activity was examined by the same method as described previously.[17]

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain number Y-56) was isolated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for 2 weeks in a seawater medium enriched with 1% ES supplement. The harvested cells of the cultured dinoflagellate (420 g, wet weight, from 580 L of culture) were extracted with MeOH/toluene (3:1, 3 L × 3). After the addition of 1 M aqueous NaCl (1 L), the mixture was extracted with toluene (4 L × 3). The toluene-soluble fraction was evaporated under reduced pressure to give a residue (3.67 g), which was partially (2.67 g) subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) and a Sep-Pak C_{18} cartridge (MeOH/H_2O, 8:2) followed by C_{18} HPLC [LUNA C18(2), 5 μm, Phenomenex, 10 mm × 250 mm; eluent, CH_3CN/H_2O, 85:15; flow rate, 2.5 mL/min; UV detection at 210 nm] to afford amphidinolide T (**1**, 0.005%, wet weight, t_R 24.4 min).

Amphidinolide T (1): colorless oil; $[\alpha]^{24}_D$ +18° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3490, 2935, and 1720 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m/z* 423 $(M + H)^+$ and 445 $(M + Na)^+$; HRFABMS *m/z* 423.3107 [calcd for $C_{25}H_{43}O_5$ $(M + H)^+$, 423.3111].

(*S*)-MTPA Ester (2a) of Amphidinolide T (1). To a CH_2Cl_2 solution (100 μL) of amphidinolide T (**1**, 1.0 mg) was added 4-(dimethylamino)pyridine (0.1 mg), triethylamine (20 μL), and (*R*)-(−)-MTPACl (2.5 μL) at room temperature, and stirring was continued for 2 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (10 μL) and evaporation of solvent, the residue was passed through a silica gel column (hexane/EtOAc, 4:1) to afford the (*S*)-MTPA ester (**2a**, 0.6 mg) of **1**. **2a**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 7.0 Hz, H_3-23), 0.89 (3H, t, J = 7.3 Hz, H_3-21), 0.92 (3H, d, J = 6.6 Hz, H_3-24), 1.15 (3H, d, J = 7.0 Hz, H_3-22), 1.15−1.55 (12 H), 1.79 (1H, m, H-9), 1.82 (1H, m, H-9), 2.02 (1H, dd, 10.3 and 13.5 Hz, H-15), 2.15 (1H, m, H-8), 2.28 (1H, dd, J = 3.5 and 13.5 Hz, H-15), 2.33 (2H, m, H_2-17), 2.41 (1H, brd, J = 14.2 Hz, H-11), 2.45 (1H, m, H-2), 2.46 (1H, m, H-14), 2.61 (1H, dd, J = 9.2 and 14.2 Hz, H-11), 3.63 (3H, s, OCH_3), 3.91 (1H, m, H-7), 4.64 (1H, H-10), 4.64 (1H, s, H-25), 4.84 (1H, s, H-25), 5.07 (1H, brs, H-13), 5.18 (1H, m, H-18), 7.43 (3H, m), and 7.77 (2H, m); FABMS *m/z* 639 $(M + H)^+$; HRFABMS *m/z* 639.3512 [calcd for $C_{35}H_{49}O_7F_3$ $(M + H)^+$, 639.3508].

(*R*)-MTPA Ester (2b) of Amphidinolide T (1). Amphidinolide T (**1**, 1.0 mg) was treated with (*S*)-(+)-MTPACl (2.5 μL) by the same procedure as described above to afford the (*R*)-MTPA ester (**2b**, 0.9 mg) of **1**. **2b**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, d, J = 7.0 Hz, H_3-23), 0.89 (3H, t, J = 7.3 Hz, H_3-21), 0.92 (3H, d, J = 6.6 Hz, H_3-24), 1.15 (3H, d, J =

(12) Mosher's method has been applied to determine the absolute stereochemistry of a methyl group at C-25 of steroids with a primary hydroxy group at C-26. In the ^{1}H NMR spectra of the (+)-(*R*)-MTPA esters, two 26-methylene protons of the 25*S* isomer are much closer ($\Delta\delta$ ca. 0.04) to each other than those ($\Delta\delta$ ca. 0.14) of the 25*R* isomer, whereas in the (−)-(*S*)-MTPA esters, the mutual relation is the reverse. (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.

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7.0 Hz, H_3-22), 1.15 ~ 1.55 (12 H), 1.77 (2H, m, H_2-9), 2.17 (1H, dd, 10.3 and 13.5 Hz, H-15), 2.13 (1H, m, H-8), 2.33 (1H, m, H-15), 2.34 (2H, m, H_2-17), 2.40 (1H, brd, J = 14.2 Hz, H-11), 2.45 (1H, m, H-2), 2.51 (1H, m, H-14), 2.59 (1H, dd, J = 9.2 and 14.2 Hz, H-11), 3.56 (3H, s, OCH_3), 3.91 (1H, m, H-7), 4.58 (1H, H-10), 4.86 (1H, s, H-25), 4.92 (1H, s, H-25), 5.12 (1H, brs, H-13), 5.18 (1H, m, H-18), 7.42 (3H, m), and 7.61 (2H, m); FABMS m/z 639 $(M + H)^+$; HRFABMS m/z 639.3502 [calcd for $C_{35}H_{49}O_7F_3$ $(M + H)^+$, 639.3508].

Oxidative Degradation of Amphidinolide T (1). Amphidinolide T (**1**, 0.75 mg) was dissolved in THF (50 μL) and treated with $LiAlH_4$ (1.5 mg) at room temperature for 1 h. The reaction mixture was partitioned between EtOAc (200 μL × 3) and 1 M phosphate buffer (100 μL). The organic phase was evaporated in vacuo to afford a crude residue. To a solution of the residue in THF/1 M phosphate buffer (1:1, 50 μL) was added $NaIO_4$ (1.0 mg), and the mixture was stirred at room temperature for 30 min. After evaporation, the reaction mixture was extracted with EtOAc (200 μL), and the extract was evaporated in vacuo. To a solution of the residue in EtOH (50 μL) was added $NaBH_4$ (0.43 mg) at 0 °C, and stirring was continued for 30 min. The mixture was evaporated and then partitioned between EtOAc (200 μL × 3) and 1 M phosphate buffer (100 μL). The organic phase was evaporated, and the residue was dissolved in 0.1% DMAP solution in CH_2Cl_2 (50 μL). To the mixture were added Et_3N (2 μL) and (*R*)-(−)-MTPACl (1 μL), and stirring was continued at room temperature for 2 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (3 μL), the solvent was evaporated in vacuo. The residue was subjected to silica gel column chromatography (hexane/EtOAc, 8:1) and then C_{18} HPLC (Wakosil-II 5C18 RS, Wako Pure Chemical Ind., Ltd., 4.6 mm × 250 mm; eluent CH_3CN/H_2O, 90:10; flow rate, 1.0 mL/min; UV detection at 230 nm) to afford compounds **3a** (0.2 mg, t_R 16.0 min) and **4a** (0.15 mg, t_R 13.6 min). **3a**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz), 0.91 (3H, d, J = 6.7 Hz), 1.1−1.45 (8H, m), 1.69 (2H, m), 1.78−1.93 (3H, m), 2.19 (1H, m), 3.54 (6H, s), 3.79 (1H, m), 4.05 (1H, m), 4.06 (1H, dd, J = 6.7 and 10.7 Hz), 4.23 (1H, dd, J = 5.6 and 10.7 Hz), 4.41 (2H, t, J = 6.9 Hz), 7.35−4.43 (6H, m), and 7.48−7.54 (4H, m); FABMS m/z 699 $(M + Na)^+$; HRFABMS m/z 699.2720 [calcd for $C_{34}H_{42}O_6F_6Na$ $(M + Na)^+$, 699.2732]. **4a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.3 Hz), 0.90 (3H, t, J = 7.5 Hz), 1.30 (1H, m), 1.37 (1H, m), 1.56 (1H, m), 1.62 (1H, m), 1.84 (1H, m), 2.00 (1H, m), 2.02 (1H, m), 2.17 (1H, dd, J = 6.0 and 14.2 Hz), 2.28 (1H, dd, J = 7.6 and 14.2 Hz), 3.52 (3H, s), 3.54 (3H, s), 4.09 (1H, dd, J = 5.8 and 10.2 Hz), 4.12 (1H, dd, J = 4.3 and 10.2 Hz), 4.68 (1H, s), 4.75 (1H, s), 5.20 (1H, m), 7.35−4.43 (6H, m), and 7.48−7.54 (4H, m); FABMS m/z 641 $(M + Na)^+$; HRFABMS m/z 641.2308 [calcd for $C_{31}H_{36}O_6F_6Na$ $(M + Na)^+$, 641.2314].

Compounds **3b** (0.18 mg, t_R 16.4 min) and **4b** (0.12 mg, t_R 13.0 min) were obtained from amphidinolide T (**1**, 0.7 mg) by the same procedure using (*S*)-(+)-MTPACl as described above. **3b**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz), 0.91 (3H, d, J = 6.8 Hz), 1.1−1.45 (8H, m), 1.69 (2H, m), 1.76−1.91 (3H, m), 2.19 (1H, m), 3.52 (6H, s), 3.79 (1H, m), 4.05 (1H, m), 4.15 (2H, m), 4.39 (1H, m), 4.44 (1H, m), 7.35−4.43 (6H, m), and 7.48−7.54 (4H, m); FABMS m/z 699 $(M + Na)^+$; HRFABMS m/z 699.2728 [calcd for $C_{34}H_{42}O_6F_6Na$ $(M + Na)^+$, 699.2732]. **4b**: ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, t, J = 7.3 Hz), 0.90 (3H, d, J = 6.3 Hz), 1.17 (1H, m), 1.25 (1H, m), 1.52 (1H, m), 1.56 (1H, m), 1.92 (1H, m), 2.08 (1H, m), 2.09 (1H, m), 2.25 (1H, dd, J = 5.5 and 14.3 Hz), 2.38 (1H, dd, J = 7.6 and 14.3 Hz), 3.52 (3H, s), 3.54 (3H, s), 4.03 (1H, dd, J = 6.0 and 10.6 Hz), 4.26 (1H, dd, J = 4.8 and 10.6 Hz), 4.83 (1H, s), 4.87 (1H, s), 5.22 (1H, m), 7.35−4.43 (6H, m), and 7.48−7.54 (4H, m); FABMS m/z 641 $(M + Na)^+$; HRFABMS m/z 641.2308 [calcd for $C_{31}H_{36}O_6F_6Na$ $(M + Na)^+$, 641.2314].

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

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