Amphidinolide W, a New 12-Membered Macrolide from Dinoflagellate *Amphidinium* sp.

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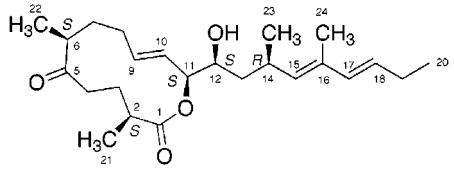
Abstract: A new cytotoxic 12-membered macrolide, amphidinolide W (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp., and the structure was elucidated by spectroscopic data including $^{13}C-^{13}C$ INADEQUATE correlations for its ^{13}C-enriched sample. The absolute stereochemistry of **1** was assigned by combination of *J*-based configuration analysis and modified Mosher method. Amphidinolide W (**1**) is the first macrolide without an exomethylene unit among all amphidinolides obtained so far.

Amphidinolides are a series of unique macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.[1] Our continuing search for bioactive secondary metabolites from laboratory-cultured marine dinoflagellates[2] resulted in the isolation of a new cytotoxic 12-membered macrolide, amphidinolide W (**1**), from extracts of the strain (Y-42) of the dinoflagellate *Amphidinium* sp. The gross structure of **1** was elucidated on the basis of the spectroscopic data including $^{13}C-^{13}C$ correlations obtained from the INADEQUATE spectrum. The absolute configurations at C-11, C-12, and C-14 of **1** were assigned by *J*-based configuration analysis and a modified Mosher method, while those at C-2 and C-6 were elucidated from NMR data of MTPA esters of a reductive product of **1**. Here, we describe the isolation and structure elucidation of **1**.



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The dinoflagellate *Amphidinium* sp. (strain Y-42) was obtained from the inside cells of a marine acoel flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The dinoflagellate was mass cultured unialgally at 25 °C for 14 days in a seawater medium enriched with 1.2 mM of ^{13}C-labeled sodium bicarbonate and 1% ES supplement. The harvested algal cells (35.9 g, wet weight, from 40 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 silica gel column chromatography ($CHCl_3$/MeOH) followed by C_{18} HPLC (CH_3CN/H_2O) to afford amphidinolide W (**1**, 0.009%, wet weight) together with two known macrolides, amphidinolides G[3] (0.0008%) and H[3] (0.0007%).

Table 1. ^1H and ^{13}C NMR Data of Amphidinolide W (1) in $CDCl_3$

position	δ_C	δ_H (m, Hz)	HMBC (H)
1	175.3 s		H-3b[c], H-11, H_3-21
2	39.4 d	2.64 m	H-3b[c], H_2-4, H_3-21
3	25.8 t	2.17 m	H_2-4, H_3-21
		1.65 m	
4	35.9 t	2.49 dt, 6.3, 18.3	H-2, H-3b[c]
		2.34 m	
5	212.8 s		H-3b[c], H_2-4, H-6, H_3-22
6	45.8 d	2.38 m	H-8a[c], H_3-22
7	32.3 t	1.89 m	H-6, H-8b[c], H_3-22
		1.50 m	
8	32.3 t	2.31	H-7a[c], H-9, H-10
		1.89 m	
9	138.2 d	5.64 ddd, 5.5, 9.7, 15.5	H-7b[c], H-8b[c], H-10, H-11
10	127.0 d	5.52 dd, 8.3, 15.5	H-8b[c], H-9, H-11
11	79.0 d	4.96 dd, 6.5, 8.3	H-9, H-10
12	70.6 d	3.56 ddd, 2.2, 6.5, 10.0	H-11, H-13a[c]
13	41.0 t	1.41 ddd, 3.3, 10.0, 13.9	H_3-23
		1.26 ddd, 2.2, 11.5, 13.9	
14	28.8 d	2.83 m	H-12, H_2-13, H_3-23
15	135.6 d	5.04 d, 10.0	H_2-13, H_3-23, H_3-24
16	133.5 s		H-15, H_3-24
17	133.6 d	6.03 d, 15.5	H-15, H-18, H_2-19, H_3-24
18	129.8 d	5.62 ddd, 6.8, 8.6, 15.5	H_2-19, H_3-20
19	25.6 t	2.11[a] m	H_3-20
20	13.8 q	1.02[b] t, 7.2	H-18, H_2-19
21	16.4 q	1.15[b] d, 6.7	
22	18.6 q	1.03[b] d, 7.2	
23	21.7 q	0.96[b] d, 6.7	H-13b, H-15
24	12.7 q	1.77[b] s	

[a] 2H. [b] 3H. [c] a and b denote low-field and high-field resonances, respectively, of a geminal pair for C-3, C-7, C-8, and C-13.

FABMS of amphidinolide W (**1**) showed a pseudomolecular ion peak at *m*/*z* 413 $(M + Na)^+$, and **1** had a molecular formula of $C_{24}H_{38}O_4$ as revealed by HRFABMS [*m*/*z* 413.2686 $(M + Na)^+$, +1.8 mmu]. IR absorptions at 3442 and 1721 cm^{-1} indicated the presence of hydroxyl(s) and carbonyl group(s), respectively. The UV spectrum showed the absorption at 235 nm (ϵ 14 000), implying the presence of a conjugated diene chromophore. In the ^1H NMR spectrum of **1**, intense satellite signals due to ^{13}C-enrichments were observed for all proton signals. The average incorporation ratio of carbon-13 in each carbon atom was estimated to be ca. 30% on the basis of intensity of proton satellite signals. ^1H and ^{13}C NMR data (Table 1) disclosed the existence of a ketone, an ester carbonyl, an sp^2 quaternary carbon, five sp^2 methines, five sp^3

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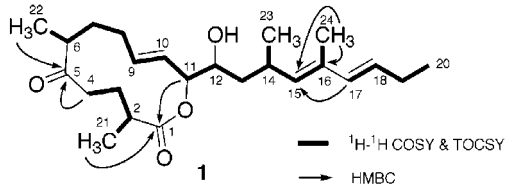

Figure 1. Selected 2D NMR correlations for amphidinolide W (**1**).

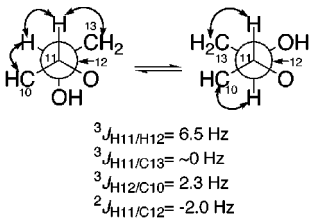

Figure 2. Rotation model for C-11−C-12 bond of amphidinolide W (**1**). NOESY correlations are illustrated by solid arrows.

methines (two of them bearing an oxygen atom), six sp^3 methylenes, and five methyls (one of them attached to an olefin). Since five out of six unsaturations were accounted for, amphidinolide W (**1**) was inferred to contain one ring. Detailed analyses of the $^1H-^1H$ COSY, TOCSY, and HMQC spectra disclosed three proton networks from H_3-21 to H_2-4, from H_3-22 to H_3-24, and from H-17 to H_3-20 (Figure 1). HMBC correlations of H_2-4 and H_3-22 to a ketone carbonyl carbon (δ_C 212.8, C-5) suggested that C-4 and C-6 were linked through the ketone carbonyl. The presence of a conjugated diene moiety at C-15−C-18 was deduced from HMBC correlations of H-17 to C-15 and H_3-24 to C-15 and C-16. The carbon chain from C-1 to C-20 containing four branched methyl groups was also supported by one-bond $^{13}C-^{13}C$ correlations observed in the INADEQUATE spectrum. The existence of an ester linkage between C-1 and C-11 was implied by HMBC correlations of H_3-21 and H-11 to C-1 (δ_C 175.3). Geometries of two disubstituted olefins at C-9−C-10 and C-17−C-18 were assigned as both *E* by $^1H-^1H$ coupling constants [J(H-9/H-10); 15.5 Hz, J(H-17/H-18); 15.5 Hz], while *E*-geometry of a trisubstituted olefin at C-15−C-16 was revealed by NOESY cross-peaks for H-14/H_3-24 and H-15/H-17. Thus, the gross structure of amphidinolide W was elucidated to be **1**.

The relative configurations at C-11, C-12, and C-14 were elucidated on the basis of $^1H-^1H$ and $^{13}C-^1H$ coupling constants[4] in addition to NOESY correlations. For the C-11−C-12 bond (Figure 2), the ^3J(H-11, H-12) (6.5 Hz) suggested that this bond underwent a conformational change between *anti* and *gauche*. Furthermore, the ^2J(C-12, H-11) showed a medium value (−2.0 Hz), also implying that H-11 had both *anti* and *gauche* relations to OH-12 due to rotation of the bond. The values for ^3J(C-13, H-11) (~0 Hz) and ^3J(C-10, H-12) (2.3 Hz) obtained from the hetero half-filtered TOCSY (HETLOC)[5] spectrum indicated that H-11 and H-12 were *gauche* to C-13 and C-10, respectively. Of the six possible rotamers

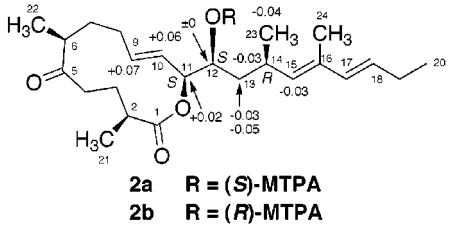

Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of amphidinolide W (**1**).

arising from *erythro* and *threo*, only one pair of threo relationship shown in Figure 2 satisfied all of these coupling data. NOESY correlations also supported the *threo* relation for the C-11−C-12 bond. For the C-12−C-13 bond, ^3J(H-12, H-13b) was a typical value (2.2 Hz) for *gauche* relationships, while ^3J(H-12, H-13a) (10.0 Hz) was indicative of an anti relation for H-12−H-13a. Combination of two-bond $^{13}C-^1H$ coupling constants of C-12/H-13a (−5.0 Hz) and C-12/H-13b (~0 Hz) with NOESY correlations for H-11/H-13a and H-11/H-13b suggested that H-13a was *gauche* to C-11 and 12-OH and that H-13b was *gauche* to C-11 and *anti* to 12-OH. On the other hand, a rotation model for the C-13−C-14 bond was deduced from the following coupling constants: ^3J(H-13a, H-14) 3.3 Hz, ^3J(H-13b, H-14) 11.5 Hz, ^3J(C-15, H-13a) 7.6 Hz, ^3J(C-15, H-13b) 2.2 Hz, ^3J(C-23, H-13a) ~0 Hz, and ^3J(C-23, H-13b) 0.9 Hz. This was also supported by NOESY correlations for H-12/H-15, H_2-13/H_3-23, and H-13a/H-14. Therefore, the 1,3-chiral center of C-12−C-14 was elucidated to have a *syn* relation.

The absolute configuration at C-12 of amphidinolide W (**1**) was determined by a modified Mosher method.[6] Treatment of **1** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) afforded the (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively). $\Delta\delta$ values ($\delta_S - \delta_R$) of H-9, H-10, and H-11 showed positve values, while those of H_2-13, H-14, H-15, and H_3-23 were negative (Figure 3), suggesting that C-12 possessed *S*-configuration. Thus, the absolute configurations at C-11 and C-14 were elucidated to be *S* and *R*, respectively. The absolute configuration at C-2 was investigated by NMR data of MTPA esters of reduction products of **1** as follows. Treatment of **1** with $LiAlH_4$ and then (*R*)- or (*S*)-MTPACl afforded tetrakis-(*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of the linear alcohol of **1** (Scheme 1). The methylene protons of C-1 for **3a** appeared as separated double doublet signals at δ_H 3.94 and 4.12, while those for **3b** were observed as overlapped 2H signals at δ_H 4.09, suggesting *S*-configuration at C-2.[7] To determine the absolute configuration at C-6 of **1**, Baeyer−Villiger reaction was applied to obtain the segment including the methine carbon at C-6. Amphidinolide W (**1**) was reduced with hydrogen gas and

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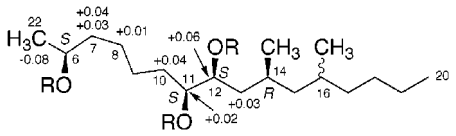
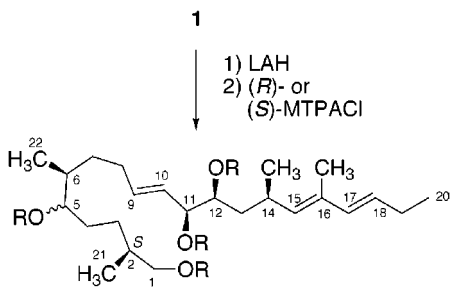
(7) Mosher's method has been applied for determination of absolute stereochemistry of a methyl group at C-25 of steroids with a primary hydroxy group at C-26. In the ^1H NMR spectra of the (+)-(*R*)-MTPA esters, two 26-methylene protons of the 25*S* isomer are much closer ($\Delta\delta$ ca. 0.04) to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



4a R = (*S*)-MTPA
4b R = (*R*)-MTPA

Figure 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for tris-(*S*)- and tris-(*R*)-MTPA esters (**4a** and **4b**, respectively) of the C-6−C-20 segment of amphidinolide W (**1**).

Scheme 1



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

rhodium−aluminum to avoid epoxidation of three olfins. Baeyer−Villiger reaction was performed by treatment of the crude hexahydro form of **1** with TFPA (trifluoroperoxyacetic acid). After reduction with $LiAlH_4$, esterification with (*R*)−(−)- and (*S*)−(+)-MTPACl and HPLC separation afforded the tris-(*S*)- and tris-(*R*)-MTPA esters (**4a** and **4b**, respectively) of the C-6−C-20 segment of **1**. $\Delta\delta$ values obtained from the ^{1}H chemical shifts of **4a** and **4b** were shown in Figure 4. The $\Delta\delta$ value of H_3-22 (−0.08) showed a negative sign, while those of H_2-7 (+0.04 and +0.03) and H_2-8 (+0.01) were positive, suggesting 6*S*-configuration. Therefore, the absolute configurations of five chiral centers in amphidinolide W (**1**) were elucidated to be 2*S*, 6*S*, 11*S*, 12*S*, and 14*R*.

Amphidinolide W (**1**) is the first macrolide without an exomethylene unit among all of the amphidinolides isolated so far. The gross structure of C-9−C-16 moiety of amphidinolide W (**1**) corresponds to that of C-6−C-15 of amphidinolide H, which was contained in this strain Y-42, suggesting that amphidinolide W (**1**) may be biogenetically related to amphidinolide H. Amphidinolide W (**1**) exhibited cytotoxicity against murine lymphoma L1210 cells in vitro with an IC_{50} value of 3.9 μg/mL.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer using 2.5 mm micro cells for $CDCl_3$ (Shigemi Co., Ltd.). 2D NMR spectra were measured on a 500 MHz spectrometer using 5 mm micro cells for $CDCl_3$ (Shigemi Co., Ltd.). HETLOC experiments were performed using the pulse sequence proposed by Woolborn and Leibfritz with composite pulses for broadband constant rotations (bandwidth ±0.60).[8] The duration of the trim pulse, the delay in the BIRD pulse, and the constant time for J_{CH} evaluation 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 1 K data points. Zero-filling to 1 K 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. FABMS spectra were recorded using *p*-nitrobenzyl alcohol containing sodium iodide as a matrix in positive mode.

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain number Y-42) was separated from the inside cells of a marine acoel flatworm *Amphiscolops* sp., which was collected off Sunabe, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for 2 weeks in seawater medium enriched with $NaH^{13}CO_3$ (1.2 mM) and 1% ES supplement. The harvested cells (36 g, wet weight, from 40 L of culture) were extracted with MeOH/toluene (3:1, 150 mL × 3). After addition of 1 M NaCl (aq) (150 mL), the mixture was extracted with toluene (150 mL × 3). The toluene-soluble fractions were evaporated under reduced pressure to give a residue (778 mg), which was subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) and then a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8:2). The fraction containing macrolides was purified by C_{18} HPLC [Mightysil RP-18, 5 μm, Kanto Chemical Co., Inc., 10 × 250 mm; eluent, CH_3CN/H_2O (85:15); flow rate, 3 mL/min; UV detection at 220 nm] to afford amphidinolide W (**1**, 3.3 mg, 0.009%, wet weight, t_R 15.4 min) together with amphidinolides G (0.0008%) and H (0.0007%).

Amphidinolide W (1): colorless oil; UV (EtOH) λ_{max} 235 nm (ϵ 14 000); IR (KBr) ν_{max} 3442, 2962, 2927, 1721 cm^{-1}; ^{1}H and ^{13}C NMR (Table S1, Supporting Information); FABMS *m*/*z* 413 $(M + Na)^+$; HRFABMS *m*/*z* 413.2686 [calcd for $C_{24}H_{38}O_4Na$ $(M + Na)^+$, 413.2668].

(*S*)-MTPA Ester (2a) of Amphidinolide W (1). To a CH_2Cl_2 solution (50 μL) of amphidinolide W (**1**, 0.5 mg) was added 4-(dimethylamino)pyridine (50 μg), triethylamine (5 μL), and (*R*)-(−)-MTPACl (1 μL) at 4 °C, and stirring was continued for 3 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (5 μL) and evaporation of solvent, the residue was passed through a silica gel column (hexane/EtOAc, 5:1) to afford the (*S*)-MTPA ester (**2a**, 0.5 mg) of **1**. **2a**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.89 (6H, m), 1.02 (3H, d, J = 6.7 Hz), 1.03 (3H, t, J = 7.0 Hz), 1.42−1.50 (2H, m), 1.55−1.65 (2H, m), 1.67 (3H, s), 1.75−1.90 (2H, m), 2.11 (2H, m), 2.22 (1H, m), 2.29−2.36 (3H, m), 2.45−2.50 (3H, m), 3.54 (3H, s), 5.00 (1H, d, J = 10.0 Hz), 5.21 (1H, m), 5.33 (1H, m), 5.34 (1H, m), 5.63 (1H, ddd, J = 6.5, 8,7, 15.5 Hz), 5.69 (1H, m), 6.00 (1H, d, J = 15.5 Hz), 7.35−7.43 (3H, m), and 7.59 (2H, m); ESIMS *m*/*z* 629 $(M + Na)^+$; HRESIMS *m*/*z* 629.3048 [calcd for $C_{34}H_{45}O_6F_3Na$ $(M + Na)^+$, 629.3066].

(*R*)-MTPA Ester (2b) of Amphidinolide W (1). Amphidinolide W (**1**, 0.5 mg) was treated with (*S*)-(+)-MTPACl (1 μL) by the same procedure as described above to afford the (*R*)-MTPA ester (**2b**, 0.3 mg) of **1**. **2b**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, J = 6.7 Hz), 0.99−1.04 (9H, m), 1.40−1.58 (4H, m), 1.63 (3H, s), 1.72 (1H, m), 1.86 (1H, m), 2.12 (2H, m), 2.17−2.35 (4H, m), 2.44 (1H, m), 2.46−2.55 (2H, m), 3.52 (3H, s), 5.03 (1H, d, J = 10.2 Hz), 5.18 (1H, m), 5.27 (1H, m), 5.33 (1H, m), 5.59−5.67 (2H, m), 6.01 (1H, d, J = 15.4 Hz), 7.37−7.43 (3H, m), and 7.60 (2H, m); ESIMS *m*/*z* 629 $(M + Na)^+$; HRESIMS *m*/*z* 629.3053 [calcd for $C_{34}H_{45}O_6F_3Na$ $(M + Na)^+$, 629.3066].

Tetrakis-(*S*)-MTPA Ester (3a) of Reduction Product of Amphidinolide W (1). Amphidinolide W (**1**, 0.5 mg) in THF solution (50 μL) was treated with $LiAlH_4$ (1.5 mg) at 4 °C for 100 min. After addition of 1 M phosphate buffer (pH 6.85) (100 μL), the mixture was extracted with EtOAc (200 μL × 3). The mixture, after evaporation, was dissolved in CH_2Cl_2 (50 μL) and treated with 4-(dimethylamino)pyridine (50 μg), triethylamine (5 μL), and (*R*)-(−)-MTPACl (3 μL) at 4 °C for 14 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (5 μL) and then evaporation of the solvent, the residue was subjected to SiO_2 column chromatography (hexane/EtOAc) followed by C_{18} HPLC (TSK-gel ODS-100S, TOSOH Co., Inc., 4.6 × 250 mm; eluent, CH_3CN/H_2O (95:5); flow rate, 1 mL/min; UV detection at 230 nm) to afford tetrakis-(*S*)-MTPA ester (**3a**, 0.4 mg, t_R 28.5 min) of reduction product of amphidinolide W (**1**). **3a**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.72 (1.5H, d, J = 6.6 Hz), 0.79 (1.5H, d, J = 6.6 Hz), 0.85 (1.5H, d, J = 6.7 Hz), 0.86 (1.5H, d, J = 6.7 Hz), 0.89 (3H, d, J = 7.2 Hz), 1.02 (3H, t, J = 7.0 Hz), 1.40−1.65 (4H, m), 1.62 (3H, s), 1.63 (1H, m), 1.76 (1H, m), 1.90−2.03 (2H, m), 2.10 (2H, m), 2.27−2.36 (2H, m), 2.45 (1H, m), 3.46 (1.5H, s), 3.47 (3H, s), 3.48 (3H, s), 3.49 (1.5H, s), 3.52 (3H, s), 4.09 (2H, m), 4.88 (0.5H, m), 4.93 (0.5H, m), 4.96 (1H, brd, J = 10.3 Hz), 5.05 (1H, m), 5.18 (1H, m), 5.49 (1H, m), 5.59−5.65 (2H, m), 5.99 (1H, d, J = 15.4 Hz), 7.30−7.42 (12H, m), and 7.44−

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7.56 (8H, m); ESIMS *m/z* 1283 $(M + Na)^+$; HRESIMS *m/z* 1283.4760 [calcd for $C_{64}H_{72}O_{12}F_{12}Na$ $(M + Na)^+$, 1283.4723].

Tetrakis-(*R*)-MTPA Ester (3b) of Reduction Product of Amphidinolide W (1). The reduction product of amphidinolide W (**1**, 0.5 mg) was treated with (*S*)−(+)-MTPACl (3 μL) by the same procedure as described above to afford the tetrakis-(*R*)-MTPA ester (**3b**, 0.3 mg) of **1**. **3b**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.79 (1.2H, d, *J* = 6.7 Hz), 0.83 (1.8H, d, *J* = 6.7 Hz), 0.86 (6H, m), 1.00 (3H, t, *J* = 7.0 Hz), 1.40−1.65 (4H, m), 1.63 (3H, s), 1.70 (1H, m), 1.81 (1H, m), 1.99 (2H, m), 2.10 (2H, m), 2.21 (0.4H, m), 2.27−2.38 (1.6H, m), 2.44 (1H, m), 3.46 (6H, s), 3.50 (1.2H, s), 3.51 (1.8H, s), 3.52 (3H, s), 3.94 (1H, dd, *J* = 6.9, 10.7 Hz), 4.12 (1H, dd, *J* = 5.4 and 10.7 Hz), 4.89 (0.6H, m), 4.96 (1.4H, m), 5.11 (1H, m), 5.17 (0.4H, m), 5.22 (0.6H, m), 5.54 (1H, m), 5.58−5.66 (2H, m), 5.94 (1H, d, *J* = 15.4 Hz), 7.33−7.42 (12H, m), and 7.44−7.57 (8H, m); ESIMS *m/z* 1283 $(M + Na)^+$; HRESIMS *m/z* 1283.4764 [calcd for $C_{64}H_{72}O_{12}F_{12}Na$ $(M + Na)^+$, 1283.4723].

Baeyer−Villiger Reaction of Amphidinolide W (1). Amphidinolide W (**1**, 0.5 mg) in MeOH/EtOAc (2:1, 75 μL) was treated with 5% rhodium−alumina (0.7 mg) under H_2 atmosphere at room temperature for 3 h. After filtration and evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (50 μL), and treated with TFPA prepared by adding trifluoroacetic anhydride (20 μL) to a 30% aqueous H_2O_2 (4 μL) in CH_2Cl_2 (25 μL) at 4 °C for 12 h. After evaporation, to the reaction mixture were added THF (50 μL) and $LiAlH_4$ (2.5 mg), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated, the residue was extracted with EtOAc (200 μL × 3), and the extract was concentrated in vacuo to give a crude C-6-C-20 segment (1.1 mg). An aliquot (0.5 mg) of the crude residue was dissolved in CH_2Cl_2 (50 μL), 4-(dimethylamino)-pyridine (50 μg), triethylamine (5 μL), and (*R*)-(−)-MTPACl (2 μL) were added to the mixture, and stirring was continued at room temperature for 14 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (4 μL), the solvent was evaporated in vacuo. The residue was passed through a silica gel column (hexane/EtOAc, 8:1) and then purified by C_{18} HPLC (TSK-gel ODS-100S, 4.6 × 250 mm; eluent, CH_3CN/H_2O (95:5); flow rate, 1 mL/min; UV detection at 230 nm) to afford a tris-(*S*)-MTPA ester **4a** (0.2 mg, t_R 30 min) of C-6−C-20 segment. **4a**: ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, d, *J* = 6.7 Hz), 0.81 (3H, d, *J* = 6.7 Hz), 0.84 (3H, t, *J* = 7.0 Hz), 1.05−1.27 (8H, m), 1.28 (3H, d, *J* = 6.0 Hz), 1.30−1.84 (12H, m), 3.48 (6H, s), 3.55 (3H, s), 5.07 (1H, m), 5.15 (1H, m), 5.28 (1H, m), 7.33−7.42 (9H, m), and 7.44−7.57 (6H, m); ESIMS *m/z* 973 $(M + Na)^+$; HRESIMS *m/z* 973.3953 [calcd for $C_{48}H_{59}O_9F_9Na$ $(M + Na)^+$, 973.3913].

A tris-(*R*)-MTPA ester **4b** (0.08 mg, t_R 27 min) was yielded by treatment of the crude C-6−C-20 segment (0.2 mg) of **1** with (*S*)-(+)-MTPACl. **4b**: ^{1}H NMR ($CDCl_3$) δ 0.78 (3H, d, *J* = 6.7 Hz), 0.84 (3H, t, *J* = 7.0 Hz), 0.85 (3H, d, *J* = 6.7 Hz), 1.05−.34 (8H, m), 1.36 (3H, d, *J* = 6.0 Hz), 1.37−1.80 (12H, m), 3.48 (6H, s), 3.50 (3H, s), 5.15 (3H, m), 7.33−7.42 (9H, m), and 7.44−7.57 (6H, m); ESIMS *m/z* 973 $(M + Na)^+$; HRESIMS *m/z* 973.3882 [calcd for $C_{48}H_{59}O_9F_9Na$ $(M + Na)^+$, 973.3913].

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Supporting Information Available: Table S1 and spectral data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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