Amphidinolides T2, T3, and T4, New 19-Membered Macrolides from the Dinoflagellate *Amphidinium* sp. and the Biosynthesis of Amphidinolide T1

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Received August 11, 2000

Three new 19-membered macrolides, amphidinolides T2 (**2**), T3 (**3**), and T4 (**4**), structurally related to amphidinolide T1 (**1**) have been isolated from two strains of marine dinoflagellates of the genus *Amphidinium*. The structures of **2**−**4** were elucidated on the basis of spectroscopic data. The absolute configurations at C-7, C-8, and C-10 of **1**−**4** were determined by comparison of NMR data of their C-1−C-12 segments with those of synthetic model compounds for the tetrahydrofuran portion. The biosynthetic origins of amphidinolide T1 (**1**) were investigated on the basis of ^{13}C NMR data of a ^{13}C enriched sample obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates and ^{13}C-labeled sodium bicarbonate in the cultures of the dinoflagellate. These incorporation patterns suggested that amphidinolide T1 (**1**) was generated from four successive polyketide chains, an isolated C_1 unit formed from C-2 of acetates, and three unusual C_2 units derived only from C-2 of acetates. Furthermore, it is noted that five oxygenated carbons of C-1, C-7, C-12, C-13, and C-18 were not derived from the C-1 carbonyl, but from the C-2 methyl of acetates.

Introduction

Amphidinolides are a series of unique cytotoxic macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.[1] Amphidinolide T1 (**1**) is a 19-membered macrolide, which has been isolated from the dinoflagellate *Amphidinium* sp. (Y-56), possessing a tetrahydrofuran ring, an *exo*-methylene, three branched methyls, a ketone, and a hydroxyl group.[2,3] Absolute configurations at four (C-2, C-13, C-14, and C-18) of seven chiral centers in amphidinolide T1 (**1**) were elucidated on the basis of the NMR data of the MTPA esters of **1** and the degradation products, and the relative stereochemistry of the tetrahydrofuran ring was assigned from NOESY data, whereas the absolute configurations at C-7, C-8, and C-10 remain unsolved. Further investigation of the extracts of two strains (Y-71 and Y-56) of the dinoflagellates resulted in the isolation of three new 19-membered macrolides, amphidinolides T2 (**2**), T3 (**3**), and T4 (**4**), together with amphidinolide T1 (**1**). The structures of **2**−**4** were elucidated on the basis of the spectroscopic data and those of their degradation products. To determine the absolute configurations at C-7, C-8, and C-10 of **1**−**4**, (*S*)-(−)- and (*R*)-(+)-MTPA esters (**11a** and **11b**, respectively) of a model compound possessing a tetrahydrofuran ring were synthsized, and the absolute configurations at C-7, C-8, and C-10 were elucidated from comparison of ^1H NMR data of **11a** and **11b** with those of bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters (**6a** and **6b**) derived from **1**−**4**.

The biosynthetic origins of amphidinolide T1 (**1**) were investigated on the basis of ^{13}C NMR data of ^{13}C-enriched sample obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in the cultures of the dinoflagellate. These incorporation patterns suggested that **1** was generated from an isolated C_1 unit (C-7) formed from C-2 of acetates and three unusual C_2 units (C-1−C-2, C-12−C-13, and C-18−C-19) derived only from C-2 of acetates in addition to three successive diketides and a monoketide. Furthermore, it was shown that five (C-12, C-7, C-11, C-12, and C-18) of six oxygenated carbons and four C_1 branches (C-22, C-23, C-24, and C-25) were derived from C-2 methyl of acetates.

Results and Discussion

Isolation of Amphidinolides T2 (2), T3 (3), and T4 (4). The dinoflagellate *Amphidinium* sp. (Y-71 strain) was obtained from an acoel flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The mass cultured algal cells (1200 g, wet weight) obtained from 1300 L of culture were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene extracts were subjected to silica gel and then successive C_{18} column chromatographies followed by C_{18} HPLC to afford amphidinolide T2 (**2**, 0.0001%) together with known macrolides, amphidinolides B,[4] C,[5] and T1[2] (**1**, 0.0009%) (Chart 1). On the other hand, the toluene-soluble materials of the Y-56 strain of *Amphidinium* sp. were subjected to silica gel column chromatography ($CHCl_3$/MeOH) followed by C_{18} HPLC (CH_3CN/H_2O) to give amphidinolides T3 (**3**, 0.0006%, wet weight) and T4

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(3) We use in this paper the following names for amphidinolide T congeners: amphidinolide T initially reported in ref 2 is referred as amphidinolide T1 (1), and the homologues reported here are amphidinolides T2 (2), T3 (3), and T4 (4).

10.1021/jo005607c CCC: $20.00
Published on Web 12/14/2000

Table 1. ^{1}H and ^{13}C NMR Data of Amphidinolides T2 (2), T3 (3), and T4 (4) in C_6D_6

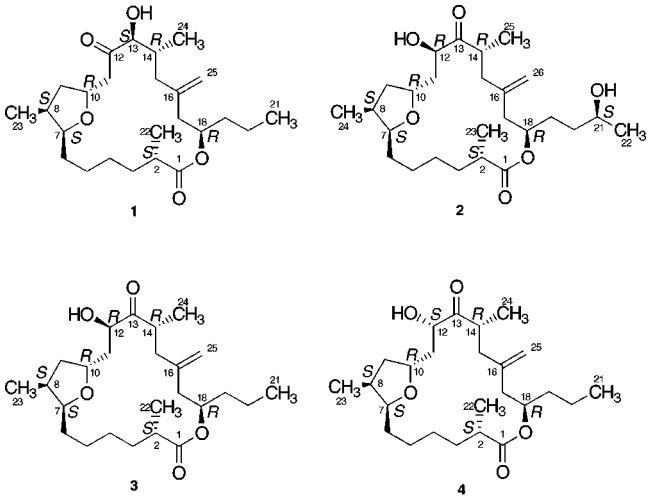
	2		**3**		**4**	
position	δ_C	δ_H (m, Hz)	δ_C	δ_H (m, Hz)	δ_C	δ_H (m, Hz)
1	175.0 s		174.9 s		175.4 s	
2	41.2 d	2.47 m	41.2 d	2.46 m	41.0 d	2.46 m
3	34.6 t	1.63 m	34.6 t	1.62 m	33.8 t	1.72 m
		1.38 m		1.37 m		1.29 m
4	26.5 t	1.51 m	26.5 t	1.53 m	26.6 t	1.48 m
		1.32 m		1.31 m		1.32 m
5	26.5 t	1.50 m	26.5 t	1.51 m	26.0 t	1.47 m
		1.39 m		1.42 m		1.43 m
6	29.7 t	1.50 m	29.7 t	1.49 m	28.7 t	1.59 m
		1.21 m		1.21 m		1.21 m
7	79.2 d	3.80 ddd, 10.0, 4.4, 3.3	79.2 d	3.80 dt, 9.6, 3.4	79.3 d	3.82 dt, 9.1, 4.4
8	36.3 d	1.79 m	36.3 d	1.79 m	36.1 d	1.93 m
9	39.9 t	1.69 m	39.3 t	1.69 dt, 15.0, 7.8	40.2 t	1.65 m
		1.39 m		1.40 m		1.50 m
10	76.1 d	4.06 m	76.1 d	4.07 ddd, 10.6, 8.1, 2.8	74.6 d	4.35 m
11	39.3 t	2.01 ddd, 14.4, 3.3, 2.8	39.3 t	2.00 dt, 14.5, 2.8	40.1 t	2.01 ddd, 14.2, 3.2, 7.7
		1.60 m		1.60 m		1.66 m
12	76.3 d	4.41 dt, 9.4, 2.8	76.3 d	4.40 dt, 9.1, 2.8	74.9 d	4.57 dt, 7.3, 3.1
12-OH		4.58 d, 2.8		4.57 d, 2.8		4.05 d, 3.1
13	215.6 s		215.7 s		215.6 s	
14	38.5 d	3.58 m	38.4 d	3.58 m	39.5 d	3.37 m
15	41.3 t	2.67 dd, 5.6, 13.3	41.4 t	2.68 dd, 5.5, 13.5	38.4 t	2.75 dd, 4.5, 14.1
		1.91 dd, 8.9, 13.3		1.89 dd, 8.7, 13.5		2.07 dd, 9.9, 14.1
16	143.3 s		143.3 s		143.1 s	
17	40.5 t	2.60 dd, 5.0, 13.9	40.6 t	2.58 dd, 5.5, 13.5	40.8 t	2.58 dd, 5.1, 13.6
		2.17 dd, 8.9, 13.9		2.17 dd, 8.5, 13.5		2.15 dd, 8.6, 13.6
18	72.2 d	5.26 m	72.1 d	5.24 m	71.9 d	5.26 m
19	29.9 t	1.68[b] m	35.9 t	1.58 m	35.5 t	1.56 m
				1.51 m		1.53 m
20	34.7 t	1.46 m	18.8 t	1.40 m	18.7 t	1.40 m
		1.38 m		1.35 m		1.35 m
21	67.2 q	3.55 m	14.1 q	0.88[a] t, 7.3	13.9 q	0.90[a] t, 7.4
21-OH		0.79 brd, 4.4				
22	23.9 q	0.99[a] d, 6.1	17.7 q	1.16[a] d, 6.9	18.0 q	1.15[a] d, 7.0
23	17.8 q	1.16[a] d, 6.7	14.2 q	0.71[a] d, 7.0	14.1 q	0.75[a] d, 7.1
24	14.2 q	0.71[a] d, 7.0	15.5 q	1.17[a] d, 6.6	15.9 q	1.02[a] d, 6.8
25	15.5 q	1.17[a] d, 6.7	114.7 t	4.92 s	115.4 t	4.87[a] s
				4.89 s		
26	114.7 t	4.93[b] s				

[a] 3H. [b] 2H.

(**4**, 0.0004%) together with known macrolides, amphidinolides C,[5] F,[6] T1 (**1**, 0.005%), and U.[1b]

Structure Elucidation of Amphidinolides T2 (2), T3 (3), and T4 (4). The FABMS spectrum of amphidinolide T3 (**3**) showed the pseudomolecular ion peak at *m*/*z* 423 $(M + H)^+$, indicating that **3** had the same molecular formula, $C_{25}H_{42}O_5$, as that of amphidinolide T1 (**1**). The ^{1}H and ^{13}C NMR data (Table 1) of **3** were analogous to those of **1** except for the resonances of C-12 (δ_H 4.40, δ_C 76.3), C-13 (δ_C 215.7), C-14 (δ_H 3.58, δ_C 38.4), and C-24 (δ_H 1.17, δ_C 15.5) of **3**. Detailed analyses of ^{1}H−^{1}H COSY and TOCSY data revealed the proton connectivities of two structural units from H_3-22 to 12-OH (δ_H 4.57) and from H_3-24 to H_3-21 through an *exo*-methylene at C-16 (Figure 1). HMBC correlations from H-12, 12-OH, H-15 (δ_H 2.68), and H_3-24 to a ketone carbonyl (C-13) suggested that the carbonyl group was located at C-13. The chemical shift of H-18 (δ_H 5.24) and the HMBC correlation from H_3-22 (δ_H 1.16) to C-1 (δ_C 174.9) indicated that an ester linkage was formed between C-2 and C-18 to construct a lactone ring. The relative stereochemistry of H-7, H-8, and H-10 were implied to be 7,8-*syn* and 7,10-*anti* by NOESY correlations for H_2-6/H_3-23, H-7/H-11, and H-10/H_3-23. Thus, amphidinolide T3 (**3**) was assigned as the 12-hydroxy-13-keto form of amphidinolide T1 (**1**).

Chart 1



(4) (a) Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127−1129. (b) Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *J. Nat. Prod.* **1989**, *52*, 1036−1041. (c) Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. *J. Am. Chem. Soc.* **1994**, *116*, 2657−2658.

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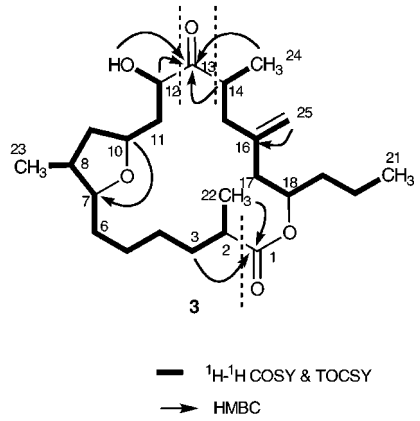


Figure 1. Selected 2D NMR correlations for amphidinolide T3 (**3**).

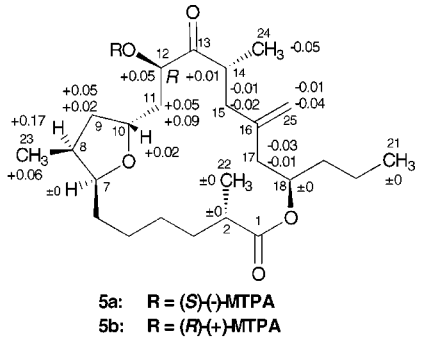
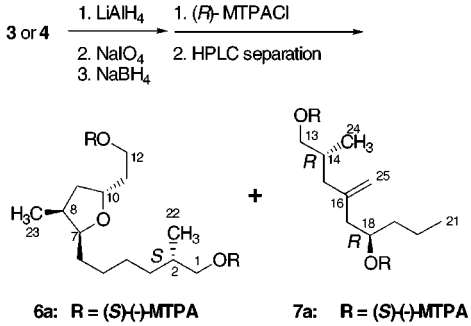


Figure 2. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $= \delta_S - \delta_R$] obtained for the (*S*)-(−)- and (*R*)-(+)-MTPA esters (**5a** and **5b**, respectively) of amphidinolide T3 (**3**).

Scheme 1



The absolute configuration at C-12 of amphidinolide T3 (**3**) was determined by modified Mosher's method. Treatment of **3** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) afforded the (*S*)-(−)- and (*R*)-(+)-MTPA esters (**5a** and **5b**, respectively). $\Delta\delta$ values ($\delta_S - \delta_R$) of H_2-15 (−0.01 and −0.02), H_2-17 (−0.03 and −0.01), H_3-24 (−0.05), and H_2-25 (−0.01 and −0.04) showed negative values, while those of H-8 (+0.17), H_2-9 (+0.05 and +0.02), H-10 (+0.02), H_2-11 (+0.05 and +0.09), and H_3-23 (+0.06) were positive (Figure 2), thus indicating a 12*R*-configuration. Treatment of **3** with $LiAlH_4$, $NaIO_4$, $NaBH_4$, and (*R*)-(−)-MTPACl afforded the bis-(*S*)-(−)-MTPA esters of C-1−C-12 (**6a**) and C-13−C-21 segments (**7a**) (Scheme 1). ^{1}H NMR data of **6a** and **7a** corresponded well to those of the bis-(*S*)-(−)-MTPA esters of C-1−C-12 and C-13−C-21 segments prepared from amphidinolide T1 (**1**), respectively. Therefore, the absolute configurations at C-2, C-14, and C-18 of amphidinolide T3 (**3**) were assigned as *S*, *R*, and *R*, respectively, and the absolute configurations at C-7, C-8, and C-10 of **3** were the same as those of amphidinolide T1 (**1**).

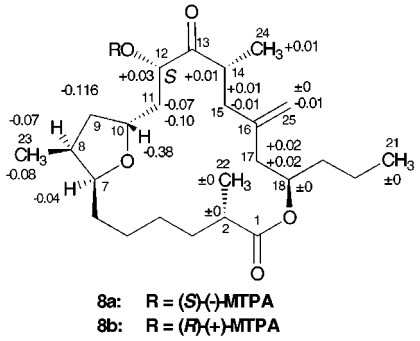


Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $= \delta_S - \delta_R$] obtained for the (*S*)-(−)- and (*R*)-(+)-MTPA esters (**8a** and **8b**, respectively) of amphidinolide T4 (**4**).

Amphidinolide T4 (**4**) had the same molecular formula, $C_{25}H_{42}O_5$, as amphidinolide T1 (**1**) and amphidinolide T3 (**3**) as revealed by HRFABMS [*m*/*z* 423.3131 $(M + H)^+$, Δ +2.1 mmu]. Extensive 2D NMR data of **4** indicated that the gross structure of **4**, including the relative stereochemistry of a tetrahydrofuran ring, was the same as that of amphidinolide T3 (**3**). The absolute configuration at C-12 in **4** was assigned as *S* by modified Mosher's method (Figure 3). Treatment of **4** with $LiAlH_4$, $NaIO_4$, $NaBH_4$, and (*R*)-(−)-MTPACl afforded the bis-(*S*)-(−)-MTPA esters of C-1−C-12 (**6a**) and C-13−C-21 segments (**7a**), of which the ^{1}H NMR data were identical with those of the bis-(*S*)-(−)-MTPA esters of C-1−C-12 (**6a**) and C-13−C-21 segments (**7a**), respectively, obtained from amphidinolides T1 (**1**) and T3 (**3**). Thus the absolute configurations at C-2, C-14, and C-18 of amphidinolide T4 (**4**) were assigned as *S*, *R*, and *R*, respectively.

The molecular formula of amphidinolide T2 (**2**) was suggested to be $C_{26}H_{44}O_6$ by HRFABMS data [*m*/*z* 453.3187 $(M + H)^+$, Δ −2.9 mmu]. ^{1}H and ^{13}C NMR data (Table 1) of **2** were close to those of amphidinolide T3 (**3**), except for the presence of an additional oxymethine carbon [δ_H 3.55 (1H, m); δ_C 67.2 (d)] and a hydroxyl group [δ_H 0.79 (1H, brd, J = 4.4 Hz)]. Analysis of the ^{1}H−^{1}H COSY and TOCSY spectra revealed connectivities from H_3-23 to H-12 and from H_3-25 to H_3-22, indicating that the hydroxyl group was located at C-21. The carbon chemical shifts of three quaternary carbons (C-1, δ_C 175.0; C-13, δ_C 215.6; C-16, δ_C 143.3) of **2** corresponded well to those (C-1, δ_C 174.9; C-13, δ_C 215.7; C-16, δ_C 143.3) of **3**. The relative stereochemistry of a tetrahydrofuran ring was the same as that of **3**. Thus the gross structure of amphidinolide T2 was concluded to be **2**.

The absolute configurations at C-12 and C-21 of amphidinolide T2 (**2**) were elucidated to be *R* and *S*, respectively, by modified Mosher's method (Figure 4). Treatment of **2** with $LiAlH_4$, $NaIO_4$, $NaBH_4$, and (*R*)-(−)- or (*S*)-(+)-MTPACl afforded the bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters (**6a** and **6b**, Scheme 2, respectively) of the C-1−C-12 segment and the tris-(*S*)-(−)- and the tris-(*R*)-(+)-MTPA esters (**10a** and **10b**, respectively) of the C-13−C-22 segment (Scheme 2). ^{1}H NMR data of the bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters (**6a** and **6b**, respectively) of the C-1−C-12 segment were identical with those of the bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters of the C-1-C-12 segment obtained from amphidinolide T1

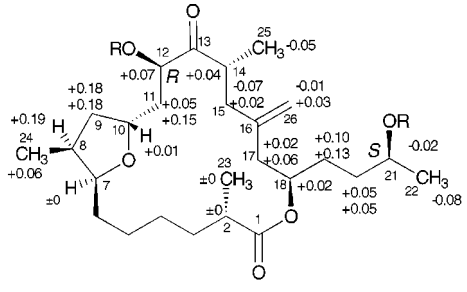
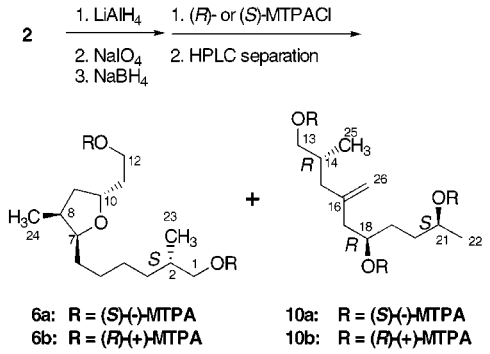


Figure 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $= \delta_S - \delta_R$] obtained for the bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters (**9a** and **9b**, respectively) of amphidinolide T2 (**2**).

Scheme 2



(**1**), indicating that the absolute stereochemistry at C-2, C-7, C-8, and C-10 of **2** was the same as that of **1**. The chemical shift differences (Figure 5) of H_2-13 for **10a** ($\Delta\delta$ 0.03; δ_H 4.09 and 4.12) and **10b** ($\Delta\delta$ 0.23; δ_H 4.03 and 4.26) suggested the 14*R*-configuration. The absolute stereochemistry of C-18 was assigned as *R* by modified Mosher's method on the basis of the ^{1}H NMR data of **10a** and **10b** (Figure 6). Therefore, the absolute configurations at C-2, C-12, C-14, C-18, and C-21 of amphidinolide T2 (**2**) were concluded to be *S*, *R*, *R*, *R*, and *S*, respectively.

To elucidate the absolute configurations at C-7, C-8, and C-10, ^{1}H NMR data of bis-(*S*)-(−)- and (*R*)-(+)-MTPA esters (**6a** and **6b**, respectively) of the C-1−C-12 segment obtained from amphidinolides T1 (**1**), T2 (**2**), T3 (**3**), and T4 (**4**) were analyzed. In the ^{1}H NMR spectrum of **6a**, the methylene protons at C-12 resonated at δ_H 4.41 (2H, t, $J = 6.9$ Hz), while in the ^{1}H NMR spectrum of **6b**, those at C-12 were observed as a 2H multiplet signal at δ_H 4.36−4.45. To determine the absolute configurations at C-7, C-8, and C-10 of amphidinolides T1 (**1**), T2 (**2**), T3 (**3**), and T4 (**4**), two model compounds (**11a** and **11b**) corresponding to the tetrahydrofuran ring portion in the C-1-C-12 segments (**6**a and **6b**) were synthesized from an *R*,*R*-iodide **12** as shown in Scheme 3. Compound **12** was prepared from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate according to the procedure reported previously.[7] Four-carbon elongation of the iodide **12** with cyanidation, reduction, Grignard reaction with allylmagnesium bromide, and then acetylation afforded a 1:1 mixture of the C-10 epimers of acetate **13**. Three-step conversion of **13** gave an alcohol **14**, which was treated with (*R*)-(−)-MTPACl to afford the (*S*)-(−)-MTPA ester.

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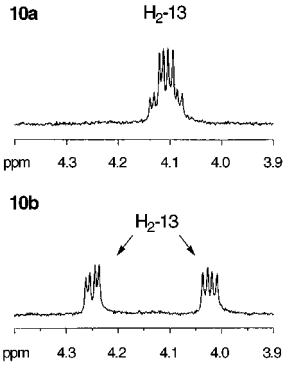


Figure 5. Proton signal patterns of H_2-13 of the tris-(*S*)-(−)- and tris-(*R*)-(+)-MTPA esters (**10a** and **10b**, respectively) of the C-13−C-22 segment of amphidinolide T2 (**2**).

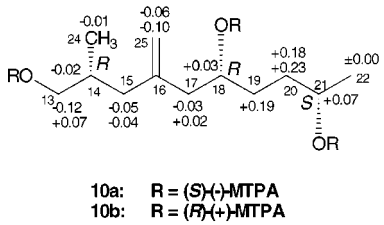
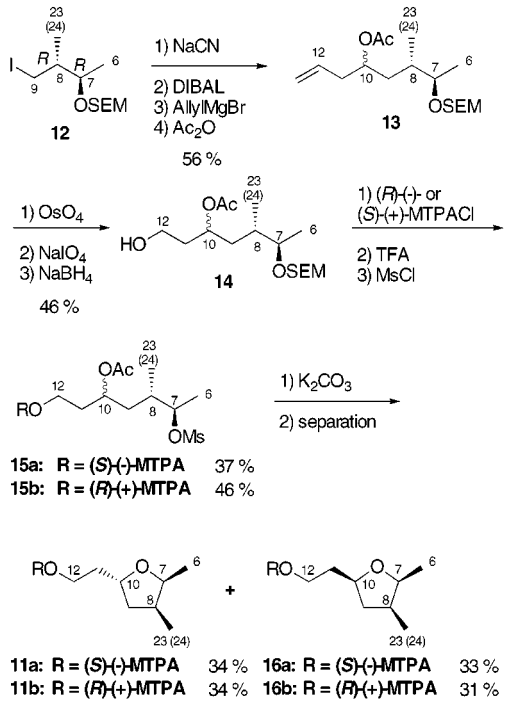


Figure 6. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $= \delta_S - \delta_R$] obtained for the tris-(*S*)-(−)- and tris-(*R*)-(+)-MTPA esters (**10a** and **10b**, respectively) of the C-13−C-22 segment of amphidinolide T2 (**2**).

Scheme 3



Selective deprotection of the 2-(trimethylsilyl)ethoxymethyl (SEM) ether was achieved by treatment with trifluoroacetic acid in CH_2Cl_2 at 0 °C. The secondary hydroxyl group was converted into mesylate **15a**. Deacetylation of **15a** with K_2CO_3 followed by C_{18} HPLC separation afforded the desired (*S*)-(−)-MTPA ester (**11a**) and its stereoisomer (**16a**). The relative streochemistry of **11a** was assigned as 7,8-*syn* and 7,10-*anti* by NOESY correlations, while that of **16a** was elucidated to be 7,8-

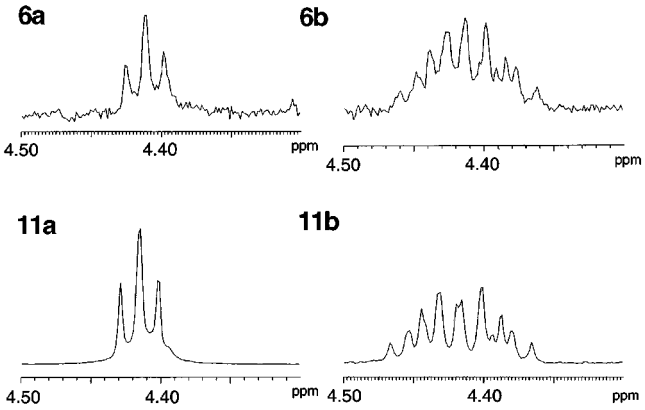


Figure 7. Proton signal patterns of H_2-12 of the bis-(*S*)-(−)- and bis-(*R*)-(+)-MTPA esters (**6a** and **6b**, respectively) of the C-1−C-12 segment and (*S*)-(−)- and (*R*)-(+)-MTPA esters (**11a** and **11b**, respectively) of the synthetic model compound for the tetrahydrofuran portion

syn and 7,10-*syn*. Therefore, the absolute configurations of **11a** and **16a** were concluded to be 7*S*, 8*S*, and 10*R*, and 7*S*, 8*S*, and 10*S*, respectively. On the other hand, the (*R*)-(+)-MTPA ester (**11b**) and its stereoisomer **16b** were prepared from **15b** in two steps.

The ^{1}H NMR spectra of **11a** and **11b** were compared with those of **6a** and **6b** to elucidate the absolute configuration of the latter compounds. Though the (*S*)-(−)- and (*R*)-(+)-MTPA esters (**11a** and **11b)** showed very similar NMR profiles, significant differences were observed in the signals of H_2-12 (Figure 7). In the ^{1}H NMR spectrum of the (*S*)-(−)-MTPA ester (**11a**), the methylene protons at C-12 resonated at δ_H 4.42 (2H, t, J = 6.9 Hz), while in ^{1}H NMR spectrum of **11b**, those at C-12 were observed as a 2H multiplet signal at δ_H 4.36−4.46. The signal pattern of H_2-12 of the bis-(*S*)-MTPA ester of the C-1-C-12 segment (**6a**) derived from natural amphidinolides T1 (**1**), T2 (**2**), T3 (**3**), and T4 (**4**) was close to that of the (*S*)-MTPA ester (**11a**), while the pattern of H_2-12 of the bis-(*R*)-MTPA ester (**6b**) derived from **1**, **2**, **3**, and **4** corresponded to that of the (*R*)-MTPA ester (**11b**), thus indicating that the absolute configurations of the tetrahydrofuran ring portion in amphidinolide T1 (**1**), T2 (**2**), T3 (**3**), and T4 (**4**) were 7*S*, 8*S*, and 10*R*-configurations.

Biosynthesis of Amphidinolide T1 (1). The dinoflagellate *Amphidinium* sp. (strain Y-71) was cultured in a 100 L nutrient-enriched seawater medium, and feeding experiments were carried out with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]sodium acetate and sodium ^{13}C-bicarbonate. In feeding experiments, the dinoflagellate was supplemented with 610 μM of labeled precursors in one portion at 4 days after inoculation, and then the culture was harvested by centrifugation after 14 days. In each case the extracts of the harvested cells were purified by a silica gel column followed by C_{18} HPLC to afford ^{13}C-labeled amphidinolide T1 (**1**) in 0.0006% yield from as an average from wet weight of the cells.

Assignments of ^{13}C NMR signals and isotope incorporation results of **1** derived from ^{13}C-labeled sodium acetate were shown in Table 2 and Figure 8. The ^{13}C NMR spectrum ($CDCl_3$) of **1** derived from [1-^{13}C]sodium acetate showed significant enrichment of seven carbons (C-3, C-5, C-8, C-10, C-14, C-16, and C-20). On the other hand, enrichment by [2-^{13}C]sodium acetate was observed

Table 2. Isotope Incorporation Results Based on the ^{13}C NMR Data of Amphidinolide T1 (1)[a]

position	δ_C	intensity ratio (labeled/unlabeled)[b] [1-^{13}C]acetate	[2-^{13}C]acetate	assignment c or m[c]
1	174.9 s	0.92	1.93	m
2	41.7 d	0.51	2.46	m
3	35.1 t	2.28	1.00	c
4	26.8 t	1.00	2.74	m
5	26.2 t	3.61	0.82	c
6	29.7 t	0.84	2.27	m
7	78.6 d	0.48	2.53	m
8	36.5 d	2.28	0.85	c
9	39.7 t	1.17	2.26	m
10	73.6 d	3.69	0.74	c
11	45.0 t	1.22	2.07	m
12	212.0 s	0.42	1.84	m
13	78.2 d	0.68	2.13	m
14	32.0 d	1.62	0.89	c
15	41.2 t	1.48	2.17	m
16	143.5 s	2.08	1.06	c
17	40.1 t	0.56	2.36	m
18	71.7 d	1.42	1.87	m
19	35.7 t	0.70	2.50	m
20	19.1 t	1.97	1.22	c
21	13.9 q	1.00	2.22	m
22	18.0 q	0.62	2.12	m
23	14.0 q	1.07	2.66	m
24	13.7 q	0.72	3.22	m
25	116.1 t	0.39	2.74	m

[a] The ^{13}C NMR spectra were recorded in C_6D_6 solution at 125 MHz with a sweep width of 35700 Hz using "zgpg30". Numbers of scans for unlabeled and labeled **1** were 24 000 and 8000, respectively. [b] Intensity of each peak in the labeled **1** divided by that of the corresponding signal in the unlabeled **1**, normalized to give a ratio of 1 for unenriched peak (C-4 for [1-^{13}C]acetate labeling and C-3 for [2-^{13}C]acetate labeling). [c] c denotes the carbon derived from C-1 of acetate, while m indicates the carbon derived from C-2 of acetate.

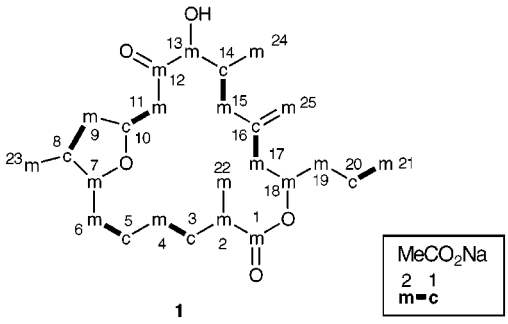


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Figure 8. Labeling patterns of amphidinolide T1 (**1**) resulting from feeding experiments with ^{13}C-labeled acetates.

for 18 carbons (C-1, C-2, C-4, C-6, C-7, C-9, C-11, C-12, C-13, C-15, C-17, C-18, C-19, C-21, C-22, C-23, C-24, and C-25). Thus, all 25 carbon signals contained in **1** were shown to be labeled by acetates. The ^{13}C NMR spectrum of **1** labeled with [1,2-$^{13}C_2$]sodium acetate showed enriched carbon signals flanked by two strong satellite signals. One-bond J_{CC} coupling constants indicated the definite incorporation of 10 acetate units for C-3/C-4 (34.5 Hz), C-5/C-6 (34.8 Hz), C-8/C-9 (32.4 Hz), C-10/C-11 (37.7 Hz), C-14/C-15 (34.4 Hz), C-16/C-17 (41.7 Hz), and C-20/C-21 (34.9 Hz). This was also supported by one-bond ^{13}C−^{13}C correlations observed in the INADEQUATE spectrum of **1** labeled with [1,2-$^{13}C_2$]sodium acetate. These results suggested that four parts from C-3 to C-6, from C-8 to C-11, from C-14 to C-17, and C-20 to C-21 were likely to be classical polyketide chains derived from two, two, two,

and one acetate units, respectively. Three irregular labeling patterns (**m**−**m**) derived only from C-2 of acetates were observed for C-1/C-2, C-12/C-13, and C-18/C-19, and an isolated C_1 unit from C-2 of acetates was observed for C-7. The C_1 branches of C-22, C-23, C-24, and C-25 were all derived from C-2 of acetates, in which the carbonyl carbons were lost.

These incorporation patterns suggested that amphidinolide T1 (**1**) was generated from four successive polyketide chains, an isolated C_1 unit from C-2 of acetates, and three unusual "**m**−**m**" units derived only from C-2 of acetates. Furthermore, it is noted that five oxygenated carbons of C-1, C-7, C-12, C-13, and C-18 were not derived from the C-1 carbonyl but from the C-2 methyl of acetates. The previous biosynthetic studies of 15- and 27-, and 26-membered macrolides, amphidinolides J, G, and H, have revealed that these are also generated through nonsuccessive mixed polyketides.[8,9] The labeling patterns "**m**−**m**(**m**)" of C-1-C-2(C-22) in amphidinolide T1 (**1**) corresponded to that of C-1-C-2(C-27) part in amphidinolides G and H. These unusual labeling patterns have been reported for okadaic acid and DTX-4,[10] isolated from dinoflagellates of other genera.

On the other hand, all carbon atoms in amphidinolide T1 (**1**) were labeled from ^{13}C-labeled sodium bicarbonate with relatively high incorporation ratio (ca. 33%), which was estimated on the basis of the ratio of intensity of proton signals and its satellite peaks, compared with those of ^{13}C-labeled acetates (2−3%). These results indicated that a main carbon source of amphidinolide T1 (**1**) was derived from carbon dioxide, and acetates were minor carbon sources. Sodium bicarbonate might be converted into carbon dioxide, which might be utilized via photosynthesis in chloroplast for biosynthsis of amphidinolide T1 (**1**). However, a small part of exogenously applied acetate may be incorporated into chloroplast, and converted into acetyl-CoA, which could be utilized for biosynthesis of **1**. These phenomena have been previously reported for the biosynthesis of fatty acids in diatoms.[11]

Cytotoxicity. Amphidinolides T1 (**1**), T2 (**2**), T3 (**3**), and T4 (**4**) exhibited cytotoxicity against murine lymphoma L1210 (IC_{50}: 18, 10, 7.0, and 11 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50}: 35, 11.5, 10, and 18 μg/mL, respectively).

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 2.5 mm micro cells for $CDCl_3$ (Shigemi Co., Ltd., Japan). FABMS spectra were recorded using diethanolamine (for **2**−**4** and their MTPA esters) or *p*-nitrobenzyl alcohol (for degradation products and synthetic products) as a matrix in positive mode. EIMS spectra were measured at 70 eV. Positive mode electrosplay ionization (ESI) mass spectra were measured using samples dissolved in MeOH with flow rate of 0.1 mL/min.

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain number Y-71) was isolated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for two weeks in a seawater medium enriched with 1% ES supplement. The harvested cells of the cultured dinoflagellate (1200 g wet weight, from 1300 L of culture) were extracted with MeOH/toluene (3:1, 600 mL × 5). After addition of 1 M NaCl aq (500 mL), the mixture was extracted with toluene (500 mL × 3). Parts (3.30 g) of the toluene-soluble materials (6.63 g) were subjected to silica gel ($CHCl_3$/MeOH, 98:2) and C_{18} column chromatographies (MeOH/H_2O, 8:2) to give a fraction (31.5 mg) containing some macrolides. The fraction was separated by C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical Co. Ltd., 10 × 250 mm; eluent, CH_3CN/H_2O (65:35); flow rate, 2.5 mL/min; UV detection at 210 nm] to afford amphidinolides T2 (**2**, 0.8 mg, 0.00013%, wet weight, t_R 22.4 min), B (0.0017%), C (0.0012%), and T1 (**1**, 0.00092%). Cultivation and separation of the dinoflagellate *Amphidinium* sp. (strain number Y-56) was previously reported. The toluene-soluble materials (3.67 g) obtained from the extract of the mass-cultured dinoflagellate (420 g wet weight, from 580 L of culture) were subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) and a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8:2) followed by C_{18} HPLC [LUNA C18(2), 5 μm, Phenomenex, 10 × 250 mm; eluent, CH_3CN/H_2O (75:25); flow rate, 2.5 mL/min; UV detection at 210 nm] to afford amphidinolides T3 (**3**, 1.5 mg, 0.0004%, wet weight, t_R 34.5 min), T4 (**4**, 1.0 mg, 0.0002%, t_R 27.5 min), C (0.0009%), F (0.0006%), T1 (**1**, 0.005%), and U (0.0002%).

Amphidinolide T2 (2): a colorless oil; IR (KBr) ν_{max} 3400, 2900, and 1720 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 453 $(M + H)^+$; HRFABMS *m*/*z* 453.3187 [calcd for $C_{26}H_{48}O_6$ $(M + H)^+$, 453.3206].

Amphidinolide T3 (3): a colorless oil; IR (KBr) ν_{max} 3450, 2935, and 1720 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 405 $(M + H - H_2O)^+$, 423 $(M + H)^+$, and 445 $(M + Na)^+$; HRFABMS *m*/*z* 423.3110 [calcd for $C_{25}H_{43}O_5$ $(M + H)^+$, 423.3111].

Amphidinolide T4 (4): a colorless oil; IR (KBr) ν_{max} 3450, 2935, and 1720 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 405 $(M + H - H_2O)^+$, 423 $(M + H)^+$, and 445 $(M + Na)^+$; HRFABMS *m*/*z* 423.3131 [calcd for $C_{25}H_{43}O_5$ $(M + H)^+$, 423.3111].

(*S*)-(−)-MTPA Ester (5a) of Amphidinolide T3 (3). To a CH_2Cl_2 solution (50 μL) of amphidinolide T3 (**3**, 0.3 mg) were added 4-(dimethylamino)pyridine (10 μg), triethylamine (3 μL), and (*R*)-(−)-MTPACl (0.5 μL) at room temperature, and stirring was continued for 2 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (2 μL) and evaporation of solvent, the residue was passed through a silica gel column (hexane/EtOAc, 4:1) to afford the (*S*)-(−)-MTPA ester (**5a**, 0.2 mg) of **3**. **5a**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 7.0 Hz, H_3-23), 0.90 (3H, t, J = 7.3 Hz, H_3-21), 0.93 (3H, d, J = 6.6 Hz, H_3-24), 1.14 (3H, d, J = 7.0 Hz, H_3-22), 1.25−1.60 (12 H), 1.63 (1H, m, H-9), 1.72 (1H, dt, J = 15.0, 7.8 Hz, H-9), 1.90 (1H, dd, J = 8.7, 13.5 Hz, H-15), 2.07 (1H, m, H-11), 2.11 (1H, m, H-11), 2.14 (1H, dd, J = 8.5, 13.5 Hz, H-17), 2.22 (1H, m, H-8), 2.37 (1H, dd, J = 5.5, 13.5 Hz, H-17), 2.41 (1H, m, H-2), 2.61 (1H, brd, J = 13.5 Hz, H-15), 3.13 (1H, m, H-14), 3.58 (3H, s, OCH_3), 3.86 (1H, m, H-7), 4.09 (1H, m, H-10), 4.83 (1H, s, H-25), 4.91 (1H, s, H-25), 4.96 (1H, m, H-18), 5.51 (1H, t, J = 5.3 Hz, H-12), 7.40 (3H, m), and 7.58 (2H, m); FABMS *m*/*z* 639 $(M + H)^+$; HRFABMS *m*/*z* 639.3488 [calcd for $C_{35}H_{49}O_7F_3$ $(M + H)^+$, 639.3508].

(*R*)-(+)-MTPA Ester (5b) of Amphidinolide T3 (3). Amphidinolide T3 (**3**, 0.3 mg) was treated with (*S*)-(+)-MTPACl (0.5 μL) by the same procedure as described above to afford the (*R*)-(+)-MTPA ester (**5b**, 0.2 mg) of **3**. **5b**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, d, J = 7.0 Hz, H_3-23), 0.92 (3H, t, J = 7.3 Hz, H_3-21), 0.98 (3H, d, J = 6.6 Hz, H_3-24), 1.16 (3H, d, J = 7.0 Hz, H_3-22), 1.25−1.65 (14 H), 1.92 (1H, dd, J = 8.7, 13.5 Hz, H-15), 1.98 (1H, m, H-11), 2.05 (1H, m, H-8), 2.08 (1H, m, H-11), 2.18 (1H, dd, J = 7.5, 13.5 Hz, H-17), 2.40 (1H, m, H-17), 2.43 (1H, m, H-2), 2.71 (1H, brd, J = 13.5 Hz, H-15), 3.12 (1H, m, H-14), 3.68 (3H, s, OCH_3), 3.81 (1H, m, H-7), 4.07 (1H, m, H-10), 4.88 (1H, s, H-25), 4.92 (1H, s, H-25),

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4.99 (1H, m, H-18), 5.46 (1H, t, J = 5.3 Hz, H-12), 7.43 (3H, m), and 7.68 (2H, m); FABMS m/z 639 $(M+H)^+$; HRFABMS m/z 639.3507 [calcd for $C_{35}H_{49}O_7F_3$ $(M + H)^+$, 639.3508].

Oxidative Degradation of Amphidinolide T3 (3). Amphidinolide T3 (**3**, 0.2 mg) was dissolved in THF (20 μL) and treated with $LiAlH_4$ (0.8 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 $NaIO_4$ (0.5 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 $NaBH_4$ (0.3 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) followed by C_{18} HPLC (Wakosil-II 5C18 RS, Wako Pure Chemical Ind., Ltd., 4.6 × 250 mm; eluent CH_3CN/H_2O, 90:10; flow rate, 1.0 mL/min; UV detection at 230 nm) to give compounds **6a** (0.08 mg, t_R 16.0 min) and **7a** (0.05 mg, t_R 13.6 min). **6a**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz, H_3-23), 0.91 (3H, d, J = 6.7 Hz, H_3-22), 1.1−1.45 (8H, m, H_2-3, H_2-4, H_2-5, and H_2-6), 1.69 (2H, m, H_2-9), 1.78−1.93 (3H, m, H-2 and H_2-11), 2.19 (1H, m, H-8), 3.54 (6H, s, 2 × OCH_3), 3.79 (1H, m, H-7), 4.05 (1H, m, H-10), 4.06 (1H, dd, J = 6.7, 10.7 Hz, H-1), 4.23 (1H, dd, J = 5.6, 10.7 Hz, H-1), 4.41 (2H, t, J = 6.9 Hz, H_2-12), 7.35−4.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); FABMS m/z 699 $(M + Na)^+$; HRFABMS m/z 699.2720 [calcd for $C_{34}H_{42}O_7F_6Na$ $(M + Na)^+$, 699.2732]. **7a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.3 Hz, H_3-24), 0.90 (3H, t, J = 7.5 Hz, H_3-21), 1.28−1.40 (2H, m, H_2-20), 1.56 (1H, m, H-19), 1.62 (1H, m, H-19), 1.84 (1H, m, H-15), 1.97−2.05 (1H, m, H-14 and H-15), 2.17 (1H, dd, J = 6.0, 14.2 Hz, H-17), 2.28 (1H, dd, J = 7.6, 14.2 Hz, H-17), 3.52 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 4.09 (1H, dd, J = 5.8, 10.2 Hz, H-13), 4.13 (1H, dd, J = 4.3, 10.2 Hz, H-13), 4.68 (1H, s, H-25), 4.75 (1H, s, H-25), 5.20 (1H, m, H-18), 7.35−4.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); FABMS m/z 641 $(M + Na)^+$; HRFABMS m/z 641.2308 [calcd for $C_{31}H_{36}O_6F_6Na$ $(M + Na)^+$, 641.2314].

(*S*)-(−)-MTPA Ester (8a) of Amphidinolide T4 (4). Amphidinolide T4 (**4**, 0.2 mg) was treated with (*R*)-(−)-MTPACl (0.5 μL) by the same procedure as described above to afford the (*S*)-(−)-MTPA ester (**8a**, 0.2 mg) of **4**. **8a**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.78 (3H, d, J = 7.1 Hz, H_3-23), 0.90 (3H, t, J = 7.3 Hz, H_3-21), 1.17 (3H, d, J = 7.0 Hz, H_3-22), 1.19 (3H, d, J = 6.8 Hz, H_3-24), 1.25−1.65 (14 H), 1.73 (1H, m, H-11), 1.92 (1H, m, H-11), 2.08 (1H, m, H-15), 2.12 (1H, m, H-8), 2.17 (1H, m, H-17), 2.37 (1H, dd, J = 5.1, 13.5 Hz, H-17), 2.43 (1H, m, H-2), 2.47 (1H, m, H-15), 3.13 (1H, m, H-14), 3.63 (3H, s, OCH_3), 3.80 (1H, m, H-7), 3.96 (1H, m, H-10), 4.90 (1H, s, H-25), 4.91 (1H, s, H-25), 4.92 (1H, m, H-18), 5.49 (1H, brd, J = 8.6 Hz, H-12), 7.42 (3H, m), and 7.65 (2H, m); FABMS m/z 639 $(M + H)^+$; HRFABMS m/z 639.3523 [calcd for $C_{35}H_{49}O_7F_3$ $(M + H)^+$, 639.3508].

(*R*)-(+)-MTPA Ester (8b) of Amphidinolide T4 (4). Amphidinolide T4 (**4**, 0.2 mg) was treated with (*S*)-(+)-MTPACl (0.5 μL) by the same procedure as described above to afford the (*R*)-(+)-MTPA ester (**8b**, 0.2 mg) of **4**. **8b**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 7.0 Hz, H_3-23), 0.90 (3H, t, J = 7.3 Hz, H_3-21), 1.17 (3H, d, J = 7.0 Hz, H_3-22), 1.17 (3H, d, J = 6.8 Hz, H_3-24), 1.25−1.65 (12 H), 1.70−1.82 (3H), 1.96 (1H, m, H-11), 2.06 (1H, dd, J = 8.7, 13.5 Hz, H-15), 2.16 (1H, m, H-8), 2.17 (1H, m, H-17), 2.36 (1H, m, H-17), 2.43 (1H, m, H-2), 2.46 (1H, brd, J = 13.5 Hz, H-15), 3.12 (1H, m, H-14), 3.54 (3H, s, OCH_3), 3.84 (1H, m, H-7), 4.19 (1H, m, H-10), 4.89 (2H, s, H_2-25), 4.92 (1H, m, H-18), 5.52 (1H, t, J = 5.3 Hz, H-12), 7.39 (3H, m), and 7.57 (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].

Oxidative Degradation of Amphidinolide T4 (4). Amphidinolide T4 (**4**, 0.2 mg) was treated with $LiAlH_4$, $NaIO_4$, $NaBH_4$, and (*R*)−(−)-MTPACl by the same procedure as described above to afford compounds **6a** (0.07 mg, t_R 16.0 min) and **7a** (0.03 mg, t_R 13.6 min). **6a**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz, H_3-23), 0.91 (3H, d, J = 6.7 Hz, H_3-22), 1.1−1.45 (8H, m, H_2-3, H_2-4, H_2-5, and H_2-6), 1.69 (2H, m, H_2-9), 1.78−1.93 (3H, m, H-2 and H_2-11), 2.19 (1H, m, H-8), 3.54 (6H, s, 2 × OCH_3), 3.79 (1H, m, H-7), 4.05 (1H, m, H-10), 4.06 (1H, dd, J = 6.7, 10.7 Hz, H-1), 4.23 (1H, dd, J = 5.6, 10.7 Hz, H-1), 4.41 (2H, t, J = 6.8 Hz, H_2-12), 7.35−4.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); FABMS m/z 699 $(M + Na)^+$; HRFABMS m/z 699.2754 [calcd for $C_{34}H_{42}O_7F_6Na$ $(M + Na)^+$, 699.2732]. **7a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.3 Hz, H_3-24), 0.90 (3H, t, J = 7.5 Hz, H_3-21), 1.28−1.40 (2H, m, H_2-20), 1.56 (1H, m, H-19), 1.62 (1H, m, H-19), 1.84 (1H, m, H-15), 1.97−2.05 (1H, m, H-14, H-15), 2.17 (1H, dd, J = 6.0, 14.2 Hz, H-17), 2.28 (1H, dd, J = 7.6, 14.2 Hz, H-17), 3.52 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 4.09 (1H, dd, J = 5.8, 10.2 Hz, H-13), 4.13 (1H, dd, J = 4.3, 10.2 Hz, H-13), 4.68 (1H, s, H-25), 4.75 (1H, s, H-25), 5.20 (1H, m, H-18), 7.35−4.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); FABMS m/z 641 $(M + Na)^+$; HRFABMS m/z 641.2284 [calcd for $C_{31}H_{36}O_6F_6Na$ $(M + Na)^+$, 641.2314].

Bis-(*S*)-(−)-MTPA Ester (9a) of Amphidinolide T2 (2). Amphidinolide T2 (**2**, 0.1 mg) was treated with (*R*)-(−)-MTPACl (0.3 μL) by the same procedure as described above to afford the bis-(*S*)-(−)-MTPA ester (**9a**, 0.2 mg) of **4**. **9a**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 7.0 Hz, H_3-24), 0.92 (3H, J = 6.7 Hz, H_3-25), 1.12 (3H, d, J = 6.9 Hz, H_3-23), 1.26 (3H, d, J = 6.1 Hz, H_3-22), 1.25−1.75 (14 H), 1.88 (1H, dd, J = 10.9, 14.5 Hz, H-15), 2.06 (1H, dt, 14.6 and 4.4 Hz, H-11), 2.10−2.15 (2H, m, H-11 and H-17), 2.22 (1H, m, H-8), 2.36 (1H, dd, J = 6.9, 13.9 Hz, H-17), 2.41 (1H, m, H-2), 2.58 (1H, br.d, J = 14.5 Hz, H-15), 3.10 (1H, m, H-14), 3.53 (3H, s, OCH_3), 3.56 (3H, s, OCH_3), 3.85 (1H, m, H-7), 4.07 (1H, m, H-10), 4.82 (1H, s, H-25), 4.88 (1H, s, H-25), 4.95 (1H, m, H-18), 5.11 (1H, m, H-21), 5.50 (1H, t, J = 5.2 Hz, H-12), 7.38−7.43 (6H, m), 7.51 (2H, m), and 7.59 (2H, m); ESIMS m/z 907 $(M + Na)^+$; HRESIMS m/z 907.3822 [calcd for $C_{46}H_{58}O_{10}F_6Na$ $(M + Na)^+$, 907.3832].

Bis-(*R*)-(+)-MTPA Ester (9b) of Amphidinolide T2 (2). Amphidinolide T2 (**2**, 0.1 mg) was treated with (*S*)-(+)-MTPACl (0.3 μL) by the same procedure as described above to afford the bis-(*R*)−(+)-MTPA ester (**9b**, 0.1 mg) of **4**. **9b**: a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, d, J = 7.0 Hz, H_3-24), 0.97 (3H, d, J = 6.6 Hz, H_3-25), 1.12 (3H, d, J = 6.9 Hz, H_3-23), 1.34 (3H, d, J = 6.3 Hz, H_3-22), 1.23−1.60 (10 H), 1.67 (1H, m, H-20), 1.87 (1H, m, H-15), 1.97 (1H, dt, J = 15.5, 6.0 Hz, H-11), 2.01−2.11 (3H, m, H-8, H-11, and H-17), 2.34 (1H, dd, J = 6.5, 14.0 Hz, H-17), 2.41 (1H, m, H-2), 2.65 (1H, br.d, J = 14.3 Hz, H-15), 3.07 (1H, m, H-14), 3.55 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.79 (1H, m, H-7), 4.06 (1H, m, H-10), 4.84 (1H, s, H-25), 4.86 (1H, s, H-25), 4.93 (1H, m, H-18), 5.13 (1H, m, H-21), 5.43 (1H, t, J = 5.4 Hz, H-12), 7.36−7.45 (6H, m), 7.52 (2H, m), and 7.67 (2H, m); ESIMS m/z 907 $(M + Na)^+$; HRESIMS m/z 907.3821 [calcd for $C_{46}H_{58}O_{10}F_6Na$ $(M + Na)^+$, 907.3832].

Oxidative Degradation of Amphidinolide T2 (2). Amphidinolide T2 (**2**, 0.2 mg) was dissolved in THF (30 μL) and treated with $LiAlH_4$ (1.0 mg) at room temperature for 1 h. The reaction mixture was partitioned between EtOAc (200 μL × 3) and 1M 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, 30 μL) was added $NaIO_4$ (0.2 mg), and the mixture was stirred at room temperature for 1 h. 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 (30 μL) was added $NaBH_4$ (0.2 mg) at 0 °C, and stirring was continued for 30 min. After addition of 1M phosphate buffer (100 μL), the mixture was partitioned between EtOAc (200 μL × 3). The organic phase was evaporated to give the residue

(0.5 mg), parts (0.25 mg) of which were dissolved in 0.1% DMAP solution in CH_2Cl_2 (15 μL). To the mixture were added Et_3N (2 μL) and (*R*)-(−)-MTPACl (1 μL), and stirring was continued at room temperature for 6 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (1 μL), the reaction mixture was partitioned between EtOAc (200 μL × 3) and 1 M phosphate buffer (100 μL), and then the organic layer was evaporated in vacuo. The residue was subjected to C_{18} HPLC (Wakosil-II 5C18 RS, 4.6 × 250 mm; eluent CH_3CN/H_2O, 83:17; flow rate, 1.0 mL/min; UV detection at 230 nm) to give compounds **6a** (0.05 mg, t_R 15.6 min) and **10a** (0.04 mg, t_R 20 min). **6a**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz, H_3-23), 0.91 (3H, d, J = 6.7 Hz, H_3-22), 1.1−1.45 (8H, m, H_2-3, H_2-4, H_2-5, and H_2-6), 1.69 (2H, m, H_2-9), 1.78−1.93 (3H, m, H-2 and H_2-11), 2.19 (1H, m, H-8), 3.54 (6H, s, 2 × OCH_3), 3.79 (1H, m, H-7), 4.05 (1H, m, H-10), 4.06 (1H, dd, J = 6.7 and 10.7 Hz, H-1), 4.23 (1H, dd, J = 5.6 and 10.7 Hz, H-1), 4.41 (2H, t, J = 6.8 Hz, H_2-12), 7.35−7.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); ESIMS *m*/*z* 699 $(M + Na)^+$; HRESIMS *m*/*z* 699.2725 [calcd for $C_{34}H_{42}O_7F_6Na$ $(M + Na)^+$, 699.2732]. **10a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.6 Hz, H_3-25), 1.23 (3H, t, J = 7.5 Hz, H_3-22), 1.56−1.72 (4H, m, H_2-19 and H_2-20), 1.84 (1H, dd, J = 6.3, 12.9 Hz, H-15), 1.96−2.04 (2H, m, H-14 and H-15), 2.14 (1H, dd, J = 5.7, 14.7 Hz, H-17), 2.30 (1H, dd, J = 7.5, 14.7 Hz, H-17), 3.47 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 4.09 (1H, dd, J = 6.0, 10.8 Hz, H-13), 4.13 (1H, dd, J = 4.8, 10.8 Hz, H-13), 4.70 (1H, s, H-26), 4.75 (1H, s, H-26), 5.10 (1H, m, H-21), 5.20 (1H, m, H-18), 7.32−7.40 (9H, m, Ph), and 7.44−7.54 (6H, m, Ph); ESIMS *m*/*z* 887 $(M + Na)^+$; HRESIMS *m*/*z* 887.2844 [calcd for $C_{42}H_{45}O_9F_9Na$ $(M + Na)^+$, 887.2818]. Crude products (0.25 mg) obtained after reactions with $LiAlH_4$, $NaIO_4$, and $NaBH_4$ of **2** described above were treated with DMAP (15 ng), Et_3N (2 μL), and (*S*)-(+)-MTPACl (1 μL) in CH_2Cl_2 at room temperature for 6 h. Workup of the reaction mixture was carried out by the same procedure described above to afford compounds **6b** (0.18 mg, t_R 16.4 min) and **10b** (0.12 mg, t_R 20.6 min). **6b**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 7.0 Hz, H_3-24), 0.91 (3H, d, J = 6.8 Hz, H_3-23), 1.1−1.45 (8H, m, H_2-3, H_2-4, H_2-5, and H_2-6), 1.69 (2H, m, H_2-9), 1.76−1.91 (3H, m, H-2 and H_2-11), 2.19 (1H, m, H-8), 3.52 (6H, s, 2 × OCH_3), 3.79 (1H, m, H-7), 4.05 (1H, m, H-10), 4.15 (2H, m, H_2-1), 4.39 (1H, m, H-12), 4.44 (1H, m, H-12), 7.35−7.43 (6H, m, Ph), and 7.48−7.54 (4H, m, Ph); ESIMS *m*/*z* 699 $(M + Na)^+$; HRESIMS *m*/*z* 699.2739 [calcd for $C_{34}H_{42}O_7F_6Na$ $(M + Na)^+$, 699.2732]. **10b**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 6.6 Hz, H_3-25), 1.23 (3H, d, J = 6.3 Hz, H_3-22), 1.33 (1H, m, H-20), 1.45−1.54 (3H, m, H_2-19 and H-20), 1.88 (1H, m, H-15), 2.01−2.09 (2H, m, H-14 and H-15), 2.12 (1H, dd, J = 5.7, 14.7 Hz, H-17), 2.32 (1H, dd, J = 7.5, 14.5 Hz, H-17), 3.49 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 4.02 (1H, dd, J = 6.0, 10.8 Hz, H-13), 4.25 (1H, dd, J = 4.8, 10.8 Hz, H-13), 4.80 (1H, s, H-26), 4.81 (1H, s, H-26), 5.03 (1H, m, H-21), 5.16 (1H, m, H-18), 7.35−7.43 (9H, m, Ph), and 7.48−7.54 (6H, m, Ph); ESIMS *m*/*z* 887 $(M + Na)^+$; HRESIMS *m*/*z* 887.2820 [calcd for $C_{42}H_{45}O_9F_9Na$ $(M + Na)^+$, 887.2818].

(2*R*,3*S*,5*RS*)-5-Acetyloxy-3-methyl-2-[2-(trimethylsilyl)-ethoxymethoxy]oct-7-ene (13). To a solution of (2*R*,3*R*)-1-iodo-2-methyl-3-[2-(trimethylsilyl)ethoxymethoxy]butane (**12**, 512 mg, 1.48 mmol) in DMSO (10 mL) was added sodium cyanide (214 mg, 4.44 mmol) at room temperature, and stirring was continued at 70 °C for 4 h. After addition of Et_2O and H_2O, the reaction mixture was extracted with Et_2O. The extract was washed with brine, dried with $MgSO_4$, and evaporated in vacuo to afford a crude cyanide (359 mg). To a stirred solution of the crude cyanide (359 mg) was added dropwise a 0.95 M solution of DIBAL in hexane (1.86 mL, 1.77 mmol) at −78 °C. After being stirred for 1 h, the reaction mixture was treated with MeOH (300 μL), allowed to warm to room temperature. After addition of saturated aqueous NH_4Cl (10 mL), the mixture was vigorously stirred for 20 min. Et_2O and saturated aqueous potassium sodium tartrate were added to the mixture, and stirring was continued at room temperature for 1 h. The reaction mixture was extracted with Et_2O, and the extract was washed with H_2O and then brine, dried with $MgSO_4$, evaporated in vacuo to give a crude aldehyde (336 mg). To a stirred solution of the crude aldehyde (336 mg) in Et_2O (10 mL) was added a 1 M Et_2O solution of allylmagnesium bromide (5.7 mL, 5.7 mmol) at −10 °C. After be stirred at room temperature for 14 h, saturated aqueous NH_4Cl and Et_2O were added to the mixture, which was extracted with Et_2O. The extract was washed with H_2O and brine, dried with $MgSO_4$, and evaporated in vacuo to afford a crude alcohol (315 mg). The crude alcohol (315 mg) was treated with Ac_2O (2 mL) and pyridine (2 mL) at room temperature for 6 h. After evaporation of the solvent, the resulting residue was subjected to silica gel column chromatography (hexane/EtOAc, 20:1) to yield compound **13** (273 mg, 826 μmol, 56% by four steps) as a colorless oil: $[\alpha]_D$ −24° (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3079, 2954, 1739, 1375, 1238, 1103, 1031, 936, and 919 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.01 (9H, s), 0.87−0.96 (5H, m), 1.06 (1.5 H, d, J = 6.6 Hz), 1.08 (1.5H, d, J = 6.6 Hz), 1.24 (0.5H, m), 1.37 (0.5H, m), 1.62−1.78 (1H, m), 2.01 (3H, s), 2.21−2.37 (3H, m), 3.50−3.65 (3H, m), 4.63 (1H, m), 4.67 (0.5H, d, J = 16.5 Hz), 4.69 (0.5H, d, J = 16.5 Hz), 4.97−5.07 (3H, m), and 5.73 (1H, m); ^{13}C NMR ($CDCl_3$) δ −1.5 (3C, q), 14.7 (0.5C, q), 15.1 (0.5C, q), 15.8 (0.5C, q), 16.3 (0.5C, q), 18.1 (1C, t), 21.1 (0.5C, q), 21.2 (0.5C, q), 34.4 (0.5C, t), 34.9 (0.5C, t), 36.6 (0.5C, d), 36.6 (0.5C, d), 38.5 (0.5C, t), 39.6 (0.5C, t), 65.0 (1C, t), 71.1 (0.5C, d), 72.2 (0.5C, d), 76.2 (0.5C, d), 76.6 (0.5C, d), 93.28 (0.5C, t), 93.34 (0.5C, t), 117.7 (1C, t), 133.6 (0.5C, d), 133.7 (0.5C, d), and 170.7 (1C, s); FABMS *m*/*z* 331 $(M + H)^+$; HRFABMS *m*/*z* 331.2312 [calcd for $C_{17}H_{34}O_4Si$ $(M + H)^+$, 331.2305].

(2*R*,3*S*,5*RS*)-5-Acetyloxy-3-methyl-2-[2-(trimethylsilyl)-ethoxymethoxy]heptan-7-ol (14). Compound **13** (294 mg, 889 μmol) was dissolved in a 1:1:1 mixture of H_2O, acetone, and CH_3CN (3 mL), and to this mixture were added microencapsulated OsO_4 (220 mg) and 4-methymorpholine *N*-oxide (270 mg, 2.32 mmol). After stirring at room temperature for 64 h, insoluble materials were filtered, and the filtrate was evaporated in vacuo to give a residue. This was purified by a silica gel column (hexane/acetone, 2:1) to afford a mixture of 1, 2-diols (248 mg, 680 μmol, 76%), and compound 13 (54 mg, 163 μmol, 18%) was recovered. To a stirring solution of the mixture (247 mg, 678 μmol) in THF (1 mL) and 1M potassium phosphate buffer (pH 7.0: 1 mL) was added $NaIO_4$ (339 mg, 1.58 mmol) at 0 °C. After stirring at 0 °C for 1 h, insoluble materials were filtered, and the filtrate was evaporated in vacuo to give a residue (254 mg). To a solution of the residue (254 mg) in EtOH (3 mL) was added $NaBH_4$ (60 mg, 1.58 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. After addition of Et_2O and 1 M aqueous HCl, the mixture was extracted with Et_2O. The extract was washed with saturated aqueous $NaHCO_3$, H_2O, and brine, dried with $MgSO_4$, and evaporated in vacuo to give a crude alcohol. This was subjected to silica gel column chromatography (hexane/EtOAc, 2:1) to afford compound **14** (135 mg, 404 μmol, 60% by two steps) as colorless oil: $[\alpha]_D$ +16° (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3461, 2954, 1740, 1376, 1248, 1055, 860, and 837 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.01 (9H, s), 0.88−0.96 (5H, m), 1.11 (1.5 H, d, J = 6.6 Hz), 1.13 (1.5 H, d, J = 6.6 Hz), 1.22 (0.5H, m), 1.50 (1H, t, J = 7.8 Hz), 1.55−1.88 (3.5H, m), 2.05 (3H, s), 3.48−3.55 (1H, m), 3.62 (2H, m), 3.71 (0.5H, m), 3.78 (0.5H, m), 4.16 (1H, m), 4.29 (1H, m), 4.65 (1H, m), and 4.72 (1H, m); ^{13}C NMR ($CDCl_3$) δ −1.48 (3C, q), 15.3 (0.5C, q), 15.7 (0.5C, q), 16.0 (0.5C, q), 16.7 (0.5C, q), 16.69 (0.5C, t), 16.81 (0.5C, t), 235.2 (0.5C, d), 35.4 (0.5C, d), 36.5 (0.5C, t), 37.4 (0.5C, t), 40.2 (0.5C, t), 40.7 (0.5C, t), 61.8 (0.5C, t), 65.1 (0.5C, t), 65.2 (0.5C, t), 65.9 (0.5C, d), 66.63 (0.5C, d), 76.7 (0.5C, d), 77.2 (0.5C, d), 93.2 (0.5C, t), 93.4 (0.5C, t), 171.3 (0.5C, s), and 171.4 (0.5C, s); FABMS *m*/*z* 335 $(M + H)^+$; HRFABMS *m*/*z* 335.2254 [calcd for $C_{16}H_{35}O_5Si$ $(M + H)^+$, 335.2254].

Compound 15a. To a solution of compound **14** (21.5 mg, 64.3 μmol) in CH_2Cl_2 (500 μL) were added DMAP (0.5 mg, 4 μmol), Et_3N (32 μL, 230 μmol), and (*R*)-(−)-MTPACl (22 μL, 114 μmol), and stirring was continued at room temperature for 3 h. The reaction mixture was partitioned between Et_2O and H_2O, and then the organic phase was washed with brine, dried with $MgSO_4$, and evaporated in vacuo. The residue was purified by a silica gel column (hexane/EtOAc, 10:1) to afford

an (*S*)-(−)-MTPA ester (27.3 mg, 49.6 μmol, 77%). A solution of the (*S*)-(−)-MTPA ester (27.0 mg, 49.0 μmol) in CH_2Cl_2 (3 mL) was treated with trifluoroaceitic acid (1 mL) at 0 °C for 1 h. The reaction mixture was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with brine, dried with $MgSO_4$, and evaporated in vacuo to give a crude product (27 mg). To a solution of the crude product (27 mg) in CH_2Cl_2 (3 mL) were added DMAP (0.5 mg, 4 μmol), Et_3N (50 μL, 358 μmol), methanesulfonyl chloride (20 μL, 25.8 μmol) at 0 °C. After stirring for 4 h, Et_2O and 1N aqueous HCl were added to the mixture, which was extracted with Et_2O. The organic phase was washed with saturated aqueous $NaHCO_3$, H_2O, and brine, dried with $MgSO_4$, and evaporated in vacuo. The residue was subjected to silica gel column chromatography (hexane/EtOAc, 6:4) to afford the mesylate **15a** (11.7 mg, 23.5 μmol, 48% by two steps) as colorless oil: $[\alpha]_D$ −36° (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2944, 1741, 1353, 1238, 1175, 1019, and 905 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (1.5H, d, J = 6.7 Hz), 0.99 (1.5H, d, J = 6.7 Hz), 1.24 (1.5H, d, J = 6.3 Hz), 1.32 (0.5H, m), 1.35 (1.5H, d, J = 6.3 Hz), 1.49 (0.5H, m), 1.59 (0.5H, m), 1.75−2.00 (3.5H, m), 2.02 (1.5H, s), 2.04 (1.5H, s), 2.93 (1.5H, s), 2.98 (1.5H, s), 3.53 (3H, s), 3.85 (0.5H, m), 3.97−4.13 (1.5H, m), 4.56 (0.5H, m), 4.68 (0.5H, m), 5.27 (1H, m), 7.36−7.43 (3H, m), and 7.47−7.54 (2H, m); ^{13}C NMR ($CDCl_3$) δ 15.0 (0.5C, q), 15.13 (0.5C), 17.1 (0.5C), 17.8 (0.5C), 20.8 (1C), 32.3 (0.5C), 33.7 (0.5C), 34.4 (0.5C), 35.0 (0.5C), 35.9 (0.5C), 36.8 (0.5C), 38.55 (0.5C), 38.57 (0.5C), 55.4 (1C), 59.9 (0.5C), 60.2 (0.5C), 71.6 (0.5C), 72.54 (0.5C), 82.4 (0.5C), 82.7 (0.5C), 127.2 (2C), 128.5 (2C), 129.7 (1C), 131.9 (1C), 166.3 (1C), 170.7 (0.5C), and 170.8 (0.5C); FABMS *m*/*z* 521 $(M + Na)^+$; HRFABMS *m*/*z* 521.1415 [calcd for $C_{21}H_{29}F_3O_8SNa$ $(M + Na)^+$, 521.1433].

Compound 15b. Compound **15b** (40.1 mg, 80.4 μmol, 46% by three steps) was obtained from compound **14** (58.2 mg, 174 μmol) by the same procedure using (*S*)-(+)-MTPACl as described above. **15b**: colorless oil; $[\alpha]_D$ +10° (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2943, 1740, 1353, 1237, 1175, 1019, and 906 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (1.5H, d, J = 6.8 Hz), 1.01 (1.5H, d, J = 6.8 Hz), 1.30 (1.5H, d, J = 6.9 Hz), 1.33 (1.5H, d, J = 6.9 Hz), 1.52 (0.5H, m), 1.66−1.80 (2H, m), 1.89−2.00 (2.5H, m), 2.04 (3H, s), 2.98 (1.5H, s), 3.13 (1.5H, s), 3.53 (3H, s), 3.92 (0.5H, m), 4.04 (1H, m), 4.12 (0.5H, m), 4.64 (1H, m), 5.21−5.32 (1H, m), 7.36−7.43 (3H, m), and 7.47−7.54 (2H, m); FABMS *m*/*z* 521 $(M + Na)^+$; HRFABMS *m*/*z* 521.1430 [calcd for $C_{21}H_{29}F_3O_8SNa$ $(M + Na)^+$,521.1433].

Compounds 11a and 16a. A solution of compound **15a** (11.0 mg, 22.1 μmol) in EtOH (1 mL) was treated with K_2CO_3 (6.5 mg, 46 μmol) at room temperature for 72 h. After evaporation of the solvent, the residue was subjected to a silica gel column (hexane/EtOAc, 6:1) to afford a mixture of 11a and 16a (6.1 mg, 16.9 μmol). The mixture was purified by C_{18} HPLC [LUNA C18(2), 5 μm, 10 × 250 mm; eluent, CH_3CN/H_2O (68:32); flow rate, 2.5 mL/min; UV detection at 230 nm] to give compounds **11a** (2.7 mg, 7.5 μmol, 34%, t_R 30 min) and **16a** (2.6 mg, 7.2 μmol, 33%, t_R 33 min). **11a**: colorless oil; $[\alpha]_D$ −32° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 2963, 1748, 1270, 1168, 1122, and 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (3H d, J = 7.0 Hz, H_3-23 or H_3-24), 1.07 (3H, d, J = 6.3 Hz, H_3-6), 1.71 (2H, t, J = 6.8 Hz, H_2-9), 1.81 (1H, m, H-11), 1.87 (1H, m, H-11), 2.20 (1H, m, H-8), 3.55 (3H, s, OCH_3), 4.04−4.11 (2H, m, H-7 and H-10), 4.42 (2H, t, J = 6.8 Hz, H_2-12), 7.40 (3H, m, Ph), and 7.51 (2H, m, Ph); EIMS *m*/*z* 189, 289, 304, and 359 $(M - H)^-$; HREIMS *m*/*z* 359.1488 [calcd for $C_{18}H_{22}F_3O_4$ $(M - H)^-$, 359.1470]. **16a**: colorless oil; $[\alpha]_D$ −16° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 2960, 1749, 1271, 1170, 1121, and 1023 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (3H, d, J = 7.0 Hz, H_3-23 or H_3-24), 1.06 (3H, d, J = 6.4 Hz, H_3-6), 1.15 (1H, dt, J = 12.3, 8.5 Hz, H-9), 1.92 (2H, m, H-9 and H-11), 2.09 (1H, dt, J = 12.3, 7.3 Hz, H-11), 2.24 (1H, m, H-8), 3.55 (3H, s, OCH_3), 3.78 (1H, m, H-10), 3.95 (1H, quint, J = 6.4 Hz, H-7), 4.36−4.48 (2H, m, H_2-12), 7.40 (3H, m, Ph), and 7.51 (2H, m, Ph); EIMS *m*/*z* 189, 289, 304, and 359 $(M - H)^-$; HREIMS *m*/*z* 359.1482 [calcd for $C_{18}H_{22}F_3O_4$ $(M - H)^-$, 359.1470].

Compounds 11b and 16b. Compounds **11b** (2.6 mg, 7.2 μmol, 34%, t_R 30 min) and **16b** (2.3 mg, 6.4 μmol, 31%, t_R 33 min) were obtained from compound **15b** (10.4 mg, 20.9 μmol) by the same procedure as described above. **11b**: colorless oil; $[\alpha]_D$ +32° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 2966, 1748, 1271, 1169, 1122, and 1024 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 7.0 Hz, H_3-23 or H_3-24), 1.13 (3H, d, J = 6.3 Hz, H_3-6), 1.70 (2H, t, J = 6.8 Hz, H_2-9), 1.81 (1H, m, H-11), 1.87 (1H, m, H-11), 2.20 (1H, m, H-8), 3.55 (3H, s, OCH_3), 4.02−4.11 (2H, m, H-7 and H-10), 4.35−4.47 (2H, m, H_2-12), 7.40 (3H, m, Ph), and 7.51 (2H, m, Ph); EIMS *m*/*z* 189, 289, 304, and 359 $(M - H)^-$; HREIMS *m*/*z* 359.1462 [calcd for $C_{18}H_{22}F_3O_4$ $(M - H)^-$, 359.1470]. **16b**: colorless oil; $[\alpha]_D$ +49° (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 2963, 1748, 1270, 1169, 1120, and 1022 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (3H, d, J = 7.0 Hz, H_3-23 or H_3-24), 1.08 (3H, d, J = 6.5 Hz, H_3-6), 1.16 (1H, dt, J = 12.3, 8.4 Hz, H-9), 1.93 (2H, m, H-9 and H-11), 2.08 (1H, dt, J = 12.3, 7.3 Hz, H-11), 2.24 (1H, m, H-8), 3.35 (3H, s, OCH_3), 3.79 (1H, m, H-10), 3.95 (1H, quint, J = 6.4 Hz, H-7), 4.42 (2H, t, J = 6.8 Hz, H_2-12), 7.40 (3H, m, Ph), and 7.52 (2H, m, Ph); EIMS *m*/*z* 189, 289, 304, and 359 $(M - H)^-$; HREIMS *m*/*z* 359.1472 [calcd for $C_{18}H_{22}F_3O_4$ $(M - H)^-$, 359.1470].

General Feeding Experiments of ^{13}C-Labeled Precursors. The dinoflagellate cultured in a 100 L nutrient-enriched seawater medium was supplemented with [1-^{13}C]-, [2-^{13}C]-, or [1,2-$^{13}C_2$]sodium acetate (610 μM) in one portion at 7 days after inoculation, and then the culture was harvested by centrifugation after 14 days. Sodium ^{13}C-bicarbonate (1.2 mM) was added to 100 L culture of the dinoflagellate at 10 days, and then the culture was harvested by centrifugation after 14 days. Extraction and isolation of amphidinolide T1 (**1**) from the harvested cells were carried out by the same procedure as described above. The ^{13}C-labeled amphidinolide T1 (**1**) was obtained in 0.0006% yield as an average from wet weight of the cells.

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

Supporting Information Available: NMR spectra of **2**, **3**, and **4**, ^1H NMR spectra of **6a**,**b**, **10a**,**b**, and **11a**,**b**, and ^{13}C NMR and INADEQUATE spectra of 13C-labeled **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO005607C