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Amphidinolide T5, a new 19-membered macrolide from a dinoflagellate and X-ray structure of amphidinolide T1

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Abstract—Amphidinolide T5 (**1**), a new 19-membered macrolide related to amphidinolide T1 (**2**), has been isolated from a marine dinoflagellate *Amphidinium* sp. The structure of **1** was elucidated on the basis of spectroscopic data and chemical means. The stereostructure of amphidinolide T1 (**2**) was confirmed by a single crystal X-ray diffraction analysis.

1. Introduction

Amphidinolide T1 (**2**) is a unique 19-membered macrolide possessing a tetrahydrofuran ring, an *exo*-methylene, three branched methyls, a ketone, and a hydroxyl group, which was first isolated from a marine dinoflagellate *Amphidinium* sp.[1] (strain Y-56). Recently, three structurally related macrolides, amphidinolides T2, T3 (**3**), and T4 (**4**), together with **2** have been isolated from two strains (strains Y-56 and Y-71) of marine dinoflagellates of the genus *Amphidinium*.[2] Their structures were elucidated on the basis of spectroscopic data and chemical means. Further investigation of the extracts of the dinoflagellate (Y-56) resulted in the isolation of a new 19-membered macrolide, amphidinolide T5 (**1**), together with amphidinolides T1 (**2**), T3 (**3**), and T4 (**4**) (Chart 1). In addition, the stereostructure of amphidinolide T1 (**2**) was established by a single crystal X-ray diffraction analysis. Here we describe the isolation and structure elucidation of amphidinolide T5 (**1**) and the X-ray structure of amphidinolide T1 (**2**).

2. Results and discussion

2.1. Isolation of amphidinolides T5 (1) and T1 (2)

The dinoflagellate *Amphidinium* sp. (strain Y-56) 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. The harvested algal cells of a marine dinoflagellate *Amphidinium* sp. 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), and followed by C_{18} HPLC (CH_3CN/H_2O) to afford amphidinolide T5 (**1**, 0.0004%, wet weight) together with known related macrolides, amphidinolides T1[1] (**2**, 0.005%), T3[2] (**3**, 0.0006%), T4[2] (**4**, 0.0004%).

2.2. Structure elucidation of amphidinolide T5 (1)

Amphidinolide T5 (**1**) had the same molecular formula, $C_{25}H_{42}O_5$, as those of amphidinolides T1 (**2**), T3 (**3**), and T4 (**4**) as revealed by HRFABMS data [*m/z* 423.3103 $(M+H)^+$, Δ −0.8 mmu]. ^1H and ^{13}C NMR data (Table 1) for **1** were analogous to those for **3** and **4**. Extensive analysis

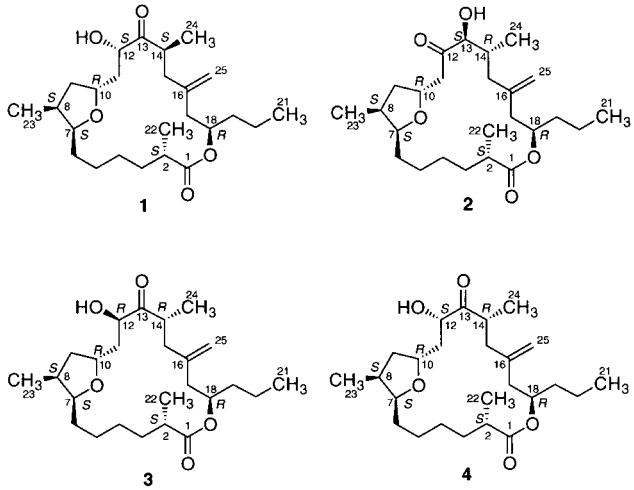

Chart 1. Structures of amphidinolides T5 (**1**), T1 (**2**), T3 (**3**), and T4 (**4**).

Keywords: macrolide; dinoflagellate; X-ray structure.
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Table 1. ^{1}H and ^{13}C NMR data for amphidinolide T5 (**1**) in C_6D_6

Positn	δ_C	δ_H (m, Hz)		δ_C	δ_H (m, Hz)
1	175.1 s		14	38.9 d	3.30 m
2	42.0 d	2.48 m	15	41.6 t	2.44 dd, 11.2, 13.3
3	35.3 t	1.72 m 1.38 m			2.24 dd, 4.3, 13.3
4	27.9 t	1.55 m 1.34 m	16	141.8 s	
5	26.9 t	1.62 m 1.45 m	17	40.3 t	2.59 dd, 3.8, 13.2
6	30.8 t	1.54 m 1.18 m			2.01 dd, 10.4, 13.2
7	78.9 d	3.70 ddd, 2.1, 4.7, 10.6	18	70.3 d	5.20 m
8	37.1 d	1.83 m	19	34.9 t	1.52 m 1.41 m
9	40.4 t	1.47 m 1.35 m	20	18.7 t	1.42 m 1.30 m
10	73.4 d	4.55 m	21	13.9 q	0.88[a] t, 7.2
11	40.7 t	1.75 m 1.17 m	22	18.4 q	1.15[a] d, 6.6
12	76.2 d	4.51 m	23	14.3 q	0.76[a] d, 7.1
12-OH		3.82 d, 5.5	24	17.2 q	1.14[a] d, 5.9
13	216.0 s		25	116.9 t	4.81 s 4.68 s

[a] 3H.

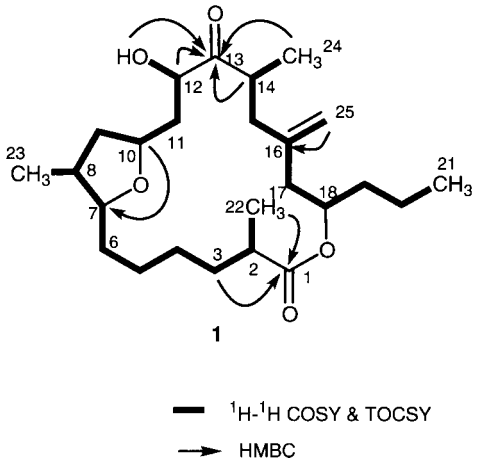


Figure 1. 2D NMR data for Amphidinolide T5 (**1**).

of 2D NMR data (Fig. 1) for **1** disclosed that the gross structure of **1** including the relative stereochemistry of the tetrahedrofuran ring was the same as those of **3** and **4**, indicating that amphidinolide T5 (**1**) was a stereoisomer of amphidinolide T3 (**3**) or T4 (**4**). The absolute stereochemistries of seven chiral centers in **1** were determined as follows. 12*S*-configuration was revealed by application of the modified Mosher's method[3] (Fig. 2). The bis-(*S*)-MTPA ester of C-1–C-12 segment (**6**), which was obtained by the successive treatments of **1** with $LiAlH_4$, $NaIO_4$, $NaBH_4$, and (*R*)-(−)-MTPACl (Scheme 1), was identical with that obtained from amphidinolide T1 (**2**) by the same procedure. Thus, the absolute configurations at C-2, C-7, C-8, and C-10 in **1** were assigned as *S*, *S*, *S*, and *R*, respectively. Treatment of amphidinolide T4 (**4**) with K_2CO_3 in MeOH yielded a 1:2 mixture of **1** and **4** due to epimerization at C-14. All spectral data for **1** isolated from this mixture were identical with those for the natural amphidinolide T5 (**1**). Thus, C-18 in **1** was suggested to have the same *R*-configuration as that in **4**, while C-14 in **1** was elucidated to possess *S*-configuration, opposite to that in **4**. Therefore, the structure of amphidinolide T5 (**1**) was concluded as the stereoisomer at C-14 of amphidinolide T4 (**4**).[4]

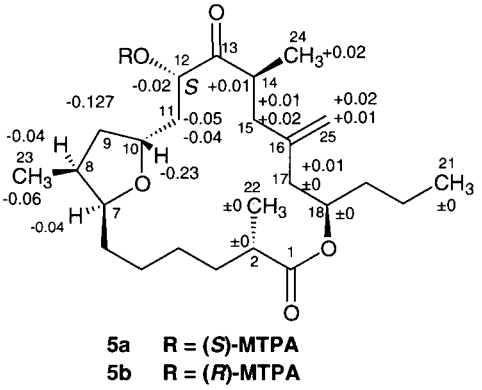
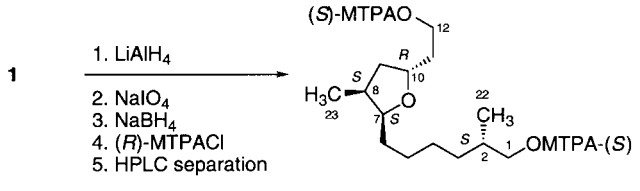


Figure 2. Δδ values [Δδ (in ppm)=$\delta_S-\delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**5a** and **5b**, respectively) of amphidinolide T5 (**1**).

2.3. X-Ray structure of amphidinolide T1 (2)

Amphidinolide T1 (**2**) was crystallized from methanol–water as colorless needles, mp 63–66°C. The single crystal X-ray diffraction analysis revealed two types of conformers (**A** and **B**) in a unit cell of the crystal. The ORTEP drawings of conformers **A** and **B** were shown in Figs. 3 and 4, respectively. The relative configurations at seven chiral centers in **2** obtained from X-ray analysis corresponded well to those proposed previously.[1,2]

Both conformers **A** and **B** were revealed to have an inequilaterally pentagonal shape, and an intramolecular hydrogen bond (1.98 and 2.1 Å for **A** and **B**, respectively) between a hydrogen atom of the hydroxyl group on C-13 and an oxygen atom of the ketone at C-12. A propyl group (C19–C-21), an *exo*-methylene (C-25), and two (C-22 and C-23) of three methyl groups were oriented outside the



Scheme 1. Preparation of bis-(*S*)-MTPA ester (**6**) of C-1–C-12 segment.

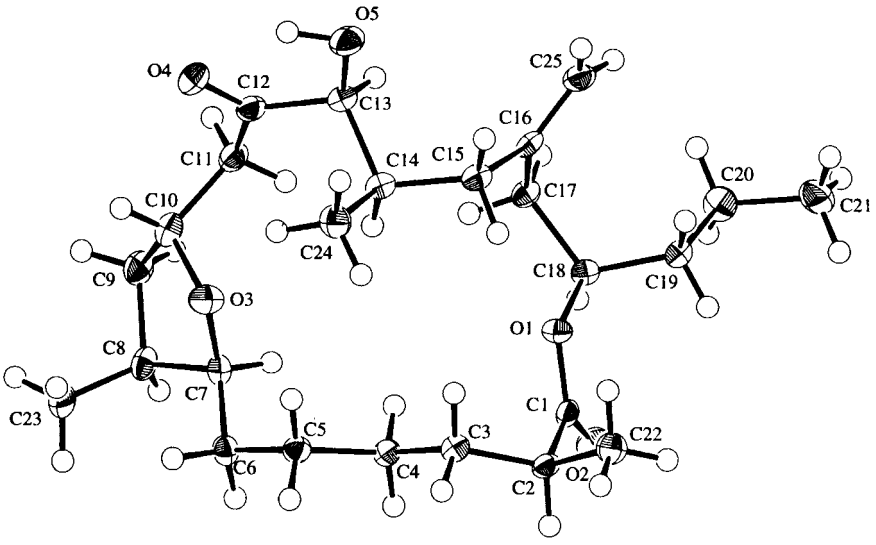


Figure 3. ORTEP drawing of conformer **A** of amphidinolide T1 (**2**).

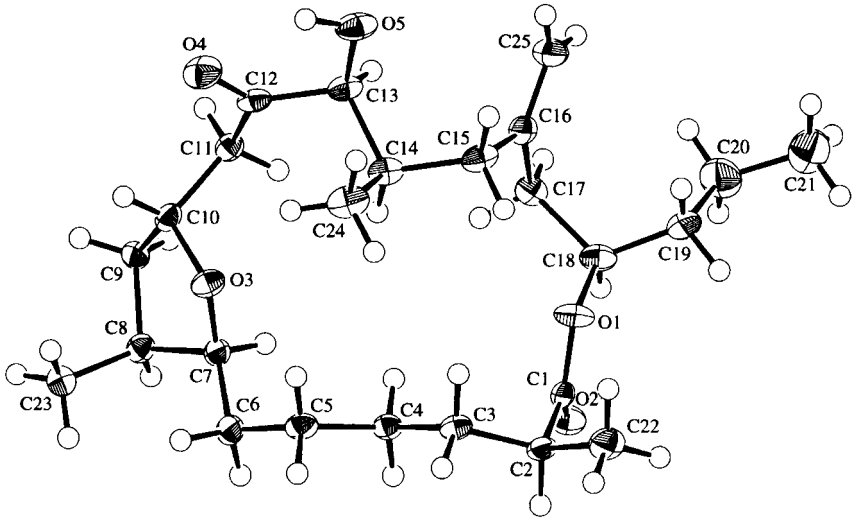


Figure 4. ORTEP drawing of conformer **B** of amphidinolide T1 (**2**).

macrocylic lactone ring, while another methyl (C-24) was oriented inside the lactone ring. Differences between the two conformations were observed in the torsion angles around the *exo*-methylene [C(14)–C(15)–C(16)–C(17), C(14)–C(15)–C(16)–C(25), C(15)–C(16)–C(17)–C(18), andC(18)–C(17)–C(16)–C(25)] and the ester linkage [O(1)–C(1)–C(2)–C(3), O(1)–C(1)–C(2)–C(22), C(1)–O(1)–C(18)–C(17), and C(1)–O(1)–C(18)–C(19)] (Table 2). Fig. 5 represented how to contact between the **A**-type and **B**-type molecules, and disclosed that the **A**-type molecule was fitted in the cage-like space of the **B**-type molecule. Three non-bonded contacts out to 3.6 Å were observed for O(2) [**A**]–C(11) [**B**] [3.486(3) Å], C(3) [**A**]–O(2) [**B**] [3.507(3) Å], C(22) [**A**]–O(2) [**B**] [3.427(4) Å].

Table 2. Parts of torsion angles (°) of conformers **A** and **B** for amphidinolide T1 (**2**)

Angles	A	B
O(1)–C(1)–C(2)–C(3)	56.5(3)	68.9(3)
O(1)–C(1)–C(2)–C(22)	−67.6(3)	−56.4(3)
O(1)–C(18)–C(19)–C(20)	62.2(3)	39.1(3)
C(1)–O(1)–C(18)–C(17)	149.0(2)	119.1(3)
C(1)–O(1)–C(18)–C(19)	−85.9(2)	−115.4(3)
C(14)–C(15)–C(16)–C(17)	−57.1(3)	−69.8(3)
C(14)–C(15)–C(16)–C(25)	125.0(3)	107.4(3)
C(15)–C(16)–C(17)–C(18)	−83.3(3)	−77.4(3)
C(16)–C(15)–C(14)–C(24)	−178.7(2)	−178.3(2)
C(18)–C(17)–C(16)–C(25)	94.7(3)	−105.3(3)

Though the solution conformation of amphidinolide T1 (**2**) was not analyzed in detail, the NOESY spectrum of **2** in benzene-d_6 showed transannular NOEs for H-3 (δ_H 1.62)/H_3-24, H-3 (δ_H 1.62)/H-15 (δ_H 2.34), and H-5 (δ_H 1.53)/H_3-24. Distances between each hydrogen atom of C-3/C-24, C-3/C-15, and C-5/C-24 in X-ray structure **A** were 2.538, 2.620, and 2.582 Å, respectively. Thus the X-ray structure may be similar to the solution conformation in benzene.

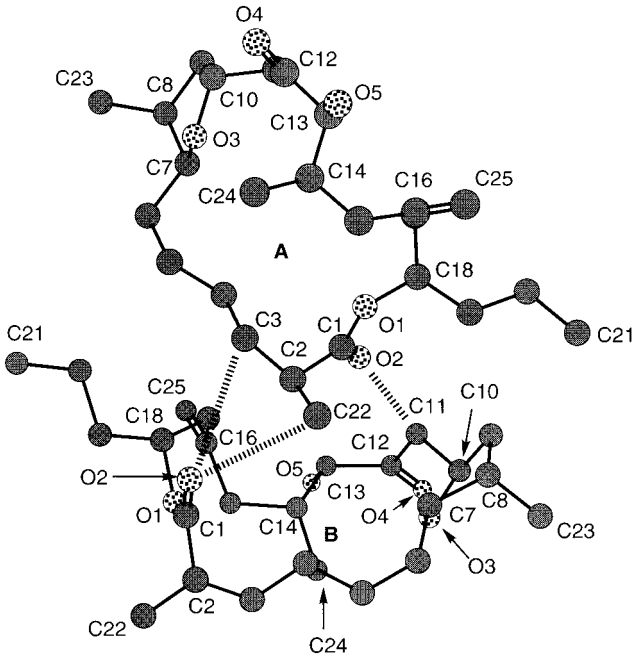


Figure 5. Contact model of conformers **A** and **B** of amphidinolide T1 (**2**). Dashed lines showed non-bonded contacts out to 3.6 Å.

3. Experimental

3.1. 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.

3.2. 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 two 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 addition of 1 M NaCl aq. (1 L), the mixture was extracted with toluene (4 L×3). The toluene soluble fractions were evaporated under reduced pressure to give a residue (3.67 g), which was 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^2; eluent, CH_3CN/H_2O (75:25); flow rate, 2.5 mL/min; UV detection at 210 nm] to afford amphidinolides T5 (**1**, 0.8 mg, 0.0002%, t_R 26.0 min), T1 (**2**, 20.3 mg, 0.005%, t_R 24.4 min), T3 (**3**, 1.5 mg, 0.0004%, t_R 34.5 min), and T4 (**4**, 1.0 mg, 0.0002%, t_R 27.5 min).

3.2.1. Amphidinolide T5 (1). 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* 423.3103 [calcd for $C_{25}H_{43}O_5$ $(M+H)^+$, 423.3111].

3.2.2. (*S*)-MTPA ester (5a) of amphidinolide T5 (1). A colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, d, *J*=7.1 Hz, H_3-23), 0.89 (3H, t, *J*=7.2 Hz, H_3-21), 1.12 (3H, d, *J*=6.8 Hz, H_3-24), 1.19 (3H, d, *J*=6.6 Hz, H_3-22), 1.25–1.75 (15H, m, H_2-3, H_2-4, H_3-5, H_2-6, H_2-9, H-11, H_2-19, and H_2-20), 1.84 (1H, m, H-11), 2.14–2.20 (2H, m, H-8 and H-15), 2.17 (1H, m, H-15), 2.41 (1H, m, H-2), 2.47 (1H, m, H-15), 2.50 (1H, m, H-17), 3.00 (1H, m, H-14), 3.68 (3H, s, OCH_3), 3.76 (1H, m, H-10), 3.88 (1H, m, H-7), 4.87 (1H, s, H-25), 4.88 (1H, s, H-25), 5.01 (1H, m, H-18), 5.53 (1H, brd, *J*=8.6 Hz, H-12), 7.42 (3H, m, Ph), and 7.65 (2H, m, Ph); FABMS *m/z* 639 $(M+H)^+$; HRFABMS *m/z* 639.3505 [calcd for $C_{35}H_{50}O_7F_3$ $(M+H)^+$, 639.3508].

3.2.3. (*R*)-MTPA ester (5b) of amphidinolide T5 (1). A colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, d, *J*=7.1 Hz, H_3-23), 0.89 (3H, t, *J*=7.2 Hz, H_3-21), 1.17 (3H, d, *J*=6.6 Hz, H_3-22), 1.11 (3H, d, *J*=6.8 Hz, H_3-24), 1.25–1.60 (11H, m, H-3, H_2-4, H_3-5, H_2-6, H_2-19, and H_2-20), 1.65–1.70 (3H, m, H-3, H-9, and H-11), 1.73 (1H, m, H-9), 1.91 (1H, m, H-11), 2.16 (1H, m, H-15), 2.21 (1H, m, H-8), 2.22 (1H, m, H-17), 2.48 (1H, m, H-17), 2.41 (1H, m, H-2), 2.48 (1H, m, H-15), 3.00 (1H, m, H-14), 3.57 (3H, s, OCH_3), 3.92 (1H, m, H-7), 4.14 (1H, m, H-10), 4.88 (2H, s, H_2-25), 5.00 (1H, m, H-18), 5.50 (1H, brd, *J*=7.6 Hz, H-12), 7.40 (3H, m, Ph), and 7.59 (2H, m, Ph); FABMS *m/z* 639 $(M+H)^+$; HRFABMS *m/z* 639.3502 [calcd for $C_{35}H_{50}O_7F_3$ $(M+H)^+$, 639.3508].

3.2.4. Oxidative degradation of amphidinolide T5 (1). Amphidinolide T5 (**1**, 0.1 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. A solution of the residue in THF/1 M phosphate buffer (1:1, 50 μL) was stirred with $NaIO_4$ (0.5 mg) at room temperature for 30 min. After evaporation, the reaction mixture was extracted with EtOAc (200 μL) and the extract was evaporated in vacuo. A solution of the residue in EtOH (50 μL) was stirred with $NaBH_4$ (0.3 mg) at 0°C for 30 min. The mixture, after evaporation, was 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). Et_3N (2 μL) and (*R*)-(−)-MTPACl (1 μL) were added to the mixture and stirred 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 compound **6** (0.03 mg, t_R 16.0 min). **6**: ^{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=6.9 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 699.2703 [calcd for $C_{34}H_{42}O_7F_6Na$ $(M+Na)^+$, 699.2732].

3.2.5. Conversion from amphidinolide T4 (4) into amphidinolide T5 (1). A solution of amphidinolide T4 (**4**, 0.3 mg) in MeOH (10 μL) was stirred with K_2CO_3 (0.1 mg) at 4°C for 30 min. After filtration and evaporation in vacuo, the residue was subjected to C_{18} HPLC (Wakosil-II 5C18 RS, 4.6×250 mm^2; eluent, CH_3CN/H_2O, 75:25; flow rate, 1 mL/min; UV detection at 240 nm) to give amphidinolides T4 (**4**, 0.13 mg, t_R 12.4 min) and T5 (**1**, 0.06 mg, t_R 11.5 min).

3.2.6. X-Ray analysis of amphidinolide T1 (2). Amphidinolide T1 (**2**) was crystallized as colorless needles (mp 63–66°C) from methanol/water. Crystal data: $C_{25}H_{42}O_5$, M_r=422.60, crystal dimensions 0.30×0.25×0.10 mm^3, monoclinic, space group $P2_1$ (no. 4), a=9.2259(2) Å, b=20.7748(4) Å, c=12.7658(3) Å, β=90.9820(4)°, V=2446.41(9) $Å^3$, Z=4, D_{calc}=1.147 g/cm^{-1}. A crystal was coated with liquid paraffin. All measurements were made on Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite monochromated MoKα radiation (λ=0.71069 Å) at a temperature of −160±1°C to maximum 2θ value of 59.8°. A total of 44 images, corresponding to 220.0° oscillation angles, were collected with two different goniometer settings. Exposure time was 0.50 min/°. The camera radius was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. Data were processed by the PROCESS-AUTO program package. Of the 28020 reflections that were collected, 7204 were unique (R_{int}=4.1%); equivalent reflections were merged. The linear absorption coefficient, μ, for MoKα radiation was 0.8 cm^{-1}. A symmetry-related absorption correction using the program ABSCOR was applied which resulted in transmission factors ranging from 0.89 to 0.99. The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods (SIR97)[5] and expanded using Fourier technique (DIRDIF94).[6] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were including but not refined. The final cycle of full matrix least-squares refinement was based on 7199 observed reflections ($I>-3.00\sigma(I)$, $2\theta<59.78$) and 541 variable parameters and converged with unweighted and weighted agreement factors of R=0.0078, R_w=0.109. The standard deviation of an observation of unit weight was 1.02. The weighting scheme was based on counting statistics and included a factor (p=0.053) to downweight the intense reflections. Plots of $\sum\omega(Fo^2-Fc^2)^2$ versus Fo^2, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.58 and −0.47 $e^-/Å^3$, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. Crystallographic data for amphidinolide T1 (**2**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 161421).

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