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Amphidinins C–F, Amphidinolide Q Analogues from Marine Dinoflagellate *Amphidinium* sp.

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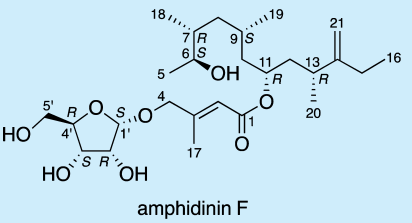
Supporting Information

ABSTRACT: Four new polyketides, amphidinins C–F (**1**–**4**), have been isolated from the culture broth of symbiotic dinoflagellate *Amphidinium* sp. The analysis of their spectral data revealed that amphidinins C–F (**1**–**4**) were 4,5-seco-analogues of amphidinolide Q (**5**). The absolute configurations of the new compounds were elucidated by the combination of *J*-based configuration analysis, modified Mosher's method, and chemical derivatization. Amphidinins D (**2**) and F (**4**) are the first glycosides related to amphidinolides. Amphidinins C–F (**1**–**4**) showed antimicrobial activity against bacteria and/or fungi.



Marine dinoflagellates have been recognized as a source of novel secondary metabolites with interesting structures and bioactivities.[1] In particular, carbon skeletons of dinoflagellate polyketides, which might be synthesized by unexplained one-carbon extension machinery, are unique and unavailable from other organisms.[2] In our continuing search for bioactive metabolites from marine dinoflagellates, we have isolated a series of macrolides, amphidinolides, and long-chain polyketides from the cells of cultured dinoflagellates *Amphidinium* spp.[3] Amphidinolide Q (**5**), a cytotoxic 12-membered macrolide, was one of them, the absolute structure of which was elucidated on the basis of spectroscopic analyses and asymmetric total synthesis.[4] Recently, we have investigated the culture medium of newly obtained dinoflagellates *Amphidinium* sp. and isolated four new amphidinolide Q analogues, amphidinins C–F (**1**–**4**) (Figure 1). Here, we describe the isolation, structure elucidation, and biological activities of **1**–**4**.

The dinoflagellates *Amphidinium* sp. (2012-7-4A strain) were isolated from the inside of a marine acoel flatworm *Amphiscolops* sp. collected at Ishigaki Island, Okinawa, Japan. The dinoflagellates were cultured at 25 °C for 3 weeks under 16 h light/8 h dark schedule in seawater medium, and the supernatant was subjected to a porous polymer gel column. The column was washed with H_2O, and the adsorbed material was eluted with MeOH, which was concentrated in vacuo and partitioned between *n*-hexane and H_2O to afford *n*-hexane-soluble materials. The *n*-hexane-soluble materials were separated by a silica gel column, a C_{18} column, and C_{18} HPLC to afford amphidinins C–F (**1**–**4**) with amphidinolides P[5] and Q (**5**)[4] and amphidinin A.[6]

Amphidinin C (**1**) was obtained as an optically active colorless amorphous solid [$[\alpha]^{22}_{D}$ −17.1 (*c* 0.50, MeOH)]. The molecular formula of **1** was established as $C_{21}H_{36}O_4$ by HRESIMS data (*m*/*z* 375.25028 $[M + Na]^+$, Δ −0.30 mmu). IR absorptions implied the existences of hydroxy (3361 cm^{-1}), ester carbonyl (1721 cm^{-1}), and keto carbonyl (1705 cm^{-1}) functionalities. Inspection of the HMQC spectrum with ^1H and ^{13}C NMR data disclosed

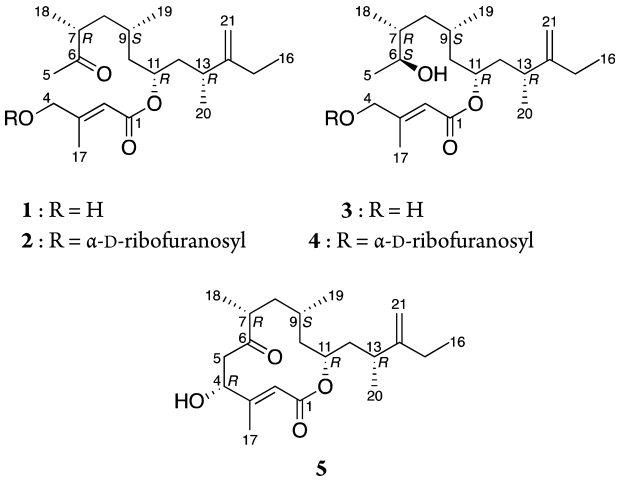


Figure 1. Structures of amphidinins C–F (**1**–**4**) and amphidinolide Q (**5**).

that **1** consists of six methyls, five sp^3 methylenes, an sp^2 methylene, four sp^3 methines, an sp^2 methine, and four sp^2 quaternary carbons including a keto carbonyl carbon and an ester carbonyl carbon (Table S1, Supporting Information). The planar structure of **1** was elucidated from 2D NMR data (Figure 2).

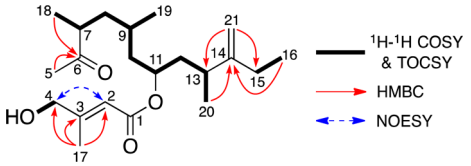


Figure 2. Selected 2D NMR correlations for amphidinin C (**1**).

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^{1}H−^{1}H COSY and TOCSY spectra of **1** revealed connectivities of C-4 to 4-OH, C-7 to C-13, C-7 to C-18, C-9 to C-19, C-13 to C-20, and C-15 to C-16. HMBC correlations between allylic methyl protons H_3-17 (δ_H 2.08) and three carbons (C-2, C-3, and C-4) indicated that the sp^2 methine (C-2, δ_C 114.0), oxymethylene (C-4, δ_C 67.1), and allylic methyl (C-17, δ_C 15.6) carbons were linked at the sp^2 quaternary carbon (C-3, δ_C 156.9). An attachment of an acetate group (C-5 and C-6, δ_C 27.9 and 212.9) to the sp^3 methine (C-7, δ_C 44.7) was implied by HMBC correlations from H_3-5 (δ_H 2.12) and H_3-18 (δ_H 1.06) to C-6. A linkage of the sp^3 methine carbon (C-13, δ_C 37.0), sp^3 methylene (C-15, δ_C 26.0), and sp^2 methylene (C-21, δ_C 107.2) carbons to the sp^2 quaternary carbon (C-14, δ_C 155.1) was suggested by the HMBC correlations from H_2-21 (δ_H 4.73 and 4.72) to C-13 and C-15 and from H_3-16 (δ_H 1.02) and H_3-20 (δ_H 1.03) to C-14. Considering the molecular formula of **1** and the chemical shifts of an oxymethine CH-11 (δ_H 4.99, δ_C 70.2), C-2 and C-11 were found to be connected through a remaining ester carbonyl carbon C-1 (δ_C 166.4). The geometry of a double bond between C-2 and C-3 was assigned as *E* by a NOESY correlation between H-2 and H-4.

The relative configuration of **1** was elucidated on the basis of *J*-based configuration analysis[7] (Figure 3). The $^{2,3}J_{C,H}$ values were measured from hetero halcf-filtered TOCSY (HETLOC)[8] and *J*-resolved HMBC-2[9] spectra of the ^{13}C-enriched sample. Relative magnitudes of coupling constants assigned from $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values (Table S2, Supporting Information) and NOESY correlations implied that C-7−C-8, C-8−C-9, C-9−C-10, C-10−C-11, and C-12−C-13 bonds existed in two major rotamers and C-11−C-12 existed in a single major rotamer as shown in Figure 3. Thus, the relative configurations at C-7, C-9, C-11, and C-13 of **1** were assigned as *R**, *S**, *R**, and *R**, respectively.

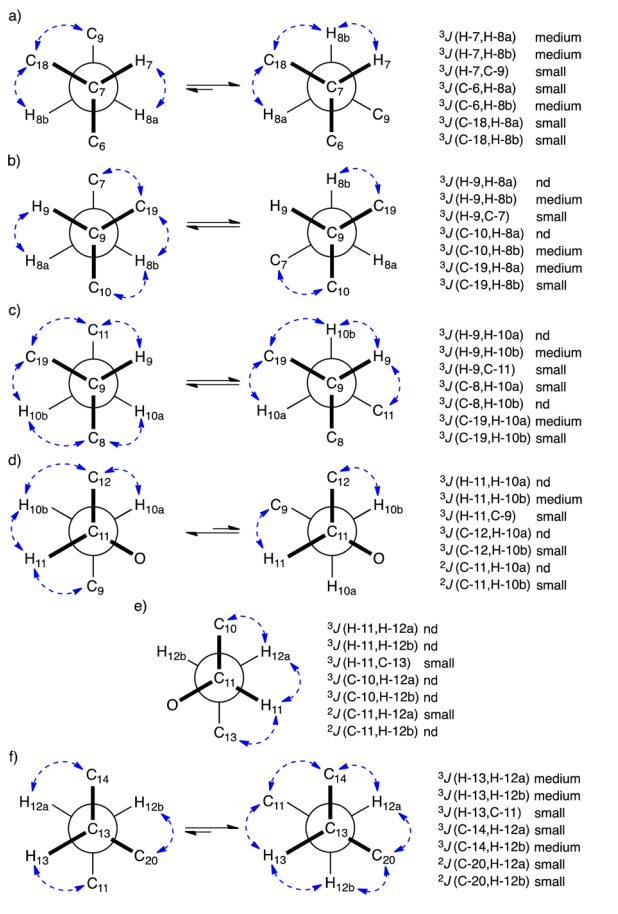
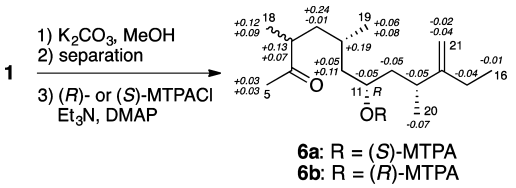


Figure 3. Rotation models for (a) C-7−C-8, (b) C-8−C-9, (c) C-9−C-10, (d) C-10−C-11, (e) C-11−C-12, and (f) C-12−C-13 bonds of amphidinin C (**1**). Protons with "a" and "b" are the germinal protons whose signals were observed in lower and higher fields, respectively. "nd" means that the magnitude was not determined. Blue dashed arrows indicate NOESY correlations. The arrows pointing "C" indicate NOESY correlations of protons attached to "C".

The absolute configuration of **1** was established by the modified Mosher's method.[10] Amphidinin C (**1**) was treated with K_2CO_3 in MeOH, and the resulting mixture was separated by HPLC to obtain the C-5−C-16 segment of **1**, which was successively treated with (*R*)- or (*S*)-2-methoxy-2-(trifluoromethyl)-2-phenylacetyl chloride (MTPACl) to obtain the (*S*)- and (*R*)-MTPA esters (**6a** and **6b**, respectively). $\Delta\delta$ values obtained from ^{1}H NMR data of **6a** and **6b** suggested that the absolute configuration at C-11 of **1** was *R* (Scheme 1). Thus, the absolute configuration of **1** was elucidated to be 7*R*,9*S*,11*R*,13*R*. Amphidinin C (**1**) corresponds to 4,5-secoamphidinolide Q.

Scheme 1. Preparation of (*S*)- and (*R*)-MTPA Esters (6a and 6b, Respectively) of the C-5−C-16 Part of Amphidinin C (1)[a]



[a] $\Delta\delta_H$ values (δ_H of **6a** − δ_H of **6b**) were indicated in italics.

Amphidinin D (**2**) was obtained as an optically active colorless amorphous solid [$[\alpha]^{20}_D$ +76.0 (*c* 0.35, MeOH)]. The molecular formula of **2** was defined as $C_{26}H_{44}O_8$ by HRESIMS data (*m*/*z* 507.29276 [M + Na]$^+$, Δ-0.08 mmu). IR absorptions suggested the presences of hydroxy (3445 cm^{-1}), ester carbonyl (1716 cm^{-1}), and keto carbonyl (1715 cm^{-1}) functionalities. Analyses of 2D NMR data revealed that **2** was the 4-*O*-pentofuranoside of **1** (Figure 4). Comparison of NMR data of **2** between methyl pentofuranosides indicated that the sugar was ribofuranose linked via an α-glycosidic bond.[11]

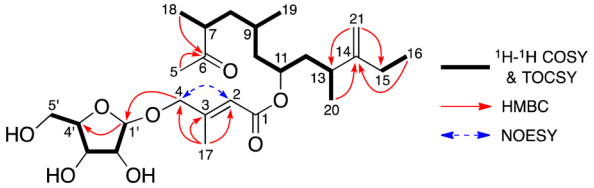


Figure 4. Selected 2D NMR correlations for amphidinin D (**2**).

Amphidinin D (**2**) was treated with Dowex (H$^+$) in MeOH, and the resulting mixture was esterified with (*R*)-MTPACl to obtain the tris-(*S*)-MTPA ester (**7a**) of a sugar moiety and the (*S*)-MTPA ester (**8a**) of an aglycon of **2** (Scheme 2). The ^{1}H NMR spectra of **7a** and **8a** were coincident with those of the tris-(*S*)-MTPA ester of methyl D-ribofuranoside and the (*S*)-MTPA ester of **1**, respectively. Thus, the absolute configuration of **2** was elucidated to be 7*R*,9*S*,11*R*,13*R*,1′*S*,2′*R*,3′*S*,4′*R*. Amphidinin D (**2**) corresponds to 4-*O*-α-D-ribofuranosyl-4,5-secoamphidinolide Q.

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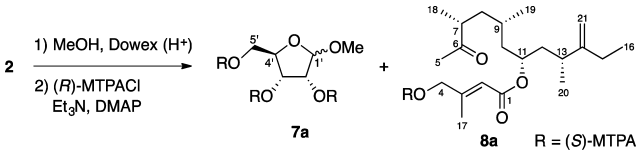
Scheme 2. Preparation of Tris-(*S*)-MTPA Ester (7a) of a Sugar Moiety and (*S*)-MTPA Ester (8a) of an Aglycon of Amphidinin D (2)



Amphidinin E (**3**) was obtained as an optically active colorless amorphous solid [$[\alpha]^{22}_{D}$ −9.6 (*c* 0.50, MeOH)]. The molecular formula of **3**, established as $C_{21}H_{38}O_4$ by HRESIMS data (*m*/*z* 377.26570 $[M + Na]^+$, Δ −0.53 mmu), was bigger than that of **1** with two protons. Comparison of NMR data between **1** and **3** with consideration of their molecular formula concluded that the planar structure of **3** was the 6-deoxo-6-hydroxy analogue of **1** (Figure 5).

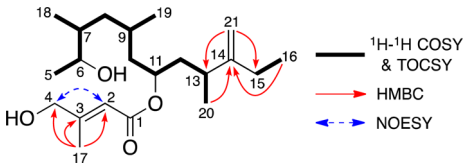
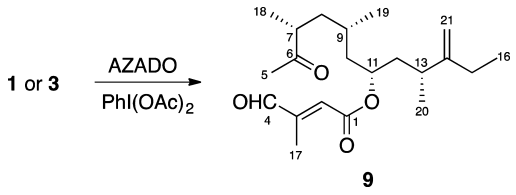


Figure 5. Selected 2D NMR correlations for amphidinin E (**3**).

Amphidinins C (**1**) and E (**3**) were independently oxidized by AZADO[12] (Scheme 3). The ^{1}H NMR spectrum of oxidized

Scheme 3. Oxidation of Amphidinins C (1) and E (3)



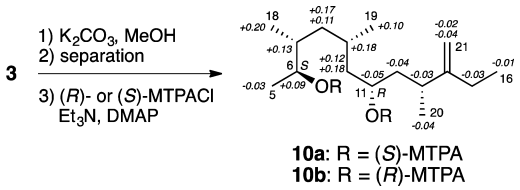
derivative (**9**) of **3** was coincident with that of the oxidized derivative of **1**. Thus, the relative configurations at C-7, C-9, C-11, and C-13 of **3** were assigned as *R**, *S**, *R**, and *R**, respectively.

The absolute configurations at C-6 and C-11 of **3** were assigned as *S* and *R*, respectively, by applying modified Mosher's method for C-5-C-16 segment of **3**, which was obtained by alkaline methanolysis of **3** (Scheme 4). Thus, the absolute configuration of **3** was elucidated to be 6*S*,7*R*,9*S*,11*R*,13*R*. Amphidinin E (**3**) corresponds to 6-deoxo-6*β*-hydroxy-4,5-secoamphidinolide Q.

Amphidinin F (**4**) was obtained as an optically active colorless amorphous solid [$[\alpha]^{20}_{D}$ +21.8 (*c* 0.50, MeOH)]. The molecular formula of **4** was defined as $C_{26}H_{46}O_8$ by HRESIMS data (*m*/*z* 509.30845 $[M + Na]^+$, Δ −0.04 mmu). Inspection of NMR data disclosed that **4** was the 4-*O*-*α*-ribofuranoside of **3** (Figure 6).

The ^{1}H NMR data of the tris-(*S*)-MTPA ester (**7a**) of a sugar moiety and the bis-(*S*)-MTPA ester (**11a**) of an aglycone of **4**, obtained by acid methanolysis of **4** and subsequent esterification with (*R*)-MTPACl (Scheme 5), were coincident with that of the tris-(*S*)-MTPA ester of methyl-D-ribofuranoside and the bis-(*S*)-MTPA ester of **3**, respectively. Thus, the absolute configuration of **4** was elucidated to be 6*S*,7*R*,9*S*,11*R*,13*R*,1′*S*,2′*R*,3′*S*,4′*R*.

Scheme 4. Preparation of Bis-(*S*)- and Bis-(*R*)-MTPA Esters (10a and 10b, Respectively) of the C-5−C-16 Part of Amphidinin E (3)[a]



[a] $\Delta\delta_H$ values (δ_H of **10a** − δ_H of **10b**) are indicated in italics.

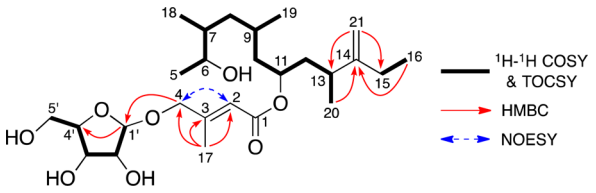
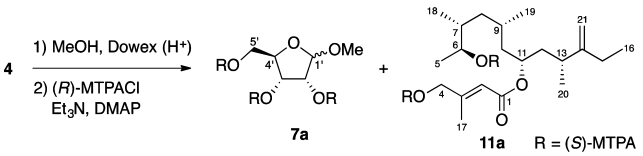


Figure 6. Selected 2D NMR correlations for amphidinin F (**4**).

Scheme 5. Preparation of Tris-(*S*)-MTPA Ester (7a) of a Sugar Moiety and Bis-(*S*)-MTPA Ester (11a) of an Aglycone of Amphidinin F (4)



Amphidinin F (**4**) corresponds to 4-*O*-*α*-D-ribofuranosyl-6-deoxo-6*β*-hydroxy-4,5-secoamphidinolide Q.

Amphidinins C−F (**1**−**4**) and amphidinolide Q (**5**) showed antimicrobial activity against several bacteria and fungi (Table 1).

Table 1. Antimicrobial Activities of Amphidinins C−F (1−4) and Amphidinolide Q (5)

	1	2	3	4	5
Escherichia coli	>32	>32	>32	>32	32
Staphylococcus aureus	32	>32	32	>32	32
Bacillus subtilis	32	>32	32	>32	16
Micrococcus luteus	>32	>32	>32	>32	>32
Aspergillis niger	32	>32	16	>32	>32
Trichophyton mentagrophytes	16	16	16	32	32
Candida albicans	>32	>32	>32	>32	32
Cryptococcus neoformans	>32	>32	>32	>32	>32

The units for values indicating activities against bacteria and fungi are MIC (*μ*g/mL) and IC_{50} (*μ*g/mL), respectively.

All compounds were active against *Trichophyton mentagrophytes*. In addition, **1** and **3** were active against *Staphylococcus aureus*, *Bacillus subtilis*, and *Aspergillis niger*, while **5** was active against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*. Amphidinin E (**3**) showed modest cytotoxicity (IC_{50} 5.8 *μ*g/mL) against murine lymphoma P388 cells in vitro, while it did not show activity (IC_{50} > 10.0 *μ*g/mL) against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro. Amphidinins C (**1**), D (**2**), and F (**3**) were not cytotoxic (IC_{50} > 10.0 *μ*g/mL) against murine lymphoma P388 and L1210 cells and human epidermoid carcinoma KB cells in vitro.

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Amphidinins C−F (**1**−**4**) were all 4,5-seco-analogues of amphidinolide Q (**5**), of which **2** and **4** were the first glycosides related to amphidinolides. Although the common biosynthetic pathway might be involved with production of amphidinins C−F (**1**−**4**) and amphidinolide Q (**5**), it is unknown which comes first. To identify the origin of each carbon composing amphidinins C−F (**1**−**4**) and amphidinolide Q (**5**), feeding experiments with ^{13}C-labeled acetates are currently in progress.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, tabulated NMR data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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