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Absolute Stereochemistry of Amphidinolide C

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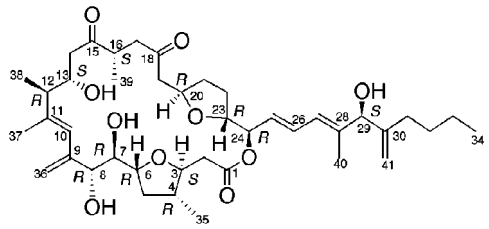
ABSTRACT

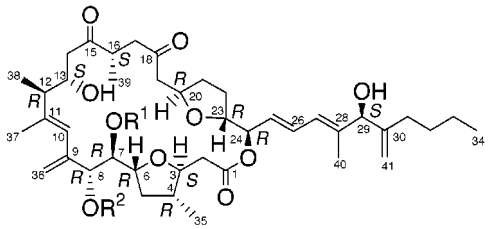


Amphidinolide C

The absolute configurations at 12 chiral centers in amphidinolide C (1), a potent cytotoxic 25-membered macrolide isolated from a marine dinoflagellate *Amphidinium* sp., were determined to be 3*S*, 4*R*, 6*R*, 7*R*, 8*R*, 12*R*, 13*S*, 16*S*, 20*R*, 23*R*, 24*R*, and 29*S* by combination of NMR analyses, degradation experiments, and synthesis of the C-1–C-7 segment.

Amphidinolides are a series of cytotoxic macrolides possessing unique structural features isolated from laboratory-cultured marine dinoflagellates *Amphidinium* sp.[1] Amphidinolides C[2] (**1**, Scheme 1)) and F,[3] isolated from dinoflagellates

Scheme 1



1 : $R^1 = R^2 = H$
2 : R^1, R^2 = $-C(CH_3)_2-$, 7*S*

Amphidinium sp. (Y-5 and Y-26 strains, respectively), are unique 25-membered macrolides having two tetrahydrofuran rings and vicinally located one-carbon branches. Particularly, amphidinolide C (**1**) exhibited potent cytotoxicity against tumor cells. The gross structure of **1** has been elucidated by 2D NMR data, and the relative stereochemistry of the C-1−C-8 and C-20−C-23 portions has been assigned tentatively by NOESY correlations of **1** and its 7,8-*O*-isopropylidene derivative (**2**).[4] During our search for bioactive metabolites from marine dinoflagellates,[5] relatively large amounts of amphidinolide C (**1**) have been recently isolated from three strains (Y-56,[1d,e] Y-59, and Y-71) of the genus *Amphidinium*, which were separated from the inside cells of the marine acoel flatworms *Amphiscolops* sp. This sample was utilized to reinvestigate the relative stereochemistry and to determine the absolute configurations at 12 chiral centers in **1**.

Investigation of Relative Stereochemistry. In our previous studies, the relative stereochemistry of H-3/H-4, H-3/

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H-6, and H-20/H-23 on the two tetrahydrofuran rings was proposed to be all *anti* from NOESY data of amphidinolide C (**1**).[6] An *erythro* relationship for the 7,8-diol was deduced from analysis of the NOESY spectrum of the 7,8-*O*-isopropylidene derivative (**2**) of **1**. The ^3J(H-12,H-13) (8.8 Hz) was a typical value for an *anti* relationship[7] (Figure 1a).

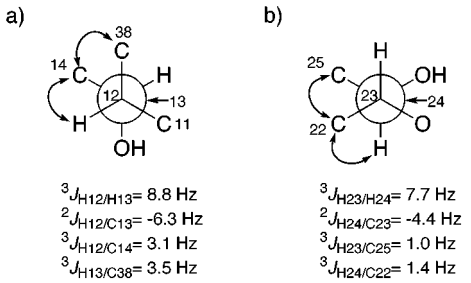


Figure 1. Rotation models for (a) C-12−C-13 and (b) C-23−C-24 bonds of amphidinolide C (**1**). NOESY correlations are illustrated by solid arrows.

The values for ^2J(C-13,H-12) (−6.3 Hz), ^3J(C-14,H-12) (3.1 Hz), and ^3J(C-38,H-13) (3.5 Hz), which were obtained from the hetero half-filtered TOCSY (HETLOC)[8] spectrum, indicated that H-12 was *gauche* to 13-OH, while H-12 and H-13 were *gauche* to C-14 and C-38, respectively. The *gauche* relation between the C-13−C-14 and C-12−C-38 bonds was deduced from the intense NOESY correlation for H-14 (δ_H 2.53)/H_3-38. Thus, the *erythro* relation for the C-12−C-13 bond was established. On the other hand, an *anti* relationship for H-23 and H-24 (Figure 1b) was inferred from the ^3J(H-23,H-24) value (7.7 Hz). The NOESY correlation for H-22 (δ_H 1.60)/H-24 as well as the J(C,H) values for C-23/H-24 (−4.4 Hz) and C-22/H-24 (1.4 Hz) indicated that C-22 and 23-O were both *gauche* to H-24. The NOESY cross-peak for H-22 (δ_H 1.87)/H-25 was suggestive of the *gauche* relation between C-22−C-23 and C-24−C-25 bonds, and the ^3J(C-25,H-23) value (1.0 Hz) was a typical one for a *gauche* relation, thus indicating that the relative configuration of C-23−C-24 was *threo*.

Absolute Configurations at C-13 and C-29. Determination of the absolute configurations of two oxymethine carbons at C-13 and C-29 was accomplished by a modified Mosher method.[9] The 7,8-*O*-isopropylidene derivative (**2**) of amphidinolide C (**1**) was treated with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) to afford the bis-(*S*)- and bis-(*R*)-MTPA esters (**3a** and **3b**, respectively), respectively. $\Delta\delta$ values ($\delta_S - \delta_R$) are shown in Figure 2. The $\Delta\delta$ values for H_2-14, H_2-17, H_2-31, H_2-32, H_2-33, H_3-34, H_3-39, and H_2-41 were negative, while

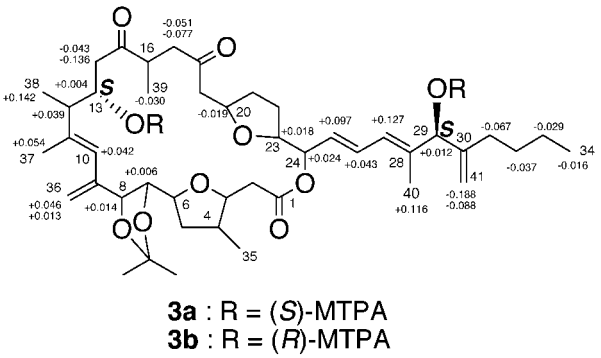
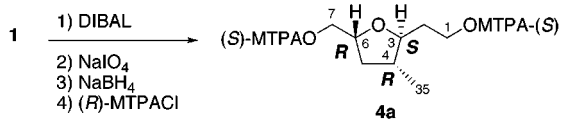


Figure 2. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the (*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of the 7,8-*O*-isopropylidene derivative (**2**) of amphidinolide C (**1**).

positive $\Delta\delta$ values were observed for H-7, H-8, H-10, H-12, H-23, H-24, H-25, H-26, H-27, H_2-36, H_3-37, H_3-38, and H_3-40, thus indicating that C-13 and C-29 both had *S*-configurations.

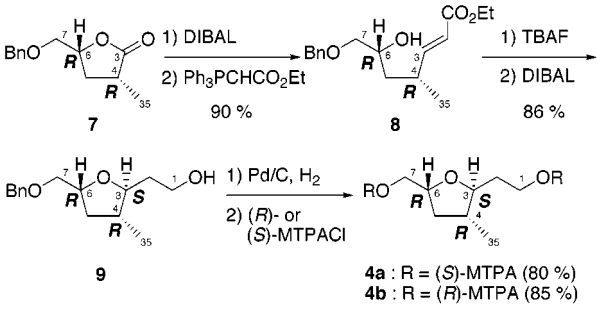
Absolute Configurations at C-3, C-4, and C-6. To investigate the absolute stereochemistry at C-3, C-4, and C-6, the oxidative degradation reaction for the 7,8-diol unit in amphidinolide C (**1**) was performed as follows. Reduction of **1** with DIBAL, oxidative cleavage of the 7,8-diol unit with $NaIO_4$, reduction with $NaBH_4$, esterification with (*R*)-(−)-MTPACl, and then HPLC separation furnished the bis-(*S*)-MTPA ester (**4a**) of the C-1−C-7 segment (Scheme 2),

Scheme 2



of which the structure was elucidated by analysis of ^{1}H−^{1}H COSY and NOESY spectra. On the other hand, both bis-(*S*)- and -(*R*)-MTPA esters (**4a** and **4b**, respectively) of the C-1−C-7 segment were prepared from the (4*R*,6*R*)-6-hydroxymethyl-4-methyl-γ-butyrolactone (**7**), which was derived from D-glutamic acid[10] (Scheme 3). Two-carbon

Scheme 3



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elongation of the γ-butyrolactone (**7**) using a Wittig reaction gave a *E*-olefin **8** in 90% yield in two steps. The unsaturated ester **8** was converted into a tetrahydrofuran (94%) by treatment with *tert*-butylammonium fluolide (TBAF) in THF through diastereoselective Michael reaction, and then the ester carbonyl group was reduced by DIBAL to give compound **9** in 91% yield. Relative configurations of H-3/H-4 and H-3/H-6 in **9** were both assigned as *anti* by NOESY correlations for H_2-2/H-6 and H-3/H_3-35. Deprotection of the benzyl group in **9** was achieved by hydrogenation using palladium−charcoal in EtOH, and then esterification with (*R*)-(−)- and (*S*)-(+)-MTPACl afforded the bis-(*S*)- and (*R*)-MTPA esters (**4a** and **4b**, respectively) of the C-1−C-7 segment.

The ^{1}H NMR spectrum of the bis-(*S*)-MTPA ester (**4a**) of the C-1−C-7 segment obtained from natural amphidinolide C (**1**) was compared with those of synthetic bis-(*S*)- and -(*R*)-MTPA esters (**4a** and **4b**) of the C-1−C-7 segment (Figure 3). Though **4a** and **4b** showed very similar NMR profiles,

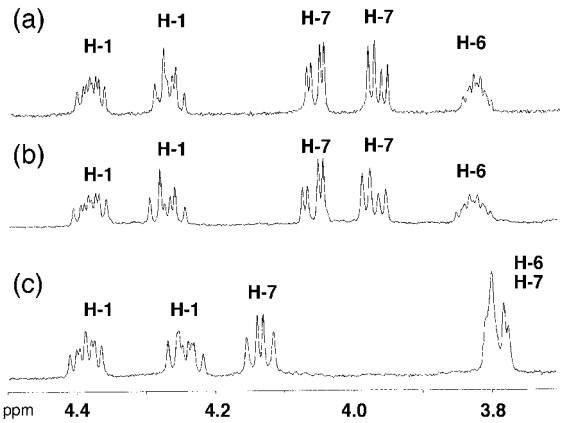


Figure 3. ^{1}H NMR spectra (partial) of (a) bis-(*S*)-MTPA ester (**4a**) of the C-1−C-7 segment derived from amphidinolide C (**1**) and (b) synthetic bis-(*S*)- and (c) bis-(*R*)-MTPA esters (**4a** and **4b**, respectively) of the C-1−C-7 segment.

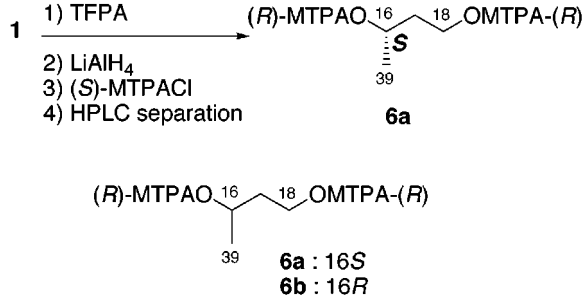
significant differences were observed for signals due to the methylene proton at C-7 (**4a**, δ_H 4.06 and 3.98; **4b**, δ_H 4.13 and 3.79). ^{1}H NMR data of the bis-(*S*)-MTPA ester (**4a**) derived from a natural specimen were identical with those of the synthetic bis-(*S*)-MTPA ester (**4a**). Therefore, the absolute configurations at C-3, C-4, and C-6 were determined to be *S*, *R*, and *R*, respectively.

Absolute Configurations at C-7, C-8, and C-24. The absolute configuration at C-24 was elucidated by application of a modified Mosher method[8] to the linear methyl esters of amphidinolide C (**1**). Treatment of amphidinolide C (**1**) with K_2CO_3 in MeOH yielded a mixture of four linear methyl esters generated by epimerization of C-16 and C-20.[11] One of the four methyl esters purified by C_{18} HPLC was treated with (*R*)-(−)- and (*S*)-(+)-MTPACl to afford the pentakis-(*S*)-(−)- and -(*R*)-(+)-MTPA esters (**5a** and **5b**, respectively). $\Delta\delta$ values obtained from the ^{1}H chemical shifts of **5a** and **5b** are shown in Figure 4. The $\Delta\delta$ values of the protons from C-19 to C-22 are negative in sign, while those of H-25, H-26, and H-27 are positive, suggesting a 24*R* configuration.

The absolute stereochemistry of C-7 and C-8 was elucidated on the basis of the application of Mosher's method for *erythro*-glycol proposed by Kusumi et al.[12] Negative $\Delta\delta$ values for H_2-2, H-3, H-4, H_2-5, and H-6 and positive ones for H-10, H_2-36, and H_3-37 obtained from **5a** and **5b** indicated that **5a** and **5b** had the 7*S* and 8*R* configurations. Therefore, the absolute configurations at C-7 and C-8 were concluded to be both *R*.

Absolute Configuration at C-16. To determine the absolute configuration at C-16 of amphidinolide C (**1**),

Scheme 4



Baeyer−Villiger oxidation using trifluoroperacetic acid[13] (TFPA) was applied to obtain the segment including the

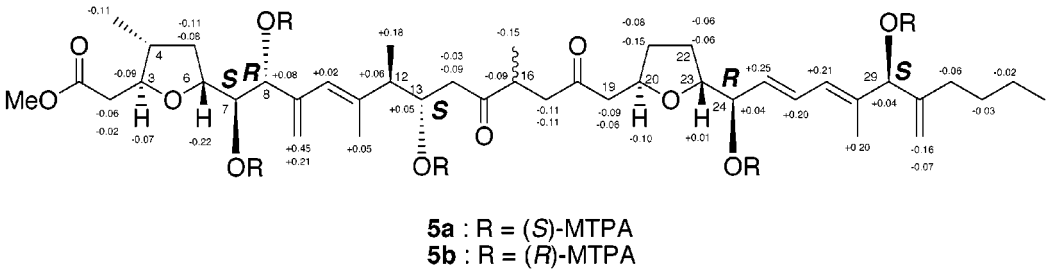


Figure 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the pentakis-(*S*)- and -(*R*)-MTPA esters (**5a** and **5b**, respectively) of the linear methyl ester of amphidinolide C (**1**).

methine carbon at C-16. Amphidinolide C (**1**) was treated with TFPA followed by reduction with $LiAlH_4$, esterification with (*S*)-(+)-MTPACl, and HPLC separation to afford a bis-(*R*)-MTPA ester (**6a**) of 1,3-butanediol corresponding to the C-16−C-18 segment of **1** (Scheme 4). On the other hand, the two authentic bis-(*R*)-MTPA esters of (*S*)-(+)- and (*R*)-(−)-1,3-butanediols (**6a** and **6b**, respectively) were prepared. ^{1}H NMR data of compounds **6a** from a natural specimen were identical with those of synthetic 16*S*-isomer (Figure 5), indicating that the absolute configuration at C-16 of

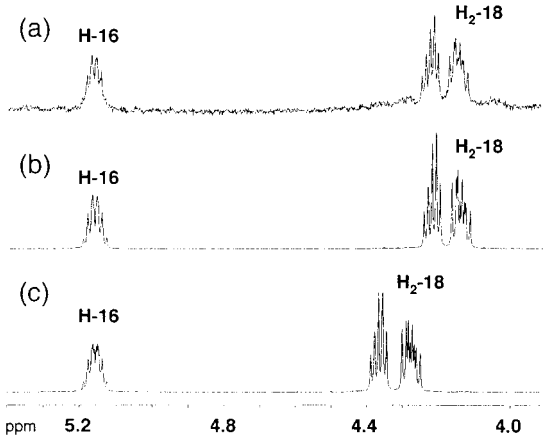


Figure 5. ^{1}H NMR spectra (partial) of (a) bis-(*R*)-MTPA ester (**6a**) of the C-16−C-18 segment derived from amphidinolide C (**1**), (b) the bis-(*R*)-MTPA ester (**6a**) of (*S*)-1,3-butanediol, and (c) the bis-(*R*)-MTPA ester (**6b**) of (*R*)-1,3-butanediol.

amphidinolide C (**1**) was determined to be *S*. Therefore, the absolute configurations of 12 chiral centers in amphidinolide C (**1**) were elucidated to be 3*S*, 4*R*, 6*R*, 7*R*, 8*R*, 12*R*, 13*S*, 16*S*, 20*R*, 23*R*, 24*R*, and 29*S*.

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Supporting Information Available: Experimental procedures, spectral data of **1**, **2**, **3a**, **3b**, **4a** (natural and synthetic), **4b**, **5a**, **5b**, **6a** (natural and synthetic), and **6b**, and Tables S1 and S2. This material is available via the Internet at http://pubs.acs.org.

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(11) Two of the four linear methyl esters were suggested to have a *syn* relationship for H-20−H-23 by NOESY correlation for H-20/H-23, while the relative stereochemistry of H-20−H-23 of the two other diastereomers was *anti*. Epimerization at C-20 is explained by inversion of C-20, generated through retro-Michael-type cleavage between the ether oxygen and C-20 followed by Michael-type re-formation of the ether linkage. Although such a retro-Michael−Michael reaction for another tetrahydrofuran ring (C-3−C-6) might occur, the *anti* relationship of H-3/H-4 in the tetrahydrofuran ring was reported to be generally more kinetically and thermodynamically stable than a *syn* relationship. Methanolysis of **1** with K_2CO_3 in MeOH-d_4 afforded four linear methyl esters labeled with deuterium. In the ^{1}H NMR data of four linear methyl esters, the signals of α-protons (H_2-2, H_2-14, H-16, H_2-17, and H_2-19) of three carbonyl groups at C-1, C-15, and C-18 disappeared. This indicated that one of the two 20,23-*syn* linear compounds and one of two 20,23-*anti* compounds were products due to epimerization at C-16. Nevertheless, the stereochemisry at C-16 of each compound was not determined. The diastereomer using the modified Mosher method possessed a 20,23-*anti* relationship.

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