

NOTE

Revised structure and stereochemical assignments of amphidinolide N

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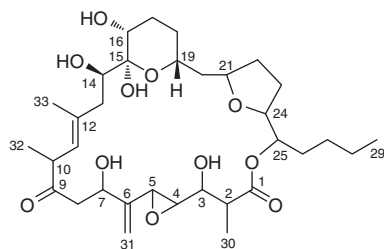
Amphidinolides are a series of cytotoxic macrolides isolated from laboratory-cultured marine dinoflagellates *Amphidinium* sp.¹ Amphidinolide N (**1**),² isolated from *Amphidinium* sp. (Y-5 strain), is a 26-membered macrolide having an allyl epoxide and a tetrahydropyran with 13 chiral centers. Among all amphidinolides isolated so far, amphidinolide N (**1**) is the most potent cytotoxic macrolide with IC₅₀ values against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro* being 0.00005 and 0.00006 μg ml⁻¹, respectively. Owing to the complex structure and the extraordinarily potent cytotoxicity, amphidinolide N (**1**) has been a challenging target for total synthesis.^{3–6} In our previous studies,² gross structure **1b** was proposed based on 2D NMR and mass spectral data, and the configuration of epoxide and the relative stereochemistry of the C-14-C-19 portions were assigned from *J*-values and NOESY data. However, the stereochemistry of the remaining part has not yet been elucidated. Here we describe a revision of the gross structure from **1b** to **1a** (Figure 1) and full assignment of the relative stereochemistry of amphidinolide N (Figure 5).

The molecular formula of amphidinolide N was established as C₃₃H₅₂O₁₁ from ESIMS (*m/z* 647 (M + Na)⁺ (pos.), *m/z* 623 (M - H)⁻ (neg.)) (in our previous studies,² pseudo molecular ion peaks of amphidinolide N, *m/z* 730 (M + DEA + H)⁺ and *m/z* 748

(M + DEA + H₃O)⁺, were misinterpreted as *m/z* 730 (M + DEA + H - H₂O)⁺ and *m/z* 748 (M + DEA + H)⁺, respectively). Deuterium-induced shifts in ¹³C NMR resonances for amphidinolide N were measured by using C₆D₆/CD₃OH (3:1) and C₆D₆/CD₃OD (3:1) as solvents. Of 11 signals observed for oxygenated sp³ carbons, six oxymethines did not show deuterium-induced upfield shifts (Table 1). Of the unchanged oxymethines, two high-field methine carbons at δ 54.92 (C-5) and 62.98 (C-4) in C₆D₆/CD₃OH (3:1) were attributed to those of an epoxide ring. Two carbons at δ 98.08 (which did show the upfield shift) and 66.73 were assigned as a hemiacetal carbon at C-15 and an oxymethine carbon at C-19, respectively, whereas an oximethine carbon (δ 75.66) was elucidated to be that forming a lactone linkage between C-1 and C-25. The remaining two unchanged oxygenated carbons at δ 75.61 and 80.22 were assigned as C-21 and C-24, respectively, suggesting that C-21 and C-24 were not connected to hydroxy groups. The cross-peak of H-24 to C-21 observed in the HMBC spectrum of **1a** indicated that C-21 and C-24 were connected to each other through an ether linkage. Thus, the gross structure of amphidinolide N was revised from **1b** to **1a** (Figure 1).

In our previous studies,² the configuration of the 4,5-epoxide was deduced to be *trans* from the coupling constant (³J_{H-4/H-5} = 1.9 Hz), and the relative stereochemistry of the C-14-C-19 portions was

1a revised gross structure of amphidinolide N



1b proposed gross structure of amphidinolide N

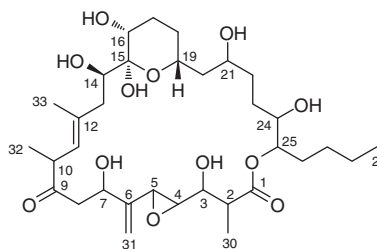


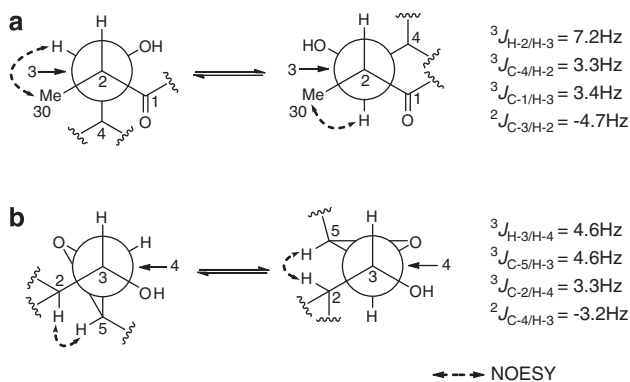
Figure 1 Revised (**1a**) and proposed (**1b**) gross structures of amphidinolide N.

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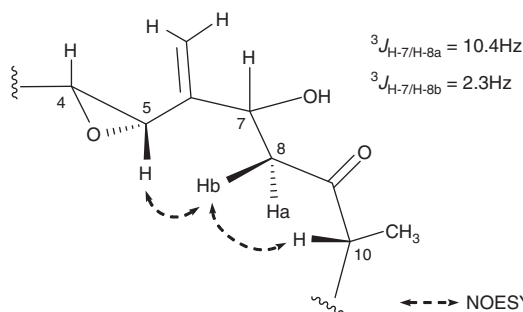
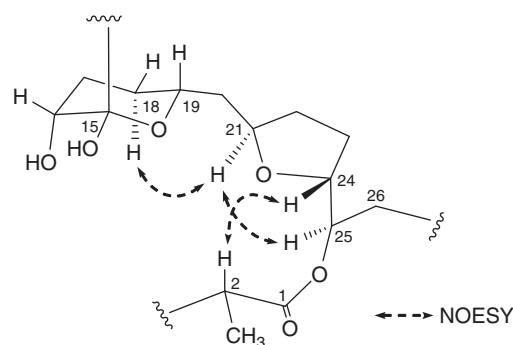
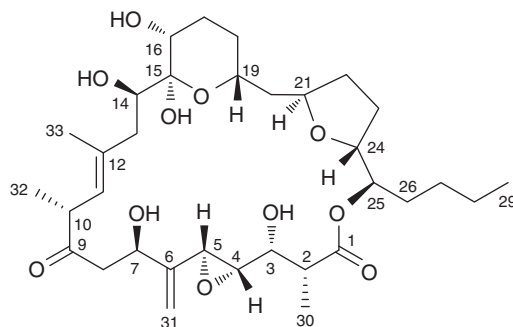
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Table 1 ^{13}C NMR data of amphidinolide N (1)

Position	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\Delta\delta_{\text{C}}^{\text{c}}$
1	174.33	174.34	-0.01
2	45.96	45.90	0.06
3	73.60	73.50	0.10
4	62.98	62.94	0.04
5	54.92	54.92	0.00
6	147.49	147.46	0.03
7	69.89	69.77	0.12
8	46.10	46.06	0.04
9	210.70	210.75	-0.05
10	47.33	47.33	0.00
11	127.18	127.13	0.05
12	136.75	136.75	0.00
13	40.45	40.39	0.06
14	71.38	71.27	0.11
15	98.08	97.98	0.10
16	65.74	65.59	0.15
17	27.37	27.34	0.03
18	25.72	25.72	0.00
19	66.73	66.74	-0.01
20	42.02	42.01	0.01
21	75.61	75.62	-0.01
22	32.99	32.98	0.01
23	28.01	28.01	0.00
24	80.22	80.22	0.00
25	75.66	75.66	0.00
26	31.38	31.38	0.00
27	27.91	27.91	0.00
28	22.74	22.74	0.00
29	14.16	14.17	-0.01
30	13.94	13.94	0.00
31	111.73	111.75	-0.02
32	15.59	15.58	0.01
33	15.05	15.05	0.00

^a δ_{C} in $\text{C}_6\text{D}_6/\text{CD}_3\text{OH}$, 3:1.^b δ_{C} in $\text{C}_6\text{D}_6/\text{CD}_3\text{OD}$, 3:1.^c $\Delta\delta_{\text{C}} = \delta_{\text{C}}$ (in $\text{C}_6\text{D}_6/\text{CD}_3\text{OH}$, 3:1) - δ_{C} (in $\text{C}_6\text{D}_6/\text{CD}_3\text{OD}$, 3:1).**Figure 2** Rotation models for (a) C-2-C-3 and (b) C-3-C-4 bonds of amphidinolide N.

elucidated from J -values and NOESY data. In this study, the relative stereochemistry of the C-2-C-4 segment was analyzed by the J -based configuration analysis method.⁷ The values for $^3J_{\text{H-2/H-3}}$ (7.2 Hz), $^3J_{\text{C-4/H-2}}$

**Figure 3** Selected NOESY correlations and relative stereochemistry for amphidinolide N (C-4-C-10 part).**Figure 4** Selected NOESY correlations and relative stereochemistry for amphidinolide N (C-15-C-26 and C-1-C-2 parts).**Figure 5** Relative stereochemistry of amphidinolide N (1).

$^4J_{\text{H-2}}$ (3.3 Hz), $^3J_{\text{C-1/H-3}}$ (3.4 Hz) and $^2J_{\text{C-3/H-2}}$ (-4.7 Hz), which were obtained from the ^1H NMR and J-HMBC⁸⁻¹² spectra, indicated that C-1 was *gauche* to C-4, H-2 was *gauche* to 3-OH and H-3 was *gauche* to C-30. The *gauche* relation between the H-3 and C-30 was deduced from the intense NOESY correlation for H-3/H₃-30 as well, suggesting the *threo* relation for the C-2-C-3 bond (Figure 2a). The values for $^3J_{\text{H-3/H-4}}$ (4.6 Hz), $^3J_{\text{C-5/H-3}}$ (4.6 Hz), $^3J_{\text{C-2/H-4}}$ (3.3 Hz) and $^2J_{\text{C-4/H-3}}$ (-3.2 Hz), and the intense NOESY correlation for H-2/H-5 indicated that C-2 was *gauche* to C-5, H-3 was *gauche* to O-4 and H-4 was

gauche to 3-OH, suggesting assignment of the relative configuration of C-3-C-4 as *threo* (Figure 2b).

The relative stereochemistry of the C-5-C-10 segment was deduced from *J*-values and NOESY data. The values for $^3J_{\text{H-7/H-8a}}$ (10.4 Hz) and $^3J_{\text{H-7/H-8b}}$ (2.3 Hz) indicated an *anti* relationship for H-7 and H-8a, while NOESY correlations of H-5/H-8b and H-8b/H-10 showed proximity of H-5, H-8b and H-10, reading to the relative configurations shown in Figure 3.

Proximity of H-18, H-21 and H-25 was indicated by NOESY correlations of H-18/H-21 and H-21/H-25, suggesting that the relative stereochemistry of H-21 and H-24 in the tetrahydrofuran ring was *anti*. The proximity of H-24 and H-2 was deduced from the NOESY correlation of H-24/H-2. The relative configurations from C-15 through C-2 as shown in Figure 4 were suggested by these NOESY correlations. Thus, the relative stereochemistry of amphidinolide N (1) was elucidated as shown in Figure 5.

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