

# Absolute Configuration of Amphidinol 3, the First Complete Structure Determination from Amphidinol Homologues: Application of a New Configuration Analysis Based on Carbon–Hydrogen Spin-Coupling Constants

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Dinoflagellates, a type of primitive unicellular algae, are a rich source of structurally and biologically intriguing natural products; e.g., okadaic acid, brevetoxins, ciguateroxins, and maitotoxin. Among these polyether-cyclic compounds, amphidinols are unique dinoflagellate metabolites since they are primarily made up of linear polyhydroxy structures. The first member of this group was isolated from the dinoflagellate *Amphidinium klebsii* as a potent antifungal substance by Yasumoto's group.<sup>1a</sup> A series of homologues<sup>1b,c</sup> has since been found in the same genus, and Kobayashi's group reported closely related compounds, luteophanols.<sup>2</sup> These long-chain polyhydroxy compounds may be one of the most challenging targets for stereostructural elucidation since chiral centers are scattered over a flexible acyclic structure. Thus, little is known of the nature of the stereogenic centers in amphidinols.

We recently developed *J*-based configuration analysis,<sup>3</sup> which has been proven to be a powerful tool for the stereochemical determination of acyclic structures.<sup>3b</sup> In this method, 1,2-diastereomeric relationships between chiral centers are determined by choosing a correct staggered rotamer among six possibilities arising from *erythro* and *threo* configurations using spin-coupling constants ( $^3J_{\text{H,H}}$  and  $^{2,3}J_{\text{C,H}}$ , e.g., see Figure 1c for  $^{2,3}J_{\text{C,H}}$ ).<sup>3a</sup> In this paper, we report the complete configuration of amphidinol 3 (**1**), a representative homologue of the amphidinol family, mainly using this *J*-based method.

From cellular extracts of *A. klebsii* obtained from 440 L of culture, 12 mg of **1** was isolated together with other amphidinol homologues.<sup>1b,c</sup> To facilitate measurements of  $^{2,3}J_{\text{C,H}}$ , we prepared a  $^{13}\text{C}$ -enriched sample of **1** (25%  $^{13}\text{C}$ , 8 mg) by making another culture (200 L) in the presence of 12 mM  $\text{NaH}^{13}\text{CO}_3$ .

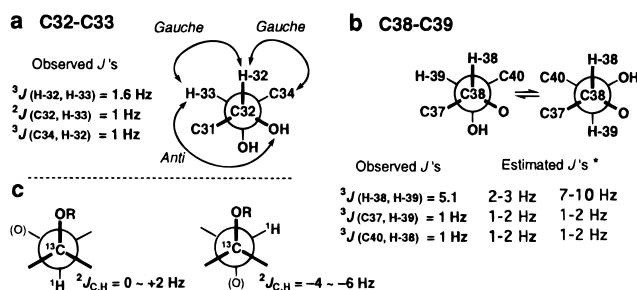
The stereochemical assignment of **1** was accomplished as follows; (a) the *J*-based method<sup>3</sup> was used for acyclic parts with 1,2- and 1,3-chiral centers, C20–C27, C32–C34, C38–C39, C44–C45, and C50–C51; (b) NOE analysis combined with *J* analysis was used for two ether cycles and their linkage C39–C44; (c) the modified Mosher method<sup>4</sup> and chromatographic/NMR comparison were used for degradation products to determine the absolute stereochemistry at C2, C6, C10, C14, C23, and C39.

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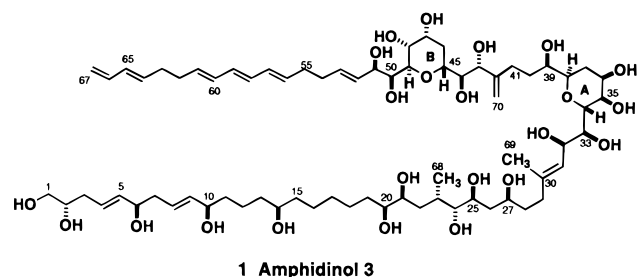
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**Figure 1.** Rotamers and coupling constants for C32–C33 (a) and C38–C39 (b). \**J* values for each rotamer estimated from stereochemically known compounds.<sup>3a</sup> (c)  $^{2,3}J_{\text{C,H}}$  values depend on the dihedral angle between an oxygen atom on a relevant carbon and a proton on a neighboring carbon; in 1,2-dioxygenated systems such as C32–C33 (a), the anti O/H orientation gives  $^{2,3}J_{\text{C,H}}$  of 0 to +2 Hz while the gauche orientation gives  $^{2,3}J_{\text{C,H}}$  of -4 to -6 Hz.<sup>8</sup>

$^3J_{\text{H,H}}$  and  $^{2,3}J_{\text{C,H}}$  values of intact **1** were measured by E.COSY<sup>5</sup>



**1 Amphidinol 3**

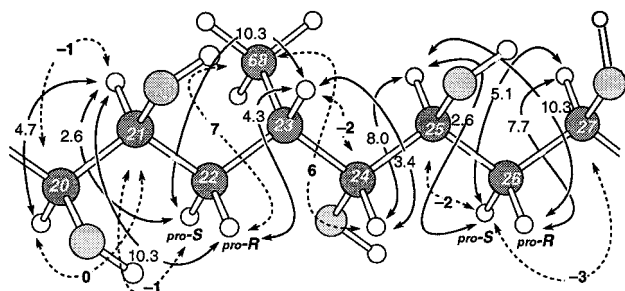
and hetero half-filtered TOCSY (HETLOC),<sup>6</sup> respectively; phase-sensitive HMBC<sup>7</sup> was also used for parts where the small magnetization transfer by TOCSY hampered the accurate measurement of  $^{2,3}J_{\text{C,H}}$  by HETLOC. C32–C33 and C38–C39 can be used as examples to see how the *J*-based analysis works in configuration assignments. As shown in Figure 1a,  $^3J(\text{H-32}, \text{H-33})$  revealed a value that is typical of gauche interaction for a 1,2-diol system.<sup>3a</sup> The values for  $^2J(\text{C32}, \text{H-33})$  and  $^3J(\text{C34}, \text{H-32})$  indicate that H-33 is anti to C32–OH<sup>8</sup> and H-32 is gauche to C34, respectively. These interactions unambiguously establish the *threo* configuration for C32–C33, as depicted in Figure 1a. For C38–C39,  $^3J(\text{H-38}, \text{H-39})$ , which is intermediate between anti and gauche, suggests that this bond undergoes a conformational change. The *J*-based analysis can even be applied to such a flexible system. The two small values for  $^3J(\text{C37}, \text{H-39})$  and  $^3J(\text{C40}, \text{H-38})$  indicate gauche C37/H-39 and gauche C40/H-38 interactions in both conformers (Figure 1b). Of the six possible pairs of alternating rotamers arising from *erythro* and *threo* configurations, only one pair in Figure 1b satisfies all of these requirements. The relative configurations of the consecutive stereogenic center in C20–C27 can be determined using this method, as shown in Figure 2. The diastereomeric relationships of C44–C45 and C50–C51 were assigned in the same manner on the basis of  $^3J_{\text{H,H}}$  and  $^{2,3}J_{\text{C,H}}$ . The configurations of rings A/B and their linkage (C39–C44) were elucidated using NOEs<sup>9</sup> in combination with  $^3J_{\text{H,H}}$  and  $^{2,3}J_{\text{C,H}}$  data (see Supporting Information).

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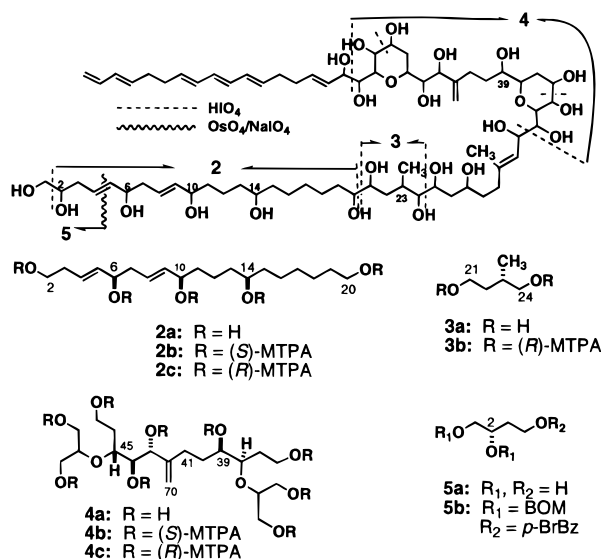
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**Figure 2.** Configuration and conformation for C20–C27 of **1** established on the basis of  $^{2,3}J_{CH}$  and  $^3J_{HH}$ . Broken lines with arrows and numbers indicate  $^{2,3}J_{CH}$  in Hz, and plain lines denote  $^3J_{HH}$  in Hz. For pairs of vicinal carbons, except C24–C25,<sup>9</sup> two anti orientations of H/H, C/H, and/or O/H can be assigned on the basis of  $^{2,3}J_{CH}/^3J_{HH}$ , which results in elucidation of the 1,2-diastereomeric relationship (for methine–methylene pairs such as C21–C22, the stereospecific assignment of methylene protons can also be attained).<sup>3a</sup>

These NMR-based analyses using intact **1** have revealed the relative configurations of C20–C27 and C32–C51. Next, their absolute configurations and those at C2, C6, C10, and C14 were investigated using degradation products; treatment of **1** with  $HIO_4/NaBH_4$ , followed by esterification with (*R*)- and (*S*)-MTPA (2-methoxy-2-trifluoromethyl-2-phenylacetic acid) and separation by HPLC, furnished MTPA esters of fragments corresponding to C2–C20 (**2b,c**), C21–C24 (**3b**) and C33–C50 (**4b,c**).<sup>10</sup> The



absolute stereochemistries of C6, C10, C14,<sup>11</sup> and C39<sup>12</sup> were elucidated by the modified Mosher method using **2b/2c** and **4b/4c**.<sup>4</sup> The configuration of **3a** was determined to be 2*SS* by comparison of the NMR data of the bis-(*R*)-MTPA esters **3b** with (*S*)- and (*R*)-MTPA esters of authentic (*R*)-methyl-1,4-butanediol.<sup>13</sup> The configuration of C2 was determined using the C1–C4 fragment obtained from the *O*-benzyloxy-methyl derivative of **1** by treatment with  $OsO_4/NaIO_4$ .<sup>14</sup> The resulting 1,2-dibenzyloxymethoxy-butyl *p*-bromobenzoate (**5b**) was chromato-

(9) Among 14 vicinal methine–methine interactions in the acyclic parts of **1**, there are four H/H-anti orientations, which were indicated by  $^3J$  (H-24, H-25) = 8.0 Hz,  $^3J$  (H-33, H-34) = 9.5 Hz,  $^3J$  (H-43, H-44) = 9.0 Hz, and  $^3J$  (H-49, H-50) = 10.0 Hz. For configuration assignments of these parts, additional NOE information is necessary.<sup>3a</sup> Diastereomeric relations between C24 and C25, C33 and C34, C43 and C44, and C49 and C50 were established by key NOEs between H-23 and C25–OH, H-32 and H-38, C43–OH and H-45, and H-45 and H-51, respectively. See Supporting Information for details.

(10) MTPA esters **2b** (690  $\mu$ g), **2c** (520  $\mu$ g), **3b** (30  $\mu$ g), **4b** (400  $\mu$ g), and **4c** (150  $\mu$ g) were obtained from 3.0 mg of **1**. See Supporting Information.

graphed on a chiral resolution column<sup>15</sup> and determined to be an (*S*)-enantiomer. Considering all of these partial configurations led to the complete structure of amphidinol **3** with the absolute stereochemistry of 2*S*, 6*R*, 10*R*, 14*R*, 20*S*, 21*S*, 23*S*, 24*R*, 25*S*, 27*S*, 32*R*, 33*S*, 34*R*, 35*R*, 36*R*, 38*R*, 39*R*, 43*R*, 44*R*, 45*R*, 47*R*, 48*R*, 49*R*, 50*S*, and 51*R*.

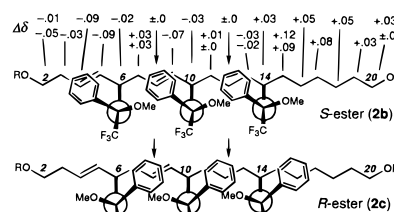
Despite recent progress in the NMR-based configuration analysis of natural products, chemical degradation in combination with partial synthesis is still considered the sole reliable method for determining the stereochemistry in acyclic structures, which often requires a fairly large amount of sample, as in the pioneering studies of mycoticins by Schreiber's group,<sup>16</sup> nystatin by Beau's group,<sup>17</sup> and roflamycin by Rychnovsky's group.<sup>18</sup> In this study, we were able to perform a complete structural determination using only 3 mg of the sample for all of the chemical degradations and 8 mg of the  $^{13}C$ -enriched sample for NMR measurements. This considerable reduction in sample size is mainly attributable to the *J*-based configuration analysis, since 12 of 17 chiral centers in the acyclic parts of **1** were established by this method. Therefore,  $^{2,3}J_{CH}$  should be reevaluated as a useful parameter for configuration assignments of acyclic stereogenic centers in natural products.

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**Supporting Information Available:** Selected spectra used for configuration assignments of **1** and their interpretations; HETLOC and HMBC for measurement of  $^{2,3}J_{CH}$ , NOESY and ROESY (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(11) As shown below, the stereochemistry at C6 and C14 can be determined to be 6*R* and 14*R* based on the distribution of  $\Delta\delta$  (**2b** – **2c**), in which positive values are observed in C15–C20, while negative ones are seen in C2–C5. The configuration of C10 could also be assigned since  $\Delta\delta$  of C8 and C12 are zero. This can only be accounted for by the *R* configuration at C10, since the shielding effect at C8 (or C12) from a phenyl group of (*S*)-MTPA at C10 (or C14) should be equal to that from an (*R*)-MTPA at C6 (or C10).



(12) The  $\Delta\delta$  values of (**4b** – **4c**) were as follows:  $CDCl_3$ , 500 MHz,  $H_2$ -37,  $-0.12/-0.06$ ; H-38,  $-0.57$ ; H-39,  $-0.14$ ; H-40,  $+0.37$ ; H<sub>2</sub>-41,  $+0.30/+0.37$ ; H<sub>2</sub>-70,  $+0.33/+0.42$ . These data show that C39 has an *R* configuration, since the clear separation of negative and positive values with respect to C39 shows that the  $\Delta\delta$  values were predominately affected by the MTPA group on C39 rather than by other MTPA groups on relatively remote sites.

(13) (*R*)-Methyl-1,4-butanediol was prepared from dimethyl-(*R*)-methylsuccinate by  $LiAlH_4$  reduction, followed by esterification with (*R*)- and (*S*)-MTPA.  $^1H$  NMR of **3b** was identical to that of the (*S*)-ester of the authentic diol; **3b** and (*S*)-ester ( $CDCl_3$ , 500 MHz)  $\delta$  3.98, 3.92, 3.82, 3.73, 1.53, 1.38, 1.05, 0.52. (*R*)-ester  $\delta$  3.97, 3.93, 3.83, 3.70, 1.52, 1.38, 1.05, 0.52.

(14) A procedure that was previously reported for ciguatoxin was followed: Satake, M.; Morohashi, A.; Oguri, H.; Oishi, T.; Hiram, H.; Harada, N.; Yasumoto, T. *J. Am. Chem. Soc.* **1997**, *119*, 11325–11326.

(15) Chiral resolution was performed on CHIRALPAK AD ( $\phi$  4.6  $\times$  250 mm, DAICEL) eluted with hexane-1-propanol (25: 1). Authentic (*S*)-ester and the derivative from **1** (**5b**) were eluted at 16 min 50 sec and 16 min 59 sec, respectively, while (*R*)-ester appeared at 19 min 13 sec.

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