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Structure Elucidation of (+)-Amphidinolide A by Total Synthesis and NMR Chemical Shift Analysis

Barry M. Trost* and Paul E. Harrington

Department of Chemistry, Stanford University, Stanford, California 93405-5080

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The cytotoxic macrolide, amphidinolide A, was isolated by Kobayashi from the culture broth of the marine dinoflagellate *Amphidinium* sp., which is symbiotically associated with an Okinawan flatworm.[1] The gross structure and relative stereochemistry were proposed after extensive 2D NMR experiments. However, subsequent efforts at total synthesis revealed that the reported structure was incorrect.[2] As shown in Table 1, there were significant deviations in the ^{1}H NMR. Because the relative stereochemistry was assigned with NOE data measured on a macrolide possessing considerable flexibility, an error in the relative stereochemistry and not gross structure seemed likely. Additionally, the differences in chemical shifts and coupling constants are not as large as would be expected for an error in connectivity. Unfortunately, only an extremely small sample of the natural material remains; thus additional NMR experiments are not possible. Therefore, total synthesis represents the only practical method by which the correct structure can be determined unambiguously. For such an approach to be feasible, the synthetic route must be convergent, efficient, and, ideally, have a minimal reliance on the chiral pool. The synthesis of **1** previously reported by this group[2c] satisfies these requirements (Figure 1).

Initially, it was assumed that the error in relative stereochemistry was in the epoxide region where the acyclic nature of the side chain would result in the least reliable NOE data. The trans stereochemistry of the epoxide was assumed to be correct on the basis of a good correlation between the reported $J_{H20/H21}$ value and other trans epoxides. The possibility of an error in correlating the tetraol and epoxide portions was also considered.[3] These were some of the assumptions made by Maleczka[2b] and Pattenden[2a] who prepared isomers **2** and **3**, respectively (Figure 2). However, neither matched the data reported for the natural product.

Along these lines, we prepared C22 epimer **4** and the isomer **5** from inversion of the C19−C21 triad. Although neither matched, the $J_{H18/H19}$ value for **5** was 10.3 Hz, whereas the values for **1**, **2**, **4**, and the isolated material were 3.3−3.8 Hz, suggesting the requirement for trans (as drawn) C18−C19 stereochemistry. Proceeding on the belief that the correlation of the tetraol to the epoxide was tenuous, isomers **6**−**8** were prepared, combining changes in the epoxide with a change in tetraol configuration. However, none matched the reported data. The $J_{H18/H19}$ value for **8** was 10.3 Hz, whereas **7** and **9** were both 3.4 Hz, further confirming the requirement for trans C18−C19 stereochemistry.

A comparison of the ^{1}H NMR data for **1**, **2**, and **4**−**8** to the natural product led us to a troubling conclusion. Initially, comparisons to the natural product focused on coupling constants, and, apart from the requirement for trans C18−C19 stereochemistry, little information was gleaned by this approach. However, as shown in Table 1, a significant departure in the chemical shifts of the H9 and H11 tetraol protons was measured. Additionally, the differences were not random in sign or magnitude. This was surprising because relatively small differences were measured in the epoxide region, the site of the variations. Therefore, an error in the relative stereochemistry of the tetraol appeared likely.

Table 1. Deviation of the ^{1}H NMR Chemical Shifts of Isomers **1**, **2**, and **4**−**11** Relative to the Values Reported for the Isolated Material[a]

Proton	δ Synthetic Isomer − δ Isolated Material									
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
2	0.00	0.00	-0.01	+0.02	+0.02	-0.03	-0.08	-0.01	+0.05	**0.00**
4	-0.07	-0.13	-0.08	-0.08	-0.11	-0.11	-0.11	-0.07	-0.01	**+0.01**
5	0.00	-0.03	-0.01	-0.01	-0.01	-0.01	-0.06	-0.01	-0.01	**+0.01**
6	+0.02	-0.01	+0.02	+0.05	+0.01	+0.01	-0.04	+0.02	0.00	**+0.01**
6'	-0.01	-0.17	-0.02	-0.13	-0.15	-0.15	-0.09	-0.19	-0.03	**-0.02**
8	-0.19	-0.05	-0.19	-0.10	-0.04	-0.04	-0.06	0.00	-0.01	**+0.01**
9	*-0.29*	*-0.49*	*-0.30*	*-0.42*	*-0.48*	*-0.46*	*-0.28*	*-0.30*	+0.04	**+0.01**
11	*-0.25*	*-0.21*	*-0.26*	-0.14	-0.20	-0.18	-0.10	-0.07	-0.05	**-0.03**
12	-0.01	-0.06	-0.02	-0.13	-0.05	-0.04	+0.05	-0.05	-0.02	**0.00**
13	*-0.23*	-0.15	*-0.25*	-0.03	-0.13	-0.10	+0.02	-0.09	-0.02	**-0.01**
14	+0.07	-0.02	+0.06	+0.03	-0.01	0.00	+0.06	-0.01	-0.01	**0.00**
15	+0.01	-0.05	0.00	-0.01	-0.03	-0.02	+0.05	-0.02	-0.03	**0.00**
15'	-0.10	-0.15	0.00	-0.01	-0.13	-0.10	-0.08	-0.09	-0.03	**0.00**
17	+0.18	+0.06	+0.16	-0.18	+0.05	-0.09	-0.20	+0.15	+0.16	**-0.01**
17'	-0.14	-	-0.13	*-0.27*	-0.10	-0.04	-0.16	-0.11	-0.07	**0.00**
18	-0.02	-	-0.04	*-0.23*	-0.06	-0.03	-0.16	-0.07	-0.05	**-0.01**
19	-0.17	-0.14	-0.11	-0.12	-0.05	-0.05	-0.16	-0.10	-0.07	**0.00**
20	+0.07	+0.05	+0.12	+0.17	+0.12	-0.01	+0.06	+0.07	+0.07	**0.00**
21	-0.06	-0.10	-0.07	-0.04	-0.10	+0.01	-0.06	-0.08	-0.08	**0.00**
26	+0.03	-0.04	+0.02	-0.01	-0.02	-0.03	-0.03	-0.01	0.00	**0.00**
27	+0.09	-0.06	+0.01	-0.02	-0.04	-0.03	-0.03	+0.01	-0.02	**0.00**
27'	+0.02	-0.03	+0.01	+0.03	-0.01	0.00	-0.01	+0.03	-0.02	**0.00**
28	+0.10	-0.03	+0.10	0.00	-0.02	-0.01	0.00	+0.02	-0.04	**0.00**
28'	*+0.21*	0.00	+0.20	+0.07	+0.02	+0.02	+0.05	+0.06	-0.03	**+0.01**
29	-0.01	-0.05	-0.02	-0.05	+0.03	-0.01	-0.14	-0.04	-0.03	**0.00**
29'	-0.01	-0.04	-0.01	-0.04	-0.02	0.00	-0.06	-0.03	-0.02	**0.00**
30	-0.07	-	-0.07	-0.02	-0.05	-0.01	-0.04	-0.08	-0.06	**0.00**
31	+0.05	-	+0.08	+0.07	+0.07	-0.04	0.00	+0.04	+0.04	**0.00**

[a] Spectra were measured in $CDCl_3$ at 500 MHz. Differences are reported in ppm. Values in black represent deviations of <0.04, blue values represent 0.04−0.10, green values represent 0.11−0.20, and red italicized values represent >0.20.

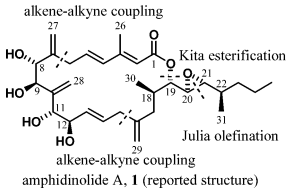

Figure 1. Retrosynthetic analysis of amphidinolide A.

Although we were concerned that errors within the epoxide and tetraol would make the possibilities so numerous and complex that we would be faced with nearly an impossible task, key spectral data provided direction. Because the $J_{H8/H9}$ and $J_{H11/H12}$ values for **1**, **2**, and **5**−**9** were consistent with the natural product, it appeared likely that the relative stereochemistry of the diols was correct, but the stereochemistry of the C8−C9 diol was incorrect relative to the C11−C12 diol. Therefore, isomer **9**, with the C8−C9 diol inverted, became the primary target.

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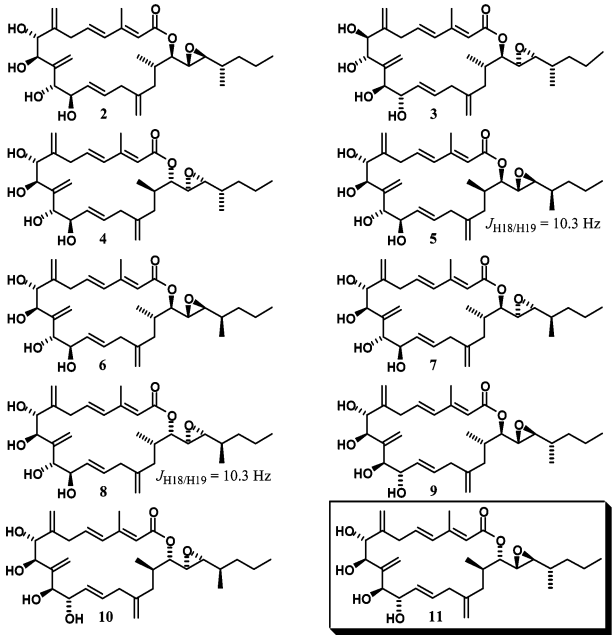


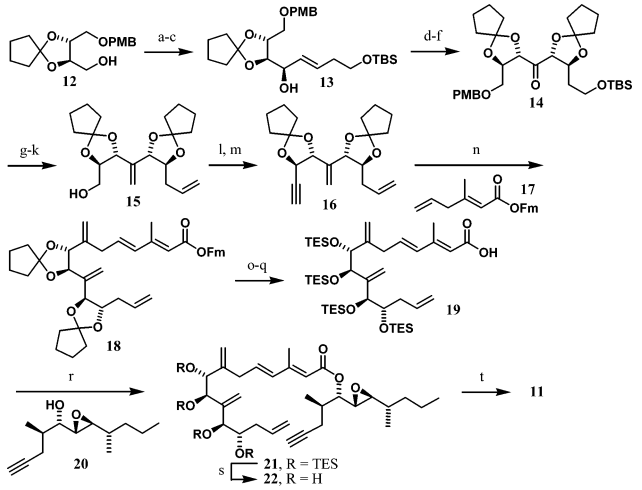
Figure 2. Amphidinolide A isomers **2**−**11**.

However, isomer **9**, with the C8−C9 diol inverted and epoxide stereochemistry of **1**, failed to match. Although the chemical shift of H11 was closer than was the case for the previous isomers, H9 was 0.30 ppm upfield from the natural product. However, as shown in Table 1, **10**, the C18−C22 epimer of **9**, provided an excellent match in the tetraol region. Only in the epoxide region were the shifts significantly different from the natural product.

Reexamination of the data in Table 1 indicated that **7** was an excellent match in the epoxide region. Therefore, isomer **11**, the combination of the relative stereochemistry found in the epoxide of **7** and the tetraol of **10**, became a priority. The more quantitative analysis of the data in Table 1 that follows also pointed to **11**. The relationship between **10** and **11** is analogous to that between **2** and **7**, inversion of the C20−C22 triad. If the changes in chemical shift that occur when the C20−C22 triad of **2** is inverted, thus yielding **7**, are applied to **10**, a nearly perfect match to the natural material is obtained. For example, the chemical shift of H19 in **2** and **7** is 4.58 and 4.67 ppm, respectively. This represents a downfield shift of 0.09 ppm. The shift of H19 in **10** is 4.65 ppm. A 0.09 ppm downfield shift yields a predicted shift of 4.74 ppm for H19 of **11**. This value compares well to the shift of 4.72 ppm for H19 of the natural product. Analysis of the other protons yields similar results.

The new tetraol required a complete redesign of nearly all stages of our original synthesis.[2c] Ester **18** was prepared in 15 steps from **12**[4] (Scheme 1). Conversion of **18** to **11** required a significant change to the end game due to the sensitivity of the epoxide in **11** to acidic hydrolysis. After deprotection of **21**, [Cp*Ru(MeCN)$_3$]-PF$_6$-catalyzed macrocyclization of **22** provided **11**, illustrating the remarkable chemoselectivity of the Ru-catalyzed alkene−alkyne addition. The spectral data for **11** provided an excellent fit to the natural product. One proton deviated by 0.03 ppm, one by 0.02 ppm, and the remainder by 0.01 ppm or less. The ^{1}H NMR spectra in C_6D_6 and CD_3OD deviated by 0.01 ppm or less from the isolated material in those solvents.[5] The ^{13}C NMR spectrum deviated by 0.1 ppm or less in $CDCl_3$.[6] The *J* values in all three solvents were also in agreement. These results are well within experimental error. Finally, the optical rotation $[\alpha]^{24}_D$ +56° (*c* 0.05, $CHCl_3$) was identical in sign, but slightly higher than the reported value $[\alpha]^{24}_D$ +46° (*c* 1.0, $CHCl_3$), therefore establishing the absolute stereochemistry.

Scheme 1. Synthesis of Amphidinolide A Isomer **11**[a]



[a] (a) (i) $(COCl)_2$, DMSO; (ii) Et_3N (Moffatt−Swern); (b) *n*-BuLi, HC≡CCH$_2$CH$_2$OTBS, ClTi(Oi-Pr)$_3$; (c) Red-Al, 51% (three steps); (d) Dess−Martin, 86%; (e) Sharpless AD; (f) 1,1-dimethoxycyclopentane, TsOH·H$_2$O; (g) Ph_3PMeBr, NaHMDS, 60% (three steps); (h) TBAF, 78%; (i) Moffatt−Swern; (j) Ph_3PMeBr, *n*-BuLi, 79%; (k) DDQ, 98%; (l) Moffatt−Swern; (m) $(MeO)_2POC(=N_2)COMe$, K_2CO_3, 87% (two steps); (n) **17** (5 equiv), [Cp*Ru(MeCN)$_3$]PF$_6$, 23% (39% brsm); (o) piperidine, 88%; (p) AcOH/H$_2$O (3:1); (q) TESOTf, *i*-Pr$_2$NEt, 83% (two steps); (r) (i) [RuCl$_2$(*p*-cymene)]$_2$, HC≡COEt; (ii) **20**, CSA, 51%;[7] (s) TBAF, AcOH, 79%; (t) [Cp*Ru(MeCN)$_3$]PF$_6$, 33% (38% brsm).

In conclusion, we have employed a combination of synthesis and NMR spectroscopy as tools to determine the correct structure of amphidinolide A. Although the lack of a sample of the natural product prevents a definitive comparison, the excellent correlation of **11** strongly suggests it is (+)-amphidinolide A.

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Supporting Information Available: Experimental procedures for **11**, **13**−**16**, and **18**−**21**. Characterization data for **4**−**11**, **13**−**16**, and **18**−**21** and spectra for **11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) The epoxide to tetraol correlation was determined sequentially by NOE between H11 and H13, H13 and H15, and H15 and H18.
(4) Alcohol **12** was prepared in three steps from (−)-diethyl tartrate: Rzepecki, P. W.; Prestwich, G. D. *J. Org. Chem.* **2002**, *67*, 5454.
(5) In C_6D_6, protons 6a, 6b, 8, 9, 11, 12, 17b, 27a, and 28a deviated by 0.01 ppm. In CD_3OD, protons 4, 6a, 6b, 12, and 28b deviated by 0.01 ppm.
(6) Carbons 3−5, 9, 13, 22, 24, 25, and 27−29 deviated by 0.1 ppm.
(7) Kita, Y.; Maeda, H.; Omori, K.; Okuno, T.; Tamura, Y. *Synlett* **1993**, 273.

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