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Absolute Stereochemistry of Amphidinolide B

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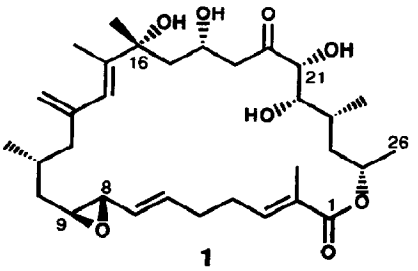
Abstract: The absolute stereochemistry of amphidinolide B (**1**), a potent cytotoxic 26-membered macrolide isolated from the cultured marine dinoflagellate *Amphidinium* sp., has been established as 8*S*, 9*S*, 11*R*, 16*R*, 18*S*, 21*R*, 22*S*, 23*R*, and 25*S*, on the basis of enantiospecific synthesis of a degradation product (**2**).

Amphidinolide B (**1**) is a potent cytotoxic 26-membered macrocyclic lactone, first isolated from a cultured marine dinoflagellate of the genus *Amphidinium*, which was originally living inside of Okinawan marine flatworms of the genus *Amphiscolops*.[1] We previously reported the planar structure of amphidinolide B (**1**) on the basis of extensive analysis of the 2D NMR data.[2] Recently, Shimizu *et al.* isolated three macrolides belonging to the amphidinolide B group (amphidinolides B_1, B_2, and B_3) from a free-swimming dinoflagellate *Amphidinium* sp. and reported their relative stereochemistry on the basis of X-ray crystal structure of amphidinolide B_1.[3] Identity of amphidinolides B and B_1 was unambiguously established by direct comparison of HPLC and ^{1}H NMR data[4] using each authentic sample. This communication describes determination of the absolute stereochemistry of amphidinolide B (**1**) based on synthesis of a degradation product (**2**) and chiral HPLC analysis.

To investigate the absolute configurations of **1**, we prepared both enantiomers of C-22 ~ C-26 fragment (**2**) as shown in Scheme 1. Tosylation of one of hydroxyl groups of (2*S*,4*S*)-(+)-pentanediol (**3**)[5] with TsCl in pyridine (19 h, rt) afforded a monotosylate (**4**), which was converted into the cyanide (**5**) with inversion of configuration by treatment with sodium cyanide in DMSO.[6] Alkaline hydrolysis of the cyano group of **5** followed by acidic work-up afforded the γ-lactone (**6**),[7] which was reduced with $LiAlH_4$ and the resulting diol (**7**)[7] was acetylated to give the diacetate (+)-**2**.[8] The enantiomer (-)-**2** was prepared from (2*R*,4*R*)-(-)-pentanediol[5] by the same procedures as those for (+)-**2**. The enantiomers (+)- and (-)-**2** were analyzed by chiral HPLC [CHIRALCEL OD, Daicel Chemical Ind., Ltd.; 4.6 x 250 mm; flow rate: 1.0 mL/min; eluent: hexane/2-propanol (500:1); UV detection at 215 nm] and found to be separable [(+)-**2**, t_R 23.2 min; (-)-**2**, t_R



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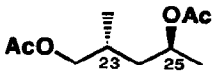
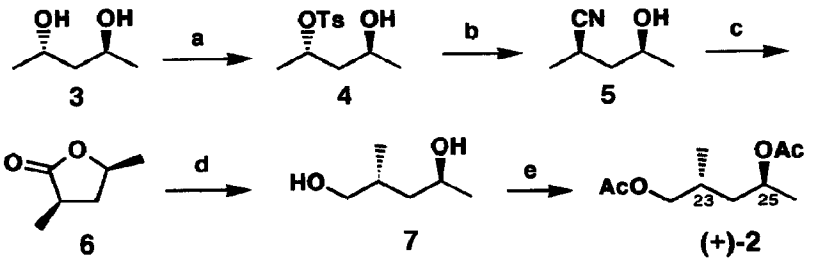


(+)-2

23*R*, 25*S*



Scheme 1. Preparation of (+)-2. (a) TsCl, pyridine, 37%; (b) NaCN, DMSO; (c) (1) NaOH, H_2O_2, EtOH; (2) 2N HCl; (d) $LiAlH_4$, Et_2O, 12% from 4; (e) Ac_2O, pyridine, 72%.

22.3 min].

A MeOH adduct of amphidinolide B (1), which was obtained as an artifact of isolation and have a structure with a methoxyl and a hydroxyl groups at C-8 and C-9, respectively,[2] was treated with $NaIO_4$ followed by $NaBH_4$ reduction and acetylation (Ac_2O/pyridine) to give the C-22 ~ C-26 fragment (2) after separation by normal-phase HPLC [YMC-Pack SIL-06; 4.6 x 250 mm; flow rate: 1.0 mL/min; eluent: hexane/EtOAc (3:1); RI detection; t_R 6.6 min]. This fragment thus obtained from natural compound (1) was subjected to chiral HPLC analysis (the same conditions as above) and proved to be identical with (+)-2 (t_R 23.2 min), thus revealing that the C-22 ~ C-26 fragment (2) derived from 1 has (23*R*, 25*S*)-configurations. Since the relative stereochemistry of amphidinolide B_1 identical with 1 is known,[3] the absolute configurations of amphidinolide B (1) were concluded as 8*S*, 9*S*, 11*R*, 16*R*, 18*S*, 21*R*, 22*S*, 23*R*, and 25*S*, which was in agreement with our recent results on the absolute configurations of amphidinolide L.[9]

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References and Notes

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2. The initially proposed planar structure was later revised: Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *J. Nat. Prod.* **1989**, *52*, 1036-1041.
3. Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. *J. Am. Chem. Soc.* **1994**, *116*, 2657-2658.
4. HPLC analysis: Develosil ODS-5, Nomura Chemical; 5 μm, 10 x 250 mm; eluent: 60% CH_3CN; flow rate: 2.5 mL/min; UV detection at 220 nm. Retention times: amphidinolide B and B_1, 30.6 min; amphidinoliode D,[2] 31.8 min. ^1H NMR spectra of amphidinolides B and B_1 were recorded on a 500 MHz spectrometer in C_6D_6 solution, and proved to be completely identical. The signs of the optical rotations of amphidinolides B and B_1 were also the same.
5. (2*S*,4*S*)-(+)-Pentanediol and (2*R*,4*R*)-(-)-pentanediol were purchased from Kanto Chemical, Co., Inc.
6. The synthesis of the cyanide with t-butyldimethylsilyl group by the similar procedure was described quite recently: Imaeda, T.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1994**, *35*, 591-594.
7. The γ-lactone (6) and diol (7) were previously prepared from (*S*)-ethyl lactate: Chiarello, J.; Joullié, M. M. *Synth. Commun.* **1989**, *19*, 3379-3383. The spectral data of 6 and 7 prepared by us were identical with those described in this literature.
8. (+)-2: colorless oil; $[\alpha]_D^{24}$ +8.8° (*c* 2.0, $CHCl_3$); IR (neat) 1745 and 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (3H, d, *J*=6.7 Hz), 1.23 (3H, d, *J*=6.3 Hz), 1.26 (1H, m), 1.72 (1H, ddd, *J*=14.0, 9.6, and 4.8 Hz), 1.87 (1H, m), 2.03 (3H, s), 2.06 (3H, s), 3.85 (2H, m), and 5.03 (1H, ddd, *J*=9.6, 6.3, and 3.3 Hz). (-)-2: colorless oil; $[\alpha]_D^{26}$ -9.4° (*c* 3.0, $CHCl_3$).
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