Asymmetric Synthesis of a Diastereomer of the Structure Proposed for Amphidinolide A and the Determination of Its Absolute Configuration

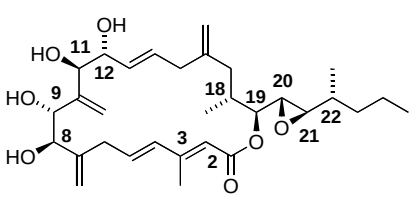
Haruaki Ishiyama, Yoichi Nakamura, and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

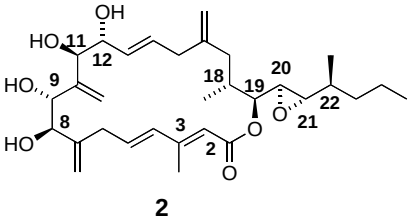
Abstract An asymmetric synthesis of a diastereomer (**2**) of the structure (**1**) proposed for amphidinolide A, a cytotoxic macrolide from the cultured dinoflagellate *Amphidinium* sp., has been accomplished. The absolute configuration of amphidinolide A was established as **3** from comparison of NMR data, HPLC analysis, and $[\alpha]_D$ values of amphidinolide A, and comparison with the synthetic diastereomers **2** and **3**, the latter of which was synthesized previously by Trost's group.

Keywords: *Amphidinium* sp.; macrolide; amphidinolide A; absolute configuration

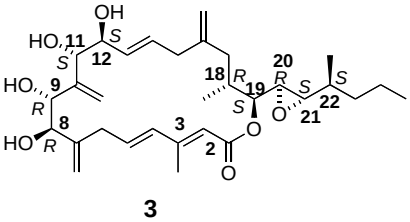
Amphidinolide A is a cytotoxic 20-membered macrolide, isolated from the cultured dinoflagellate *Amphidinium* sp., which is a symbiont of the Okinawan marine flatworm *Amphiscolops* sp.[1a] The relative stereochemistry of the nine stereogenic centers in amphidinolide A was proposed to be **1** on the basis of extensive NMR experiments by our group.[1b] The unique structure and bioactivity of amphidinolide A have prompted studies of its total syntheses. First Pattenden,[2] and later Maleczka,[3]



1



2



3

*Corresponding author. Tel. +81 11 706 4985; fax +81 11 706 4989; email; jkobay@pharm.hokudai.ac.jp

and Trost[4] accomplished total syntheses of the stereostructure (**1**) proposed for amphidinolide A, and indicated that the proposed stereostructure (**1**) was incorrect from comparison of the NMR data of their synthetic compounds with those reported for amphidinolide A. More recently, Trost's group achieved the syntheses of nine stereoisomers of the proposed stereostructure (**1**), and suggested that the diastereomer **3** may be the correct stereostructure of amphidinolide A.[4b,c,d]

In our efforts to determine the correct stereostructure of amphidinolide A, we have re-examined the relative stereochemistry of amphidinolide A. Our re-examination of the ^{1}H and ^{13}C NMR data have indicated that the correct stereostructure of amphidinolide A could be either of the diastereomer **2** or **3**. Since the diastereomer **3** has been synthesized by Trost's group,[4b] we decided to synthesize the alternative diastereomer **2** and to compare the NMR data of the synthetic diastereomers **2** and **3** with those of naturally derived amphidinolide A. In this paper, we describe an asymmetric synthesis of the diastereomer **2**, and the determination of the absolute stereochemistry of amphidinolide A to be **3**.

Although earlier we reported previously the relative stereochemistry for C-19 and C-20 in narural amphidinolide A as 19,20-*threo* from NOESY correlations of H-19 to H-21, H-20 to H_2-17, and H-20 to H_3-30 (Figure 1(a)), the proposed stereostructure **1** was not correct. Alternatively, it was

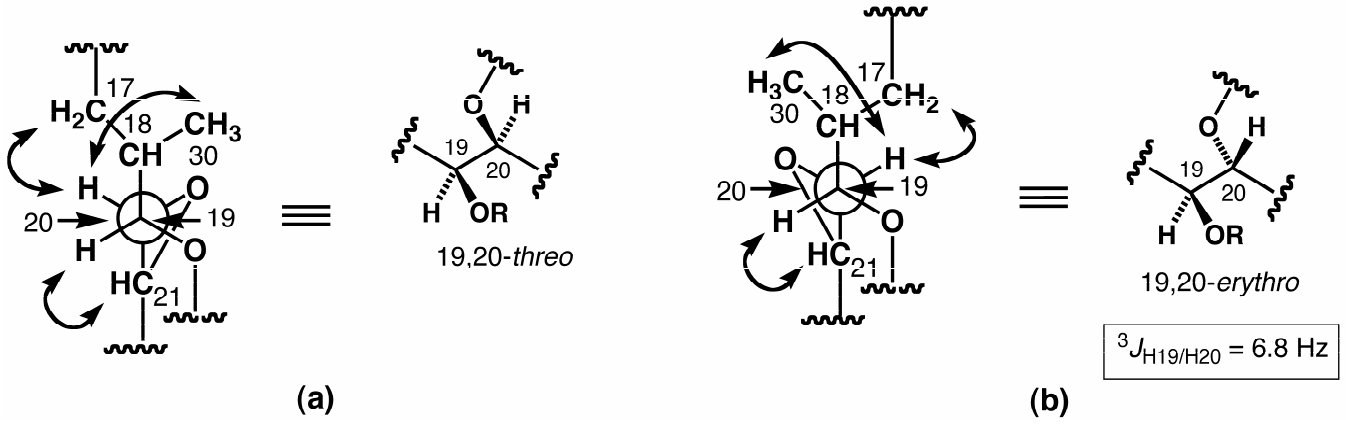
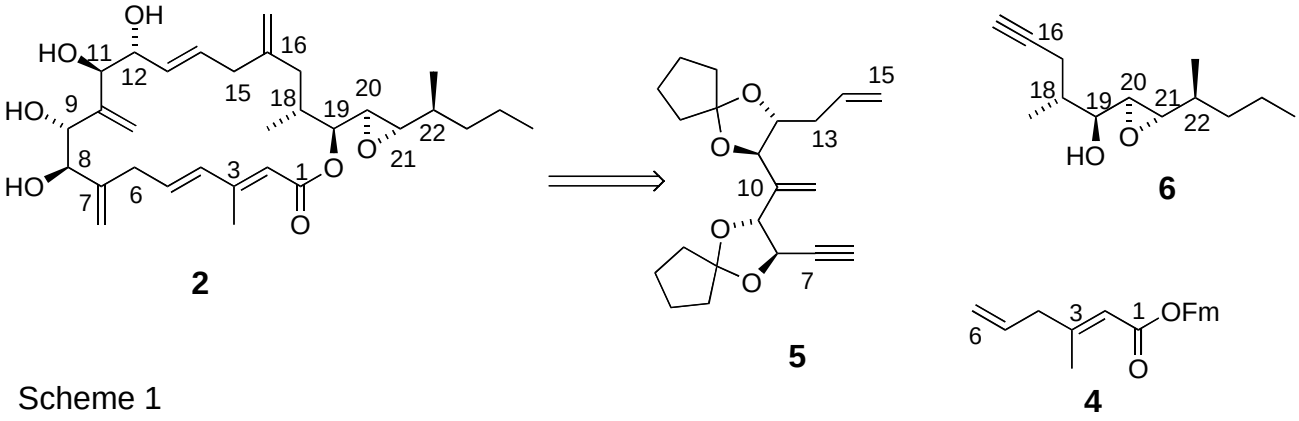


Figure 1. NOESY correlations and relative stereochemistry for C-17~C-21 segments in amphidinolide A based on the previous (a) and present (b) assignments.

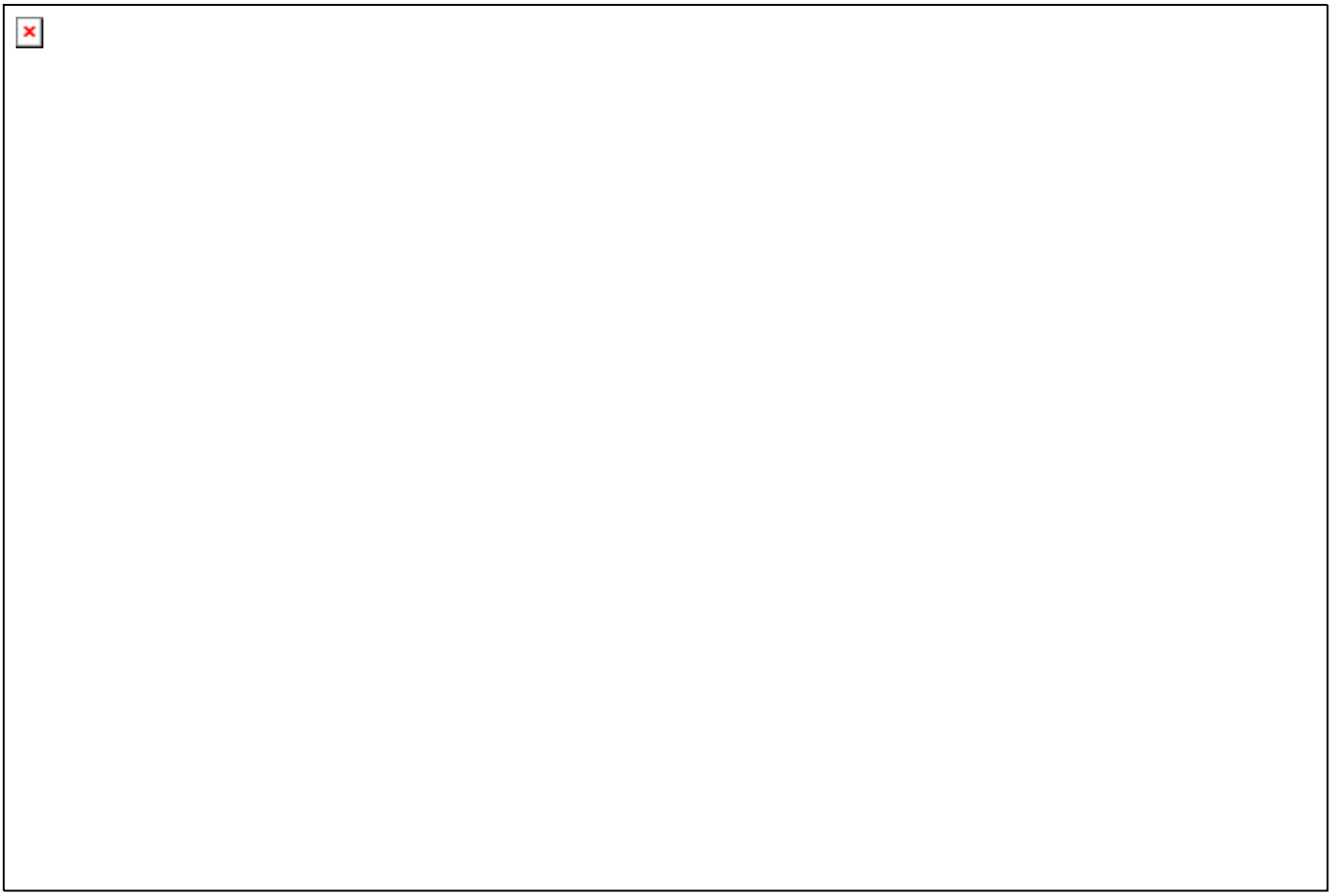
elucidated to be 19,20-*erythro* from the NOESY correlations and the ^{1}H-^{1}H coupling constant (6.8 Hz) between H-19 and H-20 (Figure 1(b)). On the other hand, the ^{13}C NMR data obtained for amphidinolide A were compared with those of the synthetic compound **1** reported by the Maleckzka group,[3] in which the difference (Δ +1.8 ppm) in chemical shifts at C-12 was slightly larger than those ($|\Delta| < 1.3$ ppm) of the other stereogenic centers.[5] The ^{1}H-^{1}H coupling constant (~0 Hz) between H-11 and H-12 indicated 11,12-*threo* as reported previously.[1b] These observations suggested that the possible stereostructure of amphidinolide A could be either diastereomer **2** or **3**.

Since Trost's group has recently reported a synthesis of the diastereomer **3**, we planned to synthesize the other possible diastereomer **2**, using the synthetic strategy by Trost[4b], but using Kita's esterification[6] and a ruthenium-catalyzed coupling reaction[7] (Scheme 1).



Scheme 1

The alkyne **5**[4b] and the alkene **4**[4b] were prepared according to the same procedure as Trost's group. Treatment of the alkyne **5** and the alkene **4** with ruthenium catalyst provided **7** and its isomer **7'** (Scheme 2). After deprotection of the fluorenymethanol (Fm) group in **7** with piperidine, the protecting group of **8** was changed from ketal to TES ether to give the acid **9**.[8] Esterification of **9** with the alchol **6** using the modified method[9] of Kita[6] provided the desired ester **10** with no isomerization of any olefin moiety.[10] After removal of the TES group, intramolecular cycloisomerization of **11** under high dilution conditions, to form the C15-C16 bond, gave the desired diastereomer **2**.



Scheme 2.

The ^{1}H and ^{13}C NMR data for synthetic compound **2** were not coincident with those of amphidinolide A, whereas the NMR data for compound **3** synthesized by Trost's group were close to those of amphidinolide A (Figure 2). Compounds **2** and **3**, and amphidinolide A were subjected to C_{18} HPLC [Mightysil RP-18, 4.6 x 250 mm; flow rate 1.0 mL/min: eluent; MeCN/H_2O (60:40); UV detection at 265 nm], and it was found that the retention time of amphidinolide A (t_R 16.5 mim) was identical with that of **3** (t_R 16.5 min) but not that of **2** (t_R 12.8 min). Thus, the relative stereostructure for amphidinolide A was assigned as **3**. The optical rotations of compounds **2** and **3**, and amphidinolide A were compared as follows; $[\alpha]_D^{21}$ -11 ° (*c* 0.6, $CHCl_3$) for **2**, $[\alpha]_D^{24}$ +56 ° (*c* 0.05, $CHCl_3$) for **3**, and $[\alpha]_D^{24}$ +46 ° (*c* 1.0, $CHCl_3$) for amphidinolide A. Therefore, it was concluded that the absolute

configurations at the nine chiral centers of amphidinolide A were 8*R*, 9*R*, 11*S*, 12*S*, 18*R*, 19*S*, 20*R*, 21*S*, and 22*S*.

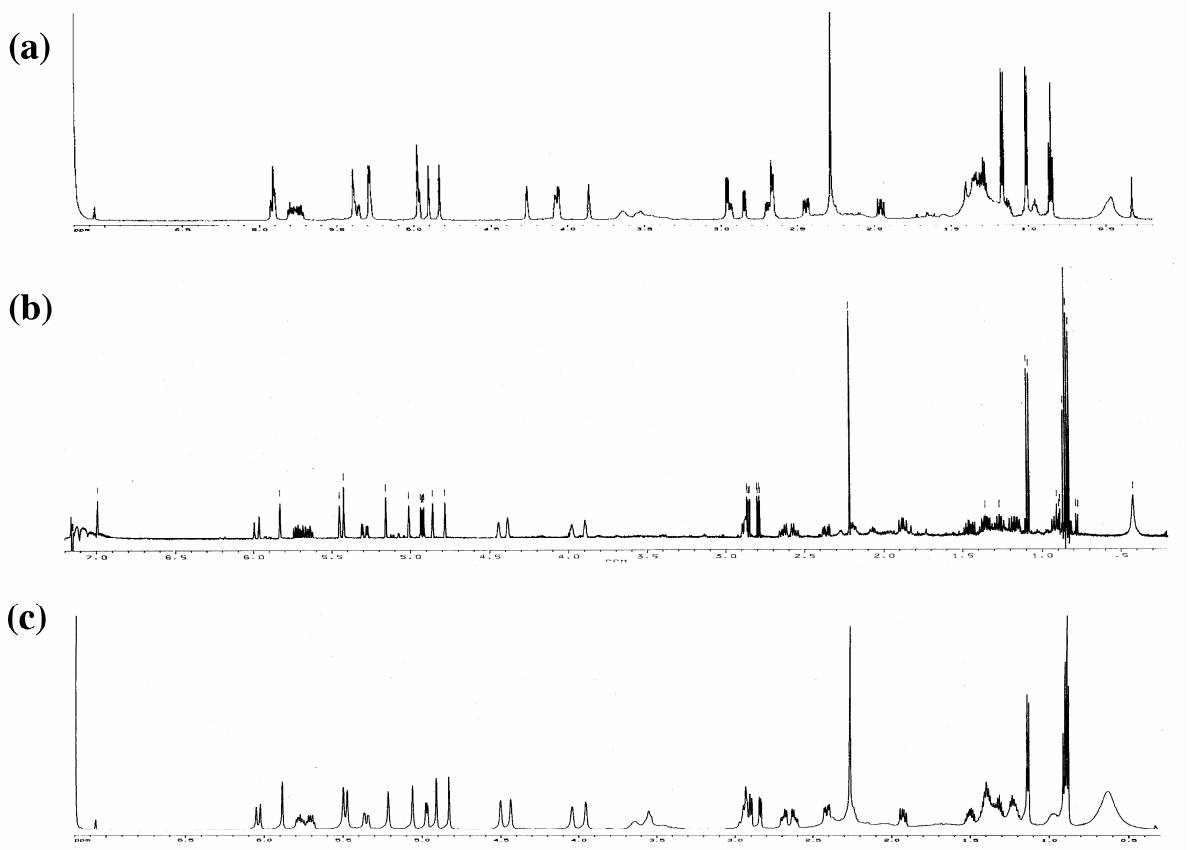


Figure 2. ^{1}H NMR profiles of synthetic diastereomers **2** (a) and **3** (c), and amphidinolide A (b) in C_6D_6.

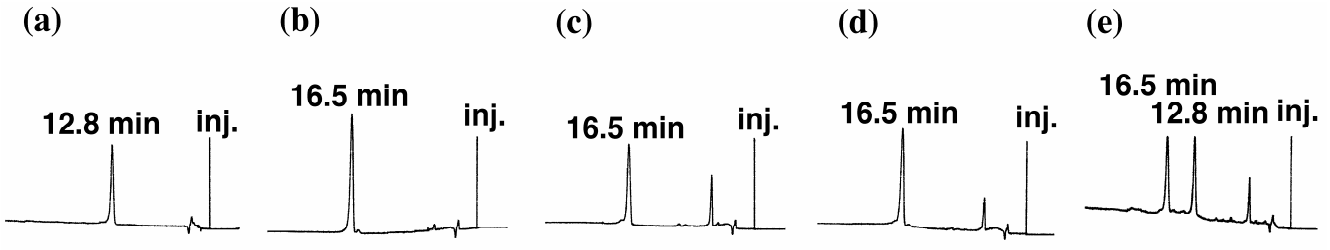


Figure 3. HPLC profiles of synthetic diastereomers **2** (a) and **3** (b), amphidinolide A (c), **3** and amphidinolide A (d), **2** and amphidinolide A (e)

Experimental Section

General Methods. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. The IR spectrum was taken on a JASCO FT/IR-5300 spectrometer. Proton and carbon NMR spectra were recorded on a Bruker 500 and/or 600 MHz and JEOL 400 MHz spectrometer. ESI mass spectra were obtained on a JEOL JMS-SX102A spectrometer.

7-{3-[1-(3-Allyl-1,4-dioxa-spiro[4,4]non-2-yl)-vinyl]-1,4-dioxa-spiro[4,4]non-2-yl}-3-methyl-octa-2, 4,7-trienoic acid 9 H-fluoren-9-ylmethyl ester (7)

A solution of alkyne **5**[4a] (208.4 mg, 0.605 mmol) and alkene **4**[4a] (916.5 mg, 3.01 mmol) in dichloroethane (DCE) (1.7 mL) was degassed by F. T. P. (Freeze-pump-thaw cycles), the reaction mixture was heated to 50ºC, and $Cp^*Ru(MeCN)_3PF_6$ (60.7 mg, 0.120 mmol) was added in one portion. After 3 h, the reaction mixture was purified by flash column chromatography on silica gel (10% to 15% Et_2O in petroleum ether) to give branched ester **7** (141.4 mg, 0.218 mmol, 36%) and linear ester **7'** (47.3 mg, 0.0729 mmol, 12%) as colorless oils. Data for branched alkene **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2 H), 7.62 (d, *J* = 8.0 Hz, 2 H), 7.41 (dd, *J* = 7.6, 7.6 Hz, 2 H), 7.32 (dd, *J* = 8.0, 7.6 Hz, 2 H), 6.16-6.23 (m, 2 H), 5.81-5.89 (m, 2 H), 5.42-5.49 (m, 2 H), 5.24 (s, 1 H), 5.09-5.14 (m, 2 H), 5.01 (s, 1 H), 4.39-4.43 (m, 3H), 4.26 (t, *J* = 7.2 Hz, 1 H), 4.20 (d, *J* = 8.8 Hz, 1 H), 4.06 (d, *J* = 8.0 Hz, 1 H), 3.89-3.94 (m, 1 H), 3.05 (dd, *J* = 16.4, 5.6 Hz, 1 H), 2.92 (m, 1 H), 2.43 (m, 1 H), 2.25-2.31 (m, 4H), 1.67-1.90 (m, 16 H); ^{13}C NMR (100 MHz, $CDCl_3$) 166.8, 152.7, 143.9, 143.3, 142.4, 141.2, 135.6, 134.1, 133.9, 127.6, 127.0, 125.0, 119.9, 119.0, 118.6, 117.9, 117.7, 117.4, 115.6, 84.0, 81.3, 79.8, 79.7, 65.8, 46.9, 37.7, 37.5, 37.4, 37.4, 36.4, 34.7, 23.6, 23.5, 23.5, 14.0; ESIMS *m/z* 480 (M-$C_{10}H_{16}O_2$).

7-{3-[1-(3-Allyl-1,4-dioxa-spiro[4,4]non-2-yl)-vinyl]-1,4-dioxa-spiro[4,4]non-2-yl}-3-methyl-octa-2, 4,7-trienoic acid (8)

To a solution of branched ester **7** (170.3 mg, 0.262 mmol) in CH_2Cl_2 (6.9 mL) at 0 °C was added piperidine (0.86 mL, 8.7 mmol). After 2.5 h at room temperature, the reaction mixture was diluted

with CH_2Cl_2 (30 mL), washed with 0.2 M H_2SO_4 (x 3), brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (hexane/EtOAc/MeOH, 30:1:2) gave acid **8** (111.2 mg, 90%) as a colorless oil.

8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14-6.20 (m, 2 H), 5.80-5.90 (m, 1 H), 5.75 (s, 1 H), 5.47 (s, 1 H), 5.46 (s, 1 H), 5.23 (s, 1 H), 5.09-5.13 (m, 2 H), 4.99 (s, 1 H), 4.40 (d, *J* = 8.4 Hz, 1 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.05 (d, *J* = 8.0 Hz, 1 H), 3.90 (td, *J* = 7.4, 4.0 Hz, 1 H), 3.02-3.07 (m, 1 H), 2.87-2.92 (m, 1 H), 2.38-2.43 (m, 1 H), 2.30 (s, 3 H), 2.24-2.33 (m, 1H), 1.64-1.91 (m, 17 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 154.4, 143.2, 142.3, 135.5, 134.7, 133.8, 119.0, 118.6, 117.7, 117.6, 117.3, 115.6, 84.0, 81.2, 79.7, 79.7, 37.6, 37.5, 37.4, 37.4, 36.3, 34.7, 23.6, 23.5, 23.5, 14.2; ESIMS *m/z* 493 (M+Na); $[\alpha]_D^{22}$ +37 ° (*c* 1.2, CH_2Cl_2).

3-Methyl-7,10-dimethylene-8,9,11,12-tetrakis-triethylsilanyloxy-pentadeca-2,4,14-trienoic acid (9)

To ketal **8** (111.2 mg, 0.236 mmol) at room temperature was added acetic acid (1.5 mL) and water (0.5 mL). The reaction mixture was heated to 40 ºC for 24 h and concentrated to give the tetraol which was used in the next step without further purification. To a solution of the tetraol in THF (5.9 mL) at 0 ºC was added *i*-Pr$_2$NEt (576 μL, 3.31 mmol) and TESOTf (530 μL, 2.36 mmol). The reaction mixture was stirred at 0 ºC for 20 min, quenched with 1 M HCl (3.3 mL), stirred for 10 min, and diluted with ether (3 mL) and water (3 mL). The aqueous phase was extracted with ether (3 x) and the combined organic extracts were washed with saturated KH_2PO_4 (1 x), brine (1 x), dried over $MgSO_4$, and concentrated. Purification by flash column chromatography on silica gel (9% EtOAc in hexanes) gave silyl ether **9** (172.9 mg, 92%) as a colorless oil

9: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.11-6.20 (m, 2 H), 5.80-5.85 (m, 1 H), 5.74 (s, 1 H), 5.32 (s, 2 H),

4.96-5.03 (m, 3 H), 4.84 (s, 1 H), 4.49 (d, *J* = 4.0 Hz, 1 H), 4.35 (d, *J* = 3.5 Hz, 1 H), 4.15 (d, *J* = 4.0 Hz, 1 H), 3.74 (ddd, *J* = 8.2, 4.0, 3.5 Hz, 1 H), 3.01 (dd, *J* = 16.3, 5.5 Hz, 1 H), 2.94 (dd, *J* = 16.3, 6.5 Hz, 1 H), 2.34-2.40 (m, 2 H), 2.30 (s, 3 H), 0.90-0.99 (m, 36 H), 0.56-0.67 (m, 24 H); ^{13}C NMR (125 MHz, $CDCl_3$) 171.9, 155.1, 148.4, 146.9, 137.1, 136.3, 135.0, 116.9, 116.1, 113.6, 113.5, 80.1, 76.8, 74.9, 74.7, 36.9, 36.6, 14.0, 7.1, 7.1, 7.0, 7.0, 5.5, 5.2, 4.9, 4.9; IR (film) ν_{max} 2955, 2874, 1686, 1609, 1092, and 742 cm^{-1}; HRESIMS calcd for $C_{42}H_{82}O_6Si_4Na$ *m/z* 817.5086, found *m/z* 817.5078; $[\alpha]_D^{22}$ +29° (*c* 1.0,$CHCl_3$).

3-Methyl-7,10-dimethylene-8,9,11,12-tetrakis-triethylsilanyloxy-pentadeca-2,4,14-trienoic acid 2-methyl-1-[3-(1-methyl-butyl)-oxiranyl]-pent-4-ynyl ester (10)

To a solution of acid **9** (17.7 mg, 22.3 μmol) in toluene (0.59 mL) at room temperature was added $[RuCl_2(p\text{-cymene})]_2$ (1.4 mg, 17.7 μmol) and a toluene (0.19 mL) solution of ethyl ethynyl ether (40 wt% in hexane, 16 μL, 66.8 μmol). The reaction mixture was stirred at room temperature for 3 h and concentrated under a stream of argon. A solution of epoxy alcohol **6** (11.7 mg, 55.6 μmol) in DCE (0.15 mL) was added via cannula followed by CSA (0.52 mg, 2.24 μmol) and MS3A (10 mg). The reaction mixture was stirred at room temperature for 2 h, filtered through silica gel, and concentrated. Purification by flash column chromatography on silica gel (5% EtOAc in hexane) gave ester **10** (9.3 mg, 42%) as a colorless oil.

10: ^{1}H NMR (600 MHz, C_6D_6) δ 6.23-6.30 (m, 2 H), 6.07 (dddd, *J* = 17.0, 9.4, 7.7, 7.2 Hz, 1 H), 5.89 (s, 1 H), 5.70 (s, 1 H), 5.67 (s, 1 H), 5.31 (s, 1 H), 5.25 (d, *J* = 17.0 Hz, 1 H), 5.15 (d, *J* = 9.4 Hz, 1 H), 5.07 (s, 1 H), 4.95 (dd, *J* = 7.1, 4.6 Hz, 1 H), 4.78 (d, *J* = 3.8 Hz, 1 H), 4.62 (d, *J* = 2.8 Hz, 1 H), 4.41 (d, *J* = 3.8 Hz, 1 H), 4.02 (ddd, *J* = 8.2, 3.9, 2.8 Hz, 1 H), 3.16-3.23 (m, 2 H), 2.89 (dd, *J* = 7.6, 1.7 Hz, 1 H),

2.75 (dd, *J* = 7.1, 1.7 Hz, 1 H), 2.70-2.73 (m, 1 H), 2.47 (s, 3 H), 2.33-2.40 (m, 3 H), 2.12-2.20 (m, 1 H), 1.82 (t, *J* = 2.3 Hz, 1 H), 1.18 (d, *J* = 6.5 Hz, 3 H), 1.09-1.32 (m, 41 H), 0.99 (d, *J* = 6.6 Hz, 3 H), 0.74-0.87 (m, 27 H); ^{13}C NMR (150 MHz, C_6D_6) δ 165.9, 153.4, 149.0, 147.5, 137.2, 135.8, 135.2, 118.1, 116.7, 114.2, 113.9, 82.3, 80.7, 77.4, 75.4, 75.3, 75.1, 70.2, 62.8, 57.0, 37.5, 37.2, 36.1, 35.9, 35.7, 22.7, 20.5, 17.3, 14.5, 14.4, 14.0, 7.5, 7.4, 7.4, 7.3, 6.0, 5.7, 5.5, 5.4; IR (neat) ν_{max} 2956, 2121, 1717, 1235, 1146 cm^{-1}; HRESIMS calcd for $C_{55}H_{102}O_7Si_4Na$ *m/z* 1009.6600, found *m/z* 1009.6638; $[\alpha]_D^{20}$ +41 ° (*c* 1.0, CH_2Cl_2).

8,9,11,12-Tetrahydroxy-3-methyl-7,10-dimethylene-pentadeca-2,4,14-trienoic acid 2-methyl-1-[3-(1-methyl-butyl)-oxiranyl]-pent-4-ynyl ester (11)

To a solution of silyl ether **10** (9.3 mg, 9.42 μmol) in THF (0.2 mL) at 0 °C was added a mixture of TBAF (190 μL, 1 M in THF, 190 μmol) and acetic acid (21.5 μL). The reaction mixture was stirred at room temperature for 24 h, diluted with water (3 mL), extracted with EtOAc (4 mL x 3), washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (13% to 67% EtOAc in hexane) gave tetraol **11** (2.3 mg, 46%) as an amorphous solid: ^{1}H NMR (600 MHz, CD_3OD) δ 6.23-6.27 (m, 2 H), 5.89 (dddd, *J* = 17.2, 10.2, 7.0, 7.0 Hz, 1 H), 5.78 (s, 1 H), 5.38 (s, 1 H), 5.34 (s, 1 H), 5.20 (s, 1 H), 5.10 (dd, *J* = 17.2, 1.5 Hz, 1 H), 5.05 (dd, *J* = 10.2, 1.5 Hz, 1 H), 4.97 (s, 1 H), 4.69 (dd, *J* = 6.6, 4.8 Hz, 1 H), 4.16 (d, *J* = 4.4 Hz, 1 H), 4.14 (d, *J* = 4.4 Hz, 1 H), 3.98 (d, *J* = 3.8 Hz, 1 H), 3.70 (ddd, *J* = 7.7, 3.8, 3.8 Hz, 1 H), 3.06 (dd, *J* = 16.4, 4.5 Hz, 1 H), 2.96 (dd, *J* = 16.4, 5.0 Hz, 1 H), 2.87 (dd, *J* = 6.7, 2.0 Hz, 1 H), 2.71 (dd, *J* = 7.4, 2.0 Hz, 1 H), 2.17-2.38 (m, 5 H), 2.29 (s, 3 H), 2.07-2.11 (m, 1 H), 1.22-1.32 (m, 5 H), 1.13 (d, *J* = 6.8 Hz, 3 H), 0.99 (d, *J* = 6.2 Hz, 3 H), 0.85 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.4, 155.8, 151.9, 149.4, 137.5, 137.5,

137.0, 119.1, 118.2, 115.0, 114.6, 83.6, 78.0, 76.7, 76.4, 75.5, 74.9, 71.9, 64.6, 59.0, 39.6, 38.4, 37.8, 37.6, 37.2, 22.9, 22.0, 18.4, 15.5, 15.4, 15.1; IR (KBr) ν_{max} 3437 (br), 2958, 2353, 1716, 1150 cm^{-1}; HRESIMS calcd for $C_{31}H_{46}O_7Na$ *m/z* 553.3141, found *m/z* 553.3138; $[\alpha]_D^{20}$ +6.7 ° (*c* 1.0, CH_2Cl_2).

Diastereomer 2

A solution of tetraol **11** (4.7 mg, 8.86 μmol) in DCE (8.9 mL) was degassed by F. T. P. The reaction mixture was heated to 50ºC and Cp*Ru(MeCN)$_3$PF$_6$ (2.2 mg, 4.4 μmol) was added. After 7 h at 50ºC, the reaction mixture was filtered through silica gel, and concentrated. Purification by C_{18} HPLC (Mightysil RP-18 250-4.6 (5 mm); eluent, 45% CH_3CN aq.; flow rate, 1.0 mL/min; UV detection at 265 nm) afforded **2** (0.96 mg, 20%, t_R = 40 .0 min).

2: ^{1}H NMR (600 MHz, C_6D_6) δ 5.91 (d, *J* = 15.6 Hz, 1 H), 5.91 (s, 1 H), 5.72-5.81 (m, 2 H), 5.39 (s, 1 H), 5.35-5.39 (m, 1 H), 5.29 (s, 1 H), 5.28 (s, 1 H), 4.97 (s, 1 H), 4.96-4.97 (m, 1 H), 4.90 (s, 1 H), 4.83 (s, 1 H), 4.26 (d, *J* = 4.3 Hz, 1 H), 4.08 (brs, 1 H), 4.06 (d, *J* = 4.3 Hz, 1 H), 3.86 (brs, 1 H), 2.96 (dd, *J* = 7.1, 1.7 Hz, 1 H), 2.93-2.97 (m, 1 H), 2.85 (dd, *J* = 7.3, 1.7 Hz, 1 H), 2.67-2.71 (m, 1 H), 2.66 (d, *J* = 7.5 Hz, 2 H), 2.44 (dd, *J* = 14.1, 5.2 Hz, 1 H), 2.24-2.38 (m, 1 H), 2.28 (s, 3 H), 1.96 (dd, *J* = 14.1, 9.1 Hz, 1 H), 1.27-1.42 (m, 5 H), 1.17 (d, *J* = 7.0 Hz, 3 H), 1.01 (d, *J* = 6.5 Hz, 3 H), 0.86 (t, *J* = 7.2 Hz, 3 H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.16 (d, *J* = 15.7 Hz, 1 H), 6.08 (ddd, *J* = 15.7, 7.0, 7.0 Hz, 1 H), 5.77 (s, 1 H), 5.69 (ddd, *J* = 15.4, 7.6, 7.6 Hz, 1 H), 5.48 (s, 1 H), 5.42 (dd, *J* = 15.4, 5.1 Hz, 1 H), 5.39 (s, 1 H), 5.33 (s, 1 H), 5.20 (s, 1 H), 4.87 (s, 1 H), 4.79 (s, 1 H), 4.63 (dd, *J* = 6.8, 3.0 Hz, 1 H), 4.38 (brs, 1 H), 4.18 (brs, 1 H), 4.14 (brs, 1 H), 3.92 (brs, 1 H), 3.21 (dd, *J* = 14.7, 7.0 Hz, 1 H), 2.98 (dd, *J* = 14.7, 7.0 Hz, 1 H), 2.88 (dd, *J* = 6.7, 1.9 Hz, 1 H), 2.71-2.76 (m, 2 H), 2.66-2.71 (m, 2 H), 2.50 (brs, 1 H), 2.47 (brs, 1 H), 2.25-2.29 (m, 2 H), 2.25 (s, 3 H), 2.12-2.17 (m, 1 H), 1.89 (dd, *J* = 14.0, 9.2 Hz, 1 H),

1.29-1.36 (m, 5 H), 1.06 (d, *J* = 7.0 Hz, 3 H), 1.00 (d, *J* = 6.4 Hz, 3 H), 0.88 (t, *J* = 7.0 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.8, 152.4, 147.0, 145.0, 144.9, 135.3, 134.9, 131.4, 130.5, 118.1, 116.1, 115.1, 113.1, 75.5, 75.4, 74.2, 73.5, 72.4, 62.3, 55.4, 40.0, 38.4, 36.3, 35.6, 35.3, 33.9, 20.1, 16.9, 14.9, 14.4, 14.2. ^{1}H NMR (600 MHz, CD_3OD) δ 6.30 (d, *J* = 15.7 Hz, 1 H), 6.21 (ddd, *J* = 15.7, 7.1, 7.1 Hz, 1 H), 5.84 (s, 1 H), 5.56 (ddd, *J* = 15.2, 6.7, 6.7 Hz, 1 H), 5.41-5.44 (m, 1 H), 5.43 (s, 1 H), 5.38 (s, 1 H), 5.27 (s, 1 H), 5.13 (s, 1 H), 4.86 (s, 1 H), 4.77 (s, 1 H), 4.61 (dd, *J* = 6.5, 3.0 Hz, 1 H), 4.22 (d, *J* = 3.2 Hz, 1 H), 4.11 (d, *J* = 3.2 Hz, 1 H), 4.05 (dd, *J* = 4.4, 3.6 Hz, 1 H), 3.87 (d, *J* = 3.6 Hz, 1 H), 3.24 (dd, *J* = 14.6, 7.1 Hz, 1 H), 2.97-3.00 (m, 1 H), 2.96 (dd, *J* = 6.4, 2.1 Hz, 1 H), 2.71 (dd, *J* = 7.4, 2.1 Hz, 1 H), 2.68-2.71 (m, 1 H), 2.60 (dd, *J* = 14.5, 6.7 Hz, 1 H), 2.34 (d, *J* = 13.9, 6.0 Hz, 1 H), 2.26 (s, 3 H), 2.10-2.16 (m, 1 H), 1.93 (dd, *J* = 13.9, 8.0 Hz, 1 H), 1.23-1.40 (m, 5 H), 1.08 (d, *J* = 7.1 Hz, 3 H), 0.99 (d, *J* = 6.5 Hz, 3 H), 0.89 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.4, 154.8, 151.3, 148.4, 148.0, 138.2, 137.1, 133.9, 131.9, 120.1, 116.1, 114.1, 113.7, 77.3, 76.6, 76.1, 75.4, 73.6, 64.3, 57.6, 41.9, 40.6, 38.8, 37.7, 37.5, 36.4, 22.0, 18.2, 16.5, 15.4, 15.4; IR (KBr) ν_{max} 3423 (br), 2925, 1634, 1151 cm^{-1}; HRESIMS calcd for $C_{31}H_{46}O_7Na$ *m/z* 553.3141, found *m/z* 553.3145; $[\alpha]_D^{21}$ -11 ° (*c* 0.6, $CHCl_3$).

Acknowledgments

We thank Professor B. M. Trost (Stanford University) for a generous offer of the sample of **3**, and S. Oka, Center for Instrumental Analysis, Hokkaido University, for ESIMS measurements. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References and Notes

1) (a) Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Sasaki, T.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27*, 5755-5758. (b) Kobayashi, J.; Ishibashi, M.; Hirota, H. *J. Nat. Prod.* **1991**, *54*, 1435-1439.

2) Lam, H. W.; Pattenden, G. *Angew. Chem. Int. Ed*. **2002**, *41*, 508-511.

3) Maleczka, R. E. Jr.; Terrell, L. R.; Geng, F.; Ward, J, S., III *Org. Lett.* **2002**, *4*, 2841-2844.

4) (a) Trost, B. M.; Chisholm, J. D.; Wrobleski, S. T.; Jung, M. *J. Am. Chem. Soc*. **2002**, *124*, 12420-12421. (b) Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc*. **2004**, *126*, 5028-5029. (c) Trost, B. M.; Wrobleski, S. T.; Chisholm, J. D.; Harrington, P. E.; Jung, M. *J. Am. Chem. Soc.* **2005**, *127*, in press (DOI: 10.1021/ja0533646). (d) Trost, B. M.; Harrington, P. E.; Chisholm, J. D.; Wrobleski, S. T. *J. Am. Chem. Soc.* **2005**, *127*, in press (DOI: 10.1021/ja053365y).

5) Assignments of some carbon chemical shifts for synthetic compound **1** by Trost's group were equivocal, while there were no assignments for them by Pattenden's group.

6) Kita, Y.; Maeda, H.; Omori, K.; Okuno, T.; Tamura, Y. *Synlett* **1993**, 273.

7) Trost, B. M.; Toste, F. D. *J. Am. Chem. Soc.* **2000**, *122*, 714

8) Ketal groups were difficult to remove at final stage.

9) Kita's method[6] does not require MS3A, but the reaction yield was low without MS3A.

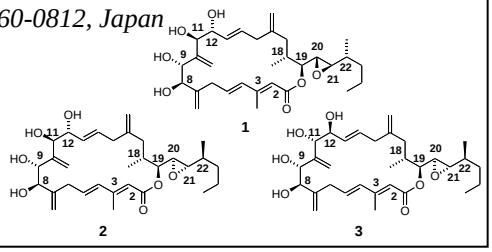
10) Esterification under Keck and Mitsunobu conditions gave a complex mixture.

Asymmetric Synthesis of a Diastereomer of Structure Proposed for Amphidinolide A and the determination of Its Absolute Configuration

Leave this area blank for abstract info.

Haruaki Ishiyama, Yoichi Nakamura, and Jun'ichi Kobayashi*
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Asymmetric synthesis of a diastereomer (**2**) of the structure (**1**) proposed for amphidinolide A, a cytotoxic macrolide from the cultured dinoflagellate *Amphidinium* sp., has been accomplished. Absolute configuration of amphidinolide A was concluded to be **3** from comparison of spectral data of amphidinolide A and synthetic diastereomers **2** and **3**, the latter of which was synthesized by Trost's group.



1

2

3