Amphidinolide L, a New Cytotoxic 27-Membered Macrolide from the Cultured Dinoflagellate *Amphidinium* sp.

Masashi Tsuda,[1a] Takuma Sasaki,[1b] and Jun'ichi Kobayashi*,[1a]

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, and Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

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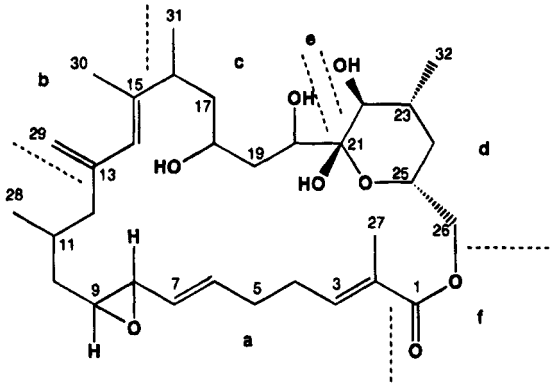
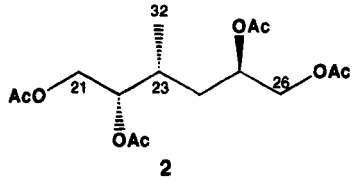
Marine microorganisms have been proven to produce a variety of chemically interesting and biologically significant secondary metabolites.[2] During our search for bioactive substances from marine organisms,[3] we isolated previously a series of cytotoxic macrolides, amphidinolides A–H, J, and K, from cultured dinoflagellates of the genus *Amphidinium*.[4] Further examination of extracts of the dinoflagellate *Amphidinium* sp. separated from the Okinawan marine flatworm *Amphiscolops breviviridis*, from which amphidinolides G and H have been obtained,[4a] led to isolation of a new cytotoxic 27-membered macrolide containing a hemiketal ring, named amphidinolide L (**1**).



1

This paper describes the isolation and structure elucidation of **1**. The relative stereochemistry was assigned by detailed analyses of NOESY data and $^1H-^1H$ coupling constants, and the absolute configurations at C-22, C-23, and C-25 were established by synthesis of the C-21–C-26 fragment (**2**) obtained through oxidation of **1** with $NaIO_4$.



2

The dinoflagellate *Amphidinium* sp. was mass cultured in a sea water medium enriched with ES nutrients[5] at 25 °C for 2 weeks. The harvested cells (ca. 1800 g, wet weight, from culture of 1750 L) were extracted with methanol/toluene (3:1), and the extracts were partitioned with toluene and water. The toluene-soluble material was subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) followed by ODS column chromatography and HPLC on ODS (both CH_3CN/H_2O, 7:3) to afford amphidinolide L (**1**, 0.9 mg, 2×10^{-4}%, wet weight) together with known compounds, amphidinolides G (0.5 mg, 1×10^{-4}%) and H (0.3 mg, 6×10^{-5}%).[4a]

Amphidinolide L [**1**, $[\alpha]^{27}_D$ −50° (*c* 0.1, C_6H_6)] was obtained as a colorless amorphous solid, and the molecular formula, $C_{32}H_{50}O_8$, was established by HREIMS (*m/z* 544.3427, $M^+ - H_2O$, Δ +2.6 mmu). UV absorption at 222 nm (ε 16 000) was indicative of α,β-unsaturated carbonyl group(s), and IR absorptions at 3400 and 1710 cm^{-1} were attributed to hydroxy and ester carbonyl groups, respectively. The ^1H and ^{13}C NMR data (Table 1) of **1** revealed the presence of an ester carbonyl, four olefins, a hemiketal, nine methines, eight methylenes, and five methyl groups. Since five out of eight unsaturations of **1** were accounted for, compound **1** was inferred to contain three rings. Detailed analyses of the $^1H-^1H$ COSY,[6] HOHAHA,[7] and HMQC spectra of **1** led to assignments of proton connectivities for four partial structures of C-2–C-12, C-27, and C-28 (**a**), C-13–C-15, C-29, and C-30 (**b**), C-16–C-20 and C-31 (**c**), and C-22–C-26 and C-32 (**d**). The carbon chemical shifts of the C-27 and C-30 vinyl methyl groups (δ_C 12.6 and 14.5, respectively) suggested that the trisubstituted $\Delta^{2(3)}$ and $\Delta^{14(15)}$ double bonds were both *E* configurations. The presence of a trans epoxide at C-8 and C-9 was deduced from the ^1H and ^{13}C chemical shifts (Table 1) as well as the $^1H-^1H$ coupling constant ($J_{8,9}$ = 2.1 Hz). Connectivities of four partial structures **a-d** were assigned on the basis of HMBC (Table 1) and NOESY[8] data. HMBC cross-peaks of H_2-12/C-13, H_2-12/C-29, H-14/C-16, H_3-30/C-16, and H_3-31/C-15 revealed connectivities of C-12 to C-13 and C-15 to C-16. The *s-cis* orientation of the diene unit (**b**) was deduced from NOESY correlations observed for H-12a/H-14, H-14/H-16, H-29b/H_3-30, and H_3-30/H_3-31. HMBC cross-peaks were observed for H-19a/C-21 and H-20/C-21, thereby revealing that the hemiketal carbon (**e**) was adjacent to C-20. The connection between C-21 and C-22 was elucidated on the basis of the NOESY cross-peak for H-20/H-22. The carbon chemical shift (δ_C 96.3) of C-21 corresponded well to those (δ_C 96–100) of the hemiketal carbons on the six-membered ring rather than those (δ_C 104–106) of the hemiketal carbons on the five-membered ring.[9] An ester carbonyl carbon (δ 169.1, C-1) showed HMBC correlations for H-3, H_2-26, and H_3-27, indicating that the ester linkage (**f**) was

(1) (a) Hokkaido University. (b) Kanazawa University.

(2) Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753–1769.

(3) Tsuda, M.; Shigemori, H.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1993**, *49*, 6785–6796 and references cited therein.

(4) (a) Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221–5224. (b) Ishibashi, M.; Sato, M.; Kobayashi, J. *J. Org. Chem.* **1993**, *58*, 6928–6929 and references cited therein.

(5) This dinoflagellate was designated strain number Y-25.

(6) The $^1H-^1H$ COSY spectrum of **1** revealed the following correlations (H/H): 3/4a, 3/4b, 3/27, 4a/4b, 4a/5a, 4a/5b, 4b/5a, 4b/5b, 5a/5b, 5a/6, 5b/6, 6/7, 7/8, 9/10a, 9/10b, 10a/11, 10b/11, 11/28, 12a/12b, 14/30, 16/17a, 16/17b, 16/31, 17a/18, 17b/18, 18/19b, 19a/19b, 19a/20, 19b/20, 22/23, 23/24a, 23/24b, 23/32, 24b/25, 25/26a, and 25/26b.

(7) HOHAHA spectrum was measured using the MLEV-17 pulse sequence for 50 ms as the spin lock interval.

(8) The NOESY spectrum of **1** revealed the following correlations (H/H): 3/27, 3/4a, 3/4b, 5a/6, 5b/6, 5a/7, 5b/7, 6/8, 7/9, 8/10a, 9/10b, 9/28, 10a/10b, 11/14, 12a/12b, 12a/14, 12a/29a, 12b/29a, 14/16, 16/17a, 16/18, 17a/17b, 17a/18, 17a/19a, 17b/18, 17b/19b, 18/19a, 18/19b, 18/20, 19a/19b, 19a/20, 19b/20, 20/22, 22/24a, 22/32, 23/25, 23/24b, 23/32, 24a/24b, 24a/26a, 24b/25, 24b/26b, 28/29a, 29a/29b, 29b/30, and 30/31.

(9) The carbon chemical shifts of hemiketal carbons of α-D-psicopyranose and β-D-psicopyranose appeared at δ 98.7 and 99.7, respectively, while those of α-D-psicofuranose and β-D-psicofuranose appeared at δ 104.4 and 106.7, respectively; see: Kalinowski, H.-O.; Berger, S.; Braun, S. In *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1988; p 442.

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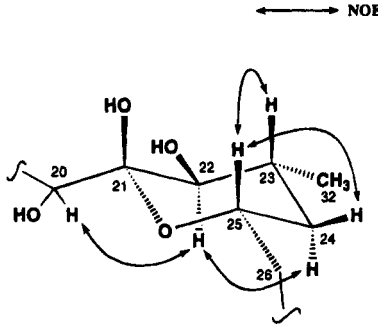
Table 1. ^1H and ^{13}C NMR Chemical Shifts of Amphidinolide L (1)

position	δ_H[a]	m	J (Hz)	δ_C[b]	m	HMBC[c]
1				167.9	s	H-3, H_2-26, H_3-27
2				128.3	s	H_3-27
3	6.96	t	6.0	141.3	d	H_3-27
4	1.98	m		27.3	t	
	1.95	m				
5	1.94	m		30.8	t	H-7
	1.91	m				
6	5.62	dt	15.2, 7.0	134.4	d	H-8
7	5.14	dd	15.2, 8.1	129.1	d	H-8
8	2.97	dd	2.1, 8.1	59.3	d	H-6, H-7
9	2.80	ddd	2.1, 3.7, 7.0	59.2	d	H-10
10	1.38	ddd	5.4, 7.0, 13.4	38.7	t	H_3-28
	1.26	ddd	3.7, 9.1, 13.4			
11	1.88	m		29.7	d	H_3-28
12	2.10	dd	6.4, 13.3	46.1	t	H_3-28, H_2-29
	2.05	dd	7.5, 13.3			
13				144.3	s	H_2-12
14	5.79	s		125.6	d	H_2-29, H_3-30
15				142.9	s	H_3-30, H_3-31
16	2.32	ddq	5.5, 10.6, 7.1	41.2	d	H-14, H_3-30, H_3-31
17	1.48	ddd	4.9, 5.5, 14.0	42.1	t	H_3-31
	1.75	ddd	7.0, 10.6, 14.0			
18	3.91	dddd	2.3, 4.9, 7.0, 8.1	70.4	d	H-17b, H-19b
19	2.23	ddd	2.3, 4.1, 14.7	37.6	t	
	1.79	dt	14.7, 8.1			
20	4.13	dd	4.1, 8.1	75.3	d	H-18, H-19b
20-OH	3.29	br s				
21				96.3	s	H-19a, H-20
22	3.27	d	10.0	73.7	d	H-26b, H_3-32
22-OH	4.51	br s				
23	1.73	dddq	3.9, 10.0, 11.5, 7.7	33.0	d	H_3-32
24	0.87	q	11.5	34.7	t	H-25, H_3-32
	1.09	ddd	1.9, 3.9, 11.5			
25	4.14	ddt	7.4, 11.5, 1.9	68.1	d	H-24a, H-26a
26	4.03	dd	7.4, 11.9	66.6	t	H-25
	4.19	dd	1.9, 11.9			
27	1.90	br s		12.6	q	
28	0.93	d	6.6	19.6	q	H_2-12
29	5.05	d	2.1	114.9	t	H_2-12, H-14
	4.98	d	2.1			
30	1.80	br s		14.5	q	H-14
31	1.00	d	7.7	18.5	q	
32	1.01	d	7.1	20.6	q	

[a] Recorded on a Bruker AMX-600 spectrometer in C_6D_6. [b] Recorded on a JEOL EX-400 spectrometer in $CDCl_3$. [c] The HMBC experiment was carried out with F1 width from 6 ppm to 94 ppm, giving a spectrum with two-folded signals.

Chart 1. Relative Stereochemistry for C-20–C-26 Part of 1 Proposed from NOESY Data[a]

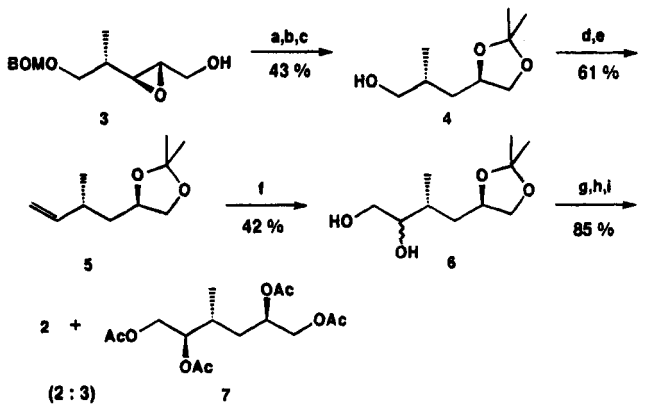


[a] The coupling constants for this moiety (H/H, in Hz) are as follows: 22/23 = 10.0, 23/24a = 11.5, 23/24b = 3.9, 24a/25 = 11.5, and 24b/25 = 1.9.

located between C-2 and C-26 to make a lactone ring. Thus, the structure of amphidinolide L was concluded to be **1**.

The relative stereochemistry of this macrocyclic compound containing 10 chiral centers was deduced from combination of the NOESY data with the $^1H-^1H$ coupling constants as shown in Chart 1. A chair form of the tetrahydropyran ring was assignable from NOESY cross-peaks for H-22/H-20, H-22/H_3-32, H-22/H-24a, and H-23/H-25.

Scheme 1. Synthesis of the C21-C26 Fragment (2)[a]



[a] Key: (a) DIBALH, benzene, rt, 1 h; (b) $(MeO)_2CMe_2$, PPTS, CH_2Cl_2, rt, 5 h; (c) $Pd(OH)_2$, EtOH, rt, 18 h; (d) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, 30 min, then Et_3N, −20 °C, 30 min; (e) Ph_3PCH_3Br, *n*-BuLi, THF, rt, 2 h; (f) OsO_4, pyridine, THF, rt, 4 h; (g) 1 N HCl, THF, rt, 5 h; (h) Ac_2O, pyridine, rt, 18 h; (i) HPLC separation.

Treatment with $NaIO_4$ of **1** followed by $NaBH_4$ reduction and acetylation afforded a degradation product (**2**) corresponding to the C-21–C-26 fragment, the structure of which was established by the $^1H-^1H$ COSY and the FABMS (*m*/*z* 333, $M^+ + H$) data. In order to determine the absolute configurations at C-22, C-23, and C-25 of **1**, the C-21–C-26 fragment (**2**) was synthesized as shown in Scheme 1. (2*S*,3*S*,4*S*)-Epoxy alcohol **3** was prepared from methyl (2*S*)-3-hydroxy-2-methylpropionate according to the procedure reported previously.[10] Treatment of the epoxy alcohol **3** with DIBALH gave a (8:1) mixture of the 1,2-diol and the 1,3-diol,[11] which were separated by a silica gel column after conversion into the acetonides. The acetonide **4** of 1,2-diol was applied to Swern oxidation followed by Wittig reaction to afford the olefin **5**. Treatment of **5** with OsO_4 gave the diol **6** as a 2:3 mixture of two diastereomers, which was separated as the tetraacetate **2** and its isomer **7**. Spectroscopic data of the synthetic tetraacetate **2** were all identical with those of **2** derived from the natural product (**1**), including the sign of optical rotations [synthetic **2**, $[\alpha]_D$ +64 ° (*c* 0.2, $CHCl_3$); **2** derived from **1**, $[\alpha]_D$ +72 ± 8° (*c* 0.01, $CHCl_3$)]. Thus, the absolute configurations at C-22, C-23, and C-25 of **1** were determined to be *S*, *R*, and *R*, respectively.

Amphidinolide L (**1**) is a new 27-membered macrolide from culture of the dinoflagellate *Amphidinium* sp. separated from the Okinawan flatworm *Amphiscolops breviviridis*. Amphidinolide L (**1**) is considered to be a congener of amphidinolides G and H,[4a] although the former (**1**) possesses a hemiketal ring (C-21–C-25) and the latter have a ketone group at C-20. Compound **1** exhibited cytotoxity against L1210 murine leukemia cells and KB human epidermoid carcinoma cells in vitro with IC_{50} values of 0.092 and 0.1 μg/mL, respectively.

Experimental Section

General Procedure. Multiplicities of proton signals were determined from the resolution-enhanced ^1H NMR spectrum as well as ^1H decoupling difference experiments. FABMS spectra were obtained using 3-nitrobenzyl alcohol. Silica gel column

(10) Horita, K.; Tanaka, K.; Yonemitsu, O. *Chem. Pharm. Bull.* **1993**, *41*, 2044–2046 and references cited therein.

(11) Suzuki, T.; Saimoto, H.; Tomioka, H.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1982**, *23*, 3597–3600.

chromatography was performed using Wako gel C-300 (Wako Pure Chemical).

Isolation. The procedure of alga cultivation was previously described.[4a] The harvested cells (1822.5 g, wet weight, from culture of 1750 L) were extracted with methanol/toluene (3:1, 1.2 L × 2). After addition of 1 M NaCl (aq) (1 L), the mixture was extracted with toluene (1.2 L × 3). The toluene-soluble fraction was evaporated under reduced pressure to give a crude extract (8.23 g), part (2.05 g) of which was subjected to silica gel column chromatography (2.5 × 40 cm) with $CHCl_3$/MeOH (98:2). The fraction eluting from 650 to 800 mL was separated on C_{18} medium-pressure liquid chromatography (Develosil LOP ODS 24S, Nomura Chemical, 2.2 × 30 cm) with CH_3CN/H_2O (70:30) followed by HPLC (Develosil ODS-HG-5, Nomura Chemical, 10 × 250 mm; flow rate 2.5 mL/min; UV detection at 240 nm; eluent CH_3CN/H_2O, 70:30) to afford amphidinolide L (**1**, 0.9 mg, 2×10^{-4}%, t_R 19 min).

Amphidinolide L (1). A colorless amorphous powder; $[\alpha]^{27}_D$ −50° (*c* 0.1, benzene); UV (EtOH) λ_{max} 222 nm (ϵ 16 000); IR (film) ν_{max} 3400, 2840, and 1710 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 544 ($M^+ - H_2O$), 526 ($M^+ - 2H_2O$), and 508 ($M^+ - 3H_2O$); HREIMS *m/z* 544.3427 ($M^+ - H_2O$, calcd for $C_{32}H_{48}O_7$, 544.3401).

Compound 2. A THF/1 M phosphate buffer (pH 7.2) solution (1:1, 600 μL) of **1** (0.5 mg, 890 nmol) was treated with $NaIO_4$ (2.1 mg, 9.8 μmol) at room temperature for 1 h. After evaporation under reduced pressure, the residue was extracted with EtOAc (3 mL × 3) and evaporated. An EtOH solution (500 μL) of the residue was added to $NaBH_4$ (2.0 mg) in EtOH (500 μL) at 0 °C. After the mixture was stirred at 0 °C for 1 h, 1 M phosphate buffer was added, and the reaction mixture was dried with N_2 gas. The residue was dissolved in pyridine (1 mL) and acetic anhydride (1 mL) and stirred at room temperature for 18 h. The residue was evaporated and partitioned between EtOAc (10 mL) and H_2O (3 mL). The EtOAc layer was washed with brine and dried over $MgSO_4$. After evaporation the residue was passed through a Sep-Pak silica cartridge (Waters) with hexane/EtOAc (2:1) followed by silica gel HPLC (YMC Pack silica-06, YMC Co., Ltd., 4.6 × 250 mm; flow rate, 1 mL/min; RI detection; eluent, hexane/EtOAc, 2:1) to afford compound **2** (0.1 mg, t_R 9.2 min): $[\alpha]^{20}_D$ +72 ± 8° (*c* 0.01, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.97 (3H, d, *J* = 6.8 Hz), 1.32 (1H, ddd, *J* = 3.2, 10.0, and 14.2 Hz), 1.76 (1H, ddd, *J* = 3.8, 10.5, and 14.2 Hz), 1.86 (1H, m), 2.05 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.09 (3H, s), 3.98 (1H, dd, *J* = 6.3 and 11.8 Hz), 4.08 (1H, dd, *J* = 7.6 and 11.8 Hz), 4.23 (1H, dd, *J* = 3.5 and 9.2 Hz), 4.25 (1H, dd, *J* = 3.7 and 11.8 Hz), 5.04 (1H, ddd, *J* = 3.7, 4.3, and 7.6 Hz), and 5.18 (1H, m); FABMS *m/z* 333 (M^+ + H) and 273 (M^+ − AcOH + H); HRFABMS *m/z* 333.1541 (M^+ + H, calcd for $C_{15}H_{25}O_8$, 333.1550).

(2*R*,4*R*)-4,5-(Isopropylidenedioxy)-2-methylpentan-1-ol (4). To a solution of (2*S*,3*S*,4*S*)-5-[[(benzyloxy)methyl]oxy]-2,3-epoxy-4-methylpentan-1-ol[10] (**3**, 1.42 g, 5.63 mmol) in benzene (15 mL) was slowly added a 1.5 M toluene solution of DIBALH (18.8 mL, 28.2 mmol), and the mixture was stirred at room temperature for 1 h. After addition of MeOH (3 mL), ether (70 mL), and saturated aqueous potassium sodium tartrate (30 mL), the mixture was stirred at room temperature for 1 h. After extraction with EtOAc (200 mL × 3), the organic phase was washed with brine and evaporated under reduced pressure to afford the residue, which was purified with a silica gel column (5 × 40 cm, hexane/EtOAc, 1:1) to give a mixture (8:1) of 1,2-diol and 1,3-diol (800 mg). To a solution of the mixture (400 mg, 1.48 mmol) in CH_2Cl_2 (30 mL) were added pyridinium *p*-toluenesulfonate (38 mg, 151 μmol) and 2,2-dimethoxypropane (1 mL, 16 mmol) at room temperature, and stirring was continued for 4 h. After addition of H_2O (20 mL), the reaction mixture was extracted with CH_2Cl_2 (50 mL × 3) and washed with brine followed by evaporation to give a residue (470 mg). To a solution of part (200 mg) of the residue in EtOAc (20 mL) was added 20% palladium hydroxide on carbon (25 mg) at room temperature, and the mixture was stirred for 5 h under H_2 atomosphere. After filtration with Celite, solvent was evaporated to give a residue, which was purified by a silica gel column (1 × 40 cm, hexane/EtOAc, 3:1) to afford compound **4** (90 mg, 43%) and 1,3-acetonide alcohol (11 mg). **4:** $[\alpha]^{20}_D$ −8° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.95 (3H, d, *J* = 6.8 Hz), 1.37 (3H, s), 1.39 (1H, m), 1.42 (3H, s), 1.58 (1H, m), 1.82 (1H, m), 3.45 (1H, dd, *J* = 6.3 and 11.8 Hz), 3.50 (1H, t, *J* = 7.8 Hz), 3.56 (1H, m), 4.08 (1H, dd, *J* = 5.9 and 7.8 Hz), and 4.19 (1H, m); FABMS *m/z* 175 (M^+ + H); HRFABMS *m/z* 175.1340 (M^+ + H, calcd for $C_9H_{19}O_3$, 175.1335).

(3*R*,5*R*)-5,6-(Isopropylidenedioxy)-3-methylhex-1-ene (5). To a solution of oxalyl chloride (0.1 mL, 1.2 mmol) in CH_2Cl_2 (5 mL) was slowly added DMSO (0.2 mL, 2.8 mmol) in CH_2Cl_2 (1 mL) at −78 °C, and successively compound **4** (80 mg, 460 μmol) in CH_2Cl_2 (2 mL) was dropwise added. After the mixture was stirred at −78 °C for 45 min, triethylamine (0.5 mL, 3.6 mmol) was added to the reaction mixture, and stirring was continued at −50 °C for 1 h. After addition of saturated aqueous NH_4Cl (10 mL) and extraction with CH_2Cl_2 (50 mL × 3), the organic phase was washed with H_2O and brine and dried over $MgSO_4$. Evaporation of the solvent afforded crude aldehyde, which was subjected to the following reaction without separation. THF (5 mL) was added to methyltriphenylphosphonium bromide (203 mg, 568 μmol) at 0 °C, and then a hexane solution of 1.6 M *n*-butyllithium (340 μL, 544 μmol) was added at 0 °C. After the solution was stirred at 0 °C for 30 min, the crude aldehyde (70 mg) in THF (2 mL) was dropwise added to the reaction mixture at 0 °C, and stirring was continued for 30 min and then at room temperature for 1 h. After addition of saturated aqueous NH_4Cl (10 mL), the reaction mixture was extracted with ether (30 mL × 3) and washed with H_2O and then brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was subjected to a silica gel column (1 × 10 cm, hexane/EtOAc, 100:1) to give compound **5** (48 mg, 61%): $[\alpha]^{20}_D$ −15° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, *J* = 6.8 Hz), 1.35 (3H, s), 1.41 (3H, s), 1.47 (1H, m), 1.76 (1H, ddd, *J* = 6.0, 7.7, and 13.2 Hz), 2.22 (1H, m, H-3), 3.50 (1H, t, *J* = 7.1 Hz), 4.02−4.14 (2H, m), 4.94 (1H, d, *J* = 1.7 and 17.6 Hz), 4.98 (1H, dd, *J* = 1.7 and 17.6 Hz), and 5.73 (1H, ddd, *J* = 7.7, 10.4, and 17.6 Hz); FABMS *m/z* 171 (M^+ + H); HRFABMS *m/z* 171.1391 (M^+ + H, calcd for calcd for $C_{10}H_{19}O_2$, 171.1386).

(2*SR*,3*R*,5*R*)-5,6-(Isopropylidenedioxy)-3-methylhexane-1,2-diol (6). To a solution of compound **5** (24 mg, 141 μmol) in THF (2 mL) were added pyridine (50 μL), and a 393 μM THF solution of osmium tetraoxide (400 μL, 157 μmol) at room temperature, and stirring was continued for 2 h. Sodium hydrosulfite (165 mg), Florisil (165 mg), acetone (1.3 mL), and H_2O (0.2 mL) were added to the reaction mixture at room temperature, and the reaction mixture was stirred for 30 h. After filtration with Celite and evaporation, the residue was partitioned between EtOAc (100 mL × 3) and H_2O (30 mL), and the organic phase was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the residue was purified with a silica gel column (hexane/EtOAc, 1:1) to give a diastereomeric mixture of diol (**6**, 12 mg, 42%): ^1H NMR ($CDCl_3$) δ 0.95 (3H, d, *J* = 6.8 Hz), 1.366 (1.2H, s), 1.373 (1.8H, s), 1.42 (1.2H, s), 1.43 (1.8H, s), 1.45−1.80 (3H, m), 1.76 (1H, ddd, *J* = 6.0, 7.7, and 13.2 Hz), 2.22 (1H, m), 3.45−3.54 (2H, m), 3.62 (1H, m), 3.73 (1H, m), 4.07 (1H, dt, *J* = 5.8 and 7.8 Hz), and 4.19 (1H, m); FABMS *m/z* 205 (M^+ + H); HRFABMS *m/z* 205.1450 (M^+ + H, calcd for $C_{10}H_{21}O_4$, 205.1440).

(2*S*,3*R*,5*R*)- and (2*R*,3*R*,5*R*)-3-Methyl-1,2,5,6-tetraacetoxyhexane (2 and 7). To a solution of the diastereomeric mixture of diol (**6**, 3.9 mg, 19 μmol) in THF (200 μL) was added 1 N HCl (50 μL), and the mixture was allowed to stand at room temperature for 3 h. After evaporation, acetic anhydride (100 μL) and pyridine (100 μL) were added to the residue, and the mixture was stirred at room temperature for 6 h. After being dried with N_2 gas, the residue was subjected to a silica gel column (0.5 × 10 cm, hexane/EtOAc, 3:1) and C_{18} HPLC (Develosil ODS-HG-5, 4.6 × 250 mm; flow rate 0.7 mL/min; RI detection; eluent MeOH/H_2O, 1:1) to give compounds **2** (2.2 mg, 35%, t_R 23.2 min) and **7** (3.2 mg, 50%, t_R 20.0 min). **2:** $[\alpha]^{19}_D$ +64° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.97 (3H, d, *J* = 6.8 Hz), 1.32 (1H, ddd, *J* = 3.2, 10.0, and 14.2 Hz), 1.76 (1H, ddd, *J* = 3.8, 10.5, and 14.2 Hz), 1.86 (1H, m), 2.05 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.09 (3H, s), 3.98 (1H, dd, *J* = 6.3 and 11.8 Hz), 4.08 (1H, dd, *J* = 7.6 and 11.8 Hz), 4.23 (1H, dd, *J* = 3.5 and 9.2 Hz), 4.25 (1H, dd, *J* = 3.7 and 11.8 Hz), 5.04 (1H, ddd, *J* = 3.7, 4.3, and 7.6 Hz), and 5.18 (1H, m); FABMS *m/z* 333 (M^+ + H) and 273 (M^+ − AcOH + H); HRFABMS *m/z* 333.1552 (M^+ + H, calcd for $C_{15}H_{25}O_8$, 333.1550). **7:** $[\alpha]^{19}_D$ +28° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.97 (3H, d, *J* = 6.8 Hz), 1.34 (1H, ddd, *J* = 3.2, 10.0, and 14.2 Hz), 1.78 (1H, ddd, *J* = 3.8, 10.5, and 14.2 Hz), 1.86 (1H, m), 2.05 (3H, s), 2.065 (3H, s), 2.072 (3H, s), 2.09 (3H, s), 4.01 (1H, dd, *J* = 6.3 and 11.8 Hz), 4.06 (1H, dd, *J* = 7.4 and 11.8 Hz), 4.23 (1H, dd, *J* = 3.5 and 9.2 Hz), 4.27 (1H, dd, *J* = 3.2 and 11.8 Hz), 4.97 (1H, ddd, *J* = 3.7, 4.3, and 7.6 Hz),

and 5.20 (1H, m); FABMS m/z 333 ($M^+ + H$) and 273 ($M^+ - AcOH + H$); HRFABMS m/z 333.1538 ($M^+ + H$, calcd for $C_{15}H_{25}O_8$, 333.1550).

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Supplementary Material Available: ^1H, ^{13}C, and 2D NMR spectra of compound **1** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.