Isolation and Structures of Haterumadioxins A and B, Cytotoxic Endoperoxides from the Okinawan Sponge *Plakortis lita*

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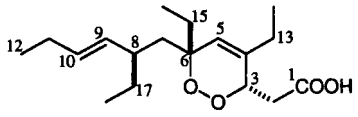
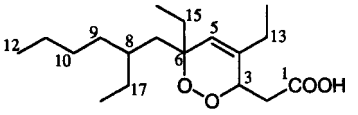
Cytotoxic endoperoxides, haterumadioxins A (**1**) and B (**2**), were isolated from the Okinawan sponge *Plakortis lita*. Their structures were determined by spectroscopic analysis. The absolute stereostructure of **1** was determined by degradation reactions and the modified Mosher's method. Haterumadioxins showed significant cytotoxicity against 38 human cancer cell lines.

In our screening for compounds that inhibited the cell division of fertilized sea urchin eggs, we previously reported the isolation and structures of haterumalides, chlorinated 14-membered macrolides, from the Okinawan sponge *Ircinia* sp.[1] In our continuing search for such compounds, we found that the extract of the Okinawan sponge *Plakortis lita* De Laubenfels (order Homosclerophorida, family Plakinidae) exhibited significant inhibitory activity. The genus *Plakortis* has yielded a wide variety of endoperoxide-containing metabolites, such as plakortin, plakinic acids, plakorin, plakortolide, plakinidone, peroxyplakoric acids, manadic acids, plakortones, and plakortides.[2] These compounds have exhibited cytotoxicity, antibacterial and antifungal activity, and Ca^{2+}-ATPase activation. We report here the isolation and structure determination of haterumadioxins A (**1**) and B (**2**), cytotoxic endoperoxides from this sponge.



Haterumadioxin A (**1**)



Haterumadioxin B (**2**)

The CH_2Cl_2-soluble fraction of the acetone extract of the Okinawan sponge *P. lita*, collected on Hateruma Island in Okinawa prefecture, was subjected to fractionation guided by cytotoxicity against P388 cells using column chromatography (SiO_2 and ODS) and reversed-phase HPLC (ODS) to give haterumadioxins A (**1**, 0.038% yield based on wet wt) and B (**2**, 0.008% yield based on wet wt) as colorless oils. Haterumadioxins A (**1**) and B (**2**) showed significant cytotoxicity against P388 cells, with IC_{50}s of 11 and 5.5 ng/mL, respectively.

The molecular formula of **1** was determined to be $C_{18}H_{30}O_4$ by HRFABMS (*m/z* 333.2050, calcd for $C_{18}H_{30}O_4Na$ $[M + Na]^+$, 333.2042). The IR spectrum indicated the presence of a carboxylic acid functionality (3400−2800 (br), 1715 cm^{-1}). The NMR data for **1** are summarized in Table 1. The ^1H NMR, ^{13}C NMR, and HMQC spectra of **1** showed the presence of four methyl carbons, six methylene carbons, two methine carbons, one quaternary carbon, four olefinic carbons (δ_C 125.5, 131.8, 134.3, 136.4), and one carbonyl carbon (δ_C 176.4). The carbon chemical shifts of **1** suggested that one methine carbon (δ_C 76.5) and the quaternary carbon (δ_C 83.0) were connected to oxygen atoms. A detailed analysis of the phase-sensitive DQF-COSY spectrum of **1** allowed four partial structures, C-2 to C-3, C-7 to C-12 including C-17 and C-18, C-13 to C-14, and C-15 to C-16, to be constructed. The remaining connectivities of **1** were clarified by the HMBC correlations H-2/C-1, H-3/C-4, H-5/C-3, H-5/C-4, H-5/C-6, H-7/C-6, H-13/C-4, H-13/C-5, H-15/C-5, and H-15/C-6. Therefore, the connectivities of the entire carbon framework were established. Furthermore, a C-1 carbonyl carbon was determined to be a carboxyl carbon on the basis of a characteristic chemical shift of C-1 (δ_C 176.4) and the IR spectrum. On the basis of the molecular formula and degree of unsaturation of **1**, the presence of an oxygen−oxygen bond was suggested. Finally, the stereochemistry of C-9 olefin was clarified to be 9*E* on the basis of the coupling constant between H-9 and H-10 (15.0 Hz). Thus, the gross structure of haterumadioxin A (**1**) was determined.

The ^1H NMR spectrum of **2** resembled that of **1**, but the absence of H-9 and H-10 vinyl protons and the molecular formula of **2** (*m/z* 335.2226, calcd for $C_{18}H_{32}O_4Na$ $[M + Na]^+$, 335.2198, determined by HRFABMS) suggested that **2** was a dihydro derivative of **1**. The NMR data for **2** are summarized in Table 1. As expected, a similar analysis of the phase-sensitive DQF-COSY, HMQC, and HMBC spectra of **2** allowed the entire carbon framework to be constructed (Figure 1). Although correlation between C-1 and C-2 was not observed, the chemical shifts of H-2a and H-2b (δ_H 2.62, 2.93) indicated that C-1 was a carbonyl carbon. Finally, the molecular formula and degree of unsaturation of **2** suggested the presence of an oxygen−oxygen bond. Thus, the gross structure of haterumadioxin B (**2**) was determined, as shown in Figure 1.

The relative stereochemistry in **1** was determined as follows. The magnitude of $J_{2a,3} = 3.0$ Hz and $J_{2b,3} = 9.5$ Hz suggested that H-2a and H-3 were located in a *gauche* arrangement and that H-2b and H-3 were located in an

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Table 1. NMR Data for Haterumadioxin A (**1**) and B (**2**)[a]

haterumadioxin A (**1**)						haterumadioxin B (**2**)					
atom	$^1H^b$	$^{13}C^{c,e}$	atom	$^1H^b$	$^{13}C^{c,e}$	atom	$^1H^b$	$^{13}C^{d,e}$	atom	$^1H^b$	$^{13}C^{d,e}$
1		176.4 s	10	5.28 dt	131.8 d	1		176.3 s	11a	1.19 m	23.0 t
2a	2.61 dd	36.7 t		(15.0, 6.6)		2a	2.62 br d	37.3 t	11b	1.26 m	
	(3.0, 16.2)		11a	1.98 m	25.7 t		(15.6)		12	0.89 t 3H	14.1 q
2b	2.94 dd		11b	1.98 m		2b	2.93 dd			(7.1)	
	(8.8, 16.2)		12	0.96 t 3H	14.1 q		(8.1, 15.6)		13	2.02 m 2H	24.9 t
3	4.59 br d	76.4 d		(7.5)		3	4.58 br d	77.2 d	14	1.08 t 3H	11.7 q
	(8.8)		13	1.98 m 2H	25.0 t		(8.1)			(7.3)	
4		136.4 s	14	1.07 t 3H	11.6 q	4		137.5 s	15a	1.66 dq	30.8 t
5	5.51 d	125.6 d		(7.3)		5	5.51 s	125.2 d		(13.9, 7.3)	
	(1.5)		15a	1.63 dq	31.0 t	6		83.5 s	15b	1.76 dq	
6		83.0 s		(14.1, 7.5)		7a	1.36 dd	39.3 t		(13.9, 7.3)	
7a	1.50 dd	40.7 t	15b	1.74 dq			(4.4, 15.0)		16	0.90 t 3H	8.2 q
	(8.1, 14.6)			(14.1, 7.5)		7b	1.50 dd			(7.3)	
7b	1.58 dd		16	0.88 t 3H	8.1 q		(5.7, 15.0)		17a	1.24 m	27.7 t
	(3.7, 14.6)			(7.5)		8	1.29 m	34.3 d	17b	1.35 m	
8	1.92 m	39.7 d	17a	1.15 m	30.0 t	9a	1.18 m	34.2 t	18	0.82 t 3H	10.7 q
9	5.08 dd	134.3 d	17b	1.37 m		9b	1.25 m			(7.3)	
	(9.1, 15.0)		18	0.79 t 3H	11.6 t	10a	1.18 m	29.0 t			
				(7.4)		10b	1.25 m				

[a] Recorded in $CDCl_3$. [b] Recorded at 800 MHz. Coupling constants (Hz) are in parentheses. [c] Recorded at 200 MHz. [d] Recorded at 100 MHz. [e] Multiplicity was based on HMQC spectrum.

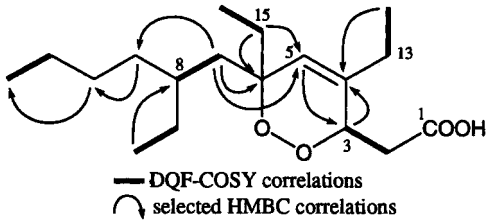

Figure 1. Partial structures of haterumadioxin B (**2**) based on 2D NMR correlations.

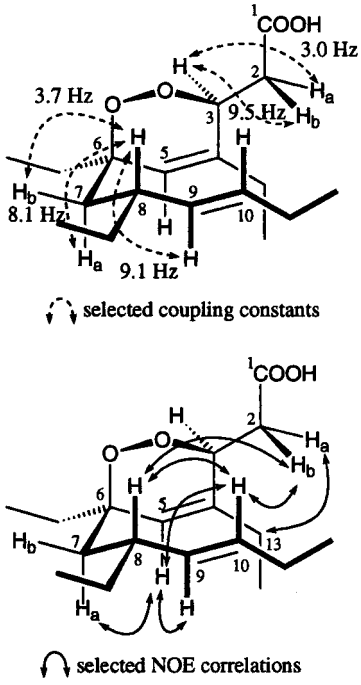

Figure 2. Relative stereochemistry of haterumadioxin A (**1**).

anti arrangement (Figure 2). Similarly, the magnitude of $J_{7a,8}$ = 8.1 Hz, $J_{7b,8}$ = 3.7 Hz, and $J_{8,9}$ = 9.1 Hz suggested that H-7a and H-8 were located in an *anti* arrangement, H-7b and H-8 were located in a *gauche* arrangement, and H-8 and H-9 were located in an *anti* arrangement. These results and the NOESY correlations H-2a/H-13, H-2b/H-8, H-2b/H-10, H-5/H-7a, H-5/H-9, H-5/H-10, and H-8/H-10 suggested that one of the stable conformations of **1** was

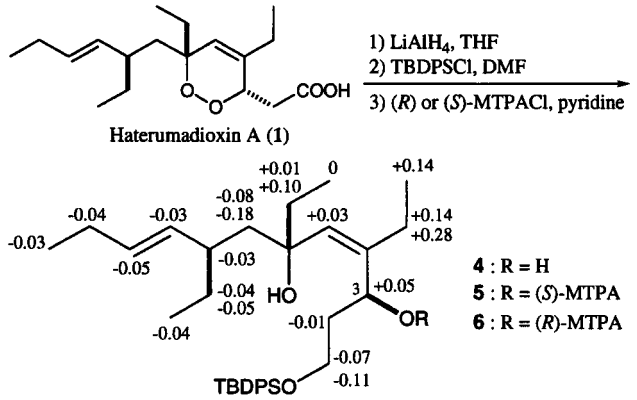

Figure 3. Degradation reactions of haterumadioxin A (**1**) and $\Delta\delta$ values ($\delta_S-\delta_R$) for the MTPA esters **5** and **6** in ppm.

as shown in Figure 2. The stability of this conformation may be due to $\pi-\pi$ stacking between C-4 olefin and C-9 olefin and steric repulsion between the C-6 ethyl group and the C-8 ethyl group. Therefore, the relative stereochemistry in haterumadioxin A (**1**) was determined to be 3*S**, 6*R**, and 8*R**. The computational calculation using Macromodel 6.0 and the MM2* force field suggested this result. To be more precise, the computational calculation gave 10 conformations having 2 kJ/mol energy greater than that of the most stable conformation. All these conformations had alkyl side chains turned at C-7, as shown in Figure 2.

The absolute stereochemistry in **1** was determined using the modified Mosher's method.[3] Reduction of **1** by $LiAlH_4$ followed by TBDPSCl (*tert*-butyldiphenylsilyl chloride)/imidazole gave TBDPS ether **4** (Figure 3). Treatment of TBDPS ether **4** with (*R*)- or (*S*)-MTPACl gave (*S*)- or (*R*)-MTPA esters **5** and **6**, respectively. The ^1H NMR signals of the two MTPA esters **5** and **6** were assigned on the basis of the 2D NMR spectra, and the $\Delta\delta$ values ($\delta_S-\delta_R$, ppm) were then calculated. The results, shown in Figure 3, established that the absolute stereochemistry of C-3 was 3*S*. Therefore, the absolute stereochemistry in haterumadioxin A (**1**) was determined to be 3*S*, 6*R*, and 8*R*, as shown in Figure 3.

Haterumadioxin A (**1**) was evaluated against a human cancer cell line panel at the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research.[4] Figure 4 shows the mean graph of **1** based on the growth inhibition, which

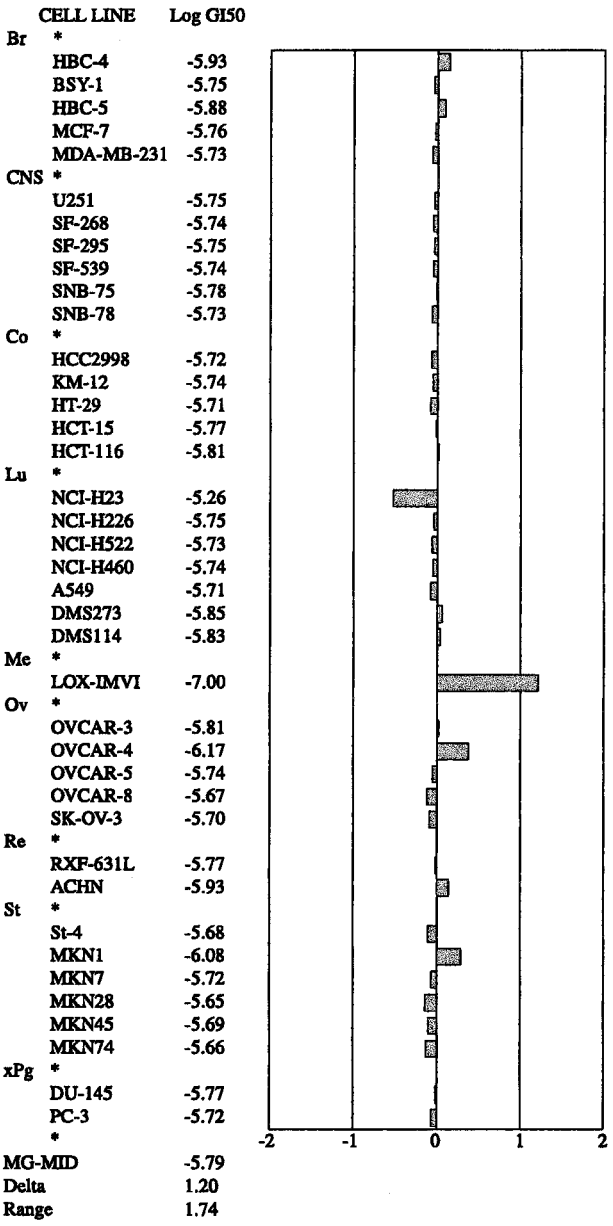

Figure 4. Growth inhibition against a panel of 38 human cancer cell lines. The log GI_{50} for each cell lines is indicated; columns extending to the right, sensitivity to haterumadioxin A; columns extending to the left, resistance to haterumadioxin A. One scale represents one logarithm difference. MG-MID, the mean of log GI_{50} values for 39 cell lines. Delta, the logarithm of difference between the MG-MID and the log GI_{50} of the most sensitive cell line. Range, the logarithm of difference between the log GI_{50} of the most resistance cell line and the log GI_{50} of the most sensitive one.

seems to be more effective against a melanoma cancer cell line. The COMPARE analysis of the mean graph revealed that **1** was correlated with doxifluridine, an antimetabolite ($r = 0.767$). This result indicated that haterumadioxin A is an antimetabolite. Secondary screening with a panel of nude mice-xenografts is underway.

Experimental Section

General Experimental Procedures. ^1H, ^{13}C, and 2D NMR spectra were recorded on 400 and 800 MHz spectrometers. FABMS spectra were recorded using *p*-nitrobenzyl alcohol as a matrix in positive mode. The starting materials were azeotropically dried with benzene before use. All reactions were conducted under a nitrogen atmosphere.

Cytotoxicity Test. Growing cells of murine P388 lymphocytic leukemia were suspended in RPMI-1640 medium containing 10% fetal bovine serum, 10 μM 2-hydroxyethyl disulfide, and kanamycin (100 μg/mL) at 2×10^4 cells/mL, and samples dissolved in MeOH were added. The mixture was incubated at 37 °C for 4 days in a CO_2 incubator with a humidified atmosphere containing 5% CO_2. The cells were counted by the MTT method.[5] The IC_{50} value (concentration required for 50% inhibition of cell growth) was determined using the growth curve.

Collection and Isolation. The Okinawan sponge *P. lita* was collected on Hateruma Island, Okinawa prefecture, Japan. This sponge (380 g, wet wt) was crushed and extracted with acetone. The aqueous acetone extract was filtered, concentrated, and then extracted with EtOAc to give 7.7 g of EtOAc extract. The EtOAc extract was subjected to fractionation guided by cytotoxicity against P388 cells. The EtOAc extract was partitioned between aqueous 90% MeOH (300 mL) and hexane (3×300 mL) to give 2.3 g of aqueous 90% MeOH extract. The aqueous 90% MeOH extract was diluted to 60% aqueous MeOH (200 mL) and partitioned with CH_2Cl_2 (2×200 mL) to give 2.2 g of CH_2Cl_2 extract. The CH_2Cl_2 extract was subjected to fractionation guided by cytotoxicity against P388 cells using SiO_2 column chromatography [benzene → benzene/EtOAc (19:1) → EtOAc → EtOAc/MeOH (7:3) → MeOH] and ODS column chromatography [MeOH/H_2O (60:40 → 70:30 → 80:20) → MeOH → $CHCl_3$/MeOH (9:1)] to give haterumadioxin A (**1**) as a colorless oil (145 mg; 0.038% yield based on wet wt). Next, about one-third of another cytotoxic fraction was subjected to fractionation using reversed-phase HPLC [ODS, MeOH/H_2O (85:15) containing 0.1% TFA, flow rate 5.0 mL/min, detection at 215 nm] to give haterumadioxin B (**2**) as a colorless oil (9.5 mg; 0.008% yield based on wet wt).

Haterumadioxin A (1): $[\alpha]^{29}_D$ −102° (*c* 1.56, MeOH); IR ($CHCl_3$) ν_{max} 3400−2800 (br), 1715 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS *m/z* 333 $[M + Na]^+$; HRFABMS *m/z* 333.2050 (calcd for $C_{18}H_{30}O_4Na$, 333.2042).

Haterumadioxin B (2): $[\alpha]^{29}_D$ −28° (*c* 0.42, MeOH); IR ($CHCl_3$) ν_{max} 3400−2800 (br), 1715 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS *m/z* 335 $[M + Na]^+$; HRFABMS *m/z* 335.2226 (calcd for $C_{18}H_{32}O_4Na$, 335.2198).

Triol 3. To a stirred solution of haterumadioxin A (4.7 mg) in THF (0.5 mL) cooled to 0 °C was slowly added 1.0 M THF solution of $LiAlH_4$ (0.06 mL). The reaction mixture was stirred under reflux for 21 h and diluted with saturated NH_4Cl_{aq} (2 mL). The reaction mixture was filtered through a pad of Celite, and the residue was washed with ether. The filtrate and washings were concentrated, and the resulting aqueous residue was extracted with $CHCl_3$ (3×3 mL). The extracts were combined, washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The oily residue was purified by SiO_2 column chromatography using benzene/EtOAc to give triol **3** (1.3 mg, 31%) as a colorless oil: $[\alpha]^{29}_D$ −66° (*c* 0.054, MeOH); IR ($CHCl_3$) ν_{max} 3500−3200 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.54 (1H, dt, $J = 15.6$, 6.3 Hz, H-10), 5.25 (1 H, dd, $J = 9.6$, 15.6 Hz, H-9), 5.03 (1 H, s, H-5), 4.76 (1 H, dd, $J = 4.6$, 9.0 Hz, H-3), 3.97 (1 H, br s, OH), 3.82−3.73 (2 H, m, H-1a, H-1b), 3.25 (1 H, br s, OH), 2.98 (1 H, br s, OH), 2.19−1.94 (5 H, m), 1.75−1.63 (3 H, m), 1.63−1.49 (2 H, m), 1.43−1.31 (1 H, m), 1.31−1.20 (2 H, m), 1.04 (3 H, t, $J = 7.6$ Hz, H-18), 0.98 (3 H, t, $J = 7.4$ Hz, H-12), 0.89 (3 H, t, $J = 7.4$ Hz, H-16), 0.83 (3 H, t, $J = 7.6$ Hz, H-14); FABMS *m/z* 321 $[M + Na]^+$.

TBDPS Ether 4. To a stirred solution of triol **3** (2.0 mg) in DMF (0.2 mL) cooled at 0 °C were added imidazole (6.1 mg) and *tert*-butyldiphenylsilyl chloride (0.01 mL). The reaction mixture was stirred at 0 °C for 3 h, and the reaction was quenched by adding ice (1 g). The mixture was extracted with $CHCl_3$ (3×2 mL), and the combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The oily residue was purified by SiO_2 column chromatography using hexane/EtOAc and ODS column chromatography using aqueous MeOH to give TBDPS ether **4** (1.6 mg, 49%) as a colorless oil: $[\alpha]^{29}_D$ −24° (*c* 0.082, MeOH); IR ($CHCl_3$) ν_{max} 3500−3300 (br), 1600, 1460, 1430 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.65−7.72 (4 H, m), 7.35−7.46 (6 H, m), 5.42 (1H, dt, $J = 15.4$, 6.4 Hz, H-10), 5.20 (1 H, dd, $J = 9.0$, 15.4 Hz, H-9), 5.01 (1 H, s, H-5), 4.65 (1 H, dd, $J = 2.7$, 9.8 Hz, H-3), 3.93−3.77 (3 H,

m), 2.17−1.90 (7 H, m), 1.78−1.60 (6 H, m), 1.05 (9 H, s, *t*-Bu), 1.03 (3 H, t, $J = 7.3$ Hz, H-18), 0.95 (3 H, t, $J = 7.4$ Hz, H-12), 0.86 (3 H, t, $J = 7.5$ Hz, H-16), 0.81 (3 H, t, $J = 7.4$ Hz, H-14); FABMS *m*/*z* 559 $[M + Na]^+$.

(*S*)-MTPA Ester 5. To a solution of TBDPS ether **4** (0.5 mg) in pyridine (0.5 mL) was added (*R*)-MTPACl (6.1 mg) at room temperature. The reaction mixture was stirred at room temperature for 8 h and concentrated. The oily residue was purified by SiO_2 preparative thin-layer chromatography using $CHCl_3$ to give (*S*)-MTPA ester **5** (1.0 mg, quant.) as a colorless oil: $[\alpha]^{29}_D$ −23° (*c* 0.051, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600−3480 (br), 1730, 1460, 1430 cm^{-1}; ^1H NMR ($CDCl_3$, 800 MHz) δ 7.62 (4 H, d, $J = 6.6$ Hz), 7.45 (2 H, d, $J = 7.3$ Hz), 7.40 (2 H, tq, $J = 7.3$, 1.3 Hz), 7.35 (4 H, t, $J = 7.8$ Hz), 7.31−7.27 (3 H, m), 7.03 (1 H, dd, $J = 3.7$, 9.9 Hz, H-3), 5.33 (1H, dt, $J = 15.4$, 6.2 Hz, H-10), 5.24 (1 H, s, H-5), 5.17 (1 H, dd, $J = 9.2$, 15.4 Hz, H-9), 3.61−3.54 (2 H, m, H-1a, H-1b), 3.44 (3 H, s, OMe), 2.08−1.92 (6 H, m, H-2b, H-8, H-11a, H-11b, H-13a, H-13b), 1.82 (1 H, m, H-2a), 1.65 (1 H, dd, $J = 8.6$, 13.9 Hz, H-7b), 1.62−1.57 (2 H, m, H-7a, H-15b), 1.54 (1 H, q, $J = 7.4$ Hz, H-15a), 1.40 (1 H, m, H-17b), 1.20 (1 H, m, H-17a), 1.04 (9 H, s, *t*-Bu), 0.97 (3 H, t, $J = 7.5$ Hz, H-14), 0.93 (3 H, t, $J = 7.5$ Hz, H-12), 0.84 (3 H, t, $J = 7.3$ Hz, H-16), 0.79 (3 H, t, $J = 7.3$ Hz, H-18); FABMS *m*/*z* 775 $[M + Na]^+$.

(*R*)-MTPA Ester 6. To a solution of TBDPS ether **4** (0.6 mg) in pyridine (0.5 mL) was added (*S*)-MTPACl (5.9 mg) at room temperature. The reaction mixture was stirred at room temperature for 8.5 h and concentrated. The oily residue was purified by SiO_2 preparative thin-layer chromatography using $CHCl_3$ to give (*R*)-MTPA ester **6** (0.6 mg, 72%) as a colorless oil: $[\alpha]^{28}_D$ −2.0° (*c* 0.031, MeOH); IR ($CHCl_3$) ν_{max} 3600−3480 (br), 1730, 1460, 1430 cm^{-1}; ^1H NMR ($CDCl_3$, 800 MHz) δ 7.64 (4 H, d, $J = 6.2$ Hz), 7.44 (2 H, d, $J = 7.8$ Hz), 7.41 (2 H, t, $J = 6.8$ Hz), 7.36 (4 H, t, $J = 7.5$ Hz), 7.31−7.26 (3 H, m), 6.98 (1 H, dd, $J = 3.4$, 9.2 Hz, H-3), 5.38 (1H, dt, $J = 15.0$, 6.2 Hz, H-10), 5.21 (1 H, s, H-5), 5.20 (1 H, dd, $J = 9.2$, 15.0 Hz, H-9), 3.69 (1 H, m, H-1b), 3.65 (1 H, m, H-1a), 3.40 (3 H, s, OMe), 2.09 (1 H, m, H-8), 2.04 (1 H, m, H-2b), 1.99 (2 H, m, H-11a, H-11b), 1.88−1.80 (3 H, m, H-2a, H-7b, H-13b), 1.72−1.65 (2 H, m, H-7a, H-13a), 1.58 (1 H, m, H-15b), 1.49−1.42 (2 H, m, H-15a, H-17b), 1.24 (1 H, m, H-17a), 1.05 (9 H, s, *t*-Bu), 0.96 (3 H, t, $J = 7.4$ Hz, H-12), 0.86 (3 H, t, $J = 7.5$ Hz, H-14), 0.84 (3 H, t, $J = 7.5$ Hz, H-16), 0.83 (3 H, t, $J = 7.3$ Hz, H-18); FABMS *m*/*z* 775 $[M + Na]^+$.

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References and Notes

(1) (a) Ueda, K.; Hu, Y. *Tetrahedron Lett.* **1999**, *40*, 6305−6308. (b) Takada, N.; Sato, H.; Suenaga, K.; Arimoto, H.; Yamada, K.; Ueda, K.; Uemura, D. *Tetrahedron Lett.* **1999**, *40*, 6309−6312.

(2) (a)Stierle, D. B.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 3396−3401. (b) Phillipson, D. W.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 7735−7736. (c) Murayama, T.; Ohizumi, Y.; Nakamura, H.; Sasaki, T.; Kobayashi, J. *Experiencia* **1989**, *45*, 898−899. (d) Gunasekera, S. P.; Gunasekera, M.; Gunawardana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod.* **1990**, *53*, 669−674. (e) De Guzman, F. S.; Schmitz, F. *J. Nat. Prod.* **1990**, *53*, 926−931. (f) Davidson, B. S. *Tetrahedron Lett.* **1991**, *32*, 7167−7170. (g) Kushlan, D. M.; Faulkner, D. J. *J. Nat. Prod.* **1991**, *54*, 1451−1454. (h) Davidson, B. S. *J. Org. Chem.* **1991**, *56*, 6722−6724. (i) Kobayashi, M.; Kondo, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 1324−1326. (j) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827−1830. (k) Rudi, A.; Talpir, R.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1993**, *56*, 2178−2182. (l) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1995**, *51*, 12195−12202. (m) Varoglu, M.; Peters, B. M.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 27−36. (n) Schmidt, E. W.; Faulkner, D. J. *Tetrahedron Lett.* **1996**, *37*, 6681−6684. (o) Patil, A. D.; Freyer, A. J.; Bean, M. F.; Carte, B. K.; Westley, J. W.; Johnson, R. K. Lahouratate, P. *Tetrahedron* **1996**, *52*, 377−394. (p) Patil, A. D.; Freyer, A. J.; Carte, B.; Johnson, R. K.; Lahouratate, P. *J. Nat. Prod.* **1996**, *59*, 219−223.

(3) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(4) Yamori, T. *Jpn. J. Cancer Chemother.* **1997**, *24*, 129−135.

(5) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589−601.

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