Cytotoxic Polyacetylenic Alcohols from the Marine Sponge *Petrosia* Species

Young Ja Lim,[†] Chong-O. Lee,[‡] Jongki Hong,[§] Dong-kyoo Kim,[⊥] Kwang Sik Im,[†] and Jee H. Jung*[,†]

College of Pharmacy, Pusan National University, Pusan 609-735, Korea, Korea Research Institute of Chemical Technology, Taejon, Korea, Korea Basic Science Institute, Taejon, Korea, and Department of Chemistry, Inje University, Kimhae, Korea

Received May 23, 2001

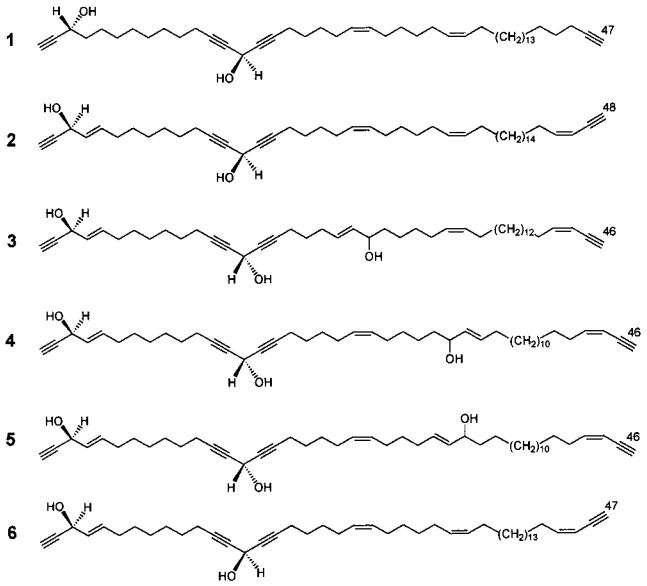
New polyacetylenic alcohols (**1**−**5**) have been isolated as cytotoxic principles from the marine sponge *Petrosia* sp. The compounds were particularly cytotoxic against a human melanoma cell line (SK-MEL-2). The gross structures were established on the basis of NMR and MS data, and the absolute configuration was determined by the modified Mosher's method.

Biologically active polyacetylenes characterized by unbranched long alkyl chains have frequently been isolated from the marine sponges of the genus *Petrosia*.[1−6] In earlier papers, we reported new C_{30}, C_{45}, C_{46}, and C_{47} polyacetylenic alcohols which displayed significant cytotoxicities against five human tumor cell lines.[7−9] In our continuing search for bioactive metabolites from the same sponge, new C_{46}, C_{47}, and C_{48} polyacetylenic alcohols (**1**−**5**) have been isolated. The structure elucidation of **1**−**5** and their cytotoxic effect and inhibitory activity on DNA replication are reported herein.

The MeOH extract of the frozen sponge was partitioned between H_2O and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 solubles between 90% aqueous MeOH and *n*-hexane. The 90% aqueous MeOH layer was then partitioned between H_2O and CH_2Cl_2 to afford 58.15 g of the CH_2Cl_2 layer, which was subjected to reversed-phase flash column chromatography to yield eight fractions. In vitro P388 and brine shrimp lethality assays were performed on these fractions. Guided by the in vitro P388 assay, fraction 7 was successively fractionated employing reversed-phase flash column chromatography and HPLC to afford compounds **1** and **2**. Compounds **3**−**5** were obtained after C_{18} flash column chromatography and HPLC of fraction 6, guided by the brine shrimp lethality assay.

Compounds **1**−**5** displayed a significant activity comparable to that of cisplatin against a panel of human tumor cell lines (Table 1). These polyacetylenes showed rather selective cytotoxicity against a skin cancer cell line (SK-MEL-2). The cytotoxicity of these polyacetylenes may be partly due to their significant inhibition of the DNA replication (Table 2).

Petrotetrayndiol F (**1**) closely resembled homo-(3*S*,14*S*)-petrocortyne A (**6**)[9] in its ^{1}H and ^{13}C NMR spectra except for the absence of the signals corresponding to the two olefinic groups (Δ^4 and Δ^{44}) (Table 3). Compared to those of homo-(3*S*,14*S*)-petrocortyne A, H-4 (δ 1.65), H-5 (δ 1.47), H-44 (δ 1.48), and H-45 (δ 2.157) signals were shifted upfield, indicating that **1** is saturated at C-4 and C-44. This interpretation was further supported by the $[M + Na]^+$ ion of **1** at *m*/*z* 695 in the FABMS, which was 4 amu higher than that of homo-(3*S*,14*S*)-petrocortyne A. The absolute configuration of **1** was determined by the modified Mosher's method. Diagnostic ^{1}H NMR chemical shift differences between the MTPA esters [$\delta\Delta = \delta_S - \delta_R$; H-1 (−0.03 ppm), H-4 (could not be calcd due to overlap with other signals), H-11 (−0.02 ppm), H-14 (+0.01 ppm)] revealed the absolute stereochemistry at C-3 and C-14 to be *R* and *S*, respectively. Although the 4-dehydro, 43-dehydro, and 4,43-didehydro analogues have previously been reported from the genus *Petrosia*,[10−13] petrotetrayndiol F (**1**) is unique for having unconjugated acetylenic groups at both termini.



The ^{1}H NMR spectrum of dihomo-(3*S*,14*S*)-petrocortyne A (**2**) was superimposable on that of homo-(3*S*,14*S*)-petrocortyne A (Experimental Section). However, when coeluted in HPLC, **2** showed a distinctly longer retention time than that of homo-(3*S*,14*S*)-petrocortyne A. In the FABMS, **2** showed the $[M + Na]^+$ ion at *m*/*z* 705, which was 14 amu higher than that of homo-(3*S*,14*S*)-petrocortyne A. The gross structure was further confirmed by FAB-CID tandem mass spectrometry (Figure 1). The fragmentation of **2** was similar to that of homo-(3*S*,14*S*)-petrocortyne A, except for an additional higher mass fragment (*m*/*z* 639), which was due to the allylic cleavage of the terminus. The absolute configuration of **2** was determined in the same manner as for petrotetrayndiol F (**1**). Diagnostic ^{1}H NMR chemical shift differences between the MTPA esters [$\delta\Delta = \delta_S - \delta_R$; H-1 (+0.02 ppm), H-4 (−0.12 ppm), H-5 (−0.08 ppm), H-6 (−0.05 ppm), H-11 (−0.06 ppm), H-14 (+0.01 ppm)] revealed the absolute stereochemistry at both C-3 and C-14 to be *S*.

(3*S*,14*R*)-Petrocortyne F (**3**) was isolated as a colorless oil. (3*S*,14*R*)-Petrocortynes G and H (**4** and **5**) were isolated as a mixture that could not be separated by semiprepara-

* To whom correspondence should be addressed. Tel: 82-51-510-2803. Fax: 82-51-510-2803. E-mail: jhjung@pusan.ac.kr.
[†] Pusan National University.
[‡] Korea Research Institute of Chemical Technology.
[§] Korea Basic Science Institute.
[⊥] Inje University.

10.1021/np010247p CCC: $20.00
Published on Web 11/16/2001

Table 1. In Vitro Cytotoxicity of **1**−**5** against a Panel of Human Solid Tumor Cell Lines[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	3.7	3.8	1.1	4.3	3.4
2	5.2	5.1	1.6	5.8	3.9
3	10.0	1.8	1.3	4.7	4.0
4, **5**	4.0	1.2	0.5	3.5	1.4
cisplatin	0.7	1.3	1.0	0.7	1.1

[a] Data as expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

Table 2. Percent Inhibitions of **1**−**5** on the SV40 DNA Replication

concentration	**1**	**2**	**3**	**4, 5**
20 μM	16	21	9	8
40 μM	48	38	36	55

Table 3. ^{1}H and ^{13}C NMR Data of Compound **1** (CD_3OD)

position	^{1}H (600 MHz)	^{13}C (75 MHz)	HMBC
1	2.75 (d, 2.1)	73.3	C-3
2		86.4	
3	4.27 (td, 6.7, 2.0)	62.6	C-1, C-2, C-4, C-5
4	1.65 (m)	39.0	C-2, C-3, C-5, C-6
5	1.47 (m)	26.3	C-3, C-4, C-6
6−9	1.30−1.40 (m)	29.2−30.7	
10	1.50 (m)	29.2−30.7	C-9, C-11
11	2.22 (td, 7.1, 2.0)	19.2[a]	C-10, C-12, C-13, C-14
12		84.5[b]	
13		80.0[c]	
14	5.01 (quint, 2.0)	52.6	C-13, C-15
15		79.9[c]	
16		84.4[b]	
17	2.23 (td, 6.8, 1.9)	19.3[a]	C-14, C-15, C-16, C-18
18	1.50 (m)	29.2−30.7	C-17, C-19, C-20
19	1.30−1.40 (m)	29.2−30.7	
20	2.06 (m)	27.7[d]	C-19, C-21, C-22
21	5.35−5.39 (m)	130.6[e]	C-19, C-20, C-23
22	5.35−5.39 (m)	131.1[e]	C-20, C-23, C-24
23	2.06 (m)	28.1[d]	C-21, C-22, C-24
24−25	1.38 (m)	29.2−30.7	
26	2.06 (m)	28.0[d]	C-25, C-27, C-28
27	5.35−5.39 (m)	131.0[e]	C-25, C-26, C-29
28	5.35−5.39 (m)	130.8[e]	C-26, C-29, C-30
29	2.06 (m)	28.0[d]	C-27, C-28, C-30
30	1.38 (m)	29.2−30.7	C-27, C-28, C-29, C-31
31−43	1.30−1.40 (m)	29.2−30.7	
44	1.48 (m)	29.2−30.7	C-43, C-46
45	2.157 (t, 7.1)	19.0	C-44, C-46, C-47
46		84.5[b]	
47	2.160 (s)	69.3	C-46

[a−e] Assignments with the same superscript in the same column may be interchanged.

tive ODS and CN HPLC. The ^{1}H and ^{13}C NMR data of **3**, **4**, and **5** suggested that these compounds have the same gross structure as petrocortynes F, G, and H,[10] respectively (Experimental Section). Analyses of the HMBC (for **3**, **4**, and **5**), TOCSY (for **3**), and FAB-CID tandem mass (for **3**, **4**, and **5**) spectral data further supported this conclusion. The absolute configuration at C-3 and C-14 of **3**, **4**, and **5** was proposed as 3*S*,14*R*, by comparing ^{1}H NMR data of *S*-MTPA esters with that of (3*S*,14*R*)-petrocortyne E,[9] while the absolute configuration at the third carbinol carbon could not be defined due to overlap of the corresponding signals. Thus it appears that **3**−**5** differed from petrocortynes F−H[10] by the C-3 absolute stereochemistry. It is interesting to note that the analogous linear polyacetylenes from *Petrosia* sp. revealed mixed stereochemistries of each allylic secondary alcohol,[3,7−10,13] possibly due to a racemization either in the animal or subsequently in the process of storage, isolation, or derivatization.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH using a UV-2401 PC Shimadzu spectrophotometer. IR spectra were recorded on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DMX600, Varian Unity Plus 300, and Inova 500 instruments. Chemical shifts are reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.26 for $CDCl_3$). FAB-CID tandem MS data were obtained using a JEOL JMS-HX110/110A. HPLC was performed with a YMC ODS-H80 (semipreparative, 250 × 10 mm i.d., 4 μm, 80 Å; preparative, 250 × 20 mm i.d., 4 μm, 80 Å) and YMC-Pack CN (250 × 10 mm i.d., 5 μm, 120 Å) column using a Shodex RI-71 detector.

Animal Material. The sponge *Petrosia* sp. was collected in July 1995 (15−25 m depth), off Komun Island, Korea (see ref 7 for description of the sponge material). A voucher specimen (J95K-11) was deposited in the Natural History Museum, Hannam University, Taejon, Korea.

Extraction and Isolation. The frozen sponge (14.5 kg) was extracted with MeOH at room temperature. The MeOH solubles were partitioned as described in our previous report.[8] Fraction 7 (7 g) was active in the P388 assay (ED_{50} 4.7 μg/mL, doxorubicin 4.0 μg/mL), and fraction 6 (4.5 g) was active in the brine shrimp lethality assay (LD_{50} < 7.3 μg/mL). Guided by the P388 assay, fraction 7 was further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with 10 → 0% H_2O/MeOH, ethyl acetate, and $CHCl_3$ to afford 13 fractions. Fraction 7-9 (762.1 mg, ED_{50} 3.7 μg/mL, doxorubicin 2.0 μg/mL), eluted with MeOH, was separated on preparative ODS HPLC eluting with 100% MeOH to afford fraction 7-9-7 (25.6 mg). Fraction 7-9-7 yielded compounds **1** (2.5 mg) and **2** (7.2 mg) by purification on semipreparative ODS HPLC eluting with 49.3% MeOH/MeCN. Fraction 6 was further subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 120 Å 230 mesh), eluting with the solvent system 17 → 0% H_2O/MeOH, 17% and 50% MeOH/EtOAc, EtOAc, and acetone to afford 12 fractions. Fraction 6-6 (760.0 mg, LD_{50} 1.4 μg/mL), which eluted at 4% H_2O/MeOH, was separated by preparative ODS HPLC eluting with 2% H_2O/MeOH to afford fraction 6-6-12 (64.2 mg) and fraction 6-6-7 (35.0 mg). Separation of fraction 6-6-12 by preparative ODS HPLC eluting with 5% H_2O/MeOH afforded compound **3** (2.4 mg), which was further purified by CN HPLC eluting with 40% H_2O/MeCN. Fraction 6-6-7 was purified by CN HPLC eluting with 40% H_2O/MeCN to afford compounds **4** and **5** (5.3 mg) as a mixture.

Petrotetrayndiol F (1): yellow oil; ^{1}H and ^{13}C NMR data, see Table 3; LRFABMS *m*/*z* 695 $[M + Na]^+$ ($C_{47}H_{76}O_2Na$).

Dihomo-(3*S*,14*S*)-petrocortyne A (2): yellow oil; $[\alpha]^{23}_D$ +10° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.9) nm; IR (film) ν_{max} 3297, 2925, 2853, 1452, 995 cm^{-1}; ^{1}H NMR data, (CD_3OD, 300 MHz) δ 5.99 (1H, dt, *J* = 10.7, 7.3, H-45), 5.85 (1H, dtd, *J* = 15.1, 6.8, 1.5, H-5), 5.54 (1H, ddt, *J* = 15.1, 6.3, 1.5, H-4), 5.44 (1H, brd, *J* = 10.7, H-46), 5.33−5.37 (4H, m, H-21, 22, 27, 28), 5.01 (1H, quint, *J* = 2.0, H-14), 4.74 (1H, brd, *J* = 6.3, H-3), 3.39 (1H, brd, *J* = 2.0, H-48), 2.86 (1H, d, *J* = 2.0, H-1), 2.31 (2H, q, *J* = 6.3, H-44), 2.21 (4H, m, H-11, 17), 2.06 (10H, m, H-6, 20, 23, 26, 29), 1.29−1.50 (44H, m, H-7−10, 18, 19, 24, 25, 30−43); LRFABMS *m*/*z* 705 $[M + Na]^+$ ($C_{48}H_{74}O_2Na$).

(3*S*,14*R*)-Petrocortyne F (3): colorless oil; ^{1}H NMR data (CD_3OD, 500 MHz) δ 5.99 (1H, dtd, *J* = 11.0, 7.5, 1.0, H-43), 5.84 (1H, dtd, *J* = 15.5, 6.5, 1.0, H-5), 5.59 (1H, dt, *J* = 15.5, 7.0, H-20), 5.55 (1H, ddt, *J* = 15.5, 6.0, 1.5, H-4), 5.46 (1H, dd, *J* = 15.5, 7.0, H-21), 5.44 (1H, ddt, *J* = 11.0, 2.0, 1.5, H-44), 5.34 (2H, m, H− 27, 28), 5.01 (1H, quint, *J* = 2.0, H-14), 4.73 (1H, brd, *J* = 6.5, H-3), 3.96 (1H, q, *J* = 6.0, H-22), 3.40 (1H, d, *J* = 2.0, H-46), 2.86 (1H, d, *J* = 2.0, H-1), 2.31 (2H, q, *J* = 7.0, H-42), 2.23 (2H, td, *J* = 7.0, 2.0, H-17), 2.21 (2H, td, *J* = 7.0, 2.0, H-11), 2.15 (2H, m, H-19), 2.03 (6H, m, H-6, 26, 29), 1.59 (2H, m, H-18), 1.50 (4H, m, H-10, 23), 1.41 (6H, m, H-7,

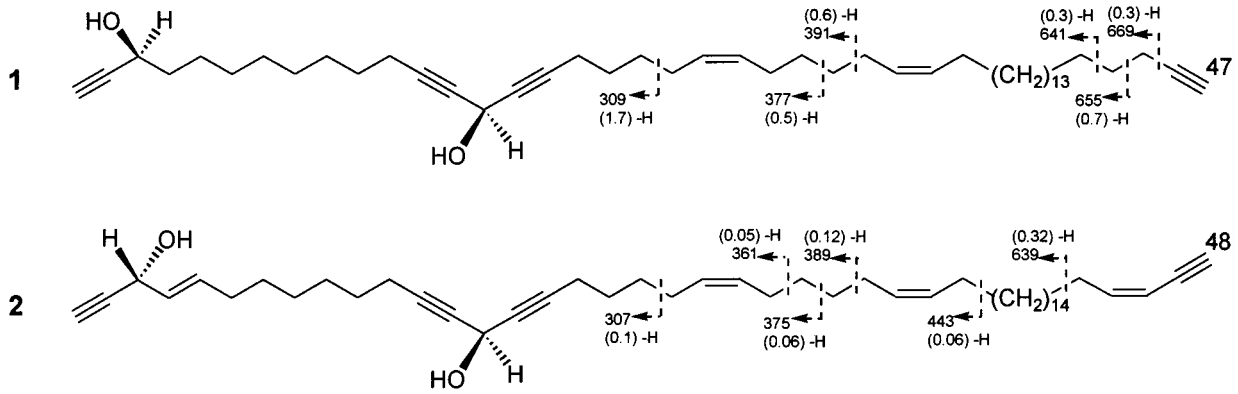


Figure 1. Key FAB-CID MS/MS fragmentations of the $[M + Na]^+$ of **1** and **2**. The % relative abundance is given in parentheses.

24, 41), 1.29 (28H, m, H-8−9, 25, 30−40); ^{13}C NMR data (CD_3OD, 75 MHz) δ 146.4 (C-43), 135.4 (C-20), 134.1 (C-5), 131.3 (C-4/21/27/28), 131.0 (C-4/21/27/28), 130.72 (C-4/21/27/28), 130.69 (C-4/21/27/28), 109.3 (C-44), 84.7 (C-2/12/16), 84.6 (C-2/12/16), 79.8 (C-13, 15), 74.6(C-1), 73.7 (C-22), 63.2 (C-3), 52.6 (C-14), 38.3 (C-23), 32.9 (C-6), 32.2 (C-19), 31.1 (C-42), 29.2−30.8 (C-7−10, 18, 24, 25, 30−41), 28.2 (C-26, 29), 19.3 (C-11, 17), (signals of C-45 and C-46 were not detected); selected TOCSY data, H-20 (H-17), H-22 (H-26); selected HMBC data, H-10 (C-17, C-21); key ^1H NMR data of (*S*)-MTPA ester of **3** ($CDCl_3$, 500 MHz), 5.98 (1H, dt, J = 16.0, 7.5, H-5), 5.48 (1H, dd, J = 16.0, 7.0, H-4), 2.62 (1H, d, J = 2.0, H-1), 2.20 (2H, t, J = 8.0, H-17), 2.19 (2H, t, J = 8.0, H-11); LRFABMS *m/z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$); FAB-CID tandem MS *m/z* 693 $[M + Na]^+$ (1.3), 627 (1.1), 613 (0.3), 599 (0.4), 585 (0.3), 571 (0.4), 557 (0.4), 543 (0.4), 529 (0.3), 515 (0.2), 501 (0.2), 487 (0.2), 473 (0.4), 459 (0.3), 405 (0.5), 293 (0.5).

(3*S*,14*R*)-Petrocortyne G and (3*S*,14*R*)-petrocortyne H (4 and 5): colorless oil; ^1H NMR data (CD_3OD, 300 MHz) δ 5.99 (1H, dt, J = 10.8, 7.8, H-43), 5.85 (1H, dtd, J = 16.5, 6.3, 0.9, H-5), 5.59 (1H, m, H-29 of **4**/H-26 of **5**), 5.55 (1H, ddt, J = 16.5, 6.3, 1.5, H-4), 5.40−5.46 (2H, m, H-44, H-28 of **4**/H-27 of **5**), 5.37 (2H, m, H− 21, 22), 5.01 (1H, quint, J = 2.1, H-14), 4.74 (1H, brd, J = 6.3, H-3), 3.95 (1H, q, J = 6.3, H-27 of **4**/H-28 of **5**), 3.41 (1H, d, J = 2.4, H-46), 2.87 (1H, d, J = 2.1, H-1), 2.31 (2H, q, J = 7.2, H-42), 2.22 (4H, m, H-11, 17), 2.06 (8H, m, H-6, 20, 23, H-30 of **4**/H-25 of **5**), 1.20−1.57 (40H, m, H-7−10, 18, 19, 24, 31−41, H-25, 26 of **4**/H-29, 30 of **5**); ^{13}C NMR data (CD_3OD, 75 MHz) δ 146.41 (C-43), 146.39 (C-43), 134.8 (C-29 of **4**/C-26 of **5**), 134.5 (C-29 of **4**/C-26 of **5**), 134.1 (C-5), 132.6 (C-28 of **4**/C-27 of **5**), 132.2 (C-28 of **4**/C-27 of **5**), 131.1 (C-4/21/22), 130.8 (C-4/21/22), 130.7 (C-4/21/22), 130.6 (C-4/21/22), 109.3 (C-44), 84.5 (C-2/12/16), 84.3 (C-2/12/16), 82.7 (C-46), 79.9 (C-13/15), 79.8 (C-13/15), 74.6(C-1), 73.8 (C-27 of **4**, C-28 of **5**), 63.1 (C-3), 52.6 (C-14), 38.5 (C-26 of **4**/C-29 of **5**), 38.3 (C-26 of **4**/C-29 of **5**), 33.3 (C-30 of **4**/C-25 of **5**), 32.9 (C-6), 32.8 (C-30 of **4**/C-25 of **5**), 31.1 (C-42), 29.2−30.8 (C-7−10, 18, 19, 24, 31−41, C-25 of **4**, C-30 of **5**), 28.2 (C-20/23), 27.8 (C-20/23), 27.7 (C-20/23), 27.6 (C-20/23), 19.3 (C-11/17), 19.2 (C-11/17) (signal of C-45 was not detected); selected HMBC data, C-17 (H-20), C-25 (H-23 of **5**); key ^1H NMR data of (*S*)-MTPA ester of **4** and **5** ($CDCl_3$, 500 MHz), 5.98 (1H, dt, J = 15.5, 6.5, H-5), 5.48 (1H, dd, J = 15.5, 6.5, H-4), 2.62 (1H, d, J = 2.0, H-1), 2.18 (2H, t, J = 7.0, H-17), 2.17 (2H, t, J = 7.0, H-11); LRFABMS *m/z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$); FAB-CID tandem MS *m/z* 693 $[M + Na]^+$ (1.3), 627 (1.1), 613 (0.3), 599 (0.4), 585 (0.3), 571 (0.4), 557 (0.4), 543 (0.4), 529 (0.3), 515 (0.2), 501 (0.2), 487 (0.2), 473 (0.4), 459 (0.3), 405 (0.5), 293 (0.5).

Preparation of MTPA Ester. The (*R*)-MTPA or (*S*)-MTPA esters of **1**−**5** were prepared as described previously.[8] To solutions of **1**−**5** in dry pyridine (20 μL) were added four times the molar excess of (*R*)-(−)- or (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (paying attention to the fact that (*R*)-MTPA-Cl gives (*S*)-MTPA ester and vice versa). Each mixture was allowed to stand at room temperature for 16 h, and an equimolar amount of 3-(dimethylamino)propylamine was added. After standing for 10 min, the solvent was evaporated. The residue was purified on silica gel in a Pasteur pipet eluting with CH_2Cl_2 and characterized by ^1H NMR ($CDCl_3$, 500 MHz).

In Vitro SV40 DNA Replication. The reaction mixtures (40 μL) included 40 mM creatine phosphate-di-Tris salt (pH 7.7), 1 μg of creatine kinase, 7 mM $MgCl_2$, 0.5 mM DTT, 4 mM ATP, 200 μM UTP, GTP, and CTP, 100 μM dATP, dGTP, and dCTP, 25 μM [^{3}H]dTTP (300 cpm/pmol), 0.6 μg of SV40 T-Ag, 0.3 μg of SV40 origin-containing DNA (pUC), and the indicated amounts of replication proteins. The reactions were performed for 90 min at 37 °C, after which the acid-insoluble radioactivity was measured. Replication products were analyzed using [α-^{32}P] dATP (30,000 cpm/pmol) instead of [^{3}H] dTTP in the reactions just described. After incubation, the reactions were stopped by addition of 40 μL of a solution containing 20 mM EDTA, 1% sodium dodecyl sulfate, and *E. coli* tRNA (0.5 mg/mL). One-tenth of the reaction mixture was used to measure the acid-insoluble radioactivity. DNA was isolated and electrophoretically separated on a 1.0% agarose gel for 12−14 h at 2 V/cm. The gel was subsequently dried and exposed to X-ray film.

Acknowledgment. The authors gratefully acknowledge Dr. C. J. Sim for the identification of the sponge. Our thanks are due to Joon Hee Park, Hyun Soo Kim, and Kyu Sik Choi for their technical assistance in the extractions and collection of the marine sponge. The 600 MHz NMR spectra were provided by the Research Equipment Share Program of the Korea Basic Science Institute. This study was supported by a grant from the Ministry of Health and Welfare.

References and Notes

(1) Guo, Y.; Gavagnin, M.; Salierno, C.; Cimino, G. *J. Nat. Prod.* **1998**, *61*, 333−337.
(2) Li, H.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1994**, *57*, 1464−1467.
(3) Ochi, M.; Ariki, S.; Tatsukawa, A.; Kotsuki, H.; Fukuyama, Y.; Shibata, K. *Chem. Lett.* **1994**, 89−92.
(4) Fusetani, N.; Li, H.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49*, 1203−1210.
(5) Issacs, S.; Kashman, Y.; Loya, S.; Hizi, A.; Loya, Y. *Tetrahedron* **1993**, *49*, 10435−10438.
(6) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *J. Nat. Prod.* **1990**, *53*, 345−353.
(7) Kim, J. S.; Im, K. S.; Jung, J. H.; Kim, Y.-L.; Kim, J.; Sim, C. J.; Lee, C.-O. *Tetrahedron* **1998**, *54*, 3151−3158.
(8) Kim, J. S.; Lim, Y. J.; Im, K. S.; Jung, J. H.; Sim, C. J.; Lee, C.-O.; Hong, J.; Lee, H. *J. Nat. Prod.* **1999**, *62*, 554−559.
(9) Lim, Y. J.; Park, H. S.; Im, K. S.; Lee, C.-O.; Hong, J.; Lee, M.-Y.; Kim, D.-K.; Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 46−53.
(10) Shin, J.; Seo, Y.; Cho, K. W. *J. Nat. Prod.* **1998**, *61*, 1268−1273.
(11) Guo, Y.; Gavagnin, M.; Salierno, C.; Cimino, G. *J. Nat. Prod.* **1998**, *61*, 333−337.
(12) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *J. Nat. Prod.* **1995**, *58*, 712−722.
(13) Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J. *Tetrahedron* **1998**, *54*, 447−462.

NP010247P