Regular Article

Six New Polyacetylenic Alcohols from the Marine Sponges *Petrosia* sp. and *Halichondria* sp.

Adeyemi Francis Gabriel, Zhen Li, Ryouhei Kusuda, Chiaki Tanaka, and Tomofumi Miyamoto*

Department of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.

Received March 5, 2015; accepted March 18, 2015; advance publication released online April 9, 2015

Six new polyacetylenic alcohols, termed strongylotriols A and B; pellynols J, K, and L; and isopellynol A, together with three known polyacetylenic alcohols, pellynols A, B, and C were isolated from the marine sponges *Petrosia* sp., and *Halichondria* sp. collected in Okinawa, Japan. Their planer structures were determined based on 2D-NMR and mass spectrometric analysis of the degraded products by $RuCl_3$ oxidation. The absolute stereochemistry of isolates was examined by their Mosher's esters. The strongylotriols were found to be optically pure compounds, whereas the pellynols are diastereomeric mixtures at the C-6 position. Proliferation experiments using the HeLa and K562 cell lines suggested that the essential structural units for activity are the "hexa-2,4-diyn-1,6-diol" and "pent-1-en-4-yn-3-ol" on the termini.

Key words polyacetylenic alcohol; marine sponge; *Petrosia* sp.; *Halichondria* sp.; strongylotriol; pellynol

Polyacetylenic compounds are diverse groups of secondary metabolites that have been isolated from various sources, including plants, marine organisms, nudibranchs, hard corals and red algae. These compounds have been shown to possess various pharmacological activities, which including anti-tumor, anti-viral, anti-microbial and anti-inflammatory activities.[1–5] In our continuing research on biologically active metabolites in marine invertebrates,[6] we have isolated six new polyacetylenic alcohols from the marine sponges, *Petrosia* sp., and *Halichondria* sp.[7] collected in Okinawa. This paper describes the isolation, structure and biological activity of acetylenic alcohols.

Results and Discussion

Ethanol (EtOH) extracts prepared from 93 specimens of marine invertebrates collected at Iriomote, Okinawa, were screened for anti-proliferative activity. Among them, an extract of the *Petrosia* sp. and an extract of the *Halichondria* sp. were able to inhibit HeLa cell proliferation. These extracts were subjected to bioassay-guided separation. The diethyl ether (Et_2O) soluble fraction of the EtOH extract of *Petrosia* sp. was fractionated using a Sephadex LH-20 column chloroform ($CHCl_3$), and reverse-phase HPLC ($5C_{22}$, 91% methanol (MeOH)/H_2O), yielding strongylotriols A (**1**) and B (**2**); and pellynols J (**4**), A (**7**), B (**8**) and C (**9**).[8] The Et_2O soluble fraction of the EtOH extract of *Halichondria* sp. was fractionated using a silica-gel column, followed by reverse-phase HPLC ($5C_{30}$, 93% MeOH/H_2O or $5C_{18}$, 95% MeOH/H_2O), yielding isopellynol A (**3**); pellynols J (**4**), K (**5**), L (**6**), A (**7**), B (**8**) and C (**9**) (Fig. 1).

Strongylotriol A (**1**) was found to be a white, amorphous solid, with electrospray ionization-time of flight (ESI-TOF)-MS showing a molecular formula of $C_{25}H_{40}O_3$. ^{1}H- and ^{13}C-NMR and heteronuclear single quantum coherence (HSQC) spectroscopic data indicated the presence of one primary methyl, one oxygenated methylene, two oxygenated methines, three di-substituted alkynes, and 15 aliphatic methylenes, suggesting that **1** was an acyclic polyacetylenic alcohol. Its heteronuclear multiple bond correlation (HMBC) spectrum showed partial structures, including the terminal "hexa-2,4-diyn-1,6-diol" and the internal "prop-2-yn-1-ol" (Fig. 2a). The location of the "prop-2-yn-1-ol" unit was determined by

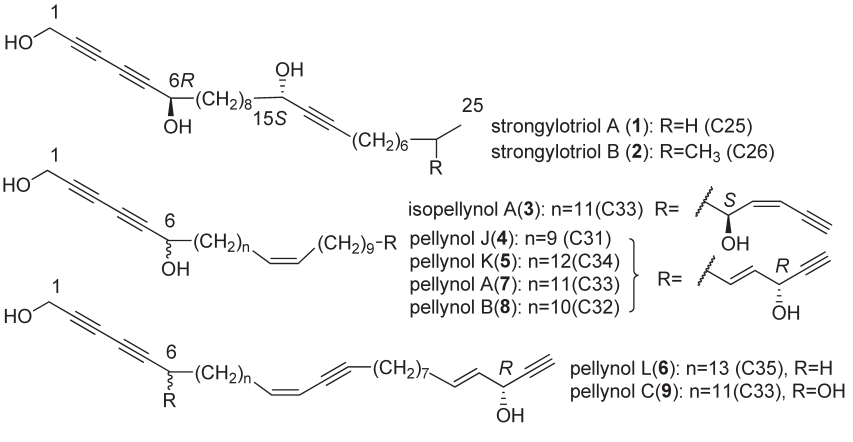

Fig. 1. Structures of Compounds **1**–**9**

* To whom correspondence should be addressed. e-mail: miyamoto@phar.kyushu-u.ac.jp

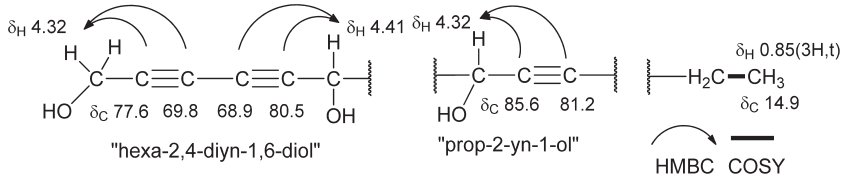

Fig. 2a. HMBC Correlations and Partial Structure of **1**

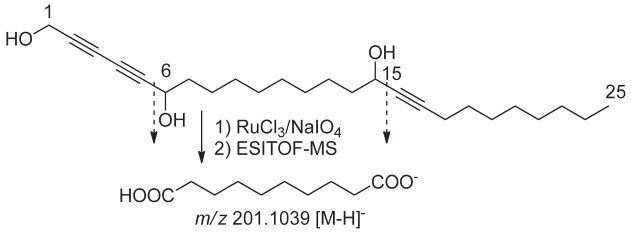

Fig. 2b. $RuCl_3$ Oxidation and Its Degradation Product of **1**

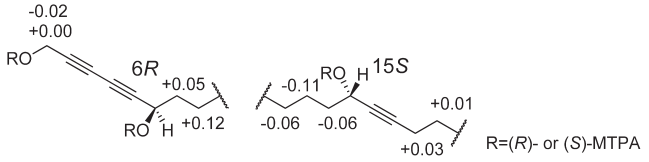

Fig. 2c. $\Delta\delta$ Values of (*R*)- and (*S*)-MTPA Esters of **1**

Table 1. ^{1}H-NMR (600 MHz) Data of Compounds **1**–**9** in $CDCl_3$

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7** (**8**)	**9**
1	4.32 (2H, s)	4.32 (2H, s)	4.33 (2H, s)	4.32 (2H, s)	4.32 (2H, s)	4.31 (2H, s)	4.32 (2H, s)	4.32 (2H, s)
6	4.41 (t, 6.6)	4.41 (t, 6.6)	4.41 (t, 6.6)	4.41 (t, 6.6)	4.41 (t, 6.6)	2.26 (t, 6.6)	4.41 (t, 6.6)	4.41 (t, 6.6)
7	1.70 (2H, m)	1.70 (2H, m)	1.70 (2H, m)	1.70 (2H, m)	1.70 (2H, m)		1.70 (2H, m)	1.70 (2H, m)
Internal	1.64 (2H, m)	1.64 (2H, m)	1.99 (2H, brd)	1.99 (2H, q)	1.99 (2H, q)	2.26 (2H, q)	1.99 (2H, q)	2.26 (2H, q)
	4.32 (brs)	4.32 (brs)	5.33 (2H, t-like)	5.33 (2H, t-like)	5.33 (2H, t-like)	5.80 (dt, 7.2, 10.8)	5.33 (2H, t-like)	5.79 (dt, 7.2, 10.8)
	2.18 (2H, t, 6.6)	2.18 (2H, t, 6.6)	1.95 (2H, brd)	1.99 (2H, q)	1.99 (2H, q)	5.43 (d, 10.8)	1.99 (2H, q)	5.41 (d, 10.8)
						2.33 (t, 6.6)		
Terminal			1.55 (2H, brd)	2.05 (q, 7.2)	2.05 (q, 7.2)	2.06 (q, 7.2)	2.05 (q, 7.2)	2.05 (q, 7.2)
			4.66 (2H, q, 7.2)	5.90 (dt, 6.0, 15.6)	5.90 (dt, 6.0, 15.6)	5.91 (dt, 6.0, 15.6)	5.90 (dt, 6.0, 15.6)	5.90 (dt, 6.0, 15.6)
			5.97 (dd, 8.4, 10.8)	5.59 (dd, 6.0, 15.6)	5.59 (dd, 6.0, 15.6)	5.59 (dd, 6.0, 15.6)	5.59 (dd, 6.0, 15.6)	5.59 (dd, 6.0, 15.6)
	1.28 (2H, m)	1.49 (1H, m)	5.51 (brd, 10.8)	4.82 (brd, 6.0)	4.82 (brd, 6.0)	4.83 (brd, 6.0)	4.82 (brd, 6.0)	4.82 (brd, 6.0)
	0.85 (3H, t, 7.8)	0.85 (6H, d, 7.2)	3.12 (d, 1.8)	2.54 (d, 1.8)	2.54 (d, 1.8)	2.54 (d, 1.8)	2.54 (d, 1.8)	2.55 (d, 1.8)

analysis of the degradation product of **1** resulting from $RuCl_3$ oxidation. Because the fragment ion peak observed at *m/z* 201.1039 ($C_{10}H_{17}O_4^-$, Δmmu−9.3) was estimated to be a decanedeoic acid, the "prop-2-yn-1-ol" unit was located at C-15 of **1**, yielding the deduced planar structure shown in Fig. 2b. The absolute stereochemistry of **1** was determined using a modified Mosher's method.[9] The proton chemical shifts of (*R*)- and (*S*)-α-methoxy(trifluoromethyl)phenyl acetic acid (MTPA) esters of **1** were assigned by correlated spectroscopy (COSY) and total correlation specroscopy (TOCSY) spectral data. The distribution of $\Delta\delta$ (δ_S–δ_R) values indicated that the absolute stereochemistry at C-6 and C-15 was 6*R* and 15*S*, respectively (Fig. 2c).

Strongylotriol B (**2**) was found to be a white, amorphous solid, with ESI-TOF-MS showing a molecular formula of $C_{26}H_{42}O_3$. ^{1}H- and ^{13}C-NMR spectra were similar to those of **1** except for the signals due to the terminal isopropyl moiety [δ_H 0.85 (6H, d), δ_C 22.6 (q), 27.2 (d)] (Tables 1, 2). Mass spectrometric analysis of the degradation product of **2** resulting from $RuCl_3$ oxidation showed that its "prop-2-yn-1-ol" unit was located in the same position as in **1**. The absolute stereochemistry of **2** was also examined using a modified Mosher's method, and the absolute configurations at C-6 and C-15 were determined to be 6*R* and 15*S* respectively, as in **1**.

Isopellynol A (**3**) was isolated as a white, amorphous solid from the Et_2O extractive of *Halichondria* sp. ESI-TOF-MS showed that its molecular formula was $C_{33}H_{52}O_3$. ^{1}H- and ^{13}C-NMR and HSQC spectroscopic data indicated the presence of one oxygenated methylene, two oxygenated methines, two di-substituted alkenes, two di-substituted alkynes, one terminal acetylene and 20 aliphatic methylenes. The HMBC spectrum of **3** showed three partial structures, a terminal "hexa-2,4-diyn-1,6-diol," a "pent-2-en-4-yn-1-ol" and an internal "*Z*-olefin" (Fig. 3a). The olefin geometry was determined to be *Z* both from its coupling constant (J=10.8 Hz) and the ^{13}C chemical shift of the allylic methylenes (δ_C 27.2).[10] The location of the internal "*Z*-olefin" was determined by analysis of the degradation products of **3** resulting from $RuCl_3$ oxidation. The fragment ion peaks derived from tridecanedioic acid ($C_{13}H_{24}O_4$) and undecanedioic acid ($C_{11}H_{20}O_4$) were observed at *m/z* 215.1305 $(M-H)^-$ and *m/z* 243.1564 $(M-H)^-$, respectively, resulting in the deduced planar structure shown in Fig. 3b. The absolute stereochemistry of **3** was examined using its Mosher's esters, and the distribution of $\Delta\delta$ (δ_S–δ_R) values closed to C-29 indicates that absolute stereochemistry at C-29 was in the *S* configuration. However, the ^{1}H-NMR spectra of (*R*)- and (*S*)-MTPA esters showed diastereomeric proton signals for H-6 in a ratio of 3/4.

Pellynol J (**4**) was obtained as a white, amorphous solid from the Et_2O extractives of both *Petrosia* sp. and *Halichondria* sp. ESI-TOF-MS showed a molecular formula of $C_{31}H_{48}O_3$. ^{1}H- and ^{13}C-NMR and HSQC spectroscopic data

Table 2. ^{13}C-NMR (150 MHz) Data of Compounds **1**–**9** in $CDCl_3$

Carbon	1	2	3	4	5	6	7 (8)	9
1	51.4 (t)	51.5 (t)	51.5 (t)	51.5 (t)	51.5 (t)	51.6 (t)	51.4 (t)	51.5 (t)
2	77.6 (s)	77.5 (s)	77.5 (s)	77.5 (s)	77.5 (s)	75.2 (s)	77.5 (s)	77.5 (s)
3	69.8 (s)	69.8 (s)	69.8 (s)	69.8 (s)	69.8 (s)	72.0 (s)	69.8 (s)	69.8 (s)
4	68.9 (s)	69.8 (s)	68.8 (s)	68.8 (s)	68.8 (s)	65.2 (s)	68.8 (s)	68.8 (s)
5	80.5 (s)	80.6 (s)	80.6 (s)	80.6 (s)	80.6 (s)	84.0 (s)	80.6 (s)	80.6 (s)
6	62.8 (d)	62.8 (d)	62.8 (d)	62.8 (d)	62.8 (d)	32.0 (t)	62.8 (d)	62.8 (d)
7	37.4 (t)	37.4 (t)	37.5 (t)	37.5 (t)	37.5 (t)		37.5 (t)	37.5 (t)
Int.	38.2 (t)	38.2 (t)	27.2 (t)	27.2 (t)	27.2 (t)	25.0 (t)	27.2 (t)	25.0 (t)
	62.8 (d)	62.8 (d)	129.9 (d)	129.9 (d)	129.9 (d)	142.5 (d)	129.9 (d)	142.5 (d)
	85.6 (s)	85.4 (s)	129.9 (d)	129.9 (d)	129.9 (d)	109.4 (d)	129.9 (d)	109.4 (d)
	81.2 (s)	81.1 (s)	27.2 (t)	27.2 (t)	27.2 (t)	94.5 (s)	27.2 (t)	94.5 (s)
	18.7 (t)	18.7 (t)				77.5 (s)		77.5 (s)
ω6			36.5 (t)	31.9 (t)	31.9 (t)	31.9 (t)	31.9 (t)	31.9 (t)
ω5			70.1 (d)	134.6 (d)	134.6 (d)	134.6 (d)	134.6 (d)	134.6 (d)
ω4			147.4 (d)	128.4 (d)	128.4 (d)	128.4 (d)	128.4 (d)	128.4 (d)
ω3		27.2 (d)	108.9 (d)	62.9 (d)	62.9 (d)	62.8 (d)	62.9 (d)	62.8 (d)
ω2		22.6 (q)	82.8 (s)	83.4 (s)	83.4 (s)	83.3 (s)	83.4 (s)	83.3 (s)
ω1	14.1 (q)	22.6 (q)	79.5 (d)	74.0 (d)	74.0 (d)	73.9 (d)	74.0 (d)	74.0 (d)

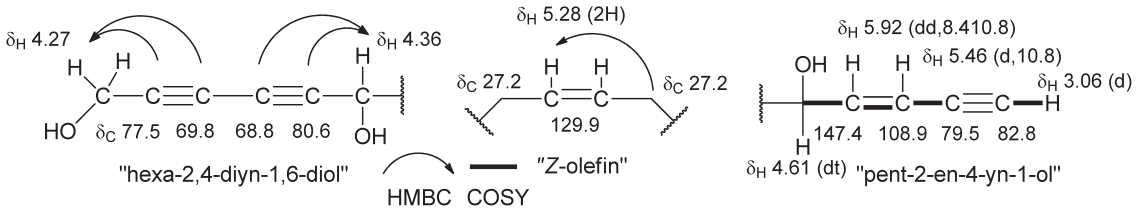

Fig. 3a. HMBC and COSY Correlations and Partial Structure of **3**

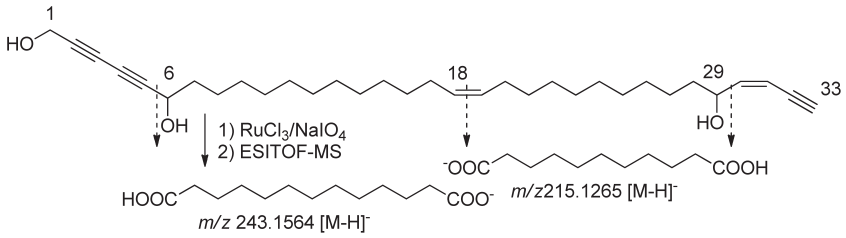

Fig. 3b. $RuCl_3$ Oxidation and Its Degradation Products of **3**

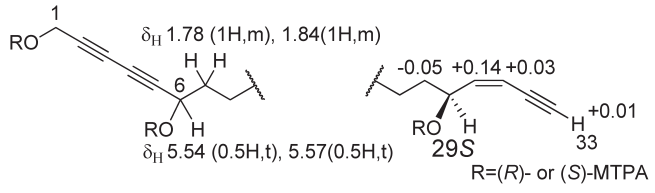

Fig. 3c. ^{1}H-NMR Chemical Shifts at H-6 and H_2-7, and $\Delta\delta$ Values of (*R*)- and (*S*)-MTPA Esters of **3**

indicated the presence of one oxygenated methylene, two oxygenated methines, two di-substituted alkenes, two di-substituted alkynes, one terminal acetylene and 18 aliphatic methylenes. Because these spectral features corresponded to those of the known polyacetylenic alcohols, pellynols A (**7**; $C_{33}H_{52}O_3$) and B (**8**; $C_{32}H_{50}O_3$), pellynol J was regarded as a C_{31} polyacetylenic alcohol possessing terminal "hexa-2,4-diyn-1,6-diol" and "pent-1-en-4-yn-3-ol" units and an internal "*Z*-olefin" unit (Tables 1, 2). The position of "*Z*-olefin" at C16 was determined by the ESI-TOF-MS analysis of **4** with $RuCl_3$ oxidation. The absolute stereochemistry of C-29 was determined to be *R* configuration, however the stereochemistry at C-6 was a diastereomeric mixture, as in isopellynol A (**3**).

Pellynol K (**5**) was obtained as a white, amorphous solid from the Et_2O extractive of *Halichondria* sp. ESI-TOF-MS gave a molecular formula of $C_{34}H_{54}O_3$. ^{1}H- and ^{13}C-NMR and HSQC spectroscopic data were similar to those of pellynols A (**7**), B (**8**), and J (**4**), suggesting that pellynol K was a C_{34} polyacetylenic alcohol possessing terminal "hexa-2,4-diyn-1,6-diol" and "pent-1-en-4-yn-3-ol" units and an internal "*Z*-olefin" unit. The position of "*Z*-olefin" at C-19 was determined by the ESITOF-MS analysis of **5** with $RuCl_3$ oxidation. A comparison of the specific rotation of **5** with those of **4**, **7**, and **8** indicated that the stereochemistry of **5** was 32*R*.

Pellynol L (**6**) was obtained as a white, amorphous solid from the Et_2O extractive of *Halichondria* sp. ESI-TOF-MS

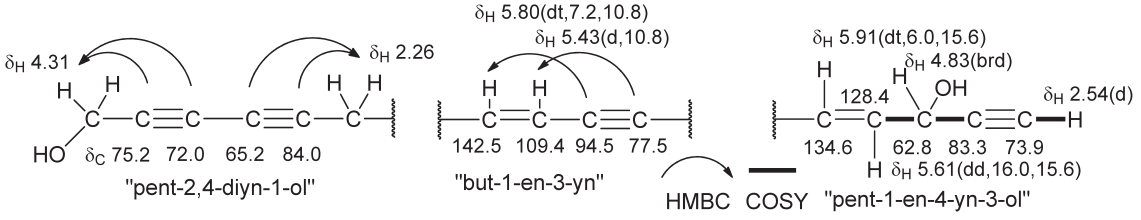

Fig. 4a. HMBC and COSY Correlations and Partial Structure of **6**

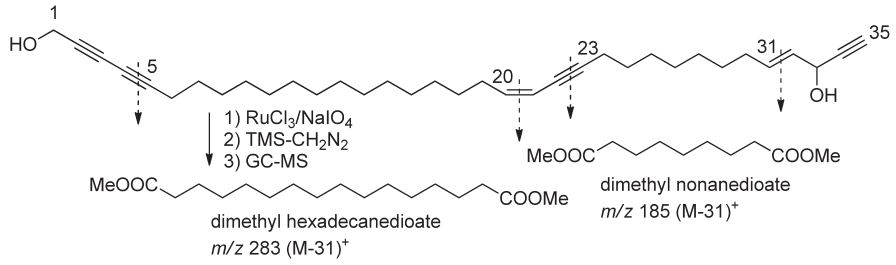

Fig. 4b. $RuCl_3$ Oxidation and Its Degradation Products of **6**

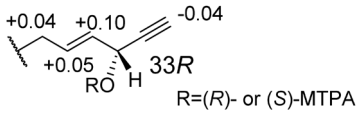

Fig. 4c. $\Delta\delta$ Values of (*R*)- and (*S*)-MTPA Esters of **6**

gave a molecular formula of $C_{35}H_{52}O_2$. ^{1}H- and ^{13}C-NMR and HSQC spectral data indicated the presence of one oxygenated methylene, one oxygenated methine, two di-substituted alkenes, three di-substituted alkynes, one terminal acetylene and 21 aliphatic methylenes. The HMBC spectrum of **6** yielded partial structures, terminal "pent-2,4-diyn-1-ol" and "pent-1-en-4-yn-3-ol" units and an internal "but-1-en-3-yn" unit (Fig. 4a). These data suggested that **6** is a 6-deoxy-type derivative of pellynol C (**9**; $C_{33}H_{48}O_3$). The coupling constants of the olefins (J=10.8, 15.6 Hz) indicated that their geometries were *Z* and *E*, respectively. The location of the internal "but-1-en-3-yn" unit was determined by the degradation products of **6** with $RuCl_3$ oxidation followed by GC-MS analysis. Mass spectral data identified two dimethyl ester derivatives, dimethyl hexadecanedioate ($C_{18}H_{34}O_4$) and dimethyl nonanedioate ($C_{11}H_{20}O_4$) indicating that the "but-1-en-3-yn" unit was located at C-20 (Fig. 4b). The absolute stereochemistry of **6** was also examined using a modified Mosher's method, with C-33 determined to have an absolute *R* configuration (Fig. 4c).

The absolute stereochemistry of the known compounds, pellynols A (**7**) and C (**9**) was also assessed using the modified Mosher's method yielding results identical to those of isopellynol A (**3**) and pellynol J (**4**) with the secondary alcohol at C-6 being a diastereomeric mixture (Fig. 5).

The ability of the isolated compounds to inhibit the proliferation of HeLa and K562 cells were examined. Pellynols J (**4**), A (**7**), and B (**8**), all of which have the same structural units "hexa-2,4-diyn-1,6-diol" and "pent-1-en-4-yn-3-ol," on both ends, showed significant activity, with almost equivalent IC_{50} values (Table 3). In contrast, strongylotriols A (**1**) and B (**2**) with structural unit "pent-1-en-4-yn-3-ol" on one-half, had nearly one-third the activity of pellynols J, A and B. Furthermore, the activities of isopellynol A (**3**) and pellynol L (**6**), with terminal "pent-2-en-4-yn-1-ol" and "pent-2,4-diyn-1-ol" units, respectively, were decreased slightly. These results were consistent with earlier findings.[6,11]

In summary, six new polyacetylenic alcohols, strongylotriols A (**1**) and B (**2**); pellynols J (**4**), K (**5**), and L (**6**); and isopellynol A (**3**), were isolated from the marine sponges *Petrosia* sp. and *Halichondria* sp. Compounds **4**, **7**, and **8** were isolated from both sponges, despite the difference in appearance of these marine sponges. Furthermore, other abundant compounds differed in these species, in that meroditerpenoids such as strongylophorines were found only in *Petrosia* sp.,[12] while polyacetylenic acids such as pellynic acids were found only in *Halichondria* sp.[8] (Supplementary material). The isolated strongylotriols (**1**, **2**) were optically pure, whereas the pellynols were diastereomeric mixtures at the C-6 position. The positions of the internal olefins differed in the pellynols, but their locations from the end terminal were identical to each other. Some symbionts may therefore biosynthsize these polyacetylenic compounds linking to two types of acetylene groups at this position.

Experimental

General Experimental Procedures Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 24°C. The NMR spectra were recorded on a Varian INOVA 600 operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$. ^{1}H- and ^{13}C-NMR chemical shifts is reported in ppm (δ) and referenced to tetramethylsilane (TMS) for ^{1}H (δ_H=0) and the solvent signal for ^{13}C ($CDCl_3$:, δ_C=77.2). ESI-TOF-MS were measured with a Bruker microTOF mass spectrometer. GC-MS was performed on Gas Chromatography Mass Spectrometer-QP2010 using INERT Cap5MS/Sil, 30 m×0.25 mm i.d., GL Science); column temp. 100–280°C, injection temp., 300°C, interface temp., 250°C. Chromatographic separations were carried out using Sephadex LH-20 (GE Healthcare), silica gel (Merck Silica gel 60), and reversed phase-HPLC (Cosmosil 5C22AR-II or 5C18AR-II, 250×4.6 mm i.d., Nakalai Tesque, Develosil C30 UG-3, 250×4.6 mm i.d., Nomura Chemicals) with a Jasco PU2089 gradient pump and PU2075

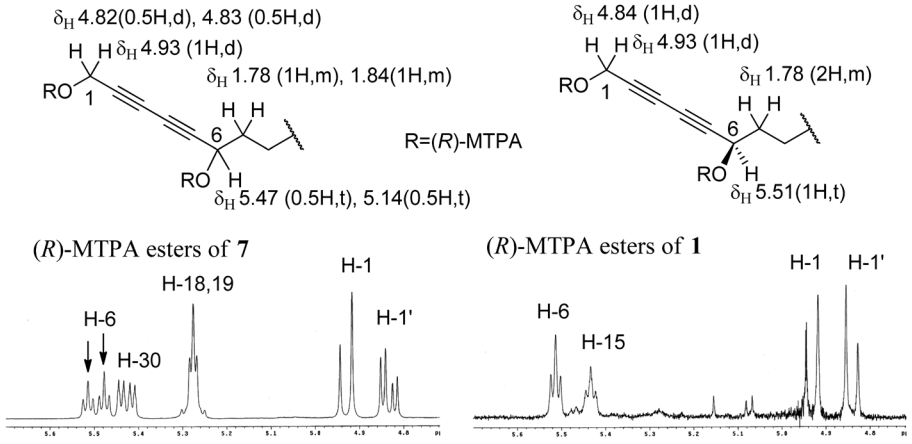

Fig. 5. ^{1}H-NMR Spectra of (*R*)-MTPA Esters of **7** and **1**

Table 3. IC_{50} Values (μM) of Compounds **1**–**9** on the Proliferation of HeLa and K562 Cells

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HeLa	18.1	14.3	4.13	0.62	nt	0.94	0.49	0.52	0.95
K562	11.5	11.3	1.95	0.55	nt	1.71	0.46	0.55	0.83

UV/Vis detector. Thin layer chromatography (TLC) analysis were carried out using Merck precoated TLC plates Silica gel $60F_{254}$ and RP-$18F_{254s}$.

Animal Material *Petrosia* sp. and *Halichondria* sp. were collected by hand at a depth of 10–15 m off the shore of Iriomote Island, Okinawa Prefecture, Japan, in Nobember 2009. Voucher specimens were deposited at the Graduate School of Pharmaceutical Sciences, Kyushu University under reg. no. 091120-36 for *Petrosia* sp., and no. 091120-35 for *Halichondria* sp.

Extraction and Isolation *Petrosia* sp. (wet weight 405.89 g) was homogenized and extracted with EtOH (3×0.5 L) and filtrated. The extract was evaporated *in vacuo*, and the resulting aqueous suspension was diluted H_2O (0.5 L) and extracted with Et_2O (3×0.2 L) and *n*-BuOH (3×0.2 L). The organic layers were evaporated to give an Et_2O extract (8.71 g) and *n*-BuOH extract (0.99 g). The part of Et_2O extract (4.99 g) was subjected to Sephadex LH-20 column chromatography with $CHCl_3$ to give twelve fractions, and fraction 8 (33.8 mg) was applied to reversed phase HPLC (Cosmosil 5C22 AR-II) with 91% MeOH/H_2O to give compounds **1** (2.0 mg), **2** (1.0 mg), **4** (2.4 mg), **7** (6.7 mg), and **8** (2.5 mg), respectively. *Halichondria* sp. (wet weight 100.0 g) was homogenized and extracted with EtOH (3×1.0 L) and filtrated. The extract was evaporated *in vacuo*, to give EtOH extract (5.0 g). The EtOH extract was partitioned between Et_2O, *n*-BuOH and H_2O to give an Et_2O extract (2.73 g) and *n*-BuOH extract (1.06 g). The part of Et_2O extract (1.43 g) was subjected to silica gel column chromatography using *n*-hexane–EtOAc (1 : 9–0 : 1) to give seven fractions. Fraction 3 (92.9 mg) was subjected to silica gel column followed by reversed phase HPLC (Cosmosil 5C18 AR-II) with 95% MeOH/H_2O to give compound **6** (5.2 mg), and part of fraction 5 (165.8 mg) was applied to reversed phase HPLC (Develosil C30 UG-3) with 100% MeOH to give compounds **3** (2.8 mg), **4** (7.2 mg), **7** (71.2 mg), **8** (30.5 mg), and **9** (13.7 mg), respectively.

$RuCl_3$–$NaIO_4$ Oxidation To a solution of each sample (*ca.* 1.0 mg) in CCl_4 (1.0 mL) was added $NaIO_4$ (10 eq), $RuCl_3$ (0.04 mg), CH_3CN (1.0 mL), and H_2O (1.0 mL). The solution was stirred vigorously for 1 h, then 1 M HCl (2.0 mL) was added and extracted with Et_2O. Et_2O layer was analyzed ESI-TOF-MS (negative ion) directly or methylated with TMS–CH_2N_2 in 20% MeOH/benzene followed by GC-MS analysis.

Preparation of the (*R*)- or (*S*)-MTPA Esters To a solution of each **6** (1.0 mg, 2 μmol) in dry CH_2Cl_2 (0.2 mL) stirring at room temperature was added (*R*)- or (*S*)-MTPA (7.4 mg, 32.0 μmol), *N*,*N*′-dicyclohexyl carbodiimide (DCC) (6.5 mg, 32.0 μmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The solution was stirred at room temperature for 8 h and filtered. The filtrate was dried with N_2 and subjected to silica gel column with *n*-hexane–EtOAc (9 : 1) to afford (*R*)-MTPA ester (1.9 mg) and (*S*)-MTPA ester (2.3 mg).

Strongylotriol A (**1**) Amorphous solid; $[\alpha]_D$ −8.3 (*c*=0.012, $CHCl_3$); ESI-TOF-MS *m*/*z*: 411.2900 $[M+Na]^+$ (Calcd for $C_{25}H_{40}NaO_3$, 411.2870); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: decanedeoic acid: *m*/*z*: 201.1039 $[M-H]^-$ (Calcd for $C_{10}H_{17}O_4$, 201.1132).

(*R*)-MTPA ester of **1**, ESI-TOF-MS *m*/*z*: 1059.4119 $[M+Na]^+$ (Calcd for $C_{55}H_{61}F_9NaO_9$, 1059.4064); ^{1}H-NMR ($CDCl_3$) δ: 0.878 (3H, t, H-25), 1.260 (2H, m, H-12), 1.280 (2H, m, H-8), 1.315 (2H, m, H-20), 1.390 (2H, m, H-13), 1.405 (2H, m, H-19), 1.770 (2H, t, H-7), 1.775 (2H, m, H-14), 2.145 (2H, t, H-18), 4.891 (1H, d, H-1), 4.981 (1H, d, H-1′), 5.484 (1H, t, H-15), 5.563 (1H, t, H-6), 3.544 (3H, s, OMe), 3.568 (6H, s, OMe), 7.3–7.7 (15H, phenyl); (*S*)-MTPA ester of **1**, ESI-TOF-MS *m*/*z*: 1059.4158 $[M+Na]^+$ (Calcd for $C_{55}H_{61}F_9NaO_9$, 1059.4064); ^{1}H-NMR ($CDCl_3$) δ: 0.878 (3H, t, H-25), 1.210 (2H, m, H-12), 1.400 (2H, m, H-8), 1.340 (2H, m, H-20), 1.290 (2H, m, H-13), 1.470 (2H, m, H-19), 1.830 (2H, t, H-7), 1.720 (2H, m, H-14), 2.175 (2H, t, H-18), 4.870 (1H, d, H-1), 4.987 (1H, d, H-1′), 5.510 (1H, t, H-15), 5.528 (1H, t, H-6), 3.540 (3H, s, OMe), 3.568 (6H, s, OMe), 7.3–7.7 (15H, phenyl).

Strongylotriol B (**2**) Amorphous solid; $[\alpha]_D$ −8.3 (c=0.013, $CHCl_3$); ESI-TOF-MS m/z: 425.3009 $[M+Na]^+$ (Calcd for $C_{26}H_{42}NaO_3$, 425.3026); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: decanedeoic acid: m/z: 201.1035 $[M-H]^-$ (Calcd for $C_{10}H_{17}O_4$, 201.1132).

(*R*)-MTPA ester of **2**, ESI-TOF-MS m/z: 1073.4332 $[M+Na]^+$ (Calcd for $C_{56}H_{63}F_9NaO_9$, 1073.4221); ^{1}H-NMR ($CDCl_3$) δ: 0.853 (6H, d, H-25, 26), 1.260 (2H, m, H-12), 1.280 (2H, m, H-8), 1.315 (2H, m, H-20), 1.390 (2H, m, H-13), 1.405 (2H, m, H-19), 1.770 (2H, t, H-7), 1.775 (2H, m, H-14), 2.145 (2H, t, H-18), 4.891 (1H, d, H-1), 4.981 (1H, d, H-1′), 5.484 (1H, t, H-15), 5.563 (1H, t, H-6), 3.544 (3H, s, OMe), 3.568 (6H, s, OMe), 7.3–7.7 (15H, phenyl); (*S*)-MTPA ester of **2**, ESI-TOF-MS m/z: 1073.4302 $[M+Na]^+$ (Calcd for $C_{56}H_{63}F_9NaO_9$, 1073.4221); ^{1}H-NMR ($CDCl_3$) δ: 0.850 (3H, t, H-25), 1.210 (2H, m, H-12), 1.400 (2H, m, H-8), 1.335 (2H, m, H-20), 1.280 (2H, m, H-13), 1.465 (2H, m, H-19), 1.830 (2H, t, H-7), 1.730 (2H, m, H-14), 2.180 (2H, t, H-18), 4.880 (1H, d, H-1), 4.982 (1H, d, H-1′), 5.517 (1H, t, H-15), 5.527 (1H, t, H-6), 3.536 (3H, s, OMe), 3.565 (3H, s, OMe), 3.586 (3H, s, OMe) 7.3–7.7 (15H, phenyl).

Isopellynol A (**3**) Amorphous solid; $[\alpha]_D$ −15.0 (c=0.022, $CHCl_3$); ESI-TOF-MS m/z: 519.3854 $[M+Na]^+$ (Calcd for $C_{33}H_{52}NaO_3$, 519.3817); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1305 $[M-H]^-$ (Calcd for $C_{11}H_{19}O_4$, 215.1289), tridecanedioic acid: m/z: 243.1564 $[M-H]^-$ (Calcd for $C_{13}H_{23}O_4$, 243.1602).

(*R*)-MTPA ester of **3**, ESI-TOF-MS m/z: 1167.5015 $[M+Na]^+$ (Calcd for $C_{63}H_{73}F_9NaO_9$, 1167.5003); ^{1}H-NMR ($CDCl_3$) δ: 1.765 (2H, m, H-28), 1.779 (1H, m, H-7), 1.839 (1H, m, H-7), 1.998–2.023 (4H, brd, H-17, 20), 3.220 (1H, s, H-33), 4.872–4.983 (2H, ddd, H-1), 5.328 (2H, brdt, H-18, 19), 5.529 (0.5H, t, H-6), 5.565 (0.5H, t, H-6), 5.611 (1H, d, H-31), 5.802 (1H, dd, H-30), 5.925 (1H, q, H-29), 3.524 (3H, s, OMe), 3.532 (3H, s, OMe), 3.553 (3H, s, OMe), 7.380–7.500 (15H, phenyl); (*S*)-MTPA ester of **3**, ESI-TOF-MS m/z: 1167.5007 $[M+Na]^+$ (Calcd for $C_{63}H_{73}F_9NaO_9$, 1167.5003); ^{1}H-NMR ($CDCl_3$) δ: 1.721 (2H, m, H-28), 1.778 (1H, m, H-7), 1.839 (1H, m, H-7), 1.994 (4H, brd, H-17, 20), 3.227 (1H, s, H-33), 4.873–4.985 (2H, ddd, H-1), 5.330 (2H, brdt, H-18,19), 5.529 (0.5H, t, H-6), 5.566 (0.5H, t, H-6), 5.642 (1H, d, H-31), 5.944 (1H, dd, H-30), 5.971 (1H, q, H-29), 3.524 (3H, s, OMe), 3.533 (3H, s, OMe), 3.553 (3H, s, OMe), 7.380–7.500 (15H, phenyl).

Pellynol J (**4**) Amorphous solid; $[\alpha]_D$ −9.6 (c=0.016, $CHCl_3$); ESI-TOF-MS m/z: 491.3541 $[M+Na]^+$ (Calcd for $C_{31}H_{48}NaO_3$, 491.3503); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1305 $[M-H]^-$ (Calcd for $C_{11}H_{19}O_4$, 215.1289).

(*R*)-MTPA ester of **4**, ESI-TOF-MS m/z: 1139.4659 $[M+Na]^+$ (Calcd for $C_{61}H_{69}F_9NaO_9$, 1139.4690); ^{1}H-NMR ($CDCl_3$) δ: 1.798 (2H, m, H-26), 1.805 (1H, m, H-7), 1.857 (1H, m, H-7), 2.012–2.034 (4H, brd, H-15, 18), 2.620 (1H, s, H-31), 4.884–4.999 (2H, ddd, H-1), 5.343 (2H, brdt, H-16, 17), 5.495 (1H, dd, H-28), 5.546 (0.5H, t, H-6), 5.583 (0.5H, t, H-6), 6.006 (1H, m, H-27), 6.030 (1H, d, H-29), 3.540 (3H, s, OMe), 3.570 (3H, s, OMe), 3.587 (3H, s, OMe), 7.392–7.516 (15H, phenyl); (*S*)-MTPA ester of **4**, ESI-TOF-MS m/z: 1139.4697 $[M+Na]^+$ (Calcd for $C_{61}H_{69}F_9NaO_9$, 1139.4690); ^{1}H-NMR ($CDCl_3$) δ: 1.793 (1H, m, H-7), 1.856 (1H, m, H-7), 1.843 (2H, m, H-26), 2.007–2.074 (4H, brd, H-15,18), 2.579 (1H, s, H-31), 4.884–4.998 (2H, ddd, H-1), 5.343 (2H, brdt, H-16, 17), 5.546 (0.5H, t, H-6), 5.582 (0.5H, t, H-6), 5.600 (1H, dd, H-28), 6.009 (1H, d, H-29), 6.067 (1H, m, H-27), 3.541 (3H, s, OMe), 3.547 (3H, s, OMe), 3.568 (3H, s, OMe), 7.398–7.519 (15H, phenyl).

Pellynol K (**5**) Amorphous solid; $[\alpha]_D$ −6.3 (c=0.027, $CHCl_3$); ESI-TOF-MS m/z: 533.3950 $[M+Na]^+$ (Calcd for $C_{34}H_{54}NaO_3$, 533.3971); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1281 $[M-H]^-$ (Calcd for $C_{11}H_{19}O_4$, 215.1289), tetradecanedioic acid: m/z: 257.1782 $[M-H]^-$ (Calcd for $C_{14}H_{25}O_4$, 257.1758) .

Pellynol L (**6**) Amorphous solid; $[\alpha]_D$ −2.6 (c=0.019, $CHCl_3$); ESI-TOF-MS m/z: 527.3964 $[M+Na]^+$ (Calcd for $C_{35}H_{52}NaO_2$, 527.3865); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: dimethyl nonanedioate ($C_{11}H_{20}O_4$), t_R=10.04 min, m/z: 185 $(M-31)^+$, 152, 111, 83, 55 (base peak), dimethyl hexadecanedioate ($C_{18}H_{34}O_4$), t_R=17.66 min, m/z: 283 $(M-31)^+$, 241, 112, 98 (base peak).

(*R*)-MTPA ester of **6**, ESI-TOF-MS m/z: 959.4668 $[M+Na]^+$ (Calcd for $C_{55}H_{66}F_9NaO_6$, 959.4656); ^{1}H-NMR ($CDCl_3$) δ: 1.798 (2H, m, H-30), 2.276 (2H, q, H-19), 2.343 (2H, t, H-24), 2.556 (1H, s, H-35), 4.886 (2H, d, H-1), 5.433 (1H, d, H-21), 5.497 (1H, dd, H-32), 5.796 (1H, q, H-20), 6.015 (1H, m, H-31), 6.036 (1H, d, H-33), 3.552 (3H, s, OMe), 3.569 (3H, s, OMe), 3.587 (3H, s, OMe), 7.394–7.516 (15H, phenyl); (*S*)-MTPA ester of **6**, ESI-TOF-MS m/z: 959.4727 $[M+Na]^+$ (Calcd for $C_{55}H_{66}F_9NaO_6$, 959.4656)); ^{1}H-NMR ($CDCl_3$) δ: 1.778 (2H, m, H-30), 2.306 (2H, q, H-19), 2.373 (2H, t, H-24), 2.516 (1H, s, H-35), 4.906 (2H, d, H-1), 5.453 (1H, d, H-21), 5.597 (1H, dd, H-32), 5.816 (1H, q, H-20), 6.065 (1H, m, H-31), 6.006 (1H, d, H-33), 3.550 (3H, s, OMe), 3.569 (3H, s, OMe), 3.587 (3H, s, OMe), 7.394–7.516 (15H, phenyl).

Pellynol A (**7**) Amorphous solid; $[\alpha]_D$ −5.0 (c=0.024, $CHCl_3$), ref. 9 $[\alpha]_D$ −8.5 (c=1.0, $CHCl_3$); ESI-TOF-MS m/z: 519.3864 $[M+Na]^+$ (Calcd for $C_{33}H_{52}NaO_3$, 519.3817); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1281 $[M-H]^-$ (Calcd for $C_{11}H_{19}O_4$, 215.1289), tridecanedioic acid: m/z: 243.1585 $[M-H]^-$ (Calcd for $C_{13}H_{23}O_4$, 243.1602).

(*R*)-MTPA ester of **7**, ESI-TOF-MS m/z:1167.4934 $[M+Na]^+$ (Calcd for $C_{63}H_{73}F_9NaO_9$, 1167.5003); ^{1}H-NMR ($CDCl_3$) δ: 1.727 (1H, m, H-7), 1.787 (1H, m, H-7), 1.925 (2H, m, H-28), 1.966–1.978 (4H, brd, H-17,20), 2.550 (1H, s, H-33), 4.814–4.944 (2H, ddd, H-1), 5.276 (2H, brdt, H-18,19), 5.427 (1H, dd, H-30), 5.478 (0.5H, t, H-6), 5.514 (0.5H, t, H-6), 5.938 (1H, m, H-29), 5.957 (1H, d, H-31), 3.472 (3H, s, OMe), 3.500 (3H, s, OMe), 3.519 (3H, s, OMe), 7.322–7.335 (15H, phenyl); (*S*)-MTPA ester of **2**, ESI-TOF-MS m/z: 1167.4978 $[M+Na]^+$ (Calcd for $C_{63}H_{73}F_9NaO_9$, 1167.5003); ^{1}H-NMR ($CDCl_3$) δ: 1.726 (1H, m, H-7), 1.787 (1H, m, H-7), 1.966 (2H, m, H-28), 1.966–1.978 (4H, brd, H-17,20), 2.512 (1H, s, H-33), 4.894–4.944 (2H, ddd, H-1), 5.277 (2H, brdt, H-18,19), 5.528 (1H, dd, H-30), 5.476 (0.5H, t, H-6), 5.514 (0.5H, t, H-6), 5.999 (1H, m, H-29), 5.767 (1H, d, H-31), 3.473 (3H, s, OMe), 3.502 (3H, s, OMe), 3.519 (3H, s, OMe), 7.322–7.335 (15H, phenyl).

Pellynol B (**8**) Amorphous solid; $[\alpha]_D$ −8.8 (c=0.019, $CHCl_3$), ref. 9 $[\alpha]_D$ −7.6 (c=0.28, $CHCl_3$) ; ESI-TOF-MS m/z: 505.3771 $[M+Na]^+$ (Calcd for $C_{32}H_{50}NaO_3$, 505.3660); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1281 $[M-H]^-$ (Calcd for $C_{11}H_{19}O_4$, 215.1289), dodecanedioic acid: m/z: 229.1393 $[M-H]^-$ (Calcd for $C_{12}H_{21}O_4$, 229.1445).

Pellynol C (**9**) Amorphous solid; $[\alpha]_D$ −8.7 (c=0.031, $CHCl_3$), ref. 9 $[\alpha]_D$ −11.2 (c=2.38, $CHCl_3$); ESI-TOF-MS m/z: 515.3496 $[M+Na]^+$ (Calcd for $C_{33}H_{48}NaO_3$, 515.3503); ^{1}H- and ^{13}C-NMR see Tables 1 and 2. Oxidative product: dimethyl nonanedioate ($C_{11}H_{20}O_4$), t_R=10.08 min, m/z: 185 $(M-31)^+$, 152, 111, 83, 55 (base peak), dimethyl tridecanedioate ($C_{15}H_{28}O_4$), t_R=14.74 min, m/z: 241 $(M-31)^+$, 199, 167, 112, 98 (base peak).

(*R*)-MTPA ester of **9**, ESI-TOF-MS m/z: 1163.5035 $[M+Na]^+$ (Calcd for $C_{63}H_{69}F_9NaO_9$, 1163.4690); ^{1}H-NMR ($CDCl_3$) δ: 1.797 (2H, m, H-28), 1.806 (1H, m, H-7), 1.857 (1H, m, H-7), 2.274 (2H, q, H-17), 2.342 (2H, t, H-22), 2.557 (1H, s, H-33), 4.886–4.998 (2H, ddd, H-1), 5.432 (1H, d, H-19), 5.498 (1H, dd, H-30), 5.548 (0.5H, t, H-6), 5.573 (0.5H, t, H-6), 5.794 (1H, q, H-18), 6.013 (1H, m, H-29), 6.033 (1H, d, H-31), 3.542 (3H, s, OMe), 3.568 (3H, s, OMe), 3.577 (3H, s, OMe), 7.392–7.516 (15H, phenyl); (*S*)-MTPA ester of **9**, ESI-TOF-MS m/z: 1163.4941 $[M+Na]^+$ (Calcd for $C_{63}H_{69}F_9NaO_9$, 1163.4690); ^{1}H-NMR ($CDCl_3$) δ: 1.837 (2H, m, H-28), 1.793 (1H, m, H-7), 1.857 (1H, m, H-7), 2.274 (2H, q, H-17), 2.343 (2H, t, H-22), 2.517 (1H, s, H-33), 4.886–4.997 (2H, ddd, H-1), 5.432 (1H, d, H-19), 5.597 (1H, dd, H-30), 5.545 (0.5H, t, H-6), 5.573 (0.5H, t, H-6), 5.795 (1H, q, H-18), 6.064 (1H, m, H-29), 5.853 (1H, d, H-31), 3.542 (3H, s, OMe), 3.568 (3H, s, OMe), 3.577 (3H, s, OMe), 7.392–7.516 (15H, phenyl).

Cell Culture and Cell Proliferation Assay Human malignant epithelial cells (HeLa) were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) kept in an incubator at 37°C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan). Human immortalized myelogenous leukemia cells (K562) were cultured in RPMI-1640 medium supplemented with 10% FBS kept in an incubator at 37°C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. Cell viability was determined by a Cell-Titer 96 AQueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI, U.S.A.) according to the manufacturer's protocol. HeLa and K562 cells (3×10^4 cells/well) were seeded in 96 well plates and incubated for 24 h, subsequently grown with compounds for additional 48 h, and then cell proliferation assay was performed.

Acknowledgments We wish to thank the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for the financial support to Mr. Gabriel Adeyemi Francis. This work was supported by JSPS KAKENHI Grant Number 24590139.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

1) Jung H. J., Im S. K., Bae H. B., "Biomaterials from Aquatic and Terrestial Organisms, Chap. 12, Bioactive Polyacetylenic Compounds from Marine Sponges," ed. by Fingerman M., Nagabhushanam R., Science Publishers, New Hampshire, 2006.
2) Bohlmann F., Lam J., Breteler H., Arnason T., Hansen L., "Chemistry and Biology of Naturally Occurring Acetylenes and Related Compounds," Elsevier, Amsterdam, 1998.
3) Bohlmann F., Burkhardt T., Zdero C., "Naturally Occuring Acetylenes," Academic Press, London and New York, 1973.
4) Perry N. B., Span E. M., Zidorn C., *Tetrahedron Lett.*, **42**, 4325–4328 (2001).
5) Allen E. H., Thomas C. A., *Phytochemistry*, **10**, 1579–1582 (1971).
6) Shirouzu T., Watari K., Ono M., Koizumi K., Saiki I., Tanaka C., van Soest R. W. M., Miyamoto T., *J. Nat. Prod.*, **76**, 1337–1342 (2013).
7) The genus of *Petrosia* was classified to *Strongylophora* and *Halichondria* was classified to *Pellina* until 2002. De Weerdt W. H., "Family Chalinidae Gary. 1867 in Systema Porifera, a Guide to the Classification of Sponges," ed. by Hooper J. N. A., Van Soest R. W. M., Kluwer Academic/Plenum Publishers, New York, 2002.
8) Fu X., Abbas S. A., Schmitz F. J., Vidavsky I., Gross M. L., Laney M., Schatzman R. C., Cabuslay R. D., *Tetrahedron*, **53**, 799–814 (1997).
9) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092–4096 (1991).
10) Fusetani N., Yasumuro K., Matsunaga S., Hirota H., *Tetrahedron Lett.*, **30**, 6891–6894 (1989).
11) Zhou G.-X., Molinski T. F., *Mar. Drugs*, **1**, 46–53 (2003).
12) Braekman J. C., Daloze D., Hulot G., Tursch B., Declercq J. P., Germain G., Van Meerssche M., *Bull. Soc. Chim. Belg.*, **87**, 917–926 (1978).