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(−)-Duryne and Its Homologues, Cytotoxic Acetylenes from a Marine Sponge *Petrosia* sp.

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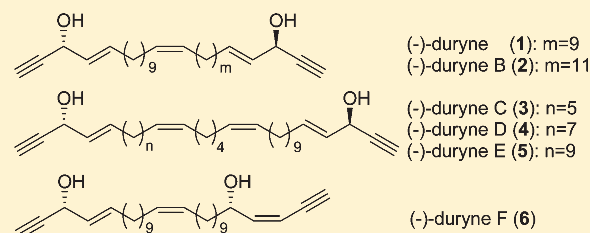
S Supporting Information

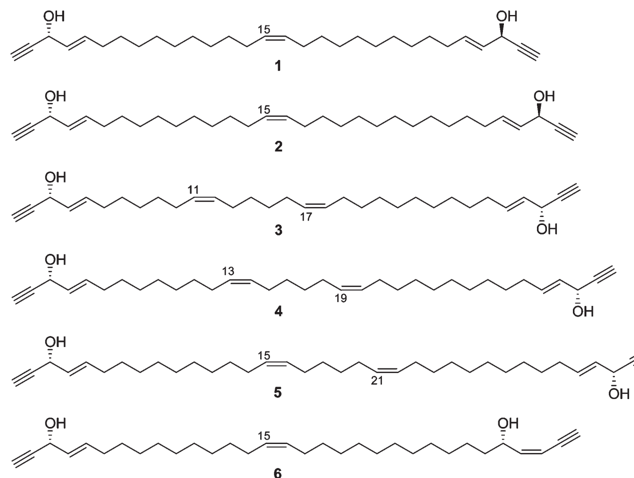
ABSTRACT: Six linear acetylenes, (−)-duryne (**1**) and (−)-durynes B−F (**2**−**6**), were isolated from the marine sponge *Petrosia* sp. Their structures were elucidated by NMR and tandem FABMS analyses. The positions of the olefinic bonds were confirmed by ozonolysis experiments, and the absolute configurations were determined by the modified Mosher's method. Compound **1** was found to be the enantiomer of duryne, a previously reported sponge metabolite. Compounds **1**−**6** show cytotoxicity against HeLa cells with IC_{50} values between 0.08 and 0.50 μM.



Linear acetylenes are prominent constituents of marine sponges of the order Haplosclerida.[1] A classification of this class of metabolites from a chemical perspective has been reported.[2] There are two distinct groups of acetylenes with both termini functionalized. One group contains compounds related to the petroformynes, which possess chains of C_{46} or longer with a carbinol-interrupted diyne in the middle of the chain.[3−7] Compounds in the other group are shorter, with chains of around C_{30}, and do not contain the carbinol-interrupted diyne moiety.[8−10] Even though their modes of action have not been elucidated, compounds in these two groups are reported to exhibit potent cytotoxic activity. In the course of our screening for cytotoxic activity against HeLa cells, the extract of a marine sponge *Petrosia* sp. exhibited significant activity. Bioassay-guided fractionation of the extract afforded six related compounds, (−)-duryne (**1**) and its homologues (−)-durynes B−F (**2**−**6**). This is the first isolation of the (−)-duryne as a natural product. Duryne, the (+)-enantiomer, was isolated from the marine sponge *Cribrochalina dura*,[8] the taxonomy of which was later suggested to be *Petrosia*,[2] and its absolute configuration was determined by synthesis of both enantiomers.[11]

RESULTS AND DISCUSSION

The sponge *Petrosia* sp. was collected by a remotely operated vehicle at Miyako sea-knoll. The sponge specimen was extracted with EtOH and MeOH/$CHCl_3$ (1:1), and the combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was further partitioned between 90% MeOH and *n*-hexane. Bioassay-guided fractionation of the 90% MeOH fraction by ODS column chromatography, silica gel column chromatography, and reversed-phase HPLC afforded six cytotoxic compounds (**1**−**6**).



Compound **1**, which analyzed for $C_{30}H_{48}O_2$ by HRESIMS, exhibited ^{1}H NMR signals characteristic of the terminal structure of sponge-derived acetylenes [δ 2.57 (d, J = 2.1 Hz; H-1, H-30), 4.84 (brd, J = 6.4 Hz; H-3, H-28), 5.61 (dd, J = 15.1, 6.4 Hz; H-4, H-27), and 5.92 (dt, J = 15.1, 6.9 Hz; H-5, H-26)]. A literature search showed that the ^{1}H and ^{13}C NMR data of **1** (Table 1) were identical with those of duryne. However, an opposite sign with a similar magnitude for the specific rotation ($[\alpha]_D$ = −19) showed that **1** was the enantiomer of the previously reported duryne ($[\alpha]_D$ = +29).[8,11] The modified Mosher's analysis of **1** supported this assignment (Figure 1).[12] Therefore, **1** was firmly established to be (−)-duryne.[13]

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Table 1. ^{1}H and ^{13}C NMR Data ($CDCl_3$) for (−)-Duryne (1), (−)-Duryne B (2), and (−)-Duryne F (6)

(−)-duryne (1)			(−)-duryne B (2)			(−)-duryne F (6)		
position	δ_C[a]	δ_H (J in Hz)	position	δ_C[b]	δ_H (J in Hz)	position	δ_C[b]	δ_H (J in Hz)
1, 30	74.1	2.57, d (2.1)	1	73.9	2.57, d (2.1)	1	74.1	2.57, d (2.1)
2, 29	83.5		2	83.4		2	83.4	
3, 28	63.0	4.84, d (6.4)	3	62.8	4.84, brd (6.2)	3	62.8	4.84, brd (6.2)
4, 27	128.3	5.61, dd (15.1, 6.4)	4	128.3	5.61, dd (15.3, 6.2)	4	128.1	5.62, dd (15.1, 6.2)
5, 26	134.8	5.92, dt (15.1, 6.9)	5	134.5	5.92, dt (15.3, 6.9)	5	134.4	5.92, dt (15.1, 6.9)
6, 25	32.1	2.07, m	6	31.9	2.07, m	6	31.8	2.07, m
7, 24	30.0[c]	1.40, m	7	28.7	1.40, m	7	28.9	1.40, m
8−12, 19−23	29.4−29.8[c]	1.27−1.30, m	8−13	28.7−29.7	1.27−1.36	8−13	28.7−30.0	1.27−1.34
13, 18	29.0[c]	1.32, m	14	27.4	2.02, m	14	27.3	2.01, m
14, 17	27.4	2.02, m	15	129.9	5.35, m	15	129.9	5.36, m
15, 16	129.9	5.35, m	16	129.9	5.35, m	16	129.9	5.36, m
			17	27.4	2.02, m	17	27.3	2.01, m
			18−25	28.7−29.7	1.27−1.36	18−26	28.7−30.0	1.27−1.34
			26	28.7	1.40, m	27α	36.3	1.63, m
			27	31.9	2.07, m	27β		1.52, m
			28	134.5	5.92, dt (15.3, 6.9)	28	70.0	4.68, m
			29	128.3	5.61, dd (15.3, 6.2)	29	147.5	6.00, dd (11.0, 8.9)
			30	62.8	4.84, brd (6.2)	30	108.7	5.54, dd (11.0, 2.5)
			31	83.4		31	82.4	
			32	73.9	2.57, d (2.1)	32	79.5	3.14, d (2.5)

[a] ^{13}C NMR spectrum was measured at 150 MHz. [b] Chemical shifts were assigned from the HSQC data. [c] Assignments with the same superscript in the same column may be interchanged.

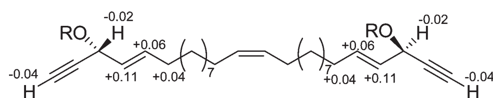


Figure 1. Modified Mosher's analysis for **1**. $\Delta\delta$ ($\delta_S - \delta_R$) values are shown.

(−)-Duryne B (**2**) had the molecular formula $C_{32}H_{52}O_2$, as assigned by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** were identical with those of **1** except for the integration of the methylene region (Table 1), suggesting that they differ only in the length of the aliphatic chains. The position of the *Z*-olefin in the middle of the chain was assigned by tandem FABMS to be between C-15 and C-16 (Figure 2). The geometry of the Δ^{15}-double bond was assigned by the chemical shifts of 27.4 ppm for C-14 and C-17.[14] The positions of the double bonds were confirmed by ozonolysis with oxidative workup, which gave FABMS ions at m/z 215 and 243 $[M - H]^-$, consistent with the formation of undecanedioic acid and tridecanedioic acid, respectively (Scheme 1). The configurations of the two secondary alcohols were both determined to be *R* by the modified Mosher's method.[12]

(−)-Durynes C−E (**3**−**5**) had molecular formulas of $C_{32}H_{50}O_2$, $C_{34}H_{54}O_2$, and $C_{36}H_{58}O_2$, respectively, as assigned by HRESIMS. The ^{1}H NMR data of **3**−**5** were identical with those of **1**, except for doubled olefinic and allylic proton peaks representing the central double bonds and an increased methylene peak at δ 1.27−1.34, suggesting that **3**−**5** have longer aliphatic chains and one additional double bond in their chains (Table 2). The geometries of the olefins in the middle of the chain in **3**−**5** were all assigned as *Z* judging from the chemical shifts of the allylic carbons.[14] The locations of the two *Z*-olefins were assigned by tandem FABMS to be $\Delta^{11,17}$ for **3**, $\Delta^{13,17}$ for **4**, and $\Delta^{15,21}$ for **5** (Figure 2). These assignments were confirmed by the ozonolysis experiments (Scheme 1). Ozonolysis of **3**, **4**, and **5** followed by oxidative workup afforded common FABMS ion peaks at m/z 145 and 215, which suggested the formation of adipic acid and undecanedioic acid, respectively. Additional ions were observed at m/z 159 and 187 in the ozonolysis products of **3** and **4**, respectively, consistent with the formation of heptanedioic acid and nonanedioic acid. The absolute configurations of the secondary alcohols in the three compounds were all determined as *R* by the modified Mosher's method.[12]

HRESIMS showed that (−)-duryne F (**6**) was an isomer of **2**. The structure of one end was identical with those in the other compounds, as judged from the ^{1}H and ^{13}C NMR data (Table 1), whereas at the other end, an acetylenic proton (δ 3.14, J = 2.5 Hz, H-32) was coupled to a *Z*-olefinic proton (δ 5.54, J = 11.0, 2.5 Hz; H-30), which was then coupled to another olefinic proton (δ 6.00, J = 11.0, 8.9 Hz; H-29). This latter proton, H-29, was further coupled to an oxymethine proton (δ 4.68, H-28), indicating a rearranged terminus relative to the commonly observed motif. There was a degenerate olefinic signal integrating for 2H, which was assumed as a *Z*-olefin in the middle of the chain. The *Z*-geometry was again assigned by the chemical shifts of the allylic carbons.[14] Tandem FABMS data were not sufficient to locate the double bond, because it was not possible to distinguish the two termini by the MS data. Again we resorted an ozonolysis experiment that afforded a reaction mixture with FABMS ion peaks at m/z 215 and 273, consistent with the formation of undecanoic acid and 2-hydroxytetradecanoic acid, respectively, demonstrating the location of the double bond between C-15 and C-16 (Scheme 1). The

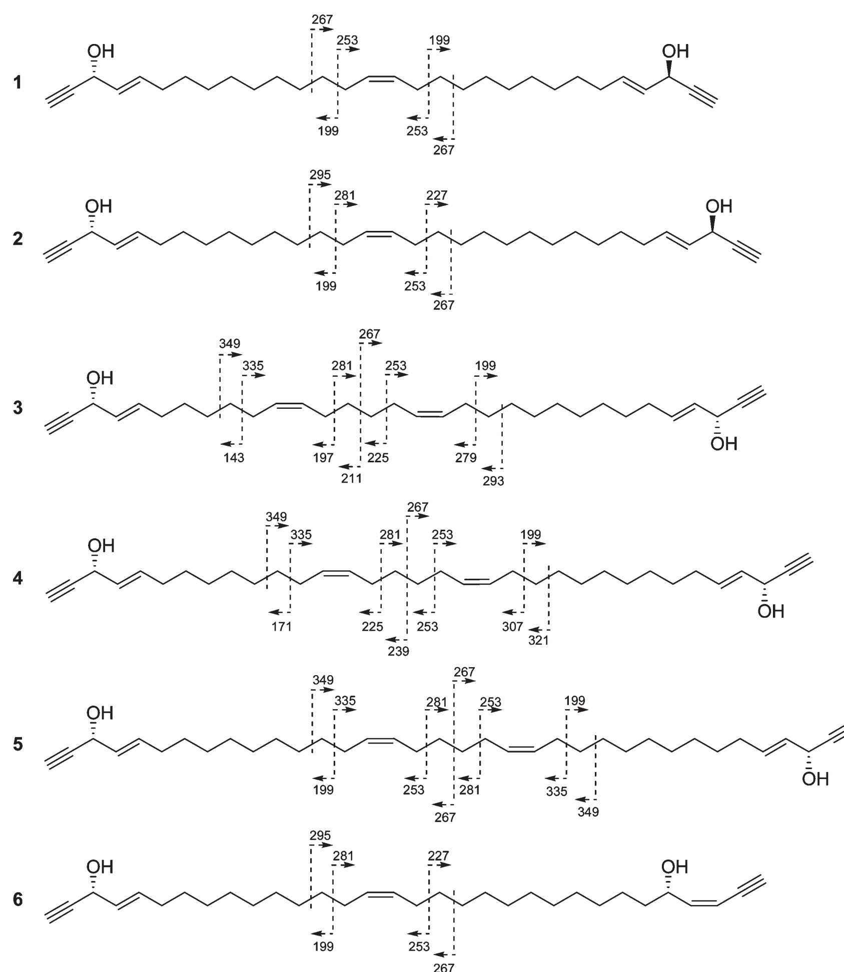


Figure 2. Key tandem FABMS fragmentations for **1**−**6** by using the $[M + Li]^+$ ion as the precursor.

absolute configuration was determined to be 3*R*, 28*S* by the modified Mosher's method.[12]

(−)-Duryne (**1**) and (−)-durynes B−F (**2**−**6**) show cytotoxic activity against HeLa cells with IC_{50} values of 0.50, 0.26, 0.26, 0.10, 0.08, and 0.34 μM, respectively. They are equipotent to duryne, whose reported IC_{50} values are 0.16−0.23 μM against P388, HCT-8, A549, and MCF7 cell lines.[8] The configuration of the allylic propargylic alcohol is variable among acetylenes from *Petrosia*. Only the *S*-isomers were reported for the petrosynols,[9,15] adociacetylenes,[16] duryne,[8] petroformynes,[17] neopetroformynes,[7] petrocortynes,[18,19] and the petroformynic acids.[20] They invariably exhibit a positive sign of optical rotation. However, the *R*-configuration was assigned for the corresponding carbinol carbons in the triangulynes, all of which showed a negative sign of optical rotation.[21] Curiously scalemic C_{30}-acetylenes were isolated from a Korean *Petrosia* sp.[22,23] It is interesting to note that (−)-duryne was equipotent to its enantiomer, suggesting that the cellular targets of these metabolites do not recognize the configuration of the two secondary alcohols. The more potent activity exhibited by the longer molecules is noticeable.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a Shimadzu Biospec 1600. IR spectra were measured with a JASCO FT-IR-230 spectrophotometer. NMR spectra were recorded on a JEOL alpha 600 NMR spectrometer at 300 K. Chemical shifts were referenced to solvent peaks: δ_H 7.27 and δ_C 77.2 for $CDCl_3$. ESI mass spectra were measured on a JEOL JMS-T100LC. FABMS and FABMSMS were measured on a JEOL JMS-700T. HPLC was carried out on a Shimadzu LC 20AT with a SCL-10Avp controller and a SPD-10Avp detector.

Animal Material. The sponge *Petrosia* sp. was collected at Miyako sea-knoll, southwestern Japan, 25°25.64′ N, 125°42.48′ E, at a depth of 415 m during a cruise of R/V *Natsushima* by using the remotely operated vehicle "Hyper-Dolphin" in October 2009. Sponge description: subglobular with an optically smooth surface; a single large oscule with a peripheral sleeve opened on top of the body; color beige; consistency hard and crumbly. The ectosomal skeleton is different from the choanosomal skeleton, consisting of a tangential unispicular network. The choanosomal skeleton consists of an isotropic network. Megascleres strongyles appeared in two size classes, with few oxeas. Microscleres were absent. The comparable species are *P. spheroida* Tanita, 1967 and *P. solusstrongyla* Hoshino, 1981 on the basis of its external morphology. *P. spheroida* is distinguished from the present species by its bean-shaped microstrongyles. *P. solusstrongyla* has strongyles only, and the form and size of strongyles are variable. The habitat of the present species is also different from the two species. *P. spheroida* is reported from the shallow water of the southwestern region of the Sea of Japan, and *P. solusstrongyla* is reported from the subtidal zone to a depth of 15 m in Kagoshima. The specimen used for the identification (NSMT-Po1954) was deposited at National Museum of Nature and Science, Tokyo.

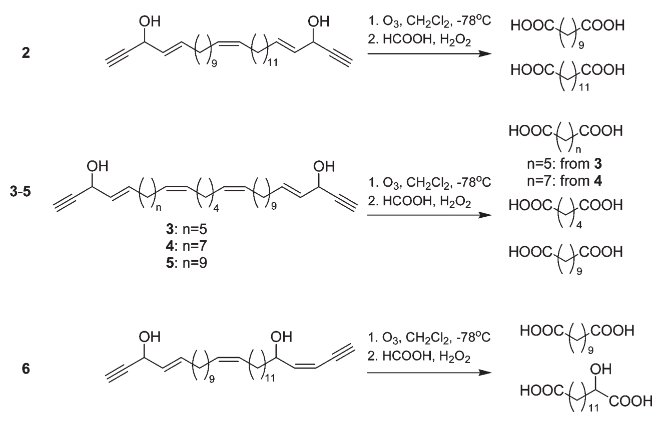
Extraction and Isolation. The sponge was frozen after collection and kept frozen until extraction. The sponge (130 g, wet weight) was

Table 2. ^1H and ^{13}C NMR Data ($CDCl_3$) for (−)-Duryne C (3), (−)-Duryne D (4), and (−)-Duryne E (5)

(−)-duryne C (3)			(−)-duryne D (4)			(−)-duryne E (5)		
position	δ_C [b]	δ_H (J in Hz)	position	δ_C [a]	δ_H (J in Hz)	position	δ_C [b]	δ_H (J in Hz)
1	73.9	2.57, d (2.1)	1	74.1	2.57, d (2.1)	1, 36	74.1	2.57, d (2.2)
2	83.3		2	83.6		2, 35	83.2	
3	62.8	4.84, brd (6.2)	3	62.9	4.84, brd (6.2)	3, 34	62.8	4.84, brd (6.2)
4	128.4	5.61, dd (15.1, 6.2)	4	128.6	5.61, dd (15.1, 6.2)	4, 33	128.2	5.61, dd (15.1, 6.2)
5	134.5	5.92, dt (15.1, 6.9)	5	134.7	5.92, dt (15.1, 6.9)	5, 32	134.5	5.93, dt (15.1,6.9)
6	31.9	2.07, m	6	32.1	2.07, m	6, 31	31.9	2.07, m
7	28.7	1.40, m	7	29.0	1.40, m	7, 30	28.7	1.40, m
8	29.1−29.4	1.28−1.33	8−10	29.3−29.9	1.28−1.32	8−12, 25−29	28.7−29.7	1.27−1.34
9	29.4	1.36,m	11	29.5	1.36, m	13, 24	29.4	1.36, m
10	27.1	2.02, m	12	27.1	2.03, m	14, 23	27.1	2.03, m
11	130.0	5.36, m	13	129.9	5.35, m	15, 22	129.7	5.36, m
12	130.0	5.36, m	14	130.2 [d]	5.35, m	16, 21	129.7	5.36, m
13	27.1	2.02, m	15	27.1	2.03, m	17, 20	27.1	2.03, m
14	29.4	1.36, m	16	29.5	1.36, m	18, 19	29.4	1.36, m
15	29.4	1.36, m	17	29.5	1.36, m			
16	27.1	2.02, m	18	27.1	2.03, m			
17	130.0	5.36, m	19	130.1 [d]	5.35, m			
18	130.0	5.36, m	20	129.9	5.35, m			
19	27.1	2.02, m	21	27.1	2.03, m			
20	29.4	1.36, m	22	29.5	1.36, m			
21−25	29.1−29.4	1.28−1.33	23−27	29.3−29.9	1.28−1.32			
26	28.7	1.40, m	28	29.0	1.40, m			
27	31.9	2.07, m	29	32.1	2.07, m			
28	134.5	5.92, dt (15.1, 6.9)	30	134.7	5.92, dt (15.1, 6.9)			
29	128.4	5.61, dd (15.1, 6.2)	31	128.6	5.61, dd (15.1, 6.2)			
30	62.8	4.84, brd (6.2)	32	62.9	4.84, brd (6.2)			
31	83.3		33	83.6				
32	73.9	2.57, d (2.1)	34	74.1	2.57, d (2.1)			

[a] ^{13}C NMR spectrum was measured at 150 MHz. [b] Chemical shifts were assigned from the HSQC data. [d] Assignments with the same superscript in the same column may be interchanged.

Scheme 1. Ozonolysis of 2−6



homogenized and extracted with EtOH (350 mL × 3) and $CHCl_3$/MeOH (1:1) (350 mL). The combined extracts were concentrated and partitioned between H_2O (200 mL) and $CHCl_3$ (200 mL × 3). The $CHCl_3$ layer (IC_{50} value against HeLa cells 0.4 μg/mL) was further partitioned between *n*-hexane (100 mL) and 90% MeOH (100 mL). The 90% MeOH layer (IC_{50} 0.4 μg/mL) was concentrated and subjected to ODS column chromatography, eluting with 50% MeOH, 70% MeOH (IC_{50} 10 μg/mL), 90% MeOH (IC_{50} 10 μg/mL), MeOH (IC_{50} 0.4 μg/mL), and $CHCl_3$/MeOH/H_2O (6:4:1, IC_{50} 22 μg/mL). The MeOH eluate was concentrated and subjected to silica gel column chromatography eluting with $CHCl_3$ (IC_{50} 0.4 μg/mL), $CHCl_3$/MeOH (98:2, IC_{50} 4.5 μg/mL), $CHCl_3$/MeOH (95:5, IC_{50} 4.5 μg/mL), $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH/H_2O (8:2:0.1), and $CHCl_3$/MeOH/H_2O (7:3:0.5). The $CHCl_3$ eluate was subjected to silica gel column chromatography, eluting with *n*-hexane/EtOAc (9:1, IC_{50} 4.5 μg/mL), *n*-hexane/EtOAc (8:2, IC_{50} 0.4 μg/mL), and *n*-hexane/EtOAc (1:1, IC_{50} 4.5 μg/mL). The *n*-hexane/EtOAc (8:2) eluate was concentrated and separated by ODS HPLC (Cosmosil AR-II; 10 × 250 mm) with 90−100% MeOH to afford 14 fractions. The second fraction was purified by ODS HPLC (Cosmosil MS-II; 10 × 250 mm) with 85% MeCN to afford **1** (6.3 mg). The third fraction was purified by ODS HPLC with 86% MeCN to afford **3** (2.0 mg). The sixth fraction was purified by ODS HPLC with 90% MeCN to afford **6** (1.8 mg). The seventh fraction was purified by ODS HPLC with 85% MeCN to afford **2** (15.6 mg). The eighth fraction was purified by ODS HPLC with 90% MeCN to afford **4** (16.0 mg). The 13th fraction was purified by ODS HPLC with 93% MeCN to afford **5** (1.2 mg).

*(−)-Duryne (**1**):* white solids; $[\alpha]^{17}_D$ −19 (*c* 0.27, $CHCl_3$); IR (film) ν_{max} 3400−3360 (br), 3282, 2917, 2852, 2118, 1654, 1467, 1088,

962 cm^{-1}; ^{1}H NMR data ($CDCl_3$), see Table 1; ^{13}C NMR data ($CDCl_3$), see Table 1; HRESIMS m/z 463.3524 [M + Na]$^+$ (calcd for $C_{30}H_{48}O_2Na$, 463.3552).

(−)-Duryne B (**2**): white solids; $[\alpha]^{26}_{D}$ −17 (c 0.34, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.57); IR (film) ν_{max} 3440−3400 (br), 2916, 2849, 2121, 1647, 1468, 1215 cm^{-1}; ^{1}H NMR data ($CDCl_3$), see Table 1; ^{13}C NMR data ($CDCl_3$), see Table 1; HRESIMS m/z 491.3857 [M + Na]$^+$ (calcd for $C_{32}H_{52}O_2Na$, 491.3865).

(−)-Duryne C (**3**): white solids; $[\alpha]^{25}_{D}$ −61 (c 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (3.60); IR (film) ν_{max} 3400−3360 (br), 3307, 3005, 2925, 2853, 2118, 1655, 1459, 1012, 968 cm^{-1}; ^{1}H NMR data ($CDCl_3$), see Table 2; ^{13}C NMR data ($CDCl_3$), see Table 2; HRESIMS m/z 489.3706 [M + Na]$^+$ (calcd for $C_{32}H_{50}O_2Na$, 489.3708).

(−)-Duryne D (**4**): white solids; $[\alpha]^{26}_{D}$ −38 (c 0.22, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.59); IR (film) ν_{max} 3400−3340 (br), 3290, 2997, 2920, 2849, 2120, 1654, 1459, 1215, 964 cm^{-1}; ^{1}H NMR data ($CDCl_3$), see Table 2; ^{13}C NMR data ($CDCl_3$), see Table 2; HRESIMS m/z 517.3986 [M + Na]$^+$ (calcd for $C_{34}H_{54}O_2Na$, 517.4021).

(−)-Duryne E (**5**): white solids; $[\alpha]^{26}_{D}$ −120 (c 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.73); ^{1}H NMR data ($CDCl_3$), see Table 2; ^{13}C NMR data ($CDCl_3$), see Table 2; HRESIMS m/z 545.4351 [M + Na]$^+$ (calcd for $C_{36}H_{58}O_2Na$, 545.4334).

(−)-Duryne F (**6**): white solids; $[\alpha]^{26}_{D}$ −32 (c 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (3.76); IR (film) ν_{max} 3400−3360 (br), 3308, 2922, 2851, 2120, 1655, 1459, 1014, 968 cm^{-1}; ^{1}H NMR data ($CDCl_3$), see Table 1; ^{13}C NMR data ($CDCl_3$), see Table 1; HRESIMS m/z 491.3846 [M + Na]$^+$ (calcd for $C_{32}H_{52}O_2Na$, 491.3865).

Preparation of MTPA Esters. To solutions of samples **1**−**6** (0.1 mg each) in dry pyridine (10 μL) was added (S)-MTPACl (2 μL). The solutions were left at room temperature for 15 h, and the reaction mixtures were diluted with H_2O and extracted with $CHCl_3$. The organic layers were purified by reversed-phase HPLC to afford the (R)-MTPA esters. (S)-MTPA esters were prepared in a similar way using (R)-MTPACl.

(R)-MTPA Ester of **1** (**1a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3, H-28), 6.02 (H-5, H-26), 5.50 (H-4, H-27), 5.35 (H-15, H-16), 2.64 (H-1, H-30), 2.05 (H-6, H-25), 2.02 (H-14, H-17), 1.26−1.36 (H-7−H-13, H-18−H-24): ESIMS m/z 895 [M + Na]$^+$.

(S)-MTPA Ester of **1** (**1b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5, H-26), 6.02 (H-3, H-28), 5.61 (H-4, H-27), 5.35 (H-15, H-16), 2.60 (H-1, H-30), 2.09 (H-6, H-25), 2.02 (H-14, H-17), 1.27−1.39 (H-7−H-13, H-18−H-24): ESIMS m/z 895 [M + Na]$^+$.

(R)-MTPA Ester of **2** (**2a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3, H-30), 6.02 (H-5, H-28), 5.50 (H-4, H-29), 5.35 (H-15, H-16), 2.64 (H-1, H-32), 2.05 (H-6, H-27), 2.02 (H-14, H-17), 1.26−1.37 (H-7−H-13, H-18−H-26); ESIMS m/z 923 [M + Na]$^+$.

(S)-MTPA Ester of **2** (**2b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5, H-28), 6.02 (H-3, H-30), 5.61 (H-4, H-29), 5.35 (H-15, H-16), 2.60 (H-1, H-32), 2.09 (H-6, H-27), 2.02 (H-14, H-17), 1.27−1.40 (H-7−H-13, H-18−H-26); ESIMS m/z 923 [M + Na]$^+$.

(R)-MTPA Ester of **3** (**3a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3, H-30), 6.02 (H-5, H-28), 5.50 (H-4, H-29), 5.35 (H-11, H-12, H-17, H-18), 2.64 (H-1, H-32), 2.05 (H-6, H-27), 2.02 (H-10, H-13, H-16, H-19), 1.27−1.38 (H-7−H-9, H-14−H-15, H-20−H-26); ESIMS m/z 921 [M + Na]$^+$.

(S)-MTPA Ester of **3** (**3b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5, H-28), 6.02 (H-3, H-30), 5.61 (H-4, H-29), 5.35 (H-11, H-12, H-17, H-18), 2.60 (H-1, H-32), 2.09 (H-6, H-27), 2.02 (H-10, H-13, H-16, H-19), 1.27−1.40 (H-7−H-9, H-14−H-15, H-20−H-26); ESIMS m/z 921 [M + Na]$^+$.

(R)-MTPA Ester of **4** (**4a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3, H-32), 6.02 (H-5, H-30), 5.50 (H-4, H-31), 5.35 (H-13, H-14, H-19, H-20), 2.64 (H-1, H-34), 2.05 (H-6, H-29), 2.02 (H-12, H-15, H-18, H-21), 1.26−1.36 (H-7−H-11, H-16−H-17, H-22−H-28); ESIMS m/z 949 [M + Na]$^+$.

(S)-MTPA Ester of **4** (**4b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5, H-30), 6.02 (H-3, H-32), 5.61 (H-4, H-31), 5.35 (H-13, H-14, H-19, H-20), 2.60 (H-1, H-34), 2.09 (H-6, H-29), 2.02 (H-12, H-15, H-18, H-21), 1.26−1.39 (H-7−H-11, H-16−H-17, H-22−H-28); ESIMS m/z 949 [M + Na]$^+$.

(R)-MTPA Ester of **5** (**5a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3, H-34), 6.02 (H-5, H-32), 5.50 (H-4, H-33), 5.35 (H-15, H-16, H-21, H-22), 2.64 (H-1, H-36), 2.05 (H-6, H-31), 2.02 (H-14, H-17, H-20, H-23), 1.26−1.37 (H-7−H-13, H-18−H-19, H-24−H-30); ESIMS m/z 977 [M + Na]$^+$.

(S)-MTPA Ester of **5** (**5b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5, H-32), 6.02 (H-3, H-34), 5.61 (H-4, H-33), 5.35 (H-15, H-16, H-21, H-22), 2.60 (H-1, H-36), 2.09 (H-6, H-31), 2.02 (H-14, H-17, H-20, H-23), 1.27−1.41 (H-7−H-13, H-18−H-19, H-24−H-30) ESIMS m/z 977 [M + Na]$^+$.

(R)-MTPA Ester of **6** (**6a**): ^{1}H NMR ($CDCl_3$) δ 6.04 (H-3), 6.02 (H-5), 5.95 (H-28), 5.83 (H-29), 5.64 (H-30), 5.36 (H-15, H-16), 5.50 (H-4), 3.25 (H-32), 2.64 (H-1), 2.05 (H-6), 2.02 (H-14, H-17), 1.81 (H-27α), 1.68 (H-27β), 1.26−1.36 (H-7−H-13, H-18−H-26); ESIMS m/z 923 [M + Na]$^+$.

(S)-MTPA Ester of **6** (**6b**): ^{1}H NMR ($CDCl_3$) δ 6.08 (H-5), 6.02 (H-3), 6.00 (H-29), 5.96 (H-28), 5.67 (H-30), 5.61 (H-4), 5.36 (H-15, H-16), 3.26 (H-32), 2.60 (H-1), 2.09 (H-6), 2.02 (H-14, H-17), 1.74 (H-27α), 1.23−1.61 (H-7−H-13, H-18−H-26, H-27β); ESIMS m/z 923 [M + Na]$^+$.

Ozonolysis. Separately, solutions of acetylenes **2**−**6** (0.5 mg each) in CH_2Cl_2 (1 mL) were treated with O_3 at −78 °C for 15 min. After excess O_3 was removed by a stream of N_2, the reaction mixtures were treated with 90% HCOOH/35% H_2O_2 (2:1, 1 mL) at room temperature for 15 min. The products were concentrated and subjected to FABMS without further separation.

Cytotoxicity Assay. The cytotoxicities of **1**−**6** against HeLa cells were evaluated by an MTT assay.[24] HeLa human cervical cancer cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 2 μg/mL gentamycin, and 2 μg/mL antibiotic−antimicotic (Gibco) at 37 °C under an atmosphere of 5% CO_2. To each well of a 96-well microplate containing 200 μL of tumor cell suspension (1 × 10^4 cells/mL) was added a sample after 24 h preincubation, and the plate was incubated for 72 h. After addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) saline solution (1 mg/mL, 50 μL) to each well, the plate was incubated for 3 h. After the incubation, the supernatant was discarded and DMSO (150 μL) was added. The absorbance was measured to determine IC_{50} values. In this assay, adriamycin was used as a positive control (IC_{50} 1.64 μM).

■ ASSOCIATED CONTENT

Supporting Information. NMR data and tandem FABMS data for **1**−**6** and photograph of the sponge. This material is available free of charge via the Internet at http://pubs.acs.org.

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