Seven New Polyacetylene Derivatives, Showing Both Potent Metamorphosis-Inducing Activity in Ascidian Larvae and Antifouling Activity Against Barnacle Larvae, from the Marine Sponge *Callyspongia truncata*

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Seven new polyacetylene derivatives, two hydrocarbons (callytetrayne and callypentayne) and five triols (callytriols A−E), were isolated from the marine sponge *Callyspongia truncata* along with the known siphonodiol and its monosulfates and disulfates. All compounds showed potent metamorphosis-inducing activity in the ascidian *Halocynthia roretzi* larvae, with ED_{100} values of 0.13−1.3 μg/mL, and the triols also showed antifouling activity against the barnacle *Balanus amphitrite* larvae, with ED_{50} values of 0.24−4.5 μg/mL.

Settlement and metamorphosis are a sequential critical events in the life cycle of sessile marine organisms to acquire a preferable habitat for food and reproduction. Although various physical factors have been documented that influence larval settlement and metamorphosis, chemical cues derived from the adults, from prey, or from bacterial films are believed to be more important.[1] In fact, metamorphosis-inducing substances have been reported from such organisms. For example, dibromomethane from the crustose coralline *Lithophyllum yessoense* induced larval settlement of the sea urchin *Strongylocentrous nudus*,[2] while epoxy-δ-tocotrienol from the brown alga *Sargassum tortile* triggered settlement of the hydroid *Coryne uchidai*.[3] We have also isolated metamorphosis-inducing substances in tadpole larvae of ascidians *Halocynthia roretzi* and *Ciona savignyi* from marine organisms; pteridine-containing pyrroloindole alkaloids from two ascidians *C. savigni* and *Botrylloides* sp.,[4,5] 3,4-dihydroxystyrene sulfate derivatives from a marine sponge *Jaspis* sp.,[6, 7] pipecolate derivatives from a marine sponge *Anthosigmella* aff. *raromicrosclera*,[8] a bisguanidinium alkaloid from a marine sponge *Stelletta* sp.,[9] and psammaplysin A[10] from the marine sponge *Pseudoceratina purpurea*.[11]

Sessile marine organisms, such as barnacles, mussels, hydroids, bryozoans, and ascidians, cause problems by settling on man-made surfaces. Organotin compounds have been successfully used to control these macrofoulants; however, recently their harmful effects on marine environments have caused worldwide concern.[12−14] Marine invertebrates are known to have developed chemical defense systems against predators and epibiosis;[15−17] hence, their secondary metabolites might be environmentally safe antifoulants. A variety of antifouling substances have been isolated: diterpenes from the sea pansy *Renilla reniformis*;[18] *p*-(hydroxysulfonyloxy)cinnamic acid from the seagrass *Zostera marina*;[19] 2,5,6-tribromo-1-methylgramine from the marine bryozoan *Zoobotryon pellucidum*;[20] and bromopyrrole dimers from the marine sponge *Agelas conifera*.[21] During our search for antifouling agents using laboratory-reared cyprid larvae of the barnacle *Balanus amphitrite*, we isolated several potent antifouling compounds: terpenoids from the marine sponge *Acanthella cavernosa*[22,23] and from nudibranchs of the familiy Phyllidiidae,[24] a bromopyrrole dimer from the marine sponge *Agelas mauritiana*,[25] and a bromopyrrole derivative[26] and bromotyrosine derivatives[11,27] from the marine sponge *Pseudoceratina purpurea*.

We have recently discovered both metamorphosis-inducing and antifouling activities in the MeOH extract of the marine sponge *Callyspongia truncata* Lindgren (Callyspongiidae) collected in Sagami Bay, Japan. Bioassay-monitored purification afforded seven new polyacetylene derivatives along with the known siphonodiol (**3**)[28,29] and its monosulfates and disulfates, callyspongins A (**9**) and B (**10**).[30] The known compounds were readily identified by comparison of their spectral data including $[\alpha]_D$ values with those in literature.

The sponge (550 g, wet wt) was extracted with MeOH. The concentrated aqueous residue was extracted with Et_2O, then *n*-BuOH. The Et_2O layer showed both metamorphosis-inducing activity in the ascidian larvae and antifouling activity against the barnacle larvae; the *n*-BuOH layer was less active. The Et_2O layer was repeatedly fractionated by Si gel column chromatography, Sephadex LH-20 gel-filtration, and reversed-phase (C_{18}) HPLC to afford ten polyacetylenes, including two new hydrocarbons callytetrayne (**1**, yield: 5.0×10^{-3}%, wet wt) and callypentayne (**2**, 7.5×10^{-3}%) and five new triols, callytriols A (**4**, 1.7×10^{-4}%), B (**5**, 1.6×10^{-4}%), C (**6**, 9.1×10^{-5}%), D (**7**, 5.5×10^{-5}%), and E (**8**, 8.4×10^{-5}%).

One of the nonpolar compounds, callytetrayne (**1**),[31] showed prominent IR bands at 3280 and 2220 cm^{-1} and characteristic UV absorptions at 215.5 (ϵ 34 900), 253.5 (8500), 267.5 (10 800), and 283.5 nm (9800), which were indicative of a diyne system. The ^{13}C-NMR spectrum (Table 1) together with DEPT and HMQC experiments revealed the presence of nine methylene carbons (δ 18.8−30.1), two terminal acetylene carbons (δ 81.2 and 81.7), six disubstituted acetylene carbons (δ 65.3−80.5), and four olefinic carbons (δ 108.1, 109.2, 144.3, and 145.9). The $^1H-^1H$ COSY spectrum led to two partial structures, C1−C7 and C12−C21, which were consistent with HMBC cross peaks, H-1/C3, H-3/C1, H-4/C2, H-5/C2 (four-bond coupling), H-17/C20 (four-bond coupling), H-18/C20, H-19/C21, and H-21/C19. 3*Z*,18*Z*-Geometry

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Table 1. ^{1}H- and ^{13}C-NMR Data for **1** and **2**[a]

	1			2		
no.	^{1}H[b]	^{13}C[c]	HMBC	^{1}H[b]	^{13}C[c]	HMBC
1	3.09 d 1.9	8.12 d	3	1.98 s	64.9 d	3
2		80.2 s			68.2 s	
3	5.48 dd 10.8, 1.9	109.2 d	1, 5		65.6 s	
4	5.96 m	144.3 d	2, 3, 5, 6		76.9 s	
5	2.42 (2H) m	29.4 t	2, 3, 4, 6, 7	2.42 (2H) t 7.0	18.2 t	2, 3, 4, 6, 7
6	1.67 (2H) m	27.5 t	4, 5, 7, 8	1.79 (2H) qunit 7.0	26.8 t	4, 7, 8
7	2.29 (2H) m	18.8 t	5, 6, 8, 9	2.47 (2H) t 7.0	18.8 t	5, 6, 8, 9, 10, 11
8		76.5 s			82.9 s	
9		65.8 s			72.5 s	
10		65.3 s			66.2 s	
11		77.4 s			78.0 s	
12	2.24 (2H) m	19.2 t	10, 11, 13, 14	5.49 d 10.8	108.31 d	10, 11, 13, 14
13	1.53 (2H) m	28.2 t	11, 12, 14, 15	6.04 dt 10.8, 7.6	147.7 d	9, 12, 14, 15
14	1.39 (2H) m	28.52 t	12, 13, 15, 16	2.36 (2H) m	30.5 t	12, 13, 15, 16
15	1.32 (2H) m	28.56 t	13, 14, 16, 17	1.46 (2H) m	28.2 t	14, 15, 17, 18
16	1.42 (2H) m	28.48 t	14, 15, 17, 18	1.46 (2H) m	28.1 t	13, 14, 16, 17
17	2.33 (2H) m	30.1 t	15, 16, 18, 19, 20	2.36 (2H) m	30.0 t	15, 16, 18, 19
18	5.99 m	145.9 d	16, 17, 19, 20	5.99 dt 10.8, 7.6	145.7 d	16, 17, 19
19	5.44 dd 10.8, 1.9	108.1 d	17, 21	5.46 dd 10.8, 1.7	108.24 d	17, 20
20		80.5 s			81.3 s	
21	3.07 d 1.9	8.17 d	19	3.08 d 1.7	80.5 d	18, 19

[a] Data recorded in MeOH-d_4 at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 27 °C. [b] Multiplicities and coupling constants in Hz are given. [c] Multiplicities were determined by an HMQC and DEPT experiments.

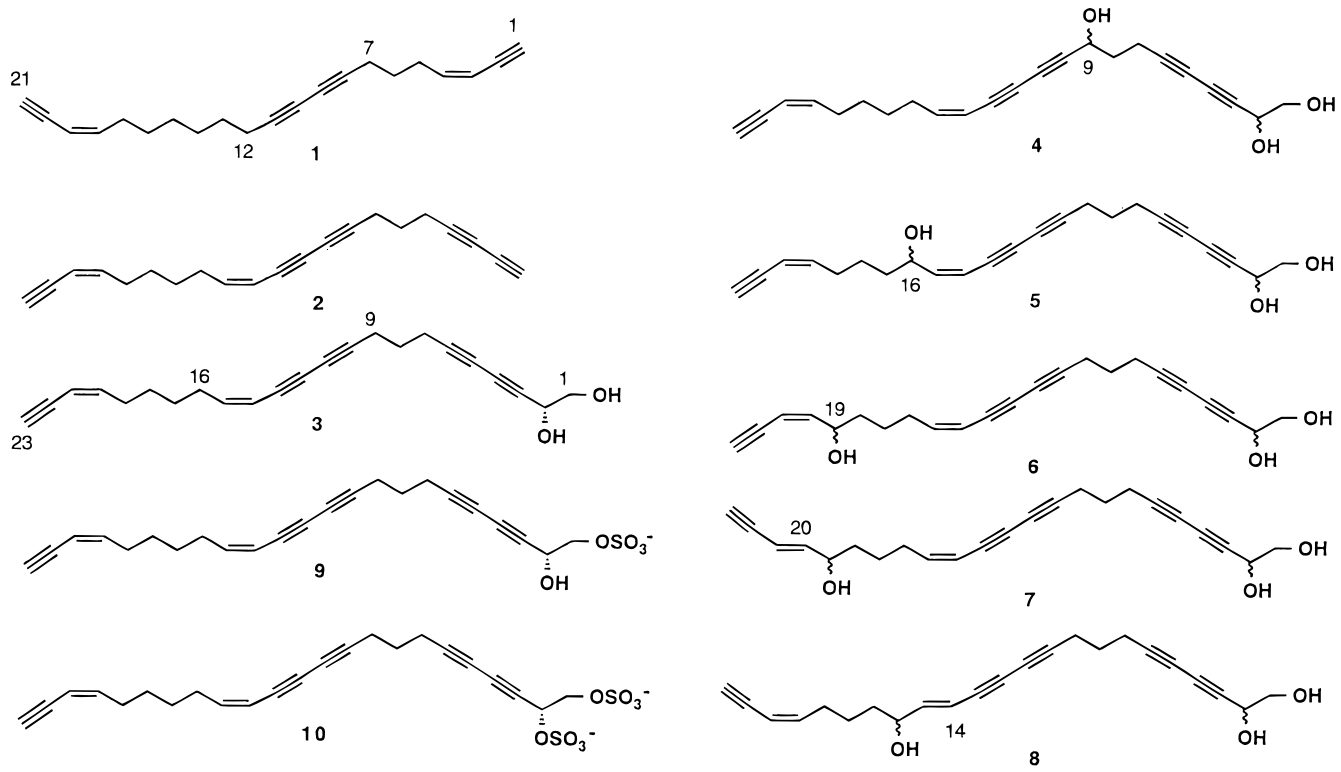

Figure 1.

was determined by ^{1}H coupling constants ($J_{3,4}$ = 10.8 Hz and $J_{18,19}$ = 10.8 Hz). The partial structures were connected via a diyne unit, which accounted for the remaining four acetylenic carbons (δ 65.3, 65.8, 76.5, and 77.4). These connectivities were supported by HMBC cross peaks, H-6/C8, H-7/C8 and C9, H-12/C10 and C11, and H-13/C11. Thus, callytetrayne (**1**) is (3*Z*,-18*Z*)-henicosadiene-1,8,10,20-tetrayne (Figure 1).

The ^{1}H- and ^{13}C-NMR spectra of callypentayne (**2**) (Table 1) were almost superimposable on those of siphonodiol except for the absence of a terminal dihydroxyethyl group. Instead, another terminal acetylene group (δ_H 1.98, s; δ_C 64.9, d) was present, thus establishing that **2** is (12*Z*,18*Z*)-henicosadiene-1,3,8,10,20-pentayne.[31]

Callytriols A−E (**4**−**8**) showed pseudomolecular ion peaks at *m*/*z* 454 (M + DEA + H)$^+$ in the FABMS, which matched a formula of $C_{27}H_{36}NO_5$, with one more oxygen atom than siphonodiol (**3**). The ^{1}H- and ^{13}C-NMR spectra of **4** revealed the presence of one additional oxymethine group (δ_H 4.49, t, J = 6.5 Hz; δ_C 61.8, d), which was incorporated into a partial structure $CH(OH)CH_2CH_2$, as implied by COSY cross peaks: δ 4.49/δ 1.86 and 1.88/δ 2.445 and 2.455 (Table 2). HMBC cross peaks, δ 4.33 (H-2), 2.445, and 2.455/δ 70.5 (C4),

Table 2. ^{1}H NMR Data for **4**-**8**.[a]

no.	4	5	6	7	8
1	3.54 dd 11.2, 6.8 3.56 dd 11.2, 5.1	3.54 dd 11.2, 6.8 3.57 dd 11.2, 5.1	3.54 dd 11.2, 6.6 3.57 dd 11.2, 5.1	3.55 dd 11.2, 6.7 3.57 dd 11.2, 5.1	3.55 dd 11.2, 6.9 3.57 dd 11.2, 5.1
2	4.33 dd 6.8, 5.1	4.34 dd 6.8, 5.1	4.33 dd 6.6, 5.1	4.33 dd 6.7, 5.1	4.34 dd 6.9, 5.1
7	2.445 m 2.455 m	2.42 t 7.0	2.42 t 7.0	2.41 t 7.0	2.42 t 7.0
8	1.86 m 1.88 m	1.74 quint 7.0	1.74 quint 7.0	1.73 quint 7.0	1.74 quint 7.0
9	4.49 t 6.5	2.46 t 7.0	2.46 t 7.0	2.44 t 7.0	2.46 t 7.0
14	5.54 d 10.7	5.57 d 10.3	5.51 d 10.8	5.72 d 16.0	5.51 d 10.1
15	6.15 dt 10.7, 7.8	6.02 m	6.09 dt 10.8, 7.4	6.24 dd 16.0, 5.7	6.09 dt 10.1, 7.3
16	2.34 m	4.55 m	2.35 m	4.11 m	2.35 m
17	1.46 m	1.49 m	1.50 m 1.61 m	1.51 m	1.44 m 1.51 m
18	1.46 m	1.49 m	1.50 m 1.62 m	1.51 m	1.51 m
19	2.34 m	2.36 m	4.62 m	2.34 m	4.11 m
20	5.99 dt 10.8, 7.4	5.99 m	5.92 dd 11.0, 8.8	5.99 dt 10.4, 7.3	6.17 dd 15.7, 5.5
21	5.46 dd 10.8, 2.0	5.47 dd 10.3, 2.0	5.53 dd 11.0, 2.0	5.46 dd 10.4, 1.7	5.67 dd 15.7, 2.2
23	3.40 d 2.0	3.40 d 2.0	3.50 d 2.0	3.41 d 1.7	3.20 d 2.2

[a] Data recorded in MeOH-d_4 at 500 MHz at 27 °C. Multiplicities and coupling constants in Hz are given.

Table 3. Metamorphosis-Inducing Activity in the Ascidian *Halocynthia roretzi* and Antifouling Activity Against the Barnacle *Balanus amphitrite* of Compounds **1**−**10**

compound	metamorphosis-inducing activity ED_{100} [μg/mL]	antifouling activity ED_{50} [μg/mL]
1	0.25	30
2	0.25	
3	0.25	4.5
4	1.3	0.43
5	1.3	0.63
6	0.13	0.24
7	1.3	0.38
8	0.13	0.63
9	0.13	3.9
10	0.25	4.1

placed the hydroxy group at C9. Hence, **4** is 9-hydroxysiphonodiol.[32]

Callytriol B (**5**) had a partial structure, C1−C15, identical with that of **3**, which was supported by ^{1}H- and ^{13}C-NMR data. The position of a hydroxyl group at C16 was deduced from ^{1}H−^{1}H COSY cross peak data. Therefore, **5** is 16-hydroxysiphonodiol.[32] Similarly, callytriol C (**6**) is 19-hydroxysiphonodiol.[32] Spectral data of callytriols D (**7**) and E (**8**) readily indicated that they were geometrical isomers of callytriols C (**6**) and B (**5**), 20*E*-callytriol C ($J_{20,21}$ = 15.7 Hz) and 14*E*-callytriol B ($J_{14,15}$ = 16.0 Hz), respectively.[32]

The polyacetylene derivatives **1**−**10** showed potent metamorphosis-inducing activity in the ascidian *H. roretzi*, with ED_{100} values ranging from 0.13 to 1.3 μg/mL (Table 3). Compounds **6**, **8**, and **9** are the most potent among compounds we have isolated so far.[4−9,11] Interestingly, the position of a hydroxyl group and the geometry of an olefin affect the activity of the five triols. Polyacetylene derivatives **3**−**10** also showed antifouling activity against barnacle larvae with ED_{50} values of 0.24−4.5 μg/mL. It is noteworthy that they had both metamorphosis-inducing activity for ascidian larvae and antifouling activity for barnacle larvae; another example was psammaplysin A (ED_{100} 1.2 μg/mL and ED_{50} 0.27 μg/mL, respectively).[11]

Acetylenic metabolites isolated from marine sponges exhibit various biological activities; for example, antibacterial/antifungal activity,[28] H^+,K^+-ATPase inhibitory activity,[29] cytotoxic activity,[33] inhibition of fertilization in the starfish *Asterina pectinifera*,[30] anti-HIV activity,[34] and inhibitory activity in neutrophil leukocyte adhesion to TNF-α-stimulated endotherial cells[35] all have been reported for sponge polyacetylenes. This is the first report of acetylenic derivatives that influence larval settlement and metamorphosis of sessile marine animals.[36]

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. IR spectra were measured on a JASCO IR-700 spectrometer. UV spectra were recorded on a Hitachi U-2000 spectrometer in EtOH or MeOH. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker ARX-500 NMR spectrometer in MeOH-d_4 or $CDCl_3$ at 27 °C. Multiplicities of ^{13}C signals were determined by HMQC and/or DEPT experiments. MS were measured on a JEOL SX-102A mass spectrometer.

Collection and Isolation. The marine sponge was collected by scuba at a depth of 15 m off Atami in Sagami Bay and identified as *Callyspongia truncata* Lindgren (Callyspongiidae) by Dr. Rob van Soest. A voucher specimen (ZMA POR. 11365) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam, the Netherlands. The frozen sponge (550 g, wet wt) was extracted with MeOH. The concentrated aqueous residue was successively extracted with Et_2O and *n*-BuOH. Half of the Et_2O layer (2.34 g) was subjected to Si gel column chromatography (Wakogel C-200, Wako Pure Chemical Industries Ltd., Osaka, 3 × 38 cm) with 0, 5, 10, 20, and 50% MeOH−$CHCl_3$. The first fraction (331.4 mg) was purified on Si gel (Wakogel C-300, 2.2 × 30 cm) with EtOAc−hexane (1:9) to afford callytetrayne (**1**, 27.5 mg, 5.0×10^{-3} %, wet wt) and callypentayne (**2**, 4.1 mg, 7.5×10^{-3} %). The third fraction (597.8 mg) was fractionated on Si gel (Wakogel C-200, 2.2 × 35 cm) with 5 and 10% MeOH−CH_2Cl_2 to furnish siphonodiol (**3**, 61.3 mg, 1.1×10^{-2} %) and a mixture of triols (6.3 mg). The triol fraction combined with a corresponding fraction from the remaining Et_2O layer was purified by HPLC (L-column ODS, 10 × 250 mm, Chemicals Inspection & Testing Institute, Tokyo) with 70% MeOH−H_2O and 50% CN_3-CN−H_2O to afford callytriols A (**4**, 0.92 mg, 1.7×10^{-4} %), B (**5**, 0.88 mg, 1.6×10^{-4} %), C (**6**, 0.50 mg, 9.1×10^{-5} %), D (**7**, 0.30 mg, 5.5×10^{-5} %), and E (**8**, 0.46

mg, 8.4×10^{-5} %). The fourth (79.4 mg) and fifth (134.1 mg) fractions were combined and gel-filtered on Sephadex LH-20 (Pharmacia, 2.2 × 70 cm) with MeOH to afford callyspongins A (**9**, 49.5 mg, 9.0×10^{-3} %) and B (**10**, 13.0 mg, 2.4×10^{-3} %). The *n*-BuOH layer (3.40 g) was subjected to reversed-phase flash column chromatography [YMC-GEL ODS-A 60-400/230 (Yamamura Chem. Co., Ltd., Kyoto), 7 × 15 cm] with 80% MeOH−H_2O followed by gel-filtration on Sephadex LH-20 (2.2 × 70 cm) with MeOH to afford **9** (10.0 mg, 1.8×10^{-3} %) and **10** (6.9 mg, 1.3×10^{-3} %).

Callytetrayne (1): yellow oil; IR (film) ν_{max} 3280, 2930, 2850, 2220, 1720, 1450, 1430, 1280, 740, and 630 cm^{-1}; UV (EtOH) λ_{max} 215.5 (ϵ 34 900), 253.5 (8500), 267.5 (10 800), and 283.5 nm (9800); ^1H and ^{13}C NMR data in $CDCl_3$, see Table 1.

Callypentayne (2): yellow oil; IR (film) ν_{max} 3280, 2930, 2850, 2220, 1450, 1430, 740, and 630 cm^{-1}; UV (EtOH) λ_{max} 207.0 (ϵ 33 200), 214.5 (41 800), 253.0 (8100), 267.5 (11 900), and 283.5 nm (9500); ^1H and ^{13}C NMR data in $CDCl_3$, see Table 1.

Callytriol A (4): yellow oil; $[\alpha]^{25}_D$ +0.16° (*c* 0.092, MeOH); neither **4** nor triols **5**−**8** gave rise to satisfactory IR spectra; UV (EtOH) λ_{max} 208.5 (ϵ 33 600), 216.0 (38 400), 254.0 (8600), 268.0 (10 600), and 284.0 nm (9000); ^1H NMR data in CD_3OD, see Table 2; ^{13}C NMR (CD_3OD) δ 16.0 (t, C7), 29.2 (2C, t, C17 and C18), 30.8 (t, C19), 31.4 (t, C16), 37.2 (t, C8), 61.8 (d, C9), 64.5 (d, C2), 66.0 (s, C5), 67.1 (t, C1), 69.0 (s, C11), 70.5 (s, C4), 76.3 (s, C3), 76.4 (s, C12 or C13), 77.9 (s, C13 or C12), 80.3 (s, C6), 81.2 (s, C22), 82.9 (d, C23), 84.3 (s, C10), 108.9 (d, C14), 109.6 (d, C21), 146.0 (d, C20), and 149.5 (d, C15); HMBC cross peaks: H_2-1/C2 and C3; H-2/C1, C3, and C4; H_2-7/C4, C5, C6, C8, and C9; H_2-8/C6, C7, C9, and C10; H-9/C7, C8, C10, and C11; H_2-18/C15, C17, C18, and C20; H_2-19/C15, C17, C18, C20, and C21; FABMS (positive, DEA) *m*/*z* 454 $(M + DEA + H)^+$; HRFABMS (positive, DEA) *m*/*z* 454.2562; calcd for $C_{27}H_{36}NO_5$, Δ −3.2 mmu.

Callytriol B (5): yellow oil; $[\alpha]^{25}_D$ +0.96° (*c* 0.078, MeOH); UV (EtOH) λ_{max} 205.5 (ϵ 30 500), 214.5 (32 300), 253.5 (8200), 268.0 (9300), and 284.0 nm (8800); ^1H NMR data in CD_3OD, see Table 2; ^{13}C NMR (CD_3OD) δ 18.9 (t),19.2 (t), 25.5 (t), 28.1 (t), 30.9 (t), 37.3 (t), 64.5 (d), 66.3 (s), 66.5 (s), 67.1 (t), 70.5 (s), 70.6 (d), 72.2 (s), 76.3 (s), 80.3 (s), 81.2 (s), 82.9 (d), 84.9 (s), 109.1 (d), 109.7 (d), 146.0 (d), and 150.6 (d); one acetylene carbon may be overlapped; FABMS (positive, DEA) *m*/*z* 454 $(M + DEA + H)^+$; HRFABMS (positive, DEA) *m*/*z* 454.2593; calcd for $C_{27}H_{36}NO_5$, Δ −0.1 mmu.

Callytriol C (6): yellow oil; $[\alpha]^{25}_D$ −4.5° (*c* 0.050, MeOH); UV (EtOH) λ_{max} 206.5 (ϵ 32 700), 214.5 (39 700), 253.0 (8100), 267.5 (11 800), and 283.0 nm (9400); ^1H NMR data in CD_3OD, see Table 2; ^{13}C NMR (CD_3OD) δ 18.9 (t),19.2 (t), 25.5 (t), 28.2 (t), 31.5 (t), 37.2 (t), 64.5 (d), 66.2 (s), 66.7 (s), 67.1 (t), 70.4 (d), 70.5 (s), 73.0 (s), 76.3 (s), 79.0 (s), 80.4 (s), 84.0 (d), 109.3 (d), 109.5 (d), 148.40 (d), and 148.44 (d); two acetylene carbons may be overlapped; FABMS (positive, DEA) *m*/*z* 454 $(M + DEA + H)^+$; HRFABMS (positive, DEA) *m*/*z* 454.2562; calcd for $C_{27}H_{36}NO_5$, Δ −3.2 mmu.

Callytriol D (7): yellow oil; $[\alpha]^{25}_D$ −1.5° (*c* 0.030, MeOH); UV (EtOH) λ_{max} 207.5 (ϵ 29 800), 214.5 (37 300), 253.0 (7300), 267.0 (10 500), and 283.0 nm (8300); ^1H NMR data in CD_3OD, see Table 2; ^{13}C NMR (CD_3OD) δ 18.87 (t), 18.93 (t), 25.6 (t), 28.2 (t), 31.3 (t), 37.2 (t), 64.5 (d), 66.1 (s), 66.2 (s), 66.6 (s), 67.1 (d), 70.4 (s), 70.5 (s), 72.2 (t), 73.0 (s), 76.3 (s), 78.8 (s), 80.4 (s), 84.1 (d), 109.4 (d), 109.5 (d), 148.4 (d), and 148.8 (d); FABMS (positive, DEA) *m*/*z* 454 $(M + DEA + H)^+$; HRFABMS (positive, DEA) *m*/*z* 454.2589; calcd for $C_{27}H_{36}NO_5$, Δ −0.5 mmu.

Callytriol E (8): yellow oil; $[\alpha]^{25}_D$ −1.6° (*c* 0.046, MeOH); UV (EtOH) λ_{max} 207.0 (ϵ 33 000), 214.0 (41 900), 253.0 (10 000), 267.5 (14 700), and 283.5 nm (11 600); ^1H NMR data in CD_3OD, see Table 2; ^{13}C NMR (CD_3OD) δ 18.9 (t), 19.1 (t), 25.6 (t), 28.2 (t), 30.9 (t), 37.2 (t), 64.5 (d), 66.2 (s), 66.7 (s), 67.1 (d), 70.5 (s), 72.2 (t), 74.4 (s), 75.1 (s), 76.3 (s), 80.4 (s), 81.2 (s), 82.9 (d), 83.3 (s), 109.0 (d), 109.7 (d), 145.9 (d), and 150.7 (d); FABMS (positive, DEA) *m*/*z* 454 $(M + DEA + H)^+$; HRFABMS (positive, DEA) *m*/*z* 454.2597; calcd for $C_{27}H_{36}NO_5$, Δ +0.4 mmu.

Antifouling Assays Against Barnacle Larvae. This assay was performed as previously described.[11]

Metamorphosis-Promoting Assays on Ascidian Larvae. This assay was performed as previously described.[11]

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