Triangulynes A−H and Triangulynic Acid, New Cytotoxic Polyacetylenes from the Marine Sponge *Pellina triangulata*

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Nine new polyacetylenes, triangulynes A−H (**1**−**8**) and triangulynic acid (**9**), have been isolated from the marine sponge *Pellina triangulata* (Oceanapiidae) through cytotoxicity-guided fractionation. Structural elucidations and stereochemistry assignments were based on chemical and spectral studies.

There have been limited chemical studies of marine sponges of the genus *Pellina*.[1−3] For the present study, we selected an extract of the previously uninvestigated marine sponge *Pellina triangulata* Desqueyroux-Faundez (Oceanapiidae), which had shown an interesting profile of differential cytotoxicity in the NCI's primary antitumor screen.[4−7] The leukemia and colon tumor lines were, in general, more sensitive to the extract than the other tumor subpanels. Bioassay-directed fractionation of the organic extract has resulted in the isolation of nine cytotoxic polyacetylenes, triangulynes A−H (**1**−**8**) and triangulynic acid (**9**). The absolute configurations of **1**, **2**, **6**, **8**, and **9** were determined by the modified Mosher method.

Results and Discussion

Samples of *P. triangulata* were collected at Kuop Atoll, Truk Island, Micronesia. The CH_2Cl_2−MeOH extract of *P. triangulata* was fractionated via an extensive solvent−solvent partition protocol[8] that concentrated the cytotoxic activity in the CCl_4 and $CHCl_3$ fractions. Further separation of the CCl_4 fraction by gel permeation on a Sephadex LH-20 column, followed by repeated reversed-phase HPLC, yielded pure triangulynes A−H (**1**−**8**), while purification of the $CHCl_3$ fraction with more polar solvent mixtures led to isolation of triangulynic acid (**9**).

Triangulyne A (**1**) was isolated as a major constituent from the CCl_4 fraction. The molecular formula of triangulyne A, $C_{32}H_{46}O_3$, as derived from HRFABMS, indicated 10 degrees of unsaturation. Its ^{1}H NMR spectral data showed the presence of two disubstituted double bonds [δ 5.90, 5.58, 5.32 (2H)], an oxymethylene (δ 4.32), two oxymethines (δ 4.81 and 4.40), and a terminal acetylene (δ 2.54). In a ^{1}H−^{1}H COSY experiment, the olefinic proton signals at δ 5.90 and 5.58 showed correlations to the oxymethine at δ 4.81 and a methylene at δ 2.04. The signal at δ 4.81 was, in turn, correlated to the terminal acetylene proton at δ 2.54 (J = 2 Hz), thus providing partial structure **A** (Figure 1). The configuration of the allylic double bond was assigned as *E* based on the vicinal coupling constant of the olefinic protons (J = 15 Hz).

The UV absorption maxima (λ_{max} 257, 242, 230, 204 mm) and molar absorptivities most closely resembled those of a triyne chromophore.[9] That observation and HMBC correlations from δ 4.32 to the acetylene carbons at δ 77.5 and 69.7, and from δ 4.40 to carbons at δ 68.8 and 80.5, led to the assignment of partial structure **B** (Figure 1). The remaining atoms were accommodated by substructure **C** (Figure 1), derived from COSY relationships (δ 5.32 and δ 1.99) and HMBC correlations between the olefinic protons at δ 5.32 and the allylic carbons (δ 27.0); the *Z* geometry of the olefin was assigned on the basis of the ^{13}C NMR chemical shifts of the allylic carbons.[10] Fragment ions at m/z 271 [**B** + $6CH_2$ + CH=$CHCH_2$] and 261 [**A** + $8CH_2$ + CH=$CHCH_2$] in the EIMS, which corresponded to the α-cleavages at the positions allylic to the isolated double bond, indicated the presence of the olefin at C-16. Ozonolysis of **1**, followed by treatment with CH_2N_2, resulted in the formation of the dimethyl ester of dodecanedioic acid, thus confirming the location of the isolated *Z* double bond. Triangulyne A was therefore identified as 1,8,30-trihydroxydotriaconta-16(*Z*), 28(*E*)-diene-2,4,6,31-tetrayne (**1**).

The ^{1}H and ^{13}C NMR spectra of triangulynes B and C showed features similar to those of **1**. The molecular formulas of triangulyne B (**2**, $C_{33}H_{48}O_3$) and C (**3**, $C_{31}H_{44}O_3$), established by HRCIMS, suggested that both compounds were homologs of **1**. Analyses of NMR data, including COSY, DEPT, HMQC, and HMBC, also led to partial structures **A**, **B**, and **C**. A fragment ion at m/z 285 [**B** + $7CH_2$ + CH_2CH=CH], observed for both **2** and **3**, suggested that m = 6; therefore a C-17 double bond was indicated for both **2** and **3**.

Triangulyne D (**4**), which eluted much later in the reversed-phase HPLC, gave $C_{41}H_{64}O_3$ by HRFABMS. The molecular formula, in combination with ^{1}H and ^{13}C NMR spectral data, indicated that **4** was a higher homolog of **1**, **2**, and **3**. The presence of the same partial substructures **A**, **B**, and **C**, as revealed by detailed analyses of COSY, DEPT, HMQC, and HMBC data, further supported this assignment. As before, the location of the isolated double bond was established by analysis of mass spectral fragments (CIMS). Accordingly, fragments at m/z 369 [**B** + $13CH_2$ + CH_2-CH=CH] and m/z 289 [**A** + $10CH_2$ + CH_2CH=CH] provided evidence for the C-23 location of the isolated *Z* double bond.

In order to determine the absolute configuration at the carbinol centers, we employed the modified Mosher method.[11−13] On the basis of the $\Delta\delta$ ($\delta_S - \delta_R$) values (Figure 2), the absolute configurations for **1** and **2** were 8*S*,30*R* and 8*S*,31*R*, respectively. Due to the limited

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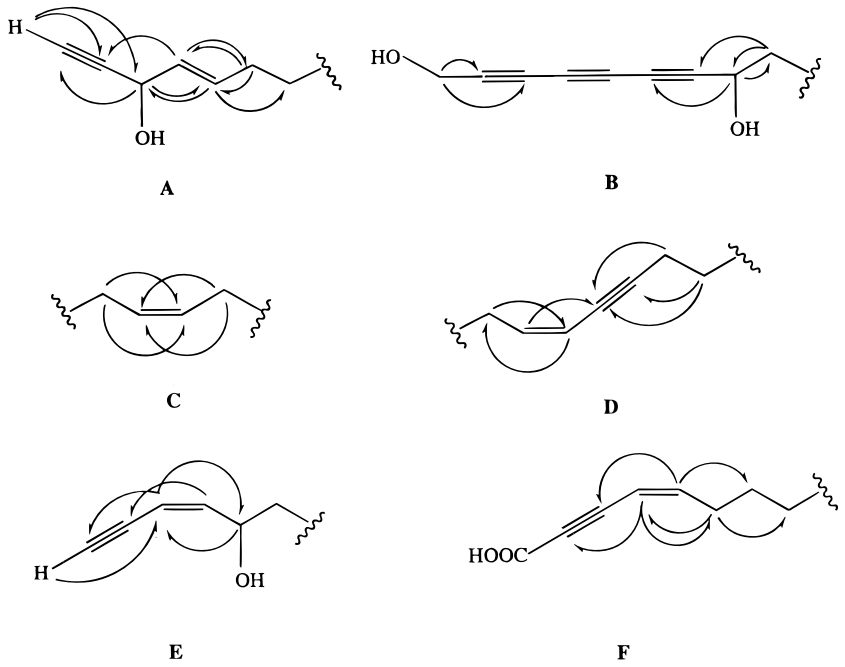

Figure 1. Partial structures with key HMBC correlations.

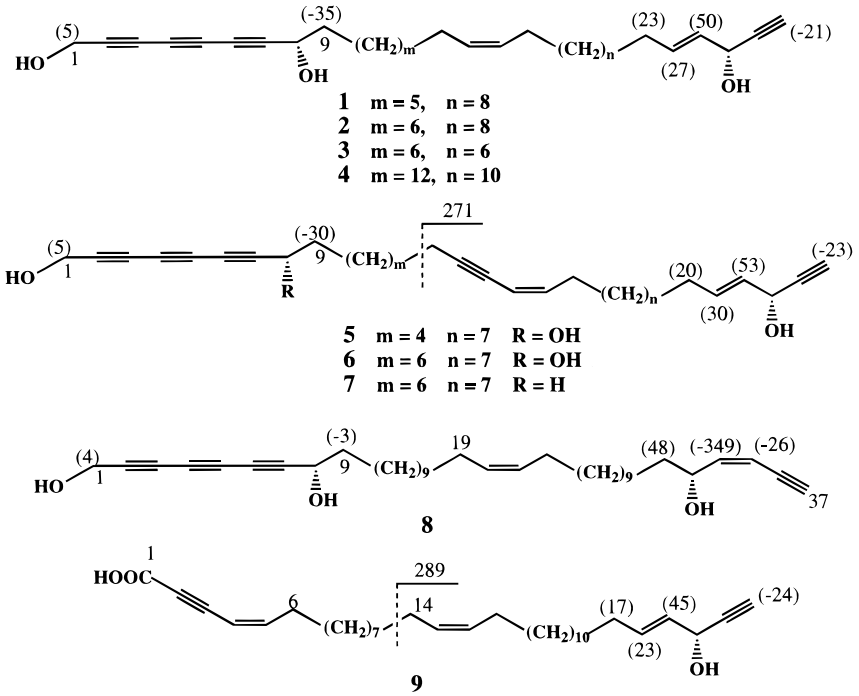

Figure 2. Structures of **1**−**9**. Key mass spectral fragmentations indicated for **5**, **6**, and **9**. Mosher ester $\Delta\delta$ values (in Hz) indicated in parentheses for **1**, **6**, **8**, and **9**.

amount of **3** and **4**, their stereochemistry was not independently determined. It is likely that they have the same configurations as **1** and **2**, since all four compounds had the same sign and magnitude of optical rotation.

Triangulyne E (**5**) analyzed for $C_{32}H_{42}O_3$ by HRCIMS. The presence of two extra degrees of unsaturation relative to triangulynes A−D was consistent with the ^{13}C NMR spectra, in which an additional acetylene functionality was observed (δ 77.5, 94.5). In addition to substructure units **A** and **B**, detailed analyses of COSY and HMBC data also revealed substructure **D** (Figure 1). The proton signal at δ 5.79 was vicinally coupled to the olefinic proton at δ 5.41 (J = 10.5 Hz). Additionally, the acetylene carbon at δ 77.5 showed HMBC correlations to proton signals at δ 5.79 (H-18) and δ 2.31 (H-14), indicating a conjugated enyne partial structure. The C-17 olefin was assigned as Z, based on the coupling constant (J = 10.5 Hz). Partial structures **A**, **B**, and **D** were connected in a linear manner to give **5**. The number of methylene units (m and n) was deduced as 4 and 7, based on the observation of mass fragments m/z 271 (Figure 2) and 207 [271 − C_5H_4].

The structure elucidation of triangulyne F (**6**) followed a similar course, as the molecular formula ($C_{34}H_{46}O_3$) obtained from HRFABMS clearly indicated that it was homologous to **5**. Analyses of NMR spectral data, including COSY, HMQC, and HMBC, established the same substructure units **A**, **B**, and **D**. The observation

of mass fragments *m/z* 271 and 207, as in **5**, suggested that $n = 7$ and $m = 6$ for **6**. Additional fragments at *m/z* 309 and 245, corresponding to α-cleavages at C-21 and C-17, respectively, supported this assignment. The stereochemistry at the carbinol centers for both **5** and **6** was also determined by the modified Mosher's method as 8*S*,30*R* and 8*S*,32*S*, respectively (Figure 2).

The molecular formula of the minor constituent triangulyne G (**7**), established by HRCIMS as $C_{34}H_{46}O_2$, indicated the presence of 12 degrees of unsaturation, the same as triangulynes E and F. Although most of the ^{1}H and ^{13}C NMR signals were similar to those of **5** and **6**, one notable difference was the lack of an oxymethine signal. Instead, an additional triplet proton signal at δ 2.27 was observed. HMBC correlations from δ 2.27 to the conjugated acetylene carbons at δ 82.0 and 64.3 revealed that a hydroxyl group was not present at C-8. Other functional group assignments followed a parallel course as described for **1**−**6** to provide **7**. Due to the paucity of pure **7**, the chirality at C-32 was not determined.

Triangulyne H (**8**) was also isolated as a minor compound. Its molecular formula ($C_{37}H_{56}O_3$, HR-FABMS) indicated the presence of 10 degrees of unsaturation, the same as triangulynes A−D (**1**−**4**). The ^{1}H NMR showed features similar to compounds **1**−**4**, except for the alkyne proton signal. A new signal at δ 3.12 appeared in place of the terminal acetylene proton signal at δ 2.55 present in all other triangulynes. In the ^{13}C NMR spectra, a pair of olefin signals at δ 108.9 (C-35) and δ 147.4 (C-34) was observed in place of those (δ 128.3 and 134.6) seen in substructure **A**. COSY, HMQC, and HMBC experiments revealed partial structure **E** (Figure 1), along with substructures **B** and **C**. In partial structure **E**, the olefin signal at δ 108.9 (C-35) showed HMBC correlations to δ 3.12 (H-37) and the oxymethine at δ 4.66 (H-33, δ_C 70.1). The geometry of the C-34 double bond was assigned as *Z* on the basis of the vicinal coupling constant of 11.5 Hz. As with triangulynes A−G, the C-20-isolated olefin in **8** was assigned the *Z* configuration on the basis of the chemical shifts of the allylic carbons (δ 26.7 and 27.1).[10] The location of the isolated *Z* double bond was deduced by analysis of mass spectral fragment ions at *m/z* 327 [**B** + $10CH_2$ + CH_2CH═CH] and 275 [**E** + $10CH_2$ + CH_2-CH═CH]. The stereochemistry was determined by employing the modified Mosher method. Thus, triangulyne H (**8**) was 1,8(*S*),33(*R*)-trihydroxyheptatriaconta-20(*Z*),34(*Z*)-diene-2,4,6,36-tetrayne.

Triangulynic acid (**9**) was isolated as a white solid from the more polar $CHCl_3$ fraction. In the IR spectrum, absorptions for acetylenic (2212 cm^{-1}) and carboxylic acid (1695 and 3400 cm^{-1}) functionalites were observed. Following treatment with CH_2N_2, the methylation product of **9** showed a singlet at δ 3.80 integrating for three protons, confirming the presence of a carboxylic acid functional group. The constitution of **9** was deduced from the molecular formula of the methyl ester, $C_{34}H_{54}O_3$, established by HRFABMS. Substructure **F** (Figure 1), along with partial structures **A** and **C**, was obtained from interpretation of ^{1}H−^{1}H COSY, DEPT, HMQC, and HMBC spectra. The *Z* olefinic protons ($J = 10.5$ Hz) at δ 5.53 (H-4) and δ 6.24 (H-5) showed HMBC correlations to the substituted acetylene carbons (δ 84.6 and 84.7), thus providing partial structure **F**. To account for the remaining atoms, the three partial structures **A**, **C**, and **F** had to be connected through methylene units to give **9**. A large coupling constant (15.5 Hz) between δ 5.89 (H-29) and δ 5.58 (H-30) led to the assignment of the *E* configuration at C-29, while the *Z* geometry at C-15 was indicated by the carbon chemical shifts of the allylic carbons (δ 27.2). Esterification with CH_2N_2, followed by derivatization with Mosher's reagent, allowed the assignment of the 31*R* configuration for **9**. FABMS of both **9** and its methyl ester produced a fragment ion at *m/z* 289 [**A** + $10CH_2$ + CH_2CH═CH], which identified C-15 as the location of the isolated double bond (Figure 2).

Triangulynic acid showed structural similarities to the corticatic acids, recently reported from the sponge genus *Petrosia*.[14] In a recently published summary of the 1994 U.S.−Japan Seminar on Marine Bioorganic Chemistry, Rinehart and Tachibana pointed to a preliminary report from the Schmitz group of a compound which strongly resembled triangulynic acid from *P. triangulata*.[15]

Triangulynes A−H (**1**−**8**) demonstrated similar *in vitro* cytotoxicity profiles and generally comparable overall potency against the NCI human tumor cell line panels.[4−7] In general, leukemia, colon, and melanoma tumor lines showed greater sensitivity to **1**−**8**. It is interesting to note that triangulynic acid (**9**) was less potent than **1**−**8** and did not exhibit differential cytotoxicity. In addition, the differential cytotoxicity patterns produced by the triangulynes (**1**−**8**) did not resemble or COMPARE[7] to those produced by the simple C_{20}−C_{23} enynols from *Cribrochalina vasculum*,[16] but they did closely match that exhibited by vasculyne, a C_{43} bis-enynol from a different collection of *C. vasculum*.[17] Repetitive testing of **1**, as representative of the series, yielded mean panel GI_{50}, TGI, and LC_{50} concentrations of 0.5, 2.0, and 12 μM, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in $CHCl_3$. UV spectra were recorded on a Beckman DU-64 spectrophotometer. FT-IR spectra were obtained on a Perkin-Elmer 267 spectrometer. High-resolution mass spectra were measured on a Finnegan MAT 90 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 spectrometer using $CDCl_3$ as solvent and internal standard (^{1}H, 7.24 ppm, and ^{13}C, 77.0 ppm). The number of attached protons for each carbon was determined from DEPT experiments. HPLC was performed on a Waters 600E system using a Waters 990 photodiode array detector.

Animal Material. Samples of *P. triangulata* Desqueyroux-Faundez were collected by the Coral Reef Foundation, under contract to the NCI, at a depth of 15 m on the south oceanside reef (151°, 6.05′ E, 7°, 0.00′ N) in Kuop Atoll, Micronesia, in September, 1992. The sponge was identified by M. Kelly-Borges; a voucher specimen is on deposit at the Smithsonian Institution Sorting Center, Suitland, MD.

Extraction and Purification. Sponge samples were kept frozen prior to extraction. The frozen sponge was ground (110.33 g) with dry ice and extracted with H_2O at 4 °C; the aqueous extract was removed by centrifugation and lyophilized. The sponge residue was

also lyophilized and extracted overnight at room temperature with MeOH−CH_2Cl_2 (1:1), followed by MeOH. Solvents from the combined organic extracts were removed *in vacuo* to give a residue (13.13 g). A portion of this extract (5.10 g) was partitioned[8] to give hexane (0.56 g), CCl_4 (2.29 g), $CHCl_3$ (1.37 g), and H_2O (0.85 g) fractions. The cytotoxic CCl_4 fraction was further fractionated by Sephadex LH-20 column chromatography to afford fractions A−D. The active fractions B and D were separated by HPLC on a Rainin Microsorb C_{18} column (5 μm, 1 × 25 cm) using MeOH−H_2O (93:7) as mobile phase to yield compounds **1** (154 mg, 1.73% wet weight), **2** (13.6 mg, 0.15%), **3** (7 mg, 0.07%), **4** (3.9 mg, 0.04%), **5** (8.6 mg, 0.08%), **6** (14.6 mg, 0.16%), **7** (3.7 mg, 0.04%), and **8** (3.3 mg, 0.04%). The cytotoxic $CHCl_3$ fraction was separated on a Sephadex LH-20 column and eluted with CH_2Cl_2−MeOH (2:1) and produced fractions E−I. Cytotoxic fraction H (70 mg) was further purified by HPLC on a Rainin cyano-bonded phase column (5 μm, 1 × 25 cm) using hexane−*i*-PrOH (17:3, 0.1% AcOH) to yield compound **9** (13.2 mg, 0.15%).

1,8(*S*),30(*R*)-Trihydroxydotriaconta-16(*Z*),28(*E*)-diene-2,4,6,31-tetrayne (1): white powder; $[\alpha]_D$ −15° (*c* 1.56, $CHCl_3$); HRFABMS *m/z* 479.3524, calcd for $C_{32}H_{47}O_3$ 479.3525; LRCIMS (methane) *m/z* 479 [MH^+] (1), 477 [$M^+ - H$] (1), 461 [479 − H_2O]$^+$ (1), 443 [479 − $2H_2O$]$^+$ (1), 399 [479 − 80]$^+$ (5), 271 (1), 261 (1), 133 (2), 115 (1), 109 (2), 103 (2), 95 (3), 81 (8), 63(100); UV (MeOH) λ_{max} (log ϵ) 257 (2.6), 242 (2.8), 230 (3.0), 204 (3.5) nm; IR (film) ν_{max} 3294, 2920, 2851, 2254, 2118, 1669, 1466, 1218, 1020, 967, 759, 721, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 7, 1.5 Hz, H-28), 5.58 (1H, ddt, *J* = 15, 6, 1.5 Hz, H-29), 5.32 (2H, dt *J* = 9, 5 Hz, H-16, 17), 4.81 (1H, ddd, *J* = 6, 2, 1.5 Hz, H-30), 4.40 (1H, t, *J* = 7 Hz, H-8), 4.32 (2H, s, H-1), 2.54 (1H, d, *J* = 2 Hz, H-32), 2.04 (2H, q, *J* = 7 Hz, H-27), 1.99 (4H, dt, *J* = 7, 5 Hz, H-15, 18), 1.69 (2H, m, H-9), 1.41 (m), 1.38 (m), 1.25 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.6 (C-28), 129.9 (C-17), 129.8 (C-16), 128.2 (C-29), 83.3 (C-31), 80.5 (C-4, 5, 7), 77.5 (C-2), 74.0 (C-32), 69.7 (C-3), 68.8 (C-6), 62.7 (C-8, 30), 51.2 (C-1), 37.4 (C-9), 31.9 (C-27), 30.0−29.2 (C-11 to C-14 and C-19 to C-26), 28.8 (C-26), 27.1 (C-15), 27.0 (C-18), 25.0 (C-10).

1,8(*S*),31(*R*)-Trihydroxytritriaconta-17(*Z*),29(*E*)-diene-2,4,6,32-tetrayne (2): white powder; $[\alpha]_D$ −14° (*c* 0.8, $CHCl_3$); HRCIMS (isobutane) *m/z* 493.3681, calcd for $C_{33}H_{49}O_3$ 493.3681; LRCIMS (isobutane) *m/z* 494 [$M^+ + 2H$] (87), 493 [MH^+] (3), 492 [M^+] (4), 476 [494 − H_2O]$^+$ (100), 474 [M − H_2O]$^+$ (3), 456 [M − $2H_2O$]$^+$ (2), 414 [494 − 80]$^+$ (88), 285 (4), 271 (2), 133 (3), 131 (5), 109 (10), 95 (26), 81 (62); UV (MeOH) λ_{max} (log ϵ) 256 (2.7), 226 (3.2), 204 (23.5) nm; IR (film) ν_{max} 3308, 2921, 2851, 2254, 2160, 1669, 1465, 1021, 968, 759, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 7, 1 Hz, H-29), 5.59 (1H, ddt, *J* = 15, 6, 1 Hz, H-30), 5.32 (2H, dt, *J* = 10, 6 Hz, H-17, 18), 4.82 (1H, ddt, *J* = 6, 2, 1 Hz, H-31), 4.41 (1H, t, *J* = 7 Hz, H-8), 4.32 (2H, s, H-1), 2.55 (1H, d, *J* = 2 Hz, H-33), 2.05 (2H, q, *J* = 7 Hz, H-28), 1.99 (4H, dt, *J* = 7, 6 Hz, H-16, 19), 1.68 (2H, q, *J* = 7 Hz, H-9), 1.41 (m), 1.37 (m), 1.24 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.7 (C-29), 129.9 (C-17, 18), 128.2 (C-30), 83.3 (C-32), 80.5 (C-4, 5, 7), 77.5 (C-2), 74.0 (C-33), 69.7 (C-3), 68.8 (C-6), 62.8 (C-8, 31), 51.4 (C-1), 37.4 (C-9), 31.9 (C-28), 30.0−29.2 (C-11 to C-15 and C-20 to C-27), 28.8 (C-27), 27.2 (C-19), 27.0 (C-16), 25.0 (C-10).

1,8,29-Trihydroxyhentriaconta-17(*Z*),27(*E*)-diene-2,4,6,30-tetrayne (3): white powder; $[\alpha]_D$ −19° (*c* 0.66, $CHCl_3$); HRFABMS *m/z* 465.3371, calcd for $C_{31}H_{45}O_3$ 465.3368; LRCIMS (methane) *m/z* 465 [MH^+] (40), 464 [M^+] (17), 447 [M + H − H_2O]$^+$ (100), 435 [M + H − CH_2OH]$^+$ (11), 429 [447 − H_2O]$^+$ (19), 409 [M + H − C_3H_4O]$^+$ (9), 385 (30), 285 (10), 193 (5), 133 (3), 115 (8), 81 (9); UV (MeOH) λ_{max} (log ϵ) 257 (2.5), 243 (2.7), 231 (2.8), 204 (3.4) nm; IR (film) ν_{max} 3308, 2919, 2852, 2254, 2161, 2119, 1669, 1464, 1217, 1020, 969, 759, 721, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 7, 1.5 Hz, H-27), 5.59 (1H, ddt, *J* = 15, 6, 1.5 Hz, H-28), 5.33 (2H, dt, *J* = 11, 5 Hz, H-17, 18), 4.82 (1H, ddd, *J* = 6, 2, 1.5 Hz, H-29), 4.41 (1H, t, *J* = 7 Hz, H-8), 4.32 (2H, s, H-1), 2.55 (1H, d, *J* = 2 Hz, H-31), 2.04 (2H, q, *J* = 7 Hz, H-26), 1.99 (4H, dt, *J* = 7, 5 Hz, H-16, 19), 1.70 (2H, m, H-9), 1.42 (m), 1.37 (m), 1.25 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.7 (C-27), 129.9 (C-17, 18), 128.3 (C-28), 83.3 (C-30), 80.5 (C-4, 5, 7), 77.5 (C-2), 74.0 (C-31), 69.8 (C-3), 68.8 (C-6), 62.8 (C-8, 29), 51.5 (C-1), 37.5 (C-9), 31.9 (C-26), 29.7−29.2 (C-11 to C-15 and C-20 to C-24), 28.8 (C-25), 27.2 (C-16, 19), 25.0 (C-10).

1,8,39-Trihydroxyhentetraconta-23(*Z*),37(*E*)-diene-2,4,6,40-tetrayne (4): colorless oil; $[\alpha]_D$ −10.7° (*c* 0.013, $CHCl_3$); HRFABMS *m/z*, 605.4919, calcd for $C_{41}H_{65}O_3$ 605.4933; LRCIMS (methane) *m/z* 605 [MH^+] (1), 477 (4), 415 (2), 369 (1), 289 (4), 249 (2), 133 (1), 115 (4), 109 (5), 95 (7), 81 (6), 55 (100); UV (MeOH) λ_{max} (log ϵ) 256 (2.8), 230 (3.1), 203 (3.6) nm; IR (film) ν_{max} 3284, 2920, 2848, 1466, 1118, 1065, 1011, 965, 722, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 7, 1.5 Hz, H-37), 5.59 (1H, ddt, *J* = 15, 6, 1.5 Hz, H-38), 5.33 (2H, dt, *J* = 10, 5 Hz, H-23, 24), 4.82 (1H, br, H-39), 4.41 (1H, dt, *J* = 7, 6 Hz, H-8), 4.33 (2H, d, *J* = 5 Hz, H-1), 2.55 (1H, d, *J* = 2 Hz, H-41), 2.05 (2H, q, *J* = 7 Hz, H-36), 1.99 (4H, dt, *J* = 7, 5 Hz, H-22, 25), 1.70 (2H, m, H-9), 1.61 (m), 1.53 (m), 1.39 (m), 1.24 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.7 (C-37), 129.9 (C-23, 24), 128.3 (C-38), 83.3 (C-40), 80.6 (C-4, 5, 7), 77.5 (C-2), 74.0 (C-41), 69.8 (C-3), 68.8 (C-6), 62.9 (C-39), 62.8 (C-8), 51.5 (C-1), 37.5 (C-9), 31.9 (C-36), 30.9−28.8 (C-10 to C-21 and C-26 to C-35), 27.2 (C-22), 27.1 (C-25).

1,8,30-Trihydroxydotriaconta-17(*Z*),28(*E*)-diene-2,4,6,15,31-pentayne (5): white powder; $[\alpha]_D$ −11.4° (*c* 0.42, $CHCl_3$); HRCIMS (isobutane) *m/z* 475.3214, calcd for $C_{32}H_{43}O_3$ 475.3212; LRCIMS (isobutane) *m/z* 476 [$M^+ + 2H$] (17), 474 [M^+] (1), 458 [476 − H_2O]$^+$ (18), 440 [476 − $2H_2O$]$^+$ (1), 396 [476 − 80]$^+$ (9), 271 (2), 207 (2), 133 (3), 109 (9), 95 (28), 81 (100); UV (MeOH) λ_{max} (log ϵ) 227 (4.0), 203 (3.7) nm; IR (film) ν_{max} 3305, 2925, 2853, 2208, 2122, 1669, 1463, 1020, 967 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 7, 1 Hz, H-28), 5.79 (1H, dt, *J* = 10.5, 7 Hz, H-18), 5.59 (1H, ddt, *J* = 15, 6, 1.5 Hz, H-29), 5.41 (1H, dt, *J* = 10.5, 2 Hz, H-17), 4.82 (1H, ddt, *J* = 6, 2, 1 Hz, H-30), 4.41 (1H, t, *J* = 7 Hz, H-8), 4.32 (2H, s, H-1), 2.55 (1H, d, *J* = 2 Hz, H-32), 2.31 (2H, dt, *J* = 7, 2 Hz, H-14), 2.26 (2H, q, *J* = 7 Hz, H-19), 2.05 (2H, q, *J* = 7 Hz, H-27), 1.68 (2H, q, *J* = 7 Hz, H-9), 1.51 (m), 1.37 (m), 1.26 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.6 (C-18), 134.6 (C-28), 128.3 (C-29), 109.4 (C-17), 94.5 (C-15), 83.3 (C-31), 80.6 (C-4, 5, 7), 77.5 (C-16), 77.4 (C-2), 74.0 (C-32), 69.8 (C-3), 68.8 (C-6), 62.8 (C-8, 30), 51.5 (C-1), 37.5 (C-9), 32.0 (C-27),

30.0 (C-19), 29.6−25.0 (C-10 to C-13 and C-20 to C-26), 19.5 (C-14).

1,8(*S*),32(*R*)-Trihydroxytetratriaconta-19(*Z*),30(*E*)-diene-2,4,6,17,33-pentayne (6): white powder; $[\alpha]_D$ −10.6° (*c* 1.15, $CHCl_3$); HRFABMS *m/z* 503.3528, calcd for $C_{34}H_{47}O_3$ 503.3525; LRCIMS (methane) *m/z* 503 $[MH^+]$ (59), 502 $[M^+]$ (9), 485 $[503 - H_2O]^+$ (55), 447 $[502 - 55]^+$ (9), 370 [503 − 133] (9), 309 (4), 295 (4), 289 $[370 - 81]^+$ (4), 271 (2), 245 (20), 207 (5), 133 (11), 115 (100), 103 (3), 95 (27), 81 (33); UV (MeOH) λ_{max} (log ϵ) 227 (4.1), 202 (3.8) nm; IR (film) ν_{max} 3299, 2921, 2849, 2159, 1668, 1463, 1075, 1030, 964, 724 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.89 (1H, ddt, *J* = 15.5, 6, 1 Hz, H-30), 5.78 (1H, dt, *J* = 10.5, 7 Hz, H-20), 5.59 (1H, ddt, *J* = 15.5, 6, 1.5 Hz, H-31), 5.41 (1H, dt, *J* = 10.5, 2 Hz, H-19), 4.82 (1H, ddt, *J* = 6, 2.5, 1 Hz, H-32), 4.41 (1H, t, *J* = 7 Hz, H-8), 4.32 (2H, s, H-1), 2.55 (1H, d, *J* = 2.5 Hz, H-34), 2.31 (2H, dt, *J* = 7, 2 Hz, H-16), 2.26 (2H, q, *J* = 7 Hz, H-21), 2.04 (2H, q, *J* = 7 Hz, H-29), 1.69 (2H, q, *J* = 7 Hz, H-9), 1.51 (m), 1.37 (m), 1.26 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.6 (C-20), 134.6 (C-30), 128.3 (C-31), 109.3 (C-19), 94.5 (C-17), 82.5 (C-33), 80.6 (C-4, 5, 7), 77.5 (C-2, 18), 74.0 (C-34), 69.8 (C-3), 68.8 (C-6), 62.8 (C-8, 32), 51.5 (C-1), 37.5 (C-9), 31.9 (C-29), 30.0 (C-21), 29.6−25.0 (C-10 to C-15 and C-22 to C-28), 19.5 (C-16).

1,32-Dihydroxytetratriaconta-19(*Z*),30(*E*)-diene-2,4,6,17,33-pentayne (7): white powder; $[\alpha]_D$ −10.5° (*c* 0.37, $CHCl_3$); HRCIMS (isobutane) *m/z* 487.3575, calcd for $C_{34}H_{47}O_2$, 487.3576; LRCIMS (isobutane) *m/z* 488 $[M^+ + 2H]$ (100), 486 $[M^+]$ (3), 470 $[488 - H_2O]^+$ (35), 452 $[488 - 2H_2O]^+$ (1), 293 (1), 271 (1), 173 (2), 159 (3), 145 (4), 131 (4), 109 (5), 95 (9), 81 (13); UV (MeOH) λ_{max} (log ϵ) 306 (2.5), 227 (4.2), 203 (4.0) nm; IR (film) ν_{max} 3287, 2915, 2849, 2341, 1652, 1558, 1470, 1029, 963, 718, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, ddt, *J* = 15, 6.5, 1 Hz, H-30), 5.79 (1H, dt, 10, 7, H-20), 5.59 (1H, ddt, 15, 6.5, 1.5, H-31), 5.41 (1H, dt, 10, 2, H-19), 4.82 (1H, ddt, 6.5, 2, 1, H-32), 4.30 (2H, s, H-1), 2.55 (1H, d, 2, H-34), 2.31 (2H, dt, 7, 2, H-16), 2.27 (2H, t, 6, H-8), 2.25 (2H, q, 7 Hz, H-21), 2.05 (2H, dt, 7, 6.5, H-29), 1.51 (m), 1.37 (m), 1.24 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.6 (C-20), 134.6 (C-30), 128.4 (C-31), 109.4 (C-19), 94.5 (C-17), 83.3 (C-33), 82.0 (C-4, 5, 7), 77.4 (C-18), 74.0 (C-34), 73.5 (C-2), 71.0 (C-3), 64.3 (C-6), 62.8 (C-32), 51.6 (C-1), 32.0 (C-29), 30.0 (C-21), 29.6−29.1 (C-10 to C-15 and C-22 to C-28), 28.8 (C-9), 19.5 (C-16), 19.3 (C-8).

1,8(*S*),33(*R*)-Trihydroxyheptatriaconta-20(*Z*),34(*Z*)-diene-2,4,6,36-tetrayne (8): colorless oil; $[\alpha]_D$ −23.7° (*c* 0.32, $CHCl_3$); HRFABMS *m/z* 549.4314, calcd for $C_{37}H_{57}O_3$ 549.4307; LRCIMS (methane) *m/z* 549 $[MH^+]$ (11), 548 $[M^+]$ (3), 531 $[549 - H_2O]^+$ (5), 513 $[549 - 2H_2O]^+$ (3), 495 $[549 - 3H_2O]^+$ (14), 479 $[M - C_4H_3 - H_2O]^+$ (66), 467 $[M - C_5H_5O]^+$ (19), 461 $[M - C_4H_3 - 2H_2O]^+$ (64), 327 (4), 275 (6), 133 (20), 115 (7), 109 (52), 103 (9), 95 (74), 81 (100); UV (MeOH) λ_{max} (log ϵ) 255 (2.7), 223 (4.1), 203 (3.8) nm; IR (film) ν_{max} 3309, 2924, 2853, 1464, 1023, 721, 668, 636 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, ddt, *J* = 11.5, 7, 0.5 Hz, H-34), 5.52 (1H, ddt, *J* = 11.5, 2.0, 0.5 Hz, H-35), 5.33 (2H, dt, *J* = 9, 6 Hz, H-20, 21), 4.66 (1H, ddd, *J* = 7, 7, 0.5 Hz, H-33), 4.41 (1H, t, *J* = 6.5 Hz, H-8), 4.33 (2H, s, H-1), 3.12 (1H, d, *J* = 2 Hz, H-37), 1.99 (4H, dt, *J* = 7, 6 Hz, H-19, 22), 1.69 (2H, dt, *J* = 7, 6.5 Hz, H-9), 1.62 (2H, m, H-32), 1.51 (m), 1.42 (m), 1.24 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.4 (C-34), 129.9 (C-20, 21), 108.9 (C-35), 82.8 (C-37), 80.6 (C-4, 5, 7), 79.5 (C-36), 77.6 (C-2), 70.1 (C-33), 69.8 (C-3), 68.8 (C-6), 62.9 (C-8), 51.5 (C-1), 37.5 (C-9), 37.0 (C-32), 36.5 (C-31), 30.2−28.7 (C-11 to C-18 and C-23 to C-30), 27.1 (C-19), 26.7 (C-22), 25.0 (C-10).

31(*R*)-Hydroxytritriaconta-4(*Z*),15(*Z*),29(*E*)-triene-2,32-diynoic acid (9): colorless oil; $[\alpha]_D$ −12.9° (*c* 1.20, $CHCl_3$); negative HRFABMS of methyl ester of **9** *m/z* 509.3971, calcd for $C_{34}H_{53}O_3$ 509.3995; positive FABMS of **9** *m/z* 479 $[M - OH]^+$ (7), 289 $[\mathbf{A} + 11CH_2 + HC{=}CHCH_2]^+$ (3); positive FABMS of methyl ester of **9** *m/z* 493 $[M - OH]^+$ (9), 479 $[M - OCH_3]^+$ (7), 433 $[M - COOCH_3 - H_2O]^+$ (3), 289 $[\mathbf{A} + 11CH_2 + HC{=}CHCH_2]^+$ (17); UV (MeOH) λ_{max} (log ϵ) 247 (4.0), 241 (4.0), 203 (4.0) nm; IR (film) ν_{max} 3400, 3309, 3004, 2924, 2853, 2212, 1695, 1682, 1464, 1402, 1369, 1276, 1087, 1009, 968, 722, 660, 587 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.24 (1H, dt, *J* = 10.5, 8 Hz, H-5), 5.89 (1H, dt, *J* = 15.5, 6.5 Hz, H-29), 5.58 (1H, dd, *J* = 15.5, 6.5 Hz, H-30), 5.53 (1H, d, *J* = 10.5 Hz, H-4), 5.32 (2H, dt, *J* = 9, 5.5 Hz, H-15, 16), 4.83 (1H, m, H-31), 2.54 (1H, d, *J* = 2 Hz, H-33), 2.35 (2H, dt, *J* = 8, 7 Hz, H-6), 2.04 (2H, dt, *J* = 7, 6.5 Hz, H-28), 1.99 (4H, dt, *J* = 7, 6.5 Hz, H-14, 17), 1.39 (m), 1.25 (m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.0 (C-1), 152.0 (C-5), 134.7 (C-29), 129.9 (C-15, 16), 128.2 (C-30), 106.5 (C-4), 84.7 (C-3), 84.6 (C-2), 83.2 (C-32), 74.1 (C-33), 62.8 (C-31), 31.9 (C-28), 31.0 (C-6), 30.0−28.6 (C-7 to C-13, and C-18 to C-27), 27.2 (C-14, 17).

MTPA Esters of Compounds 1, 2, 6, 8, and 9. The polyacetylene (2−6 mg) was dissolved in distilled pyridine (0.5 mL). Then, 4× molar excess of α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) and a catalytic amount (few granules) of 4-(dimethylamino)-pyridine were added to the solution. The mixture was stirred at room temperature overnight under argon. The progress of the reaction was monitored by TLC (cyano-bonded phase, hexane−*i*-PrOH, 17:3). The reaction was quenched by removal of solvent *in vacuo*, and the residue obtained was redissolved in 0.5 mL of CH_2Cl_2 and applied to a small cyano-bonded phase column (2 × 4 cm) equilibrated with hexane. Vacuum−liquid chromatography, eluted by hexane (40 mL), gave pure Mosher's ester derivatives. Both *R*- and *S*-esters were prepared for each compound and characterized by 500 MHz ^1H and $^1H-^1H$ COSY NMR spectral data.

Ozonolysis of Compound 1. Ozone (O_3) was introduced for 5 min (−78 °C) to a 10 mL CH_2Cl_2 solution of **1** (10 mg). After removal of CH_2Cl_2, the reaction products were dissolved in 10 mL of distilled H_2O, followed by addition of six drops of a 30% solution of H_2O_2. The mixture was brought to reflux for 1 h. After it was cooled to room temperature, the reaction mixture was extracted with Et_2O (3 × 15 mL). The combined organic extract was concentrated to about 5 mL and then was treated with freshly generated CH_2N_2 at room temperature for 1 h. Removal of solvent gave the methyl ester product.

Antitumor Testing Data of 1. The tumor cell line subpanels are identified as follows: 1 (leukemia); II (lung, non small-cell); III (colon); IV (CNS); V (melanoma); VI (ovarian); VII (renal); VIII (prostate); IX (breast). The subpanel and individual cell line identi-

fiers are given, along with the corresponding negative $\log_{10}$ GI_{50}, TGI, and LC_{50} values, respectively. The results for compound **1** (average of quadruplicate tests) are representative of the series: [I], CCRF-CEM (6.77, 6.11, 4.47), HL-60 (TB) (7.08, 5.44, 4.60), K-562 (6.43, 5.64, 4.43), MOLT-4 (6.68, 6.09, 4.17), RPMI-8226 (7.51, 6.00, 4.00), SR (6.74, 5.49, 4.00); [II], A549/ATCC (6.47, 5.24, 4.32), EKVX (6.06, 5.62, 5.17), HOP-62 (5.80, 5.27, 4.12), HOP-92 (6.49, 5.92, 4.82), NCI-H226 (6.22, 5.72, 5.09), NCI-H23 (6.59, 5.89, 4.85), NCI-H322M (5.48, 4.92, 4.24), NCI-H460 (6.46, 5.74, 4.37), NCI-H522 (6.82, 6.42, 6.19); [III], COLO 205 (6.70, 6.38, 5.64), HCC-2998 (6.59, 6.16, 5.66), HCT-116 (6.89, 6.18, 5.46), HCT-15 (6.39, 5.68, 5.14), HT29 (6.44, 5.89, 5.44), KM12 (6.77, 6.42, 5.96), SW-620 (6.47, 5.89, 5.03); [IV], SF-268 (6.46, 5.41, 4.89), SF-295 (5.54, 5.02, 4.29), SF-539 (5.96, 5.48, 4.72), SNB-19 (5.47, 4.92, 4.28), SNB-75 (5.70, 5.18, 4.80), U251 (6.09, 5.66, 5.28); [V], LOX IMVI (6.85, 6.44, 5.10), MALME-3M (6.60, 6.19, 5.72), M14 (6.80, 6.09, 5.46), SK-MEL-2 (6.60, 6.05, 5.11), SK-MEL-28 (6.15, 5.64, 4.92), SK-MEL-5 (7.14, 6.70, 6.32), UACC-257 (6.29, 5.68, 5.09), UACC-62 (6.64, 6.19, 5.04); [VI], IGR-OV1 (6.31, 5.74, 5.09), OVCAR-3 (6.43, 5.82, 5.28), OVCAR-4 (6.07, 5.20, 4.42), OVCAR-5 (5.35, 4.92, 4.46), OVCAR-8 (6.89, 6.07, 4.66), SK-OV-3 (5.55, 5.10, 4.43); [VII], 786−0 (6.54, 5.96, 5.40), A498 (6.28, 5.80, 5.28), ACHN (6.11, 5.14, 4.49), CAKI-1 (6.60, 6.17, 5.47), RXF-393 (6.47, 5.89, 4.74), SN12C (5.85, 4.96, 4.49), TK-10 (6.19, 5.68, 4.96), UO-31 (5.89, 5.57, 5.26); [VIII], PC-3 (6.72, 5.96, 5.00), DU-145 (5.85, 5.26, 4.74); [IX], MCF-7 (5.80, 5.22, 4.59), MCF7/ADR-RES (6.52, 5.29, 4.49), MDA-MB-231/ATCC (6.64, 6.22, 5.04), HS 578T (5.62, 5.08, 4.24), MDA-MB-435 (6.77, 6.08, 5.35), MDA-N (6.62, 5.51, 5.07), BT-549 (6.10, 5.62, 4.96), T-47D (5.82, 5.14, 4.32).

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