DNA Cleavage Potency, Cytotoxicity, and Mechanism of Action of a Novel Class of Enediyne Prodrugs

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Supporting Information:

DNA binding constants *K'* of selected compounds, and spectral, analytical, and LC-MS data

Supporting Information

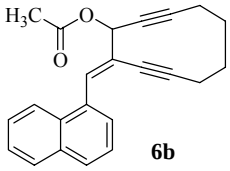
DNA Binding Constants. The DNA binding constants *K'* of selected compounds were measured in the buffer solution containing 5% DMSO using ΦX174 RFI DNA according to the reported procedure (Strothkamp, K. G.; Strothkamp, R. E. *J. Che. Edu.* **1994**, *71*, 77).

DNA binding constants *K'* (in units of 10^4 M^{-1}):

Ar	**5**	**6**	**7**	**8**
Ph (a)	3.5	6.4	0.8	3.4
1-Naph (b)	5.0	3.6	4.5	5.8
2-Naph (c)	n.d.	1.8	2.3	1.8

General Procedure for Synthesis of Acetates 6.

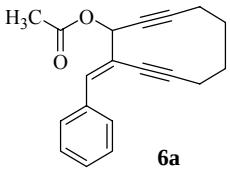
(*E*)-3-Acetoxy-4-(1'-naphtylmethylidene)cyclodeca-1,5-diyne (6b).



6b

To a solution of the alcohol **5b** (11.4 mg, 3.99 x 10^{-2} mmol) and DMAP (48.7 mg, 0.40 mmol) in dry CH_2Cl_2 (5 mL) was added acetic anhydride (20.3 mg, 0.20 mmol) followed by stirring at room temperature for 2.5 h. The reaction was then quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2, washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 14% EtOAc-hexane) to give **6b** (9.4 mg, 67%): pale yellow oil; R_f = 0.34 (14% EtOAc-hexane); IR (neat) 2934, 2239, 2229, 1740, 1226, 1012 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.17 Hz, 1 H), 8.02 (d, *J* = 7.65 Hz, 1 H), 7.83 (t, *J* = 7.74 Hz, 2 H), 7.55-7.48 (m, 3 H), 7.46 (s, 1 H), 6.22 (s, 1 H), 2.55-2.23 (m, 4 H), 2.18 (s, 3 H), 1.98-1.67 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 134.2, 132.9 (x 2), 132.2, 129.7, 129.3, 127.0 (x 2), 126.5, 125.9, 124.1, 123.6, 103.3, 93.8, 80.9, 79.4, 69.7, 28.2, 28.1, 22.5, 22.0, 21.4; MS (+CI) *m/z* (relative intensity) 329 ($M + H^+$, 20), 269 (100); HRMS (+EI) calcd for $C_{23}H_{20}O_2$ (M^+) 328.1463, found 328.1461.

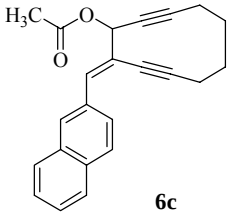
(*E*)-3-Acetoxy-4-(phenylmethylidene)cyclodeca-1,5-diyne (6a).



6a

Prepared from **5a** in 81% yield. **6a**: pale yellow oil; R_f = 0.29 (10% EtOAc-hexane); IR (neat) 2934, 2234, 2210, 1736, 1448, 1226 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91-7.88 (m, 2 H), 7.38-7.27 (m, 3 H), 6.69 (s, 1 H), 6.05 (t, J = 1.05 Hz, 1 H), 2.60-2.20 (m, 4 H), 2.12 (s, 3 H), 2.00-1.71 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 135.4, 135.3, 128.7, 128.7, 128.2, 120.4, 104.0, 93.0, 80.4, 78.6, 69.2, 27.6, 27.5, 22.0, 21.4, 20.8; MS (+CI) *m/z* (relative intensity) 279 (M + H$^+$, 15), 219 (100); HRMS (+EI) calcd for $C_{19}H_{18}O_2$ (M$^+$) 278.1307, found 278.1298.

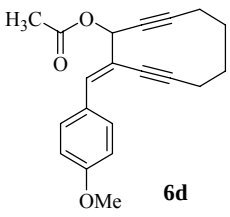
(*E*)-3-Acetoxy-4-(2'-naphtylmethylidene)cyclodeca-1,5-diyne (6c).



6c

Prepared from **5c** in 68% yield. **6c**: pale yellow oil; R_f = 0.52 (25% EtOAc-hexane); IR (neat) 2934, 2239, 2229, 1740, 1224, 1012 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23-8.19 (m, 2 H), 7.85-7.79 (m, 3 H), 7.53-7.43 (m, 2 H), 6.86 (s, 1 H), 6.11 (s, 1 H), 2.65-2.42 (m, 2 H), 2.39-2.25 (m, 2 H), 2.15 (s, 3 H), 2.00-1.67 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 136.2, 134.1, 133.9, 133.8, 129.6, 129.1, 128.5, 128.3, 127.2, 126.8, 126.4, 121.5, 104.8, 93.7, 81.2, 79.3, 70.0, 28.2, 28.1, 22.6, 22.0, 21.4; MS (+CI) *m/z* (relative intensity) 329 (M + H$^+$, 23), 269 (100); HRMS (+EI) calcd for $C_{23}H_{20}O_2$ (M$^+$) 328.1463, found 328.1468.

(*E*)-3-Acetoxy-4-[(4'-methoxyphenyl)methylidene]cyclodeca-1,5-diyne (6d).



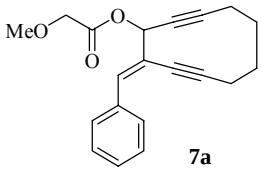
6d

Prepared from **5d** in 44% yield. **6d**: pale yellow oil; R_f = 0.44 (20% EtOAc-hexane); IR (neat) 2934, 2198, 1738, 1606, 1512, 1232, 1176, 1030 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.10-8.06 (AA'BB', 2 H), 7.12-7.07 (AA'BB', 2 H), 6.86 (s, 1 H), 6.12 (s, 1 H), 3.98 (s, 3 H), 2.68-2.36 (m, 4 H), 2.21 (s, 3 H), 2.10-1.75 (m, 4 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 169.2, 160.0, 134.3, 130.0, 128.4, 118.4, 113.5, 103.3, 92.2, 80.4, 78.8, 68.6, 54.6, 27.4, 27.3, 21.1, 20.9, 19.9; MS (+CI) *m/z*

(relative intensity) 309 (M + H^+, 8), 249 (100); HRMS (+EI) calcd for $C_{20}H_{20}O_3$ (M^+) 308.1412, found 308.1405.

General Procedure for Synthesis of Methoxyacetates 7.

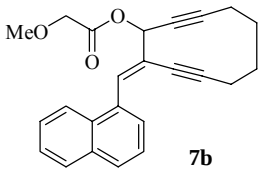
(*E*)-3-Methoxyacetoxy-4-(phenylmethylidene)cyclodeca-1,5-diyne (7a).



7a

To a solution of compound **5a** (50.0 mg, 0.212 mmol), DCC (87.3 mg, 0.432 mmol) and DMAP (259.0 mg, 2.12 mmol) in dry CH_2Cl_2 (10 mL) cooled in an ice-water bath was added methoxyacetic acid (30.6 mg, 0.339 mmol) followed by stirring at room temperature for 12 h. The reaction mixture was filtered through a short plug of Celite with rinsing by EtOAc. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 20% EtOAc-hexane) to give **7a** (51.6 mg, 79%): pale yellow oil; R_f = 0.40 (20% EtOAc-hexane); IR (neat) 2932, 2234, 2210, 1754, 1182, 1124 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.08 Hz, 2 H), 7.39-7.26 (m, 3 H), 6.72 (s, 1 H), 6.16 (d, *J* = 0.93 Hz, 1 H), 4.12 (s, 2 H), 3.45 (s, 3 H), 2.60-2.25 (m, 4 H), 1.94-1.66 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 136.5, 136.0, 129.5, 129.4, 129.0, 120.9, 104.8, 94.3, 80.9, 78.8, 70.4, 70.3, 60.1, 28.1, 28.0, 22.5, 21.3; MS (+CI) *m/z* (relative intensity) 309 (M + H^+, 20); HRMS (+EI) calcd for $C_{20}H_{20}O_3$ (M^+) 308.1412, found 308.1403.

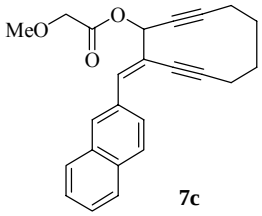
(*E*)-3-Methoxyacetoxy-4-(1'-naphtylmethylidene)cyclodeca-1,5-diyne (7b).



7b

Prepared from **5b** in 65% yield. **7b**: pale yellow oil; R_f = 0.38 (20% EtOAc-hexane); IR (neat) 2932, 2239, 2229, 1756, 1182, 1126 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.29 Hz, 1 H), 8.14-8.00 (m, 1 H), 7.92-7.81 (m, 2 H), 7.58-7.44 (m, 3 H), 7.50 (s, 1 H), 6.33 (t, *J* = 1.05 Hz, 1 H), 4.15 and 4.13 (AB q, *J* = 16.50 Hz, 2 H), 3.48 (s, 3 H), 2.55-2.20 (m, 4 H), 2.00-1.60 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 134.2, 133.4, 132.8, 132.2, 129.8, 129.3, 127.1, 127.0, 126.5, 125.9, 124.1, 123.3, 103.5, 94.4, 80.8, 79.0, 70.6, 70.2, 60.1, 28.2, 28.1, 22.5, 21.4; MS (+CI) *m/z* (relative intensity) 359 (M + H^+, 4), 269 (100); HRMS (+EI) calcd for $C_{24}H_{22}O_3$ (M^+) 358.1569, found 358.1574.

(*E*)-3-Methoxyacetoxy-4-(2'-naphtylmethylidene)cyclodeca-1,5-diyne (7c).

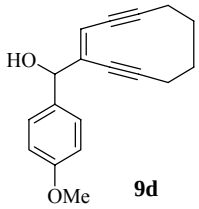


7c

Prepared from **5c** in 65% yield. **7c**: pale yellow oil; R_f = 0.50 (25% EtOAc-hexane); IR (neat) 2932, 2222, 2212, 1754, 1182, 1124 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.25-8.19 (m, 2 H), 7.95-7.77 (m, 3 H), 7.57-7.45 (m, 2 H), 6.90 (s, 1 H), 6.23 (s, 1 H), 4.13 (s, 2 H), 3.47 (s, 3 H), 2.68-2.20 (m, 4 H), 2.03-1.67 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 136.7, 134.2, 133.8, 133.7, 129.7, 129.1, 128.5, 128.3, 127.2, 126.9, 126.4, 121.2, 104.9, 94.4, 81.1, 78.9, 76.2, 70.5, 60.1, 28.2, 28.1, 22.6, 21.4; MS (+CI) *m/z* (relative intensity) 359 ($M + H^+$, 18), 269 (100); HRMS (+EI) calcd for $C_{24}H_{22}O_3$ (M^+) 358.1569, found 358.1594.

Formation of Methoxyacetate 7d and Rearrangement to 9d.

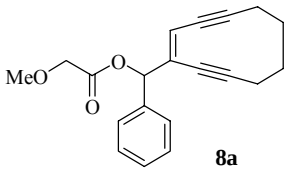
3-[1'-Hydroxy-1'-(4"-methoxyphenyl)methyl]cyclodeca-3-en-1,5-diyne (9d).



9d

To a solution of compound **5d** (20.4 mg, 7.67 x 10^{-2} mmol), DCC (31.6 mg, 0.15 mmol), and DMAP (93.6 mg, 0.77 mmol) in dry CH_2Cl_2 (10 mL) cooled in an ice-water bath was added methoxyacetic acid (11.1 mg, 0.12 mmol) followed by stirring at room temperature for 6 h. The reaction mixture was filtered through a short plug of Celite with rinsing by EtOAc. The filtrate was concentrated under reduced pressure to give the crude product of **7d**, which was converted, during flash column chromatographic purification over silica gel, into **9d** (8.8 mg) in 43% yield calculated from **5d**. **9d**: pale yellow oil; R_f = 0.37 (20% EtOAc-hexane); IR (neat) 3426 (br), 2934, 2194, 1610, 1512, 1248, 1174, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37-7.32 (AA'BB', 2 H), 6.92-6.87 (AA'BB', 2 H), 5.88 (s, 1 H), 5.20 (br s, 1 H), 3.80 (s, 3 H), 2.42-2.30 (m, 4 H), 2.11 (d, J = 3.66 Hz, 1 H), 1.94-1.85 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 142.4, 134.2, 128.7, 118.5, 114.6, 106.5, 103.4, 83.5, 81.9, 75.1, 56.0, 29.5, 29.3, 22.5, 22.4; MS (+CI) *m/z* (relative intensity) 267 ($M + H^+$, 58), 249 (100); HRMS (+EI) calcd for $C_{18}H_{18}O_2$ (M^+) 266.1307, found 266.1300.

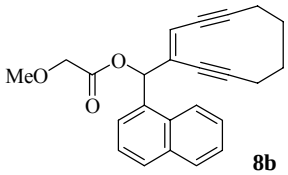
General Procedure for $Eu(fod)_3$-Catalyzed Allylic Rearrangement. 3-[(1'-Methoxyacetoxy-1'-phenyl)methyl]cyclodeca-3-en-1,5-diyne (8a).



8a

To a solution of **7a** (64.0 mg, 2.08 x 10^{-1} mmol) in $CHCl_3$ (5 mL) was added $Eu(fod)_3$ (21.5 mg, 2.08 x 10^{-2} mmol) followed by stirring at room temperature for 48 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 20% EtOAc-hexane) to give **8a** (50.6 mg, 79%): colorless oil; R_f = 0.43 (20% EtOAc-hexane); IR (neat) 2932, 2194, 1758, 1182, 1126 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42-7.29 (m, 5 H), 6.39 (s, 1 H), 5.85 (s, 1 H), 4.15 and 4.07 (AB q, *J* = 16.50 Hz, 2 H), 3.45 (s, 3 H), 2.40-2.32 (m, 4 H), 1.93-1.83 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 138.0, 137.8, 129.2, 128.0, 120.7, 106.4, 104.5, 83.1, 81.6, 77.9, 76.1, 70.5, 60.1, 29.4, 29.2, 22.4, 22.3; MS (+CI) *m/z* (relative intensity) 326 ($M + NH_4^+$, 10), 219 (100); HRMS (+EI) calcd for $C_{20}H_{20}O_3$ (M^+) 308.1412, found 308.1428.

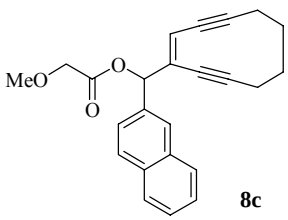
3-[(1'-Methoxyacetoxy-1'-(1"-naphthyl))methyl]cyclodeca-3-en-1,5-diyne (8b).



8b

Prepared from **7b** in 59% yield. **8b**: pale yellow oil; R_f = 0.35 (14% EtOAc-hexane); IR (neat) 2932, 2194, 1758, 1182, 1126 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.01 Hz, 1 H), 7.88-7.81 (m, 2 H), 7.67 (d, *J* = 7.02 Hz, 1 H), 7.56-7.43 (m, 3 H), 7.17 (s, 1 H), 5.79 (s, 1 H), 4.19 and 4.09 (AB q, *J* = 16.47 Hz, 2 H), 3.48 (s, 3 H), 2.343-2.30 (m, 4 H), 1.97-1.84 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 137.7, 134.5, 133.5, 131.3, 130.1, 129.5, 127.3, 126.6, 126.5, 125.9, 124.2, 121.3, 106.4, 104.8, 83.2, 81.9, 73.2, 70.5, 60.1, 29.4, 29.2, 22.4, 22.4; MS (+CI) *m/z* (relative intensity) 359 ($M + H^+$, 7), 271 (100); HRMS (+EI) calcd for $C_{24}H_{22}O_3$ (M^+) 358.1569, found 358.1614.

3-[(1'-Methoxyacetoxy-1'-(2"-naphthyl))methyl]cyclodeca-3-en-1,5-diyne (8c).

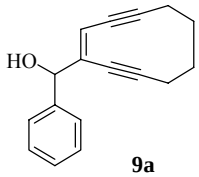


8c

Prepared from **7c** in 79% yield. **8c**: pale yellow oil; R_f = 0.33 (20% EtOAc-hexane); IR (neat) 2932, 2194, 1760, 1184, 1124 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.93-7.80 (m, 4 H), 7.53-7.46 (m, 3 H), 6.57 (s, 1 H), 5.92 (s, 1 H), 4.19 and 4.10 (AB q, J = 16.50 Hz, 2 H), 3.45 (s, 3 H), 2.42-2.38 (m, 4 H), 1.97-1.83 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 137.8, 135.4, 134.0, 133.8, 129.1, 128.9, 128.4, 127.4, 127.1, 127.0, 125.5, 120.8, 106.6, 104.6, 83.1, 81.6, 76.3, 70.5, 60.2, 29.4, 29.2, 22.5, 22.4; MS (+CI) *m/z* (relative intensity) 359 ($M + H^+$, 36), 269 (100); HRMS (+EI) calcd for $C_{24}H_{22}O_3$ (M^+) 358.1569, found 358.1591.

Hydrolysis of Ester 8a.

3-[(1'-Hydroxy-1'-phenyl)methyl]cyclodeca-3-en-1,5-diyne (9a).

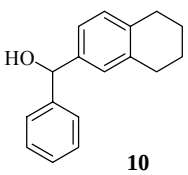


9a

To a solution of **8a** (387.8 mg, 1.26 mmol) in MeOH (60 mL) cooled in an ice-water bath (ca. 0 °C) was added a solution of K_2CO_3 (350.0 mg, 2.53 mmol) in water (60 mL) followed by stirring at the same temperature for 30 min. The reaction was then quenched with saturated aqueous NH_4Cl (50 mL) and extracted with EtOAc (100 x 3 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, 20% EtOAc-hexane) to give **9a** (250.0 mg, 84%): pale yellow oil; R_f = 0.20 (10% EtOAc-hexane); IR (neat) 3412, 2931, 2859, 2195, 1045; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44-7.27 (m, 5 H), 5.89 (s, 1 H), 5.24 (s, 1 H), 2.36 (m, 4 H), 2.22 (s, 1 H), 1.92-1.88 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.5, 141.2, 128.5, 128.1, 126.6, 118.0, 106.0, 102.8, 82.7, 81.1, 74.7, 28.8, 28.6, 21.8, 21.7; MS (+CI) *m/z* (relative intensity) 237 ($M + H^+$, 10), 219 (M^+ -OH, 12), 154 (100).

Cycloaromatization of 9a.

2-[(1'-Hydroxy-1'-phenyl)methyl]-5,6,7,8-tetrahydronaphthalene (10).



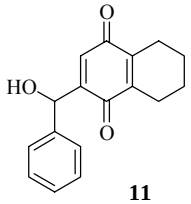
10

A solution of **9a** (27.7 mg, 0.117 mmol) in degassed 1,4-cyclohexadiene and toluene (1:5, 3.0 mL) was heated at 90 °C for 3 h under nitrogen atmosphere. After cooling to room temperature, the

solvent was removed in vacuo. The residue was then purified by preparative TLC (silica gel, 10% EtOAc-hexane) to give **10** (23.8 mg, 85%): pale yellow oil; R_f = 0.33 (10% EtOAc-hexane); IR (neat) 3369, 2928, 1035, 1022 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42-7.23 (m, 5 H), 7.09-7.02 (m, 3 H), 5.78 (s, 1 H), 2.77-2.73 (m, 4 H), 2.19 (s, 1 H), 1.82-1.75 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.9, 141.0, 137.3, 136.6, 129.3, 128.4, 127.4, 127.2, 126.4, 123.7, 76.2, 29.4, 29.1, 23.1, 23.1; MS (+CI) *m/z* (relative intensity) 238 (M^+, 26), 221 (M^+ -OH, 100).

Reaction of 9a with TEMPO.

2-[(1'-Hydroxy-1'-phenyl)methyl]-5,6,7,8-tetrahydro-1,4-naphthoquinone (11).



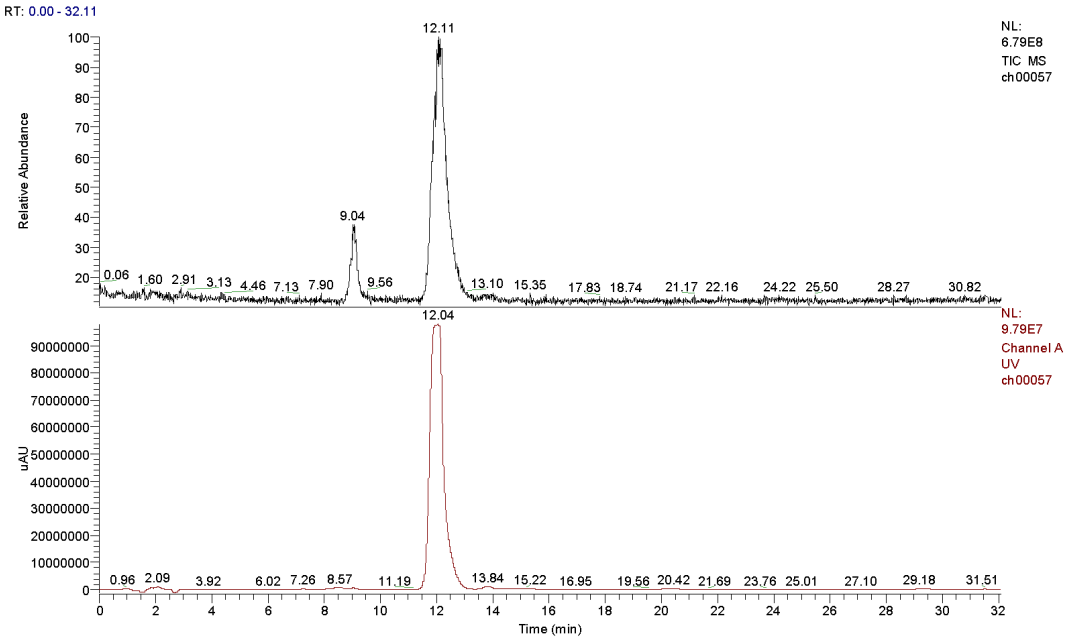
11

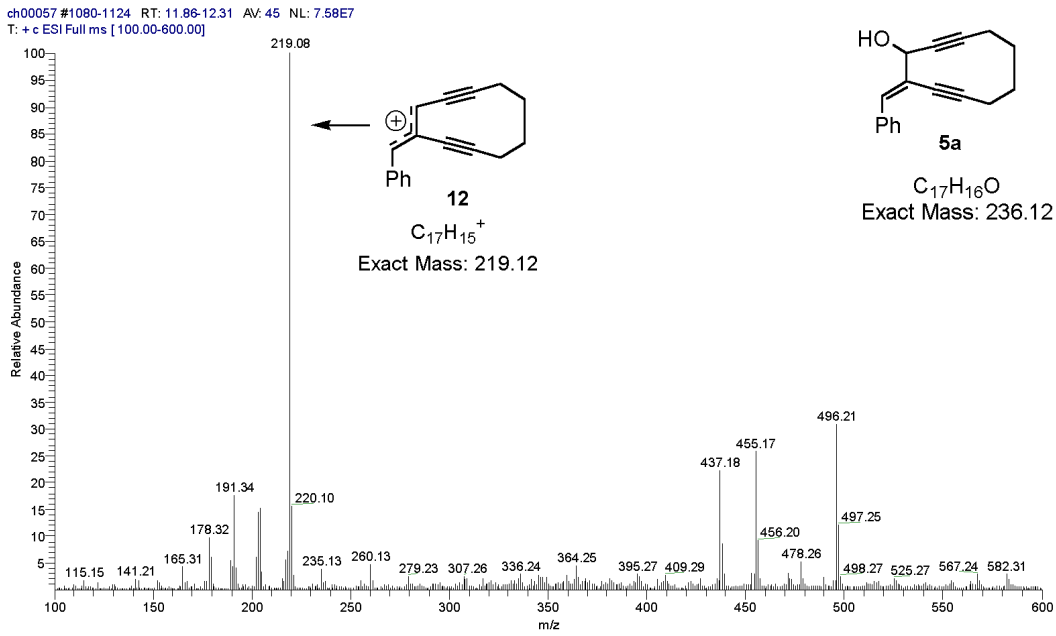
A solution of enediyne **9a** (79.4 mg, 0.336 mmol) and 2,2,6,6-tetramethylpiperidinooxy [TEMPO] (262.6 mg, 1.68 mmol) in benzene (7 mL) was heated under reflux for 6 h. After cooling to room temperature the solvent was removed in vacuo and the residue was purified by flash column chromatography (silica gel, 20% EtOAc-hexane) to give **11** (37.9 mg, 42%): brown oil; R_f = 0.26 (20% EtOAc-hexane); IR (neat) 3501, 3063, 3033, 2938, 2863, 1645, 1632, 1455, 1428, 1337, 1299 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.25 (m, 5 H), 6.71 (d, *J* = 1.20 Hz, 1 H), 5.76 (s, 1 H), 3.06 (s, 1 H), 2.38-2.32 (m, 4 H), 1.66-1.64 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.8, 187.3, 148.4, 142.4, 142.4, 140.3, 130.9, 128.6, 128.2, 126.8, 70.5, 22.3, 22.3, 20.9, 20.8; MS (+CI) *m/z* (relative intensity) 269 ($M + H^+$, 21), 251 (M^+ -OH, 100).

LC-MS Analysis Conditions. A reverse-phase LiChrospher RP-select B column was used in all LC-MS analyses eluted with $CH_3CN–H_2O$ (50:50 containing 0.1% acetic acid) at 1 mL/min. UV detection at 230 nm was used.

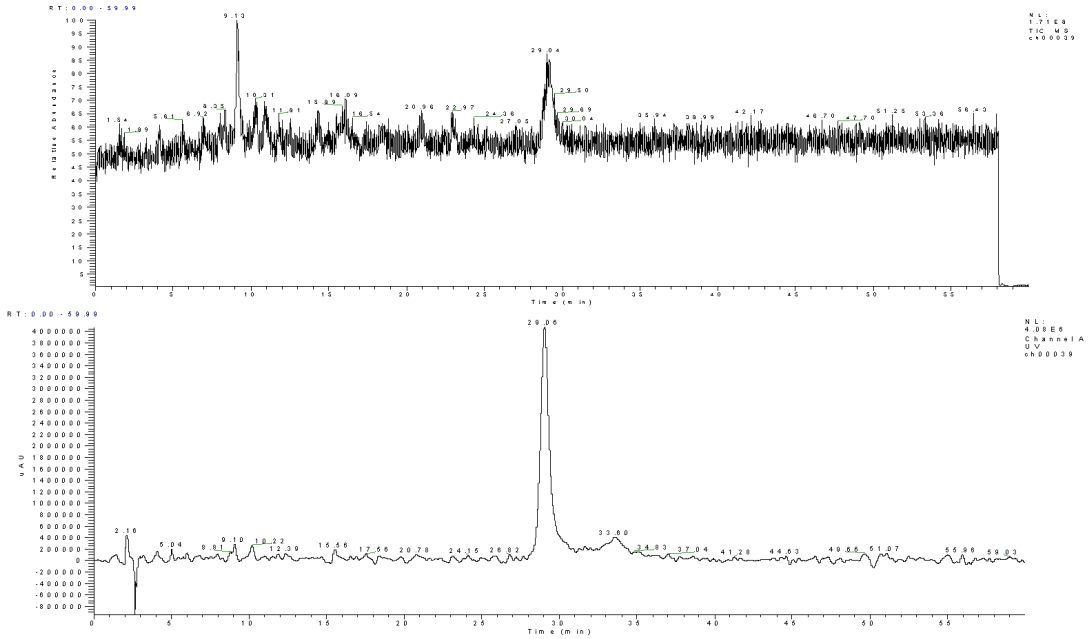
LC-MS Data for Authentic Compounds 5a, 6a, 9a, 10, and 11.

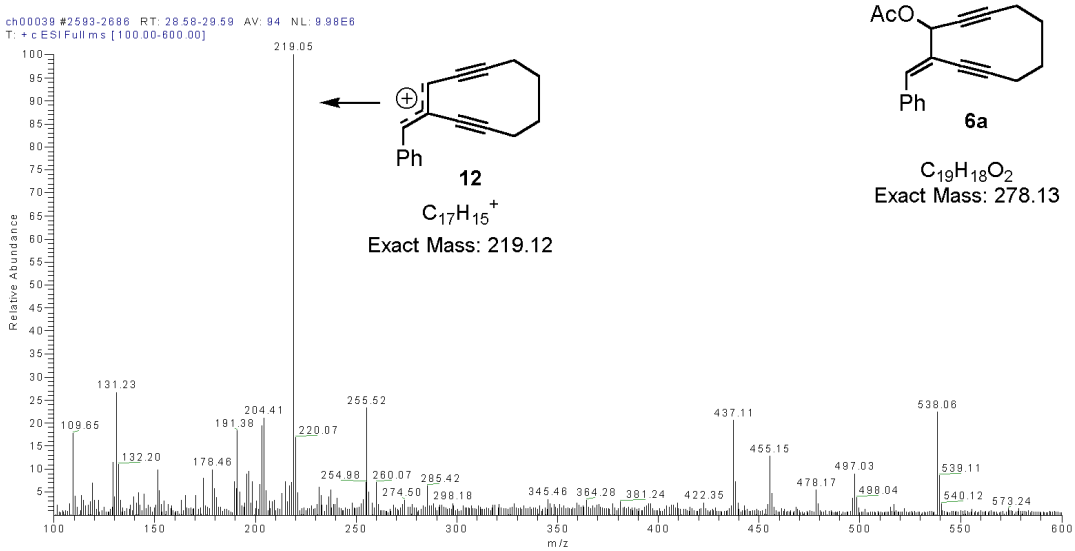
Compound 5a:





Compound 6a:





12

$C_{17}H_{15}^{+}$

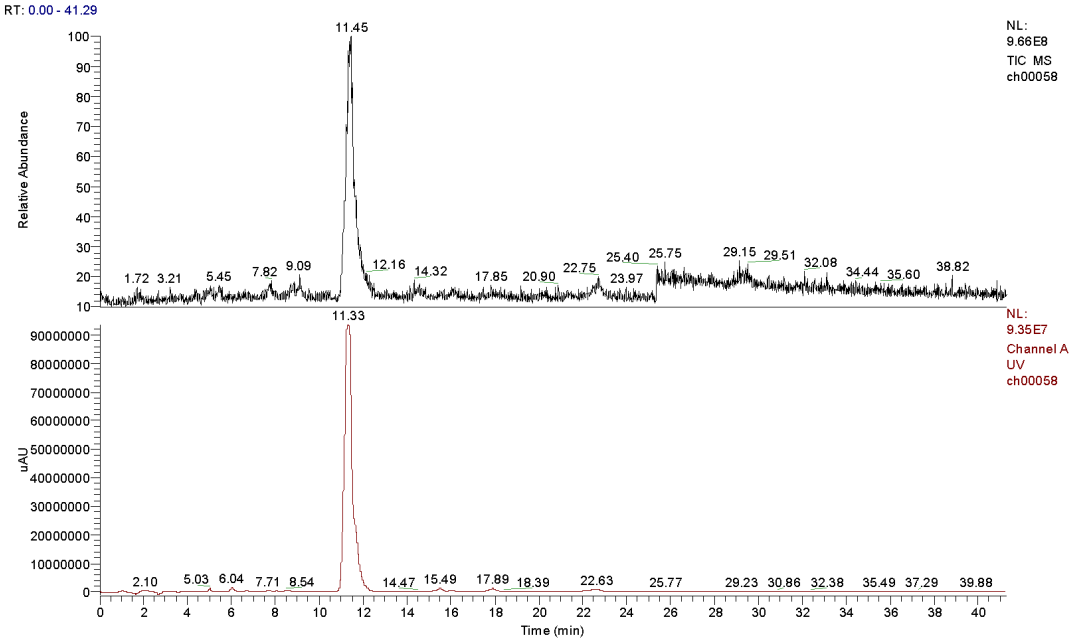
Exact Mass: 219.12

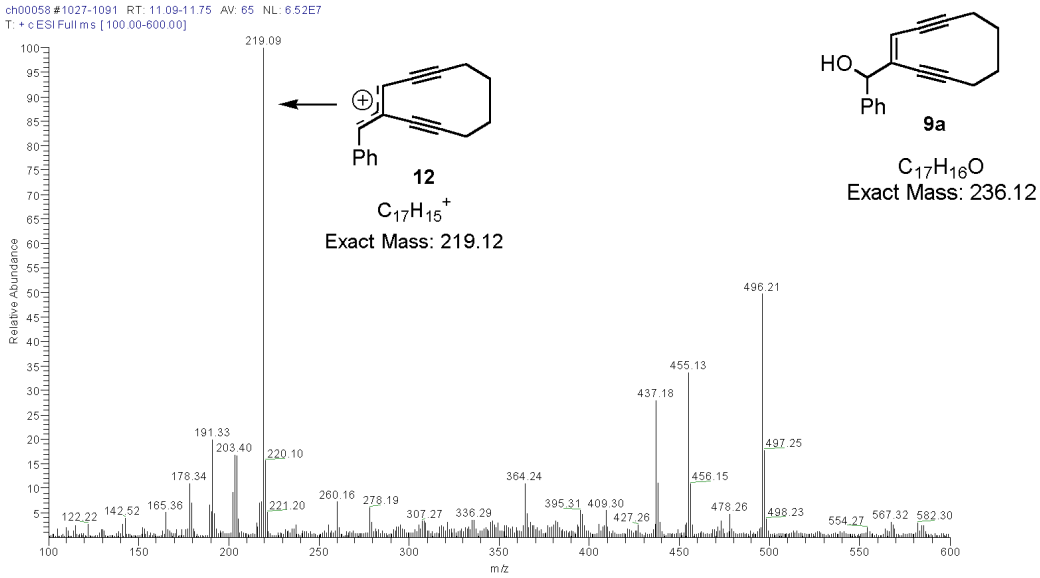
6a

$C_{19}H_{18}O_2$

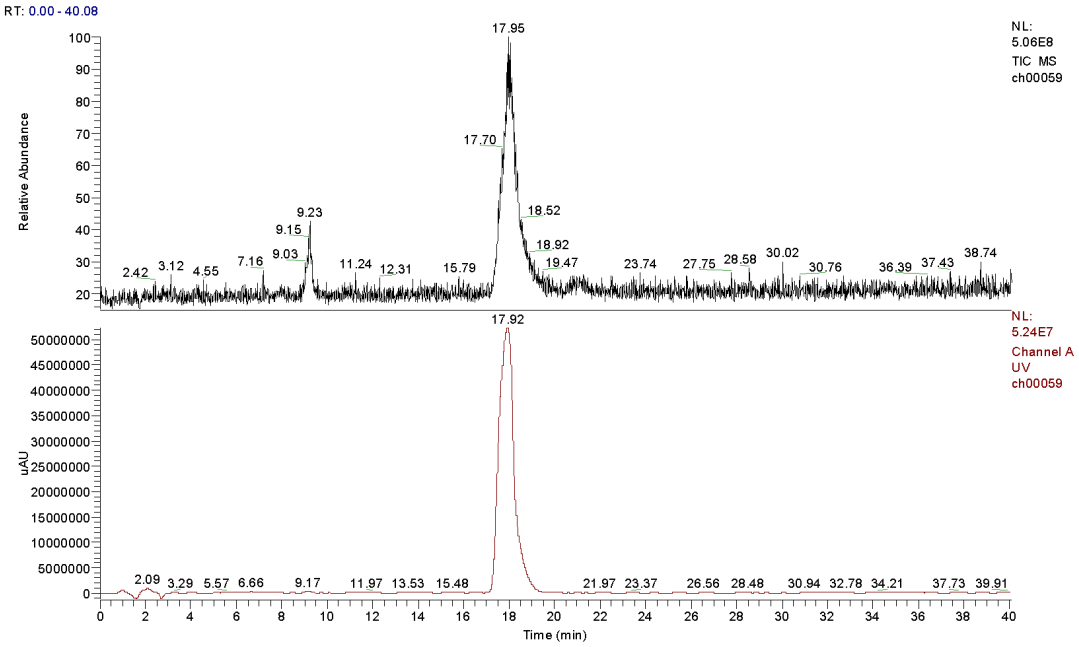
Exact Mass: 278.13

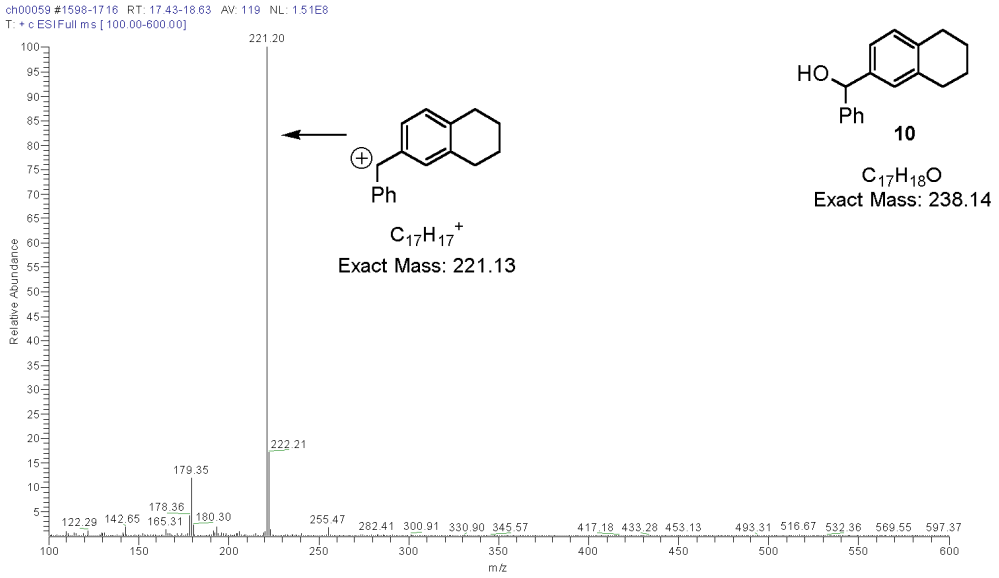
Compound 9a:


RT: 0.00 - 41.29
Relative Abundance
11.45
NL:
9.66E8
TIC MS
ch00058
1.72 3.21 5.45 7.82 9.09 12.16 14.32 17.85 20.90 22.75 23.97 25.40 25.75 29.15 29.51 32.08 34.44 35.60 38.82
11.33
NL:
9.35E7
Channel A
UV
ch00058
uAU
2.10 5.03 6.04 7.71 8.54 14.47 15.49 17.89 18.39 22.63 25.77 29.23 30.86 32.38 35.49 37.29 39.88
Time (min)


ch00058 #1027-1091 RT: 11.09-11.75 AV: 65 NL: 6.52E7
T: + c ESI Full ms [100.00-600.00]
219.09
Ph
12
C17H15+
Exact Mass: 219.12
HO
Ph
9a
C17H16O
Exact Mass: 236.12
Relative Abundance
496.21
455.13
437.18
497.25
191.33
203.40
220.10
178.34
456.15
364.24
260.16
278.19
221.20
122.22
142.52
165.36
307.27
336.29
395.31
409.30
427.26
478.26
498.23
554.27
567.32
582.30
m/z

Compound 10:





$C_{17}H_{17}^{+}$

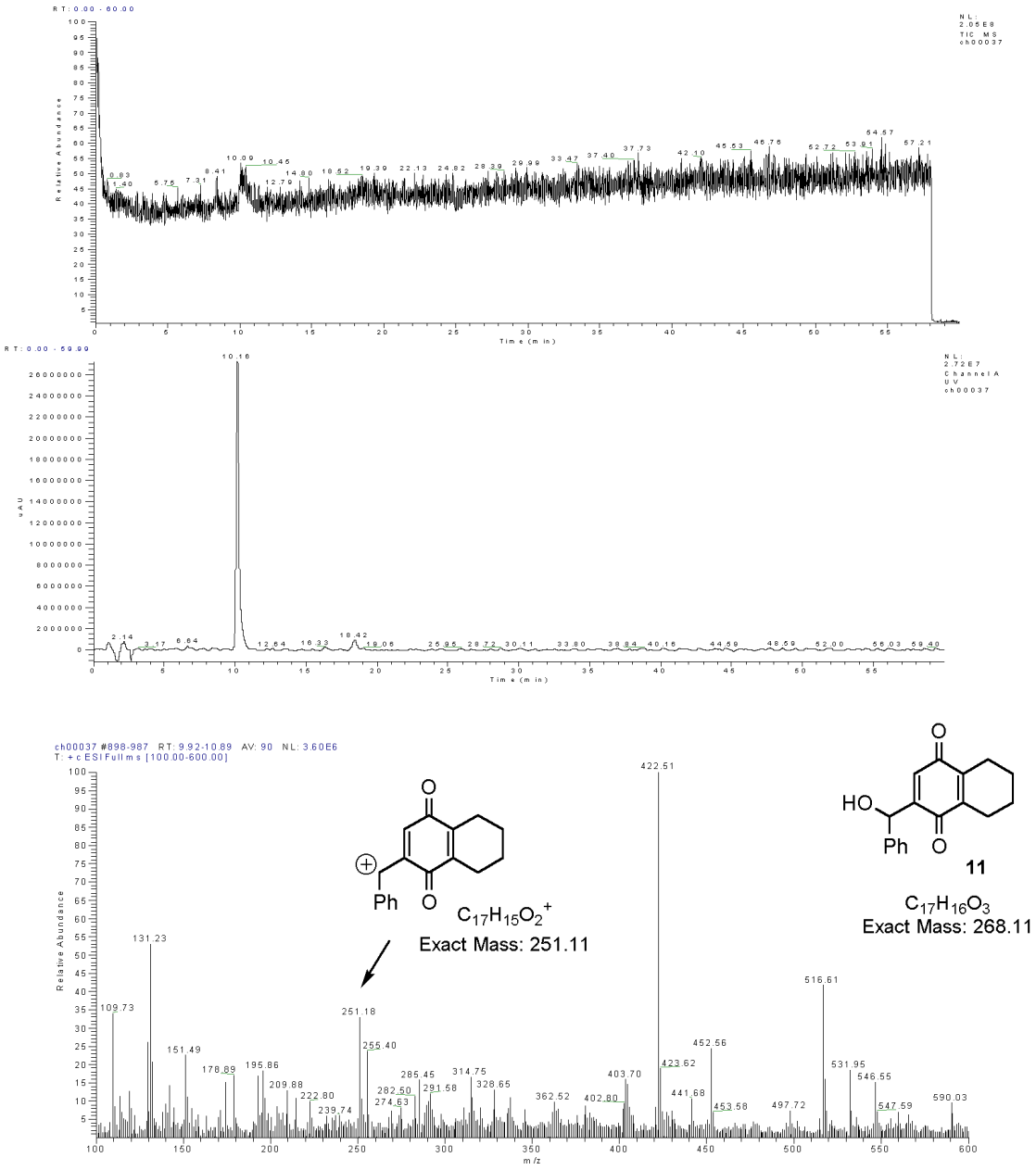
Exact Mass: 221.13

10

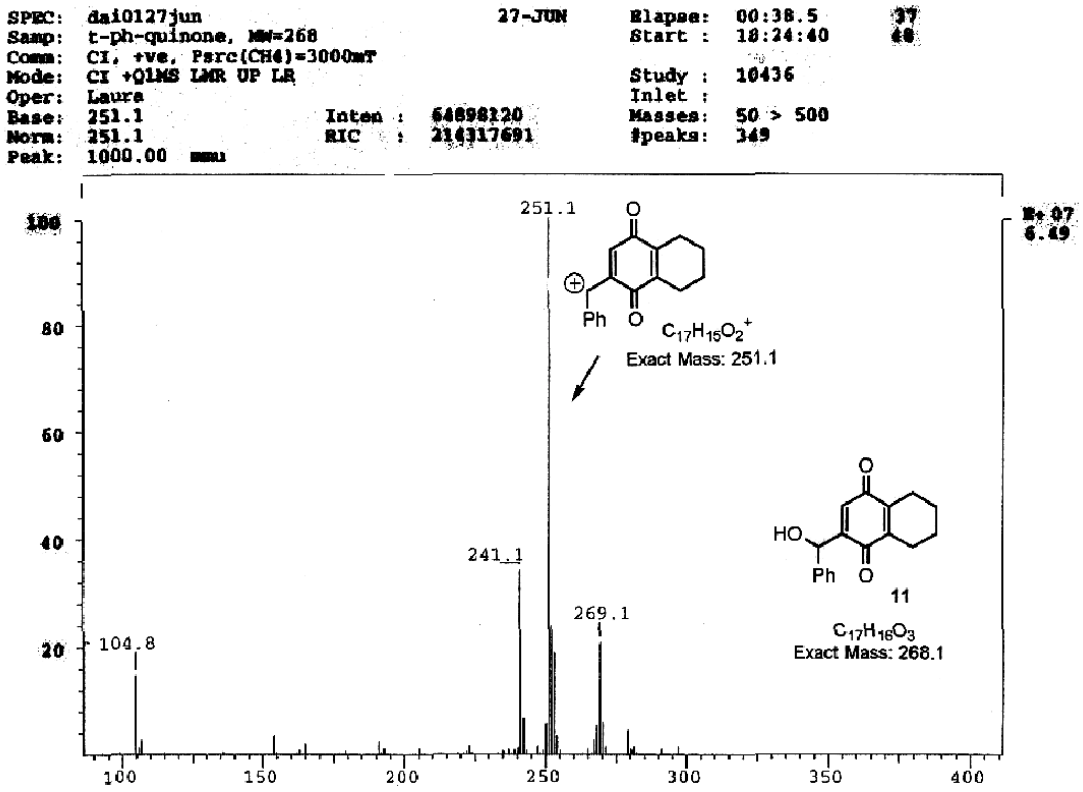
$C_{17}H_{18}O$

Exact Mass: 238.14

Compound 11:



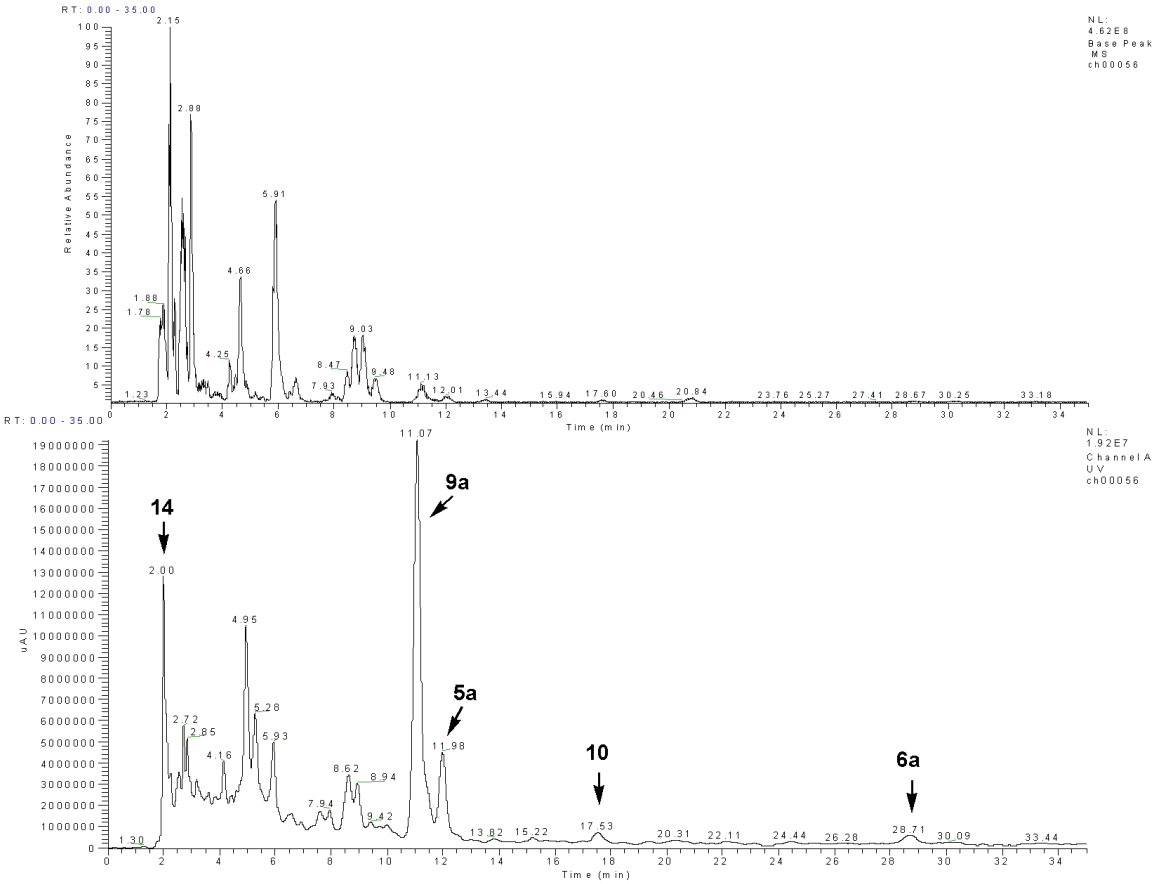
Note: The mass spectrum of quinone **11** obtained using +CI condition clearly shows the M^++1 (m/z 269) and M^+-OH (251) peaks (see next page). The HPLC chromatogram of **11** illustrated above confirms it in good purity. However, we could not obtain satisfactory result in the LC-MS analysis. We have tried LC-MS analyses of **11** under different conditions and always observed the base peak having m/z of 422. The source of this ion is not clear.



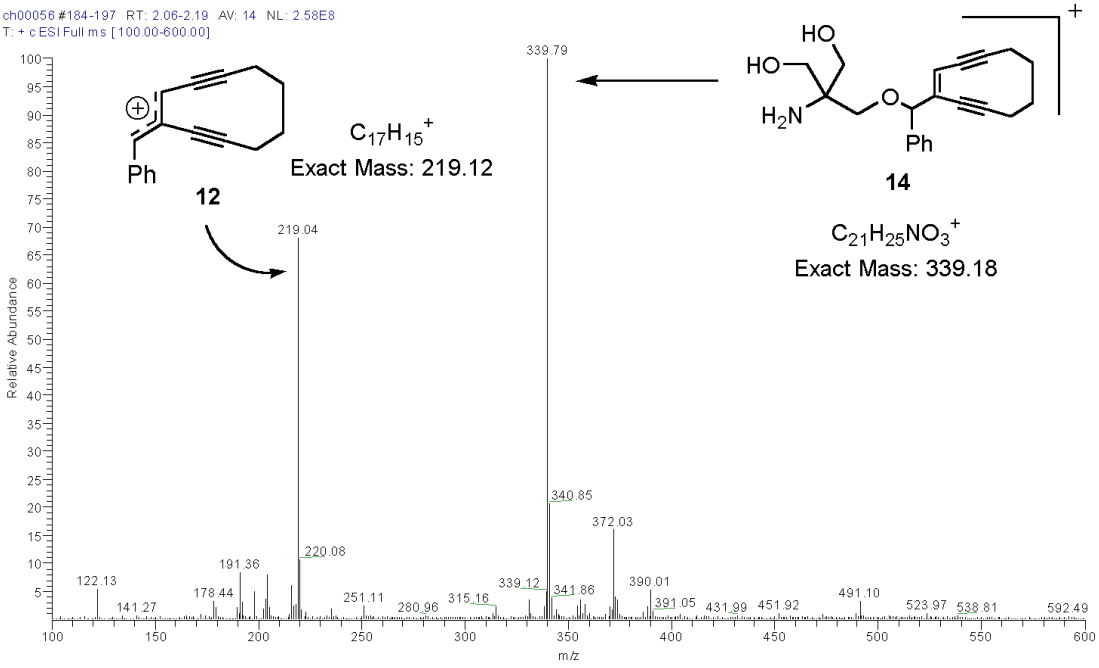
Formation of Enediyne 9a from 6a in Buffer Solution Monitored by LC-MS Analysis.

Acetate **6a** (21.4 mg, 76.9 x 10^{-3} mmol) was incubated in pH 8.5 TEA buffer (5 mL) containing 20% DMSO at 37 °C. Aliquot of the reaction mixture (1 mL) was drawn at 48 h and extracted with EtOAc (1 x 3 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude reaction mixture. The latter was dissolved in acetonitrile (1 mL) and analyzed by LC-MS using a reverse-phase LiChrospher RP-select B column. The sample was eluted with CH_3CN–H_2O (50:50 containing 0.1% acetic acid) at 1 mL/min and detected by UV at 230 nm. The LC-MS data are presented as follows. The peaks corresponding to compounds **5a**, **6a**, **9a**, and **10** are identified according to the retention times and mass spectra in comparison with those of the authentic samples given above. [Note: Because the UV absorption profiles are different, the peak areas for compounds **5a**, **6a**, **9a**, and **10** are not directly proportional to their actual concentrations in the sample. TEA = *T*ris(hydroxymethyl)-aminomethane–*E*DTA–*A*cetic acid]

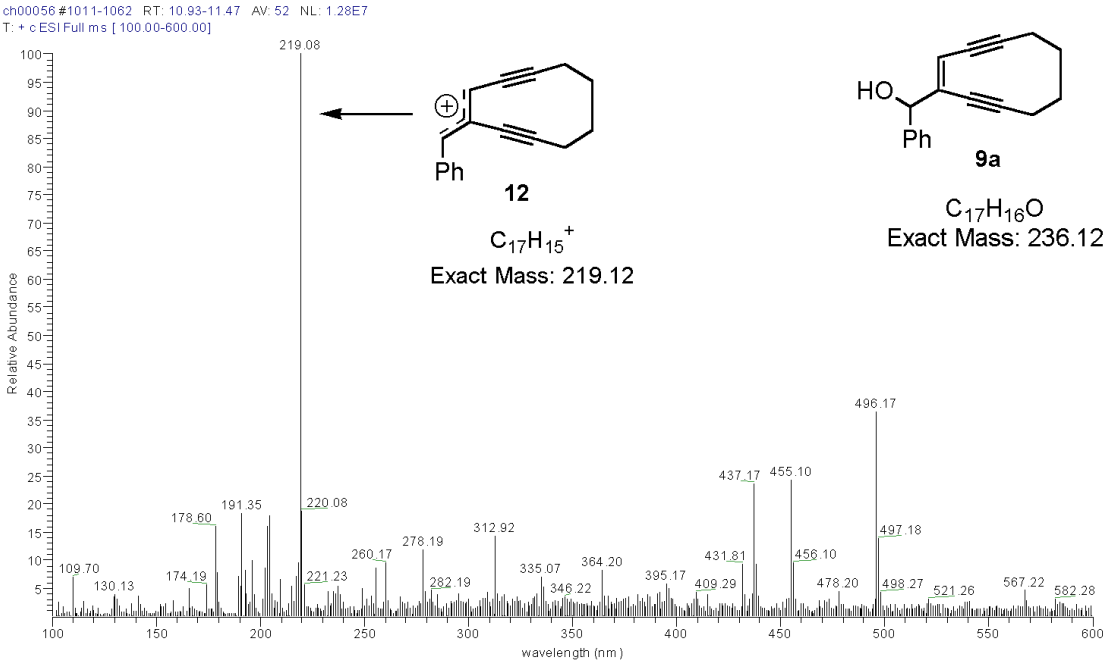
LC-MS Data of the Crude Reaction Mixture of 6a.



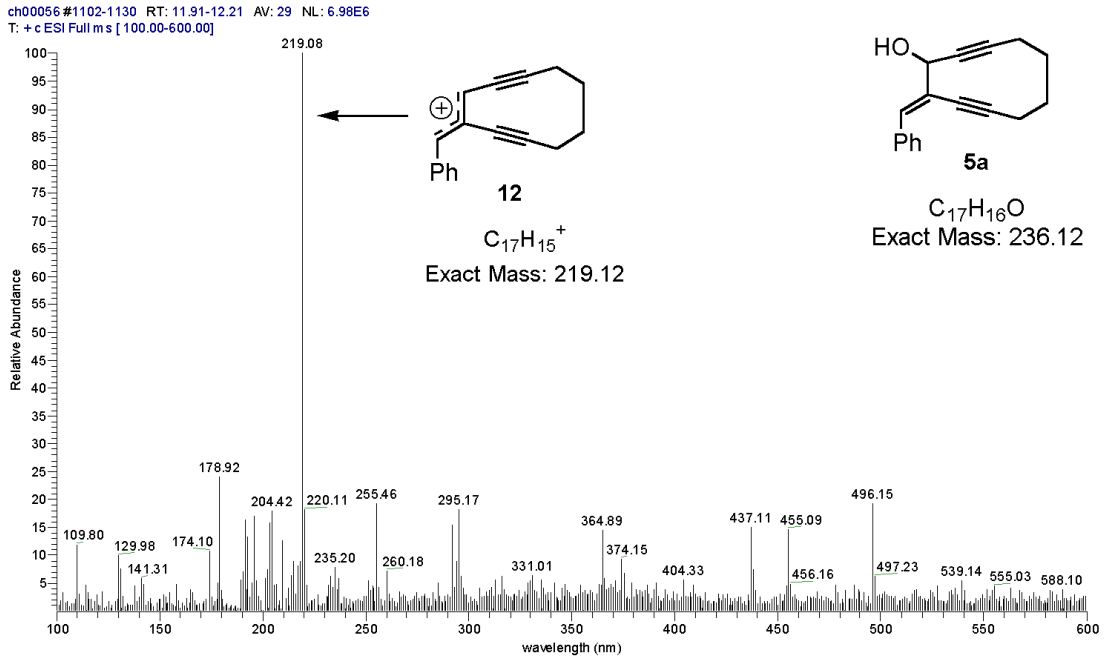
Compound 14 from the Reaction Mixture:



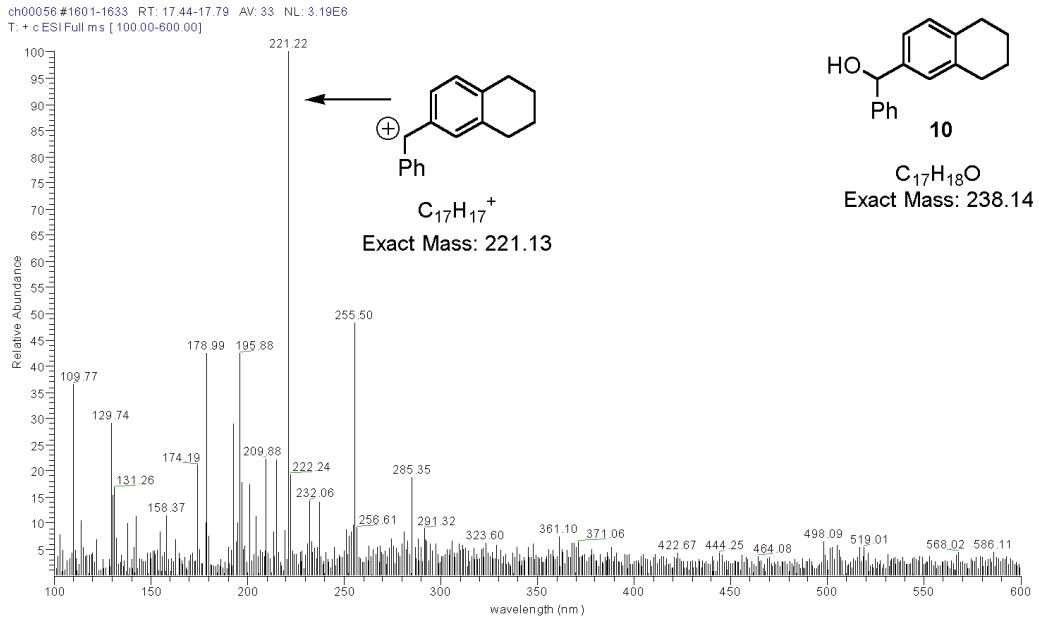
Compound 9a from the Reaction Mixture:



Compound 5a from the Reaction Mixture:



Compound 10 from the Reaction Mixture:



Compound 6a from the Reaction Mixture: