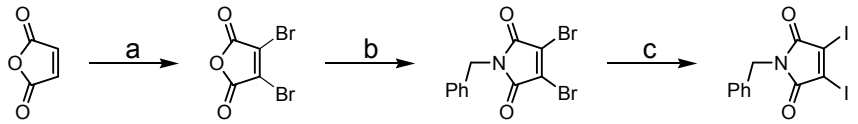
Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for

Maleimide-based acyclic enediyne for efficient DNA-cleavage and tumor cell suppression

Synthesis.

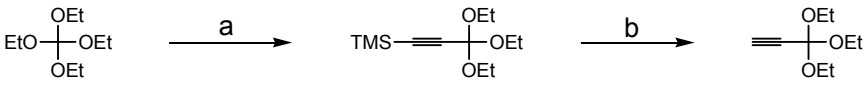


Scheme S1. Synthesis of compounds **3**. (a) Br_2 , $AlCl_3$, 120 °C, 16 h; (b) $PhCH_2NH_2$, acetic acid, 22 h; (c) NaI, acetic acid, 5 h.

3,4-dibromomaleic anhydride (Compound 6) [1]**.** A mixture of maleic anhydride (9.0 g, 91.8 mmol), aluminum chloride (0.188 g, 1.41 mmol) and bromine (28.07 g, 175.6 mmol) were added to a sealed flask under nitrogen. The flask was kept airtight and heated at 120 °C for 16 h. After cooling down to room temperature, the mixture was dissolved in ethyl acetate and filtered. Compound **6** was obtained by removing the solvent under vacuum, followed with recrystallization by ethyl acetate and petroleum ether (21.84 g, 93%).

3,4-dibromo-N-benzylmaleimide (Compound 7) [1]. Compound **6** (9.21 g, 36.0 mmol) was dissolved in acetic acid (40 ml) with slow addition of benzylamine (4 ml, 36.6 mmol); the solution was heated at 120 °C for 22 h. After removal of solvent, the crude residue was separated by column chromatography on silica gel (hexane/ethyl acetate = 15:1) to give compound **7** (8.69 g, 70.1%).

3,4-diiodo-N-benzylmaleimide (Compound 3)[1]**.** A solution of **7** (3.088 g, 8.95 mmol) and sodium iodide (5.05 g, 35.8 mmol) in acetic acid (40 ml) was heated at 120 °C for 5 h. Then the solution was added to water with yellow floccule precipitated. After filtering, washing with water and drying at 60 °C, the compound **3** was obtained (2.69 g, 68.4 %).

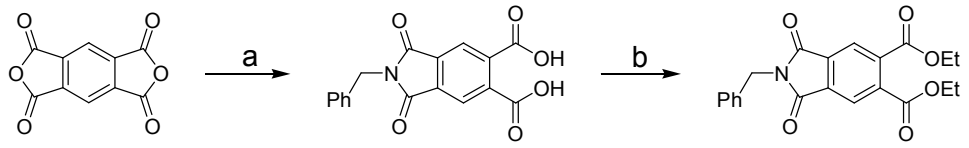


8 **4**

Scheme S2. Synthesis of compounds **4**. (a) TMSA, EtMgBr, diethyl ether; (b) TBAF, THF, 15 min.

1,1,1-triethoxy-3-trimethylsilypropyne (Compound 8)[2]**.** Trimethylsilylacetylene (TMSA, 18.17 g, 0.185 mol) was added to a solution of Grignard reagent prepared from Mg (5 g) and bromoethane (22.23 g, 0.204 mol) in diethyl ether (55 ml), the solution was stirred at room temperature for 1 h, followed by dropwise addition of tetraethyl orthocarbonate (42.7 g, 0.222 mol) dissolved in diethyl ether (35 ml) and stirred at reflux overnight. After completion of the reaction, the mixture was extracted and washed by diethyl ether and saturated ammonium chloride solution. The diethyl ether layer was dried over $MgSO_4$. After removal of solvent, the crude residue was purified by distillation under vacuum to give compound **8** (31.46 g, 69.7 %).

3,3,3-triethoxy-1-propyne (Compound 4). To a degassed solution of tetra-n-butyl ammonium fluoride (TBAF, 5.23 g, 20 mmol) in anhydrous THF (20 mL), compound **8** (2.54 g, 10 mmol) was added under a nitrogen atmosphere. The solution was stirred for 15 min at room temperature and purified by silica chromatography with hexane/ethyl acetate = 15/1 as eluent to give compound as a yellow liquid (1.30 g 72.6%).



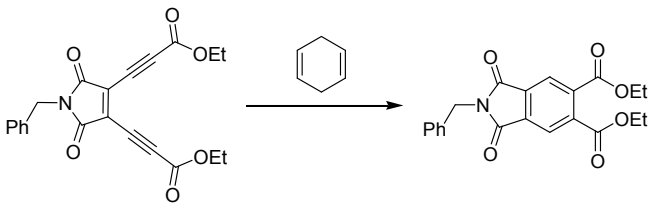
9 **5**

Scheme S3. Synthesis of model compound **9**. (a) benzyl amine, acetic acid, reflux; (b) dichloromethane, ethanol, DCC, DMAP, room temperature.

2-Benzyl-1,3-dioxoisoindoline-5,6-dicarboxylic acid (compound 9): 1,2,4,5-Benzenetetracarboxylic anhydride (10 g, 45.8 mmol) was added to acetic acid (100 ml), the mixture was stirred at room temperature with slow addition of benzylamine (5 ml, 45.7 mmol). The solution was heated at 120 °C for 22 h and then poured into

water (1 L) and stirred at room temperature for 1 h. After filtering, the white filter cake was washed with water and dried under vacuum at 60 °C for 4 h, the compound **9** was obtained as white solid (11.83 g, 79.3 %). ^{1}H NMR (DMSO-d_6, ppm): δ 8.08 (s, 2 H, Bn), 7.34-7.25 (m, 5 H, Bn), 4.80 (s, 2 H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 167.21, 166.39, 138.26, 136.24, 133.29, 128.51, 127.41, 127.36, 122.83, 41.17.

Diethyl 2-benzyl-1,3-dioxoisoindoline-5,6-dicarboxylate (compound 5)[3]: In dichloromethane (60 ml), 2-benzyl-1,3-dioxoisoindoline-5,6-dicarboxylic acid (5 g, 15.4 mmol) was added, followed by DCC (6.65 g, 32.25 mmol). After stirring for 2 h at room temperature, the solution was treated with DMAP (0.457 g, 3.74 mmol) and ethanol (1.5 g, 32.6 mmol), and stirred for another 4 h. The reaction mixture was filtered and washed with dichloromethane. With the concentration of filtrate and filtration again to remove insoluble solid, a column chromatography over silica gel (hexane/ethyl acetate = 8:1) yielded white solid compound **5** (0.405 g, 19.8 %). ^{1}H NMR ($CDCl_3$, ppm): δ 8.16 (s, 2 H, Bn), 7.42-7.27 (m, 5 H, Bn), 4.87 (s, 2 H, CH_2), 4.41 (q, 4 H, CH_2, J = 7.2 Hz), 1.39 (t, 6 H, CH_3, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, ppm): δ 166.3, 165.8, 137.8, 135.7, 133.7, 128.8, 128.6, 128.1, 123.9, 62.5 HRMS (ESI): m/z calcd. For $C_{21}H_{19}NO_6$ $(M)^+$: 381.1212; found: 381.1211.

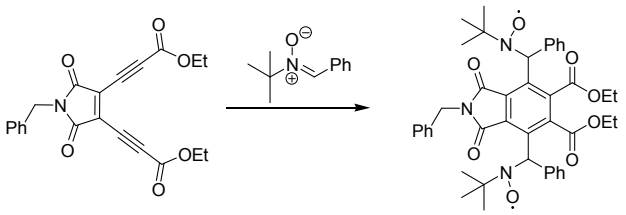


2 (Reactive Enediyne) **5**

Scheme S4. Bergman cyclization of enediyne 2 in the presence of CHD.

A solution of enediyne **1** (200 mg) in toluene (50 ml) was added with TFA (0.1ml) and stirred for 30 s; the excess acid was neutralized by the addition of potassium carbonate. After filtration, the solution was added with 1,4-cyclohexadiene (CHD, 2 ml), transferred to a sealed flask, and incubated at 130 °C for 3 h. The solvent was removed under vacuum and the residue was purified by silica chromatography to give compound **5** as off-white solid (15 mg, 10%). This H-abstraction was also tried with large excess of CHD at room temperature. However, only trace (and inseparable)

amount of H-abstraction product (compound **5**) was detected with the rest of enediynes converted to polymeric product, which indicates that the intermolecular radical coupling reaction is more favored over intermolecular H-abstraction.



2 (Reactive Enediyne) **10**

Scheme S5. Bergman cyclization of enediyne 2 in the presence of PBN.

A solution of enediyne **1** (20 mg) in chloroform (10 ml) was added with TFA (0.1ml) and stirred for 30 s; the excess acid was neutralized by the addition of potassium carbonate. After filtration, the solution was added with PBN (26 mg) and incubated at 37 °C for 12 h. The mixture was then purified by column chromatography with magnesium silicate (cyclohexane/ethyl acetate=30:1) to give an orange viscous liquid. HRMS (ESI): m/z calcd. for $C_{43}H_{47}N_3O_8Na^2 \cdot (M+Na)^+$: 756.3261; found: 756.3265.

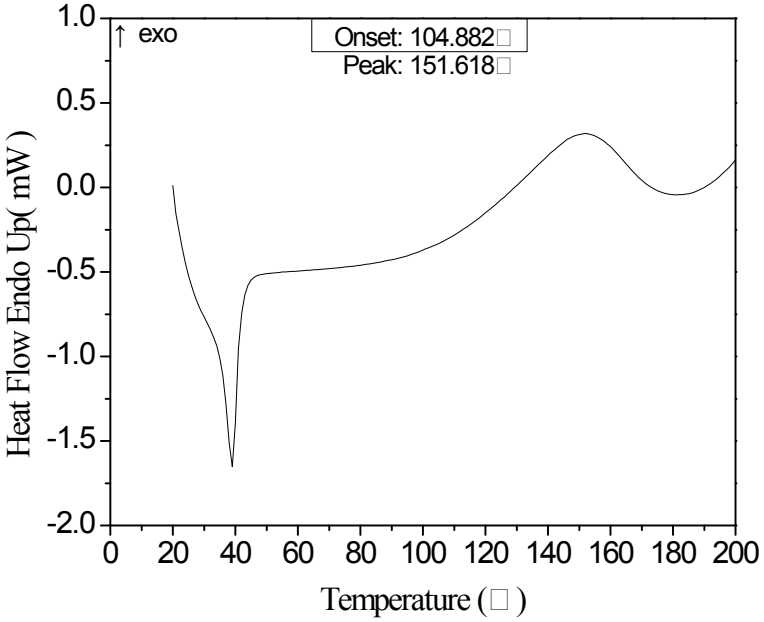


Figure S1. DSC curves of enediyne **1**.

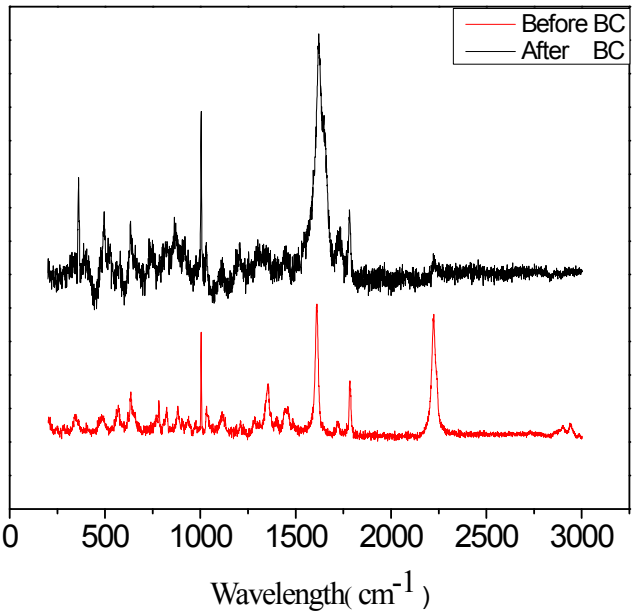


Figure S2. Raman spectra of enediyne **1**. Red line: before Bergman cyclization; Black line: after Bergman cyclization.

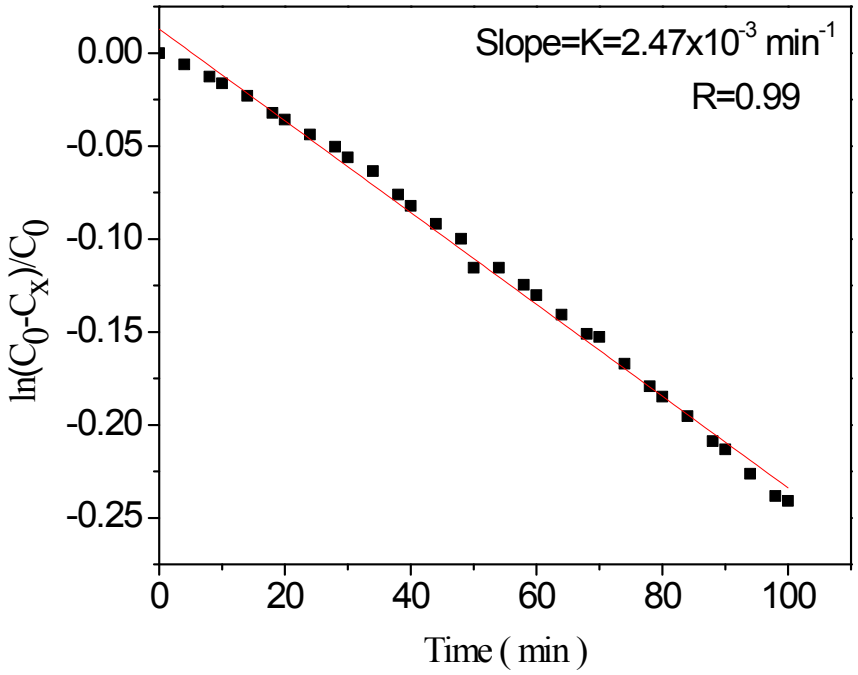


Figure S3. Rate profile for the Bergman cyclization of enediyne **2** in methanol. The line was drawn by least-squares fitting in single exponential equation. C_0 is the initial concentration of enediyne while C_x is the concentration of enediyne at the given time.

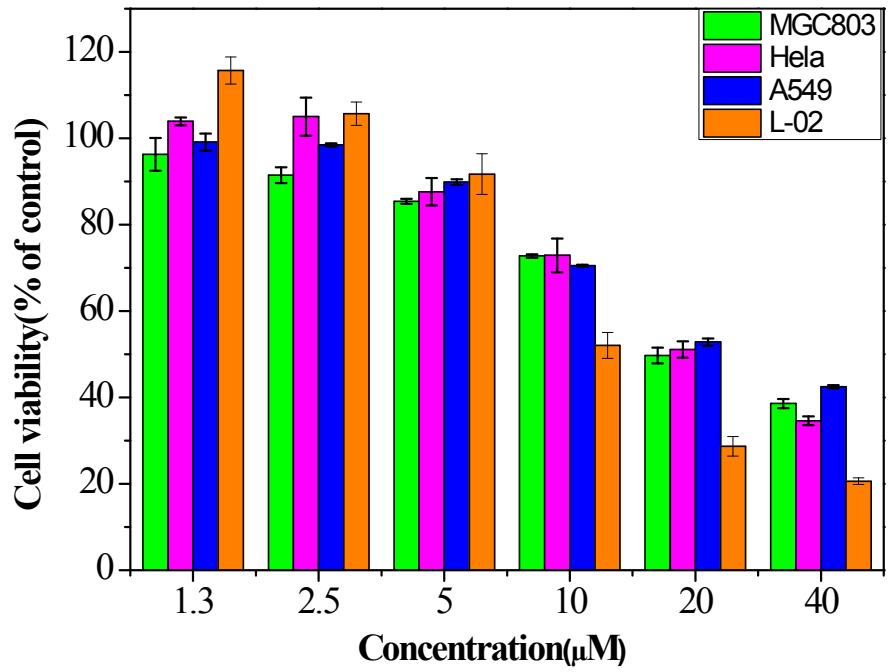


Figure S4. Effects of enediyne **1** on cell viability of three different tumor cells and one normal cell. The cells were incubated with varies of concentrations of enediyne **1** for 24 h and cell viability was determined and analyzed by MTT assay.

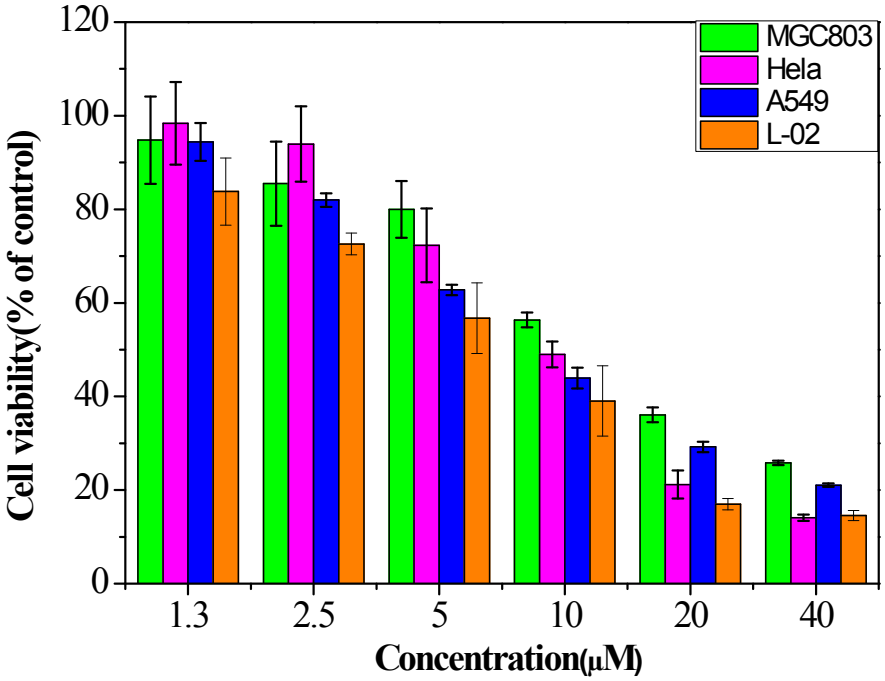


Figure S5. Effects of enediyne **1** on cell viability of three different tumor cells and one normal cell. The cells were incubated with varies of concentrations of enediyne **1** for 48 h and cell viability was determined and analyzed by MTT assay.

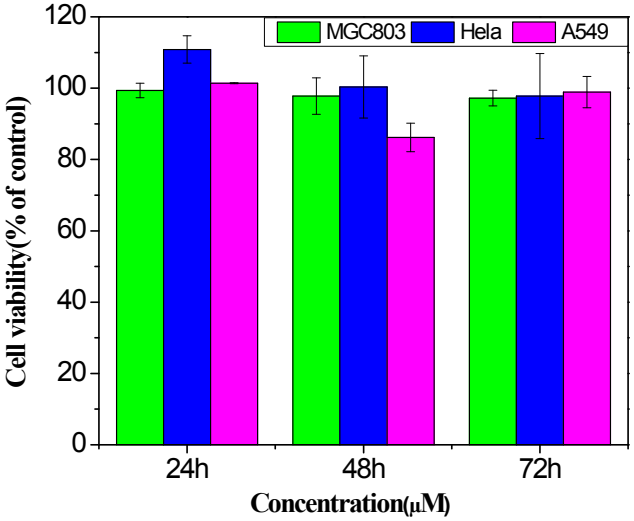


Figure S6. Effects of acetone on cell viability of three different tumor cells. The cells were incubated with acetone for 24 h, 48 h, and 72 h respectively. Cell viability was determined and analyzed by MTT assay.

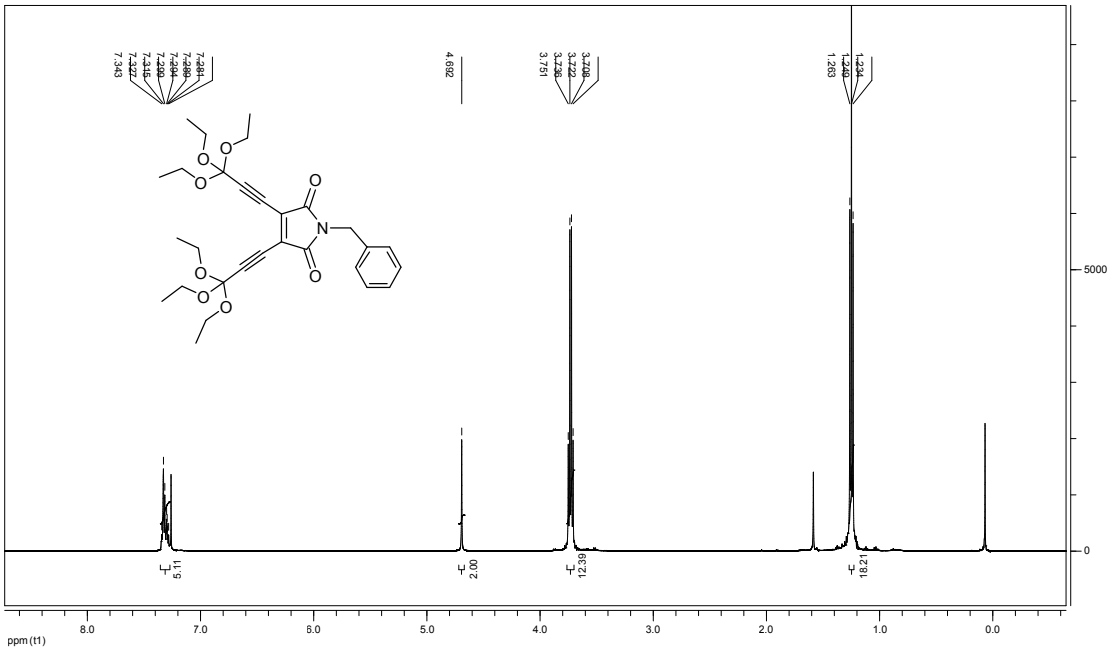


Figure S7. ^{1}H NMR spectrum of enediyne **1**

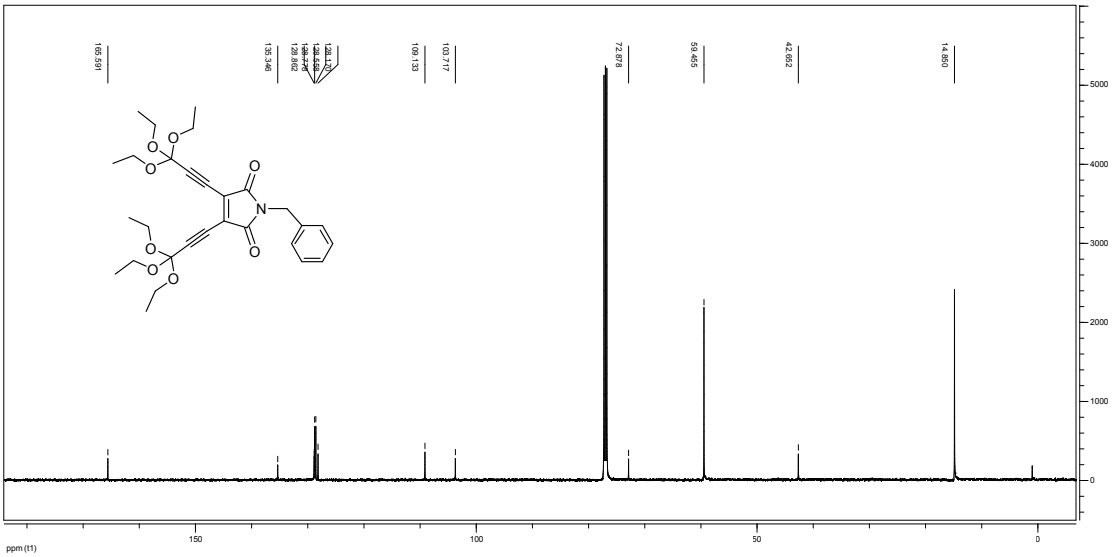


Figure S8. ^{13}C NMR spectrum of enediyne **1**

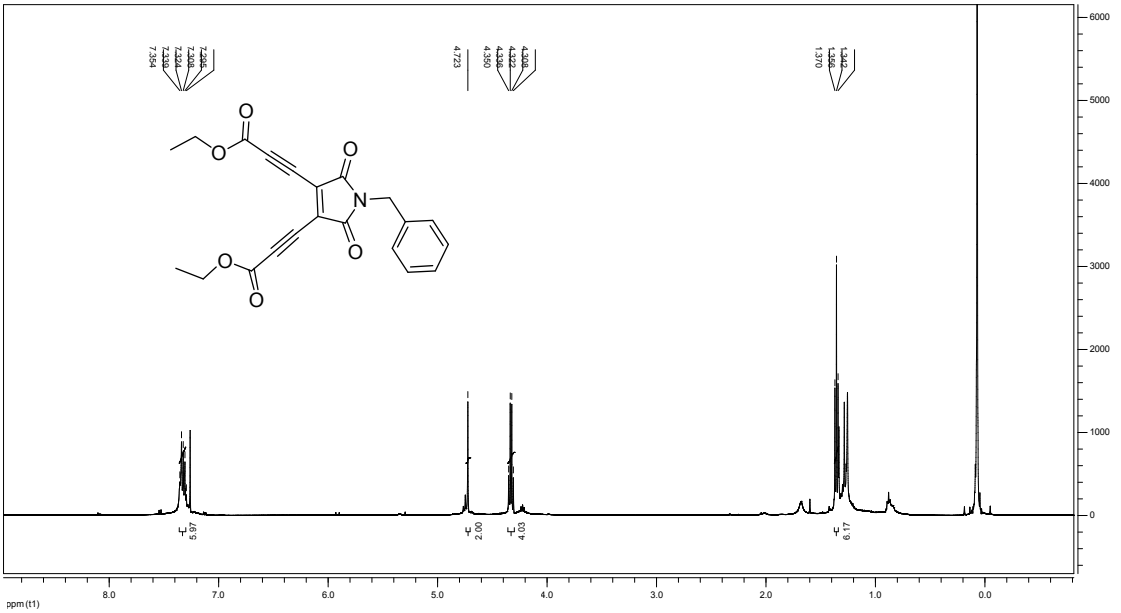


Figure S9. ^{1}H NMR spectrum of enediyne **2**

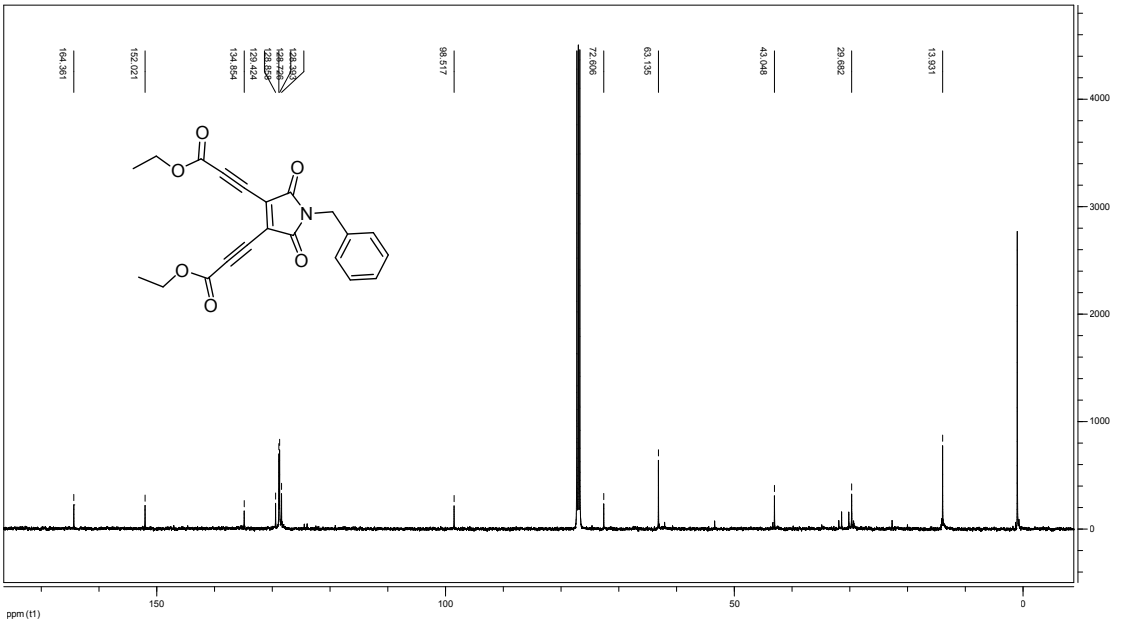


Figure S10. ^{13}C NMR spectrum of enediyne **2**

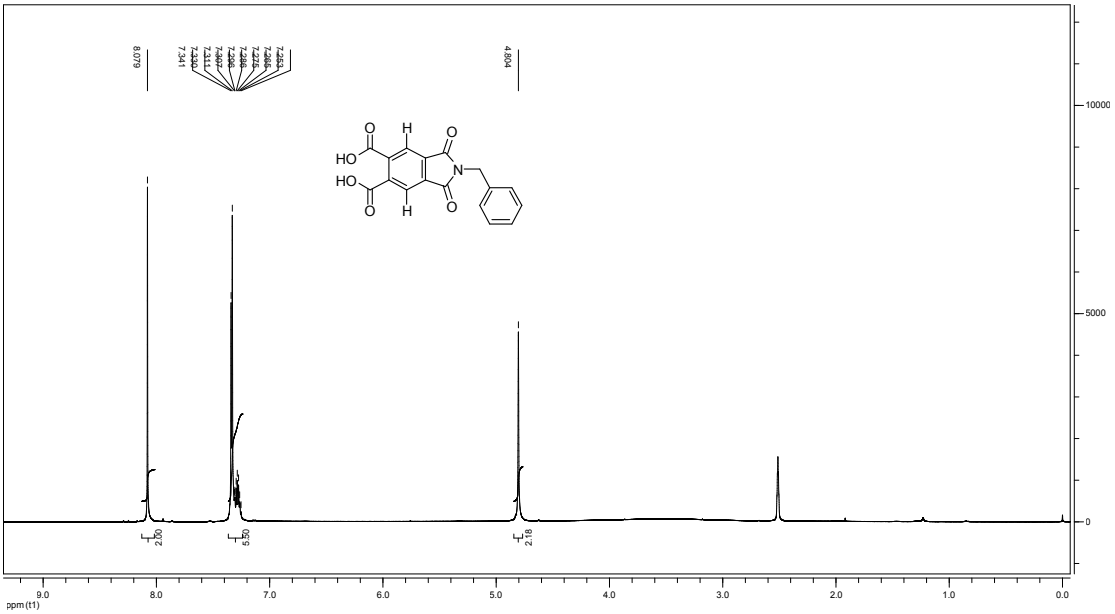


Figure S11. ^{1}H NMR spectrum of compound **9**

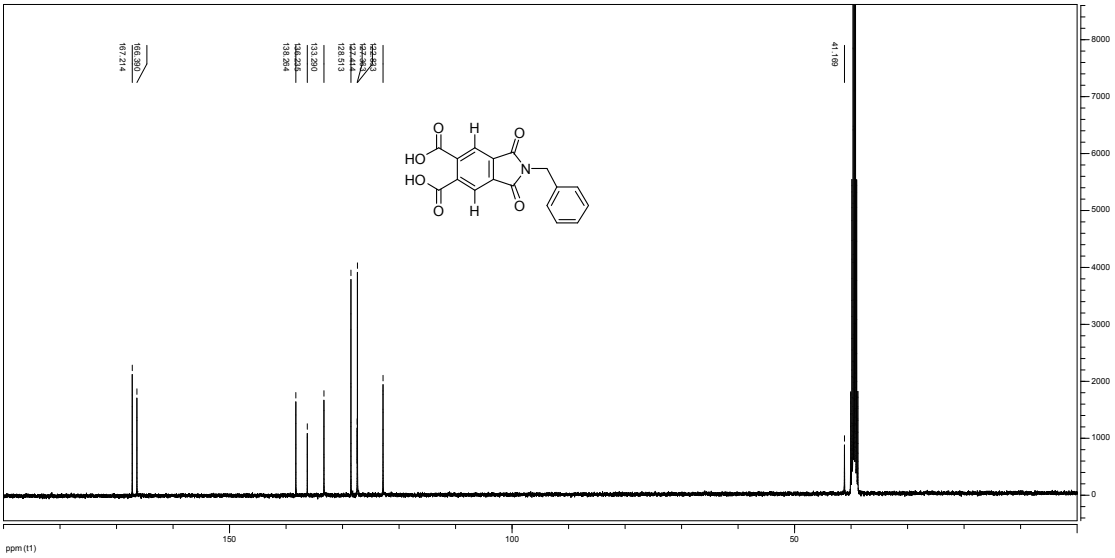


Figure S12. ^{13}C NMR spectrum of compound **9**

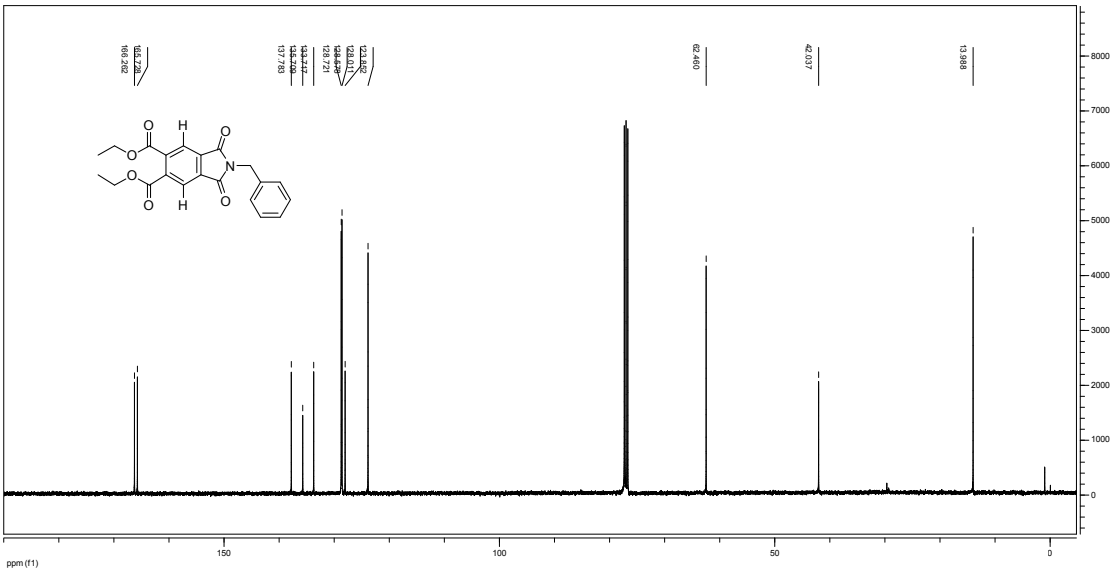


Figure S13. ^{13}C NMR spectrum of compound **5**

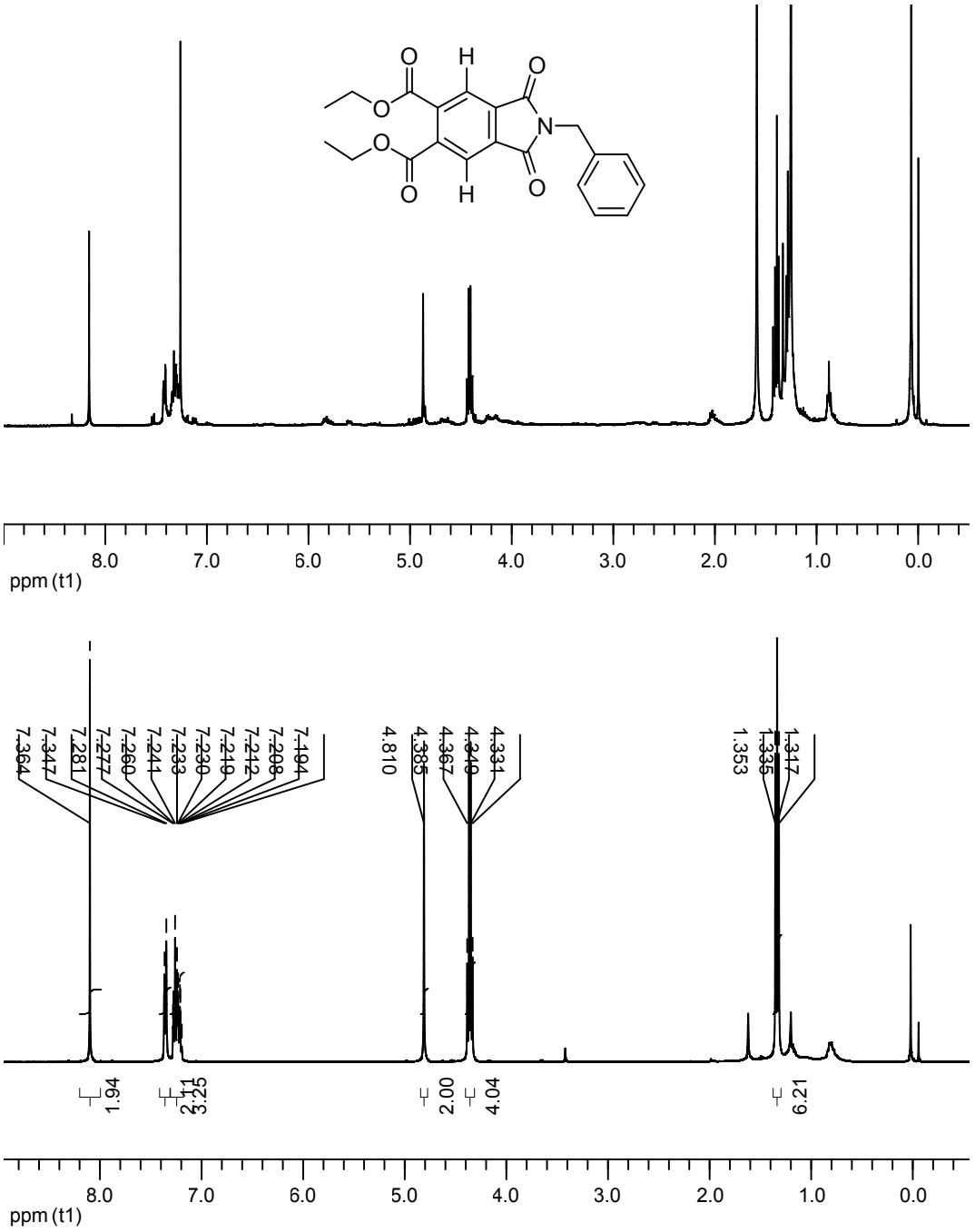


Figure S14. Comparison of ^{1}H NMR spectra of compound **5** obtained from Bergman cyclization (upper panel) and from 1,2,4,5-Benzenetetracarboxylic anhydride (lower panel)

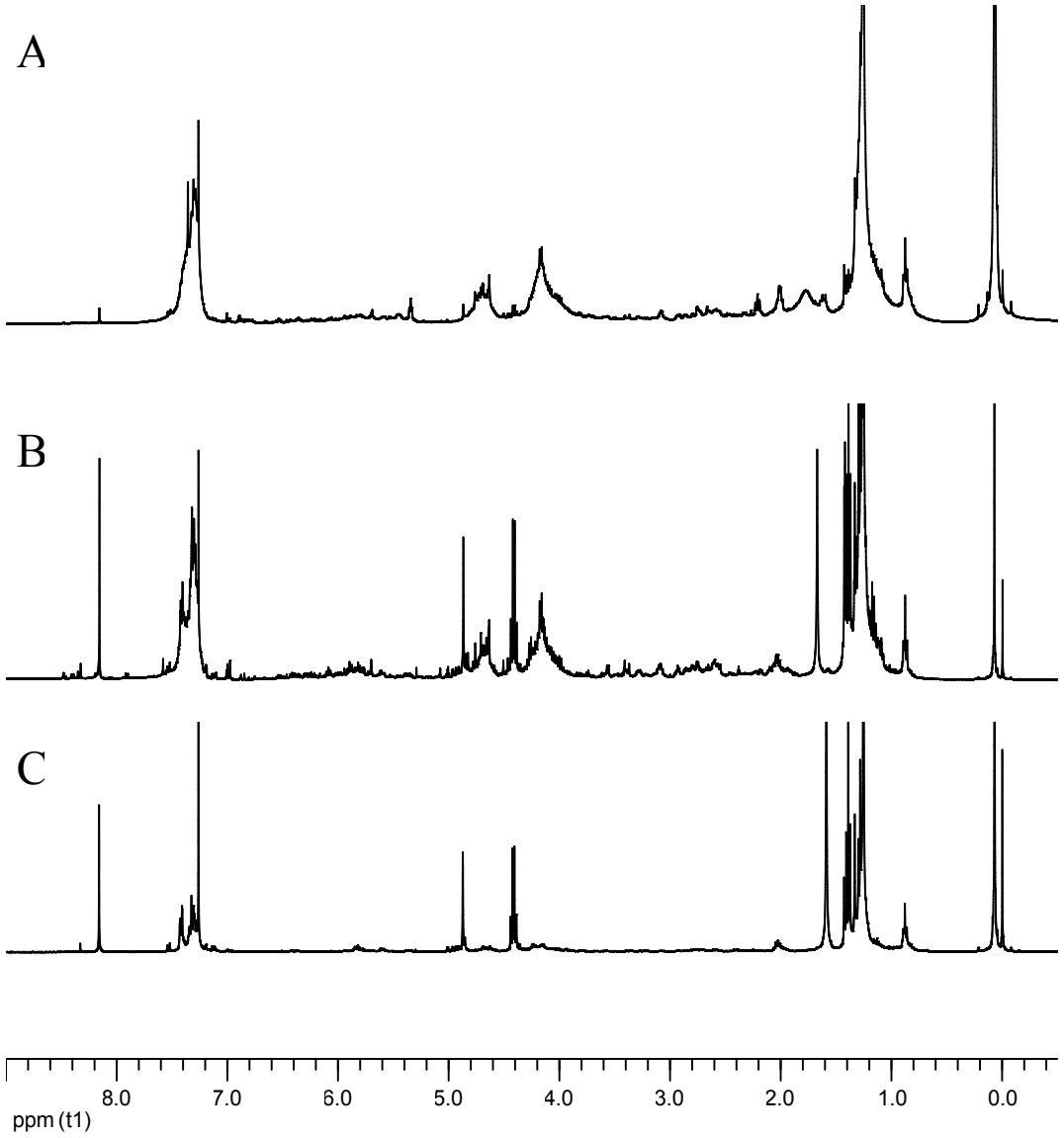


Figure S15. Comparison of ^{1}H NMR spectra of Bergman cyclization products. A) polymer, B) oligomer, C) monomer (compound **5**).

References:

1. M. Dubernet, V. Caubert, J. Guillard and M.-C. Viaud-Massuard, *Tetrahedron*, 2005, **61**, 4585-4593.
2. J. F. Mike, A. J. Makowski and M. Jeffries-El, *Org. Lett.*, 2008, **10**, 4915-4918.
3. H. W. Lee, S. J. Shin, H. Yu, S. K. Kang and C. L. Yoo, *Org. Process Res. Dev.*, 2009, **13**, 1382-1386.