Supporting Information

Synthesis and biological properties of maleimide-based macrocyclic lactone enediyne

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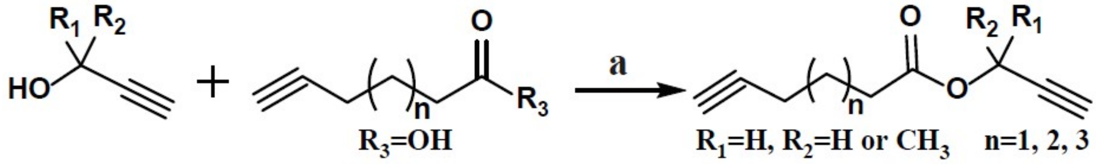
Materials

Common organic solvents and reagents were obtained from commercial suppliers and used as received without further purification. Toluene, tetrahydrofuran (THF), N, N-dimethylformamide (DMF) and dichloromethane (DCM) were dried over calcium hydride (CaH_2) and distilled before use. Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS) and fetal bovine serum (FBS) were obtained from BBI Life Sciences Corporation. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) were purchased from Macklin (Shanghai, China). Annexin V-FITC/PI apoptosis detection kit, phospho-histone H2A.X (Ser139) rabbit monoclonal antibody and Alexa Fluor 555-labeled Donkey Anti-Rabbit IgG (H+L) was purchased from Beyotime Biotechnology. Sonogashira reactions were performed with dry Schlenk technique under nitrogen atmosphere. Pd@DMSN catalyst was synthesized according to our previous work.[1, 2] Detailed synthesis of all intermediates is listed below.

Characterization

1H NMR and 13C NMR spectra were obtained on either Bruker DRX-400, or DRX-600 instruments and calibrated using chloroform (CDCl3) as an internal reference. High-resolution mass spectra (HR-MS) were obtained on a Micromass LCTTM mass spectrometer using the ESI method. Differential scanning calorimetry (DSC) was carried out with a Pyris Diamond thermal analysis workstation equipped with a model 822e DSC module under a constant nitrogen flow. Fluorescence spectra were recorded on a PerkinElmer LS-55 (excited at 256 nm). Electron paramagnetic resonance (EPR) measurements were performed with an X-band EMX-8/2.7C EPR spectrometer (Bruker, Germany). Cytotoxicity assay was measured by a microplate reader (Thermo Scientific). Fluorescence microscopy images were taken using a confocal laser scanning microscope (CLSM, C1-Si, Nikon, Japan). Quantitative flow cytometry was recorded by a flow cytometer (Beckman).

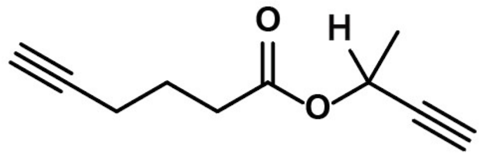
Synthesis



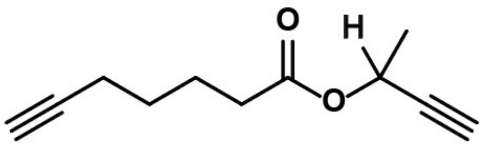
Scheme S1 Synthesis of Diynyl compounds. (a) 4-dimethylaminopyridine (DMAP), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), DMF, rt, 2h.

General procedure for the synthesis of terminal dialkynes[3]

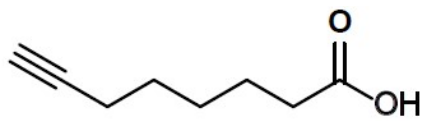
To a stirred solution of alkynyl carboxylic acid (441 mg, 4.5 mmol) in DMF (2.5 mL) were added DMAP (55 mg, 0.45 mmol), alkynyl alcohol (379 mg, 5.4 mmol), EDC·HCl (1.035 g, 5.4 mmol) and DMF (5 mL). After the mixture was stirred at room temperature for 2 h, it was poured into brine and extracted with ethyl acetate. The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude material was purified by flash chromatography (ethyl acetate/n-hexane=1/5) to afford the desired compound.



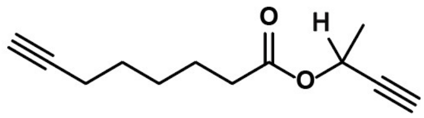
But-3-yn-2-yl hex-5-ynoate (A1). 70 % (515 mg); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.43 (qd, *J* = 6.7, 2.1 Hz, 1H), 2.47 (t, *J* = 7.4 Hz, 2H), 2.44 (d, *J* = 2.1 Hz, 1H), 2.26 (td, *J* = 6.9, 2.6 Hz, 2H), 1.96 (t, *J* = 2.6 Hz, 1H), 1.85 (p, *J* = 7.2 Hz, 2H), 1.49 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.86, 82.13, 81.10, 71.86, 68.20, 58.98, 31.83, 22.48, 20.19, 16.76. HRMS (ESI) m/z: calcd for $C_{10}H_{12}O_2Na$ [M + Na]$^+$: 187.0735, found: 187.0737.



But-3-yn-2-yl hept-6-ynoate (B1). 81 % (650 mg); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.42 (qd, *J* = 6.7, 3.3 Hz, 1H), 2.43 (d, *J* = 2.1 Hz, 1H), 2.35 – 2.31 (m, 2H), 2.21 – 2.16 (m, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.77 – 1.70 (m, 2H), 1.58 – 1.51 (m, 2H), 1.47 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.15, 82.85, 81.15, 71.83, 67.66, 58.88, 32.63, 26.72, 22.84, 20.20, 17.09. HRMS (ESI) m/z: calcd for $C_{11}H_{14}O_2Na$ [M + Na]$^+$: 201.0891, found: 201.0892.

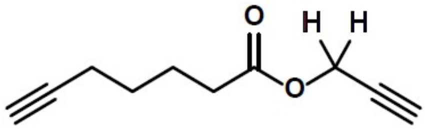


The synthesis of oct-7-ynoic acid (C0)[4]: A 50 mL round bottom flask was charged with lithium acetylide-ethylenediamine complex (994 mg, 10.8 mmol) and DMSO (2.3 mL) at 25 °C. The mixture was then cooled to 0 °C in an ice bath and a solution of bromocaproic acid (702 mg, 3.6 mmol) in DMSO (3.1 mL) was added dropwise. After 10 min, the ice bath was removed and the stirring solution was allowed to warm to 25 ℃. After 2 h, the reaction mixture was poured over an ice/brine mixture (~100 mL), acidified with 1N HCl and extracted with CH_2Cl_2. The organic layer was dried with MgSO4, filtered, and concentrated in vacuo. The crude residue was purified via flash column chromatography (ethyl acetate) to yield C0 as a clear oil (200 mg, 40 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (t, *J* = 7.5 Hz, 2H), 2.18 (td, *J* = 6.9, 2.6 Hz, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.64 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.54 (dt, *J* = 21.0, 6.8 Hz, 2H), 1.49 – 1.39 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.19, 84.27, 68.41, 33.93, 28.10, 28.05, 24.14, 18.23.

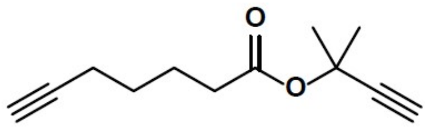


This compound was synthesized according to the general method for the synthesis of terminal dialkynes.

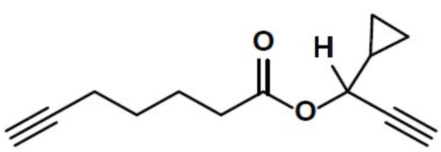
But-3-yn-2-yl oct-7-ynoate (C1). 77 % (500 mg); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.43 (qd, *J* = 6.7, 2.1 Hz, 1H), 2.43 (d, *J* = 2.1 Hz, 1H), 2.33 (td, *J* = 7.4, 1.7 Hz, 2H), 2.18 (td, *J* = 7.0, 2.6 Hz, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.65 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.54 (dt, *J* = 14.5, 7.1 Hz, 2H), 1.49 (d, *J* = 6.7 Hz, 3H), 1.46 – 1.40 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.40, 84.30, 82.21, 72.78, 68.38, 59.84, 34.08, 28.09, 28.06, 24.32, 21.22, 18.23. HRMS (ESI) m/z: calcd for $C_{12}H_{16}O_2Na$ $[M + Na]^+$: 215.1048, found: 215.1047.



prop-2-yn-1-yl hept-6-ynoate (D1). 88 % (650 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 2H), 2.46 (s, 1H), 2.38 (s, 2H), 2.20 (s, 2H), 1.94 (s, 1H), 1.76 (s, 2H), 1.56 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.50, 82.81, 76.69, 73.81, 67.68, 50.84, 32.39, 26.71, 22.81, 17.08. HRMS (ESI) m/z: calcd for $C_{10}H_{12}O_2Na$ $[M + Na]^+$: 187.0735, found: 187.0736.

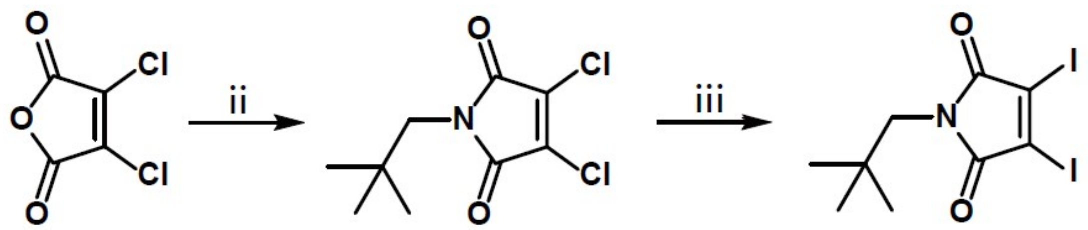


The synthesis of 2-methylbut-3-yn-2-yl hept-6-ynoate (E1): To 6-heptynoic acid (0.76 g, 6.0 mmol) in 12 mL of dichloromethane was added oxalyl chloride (4.5 g, 36 mmol) and the mixture was refluxed at 70 ˚C for an hour. DCM and oxalyl chloride were removed by evaporation affording a colorless oil[5]. Then the obtained hept-6-ynoyl chloride (5.7 mmol) was added to the 2-methylbut-3-yn-2-ol (57 mmol) and the mixture was stirred in a 25 mL round-bottom flask equipped with a magnetic stir bar during 1 hour. The crude product was purified by column chromatography (ethyl acetate/n-hexane=1/5) to achieve E1 as a colorless oil (950 mg, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.23 – 2.14 (m, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.76 – 1.68 (m, 2H), 1.65 (s, 6H), 1.55 (dt, *J* = 14.5, 7.1 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.67, 71.23, 70.51, 67.56, 33.34, 27.90, 26.73, 22.89, 17.12. HRMS (ESI) m/z: calcd for $C_{12}H_{16}O_2Na$ $[M + Na]^+$: 215.1048, found: 215.1049.



The synthesis of 1-cyclopropylprop-2-yn-1-yl hept-6-ynoate (F1): A solution of ethynyl-magnesium bromide (100 mL, 50 mmol) was added slowly to a solution of cyclopropane-carboxaldehyde (42 mmol) in THF (30 mL) under a nitrogen atmosphere at -78 °C. The cold bath was then removed and the reaction mixture was stirred at room temperature. After 2 h, the reaction was quenched with saturated NH_4Cl aqueous solution and extracted with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation to furnish 1-cyclopropylprop-2-yn-1-ol, which was used in the next step without further purification[6].

The hept-6-ynoyl chloride (6 mmol, 870 mg) and N, N-Diisopropylethylamine (DIPEA， 9 mmol, 1.48 mL) was added to the solution of 1-cyclopropylprop-2-yn-1-ol (17 mmol, 1.6 g) in DCM (3 mL) and the mixture was stirred in a 25 mL round-bottom flask equipped with a magnetic stir bar during 12 h. After completion of the reaction, the solvent was removed by rotary evaporation. The crude residue was purified via flash column chromatography (ethyl acetate/n-hexane=1/5) to yield F1 as a clear oil (762 mg, 62 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.18 (dd, *J* = 7.1, 1.9 Hz, 1H), 2.41 (d, *J* = 2.2 Hz, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.20 (td, *J* = 7.0, 2.5 Hz, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.81 – 1.70 (m, 2H), 1.61 – 1.50 (m, 2H), 1.3 – 1.19 (m, 1H), 0.6 - 0.44 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.18, 81.71, 77.18, 71.37, 66.48, 64.88, 31.51, 25.57, 21.75, 15.94, 12.14, 1.37, -1.16. HRMS (ESI) m/z: calcd for $C_{13}H_{16}O_2Na$ $[M + Na]^+$: 227.1048, found: 227.1049.



S1 S2

Scheme S2 Synthesis of compound C2. (a) Neopentylamine, acetic acid, 120 ℃, 24 h. (b) NaI, acetonitrile, 85 ℃, 20 h.

3,4-Dichloro-1-(2,2-dimethyl-propyl)-pyrrole-2,5-dione (S1)

This compound was synthesized following the procedure described in our previous work.[7] Dichloromaleic anhydride (8.35 g, 50 mmol) was dissolved in acetic acid (40 mL) with slow addition of neopentylamine (3.92 g, 45 mmol) at 0℃; the solution was refluxed at 120 °C for 24 h. After removal of solvent, the crude residue was separated by column chromatography on silica gel (hexane/ethyl acetate = 9:1) to give the product S1 (8.02 g, 75.5%).

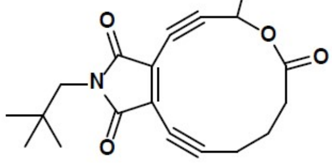
3,4-diiodo-1-(2,2-dimethyl-propyl)-pyrrole-2,5-dione (S2)

This compound was synthesized following the procedure described in our previous work.[7] A solution of sodiumiodide (7.79 g, 132 mmol) and 3,4-dichloro-1-(2,2-dimethyl-propyl)-pyrrole-2,5-dione (19.79 g, 33 mmol) in acetonitrile (70 mL) was refluxed at 85 °C for 20 h. Then the solution was added to water with yellow floccule precipitated. After completion of the reaction, the solvent was removed by rotary evaporation. The crude residue was purified via flash column chromatography using DCM as eluent and drying at 50 °C, the compound S2 was obtained (12.64 g, 91.4 %).

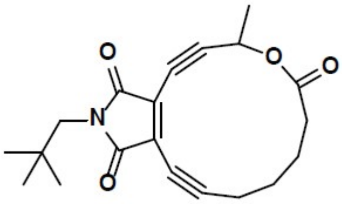
General procedure for the synthesis of macrocyclic enediynes

Compound S2 (209.5 mg, 0.5 mmol), 1.67 equivalent of 6% Pd@DMSN (88.7mg), CuI (38 mg, 0.2 mmol), and DIPEA (0.25 mL, 1.5 mmol) were successively added into a solvent mixture of dry THF (1.5 mL) and toluene (5 mL) under a nitrogen atmosphere. Then, the solution of terminal dialkyne (0.75 mmol) in THF (1 mL) was added dropwisely.

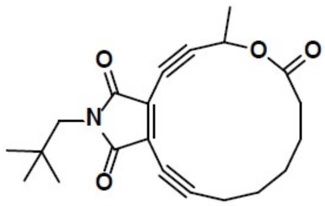
The mixture was stirred at room temperature and monitored with TLC. After completion of the reaction, the mixture was purified through column chromatograph to give the desired compound.



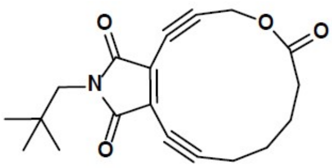
6-methyl-2-neopentyl-6,9,10,11-tetrahydro-4,5,12,13-tetradehydro-[1]oxacyclododecino[5,6-*c*]pyrrole-1,3,8(2*H*)-trione (EDY-A). 15 mg (9.2%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.50 (q, *J* = 6.9 Hz, 1H), 3.31 (s, 2H), 2.81 (ddd, *J* = 18.3, 5.6, 3.0 Hz, 1H), 2.63 – 2.53 (m, 2H), 2.43 (dd, *J* = 15.9, 10.4 Hz, 1H), 2.31 (dt, *J* = 23.5, 9.3 Hz, 1H), 1.91 (ddd, *J* = 18.2, 8.9, 4.1 Hz, 1H), 1.59 (d, *J* = 7.0 Hz, 3H), 0.88 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.31, 166.89, 166.79, 134.63, 129.76, 112.08, 111.36, 77.45, 72.99, 61.95, 49.75, 33.55, 33.52, 27.83, 21.98, 21.27, 18.60. HRMS (ESI) m/z: calcd for $C_{19}H_{21}NO_4Na$ $[M + Na]^+$: 350.1368, found: 350.1367.



6-methyl-2-neopentyl-9,10,11,12-tetrahydro-1*H*-4,5,13,14-tetradehydro-[1]oxacyclotridecino[5,6-c]pyrrole-1,3,8(2*H*,6*H*)-trione (EDY-B). 28 mg (16.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (q, *J* = 6.9 Hz, 1H), 3.32 (s, 2H), 2.71 (d, *J* = 20.3 Hz, 1H), 2.51 (dd, *J* = 12.1, 5.4 Hz, 1H), 2.44 – 2.33 (m, 1H), 2.15 (t, *J* = 12.0 Hz, 1H), 2.02 – 1.93 (m, 1H), 1.81 (dd, *J* = 8.1, 5.7 Hz, 3H), 1.60 (d, *J* = 6.9 Hz, 3H), 0.89 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.97, 166.22, 166.20, 131.31, 128.39, 113.35, 106.95, 74.20, 71.23, 59.41, 48.87, 32.89, 32.55, 26.85, 26.32, 22.98, 19.46, 18.78. HRMS (ESI) m/z: calcd for $C_{20}H_{23}NO_4Na$ $[M + Na]^+$: 364.1525, found: 364.1526.

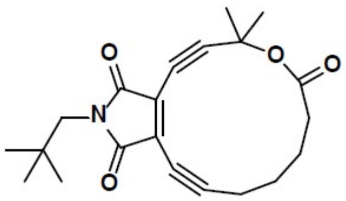


6-methyl-2-neopentyl-6,9,10,11,12,13-hexahydro-4,5,14,15-tetradehydro-[1]oxacyclotetradecino[5,6-c]pyrrole-1,3,8(2*H*)-trione (EDY-C). 21 mg (11.8%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.68 (q, *J* = 6.9 Hz, 1H), 3.33 (s, 2H), 2.65 – 2.56 (m, 2H), 2.49 – 2.42 (m, 1H), 2.40 – 2.35 (m, 1H), 1.78 – 1.72 (m, 2H), 1.66 – 1.59 (m, 7H), 0.90 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.66, 166.44, 166.42, 129.46, 127.31, 112.09, 106.17, 74.09, 71.97, 59.25, 48.95, 33.18, 32.55, 26.85, 26.08, 25.83, 24.54, 19.48, 18.99. HRMS (ESI) m/z: calcd for $C_{21}H_{25}NO_4Na$ $[M + Na]^+$: 378.1681, found: 378.1682.

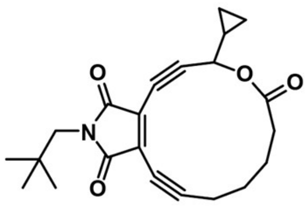


2-neopentyl-9,10,11,12-tetrahydro-4,5,13,14-tetradehydro-1*H*-[1]oxacyclotridecino[5,6-c]pyrrole-1,3,8(2*H*,6*H*)-

trione (EDY-D). 17 mg (10.4%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.00 (s, 2H), 3.33 (s, 2H), 2.58 – 2.54 (m, 2H), 2.38 – 2.34 (m, 2H), 1.90 (dt, *J* = 10.8, 6.9 Hz, 2H), 1.82 (dt, *J* = 12.5, 6.9 Hz, 2H), 0.90 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.06, 166.16, 131.37, 113.36, 103.12, 76.13, 71.16, 51.01, 48.89, 32.72, 32.55, 28.68, 26.85, 26.25, 19.43. HRMS (ESI) m/z: calcd for $C_{19}H_{21}NO_4Na$ $[M + Na]^+$: 350.1368, found: 350.1360.



6,6-dimethyl-2-neopentyl-9,10,11,12-tetrahydro-4,5,13,14-tetradehydro-1*H*-[1]oxacyclotridecino[5,6-c]pyrrole-1,3,8(2*H*,6*H*)-trione (EDY-E). 30 mg (16.9%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.32 (s, 2H), 2.55 – 2.51 (m, 2H), 2.26 – 2.22 (m, 2H), 1.91 (ddd, *J* = 14.1, 9.1, 4.2 Hz, 2H), 1.88 – 1.84 (m, 2H), 1.77 (s, 6H), 0.89 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.24, 167.37, 167.34, 131.33, 129.54, 113.98, 110.49, 75.05, 72.87, 72.10, 49.81, 34.22, 33.54, 28.34, 27.84, 27.42, 24.23, 20.48. HRMS (ESI) m/z: calcd for $C_{21}H_{25}NO_4Na$ $[M + Na]^+$: 378.1681, found: 378.1683.



6-cyclopropyl-2-neopentyl-9,10,11,12-tetrahydro-4,5,13,14-tetradehydro-1*H*-[1]o-xacyclotridecino[5,6-c]pyrrole-1,3,8(2*H*,6*H*)-trione (EDY-F). 18 mg (9.8%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.36 (d, *J* = 7.7 Hz, 1H), 3.32 (s, 2H), 2.71 (ddd, *J* = 18.3, 5.2, 2.3 Hz, 1H), 2.58 – 2.53 (m, 1H), 2.39 (ddd, *J* = 18.4, 10.9, 2.6 Hz, 1H), 2.20 – 2.13 (m, 1H), 2.03 – 1.96 (m, 1H), 1.82 – 1.77 (m, 2H), 1.37 – 1.31 (m, 1H), 0.98 (t, *J* = 6.5 Hz, 1H), 0.89 (s, 9H), 0.73 – 0.63 (m, 2H), 0.60 – 0.50 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.07, 167.22, 167.15, 132.63, 129.41, 114.45, 105.76, 75.79, 72.29, 68.07, 49.91, 34.00, 33.56, 27.88, 27.37, 24.01, 20.49, 13.65, 3.77, 2.94. HRMS (ESI) m/z: calcd for $C_{21}H_{25}NO_4Na$ $[M + Na]^+$: 390.1681, found: 390.1680.

EPR Measurements

A series of enediynes (20 mM) with an excess of PBN (100 mM) were respectively incubated in DMSO at 37℃ for 12 h. The solution of PBN (100 mM) in DMSO without addition of enediyne was used as the control. Measurements were performed with an X-band EMX-8/2.7C EPR spectrometer (Bruker, Germany). The settings of the spectrometer were as follows: sweep width, 150 G; time constant, 163.84 ms; conversion time, 40.96 ms; resolution, 1024 points; modulation frequency, 100.00 kHz; modulation amplitude, 1.00 G; and microwave power, 6.420 mW.

DNA Cleavage Assay

Freshly prepared enediynes were dissolved in DMSO to a concentration of 20 mM respectively. 1 μL PUC19 plasmid DNA (200 ng/μL, in pH 8 TE solution) was added to 17 μL ultra-pure water, then EDYs in DMSO were added. Extra DMSO were added if it is necessary to maintain a total volume of 17 μL. Control samples consisting a solution of pUC19 DNA (200 ng/μL) in ultra-pure water (17 μL) were separately incubated with 8 μL DMSO. All samples were incubated at 37 °C for 48 h. After incubation, each system (8 μL) was mixed with 6 × loading buffer (2 μL) and subjected to 1% agarose gel electrophoresis at 90 V for 1 h, stained by Dured and then the gel was photographed on a UV transilluminator (FR-200A) and analyzed by scanning densitometry.

Cytotoxicity Assay

The cytotoxicity of enediynes were investigated by 3-(4,5-dimethyl-2-thiazole)-3,5-diphenyl-2H-tetrazol-3-ium bromide (MTT) assay. Hela cells were obtained from the Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China) and cultured in DMEM medium supplemented with 10% FBS and 1% antibiotics (penicillin–streptomycin, 10000 U/mL) in a humidified incubator at 37 °C with 5% CO_2. The cells were trypsinized until they reached 70% confluence in the tissue culture flasks with a buffered saline solution containing 0.25% trypsin and 0.03% EDTA. Cells were seeded into a 96-well plate (5000 cells and 100 µL cell culture medium per well) and incubated overnight for adherence. After incubation, the culture medium was removed and the cells were then exposed to 0.16, 0.31, 0.62, 1.25, 2.5, 5, 10, 20 µM concentrations of EDY. The culture medium (with 0.1% DMSO) was used as a blank control. After incubation for another 48 h, 10 µL of sterile filtered MTT stock solution (5 mg/mL) in PBS (pH 7.4) was added to each well and the cells were further incubated at 37 °C for 4 h to allow the yellow dye to transform into blue crystals. Then, the medium was replaced with 150 µL of DMSO to dissolve the purple MTT-formazan crystals. Finally, the optical density was measured using a microplate reader at a wavelength of 570 nm. The spectrophotometer was normalized using a culture medium without cells. Cell viability (%) related to control wells containing cell culture medium was calculated by $[OD]_{test}/[OD]_{control}$.

Cellular Uptake

The cellular uptake of enediynes in vitro were examined using HeLa cells as a model cell line with Confocal Laser Scanning Microscopy (CLSM). Hela cells were seeded in glass bottom confocal dishes at a density of 2×10^5 per well in 2 mL of DMEM and incubated for 24 h. After removal of culture medium, cells were incubated with EDY-A (4 µM) or EDY-B (1 µM) in 2 mL of DMEM respectively. Hela cells without any drug were incubated as a blank control. After 14 h of incubation at 37 °C, the cells are gently washed three times with PBS. Subsequently, the cells were fixed with 2.5% glutaraldehyde at room temperature for 20 min, and the slides were rinsed with PBS three times. After permeated by 0.5% Triton solution for 10 min, washed three times with PBS again. Then, 400 µL of propidium iodide (PI) solution (15 µg/mL) was added and the cells were cultured at 37 °C in the dark for 10 min, followed by washing with cold PBS for three times. The resulting slides were mounted and observed using a LEICA TCS SP8 fluorescence microscope.

Intracellular DNA Damage Assay

Cells were cultured using the same procedure as the cellular uptake assay. After fixation and permeation, cells were blocked by 8% BSA/PBS for 2 h at room temperature. Then, cells were stained with phospho-histone H2A.X (Ser139) rabbit monoclonal antibody (1:200 dilution in 1% BSA/PBS, at 4 °C, overnight) and labeled with secondary antibodies (Alexa Fluor® 488 dye, 1:1000 dilution, RT, 2 h). The nuclei were further stained with PI for visualizing DNA. After incubation, cells were washed three times with PBS (5 min each), and the γ-H2AX foci was imaged via CLSM.

Cell Cycle Arrest Assay

HeLa cells were seeded in 6-well plates at a density of 2×10^5 per well in 2 mL of DMEM and incubated overnight. After removing the medium and washing with PBS, the cells were incubated with different concentrations of EDY-B at 37 °C for 24 h. HeLa cells without drug were used as a control. Cells were harvested by trypsinization, rinsed and resuspended in cold PBS, and fixed with 70% ethanol overnight at 4 °C. Then the cells were washed with PBS, stained with 400 µL PI solution (10 µg/mL PI and 0.1% Triton-X100 in PBS) after centrifugation (4 °C, 1000 r.p.m., 5 min) and incubated at 37 °C in the dark for 30 min. Finally, DNA content was measured by Flow Cytometry (BDFACSCalibur, USA), the percentage of cells in each phase of the cell cycle was calculated using the Flow Jo software.

Apoptosis Assay

HeLa cells were seeded in 6-well plates at a density of 2×10^5 per well in 2 mL of DMEM and incubated overnight. After removing the medium and washing with PBS, the cells were incubated with different concentrations of EDY-B at 37 °C for 24 h. HeLa cells without drug were used as a control. Then, the treated cells were harvested by trypsinization, washed twice with cold PBS. After that, the cells were resuspended in 400 µL annexin binding buffer and stained with Annexin V-FITC (5 µL) and PI (5 µL) at room temperature for 15 min and analyzed by flow cytometry (BDFACSCalibur, USA)

Table S1 Physicochemical properties and reactivity evaluation in the Myers-Saito Cyclization of enediyne compounds

Compound	Molecular formular	Molecular weight (g/mol)	Cycle size	Log p	△G (Kcal/mol)
EDY-A	$C_{19}H_{21}NO_4$	327	12	2.12	21.6
EDY-B	$C_{20}H_{23}NO_4$	341	13	2.53	25.7
EDY-C	$C_{21}H_{25}NO_4$	355	14	2.95	26.7
EDY-D	$C_{19}H_{21}NO_4$	327	13	2.22	27.9 & 18.5[a]
EDY-E	$C_{21}H_{25}NO_4$	355	13	2.75	
EDY-F	$C_{22}H_{25}NO_4$	367	13	2.92	25.5

[a] The two data are the energy barriers of MSC after single and multiple 1,3-proton transfer steps[8], respectively.

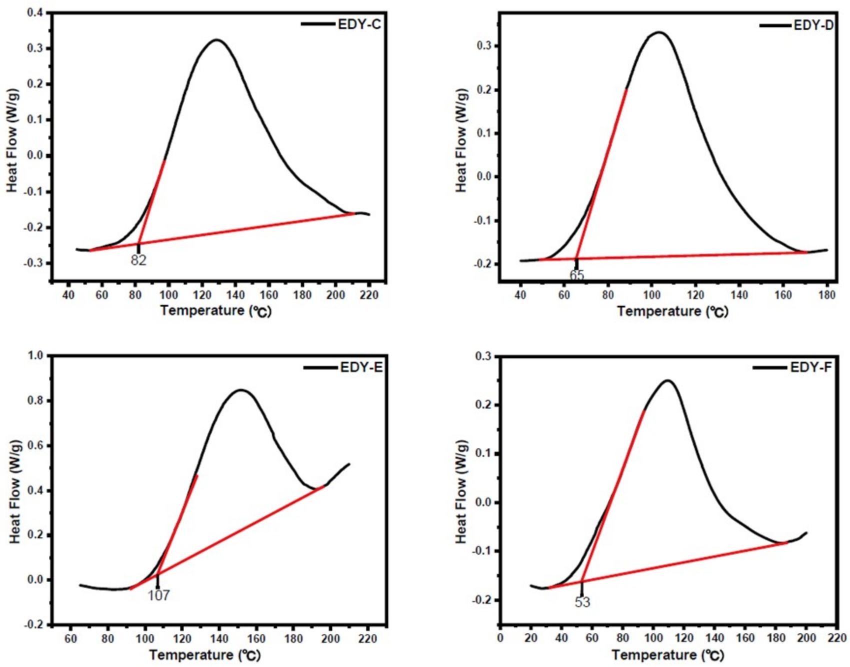


Fig. S1 DSC curves of neat enediynes at a heating rate of 10 ℃/min. The baselines are marked in red for guide of view.

Table S2 Onset temperature of enediynes measured by DSC.

EDY	A	B	C	D	E	F
T_{onset} (℃)	47	73	82	65	107	53

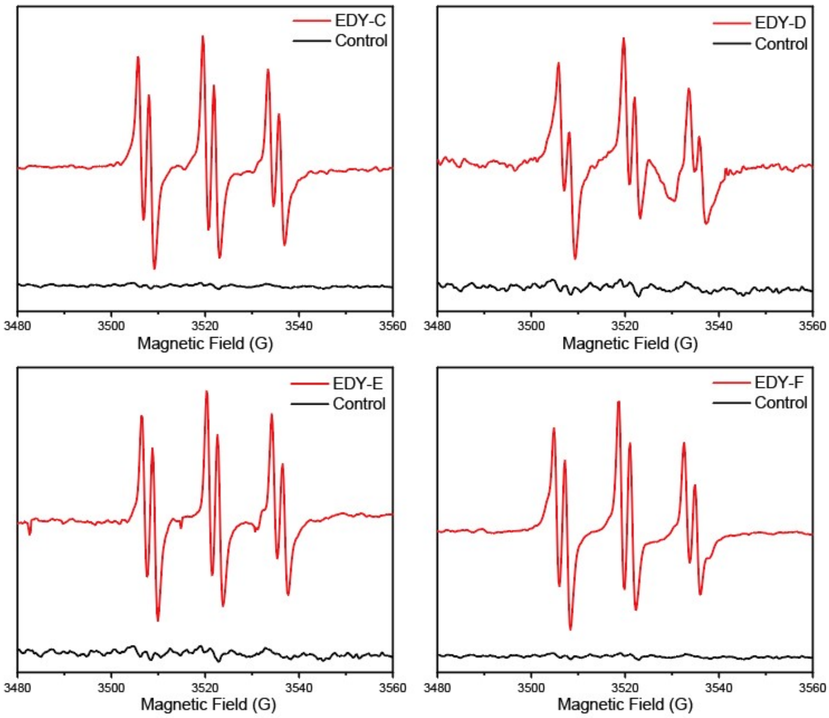


Fig. S2 EPR curves of enediynes.

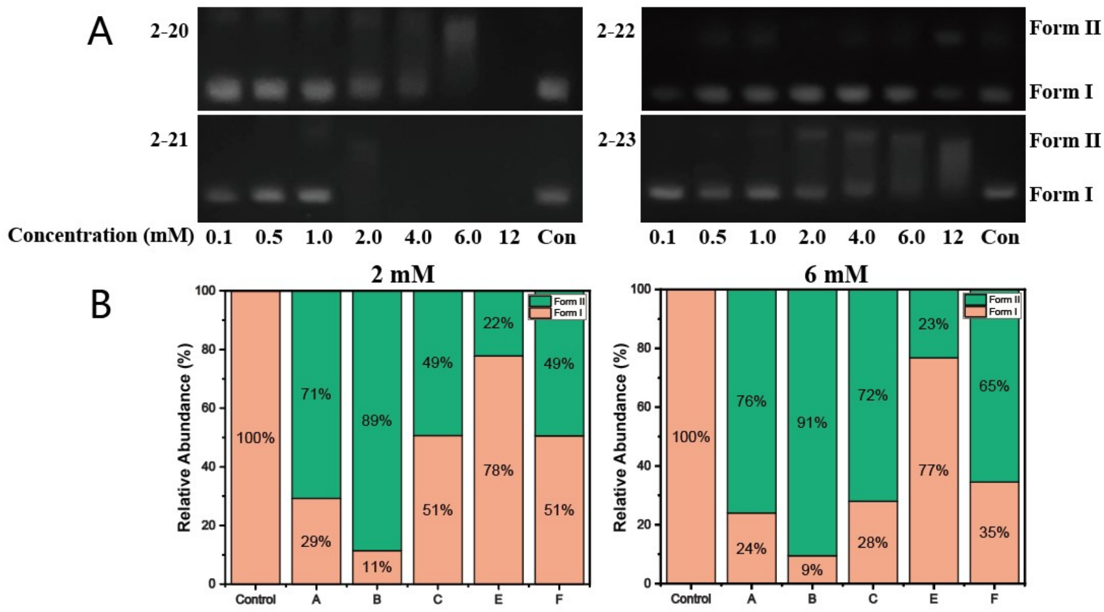


Fig. S3 (A) Agarose gel electrophoretic image of enediynes. (B) Quantified cleavage data for DNA cleavage assay of enediynes at the concentration of 2 μM and 6 μM. Control: pUC19 (10 μg mL^{-1}) alone.

Table S3 IC_{50} values of enediynes against Hela cells obtained by MTT assay.

EDY	A	B	C	D	E	F
IC_{50} (μM)	3.90	0.63	0.57	3.85	12.80	5.29

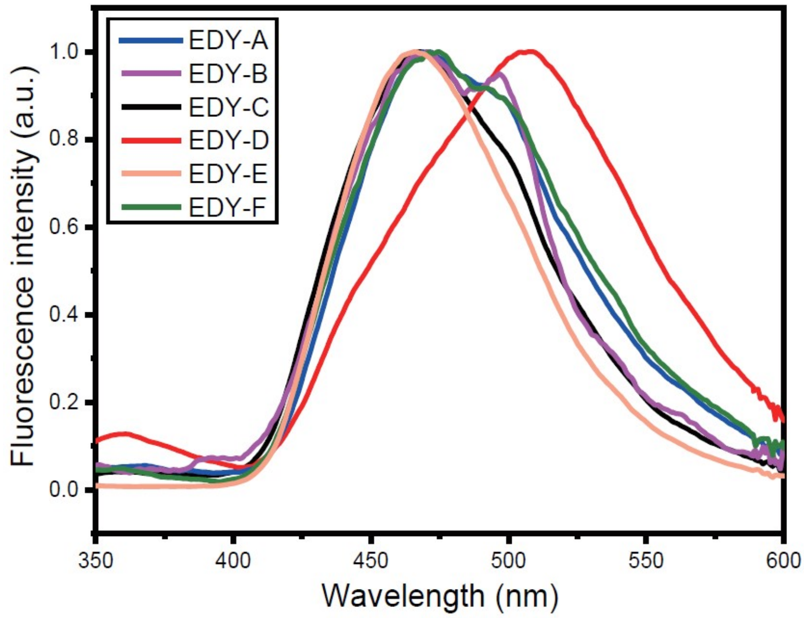


Fig. S4 Fluorescence emission spectra of enediynes in DCM (λ_{ex} = 254 nm).

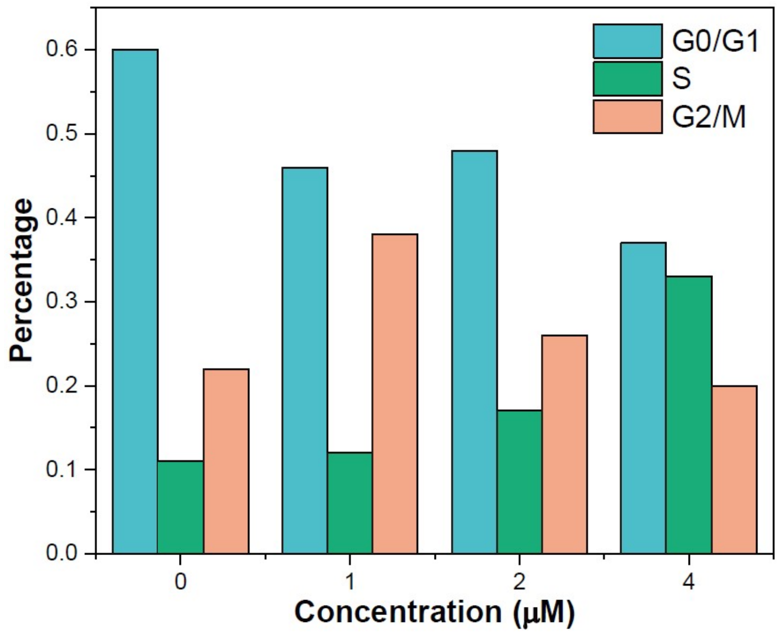


Fig. S5 Concentration dependence of cell cycle distributions of Hela cells treated with EDY-B.

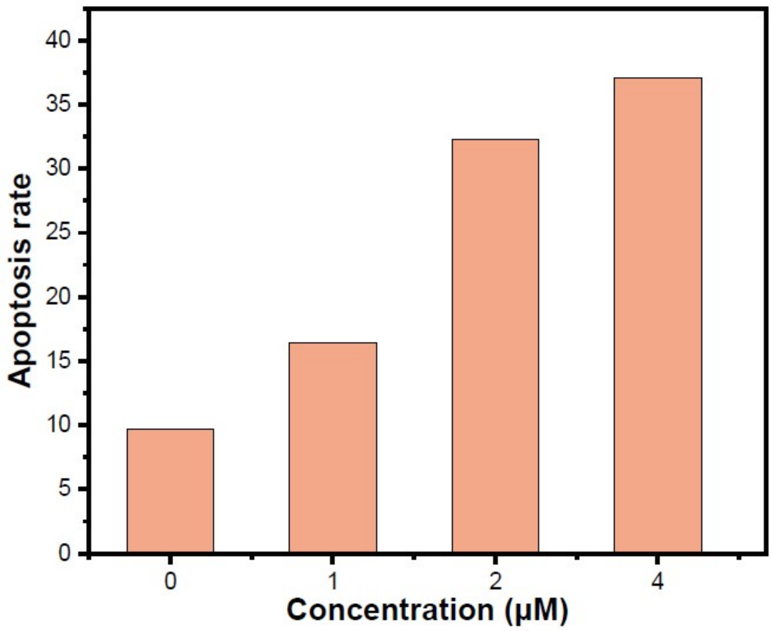
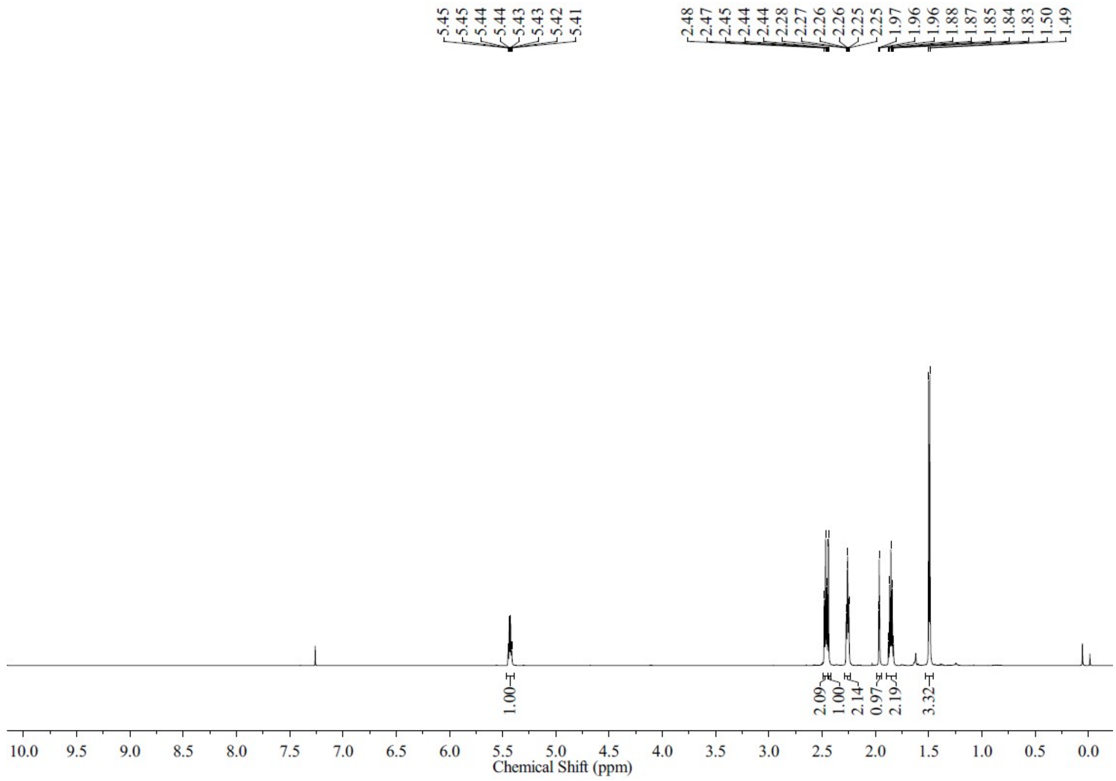


Fig. S6 Concentration dependence of the apoptosis rate of Hela cells treated with EDY-B.



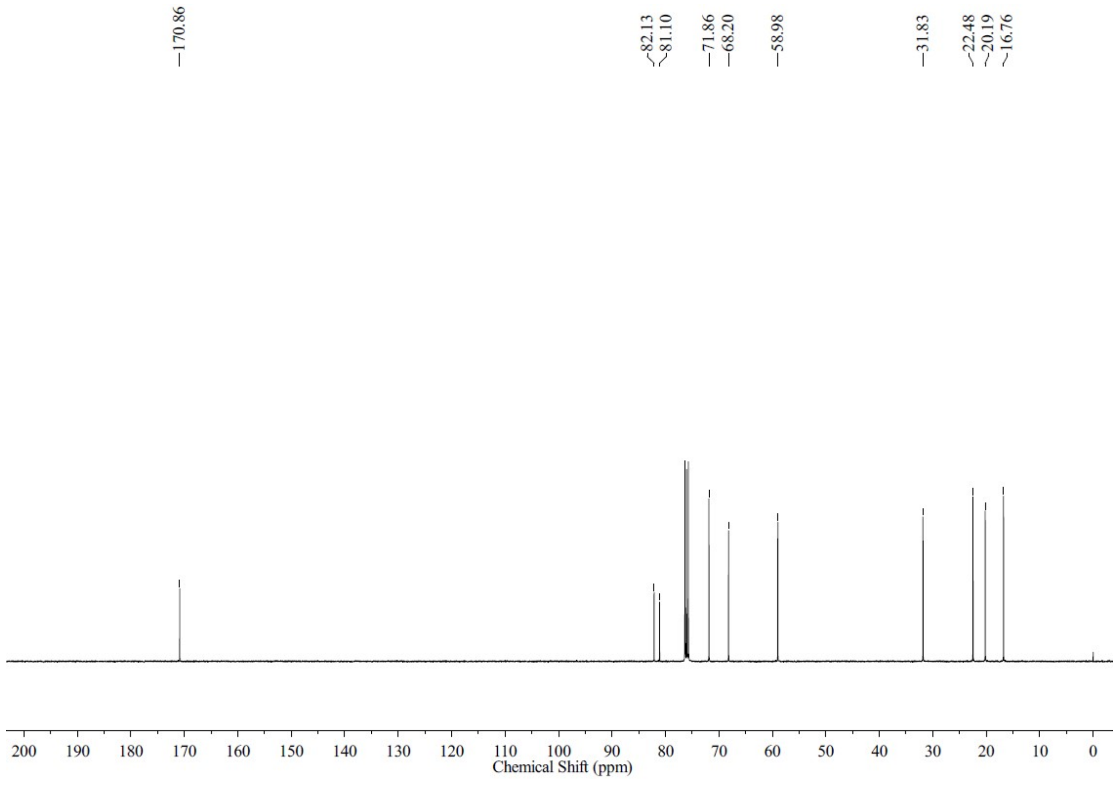
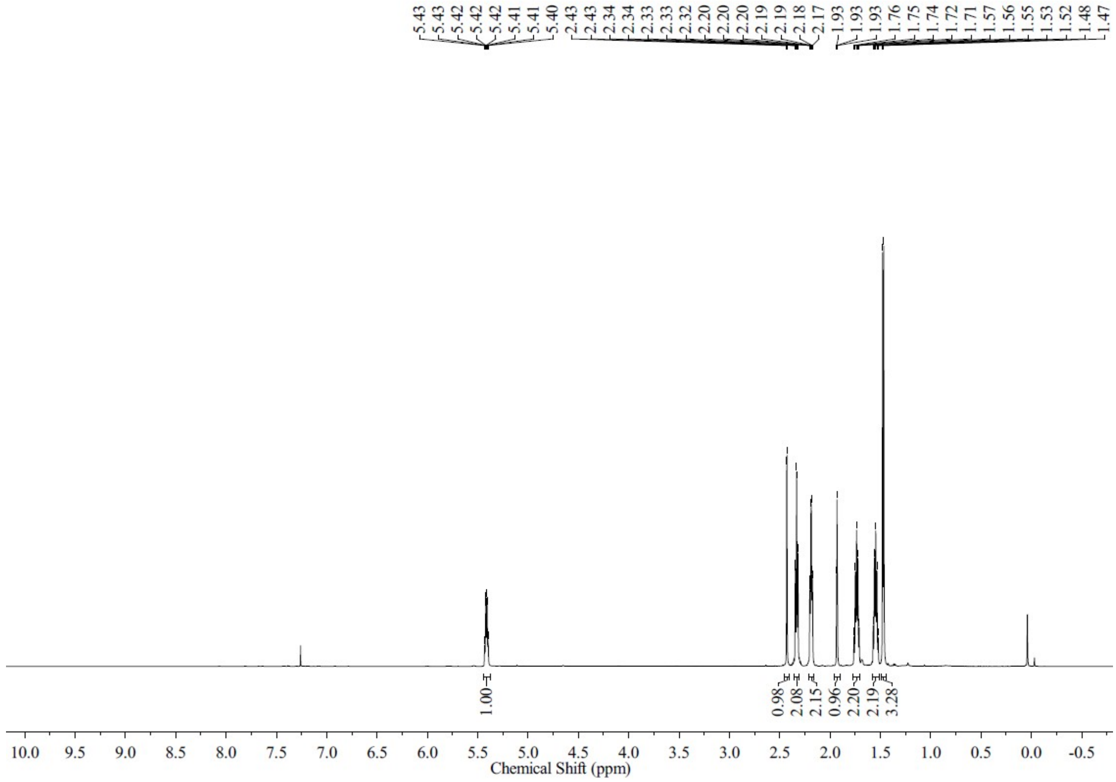


Fig. S7 ^{1}H NMR and ^{13}C NMR spectra of compound A1



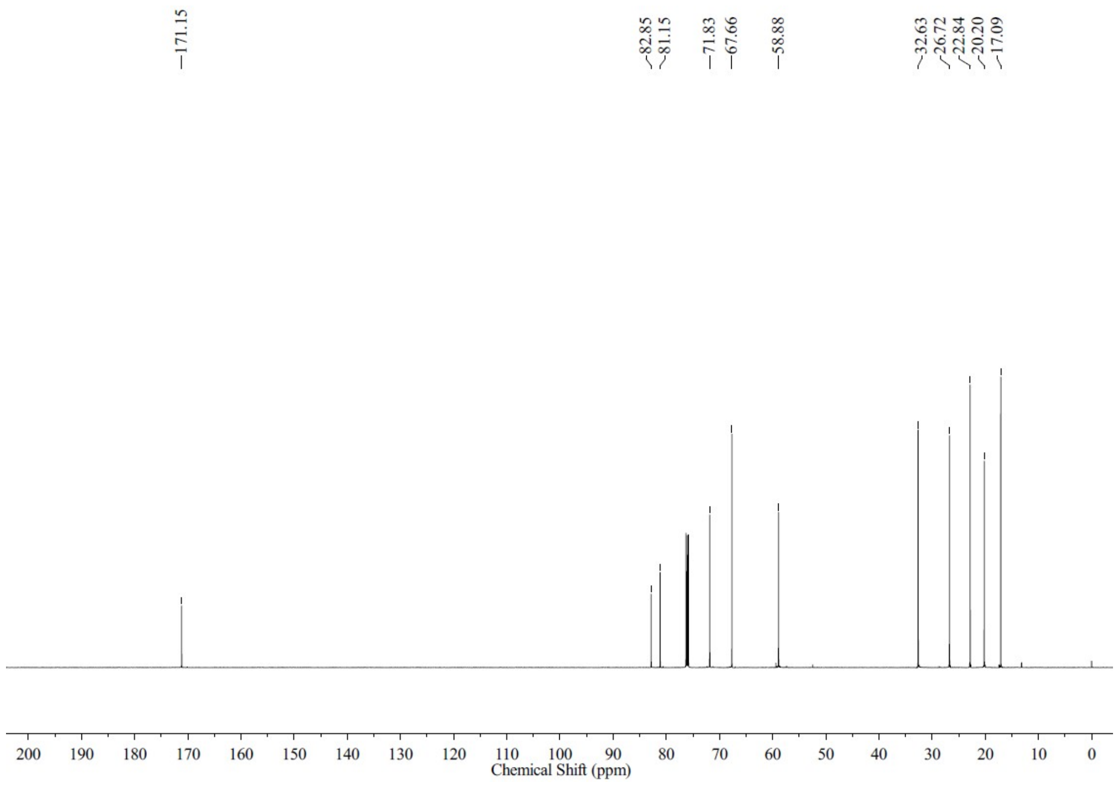


Fig. S8 ^{1}H NMR and ^{13}C NMR spectra of compound B1

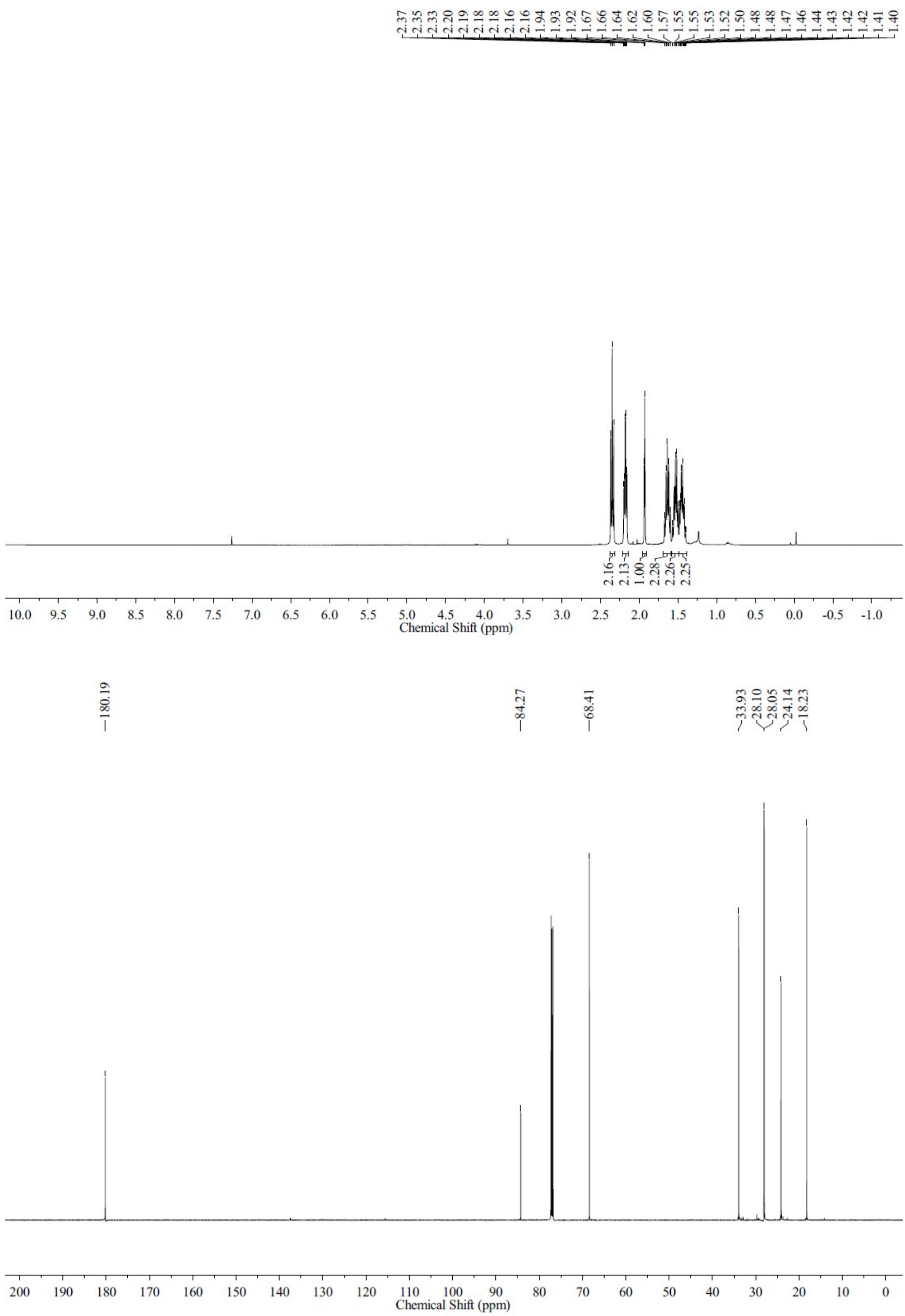


Fig. S9 ^{1}H NMR and ^{13}C NMR spectra of compound C0

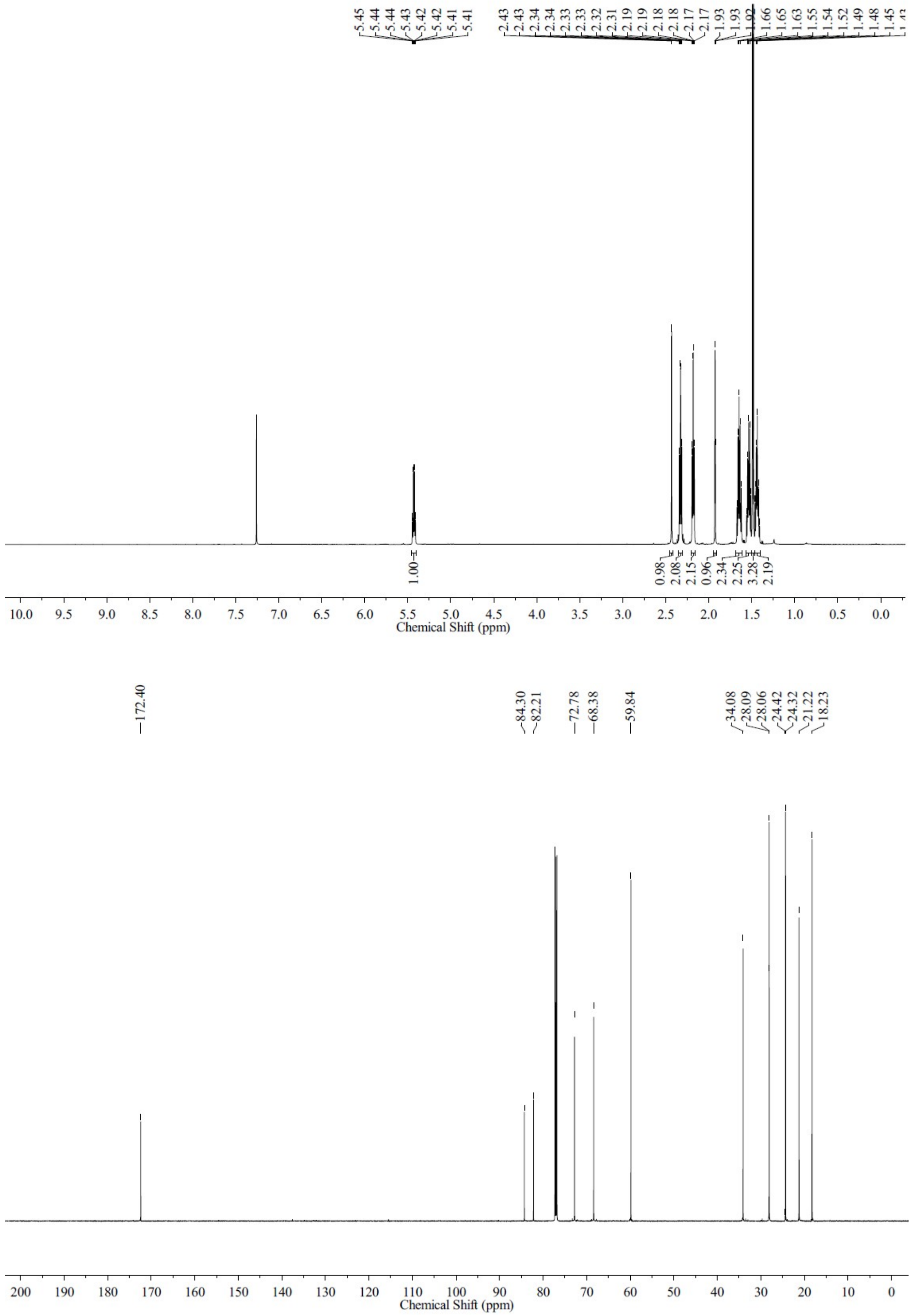


Fig. S10 ^{1}H NMR and ^{13}C NMR spectra of compound C1

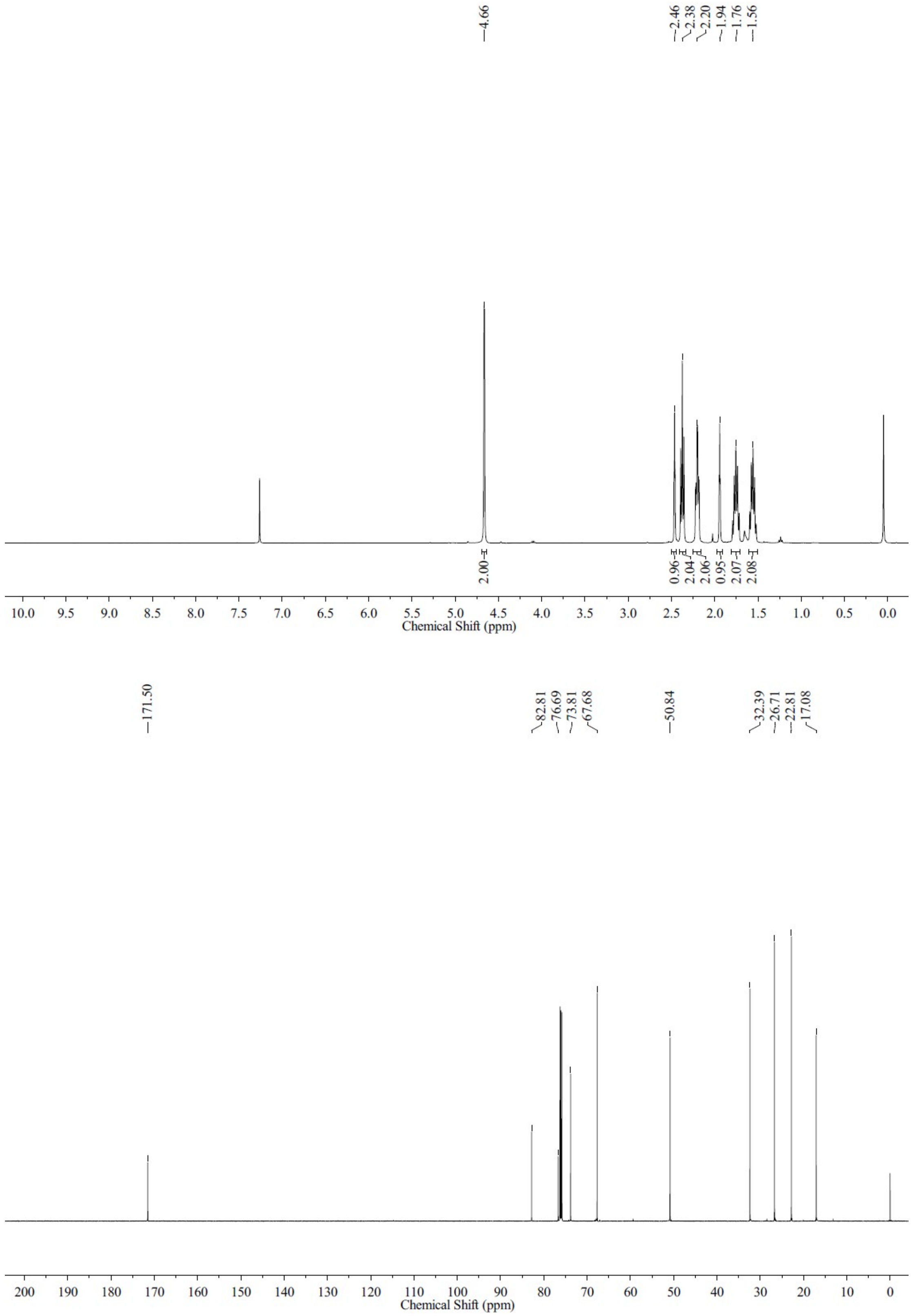
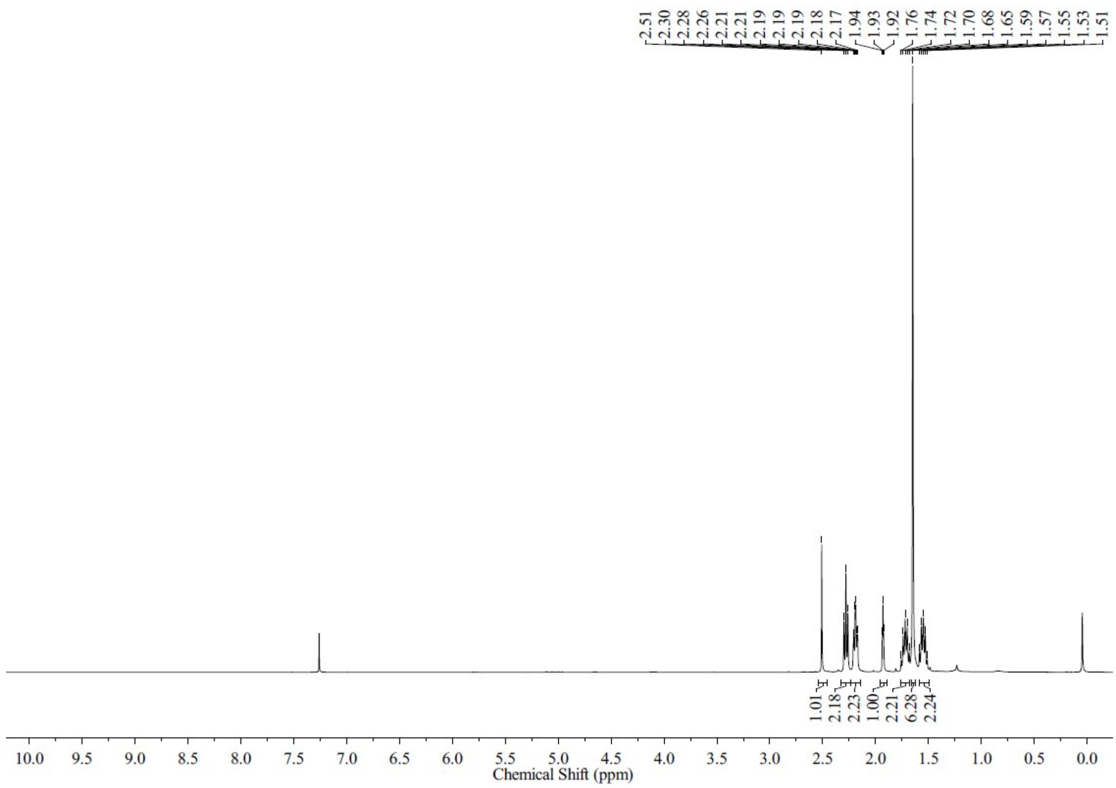


Fig. S11 ^{1}H NMR and ^{13}C NMR spectra of compound D1



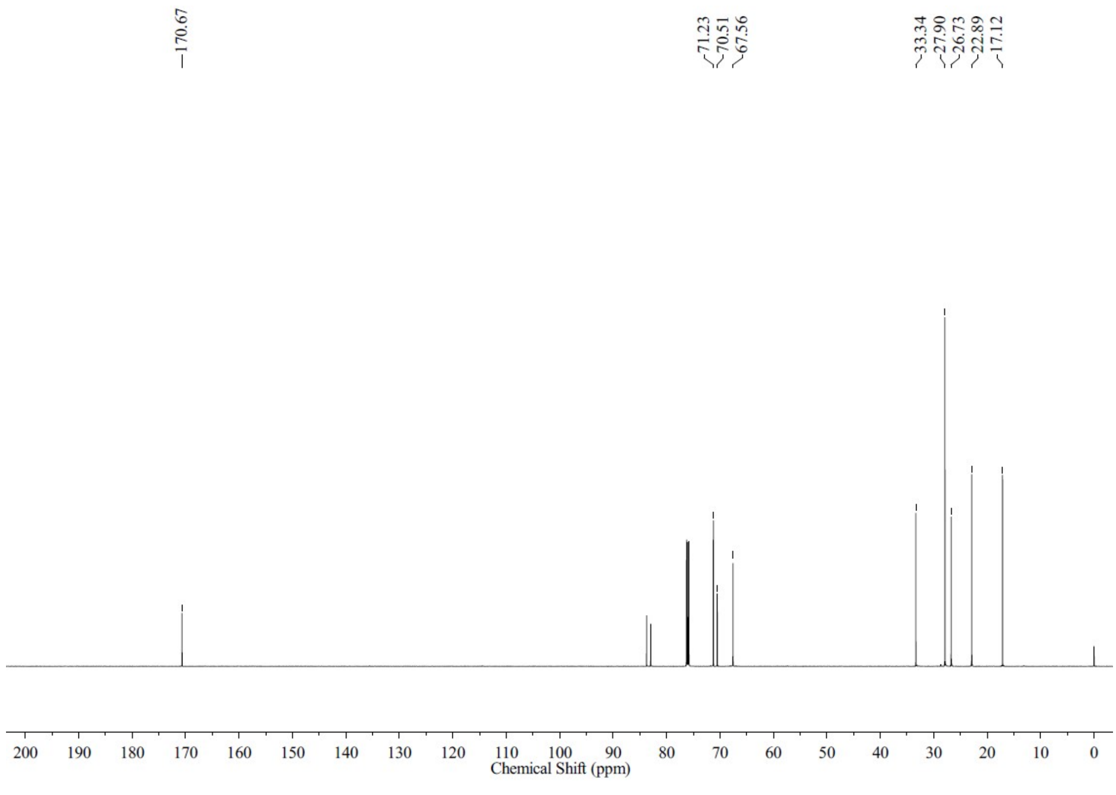


Fig. S12 ^{1}H NMR and ^{13}C NMR spectra of compound E1

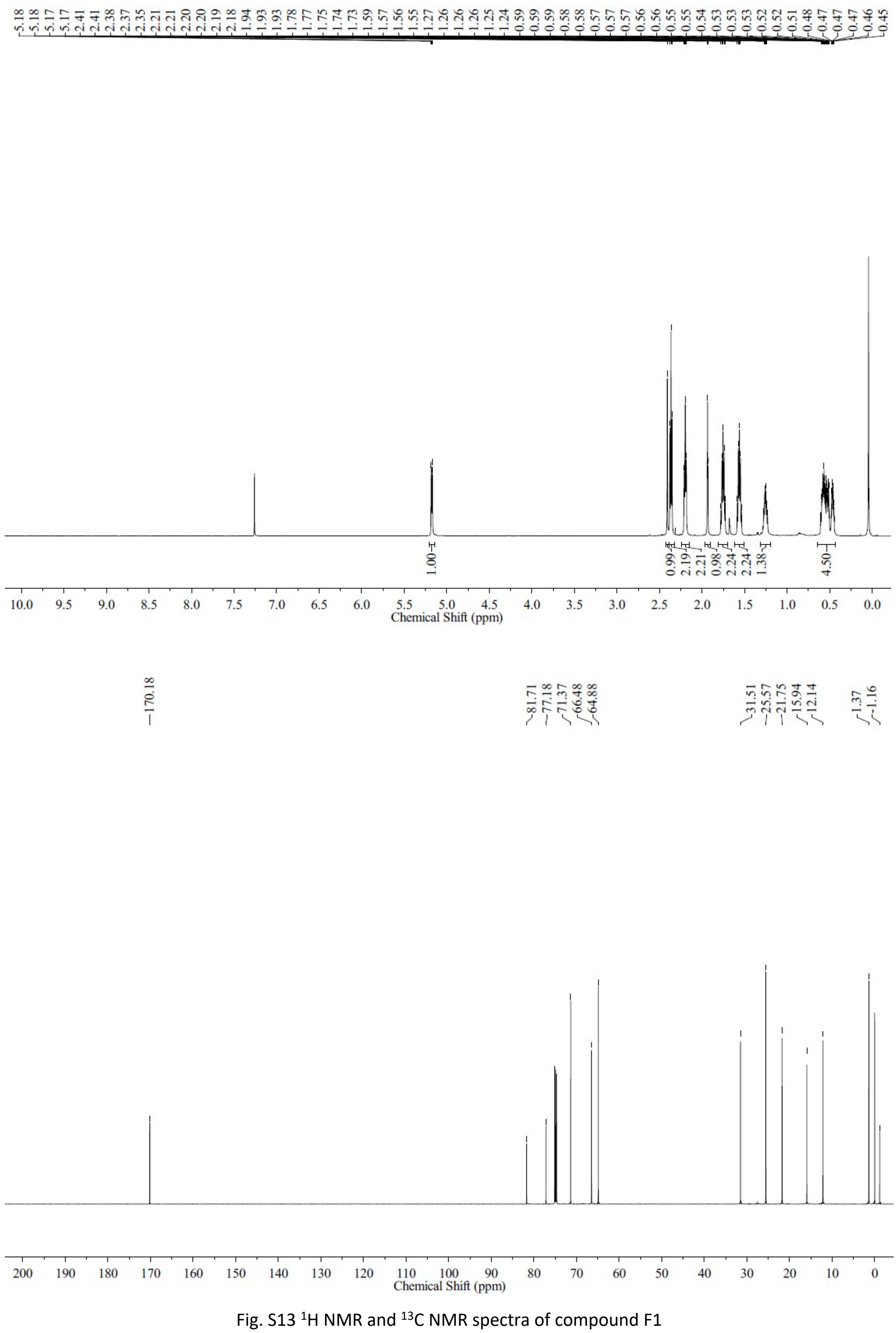
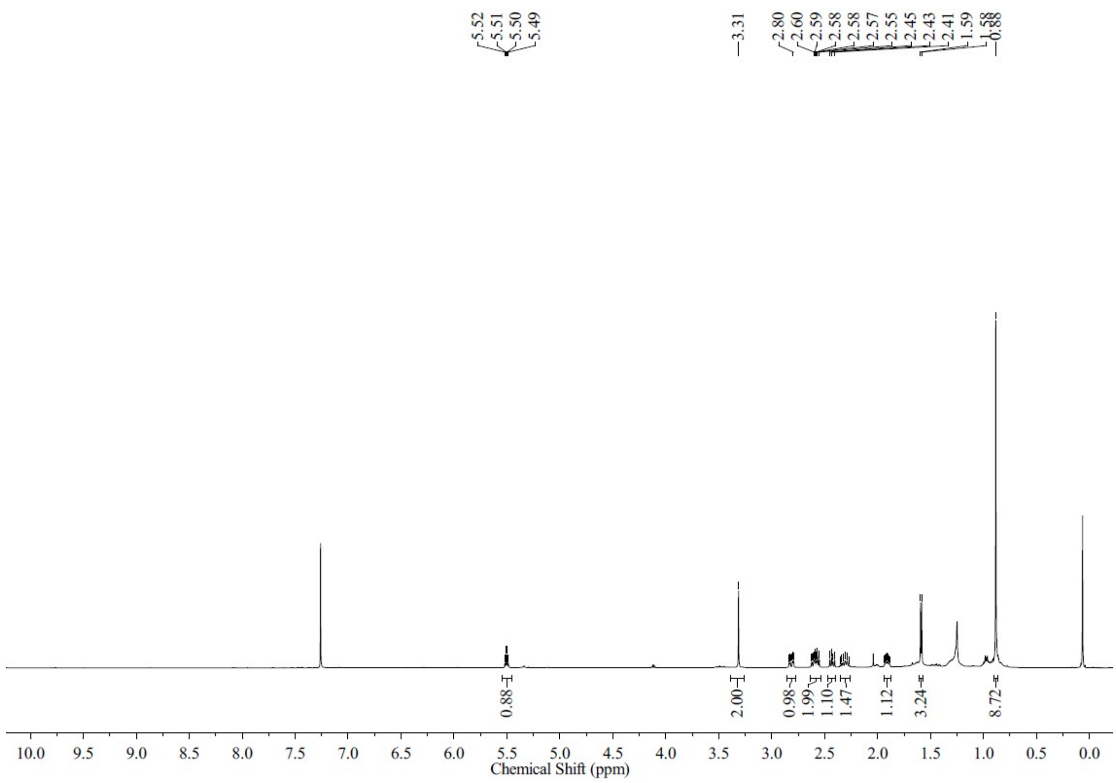


Fig. S13 ^{1}H NMR and ^{13}C NMR spectra of compound F1



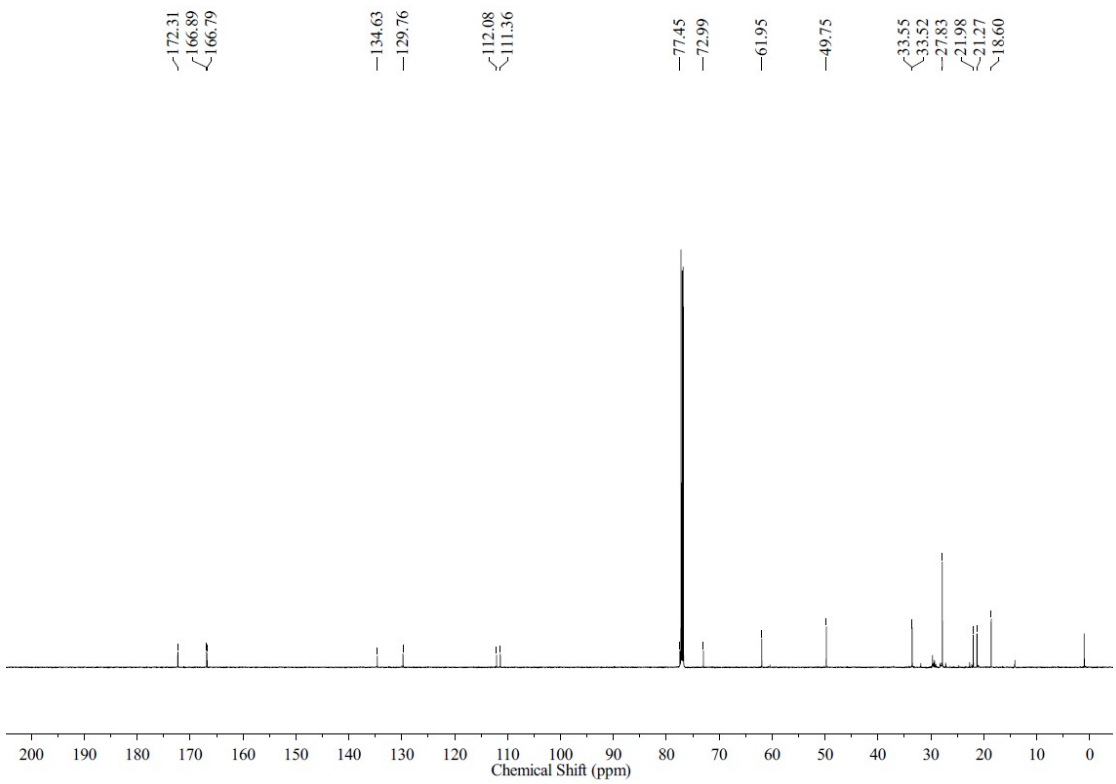
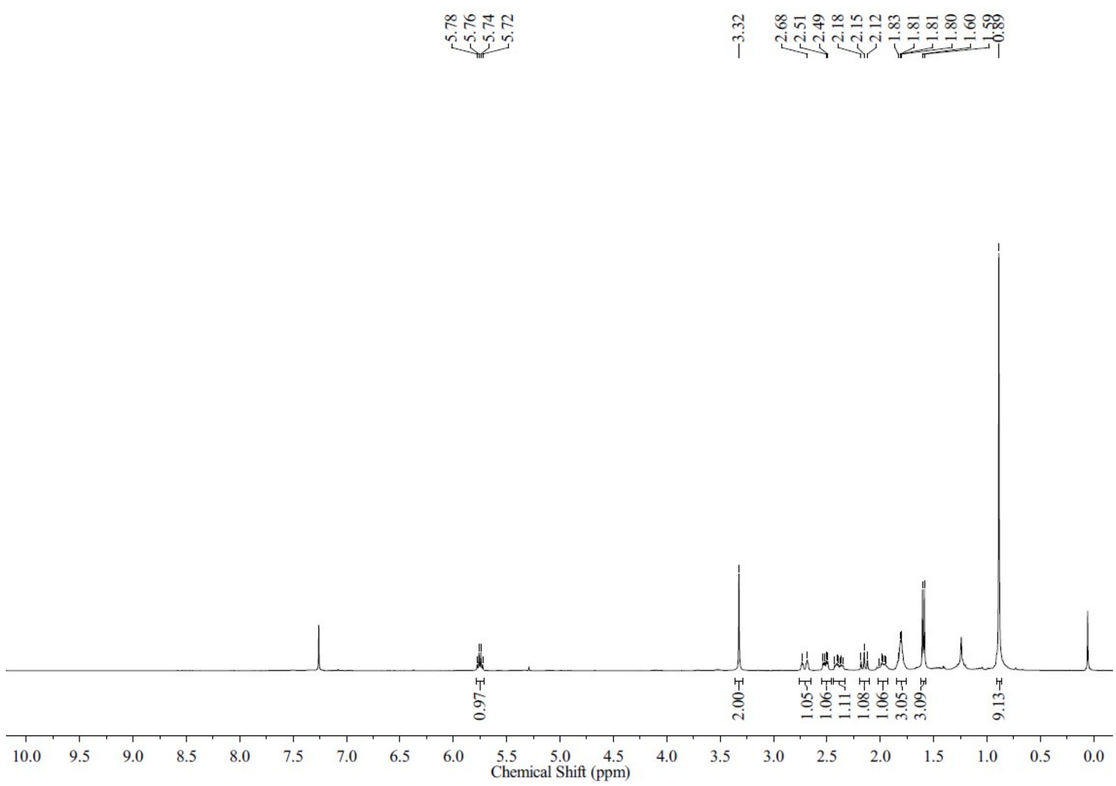


Fig. S14 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-A



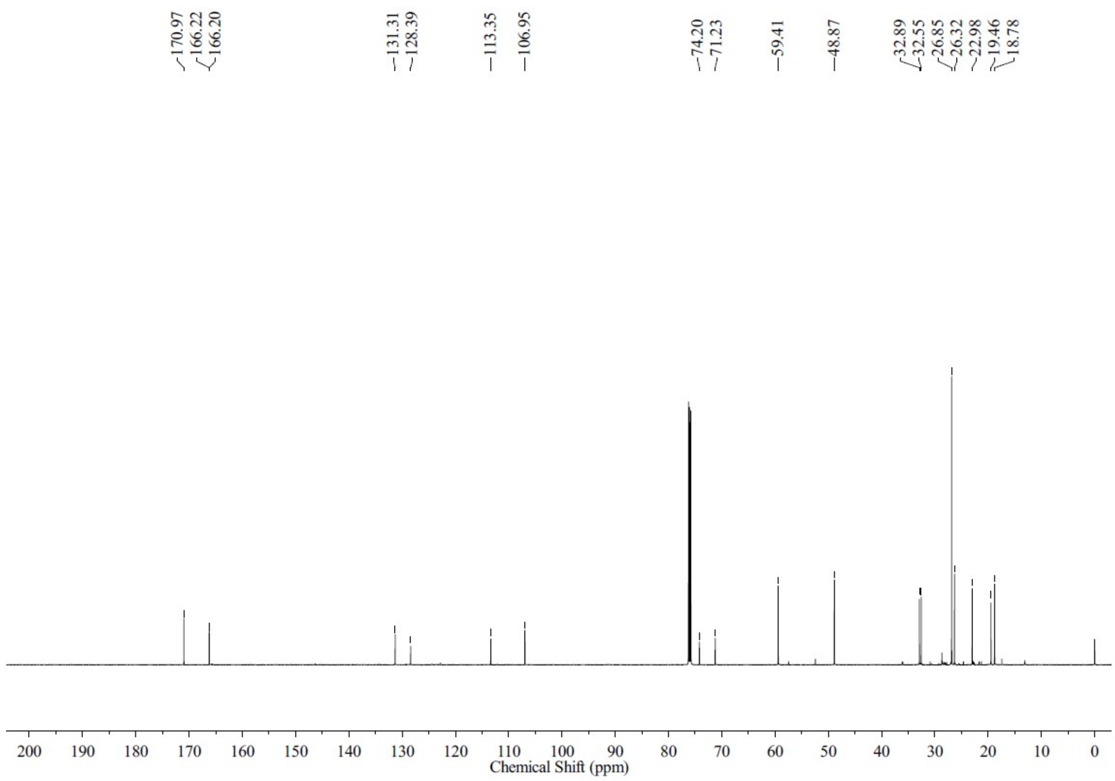


Fig. S15 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-B

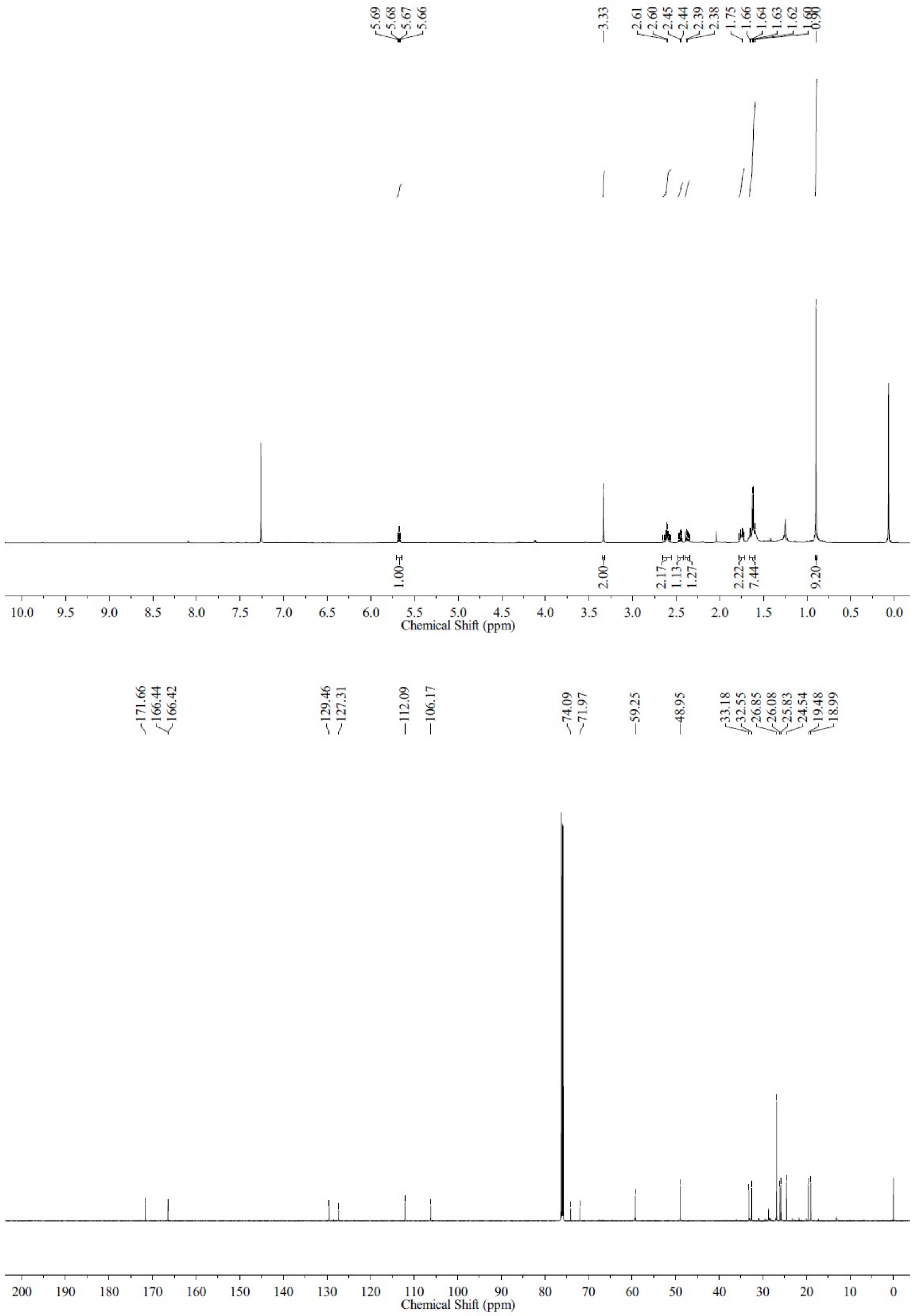
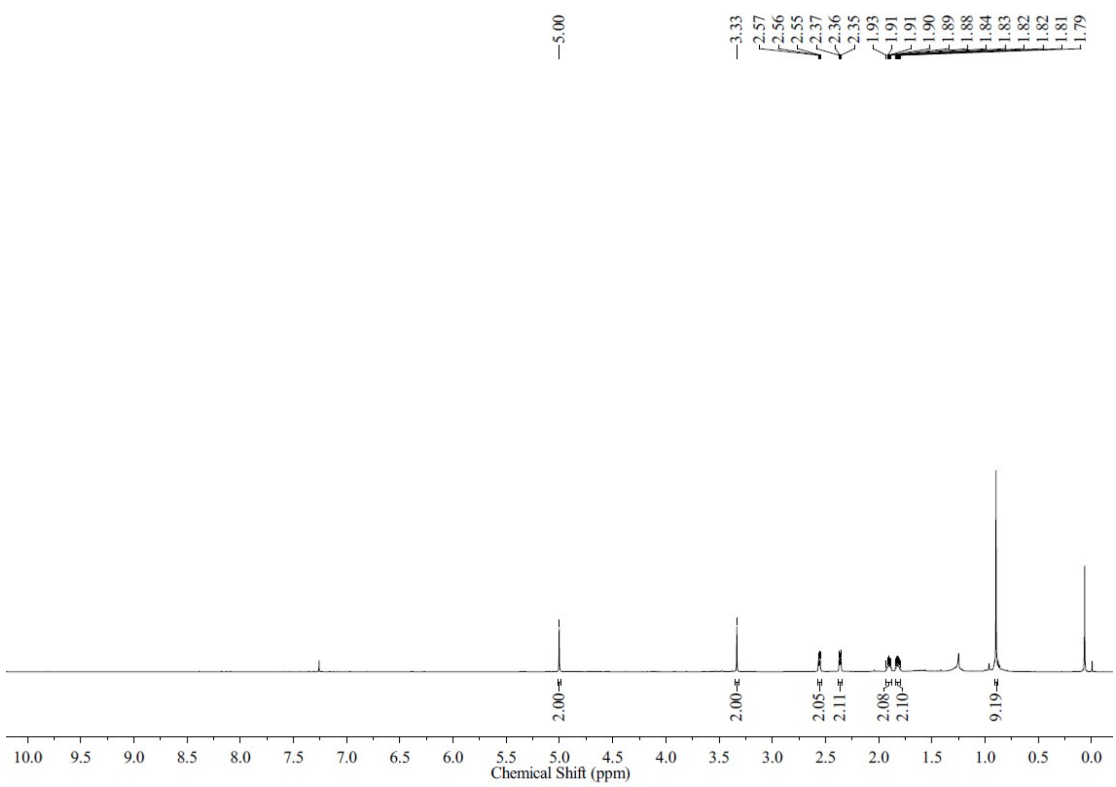


Fig. S16 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-C



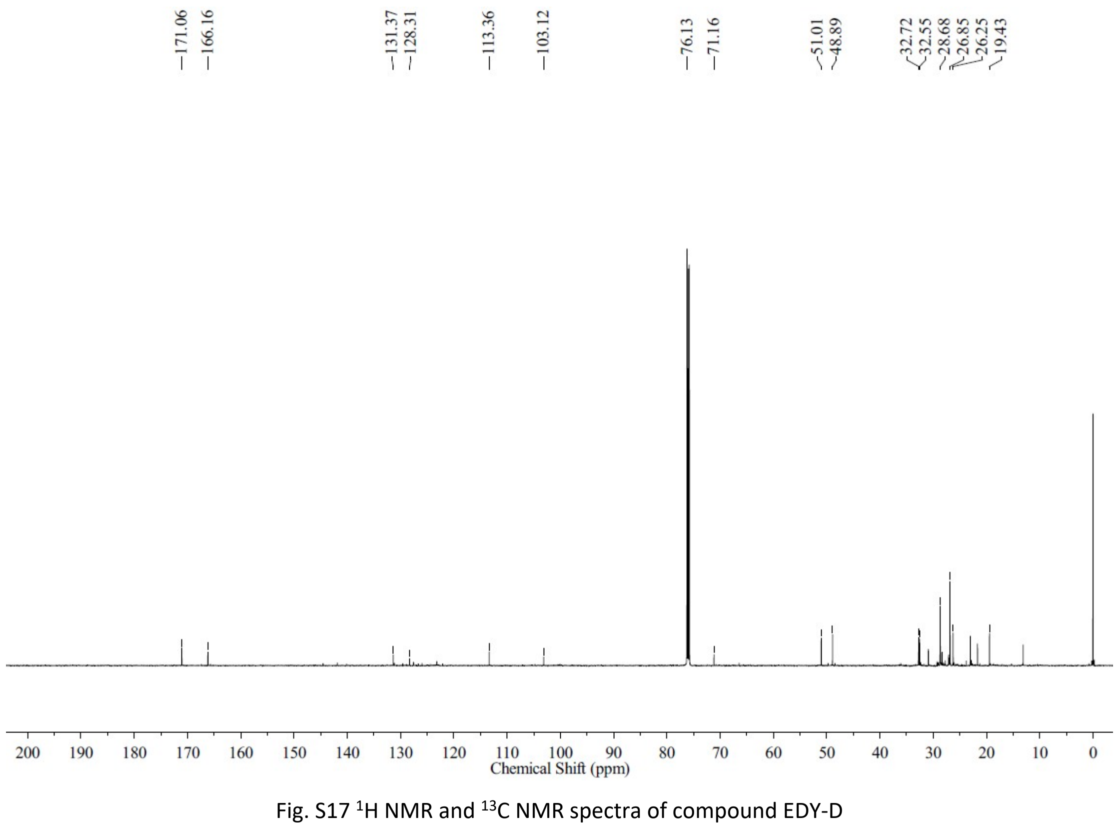
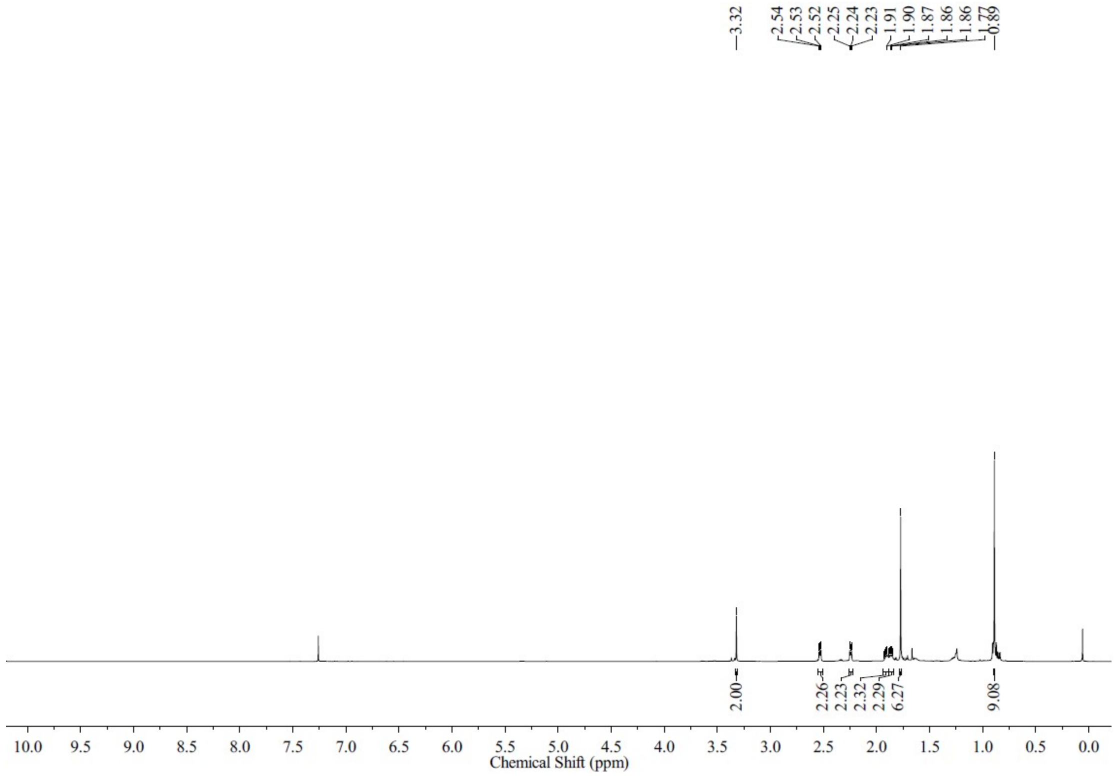


Fig. S17 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-D



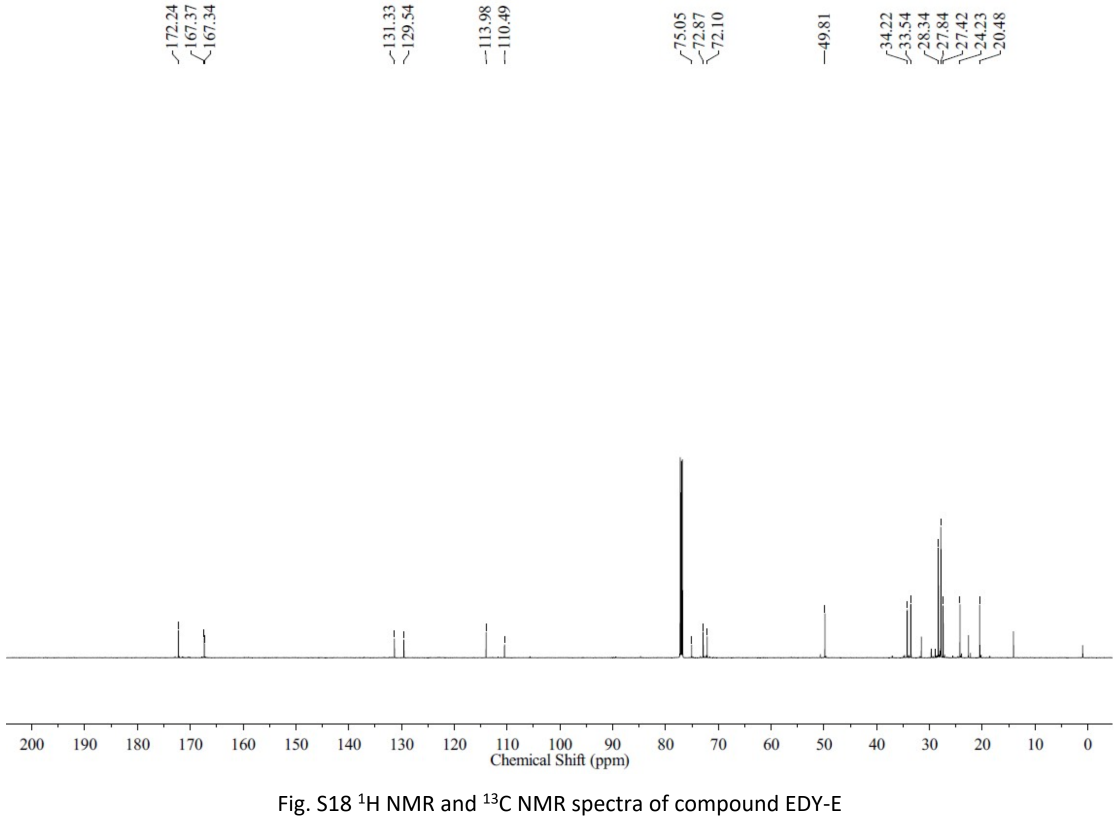


Fig. S18 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-E

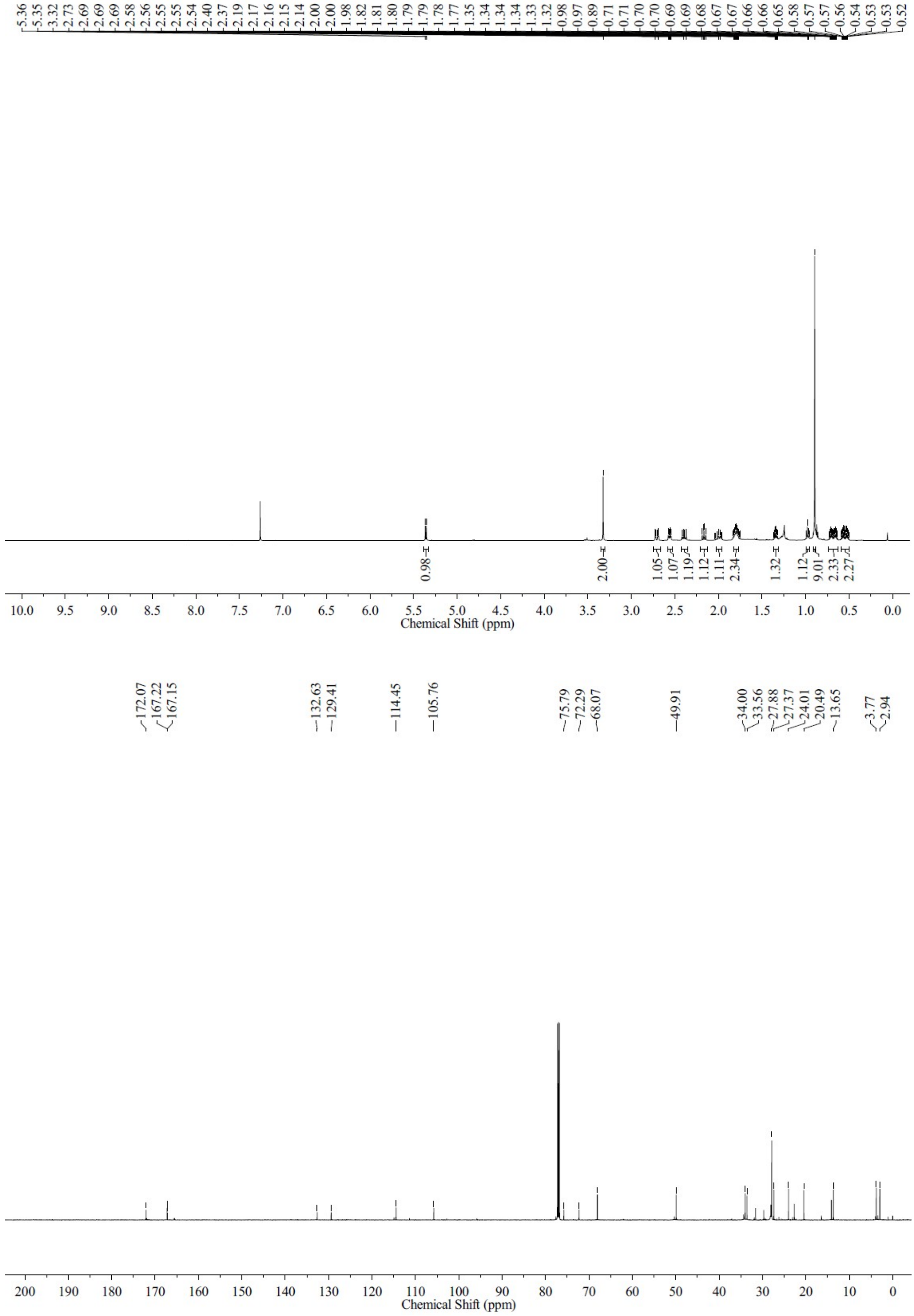


Fig. S19 ^{1}H NMR and ^{13}C NMR spectra of compound EDY-F

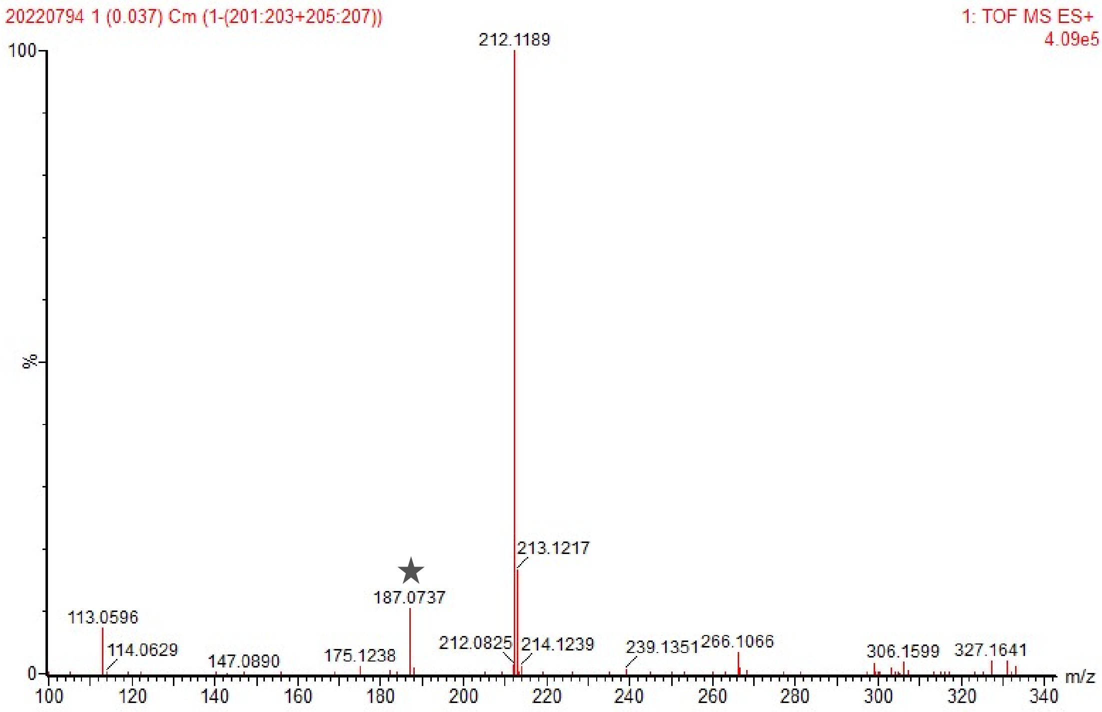


Fig. S20 HR-MS spectrum of A1

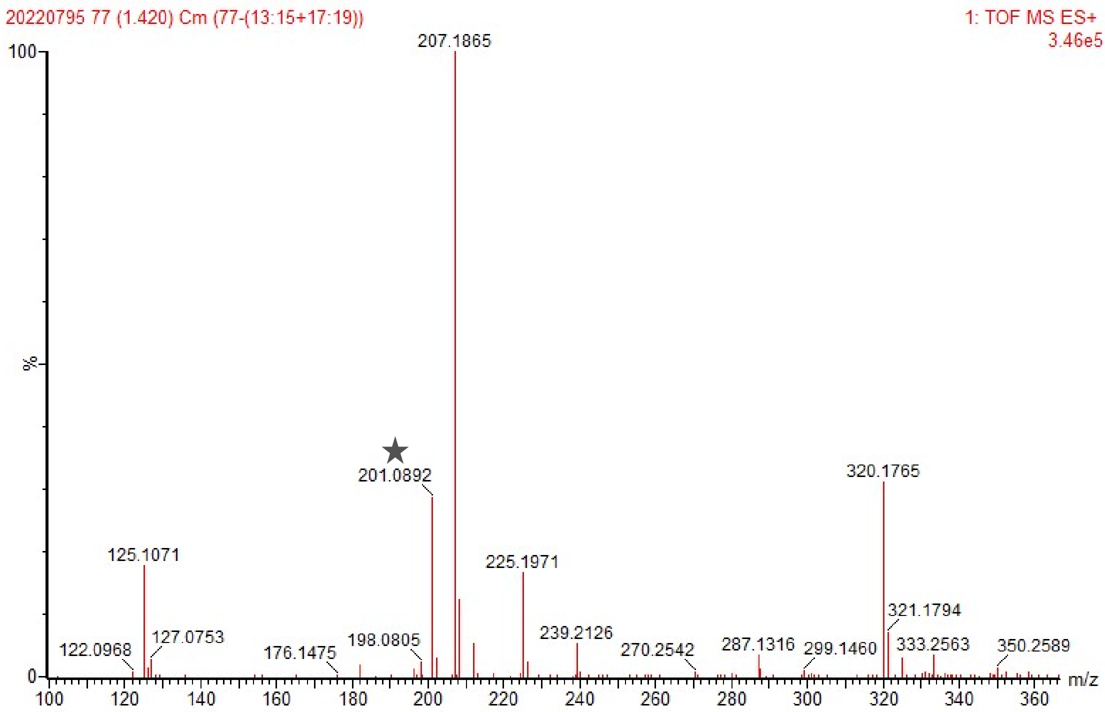


Fig. S21 HR-MS spectrum of B1

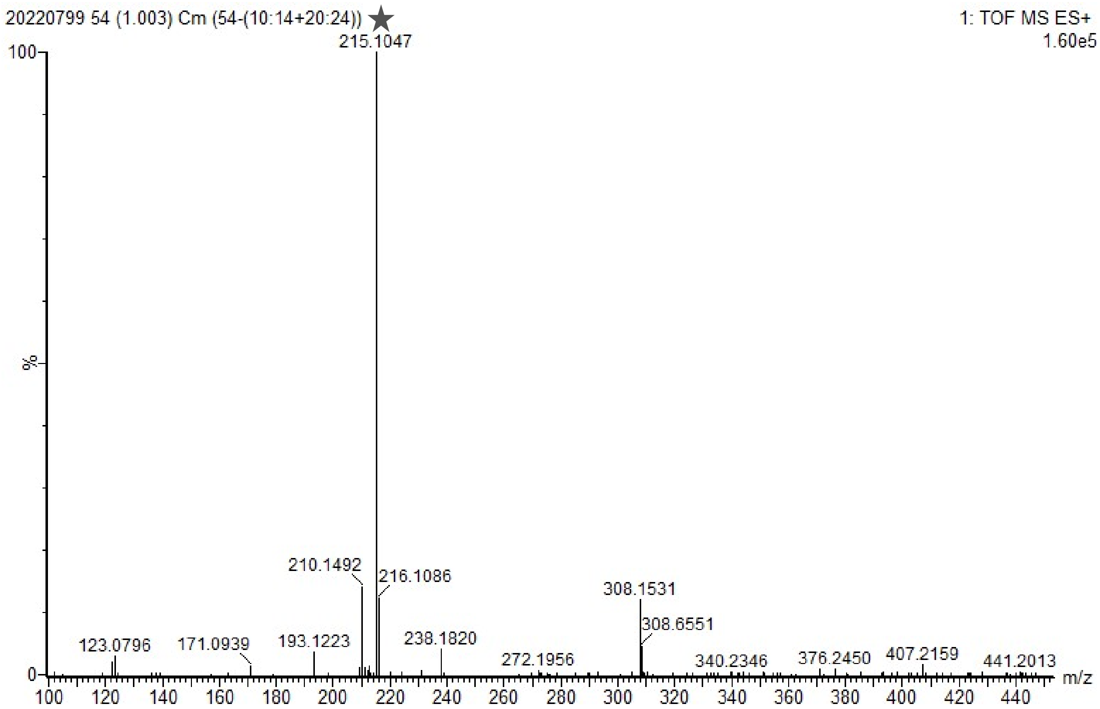


Fig. S22 HR-MS spectrum of C1

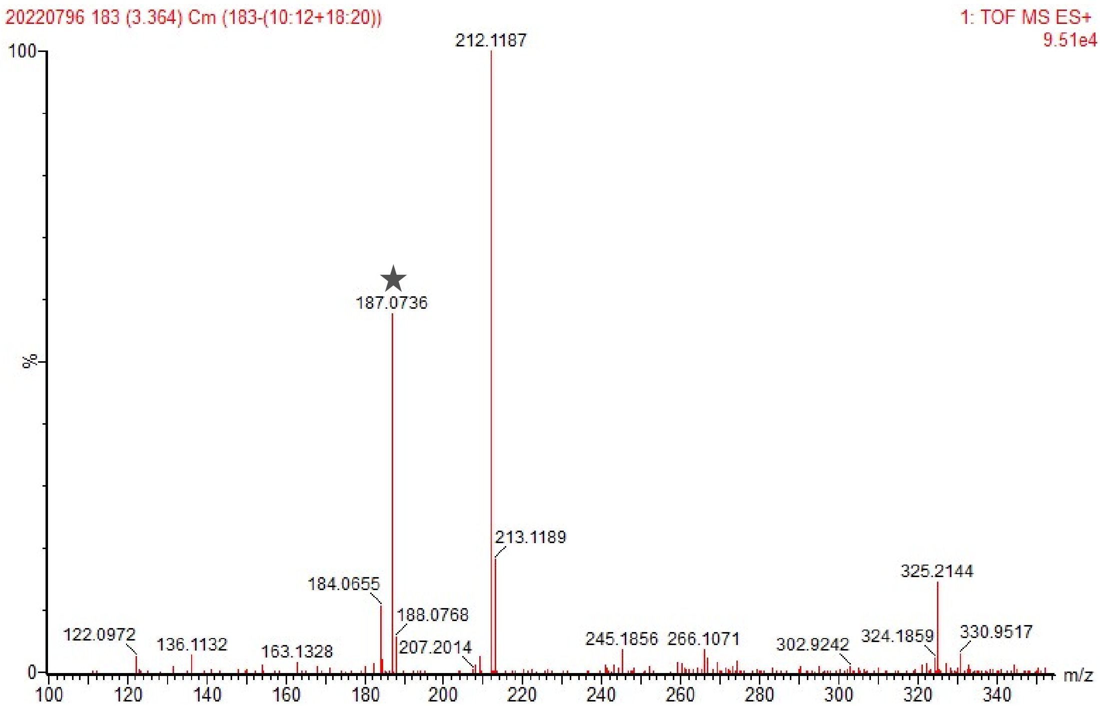


Fig. S23 HR-MS spectrum of D1

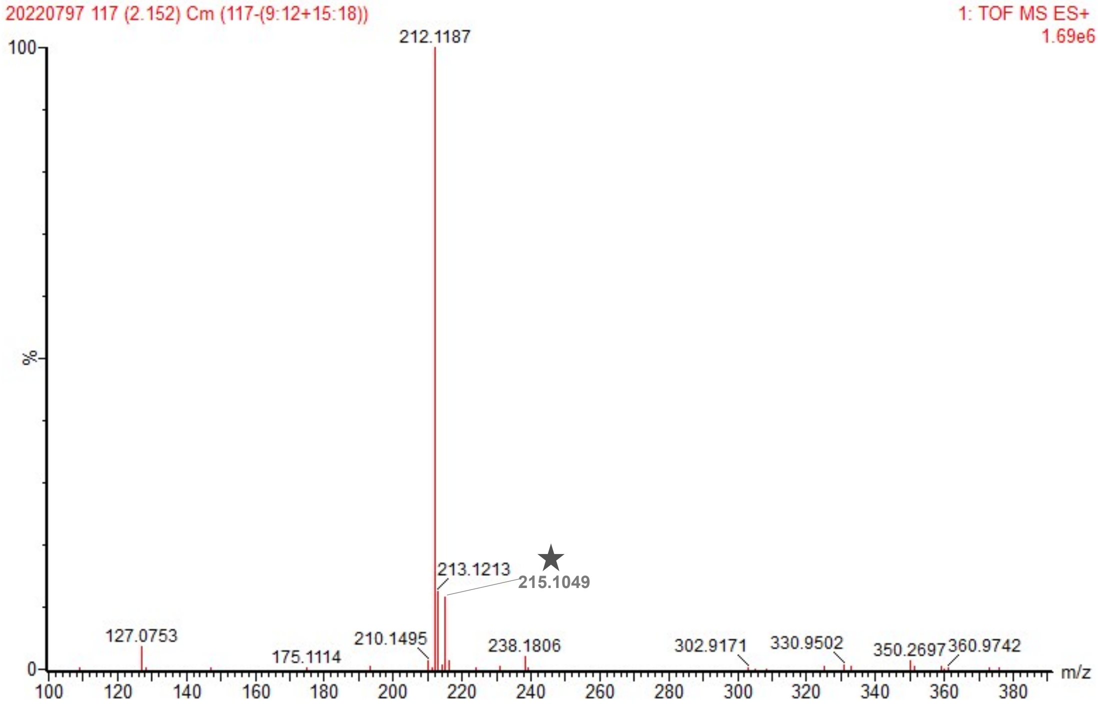


Fig. S24 HR-MS spectrum of E1

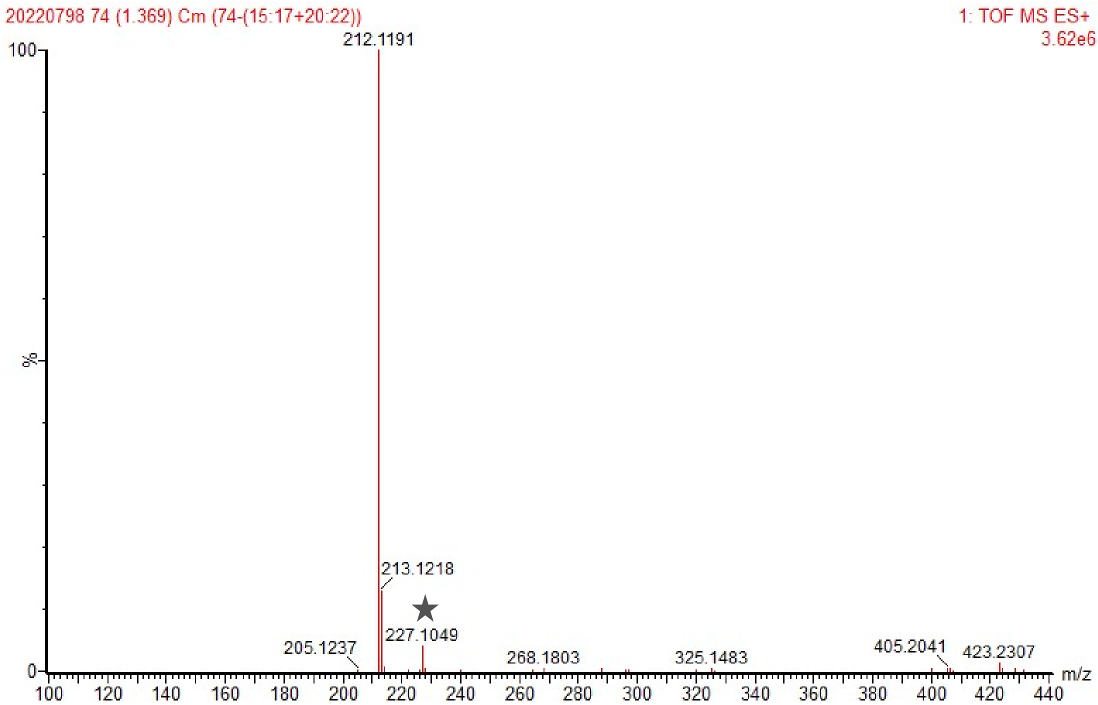


Fig. S25 HR-MS spectrum of F1

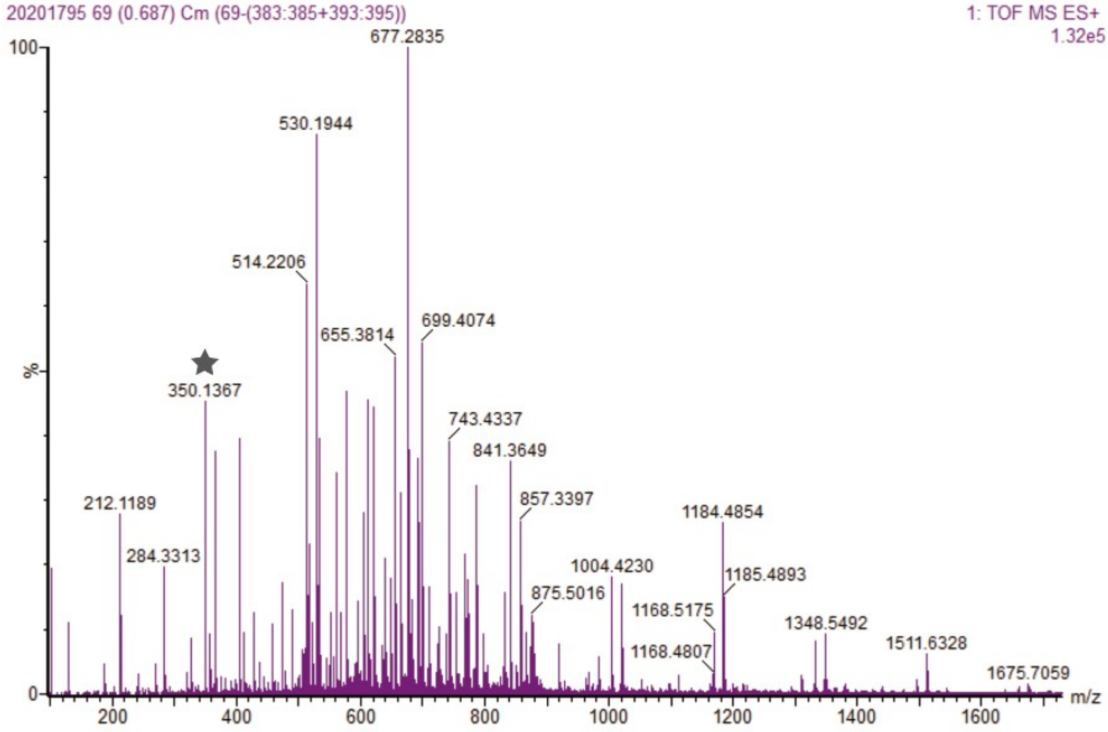


Fig. S26 HR-MS spectrum of EDY-A

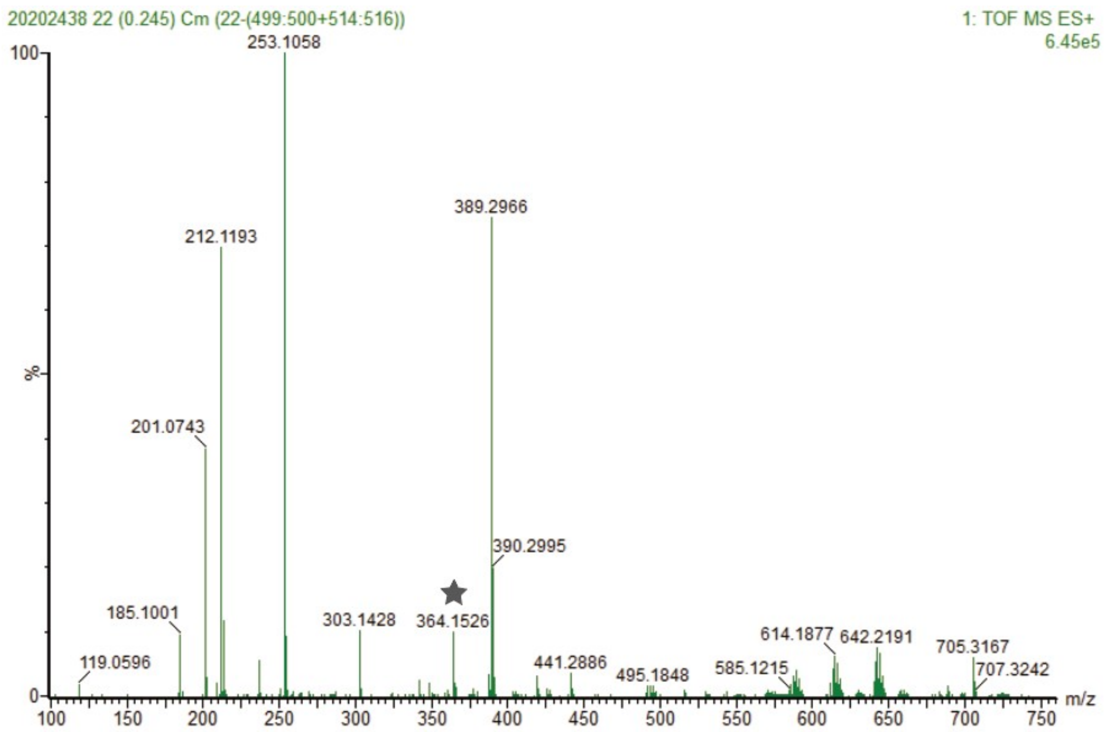


Fig. S27 HR-MS spectrum of EDY-B

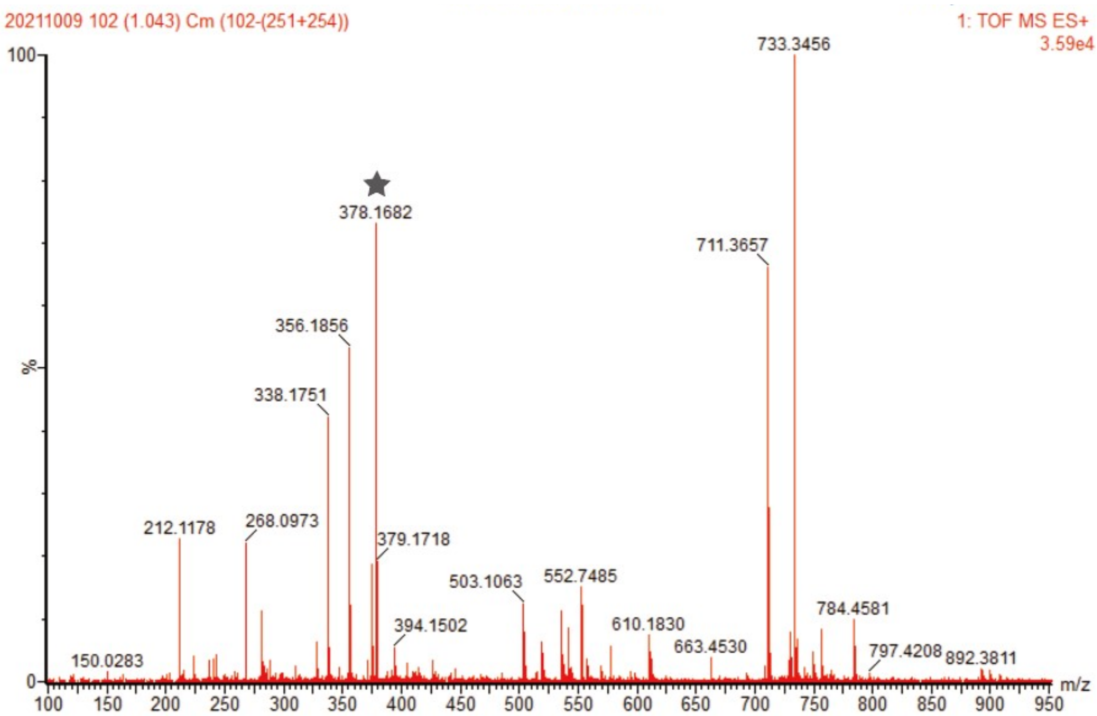


Fig. S28 HR-MS spectrum of EDY-C

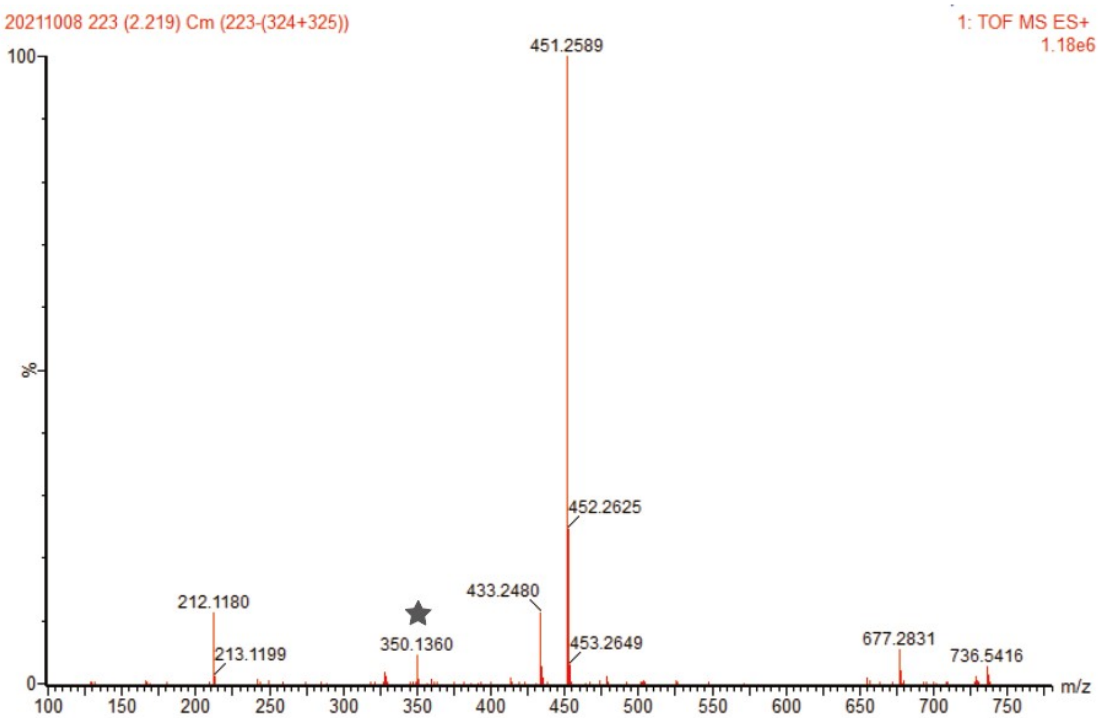


Fig. S29 HR-MS spectrum of EDY-D

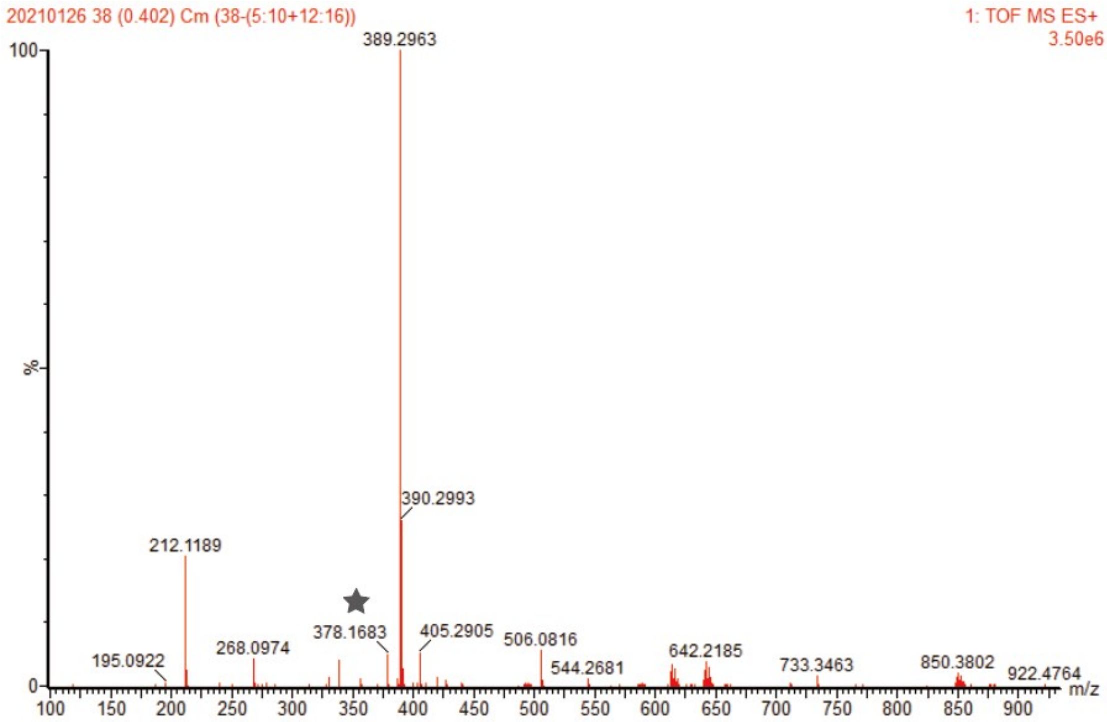


Fig. S30 HR-MS spectrum of EDY-E

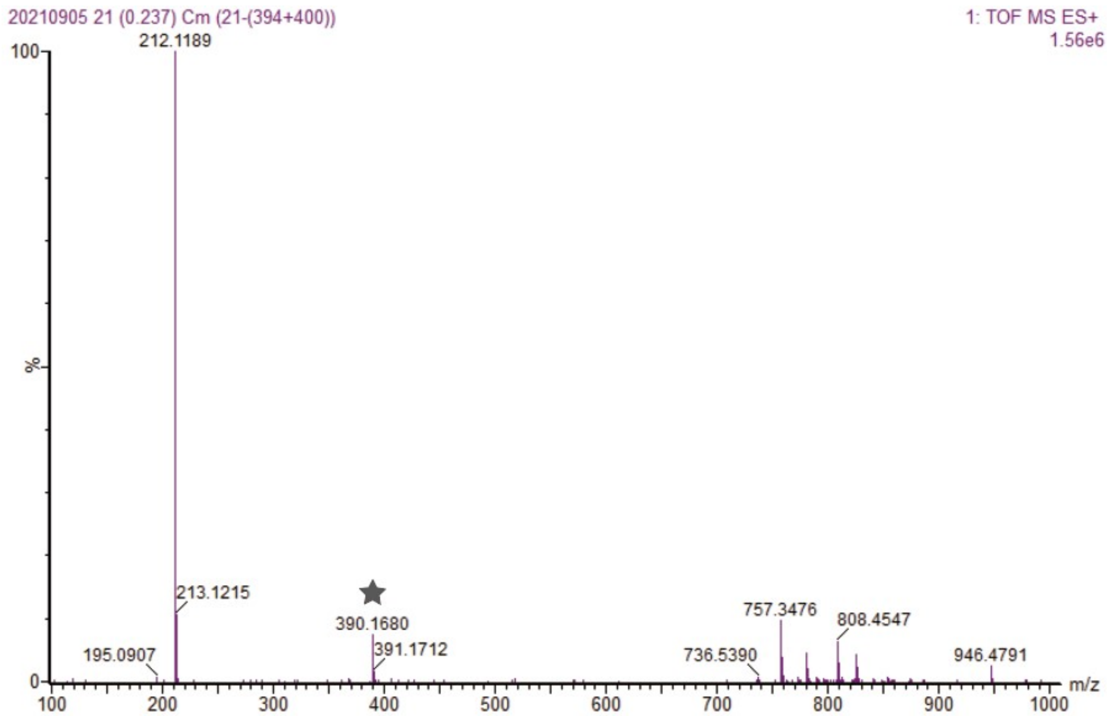


Fig. S31 HR-MS spectrum of EDY-F

Computational Details

Below is a list of the calculated total energies and Gibbs free energy in atomic unit at (U)B3LYP/631G(d) as well as the Cartesian coordinates of all the optimized structures. The nature of the stationary point was characterized by vibrational frequency analysis. The Gaussian 09[9] package was employed to perform all the calculations.

Table S4 Cartesian Coordinates of stationary points for model compound EDY-B in the rearrangement and cycloaromatization process, Absolute Energies (A.U.).

EDY-B					**EDY-B**				
I					**TS1**				
E(RB3LYP) = -1617.95406427 A.U.					E(RB3LYP) = -1617.93610119 A.U.				
G(298.15 K) = -1617.691661 A.U.					G(298.15 K) = -1617.678163 A.U.				
Imaginary frequency = 0					Imaginary frequency = 1				
1	C	-1.98238	-1.28971	0.44067	1	C	-0.85477	2.22301	-0.07146
2	C	-0.13405	-2.85293	-0.51756	2	C	1.31495	2.56713	-1.4752
3	C	0.80619	0.71913	1.39744	3	C	0.71553	-0.85467	0.79857
4	C	-2.86071	0.73787	1.15945	4	C	-2.37385	1.12331	1.30754
5	C	-3.45442	-1.26011	0.17046	5	C	-2.11977	3.01819	0.00988
6	N	-3.9114	-0.0282	0.65826	6	N	-2.97332	2.29862	0.85925
7	C	-5.25358	0.47873	0.47181	7	C	-4.34049	2.66815	1.15709
8	H	-5.85546	-0.33158	0.05623	8	H	-4.54324	3.6114	0.64647
9	H	-5.67379	0.80608	1.42752	9	H	-5.02765	1.89452	0.80086
10	H	-5.24796	1.3289	-0.21849	10	H	-4.4782	2.79126	2.23611
11	O	-4.15	-2.09988	-0.3672	11	O	-2.39432	4.07454	-0.52607
12	O	-2.96629	1.87799	1.56915	12	O	-2.8898	0.312	2.0504
13	C	1.07849	-3.4476	-1.06453	13	C	2.60361	2.46954	-2.15225
14	H	1.64877	-3.90461	-0.24325	14	H	2.62547	1.51506	-2.69694
15	H	0.82963	-4.26549	-1.75441	15	H	2.70969	3.25833	-2.90897
16	C	3.73531	-0.46503	1.09511	16	C	3.81805	-0.73666	0.94738
17	O	4.90677	-0.72207	1.28393	17	O	4.84179	-0.58961	1.58308
18	O	3.26737	0.71037	1.5869	18	O	2.93352	-1.66698	1.36993
19	C	2.80156	-1.4672	0.42904	19	C	3.54649	0.10744	-0.30056
20	H	1.92288	-1.61765	1.06569	20	H	2.56656	-0.076	-0.74253
21	H	3.3841	-2.39449	0.42595	21	H	4.3074	-0.18902	-1.03574
22	C	-1.07358	-2.23201	-0.06435	22	C	0.27412	2.52275	-0.85195
23	C	-0.3428	0.32996	1.3438	23	C	-0.08085	0.0602	0.85749
24	C	-1.63328	-0.12415	1.06153	24	C	-1.00164	1.0968	0.69355
25	C	2.07044	1.40236	1.12579	25	C	1.67342	-1.95029	0.67283
26	C	1.97761	-2.4144	-1.80126	26	C	3.80533	2.53111	-1.17521
27	H	1.47957	-2.11463	-2.73037	27	H	3.91059	3.56214	-0.81387
28	H	2.89663	-2.94808	-2.08323	28	H	4.71301	2.3019	-1.75085
29	C	2.3328	-1.14247	-1.00763	29	C	3.71222	1.60014	0.04123
30	H	3.12664	-0.62049	-1.55507	30	H	4.61951	1.71356	0.64336

31	H	1.48356	-0.45462	-1.00094
32	P	-0.35541	2.39899	-1.68173
33	O	-0.83208	3.28497	-2.79571
34	O	-1.57358	1.23768	-1.47997
35	H	-2.13306	1.37494	-2.26009
36	O	-0.50117	3.13113	-0.19163
37	H	-1.40356	3.01345	0.14588
38	O	0.99619	1.73047	-1.68837
39	C	2.11667	2.78143	1.77918
40	H	3.05616	3.27273	1.50837
41	H	1.27317	3.36585	1.40446
42	H	2.06339	2.69478	2.86989
43	H	2.08464	1.51958	0.02929

EDY-B

Ⅱ

E(RB3LYP) = -1617.95699808 A.U.

G(298.15 K) = -1617.697498 A.U.

Imaginary frequency = 0

1	C	-1.99079	-1.41387	0.3191
2	C	-0.13289	-3.08699	-0.42896
3	C	0.86359	0.68878	1.26949
4	C	-2.7635	0.73422	0.89106
5	C	-3.41552	-1.3789	0.15341
6	N	-3.8322	-0.04516	0.58452
7	C	-5.18244	0.44595	0.45347
8	H	-5.78881	-0.38461	0.08527
9	H	-5.56992	0.79085	1.41869
10	H	-5.2235	1.28372	-0.25249
11	O	-4.23582	-2.1955	-0.25221
12	O	-2.82695	1.94446	1.16292
13	C	1.10445	-3.7077	-0.89032
14	H	1.67518	-4.10118	-0.03441
15	H	0.89494	-4.57786	-1.52938
16	C	3.74321	-0.47257	0.91289
17	O	4.94335	-0.65066	0.89416
18	O	3.32179	0.7598	1.32429
19	C	2.76739	-1.54626	0.47865
20	H	1.90344	-1.60638	1.14476
21	H	3.34105	-2.47407	0.56622
22	C	-1.08204	-2.39732	-0.10456
23	C	-0.31634	0.2918	1.09403
24	C	-1.55497	-0.14338	0.78982
25	C	2.0374	1.31244	1.42772
26	C	2.00444	-2.72087	-1.68035

31	H	2.86625	1.90714	0.66791
32	P	0.36463	-5.48498	2.38122
33	O	-0.22742	-6.59277	1.56742
34	O	1.66098	-6.10029	3.26315
35	H	1.51911	-7.05936	3.28126
36	O	-0.67952	-5.17197	3.65068
37	H	-0.16072	-4.8078	4.38592
38	O	0.84993	-4.18262	1.7933
39	C	1.92206	-2.18627	-0.82853
40	H	2.93486	-2.49752	-0.97773
41	H	1.25905	-2.94687	-1.18463
42	H	1.74502	-1.27838	-1.36639
43	H	1.16238	-3.14126	1.2024

EDY-B

TS2

E(RB3LYP) = -1617.93016456 A.U.

G(298.15 K) = -1617.675719 A.U.

Imaginary frequency = 1

1	C	-0.57039	2.68111	-0.01679
2	C	1.86775	3.44557	0.51905
3	C	1.0203	-0.52889	-0.78894
4	C	-2.22773	1.17358	-0.7399
5	C	-1.83389	3.35873	-0.01993
6	N	-2.80695	2.37444	-0.48573
7	C	-4.22074	2.63783	-0.59283
8	H	-4.37622	3.66987	-0.27058
9	H	-4.56588	2.51447	-1.62582
10	H	-4.79301	1.95382	0.04418
11	O	-2.1791	4.49975	0.27026
12	O	-2.84755	0.17089	-1.13209
13	C	3.30262	3.56596	0.75284
14	H	3.8406	3.56022	-0.20891
15	H	3.54934	4.5288	1.22343
16	C	4.1566	-0.76206	-0.65491
17	O	5.26999	-1.17261	-0.40124
18	O	3.17887	-1.70476	-0.79189
19	C	3.7813	0.68748	-0.27156
20	H	2.96342	1.01699	-0.91466
21	H	4.69561	1.21599	-0.56212
22	C	0.69681	3.19427	0.30239
23	C	0.17428	0.34193	-0.45922
24	C	-0.76504	1.34077	-0.46118
25	C	1.80174	-1.59065	-1.02298
26	C	3.85725	2.42383	1.64037

27	H	1.53401	-2.51513	-2.64995
28	H	2.96034	-3.22516	-1.88477
29	C	2.278	-1.37579	-0.98282
30	H	3.03286	-0.83616	-1.56857
31	H	1.36988	-0.76683	-0.98887
32	P	-0.50146	3.03724	-1.38355
33	O	-0.66709	4.11094	-2.38894
34	O	-1.45792	1.74483	-1.68053
35	H	-1.72721	1.77441	-2.61182
36	O	-0.82623	3.35431	0.14121
37	H	-1.58223	2.82686	0.54712
38	O	1.00882	2.475	-1.35696
39	C	2.15451	2.75742	1.82355
40	H	2.81097	3.29195	1.12511
41	H	1.17062	3.23078	1.80521
42	H	2.59123	2.85637	2.82771
43	H	1.16638	1.91025	-0.57094

27	H	3.51387	2.58032	2.67086
28	H	4.95445	2.50023	1.65073
29	C	3.45077	1.00504	1.2101
30	H	3.97706	0.29307	1.85777
31	H	2.3772	0.86447	1.36724
32	P	-0.68227	-3.4859	-2.00621
33	O	0.45316	-4.28363	-2.52221
34	O	-1.84006	-3.23784	-3.13507
35	H	-1.64281	-3.81546	-3.88879
36	O	-0.40074	-2.01806	-1.46753
37	H	-0.09465	-0.76179	-0.93079
38	O	-1.42807	-4.22594	-0.78314
39	C	1.91268	-2.0085	-2.46206
40	H	1.72596	-3.08516	-2.56582
41	H	1.1821	-1.46998	-3.0688
42	H	2.92433	-1.81134	-2.8443
43	H	-2.0745	-3.63403	-0.34738

EDY-B

Ⅲ

E(RB3LYP) = -1617.94629184 A.U.

G(298.15 K) = -1617.685414 A.U.

Imaginary frequency = 0

1	C	-2.49077	0.37445	-0.07642
2	C	-1.32651	2.65168	0.47975
3	C	0.51276	-0.68588	-0.97215
4	C	-2.93476	-1.88091	-0.3427
5	C	-3.97872	0.15637	-0.08108
6	N	-4.1638	-1.21704	-0.24534
7	C	-5.44668	-1.88042	-0.31445
8	H	-6.21438	-1.13981	-0.08213
9	H	-5.62171	-2.2913	-1.31501
10	H	-5.48289	-2.70111	0.4081
11	O	-4.86892	0.97695	0.03934
12	O	-2.81369	-3.07869	-0.49589
13	C	-0.43678	3.7618	0.80054
14	H	-0.11729	4.23736	-0.13775
15	H	-0.9644	4.53801	1.37104
16	C	2.91607	1.25135	-0.92465
17	O	4.05216	1.62686	-0.75712
18	O	2.7217	0.10991	-1.64246
19	C	1.73002	2.0903	-0.48987
20	H	0.81718	1.81989	-1.0265
21	H	2.00972	3.11273	-0.77416
22	C	-1.8996	1.62133	0.19557

EDY-B

TS3

E(RB3LYP) = -974.29843908 A.U.

G(298.15 K) = -974.064175 A.U.

Imaginary frequency = 1

1	C	1.9647	-0.8005	-0.1438
2	C	-0.47694	-0.96704	-0.28983
3	C	-0.44752	0.90428	0.03772
4	C	3.39691	1.05678	0.05011
5	C	3.3673	-1.28399	-0.05635
6	N	4.15571	-0.11912	0.04308
7	C	5.6029	-0.15228	0.13151
8	H	5.90703	-0.89227	0.87601
9	H	6.04445	-0.42385	-0.83302
10	H	5.94239	0.84337	0.4217
11	O	3.81048	-2.41456	-0.06959
12	O	3.83562	2.18697	0.14552
13	C	-1.72345	-1.4978	-0.68256
14	H	-2.31986	-0.83531	-1.3191
15	H	-1.4068	-2.31922	-1.3341
16	C	-3.87453	0.56982	-0.08262
17	O	-4.10613	1.15835	-1.10839
18	O	-2.72273	0.74109	0.6597
19	C	-4.68749	-0.5303	0.55245
20	H	-5.71736	-0.4455	0.19724
21	H	-4.67033	-0.39268	1.63872
22	C	0.73706	-1.40093	-0.24184

23	C	-0.45749	-1.19611	-0.23226
24	C	-1.86275	-0.82498	-0.24065
25	C	1.59805	-0.69691	-1.71632
26	C	0.8158	3.29282	1.58938
27	H	0.53035	3.10869	2.63255
28	H	1.52951	4.12805	1.59569
29	C	1.49462	2.03167	1.03251
30	H	2.45559	1.89118	1.53786
31	H	0.88526	1.15048	1.24924
32	P	2.55416	-2.59231	1.16363
33	O	3.16448	-2.96613	-0.15609
34	O	3.56649	-3.14435	2.36697
35	H	4.41645	-3.29665	1.92547
36	O	1.14517	-2.92019	1.5941
37	H	-0.15984	-2.01887	0.44648
38	O	2.71749	-0.92734	1.33475
39	C	1.82796	-1.74875	-2.76655
40	H	2.58696	-2.42192	-2.34997
41	H	0.91442	-2.31648	-2.9549
42	H	2.18895	-1.30122	-3.7007
43	H	2.08967	-0.68452	2.03215

23	C	0.82424	1.34993	0.02594
24	C	1.97634	0.61279	-0.06584
25	C	-1.62824	1.44247	0.18192
26	C	-1.81343	2.91608	0.05122
27	H	-2.07074	3.36083	1.02263
28	H	-0.92456	3.41287	-0.34018
29	H	-2.64902	3.11251	-0.63029
30	H	0.89212	2.42116	0.17861
31	C	-2.59918	-2.08664	0.46505
32	H	-2.3414	-1.61242	1.41687
33	H	-2.36235	-3.15058	0.57567
34	C	-4.11096	-1.93237	0.20793
35	H	-4.33009	-2.15943	-0.84321
36	H	-4.66771	-2.66217	0.80701

EDY-B

Ⅳ

E(RB3LYP) = -974.35049077 A.U.

G(298.15 K) = -974.113798 A.U.

Imaginary frequency = 0

1	C	-1.96431	-0.80113	0.14502
2	C	0.47381	-0.75219	0.25273
3	C	0.45065	0.68959	0.00001
4	C	-3.39769	1.05443	-0.04808
5	C	-3.36601	-1.28649	0.05518
6	N	-4.15549	-0.12107	-0.04396
7	C	-5.5657	-0.16847	0.29134
8	H	-6.03362	-0.97137	-0.28167
9	H	-5.70934	-0.36425	1.35972
10	H	-6.01268	0.79592	0.04239
11	O	-3.80486	-2.41874	0.06464
12	O	-3.84027	2.18311	-0.14402
13	C	1.72396	-1.49666	0.68407
14	H	2.32007	-0.83319	1.31984
15	H	1.40773	-2.31752	1.33652
16	C	3.87401	0.57105	0.08143
17	O	4.10547	1.16019	1.10689
18	O	2.72209	0.74149	-0.66086

19	C	4.68735	-0.52923	-0.55292
20	H	5.71735	-0.44341	-0.19835
21	H	4.6695	-0.39299	-1.63934
22	C	-0.73648	-1.40133	0.242
23	C	-0.82482	1.3497	-0.0247
24	C	-1.97657	0.6123	0.06841
25	C	1.62748	1.44283	-0.18293
26	C	1.81254	2.91652	-0.05292
27	H	2.06912	3.36084	-1.0247
28	H	0.92384	3.41331	0.33886
29	H	2.64855	3.11336	0.62794
30	H	-0.89306	2.42087	-0.17761
31	C	2.59987	-2.08639	-0.46299
32	H	2.34178	-1.61323	-1.41524
33	H	2.36338	-3.15052	-0.57249
34	C	4.11163	-1.93113	-0.20626
35	H	4.33106	-2.15649	0.84518
36	H	4.66864	-2.66156	-0.80434

Table S5 Cartesian Coordinates of stationary points of other enediynes for MSC, Absolute Energies (A.U.).

EDY-A

A-Ⅲ

E(RB3LYP) = -935.00962041 A.U.

G(298.15 K) = -934.804460 A.U.

Imaginary frequency = 0

1	C	-1.65515	-0.45666	-0.24951
2	C	0.54804	-1.6043	-1.02888
3	C	0.72491	1.56362	0.23629
4	C	-3.02039	1.11916	0.76334
5	C	-3.08404	-0.94082	-0.26184
6	N	-3.83466	0.06503	0.35619
7	C	-5.27455	0.02777	0.53529
8	H	-5.52529	0.56496	1.45223
9	H	-5.78856	0.50224	-0.30762
10	H	-5.5883	-1.01532	0.60456
11	O	-3.52376	-1.98323	-0.69697
12	O	-3.39271	2.12688	1.33283
13	C	1.95004	-1.99256	-1.12756
14	H	2.07568	-2.91053	-1.71406
15	H	2.50234	-1.20187	-1.65582
16	C	3.45404	0.08682	0.88126
17	O	4.61085	-0.01643	1.2014
18	O	3.12731	1.18183	0.11131

EDY-A

A-TS3

E(RB3LYP) = -934.97524290 A.U.

G(298.15 K) = -934.770057 A.U.

Imaginary frequency = 1

1	C	1.55527	0.70928	-0.19497
2	C	-0.87541	0.92507	-0.49916
3	C	-0.88628	-0.96424	-0.2982
4	C	2.93671	-1.1624	0.15022
5	C	2.96402	1.16869	-0.08385
6	N	3.71967	-0.005	0.12223
7	C	5.16279	-0.00783	0.27358
8	H	5.44292	-0.82939	0.93579
9	H	5.65776	-0.14475	-0.69399
10	H	5.46716	0.951	0.69705
11	O	3.42603	2.28978	-0.14046
12	O	3.34712	-2.29438	0.3156
13	C	-2.12855	1.52599	-0.68802
14	H	-1.83571	2.50629	-1.07565
15	H	-2.75969	1.06197	-1.45467
16	C	-4.11363	-0.41571	0.22517
17	O	-5.28203	-0.35255	-0.0372
18	O	-3.323	-1.25267	-0.56591

19	C	2.42444	-0.97284	1.22225
20	H	1.40331	-0.59154	1.21902
21	H	2.66812	-1.28848	2.2404
22	C	2.5516	-2.19753	0.28891
23	H	3.60951	-2.46456	0.1883
24	H	2.04248	-3.04389	0.76156
25	C	-0.5436	-1.16828	-0.72356
26	C	-0.52449	1.6648	0.66219
27	C	-1.61616	0.75961	0.36797
28	C	1.89296	1.63433	-0.35288
29	H	-0.80916	2.52062	1.27716
30	C	2.09939	2.36981	-1.65264
31	H	2.54091	1.69891	-2.39895
32	H	1.15592	2.76311	-2.03625
33	H	2.79893	3.19936	-1.49594

19	C	-3.40547	0.41681	1.26505
20	H	-2.55557	-0.1217	1.6927
21	H	-4.1242	0.62577	2.06057
22	C	-2.94245	1.73631	0.60678
23	H	-3.826	2.34006	0.37026
24	H	-2.34204	2.29818	1.33071
25	C	0.34716	1.3257	-0.3955
26	C	0.36779	-1.41701	-0.11462
27	C	1.53123	-0.69734	-0.05455
28	C	-2.01658	-1.57995	-0.35435
29	H	0.39369	-2.49725	-0.00457
30	C	-1.82369	-3.10682	-0.29922
31	H	-1.17982	-3.41401	-1.0967
32	H	-1.38434	-3.37721	0.6382
33	H	-2.77288	-3.59014	-0.40087

EDY-C

C-Ⅲ

E(RB3LYP) = -1013.65868101 A.U.

G(298.15 K) = -1013.396627 A.U.

Imaginary frequency = 0

1	C	2.40572	0.30435	0.29723
2	C	0.08403	1.23629	1.05164
3	C	0.30897	-1.98132	-0.29156
4	C	4.01004	-1.14737	-0.54316
5	C	3.76636	0.96943	0.32707
6	N	4.66851	0.02285	-0.17629
7	C	6.09473	0.2339	-0.34826
8	H	6.6618	-0.19027	0.48739
9	H	6.41352	-0.25491	-1.27207
10	H	6.28043	1.30794	-0.40176
11	O	4.05214	2.09283	0.68104
12	O	4.53353	-2.1346	-1.02429
13	C	-1.25378	1.6321	1.48407
14	H	-1.86953	0.73109	1.60091
15	H	-1.17774	2.08344	2.48231
16	C	-3.01213	-0.9653	-0.19427
17	O	-3.29695	-1.03766	0.98185
18	O	-1.90992	-1.52334	-0.76547
19	C	-3.80745	-0.15272	-1.19218
20	H	-4.83368	-0.53899	-1.18936
21	H	-3.39639	-0.26316	-2.19895
22	C	1.58517	-1.94086	-0.6258
23	C	2.5563	-0.94006	-0.24066
24	C	-0.94367	-2.18524	0.02977

EDY-C

C-TS3

E(RB3LYP) = -1013.61560022 A.U.

G(298.15 K) = -1013.354021 A.U.

Imaginary frequency = 1

1	C	1.9682	-0.70405	-0.06773
2	C	-0.39547	-0.95448	-0.77301
3	C	-0.30689	1.1488	-0.543
4	C	3.46706	1.06266	0.22462
5	C	3.36728	-1.23749	-0.06398
6	N	4.19414	-0.12863	0.17272
7	C	5.63624	-0.21111	0.30981
8	H	6.0652	0.7441	0.00126
9	H	5.92187	-0.41611	1.34727
10	H	6.0018	-1.02023	-0.32564
11	O	3.75041	-2.37989	-0.2045
12	O	3.93745	2.17742	0.33752
13	C	-1.70044	-1.52561	-1.19112
14	H	-2.43034	-0.75235	-1.40105
15	H	-1.51334	-2.00189	-2.16385
16	C	-3.06487	1.26334	1.0402
17	O	-3.83176	1.68279	0.21268
18	O	-1.78018	1.77543	1.04763
19	C	-3.42853	0.17412	2.0373
20	H	-4.31417	0.55377	2.56169
21	H	-2.64518	0.02528	2.78282
22	C	0.99809	1.54223	-0.32362
23	C	2.02559	0.67737	0.07755
24	C	-1.47077	1.76779	-0.2981

25	C	-1.40254	-3.16152	1.0798
26	H	-0.5483	-3.74899	1.4245
27	H	-1.87501	-2.66184	1.9264
28	H	-2.14175	-3.84111	0.63834
29	H	1.97868	-2.70958	-1.29323
30	C	-1.94751	2.6333	0.52432
31	H	-2.80327	3.06856	1.05575
32	H	-1.24985	3.45481	0.32795
33	C	-3.81467	1.32362	-0.73906
34	H	-4.2056	1.34908	0.2849
35	H	-4.53856	1.86453	-1.35907
36	C	-2.42853	2.02493	-0.80927
37	H	-1.66573	1.32625	-1.1783
38	H	-2.47725	2.82708	-1.55425
39	C	1.19987	0.89247	0.71202

EDY-D

D-MSC1-Ⅲ

E(RB3LYP) = -935.00819591 A.U.

G(298.15 K) = -934.801996 A.U.

Imaginary frequency = 0

1	C	-1.89033	0.23751	-0.22481
2	C	0.20446	1.76396	-0.53379
3	C	0.48708	-1.71171	-0.99125
4	C	-3.22333	-1.65962	-0.17698
5	C	-3.29875	0.61219	0.1812
6	N	-4.02443	-0.58324	0.19606
7	C	-5.43018	-0.69617	0.54138
8	H	-5.56304	-0.80737	1.62282
9	H	-5.95061	0.20367	0.20805
10	H	-5.83292	-1.57883	0.0406
11	O	-3.74432	1.71072	0.43276
12	O	-3.58301	-2.81691	-0.27279
13	C	1.51735	2.38998	-0.64787
14	H	2.05938	1.90711	-1.47416
15	H	1.42309	3.44814	-0.92024
16	C	3.70656	-1.37097	0.39002
17	O	4.53747	-1.97891	1.01119
18	O	2.6391	-2.0836	-0.12234
19	C	3.69885	0.13296	0.20408
20	H	3.55401	0.38198	-0.85305
21	H	4.69034	0.48974	0.49407
22	C	-0.80126	-1.98557	-0.90916
23	C	-1.85263	-1.10805	-0.44094
24	C	2.33169	2.25838	0.66686

25	C	-1.99129	2.93212	-1.10422
26	H	-1.26099	3.26172	-1.84477
27	H	-2.90747	2.61697	-1.6209
28	H	-2.26007	3.7887	-0.47417
29	H	1.29828	2.55206	-0.60454
30	C	-2.26167	-2.59887	-0.23448
31	H	-3.1645	-3.01308	-0.70275
32	H	-1.52877	-3.41322	-0.17829
33	C	-3.77697	-1.17701	1.37271
34	H	-4.27534	-0.98765	0.41461
35	H	-4.5165	-1.66863	2.01507
36	C	-2.59346	-2.14989	1.19527
37	H	-1.69333	-1.72186	1.65925
38	H	-2.82273	-3.05735	1.76842
39	C	0.77886	-1.30012	-0.4392

EDY-D

D-MSC1-TS3

E(RB3LYP) = -934.96515475 A.U.

G(298.15 K) = -934.757521 A.U.

Imaginary frequency = 1

1	C	1.79596	0.7892	0.19353
2	C	-0.46987	1.5375	0.72067
3	C	-0.80731	-0.31899	0.94336
4	C	2.72997	-1.3509	-0.08926
5	C	3.25449	0.92998	-0.08992
6	N	3.72891	-0.38272	-0.25673
7	C	5.11282	-0.68788	-0.56855
8	H	5.37124	-0.31911	-1.56597
9	H	5.77237	-0.2124	0.16233
10	H	5.22848	-1.77225	-0.53342
11	O	3.93735	1.92885	-0.18058
12	O	2.88441	-2.55294	-0.16924
13	C	-1.6647	2.29103	0.63564
14	H	-2.40595	2.11485	1.4228
15	H	-1.29212	3.30817	0.79288
16	C	-3.74903	-1.2723	-0.22107
17	O	-4.36435	-2.19389	-0.67592
18	O	-2.68893	-1.55207	0.65489
19	C	-3.99092	0.19918	-0.53592
20	H	-4.20449	0.73542	0.39624
21	H	-4.90708	0.22361	-1.12897
22	C	0.23591	-1.04726	0.57656
23	C	1.48077	-0.58757	0.2101
24	C	-2.34915	2.24707	-0.76162

25	H	1.77406	2.75867	1.46625
26	H	3.27556	2.80479	0.54284
27	C	2.61085	0.80397	1.07394
28	H	2.93586	0.76719	2.11961
29	H	1.6781	0.23289	1.0168
30	H	-1.14676	-2.97234	-1.22178
31	C	1.78195	-1.56394	-1.08887
32	H	2.25629	-1.12062	-1.96309
33	C	-0.82048	1.13401	-0.36741

25	H	-1.63867	2.66	-1.48842
26	H	-3.20403	2.93628	-0.73579
27	C	-2.81404	0.88035	-1.30485
28	H	-3.13684	1.02966	-2.34077
29	H	-1.95616	0.20366	-1.36579
30	H	0.0273	-2.1097	0.6444
31	C	-2.08029	-0.49714	1.27738
32	H	-2.71423	0.11171	1.91148
33	C	0.78371	1.67599	0.44609

EDY-D

D-MSC2-Ⅲ

E(RB3LYP) = -934.99999894 A.U.

G(298.15 K) = -934.792666 A.U.

Imaginary frequency = 0

1	C	-1.7455	0.2202	0.07249
2	C	-3.82011	-0.94048	-0.02832
3	C	-2.88736	1.19789	0.14358
4	N	-4.05722	0.43185	0.10416
5	C	-5.38102	1.01926	0.19504
6	H	-6.10317	0.20269	0.23615
7	H	-5.45075	1.63611	1.09521
8	H	-5.57831	1.6505	-0.67661
9	O	-2.86109	2.40776	0.23749
10	O	-4.68065	-1.79922	-0.09285
11	C	1.73788	1.1648	-0.82386
12	H	1.7861	0.25323	-1.43317
13	H	1.33653	1.94159	-1.48567
14	C	2.71671	-1.57109	0.49918
15	O	2.20756	-1.60815	1.58007
16	O	2.12109	-2.19764	-0.63112
17	C	3.98534	-0.85232	0.11898
18	H	4.18025	-0.95279	-0.95193
19	H	4.80051	-1.34462	0.66245
20	C	-0.48084	0.58115	0.18689
21	C	-1.77249	-2.31743	-0.32976
22	C	-2.33988	-1.1117	-0.08597
23	C	0.82953	-2.42084	-0.53957
24	C	3.15797	1.5764	-0.39493
25	H	3.11845	2.56618	0.0784
26	H	3.75389	1.69698	-1.30937
27	C	3.91001	0.63053	0.56148
28	H	4.93413	1.00518	0.66934
29	H	3.46811	0.65772	1.56399
30	H	-2.43359	-3.17263	-0.45579

EDY-D

D-MSC2-TS3

E(RB3LYP) = -934.97021379 A.U.

G(298.15 K) = -934.763137 A.U.

Imaginary frequency = 1

1	C	-0.10878	-0.58398	0.62847
2	C	-1.54839	-2.52241	0.40826
3	C	-0.87824	-0.84747	1.92765
4	N	-1.93467	-1.87954	1.69918
5	C	-3.24882	-1.23421	1.56705
6	H	-3.99057	-1.97567	1.35502
7	H	-3.49456	-0.73504	2.48102
8	H	-3.21791	-0.5222	0.76893
9	O	-0.64999	-0.2809	3.02786
10	O	-1.98637	-3.62519	-0.01081
11	C	2.52957	1.80241	1.89587
12	H	2.90755	2.37629	1.07569
13	H	2.5234	2.40263	2.78164
14	C	3.5966	0.74882	-1.50248
15	O	4.28417	0.11653	-2.3457
16	O	2.30112	1.2637	-1.73276
17	C	4.01475	1.04558	-0.11569
18	H	3.56492	1.97824	0.15393
19	H	5.07646	1.12197	-0.00681
20	C	0.72907	0.3856	0.53689
21	C	-0.05159	-1.73567	-1.56442
22	C	-0.53492	-1.64187	-0.32582
23	C	1.44781	0.32805	-1.32861
24	C	3.44199	0.57538	2.11735
25	H	3.05004	-0.0753	2.87093
26	H	4.4301	0.86842	2.40488
27	C	3.45595	-0.05337	0.77619
28	H	4.04162	-0.94676	0.71515
29	H	2.44755	-0.288	0.50604
30	H	-0.36211	-2.5159	-2.22753

31	C	0.76604	0.93061	0.32749
32	H	1.15768	0.99168	1.34411
33	C	-0.37579	-2.47343	-0.43761

EDY-F

F-Ⅲ

E(RB3LYP) = -1051.72222216 A.U.

G(298.15 K) = -1051.455377 A.U.

Imaginary frequency = 0

1	C	-2.48233	0.29715	-0.20453
2	C	-0.48729	1.90583	-0.6905
3	C	-3.64641	-1.63171	0.3383
4	C	-3.95794	0.60404	-0.11019
5	N	-4.57884	-0.60275	0.22891
6	C	-6.00597	-0.77163	0.43234
7	H	-6.27017	-0.65473	1.48892
8	H	-6.53142	-0.01617	-0.15453
9	H	-6.28793	-1.77481	0.10579
10	O	-4.52277	1.66015	-0.29767
11	O	-3.89625	-2.79148	0.60395
12	C	0.77762	2.63116	-0.69245
13	H	1.52801	2.02976	-1.22526
14	H	0.69266	3.57705	-1.24072
15	C	2.88631	-0.50794	1.67477
16	O	3.50815	-0.81858	2.6569
17	O	2.22644	-1.5183	0.99883
18	C	2.77768	0.90811	1.14914
19	H	2.85247	0.89919	0.06029
20	H	3.64551	1.44453	1.54293
21	C	-1.12289	-1.86822	0.0219
22	C	-2.2998	-1.02812	0.058
23	H	-1.34513	-2.93747	0.04043
24	C	2.1443	-1.17493	-1.4614
25	H	1.52365	-0.69653	-2.2144
26	C	1.24662	2.90534	0.7634
27	H	0.48735	3.52252	1.2557
28	H	2.1686	3.50004	0.72081
29	C	1.48338	1.62687	1.58342
30	H	1.55362	1.87314	2.64838
31	H	0.62241	0.95999	1.47045
32	C	3.63804	-0.92858	-1.56896
33	H	4.22396	-0.89311	-0.65593
34	H	3.97366	-0.24182	-2.34172
35	C	3.07752	-2.26248	-1.96704
36	H	3.0215	-2.5225	-3.02028

31	C	1.05863	1.34516	1.60061
32	H	0.73903	0.8109	2.47085
33	C	0.97281	-0.66979	-2.00016

EDY-F

F-TS3

E(RB3LYP) = -1051.68038785 A.U.

G(298.15 K) = -1051.414697 A.U.

Imaginary frequency = 1

1	C	-2.20099	-0.73986	0.38433
2	C	0.09083	-1.50689	0.71962
3	C	-3.08716	1.34311	-0.25065
4	C	-3.68894	-0.83555	0.35915
5	N	-4.13491	0.43777	-0.0431
6	C	-5.53562	0.78075	-0.20058
7	H	-5.61951	1.5574	-0.96313
8	H	-6.08129	-0.11585	-0.50059
9	H	-5.95579	1.15605	0.73901
10	O	-4.41267	-1.77934	0.60178
11	O	-3.21389	2.50405	-0.58664
12	C	1.21567	-2.34578	0.53651
13	H	2.15208	-2.03069	1.00493
14	H	0.90604	-3.26843	1.0376
15	C	2.813	0.85236	-1.52758
16	O	3.06346	1.60177	-2.42914
17	O	2.3205	1.40274	-0.3361
18	C	3.01357	-0.65587	-1.55782
19	H	3.42775	-0.98394	-0.60089
20	H	3.77346	-0.83163	-2.32247
21	C	-0.53375	1.01953	0.10362
22	C	-1.83581	0.57837	0.03046
23	H	-0.28245	2.05428	-0.10413
24	C	2.69665	0.33587	1.8035
25	H	2.25874	-0.30817	2.56006
26	C	1.46099	-2.66573	-0.9657
27	H	0.58028	-3.19617	-1.34821
28	H	2.30448	-3.36674	-1.03008
29	C	1.71945	-1.46212	-1.89111
30	H	1.79676	-1.82931	-2.91972
31	H	0.8455	-0.80402	-1.88068
32	C	4.21986	0.34074	1.70009
33	H	4.65452	0.49669	0.71727
34	H	4.75788	-0.36485	2.327
35	C	3.53518	1.50559	2.33786
36	H	3.57676	1.62132	3.41686

37	H	3.26563	-3.0973	-1.29836	37	H	3.48583	2.43116	1.77313
38	C	-1.47658	1.22962	-0.49426	38	C	-1.19365	-1.61779	0.67942
39	C	0.14387	-1.499	-0.05346	39	C	0.52927	0.32797	0.49371
40	C	1.44278	-1.33634	-0.1519	40	C	1.83214	0.55944	0.64459

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