Electronic supplementary materials *Mendeleev Commun.*, 2019, **29**, 503–505

Synthesis of a new alkynylated deoxyadenosine phosphoramidite for the click reaction-mediated DNA labeling

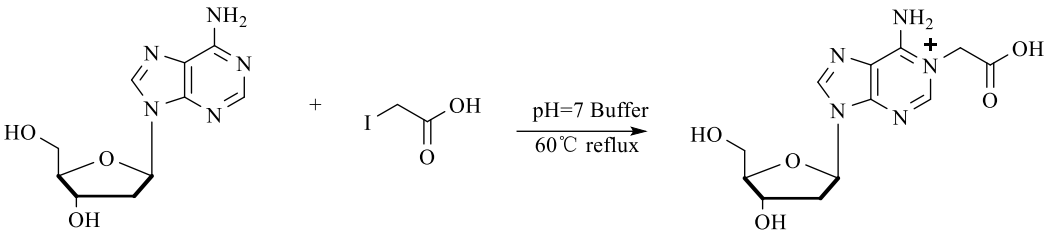
Shiyu Wang, Feifan Liu, Jiaobing Fang, Yashu Li, Jun Liu and Mingzhe Liu

General

Solvents were purified and dried by standard procedures. Reagents were generally the best quality commercial grade ones and were used without further purification, unless otherwise indicated. All reactions were carried in oven-dried glassware. TLC was performed using plates with GF-254 110 μm layer of 5–17 mesh silica gel. Column chromatography was performed using 200–300 mesh silica gel. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AVANCE III spectrometer (600 MHz for ^{1}H and 100 MHz for ^{13}C). Mass spectra were obtained using an Agilent 1100 Series LC/MSD Trap.

Table of contents:

1. Synthetic procedure for compound **1**

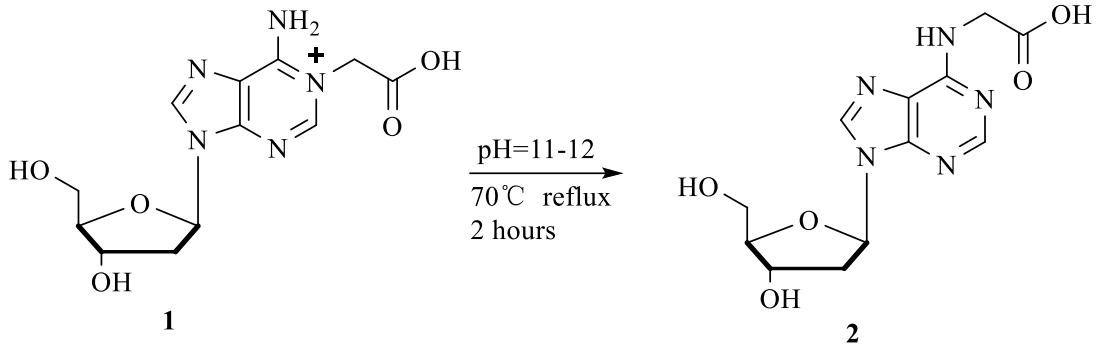


1

N^{1}-carboxymethyl-2'-deoxyadenosine **1**

To 2'-deoxyadenosine (1 g, 3.98 mmol) in a 10 ml round bottom flask, iodoacetic acid (4.5 g, 24.20 mmol, 6.08 eq) and 0.1 M phosphate buffer pH 7.0 (5 ml) were added. The mixture was stirred at 60 ℃ for 8 h. Then the mixture was allowed to cool to room temperature and pH was adjusted to 3.0 using 5 M aq. HCl. The mixture was poured into acetone (50 ml) at -5 ℃. After 30 min, the suspension was centrifuged at 8000 rpm. The supernatant was poured out and the residue was dried *in vacuo* to give a pink solid (1.23 g, yield 99%). ^{1}H NMR (600 MHz, D_2O) δ: 2.54–2.58 (m, 1H), 2.81 (ddd, 1H, *J* 13.12 Hz), 3.73 (d, 2H, CH_2, *J* 25.31 Hz), 4.07–4.10 (m, 1H), 4.59 (dd, 1H, *J* 6.16 Hz), 4.85 (s, 2H, C*H*$_2$COOH), 4.96 (s, 1H, OH), 5.03 (s, 1H, OH), 6.47 (t, 1H, *J* 4.76 Hz), 8.38 (s, 1H, HetAr–H), 8.43 (s, 1H, HetAr–H). ^{13}C NMR (100 MHz, D_2O) δ: 38.96, 53.02, 61.20, 70.68, 84.76, 87.39, 119.31, 143.13, 146.52, 147.67, 148.19, 170.50. MS (EI, ion trap), *m*/*z*: 310.2 $[M]^+$ (calc. for $C_{12}H_{16}N_5O_5$, *m*/*z*: 310.2).

2. Synthetic procedure for compound **2**

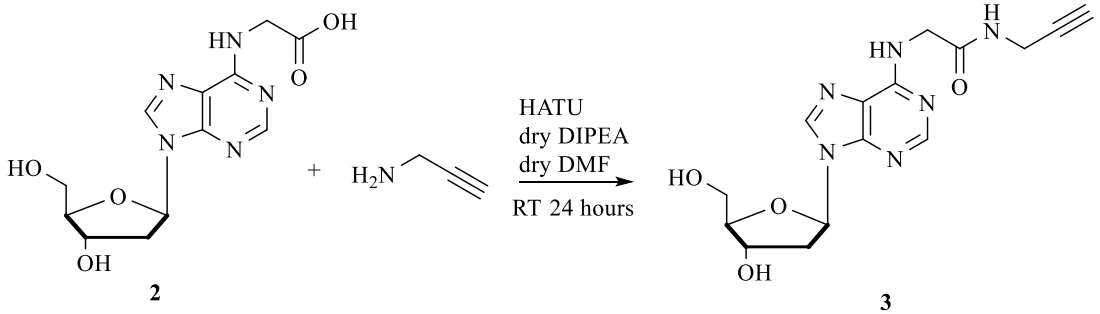


1 2

N^6-carboxymethyl-2'-deoxyadenosine **2**

To N^1-carboxymethyl-2'-deoxyadenosine **1** (1.23 g, 3.98 mmol) in a 10 ml round bottom flask, distilled water (5 ml) was added and pH of the water solution was adjusted to 11–12 using 5 M aq. NaOH. The mixture was stirred at 70 ℃ for 2 h. Then the mixture was allowed to cool to room temperature and pH was adjusted to 3.0 using 5 M aq. HCl. Water was removed *in vacuo* and the residue was mixed with methanol (20 ml). The resulting suspension was filtered and concentrated *in vacuo* to the volume of 5 ml. The residue was poured into diethyl ether (50 ml) at -5 ℃. After 10 min, the suspension was centrifuged in 8000 rpm. The supernatant was poured out and the residue was dried *in vacuo* to give a pink solid (0.74 g, yield 60%). ^{1}H NMR (600 MHz, D_2O) δ: 2.55–2.59 (m, 1H), 2.81 (ddd, 1H), 3.80 (d, 1H, *J* 4.32 Hz), 3.85 (d, 1H, *J* 3.13 Hz), 3.97 (s, 2H, CH_2COOH), 4.09 (d, 2H, CH_2, *J* 8.47 Hz), 4.19 (q, 1H, *J* 3.34 Hz), 4.65 (m, 1H), 4.91 (d, 1H, OH, *J* 9.50 Hz), 5.06 (d, 1H, OH, *J* 24.60 Hz), 6.42 (t, 1H, *J* 6.87 Hz), 7.92 (s, 1H, HetAr–NH), 8.15 (s, 1H, HetAr–H), 8.24 (s, 1H, HetAr–H). ^{13}C NMR (100 MHz, D_2O) δ: 39.00, 61.12, 61.61, 71.18, 84.50, 87.31, 139.60, 144.25, 149.27, 152.22, 170.97, 179.79. MS (EI, ion trap), *m*/*z*: 310.2 $[M+H]^+$, 307.9 $[M-H]^-$ (calc. for $C_{12}H_{15}N_5O_5$, *m*/*z*: 309.2).

3. Synthetic procedure for compound **3**



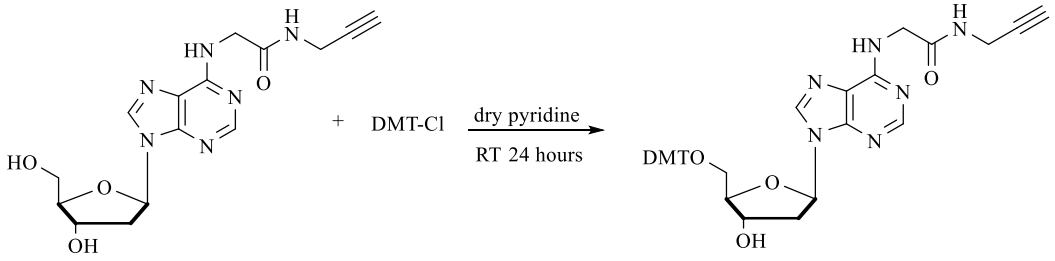
2 3

N^6-(propargylcarbamoylmethyl)-2'-deoxyadenosine **3**

To N^6-carboxymethyl-2'-deoxyadenosine **2** (0.9 g, 2.91 mmol) in a 50 ml round bottom flask, dry DMF (10 ml) was added. Then dry *N*,*N*-diisopropylethylamine (0.6 ml, 3.0 eq.) was added to the above mixture dropwise in ice bath followed by propargylamine (0.65 ml, 2.0 eq.). 2-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-

tetramethyluronium hexafluorophosphate (1.66 g, 4.36 mmol, 1.5 eq.) was dissolved in dry DMF (5 ml) and added to the mixture dropwise. The resulting mixture was stirred at room temperature for 24 h and evaporated to dryness. The residue was treated with methanol (10 ml) and the resulting suspension was filtered. The filtrate was concentrated to the volume of 5 ml and poured into diethyl ether (50 ml). The resulting suspension was filtered and dried to afford the crude product, which was purified by flash column chromatography using methanol–dichloromethane (1 : 3 v/v) to give a brown solid (0.7 g, yield 70%). ^{1}H NMR (600 MHz, CD_3OD) δ: 2.58 (s, 1H), 2.62 (s, 1H), 2.99–3.01 (m, 2H, CH_2), 3.05–3.08 (m, 2H, CH_2), 3.11 (d, 2H, CH_2, *J* 6.22 Hz), 3.76 (d, 1H, *J* 2.17 Hz), 3.89–3.96 (m, 1H), 6.12 (s, 1H, NH), 6.23 (t, 1H, *J* 5.37 Hz), 7.45–7.47 (m, 1H, HetAr–NH), 8.65 (s, 1 H, HetAr–H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.12, 20.98, 29.05, 29.62, 55.17, 60.32, 61.97, 63.60, 87.66, 113.08, 113.36, 127.83, 129.04, 129.87, 158.75. MS (EI, ion trap), *m*/*z*: 384.4 $[M+K]^+$ (calc. for $C_{15}H_{18}N_6O_4$, *m*/*z*: 346.3).

4. Synthetic procedure for compound **4**



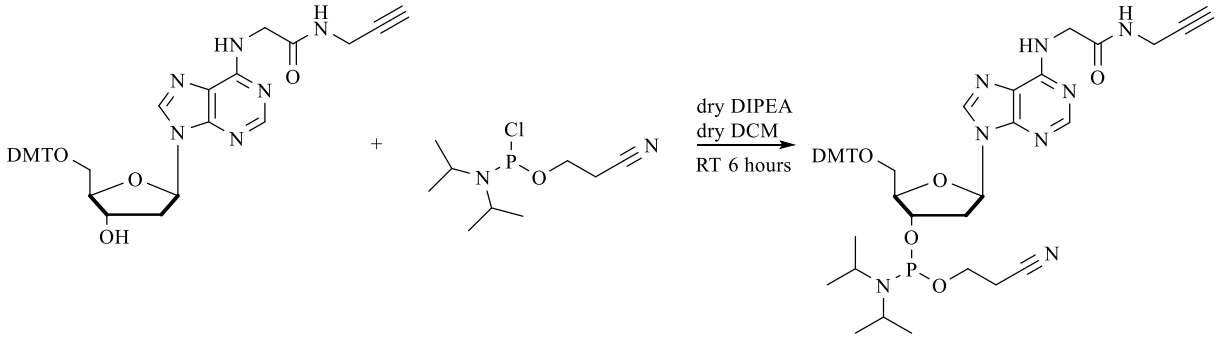
3 **4**

5'-(4,4'-Dimethoxytrityl)-N^6-(propargylcarbamoylmethyl)-2'-deoxyadenosine **4**

To N^6-propargylcarbamoylmethyl-2'-deoxyadenosine **3** (741 mg, 2.14 mmol) in a 50 ml round bottom flask, 4,4'-dimethoxytrityl chloride (870 mg, 2.57 mmol, 1.2 eq.), 4-dimethylaminopyridine (13.0 mg, 0.107 mmol, 0.05 eq.) and dry pyridine (20 ml) were added. The mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was dissolved in dichloromethane and extracted by aq. $NaHCO_3$–NaCl. The crude product was purified by flash column chromatography using methanol–dichloromethane–triethylamine (1 : 9 : 0.5 v/v) to give a yellow solid (1.0 g, yield 73%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 2.45–2.49 (m, 1H), 2.75 (dt, 1H, *J* 13.02 Hz), 2.97 (s, 1H), 3.30–3.37 (m, 2H, CH_2), 3.71 (s, 6H, Ar–OCH_3), 3.98 (dd, 2H, CH_2, *J* 5.22 Hz), 4.03–4.08 (m, 2H, CH_2), 4.26 (s, 2H, CH_2), 4.59–4.62 (m, 1H), 6.36 (t, 1H, *J* 6.36 Hz), 6.73 [m , 4H, (Het)Ar–H], 7.12 [dd, 4H, (Het)Ar–H, *J* 22.35 Hz], 7.34 [dd, 5H, (Het)Ar–H, *J* 26.97 Hz], 7.62 [t, 1H, NH, *J* 7.57 Hz], 7.90 (s, 1H, HetAr–NH), 8.26 [s, 1H, (Het)Ar–H], 8.56 [s, 1H, (Het)Ar–H]. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.61, 29.61, 40.16, 55.16, 63.63, 71.55, 72.40, 79.16, 84.32, 85.97, 86.53,

113.09, 127.81, 127.98, 129.04, 136.01, 139.39, 144.41, 149.57, 152.78, 154.19, 158.53, 169.26. MS (EI, ion trap), *m*/*z*: 649.8 $[M+H]^+$, 671.8 $[M+Na]^+$, 687.8 $[M+K]^+$ (calc. for $C_{36}H_{36}N_6O_6$, *m*/*z*: 648.7).

5. Synthetic procedure for compound **5**



4 **5**

5'-(4,4'-Dimethoxytrityl)-N^6-(propargylcarbamoylmethyl)-2'-deoxyadenosin-3'-*O*-yl 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite **5**

To 5'-(4,4'-Dimethoxytrityl)-N^6-(propargylcarbamoylmethyl)-2'-deoxyadenosine **4** (165 mg, 0.25 mmol) in a 50 ml round bottom flask, dry dichloromethane (5 ml) was added. The flask was put into ice-bath. Dry *N*,*N*-diisopropylethylamine (0.43 ml, 2.0 eq.) was added dropwise to the mixture and then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite was also added dropwise at 0 °C. The mixture was stirred at room temperature for 6 h, evaporated to dryness and the residue was dissolved in dichloromethane (10 ml). The organic layer was washed with saturated aq. $NaHCO_3$–NaCl. The crude product was purified by flash column chromatography using methanol–dichloromethane–triethylamine (1 : 9 : 0.5 v/v) to give a yellow solid (190.8 mg, yield 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.20 (s, 12H, Me), 2.38 (d, 1H, *J* 6.52 Hz), 2.52–2.54 (m, 1H), 2.57–2.62 (m, 2 H), 2.67 (td, 2H, CH_2, *J* 6.17 Hz), 3.43 (dq, 2H, CH_2, *J* 18.32 Hz), 3.67 (s, 2H, CH_2), 3.69 (s, 6H, OMe), 3.92–3.99 (m, 2H), 4.02–4.07 (m, 2H, CH_2), 4.09–4.14 (m, 2H, CH_2, *J* 9.75 Hz), 4.28–4.31 (m, 1H), 5.20 (s, 1H), 6.34 (s, 1H), 6.68–6.72 [m, 4H, (Het)Ar–H], 7.08–7.11 [m, 5H, (Het)Ar–H], 7.20–7.40 [m, 7H, (Het)Ar–H, HetAr–NH, NH], 7.87 [d, 1H, (Het)Ar–H, *J* 15.18 Hz], 8.24 [s, 1H, (Het)Ar–H]. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.04, 20.00, 22.57, 22.91, 24.54, 28.80, 31.50, 40.42, 41.44, 45.21, 47.39, 55.16, 58.08, 86.47, 113.01, 116.81, 126.83, 127.73, 128.09, 130.00, 135.47, 142.27, 144.35, 152.12, 158.42, 161.77, 165.72, 175.82. MS (EI, ion trap), *m*/*z*: 849.8 $[M+H]^+$, 871.8 $[M+Na]^+$ (calc. for $C_{45}H_{53}N_8O_7P$, *m*/*z*: 848.8).

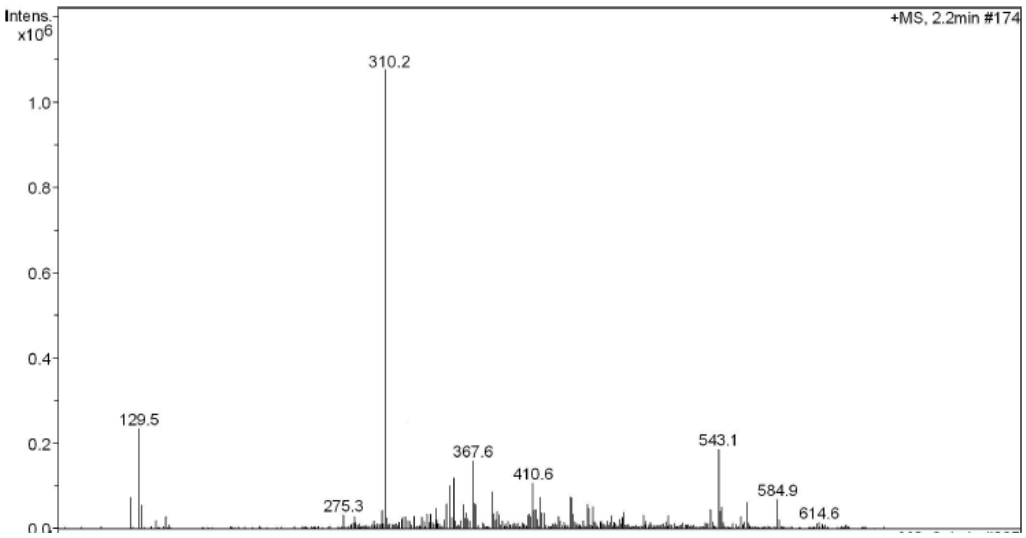


Figure S1 Mass spectrum of compound **1**.

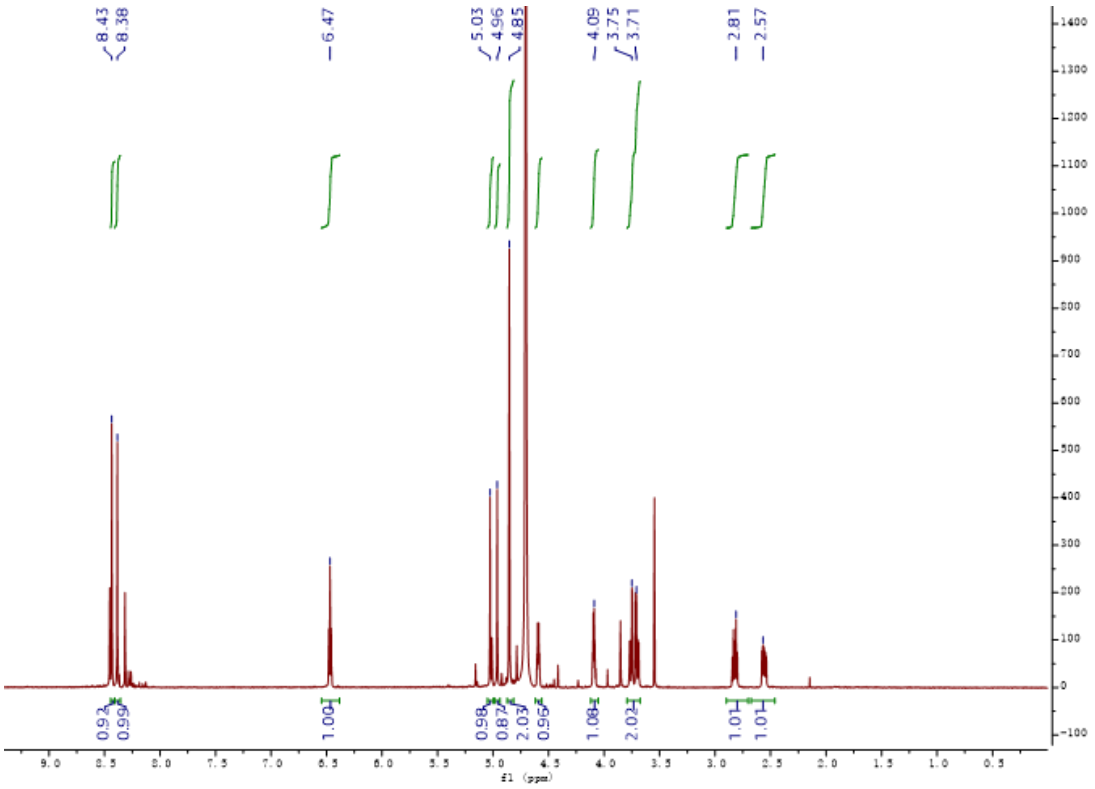


Figure S2 ^{1}H NMR spectrum of compound **1**.

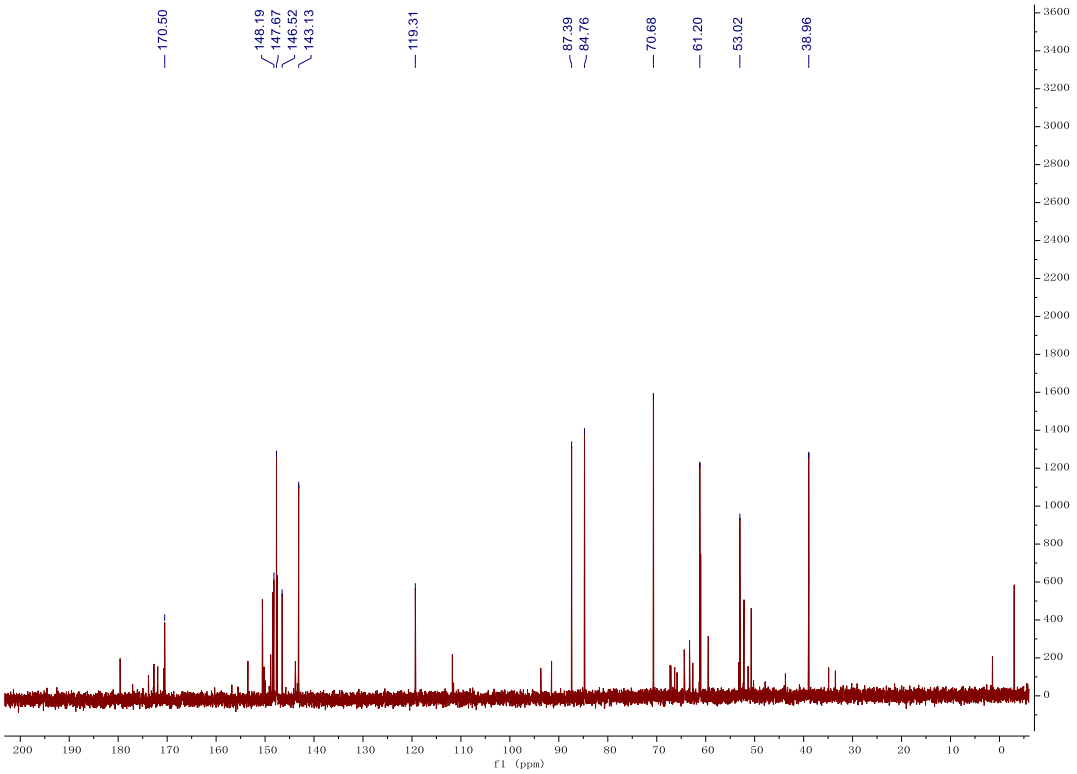


Figure S3 ^{13}C NMR spectrum of compound **1**.

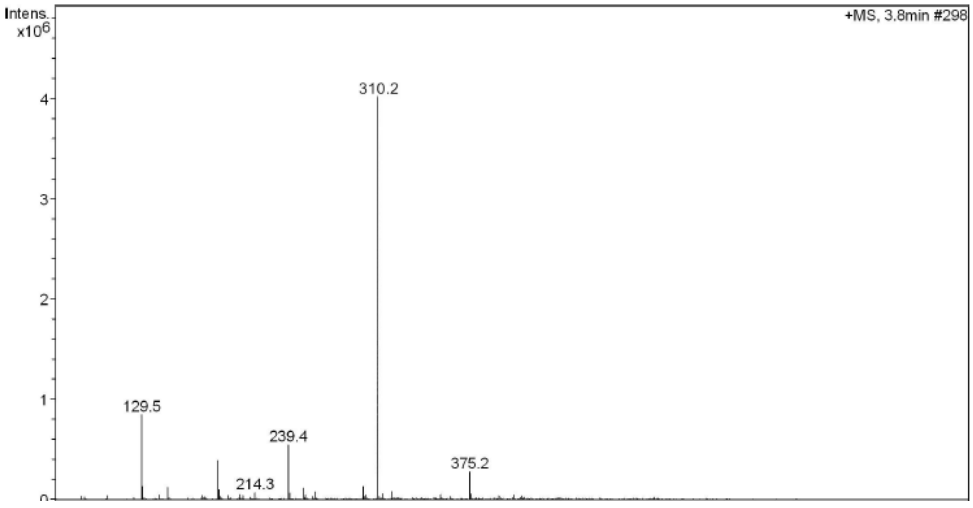


Figure S4 Mass spectrum of compound **2** (positive ion mode).

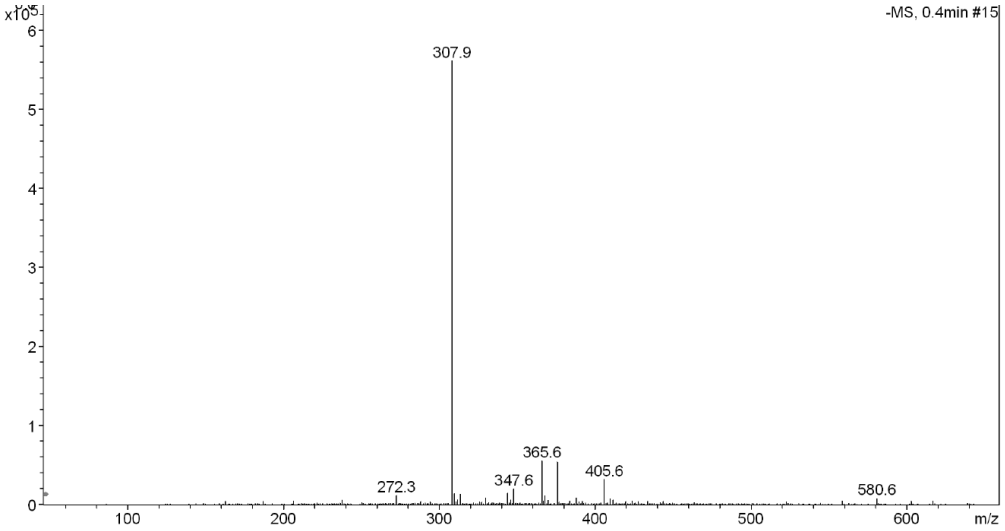


Figure S5 Mass spectrum of compound **2** (negative ion mode).

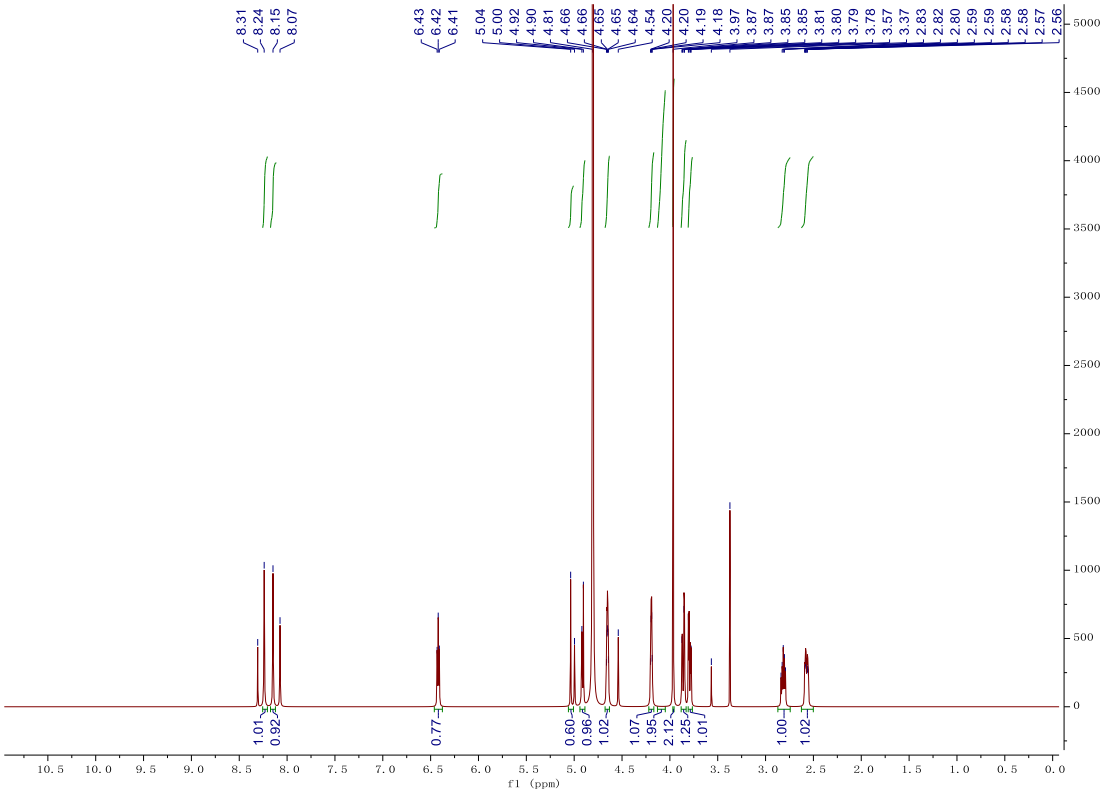


Figure S6 ^{1}H NMR spectrum of compound **2**.

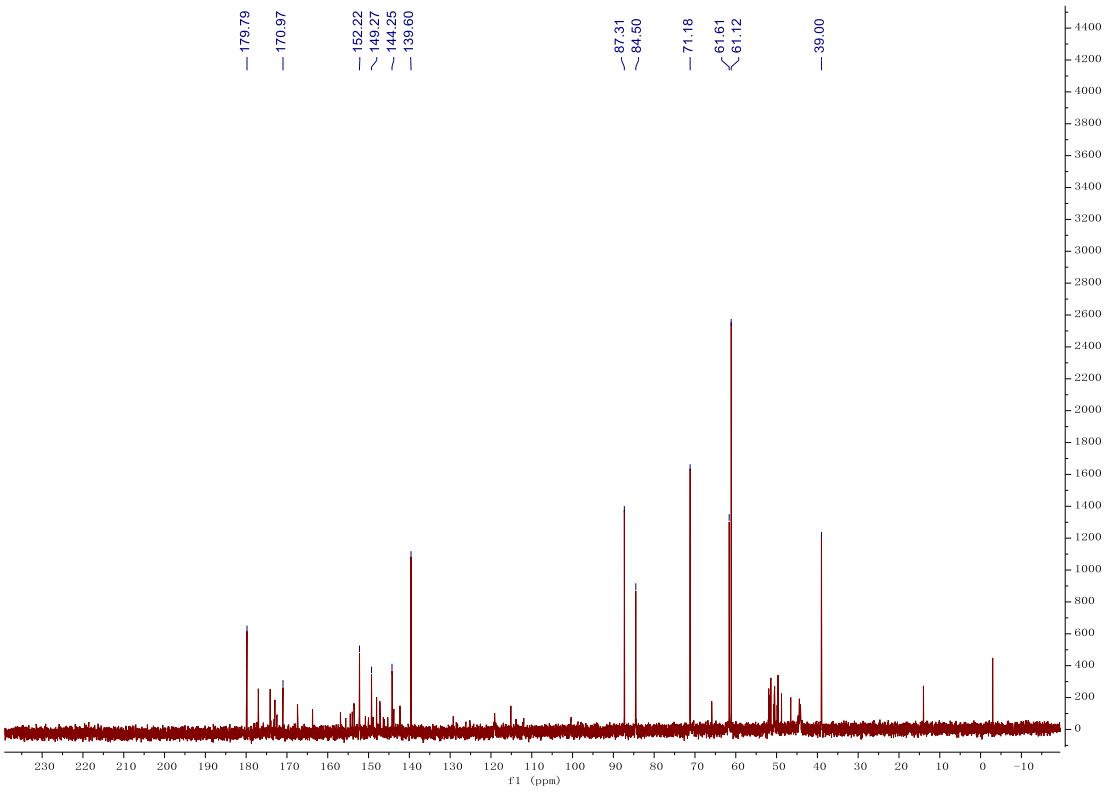


Figure S7 ^{13}C NMR spectrum of compound **2**.

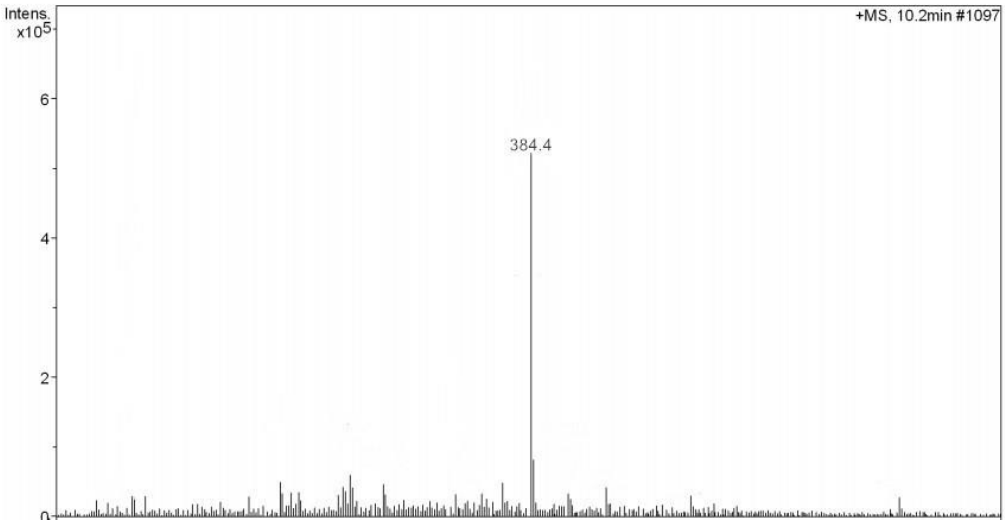


Figure S8 Mass spectrum of compound **3**.

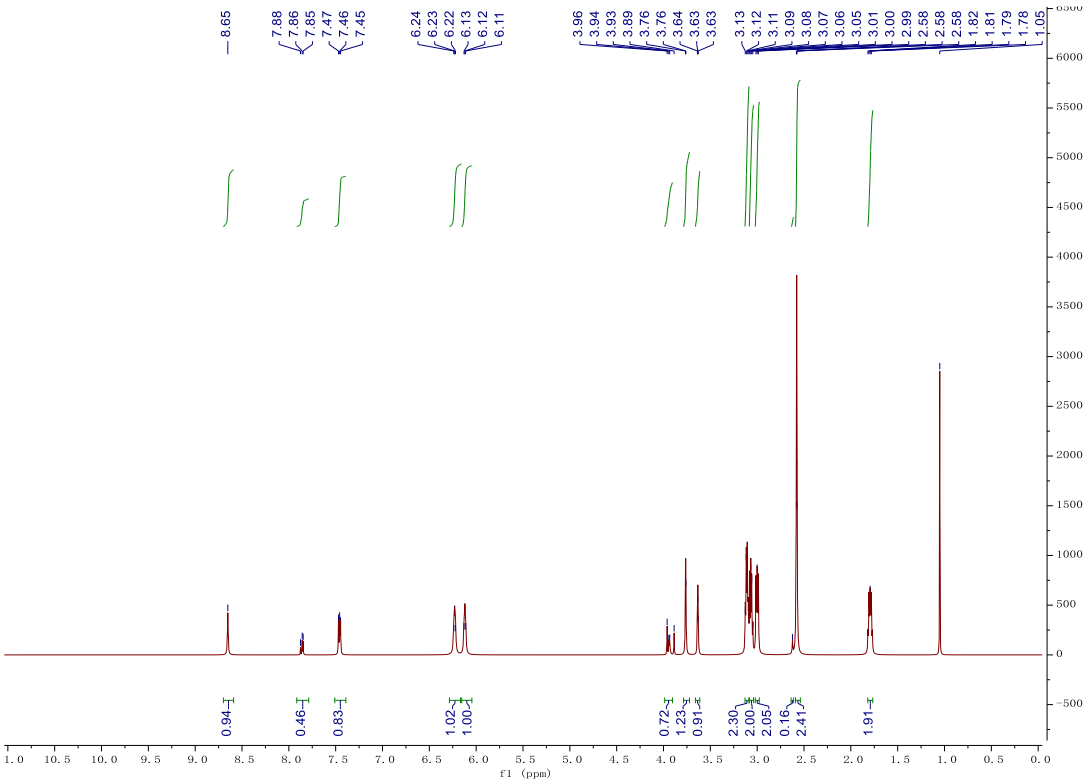


Figure S9 ^{1}H NMR spectrum of compound **3**.

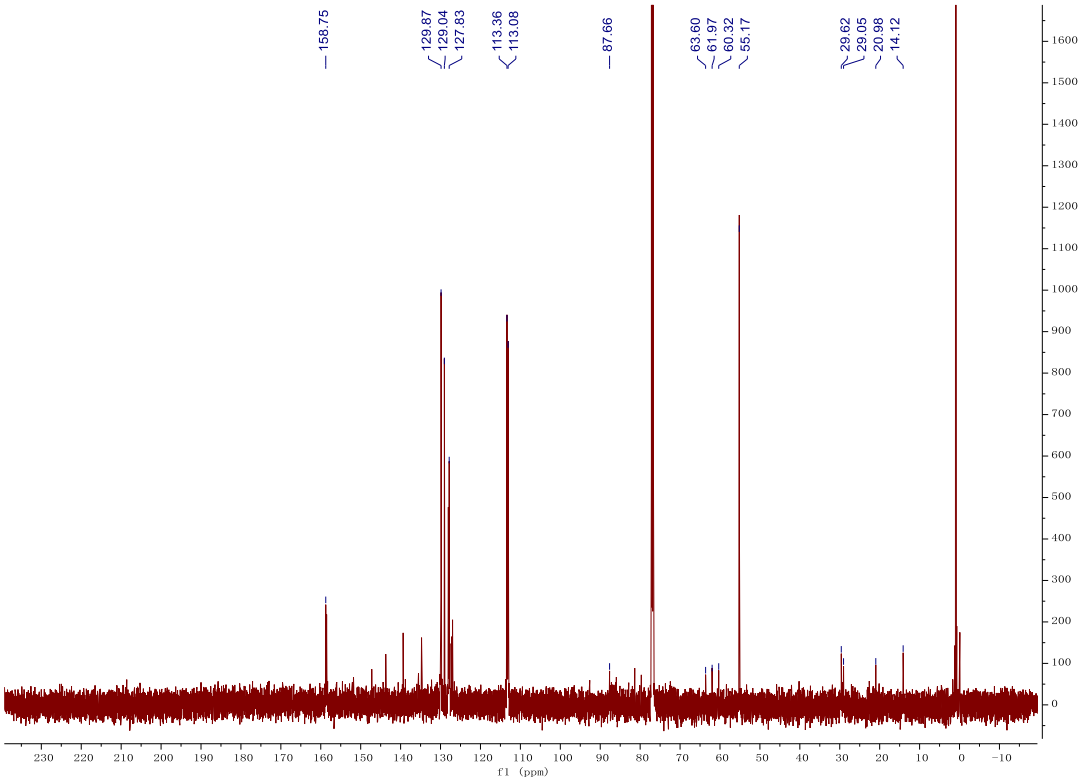


Figure S10 ^{13}C NMR spectrum of compound **3**.

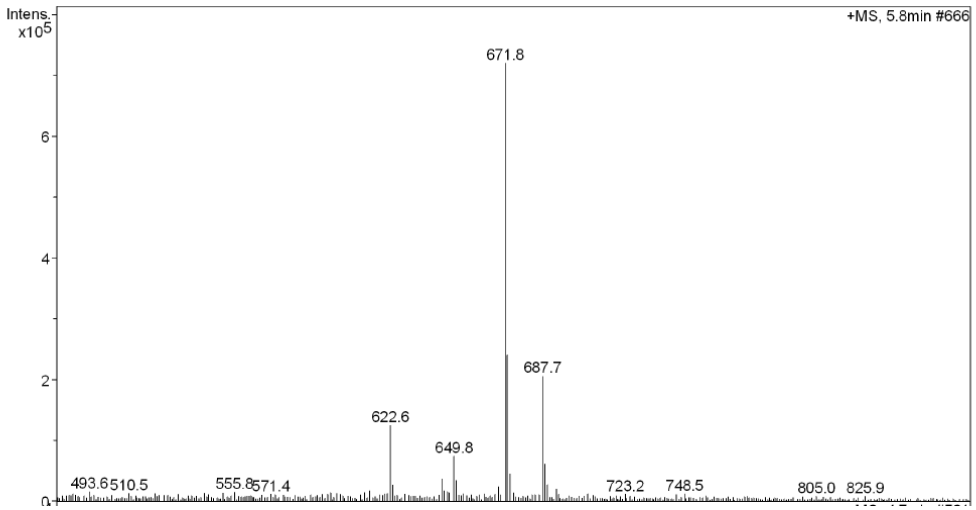


Figure S11 Mass spectrum of compound **4**.

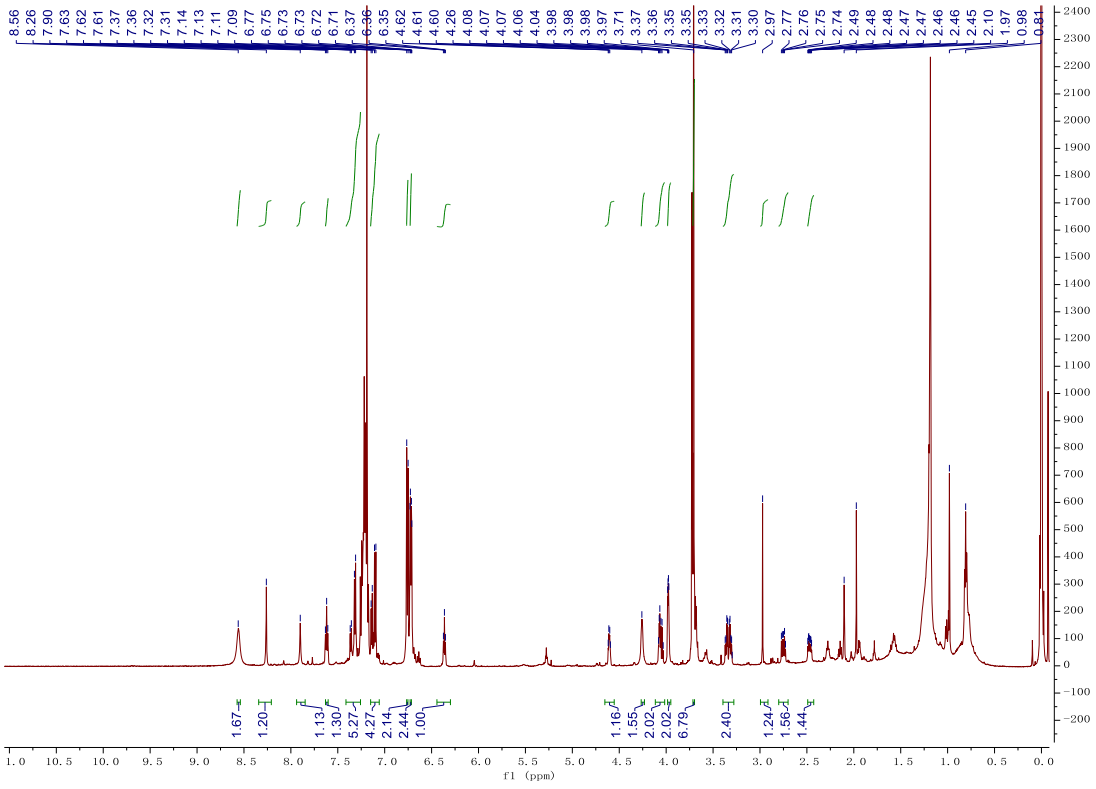


Figure S12 ^{1}H NMR spectrum of compound **4**.

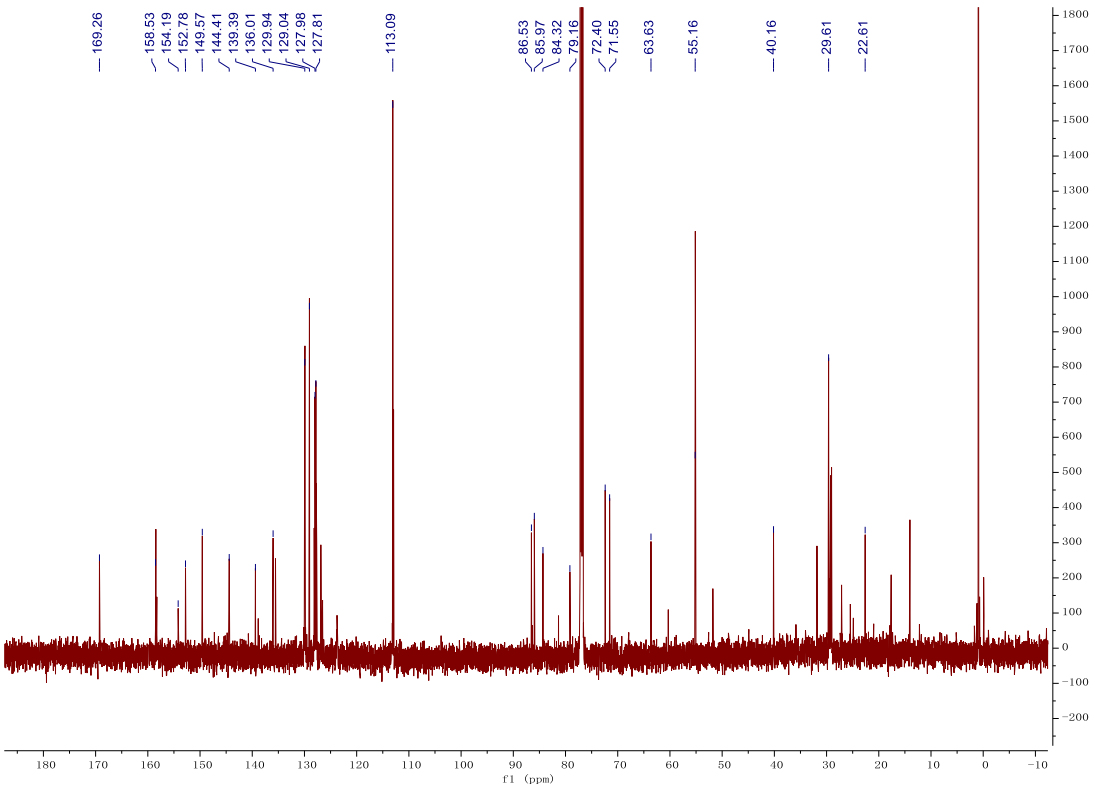


Figure S13 ^{13}C NMR spectrum of compound **4**.

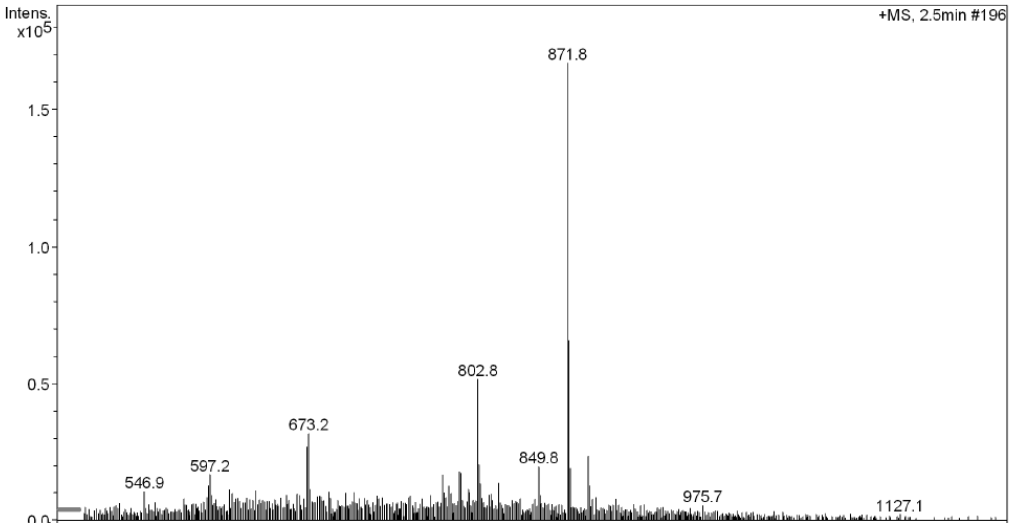


Figure S14 Mass spectrum of compound **5**.

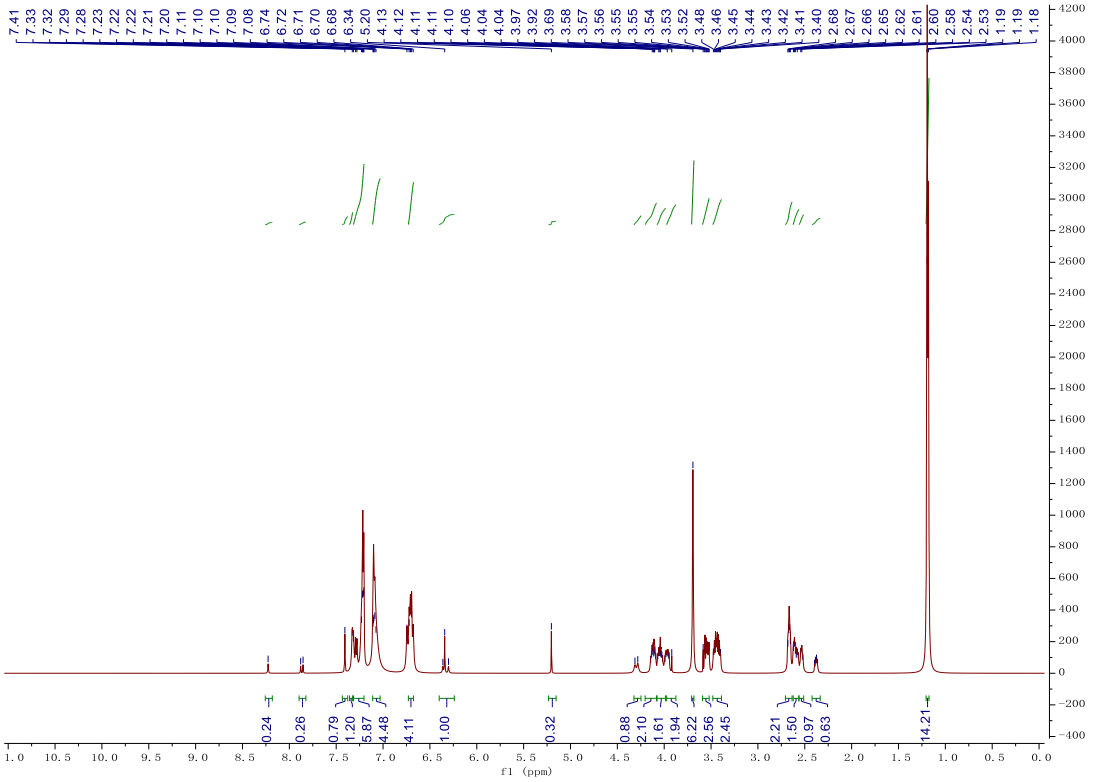


Figure S15 ^{1}H NMR spectrum of compound **5**.

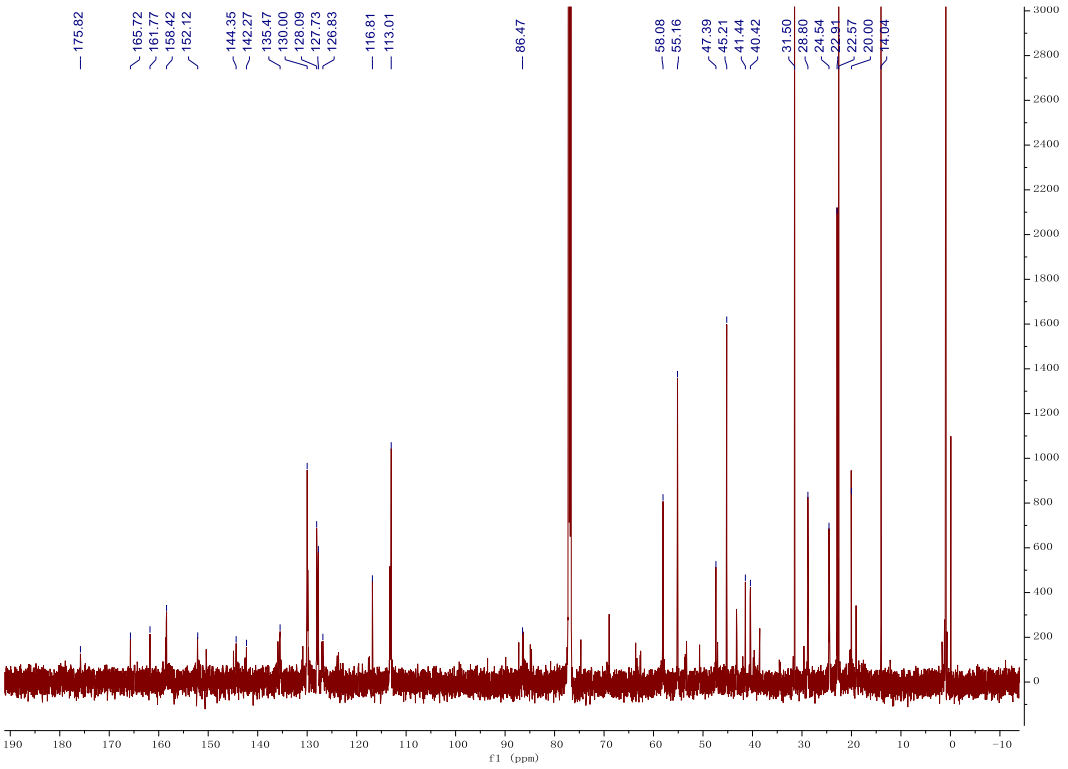


Figure S16 ^{13}C NMR spectrum of compound **5**.