Supporting Information for

Naproxen platinum(IV) hybrids inhibiting cycloxygenases, matrix metalloproteinases and causing DNA damage: Synthesis and biological evaluation as antitumor agents *in vitro* and *in vivo*

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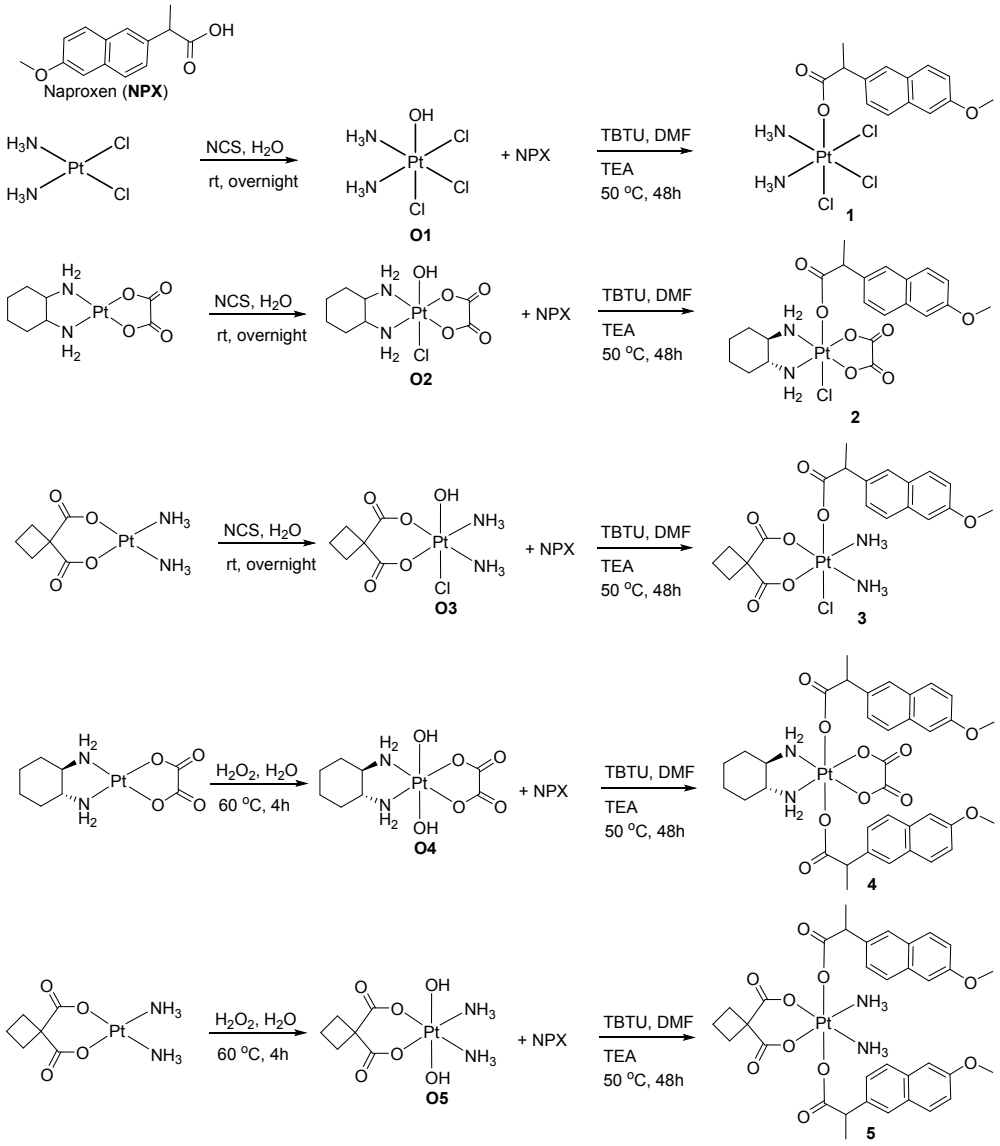
Contents

1. Synthetic procedures



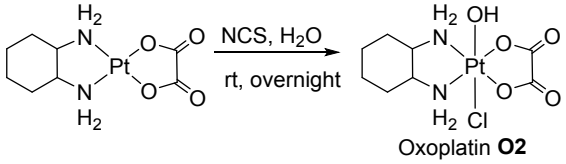
Scheme S1. Synthetic route for naproxen platinum(IV) compounds **1–5**.

1.1 Preparation of compound O1:

Cisplatin (0.75 g, 2.5 mmol) was suspended in distilled water 150 mL and stirred at room temperature. *N*-chloro succinimide (NCS) solution (0.40 g, 3 mmol) in distilled water 150 mL was added to reaction system dropwise and the resultant mixture was stirred overnight at room temperature. After that, the solid was filtrated and the solution was concentrated under vacuum.

The residue was washed with ethanol and ethylether. The product was obtained as a yellow solid (0.46 g, 52%).

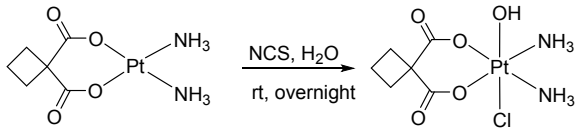
1.2 Preparation of compound O2:



Oxoplatin **O2**

Oxaliplatin (1.0 g, 2.5 mmol) was suspended in distilled water 150 mL and stirred at room temperature. *N*-chloro succinimide (NCS) solution (0.40 g, 3.0 mmol) in distilled water 150 mL was added to reaction system dropwise and the resultant mixture was stirred overnight at room temperature. After that, the solid was filtrated and the solution was concentrated under vacuum. The residue was washed with ethanol and ethylether. The product was obtained as a yellow solid (0.65 g, 58%).

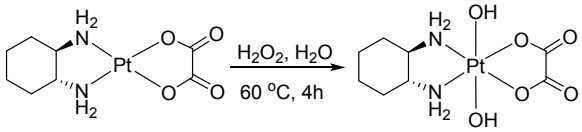
1.3 Preparation of compound O3:



O3

Carboplatin (0.93 g, 2.5 mmol) was suspended in distilled water 150 mL and stirred at room temperature. *N*-chloro succinimide (NCS) solution (0.40 g, 3.0 mmol) in distilled water 150 mL was added to reaction system dropwise and the resultant mixture was stirred overnight at room temperature. After that, the solid was filtrated and the solution was concentrated under vacuum. The residue was washed with ethanol and ethylether. The product was obtained as a yellow solid (0.58 g, 55%).

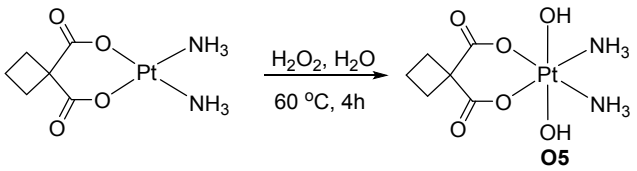
1.4 Preparation of compound O4:



O4

A suspension of oxaliplatin (0.99 g, 2.5 mmol) in distilled water 30 mL was stirred at room temperature. Then H_2O_2 (30%) 50 mL was added drop wise. The mixture was kept stirring for 4 h at 60 °C. Then the resultant mixture was recrystallized at 4 °C. Crude product as yellow solid was obtained after filtration. Then recrystallization in water afford pure oxoplatin **O4** as white solid (0.82 g, 76%).

1.5 Preparation of compound O5:



A suspension of carboplatin (0.93 g, 2.5 mmol) in distilled water 30 mL was stirred at room temperature. Then H_2O_2 (30%) 50 mL was added drop wise. The mixture was kept stirring for 4 h at 60 °C. Then the resultant mixture was recrystallized at 4 °C. Crude product as yellow solid was obtained after filtration. Then recrystallization in water afford pure oxoplatin **O5** as white solid (0.84 g, 83%).

2. Reduction of platinum(IV) complexes by AsA

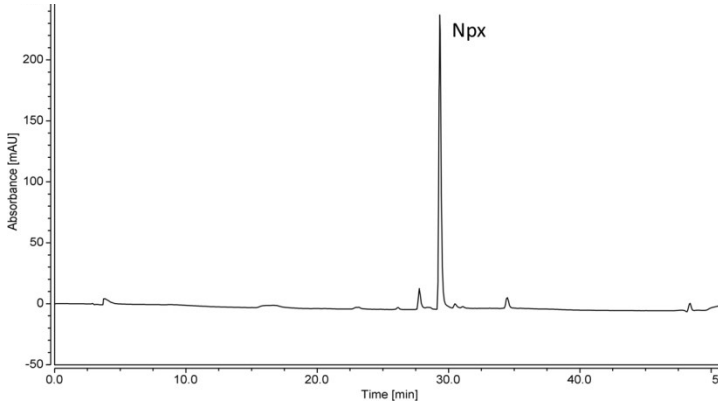


Fig. S1. HPLC spectrum for naproxen (500 μM).

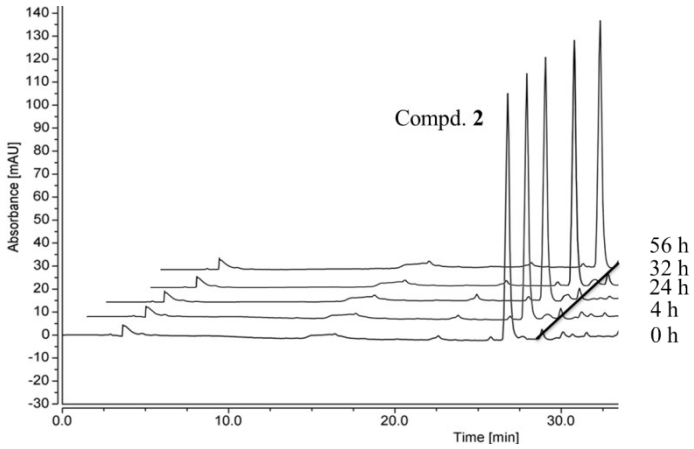


Fig. S2. HPLC spectra for solution of compound **2** (500 μM).

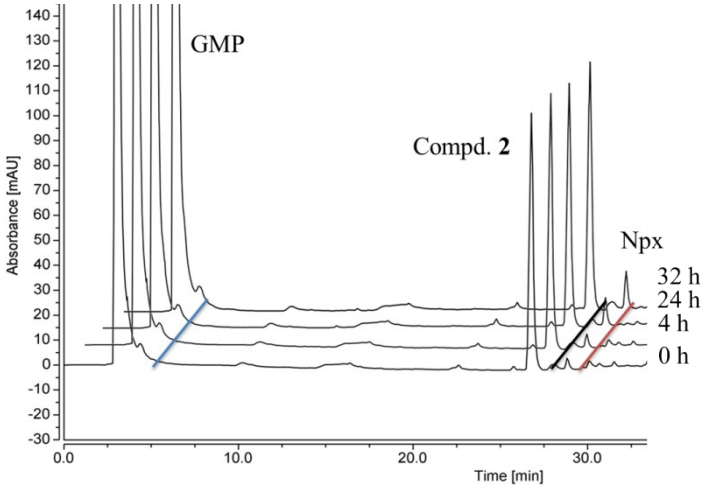


Fig. S3. HPLC spectra for solution of compound **2** (500 μM) and 5′-GMP (3 mM).

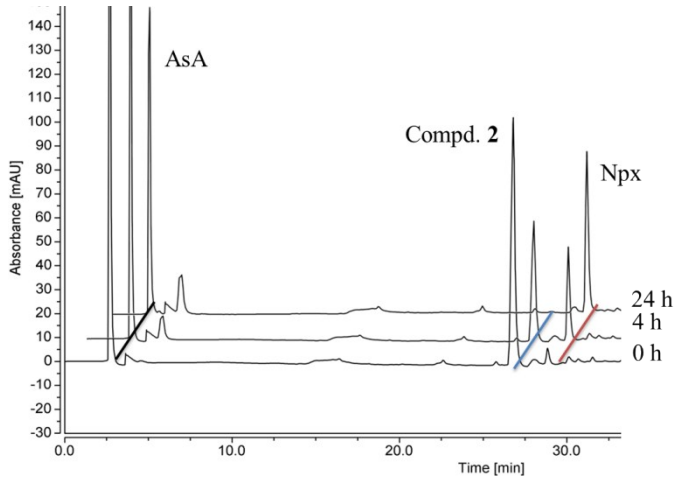


Fig. S4. HPLC spectra for solution of compound **2** (500 μM) and AsA (1 mM) without GMP.

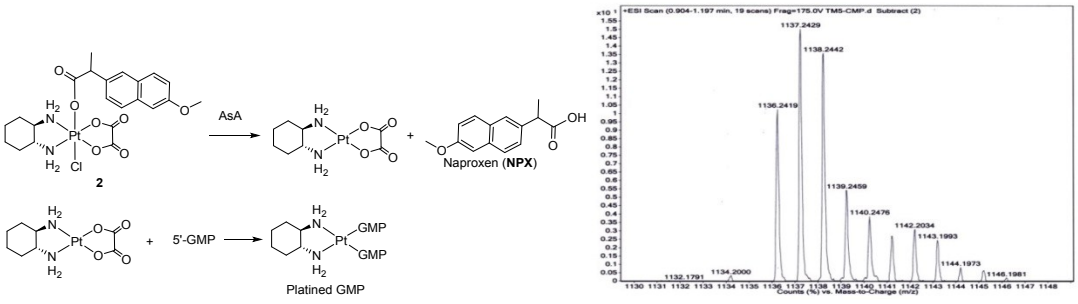


Fig. S5. The formation of platined GMP (The adduct of oxaliplatin with 5'-GMP).[S1]

3. Fluorescence spectra assay for HSA binding detection

The fluorescence spectra of HSA in the absence and presence of platinum(IV) complex **2** at three temperatures were supplied as Fig. 11 and Fig. S6.

The quenching trend of the HSA by the platinum(IV) complex **2** is in agreement with the linear Stern-Volmer equation (eq S1):

$$\frac{F_0}{F} = 1 + K_{SV}[C] = 1 + K_q\tau_0[C] \quad \text{(eq S1)}$$

F_0 and F are the fluorescence intensities in the absence and presence of the compound respectively; K_{SV} is linear Stern-Volmer quenching constant; K_q is the quenching rate constant; τ_0 is the average lifetime of molecules in the absence of quencher and its value is about 10^{-8} s; [C] is the concentration of the compound.

The Stern-Volmer plots at three different temperatures were presented as Fig. S7. The K_{sv} was calculated and given in Table S1.

For static quenching, when molecules bind independently to a set of equivalent sites on a macromolecule, the binding constant (K_b) and the number of binding sites (n) can be determined by eq S2:

$$log\frac{F_0 - F}{F} = log\, K_b + n\, log\, [C] \quad \text{(eq S2)}$$

F_0 and F are the fluorescence intensities in the absence and presence of the compound respectively; [C] is the concentration of the compound; K_b is the binding constant to a site; n is the number of binding sites per HSA. The plots of log[(F_0 - F)/F] vs log[C] are shown in Fig. S8. The values of K_b and n are obtained and given in Table S2.

The interaction forces between bioactive molecules and HSA are mainly ascribed to electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic and steric contacts. If the enthalpy change (ΔH) does not vary significantly over the studied temperature range, then its value and that of entropy change (ΔS) can be evaluated from the van't Hoff equation (eq S3):

$$lnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \text{(eq S3)}$$

In eq S3, K is analogous to the associative binding constants at the corresponding temperature; R is the gas constant; ΔH is the enthalpy change; ΔS is the entropy change. The van't Hoff plots of compound **2**-HSA system were displayed in Fig. S9.

The free energy change (ΔG) was then calculated from the following equation (eq S4):

$$\Delta G = \Delta H - T\Delta S \quad \text{(eq S4)}$$

The thermodynamic parameters ΔH, ΔG and ΔS were calculated and given in Table S2.

It has been manifested that the sign and magnitude of the thermodynamic parameter are associated with various individual kinds of interaction that may take place in protein association processes. From the thermodynamic standpoint, $\Delta H > 0$ and $\Delta S > 0$ imply a hydrophobic interaction, $\Delta H < 0$ and $\Delta S < 0$ reflect the van der Waals force or hydrogen bond formation, and $\Delta H < 0$ and $\Delta S > 0$ are characteristics for electrostatic interactions.

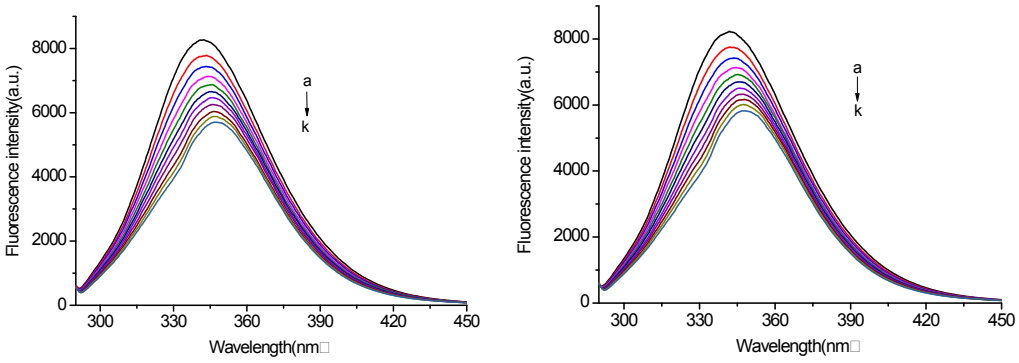


Fig. S6. Fluorescence spectra of HSA in the absence and presence of platinum(IV) complex **2** (λex= 280 nm, T= 301 K, 304 K). **a–k**: *c*(HSA) = 4.0 μM, *c*(complex **2**) = 0.0–20.0 μM, at increments of 2.0 μM.

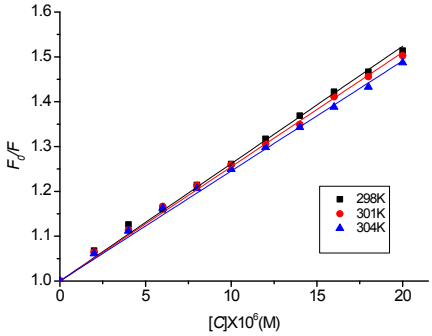


Fig. S7. Stern-Volmer plots at three different temperatures.

Table S1 Stern-Volmer quenching constants for the interaction of compound **2** with HSA at different temperatures.

T (K)	K_{sv} (M^{-1})	K_q	R[a]	SD[b]
298	0.262×10^5	0.262×10^{13}	0.9988	0.009
301	0.255×10^5	0.255×10^{13}	0.9989	0.008
304	0.246×10^5	0.246×10^{13}	0.9983	0.009

[a] R is the correlation coefficient.

[b] SD is standard deviation.

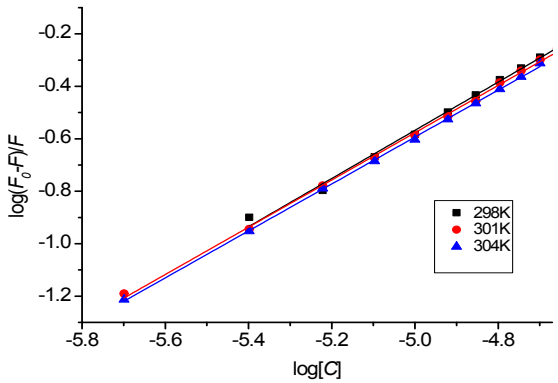


Fig. S8. The plots of log (F_0 - F)/F vs log [C] for HSA-complex **2** system at different temperatures.

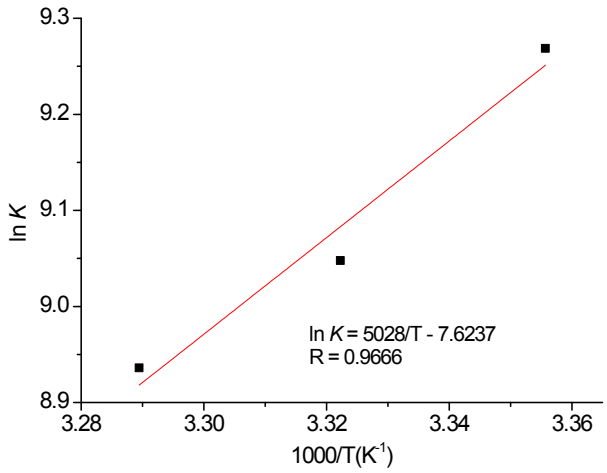


Fig. S9. Van't Hoff plots of compound **2**-HSA system.

Table S2 Binding constants, binding sites of compound **2** with HSA and the thermodynamic parameters at different temperatures.

T (K)	K_b (M^{-1})	R	*n*	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol K)
298	1.06×10^4	0.9977	0.92	-41.80 ±0.92	-22.91 ±0.10	-63.38 ±3.06
301	0.85×10^4	0.9994	0.90		-22.72 ±0.05	
304	0.76×10^4	0.9998	0.89		-22.54 ±0.03	

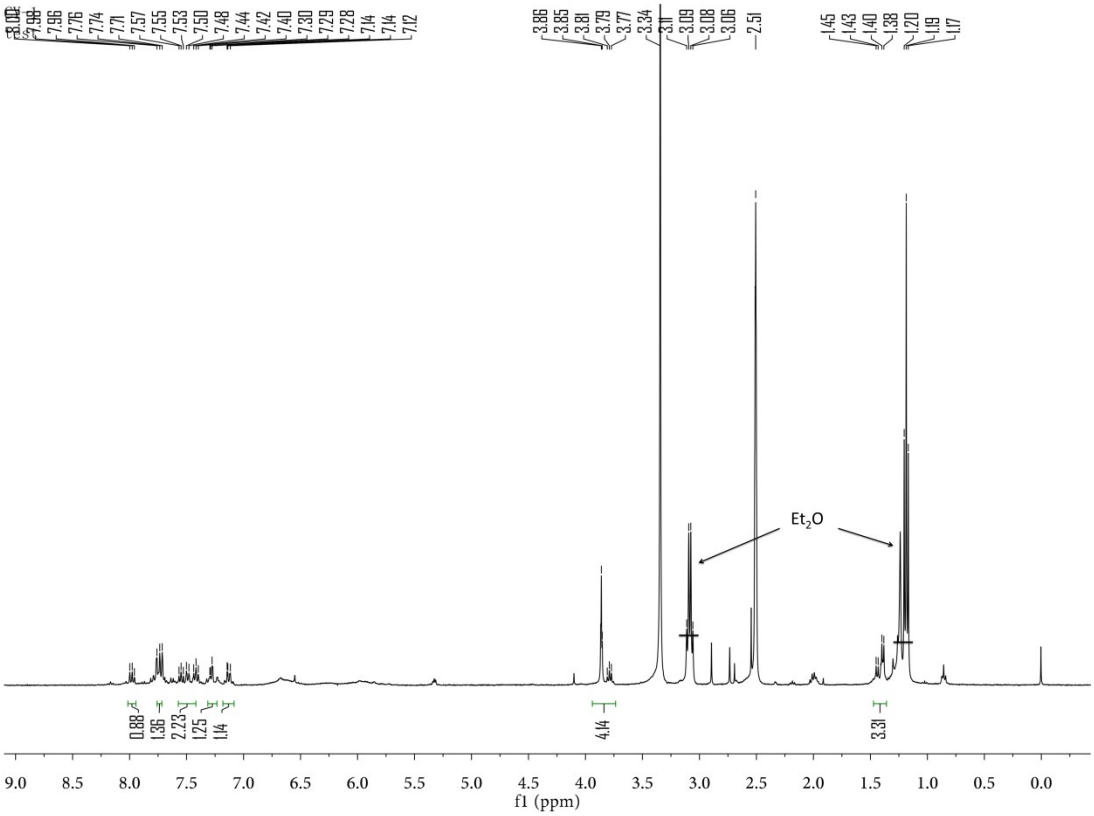
4. Data for Figure 4A

Table S3 Data for Figure 4A. *In vivo* toxicities of compound **2**, cisplatin and oxaliplatin to BALB/c mice. Body weight of the mice during the treatment.

Day	**Cisplatin**		**Oxaliplatin**		**Compd. 2**		**Saline**	
	RBW[a] (%)	SD	RBW (%)	SD	RBW (%)	SD	RBW (%)	SD
6	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
8	101.4	2.1	101.1	2.9	102.9	1.4	105.4	1.8
10	101.6	2.3	99.6	6.8	104.2	1.3	105.9	2.9
11	100.0	3.0	100.2	2.9	102.7	2.4	105.1	3.3
12	99.4	2.3	100.0	3.4	103.0	2.0	107.0	2.9
13	97.5	3.3	98.7	4.1	102.0	1.8	109.7	3.4
14	98.7	4.0	101.6	4.5	105.8	1.8	113.1	3.6
15	94.5	3.4	98.2	3.5	102.4	2.8	114.3	3.3

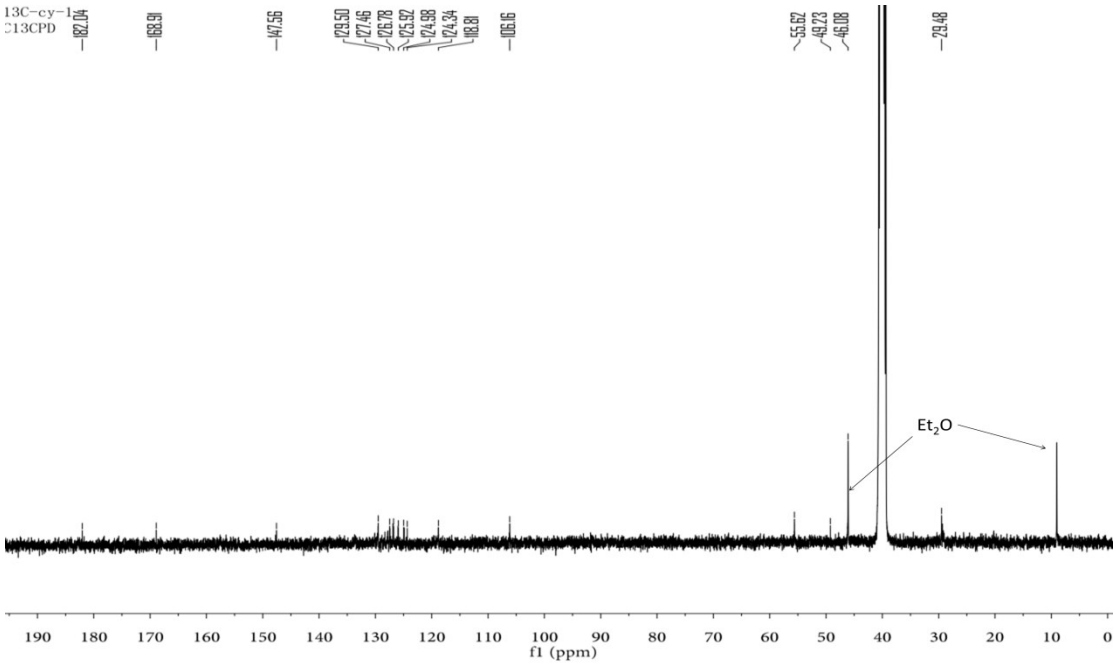
[a] RBW: relative body weight = (body weight on the indicated day / body weight on day 6) × 100%.

5. NMR spectra



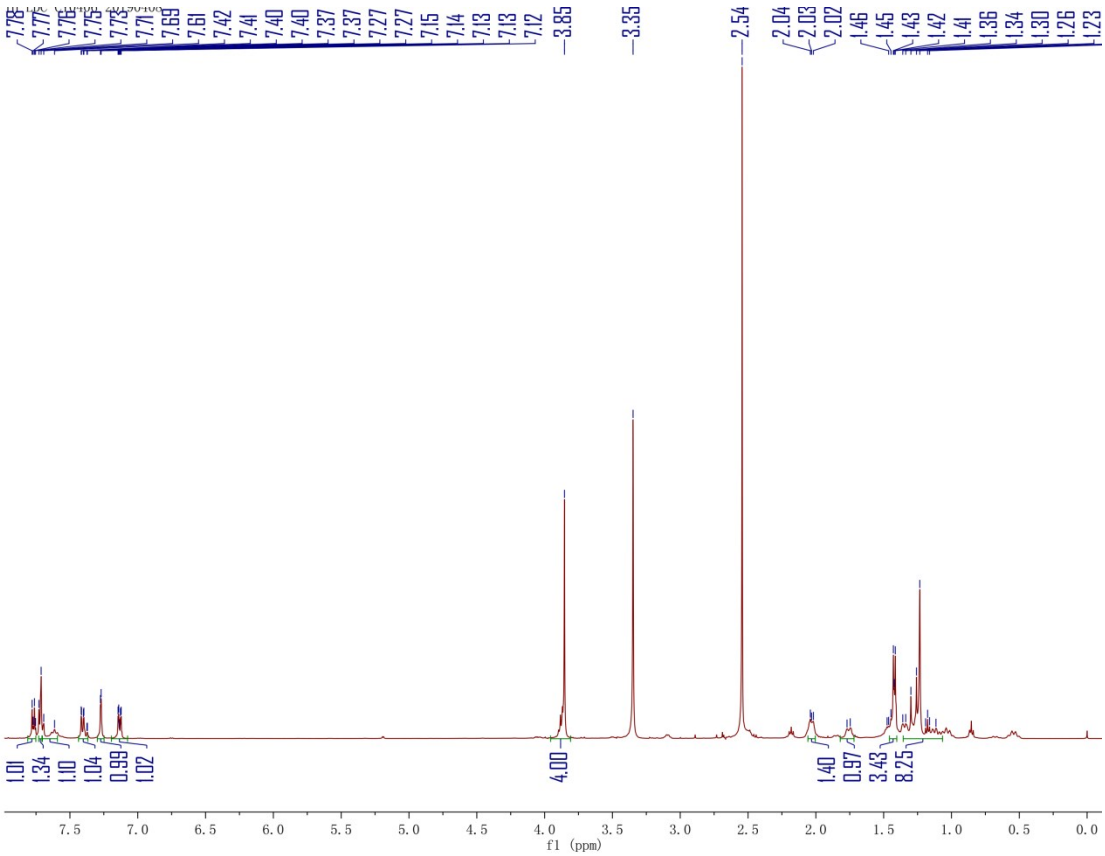
***** Signals at 1.19 (t) and 3.08 ppm (q) were ascribed to the peaks of solvent Et_2O.

^{1}H NMR for Compound **1.**

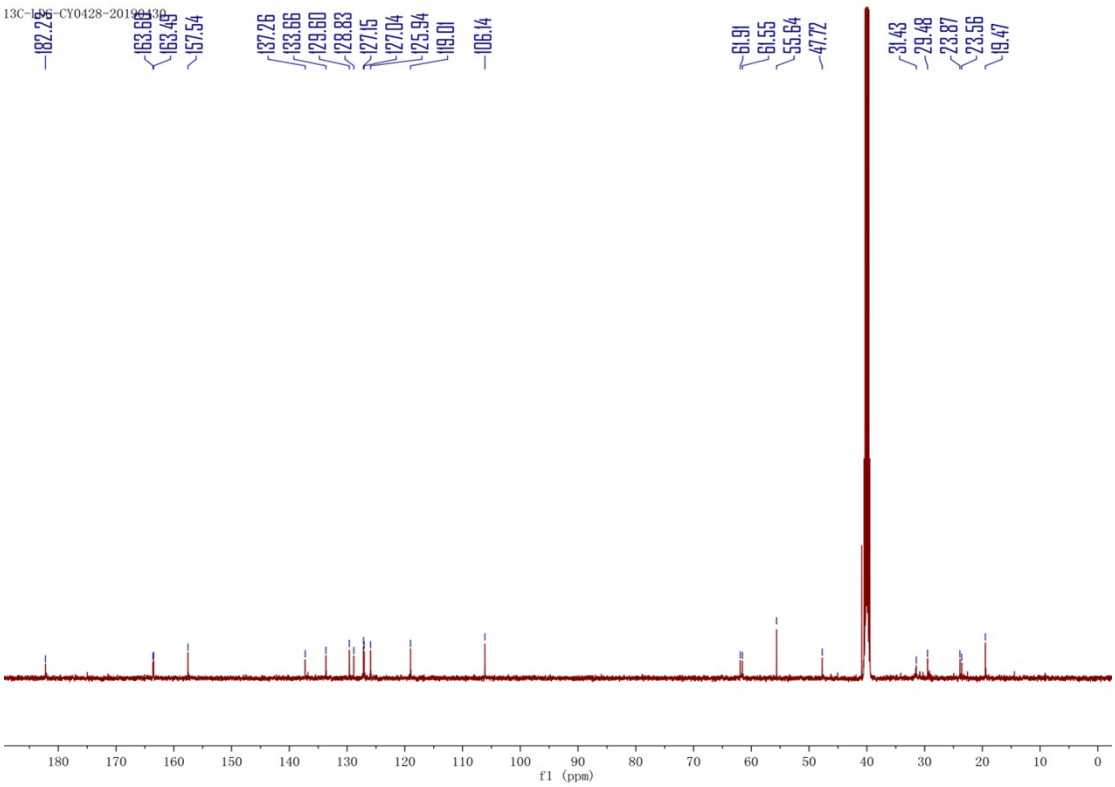


***** Signals at 9 ppm and 46 ppm were ascribed to the peaks of solvent Et_2O.

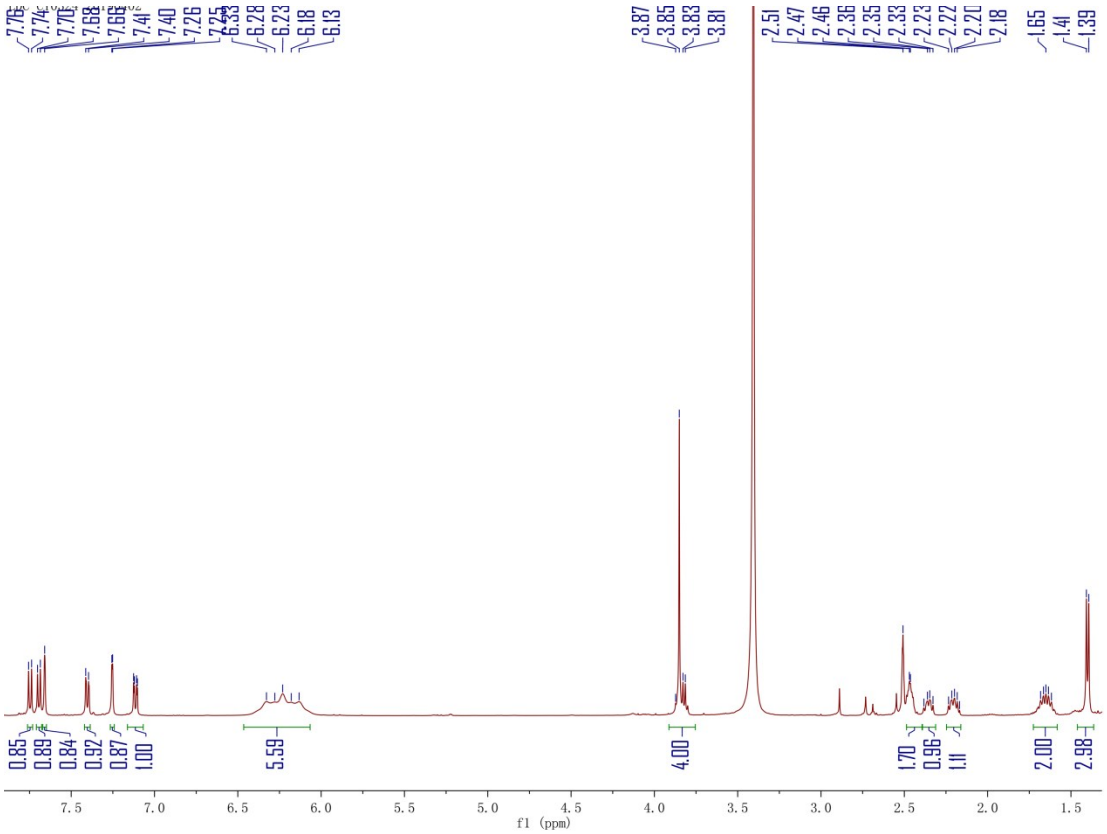
^{13}C NMR for Compound **1.**



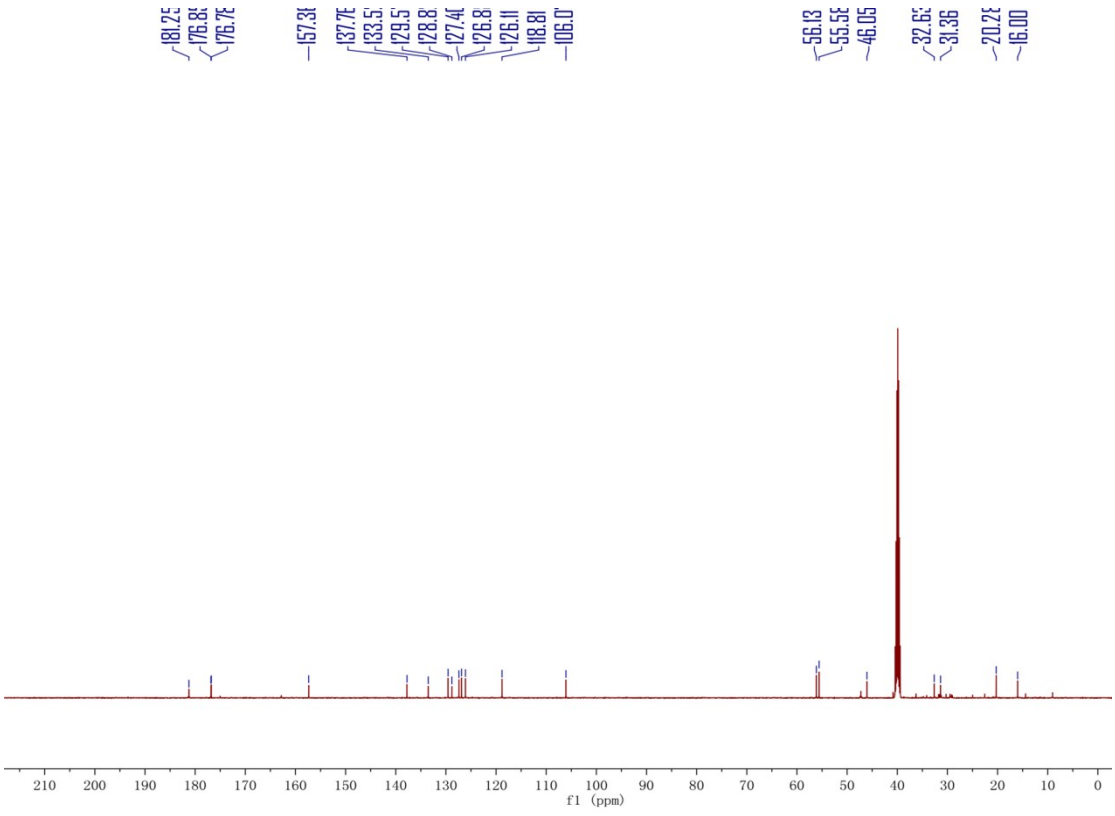
^{1}H NMR for Compound **2.**



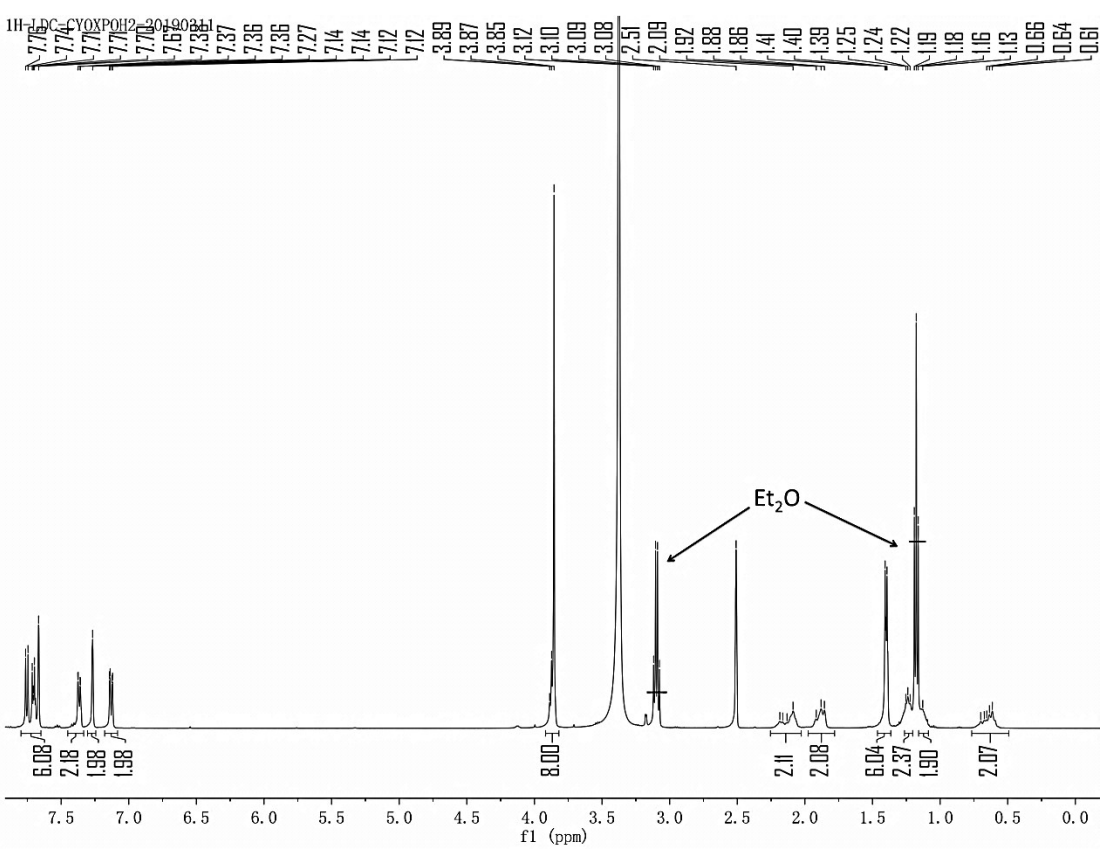
^{13}C NMR for Compound **2.**



^{1}H NMR for Compound **3.**

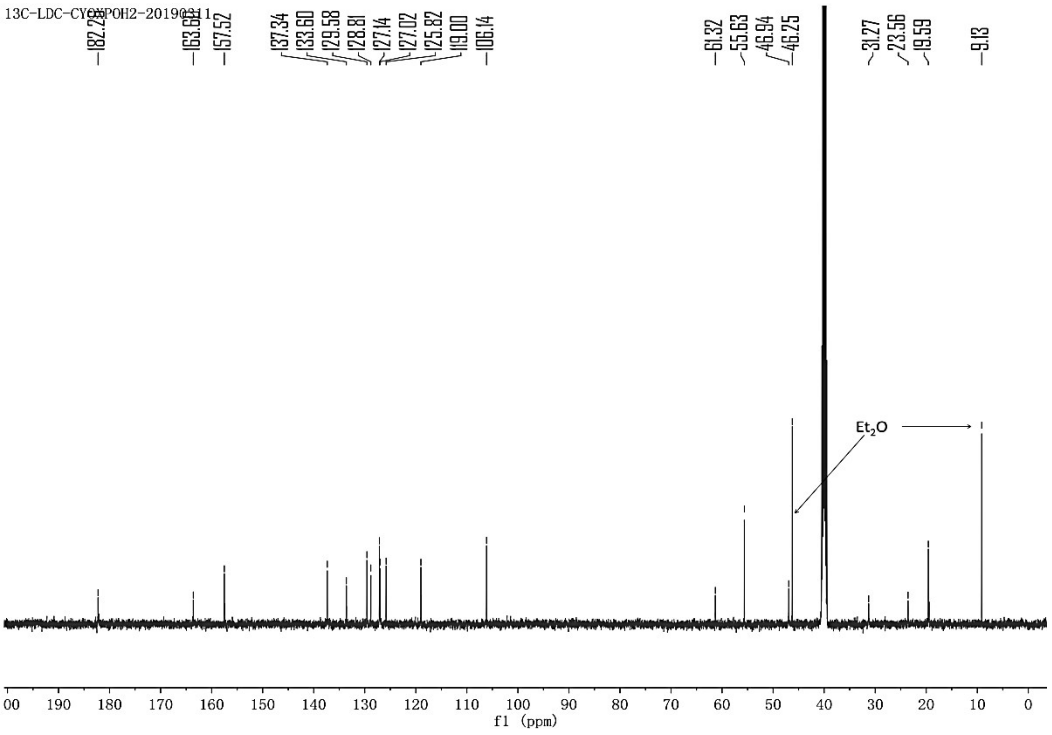


^{13}C NMR for Compound **3.**



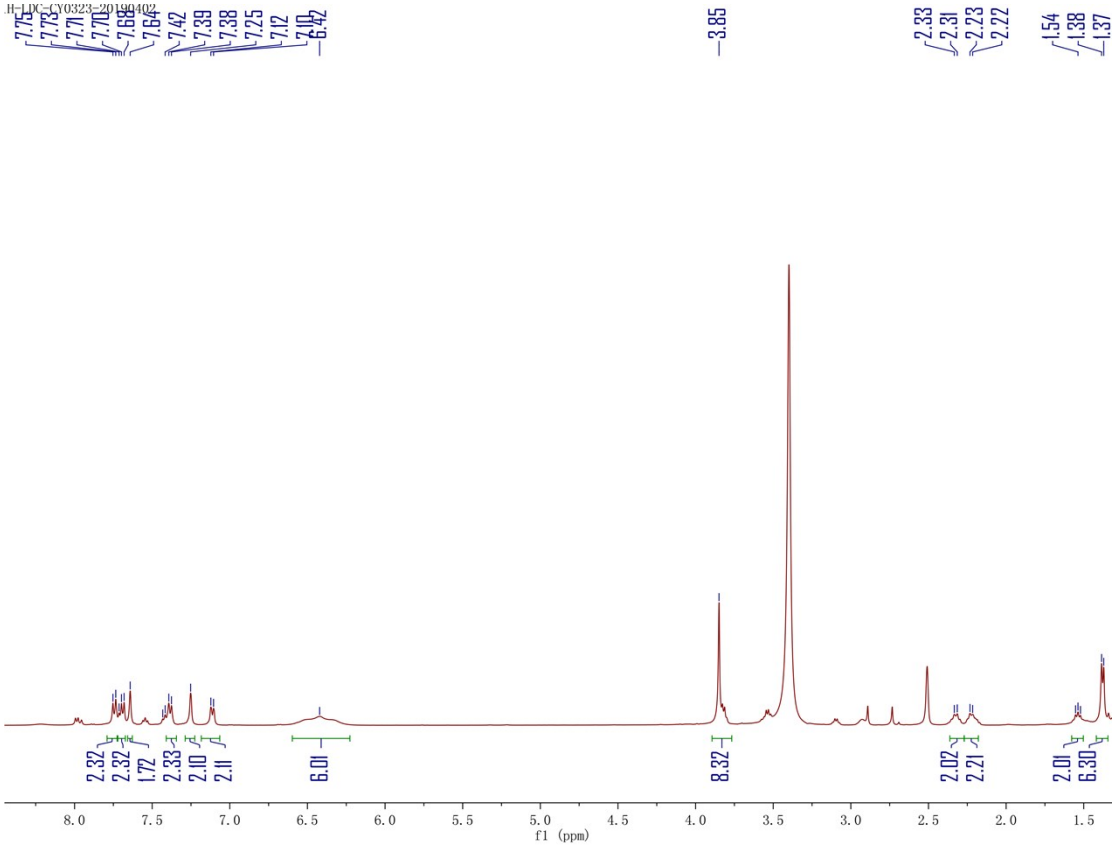
***** Signals at 1.19 (t) and 3.08 ppm (q) were ascribed to the peaks of solvent Et_2O.

^{1}H NMR for Compound **4.**

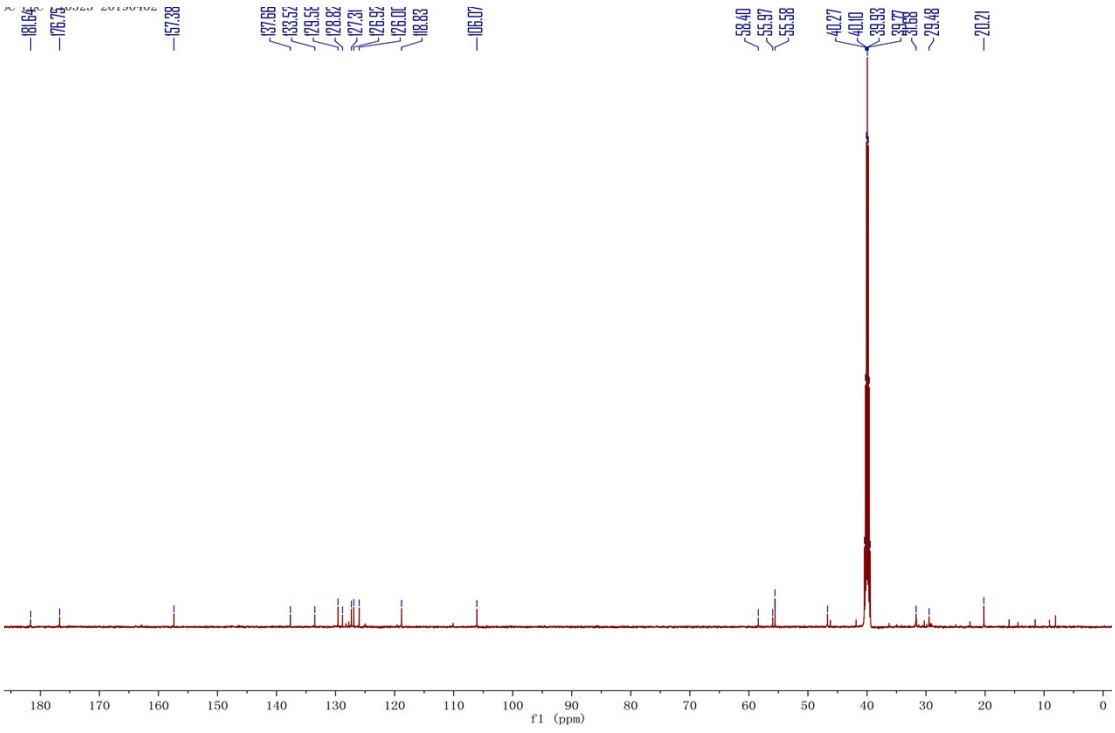


***** Signals at 9 ppm and 46 ppm were ascribed to the peaks of solvent Et_2O.

^{13}C NMR for Compound **4.**



^{1}H NMR for Compound **5.**



^{13}C NMR for Compound **5.**

References

[S1] Q. P. Wang, Z. L. Huang, J. Ma, X. L. Lu, L. Zhang, X. Wang, P. G. Wang, *Dalton Trans*. **2016**, *45*, 10366–10374.