

Supporting information

An efficient condensation of substituted salicylaldehyde and malononitrile catalyzed by lipase under microwave irradiation

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1 Materials

Porcine pancreas lipase (PPL), *Candida antarctica* lipase B (CALB), Bovine serum albumin (BSA) and substituted salicylaldehydes used in this study were purchased from Sigma (Beijing, China). Lipase from *Candida sp.* 99-125 (CSL) was obtained from Beijing CTA New Century Biotechnology Co., Ltd. (Beijing, China). *Bacillus subtilis* lipase (BSL2) was expressed from a homely constructed *Bacillus subtilis* strain [24]. Aeropyrum *pernix* esterase (APE1547) was produced from a hyperthermophilic archaeon strain [25]. These enzymes were used after lyophilization for enzymatic reaction without further purification. Phenylmethanesulfonyl fluoride (PMSF) was purchased from Sigma (Beijing, China). All the other chemical reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Commercially available reagents and solvents were used without further purification. Microwave equipment Reactions were carried out in a commercial multimode microwave reactor (MCR-3, Shanghai Bocai Instrument and Equipment Co. Ltd.,). NMR spectra were recorded on an Inova 500 (500 MHz) spectrometer. High-resolution mass spectra (FAB) were obtained using Jeol JMS700 high-resolution mass spectrometer.

2 The denaturation of lipase treated by serine protease inhibitor (PMSF)

The lyophilized lipase was dissolved in the deionized water (1mg/mL), and the enzyme solution was incubated with the PMSF stock solution (10 mM in ethanol) at room temperature to denaturate the lipase. The unbound PMSF was removed by ultrafiltration and the enzyme solution was lyophilized when the active center of BSL2 had been fully destroyed. Hydrolysis of *p*-nitrophenyl octanoate was used to check that BSL2 was fully inactivated by PMSF.

3 Microwave and conventional heating equipments

Microwave equipment: The microwave reactor consisted of a continuous focused microwave power delivery system with an operator-selectable power output from 0 to 800 W (320 W was used in this work). The temperature of the reaction mixture was monitored and kept constant ($\pm 1^\circ\text{C}$) by using a contact Teflon platinum resistance temperature transducer inserted directly into the reaction mixture. The ramp time was about 1 min to reach the designated temperature. The contents of the round bottom glass flask were stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stirring bar in the round bottom glass flask.

Conventional heating equipment: The reactor (a three-necked, round bottom glass flask) was placed in a thermostat controlled water bath containing magnetic stirrer when the temperature of water bath was increased to the specified temperature. The temperature of the reaction mixture was monitored by a mercury thermometer which was inserted directly in to the reaction mixture. The ramp time of conventional heating was about 1 min to reach the designated temperature.

4 Calculations of apparent kinetic parameters

We designed the experiment to calculate the kinetic parameters according to the references (M. Svedendahl, K. Hult, P. Berglund, J. Am. Chem. Soc., 2005, 127, 17988; A. Radzicka, R. Wolfenden, Science. 267, 90.).

In this study, we kept the concentration of malononitrile (**2**) at a low constant (0.05M), and salicylaldehyde (**1a**) was selected three concentrations (0.1M, 0.5M, 1.0M) to determine the initial rate and $k_{app\ cat}/K_{app\ M}$. The reaction was conducted in the microwave reactor for 30 seconds. The value of $k_{app\ cat}/K_{app\ M}$ was determined by using equation (1): $k_{app\ cat}/K_{app\ M} = V/[E][S_2]$, where the enzyme concentration was 0.066M in this reaction.

Table 1 Data of the calculation of $k_{app\ cat}/K_{app\ M}$ *

Salicylaldehyde(S_{1a})	Conversion of 2	V(M. s-1)	$k_{app\ cat}/K_{app\ M}$
0.1M	88%	1.47×10^{-3}	0.44
0.5M	62%	1.03×10^{-3}	0.30
1.0M	42%	7.00×10^{-4}	0.21

* [E] was 0.066M and $[S_2]$ was 0.05M in this reaction.

The value of k_{non} was calculated by using equation (2): $k_{non} = V/[S_{1a}][S_2]$, where $[S_{1a}]$ and $[S_2]$ are the concentrations of the substrates in the reaction.

Table 2 Data of the calculation of k_{non}

The value of k_{non} under microwave irradiation				
malononitrile(S_2)	Salicylaldehyde (S_{1a})	Time (S)	Conversion	k_{non}
0.05M	0.1M	120	5.6%	4.7×10^{-3}
The value of k_{non} without microwave irradiation				
malononitrile(S_2)	Salicylaldehyde (S_{1a})	Time (h)	Conversion	k_{non}

0.05M	0.1M	)	1	4.2%	1.2*10 ⁻⁴
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Because the value of $k_{app\ cat}/K_{app\ M}$ was obtained when the reaction was preceded under the microwave irradiation, we selected the value of k_{non} ($4.7 \cdot 10^{-3}$) at the same reaction condition to access the catalytic proficiency.

5 Data of NMR

3a: ¹H NMR (500 MHz, DMSO-d₆) 4.60 (d, *J* = 4.0 Hz, 1H, CH), 5.08 (d, *J* = 4.0 Hz, 1H, CN-CH-CN), 7.14 (d, *J* = 7.5 Hz, 1H, ph-H), 7.29 (t, *J* = 7.5 Hz, 1H, ph-H), 7.43 (t, *J* = 7.5 Hz, 1H, ph-H), 7.47 (d, *J* = 7.5 Hz, 1H, ph-H), 7.53 (s, 2H, NH₂); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.54, 38.44, 49.75, 112.71, 112.92, 116.81, 117.96, 119.53, 125.23, 129.09, 130.28, 150.44, 163.86; HRMS (FAB) *m/z*: 237.0 [M+H]⁺.

3b: ¹H NMR (500 MHz, DMSO-d₆) 3.84 (s, 3H, OCH₃), 4.57 (d, *J* = 4.0 Hz, 1H, CH), 5.05 (d, *J* = 4.0 Hz, 1H, CN-CH-CN), 7.00 (d, *J* = 7.5 Hz, 1H, ph-H), 7.11 (d, *J* = 8.0 Hz, 1H, ph-H), 7.19 (t, *J* = 8.0 Hz, 1H, ph-H), 7.53 (s, 2H, NH₂); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.58, 37.43, 48.91, 55.97, 112.87, 113.12, 113.30, 118.95, 119.57, 119.86, 125.11, 139.32, 147.28, 163.65; HRMS (FAB) *m/z*: 267.1 [M+H]⁺.

3c: ¹H NMR (500 MHz, DMSO-d₆) 2.31 (s, 3H, CH₃), 4.54 (d, *J* = 4.0 Hz, 1H, CH), 5.07 (d, *J* = 4.0 Hz, 1H, CN-CH-CN), 7.03 (d, *J* = 8.5 Hz, 1H, ph-H), 7.23 (d, *J* = 8.5 Hz, 1H, ph-H), 7.27 (s, 1H, ph-H), 7.48 (s, 2H, NH₂); ¹³C NMR (500 MHz, DMSO-d₆) δ: 20.81, 32.86, 37.66, 49.35, 113.35, 113.52, 116.58, 118.07, 119.86, 129.33, 131.08, 134.61, 148.20, 164.00; HRMS (FAB) *m/z*: 251.0 [M+H]⁺.

3d: ¹H NMR (500 MHz, DMSO-d₆) 4.50 (d, *J* = 4.0 Hz, 1H, CH), 5.00 (d, *J* = 4.0 Hz, 1H, CN-CH-CN), 6.84 (d, *J* = 7.5 Hz, 1H, ph-H), 6.92 (d, *J* = 7.0 Hz, 1H, ph-H), 7.03 (t, *J* = 6.0 Hz, 1H, ph-H), 7.38 (s, 2H, NH₂), 9.99 (s, 1H, OH); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.39, 37.55, 48.99, 113.12, 113.33, 116.49, 118.24, 119.26, 119.69, 124.92, 138.51, 145.50, 163.56; HRMS (FAB) *m/z*: 253.1 [M+H]⁺.

3e: ¹H NMR (500 MHz, DMSO-d₆) 4.81 (d, *J* = 3.5 Hz, 1H, CH), 5.23 (d, *J* = 3.5 Hz, 1H, CN-CH-CN), 7.41 (d, *J* = 9.0 Hz, 1H, ph-H), 7.81 (s, 2H, NH₂), 8.30 (d, *J* = 8.5 Hz, 1H, ph-H), 8.52 (s, 1H, ph-H); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.89, 37.20, 49.03, 113.07, 113.24, 118.39, 119.22, 119.73, 125.73, 126.33, 144.33, 154.56, 163.19; HRMS (FAB) *m/z*: 282.1 [M+H]⁺.

3f: ¹H NMR (500 MHz, DMSO-d₆) 4.62 (s, 1H, CH), 5.14 (s, 1H, CN-CH-CN), 7.21 (s, 1H, ph-H), 7.30 (s, 1H, ph-H), 7.39 (s, 1H, ph-H), 7.57 (s, 2H, NH₂); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.74, 37.65, 48.66, 113.23, 113.38, 118.69, 118.75, 119.69, 120.08, 146.57, 157.74, 159.66, 163.91; HRMS (FAB) *m/z*: 255.1 [M+H]⁺.

3g: ¹H NMR (500 MHz, DMSO-d₆) 4.62 (d, *J* = 4.0 Hz, 1H, CH), 5.15 (d, *J* = 4.0 Hz, 1H, CN-CH-CN), 7.18-7.20 (d, *J* = 9.0 Hz, 1H, ph-H), 7.49 (dd, *J* = 2.0 Hz, 9.0 Hz, 1H, ph-H), 7.59 (s, 3H, NH₂, ph-H); ¹³C NMR (500 MHz, DMSO-d₆) δ: 32.81, 37.32, 48.91, 113.20, 113.36, 118.80, 119.59, 120.40, 128.97, 128.99, 130.53, 149.06, 163.72; HRMS (FAB) *m/z*: 271.0 [M+H]⁺.

4a: ¹H NMR (500 MHz, DMSO-d₆) 4.86 (d, *J* = 4.0 Hz, 1H, CH), 4.93 (d, *J* = 4.0 Hz, 1H, CH-CN), 6.71 (s, 2H, NH₂), 7.11 (s, 2H, NH₂), 7.22 (d, *J* = 8.5 Hz, 1H, ph-H),

7.27 (t, $J = 7.5$ Hz, 1H, ph-H), 7.45 (m, 2H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 30.90, 35.17, 71.04, 84.28, 113.33, 113.91, 116.73, 117.31, 118.39, 124.59, 129.49, 130.61, 152.07, 157.43, 160.84, 160.91; HRMS (FAB) m/z : 303.0 $[\text{M}+\text{H}]^+$.

4b: ^1H NMR (500 MHz, DMSO- d_6) 3.86 (s, 3H), 4.85 (d, $J = 4.0$ Hz, 1H, CH), 4.93 (d, $J = 4.0$ Hz, 1H, CH-CN), 6.69 (s, 2H, NH_2), 6.97 (d, $J = 7.5$ Hz, 1H, ph-H), 7.08 (s, 2H, NH_2), 7.14 (d, $J = 8.5$ Hz, 1H, ph-H), 7.20 (t, $J = 8.0$ Hz, 1H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 30.46, 34.91, 55.93, 70.63, 83.93, 112.89, 112.97, 113.50, 116.41, 118.74, 120.11, 124.11, 141.14, 147.81, 157.12, 160.49, 160.56; HRMS (FAB) m/z : 333.1 $[\text{M}+\text{H}]^+$.

4c: ^1H NMR (500 MHz, DMSO- d_6) 2.33 (s, 3H), 4.84 (d, $J = 4.0$ Hz, 1H, CH), 4.86 (d, $J = 4.0$ Hz, 1H, CH-CN), 6.69 (s, 2H, NH_2), 7.04-7.15 (m, 3H, ph-H, NH_2), 7.20 (s, 1H, ph-H), 7.26 (d, $J = 8.0$ Hz, 1H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 20.83, 30.92, 35.14, 70.94, 84.26, 113.34, 113.91, 116.78, 117.07, 118.04, 129.50, 131.12, 133.58, 149.96, 157.42, 160.87, 160.97; HRMS (FAB) m/z : 317.0 $[\text{M}+\text{H}]^+$.

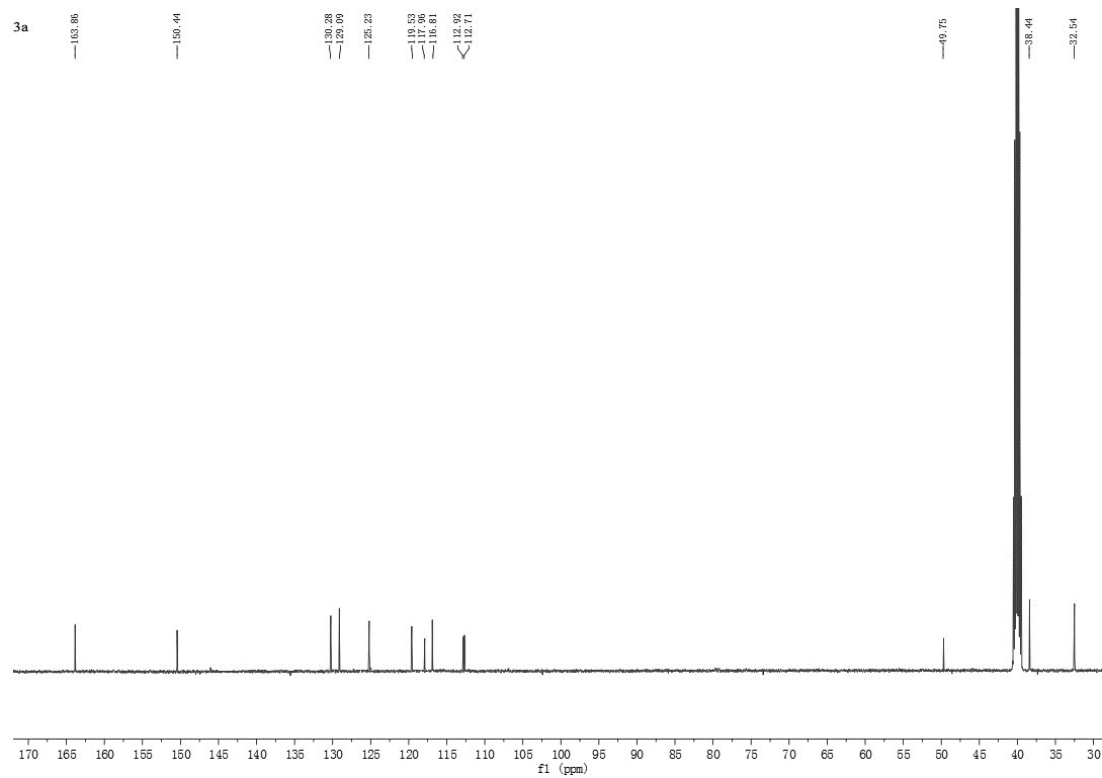
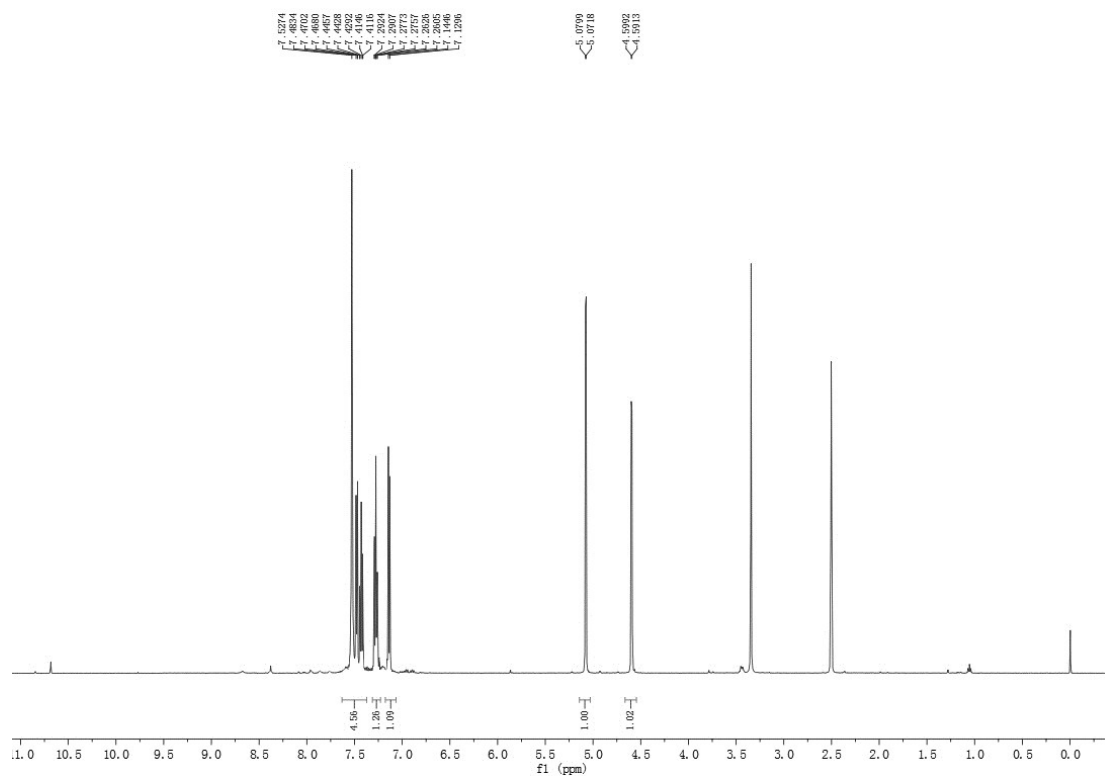
4d: ^1H NMR (500 MHz, DMSO- d_6) 4.83 (d, $J = 4.0$ Hz, 1H, CH), 4.89 (d, $J = 4.0$ Hz, 1H, CH-CN), 6.66 (s, 2H, NH_2), 6.83 (d, $J = 8.0$ Hz, 1H, ph-H), 6.94 (d, $J = 8.0$ Hz, 1H, ph-H), 7.01-7.10 (m, 3H, ph-H, NH_2), 9.88 (s, 1H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 30.81, 35.30, 70.97, 84.41, 113.43, 113.96, 116.83, 117.15, 119.04, 119.44, 124.35, 140.70, 146.13, 157.40, 160.79, 161.01; HRMS (FAB) m/z : 319.1 $[\text{M}+\text{H}]^+$.

4f: ^1H NMR (500 MHz, DMSO- d_6) 4.91 (d, $J = 4.0$ Hz, 1H, CH), 4.94 (d, $J = 4.0$ Hz, 1H, CH), 6.76 (s, 2H, NH_2), 7.13 (s, 2H, NH_2), 7.18 (dd, $J = 2.5$ Hz, 8.5 Hz, 1H, ph-H), 7.28-7.36 (m, 2H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 30.80, 35.32, 71.11, 83.46, 113.17, 113.83, 115.38, 115.57, 116.66, 117.54, 119.08, 119.15, 148.48, 157.46, 160.75, 160.95; HRMS (FAB) m/z : 321.1 $[\text{M}+\text{H}]^+$.

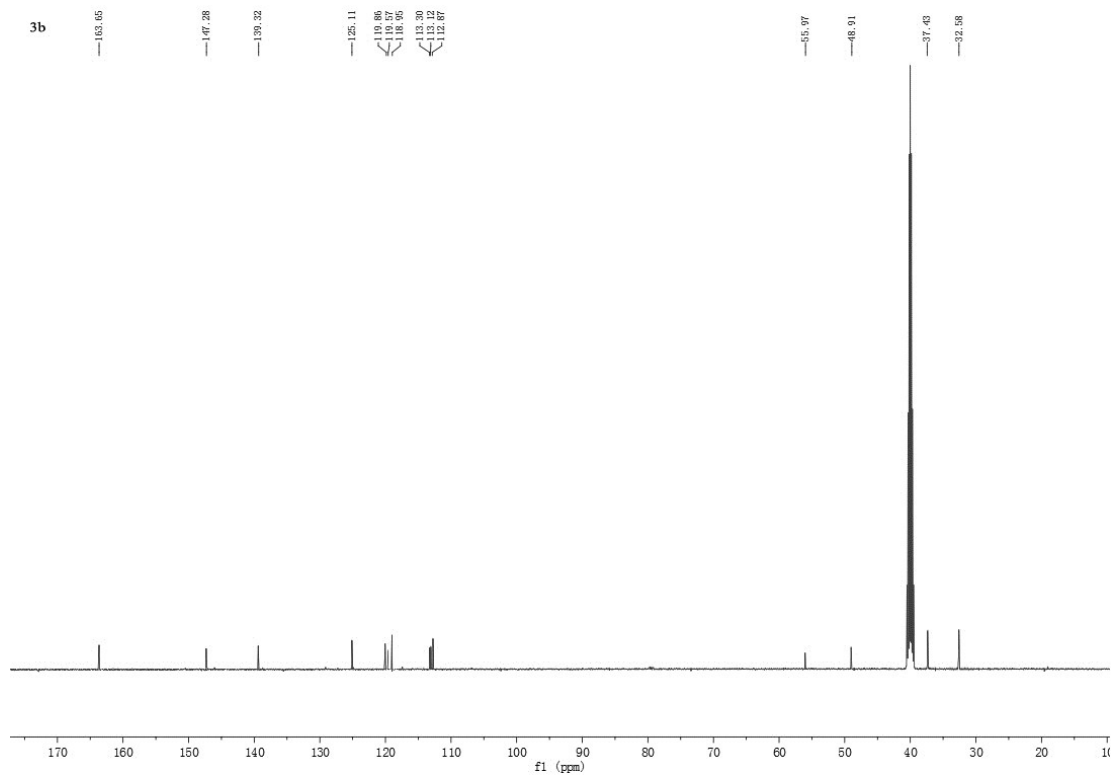
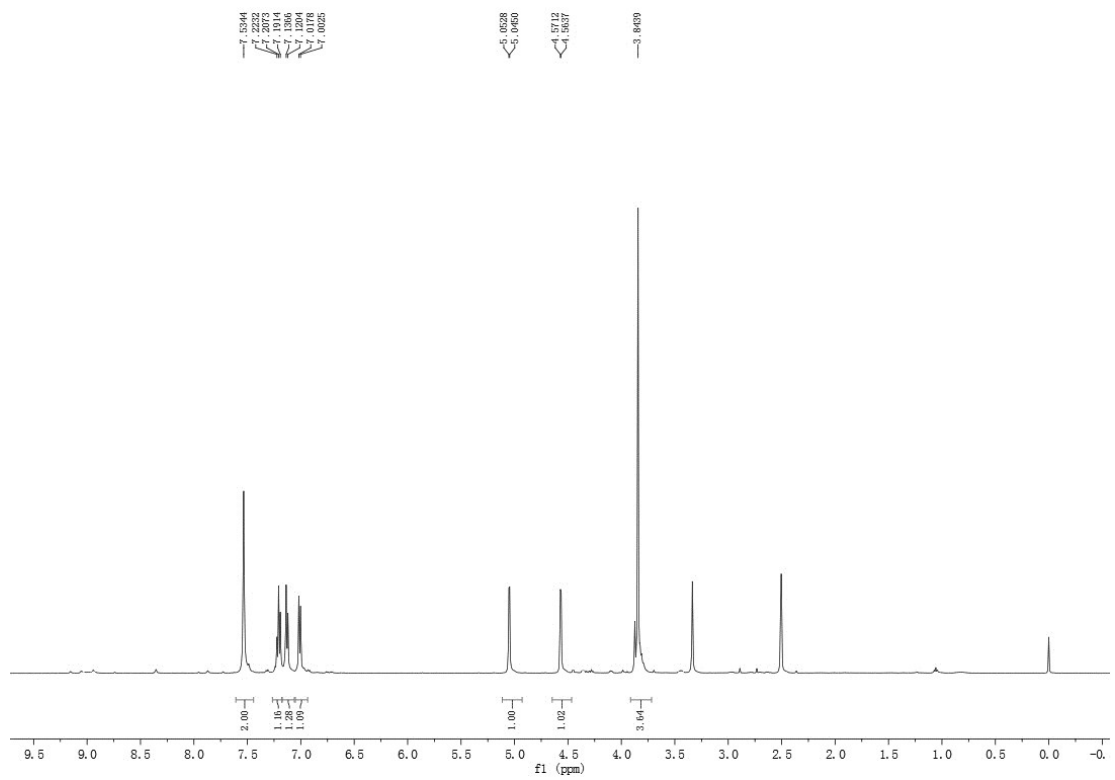
4g: ^1H NMR (500 MHz, DMSO- d_6) 4.92 (d, $J = 3.5$ Hz, 2H, CH), 6.77 (s, 2H, NH_2), 7.14 (s, 2H, NH_2), 7.28 (d, $J = 9.0$ Hz, 1H, ph-H), 7.40 (d, $J = 2.0$ Hz, 1H, ph-H), 7.53 (d, $J = 9.0$ Hz, 1H, ph-H); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 30.89, 35.06, 71.18, 83.62, 113.15, 113.74, 116.61, 119.28, 127.95, 128.86, 128.95, 130.57, 150.96, 157.46, 160.49, 160.93; HRMS (FAB) m/z : 337.1 $[\text{M}+\text{H}]^+$.

2 ¹H-NMR Spectra

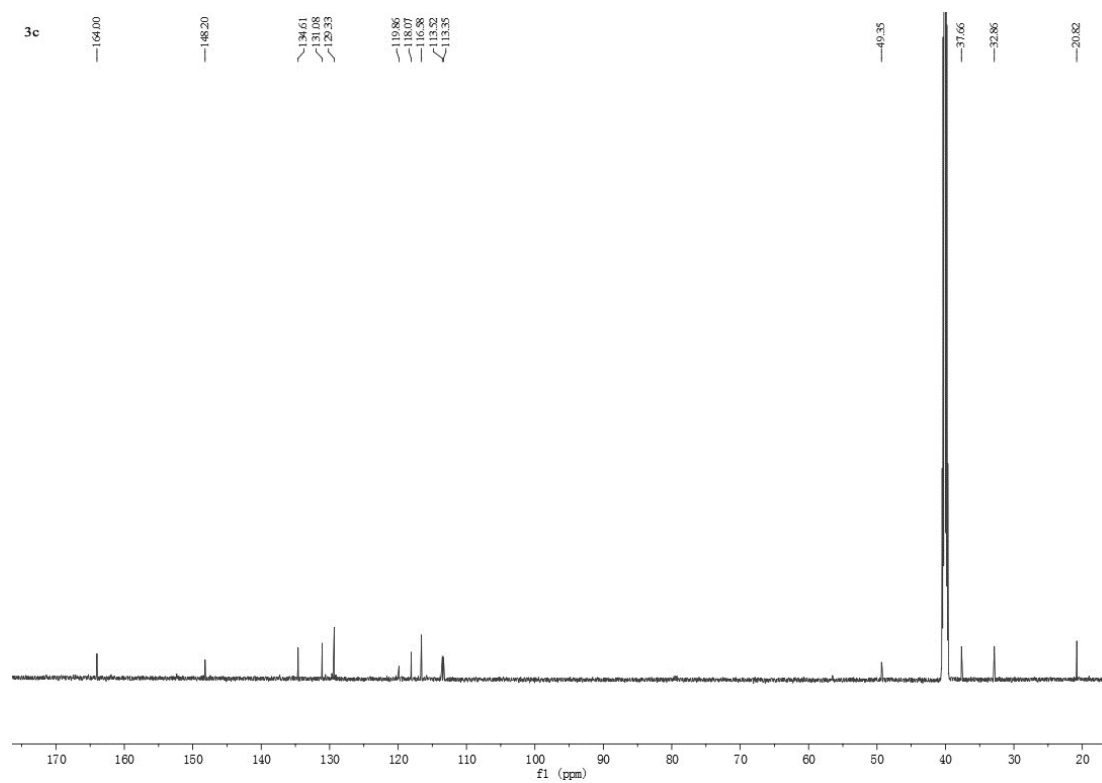
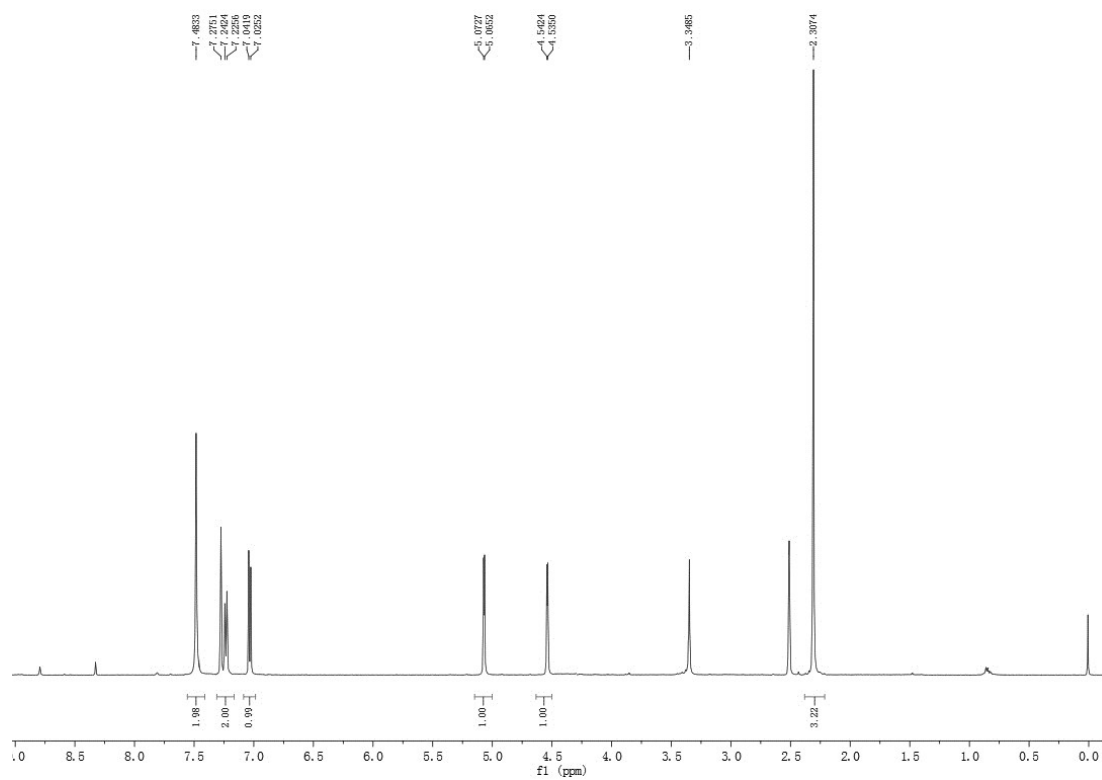
3a



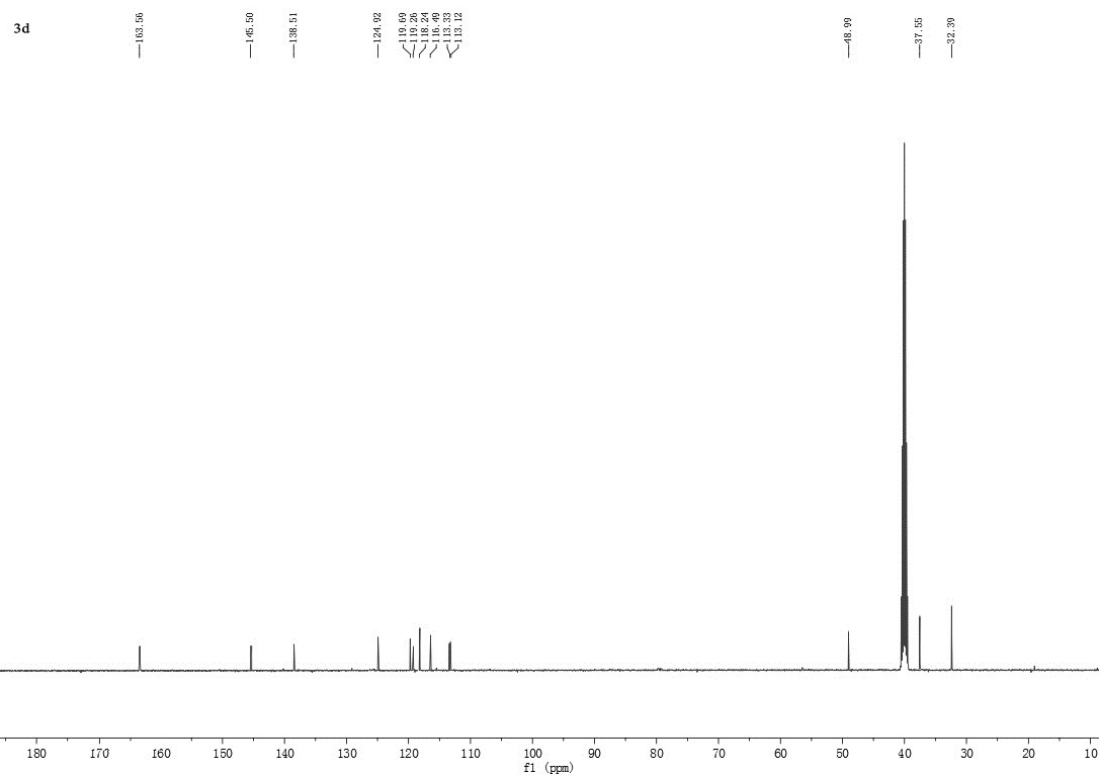
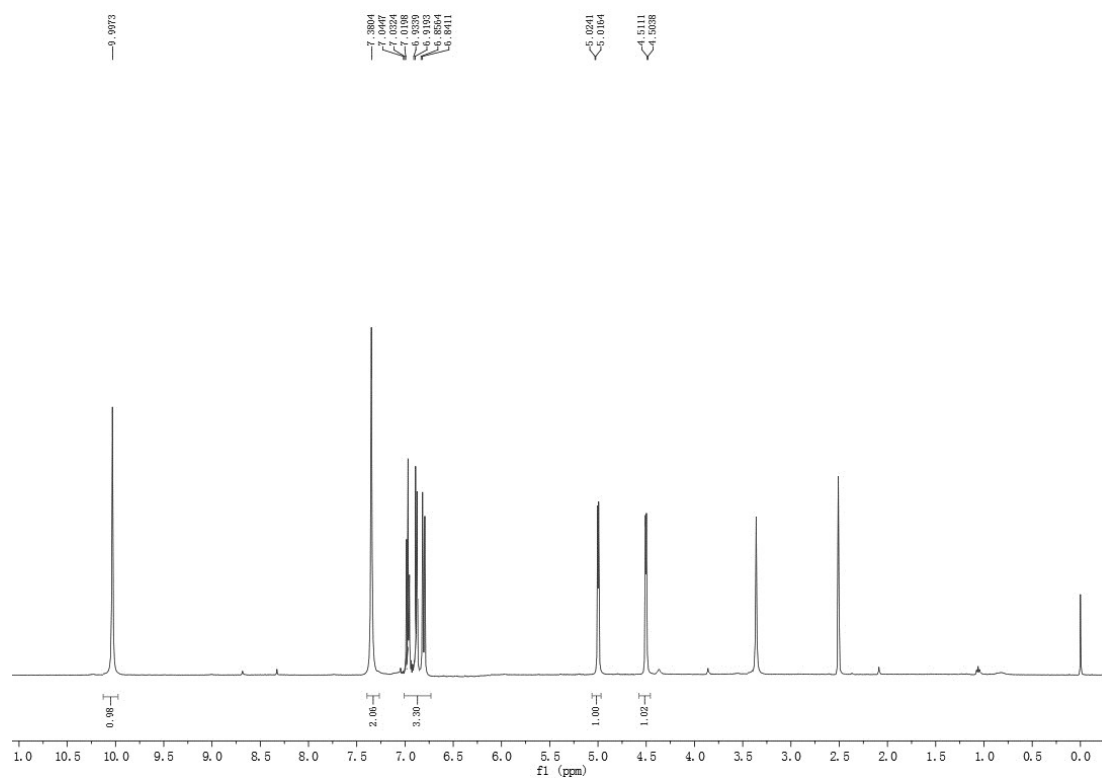
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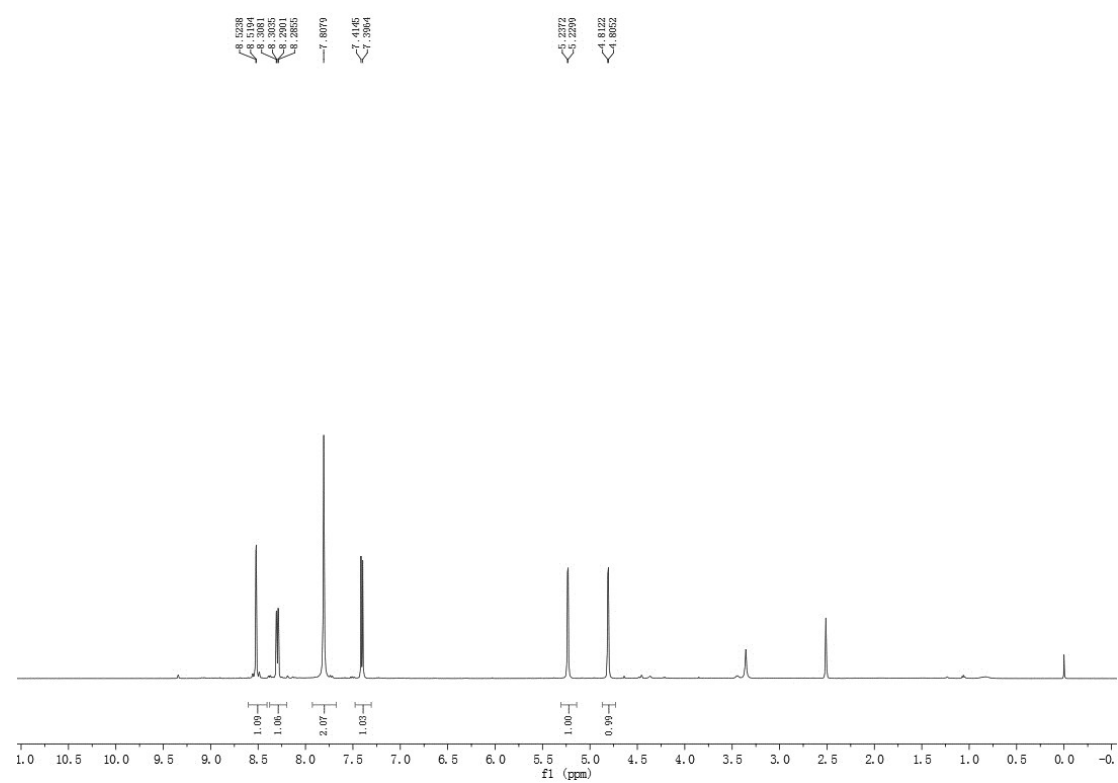
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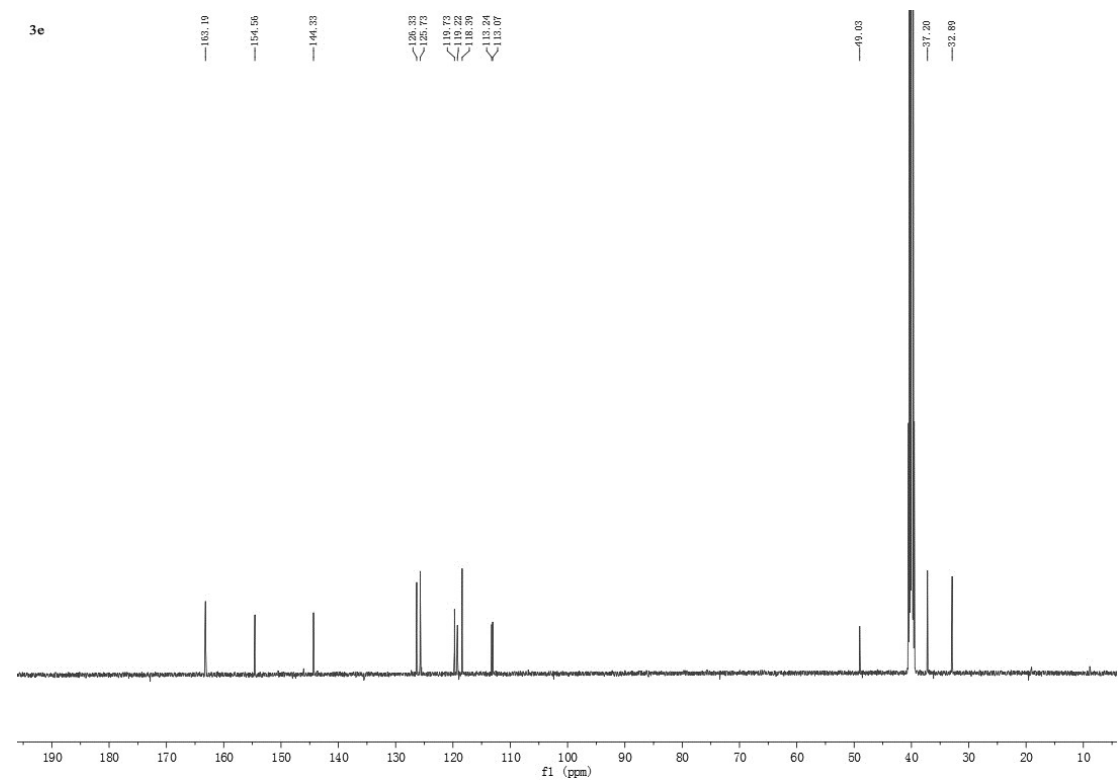
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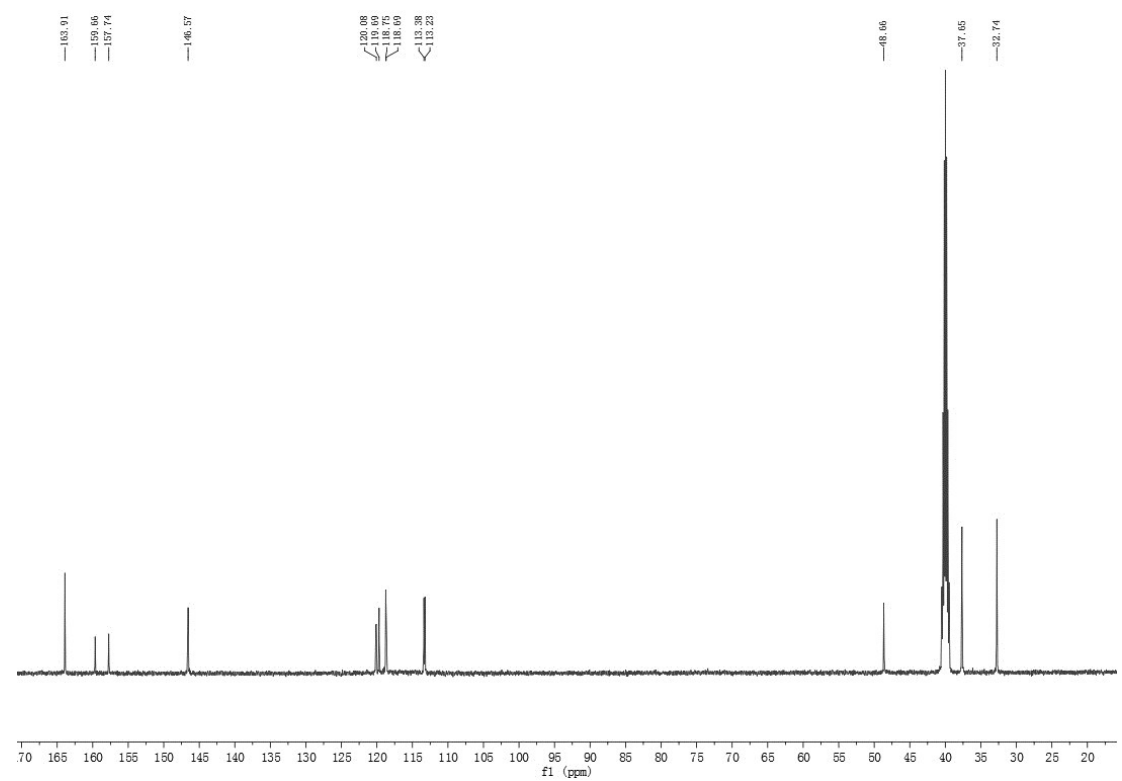
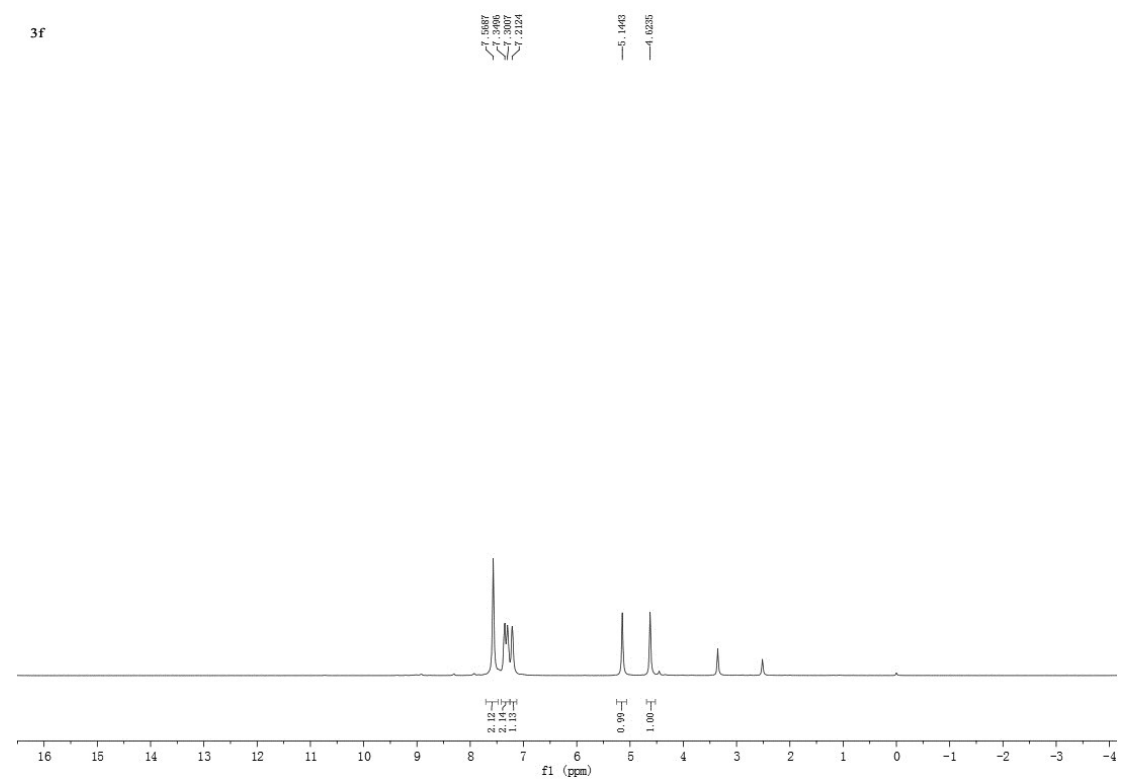
3e



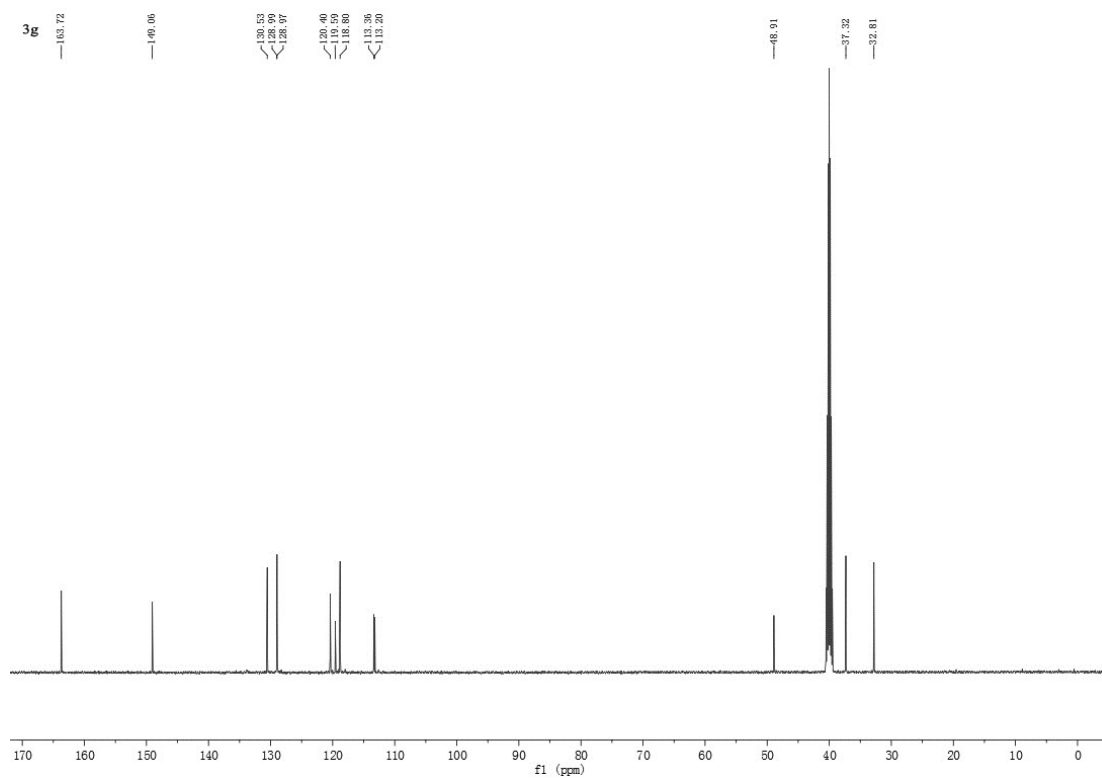
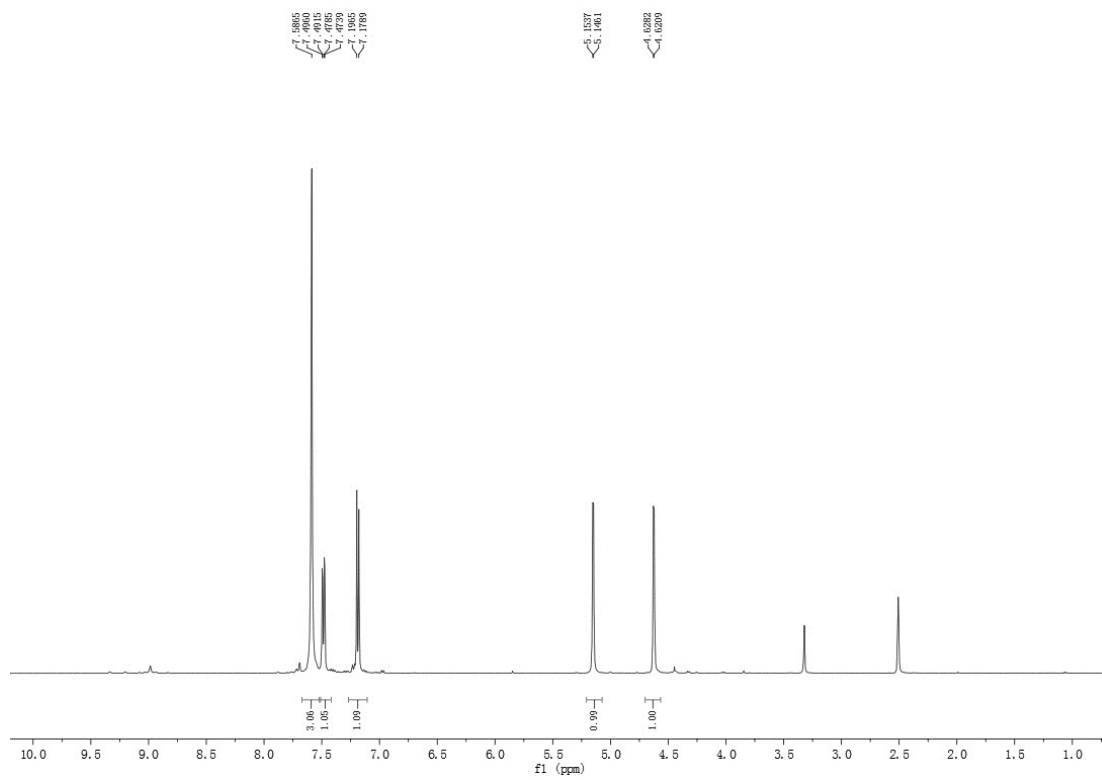
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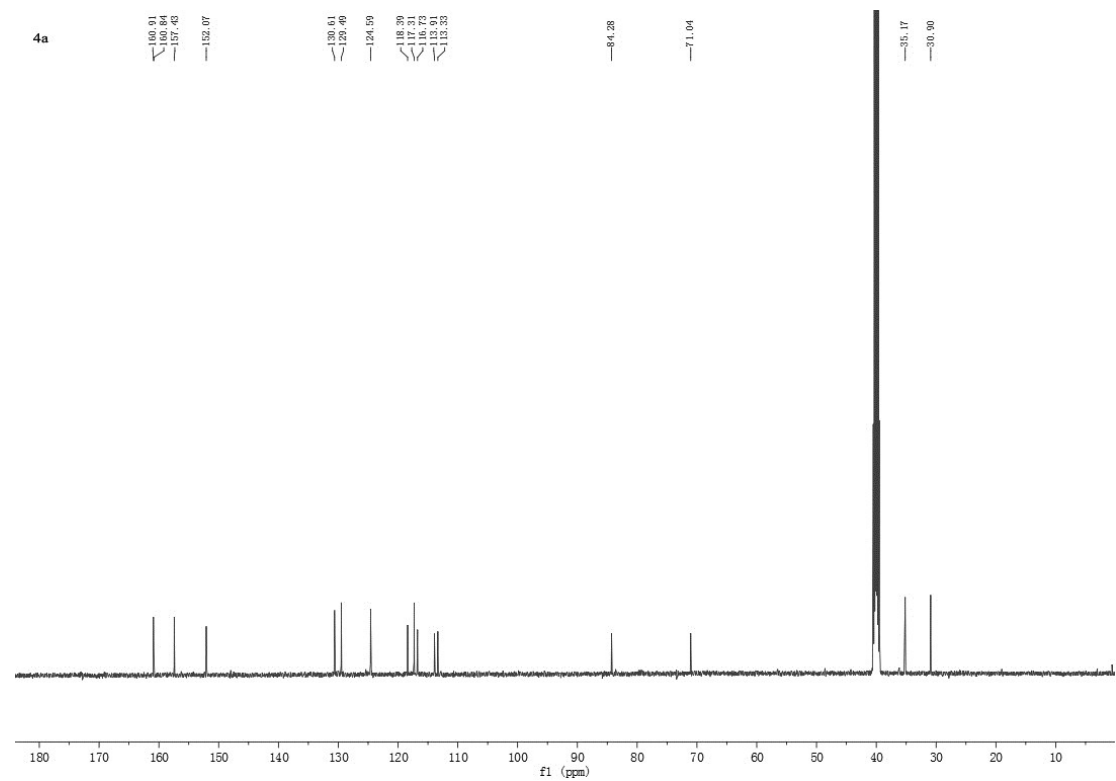
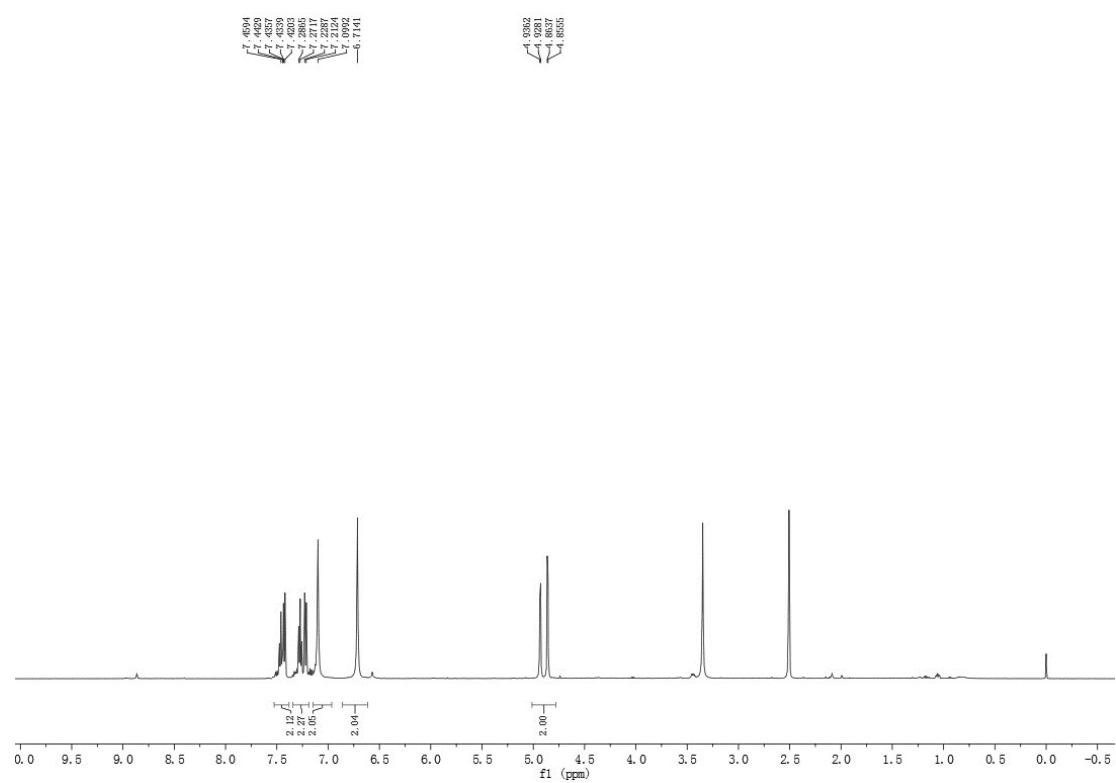
3f



3g



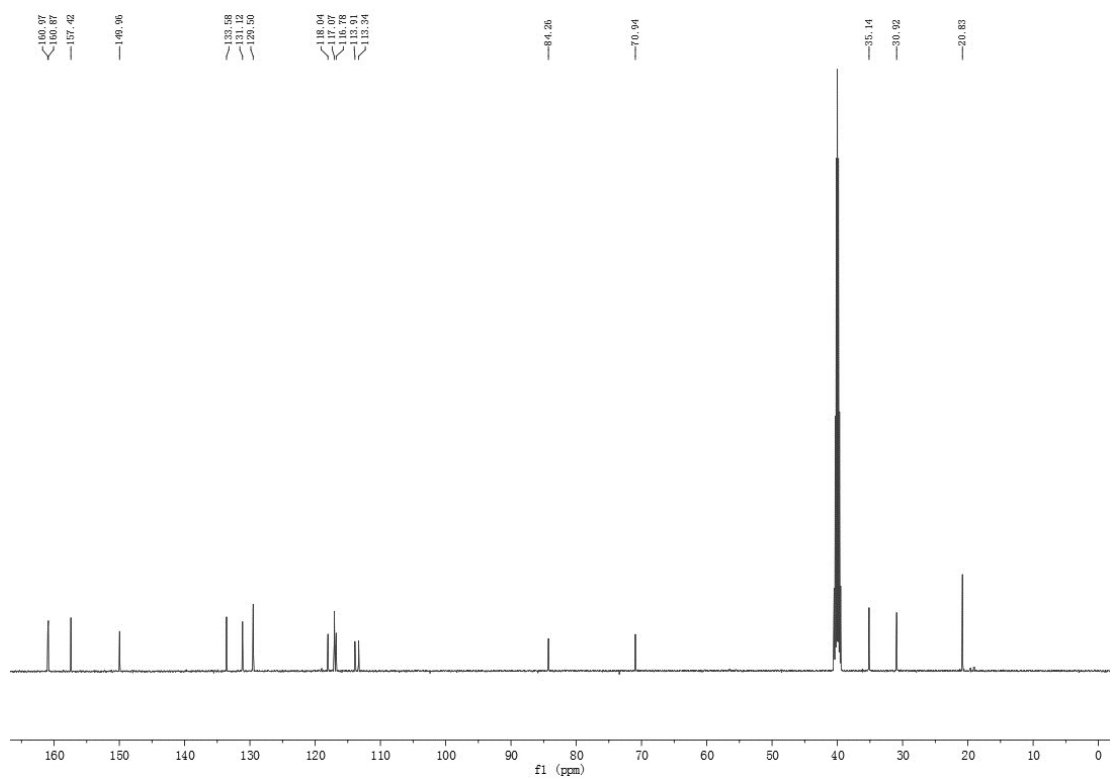
4a



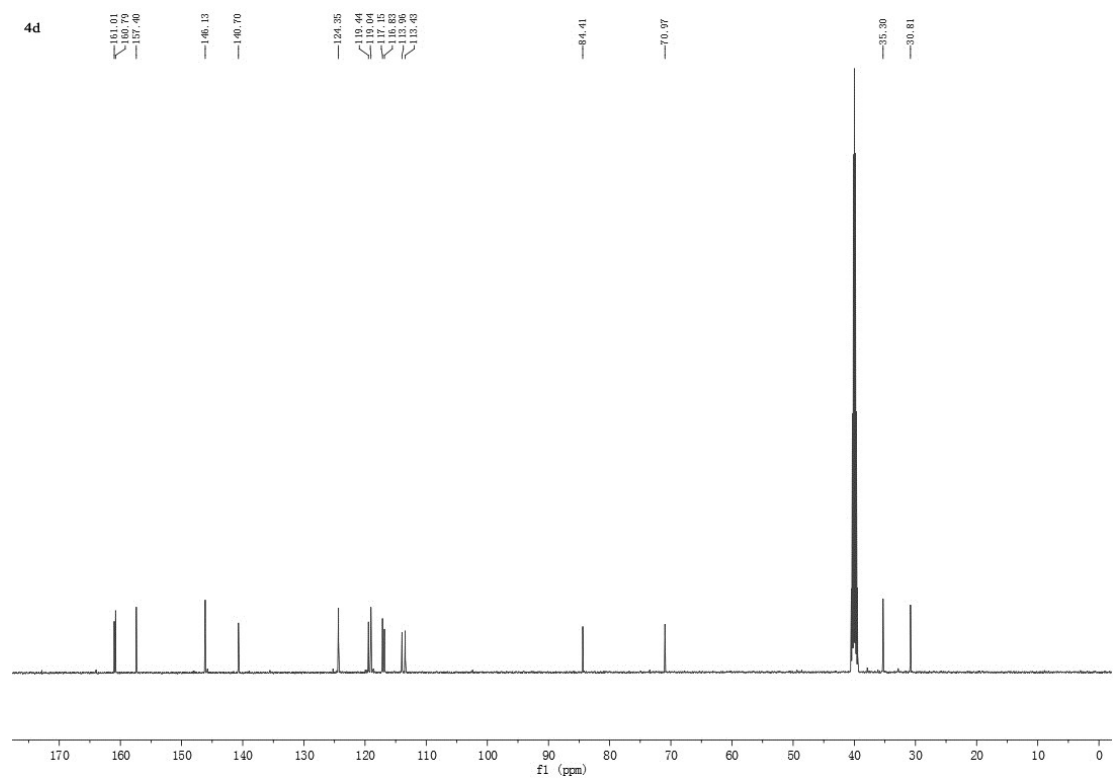
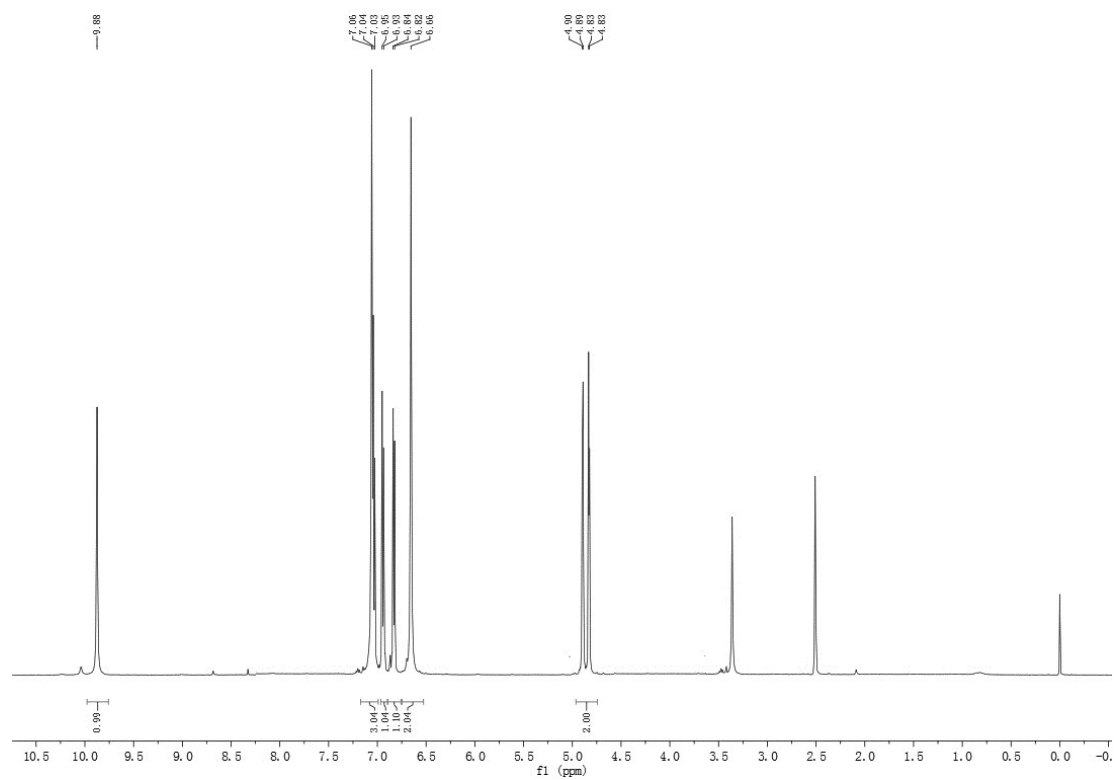
¹H NMR spectrum of compound 10 in CDCl₃. The spectrum shows peaks at 7.21, 7.14, 7.08, 7.06, 7.03, 7.02, 6.99, 6.93, 6.69 ppm (aromatic region), 4.93, 4.92, 4.85, 4.84 ppm (multiplet), 3.85 ppm (singlet), 3.58 ppm (triplet), 2.58 ppm (singlet), and 0.00 ppm (TMS). Integration values are shown below the baseline: 3.91, 1.02, 1.96, 1.90, 3.00.

4b

Chemical shifts (ppm): 160.56, 159.59, 157.12, 147.81, 141.14, 124.11, 120.11, 118.74, 118.51, 117.81, 115.99, 112.97, 112.89, 83.93, 77.03, 55.93, 34.91, 30.46.



4d



4f

