Supporting Information

A new label-free continuous fluorometric assay for trypsin and inhibitor screening with tetraphenylethene compounds

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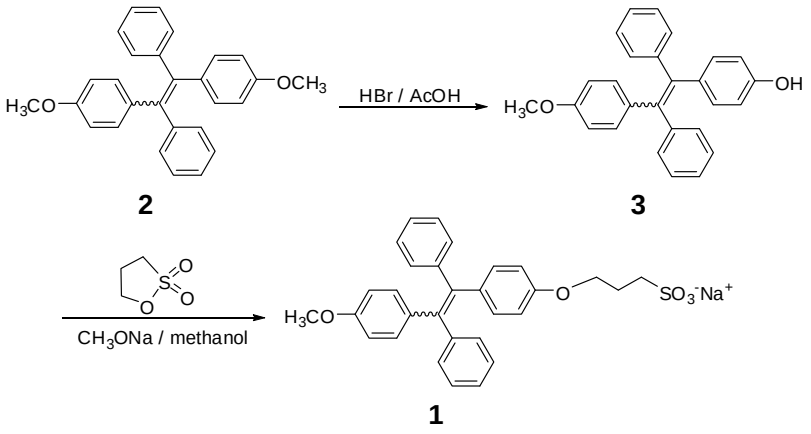
Contents

1. Synthesis and characterization

Materials: The Arg_6 peptide was purchased from GL Biochem. Ltd. (Shanghai) and purified with HPLC. The trypsin (1:250) from porcine pancreas was purchased from Biodee. Biochem. Ltd. (Beijing) and the trypsin-chymotrypsin inhibitor from *Glycine max* (soybean, Bowman-Birk Type) were purchased from Sigma-Aldrich. All the chemicals obtained from commercial sources were analytical pure and used without further purification. Stock solutions of compound **1**, trypsin and BBI were prepared with pure water. The pure water used was purified by a Millipore filtration system. The sample solutions containing PBS (phosphate buffer solution, 2.0 mM, pH = 8.5) buffer for spectral studies were further prepared.

Characterization techniques: ^{1}H-NMR and ^{13}C-NMR were collected on Bruker Avance 400-MHz spectrometer. Mass spectra were determined with Bruker Apex IV FTMS. Fluorescence spectra were recorded on a Hitachi F-4500 spectrometer. Fluorescence confocal laser scanning images were recorded with an Olympus FV1000-IX81. Dynamic light scatting (DLS) experiments were carried out with ALV5000 Laser Light Scattering Instrument.



Scheme S1. The synthetic route for compound **1**

Synthesis of compound 3. Into a 100 mL flask were added 2.80 g (7.13 mmol) of **2**[s1] and 20 mL of acetic acid (AcOH). 0.4 mL (21.40 mmol) of hydrobromic acid

(33% in AcOH w/w) was added carefully to the mixture under stirring. The resultant suspension was refluxed for 3.0 hr. After cooling to room temperature, the reaction mixture was poured into 400 mL of dichloromethane (DCM). The organic layer was separated and the aqueous portion was extracted twice with 100 mL of DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), brine (1 × 50 mL), and dried over aqueous Na_2SO_4. The organic phase was concentrated by a rotary evaporator. The crude product was purified by column chromatography on silica (petroleum ether /ethyl acetate = 6:1, v/v) to give compound **3** as a pale yellow solid powder (0.9 g, 33% yield): ^{1}H-NMR (400MHz, $CDCl_3$), δ (TMS, ppm): 7.11-7.02 (m, 10H), 6.92-6.83 (m, 4H), 6.63-6.59 (m, 2H), 6.54-6.51 (m, 2H), 4.71-4.67 (d, 1H), 3.76-3.74 (d, 3H); ^{13}C-NMR (100MHz, $CDCl_3$) δ(TMS, ppm): 158.25, 154.02, 144.40, 144.28, 139.7, 138.69, 136.77, 136.49, 136.49, 132.89, 132.67, 131.54, 127.83, 127.73, 126.37, 114.81, 114.71, 113.26, 113.18, 55.25; EI-MS: *m/e* = 378 (M^+); Anal. calcd. for $C_{27}H_{22}O_2$: C, 85.69; H, 5.86; Found: C, 85.70; H, 6.10. Since two isomers exist for compound **2**,[s1] there are also two isomers for compound **3**, which is in agreement with the ^{1}H-NMR data. Separation of these two isomers with column chromatography was not successful.

Synthesis of compound 1. Into a 100 mL round-bottom flask were added 0.5 g (1.32 mmol) of **3** and 20 mL of anhydrous methanol under nitrogen. NaOMe (0.10 g, 1.85 mmol) in 10 mL of methanol was added dropwise and stirred for 1.0 hr. To the above solution was added 0.15 g of 1, 3-propanesultone (1.27 mmol) in 5 mL of methanol. The mixture was vigorously stirred for 12.0 hr, during which time a white product was precipitated out from the solution. The product was collected by filtration and washed with ethanol and acetone twice to give a white solid. Further crystallization from methanol / *n*-hexane led to compound **1** as a white powder (0.3 g, 43% yield). ^{1}H NMR(d_6-DMSO, 400 MHz), δ(TMS, ppm): 7.11-7.06 (m, 6H), 7.04-6.94 (m, 4H), 6.92-6.81 (m, 4H), 6.79-6.60 (m, 4H), 3.95-3.90 (m, 2H), 3.64-3.62 (d, 3H), 2.54-2.50 (m, 2H), 1.97-1.90 (m, 2H). ^{13}C NMR (d_6-DMSO, 100 MHz), δ(TMS, ppm):, 157.57, 157.18, 143.78, 139.33, 139.20, 135.76, 135.54, 135.48, 131.91, 131.89, 130.75, 127.80, 127.69, 126.31, 126.24, 113.75, 113.64,

113.32, 113.18, 66.43, 54.91, 47.79, 25.23. MS (ESI) *m/e*: 499.2 ($M\text{-}Na^+$); Anal. calcd. for $C_{30}H_{27}NaO_5S \cdot 0.3C_6H_{12}$: C, 68.69; H, 5.82; Found: C, 69.00; H, 6.21. Similarly, two isomers exist for compound **1**, but it will not affect the fluorescent spectral studies.

S1. Tong, H.; Hong, Y. N.; Dong, Y. Q.; Haussler, M.; Li, Z.; Lam, J. W. Y.; Dong, Y. P.; Sung, H. H. Y.; Williams, I. D.; Tang, B. Z. *J. Phys. Chem. B* **2007**, *111*, 11817.

2. Fluorescence confocal laser scanning images of the ensemble of 1 and Arg_6 peptide in the absence and presence of trypsin

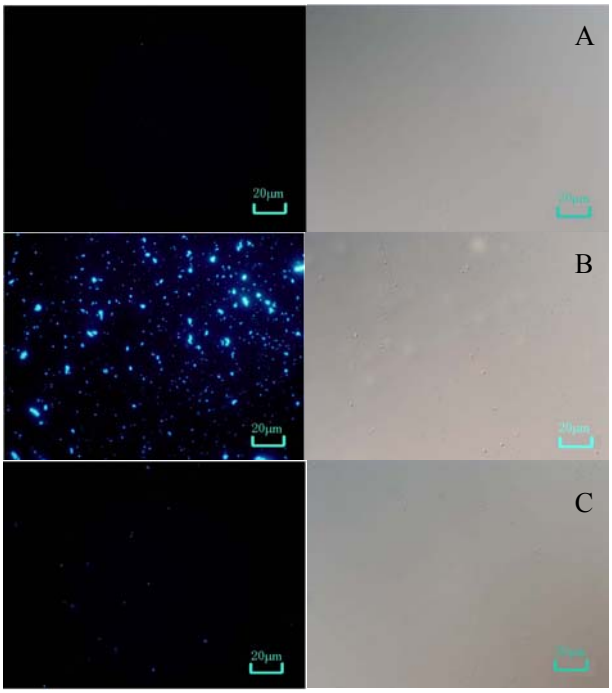


Figure S1. Confocal Laser Scanning images of A) **1** (0.1 mM) in PBS buffer (2.0 mM PBS，pH = 8.5); B) **1** (0.1 mM) in PBS buffer (2.0 mM PBS，pH = 8.5) and Arg_6 peptide (20.0 μM); C) **1** (0.1 mM) in PBS buffer (2.0 mM PBS，pH = 8.5) containing $CaCl_2$ (10.0 μM)), Arg_6 peptide (20.0 μM) and trypsin (8.0 μg/mL) after incubation for 30 min.

3. Dynamic light scattering results for the ensemble of 1 and Arg_6 peptide in the presence and absence of trypsin

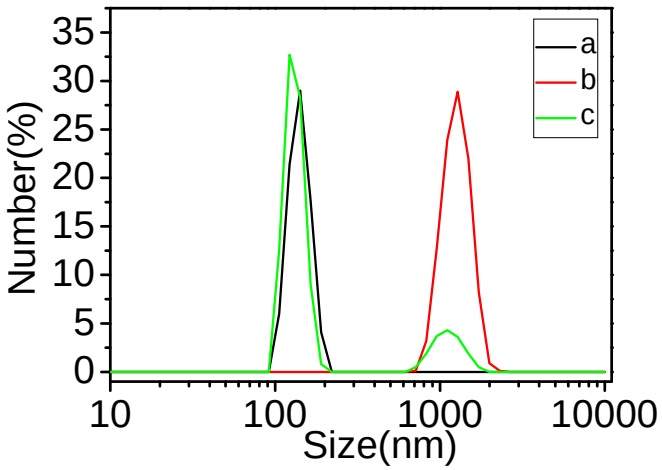


Figure S2. Dynamic light scattering results for the solution of **1** and Arg_6 peptide in the presence and absence of trypsin: (a) **1** (60.0 μM) in PBS buffer (2.0 mM, pH = 8.5) solution; (b) **1** (60.0 μM) in PBS buffer solution (2.0 mM, pH = 8.5) and Arg_6 peptide (10.0 μM); (c) **1** (60.0 μM) in PBS buffer solution (2.0 mM, pH = 8.5) containing $CaCl_2$ (10.0 μM) and Arg_6 peptide (10.0 μM) in the presence of trypsin (8.0 μg/mL) after incubation for 30 min.

3. The Lineweaver-Burk plot for the hydrolysis of Arg_6 peptide catalyzed by trypsin

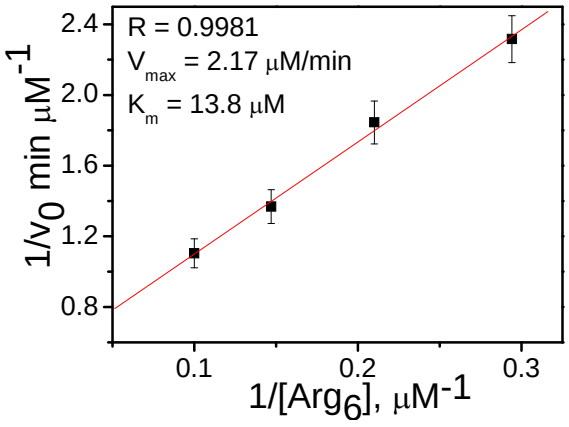


Figure S3. The Lineweaver-Burk plot for the hydrolysis of Arg_6 peptide catalyzed by trypsin (3.2 μg/mL) in the presence of **1** (60.0 μM) in PBS buffer (2.0 mM, pH = 8.5, containing $CaCl_2$ (10.0 μM)) solution; the data were obtained with different concentrations of Arg_6 peptide (3.4, 4.7, 6.8, 10.8 μM); the fluorescence intensity was monitored at 475 nm, and the excitation wavelength was 340 nm.

4. The plot of the inhibition efficiency of BBI toward trypsin

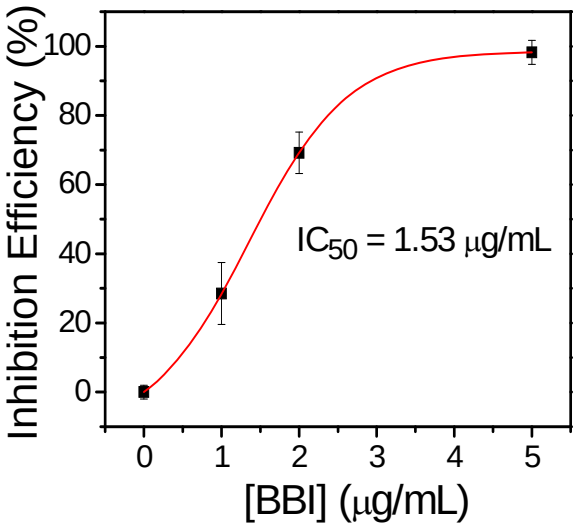
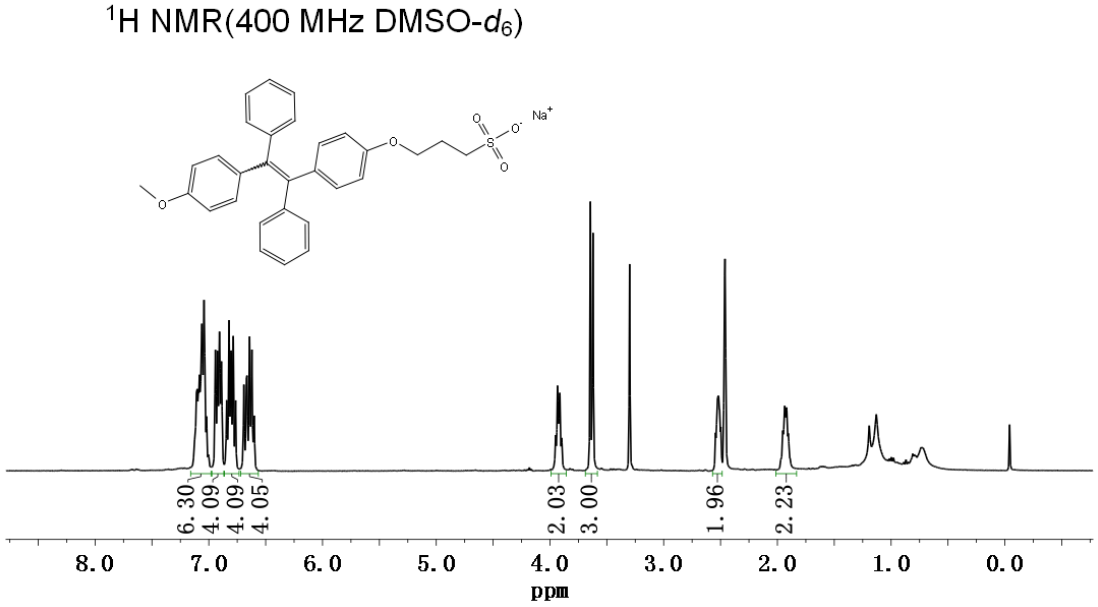
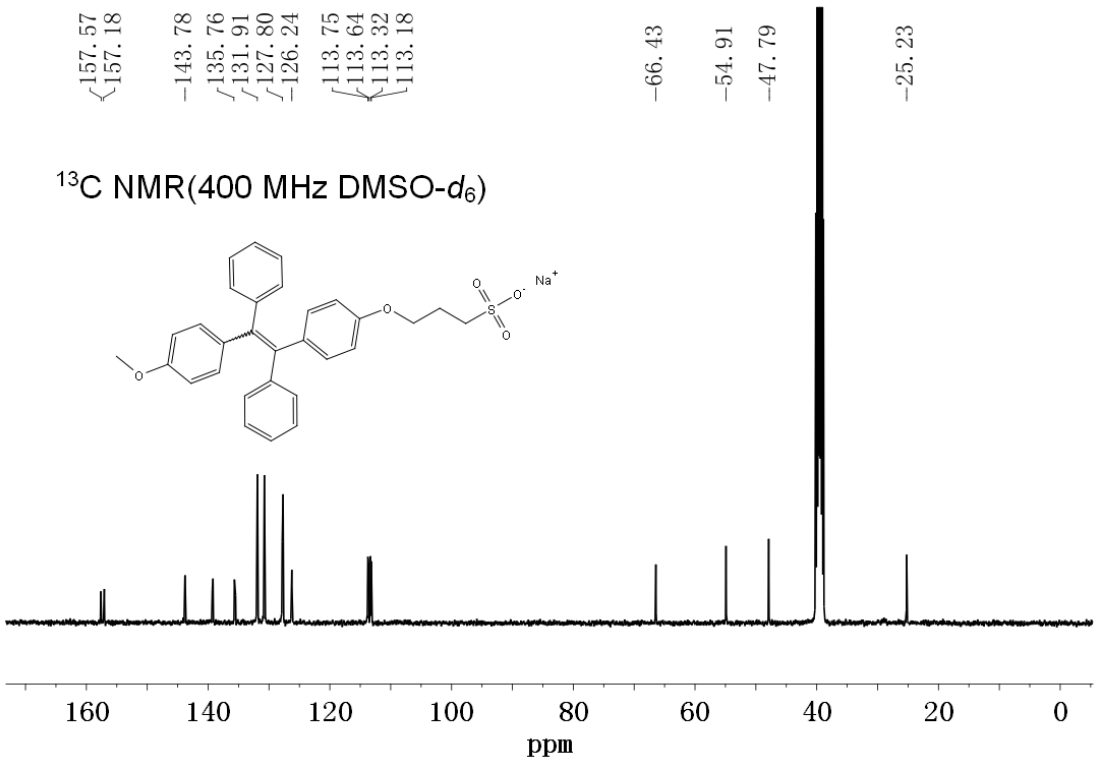
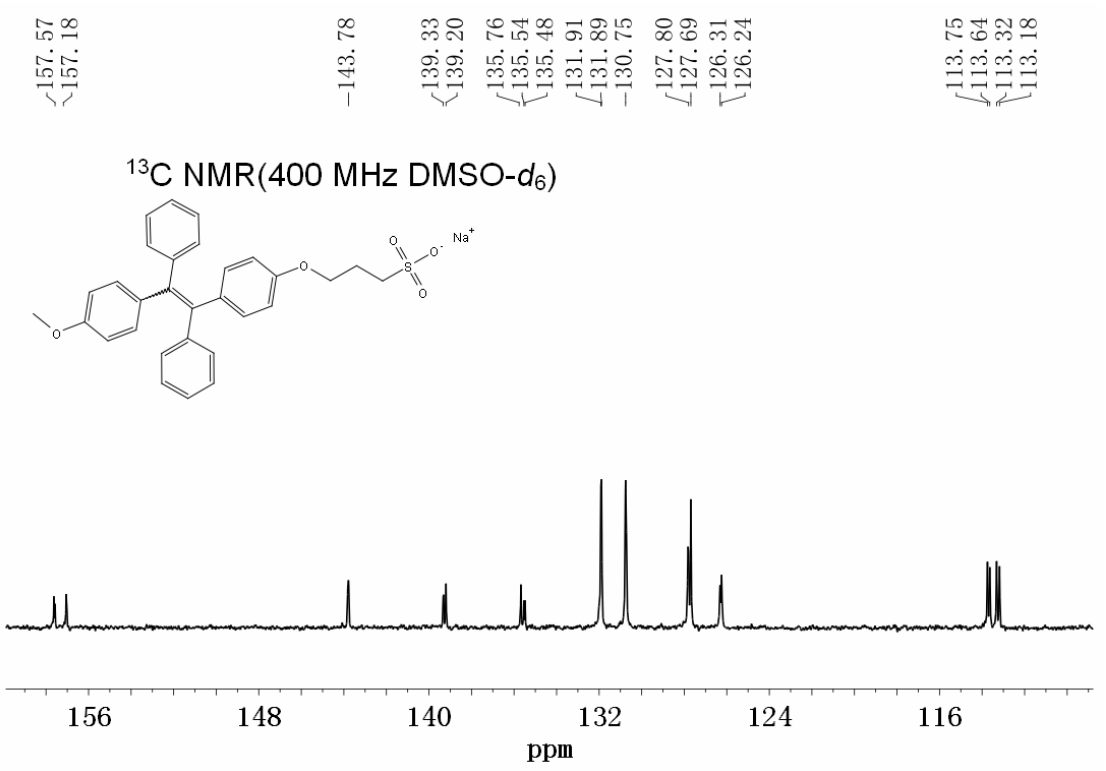


Figure S4. The plot of the inhibition efficiency of BBI toward trypsin vs. the concentration of BBI; the measurements were performed with **1** (60.0 μM) in PBS buffer solution (2.0 mM, pH = 8.5, containing $CaCl_2$ (10.0 μM)), Arg_6 peptide (10.0 μM), trypsin(3.2 μg/mL) and different amounts of BBI concentrations (0.0, 1.0, 2.0, 5.0 μg/mL).

5. ^{1}H-NMR and ^{13}C-NMR spectra of compounds 1 and 3



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143.78
139.33
139.20
135.76
135.54
135.48
131.91
131.89
130.75
127.80
127.69
126.31
126.24
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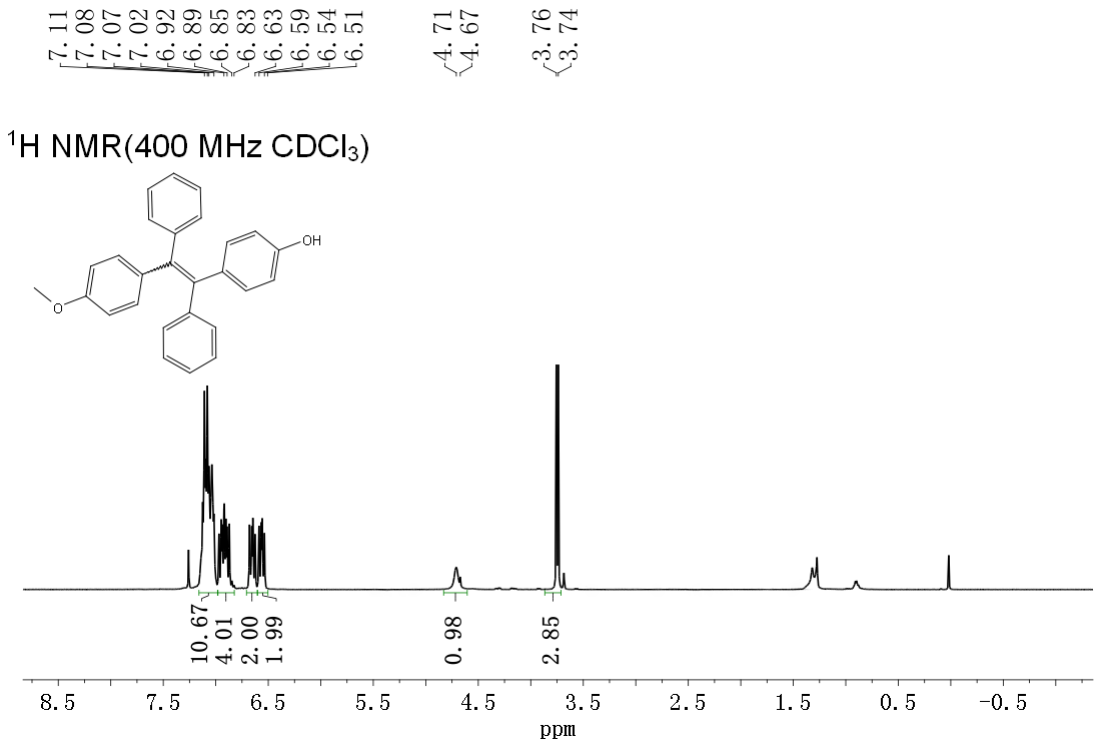
113.32
113.18
13C NMR(400 MHz DMSO-d6)
Na+
156
148
140
132
124
116
ppm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6.89
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6.54
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4.67
3.76
3.74
1H NMR(400 MHz CDCl3)
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10.67
4.01
2.00
1.99
0.98
2.85
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7.5
6.5
5.5
4.5
3.5
2.5
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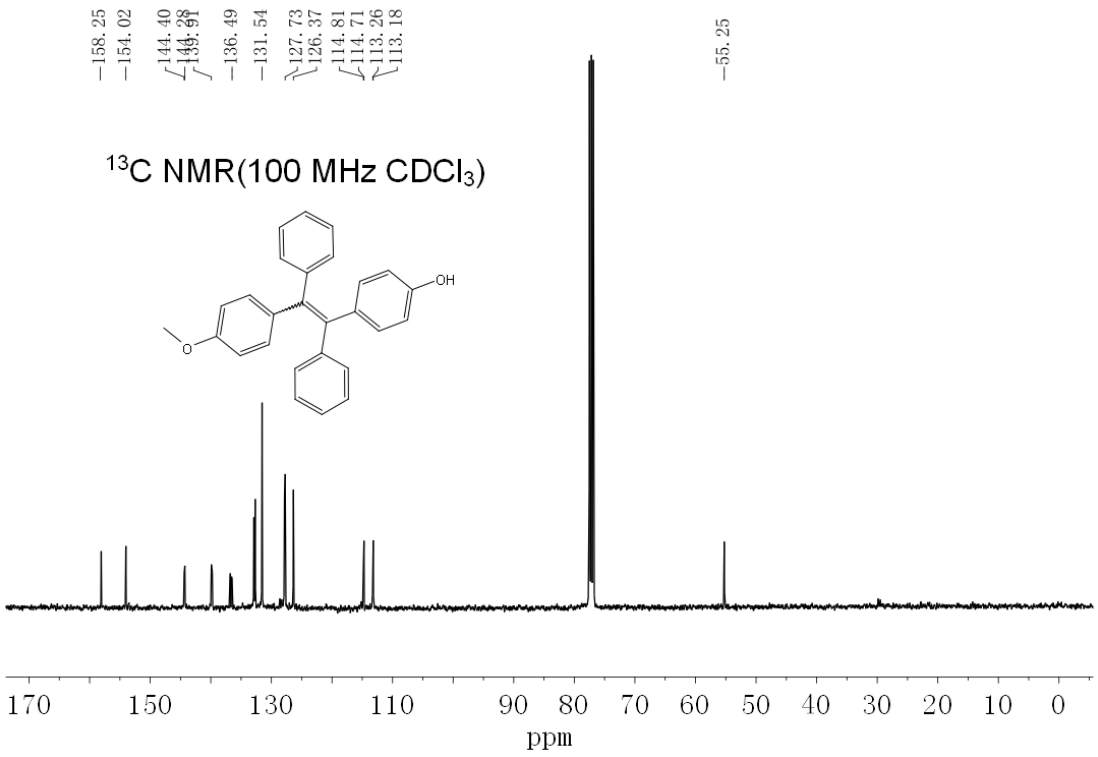
0.5
-0.5
ppm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139.74
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127.73
126.37
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114.71
113.26
113.18
55.25
13C NMR(100 MHz CDCl3)
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O
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60
50
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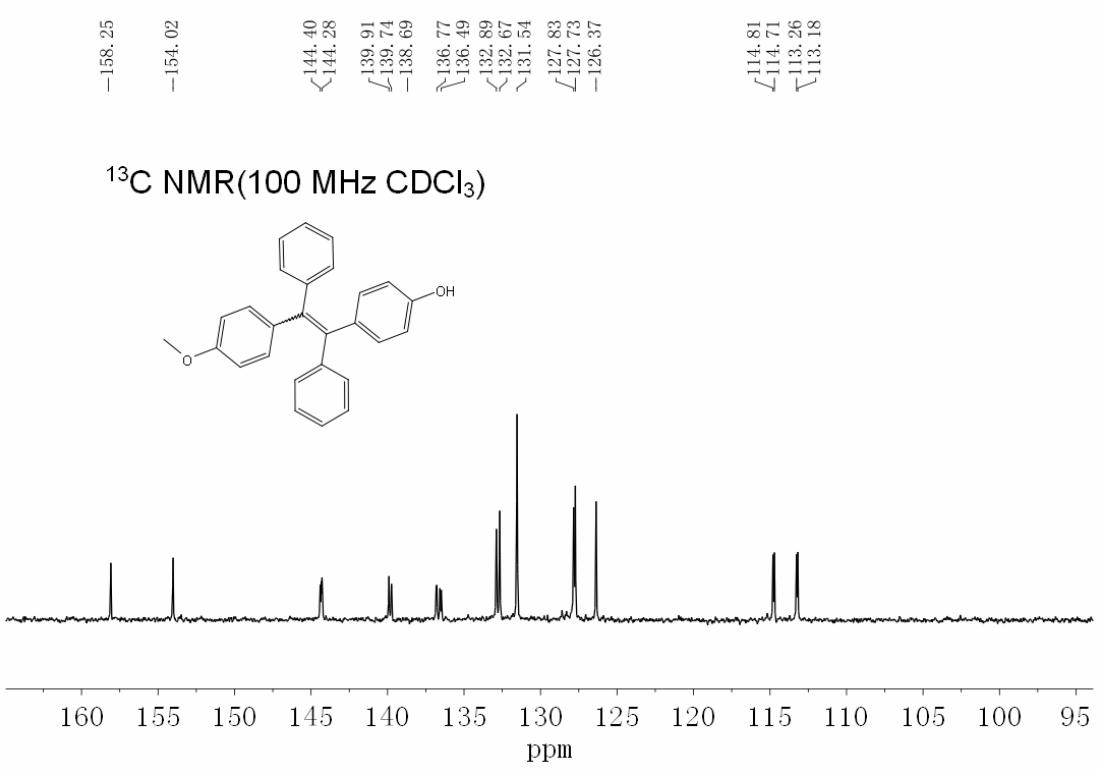
30
20
10
0
ppm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114.81
114.71
113.26
113.18
13C NMR(100 MHz CDCl3)
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O
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155
150
145
140
135
130
125
120
115
110
105
100
95
ppm