Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Development of improved dual-diazonium reagents for faster crosslinking of tobacco mosaic virus as hydrogels

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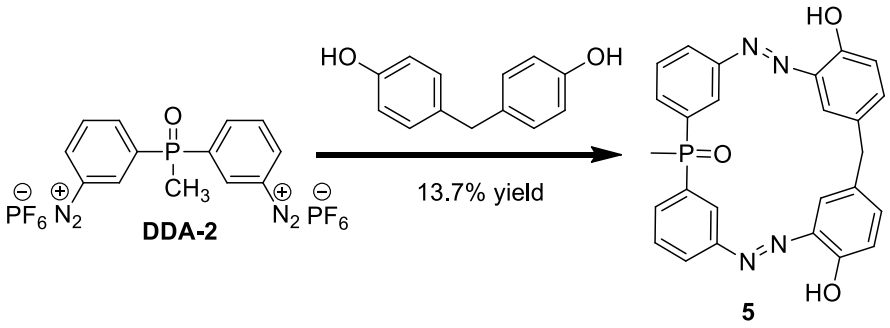
‡ The authors contribute equally to the work.

1. Synthesis.



Scheme S1. Synthesis of **5**.

Synthesis of 5

4,4'-methylenediphenol (203 mg, 1.0 mmol) was dissolved in 140 mL CH_3CN and 60 mL phosphate buffer (200 mM, pH 6.0). **DDA-2** (584 mg, 1.0 mmol) was dissolved in 50 mL CH_3CN and the reaction mixture was stirred at room temperature for 1 h. After removing CH_3CN under reduced pressure, the mixture was extracted with EtOAc. Next, the combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4. After the solution was removed, the product was purified by silica gel column chromatography (CH_2Cl_2 : MeOH = 99 : 1) to give **5** (64 mg, 13.7%).^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 2H), 8.69 (d, J = 11.2 Hz, 2H), 8.07 (d, J = 8.1 Hz, 4H), 8.00 (dd, J = 11.0, 7.6 Hz, 2H), 7.70 (td, J = 7.8, 2.4 Hz, 2H), 7.33 (dd, J = 8.4, 2.2 Hz, 2H), 6.91 (d, J = 8.3 Hz, 2H), 4.03 (d, J = 14.6 Hz, 1H), 3.80 (d, J = 14.6 Hz, 1H), 2.25 (d, J = 13.7 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.2, 151.8, 151.7, 139.3, 137.0, 136.0, 134.0, 132.6, 132.4, 132.4, 130.0, 129.8, 127.4, 127.3, 124.1, 123.1, 117.6, 37.8, 17.9, 17.2; ^{31}P NMR (162 MHz, DMSO-d_6) δ 26.62 (s). HRMS (ESI): *m/z* $[M+H]^+$ calculated for $C_{26}H_{22}N_4O_3P^+$: 469.1424; found: 469.1423.

2. Supplementary figures

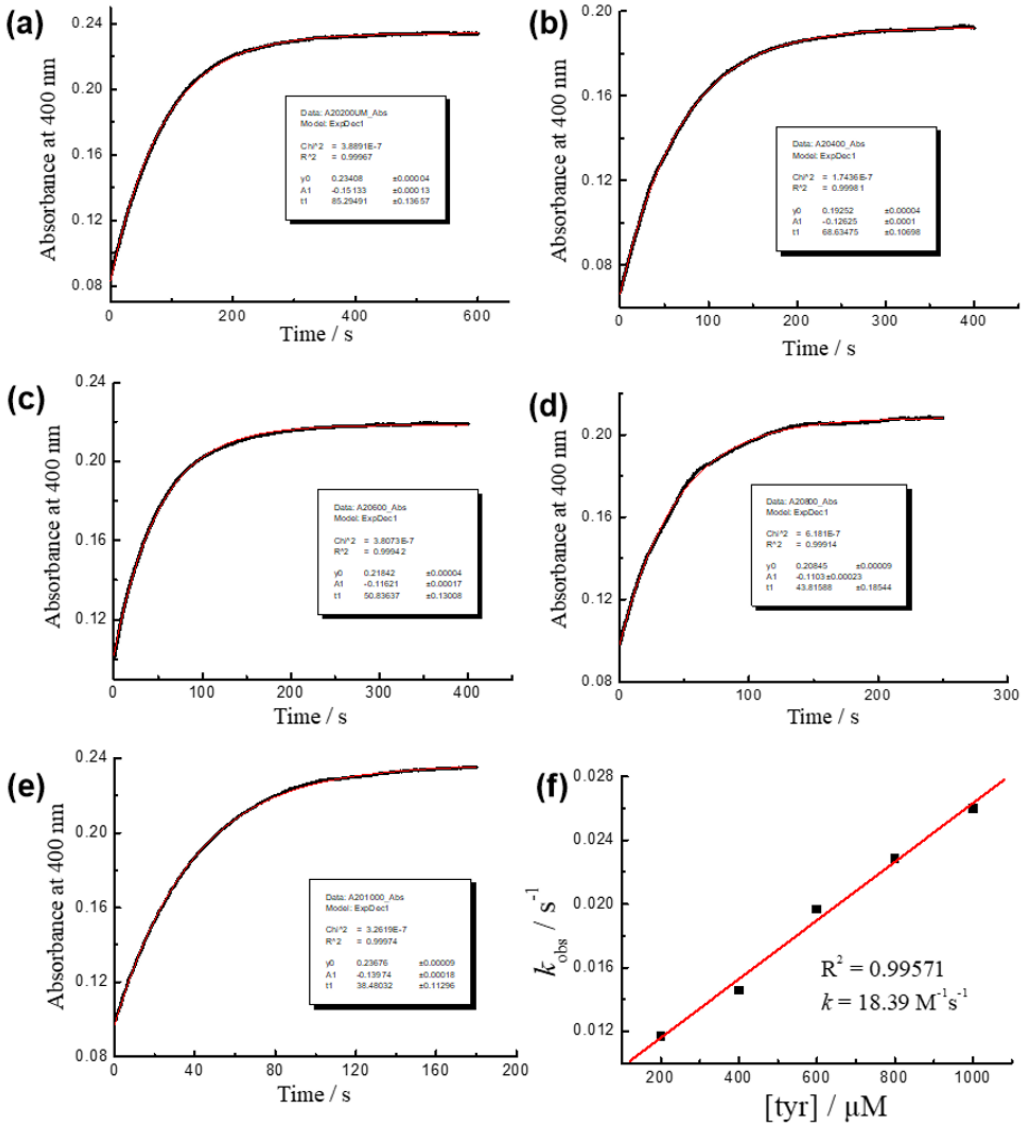


Fig. S1. Time-dependent UV-Vis absorbance spectra change of **DDA-2** (20 μM) at 400 nm, when treated with different concentrations of H-Tyr-OBzl·Tos in PBS buffer. (a. 200 μM; b. 400 μM; c. 600 μM; d. 800 μM; e. 1000 μM). The second-order rate constant was obtained by plotting the pseudo first-order rates against the concentration of tyrosine used (f).

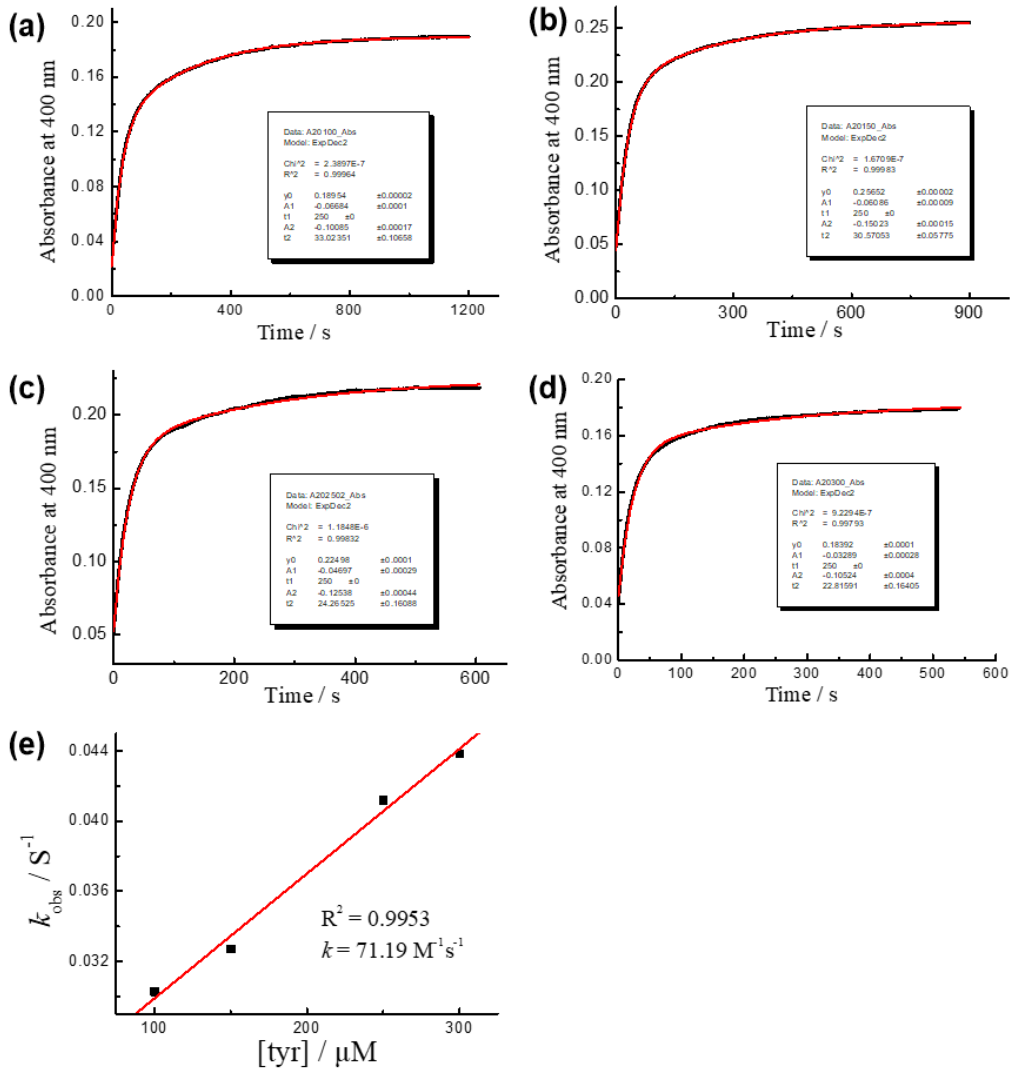


Fig. S2. Time-dependent UV-Vis absorbance spectra change of **DDA-3** (20 μM) at 400 nm, when treated with different concentrations of H-Tyr-OBzl·Tos in PBS buffer. (a. 100 μM; b. 150 μM; c. 250 μM; d. 300 μM). The second-order rate constant was obtained by plotting the pseudo first-order rates against the concentration of tyrosine used (e).

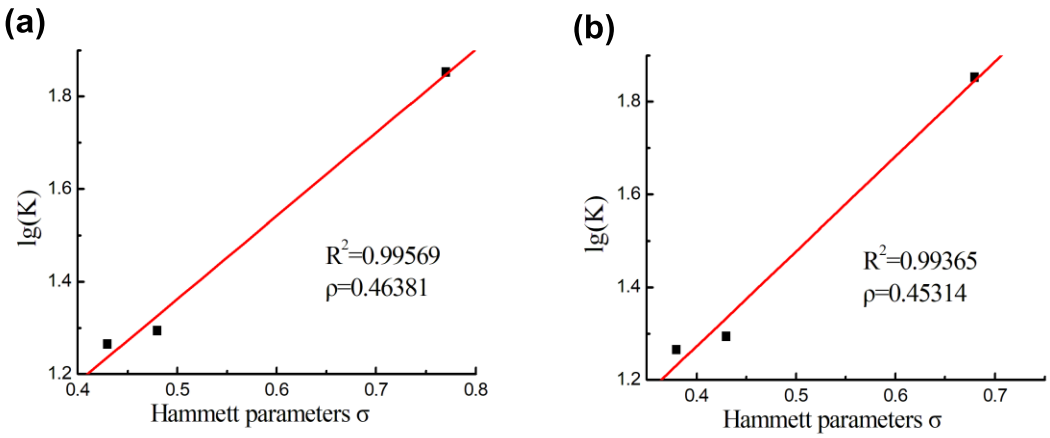


Fig. S3. Plots of the Hammett parameters vs. rate constant for three azo coupling reactions. We obtain the corresponding Hammett constant by two kinds of similar structural substituents. a) The Hammett constants of the substituent COEt, $PO(Me)_2$, SO_2Et are 0.48, 0.43, 0.77, respectively; b) The Hammett constants of the substituent COC_6H_5, $PO(C_6H_5)_2$, $SO_2C_6H_5$ are 0.43, 0.38, 0.68, respectively. The σ value is plotted against lg K, ρ is the slope of the Hammett line and is called the reaction constant, depending on the type and condition of the reaction; K is the reaction rate constant of the substituted homologue.

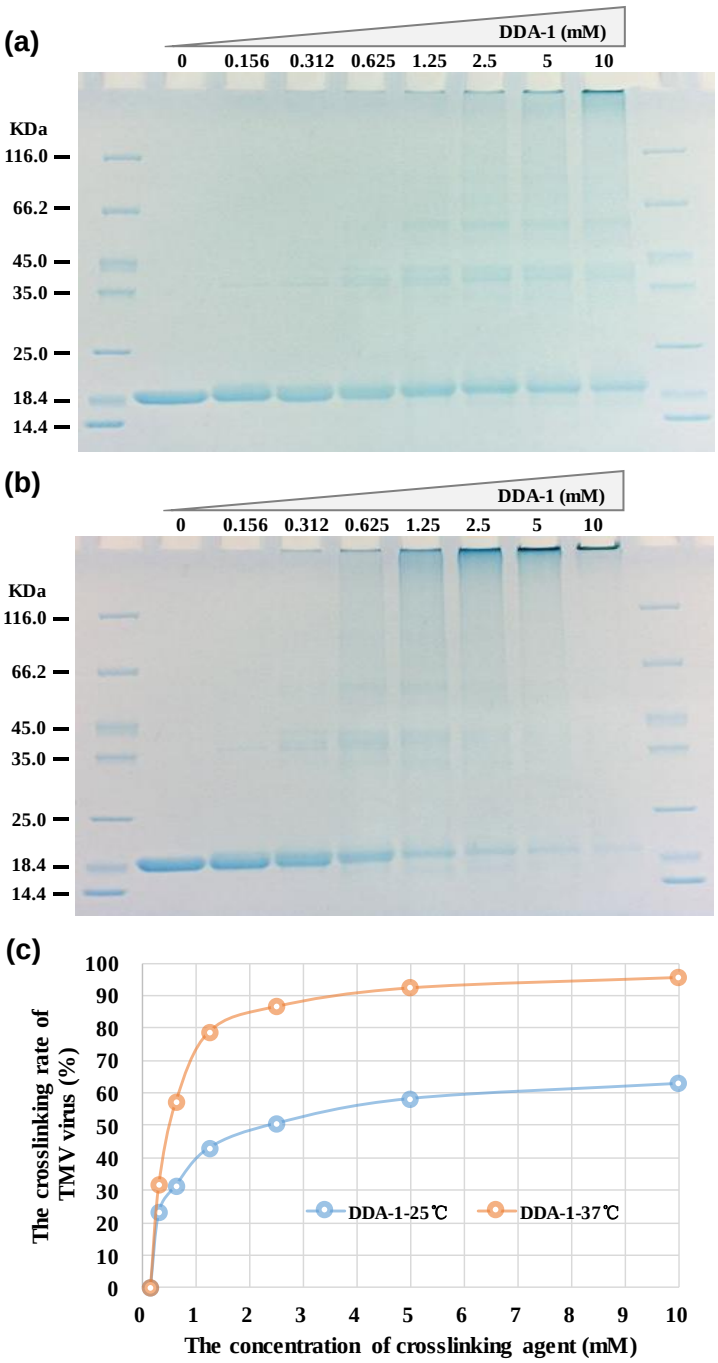


Fig. S4. The comparison of TMV gelation at 25 ºC (a) and 37 ºC (b). The reaction was performed by mixing 9 μL TMV virus (1 μg/μL) and 1 μL **DDA-1** with various concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, mM). The reaction was incubated at 25 ºC or 37 ºC for 60 min. After reaction, 10 μL sample for each treatment was directly used for 12% SDS-PAGE assay. (c) The crosslinking rate of TMV virus (%) was calculated based on the group without the addition of **DDA-1**.

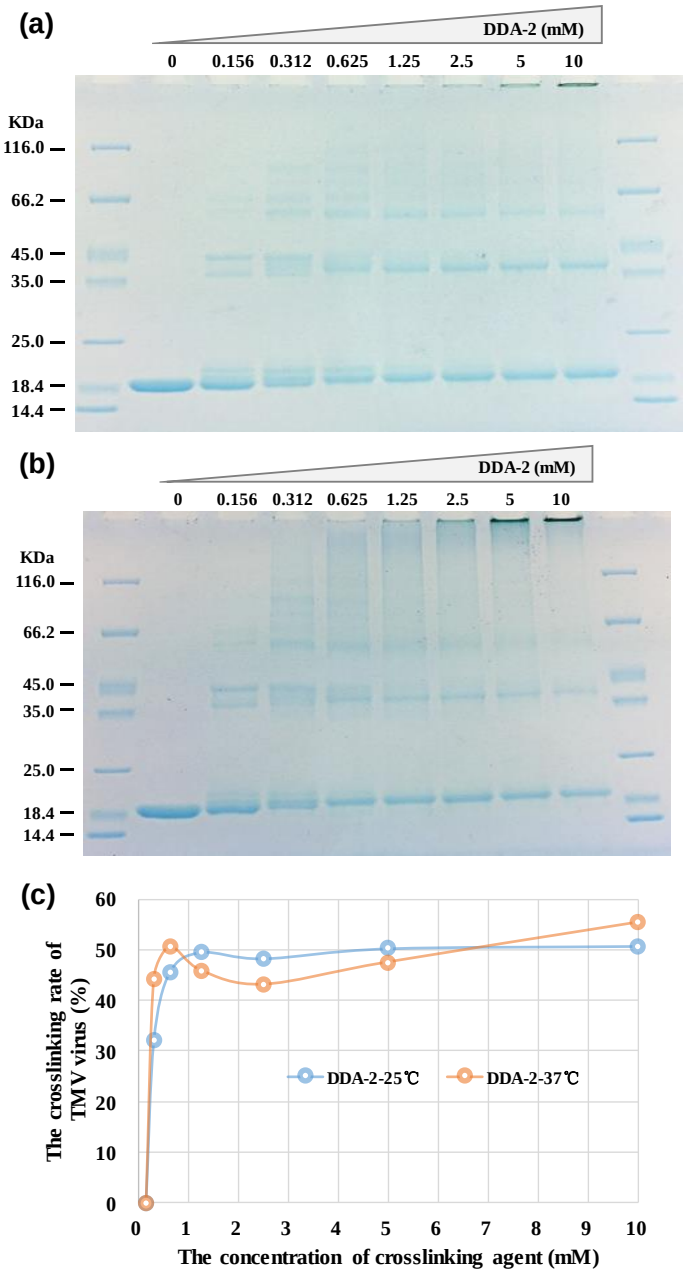


Fig. S5. The comparison of TMV gelation at 25 ºC (a) and 37 ºC (b). The reaction was performed by mixing 9 μL TMV virus (1 μg/μL) and 1 μL **DDA-2** with various concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, mM). The reaction was incubated at 25 ºC or 37 ºC for 60 min. After reaction, 10 μL sample for each treatment was directly used for 12% SDS-PAGE assay. (c) The crosslinking rate of TMV virus (%) was calculated based on the group without the addition of **DDA-2**.

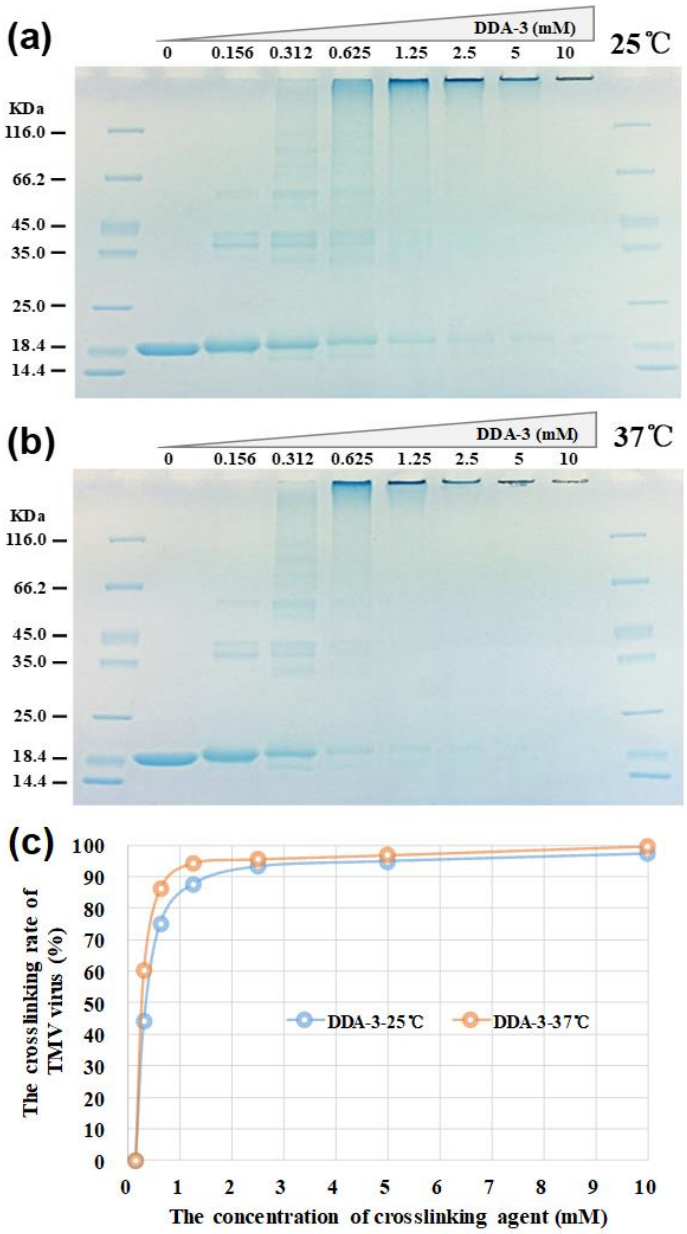


Fig. S6. The comparison of TMV gelation at 25 ºC (a) and 37 ºC (b). The reaction was performed by mixing 9 μL TMV virus (1 μg/μL) and 1 μL **DDA-3** with various concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, mM). The reaction was incubated at 25 ºC or 37 ºC for 60 min. After reaction, 10 μL sample for each treatment was directly used for 12% SDS-PAGE assay. (c) The crosslinking rate of TMV virus (%) was calculated based on the group without the addition of **DDA-3**.

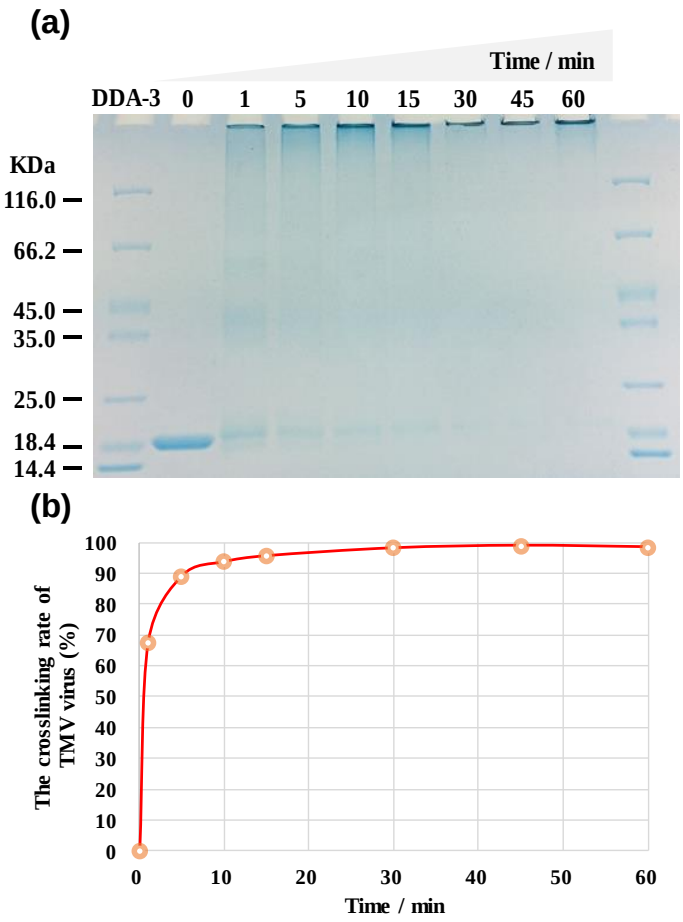


Fig. S7. Time-dependent crosslinking of TMV with **DDA-3**. (a) The reaction was performed by mixing 9 μL TMV virus (1 μg/μL) and 1 μL **DDA-3** (100 mM). The reaction was incubated at 25 ºC for various time (0, 1, 5, 10, 15, 30, 45, 60, min). At the indicated time, the reaction was stopped with 5×SDS loading buffer and then used for 12% SDS-PAGE assay. (b) The crosslinking rate of TMV virus (%) was calculated based on the group at 0 min.

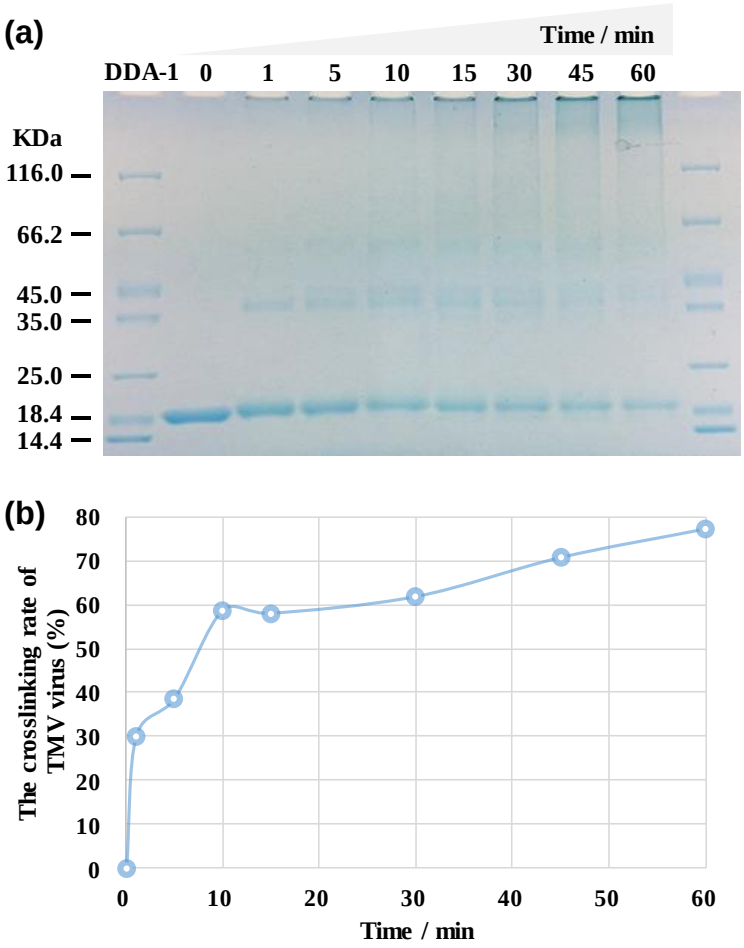


Fig. S8. Time-dependent crosslinking of TMV with **DDA-1**. (a) The reaction was performed by mixing 9 μL TMV virus (1 μg/μL) and 1 μL **DDA-1** (100 mM). The reaction was incubated at 25 ºC for various time (0, 1, 5, 10, 15, 30, 45, 60, min). At the indicated time, the reaction was stopped with 5×SDS loading buffer and then used for 12% SDS-PAGE assay. (b) The crosslinking rate of TMV virus (%) was calculated based on the group at 0 min.

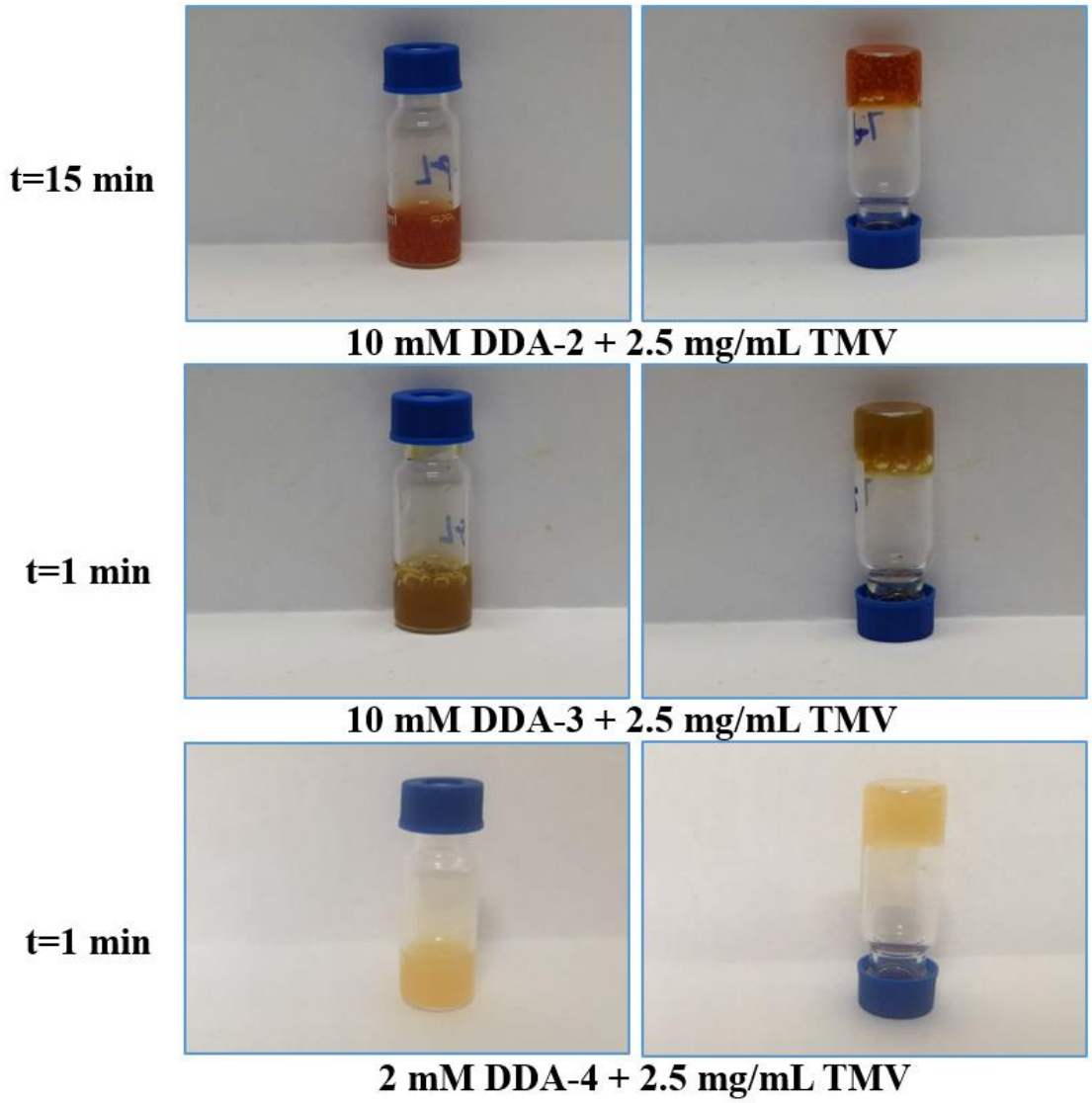


Fig. S9. The minimum gelation time of three TMV hydrogels at room temperature. The concentration of **DDA-2** or **DDA-3** was 10 mM and the concentration of **DDA-4** was 2 mM; The concentration of TMV matrix was 2.5 mg/mL.

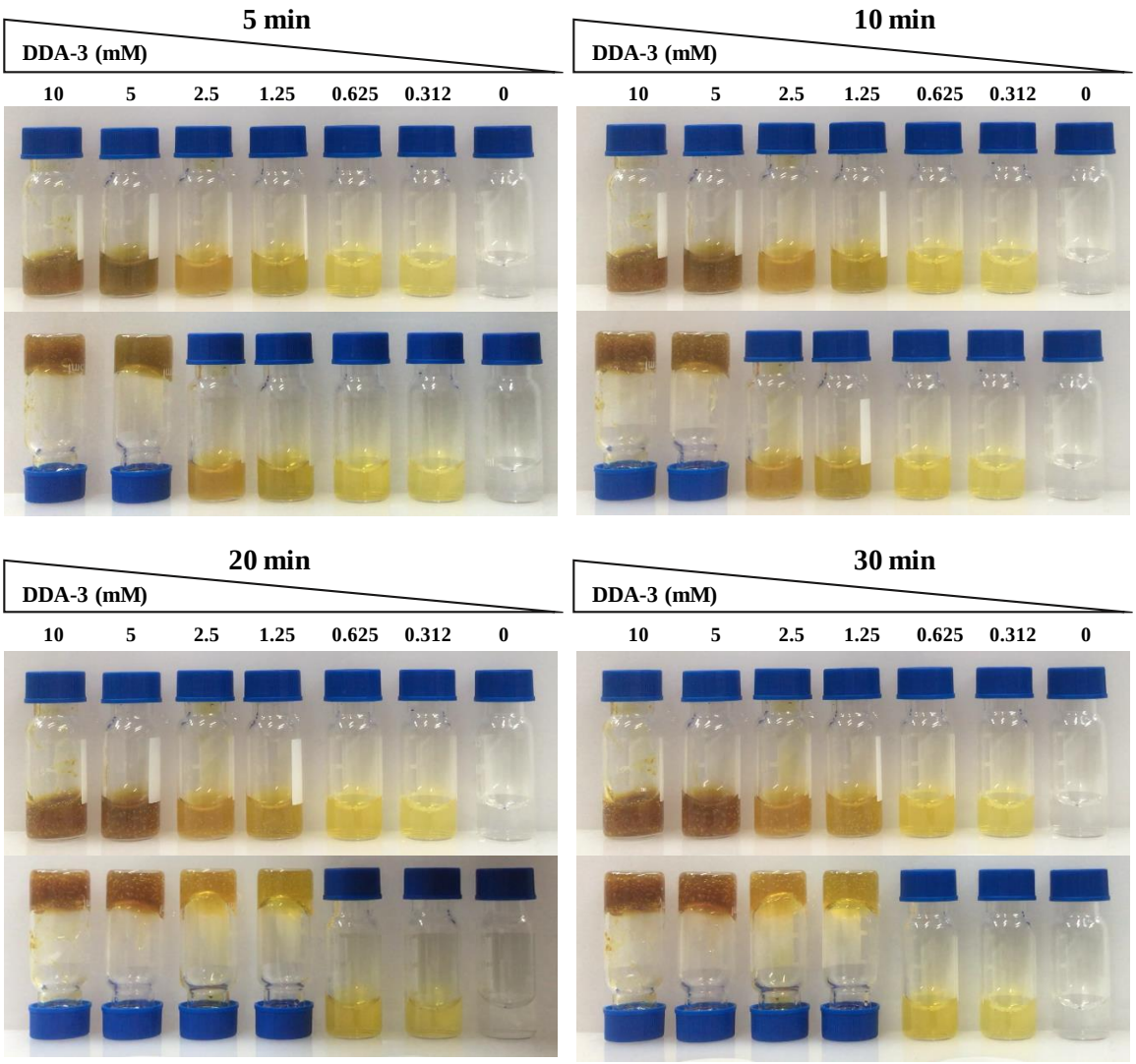


Fig. S10. The effect of the concentration of **DDA-3** on TMV gelation. The final concentration of **DDA-3** was varying from 0~10 mM and the concentration of TMV matrix was 2.5 mg/mL. The gelation time was kept within 0-30 min at room temperature.

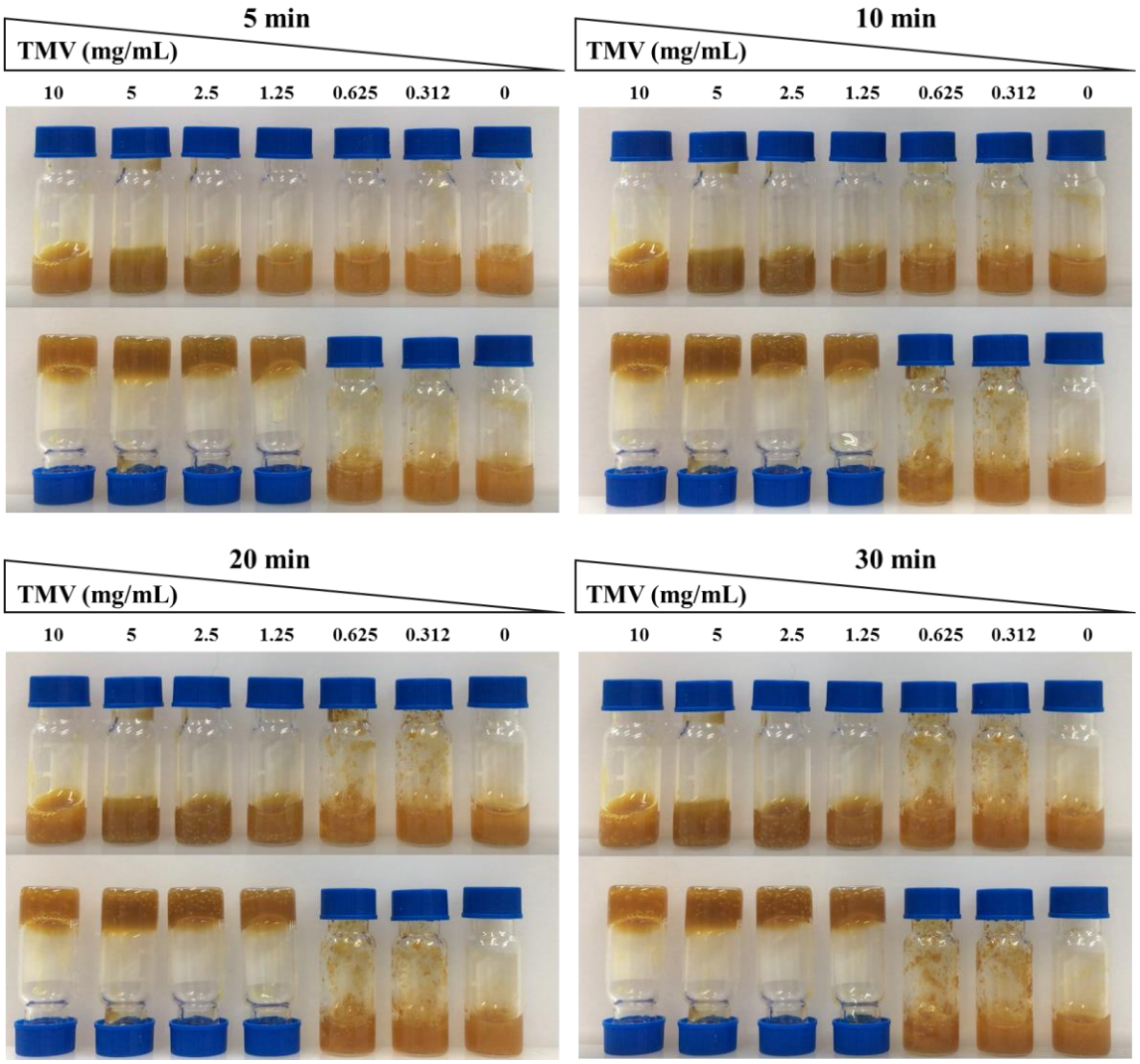


Fig. S11. The effect of the concentration of TMV matrix on TMV gelation. The final concentration of TMV matrix was varying from 0~10 mg/mL and the concentration of **DDA-3** was 10 mM. The gelation time was kept within 0-30 min at room temperature.

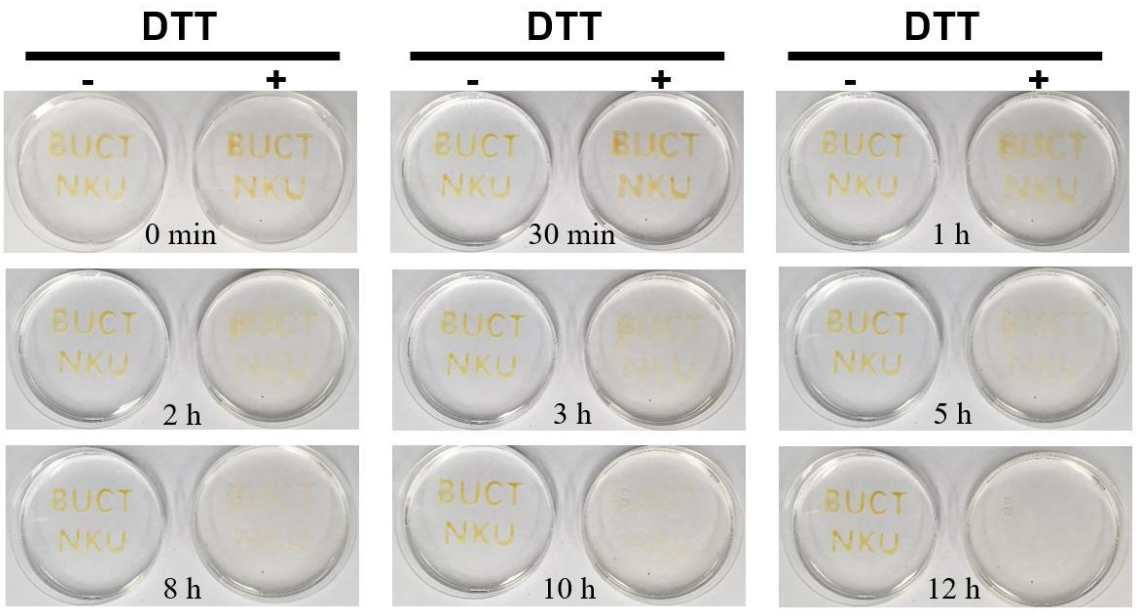
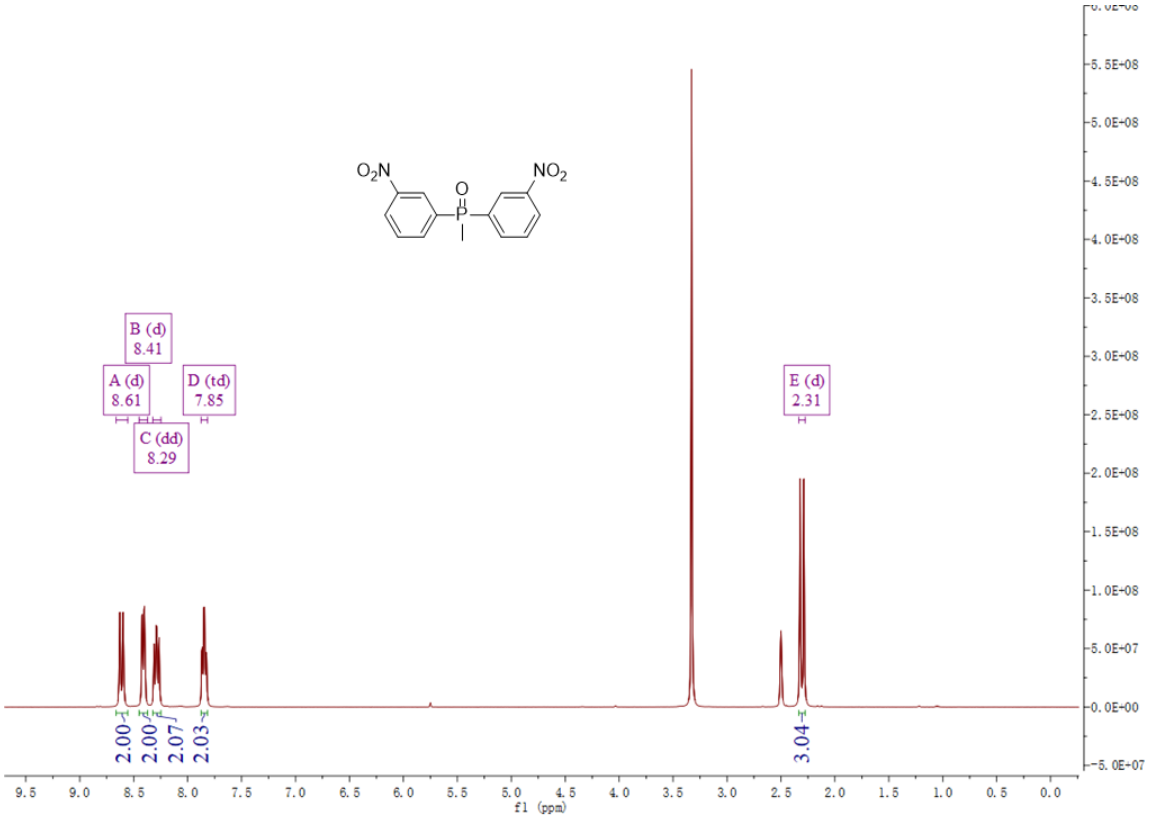


Fig. S12. Degradation of virus-based hydrogel by DTT. Both gels were prepared by 2 mM **DDA-4** and 2.5 mg/mL TMV. 20 mL DTT (deionized water, 500 mM) was added to the petri dish on the right as the experimental group and 20 mL of deionized water was added to the petri dish on the left as a control. The gel degradation was imaged at different time (0 min, 30 min, 1 h, 2h, 3 h, 5h, 8 h, 10h, 12 h).


B (d)
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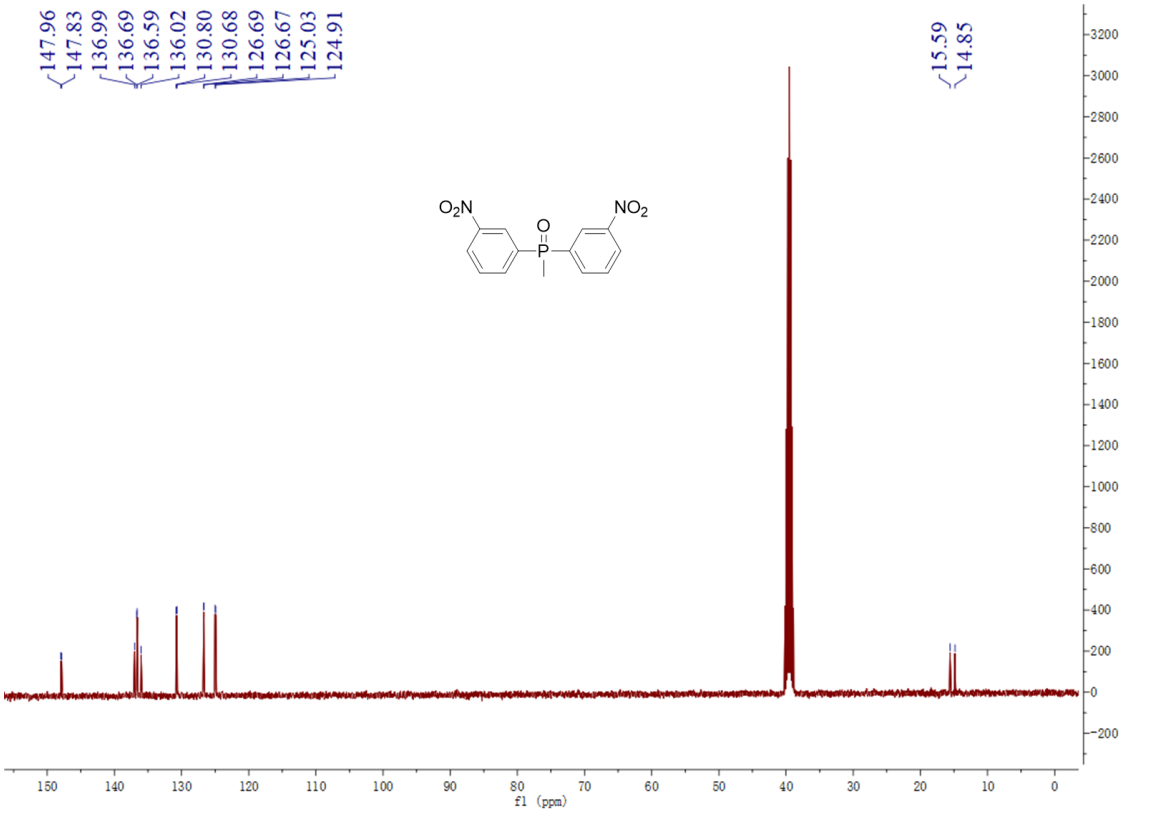
A (d)
8.61
D (td)
7.85
C (dd)
8.29
E (d)
2.31
2.00
2.00
2.07
2.03
3.04
f1 (ppm)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147.83
136.99
136.69
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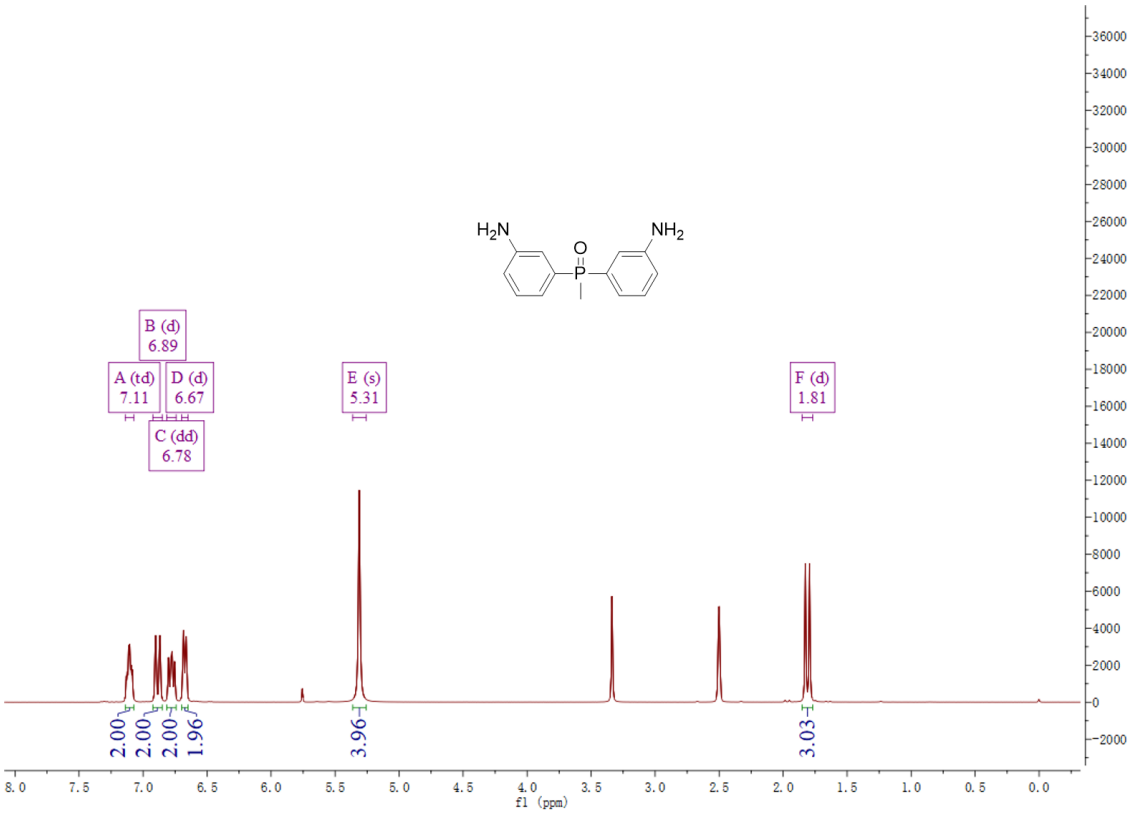
15.59
14.85
f1 (ppm)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A (td)
7.11
D (d)
6.67
C (dd)
6.78
E (s)
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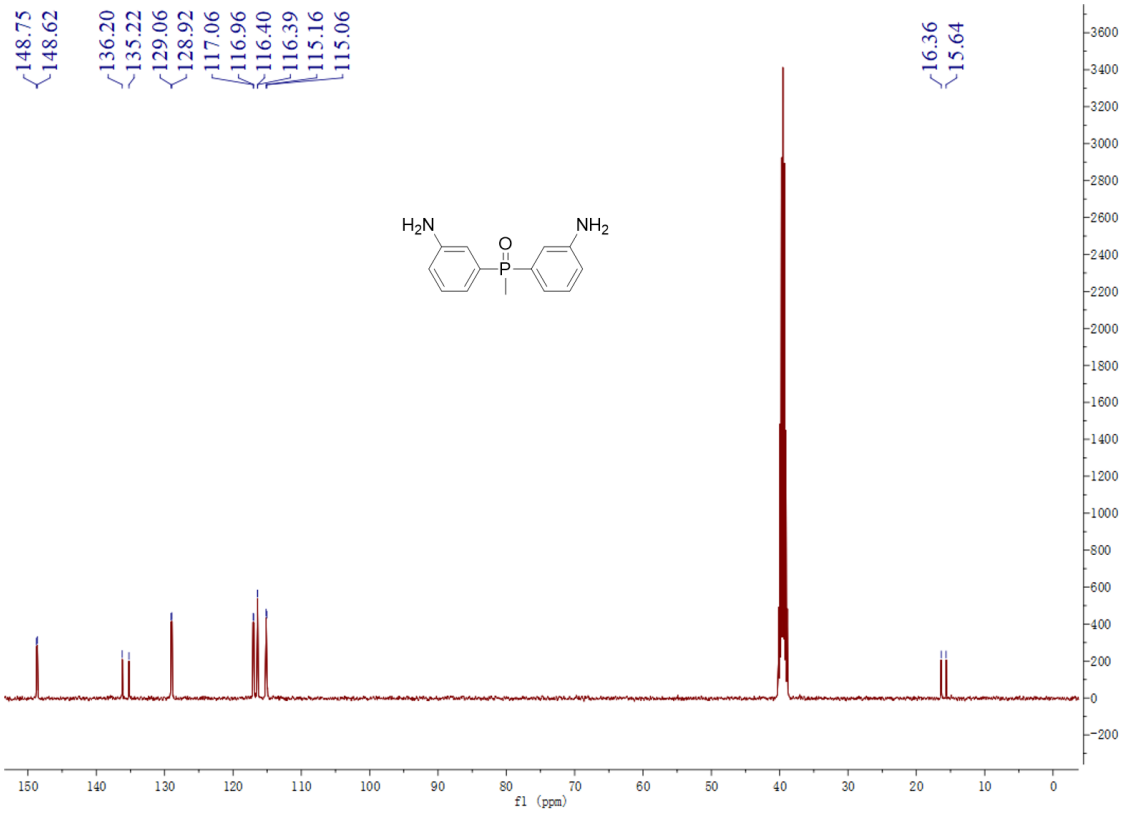
2.00
1.96
3.96
3.03
f1 (ppm)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148.62
136.20
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128.92
117.06
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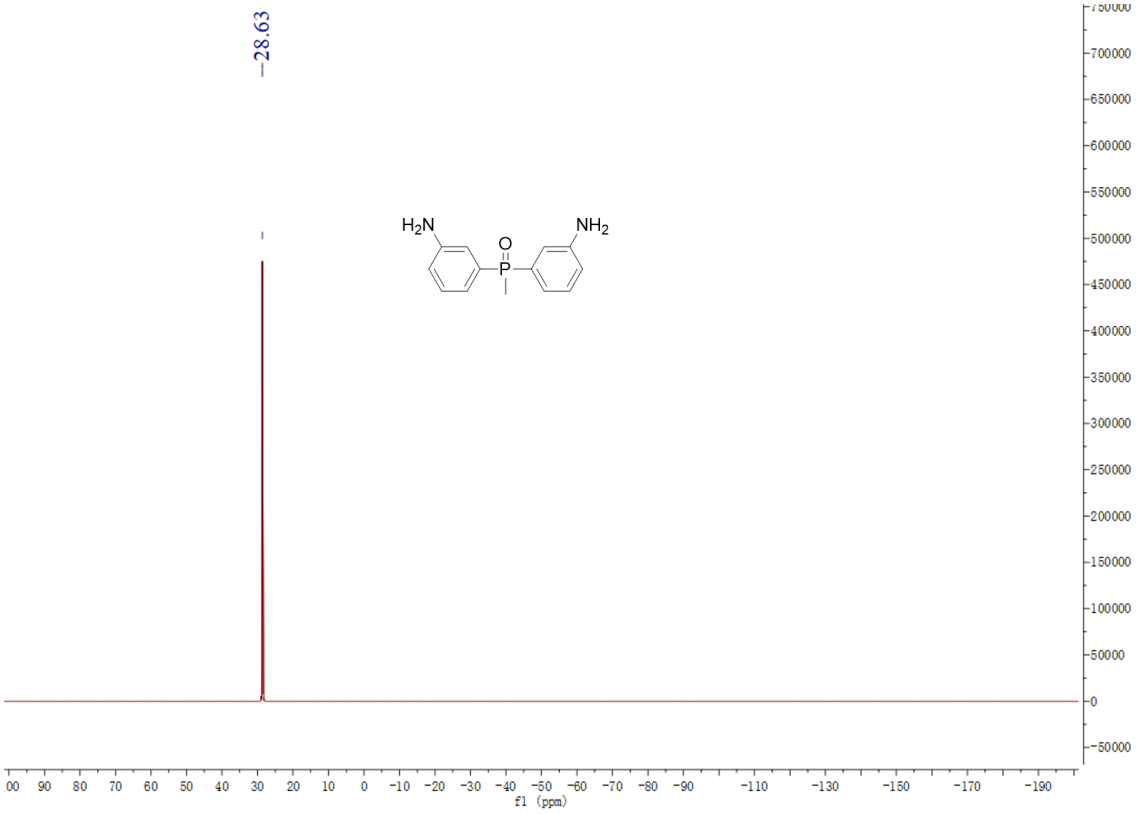
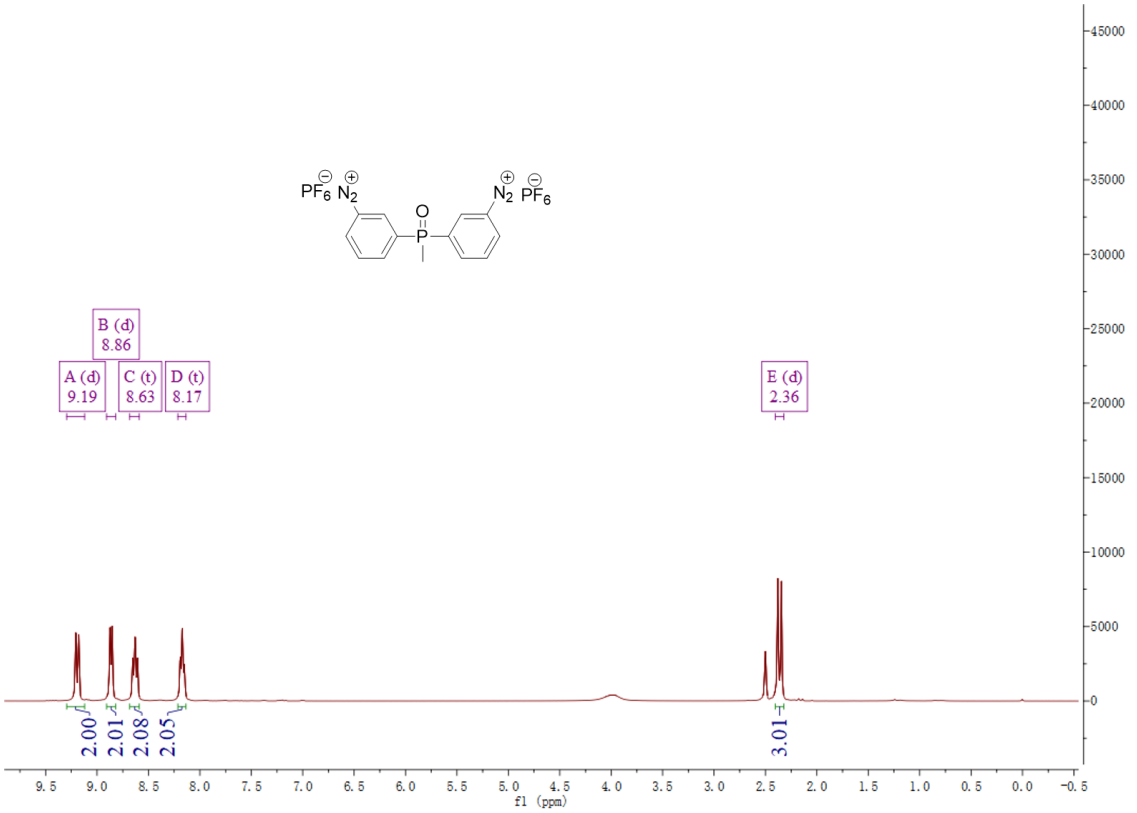
116.39
115.16
115.06
16.36
15.64
f1 (ppm)


28.63
H2N
NH2
O
P
f1 (ppm)


PF6 N2
N2 PF6
O
P
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A (d)
9.19
C (t)
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D (t)
8.17
E (d)
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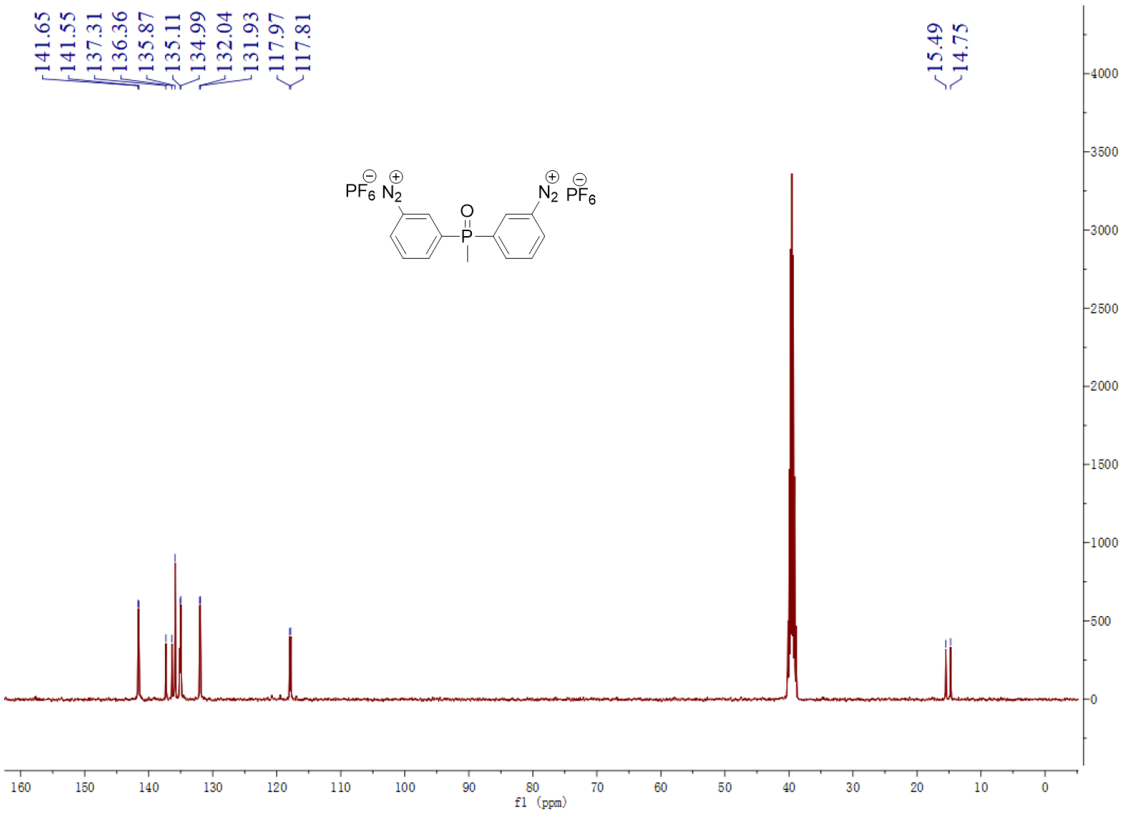
2.00
2.01
2.08
2.05
3.01
f1 (ppm)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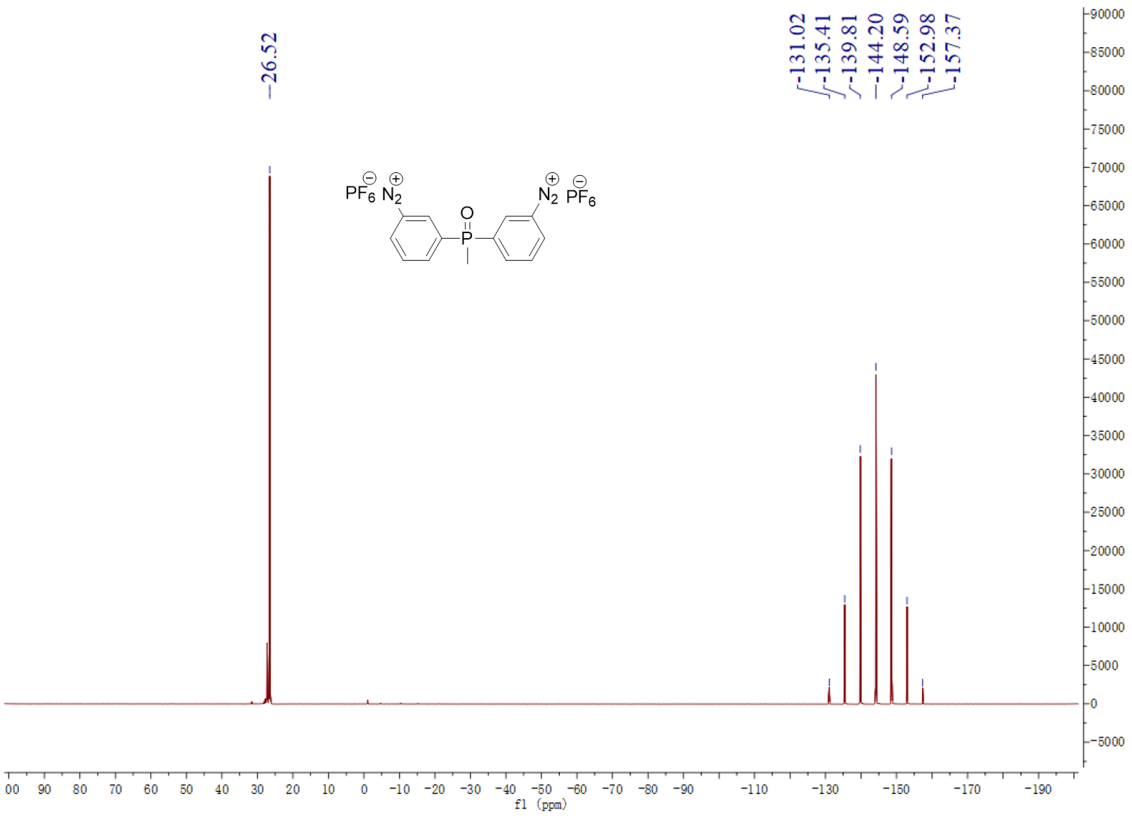
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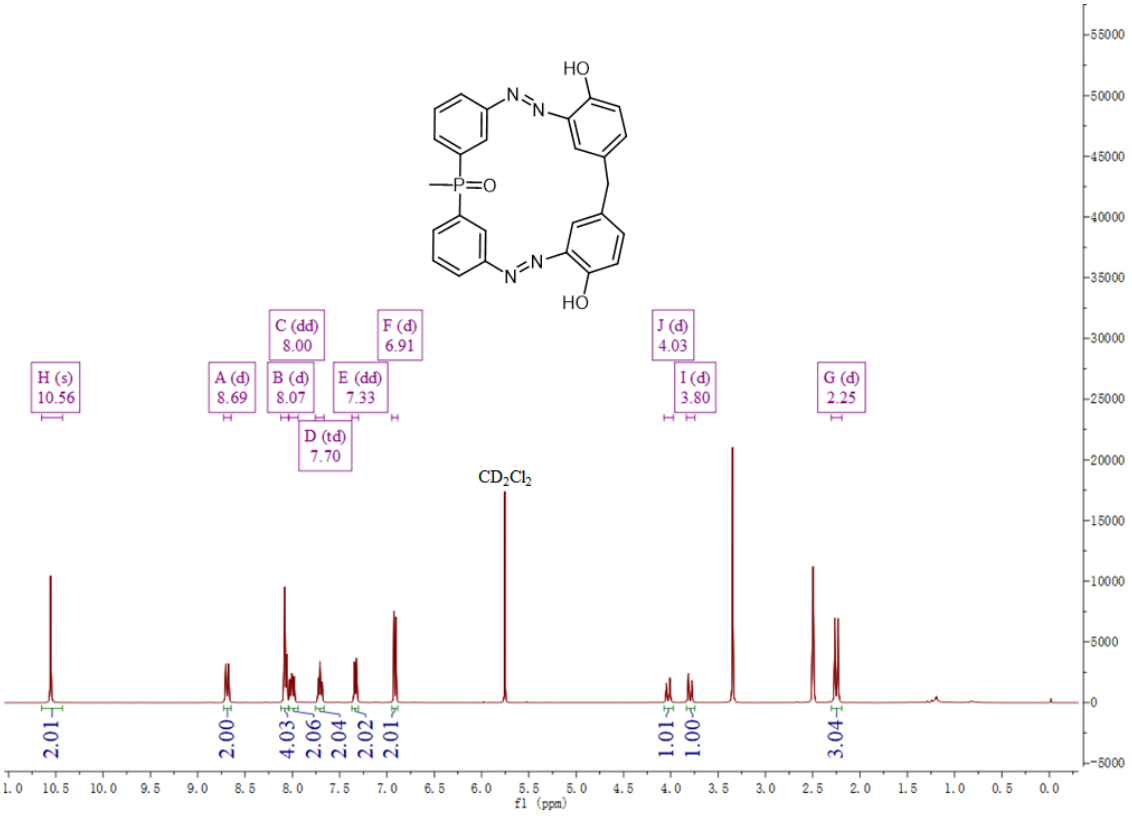
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P=O
N=N
HO
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8.69
C (dd)
8.00
B (d)
8.07
D (td)
7.70
E (dd)
7.33
F (d)
6.91
J (d)
4.03
I (d)
3.80
G (d)
2.25
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4.03
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2.04
2.02
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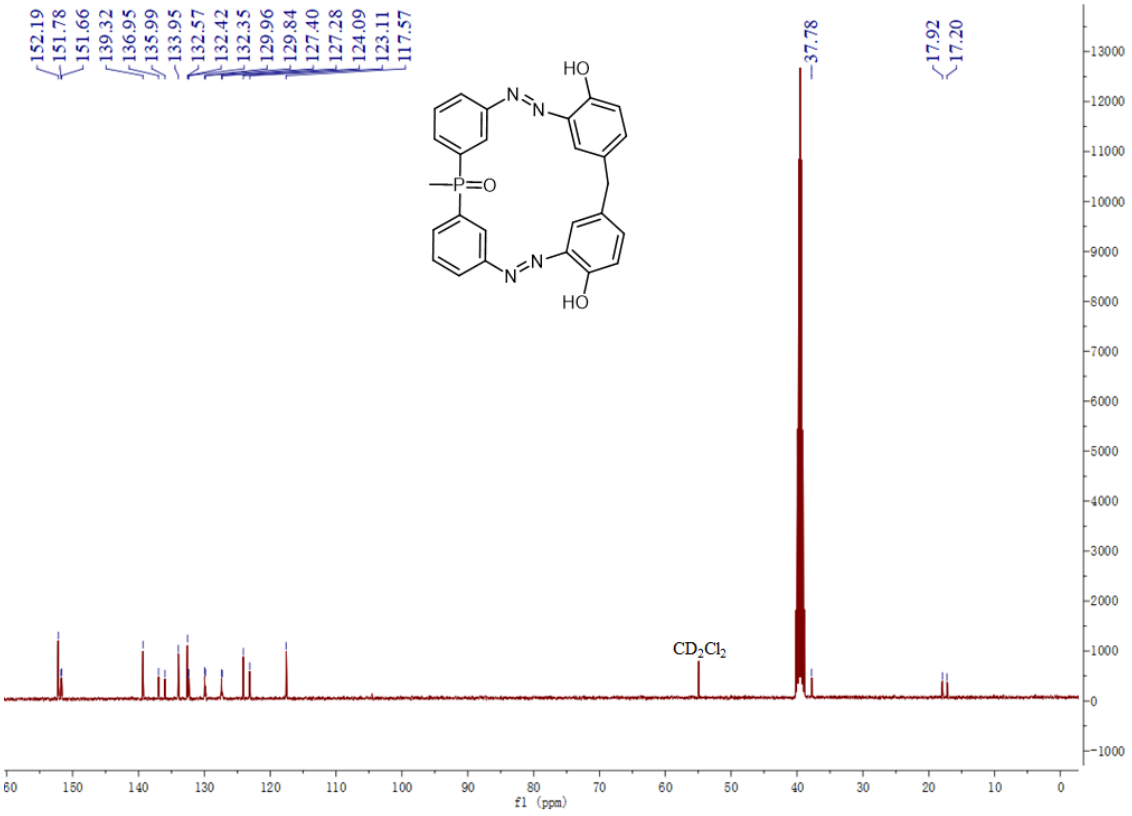
1.01
1.00
3.04
f1 (ppm)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127.40
127.28
124.09
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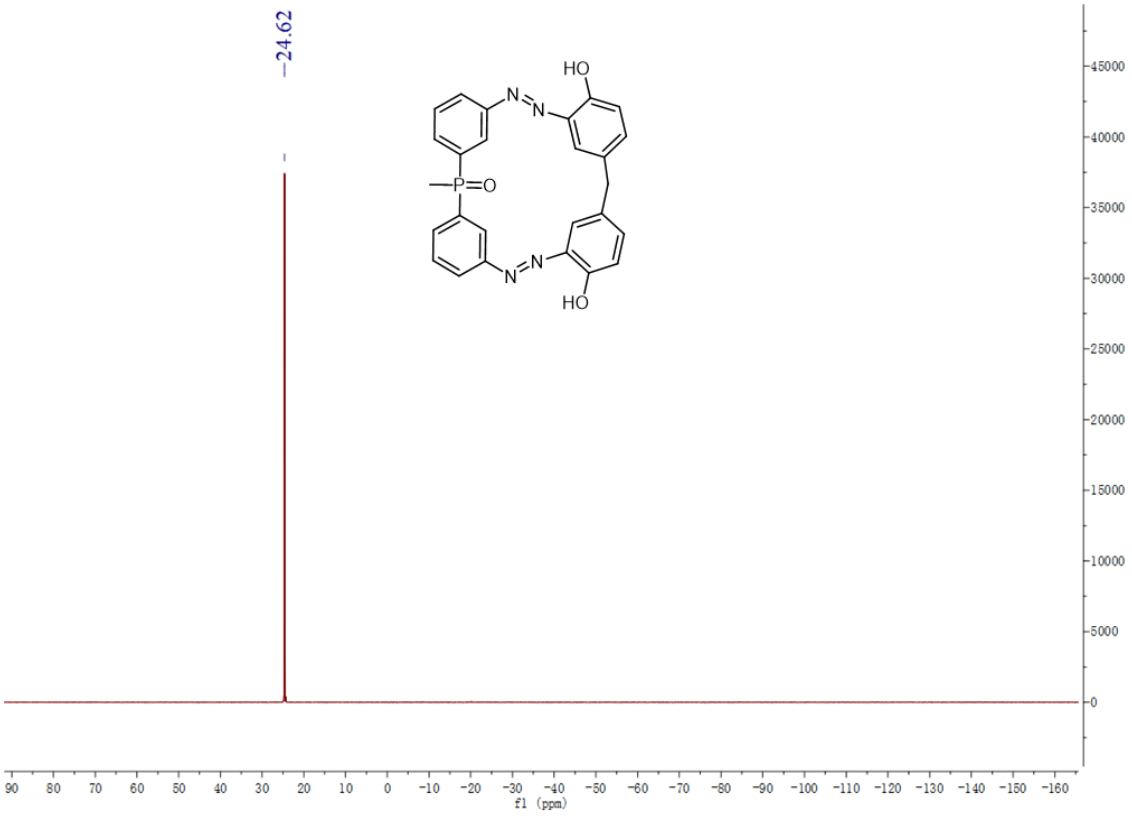
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N
P=O
N
N
HO
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40000
35000
30000
25000
20000
15000
10000
5000
0
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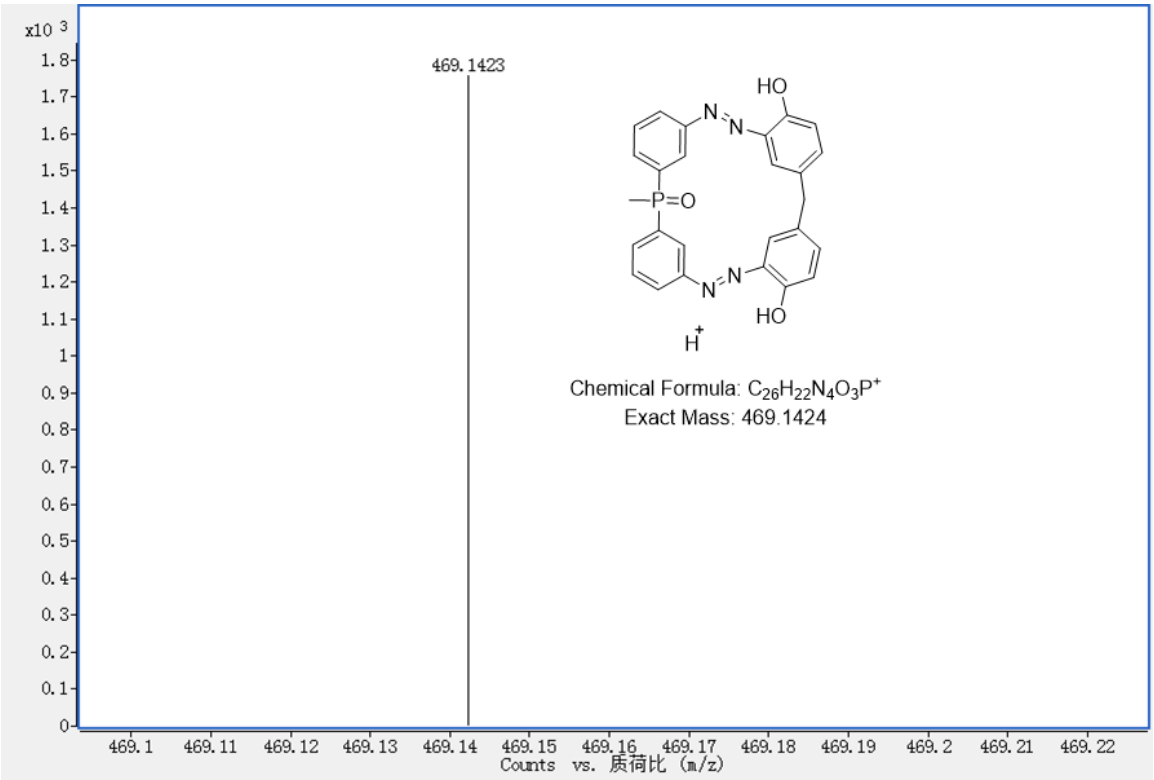
f1 (ppm)


x10 3
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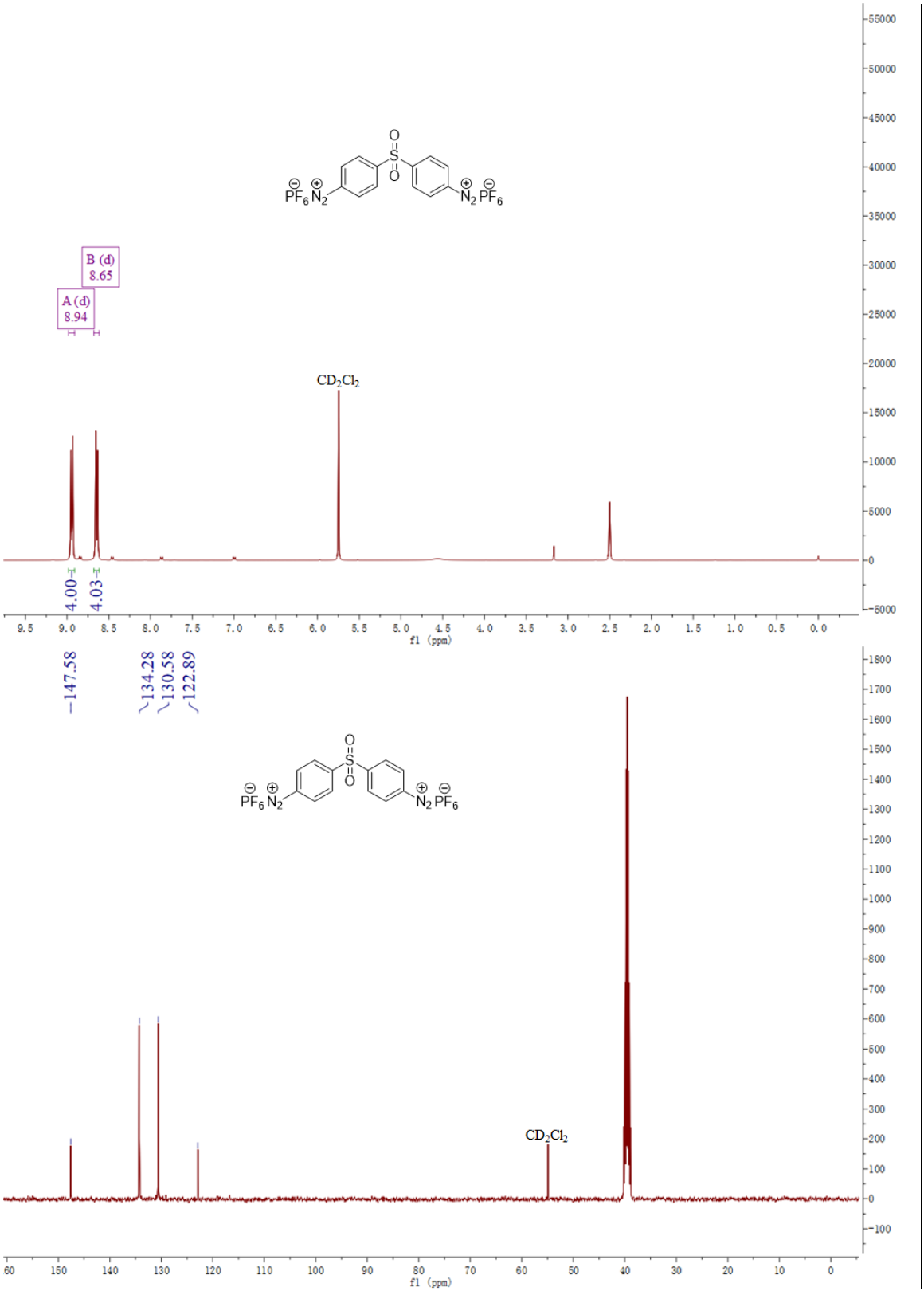
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
469.1423
HO
N
N
P=O
N
N
HO
H+
Chemical Formula: C26H22N4O3P+
Exact Mass: 469.1424
469.1 469.11 469.12 469.13 469.14 469.15 469.16 469.17 469.18 469.19 469.2 469.21 469.22
Counts vs. 质荷比 (m/z)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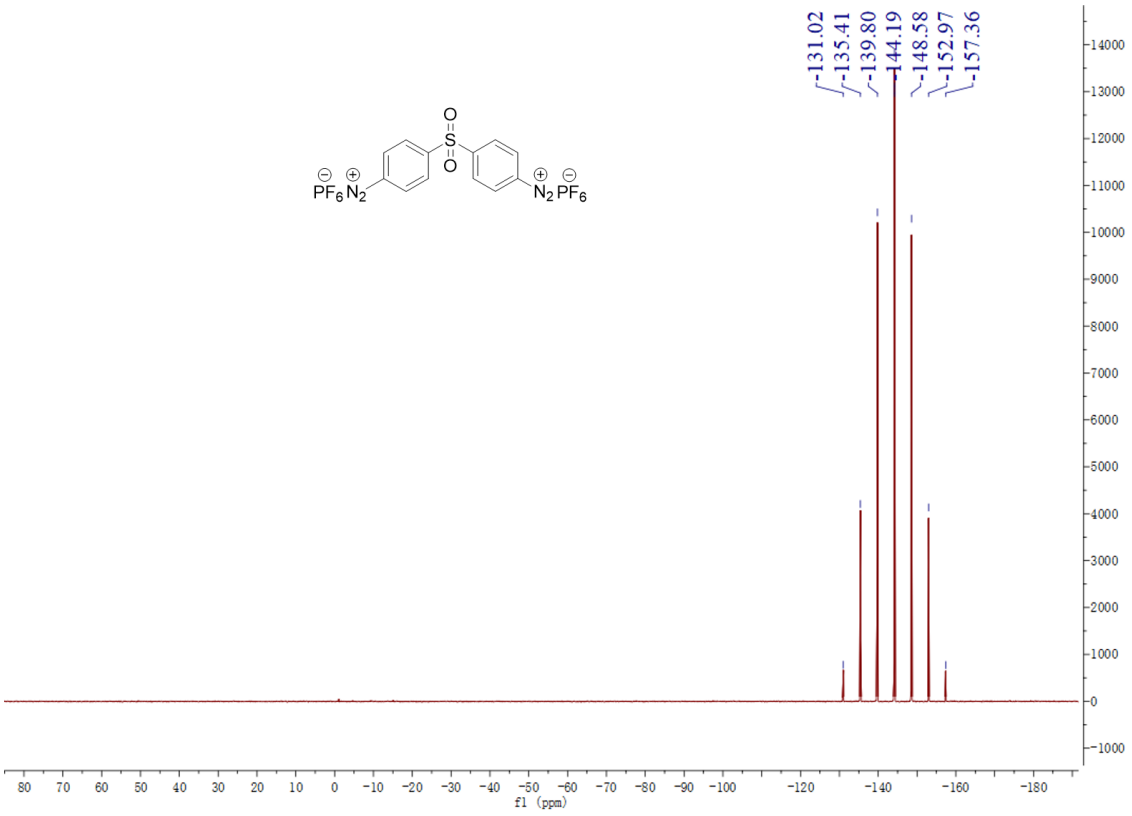
CD2Cl2
f1 (ppm)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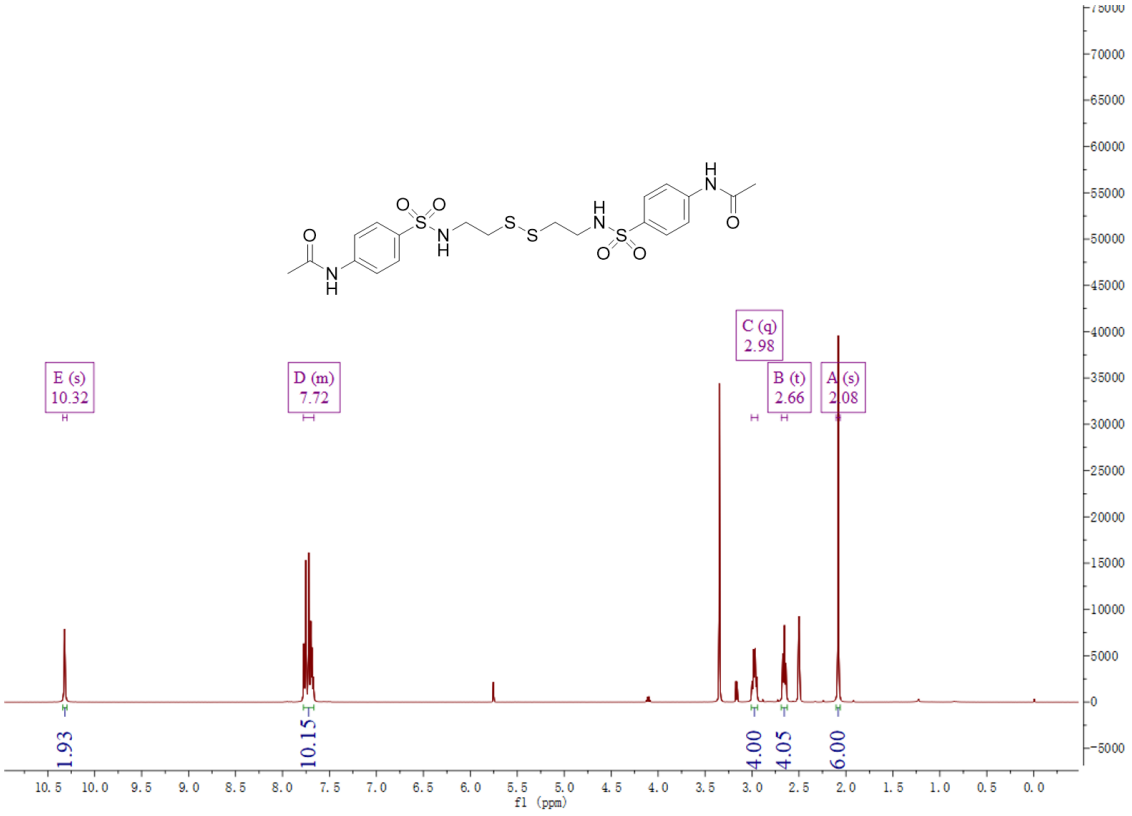
-157.36
f1 (ppm)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C (q)
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B (t)
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A (s)
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1.93
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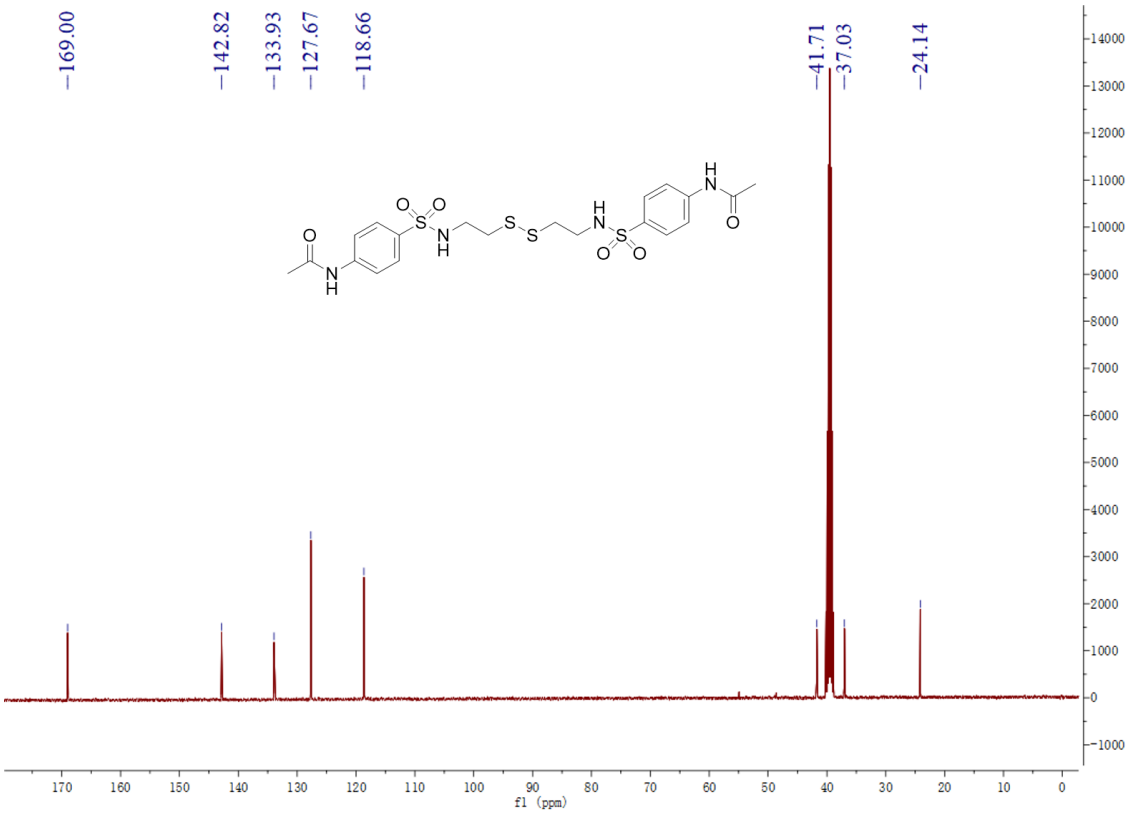
4.05
6.00
f1 (ppm)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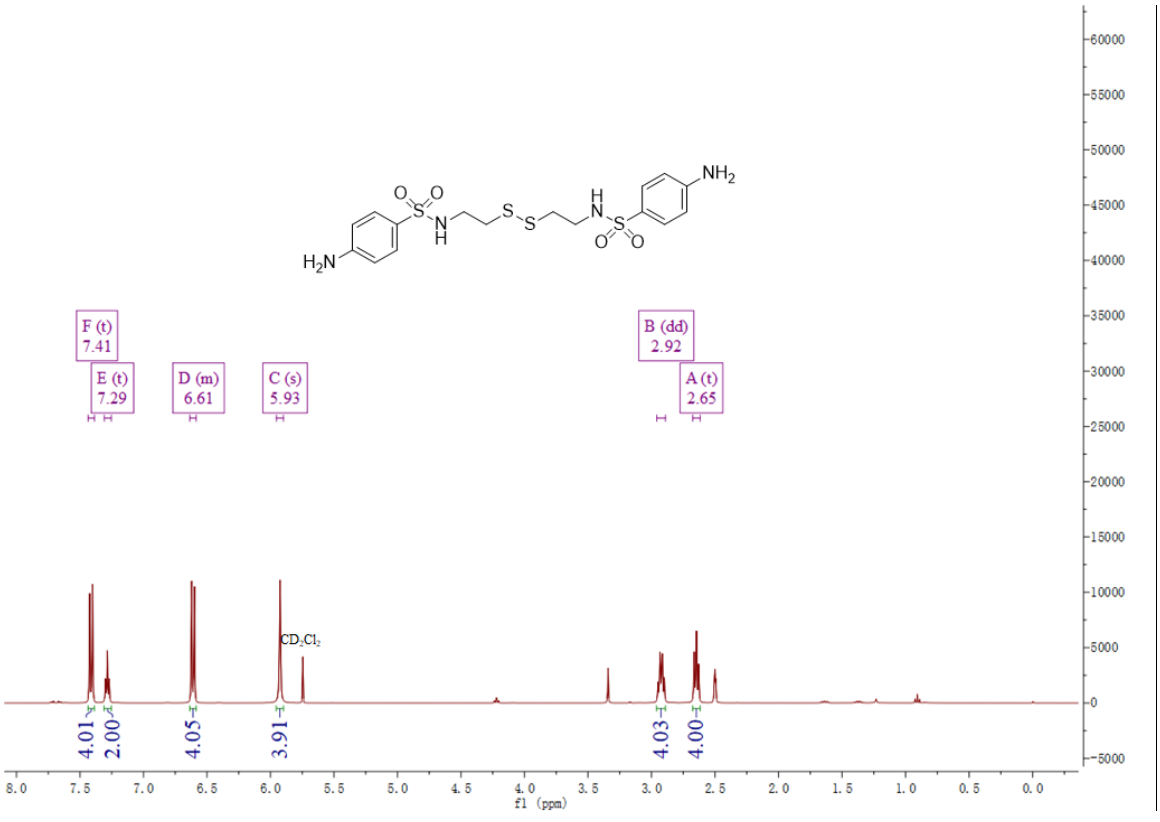
37.03
24.14
f1 (ppm)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E (t) 7.29
D (m) 6.61
C (s) 5.93
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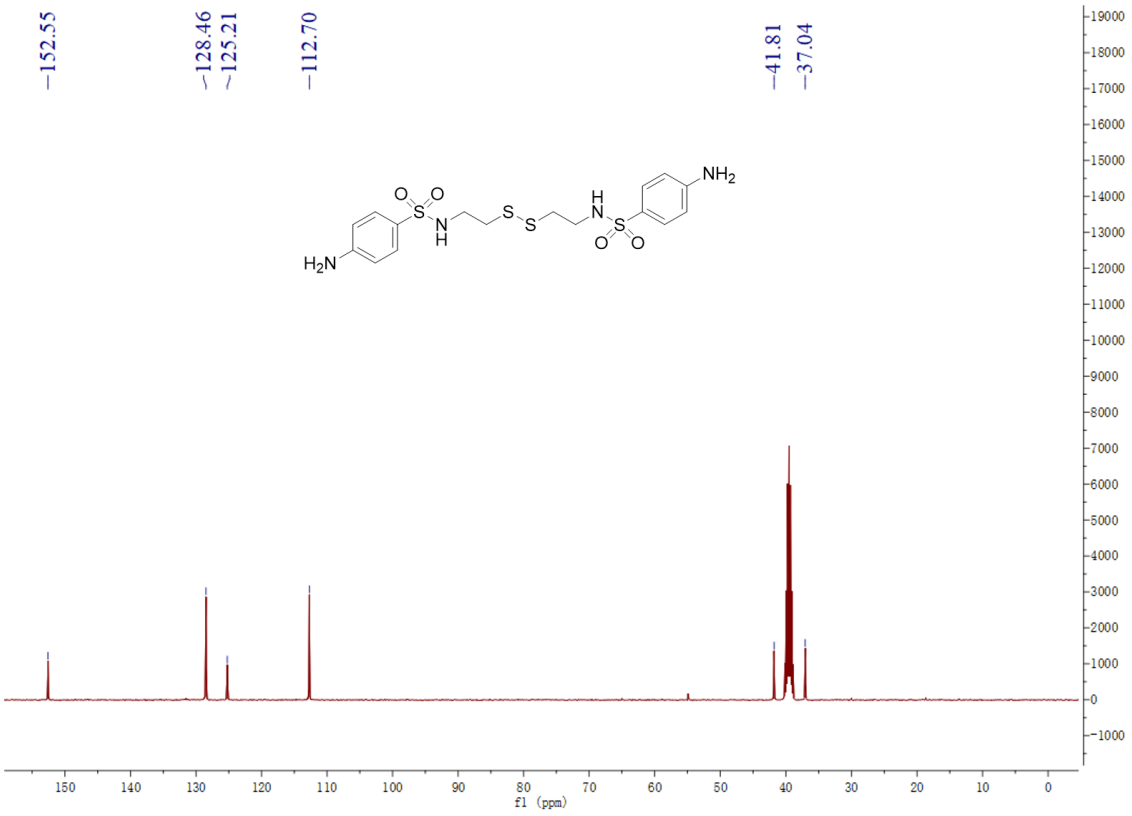
4.00
f1 (ppm)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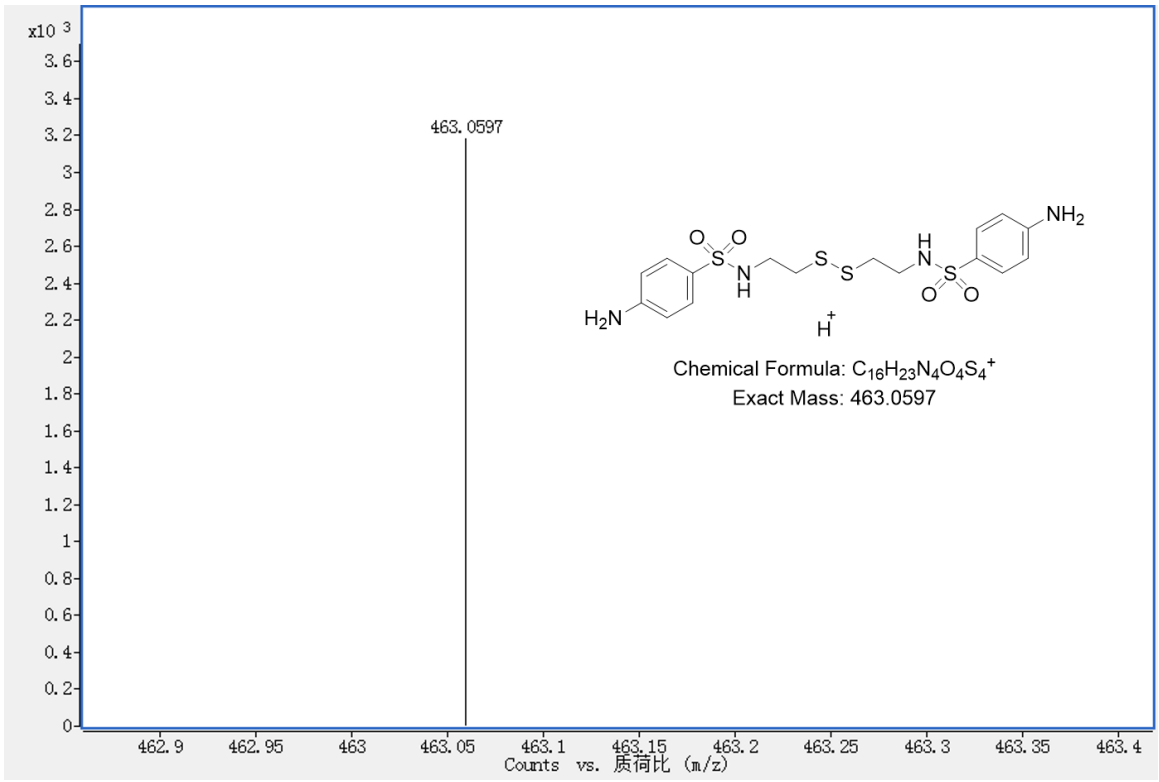
37.04
NH2
H2N
f1 (ppm)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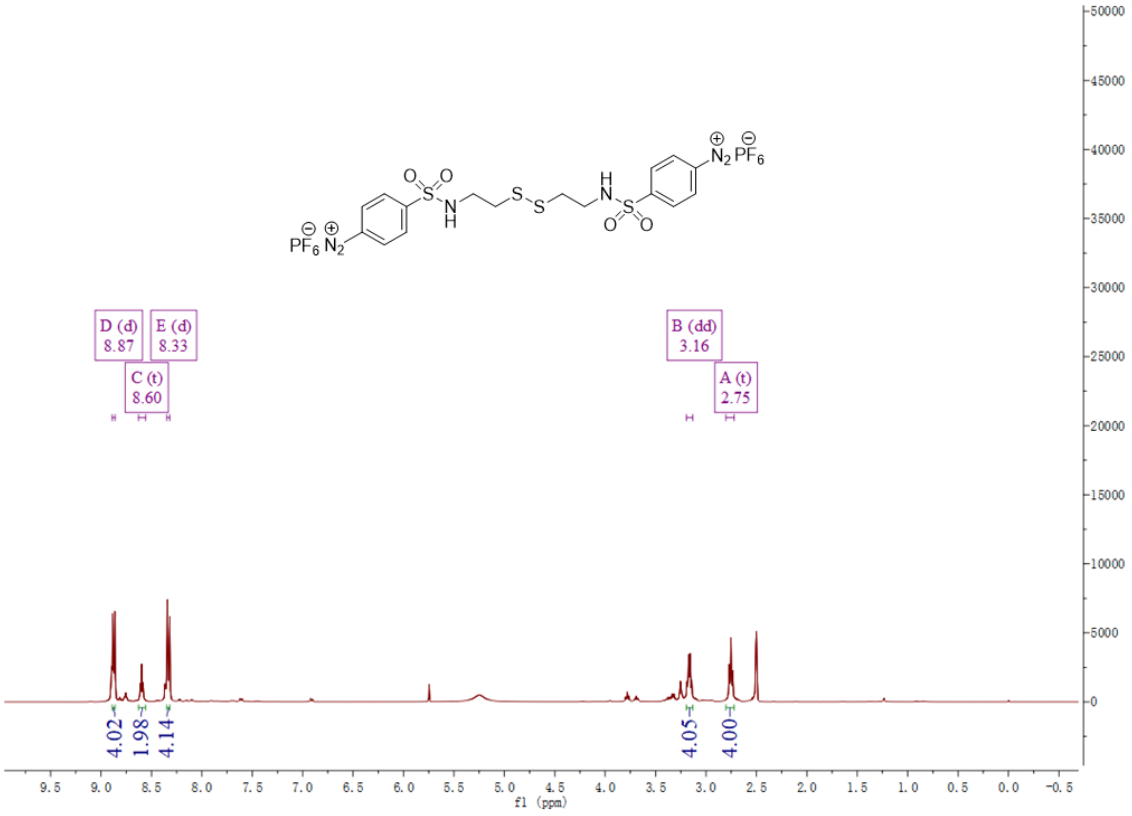
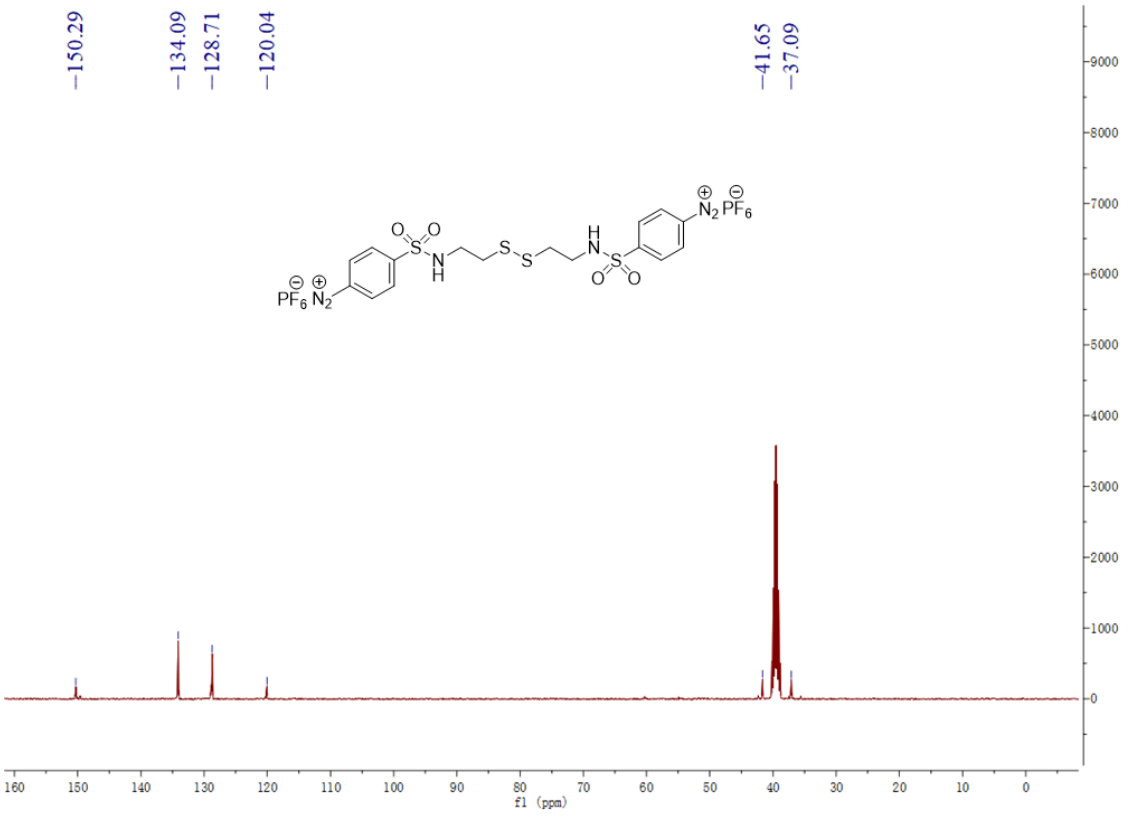
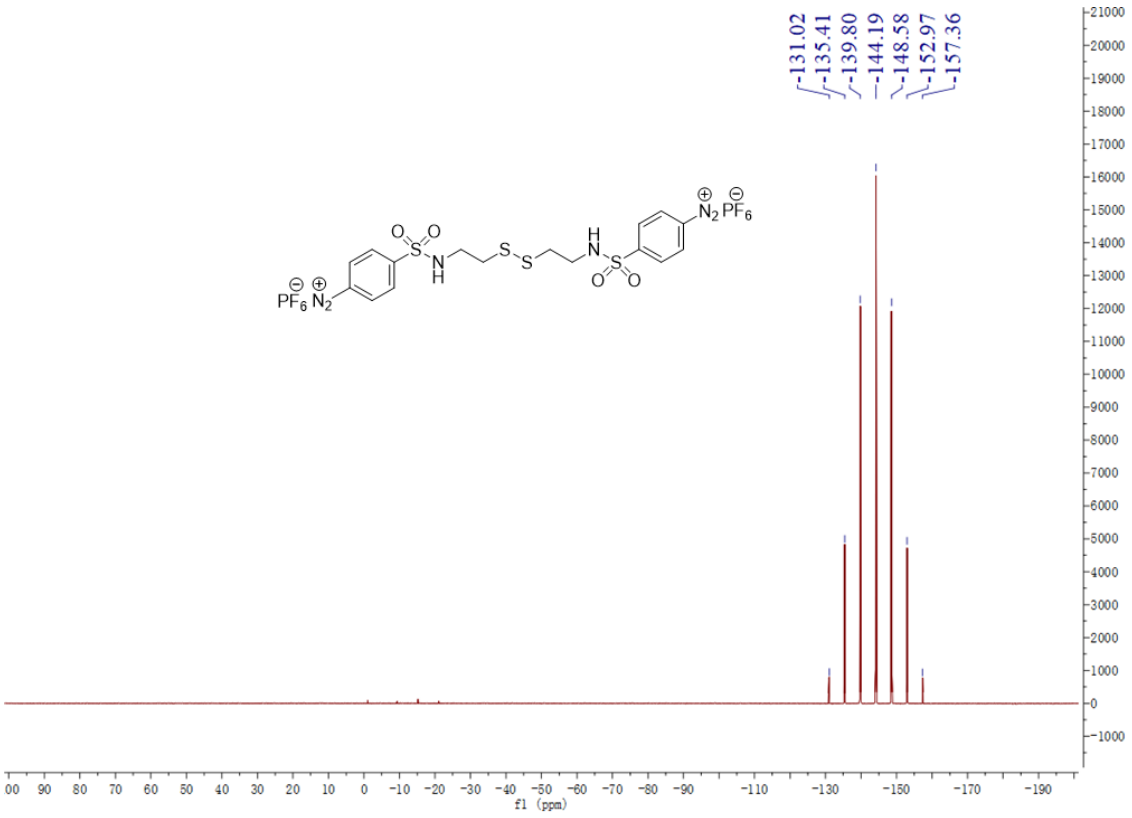
H+
Chemical Formula: C16H23N4O4S4+
Exact Mass: 463.0597
Counts vs. 质荷比 (m/z)


D (d)
8.87
E (d)
8.33
C (t)
8.60
B (dd)
3.16
A (t)
2.75
4.02
1.98
4.14
4.05
4.00
f1 (ppm)


150.29
134.09
128.71
120.04
41.65
37.09
f1 (ppm)


-131.02
-135.41
-139.80
-144.19
-148.58
-152.97
-157.36
f1 (ppm)