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Supporting Information

Aromatic Primary Monoamine-Based Fast-Response and Highly Specific Fluorescent Probes for Imaging Biological Signaling Molecule Nitric Oxide in Living cells and Organisms

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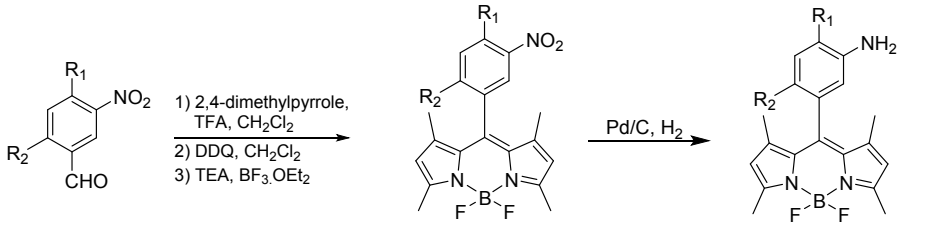
1. Synthesis of Compounds 1a-e



3a: $R_1 = R_2 = H$
3b: R_1 = OMe, $R_2 = H$
3c: $R_1 = H$, R_2 = OMe
3d: R_1 = OH, $R_2 = H$

2a: $R_1 = R_2 = H$
2b: R_1 = OMe, $R_2 = H$
2c: $R_1 = H$, R_2 = OMe
2d: R_1 = OH, $R_2 = H$

1a: $R_1 = R_2 = H$
1b: R_1 = OMe, $R_2 = H$
1c: $R_1 = H$, R_2 = OMe
1d: R_1 = OH, $R_2 = H$

1.1 Compound 1a

Compound **3a** (2.00 g, 13.2 mmol), 2,4-dimethylpyrrole (1.84 g, 26.5 mmol) and CH_2Cl_2 (400 mL) was added to a 1 L reaction flask. The mixture was stirred for 20 min at room temperature under nitrogen. Trifluroacetic acid (60 μL) was then added and stirred overnight. After TLC monitoring showed complete consumption of the starting material, a solution of DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoduinone) (3.00 g, 13.2 mmol) in 40 ml of CH_2Cl_2 was added, and stirring was continued for 2 h. The reaction mixture was washed with water, dried over Na_2SO_4, filtered and evaporated. The residue and triethylamine (26 mL) were dissolved in 100 mL of anhydrous CH_2Cl_2, and the solution was stirred at room temperature for 30 min. BF_3•OEt_2 (26 mL) was added, and stirring was continued for 10 min. The reaction mixture was washed with water and 2 N NaOH. The aqueous solution was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The crude compound was purified by column chromatography (EtOAc/PE = 1:10) to afford compound **2a** as an orange solid. ^{1}H NMR (600 MHz, DMSO) δ 8.42 (d, J = 8.3 Hz, 1H), 8.28 (s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.87 (t, J = 7.9 Hz, 1H), 6.22 (s, 2H), 2.47 (s, 6H), 1.34 (s, 6H);^{13}C NMR (151 MHz, DMSO) δ 156.14, 148.95, 143.04, 139.18, 135.87, 135.43, 131.59, 130.96, 124.71, 123.69, 122.29, 14.91, 14.82.

A mixture of **2a** (500 mg, 1.47 mmol) and 10% Pd-C (100 mg) in 50 mL CH_2Cl_2 was stirred at room temperature under H_2 for 5 h. The reaction mixture was filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel

flash chromatography (EtOAc/CH_2Cl_2 = 1:3) to give compound **1a** as an orange solid. Yield: 77%. ^{1}H NMR (600 MHz, DMSO) δ 7.19 (t, J = 7.8 Hz, 1H), 6.69 (dd, J = 8.1, 1.6 Hz, 1H), 6.47 (d, J = 1.8 Hz, 1H), 6.42 (d, J = 7.4 Hz, 1H), 6.16 (s, 2H), 5.33 (s, 2H), 2.44 (s, 6H), 1.52 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 154.92, 150.16, 143.57, 143.23, 134.91, 131.02, 130.33, 121.57, 114.87, 114.68, 112.84, 14.67, 14.29 ; ESI-MS: calcd for [M+H$^+$] 340.1791, Found 340.1794.

1.2 Compound 1b

Compound **2b** was synthesized starting from compound **3b** according to the similar procedure to that of **2a**. Yield: 62%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.48 (d, J = 7.9 Hz, 1H), 7.24 (d, J = 8.5 Hz, 1H), 6.02 (s, 2H), 4.05 (s, 3H), 2.56 (s, 6H), 1.46 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.47, 153.27, 142.59, 140.04, 137.87, 134.01, 131.45, 127.04, 125.85, 121.78, 114.19, 56.73, 15.04, 14.66.

Compound **1b** was synthesized starting from compound **2b** according to the similar procedure to that of **1a**. Yield: 65%. ^{1}H NMR (600 MHz, DMSO) δ 6.94 (d, J = 7.8 Hz, 1H), 6.54 (s, 1H), 6.45 (d, J = 7.2 Hz, 1H), 6.16 (s, 2H), 4.97 (s, 2H), 3.83 (s, 3H), 2.44 (s, 6H), 1.51 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 154.73, 147.23, 143.79, 143.26, 139.18, 131.47, 126.63, 121.47, 115.37, 112.88, 111.18, 55.75, 14.66, 14.46; ESI-MS: calcd for [M+H$^+$] 370.1897, Found 370.1902.

1.3 Compound 1c (also named MA)

Compound **2c** was synthesized starting from compound **3c** according to the similar procedure to that of **2a**. ^{1}H NMR (600 MHz, DMSO) δ 8.47 (dd, J = 9.2, 2.8 Hz, 1H), 8.15 (d, J = 2.8 Hz, 1H), 7.45 (d, J = 9.3 Hz, 1H), 6.20 (s, 2H), 3.94 (s, 3H), 2.46 (s, 6H), 1.42 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 161.93, 155.80, 142.50, 142.00, 136.13, 130.97, 127.85, 125.75, 123.74, 122.00, 113.09, 14.73, 14.29.

Compound **1c** (also named **MA**) was synthesized starting from compound **2c** according to the similar procedure to that of **1a**. Yield: 72%. ^{1}H NMR (600 MHz, DMSO) δ 6.91 (d, J = 8.9 Hz, 1H), 6.71 (dd, J = 8.8, 2.8 Hz, 1H), 6.39 (d, J = 2.8 Hz, 1H), 6.14 (s, 2H), 4.84 (s, 2H), 3.62 (s, 3H), 2.43 (s, 6H), 1.52 (s, 6H); ^{13}C NMR (151

MHz, DMSO) δ 157.48, 150.02, 146.93, 145.74, 143.49, 134.20, 126.19, 124.19, 119.25, 117.55, 116.56, 59.18, 17.52, 16.73; ESI-MS: calcd for [M+H$^+$] 370.1897, Found 370.1898.

1.4 Compound 1d

Compound **2d** was synthesized starting from compound **3d** according to the similar procedure to that of **2a**. ^{1}H NMR (600 MHz, DMSO) δ 11.49 (s, 1H), 7.91 (s, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H), 6.21 (s, 2H), 2.45 (s, 6H), 1.47 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 155.75, 152.66, 143.03, 139.75, 138.30, 134.90, 131.41, 125.48, 124.82, 122.08, 120.40, 15.02, 14.71.

Compound **1d** was synthesized starting from compound **2d** according to the similar procedure to that of **1a**. ^{1}H NMR (600 MHz, DMSO) δ 9.20 (s, 1H), 6.77 (d, J = 7.7 Hz, 1H), 6.49 (s, 1H), 6.29 (d, J = 7.8 Hz, 1H), 5.96 (s, 2H), 4.51 (s, 2H), 2.41 (s, 6H), 1.50 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 154.26, 145.26, 143.88, 143.34, 137.20, 131.68, 125.49, 120.91, 116.43, 115.23, 113.97, 14.51, 14.56; ESI-MS: calcd for [M+H$^+$] 356.1740, Found 356.1745.

1.5 Compound 1e

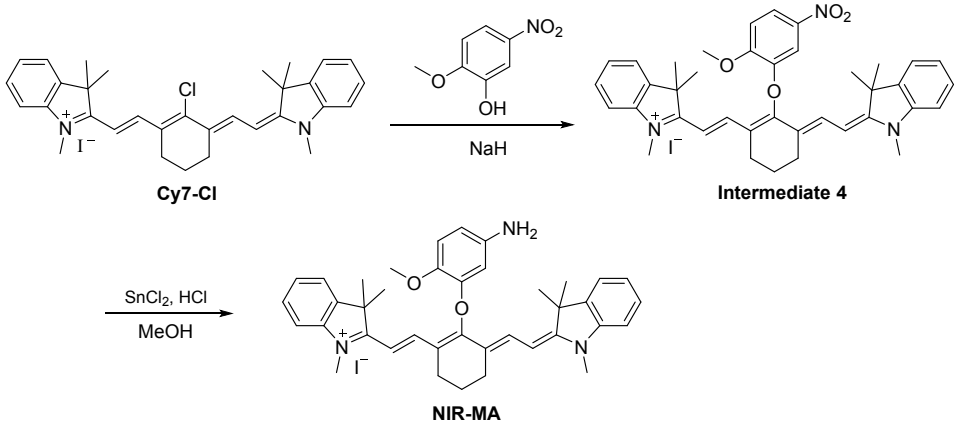
Compound **1e** was synthesized according to a reported procedure by us.[1] Yield: 77%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.77 (s, 1H), 6.70 (d, J = 8.6 Hz, 1H), 6.57 (dd, J_1= 8.6, J_2=2.7 Hz, 1H), 6.30 (d, J = 2.6 Hz, 1H), 6.13 (s, 2H), 4.64 (br, 2H), 2.43 (s, 6H), 1.58 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.16, 147.65, 145.85, 145.58, 144.30, 134.33, 124.36, 123.98, 120.31, 119.86, 116.96, 17.55, 16.77; ESI-MS: calcd for [M+H$^+$] 356.1740, Found 356.1745.

1.6 The deamination product dA-1c (also named dA-MA)

A THF solution of **1c** (40 mg, 0.108 mmol) was bubbled NO gas under aerobic conditions at room temperature. The reaction was monitored using TLC to ensure the complete consumption of **1c**. Then the solvent was removed on a rotary evaporator. The residue was dissolved in CH_2Cl_2, and the organic phase washed three times with water, and dried over $MgSO_4$. After the solvent was removed on a rotary evaporator,

dA-1c was obtained as a yellow powder. Yield: 95%. ^{1}H NMR (600 MHz, CDCl3) δ 7.44 (s, 1H), 7.13 (s, 1H), 7.08 (d, J = 4.6 Hz, 1H), 6.99 (d, J = 6.3 Hz, 1H), 5.96 (s, 2H), 3.77 (s, 3H), 2.55 (s, 6H), 1.43 (s, 6H); ^{13}C NMR (151 MHz, CDCl3) δ 156.43, 154.93, 142.60, 138.96, 131.57, 130.62, 129.54, 123.83, 121.50, 120.85, 111.16, 55.62, 14.59, 13.81; ESI-MS: calcd for [M+H^{+}] 355.1788, Found 355.1785.

1.7 Compound NIR-MA



2-methoxy-5-nitrophenol (85 mg, 0.5 mmol) and NaH (60% in mineral oil) (20 mg, 0.5 mmol) were dissolved in anhydrous N,N-dimethylformamide (DMF) (15 mL). The mixture was stirred at room temperature for 10 min under an N_2 atmosphere. Then a solution of **Cy7-Cl** (122 mg, 0.20 mmol) in anhydrous DMF (2 mL) was added to the mixture in via a syringe. The reaction mixture was further stirred for 4 h. The solvent was removed under reduced pressure, then the crude product was purified by silica gel chromatography with MeOH/DCM=1/50 to afford intermediate **4** as a dark green solid. Yield: 60%. ^{1}H NMR (600 MHz, DMSO) δ 8.07 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 14.4 Hz, 2H), 7.55 – 7.49 (m, 4H), 7.39 (s, 4H), 7.23 (s, 2H), 6.23 (d, J = 14.3 Hz, 2H), 4.23 (s, 3H), 3.63 (s, 6H), 2.76 (s, 4H), 1.94 (s, 2H), 1.36 – 1.28 (m, 12H); ^{13}C NMR (151 MHz, DMSO) δ 172.61, 167.45, 161.60, 154.27, 147.82, 143.23, 141.38, 141.03, 139.91, 132.12, 128.93, 125.42, 122.72, 121.27, 120.21, 113.21, 111.76, 108.70, 101.58, 63.47, 48.96, 30.26, 29.11, 27.37, 23.48. ESI-MS: calcd for [M-I^{-}]$^{+}$ 616.3175, Found 616.3174.

To a solution of **4** (82 mg, 0.11 mmol) in MeOH (4 mL) and concentrated HCl (0.6

mL) was added $SnCl_2$-H_2O (500 mg, 2.2 mmol). The solution was stirred at room temperature overnight under an N_2 atmosphere, then neutralized with 2 N NaOH. The precipitate was removed by filtration and washed with DCM. The filtrate and washing were extracted with DCM and the organic phase was washed with water. The organic extract was dried over Na_2SO_4, filtered and evaporated to give a dark green solid. The crude product was purified by silica gel chromatography with MeOH/DCM=1/20 to afford **NIR-MA** as dark green solid. Yield: 25%. ^{1}H NMR (600 MHz, MeOD) δ 8.04 (d, J = 14.2 Hz, 2H), 7.43 – 7.37 (m, 4H), 7.27 (d, J = 8.0 Hz, 2H), 7.23 (t, J = 7.4 Hz, 2H), 6.99 (d, J = 8.7 Hz, 1H), 6.40 (dd, J = 8.7, 2.5 Hz, 1H), 6.33 (d, J = 2.4 Hz, 1H), 6.14 (d, J = 14.2 Hz, 2H), 4.02 (s, 3H), 3.61 (s, 6H), 2.75 (t, J = 5.9 Hz, 4H), 2.10 – 2.04 (br, 2H), 1.45 (s, 12H); ^{13}C NMR (151 MHz, MeOD) δ 177.15, 168.95, 154.65, 148.81, 148.03, 146.14, 145.98, 145.85, 143.98, 133.60, 129.88, 127.56, 127.44, 126.8, 120.56, 116.22, 112.68, 105.75, 105.58, 62.33, 53.65, 36.36, 35.00, 32.24, 29.09. ESI-MS: calcd for $[M\text{-}I^-]^+$ 586.3434, Found 586.3437.

2. Supplementary Spectra

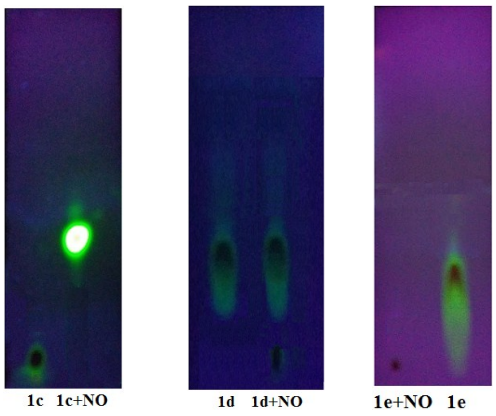


Figure S1 TLC analysis of the reaction products of **1c**, **1d**, and **1e** with NO, obtained by bubbling excess NO gas to their THF solutions, respectively.

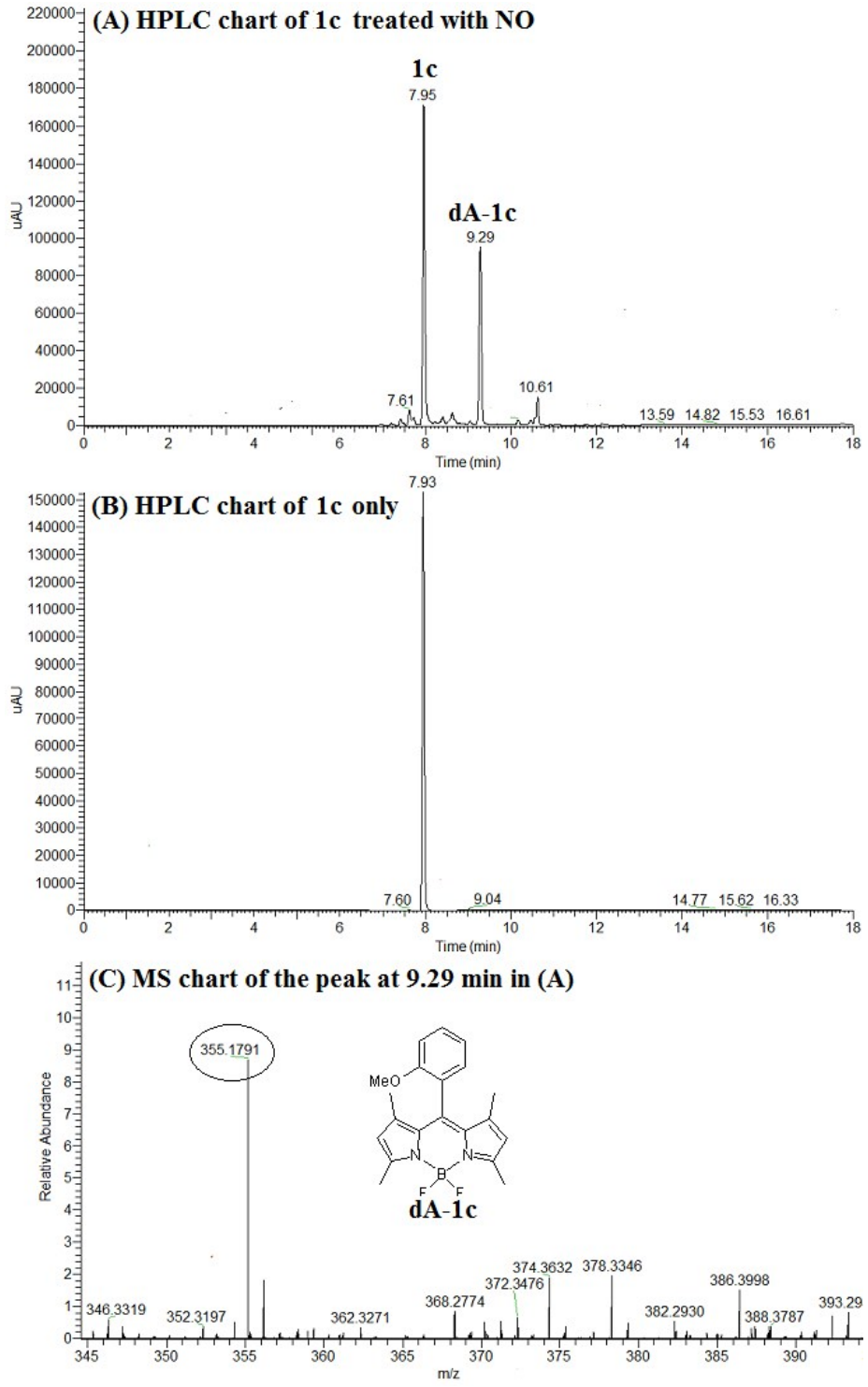


Figure S2 HPLC-MS assay of **1c** treated with NO solution.

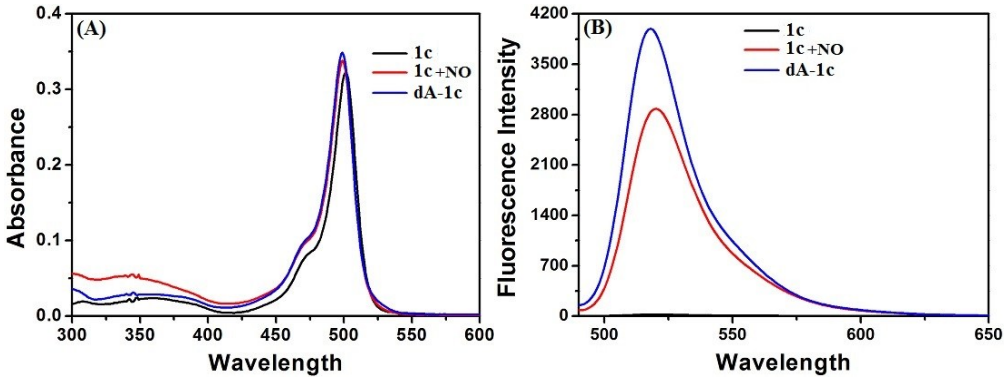


Figure S3 The absorption (A) and fluorescence (B) spectra of **1c**, **1c** treated with NO, and **dA-1c**. Conditions: PBS buffer (50 mM, pH 7.4, containing 20% DMSO); λ_{ex} = 475 nm; λ_{em} = 519 nm; Slits: 5/5 nm; voltage: 650 V; T = 25 °C.

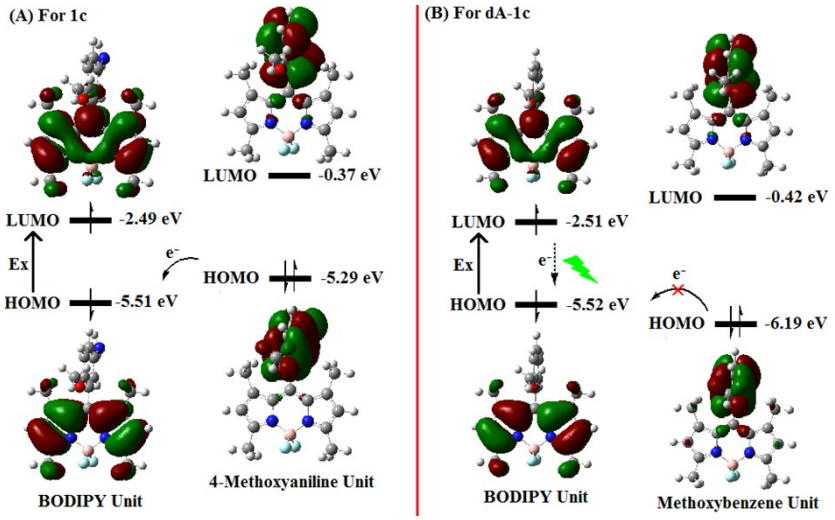
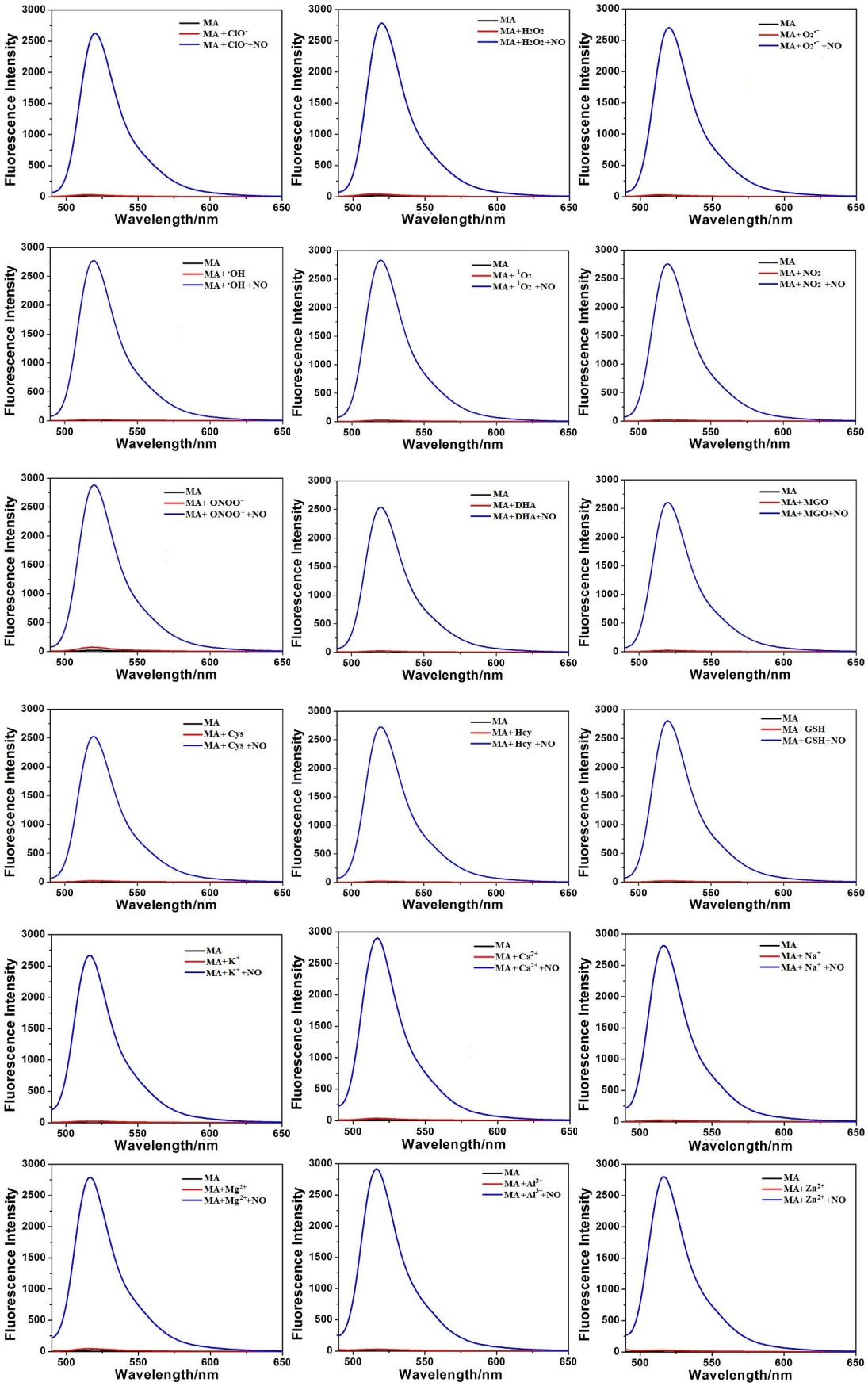


Figure S4 (A) Frontier orbital energy representation of the PeT process in **1c**. (B) Frontier orbital energy representation of the PeT inhibition in **dA-1c**.

Note that: compound 1c and its deamination product dA-1c were named as "MA" and "dA-MA" afterward.



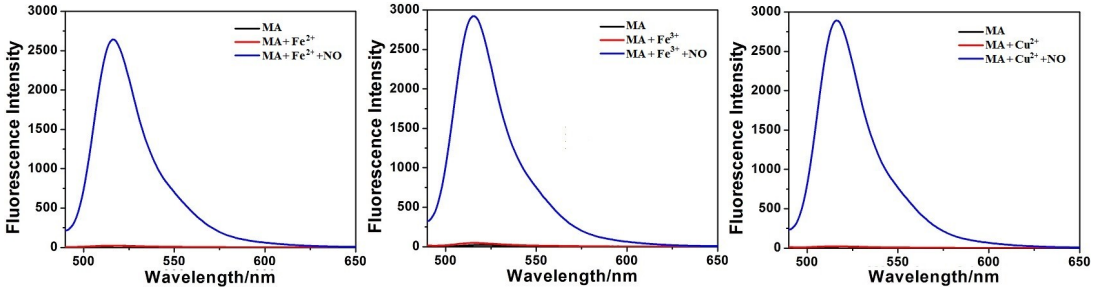


Figure S5 Fluorescence spectra of **MA** (4 μM) treated with various ROS (40 μM), DHA/MGO (1 mM), Cys/Hcy (100 μM), GSH (1 mM), and various metal ions (100 μM) for 10 min, respectively, and then treated with NO (40 μM) for 10 min in PBS buffer (50 mM, pH 7.4, **containing 20% DMSO**). λ_{ex} = 475 nm; λ_{em} = 519 nm; Slits: 5/5 nm; voltage: 650 V; T = 25 °C.

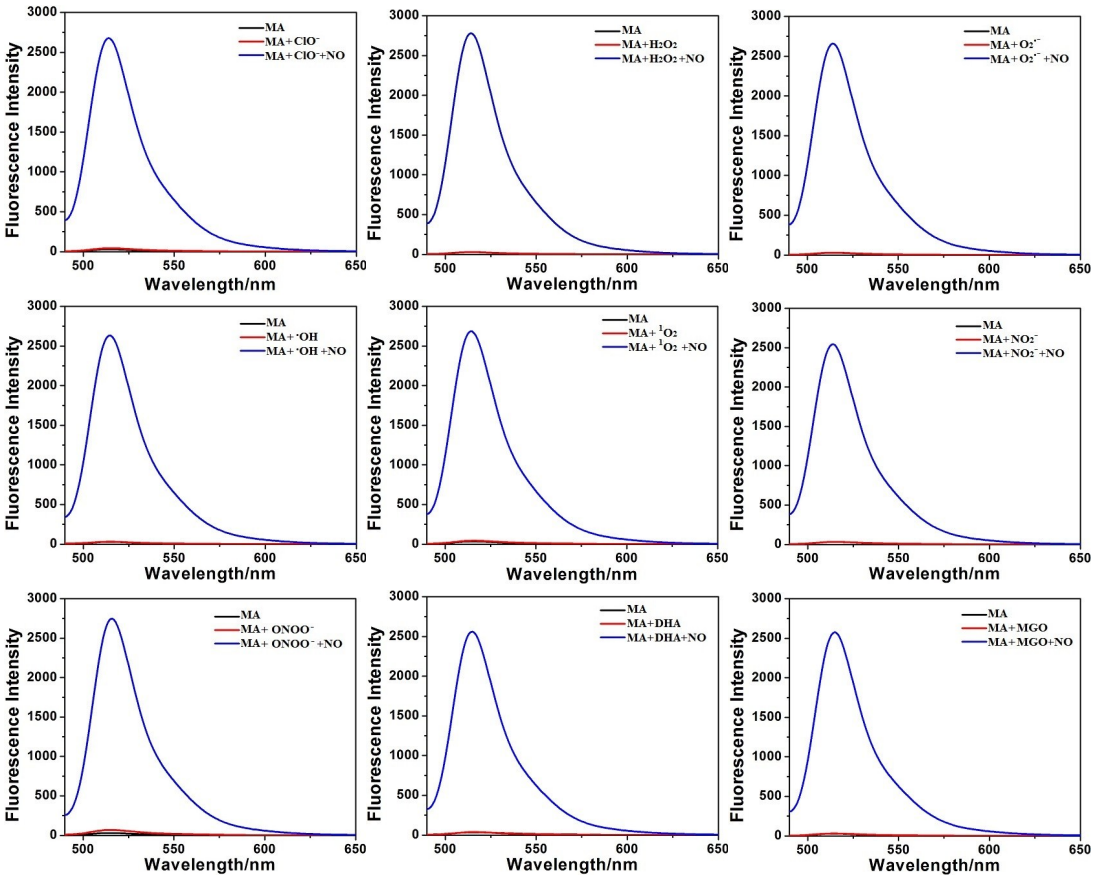


Figure S6 Fluorescence spectra of **MA** (4 μM) treated with various ROS (40 μM) and DHA/MGO (1 mM) for 10 min, respectively, and then treated with NO (40 μM) for 10 min in PBS buffer (50 mM, pH 7.4, **containing 20% CH_3CN**). λ_{ex} = 475 nm; λ_{em} = 519 nm; Slits: 5/5 nm; voltage: 650 V; T = 25 °C.

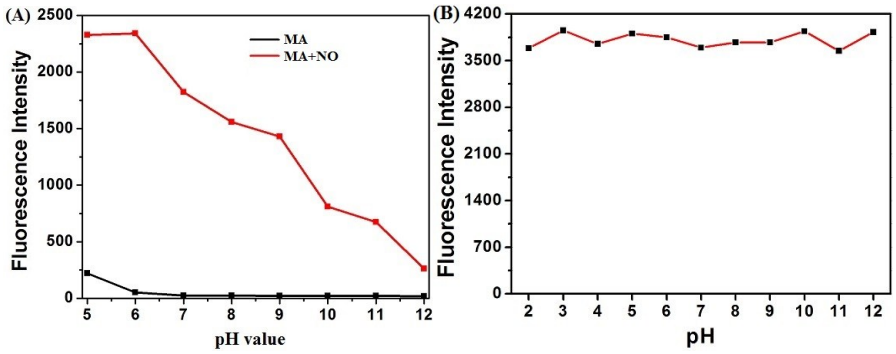


Figure S7 (A) Fluorescence intensities of **MA** in the absence and presence of 10 equiv of NO at different pH values. Note that the decreased fluorescence intensities in the high pH values are possibly because the reactive species N_2O_3 (nitrous anhydride), produced via the reaction of NO with O_2, can easily be converted to NO_2^- in the basic condition, and thus loses its reactivity with MA. (B) Fluorescence intensities of **dA-MA** at different pH values.

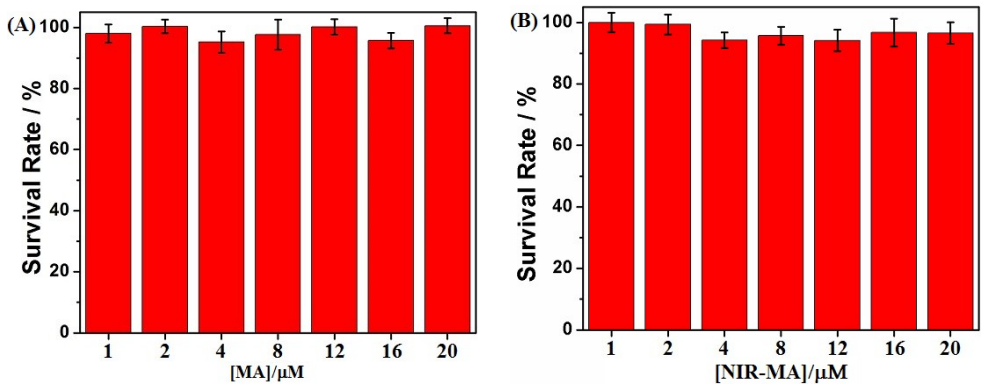


Figure S8 Percentage of viable HeLa cells after treated with increasing concentrations of **MA** (A) or **NIR-MA** (B) for 24 hours.

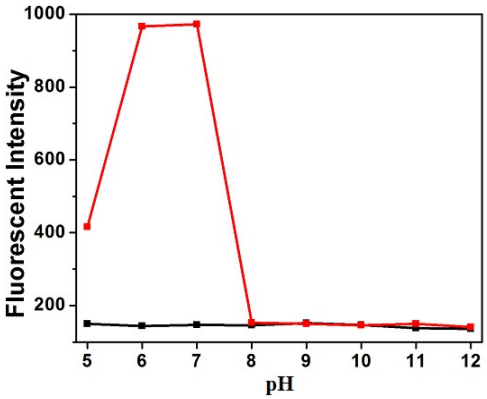


Figure S9 Fluorescence intensities of **NIR-MA** in the absence and presence of 10 equiv of NO at different pH values.

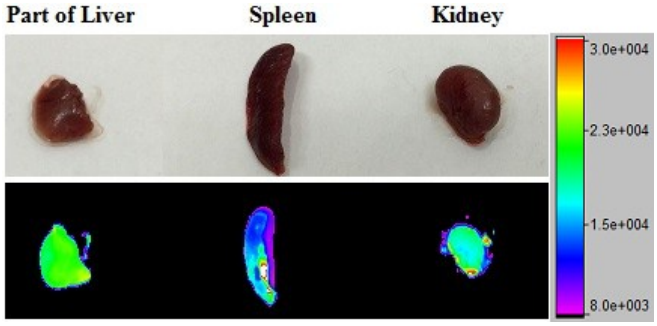


Figure S10 The brightfield images (above) and fluorescence images (below) of the dissected organs from the abdominal region of an inflamed mouse model treated with **NIR-MA**. The mouse was first i.p. injected with LPS (1 mg/ml, 100 μL) for 24 h, and then i.p. injected with **NIR-MA** (100 μL, 10 μM) for 30 min. Images were obtained by Bruker In-Vivo FX Pro small animal optical imaging system with an excitation filter 720 nm and an emission filter 790 nm.

3. ^{1}H NMR, ^{13}C NMR and HRMS Charts

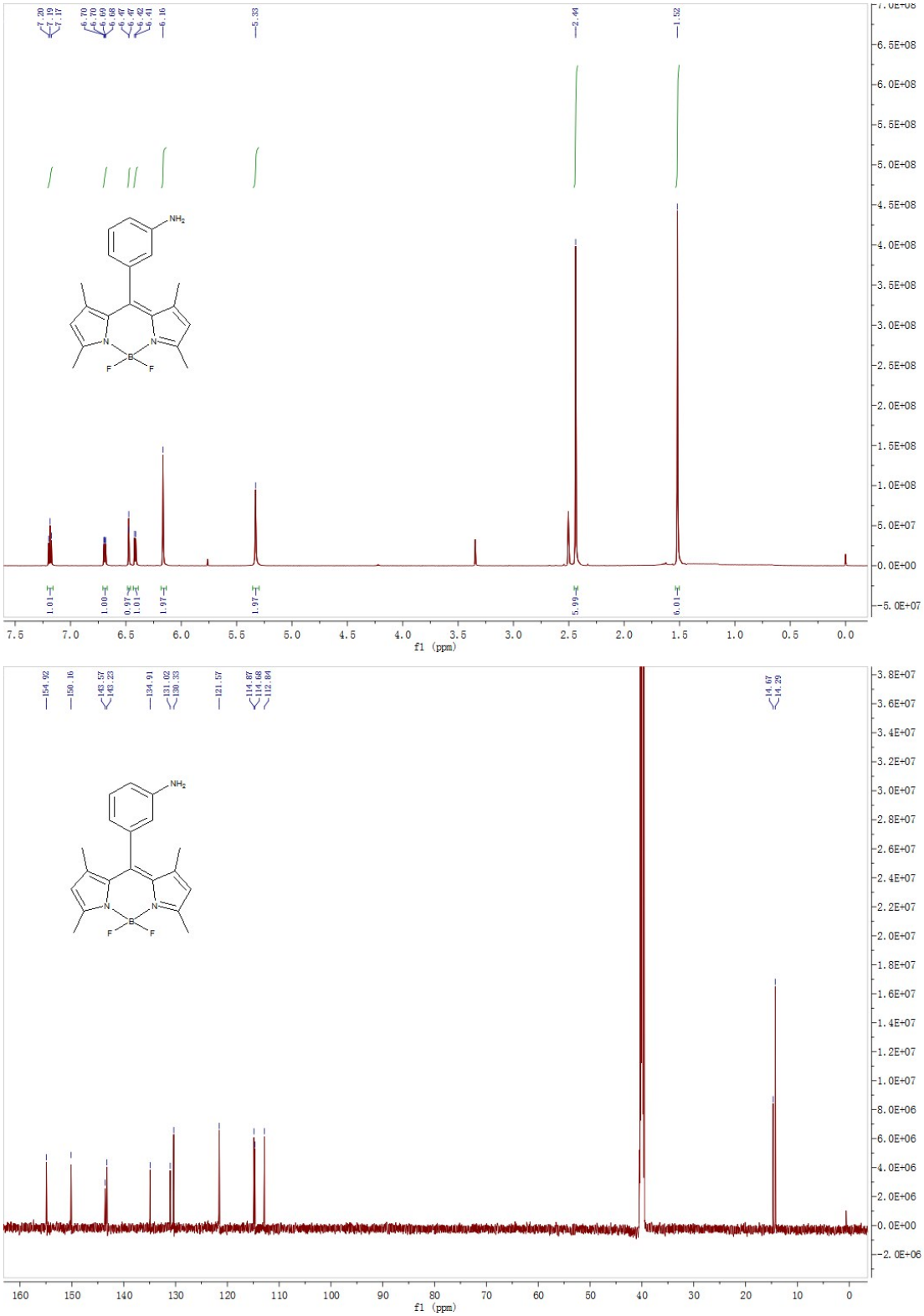


Figure S11. ^{1}H NMR and ^{13}C NMR charts of **1a** (DMSO-d_6, 600 MHz).

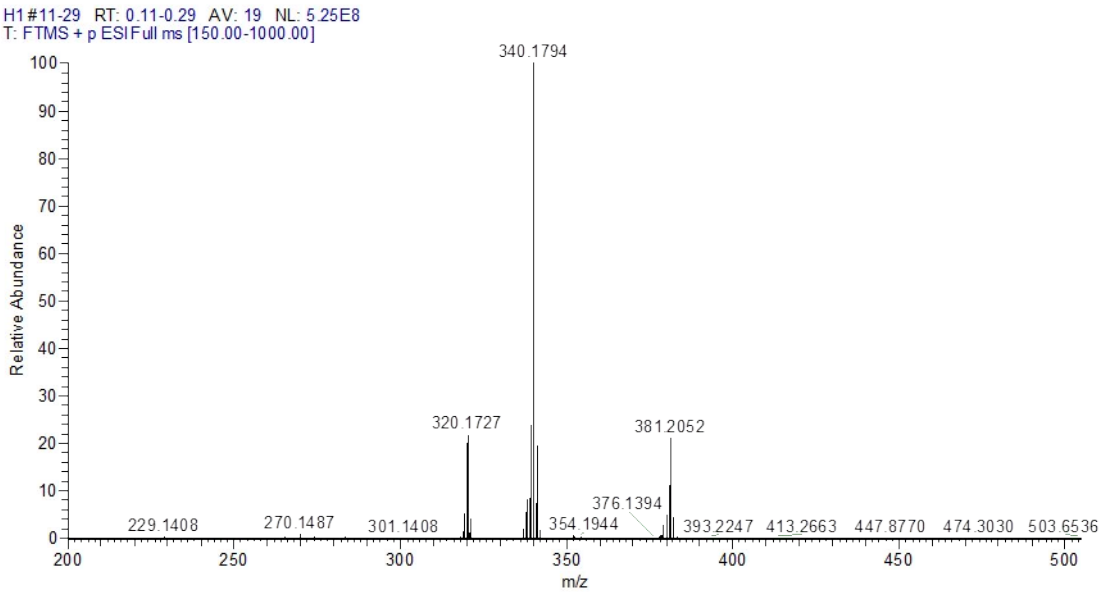


Figure S12. HRMS chart of compound **1a**.

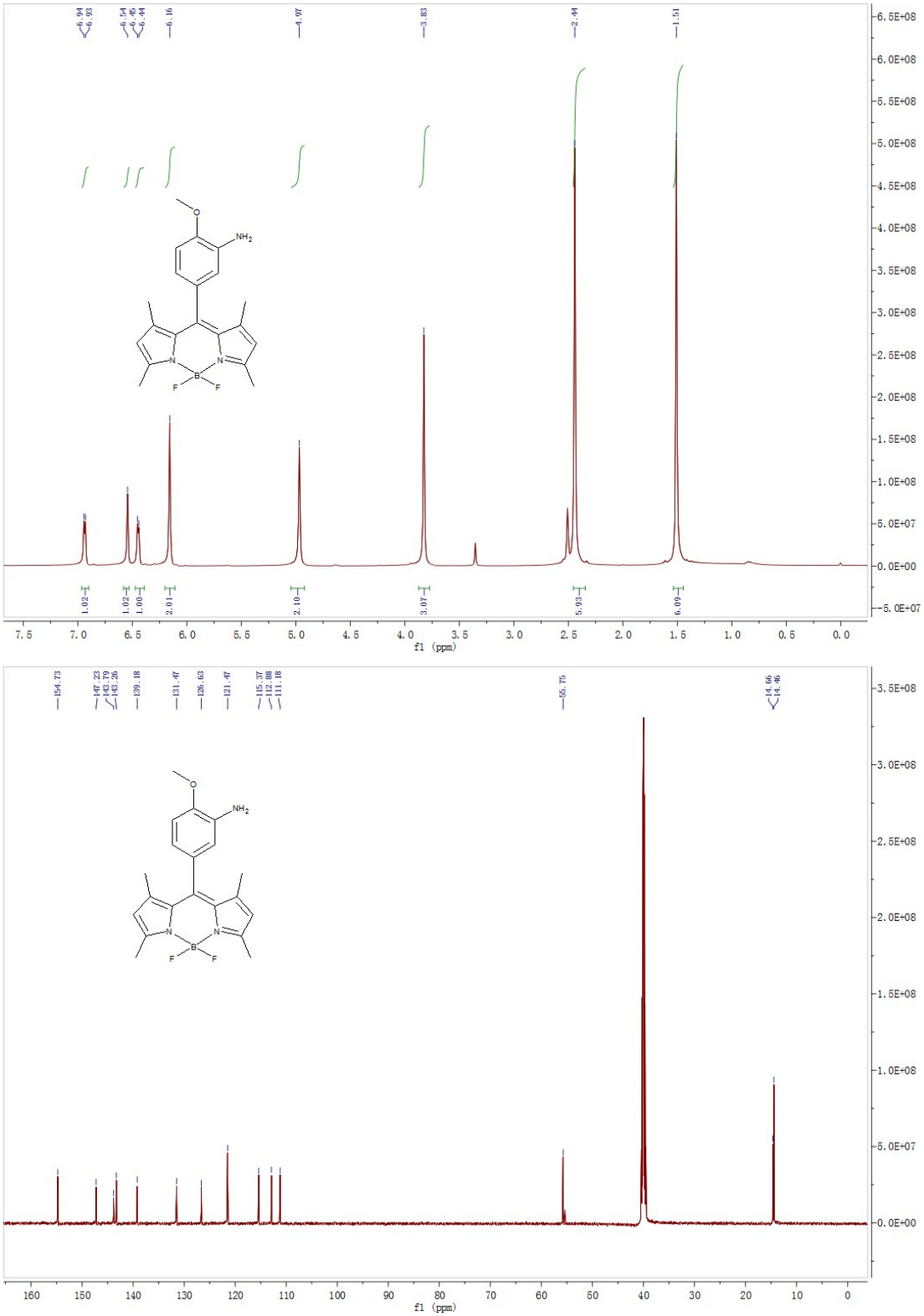


Figure S13. ^{1}H NMR and ^{13}C NMR charts of **1b** (DMSO-d_6, 600 MHz).

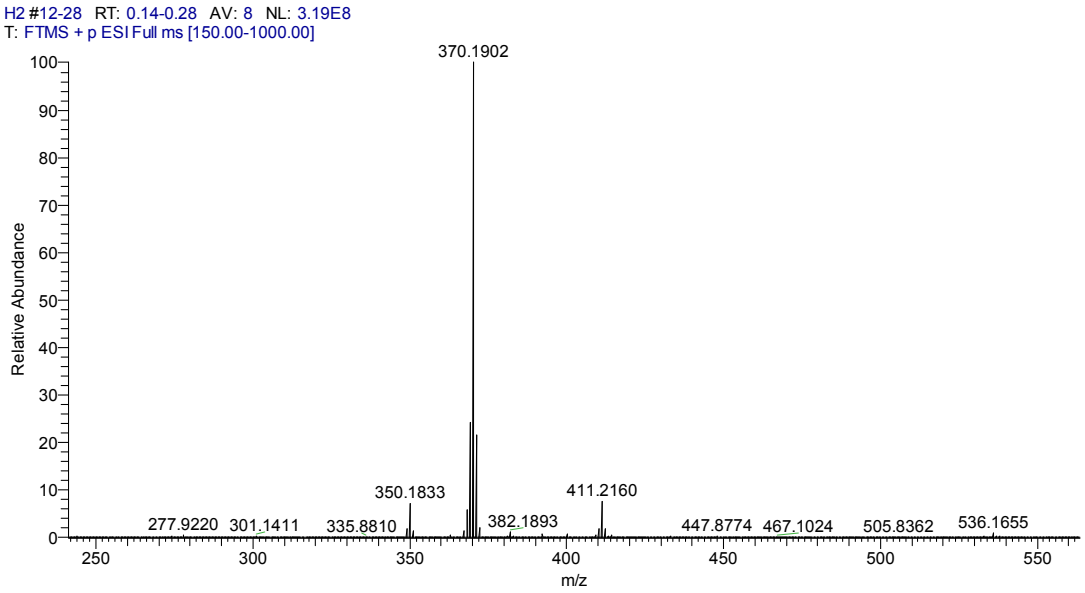


Figure S14. HRMS chart of compound **1b**.

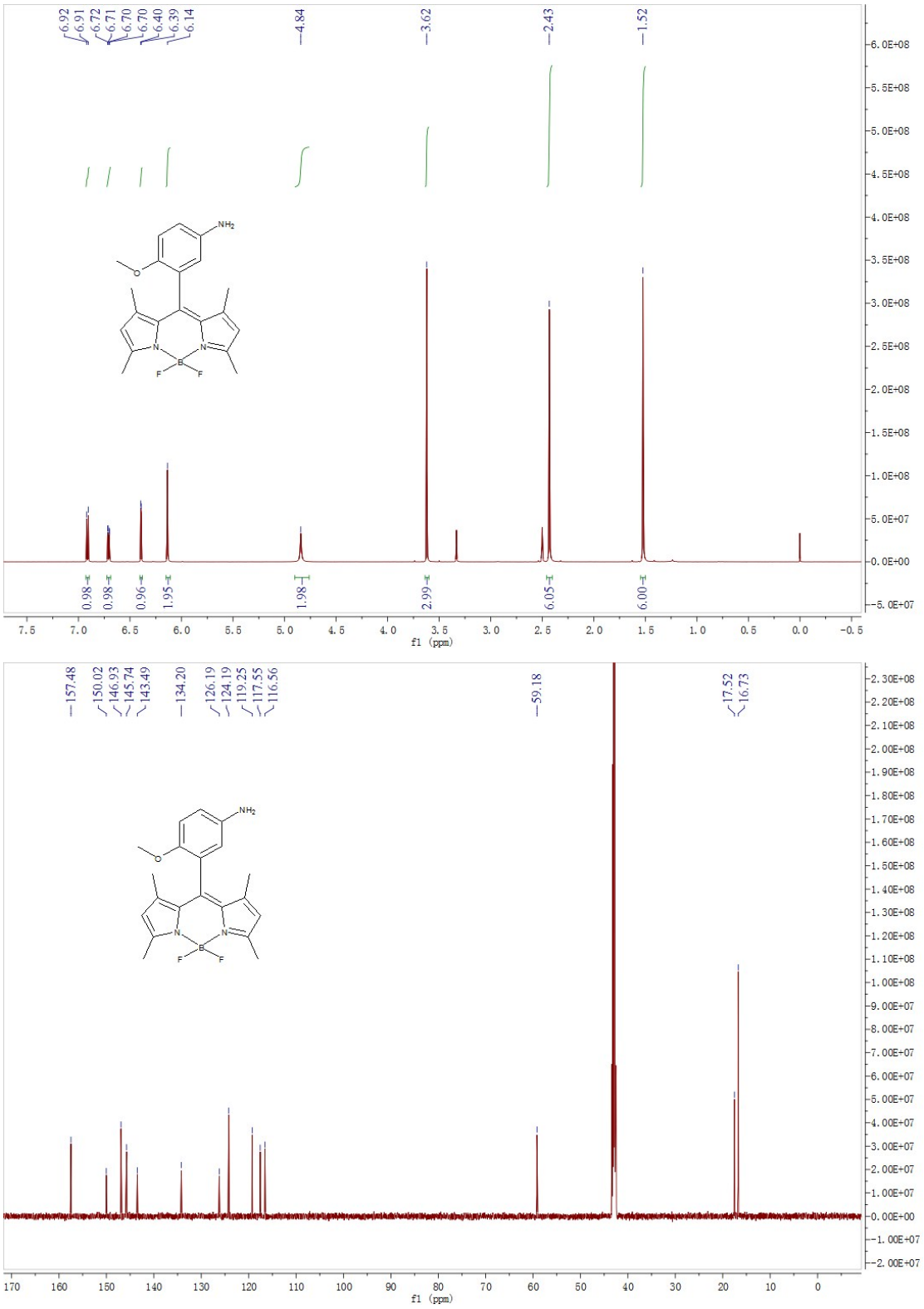


Figure S15. ^{1}H NMR and ^{13}C NMR charts of **1c** (**MA**) (DMSO-d_6, 600 MHz).

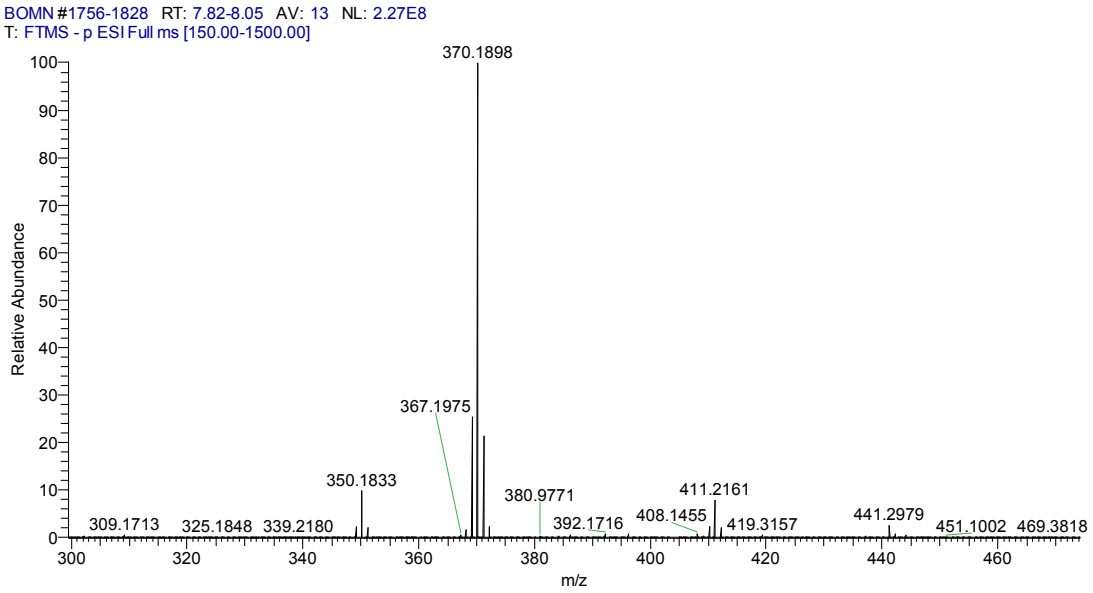


Figure S16. HRMS chart of **1c** (**MA**).

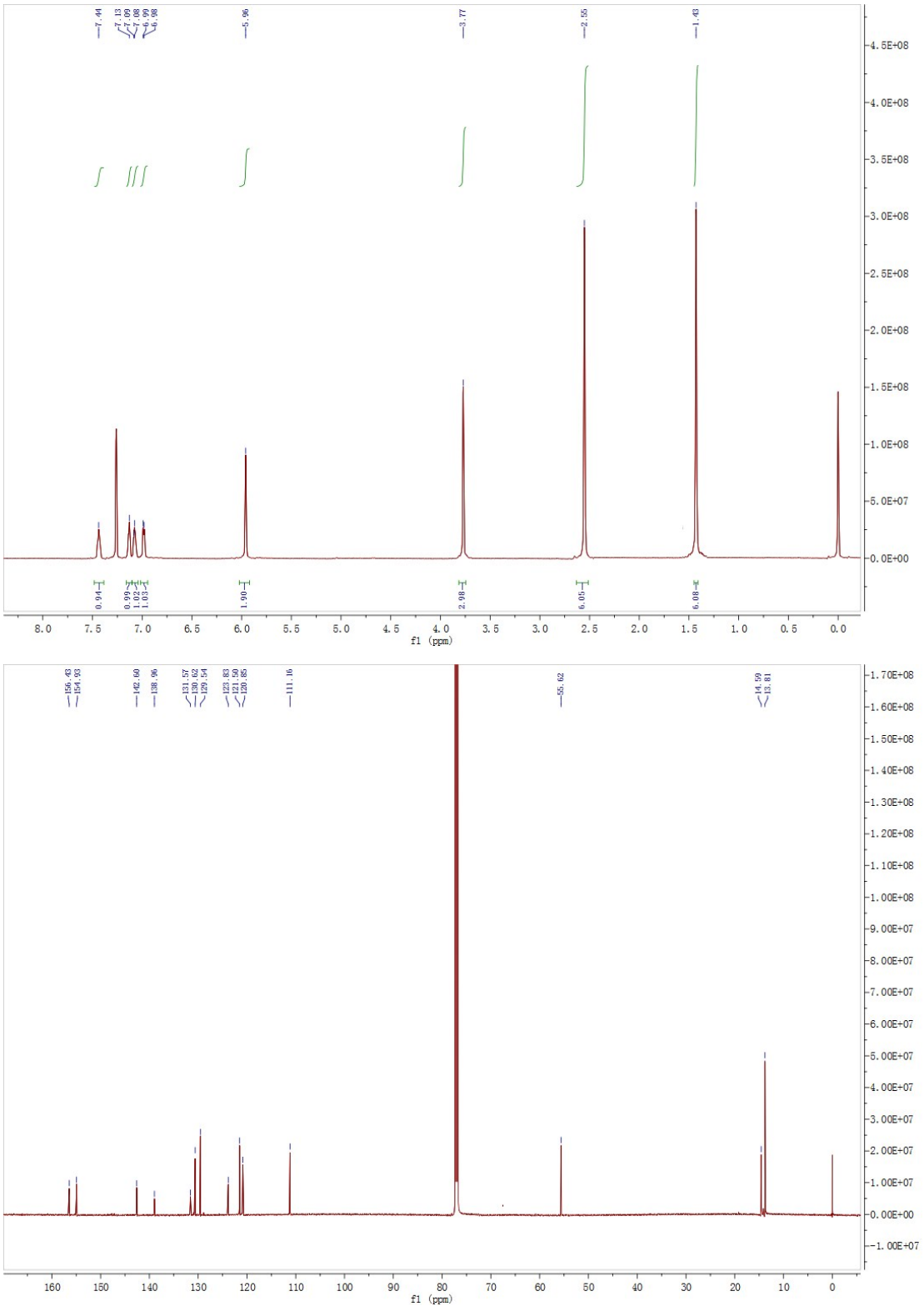


Figure S17. ^{1}H NMR and ^{13}C NMR charts of **dA-1c** (**dA-MA**) (DMSO-d_6, 600 MHz).

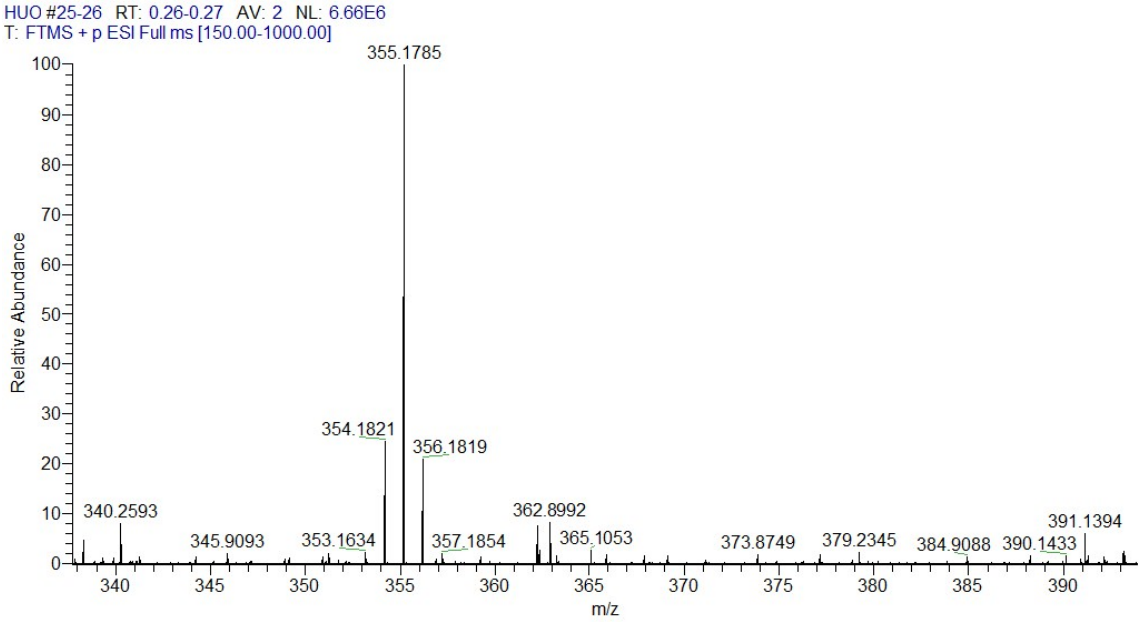


Figure S18. HRMS chart of **dA-1c** (**dA-MA**).

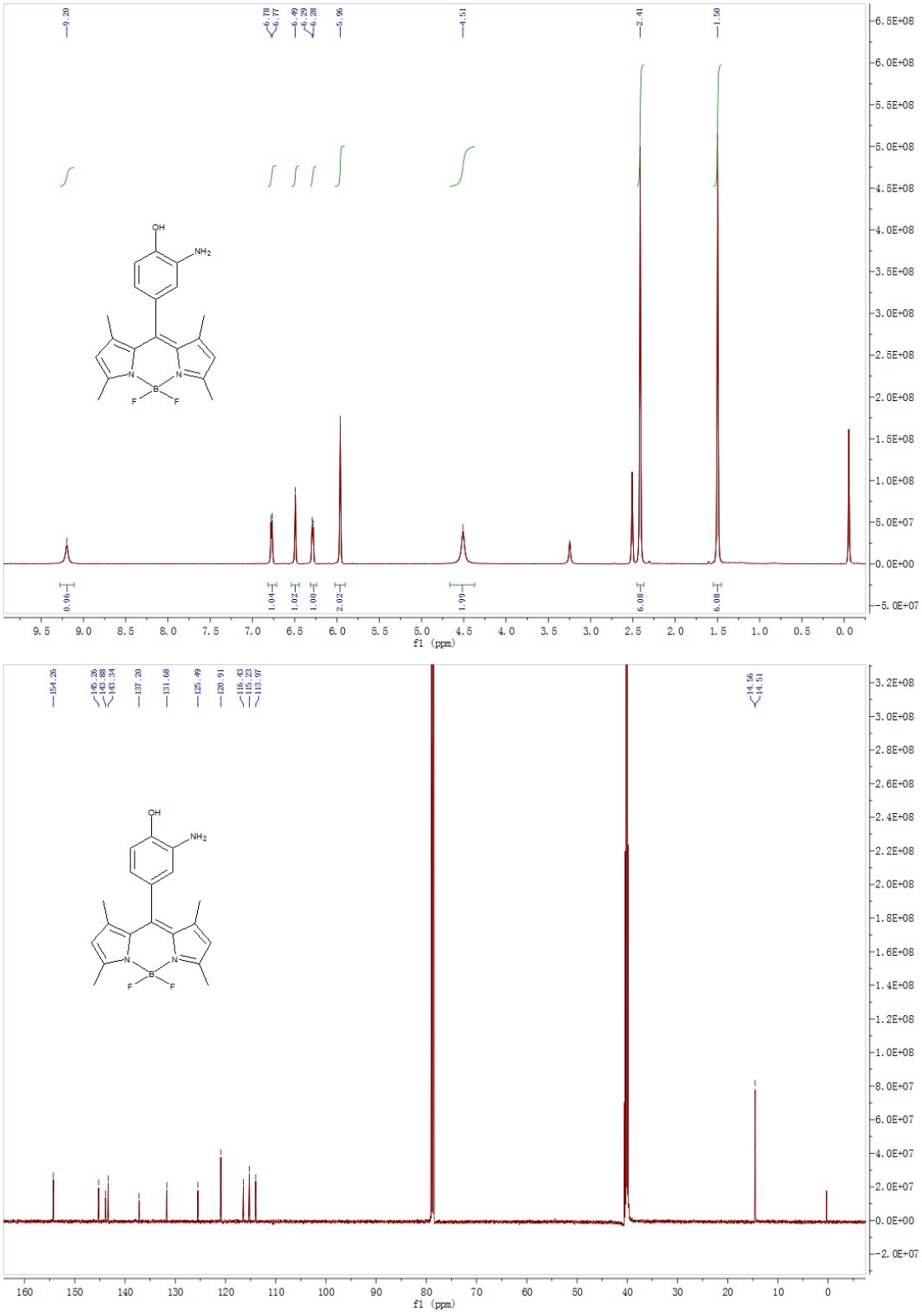


Figure S19. ^{1}H NMR and ^{13}C NMR charts of **1d** (DMSO-d_6, 600 MHz).

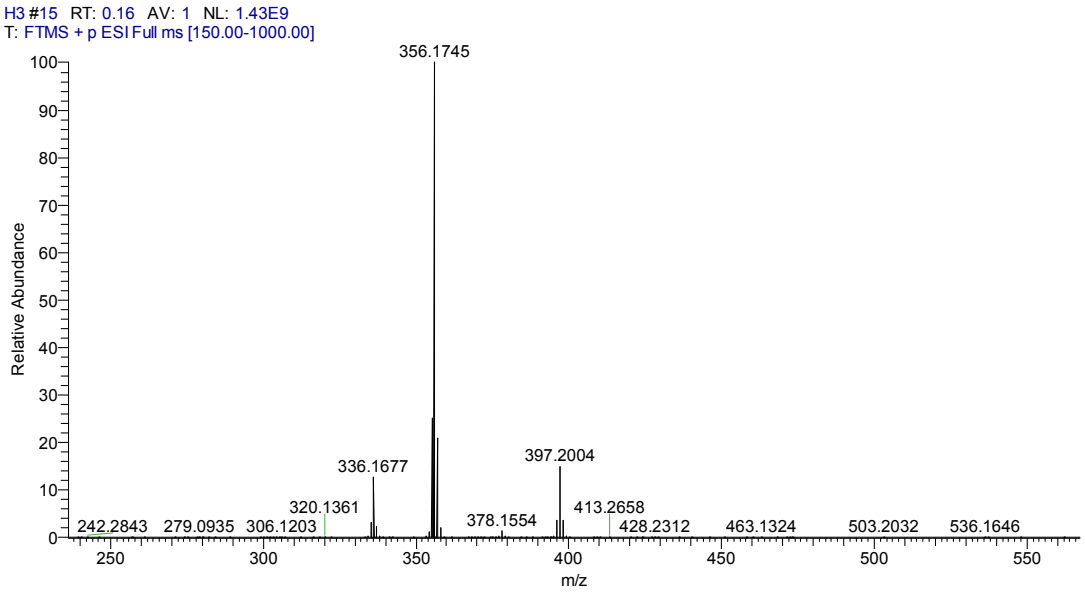


Figure S20. HRMS chart of **1d**.

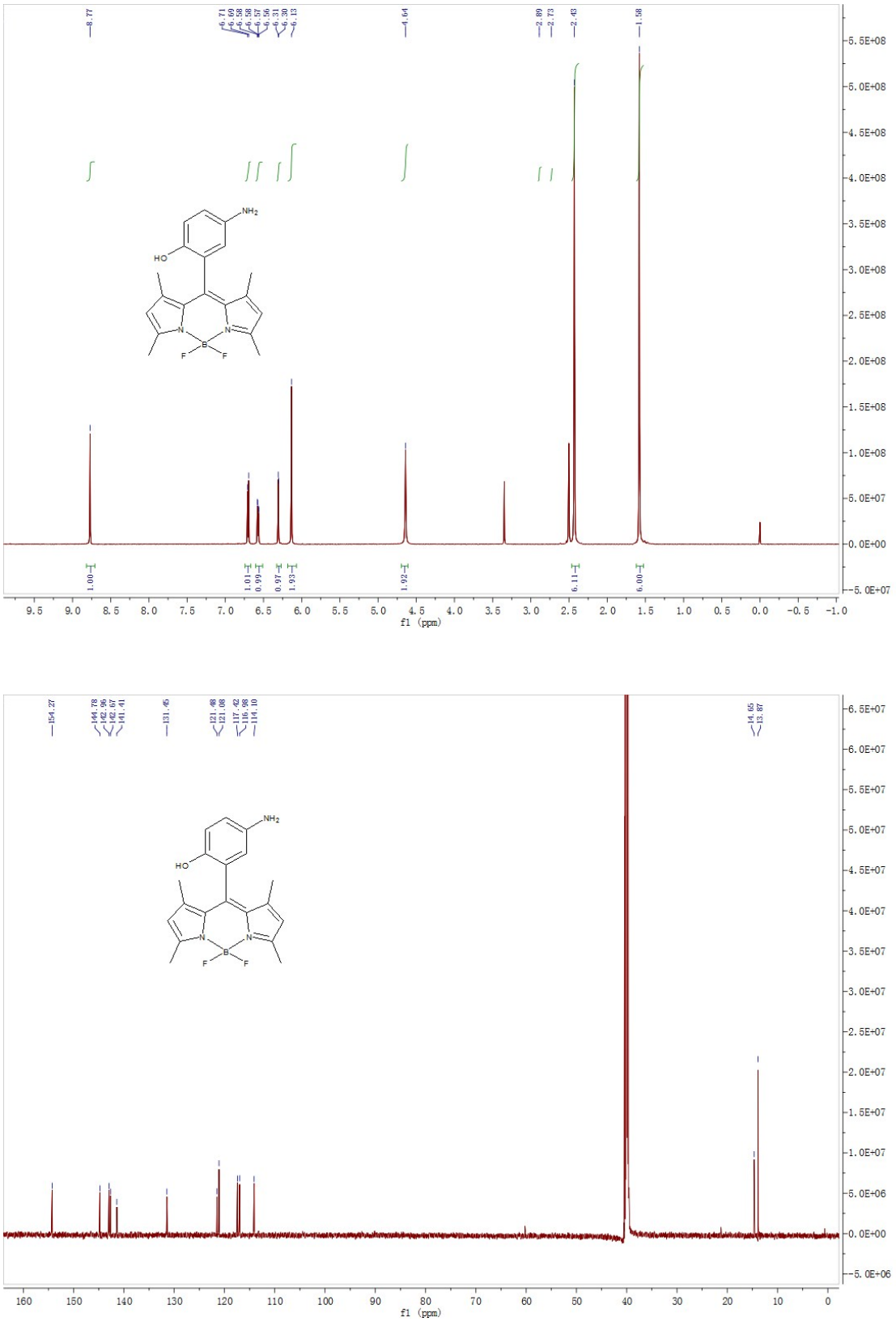


Figure S21. ^{1}H NMR and ^{13}C NMR charts of **1e** (DMSO-d_6, 600 MHz).

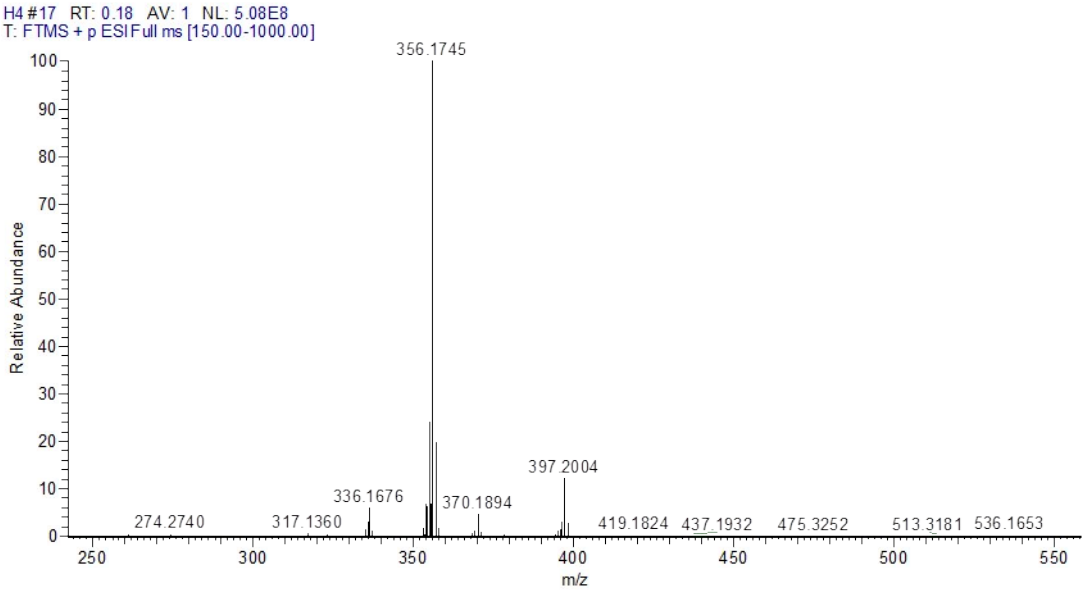


Figure S22. HRMS chart of **1e**.

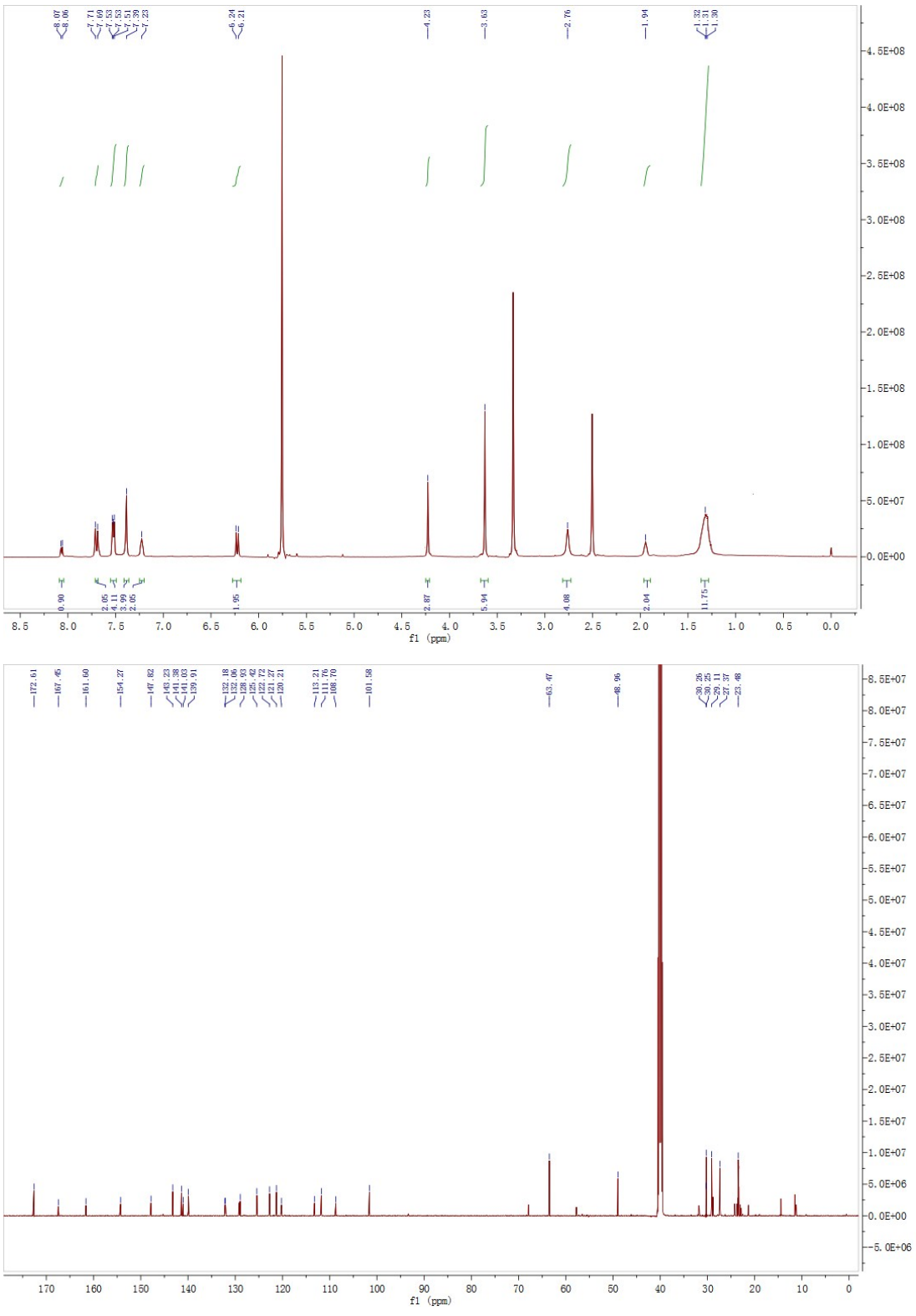


Figure S23. ^{1}H NMR and ^{13}C NMR charts of intermediate **4** (DMSO-d_6, 600 MHz).

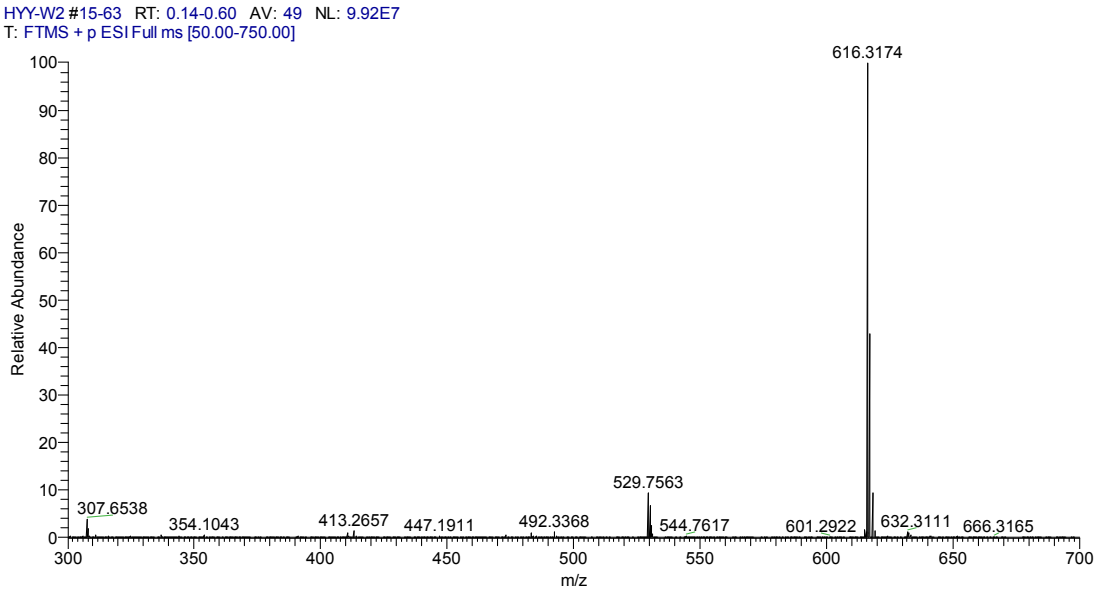


Figure S24. HRMS chart of intermediate **4**.

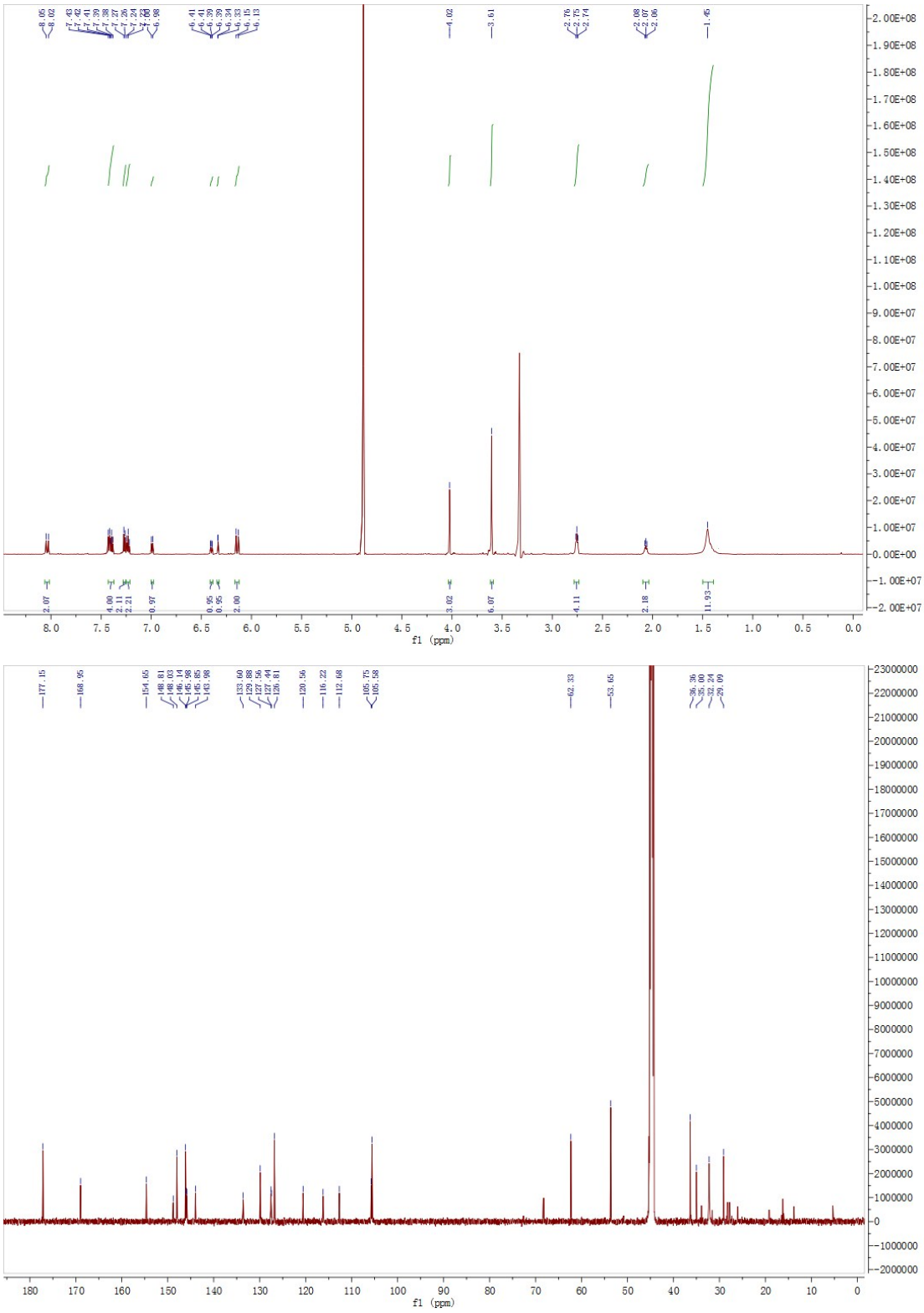


Figure S25. ^{1}H NMR and ^{13}C NMR charts of **NIR-MA** (DMSO-d_6, 600 MHz).

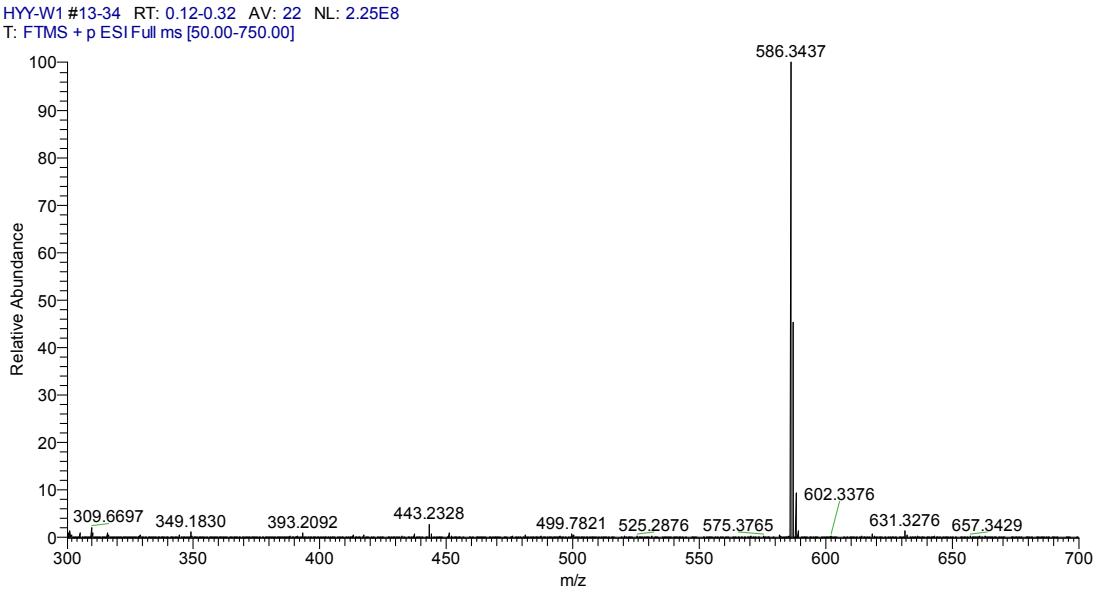


Figure S26. HRMS chart of **NIR-MA**.

4. Reference

1. J. Miao, Y. Huo, X. Lv, Z. Li, H. Cao, H. Shi, Y. Shi, W. Guo, *Biomaterials*, **2016**. *78*, 11–19.