Supplementary Information

A novel two-photon fluorescent probe for non-destructive imaging of Hg^{2+} in fresh plant tissues

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Materials and Methods

All commercially chemicals were used without any secondary purification. Deuterated solvents for NMR measurements were purchased from Annaiji. NMR datas were obtained on a Bruker spectrometer with tetramethyl silane (TMS) as the internalandard. UV-vis (Ultraviolet–visible) absorption spectra were acquired on a UV-2600 spectrophotometer and fluorescence spectra were recorded on a shimadu RF-5301pc spectrofluorometer.

Optical studies

8.42 mg **LJTP3** was dissolved in DMSO (1.0 mL), and diluted with HEPES to a concentration of 1 μM, and the absorption and emission spectra were investigated. The concentration of the reserve solution of various metal ions (including Hg^{2+}, Ag^{+}, Ba^{2+}, Ca^{2+}, Cr^{3+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Na^{+}, Pd^{2+}, Zn^{2+}) is 1.0×10^{-4} M, and the corresponding metal ions are all dissolved in HEPES as a solvent. Unless otherwise indicated, the probe **LJTP3** concentration was maintained at 1 μM, λex = 355 nm, and slit width: 3 nm/3 nm.

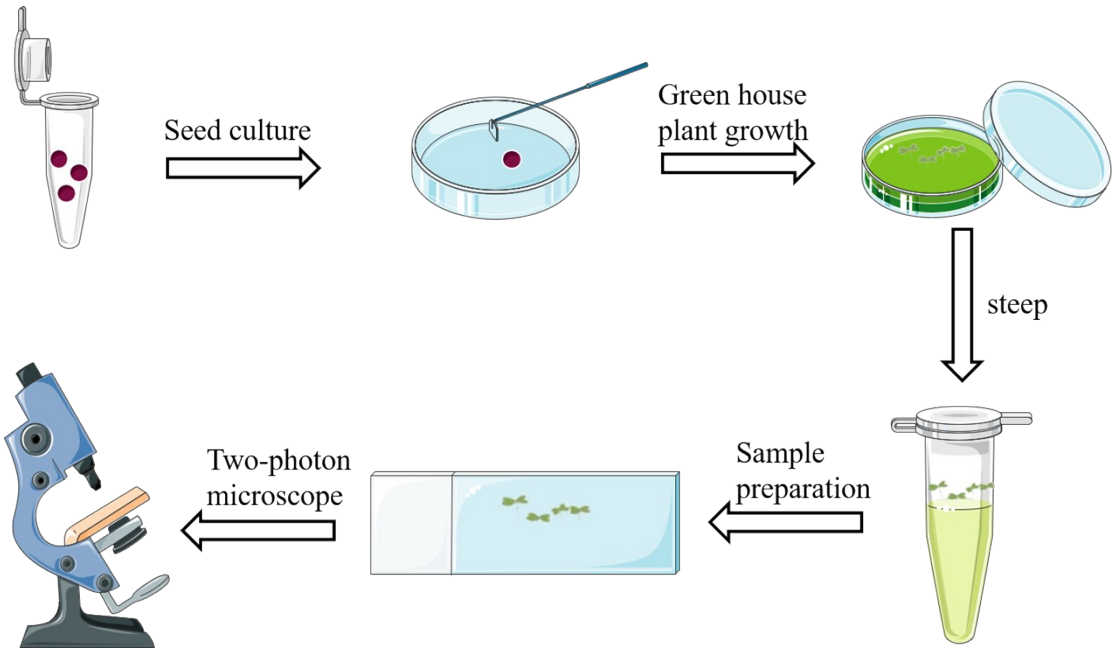
Plant material and growth conditions

Arabidopsis thaliana was used. The sterilized Arabidopsis seeds were refrigerated at 4℃ for 3 days, then placed on 1/2MS (Murashige and Skoog) medium with a pipette and cultured in controlled environment growth chamber with conditions set at 22/20 ℃ for day and night with a 16-hour photoperiod, and illumination of approximately 500 Lux from a white cool fluorescent lamp and maintained for seven days.

***Arabidopsis thaliana* Imaging**

The grown *Arabidopsis thaliana* medium was removed from the artificial climatic chamber and was taken out with forceps. To confirm Hg^{2+} sub-cellular localization under two-photon imaging, the *Arabidopsis thaliana (A. thaliana)* were soaked in different concentrations of Hg^{2+} solution (0μM, 10μM, 100μM, 1mM), or in Hg^{2+} solution for different time points (0h, 1h, 3h and 5h). Samples were removed from the Corresponding solutions and washed with double distilled (dd) water to remove excessive Hg^{2+}. Then, samples were immersed in the prepared probe solution (10μM) for 1 hour, and removed from the probe solution using tweezers. Therefore, Slides were

prepared and observed under a two-photon microscope with wavelengths (Excitation 750 nm).

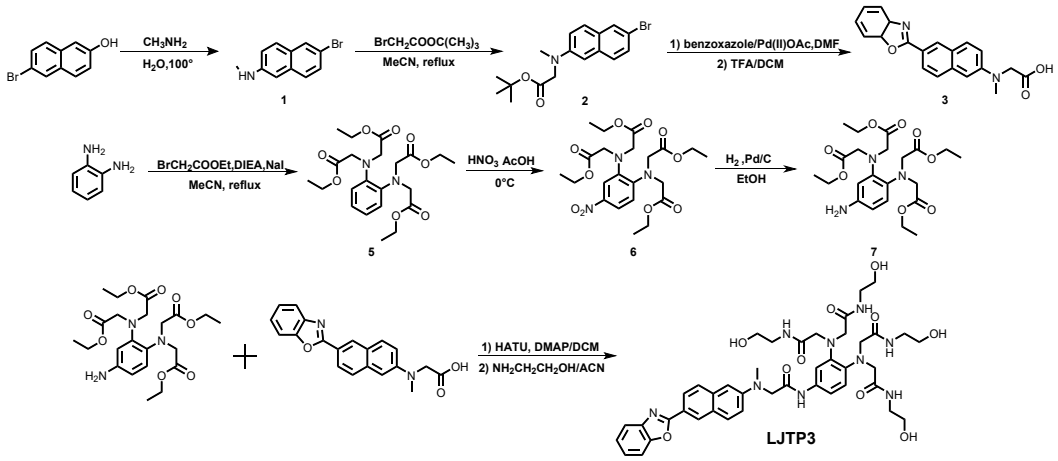


Scheme 1. **An Integrated Methodology for Arabidopsis thaliana Growth Protocols and Two-Photon Microscopy Imaging: A Schematic Workflow**

Measurement of Quantitative Data and Statistical Analysis

The fluorescence intensity of all images were calculated using ImageJ software. Subsequently, data was statistically analyzed, means and standard deviation were calculated, and the ANOVA test was applied to check the significance levels. Statistical analyses and graphs were performed and generated using GraphPad Prism software.

Synthesis of LJTP3



Scheme 2. Synthetic route of probe **LJTP3**.

Synthesis of compound 1. 6-Bromo-2-naphthol (10 g, 42.6 mmol), sodium dithionite (16.5 g, 86.8 mmol), sodium hydroxide (9 g, 22.5 mmol) and methylamine hydrochloride (15 g, 22.2 mmol) were added into 40 ml ultrapure water. The mixture was stirred at 140 °C for 96 h. After the reaction was cooled to room temperature, the crude product was washed with aqueous sodium hydroxide. After drying, the crude product was recrystallized from methanol to give a brown-yellow product (8.3 g, 83.2%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (d, *J* = 2.0 Hz, 1H), 7.52 (dd, *J* = 8.7, 1.8 Hz, 2H), 7.45 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.74 (d, *J* = 2.4 Hz, 1H), 3.91 (s, 1H), 2.91 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 147.33, 133.85, 129.66, 129.49, 128.51, 127.98, 127.68, 118.84, 114.96, 103.47, 30.67.

Synthesis of compound 2. A mixture of 6- bromo-N-methyl-2-naphthylamine (2.0 g, 8.5 mmol), proton-sponge (2.0 g, 9.4 mmol), and tert-butyl bromoacetate (2.0 g, 1.5 mL, 10.2 mmol) in MeCN was refluxed for 12 h under N^2. The product was extracted with ethyl acetate, washed with brine, dried with $MgSO_4$, concentrated, and purified on a silica gel column using hexane/ethyl acetate (5:1) as eluent. Yield 2.6 g (87 %) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, *J* = 2.0 Hz, 1H), 7.60 (d, *J* = 9.0 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.42 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.08 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.85 (d, *J* = 2.6 Hz, 1H), 4.07 (s, 2H), 3.16 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.09, 147.30, 133.50, 129.49, 129.46, 128.08, 128.06, 116.35, 115.35, 106.34, 81.83, 55.53, 39.96, 28.17.

Synthesis of compound 3. A mixture of compound 2 (1.0 g, 2.9 mmol), benzoxazole (0.41 g, 3.4 mmol), Pd(II)OAc (0.033 g, 0.15 mmol), PPh_3 (0.073 g, 0.29 mmol), CuI (0.11 g, 0.58 mmol), and $CsCO_3$ (1.1 g, 3.5 mmol) in DMF was stirred at 140 °C for 12 h under N^2. The reaction mixture was filtered, diluted with ethyl acetate, washed with brine, dried with $MgSO_4$, concentrated. The crude product was purified on a silica gel column using hexane/ethyl acetate (3:1) as eluent. Yield 0.64 g (57 %) ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.65 – 8.60 (m, 1H), 8.19 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.84 (d, *J* = 9.1 Hz, 1H), 7.78 – 7.76 (m, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.60 – 7.58 (m, 1H), 7.37 – 7.32 (m, 2H), 7.12 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 4.11 (s, 2H), 3.21

(s, 3H), 1.43 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.93, 164.07, 150.90, 148.60, 142.56, 136.77, 130.30, 128.16, 127.02, 126.33, 124.75, 124.59, 124.55, 120.54, 119.78, 116.13, 110.52, 106.19, 82.05, 55.47, 40.03, 28.22.

Synthesis of compound 4. To a solution of compound 3 (0.50 g, 1.3 mmol) in CH_2Cl_2 (10 mL) was added CF_3CO_2H (2 mL) and the mixture was stirred for 24 h under N^2. The solvent was removed under vacuo. The product was washed with hexane and filtered. Yield 0.29 g (68 %) ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (d, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.85 – 7.73 (m, 3H), 7.40 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.25 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.00 (d, *J* = 2.6 Hz, 1H), 4.30 (s, 2H), 3.12 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.27, 163.56, 150.66, 149.34, 142.38, 142.34, 136.82, 130.44, 128.06, 127.29, 125.81, 125.47, 125.18, 124.33, 119.89, 116.87, 111.12, 105.64, 53.68.

Synthesis of compound 5.
A solution of 1,2-diaminobenzene (2g, 18.5 mmol), ethyl bromoacetate (13.4 mL, 120 mmol) sodium iodide (2.5 g, 17 mmol) and diisopropylethylamine (17 ml, 100 mmol) in 20 mL acetonitrile was refluxed under nitrogen for 7 h, then cooled and poured into 100 mL water. The resulting mixture was extracted with dichloromethane. The extract was dried over sodium sulfate and was concentrated to give brown oil, which was purified by column chromatography using petroleum ether–EtOAc 5 : 1 (v/v) as eluant. (8.3 g, 85%) was yielded as a white solid. 1H NMR (400 MHz, Chloroform-d) δ 7.03 (dt, J = 7.3, 3.6 Hz, 2H), 6.94 (dd, J = 6.0, 3.5 Hz, 2H), 4.29 (s, 8H), 4.10 (q, J = 7.1 Hz, 8H), 1.19 (t, J = 7.1 Hz, 12H). 13C NMR (101 MHz, Chloroform-d) δ 171.13, 141.54, 123.25, 121.54, 60.64, 52.54, 14.25.

Synthesis of compound 6.
To a solution of compound 6 in AcOH (15 mL) was added fuming HNO_3 (0.3 mL, 5 mmol) dissolved in AcOH (2.5 mL) in a dropwise manner at 0 °over 5–10 min. The reaction was monitored by the disappearance of the starting material on a silica TLC plate. After completion, the reaction mixture was poured on to ice water. After extraction with CH_2Cl_2, the organic layer was dried over anhydrous Na_2SO_4 and filtered. After removing the solvent under reduced pressure, the residue was purified by column chromatography using petroleum ether–EtOAc 4:1 (v/v) as eluant. Yield 1.8 g (82%) as yellow crystals. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (d, *J* = 2.6 Hz, 1H), 7.86 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.05 (d, *J* = 8.9 Hz, 1H), 4.41 (s, 4H), 4.28 (s, 4H), 4.13 (qd, *J* = 7.2, 2.7 Hz, 8H), 1.23 (dt, *J* = 7.2, 3.6 Hz, 12H). 13C NMR (101 MHz, Chloroform-d) δ 170.16, 170.10, 147.68, 142.64, 140.79, 120.43, 119.17, 117.74, 61.04, 60.94, 52.24, 52.07, 14.14.

Synthesis of compound 7.
Compound 6 (420 mg, 0.85 mmol) was dissolved in 20 mL of EtOH. 5% Pd/C (200 mg) was added to the solution and the mixture was stirred overnight under a hydrogen atmosphere. The reaction mixture was filtered and dried over anhydrous Na_2SO_4, purified by column chromatography using petroleum ether–EtOAc 3 : 1 (v/v) as eluant. Yield 308 mg (78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.68 (d, *J* = 8.4 Hz, 1H), 6.20 (d, *J* = 2.4

Hz, 1H), 6.07 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.65 (s, 2H), 4.26 (s, 4H), 4.10 (s, 4H), 4.01 (dq, *J* = 9.8, 7.1 Hz, 8H), 1.12 (q, *J* = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.74, 170.69, 144.40, 142.01, 130.27, 121.51, 107.77, 106.44, 59.86, 59.78, 52.53, 52.12, 14.00.

Synthetic of probe LJTP3.

Compound **4** (200 mg, 0.6 mmol) and 2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (457 mg, 1.2 mmol) were dissolved in 10 mL of anhydrous CH_2Cl_2, and 400 μL of *N*,*N*-diisopropylethylamine (DIPEA) was injected slowly into the reaction system at ice bath under the protection of nitrogen. After stirring for 10 min, Compound **7** (225 mg, 0.48 mmol) was added to the reaction solution and reacted for another 8 h. After the reaction was completed, evaporated the solvent, and the reaction product was purified via column chromatography using DCM/MeOH (100:1, v/v) to gain the intermediate compound **8** as a white solid which reacted with ethanolamine (6 mL) in 10 mL ACN solution at 80 ° for 8 hours. After completing the reaction, the mixture was washed with saturated NaCl and extracted by CH_2Cl_2. The organic layer was purified via column chromatography to get **LJTP3** as yellow oil 42 mg (8.3%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.59 (s, 1H), 8.12 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.91 (d, *J* = 9.1 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.74 – 7.70 (m, 1H), 7.70 – 7.66 (m, 1H), 7.43 – 7.35 (m, 3H), 7.27 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.12 – 7.04 (m, 2H), 6.97 (d, *J* = 8.6 Hz, 1H), 4.29 (d, *J* = 4.0 Hz, 2H), 4.06 (d, *J* = 14.4 Hz, 8H), 3.47 (q, *J* = 5.6 Hz, 8H), 3.26 (s, 3H), 3.25 – 3.17 (m, 8H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 171.57, 169.34, 164.07, 150.58, 149.25, 142.09, 141.57, 139.34, 138.18, 136.87, 134.07, 133.50, 129.86, 127.75, 126.86, 126.23, 124.91, 124.56, 123.67, 121.66, 119.56, 118.74, 116.37, 110.23, 105.86, 60.10, 55.43, 41.43, 41.35, 30.17. HRMS calcd for [M + H]$^+$: 842.3842, found : 842.3809.

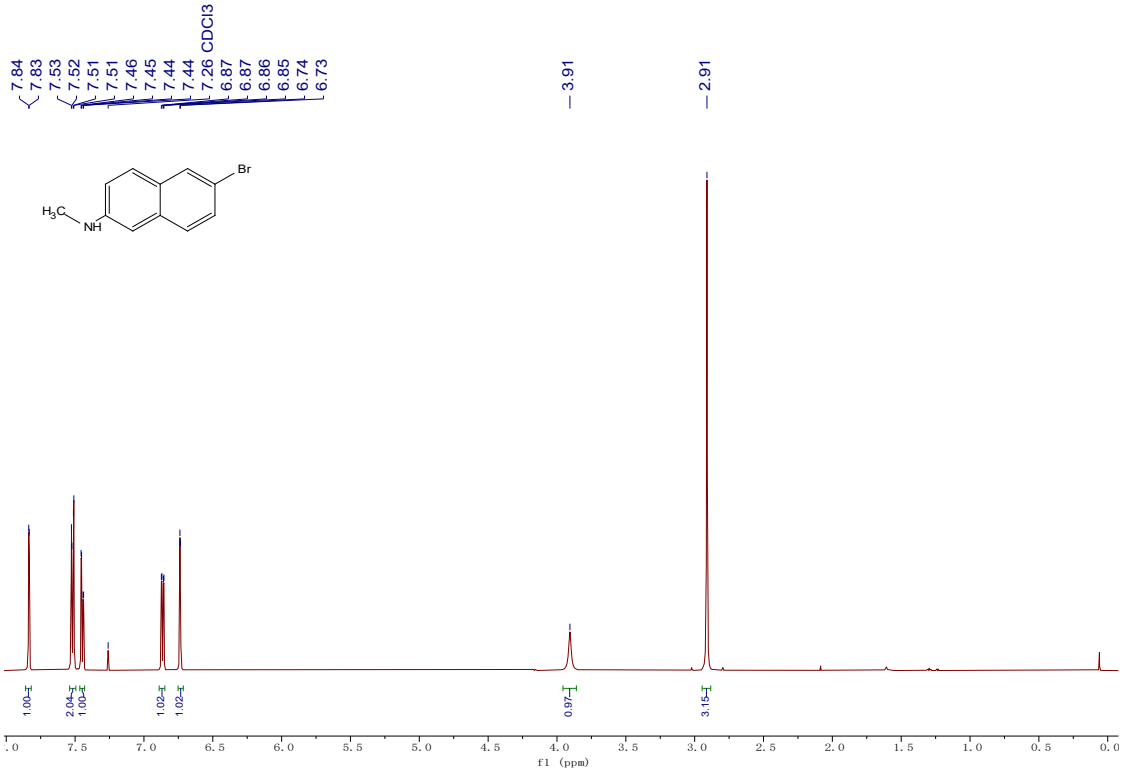


Figure S1. [1]H NMR spectrum of **compound 1.**

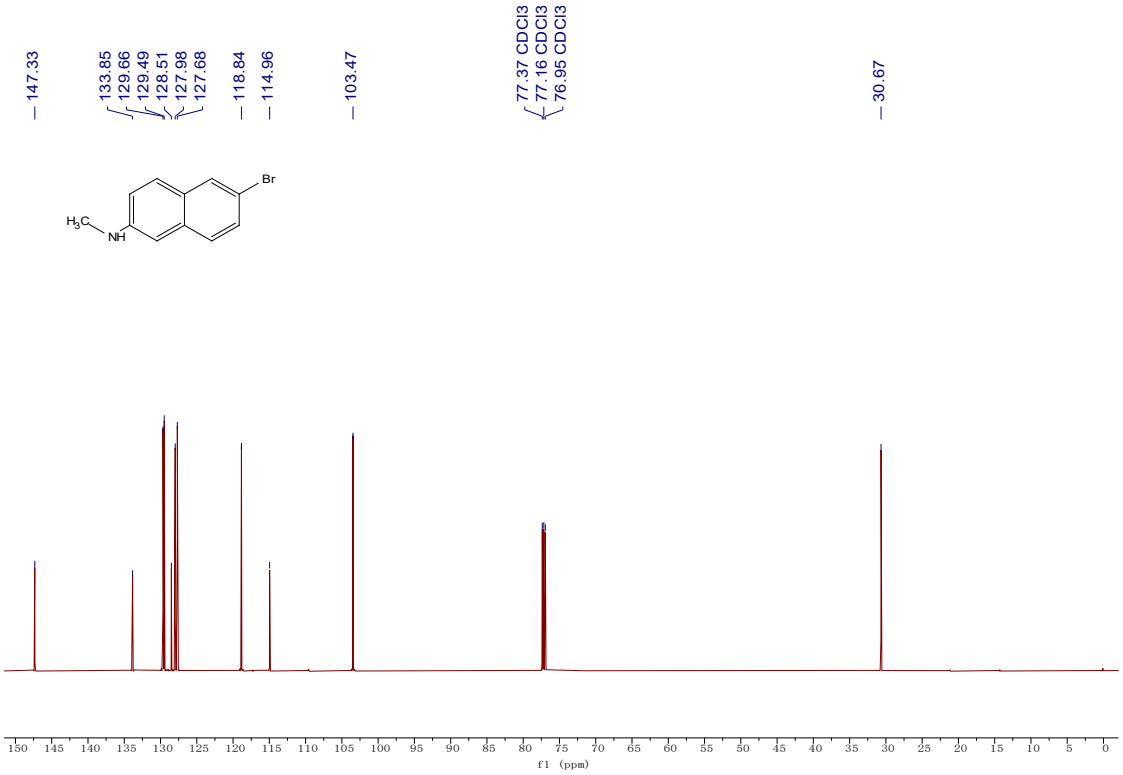


Figure S2. [13]C NMR spectrum of **compound 1.**

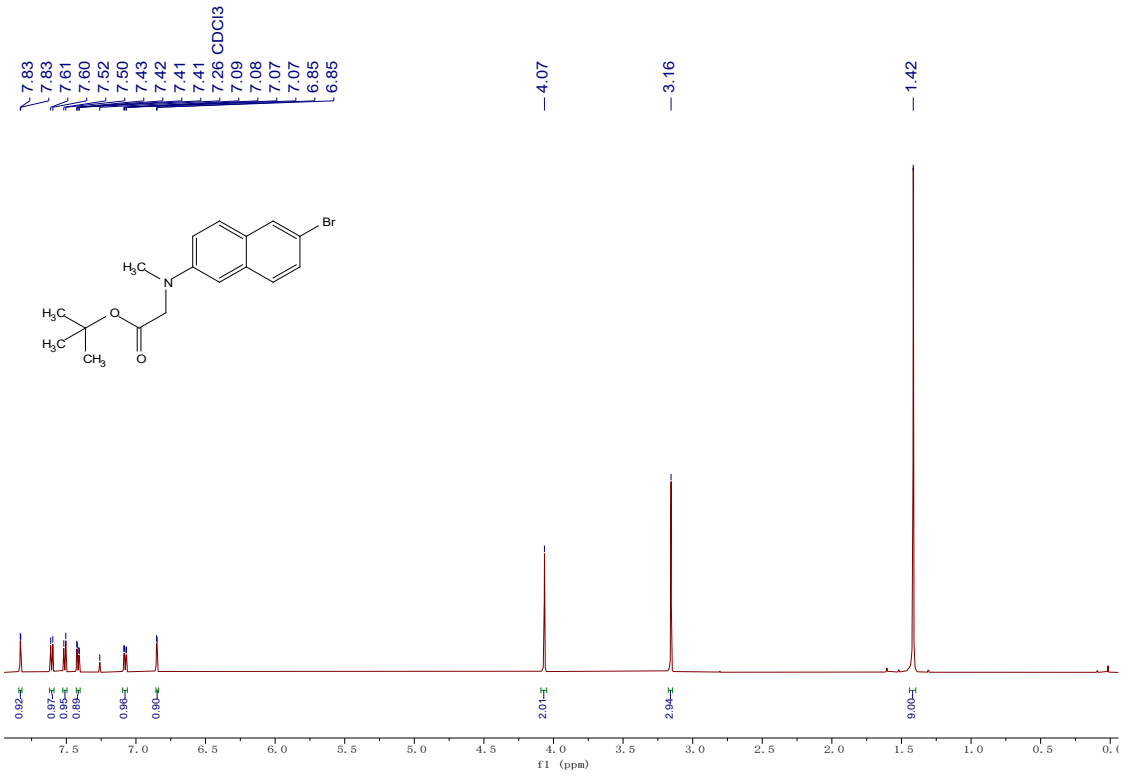


Figure S3. [1]H NMR spectrum of **compound 2.**

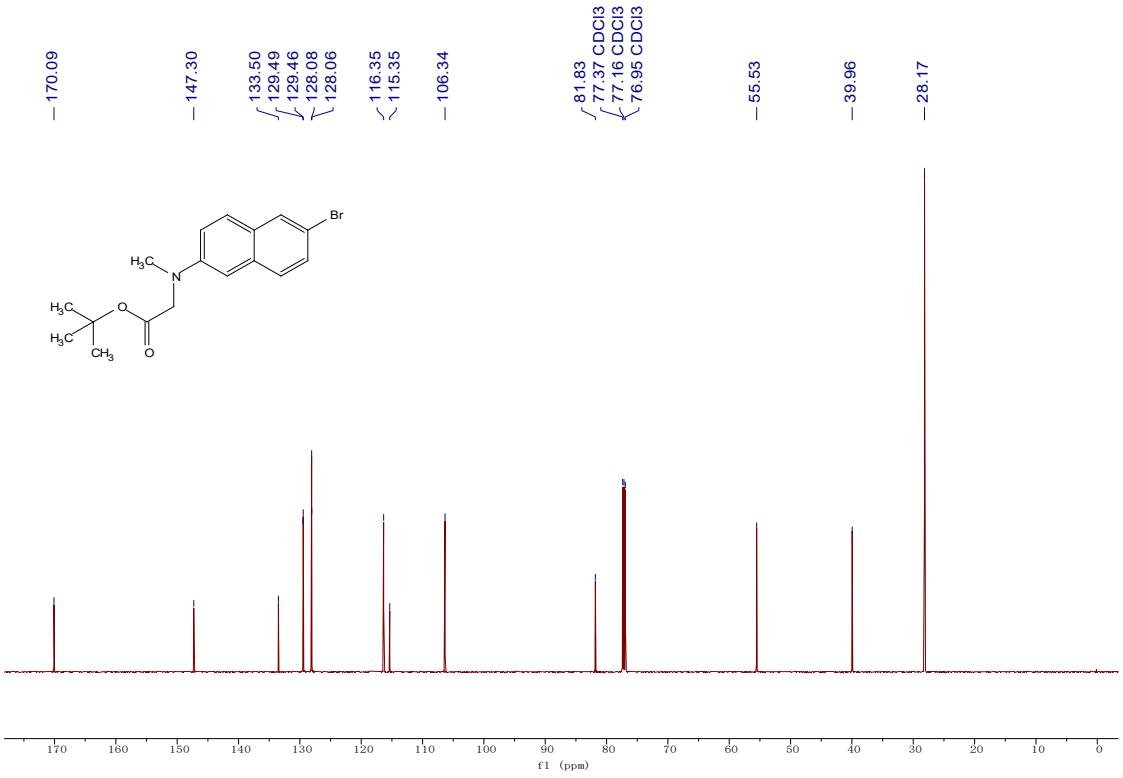


Figure S4. ^{13}C NMR spectrum of **compound 2.**

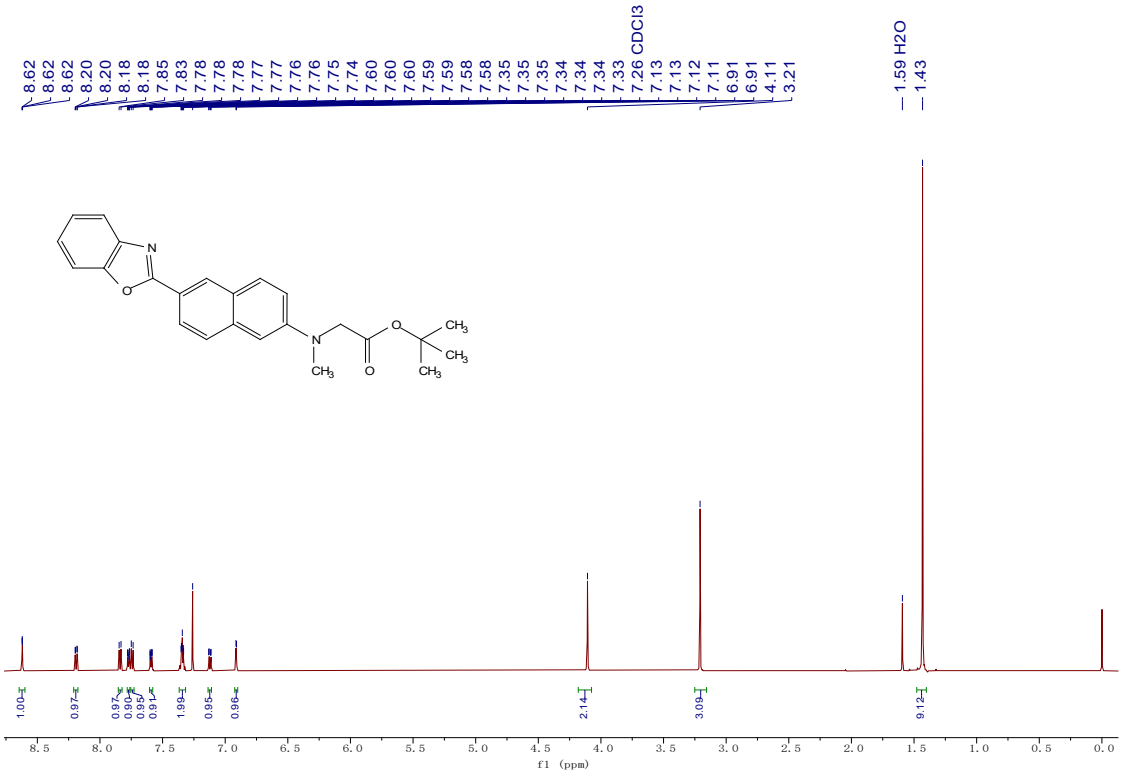


Figure S5. ^{1}H NMR spectrum of **compound 3.**

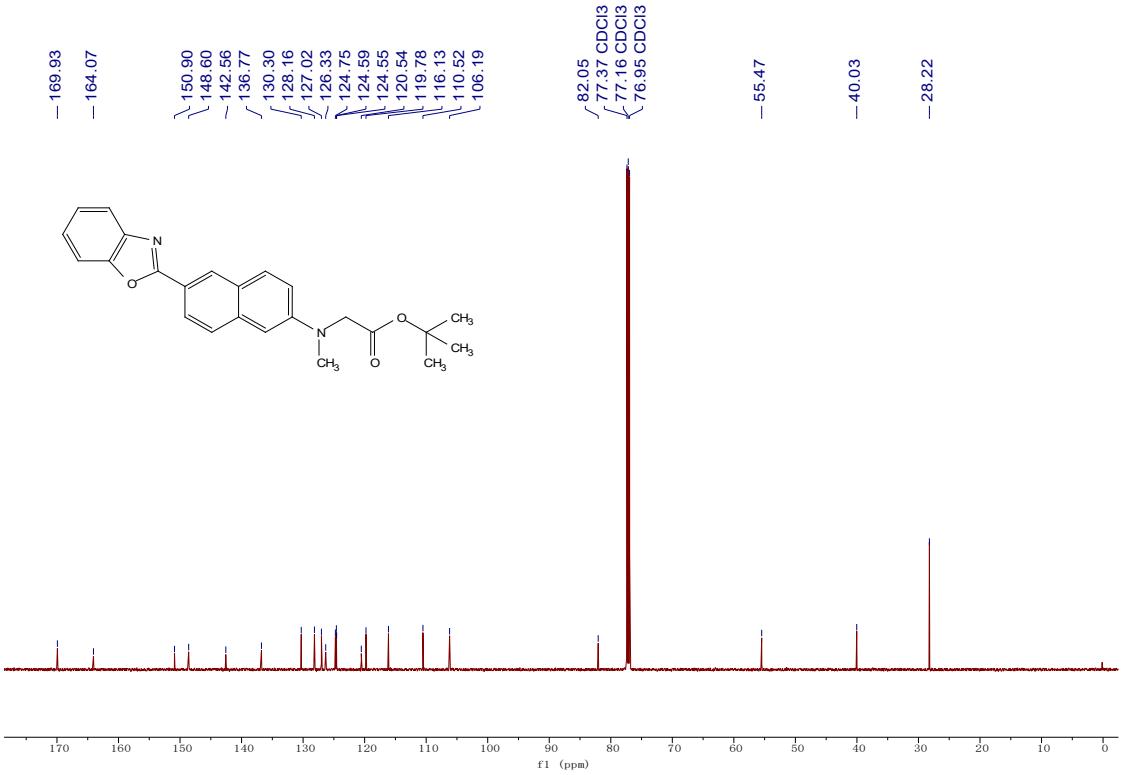


Figure S6. ^{13}C NMR spectrum of **compound 3.**

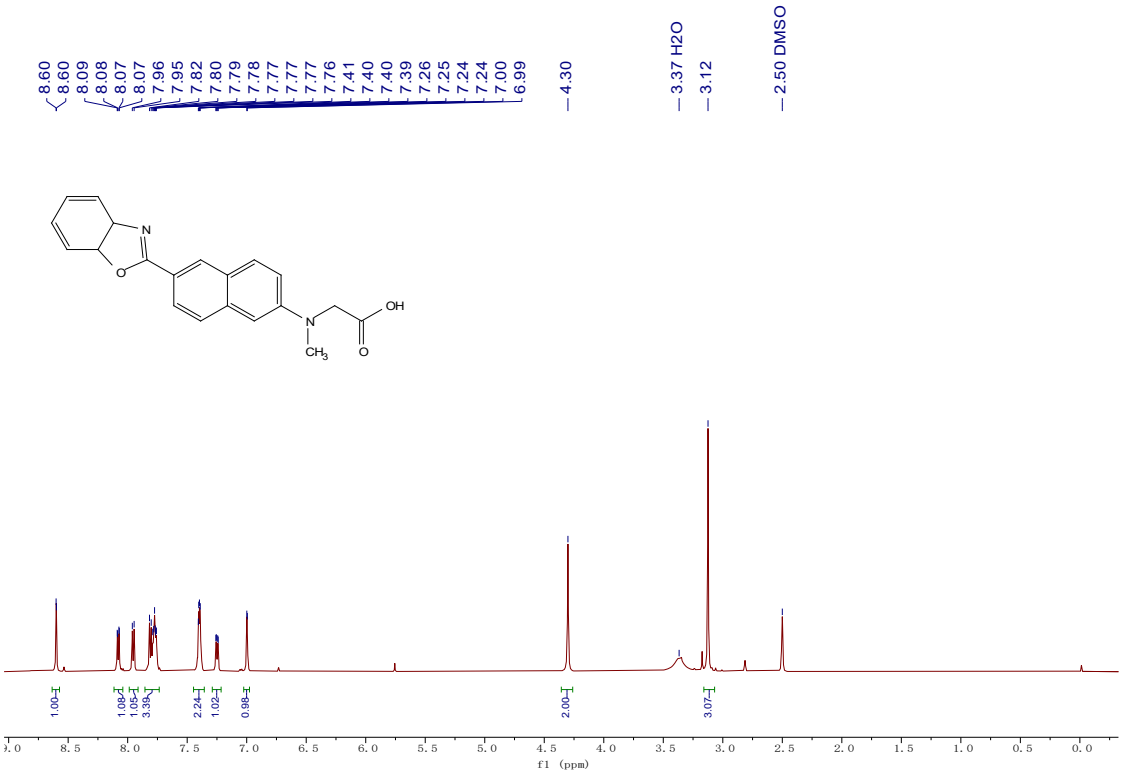


Figure S7. ^{1}H NMR spectrum of **compound 4.**

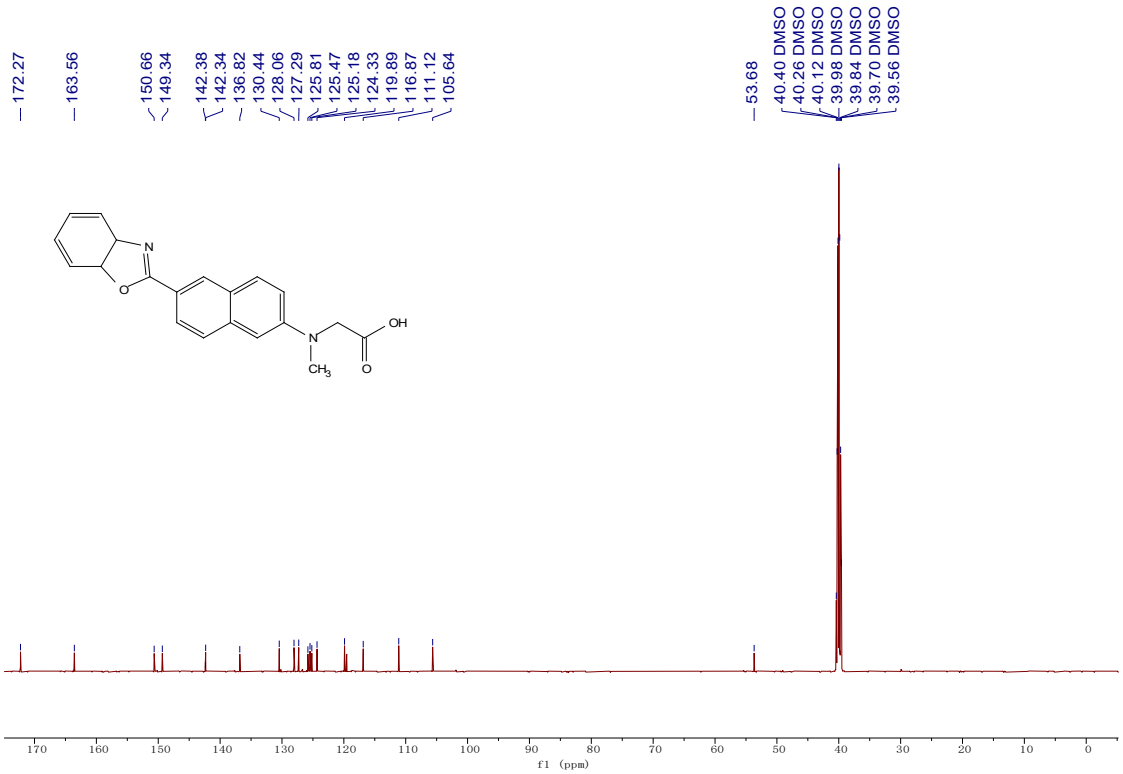


Figure S8. ^{13}C NMR spectrum of **compound 4.**

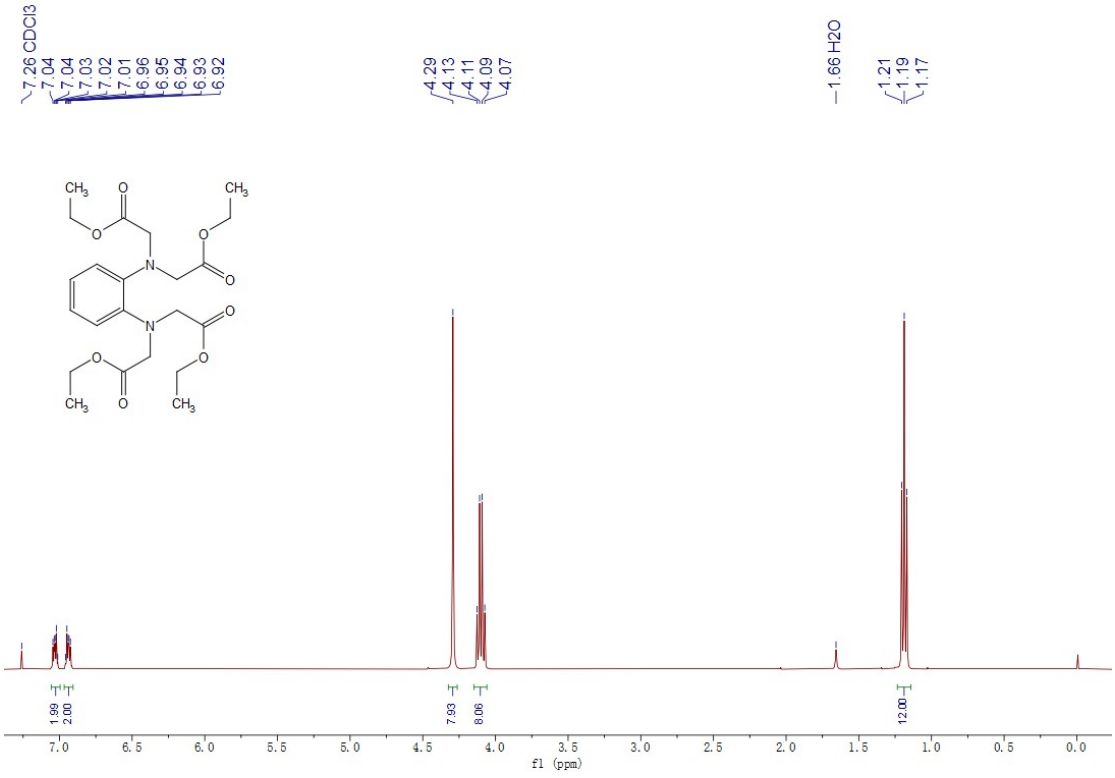


Figure S9. ^{1}H NMR spectrum of **compound 5.**

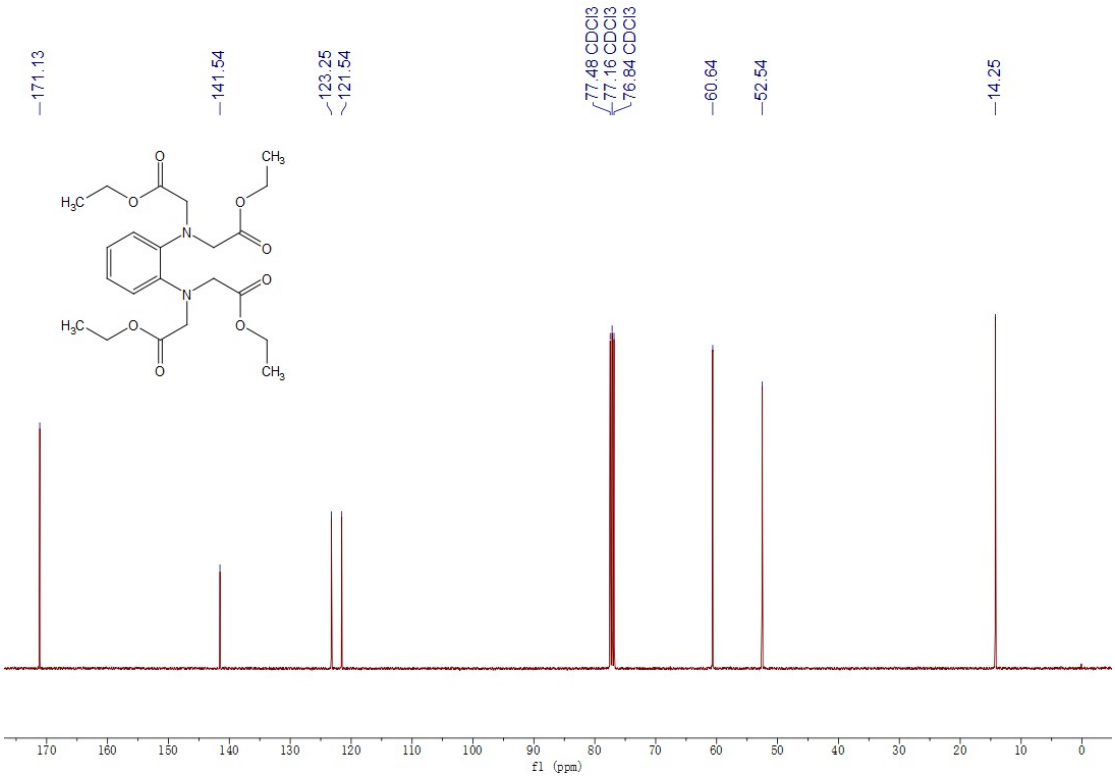


Figure S10. ^{13}C NMR spectrum of **compound 5.**

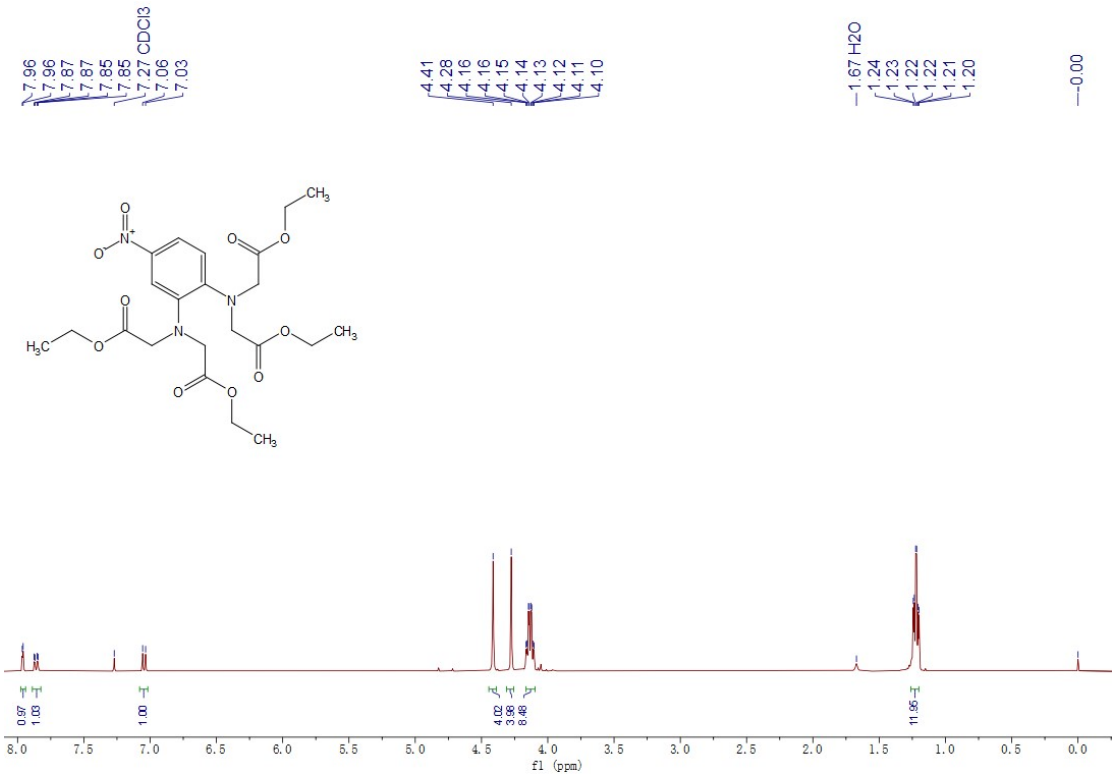


Figure S11. ^{1}H NMR spectrum of **compound 6.**

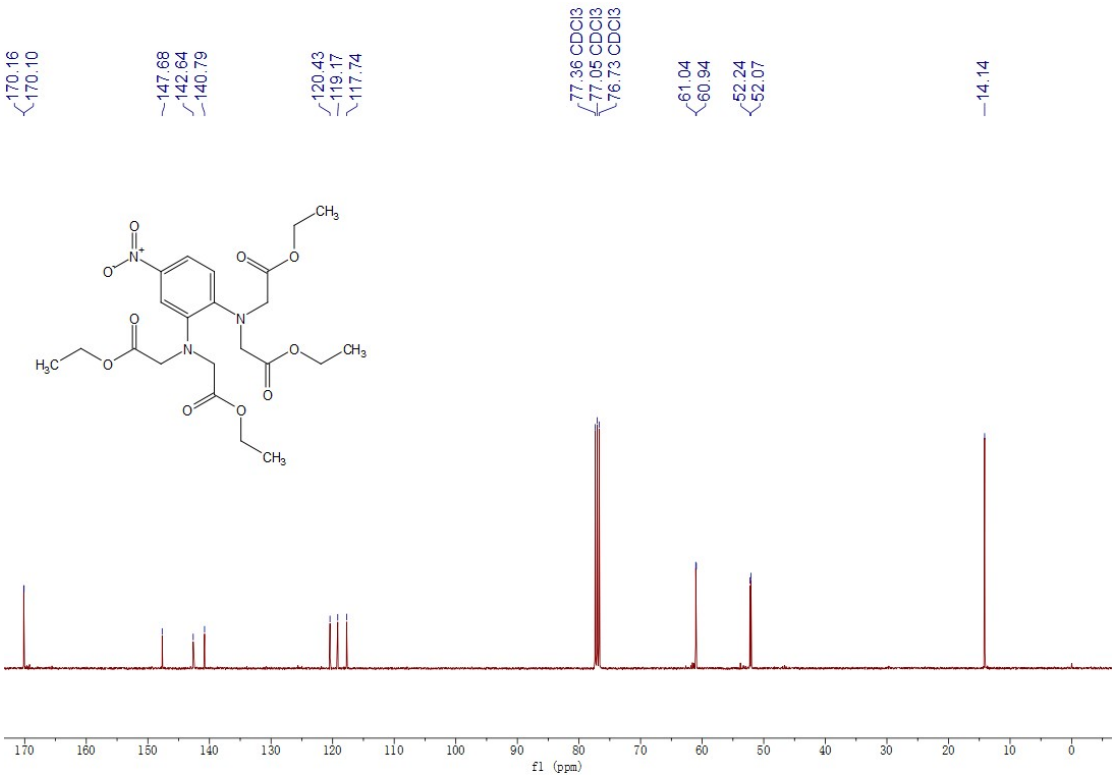


Figure S12. ^{13}C NMR spectrum of **compound 6.**

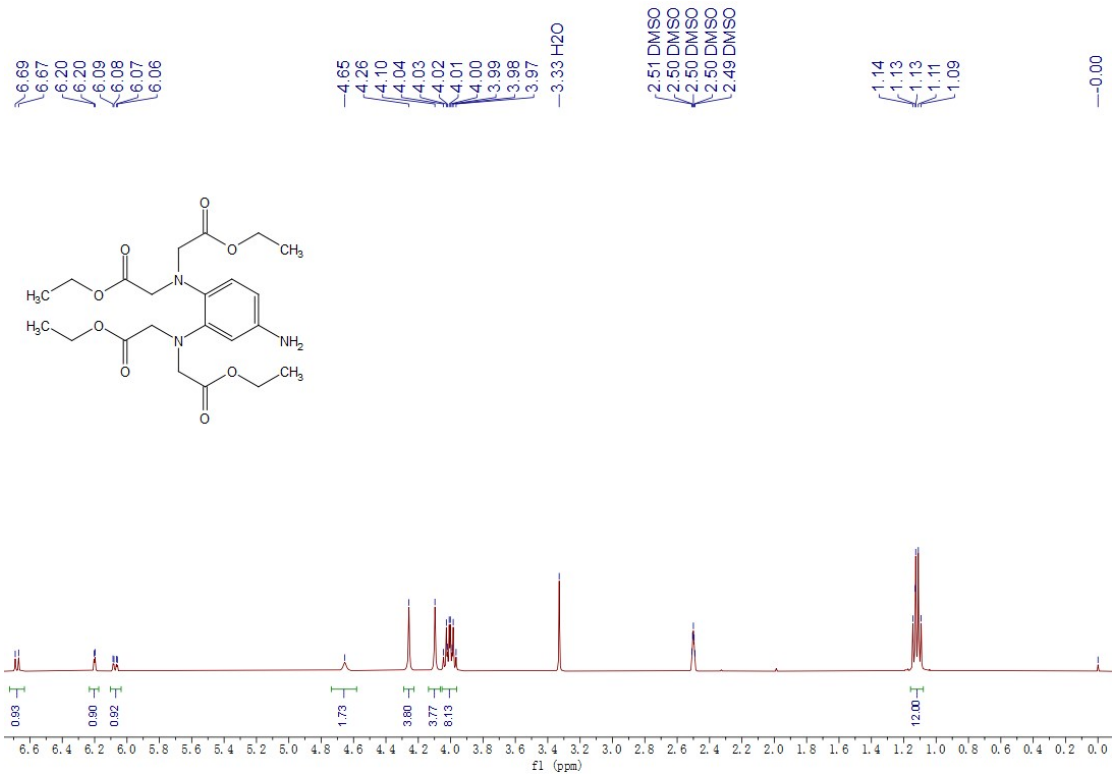


Figure S13. ^{1}H NMR spectrum of **compound 7.**

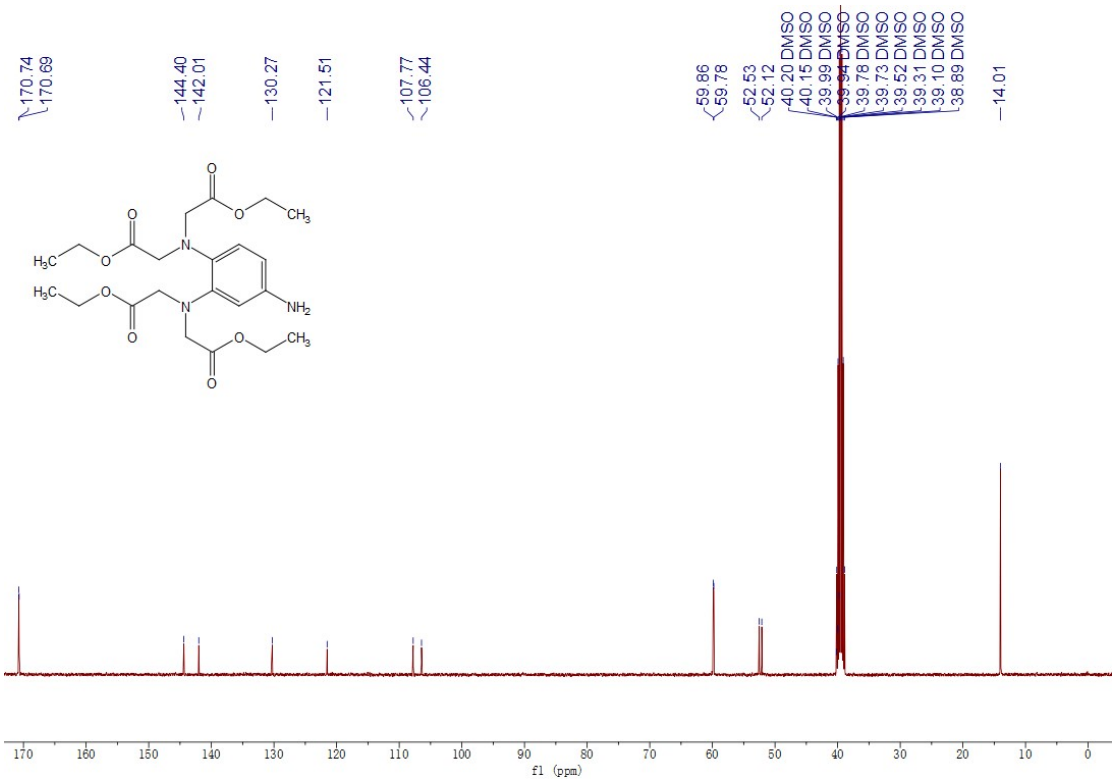


Figure S14. ^{13}C NMR spectrum of **compound 7.**

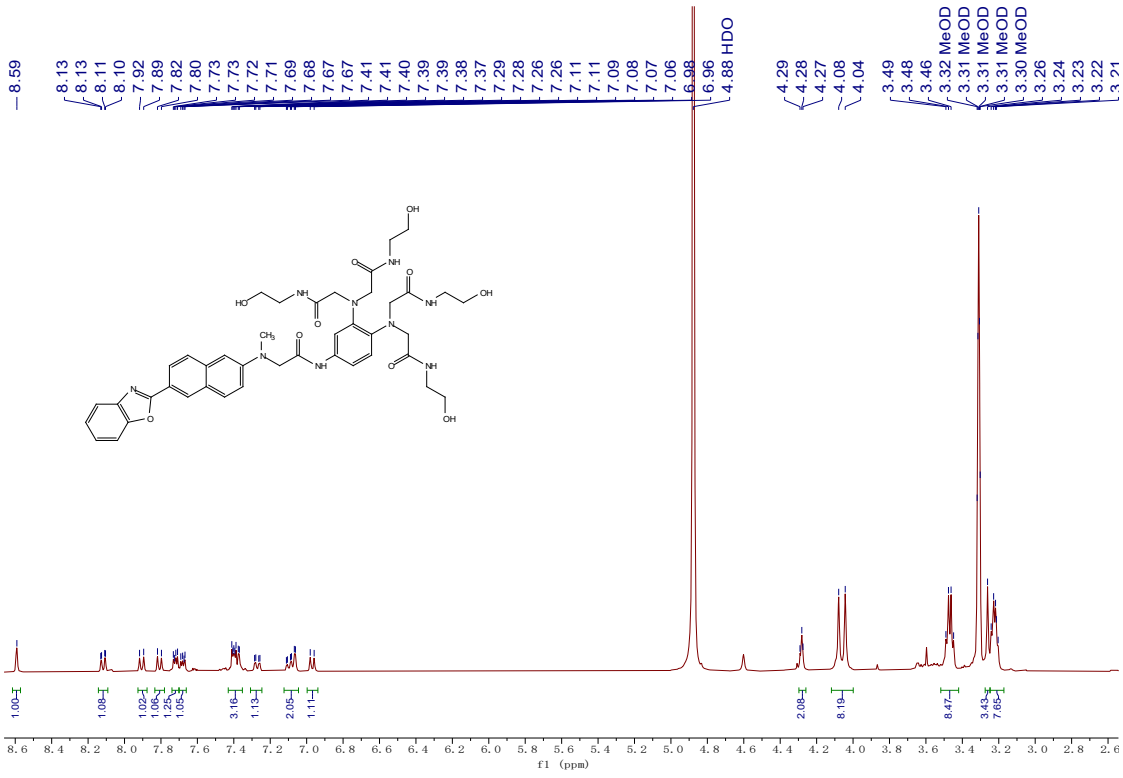


Figure S15. ^{1}H NMR spectrum of **LJTP3.**

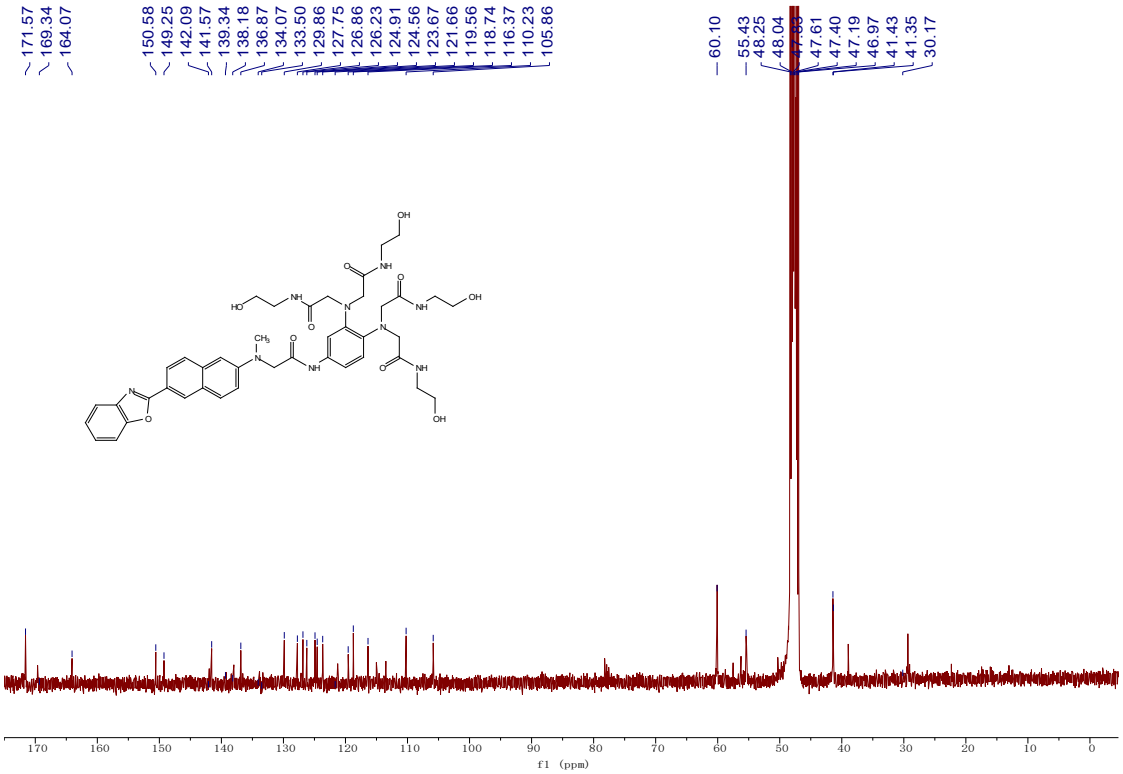


Figure S16. ^{13}C NMR spectrum of **LJTP3.**

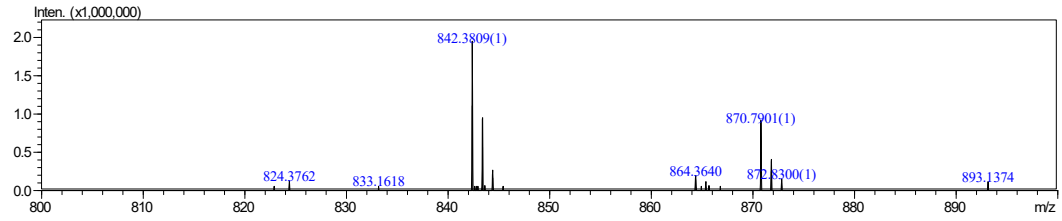


Figure S17. HRMS spectrum of **LJTP3.**

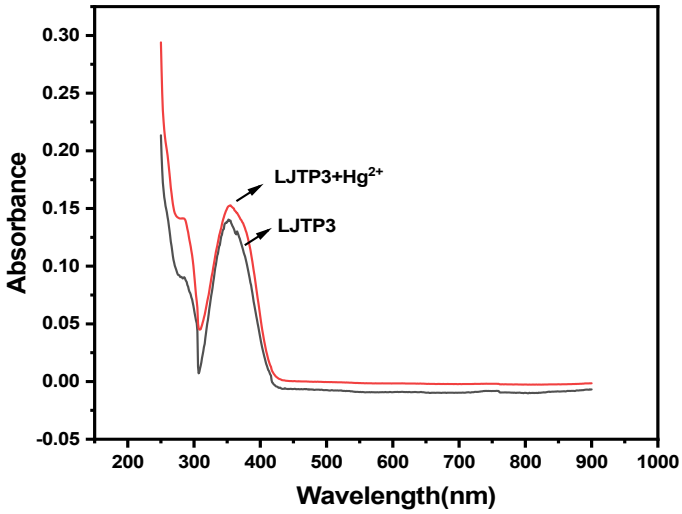


Figure S18. The UV spectrum of LJTP3 with (red line) and without (black line) of Hg^{2+} in HEPES（pH 7.0 40mM）

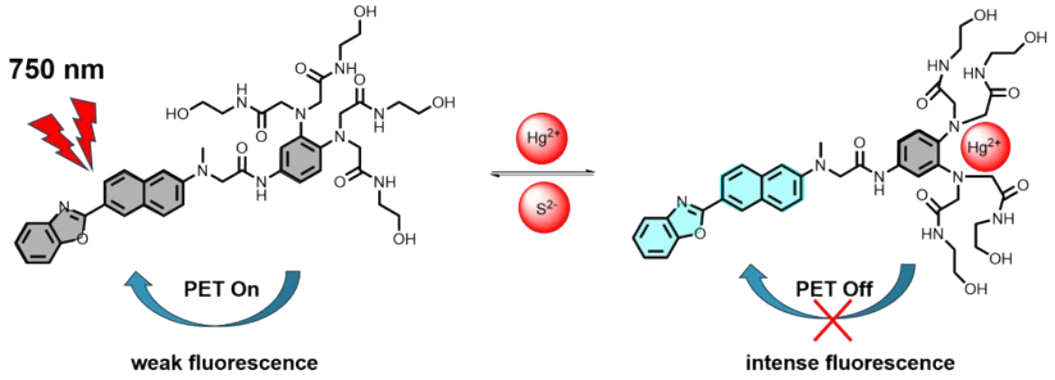


Figure S19. The sensing mechanism of LJTP3 towards Hg^{2+}.

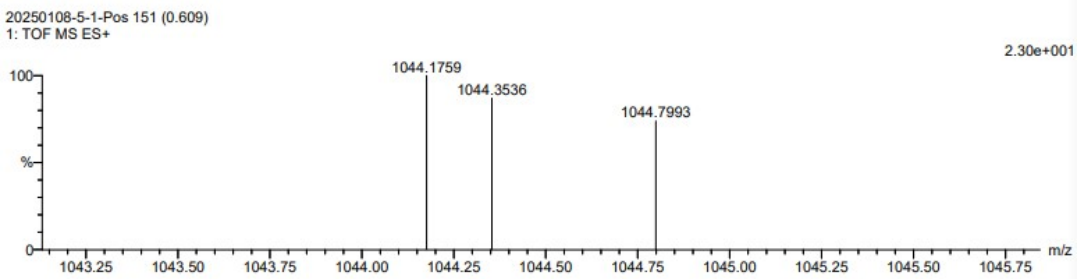


Figure S20. HRMS spectrum of **LJTP3** + Hg^{2+}

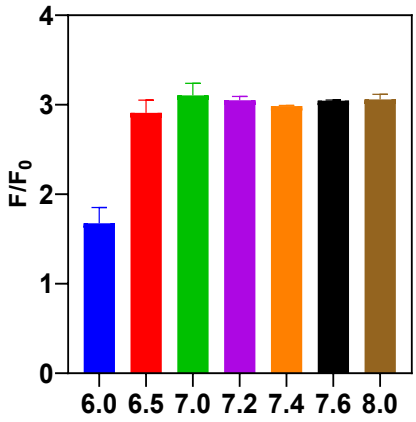


Figure S21. pH measurement against fluorescent intensity of probe and probe with mercury.

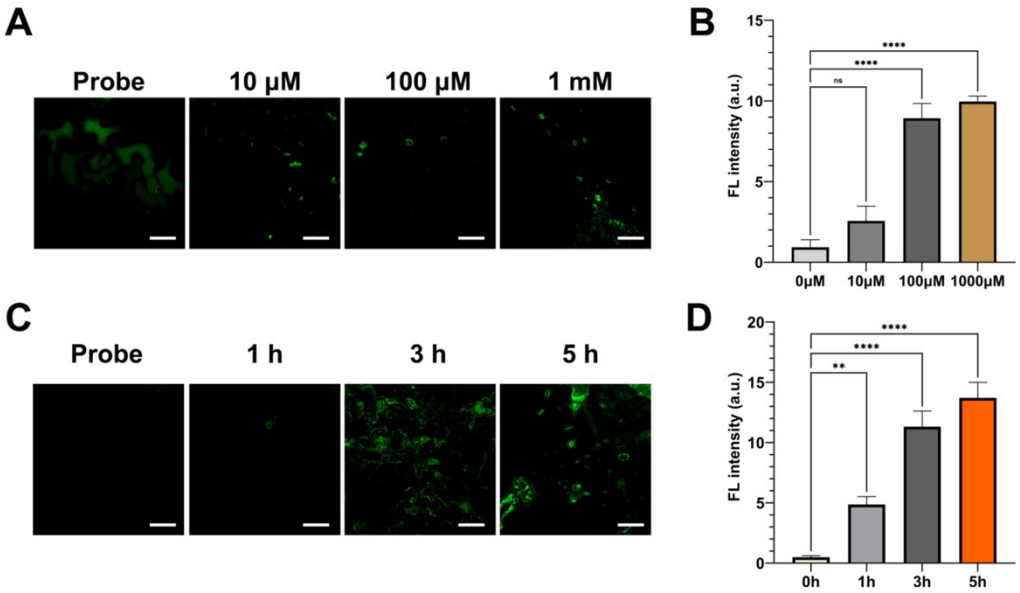


Figure S22. In Vivo Imaging of Hg^{2+} with **LJPT3** in Arabidopsis leaf epidermis. (A-B) Fluorescence pictures of Arabidopsis leaf epidermis treated with different concentrations of Hg^{2+} and their quantitative data. (C-D) Fluorescence pictures of Arabidopsis leaf epidermis under different Hg^{2+} treatment times and their quantitative data.

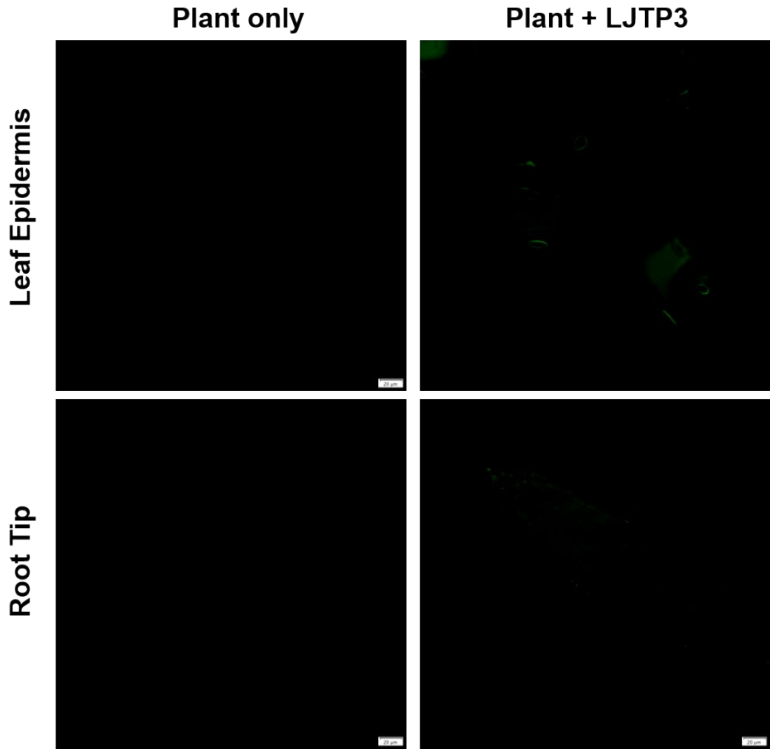


Figure S23. In Vivo Imaging of Arabidopsis with or without **LJPT3 (Scale bar: 20μm).**

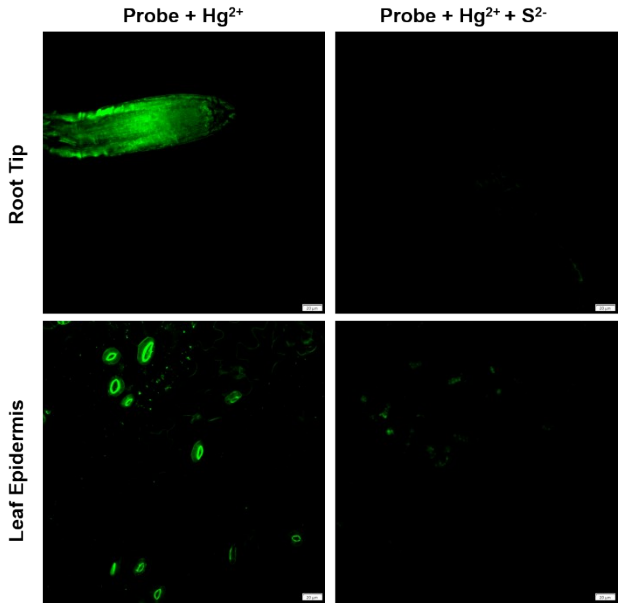


Figure S23. Two-photon fluorescence imaging comparison before and after S^{2-} (100 μM) addition (Scale bar: 20μm).

Table S1 Atomic coordinates of **LJTP3** molecule

C	2.71739000	-3.58001000	0.59775200
C	2.68547300	-4.27459000	-0.65263300
C	3.46839700	-3.78732600	-1.69264500
C	4.25980500	-2.62484600	-1.53991500
C	4.25737000	-1.92383500	-0.29345200
C	3.47289200	-2.45044700	0.76651300
C	5.04136400	-2.09696900	-2.60650800
C	5.76158000	-0.94006300	-2.45420200
C	5.74363100	-0.23654900	-1.21571200
C	5.00310900	-0.73336200	-0.15447700
N	1.89889400	-5.42813300	-0.77919600
C	1.92835300	-6.12461000	-2.05976600
C	0.59863300	-5.42631500	-0.11135800
C	-0.33788900	-4.36391400	-0.70894100
O	-0.64748500	-4.40801800	-1.89389300
N	-0.72821500	-3.39217500	0.17294000
C	6.49825600	0.99824100	-1.10017600
O	6.43838600	1.67318300	0.10773600
C	7.22484900	2.78223600	-0.08251700
C	7.72297000	2.72459800	-1.39432400
N	7.23548800	1.57254700	-2.00774700
C	7.51166500	3.80309500	0.80821500
C	8.35132800	4.81142800	0.32525100
C	8.86478900	4.77817300	-0.98355200
C	8.56118400	3.73886300	-1.86295300
H	0.72257000	-5.30062100	0.96726200
H	0.13855300	-6.40484200	-0.27293600
H	2.13834300	-3.95476300	1.43155800
H	3.46240800	-4.26964300	-2.66219000

H	3.46266100	-1.91816700	1.71116200
H	5.05156500	-2.62398400	-3.55703400
H	6.35161800	-0.53352400	-3.26815700
H	4.96951100	-0.19797500	0.78888400
H	1.49404800	-5.53426500	-2.87702300
H	2.96228700	-6.38457200	-2.30596000
H	1.35547100	-7.05011200	-1.96608300
H	-0.37910800	-3.47212500	1.12378600
H	7.10886100	3.81780600	1.81498400
H	8.61177700	5.63948900	0.97757700
H	9.51324300	5.58367100	-1.31528000
H	8.95481700	3.71041100	-2.87348600
C	-1.52361800	-2.25831000	-0.08559900
C	-1.69130400	-1.34798000	0.96666000
C	-2.12507000	-1.99443500	-1.31928000
C	-2.45792500	-0.18552200	0.83466700
H	-1.18245300	-1.56140400	1.89595100
C	-2.92224300	-0.86137900	-1.44137500
H	-1.99293500	-2.67869300	-2.14360100
C	-3.13030000	0.04021700	-0.39323500
N	-2.57036600	0.72956300	1.91235600
H	-3.43683300	-0.69774400	-2.38166300
N	-3.99588700	1.15623900	-0.52917300
C	-2.43879300	2.17181900	1.67524300
C	-2.47314200	0.21743900	3.25790600
C	-5.22429100	1.09434400	0.28355500
C	-4.16644400	1.68580700	-1.88203900
H	-3.33853300	2.59130400	1.22718700
H	-2.27879100	2.65949900	2.64129100

C	-1.26849700	2.53643300	0.74386800
H	-2.83908700	0.98240000	3.95212100
H	-3.13584800	-0.65139700	3.35667500
C	-1.05784600	-0.16449500	3.73345100
H	-4.94859500	0.76653500	1.28564000
H	-5.65577600	2.09704300	0.34683200
C	-6.23685900	0.09130400	-0.27184500
H	-4.81122300	1.07003900	-2.52340000
H	-3.17453500	1.74472700	-2.33727900
C	-4.79596300	3.07561100	-1.78218900
O	-1.41524200	3.27775500	-0.24527400
N	-0.10638800	1.98988500	1.10145800
O	-0.07122300	0.55137800	3.51396700
N	-0.95318600	-1.31427500	4.43428400
O	-6.22342000	-1.08560200	0.11256500
N	-7.05938400	0.58084100	-1.22384400
O	-6.01749900	3.24281500	-1.90455500
N	-3.91828000	4.05506400	-1.48717300
C	1.11231300	1.95874100	0.30901300
C	0.34658700	-1.86435700	4.80357900
C	-8.08877600	-0.20412900	-1.90614700
C	-4.34371000	5.36688600	-1.01514800
H	1.80486000	2.74675000	0.63011100
H	0.84160800	2.14587400	-0.73393200
H	0.97196100	-1.04858600	5.17536300
H	0.19063800	-2.57436100	5.62268400
H	-8.83087700	-0.55492500	-1.17823500
H	-8.58758400	0.48809800	-2.59105300
H	-5.38140600	5.50234600	-1.32812700

H	-3.72602200	6.13915600	-1.48623200
H	-6.91957900	1.55168600	-1.51104600
H	-2.97118300	3.75625900	-1.25062600
H	-1.74354700	-1.94429100	4.42757200
H	-0.11119000	1.48403000	1.98557100
C	1.78229100	0.59276700	0.45950700
H	1.07366200	-0.19716600	0.17213800
H	2.64260600	0.53398600	-0.21027700
C	1.04105900	-2.54102800	3.61990700
H	2.01234900	-2.94483200	3.93395500
H	1.22498600	-1.81851800	2.82288800
C	-4.22448700	5.47142800	0.50794300
H	-4.66901300	6.41521100	0.84299500
H	-4.81120000	4.65100900	0.95788500
C	-7.56973400	-1.42566700	-2.68381700
H	-6.64665400	-1.13173900	-3.21700900
H	-8.31365300	-1.68815900	-3.44601500
O	0.21943300	-3.56250600	3.04085800
H	0.22134900	-4.33985200	3.62053600
O	-2.88763400	5.47216800	0.97320200
H	-2.39072400	4.76225800	0.52327900
O	2.29909400	0.37079200	1.77234700
H	1.56861100	0.39042800	2.41552800
O	-7.38998600	-2.57015500	-1.88642200
H	-6.89854800	-2.27086600	-1.09171700

Table S2 Atomic coordinates of **LJTP3**-Hg^{2+}

C	5.10942500	-2.23416400	-0.90261000
C	5.47844000	-3.51504800	-0.36805800
C	6.75669300	-3.64452100	0.19101600
C	7.67654500	-2.57479400	0.19758700
C	7.30048400	-1.30679600	-0.36078100
C	5.98854300	-1.18064500	-0.89654900
C	8.98932700	-2.70356100	0.74589500
C	9.87305000	-1.65339300	0.73894300
C	9.49570000	-0.39407200	0.18308100
C	8.22207300	-0.23748600	-0.35274700
N	4.60182300	-4.57853800	-0.41188700
C	4.86433400	-5.74224600	0.41055100
C	3.26412400	-4.40132600	-0.92079800
C	2.34273900	-3.66091700	0.07451900
O	2.56446400	-3.72336600	1.27243000
N	1.31258000	-3.01462500	-0.52712100
C	10.45450500	0.69471500	0.19240500
O	10.05995300	1.90324300	-0.34509500
C	11.13999900	2.72301400	-0.20253400
C	12.15595000	1.96965500	0.42025100
N	11.67563000	0.68990000	0.65107000
C	11.28273200	4.05259300	-0.57346200
C	12.52514600	4.63651700	-0.29391800
C	13.55742700	3.90711100	0.32655400
C	13.39204300	2.56841600	0.69271500
H	2.82664600	-5.39660000	-1.09801800
H	3.28616200	-3.89876900	-1.90148200
H	4.10608700	-2.07023600	-1.29129600
H	7.07539600	-4.58905000	0.62896100

H	5.67091400	-0.21476200	-1.29718400
H	9.28379400	-3.66432300	1.17669600
H	10.87611300	-1.75264500	1.15644200
H	7.92423300	0.72434700	-0.77418500
H	4.97616400	-5.46987900	1.47397900
H	4.02038400	-6.43779000	0.33084700
H	5.77637300	-6.27252200	0.08334300
H	1.29338900	-3.04643800	-1.54008200
H	10.47282600	4.60596700	-1.05087800
H	12.69455300	5.68191000	-0.56225900
H	14.51003800	4.40379100	0.52693800
H	14.18890000	2.00001600	1.17546900
C	0.21554000	-2.31850400	0.04204400
C	-0.74290500	-1.82014300	-0.84408100
C	0.05716900	-2.11599700	1.41840700
C	-1.86423200	-1.10852600	-0.40512800
H	-0.62669800	-1.99378800	-1.91693000
C	-1.05267300	-1.39680800	1.85418800
H	0.79393900	-2.50646200	2.11611600
C	-2.01354500	-0.87003900	0.97984900
N	-2.83811200	-0.68259800	-1.36028800
H	-1.17913200	-1.22202500	2.92430500
N	-3.06023700	-0.03560900	1.49303800
C	-2.31573300	0.07949400	-2.48753400
C	-3.78203900	-1.73110900	-1.74584900
C	-2.53164500	1.16802100	2.13832100
C	-4.09153300	-0.70574100	2.30785200
H	-1.52998700	-0.45888400	-3.05185100
H	-3.15503100	0.23775200	-3.18403200

C	-1.74451300	1.45982600	-2.12208200
H	-3.38502700	-2.36545000	-2.56347500
H	-3.94409400	-2.38445400	-0.87291800
C	-5.17091100	-1.25549700	-2.20018700
H	-2.15037800	0.97476500	3.16032300
H	-1.68796000	1.53333300	1.53369600
C	-3.51539200	2.33441600	2.27605200
H	-4.80561900	0.06622200	2.61487700
H	-3.63553300	-1.15778900	3.21405700
C	-4.82880800	-1.80820100	1.54711500
O	-0.74219900	1.83180200	-2.79016700
N	-2.39790200	2.11873300	-1.18363400
O	-5.81890900	-2.02533300	-2.92171000
N	-5.62149700	-0.08623800	-1.71581800
O	-3.27055000	3.18558900	3.16116400
N	-4.54451000	2.36692100	1.43585500
O	-4.16806400	-2.87850500	1.34092400
N	-6.06044300	-1.52812200	1.19019300
Hg	-4.46320200	1.14457000	-0.37900300
C	-2.01070200	3.48591200	-0.86446500
C	-7.02626100	0.21468500	-1.91507900
C	-5.57081200	3.37289400	1.65250700
C	-6.80524300	-2.55952900	0.47537600
H	-2.14668200	4.15155000	-1.74345100
H	-2.68958200	3.85405300	-0.07913000
H	-7.58883100	-0.73219100	-1.97607300
H	-7.19125900	0.73519600	-2.88366600
H	-6.51151900	3.00511700	1.21180500
H	-5.73788000	3.48737600	2.74052400

H	-6.30458100	-2.86892000	-0.46171900
H	-7.76622900	-2.10481800	0.17950000
C	-7.60441400	1.10185000	-0.81423000
H	-8.62517200	1.40022100	-1.12904500
H	-7.01084500	2.05034700	-0.80605800
C	-0.57085600	3.70187500	-0.37237600
H	-0.34386300	2.91135400	0.38083700
H	-0.54070600	4.66448800	0.17410500
C	-5.22370600	4.74344200	1.07972300
H	-6.11142200	5.40493700	1.19035600
H	-5.04613800	4.63373200	-0.01174600
C	-7.11149600	-3.83665200	1.26807800
H	-7.48445600	-3.52853100	2.27445100
H	-7.95798800	-4.35638100	0.77053700
O	-6.03975600	-4.73793700	1.35540700
H	-5.22293600	-4.17218200	1.34109800
O	0.39208900	3.75304000	-1.39138100
H	0.10678500	3.05593100	-2.03733400
O	-4.10445000	5.33531500	1.69373700
H	-3.73676100	4.67845700	2.33060000
O	-7.69933500	0.54861400	0.46311300
H	-6.95487300	-0.08233300	0.71260100

Conformation search process:

Firstly, a series of possible conformations were obtained by 500ps molecular dynamics simulation using GFN0-xTB [1] method of xtb [2]. Then, using Molclus [3] to call xtb, then the molecular structure was optimized step by step under the methods of GFN0-xTB and GFN2-xTB [4] (with GBSA implicit water model [5]) and the weight was removed to obtain more possible molecular conformation. Finally, the structures were optimized and analyzed the vibration under B3LYP[6]/def2-SVP [7][8][9][10] level, and the final molecular structure and free energy thermal correction were obtained. And in ORCA 6.0.0[11], the RIJCOSX [12] technique and the auxiliary basis def2/J

[13][14][15] were enabled to accelerate all calculations. ωB97M-V [16] functionals and the def2-TZVP [7][8][9][10] basis set was used to calculate the single point energy under GBSA implicit water solvent model. Ultimately, the free energy of compound under the solvent is obtained from the free energy thermal correction and the single point energy.

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