Supplementary Information (SI) for Analytical Methods.

Supporting Information

A NIR-II Fluorescence Probe for Tracking Oxidative Stress in Plants Induced by Metal Contaminants

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Table of Contents

Experimental section

1.Reagents.

Cyclohexanone, phosphorus oxychloride ($POCl_3$), acetic anhydride (Ac_2O), 1,4-dioxane, tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$), sodium acetate, resorcinol, potassium carbonate (K_2CO_3), cyclohexanone, 4-(diethylamino) salicylaldehyde and cesium carbonate (Cs_2CO_3) were purchased from Energy Chemical Reagents. $CuCl_2$, $FeCl_3$, KCl, $CaCl_2$, $MgCl_2$, $NaNO_3$, Na_2SO_3, HClO, H_2O_2 and GSH were obtained from Aladdin Reagent. (Z)-1-[N-Methyl-N-[6-(N-methylammoniohexyl) amino]] diazen-1-ium-1,2-diolate, also known as methylamine hexamethylene methylamine NONOate, or MAHMA NONOate), was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-(4-Carboxyphenyl)-4,4,5,5-tetramethy-limidazoline-1-oxyl-3-oxide, monopotassium salt (cPTIO) and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich. 10% phosphate buffer saline (PBS) was purchased from KeyGen Bio Tech. The solvents dimethylformamide (DMF), dichloromethane (DCM), dioxane, ethanol, methanol, tetrahydrofuran (THF) and chloroform were purchased from Aladdin Regents and dried with molecular sieve and distilled before use. Other solvents used in this study were analytical grade reagents and used without further purification.

2. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on an AVANCE III HD 400 NMR spectrometer. High-resolution mass spectrometry was conducted on a Bruker MAXIS IMPACT mass spectrometer. The absorption spectra were measured on a Hitachi U-3010 spectrophotometer; and Near-Infrared II (NIR-II) fluorescence spectra were determined on a NIRQUEST512 spectrometer (excitation: 808 nm laser, emission range: 900-1700 nm). The NIR-II fluorescence imaging in plant was performed by using a NIR-II animal imaging system produced by Suzhou NIR-Optics Technologies Co., Ltd.

3. Syntheses

The synthetic route for the probe is outlined in Scheme S1 in Supporting

Information.

Compound **1** was synthesized according to previous literature reports.[1]

Compound **3** was synthesized according to previous literature reports.[2]

Compound **8** was synthesized according to previous literature reports.[3]

Synthesis of Compound 2

A mixture of 2,3,3-trimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-indole (compound 1, 2.00 g, 7.01 mmol, 1.00 eq), 1-bromo-1,2,2-triphenylethylene (3.05 g, 9.13 mmol, 1.30 eq), potassium acetate (1.94 g, 14.02 mmol, 1.30 eq), $Pd(PPh_3)_4$ (405.04 mg, 0.35 mmol, 0.05 eq) and 1,4-dioxane/H_2O (30 mL, V/V=5/1) was heated to 105 °C under stirring and then refluxed for 24 h. Upon completion of reaction, the mixture was cooled to room temperature, and the solvents were removed under vacuum. Afterward, the resultant solid was dissolved in DCM, washed three times with water and dried with anhydrous sodium sulfate. The organic layer was collected and the solvent was evaporated under vacuum. The resulting crude solid was purified by flash column chromatography over silica gel with EA/PE (4/1, v/v) and collected as buff yellow solid (2.48 g, 85.6%). ^1H NMR (400 MHz, CDCl3) δ 7.29 (d, J = 8.1 Hz, 1H), 7.18 – 7.04 (m, 12H), 7.04 – 6.99 (m, 3H), 6.93 (d, J = 8.0, 1H), 6.87 (s, 1H), 2.21 (s, 3H), 1.03 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 188.33, 151.92, 144.73, 144.12, 143.70, 141.23, 140.86, 140.75, 131.47, 131.36, 130.07, 127.67, 126.51, 126.39, 126.28, 125.07, 118.91, 53.16, 22.87, 15.43. $[M+H]^+$ $[C31H28N]^+$ calcd. 414.2222, found 414.2220.

Synthesis of Compounds 4-7

Compound **2** (2.00 g, 4.84 mmol, 1.00 eq) and compound **3** (2.65 g, 9.68 mmol, 2.00 eq) were dissolved in anhydrous acetonitrile (5 ml). The mixture was heated to 90 °C and refluxed for 12 h. After completion of the reaction, the mixture was cooled to ambient temperature and acetonitrile was evaporated under vacuum. DCM (50 mL) was added to dissolve the obtained solid and the solution was washed three times with water and then dried over anhydrous sodium sulfate. Afterward, the solvent of the solution was evaporated under vacuum to yield compound 4 as yellowish brown oil. The crude product was directly used in the next step without purification.

4-Hydroxybenzylamine (2.47 g, 20.00 mmol, 1.00 eq) and di-tert-butyl decarbonate (4.80 g, 22.00 mmol, 1.10 eq) was dissolved in 30 mL THF, the mixture was heated to 50 °C and stirred for 12 h. The solvent of the resultant mixture was removed under vacuum. The obtained colorless oil (compound **5**) was directly used in the next step.

Compound **5** (4.46 g, 20 mmol, 1.00 eq) and Cs_2CO_3 (7.82 g, 24 mmol, 1.20 eq) were dissolved in 40 mL DMF, and the solution mixture was heated to 60 °C and stirred for 0.5 h. Afterward, compound 4 (6.03 g, 22 mmol, 1.10 eq) was slowly added into the solution. After stirring at 60 °C for 12 h, the solution was cooled to ambient temperature, and poured into 200 mL ice water, followed by extraction with 200 mL ethyl acetate. The organic layer was washed with water for three times, and then dried over anhydrous sodium sulfate. The solvent of the obtained solution was evaporated under vacuum. The resultant light-yellow oil (compound 6) was directly used in the next step.

Compound 6 (7.38 g, 20 mmol) was dissolved in 40 mL THF. After the solution was heated to 60 °C, concentrated hydrochloric acid (5 mL, 37%) was slowly added into the reaction medium, which was then stirred at 60 °C for 4 h. Afterward, the reaction medium was cooled to ambient temperature and poured into 200 mL ice water. The mixture was neutralized with sodium bicarbonate to pH 8-9, and the organic phase was extracted with 200 mL ethyl acetate, and then was washed with water for three times, dried over anhydrous sodium sulfate. The solvent of the solution was then evaporated under a vacuum, and the resulting crude oil (compound 7) was purified by flash column chromatography over silica gel with DCM/MeOH (50/1, v/v) as straw yellow oil (3.31 g, 61.5%).^{1}H NMR (400 MHz, CDCl3) δ 7.20 (d, J = 8.9 Hz, 2H), 6.86 (d, J = 12.4 Hz, 2H), 4.14 – 4.07 (m, 2H), 3.87 – 3.82 (m, 2H), 3.79 (s, 2H), 3.76 – 3.70 (m, 2H), 3.69 – 3.62 (m, 4H), 3.57 – 3.51 (m, 2H), 3.37 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 158.64, 129.88, 128.53, 114.89, 71.87, 70.75, 70.55, 70.46, 69.67, 67.40, 59.00, 43.51. $[M+H]^+$ $[C_{14}H_{24}NO_4]^+$ calcd. 270.1705, found 270.1707.

Synthesis of Compound 9

Compound **4** (1.23 g, 2.20 mmol, 2.2 eq), compound **8** (0.172 g, 1.00 mmol, 1.00 eq) and sodium acetate (0.205 g, 2.5 eq, 2.5 eq) were dissolved in 20 mL acetic anhydride. The mixture was heated to 85 °C and stirred for 3 h. Afterward, the reaction medium was cooled to ambient temperature and the solvent was removed by under vacuum, and the obtained crude product (compound **9**) was purified by flash column chromatography over silica gel with DCM/MeOH (20/1, v/v). (0.582 g, 46.4%). ^{1}H NMR (400 MHz, CDCl3) δ 8.17 (d, J = 14.2 Hz, 2H), 7.15 – 6.96 (m, 34H), 6.92 (d, J = 1.7 Hz, 2H), 6.30 (d, J = 14.0 Hz, 2H), 4.38 (t, J = 5.3 Hz, 4H), 3.90 (t, J = 5.4 Hz, 4H), 3.62 (dd, J = 5.6, 3.7 Hz, 4H), 3.58 – 3.49 (m, 8H), 3.45 (dd, J = 5.6, 3.7 Hz, 4H), 3.32 (s, 6H), 2.70 (t, J = 6.1 Hz, 4H), 1.93 (t, J = 5.7 Hz, 2H), 1.39 (s, 12H). ^{13}C NMR (101 MHz, CDCl3) δ 172.70, 143.86, 143.50, 143.19, 141.76, 141.03, 140.16, 131.73, 131.45, 131.28, 127.99, 127.73, 126.85, 126.68, 125.69, 110.66, 102.60, 71.88, 70.96, 70.58, 68.03, 59.05, 48.65, 45.69, 27.96, 26.89. HR-MS (ESI, m/z) $[M]^+$ $[C_{84}H_{88}ClN_2O_6]^+$ calcd. 1255.6331, found 1255.6337**.**

Synthesis of Cy-TPE-NH

Compound **9** (0.400 g, 0.32 mmol, 1.00 eq) was dissolved in 10 mL DMF, and the solution was then heated to 80 °C. Compound 7 (0.172 g, 0.64 mmol, 2.00 eq) was dissolved in 0.5 mL DMF and then slowly added into the solution of compound 9. The mixture was heated to 80 °C and stirred for 3 h before being cooled to ambient temperature. The resultant solution was poured into 50 mL ice water and extracted with 50 mL DCM. The extracted organic phase was washed with water for three times and dried over anhydrous sodium sulfate. The solvent of the resultant mixture was evaporated under vacuum to obtain the crude product as dark blue solid, which was then purified by flash column chromatography over silica gel with DCM/MeOH (15/1, v/v) as blue solid (0.347 g, 74.3%). ^{1}H NMR (400 MHz, DMSO) δ 8.78 (s, 1H), 7.42 (d, J = 13.1 Hz, 2H), 7.23 (d, J = 8.7 Hz, 2H), 7.19 – 7.06 (m, 18H), 7.04 – 6.91 (m, 16H), 6.82 (s, 2H), 6.76 (d, J = 8.1 Hz, 2H), 5.81 (d, J = 12.5 Hz, 2H), 4.75 (s, 2H), 4.14 – 4.09 (m, 2H), 4.09 – 4.00 (m, 4H), 3.82 – 3.75 (m, 2H), 3.67 (t, J = 5.3 Hz, 4H), 3.59 (dd, J = 6.0, 3.6 Hz, 2H), 3.51 (dd, J = 6.0, 3.6 Hz, 2H), 3.49-3.45 (m, 6H), 3.44 – 3.33 (m, 12H), 3.31 (s, 2H), 3.19 (s, 3H), 3.17 (s, 6H), 2.47 (d, J = 6.6 Hz, 4H), 1.72 (t, J = 6.4 Hz, 2H), 1.06 (s, 12H). ^{13}C NMR (101 MHz, DMSO) δ 167.57, 158.64, 143.73, 143.58, 140.85, 140.58, 139.13, 137.87, 137.66, 131.33, 131.13, 130.88, 130.57, 128.74, 128.36, 128.32, 127.15, 126.85, 125.18, 71.74, 71.71, 70.69, 70.50, 70.30, 70.16, 70.08, 69.47, 67.63, 28.09, 25.55, 25.44. HR-MS (ESI, m/z) $[M]^+$ $[C_{98}H_{110}N_3O_{10}]^+$ calcd. 1489.8225, found 1489.8242.

3. Optical response of the probe Cy-TPE-NH towards NO.

The stock solutions of the probe with the concentration of 10 mM were prepared. The test solutions were prepared by mixing the stock solution, PBS and the NO donor MAHMA NONOate of different concentrations at 25 °C. For the time-dependent experiments, the test solutions were kept at 25 °C for different time periods before spectral measurements. For specificity experiments, the spectra of the test solutions were measured 6 minutes after the addition of NO or other substances into the probe solutions. The absorption and fluorescence spectra were determined at 25 °C.

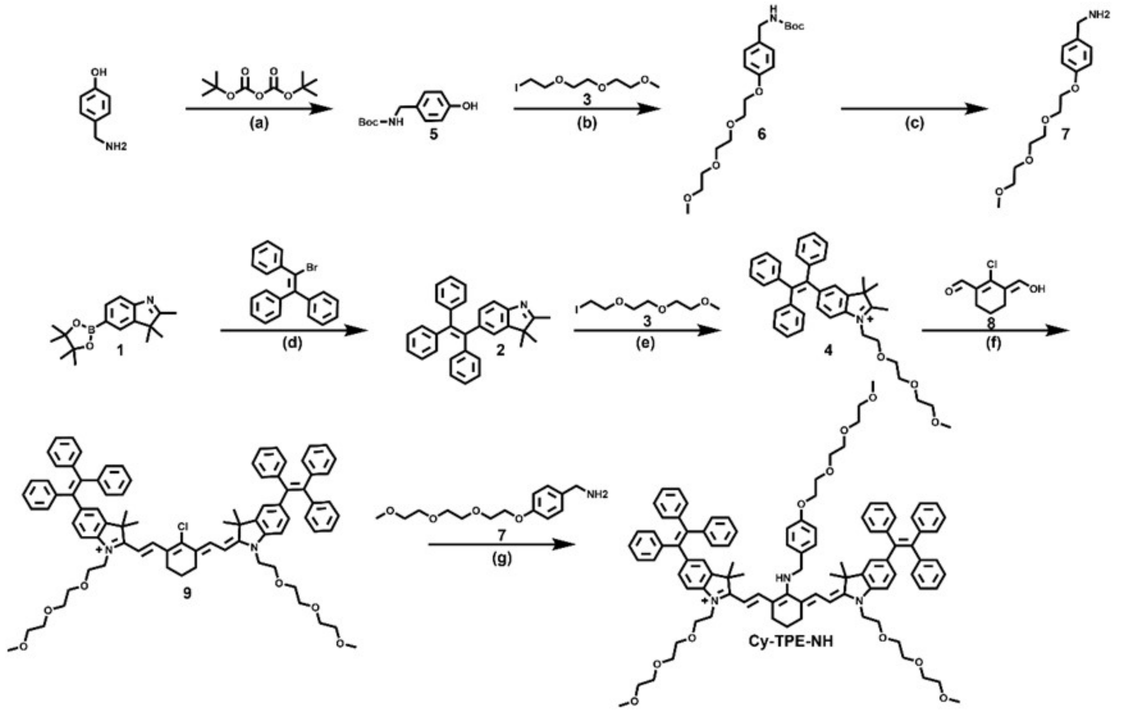
5. Establishment of Al^{3+} and Cd^{2+} -induced stress in wheat and in soybean models and fluorescence imaging.

For the Al^{3+} -induced oxidative stress experiment, wheat and soybean seeds were sterilized and germinated in the dark for 3 days at 25 °C on plates filled with clean water. The water in plates was refreshed every day. Three days later, selected the uniform seedlings for various treatments. To induce oxidative stress, wheat seedlings and soybean sprouts were cultured in clean water containing $AlCl_3$ (30 μM) for 12 h. To investigate the effects of NO donor and scavenger, wheat and soybean sprouts were respectively pretreated with aqueous solutions containing 30 μM $AlCl_3$ with a NO scavenger cPTIO (2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3-oxide, monopotassium salt, 100 μM) or a NO donor sodium nitroprusside (SNP, 100 μM) for 12 h under the same conditions as described above. To investigate the effect of incubation time on the oxidative stress, the wheat seedlings and soybean sprouts were co-cultured with $AlCl_3$ solution (30 μM) for 0 h, 3 h, 6 h, 9 h and 12 h, respectively. To investigate the Al^{3+} concentration on the oxidative stress, the wheat seedlings and soybean sprouts were co-cultured with $AlCl_3$ solutions with varied concentrations (0 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM and 100 μM) respectively for 12 h. Before imaging, the wheat seedlings were incubated with probe (Cy-TPE-NH) for 2 h in water containing 10% ethanol and were then placed into the imaging chamber of a NIR-II small animal imaging system (Series II, Suzhou NIR-Optics Technologies Co., Ltd.).

To explore the effect of Cd^{2+} of varied concentrations on germination of wheat and soybean, wheat and soybean seeds were germinated in plastic dishes filled with clean water containing Cd^{2+} of varied concentrations (0 μM, 20 μM, 80 μM, 140 μM and 200 μM) respectively for 3 days. After that, the wheat seedlings and soybean sprouts were incubated with the probe (Cy-TPE-NH) for 2 h in water contain 10% ethanol, and placed into the imaging chamber of the small animal imaging system for imaging.

For the investigation on oxidative stress induced by short-term exposure to Al^{3+} and Cd^{2+}, wheat and soybean seeds were cultured in clean water for 3 days to germinate into wheat seedlings and soybean sprouts. The wheat seedlings and soybean sprouts were then cultured with various levels of Al^{3+} or Cd^{2+} (0 μM, 30 μM, 60 μM or 90 μM) for 3 h. Then, wheat seedlings were incubated with the probe (Cy-TPE-NH) for 2 h in water containing 10% ethanol and were then placed into the imaging chamber of a NIR-II small animal imaging system (Series II, Suzhou NIR-Optics Technologies Co., Ltd.).

For fluorescence imaging measurement, the excitation laser is 808 nm with its power density at 60 mW/cm^2, and the fluorescence signals were collected through a 900 nm long-pass filter.



Scheme S1. Synthetic route for the probe Cy-TPE-NH.

Reagents and conditions: (a) THF, 50 °C,12 h; (b) Cs_2CO_3, DMF, 60 °C, 0.5 h; 60 °C, 12 h; (c) HCl, THF, 60 °C, 4 h, yield 61.5%; (d) CH_3COOK, Pd(PPh3)4, 1,4-dioxane/H_2O, 105 °C, 24 h, N_2, yield 85.6%; (e) CH_3CN, 90 °C, 12 h.

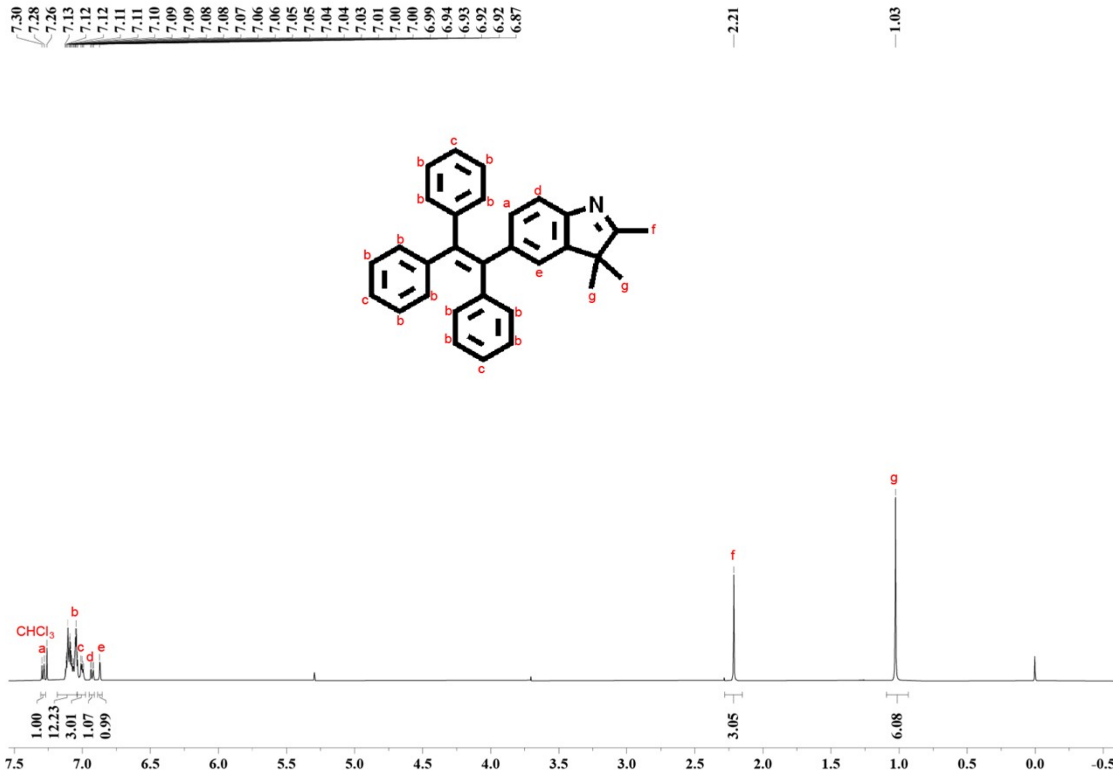


Fig. S1. ^{1}H NMR spectrum of Compound 2 in $CDCl_3$.

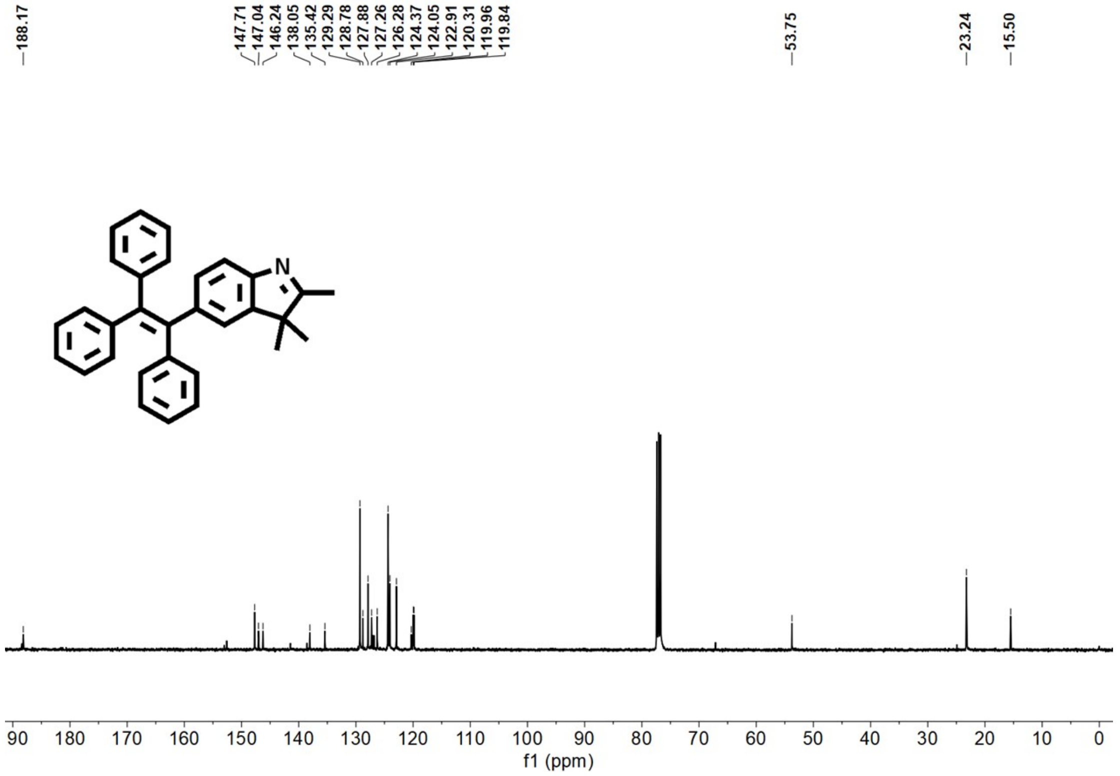


Fig. S2. ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

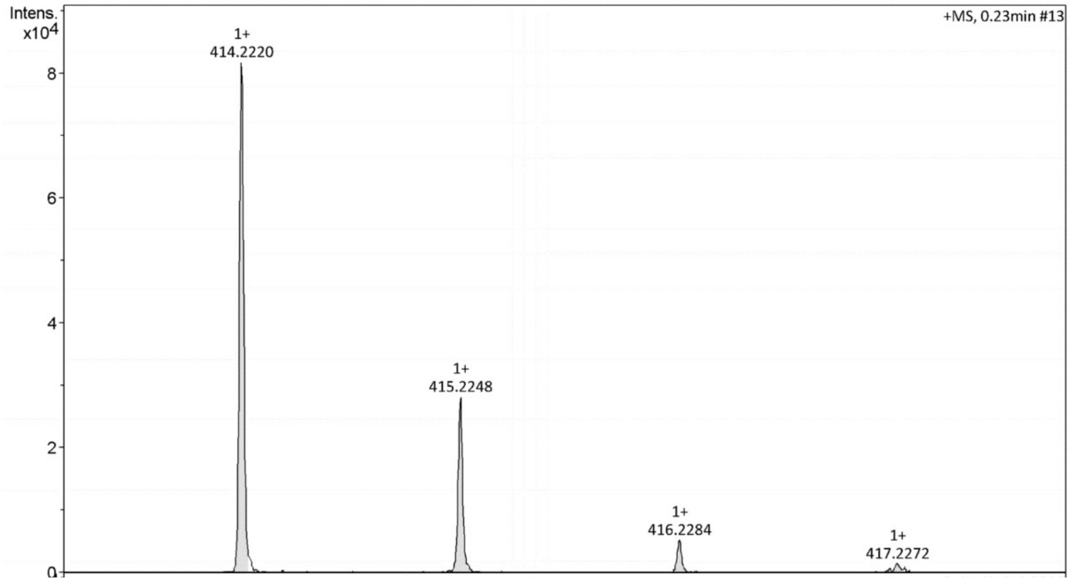


Fig. S3.HR Mass spectrum of compound **2**.

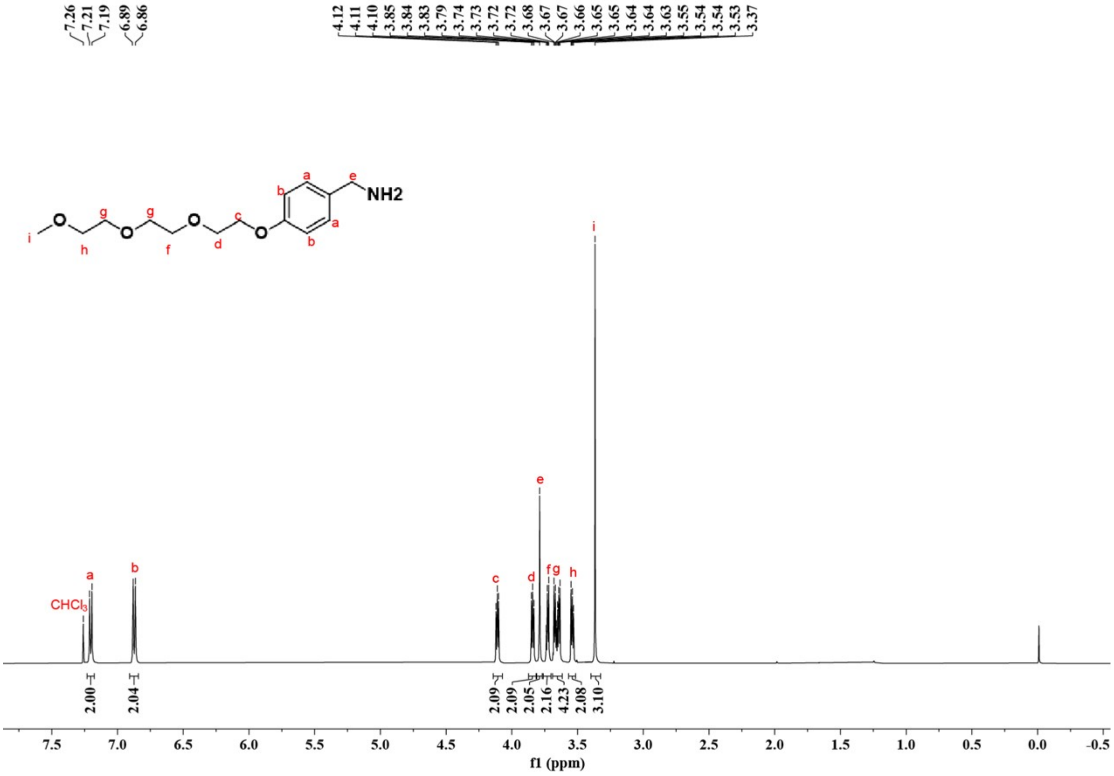


Fig. S4. ^{1}H NMR spectrum of compound **7** in $CDCl_3$.

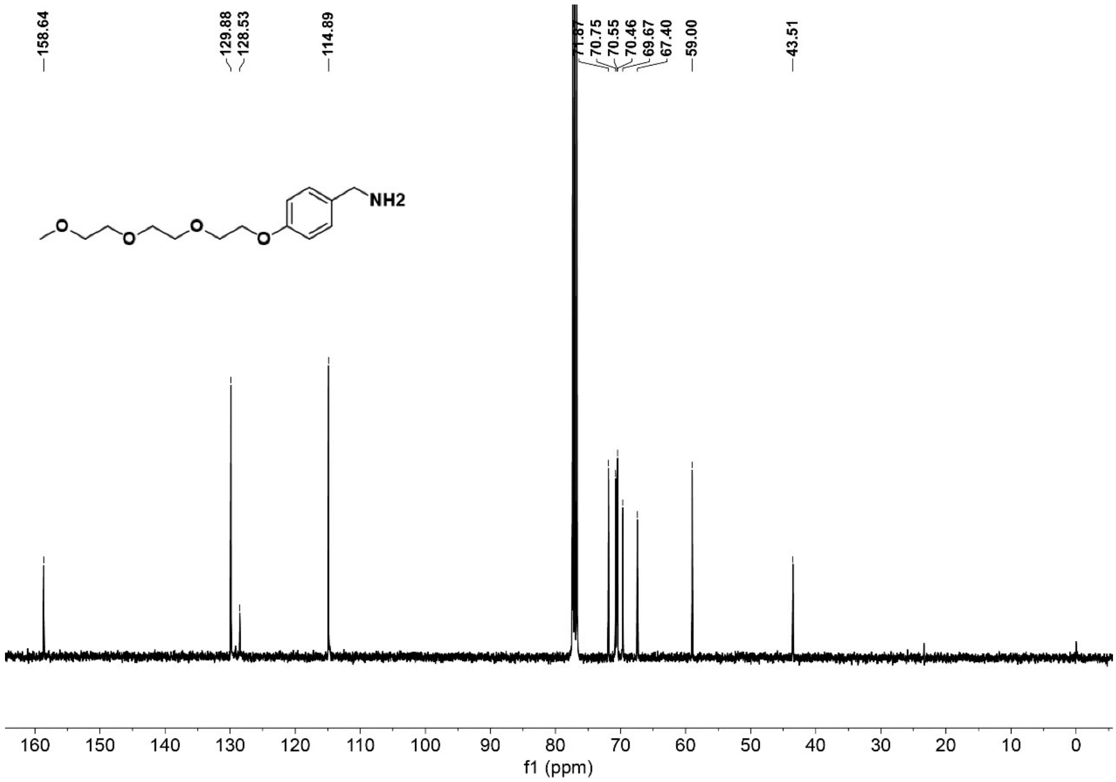


Fig. S5.^{13}C NMR spectrum of compound **7** in $CDCl_3$.

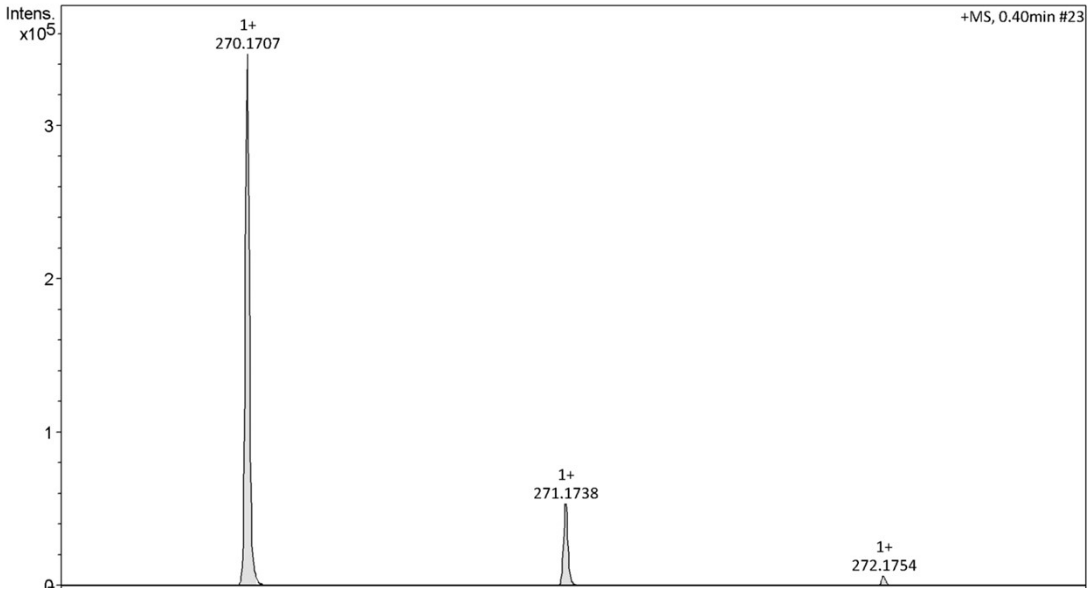


Fig. S6. HR Mass spectrum of compound **7**.

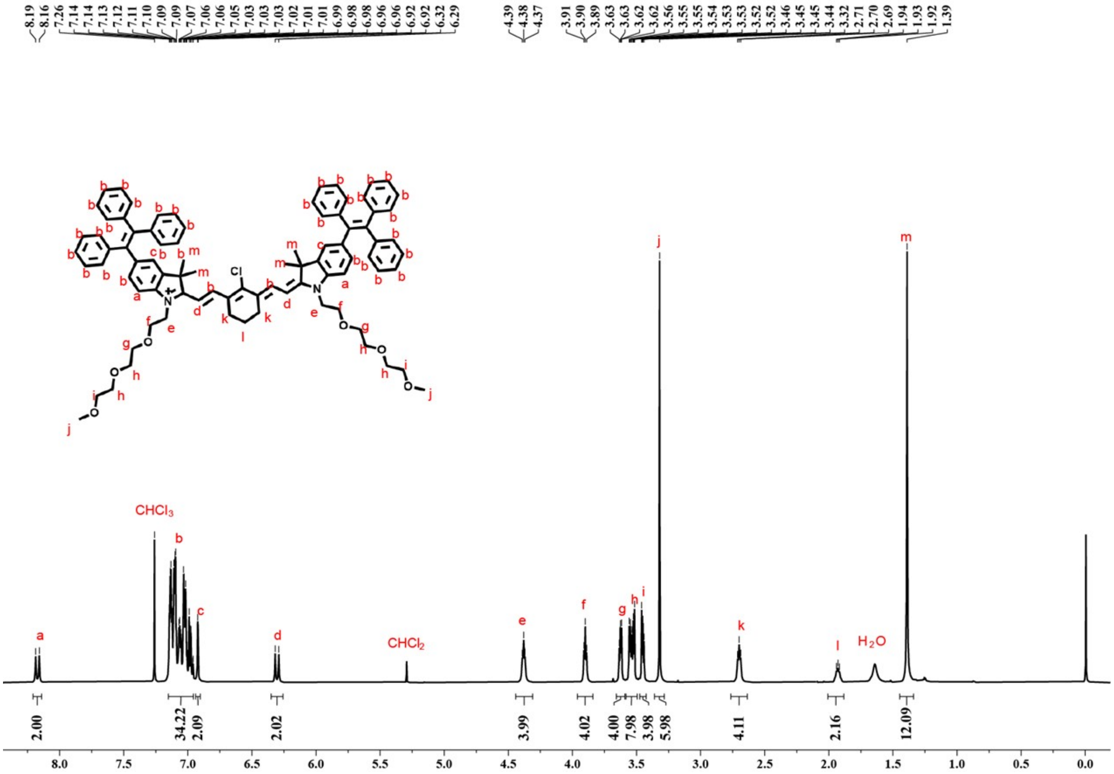


Fig. S7. ^{1}H NMR spectrum of Compound 9 in $CDCl_3$.

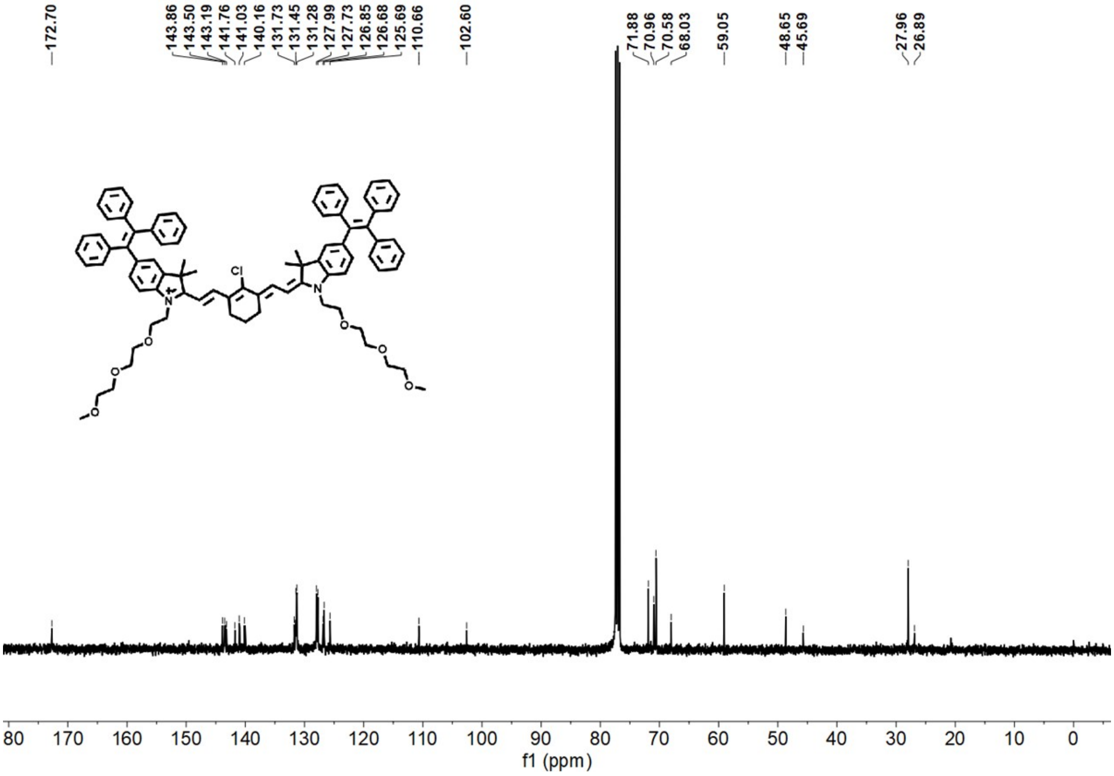


Fig. S8. ^{13}C NMR spectrum of Compound 9 in $CDCl_3$.

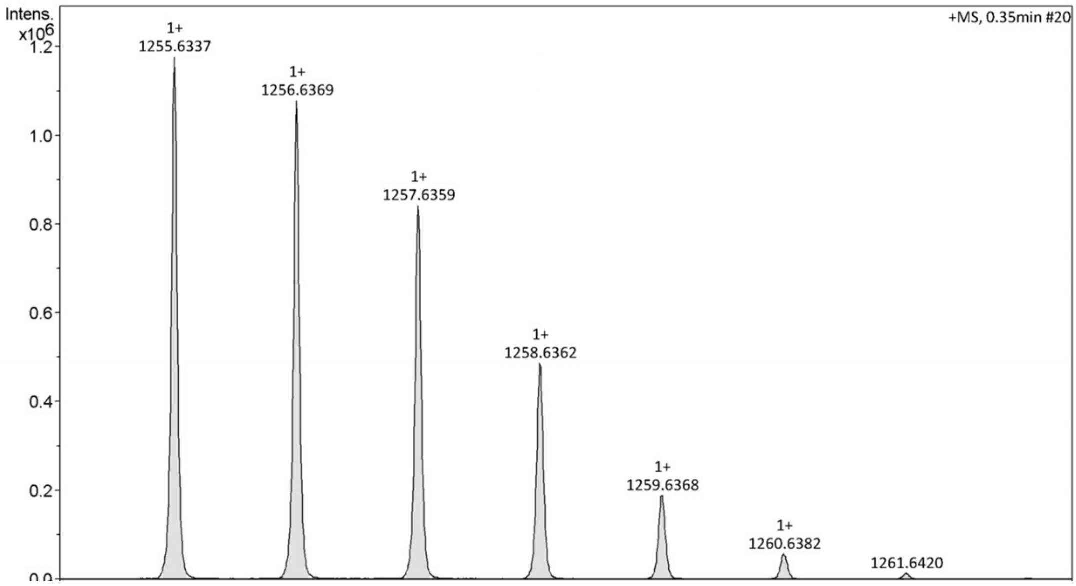


Fig. S9.HR Mass spectrum of compound **9**.

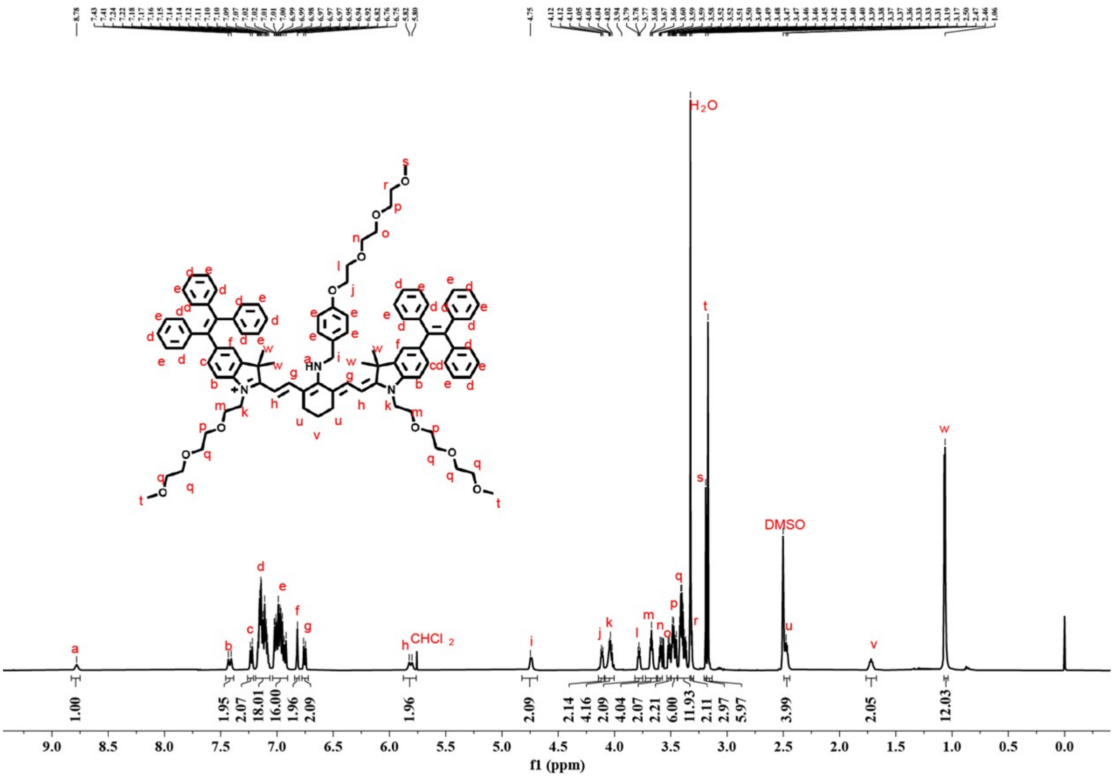


Fig. S10. ^{1}H NMR spectrum of Cy-TPE-NH in DMSO-d_6.

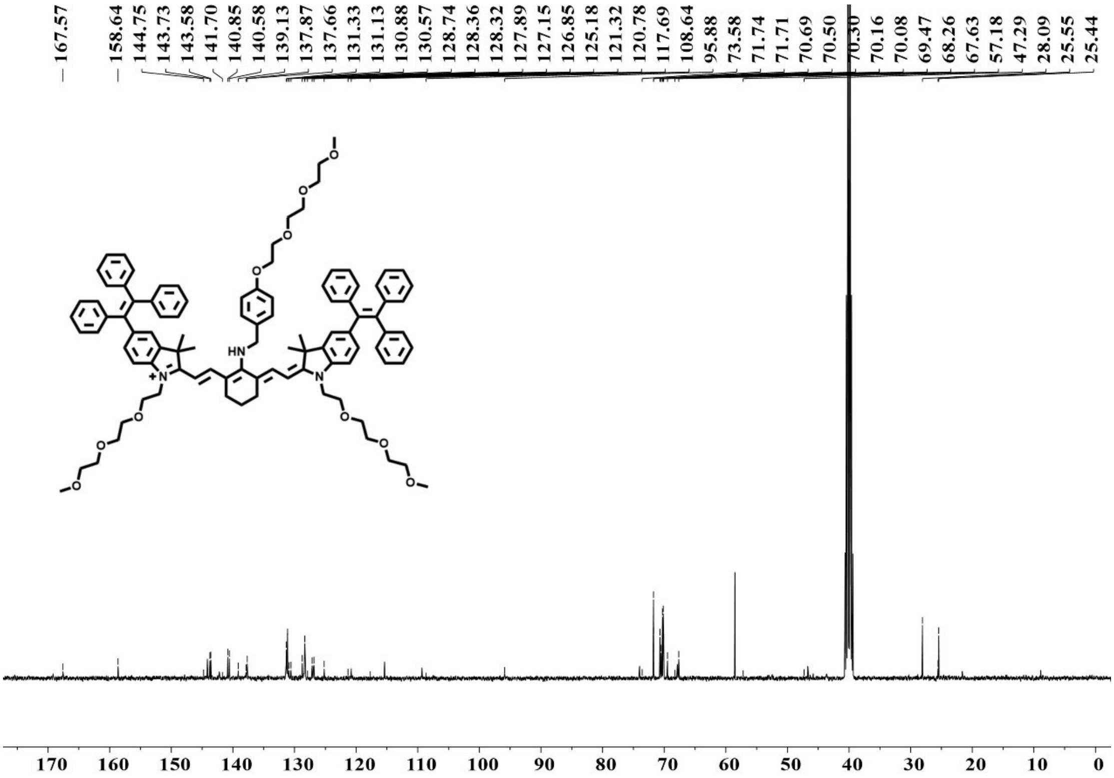


Fig. S11. ^{13}C NMR spectrum of Cy-TPE-NH in DMSO-d$_6$.

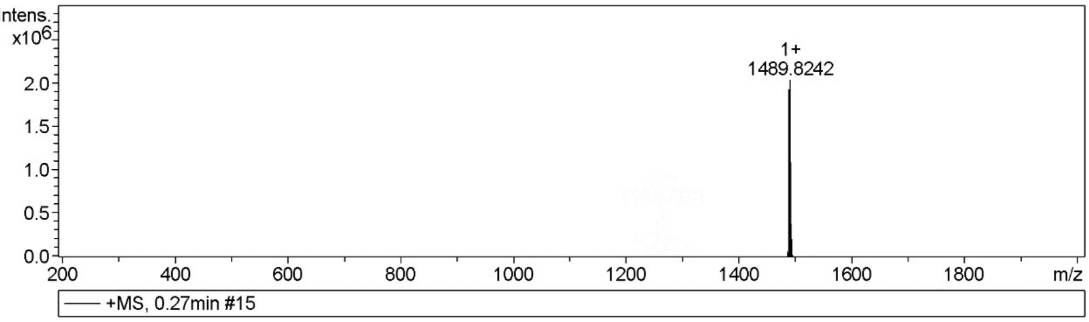


Fig S12. HR Mass spectrum of Cy-TPE-NH.

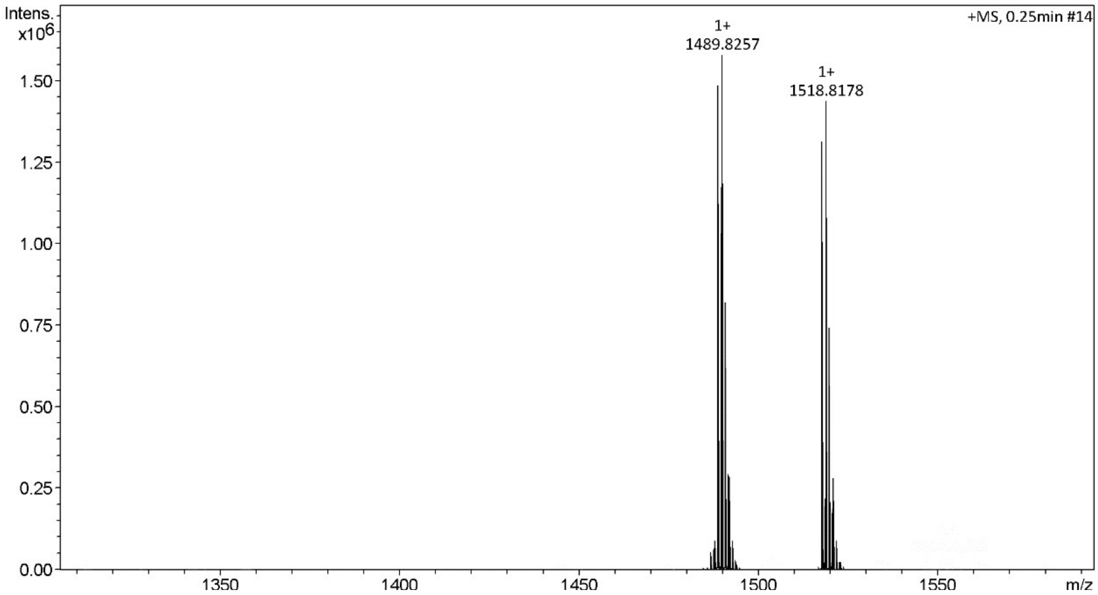


Fig. S13. HR Mass spectrum of Cy-TPE-NH upon treatment with MAHMA NONOate (incomplete reaction).

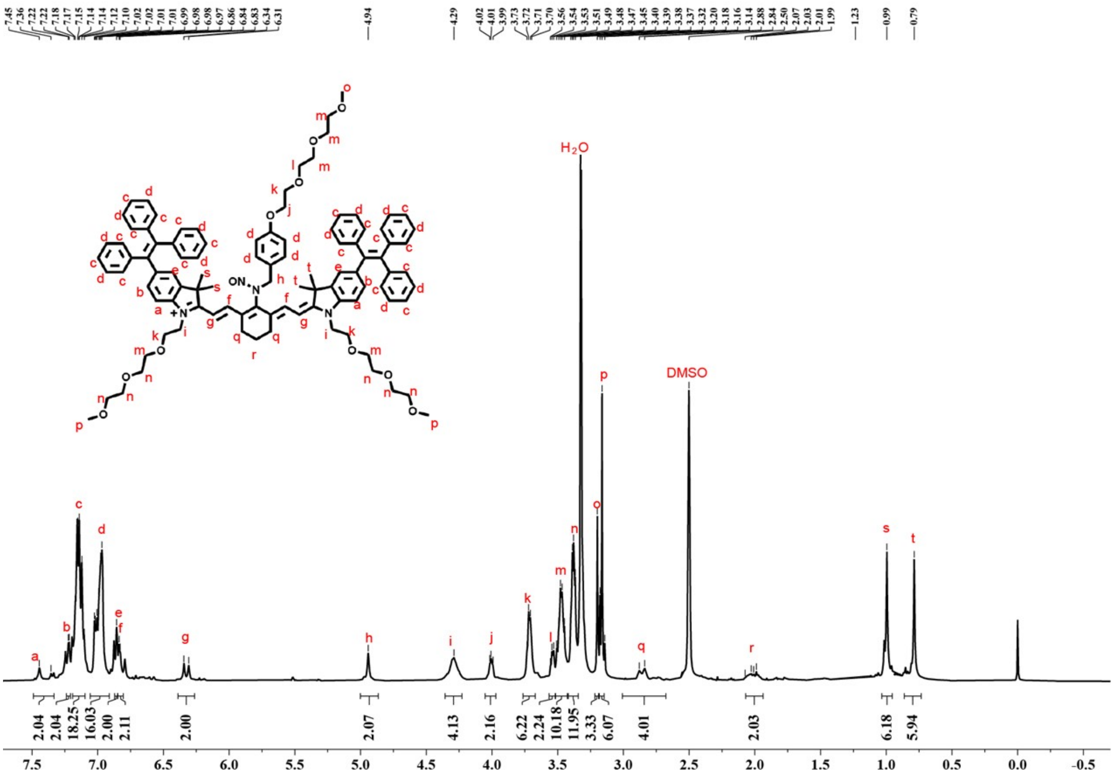


Fig. S14. ^{1}H NMR spectrum of the response product (Cy-TPE-NO) in DMSO-d$_6$.

^{1}H NMR (400 MHz, DMSO) δ 7.45 (s, 2H), 7.22 (d, J = 2.8 Hz, 2H), 7.19 – 7.10 (m, 18H), 7.06 – 6.91 (m, 16H), 6.86 (s, 2H), 6.83 (d, J = 3.2 Hz, 2H), 6.33 (d, J = 14.5 Hz, 2H), 4.94 (s, 2H), 4.29 (s, 4H), 4.05 – 3.97 (m, 2H), 3.73-3,70 (m, 6H), 3.54 (d, J = 5.5 Hz, 2H), 3.52 – 3.43 (m, 10H), 3.40-3.37 (m, 12H), 3.20 (s, 3H), 3.16 (s, 6H), 2.88-2.84 (m, 4H), 2.07 – 1.94 (m, 2H), 0.99 (s, 6H), 0.79 (s, 6H).

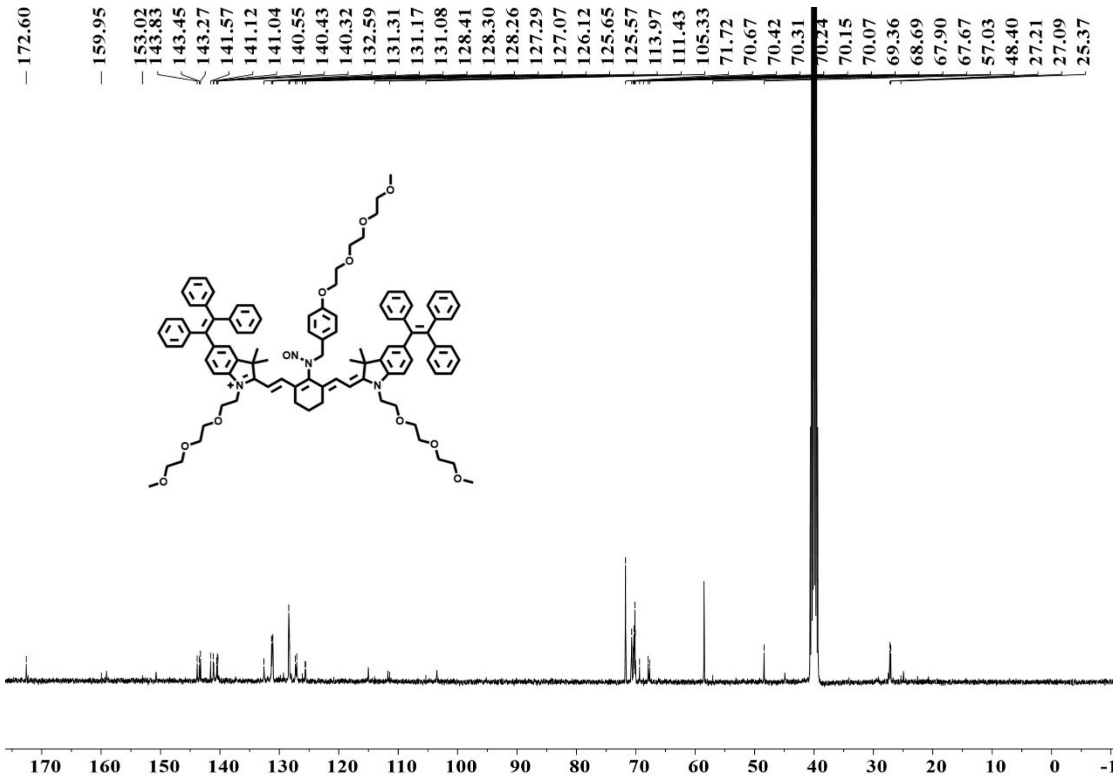


Fig. S15. ^{13}C NMR spectrum of the response product (Cy-TPE-NO) in DMSO-d$_6$.

^{13}C NMR (101 MHz, DMSO) δ 172.60, 143.83, 143.45, 143.27, 141.57, 141.12, 141.04, 140.55, 140.43, 140.32, 132.59, 131.31, 131.17, 131.08, 128.41, 128.30, 128.26, 127.29, 127.07, 125.65, 125.57, 71.72, 70.67, 70.42, 70.31, 70.24, 70.15, 70.07, 69.36, 67.90, 67.67, 48.40, 27.21, 27.09.

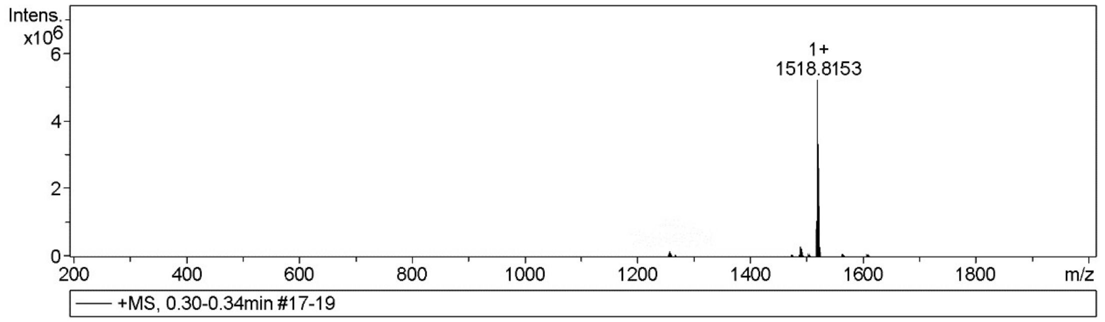


Fig. S16. HR Mass spectrum of the response product (Cy-TPE-NO).

HR-MS (ESI, m/z) [$C_{98}H_{109}N_4O_{11}$]$^+$ [M]$^+$ 1518.8121, found 1518.8153.

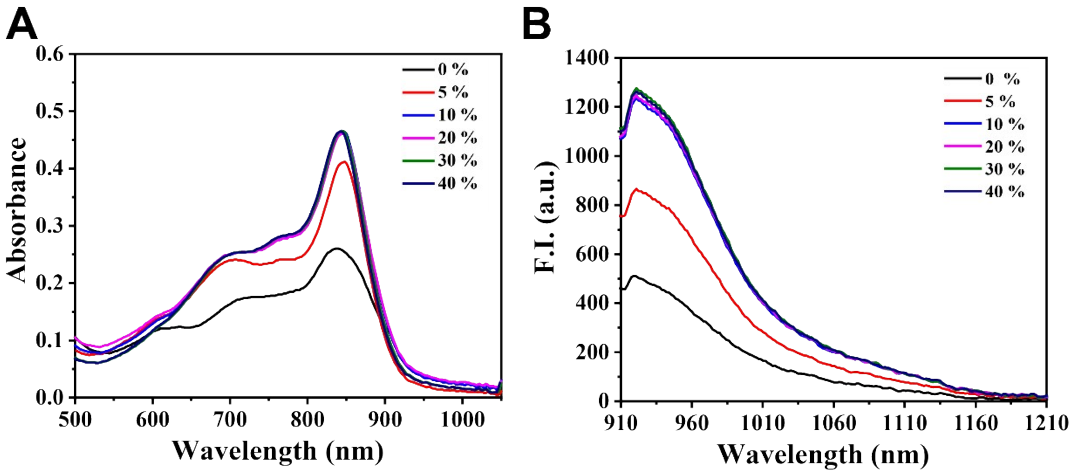


Fig. S17. (A) Absorption spectra for the probe Cy-TPE-NH (10 μM) in water/ethanol mixture with varied ethanol percentages (v/v) upon reaction with MAHMA NONOate (100 μM). (B) Fluorescence spectra for the probe Cy-TPE-NH (10 μM) in water/ethanol mixture with varied ethanol percentages (v/v) upon reaction with MAHMA NONOate (100 μM). Excitation Wavelength: 808 nm.

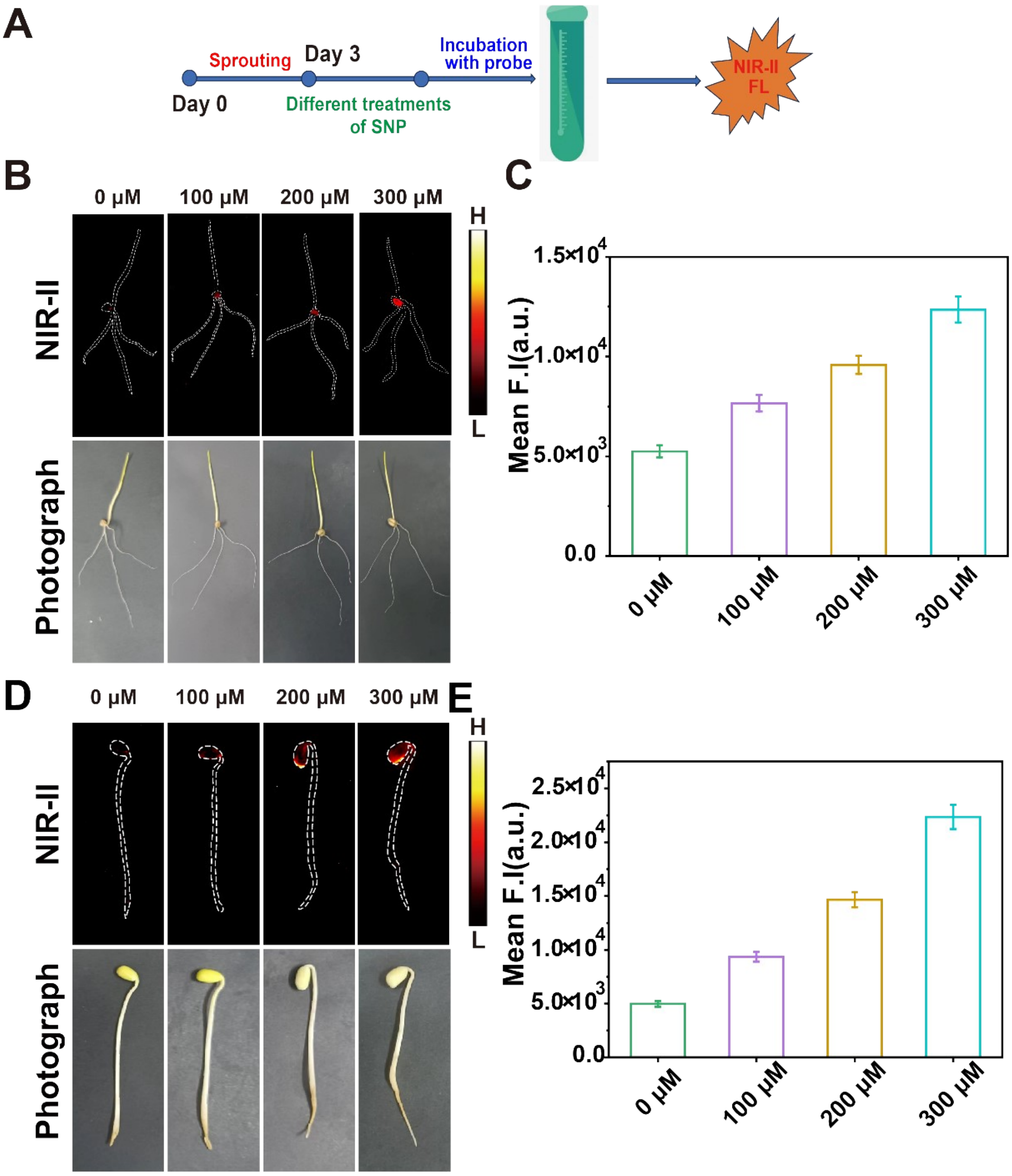


Fig. S18. (A) Experiment outline for investigation of the effect of SNP concentration in wheat seedlings and soybean sprouts. (B) NIR-II fluorescent images and photographs of wheat seedlings upon treatment with SNP of varied concentrations. (C) Average NIR-II fluorescent intensities of wheat seedlings from different groups in (B). (for each group, n=5) (D) NIR-II fluorescent images and photographs of soybean sprouts upon treatment with SNP of varied concentrations. (E) Average NIR-II fluorescent intensities of soybean sprouts from different groups in (D). (for each group, n=5)

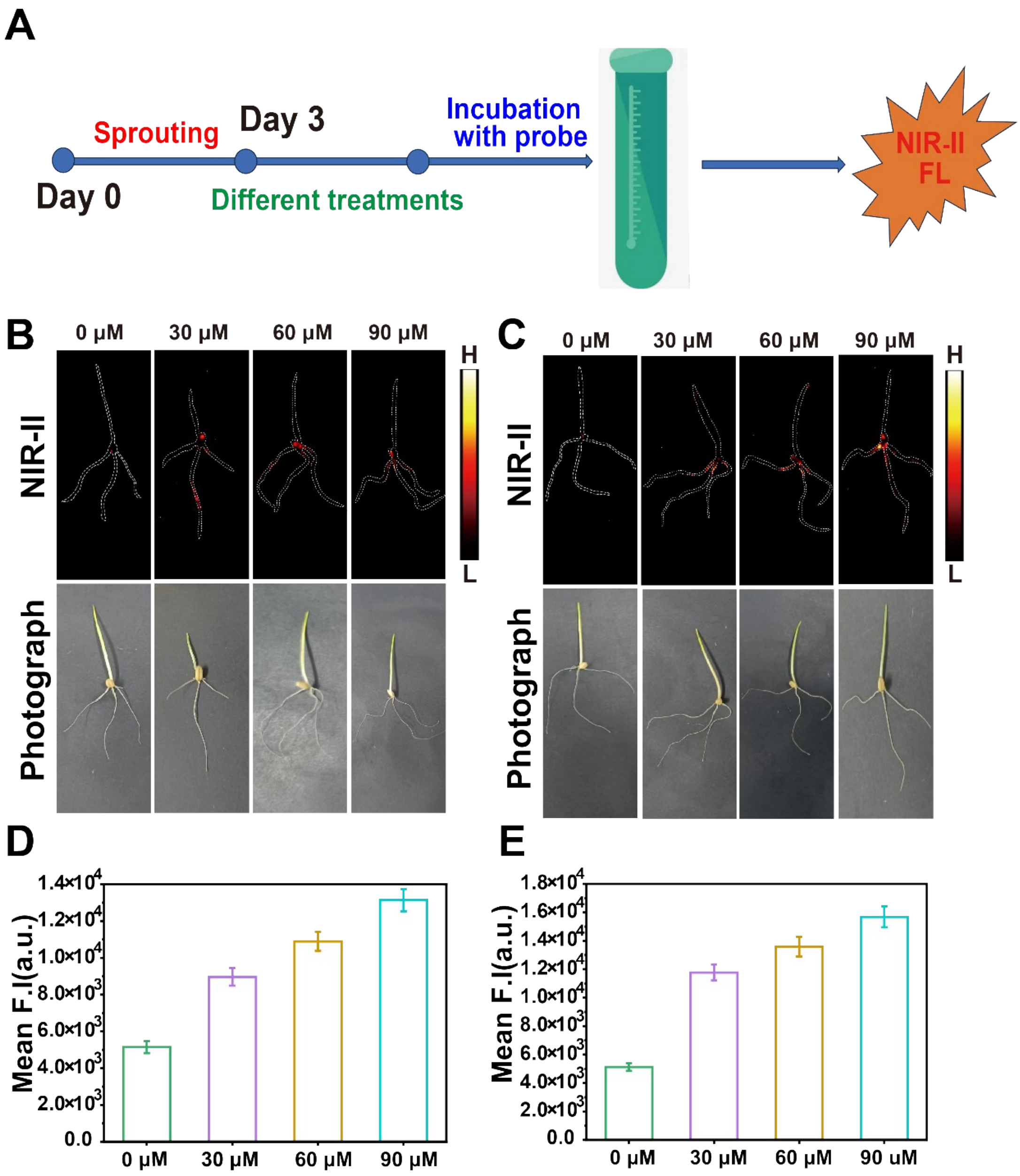


Fig. S19. (A) Experiment outline for investigation on the effect of Al^{3+} or Cd^{2+}concentration in wheat seedlings after short-time (3 h) exposure. (B) NIR-II fluorescent images and photographs of wheat seedlings upon treatment with Al^{3+} of varied concentrations. (C) NIR-II fluorescent images and photographs of wheat seedlings upon treatment with Cd^{2+} of varied concentrations. (D) Average NIR-II fluorescent intensities of wheat seedlings from different groups in (B). (E) Average NIR-II fluorescent intensities of wheat seedlings from different groups in (C). (for each group, n=5)

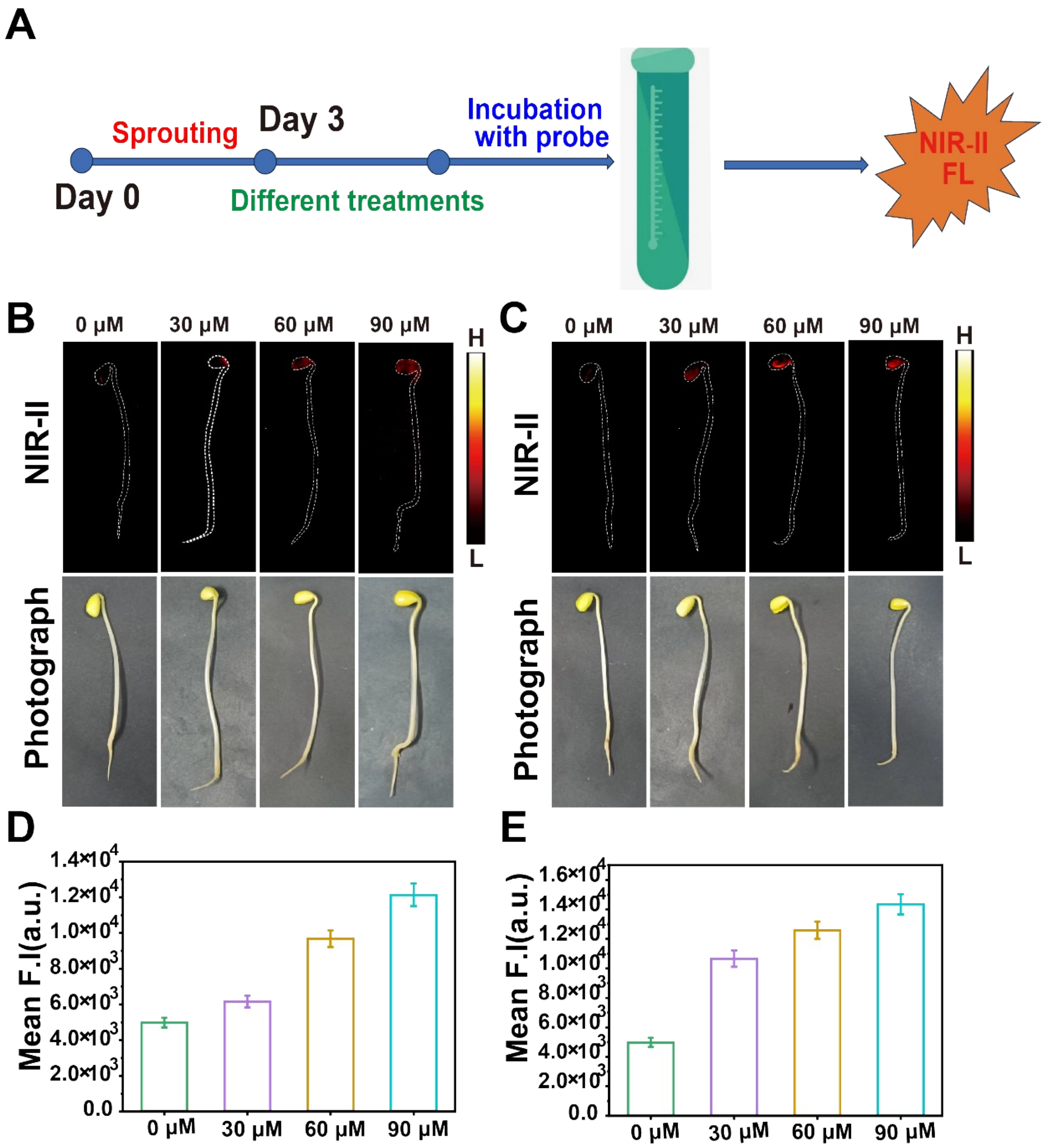


Fig. S20. (A) Experiment outline for investigation on the effect of Al^{3+} or Cd^{2+} concentration in soybean sprouts after short-time (3 h) exposure. (B) NIR-II fluorescent images of soybean sprouts upon treatment with Al^{3+} of varied concentrations. (C) NIR-II fluorescent images of soybean sprouts upon treatment with Cd^{2+} of varied concentrations. (D) Average NIR-II fluorescent intensities of soybean sprouts from different groups in (B). (For each group, n=5) (E) Average fluorescent intensities of soybean sprouts from different groups in (C). (For each group, n=5)

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