Supporting Information

A Near-infrared Fluorescent Probe for Detection of H2S and Its Applications in Monitoring Meat Freshness and Plant Growth under Aluminum-induced Stress

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Experimental section

Materials

Cyclohexanone, phosphorus oxychloride (POCl$_3$), p-toluenesulfonyl chloride, 1,2,3,3-tetramethyl-3H-indolium iodide, triethylene glycol monomethyl ether, ethanedithiol, 2,4-Dihydroxybenzaldehyde, sodium acetate, potassium carbonate (K$_2$CO$_3$) were purchased from Aladdin Reagent. NaHS (H$_2$S donor), CaCl$_2$, MgCl$_2$, KCl, AlCl$_3$, NaCl, Na$_2$SO$_4$, Na$_2$SO$_3$, NaSCN, NaHPO$_4$, NaNO$_2$, CH$_3$COONa, Hcy, Cys and GSH were also obtained from Aladdin Reagent. The solvents dimethylformamide, dichloromethane, ethanol, acetonitrile and methanol were analytical grade reagents and used without further purification.

Instrumentation

High resolution mass spectra (HRMS) were recorded on a Bruker MAXIS IMPACT mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance 600 MHz NMR Spectrometer. Absorption spectra were determined on a Hitachi U-3010 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4700 fluorescence spectrophotometer. The NIR-I fluorescence in plant imaging were performed by using Ami small animal imaging system (Spectral Instruments Imaging Co.).

Synthesis of the probe (CHO-OH-NO$_2$) and the activated probe (CHO-OH)

The probe and the activated probe (the reaction product of the probe and H$_2$S) were synthesized according to Scheme S1.
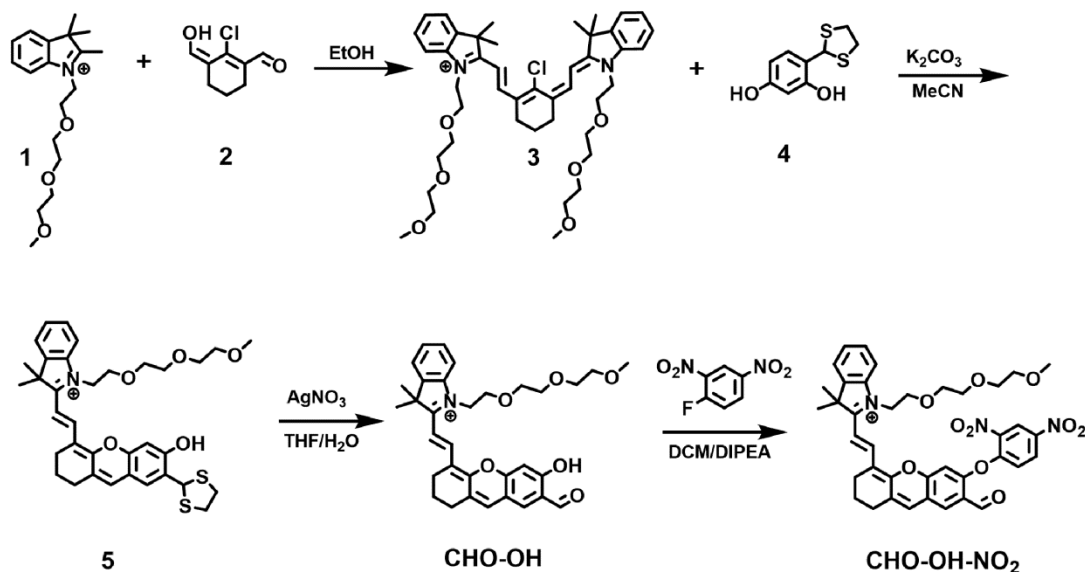
Scheme S1 Synthesis route of CHO-OH and CHO-OH-NO₂.

Compound **1**, compound **2**, compound 3, compound **4** and compound 5 were synthesized according to the previous literature reports.[1-3]

Synthesis of **3**. A mixture of **2** (344 mg, 2 mmol), **1** (1.836 g, 6 mmol), potassium acetate (108 mg, 1.1 mmol) and ethanol (20 mL) were heated at 80 °C and stirred for 6 h. The reaction mixture was then cooled to room temperature. After ethanol was evaporated under vacuum, DCM (50 mL) was added and the organic layer was washed with water, dried over anhydrous sodium sulfate, and then evaporated under vacuum. The resulting crude solid was purified by column chromatography over silica gel using DCM/MeOH (30/1, v/v) as eluent solvent to afford compound **3** as green solid (479.6 mg, 64.2%). ^{1}H NMR (CDCl₃, 500 MHz) δ (ppm): 8.30 (d, J = 14.1 Hz, 2H), 7.36-7.34 (m, 4H), 7.28 (d, J = 7.9 Hz, 2H), 7.20 (t, J = 7.4 Hz, 2H), 6.35 (d, J = 14.1 Hz, 2H), 4.44 (t, J = 4.2 Hz, 4H), 3.92 (t, J = 5.3 Hz, 4H), 3.61-3.57 (m, 4H), 3.53-3.50 (m, 4H), 3.49-3.44 (m, 6H), 3.42-3.37 (m, 4H), 3.28 (s, 6H), 2.68 (t, J = 6.1 Hz, 4H), 1.93 (m, 2H), 1.69 (s, 12H).

Synthesis of **5.** A mixture of compound **3** (747 mg, 1.0 mmol), compound **4** (428 mg, 2.0 mmol) and K₂CO₃ (138mg, 1.0 mmol) were dissolved in anhydrous acetonitrile (10 ml). The mixture was heated to 50 °C and stirred for 12 h under nitrogen atmosphere. Then the mixture was cooled to room temperature, and the solvent acetonitrile was evaporated under

vacuum. DCM (50 mL) was then added and the organic layer was washed with water, dried over anhydrous sodium sulfate and then evaporated under vacuum. The crude product was then purified by silica column chromatography (MeOH/DCM=1:40) to afford compound **5** (310 mg, 50 %) as a blue solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ (ppm): 8.10 (s, 1H), 7.68 (s, 1H), 7.39 (s, 1H), 7.30 (d, J = 7.3 Hz, 1H), 7.07 (d, J = 7.4 Hz, 1H), 6.93 (d, J = 7.9 Hz, 1H), 6.69 (d, 1H), 6.07 (d, 1H), 5.75 (d, J = 13.4 Hz, 1H), 4.79 (s, 1H), 4.04 (t, J = 6.1 Hz, 2H), 3.81 (t, J = 6.0 Hz, 2H), 3.65 (t, J = 6.1, 3.2 Hz, 2H), 3.60 (t, J = 6.4, 3.7 Hz, 2H), 3.59-3.56 (m, 2H), 3.50-3.47 (m, 2H), 3.35 (s, 3H), 3.33 (d, J = 1.8 Hz, 4H), 2.69 (t, J = 6.0 Hz, 2H), 2.61 (t, J = 6.3 Hz, 2H), 1.89 (m, 2H), 1.69 (s, 6H).

Synthesis of **CHO-OH.** Compound **5** (620 mg, 1.0 mmol) was dissolved in 5 mL THF/H$_2$O (v:v = 1:1). AgNO$_2$ (392 mg, 2 mmol) was then added to the solution, and the mixture was stirred at 25°C for 30 min. After the mixture was filtrated, it was dissolved in dichloromethane. The organic layer was washed with water, dried over anhydrous sodium sulfate and then evaporated under vacuum. The resultant substance was purified by silica column chromatography (MeOH/DCM=1:30) to afford **CHO-OH** (435.2 mg, 80%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ (ppm): 10.04 (s, 1H), 8.62 (d, J = 15.2 Hz, 1H), 7.67 (s, 1H), 7.55-7.49 (m, 3H), 7.44 (d, J = 13.2 Hz, 1H), 7.05 (s, 1H), 6.93 (s, 1H), 6.83 (d, J = 15.2 Hz, 1H), 4.76 (m, 2H), 4.04 (m, 2H), 3.62-3.60 (m, 2H), 3.52-3.50 (m, 2H), 3.48-3.43 (m, 2H), 3.42-3.37 (m, 2H), 3.30 (s, 3H), 2.76-2.64 (m, 4H), 1.92 (m, 2H), 1.83 (s, 6H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 192.24, 179.03, 166.77, 163.68, 158.03, 156.76, 145.14, 141.07, 140.53, 130.57, 130.01, 128.19, 126.96, 126.82, 121.38, 114.66, 114.22, 112.84, 106.20, 102.91, 70.75, 69.77, 69.46, 69.36, 67.13, 57.95, 50.22, 45.63, 28.67, 28.12, 27.06, 22.95, 22.72. HR-MS (ESI, m/z) $[C_{33}H_{38}NO_6]^+$ calcd for. 544.2700, found 544.2704.

Synthesis of **CHO-OH-NO$_2$.** A mixture of **CHO-OH** (544 mg, 1.0 mmol), 2,4-dinitrofluorobenzene (186 mg, 1.0 mmol) and two drops of DIPEA were dissolved in anhydrous DMF (10 ml), and the mixture was heated to 50 °C and stirred for 6 h under a nitrogen atmosphere. The mixture was then cooled to room temperature, and the solvent DMF was evaporated under vacuum. Then DCM (50 mL) was added and the organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated under vacuum. Purification by silica column chromatography (MeOH/DCM=1:30) afforded

CHO-OH-NO$_2$ (355 mg, 50%) as a blue solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ (ppm): 10.16 (s, 1H), 8.96 (d, J = 2.7 Hz, 1H), 8.54 (d, J = 15.5 Hz, 1H), 8.49 (d, J = 11.9 Hz, 1H), 7.92 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.50 (s, 1H), 7.47 (s, 2H), 7.28 (s, 1H), 7.19 (d, J = 15.5 Hz, 1H), 7.03 (s, 1H), 6.95 (s, 1H), 5.12 (t, J = 4.8 Hz, 2H), 4.08 (t, J = 4.8 Hz, 2H), 3.62 (t, J = 5.5, 3.7 Hz, 2H), 3.49 (t, J = 9.3 Hz, 2H), 3.46-3.42 (m, 2H), 3.38 (t, J = 5.9, 2.9 Hz, 2H), 3.29 (s, 3H), 2.88 (d, J = 6.0 Hz, 2H), 2.73 (t, J = 6.4 Hz, 2H), 1.98-1.93 (m, 2H), 1.77 (s, 6H). ^{13}C NMR (151 MHz, CDCl$_3$) δ 192.39, 186.38, 179.85, 165.00, 159.37, 158.08, 157.18, 156.04, 154.59, 146.13, 145.89, 142.03, 141.58, 130.69, 129.91, 129.20, 128.63, 127.90, 127.61, 124.50, 122.37, 120.72, 115.02, 113.88, 109.93, 107.17, 104.10, 71.76, 70.82, 70.70, 70.42, 70.34, 68.26, 58.98, 29.61, 29.19, 28.14, 27.83, 24.47. HR-MS (ESI, m/z) [C$_{39}$H$_{40}$N$_3$O$_{10}$]$^+$ calcd for. 710.2714, found 710.2710.

Preparation of the probe-loaded test strips and the evaluation of the freshness of prawn, beef and pork.

The test strips were obtained by immersing oblong filter paper in the probe solution (50 μM in DCM) for 10 min and drying in air. The fresh prawn, beef and pork were purchased from supermarket. The fresh prawn, beef and pork about 20 g were placed in 125 mL airtight and transparent PET boxes with the test strips at 30 ℃ respectively. Then the color change and fluorescence intensity of the test strips were recorded by camera in varied time. The test strips were then placed in the chamber of the small animal imaging system for fluorescence imaging.

Establishment of Al^{3+}-induced stress model and fluorescence imaging

Wheat grains (Triticum aestivum L.) in this research were bought from the Anhui Aidi Agricultural Technology Co., Ltd., Anhui Province, China. Firstly, wheat grains were sterilized by 0.1% HgCl$_2$ for 3 min and washed extensively with H$_2$O and then dried with filter papers. Subsequently, wheat grains were germinated in H$_2$O or aqueous solutions of AlCl$_3$ (0 mM, 5 mM, 10 mM and 25 mM) for 48 h at 30 ℃. AlCl$_3$ solutions were renewed every 12 h. Before imaging, the wheat grains were co-cultured with the probe CHO-OH-NO$_2$ (50 μM) for 2 h in DMSO/PBS (v/v =5:95) and then placed in the chamber

of the small imaging system.

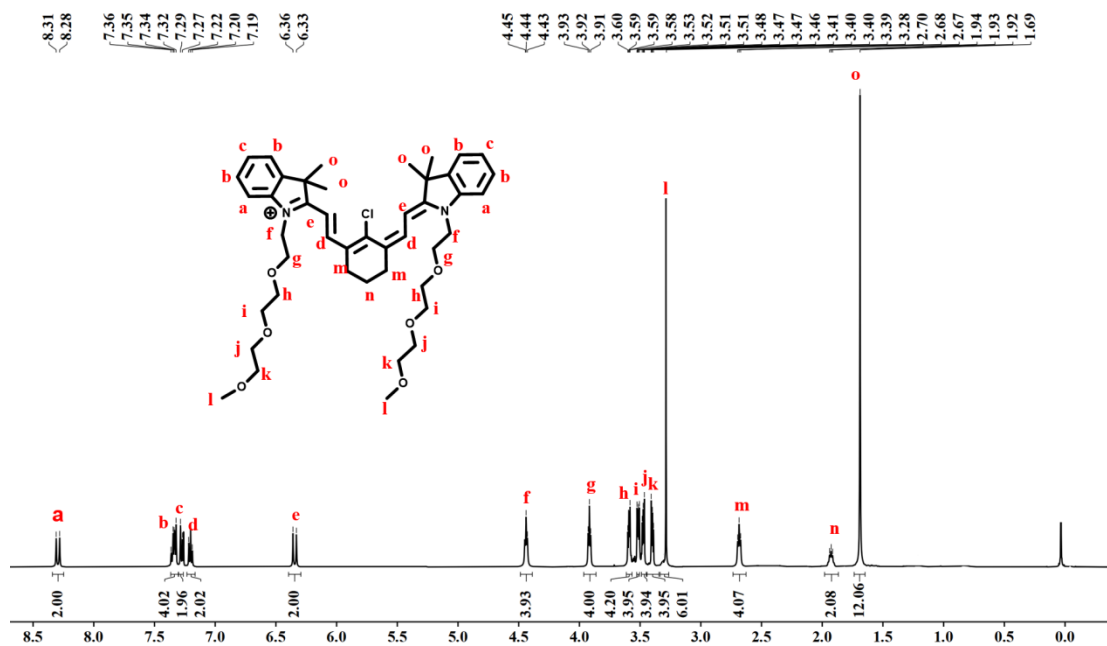**Fig. S1.** ^{1}H NMR spectrum of compound 3 in CDCl$_3$.
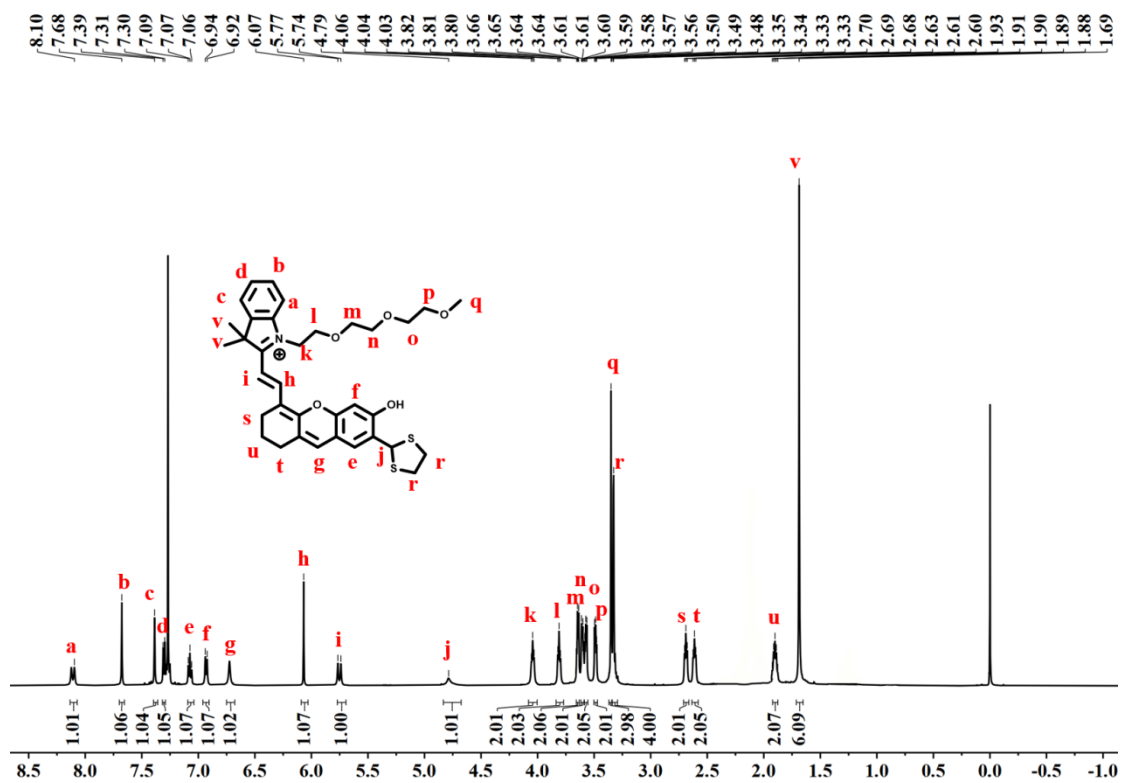
Fig. S2. ^{1}H NMR spectrum of compound **5** in CDCl$_3$.
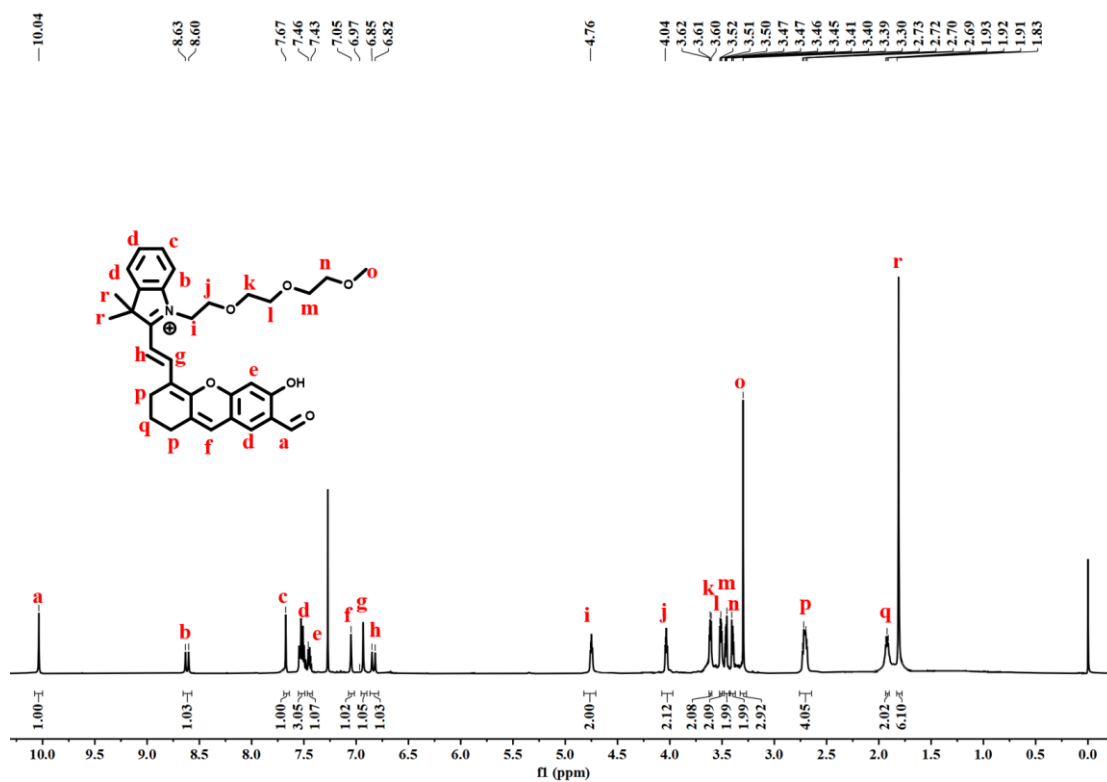
Fig. S3. ^{1}H-NMR spectrum of compound **CHO-OH** in CDCl$_3$.
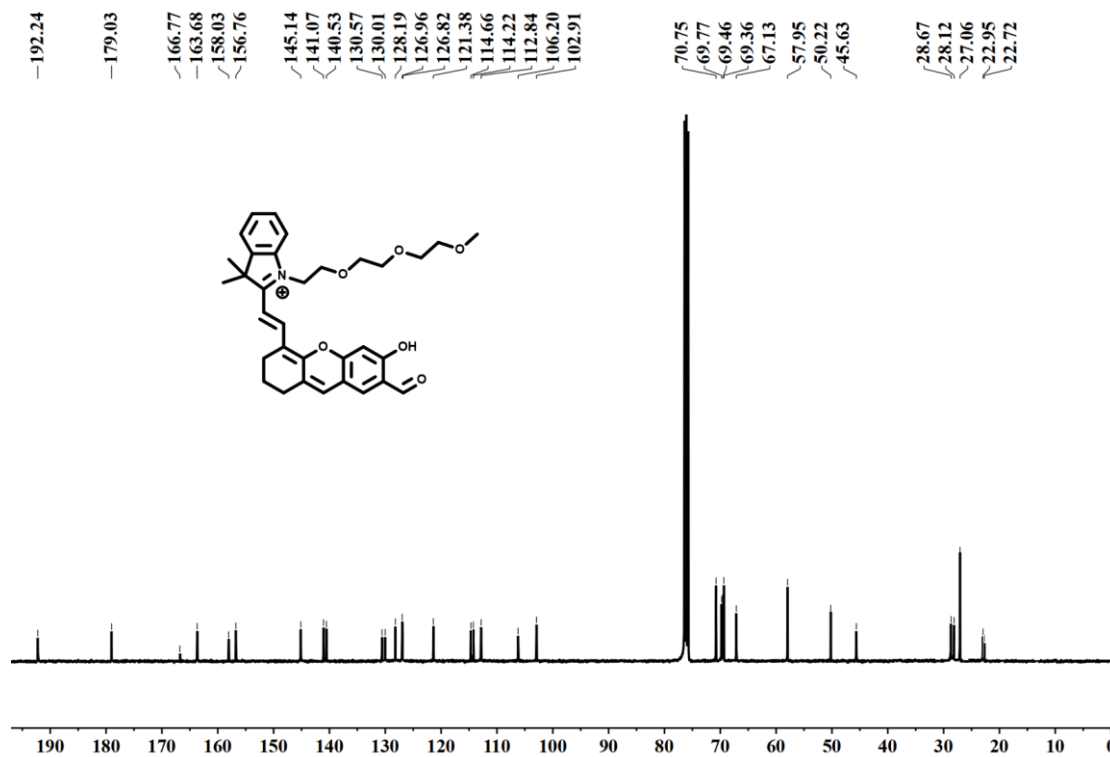
Fig. S4. ^{13}C NMR spectrum of **CHO-OH** in CDCl$_3$.
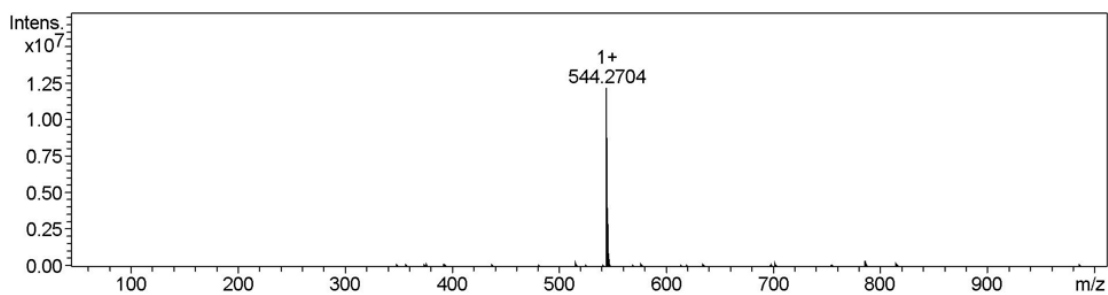
Figure S5. HR Mass spectrum of **CHO-OH**.
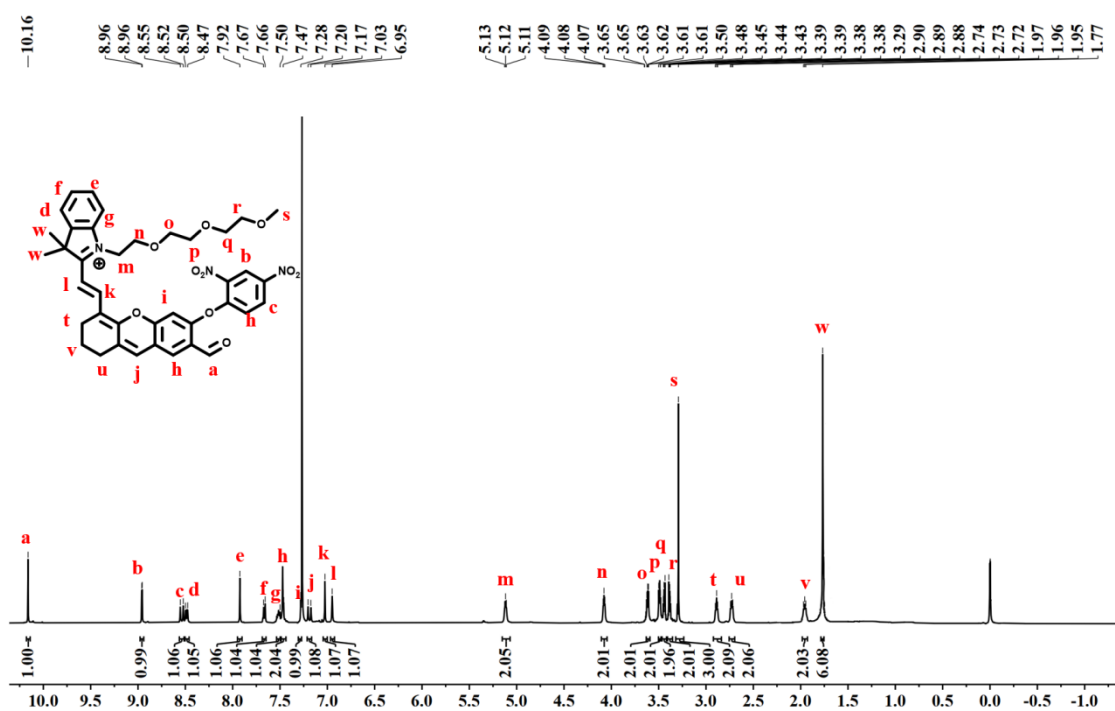
Fig. S6. ^{1}H-NMR spectrum of the probe **CHO-OH-NO$_2$** in CDCl$_3$.

Fig. S7. ^{13}C NMR spectrum of the probe **CHO-OH-NO₂** in CDCl₃.

Figure S8. HR-MS spectrum of the probe **CHO-OH-NO₂**.

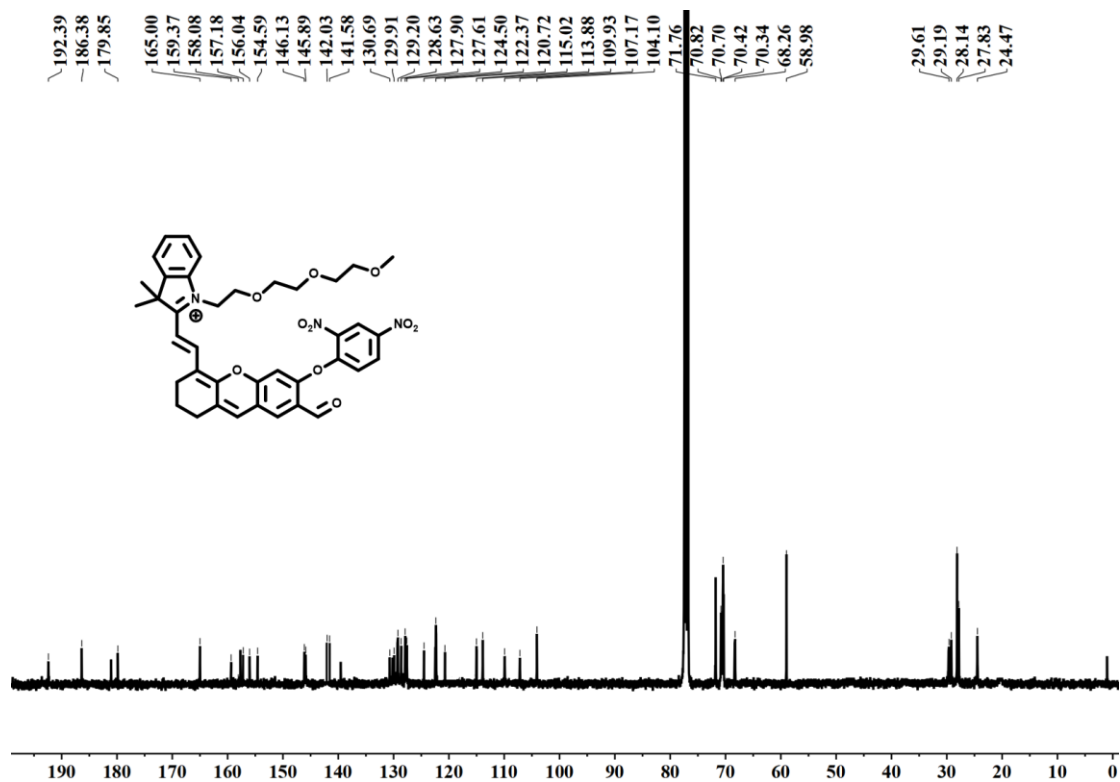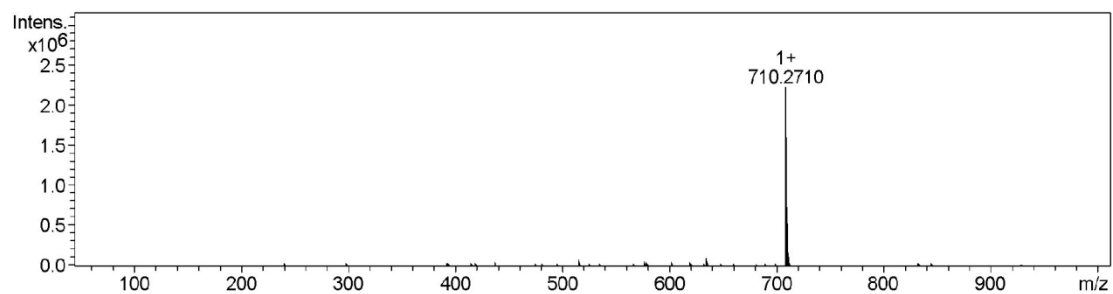**Table S1.** Fluorescence intensity data (a.u.) recorded using fluorescence imaging system and their mean values, standard deviation (SD) and mean absolute errors (MAE) for probe-loaded test papers upon exposure to different meats for varied times. (A): pork; (B): beef; (C) prawn.

(A)

Time (h)	Fluorescence intensity (a.u.)					Mean	SD	MAE
0	3.27×10^5	3.76×10^5	3.70×10^5	3.28×10^5	3.51×10^5	3.50×10^5	2.28×10^4	1.84×10^4
3	7.11×10^5	7.07×10^5	6.39×10^5	6.54×10^5	6.93×10^5	6.81×10^5	3.25×10^4	2.74×10^4
6	9.45×10^5	8.93×10^5	8.41×10^5	8.85×10^5	8.83×10^5	8.89×10^5	3.71×10^4	2.36×10^4
9	1.36×10^6	1.29×10^6	1.38×10^6	1.31×10^6	1.27×10^6	1.32×10^6	4.66×10^4	3.80×10^4
12	1.49×10^6	1.46×10^6	1.47×10^6	1.47×10^6	1.37×10^6	1.45×10^6	4.71×10^4	3.40×10^4

(B)

Time (h)	Fluorescence intensity (a.u.)					Mean	SD	MAE
0	3.62×10^5	3.67×10^5	3.55×10^5	3.18×10^5	3.86×10^5	3.58×10^5	2.49×10^4	1.68×10^4
3	6.33×10^5	6.59×10^5	6.07×10^5	5.84×10^5	5.74×10^5	6.11×10^5	3.50×10^4	2.76×10^4
6	8.03×10^5	7.89×10^5	8.42×10^5	7.63×10^5	8.16×10^5	8.03×10^5	2.95×10^4	2.12×10^4
9	1.12×10^6	1.21×10^6	1.22×10^6	1.14×10^6	1.19×10^6	1.18×10^6	4.39×10^4	3.60×10^4
12	1.43×10^6	1.34×10^6	1.35×10^6	1.40×10^6	1.35×10^6	1.37×10^6	3.91×10^4	3.20×10^4

(C)

Time (h)	Fluorescence intensity (a.u.)					Mean	SD	MAE
0	3.69×10^5	3.35×10^5	3.25×10^5	3.69×10^5	3.29×10^5	3.45×10^5	2.18×10^4	1.88×10^4
1	7.90×10^5	7.14×10^5	7.64×10^5	7.22×10^5	7.72×10^5	7.52×10^5	3.29×10^4	2.76×10^4
2	9.82×10^5	9.92×10^5	9.78×10^5	1.05×10^6	9.45×10^5	9.89×10^5	3.82×10^4	2.52×10^4
3	1.36×10^6	1.30×10^6	1.25×10^6	1.35×10^6	1.30×10^6	1.31×10^6	4.44×10^4	3.40×10^4
4	1.49×10^6	1.56×10^6	1.47×10^6	1.56×10^6	1.53×10^6	1.52×10^6	4.09×10^4	3.40×10^4

Note: we cannot obtain the true values of fluorescence intensities for the H_2S, thus we used the mean value of 5 measured fluorescence intensities as the true value.

Table S2. Fluorescence intensity data (a.u.) recorded using fluorescence imaging system, as well as their mean values, standard deviation (SD) and mean absolute errors (MAE) for the probe upon incubation with the wheat seedlings treated with Al(III) of varied concentrations.

Al^{3+} Conc. mM	Fluorescence intensity (a.u.)					Mean	SD	MAE
0	2.70×10^6	2.76×10^6	2.96×10^6	3.00×10^6	2.87×10^6	2.86×10^6	1.28×10^5	1.02×10^5
5	3.64×10^6	3.72×10^6	3.45×10^6	3.73×10^6	3.42×10^6	3.59×10^6	1.48×10^5	1.26×10^5
10	4.99×10^6	4.61×10^6	4.95×10^6	4.83×10^6	4.79×10^6	4.83×10^6	1.50×10^5	1.08×10^5
25	1.22×10^6	1.56×10^6	1.25×10^6	1.31×10^6	1.42×10^6	1.35×10^6	1.39×10^5	1.10×10^5

Note: we cannot obtain the true values of fluorescence intensities for the H_2S, thus we used the mean value of 5 measured fluorescence intensities as the true value.

References

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