Charge-Tunable Hemicyanine NIR Probe for Bacterial Imaging and Bacterial Infection Tracking

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Materials and instruments

All raw materials were analytical grade and were used directly unless otherwise stated. All tetrahydrofuran and ethyl ether in the synthesis step were further dried. UV–Vis absorption and fluorescence spectra were obtained on HITACHI U-3900H and HITACHI F-4600 spectrophotometers, respectively. The MTT cytotoxicity test kit was purchased from Solarbio. PC12 cells were purchased from Chinese Academy of Medical Sciences. In vitro imaging was carried out using a laser scanning confocal microscope (Leica SP8). Bacteria were obtained from Beijing Tiantan Hospital.

Culture and Preparation of Microorganism

Individual *E. coli* colonies on the solid medium were transferred to 10 mL of liquid LB medium and cultured in a shaking incubator for 12 hours. The microorganism concentration was determined by measuring the optical density at 600 nm (OD_{600}) using a UV spectrophotometer. The microorganism was then diluted to $OD_{600} = 1.0$ and transferred to a 1.5 mL centrifuge tube. It was centrifuged at 8,000 rpm for 3 minutes. After centrifugation, the supernatant was removed, and the pellet was washed three times with phosphate-buffered saline (PBS) to remove the medium. The precipitated *E. coli* was resuspended in PBS for later use. Other microorganisms were cultured under the same conditions and treated using the same procedures as *E. coli*.

***In vitro* cytotoxicity assay**

The cytotoxicity of the 3 probes in PC12 cells was tested by the methyl thiazolyl tetrazolium (MTT) method. The cultured cells were digested and dispersed with trypsin, and counted under a microscope using a hemocytometer. The cells were then diluted into a suspension at a concentration of 10^5/mL and added to a 96-well plate. After 24 hours of incubation, the original medium was removed, and the cells containing 3 probes (0, 5, 10, 15, 20, 25 μM) DMEM were added to the plate. Finally, the cell viability was determined by measuring the ultraviolet absorption value in different well plates by a microplate reader.

Photostability test

Photostability testing was performed by continuous laser irradiation for 300 s. Confocal fluorescence imaging was performed every 10 s during laser irradiation, and the fluorescence images were batch processed using ImageJ to quantify fluorescence intensity.

Confocal and co-localization imaging of bacteria and PC12 cell

The bacterial samples were incubated with 3 probes (15 μM) in PBS for 45 min, followed by centrifugation to remove the supernatant and two washes with PBS. The bacteria were then resuspended in 80 μL PBS, and 7 μL of the suspension was placed on a coverslip for confocal microscopy observation.

For co-localization experiments, the bacteria were first stained with the commercial nuclear probe Hoechst 33342 for 30 min, centrifuged, and washed before incubation with 3 probes, followed by the same post-treatment steps as above.

PC12 cells were digested with trypsin and dispersed in 35 mm culture dishes, each containing approximately $2*10^5$ cells. The culture dishes were then placed in a 37°C, 5% CO2 incubator for 24 h until the cells adhered. First the commercial probe Rhodamine 123 (5 μM, 15 min) or Dio (3 μM, 17 min) was incubated with PC12 cells and the medium was removed and incubated with 3 probes (15 μM, 15 min).

Excitation/emission wavelengths:

- Hoechst 33342: Ex. 405 nm, Em. 425–475 nm
- 3 probes: Ex. 488 nm, Em. 650–800 nm
- Rhodamine 123 Ex. 488 nm, Em. 508–650 nm
- Dio Ex. 488 nm, Em. 508–600 nm

Light Stability Test

3 probes and the commonly used commercial probe Rhodamine 123 (Dio) were dissolved in 2 mL of PBS solution (20 μM). A white light source was used for irradiation, starting at 0 seconds. Fluorescence intensity was measured every 50 seconds of irradiation, and the test was stopped after 300 seconds. The experiment was conducted using a white light source with a power density of 50 mW/cm².

Synthesis of the OEM

Under N_2 protection, PBr_3 (5.2 mL, 55.0 mmol) was slowly added dropwise to a solution containing DMF (4.6 mL, 60.0 mmol) and $CHCl_3$ (40 mL). The mixture was stirred at 0 °C. Initially, the solution appeared yellow, and after 1 hour, it turned milky white. Cyclohexanone (2.12 mL, 20.4 mmol) was then added dropwise to the reaction mixture, causing the solution to change from milky white to orange-yellow. The mixture was stirred at 25 °C for 16 hours. Upon completion of the reaction, ice water (50 mL) was added to quench the reaction. $NaHCO_3$ powder was then added to adjust the pH of the reaction mixture to 7. The organic layer was extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated to yield a yellow oily liquid, compound 1. 2-hydroxy-4-methoxybenzaldehyde (0.5 g, 2.6 mmol) and Cs_2CO_3 (1.88 g, 6.0 mmol) were dissolved in DMF (10 mL). Under N_2 protection, compound 1 dissolved in DMF (2 mL) was added to the reaction mixture. The mixture was stirred at 25°C for 16 hours, during which the reaction solution turned grayish-yellow. Upon completion of the reaction, the mixture was filtered to remove Cs_2CO_3. The filtrate was extracted with CH_2Cl_2, and the organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated, and the crude product was purified by column chromatography (petroleum ether: ethyl acetate = 15:1) to yield a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.32 (s, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.67 (s, 3H), 3.86 (s, 3H), 2.58 (t, J = 6.2 Hz, 2H), 2.46 (t, J = 6.1 Hz, 2H), 1.73 (p, J = 6.1 Hz, 2H). EI-MS Spectrum of OEM. (Positive mode, m/z: $[M]^+$ calcd 242.0943 found 242.1032.)

Molecular Calculation

Using Gaussian 16 software, the vertical ionization energy (VIE, I) and electron affinity (EA, A) of the peptidoglycan monomer and phospholipid monomer were calculated at the B3LYP/6-311+G(d,p) level. The results were then used to compute the electrophilicity index (ω) using Formula S1-S3.

$$\eta = I - A \quad \text{----------(S1)}$$

$$\mu = -(I + A)/2 \quad \text{----------(S2)}$$

$$\omega = \mu^2 / 2\eta \quad \text{----------(S3)}$$

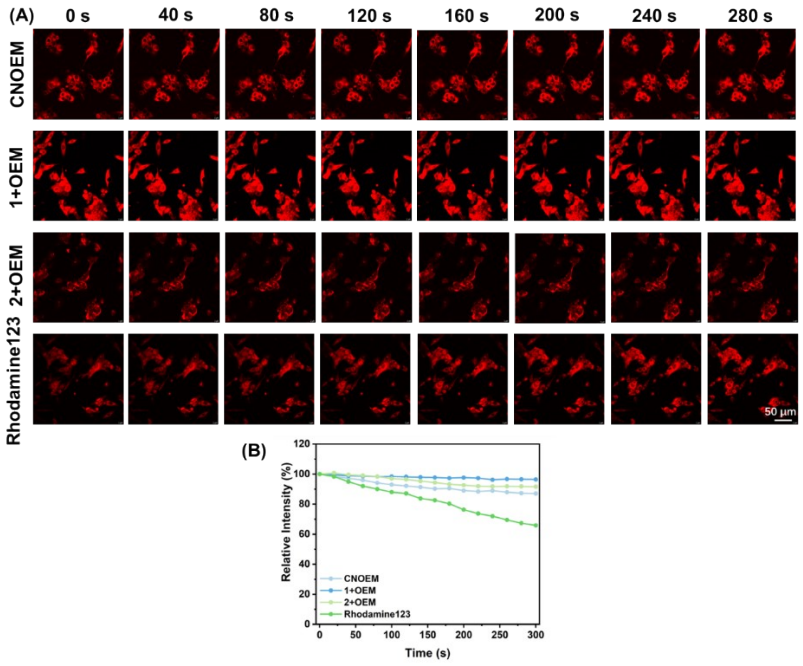

Figure S1. (a) Confocal fluorescence imaging of 3 probes (15 μM), Rhodamine 123 (5 μM) continuously irradiated with a semiconductor laser for 300 s in PC12 cells, excited at 488 nm (b) Normalized intensity profiles of average intracellular signals calculated from the confocal fluorescence images in (A) by applying ImageJ.

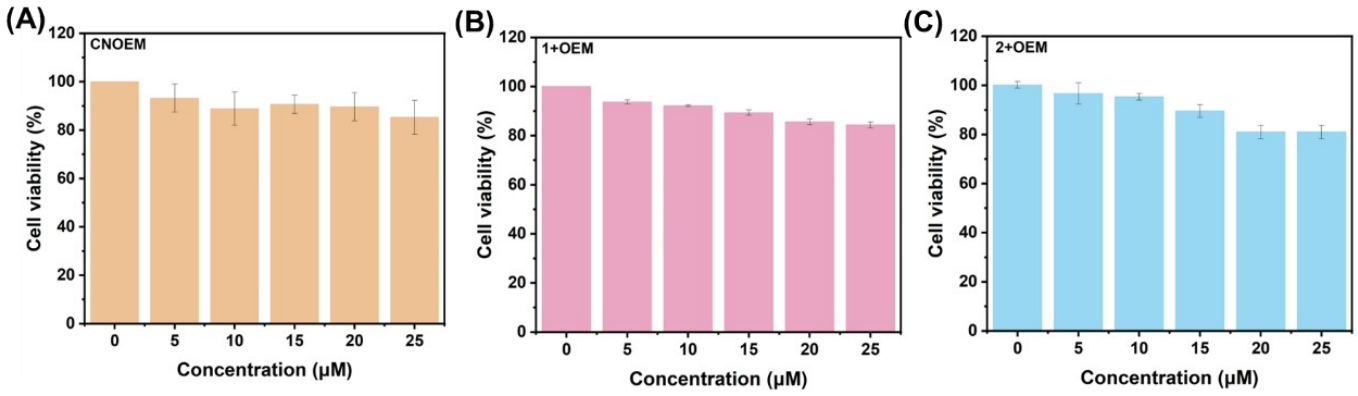

Figure S2. (A-C) Cytotoxicity of probes CNOEM, 1+OEM, and 2+OEM (0, 5, 10, 15, 20, 25 μM) on PC12 cells after 24-hour incubation, as determined by the MTT assay.

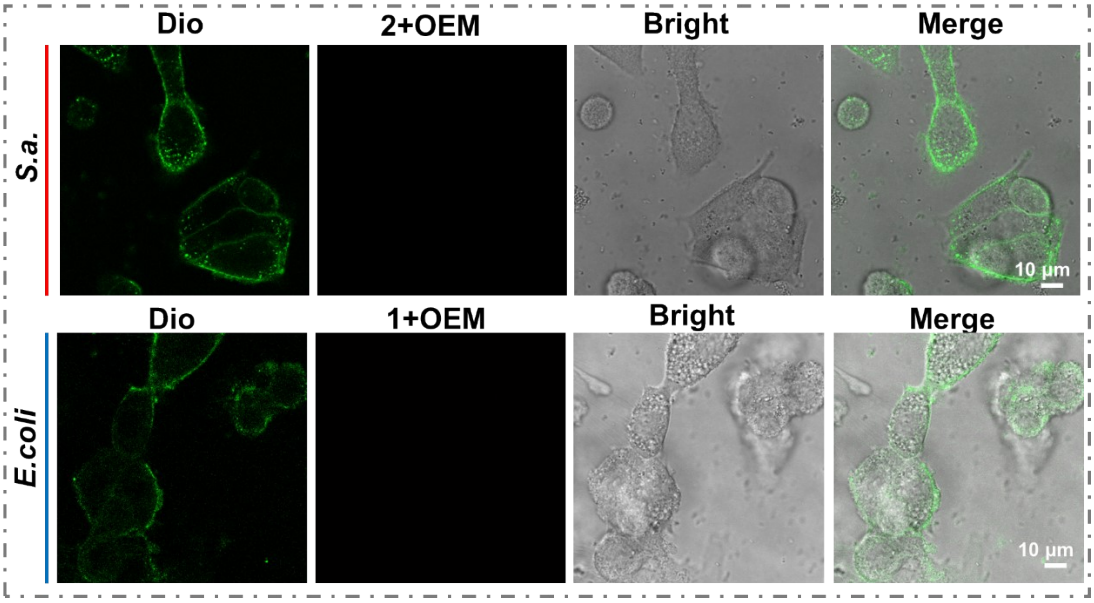

Figure S3. The confocal microscopy image shows S.a. (red, 15 μM, 45 min) labeled with 2+OEM marker infecting Dio-labeled PC12 cell (green, 3 μM, 7 min) during 0-45 minutes of observation and the confocal microscopy image shows E.coli (red, 15 μM, 45 min) labeled with 1+OEM marker infecting Dio-labeled PC12 cell (green, 3 μM, 7 min) during 0-40 minutes of observation.

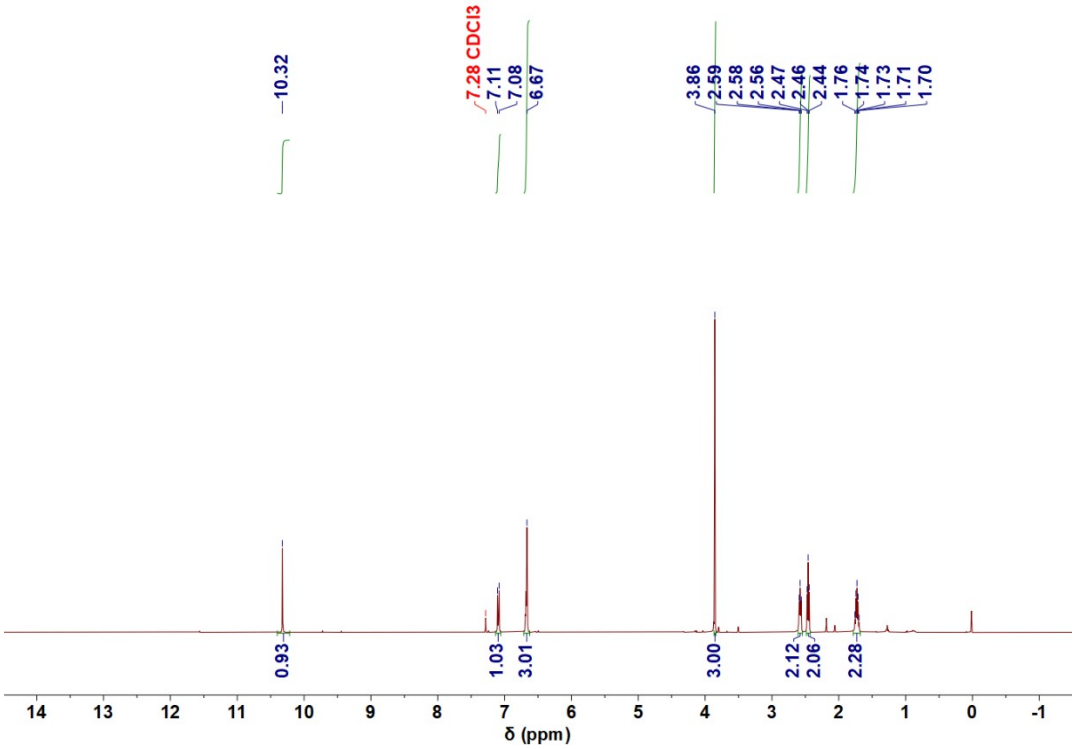

Figure S4. ^{1}H NMR spectrum of OEM in $CDCl_3$.

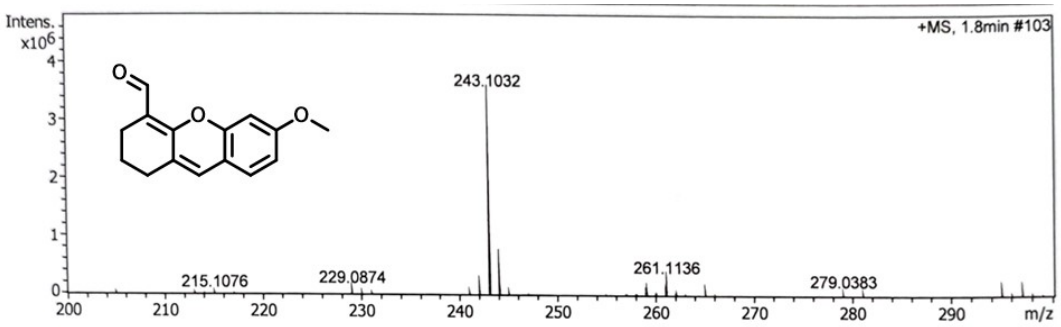

Figure S5. EI-MS Spectrum of OEM. (Positive mode, m/z: $[M]^{+}$ calcd 242.0943 found 242.1032.)

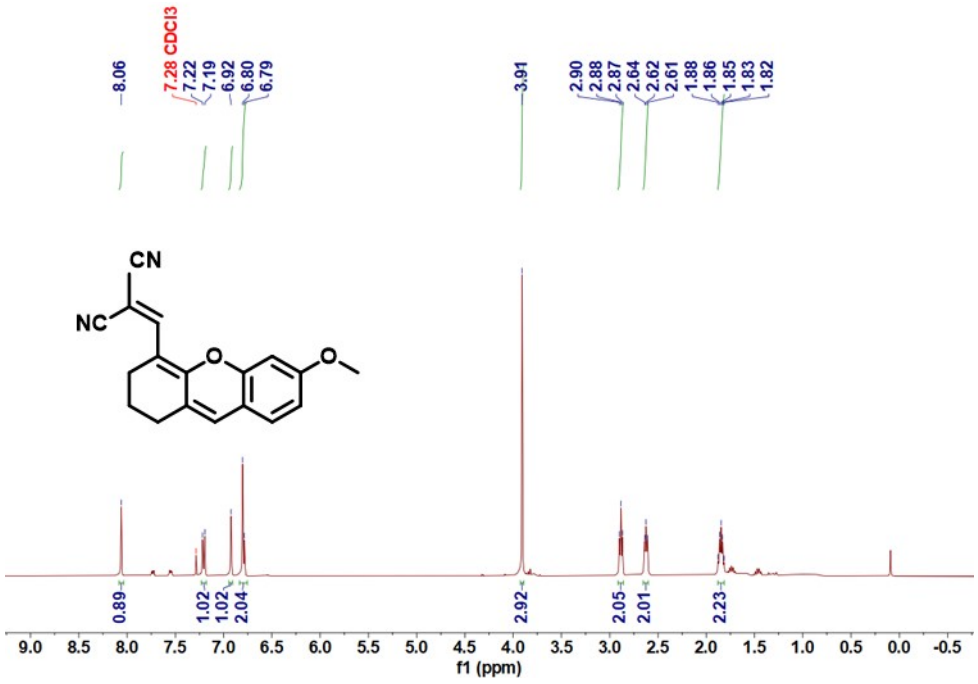

Figure S6. ^{1}H NMR spectrum of CNOEM in $CDCl_3$.

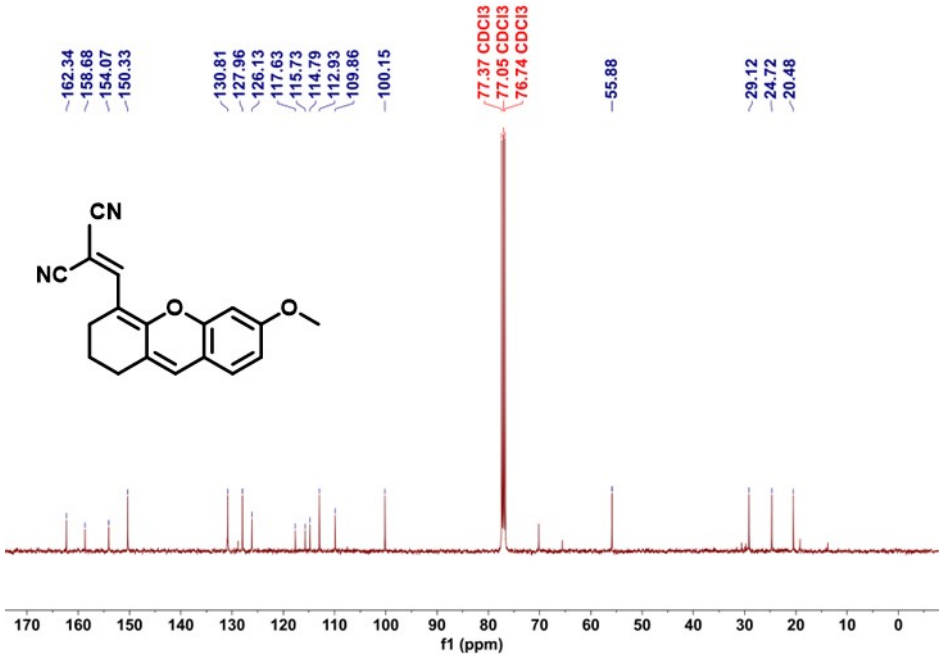

Figure S7. ^{13}C NMR spectrum of CNOEM in $CDCl_3$.

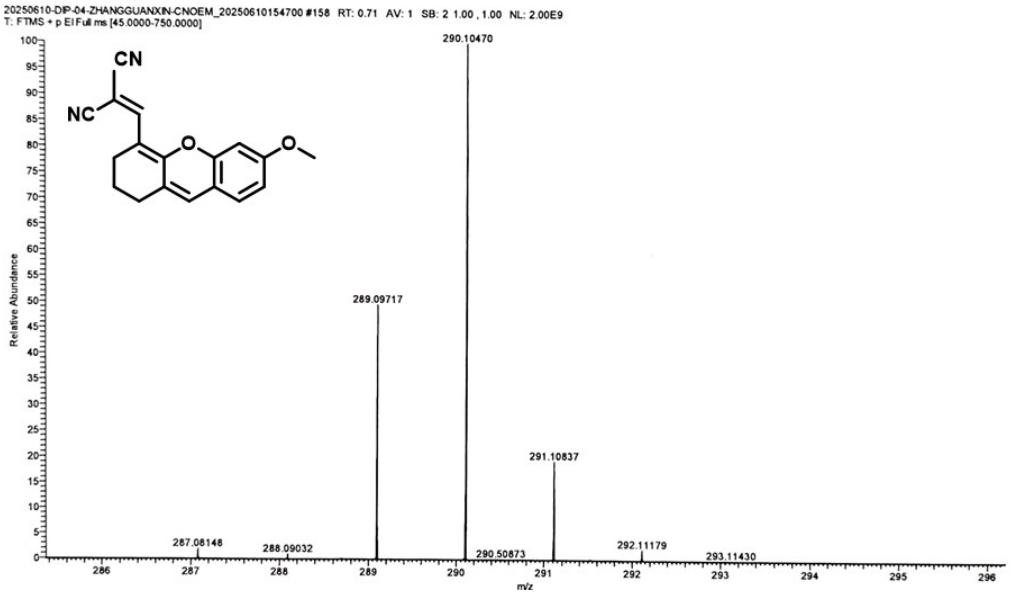

Figure S8. APCI-MS Spectrum of CNOEM. (Positive mode, m/z: $[M]^+$ calcd 290.1055 found 290.1047.)

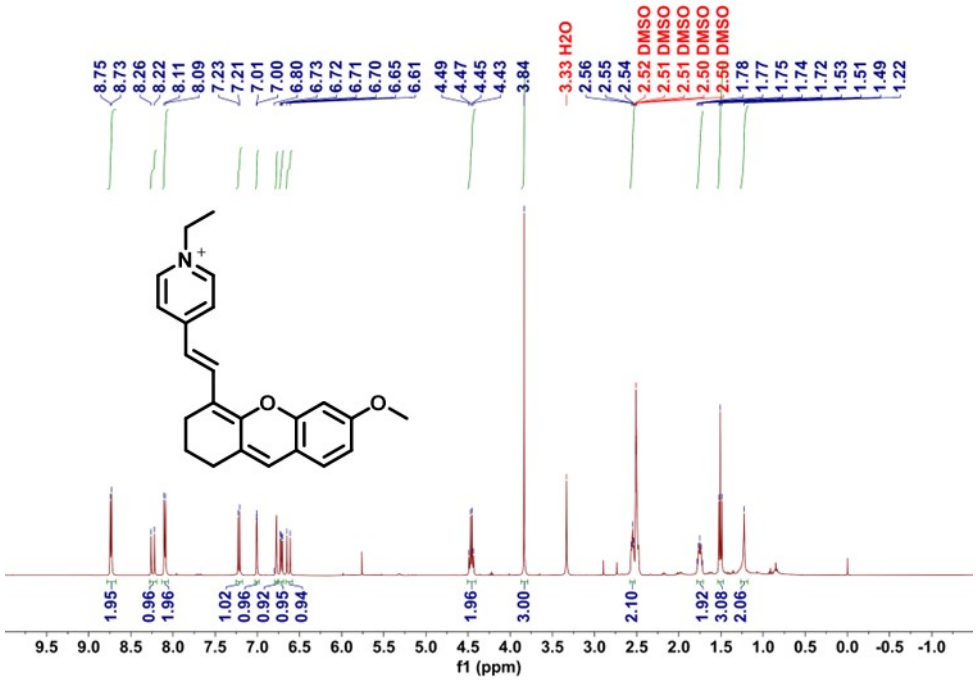

Figure S9. ^{1}H NMR spectrum of 1+OEM in D_6-DMSO

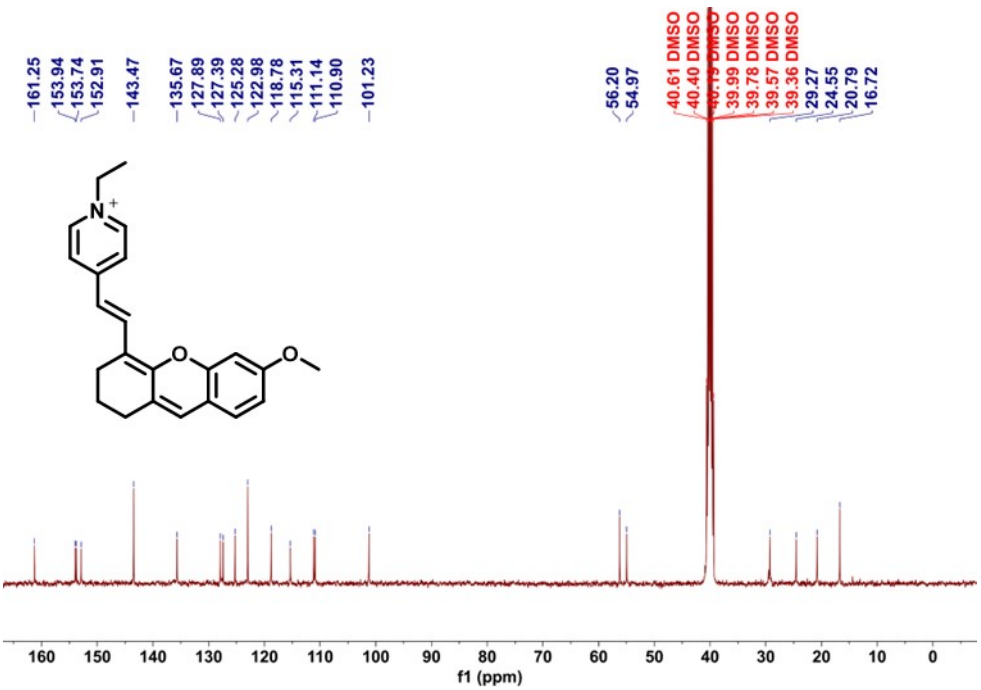

Figure S10. ^{13}C NMR spectrum of 1+OEM in D_6-DMSO

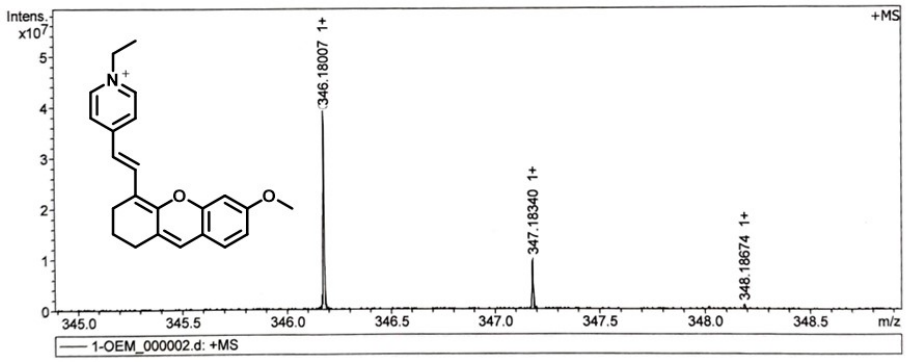

Figure S11. ESI-MS Spectrum of 1+OEM. (Positive mode, m/z: $[M\text{-}Br]^+$ calcd 346.1082 found 346.1800.)

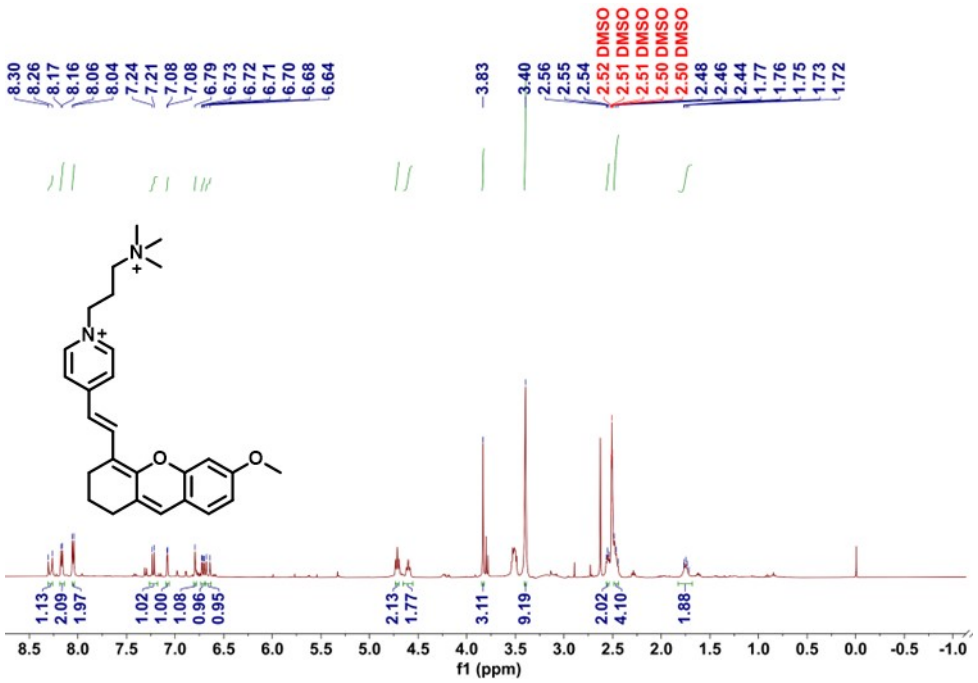

Figure S12. ^{1}H NMR spectrum of 2+OEM in D_6-DMSO

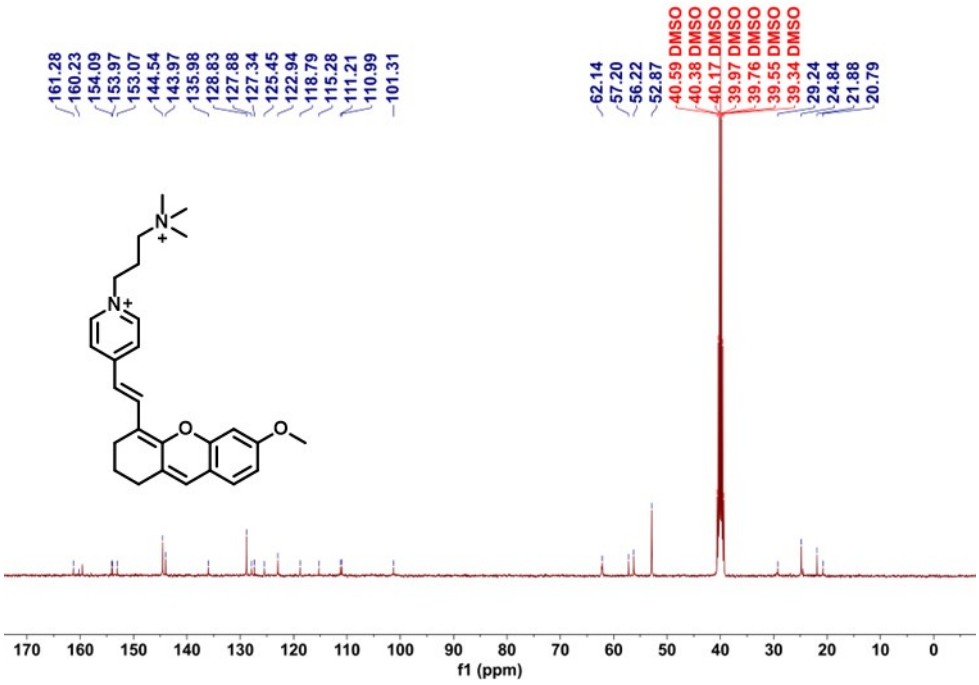

Figure S13. ^{13}C NMR spectrum of 2+OEM in D_6-DMSO

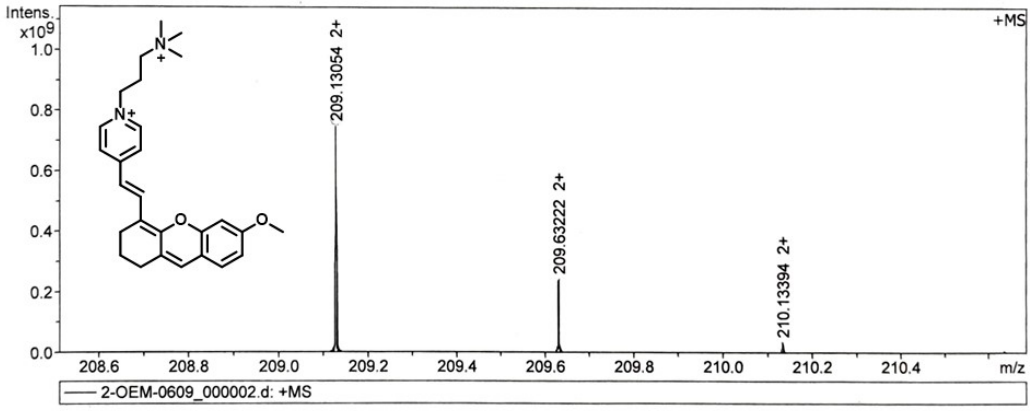

Figure S14. ESI-MS Spectrum of 2+OEM. (positive mode, m/z: $[(M-2Br)/2]^+$ calcd 209.1035 found 209.1035.)

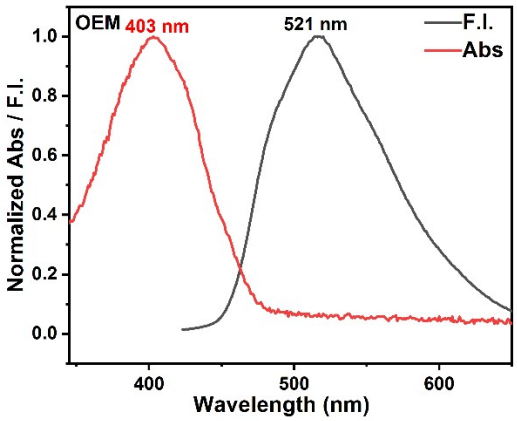

Figure S15.Normalized UV-vis absorption and normalized fluorescence emission spectra of the intermediate OEM (20 μM) in DMSO.

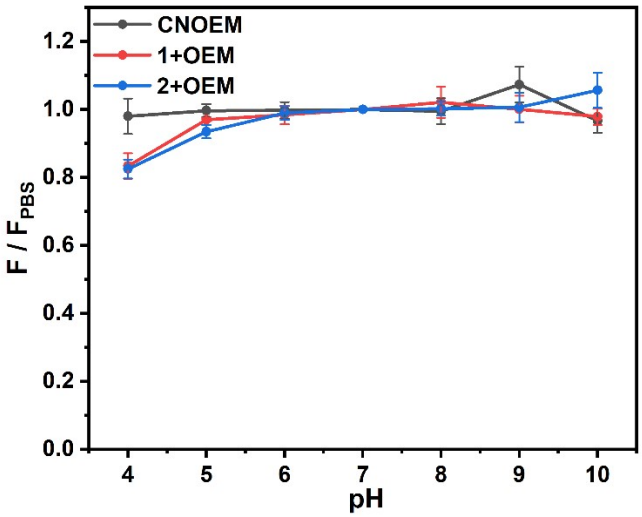

Figure S16. Relative fluorescence intensity of three probes (20 μM) in aqueous solutions measured across different pH conditions.