

Supporting Information

Acid-promoted fluorescent probe for monitoring endogenous methylglyoxal in tumors and gastritis

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Materials and instruments

All reagents and solvents were purchased from Aladdin, Bidepharm, and J&K and used without further purification. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Tianjin Kaimeihong Biological Technology Co., Ltd. LysoTracker Green, MitoTracker Green were purchased from Yingwei Jieji (Shanghai) Trading Co., Ltd. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM 400 and 100 MHz spectrometers in CDCl_3 , CD_3OD , or DMSO-d_6 . The absorbance spectra and fluorescence spectra were obtained on a UV-vis spectrophotometer (Shimadzu, UV-2600) and a Hitachi F4700 fluorometer (1 cm quartz cell), respectively. The fluorescence images of cells were acquired using a NIKON A1+ confocal, and images were analyzed using ImageJ (NIH). The whole body and ex vivo organ fluorescence imaging was performed on an IVIS Lumina System. Cell viability was measured by the CCK-8 assay on a Tecan Infinite M Plex microplate reader.

Supplemental figures

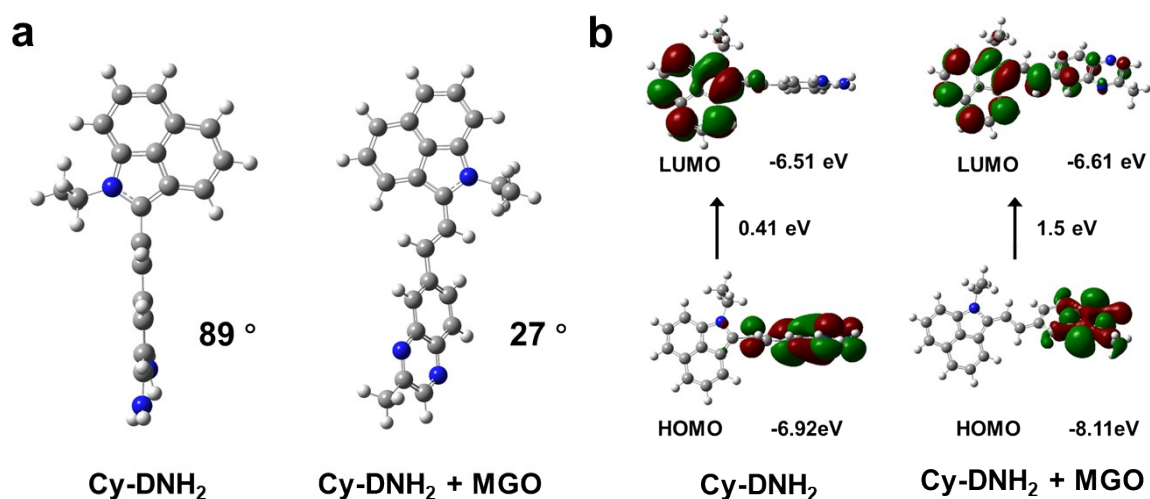


Figure S1. (a) The optimized geometry of Cy-DNH₂ and Cy-DNH₂ with MGO. (b) The excited state energy levels of Cy-DNH₂ and Cy-DNH₂ with MGO were predicted by TD-DFT/B3LYP/6-31G (d, p) method.

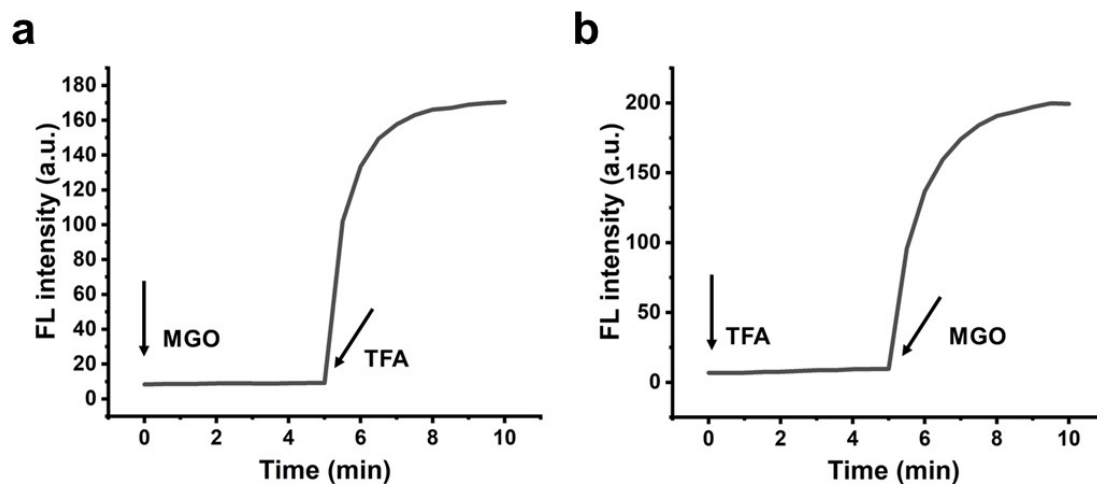


Figure S2. Time-dependent fluorescence response of 10 μM Cy-DNH₂ in the presence of TFA (5%) and MGO (100 μM) in DMSO solution.

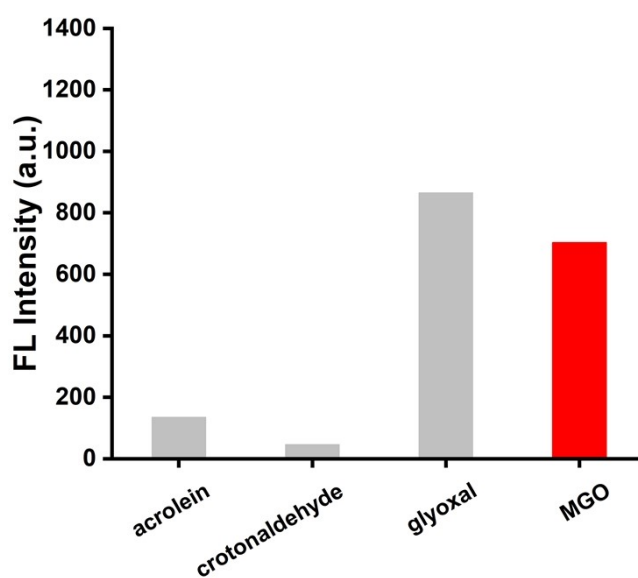


Figure S3. Fluorescence intensity at 545 nm of 10 μM Cy-DNH₂ treated with other reactive carbonyl species (200 μM) in pH 4.2 citrate buffer solutions.

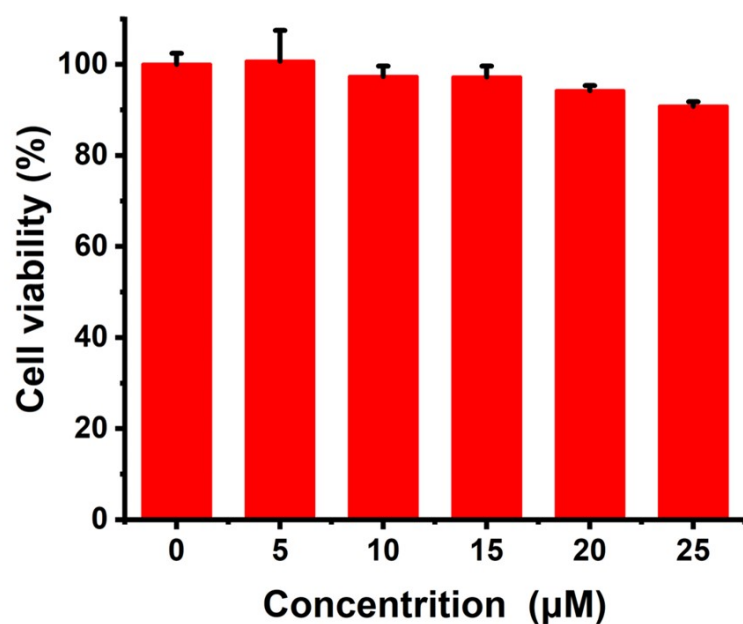


Figure S4. Cell viability of HeLa cells incubated with different concentrations of Cy-DNH₂ by CCK-8 assay (n = 3).

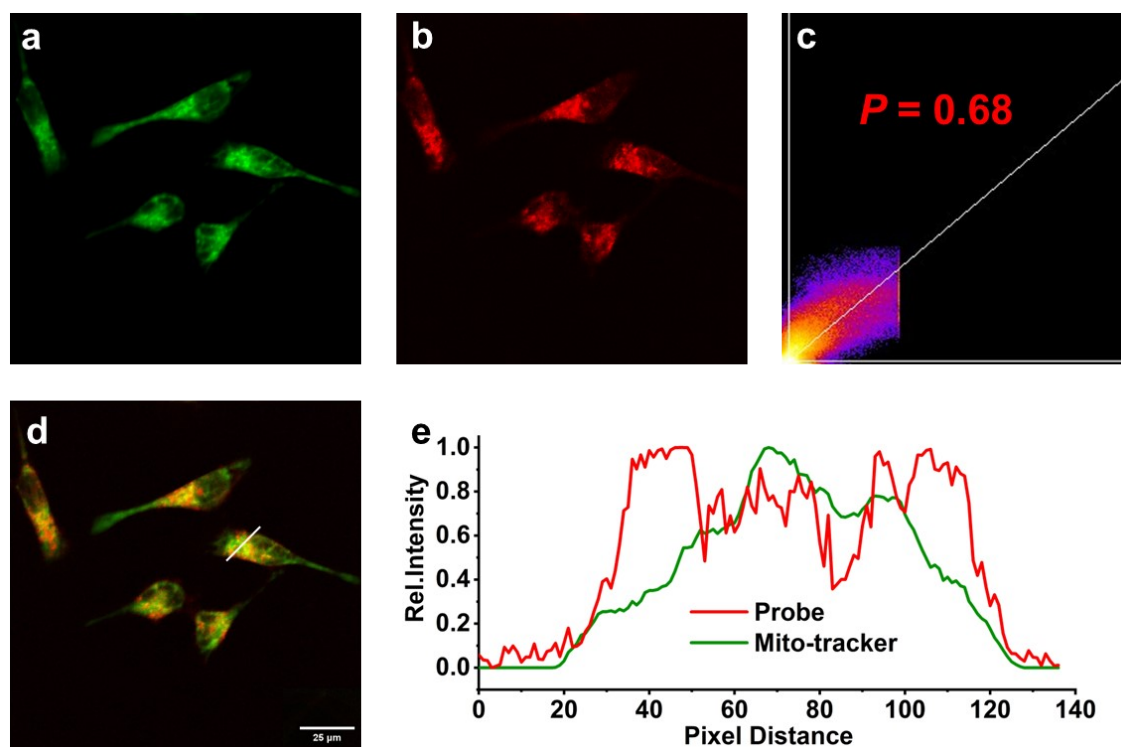


Figure S5. Co-localization of Cy-DNH₂ and mitochondria. (a) Green channel (Mito-Tracker Green). (b) Red channel (Cy-DNH₂). (c) Intensity scatter plot. (d) Merged image of (a, b). (e) Relative intensity profile of the white line across HeLa cells. Scale bar = 25 µm.

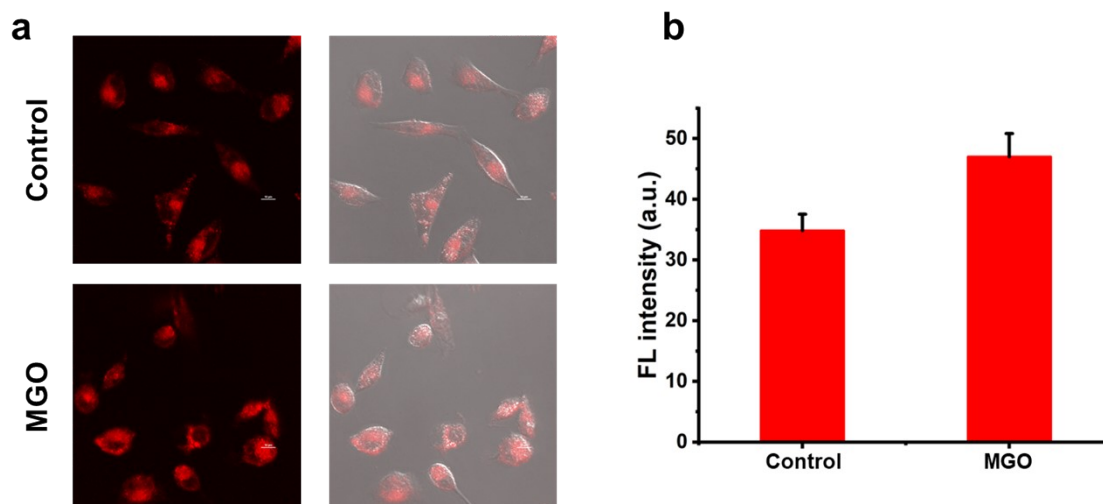


Figure S6. Confocal fluorescence images of 5 μM Cy-DNH₂-loaded HeLa cells incubated with MGO (10 μM). Scale bar = 10 μm . (b) Relative fluorescence intensity of images in (a).

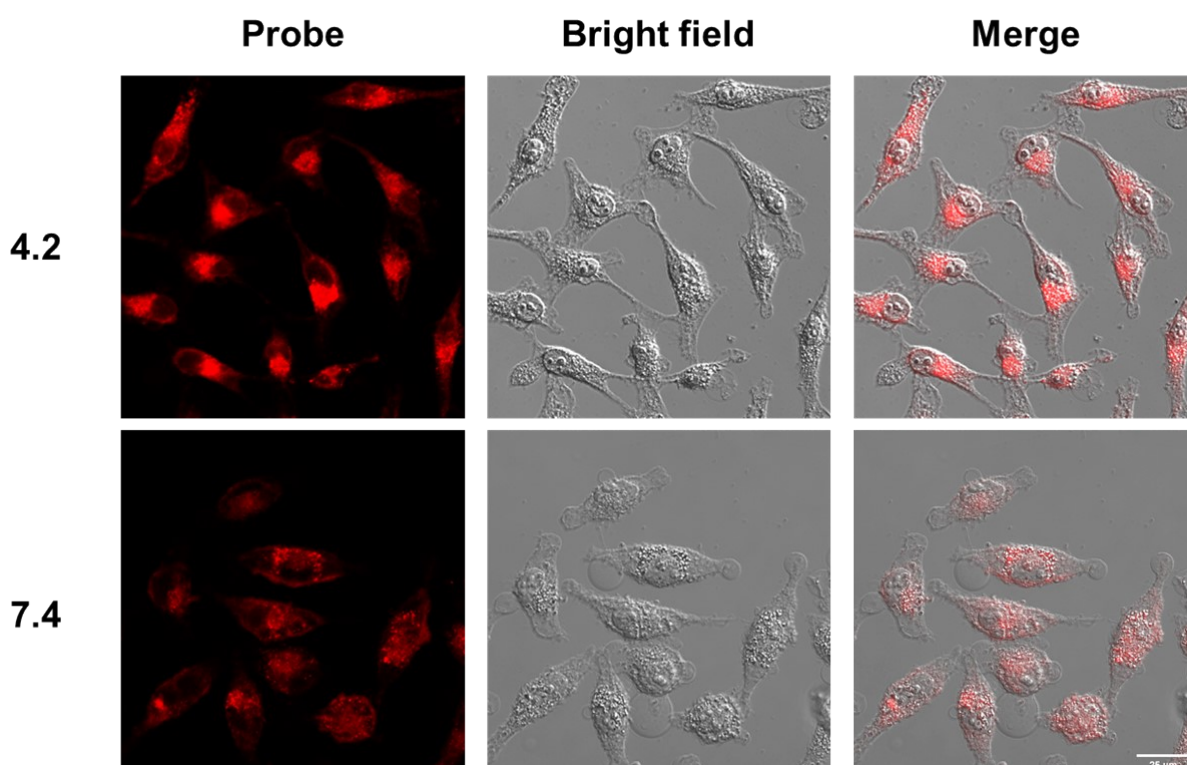


Figure S7. Confocal fluorescence images of 5 μM Cy-DNH₂-loaded HeLa cells treated with 10 μM MGO incubated in different pH buffers, Scale bar = 25 μm .

Computational methods

The molecular geometries of Cy-DNH₂ and Cy-DNH₂ with MGO were optimized at the B3LYP/6-31G(d,p) level in the gas-phase level based on density functional theory (TD-DFT). Then, the corresponding highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular

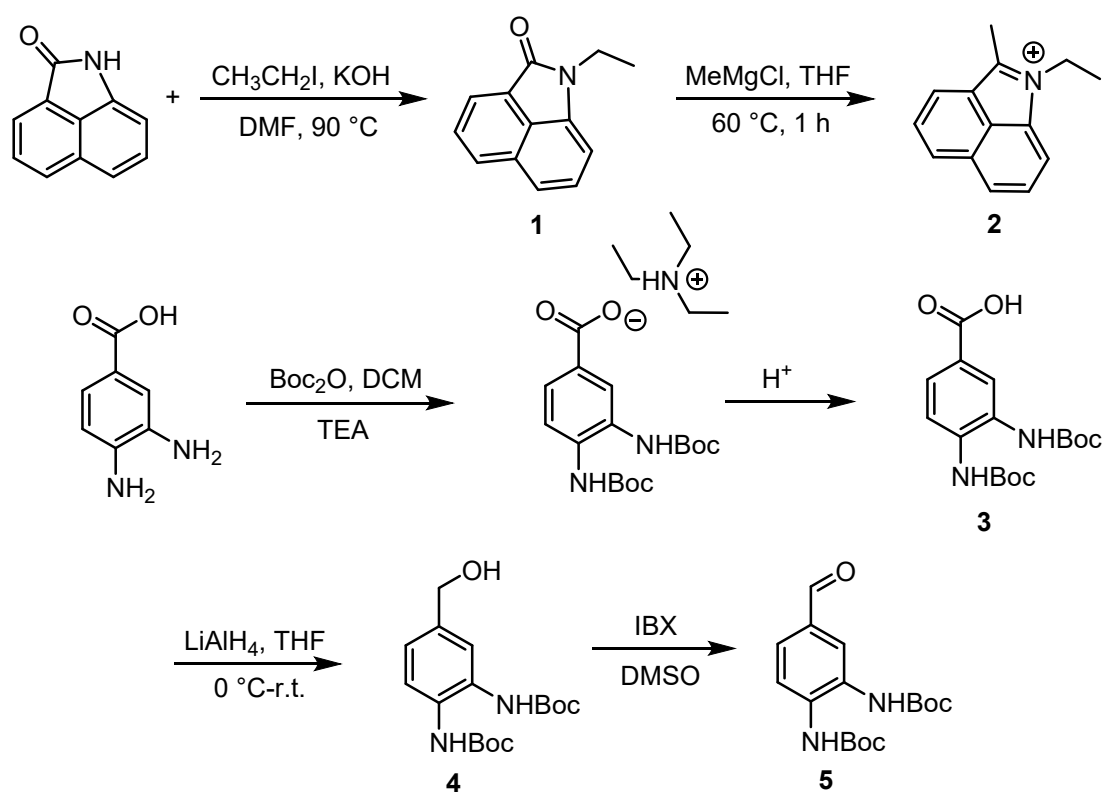
orbital (LUMO) were also calculated at the same level of theory.

Cytotoxicity assay and colocalization imaging

HeLa cells were cultured in high glucose DMEM medium containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). The cells were maintained at 37 °C under a humidified atmosphere with 5% CO₂. The cytotoxicity of probes was evaluated by CCK-8 assay. Cells were seeded in a white opaque flat-bottom 96-well plate at a cell density of 10,000 cells per well in 100 μL growth medium. After 24 h to allow cell adhesion, the medium was exchanged for 200 μL fresh growth medium. Then probes were added to the cells for final concentrations of 0 to 25 μM, followed by incubation for ~20 hours. After that, the medium was removed, and cells were washed twice with 100 μL PBS buffer. Subsequently, 100 μL fresh medium and 10 μL of CCK-8 was added, followed by incubation for 1 hour. Finally, the absorbance value was recorded at 450 nm on a Tecan Infinite M Plex microplate reader.

For confocal fluorescence imaging studies, HeLa cells were seeded in confocal petri dishes and glued to walls for 24 hours. The next day, the old medium was removed and replaced with fresh DMEM. **Cy-DNH₂** (5 μM) was added and incubated for 2 h, then the medium was removed and washed twice with phosphate-buffered saline (PBS) and incubated with Lyso Tracker Green (1 μM) or Mito-Tracker Green (200 nM) for 30 min. Finally, cells were washed twice with PBS and incubated with fresh medium before imaging.

Synthetic route



Synthesis of compound 1

1,8-Naphthoalactam (3.00 g, 18 mmol) and potassium hydroxide (3.36 g, 60 mmol) were dissolved in anhydrous DMF (60 mL) under nitrogen atmosphere. Then the mixture was cooled to 0 °C, ethyl iodide (9.36 g, 60 mmol) was added into the reaction solution. The reaction was stirred at 90 °C for 12 hours and then extracted with ethyl acetate. The crude product was purified by flash column chromatography over silica gel using ethyl acetate/petroleum ether as the eluent, affording a yellow-green solid (yield, 85.5%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.18 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 6.8 Hz, 1H), 7.80 (t, *J* = 7.2 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.2 (d, *J* = 7.2 Hz, 1H), 3.93 (q, *J* = 7.2 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 3H).

Synthesis of compound 2

Compound 1 (1.00 g, 5 mmol) dissolved in anhydrous THF (20 mL), the mixture was cooled to 0 °C, and then methyl magnesium chloride (3 M solution in THF, 3 mL, 9 mmol) was added into the reaction solution. The reaction was stirred at 60 °C for 1 hours under nitrogen atmosphere. The mixture was cooled and hydrochloric acid (1 M, 15 mL) was added to the mixture. Removed the THF under reduced pressure and added saturated sodium iodide solution into mixture, obtaining orange precipitates. The crude product was filtered, washed by ethyl acetate and water, and then dried in vacuum (yield, 73.5%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.98 (d, *J* = 7.2 Hz, 1H), 8.80 (d, *J* = 8.4 Hz, 1H), 8.54 (d, *J* = 7.2 Hz, 1H), 8.45 (d, *J* = 8.8 Hz, 1H), 8.18 (t, *J* = 7.6 Hz, 1H), 8.01 (t, *J* = 8.0 Hz, 1H), 4.72 (q, *J* = 7.2 Hz, 2H), 3.24 (s, 3H), 1.55 (t, *J* = 7.6 Hz, 3H).

Synthesis of compound 3

3, 4-diaminobenzoic acid (2.0 g, 13.2 mmol) dissolved in 40 mL dry dichloromethane. The reaction mixture was stirred at 0 °C and added triethylamine (4.05 g, 40mmol) under nitrogen atmosphere, then di-*tert*-butyl dicarbonate (8.7 g, 40mmol) was added into the reaction solution and stirred at room temperature for 12 hours. The crude product was purified by flash column chromatography over silica gel using ethyl acetate/petroleum as the eluent. Finally, washed to neutral with saturated ammonium chloride solution, and then extracted by dichloromethane, yielding the product as yellow solid (yield, 34.6%). ¹H NMR (400 MHz, CDCl₃): δ 8.01 (s, 1H), 7.88 (m, 1H), 7.22 (s, 1H), 6.61 (s, 1H), 1.52 (s, 18H).

Synthesis of compound 4

Compound 3 (1.6 g, 4.5 mmol) dissolved in THF (50 mL), LiAlH₄ (360 mg, 9.5 mmol) was added into this system at 0 °C, then raised the reaction temperature to 25 °C and stirred for 12 h under nitrogen atmosphere. Under ice bath conditions, the reaction was quenched with H₂O, the crude product was purified by flash column chromatography over silica gel using methanol/dichloromethane obtain a yellow oily substance (yield, 16.2%). ¹H NMR (400 MHz, CDCl₃): δ 7.51-7.41 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.79-6.68 (m, 2H), 4.62 (s, 2H), 1.51 (s, 18H).

Synthesis of compound 5

Compound 4 (250 mg, 0.74 mmol) dissolved in DMSO (5 mL), IBX (310 mg, 1.1 mmol) was added into this solution, and stirred at 25 °C under nitrogen atmosphere. The reaction was monitored by TLC, after the reaction was complete, added 50 mL of H₂O and extracted with DCM, the organic solvent was removed under reduced pressure to obtain a yellow powder (yield, 84.5%). ¹H NMR (400 MHz, CD₃OD): δ 9.87 (s, 1H), 7.98 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 1.54 (s, 18H).

^1H NMR and ^{13}C NMR spectra

