Supplementary Information

Rapid Synthesis of γ-halide/pseudohalide Substituted Cyanine Sensors with Programmed Generation of Singlet Oxygen

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1. **Abbreviations**

ATCC = American Type Culture Collection  
BHI = Brain Heart Infusion Broth  
CICC = China Center of Industrial Culture Collection  
DCM = Dichloromethane  
DMF = Dimethylformamide  
equiv = equivalents  
ESI = Electrospray Ionisation  
HRMS = High Resolution Mass Spectrometry  
LB = Luria-Bertani  
MeOH = Methanol  
MRS = M.R.S. Broth  
NB = Nutrient Broth  
NBS = N-Bromosuccinimide  
NCS = N-Chlorosuccinimide  
NFIS = N-fluoro-N-(phenylsulfonyl)-benzenesulphonamide  
NIS = N-Iodosuccinimide  
NMR = Nuclear Magnetic Resonance  
OD = Optical Density  
PBS = Phosphate Buffered Saline  
PI = Propidium Iodide  
rpm = Revolutions Per Minute  
rt = Room Temperature  
TBAB = Tetrabutylammonium bromide  
TBAI = Tetrabutylammonium iodide  
THF = Tetrahydrofuran  
TSB = Tryptone Soya Broth
2. Experimental Procedures

2.1 General methods

All the chemicals were purchased from J&K, Energy Chemical or Innochem. Commercially available reagents were used without further purification. Four bacterial strains (Staphylococcus aureus (S. aureus) (ATCC 29213), Klebsiella pneumoniae (K. pneumoniae) (ATCC 700603), Pseudomonas aeruginosa (P. aeruginosa) (ATCC 27853), Enterobacter cloacae (E. cloacae) (ATCC 13047)) were purchased from American Type Culture Collection (ATCC), USA. Enterococcus faecium (E. faecium) (CICC 10840) and Acinetobacter baumannii (A. baumannii) (CICC 22933) were purchased from China Center of Industrial Culture Collection, CICC©. The synthesis of Cy 5 (1a) and Cy 5.5 (5) were according to the previous literature of our lab.[1] Fluorescence emission spectra and full wavelength absorption spectra were performed on Tecan Spark™ 10M Multimode Microplate Reader. Fluorescence quantum yields were measured with Edinburgh photonics FLS980. OD values were recorded in a 10 mm path quartz cell on a Metash UV-5100B spectrometer. Confocal laser scanning microscope imaging were conducted with Leica TCS SP8 X Confocal Microscope. All 1H NMR spectra were recorded at 400 MHz or 500 MHz, respectively. 13C NMR spectra were recorded at 100 MHz or 150 MHz, respectively. HRMS was measured with Thermo LCQ Deca XP Max mass spectrometer for ESI.

The preparations of compound 1b-1i were according to the literature.[2] Compound 1a was dissolved in 1 : 1 corresponding aqueous NaX (1.0 M) and DCM, and stirred vigorously at room temperature for 24 hours. The biphasic mixture was separated, extracted with DCM, dried over Na2SO4, and the residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid.

2.2 Synthetic procedures and characterized data

![Scheme S1. Synthesis of compound 1a. Reagents and conditions: (i) compound a and compound b; acetic anhydride, reflux, 2 h, evaporate, then with compound c, anhydrous ethanol, anhydrous sodium acetate, reflux, 6 h, for 1a.](image)

Compound 1a

The compound a (1.30 g, 5.0 mmol) and compound b (1.34 g, 5.0 mmol) were added to the acetic anhydride (80 mL) and the mixture was stirred and refluxed for 2 h. The solution was cooled down to rt and the solvent was evaporated under reduced pressure. Then anhydrous ethanol (80 mL), compound c (1.79 g,
5.0 mmol), and anhydrous sodium acetate (0.66 g, 8.0 mmol) were added to the residue and stirred and refluxed for 6 h. After the reaction was complete, the product was purified by silica gel column chromatography (DCM : MeOH = 30 : 1) to give deep blue solid 1.18 g, yield 54 %. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.27 (t, $J = 12.5$ Hz, 2H, -CH$_2$), 7.50 – 7.49 (m, 2H, -Ar), 7.41 – 7.39 (m, 2H, -Ar), 7.30 – 7.26 (m, 4H, -Ar), 6.66 (t, $J = 12.0$ Hz, 1H, -CH$_2$), 6.34 – 6.29 (m, 2H, -CH$_2$), 4.17 – 4.12 (m, 4H, -CH$_2$), 2.31 (t, $J = 6.4$ Hz, 2H, -CH$_2$), 1.85 – 1.81 (m, 2H, -CH$_2$), 1.73 (s, 12H, -CH$_3$), 1.69 – 1.65 (m, 2H, -CH$_2$), 1.54 – 1.49 (m, 2H, -CH$_2$), 1.39 (t, $J = 6.8$ Hz, 3H, -CH$_3$). $^{13}$C NMR (100 MHz, Methanol-$d_4$) δ 177.7, 174.6, 174.3, 155.6, 155.5, 143.5, 143.0, 142.8, 142.6, 129.7, 129.7, 126.6, 126.2, 126.2, 123.4, 123.4, 123.4, 112.0, 111.8, 104.3, 104.0, 50.6, 50.5, 39.9, 35.0, 28.1, 27.9, 27.4, 25.8, 12.5. HRMS (m/z) (M$^+$): calcd. for C$_{33}$H$_{41}$O$_2$N$_2$+ 497.3163, found 497.3158.

**Scheme S2.** Synthesis of compound 2a. Reagents and conditions: (i) NCS, DCM, rt, 1 h.

**Compound 2a**

A mixture of compound 1a (100 mg, 173 μmol) and NCS (28 mg, 210 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 96 mg, yield 90 %. $^1$H NMR (400 MHz, Methanol-$d_4$) δ 8.41 (t, $J = 13.2$ Hz, 2H, -CH$_2$), 7.78 – 7.76 (d, $J = 6.8$ Hz, 2H, -Ar), 7.49 – 7.35 (m, 4H, -Ar), 7.22 – 7.12 (m, 2H, -Ar), 6.53 – 6.47 (m, 2H, -CH$_2$), 4.30 – 4.20 (m, 4H, -CH$_2$), 2.35 (t, $J = 7.2$ Hz, 2H, -CH$_2$), 1.92 (t, $J = 6.8$ Hz, 2H, -CH$_2$), 1.78 (s, 12H, -CH$_3$), 1.75 – 1.72 (m, 2H, -CH$_2$), 1.59 – 1.54 (m, 2H, -CH$_2$), 1.47 (t, $J = 6.8$ Hz, 3H, -CH$_3$). $^{13}$C NMR (100 MHz, Methanol-$d_4$) δ 174.8, 174.5, 149.8, 141.8, 141.6, 141.5, 141.4, 130.9, 128.5, 125.6, 122.2, 122.1, 115.6, 111.2, 111.0, 102.3, 101.9, 99.9, 99.6, 65.2, 49.6, 43.8, 39.1, 33.3, 26.6, 26.0, 25.9, 24.2, 18.8, 12.6, 10.9. HRMS (m/z) (M$^+$): calcd. for C$_{33}$H$_{40}$O$_2$N$_2$Br$^+$ 575.2268, found 575.2270.

**Scheme S3.** Synthesis of compound 2c, 2d, 2e and 2f. Reagents and conditions: (i) aqueous NaX (1.0 M) / DCM = 1 : 1, rt, 24 h. (ii) NCS, DCM, rt, 1 h.

**Compound 2c**
Compound **1a** (200 mg, 345 μmol) was dissolved in 1:1 aqueous NaCl (1.0 M, 15 mL) and DCM (15 mL), and stirred vigorously at rt for 24 hours. The biphasic mixture was separated, extracted with DCM (2 x 10 mL), and dried over Na₂SO₄. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 185 mg. A mixture of compound **1b** (100 mg, 186 μmol) and NCS (30 mg, 223 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 47 mg, yield 44 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.37 (d, J = 13.6 Hz, 2H, -CH₂), 7.56 – 7.53 (m, 2H, -Ar), 7.48 – 7.43 (m, 2H, -Ar), 7.39 (d, J = 8.0 Hz, 2H, -Ar), 7.35 – 7.30 (m, 2H, -Ar), 6.49 – 6.45 (m, 2H, -CH₃), 4.27 – 4.18 (m, 4H, -CH₂), 2.32 (t, J = 7.6 Hz, 2H, -CH₂), 1.91–1.86 (m, 2H, -CH₂), 1.76 (s, 12H, -CH₂), 1.74 – 1.68 (m, 2H, -CH₂), 1.56 – 1.50 (m, 2H, -CH₂), 1.44 (t, J = 7.6 Hz, 3H, -CH₃).¹³C NMR (150 MHz, Methanol-d₄) δ 176.1, 175.7, 149.2, 149.1, 143.4, 143.1, 143.0, 142.9, 130.0, 129.9, 127.1, 127.0, 124.2, 123.6, 112.6, 112.4, 101.4, 101.0, 51.1, 51.0, 45.2, 40.5, 34.9, 28.1, 27.5, 27.4, 25.8, 12.4. HRMS (m/z) (M⁺): calcd. for C₃₆H₅₀O₂N₃Cl⁺ 531.2773, found 531.2779.

**Compound 2d**

Compound **1a** (200 mg, 345 μmol) was dissolved in 1:1 aqueous NaI (1.0 M, 15 mL) and DCM (15 mL), and stirred vigorously at rt for 24 hours. The biphasic mixture was separated, extracted with DCM (2 x 10 mL), and dried over Na₂SO₄. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 185 mg. A mixture of compound **1d** (100 mg, 160 μmol) and NCS (26 mg, 195 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 88 mg, yield 83 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.14 (s, 1H, -CH), 8.11 (s, 1H, -CH), 7.54 – 7.51 (m, 2H, -Ar), 7.46 – 7.41 (m, 2H, -Ar), 7.39 – 7.37 (m, 2H, -Ar), 7.33 – 7.29 (m, 2H, -Ar), 6.48 – 6.44 (m, 2H, -CH), 4.28 – 4.16 (m, 4H, -CH₂), 2.32 (t, J = 7.6 Hz, 2H, -CH₂), 1.94 – 1.86 (m, 2H, -CH₂), 1.73 (s, 12H, -CH₂), 1.70 – 1.67 (m, 2H, -CH₂), 1.56 – 1.52 (m, 2H, -CH₂), 1.44 (t, J = 7.2 Hz, 3H, -CH₃).¹³C NMR (100 MHz, Methanol-d₄) δ 177.2, 176.4, 176.1, 155.6, 155.4, 143.3, 143.1, 142.9, 142.8, 132.4, 130.0, 127.1, 127.1, 123.7, 123.6, 112.6, 112.4, 108.3, 108.0, 97.8, 66.7, 51.2, 51.1, 45.4, 40.7, 34.7, 28.0, 27.6, 27.5, 27.4, 25.7, 20.3, 12.4. HRMS (m/z) (M⁺): calcd. for C₃₆H₄₀O₂N₃I⁺ 623.2129, found 623.2128.

**Compound 2e**

Compound **1a** (200 mg, 345 μmol) was dissolved in 1:1 aqueous NaSCN (1.0 M, 15 mL) and DCM (15 mL), and stirred vigorously at rt for 24 hours. The biphasic mixture was separated, extracted with DCM (2 x 10 mL), and dried over Na₂SO₄. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 150 mg. A mixture of compound **1e** (100 mg, 180 μmol) and NCS (29 mg, 217 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 81 mg, yield 76 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.55 (s, 1H, -CH), 8.51 (s, 1H, -CH), 7.77 – 7.55 (m, 2H, -
Compound 2f

Compound 1a (200 mg, 345 μmol) was dissolved in 1 : 1 aqueous NaSeCN (1.0 M, 15 mL) and DCM (15 mL), and stirred vigorously at rt for 24 hours. The biphasic mixture was separated, extracted with DCM (2 x 10 mL), and dried over Na₂SO₄. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 160 mg. A mixture of compound 1f (100 mg, 166 μmol) and NCS (27 mg, 202 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 46 mg, yield 43 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.50 (s, 1H, -CH), 8.46 (s, 1H, -CH), 7.59 – 7.57 (m, 2H, -Ar), 7.50 – 7.43 (m, 4H, -Ar), 7.38 – 7.34 (m, 2H, -Ar), 6.70 – 6.66 (m, 2H, -CH), 4.32 – 4.24 (m, 4H, -CH₂), 2.32 (t, J = 7.2 Hz, 2H, -CH₂), 1.99 – 1.92 (m, 2H, -CH₂), 1.79 (s, 12H, -CH₃), 1.76 – 1.73 (m, 2H, -CH₂), 1.60 – 1.54 (m, 2H, -CH₂), 1.50 (t, J = 7.2 Hz, 3H, -CH₃). ¹³C NMR (150 MHz, Methanol-d₄) δ 177.0, 176.7, 155.8, 155.7, 143.3, 143.2, 142.8, 130.0, 130.0, 127.4, 123.7, 123.6, 114.2, 112.9, 112.7, 105.1, 104.6, 103.2, 51.3, 51.3, 45.7, 40.8, 28.3, 27.8, 27.6, 27.5, 26.5, 12.5. HRMS (m/z) (M⁺): calcd. for C₃₄H₅₀O₂N₅Se²⁺ 602.2280, found 602.2274.

Scheme S4. Synthesis of compound 3 and 4. Reagents and conditions: (i) AcONa, Ac₂O, 60 °C, 4 h.
(ii) NCS, DCM, rt, 1 h.

Compound 3

The compound a (500 mg, 1.93 mmol), compound b (1.10 g, 4.10 mmol) and AcONa (476 mg, 5.80 mmol) were added to Ac₂O (15 mL) and the mixture was stirred at 60 °C for 2 h. The solution was cooled down to rt and the solvent was evaporated under reduced pressure. The product was purified by silica gel
column chromatography (DCM : MeOH = 30 : 1) to give deep blue solid 670 mg, yield 73 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.28 (t, J = 12.8 Hz, 2H, -CH₃), 7.53 – 7.51 (m, 2H, -Ar), 7.46 – 7.42 (m, 2H, -Ar), 7.33 – 7.27 (m, 4H, -Ar), 6.65 (t, J = 12.4 Hz, 1H, -CH₃), 6.32 (d, J = 13.6 Hz, 2H, -CH₂), 4.18 (dd, J₁ = 14.4 Hz, J₂ = 7.2 Hz, 4H, -CH₂), 1.75 (s, 12H, -CH₃), 1.41 (t, J = 7.2 Hz, 6H, -CH₃). ¹³C NMR (100 MHz, Methanol-d₄) δ 174.3, 155.7, 143.1, 142.8, 129.8, 126.5, 126.3, 123.5, 111.8, 104.0, 50.6, 40.00, 27.9, 12.6. HRMS (m/z) (M⁺): calcd. for C₂₉H₃₅N₂ + 411.2795, found 411.2799.

Compound 4

A mixture of compound 3 (100 mg, 203 μmol) and NCS (33 mg, 247 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 80 mg, yield 75 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.38 (d, J = 13.6 Hz, 2H, -CH₃), 7.54 – 7.52 (m, 2H, -Ar), 7.46 – 7.42 (m, 2H, -Ar), 7.38 – 7.30 (m, 4H, -Ar), 6.47 (d, J = 13.2 Hz, 2H, -CH₂), 4.27 – 4.21 (m, 4H, -CH₂), 1.73 (s, 12H, -CH₃), 1.44 (t, J = 7.2 Hz, 3H, -CH₃). ¹³C NMR (150 MHz, Methanol-d₄) δ 174.5, 149.8, 149.8, 141.6, 141.4, 128.5, 125.6, 122.2, 111.0, 101.8, 49.6, 49.6, 39.1, 26.0, 10.9. HRMS (m/z) (M⁺): calcd. for C₂₉H₃₄N₂Br + 489.1900, found 489.1901.

Scheme S5. Synthesis of compound 6. Reagents and conditions: (i) NCS, DCM, rt, 1 h.

Compound 6

The synthesis of compound 5 were according to the previous literature. A mixture of compound 5 (100 mg, 148 μmol) and NCS (24 mg, 180 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 87 mg, yield 83 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.52 (d, J = 13.2 Hz, 2H, -CH₃), 8.27 (d, J = 8.8 Hz, 2H, -Ar), 8.08 – 8.01 (m, 4H, -Ar), 7.69 – 7.66 (m, 4H, -Ar), 7.54 – 7.51 (m, 2H, -Ar), 6.54 (d, J = 13.2 Hz, 2H, -CH₂), 4.39 – 4.30 (m, 4H, -CH₂), 2.35 (t, J = 7.2 Hz, 2H, -CH₂), 2.05 (s, 12H, -CH₃), 2.00 – 1.95 (m, 2H, -CH₂), 1.80 – 1.72 (m, 2H, -CH₂), 1.62 – 1.56 (m, 2H, -CH₂), 1.52 (t, J = 7.2 Hz, 3H, -CH₃). ¹³C NMR (100 MHz, Methanol-d₄) δ 175.9, 175.6, 148.7, 148.5, 139.2, 138.8, 134.5, 134.3, 132.3, 132.3, 130.9, 130.4, 129.7, 128.4, 127.9, 127.4, 125.0, 122.0, 115.6, 110.8, 110.6, 101.8, 101.5, 65.2, 51.5, 51.4, 48.0, 44.0, 39.4, 33.3, 26.9, 26.0, 25.8, 25.7, 24.3, 18.8, 11.2. HRMS (m/z) (M⁺): calcd. for C₄₁H₄₄O₂N₂Br⁺ 675.2581, found 675.2582.
Scheme S6. Synthesis of compound 7 and 8. Reagents and conditions: (i) AcONa, Ac₂O, 60 °C, 2 h.

(ii) NCS, TBAB, DCM, rt, 1 h.

Compound 7

The compound d (500 mg, 1.76 mmol), compound b (1.00 g, 3.73 mmol) and AcONa (432 mg, 5.27 mmol) were added to Ac₂O (25 mL) and the mixture was stirred at 60 °C for 2 h. The solution was cooled down to rt and the solvent was evaporated under reduced pressure. The product was purified by silica gel column chromatography (DCM : MeOH = 30 : 1) to give deep blue solid 860 mg, yield 95 %. ¹H NMR (400 MHz, Methanol-d₄) δ 7.97 (t, J = 12.8 Hz, 2H, -C₆H₄), 7.64 (t, J = 12.8 Hz, 1H, -C₆H₄), 7.51 – 7.49 (m, 2H, -Ar), 7.44 – 7.40 (m, 2H, -Ar), 7.30 – 7.24 (m, 4H, -Ar), 6.58 (t, J = 12.8 Hz, 2H, -C₆H₄), 6.31 (d, J = 13.6 Hz, 2H, -C₆H₄), 4.15 (dd, J₁ = 14.8 Hz, J₂ = 7.2 Hz, 4H, -C₆H₄₂), 1.71 (s, 12H, -C₆H₃), 1.39 (t, J = 7.2 Hz, 6H, -C₆H₃). ¹³C NMR (100 MHz, Methanol-d₄) δ 172.8, 157.9, 153.2, 143.3, 142.6, 129.8, 127.0, 126.0, 123.5, 111.6, 104.5, 50.4, 39.9, 28.0, 20.9, 12.6. HRMS (m/z) (M⁺): calcd. for C₃₁H₃₇N₂⁺ 437.2951, found 437.2952.

Compound 8

A mixture of compound 7 (50 mg, 97 μmol), TBAB (156 mg, 484 μmol) and NCS (13 mg, 97 μmol) was stirred in DCM (10 mL) at rt for 1 h and DCM was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 44 mg, yield 83 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.19 (t, J = 12.4 Hz, 1H, -C₆H₄), 7.97 (d, J = 13.2 Hz, 1H, -C₆H₄), 7.97 (d, J = 13.2 Hz, 1H, -C₆H₄), 7.84 (d, J = 12.8 Hz, 1H, -C₆H₄), 7.59 (d, J = 7.6 Hz, 1H, -Ar), 7.52 – 7.45 (m, 3H, -Ar), 7.44 – 7.37 (m, 4H, -Ar), 6.88 (t, J = 12.4 Hz, 1H, -C₆H₄), 6.65 (d, J = 14.4 Hz, 1H, -C₆H₄), 6.30 (d, J = 13.2 Hz, 1H, -CH₃), 4.30 (dd, J₁ = 14.4 Hz, J₂ = 7.2 Hz, 2H, -CH₃, 2H, -CH₃), 4.15 (dd, J₁ = 14.4 Hz, J₂ = 7.2 Hz, 2H, -CH₃, 2H, -CH₃), 1.75 (s, 6H, -CH₃), 1.71 (s, 6H, -CH₃), 1.45 (t, J = 7.2 Hz, 3H, -CH₃), 1.42 (t, J = 7.2 Hz, 3H, -CH₃). ¹³C NMR (100 MHz, Methanol-d₄) δ 176.4, 172.3, 155.4, 153.8, 147.1, 143.4, 143.4, 142.8, 142.3, 130.1, 129.8, 127.6, 126.0, 125.8, 123.7, 123.5, 116.3, 113.0, 111.4, 107.7, 101.3, 51.4, 50.2, 40.9, 39.9, 27.9, 27.6, 13.0, 12.2. HRMS (m/z) (M⁺): calcd. for C₃₁H₃₆N₂Br⁺ 515.2056, found 515.2059.
Scheme S7. Synthesis of compound 11 and 12. Reagents and conditions: (i) AcONa, Ac₂O, 60 °C, 2 h.

Compound 11

The compound a (73 mg, 238 μmol), compound e (200 mg, 477 μmol) and AcONa (60 mg, 714 μmol) were added to Ac₂O (5 mL) and the mixture was stirred at 60 °C for 2 h. The solution was cooled down to rt and the solvent was poured into DCM and evaporated to dryness to give deep blue solid 186 mg, yield 88 %. ¹H NMR (400 MHz, Methanol-d₄) δ 8.31 (t, J = 12.8 Hz, 2H, -CH₂), 7.89 (d, J = 1.6 Hz, 3H, -Ar), 7.87 (d, J = 1.6 Hz, 1H, -Ar), 7.33 (d, J = 9.2 Hz, 2H, -Ar), 6.67 (t, J = 12.4 Hz, 1H, -CH₂), 6.32 (d, J = 13.6 Hz, 2H, -CH₂), 3.64 (s, 6H, -CH₃), 1.75 (s, 12H, -CH₃). ¹³C NMR (150 MHz, Methanol-d₄) δ 176.0, 156.3, 145.7, 143.3, 142.5, 128.0, 127.7, 121.3, 111.4, 105.3, 50.5, 31.8, 27.7. HRMS (m/z) (M+2H⁺): calcd. for C₂₇H₂₁N₂O₆S₂ ± 543.1618, found 543.1622.

Compound 12

A mixture of compound 11 (50 mg, 67 μmol), NCS (9 mg, 67 μmol) was stirred in DCM (1 mL)/MeOH (1 mL) at rt for 1 h and solvent was evaporated to dryness. The residue was purified by silica gel column chromatography (DCM : MeOH = 20 : 1) to give dark blue solid 20 mg, yield 38 %. ¹H NMR (500 MHz, Methanol-d₄) δ 8.22 (d, J = 13.0 Hz, 2H, -CH₂), 7.94 (s, 2H, -Ar), 7.93 (s, 1H, -Ar), 7.92 (s, 1H, -Ar), 7.44 (d, J = 8.5 Hz, 2H, -Ar), 6.48 (d, J = 13.0 Hz, 2H, -CH₂), 3.74 (s, 6H, -CH₃), 1.78 (s, 12H, -CH₃). HRMS (m/z) (M+2H⁺): calcd. for C₂₇H₂₁N₂O₆S₂ ± 669.0584, found 669.0570.
3. Results and Discussion

3.1 H-H COSY of 1a and 2a

Figure S1. H-H COSY of 1a and 2a. (A) H-H COSY of 1a. (B) H-H COSY of 2a.
3.2 HPLC analysis for compounds 2a, 2c, 2d, 2e and 2f

To clarify whether the obtained products are halogenated (Br or Cl) mixtures or not, we investigated the retention time of compounds 2a, 2c, 2d, 2e and 2f by HPLC. As shown in Figure S2a, compound 2a and 2c could be separated clearly by HPLC. HPLC condition: ACQUITY UPLC®BEH C₁₈ 1.7 μm (2.1 × 100 mm Column); Mobile phase A: water, 0.1 % (v / v) formic acid; Mobile phase B: acetonitrile, 0.1 % (v / v) formic acid; Gradient condition: 20 % - 95 % B for 10 min, 95 % - 95 % B for 3 min, 95 % - 20 % B for 0.5 min, 20 % - 20 % B for 1.5 min; Flow rate: 1 mL / min. The retention time of compound 2a and 2c was 9.591 min and 9.555 min respectively. Similarly, compound 2d and 2c, compound 2e and 2c, compound 2f and 2c could be also separated by HPLC (Figure S2b-d). The HPLC analysis showed the retention time of the impurities of each reaction are different form 2c, which indicated each γ-halide/pseudohalide substituted reaction yields a signal product.
Figure S2. The HPLC chromatogram profiles of γ-halide/pseudohalide substituted Cy 5. (a) The retention time of compound 2a, 2c and the mixture of 2a and 2c (1:1), respectively; (b) the retention time of compound 2d, 2c and the mixture of 2d and 2c (1:1), respectively; (c) the retention time of compound 2e, 2c and the mixture of 2e and 2c (1:1), respectively; (d) the retention time of compound 2f, 2c and the mixture of 2f and 2c (1:1), respectively. The assignments of the peaks: 9.591 min, compound 2a; 9.555 min, compound 2c; 9.821 min, compound 2d; 8.704 min, compound 2e and 8.840 min, compound 2f, respectively.
3.3 γ-Halogenation/pseudohalogenation of Cy 5

Table S1. γ-Halogenation/pseudohalogenation of Cy 5.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Reagent</th>
<th>Solvent</th>
<th>X'</th>
<th>Yield(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cl</td>
<td>NFIS(^b)</td>
<td>DCM</td>
<td>Cl</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2</td>
<td>Br</td>
<td>NFIS</td>
<td>DCM</td>
<td>Br</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>NFIS</td>
<td>DCM</td>
<td>I</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>OCN</td>
<td>NCS</td>
<td>DCM</td>
<td>Cl</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>SCN</td>
<td>NCS</td>
<td>DCM</td>
<td>SCN</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>SeCN</td>
<td>NCS</td>
<td>DCM</td>
<td>SeCN</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>N(_3)</td>
<td>NCS</td>
<td>DCM</td>
<td>Cl</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>NO(_2)</td>
<td>NCS</td>
<td>DCM</td>
<td>Cl</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) The reaction was run with 1 (100 mg) and NFIS (1.2 equiv) in DCM at rt for 1 h. \(^b\) NFIS = N-fluoro-N-(phenylsulfonyl)-benzenesulfonamide

3.4 Various oxidants and halogen anion sources in γ-halogenation of Cy 5

To gain further insight into the reagent dependency and underlying reaction mechanism, various oxidants instead of NCS and a diverse set of additional competing halogen anion sources were examined (Table S2). First, we performed the reaction with NCS under oxygen-free condition, which resulted in the expected γ-bromide product with good yield. This result showed that this oxidation halogenation was not dependent on the presence of oxygen (Table S2, Entry 1). Conversely, the attempted use of molecular oxygen as an oxidant instead of NCS for the oxidative halogenation,\(^{[3]}\) resulted in no reaction with only starting material recovered (Table S2, Entry 2). Interestingly, also the use of H\(_2\)O\(_2\) and DMSO as an oxidant under different condition (Table S2, Entry 3-5) did not promote the γ-bromination. Thus, NCS was proved to be unique oxidant for this γ-substitution. Subsequently, we employed tetrabutylammonium halide salts to explore the impact of additional halide counterion sources present in the reaction mixture. Gratifyingly, we obtained the γ-bromide product from the Cy 5 chloride in 79% yield, when tetrabutylammonium bromide was used (Table S2, Entry 6). However, when tetrabutylammonium iodide was added as iodine source, the yields of the γ-iodination were significantly reduced, independently whether Cy 5 chloride or Cy 5 bromide were employed as substrates (Table S2, Entry 7-8).
<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Reaction condition</th>
<th>X'</th>
<th>Yield(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Br</td>
<td>Oxygen-free, NCS</td>
<td>Br</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>Br</td>
<td>O(_2)(^b)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>H(_2)O(_2) (2.0 equiv)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>H(_2)O(_2) (2.0 eq), conc. H(_2)SO(_4) (2.0 equiv)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Br</td>
<td>DMSO (2.0 eq), conc. H(_2)SO(_4) (2.0 equiv)</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cl</td>
<td>TBAB(^c) (5.0 eq), NCS (1.2 equiv)</td>
<td>Br</td>
<td>79</td>
</tr>
<tr>
<td>7</td>
<td>Cl</td>
<td>TBAI(^d) (5.0 eq), NCS (1.2 equiv)</td>
<td>I</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>Br</td>
<td>TBAI (5.0 eq), NCS (1.2 equiv)</td>
<td>I</td>
<td>42</td>
</tr>
</tbody>
</table>

\(^a\) The reaction was run with 1a or 1c (100 mg) in DCM at rt for 1 h under corresponding reaction conditions. \(^b\) The reaction was run with 1a (100 mg) and excess O\(_2\) in DCM at rt for 1 h. \(^c\) TBAB = Tetrabutylammonium bromide. \(^d\) TBAI = Tetrabutylammonium iodide.
3.5 H-H COSY of 7 and 8

Figure S3. H-H COSY of 7 and 8. (A) H-H COSY of 7. (B) H-H COSY of 8.
3.6 Proposed reaction mechanism research for $\gamma$-substituted Cy 5

In our knowledge, there is no similar halogenation chemistry could be found in the literature. However, it is reported that NCS could be used as an oxidizing agent.[4]

Furthermore, in order to further study the reaction mechanism and according to referee 3’s comments, following experiments and literature studies have been carried out.

1. the proposed intermediate I-Cl could be used as $\gamma$-I substitution reagent of Cy 5.

Table S3. The reaction mechanism research by the proposed intermediate I-Cl used as $\gamma$-I substitution reagent of Cy 5

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Reaction condition</th>
<th>X'</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>ICl (1.0 equiv)</td>
<td>I</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>OCN</td>
<td>ICl (1.0 equiv)</td>
<td>I</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>N$_3$</td>
<td>ICl (1.0 equiv)</td>
<td>I</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>NO$_2$</td>
<td>ICl (1.0 equiv)</td>
<td>I</td>
<td>44</td>
</tr>
</tbody>
</table>

Reacting compound 1d with our proposed intermediate I-Cl (1.0 equiv) in DCM at room temperature, $\gamma$-I substituted Cy 5 was obtained as main product. Similarly, when we use compound 1g, 1h or 1i reacted with I-Cl (1.0 eq.) in DCM at room temperature, $\gamma$-I substituted Cy 5 was also obtained as main product. These results indicated that the reaction mechanism of halogen-Cl as intermediate is feasible.

2. the proposed intermediate SCN-Cl and SeCN-Cl could be used as pseudohalide substitution reagents, according to literatures.

Regarding with pseudohalide substitution, unfortunately, we couldn’t purchase or prepare the proposed intermediate SCN-Cl or SeCN-Cl, since the indispensable reagent for preparing them, Cl$_2$, is highly toxic and strictly regulated in Beijing. However, from literatures searching,[5] we found SCN and SeCN group could be introduced to unsaturated structures by SCN-Cl and SeCN-Cl respectively.

These results indicated that the reaction mechanism of $\gamma$-halide/pseudohalide substituted cyanine dyes with the corresponding halogen-Cl and pseudohalogen-Cl as intermediate is reasonable.
3.7 The optical properties of γ-halide/pseudohalide substituted cyanine compounds

Table S4. The optical properties of γ-halide/pseudohalide substituted cyanine compounds.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>λ&lt;sub&gt;ab&lt;/sub&gt; (nm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt; (nm)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt; (nm)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>FL. Intensity (a.u.)(PBS)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>ε (M&lt;sup&gt;-1&lt;/sup&gt; cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Φ&lt;sub&gt;F&lt;/sub&gt; (%)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>640</td>
<td>620</td>
<td>661</td>
<td>4903</td>
<td>83795</td>
<td>33.1</td>
</tr>
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<td>2a</td>
<td>640</td>
<td>615</td>
<td>656</td>
<td>1310</td>
<td>108263</td>
<td>5.6</td>
</tr>
<tr>
<td>2c</td>
<td>640</td>
<td>621</td>
<td>662</td>
<td>1410</td>
<td>134487</td>
<td>9.6</td>
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<td>2d</td>
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<td>615</td>
<td>656</td>
<td>1269</td>
<td>93660</td>
<td>3.1</td>
</tr>
<tr>
<td>2e</td>
<td>615</td>
<td>597</td>
<td>638</td>
<td>945</td>
<td>62160</td>
<td>5.1</td>
</tr>
<tr>
<td>2f</td>
<td>620</td>
<td>601</td>
<td>642</td>
<td>520</td>
<td>37490</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>640</td>
<td>620</td>
<td>661</td>
<td>4648</td>
<td>171386</td>
<td>30.6</td>
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<td>4</td>
<td>635</td>
<td>615</td>
<td>656</td>
<td>913</td>
<td>139397</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>675</td>
<td>658</td>
<td>699</td>
<td>594</td>
<td>43353</td>
<td>27.7</td>
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<tr>
<td>6</td>
<td>675</td>
<td>658</td>
<td>699</td>
<td>47</td>
<td>26250</td>
<td>6.0</td>
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<tr>
<td>7</td>
<td>740</td>
<td>726</td>
<td>767</td>
<td>658</td>
<td>136694</td>
<td>18.8</td>
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<tr>
<td>8</td>
<td>735</td>
<td>718</td>
<td>759</td>
<td>238</td>
<td>76438</td>
<td>16.0</td>
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<tr>
<td>11</td>
<td>645</td>
<td>625</td>
<td>666</td>
<td>7981</td>
<td>243687</td>
<td>20.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>640</td>
<td>620</td>
<td>661</td>
<td>1371</td>
<td>148323</td>
<td>3.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>λ<sub>ab</sub> = Absorption maximum. <sup>b</sup>λ<sub>ex</sub> = Excitation maximum. <sup>c</sup>λ<sub>em</sub> = Emission maximum. <sup>d</sup>FL. Intensity = Fluorescence intensity. <sup>e</sup>Φ<sub>F</sub> = Fluorescence quantum yield (the data were tested in DCM). <sup>f</sup>The data were tested in MeOH.
Figure S4. (a) Absorption and (b-g) fluorescence emission spectra of 10 µM γ-halide/pseudohalide substituted Cy 5 compounds 1a, 2a, 2c, 2d, 2e, 2f in DMSO solution. The excitation wavelength are 600, 595, 600, 595, 575 and 580 nm, respectively.

3.8 Singlet oxygen quantum yield (Φₛ) measurements

To determine the photodynamic efficacy of the γ-substituted Cy 5 derivatives, we have determined its singlet oxygen generation efficiency in ethanol. Singlet oxygen quantum yields [Φ(1O₂)] of the γ-substituted Cy 5 derivatives were determined by chemical trapping method using 1,3-diphenylisobenzofuran (DPBF, 30 µM) as the singlet oxygen scavenger. The mixture of sensitizer and DPBF was irradiated with a 650 nm laser beam (100 mW/cm²). Singlet oxygen quantum yields were measured at low dye concentrations (optical density around 0.2) to minimize the possibility of singlet oxygen quenching by the dyes. The quantum yields of singlet oxygen generation [Φ(1O₂)] were calculated by a relative method using optically matched solutions and comparing the quantum yield of photo-oxidation of DPBF sensitized by the dye of interest to the quantum yield of methylene blue (MB) (Φ(1O₂) = 0.52) in ethanol as the reference. Φₛ values were calculated using the following equation,

\[ \Phi_\psi = \Phi_{MB} \times \left( \frac{k_\psi}{k_{MB}} \right) \left( \frac{F_{MB}}{F_\psi} \right) \]

where k is the bleach rate of DPBF absorbance (410 nm) with irradiation time, and F is the absorption correction factor, which is given by \( F = 1 - 10^{-OD} \) (OD at the irradiation wavelength).
Figure S5. Changes in the absorption spectra of DPBF (30 μM) upon irradiation in the presence of the γ-substituted Cy 5 derivatives, MB and Foscan (2 μM) for different irradiation time in ethanol (λ_{irradiation} = 650 nm).
Table S5. Singlet oxygen quantum yield of the γ-substituted Cy5 derivatives.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>1a</th>
<th>2a</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
<th>2f</th>
<th>Foscan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φ(1O2)</td>
<td>0.007</td>
<td>0.042</td>
<td>0.007</td>
<td>0.103</td>
<td>N.D.</td>
<td>0.031</td>
<td>0.320</td>
</tr>
</tbody>
</table>

*The singlet oxygen quantum yield of the Cy 5 derivatives in ethanol were determined compared to methylene blue (MB), MB Φ(1O2) = 0.52. N.D. = not detectable.

### 3.9 Photobleaching experiments

The PBS solutions of γ- substituted Cy 5 derivatives (2 μM, containing 1.5% (v/v) DMSO as a cosolvent) were continuously illuminated by a laser beam at 650 nm (100 mW/cm²) for 0, 5, 10, 20, 30, 40, 50, 60 min, respectively. UV-vis spectra were obtained after each illumination on Tecan Spark™ 10M Multimode Microplate Reader.

![Figure S6. Comparison of photostability of γ- substituted Cy 5 derivatives. The change of the absorption at respective maximum absorption peak of 1a, 2a, 2c, 2d, 2e, 2f in PBS solutions (2 μM) upon different time of laser illumination (650 nm, 100 mW/cm²) is shown from 0 to 60 min.](image)

### 3.10 HepG 2 cell line and culture conditions

HepG 2 cell line was purchased from American Type Culture Collection (ATCC), USA. HepG 2 cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium) (Corning) containing 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Coming). All cell lines were maintained at a humidified incubator with 5% CO₂ at 37 °C.

### 3.11 Stability studies

DMSO stock solutions of γ- substituted Cy 5 derivatives (5 mM) were diluted to 10 μM of final concentration in cell growth media [Dulbecco’s modified Eagle’s cell growth medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin] and maintained at 37 °C in the dark. Absorption spectra were recorded every 4 h for 24 h at 37 °C on Tecan Spark™ 10M Multimode Microplate Reader, respectively.
Figure S7. Absorbance scan for 1a, 2a, 2c, 2d, 2e, 2f at 37 °C in cell growth media. The spectra were taken at t = 0 (black), 4 (red), 8 (green), 12 (blue), 16 (yellow), 20 (purple) and 24 (orange) h, respectively.

3.12 Cytotoxicity assay

The cytotoxicity of 1a and 2d was determined by MTS assay in vitro. HepG 2 cells were seeded in 96-well flat bottom microtiter plates at a density of 1×10^4 cells/mL with 100 μL per well, incubated in a humidified 5% CO₂ atmosphere at 37 °C for 24 h, then exposed to different concentrations (0-50 μM) of 1a or 2d for 12 h. After treatment, 20 μL MTS solution was added to each well and continued to incubate for 3 h. After 3 h incubation at 37 °C, the absorbance was measured at 490 nm with a Tecan Spark™ 10M Multimode
Microplate Reader. Cell viability was calculated according to the following formula: Cell viability (%) = \( \frac{(A - A_0)}{(A_s - A_0)} \times 100 \), where \( A \) is the absorbance of the experimental group, \( A_s \) is the absorbance of the control group, and \( A_0 \) is the absorbance of the blank group (no cells). The experiment was repeated three times.

**Figure S8.** Cytotoxicity of different concentrations of 1a (a) and 2d (b) to HepG 2 cells by a standard MTS assay. The experiment was repeated three times and the data are shown as mean ± SD.

**3.13 Confocal imaging of HepG 2 cells treated with 2d**

Upon reaching 80 % confluence, HepG 2 cells (300 μL, 1 × 10^5 cells/mL) were transferred into an 8-well chamber containing sterile coverslips at the bottom. After overnight culture at 37 °C, cells were washed with PBS, then incubated with 2d (5 μM or 10 μM) in DMEM medium. After incubation for 2 h, the medium was changed to phenol-red-free DMEM, and the cells were irradiated at 650 nm (100 mW/cm^2) for different time. Then the cells were stained with Hoechst 33342 and PI (4 μM) for 20 min. Next, the cells were washed once with PBS and were observed with Leica TCS SP8 X Confocal Microscope using 60× or 20× magnification. Hoechst 33342 was excited at 405 nm and its fluorescence was monitored at 430−490 nm, PI was excited at 543 nm and its fluorescence was monitored at 550−650 nm, while 2d were excited at 633 nm and the fluorescence was monitored at 650−750 nm. Relative death rate was calculated as follows: relative death rate = PI-positive cells/Hoechst 33342-positive cells.

**Figure S9.** Confocal fluorescence images of HepG 2 cells. (a) Untreated cells with 5 min photoirradiation; (b) 1a (10 μM) treated cells irradiated for 5 min. Hoechst signal (blue): \( \lambda_{ex} = 405 \) nm and \( \lambda_{em} = 460 ± 30 \) nm; PI signal (green): \( \lambda_{ex} = 543 \) nm and \( \lambda_{em} = 600 ± 50 \) nm; probe signal (red): \( \lambda_{ex} = 633 \) nm and \( \lambda_{em} = 700 ± 50 \) nm. The green color shown in the PI channel indicates the dead cells. Scale bar = 25 μm.
Figure S10. Fluorescence images of HepG 2 cells staining with 2d. (a-d) 2d (5 μM) treated cells with photoirradiation for 0 min, 1 min, 3 min, 5 min, respectively; (e-h) 2d (10 μM) treated cells with photoirradiation for 0 min, 1 min, 3 min, 5 min, respectively. Hoechst signal (blue) $\lambda_{ex} = 405$ nm and $\lambda_{em} = 460 \pm 30$ nm; PI signal (green): $\lambda_{ex} = 543$ nm and $\lambda_{em} = 600 \pm 50$ nm; probe signal (red): $\lambda_{ex} = 633$ nm and $\lambda_{em} = 700 \pm 50$ nm. The experiment was repeated three times. Scale bar = 75 μm. (i) Relative death rate of images (b-d) and (f-h), and the data are shown as mean ± SD.
3.14 *in vivo* PDT evaluation

The experiment involving animals were in accordance with the guidelines of the Committee on Animals of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. Specific ICR mice, 6–8 weeks of age, were obtained from Beijing Huafukang Bioscience Co. Inc.. Then, 5 × 10^6 H22 cells were injected subcutaneously into the selected armpit positions to establish the liver tumor model of ICR mice. Tumors were allowed to grow to about 200 mm³ in volume. In order to evaluate the *in vivo* PDT efficacy of 2d, all mice were divided into four groups and performed with the following different treatments: group 1, PBS injection; group 2, PBS injection and irradiation; group 3, only 2d injection; group 4, 2d injection and irradiation. Each group contained six mice, and 2d (100 μL, 3.5 mg/kg) was injected subcutaneously. After 20 min post-injection, tumour region was irradiated with 650 nm laser light at a power density of 100 mW/cm² for 20 min. The body weights and tumor sizes were monitored every two or three days. The tumour volume of all mice was measured using a vernier caliper. Then, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were used to calculate the tumour volume. Tumour volume = length × width²/2. After 10 days post-treatment, the mice were sacrificed. Then, tumour tissues of mice were harvested for analysis.

![Figure S11](image-url)

**Figure S11.** The antitumour evaluation of 2d based PDT. (a) Mouse body weight curves after relevant treatments with PBS as control, PBS + light, 2d and 2d + light, respectively; (b) The relative tumor volume of different groups after treatments; (c) Mean tumor weights and (d) photographs of excised tumors with different treatments at the last day of experiments. The data are shown as mean ± SD (n = 6). *P < 0.05.
3.15 Bacteria cell culture

Six wild-type bacteria strains: *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. cloacae* were used in this study. Tryptone Soya Broth (TSB) medium was used for culture of *S. aureus* and *P. aeruginosa*. Nutrient broth (NB) was used for culture of *K. pneumoniae* and *E. cloacae*. Brain Heart Infusion Broth (BHI) was used for culture of *A. baumannii*. M.R.S. Broth (MRS) was used for culture of *E. faecium*. Single colony from the stock agar plate was added to 10 mL of liquid medium, then was grown at 37 °C on a shaker incubator (180 rpm) overnight followed by a subculture until an OD₆₀₀ of approximately 0.5 - 0.7 was reached.

3.16 Minimum inhibitory concentration (MIC) test

To investigate the photobiological effects of 2d on bacteria, the PDT activity of 2d was then examined in ESKAPE pathogens. First, we tested the antibacterial capability of 2d in dark by determining the minimal inhibitory concentration (MIC) against the pathogens. The results showed 2d hardly inhibits bacteria that indicated self-cytotoxicity of 2d had minimal effect on PDT efficacy (Table S5). 1.0 mL aliquots of bacterial strains cultured in respective solution were collected and centrifuged. The cell pellets were washed twice and resuspended in sterile PBS buffer (pH 7.4) at OD₆₀₀ of 0.5, then further diluted to OD₆₀₀ of 5×10⁻⁴. Aliquots of this suspension (100 μL) were placed into a 96-well plate. 2d was diluted in PBS buffer and then added into the bacteria suspensions to give the desired concentration. The cultures were then added respective solutions and further incubated at 37 °C for 24 h. The wells containing the same number of cells but no compounds and the wells containing the same culture solution but without bacterial cells were set as control groups. Then the absorbance was measured at 600 nm with a Tecan Spark™ 10M Multimode Microplate Reader. Each concentration had triplicate values, and the whole experiment was done at three times and the MIC value was determined by taking the average of triplicate OD₆₀₀ values for each concentration and plotting it against concentration. The data was then subjected to sigmoidal fitting. The MIC value was determined as the point in the curve where the OD₆₀₀ is similar to that of control having no bacteria (Table S6). OD₆₀₀ measurement was done by using the Metash UV-5100B spectrometer.

Table S6. The results of minimum inhibitory concentration (MIC) tests for ESKAPE strains, unit: μM.

<table>
<thead>
<tr>
<th>Strains</th>
<th><em>E. faecium</em>⁠pes</th>
<th><em>S. aureus</em>⁠pes</th>
<th><em>K. pneumoniae</em>⁠pes</th>
<th><em>A. baumannii</em>⁠pes</th>
<th><em>P. aeruginosa</em>⁠pes</th>
<th><em>E. cloacae</em>⁠pes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2d</td>
<td>25</td>
<td>25</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

⁠pes CICC 10840;⁠pes ATCC 29213;⁠pes ATCC 700603;⁠pes CICC 22933;⁠pes ATCC 27853;⁠pes ATCC 13047.

3.17 *In vitro* study of PDT activities on ESKAPE pathogens

The PDT activity of 2d was then examined in ESKAPE pathogens and the bacteria viability was assessed using propidium iodide (PI) via fluorescence of apoptotic bacteria. *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *E. cloacae* cells were cultured for 12 h in respective media at 37 °C. Bacterial strains cultured overnight in respective solution were harvested and washed twice with PBS buffer (pH 7.4).
The washed cells were resuspended in PBS buffer with an OD$_{600}$ of 0.5 - 0.7. Then 1 mL aliquots of ESKAPE pathogens were incubated in the presence or absence of 2d (10 µM) at 37 °C for 2 h respectively. After incubation for 2 h, these corresponding bacteria in culture were placed in 24-well plate and were irradiated by 650 nm laser light (100 mW/cm$^2$) for 0 min, 3 min, 10 min and 20 min from the top of the plate, respectively. After the photoirradiation, the samples were incubated with PI (10 µg/mL) for 20 min. Finally, fluorescence of the samples were measured using Tecan Spark™ 10M Multimode Microplate Reader at the excitation wavelength of 535 nm. PI-DNA: $\lambda_{ex} = 535$ nm and $\lambda_{em} = 615$ nm. Wavelength interval: 5.0 nm.

**Figure S12.** Fluorescence emission spectra of photoirradiation treated ESKAPE bacteria for different time with or without 2d (10 µM, 2 h). $\lambda_{ex} = 535$ nm.

3.18 Confocal imaging of S. aureus treated with 2d

S. aureus cells were cultured on a shaker incubator (180 rpm) for 12 h in TSB media at 37 °C. After overnight culture, bacterial cells were harvested and washed twice with PBS buffer (pH 7.4). The washed
cells were resuspended in PBS buffer with an OD$_{600}$ of 0.5 - 0.7. Then 1 mL aliquots were treated with or without 2d (10 μM) at 37 °C for 2 h. Next, the 1 mL 2d stained bacterial cells were placed in 24-well plate and were irradiated by 650 nm laser light (100 mW/cm$^2$) for 0 min, 10 min and 20 min from the top of the plate, respectively. After irradiation, the samples were incubated with Hoechst 33258 (20 μg/mL) and PI (10 μg/mL) for 20 min. Then a drop of the suspension was added into an 8-well chamber followed by covering with agarose pads. Fluorescence images were taken with Leica TCS SP8 X Confocal Microscope (60× magnification).

![Fluorescence images](image)

**Figure S13.** Fluorescence images of *S. aureus* cells. (a) Untreated cells with 20 min photoirradiation; (b) 2d (10 μM) treated cells in the dark; (c and d) 2d (10 μM) treated cells irradiated for 10 min and 20 min, respectively. Hoechst signal (blue) $\lambda_{\text{ex}} = 405$ nm and $\lambda_{\text{em}} = 460 \pm 30$ nm; PI signal (green): $\lambda_{\text{ex}} = 543$ nm and $\lambda_{\text{em}} = 600 \pm 50$ nm; probe signal (red): $\lambda_{\text{ex}} = 633$ nm and $\lambda_{\text{em}} = 700 \pm 50$ nm. Scale bar = 8 μm.
4. Copies of NMR spectrum of compounds

$^1$H NMR (500 MHz, Methanol-$d_4$) of compound 1a

$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 1a
H-H COSY (100 MHz, Methanol-d4) of compound 1a

\[ \text{H NMR (400 MHz, Methanol-d4) of compound 2a} \]

S32
$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 2a

H-H COSY (100 MHz, Methanol-$d_4$) of compound 2a
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 2c

$^{13}$C NMR (150 MHz, Methanol-$d_4$) of compound 2c
$^{1}H$ NMR (400 MHz, Methanol-$d_{4}$) of compound 2d

$^{13}C$ NMR (100 MHz, Methanol-$d_{4}$) of compound 2d
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 2e

$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 2e
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 2f

$^{13}$C NMR (150 MHz, Methanol-$d_4$) of compound 2f
\(^1\)H NMR (400 MHz, Methanol-\(d_4\)) of compound 3

\(^{13}\)C NMR (100 MHz, Methanol-\(d_4\)) of compound 3
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 4

$^{13}$C NMR (150 MHz, Methanol-$d_4$) of compound 4
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 6

$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 6
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 7

$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 7
$^1$H NMR (400 MHz, Methanol-\text{d}_4) \text{ of compound 8}$
$^{13}$C NMR (100 MHz, Methanol-$d_4$) of compound 8

H-H COSY (100 MHz, Methanol-$d_4$) of compound 8
$^1$H NMR (400 MHz, Methanol-$d_4$) of compound 11

$^{13}$C NMR (150 MHz, Methanol-$d_4$) of compound 11
$^{1}$H NMR (500 MHz, Methanol-$_d_4$) of compound 12
5. References


