Supplementary Information

A turn-on fluorescence probe for imaging iodide in living cells based on an elimination reaction

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1. General Experimental Section

Materials.

Solvents were dried by distillation before use. All other reagents were of commercial quality and used without further purification. L-Selenocystine, N-bromosuccinimide (NBS) and benzoyl peroxide (BPO) was purchased from Bai Ling Wei Chemical Company. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DAPI, PI working solution, HepG2 (Human hepatocellular liver carcinoma cell line) cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Sartorius ultrapure water (18.2 MΩ cm) water was used throughout the analytical experiments.

Instruments.

Cary Eclipse Fluorescence Spectrometer (Varian, Inc. USA) with a 1.0 cm quartz cells at the slits of 10/10 nm. High-resolution mass spectral analyses were carried out on Bruker maxis UHR-TOF Ultra High Resolution Quadrupole-time of flight mass spectrometer (Bruker Co., Ltd., Germany). Melting points were measured using X-6 micro-melting point apparatus and are uncorrected. IR spectra were measured using a Bruker Tensor-27 FTIR spectrometer using the KBr pellet. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Varian Advance 600-MHz or Bruker Advance 300-MHz

spectrometer, δ values are in ppm relative to TMS. The fluorescence images of cells were taken using a TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany) with an objective lens (× 40). All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Absorbance was measured in a TRITURUS microplate reader in the MTT assay.

Preparation of the test solution.

A stock solution of HCy-OMe-Br (1.0×10^{-2} M) was prepared in CH_3CN. The test solution of HCy-OMe-Br (10 μM) in 2 mL neutral aqueous conditions (10 mM HEPES buffer, pH 7.4, containing 60% CH_3CN as a co-solvent) was prepared by placing 0.002 mL of the probe stock solution, 1.198 mL CH_3CN, and 0.8 mL of 20 mM HEPES buffer (pH = 7.4). The solutions of various testing species were prepared from KI, NaCl, KBr, NaF, NaH_2PO_4, Na_2HPO_4, $NaNO_3$, NaAcO, Na_2SO_4, $NaHSO_3$, Na_2SO_3, Na_2CO_3, GSH, Cys, NAC, Hcy, KCl, $CaCl_2$, $MgCl_2$, $Al(NO_3)_3$, $ZnCl_2$, $CoCl_2$, $Ni(NO_3)_2$, $FeCl_2$, respectively. Cr (II) solutions were prepared in the glovebox by the method of Castro[2].

Fluorescence analysis.

Fluorescence spectra were obtained with Cary Eclipse Fluorescence Spectrometer (Varian, Inc. USA) with a 1.0 cm quartz cells at the slits of 10/10 nm. After dilution to 10 μM HCy-OMe-Br with 10 mM HEPES buffer solution, various amounts of iodide or interferent were added. The fluorescence intensity was measured at $\lambda_{ex}/_{em}$ = 480 /680 nm.

Cell culture.

HepG2 was maintained following the protocols provided by the American Type Tissue Culture Collection. Cells were first grown in a circular petri dish (60 mm) using high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose / L) supplemented with 10 % fetal bovine serum (FBS), $NaHCO_3$ (2 g / L) and 1% antibiotics (penicillin/streptomycin, 100 U / mL). Cultures were maintained in a humidified incubator at 37 °C, in 5 % CO_2 / 95 % air. One day before imaging, cells were passed and plated on 18 mm glass coverslips in culture dish. The culture

medium was refreshed every 24 h. All cells used were in the exponential growth phase.

Cytotoxicity assay

Cytotoxicity was measured by using the MTT assay in the logarithmic phase of cell growth. HepG2 cells were seeded at a density of 5×10^4 cells / well in a 96 well-plate and incubated for 24 h before adding the test substance. Then fresh medium containing increasing concentrations of HCy-OMe-Br was added to each well. After 24 h incubation, medium was removed and replaced with medium containing MTT (0.5 mg / mL). Cells were incubated at 37 °C for another 5 h after which medium was removed. DMSO (100μL) was added to lyse the cells and dissolve the formazan produced. The absorbance at 570 nm of each well was monitored using a microplate reader. Viability was calculated based on the recorded data.

Confocal imaging. Fluorescence imaging studies were performed with a TCS SP5 confocal laser scanning microscope (Germany Leica Co., Ltd) with an objective lens (× 40). Excitation of probe-loaded cells at 488 nm was carried out with an Argon laser, and emission was collected using a META detector between 600 and 700 nm. Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with HEPES (pH 7.4, 0.10 M) three times.

Mass spectrometry analysis: MS spectra were obtained with LCQ Deca XP MAX (Thermo Finnigan).

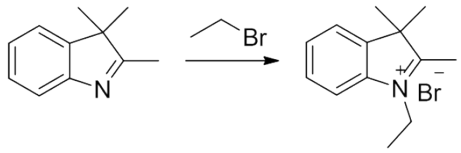
The quantum yields of the probe before and after reacting with iodide were determined according to the literature[1]:

$$\Phi_x=\Phi_s(F_x/F_s)(A_s/A_x)(\lambda_{exs}/\lambda_{exx})(n_x/n_s)^1$$

Where Φ is quantum yield; F is the integrated area under the corrected emission spectrum; A is the absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution; the subscripts x and s refer to the unknown and the standard, respectively. Fluorescein (Φ_F = 0.90) in 0.1 mol/L NaOH was used as the standard[1].

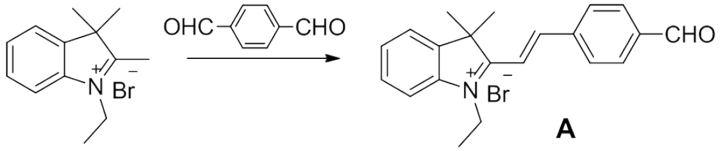
2. Synthesis of HCy-OMe-Br

Ethyl-2, 3, 3-trimethylindoleninium:



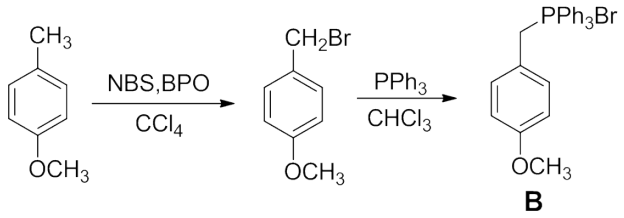
A mixture of 2, 3, 3-Trimethylindolenine (7.96 g, 50 mmol) and ethyl bromide (8.10 g, 75 mmol) in CH_3CN was heated to boiling for 24 h under an atmosphere of nitrogen. The reaction mixture was cooled to room temperature, and collected the purple color solid by suction filtration using Buchner funnel and wash them with a few milliliters of cold ethyl acetate. The desired product is obtained as an purple solid (12.01 g , yield: 90 %). The purple powder was used in further experiments without additional purification.

The synthesis of compound A:



To a solution of 1, 4 -Phthalaldehyde (2.68 g, 20.0 mmol） and sodium acetate (0.47 g) in acetic anhydride (20 mL) was added a solution of l-Ethyl-2, 3, 3-trimethylindoleninium (2.67 g, 10.0 mmol) in acetic anhydride (20 mL). The solution was heated for an additional 4 h under an atmosphere of Argon. After cooling to room temperature, diethyl ether (35 mL) was added to this solution, and the solid was collected by filtration. The orange solid is purified by column chromatography on silica gel flash chromatography using CH_2Cl_2: MeOH =10:1(v/v) as eluent to give compound A as a orange solid (3.0 g, yield 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49(t, *J* =6 Hz, 3H), 1.83(s, 6H), 4.79(d, *J* =6 Hz, 2H), 7.66-7.68(m, 2H), 7.85(d, *J* =18 Hz, 1H), 7.92-8.03(m, 2H), 8.09(d, *J* =9 Hz, 2H), 8.45(d, *J* =6Hz, 2H), 8.42(d, *J* =15 Hz, 1H), 10.12(s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 21.5, 43.2, 53.1, 115.7, 116.1, 123.6, 129.7, 130.2, 130.4, 131.3, 138.8, 139.9, 140.8, 144.8, 152.0, 182.0, 193.2.; ESI-MS：$[M-Br]^+$ ($C_{21}H_{22}NO$): m/z Calcd 304.1696，Found 304.1716；

The synthesis of compound B:



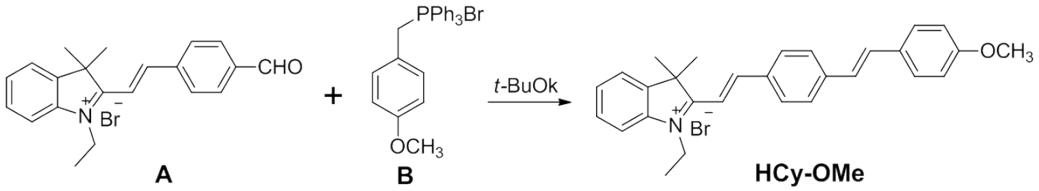
B

A mixture of p-methyl anisole (0.61 g, 5.0 mmol), NBS (1.33 g, 7.5 mmol) and catalytic amount of BPO in 20 mL of CCl_4 was refluxed at 78 °C for 12 h under an Argon atmosphere. After cooling, the solid was removed by filtration and washed with CCl_4. The solution was concentrated on a rotary evaporator. The pale yellow precipitate was collected and used in further experiments without additional purification.

A mixture of the pale yellow precipitate (1.0 g, 3.62 mmol) and PPh_3 (1.14 g, 4.34 mmol) in 20 mL of CH_2Cl_2 was refluxed at 78 °C for 4 h under an Argon atmosphere. After cooling, diethyl ether (35 mL) was added to this solution, and the solid was collected by filtration. The resultant crude material was purified by flash column chromatography (Petroleum ether: MeOH = 5 : 1 v/v) to afford compound B in 52% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.68(s, 3H), 5.12(d, *J* =15Hz, 2H), 6.79(d, *J* =9 Hz, 2H), 6.89(d, *J* =6 Hz, 2H), 7.55-7.92(m, 15H). ^{13}C NMR(75 MHz, DMSO-d_6): δ 55.2, 114.2, 117.3, 118.4, 128.8, 129.0, 130.2, 132.4, 132.6, 134.4, 134.9, 159.6。

ESI-MS：m/z $[M-Br]^+$ ($C_{26}H_{24}OP$）：Calcd 383.1559，Found 383.1585；

The synthesis of HCy-OMe:

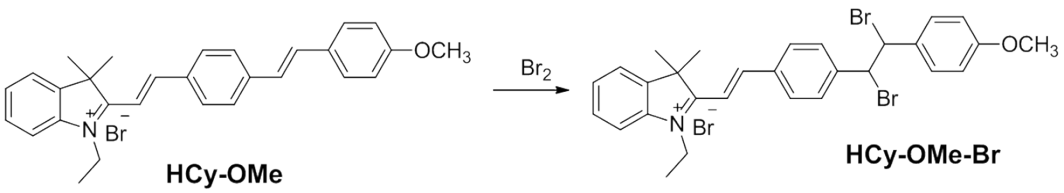


A B HCy-OMe

To a solution of Compound A (3.83 g，10 mmol)and Compound B (4.62 g，10 mmol) in CH_2Cl_2 (20 mL) was added a solution of Potassium *t*-butoxide (2.78 g, 25 mmol) in CH_2Cl_2 (20 mL). The mixture was heated for an additional 2 h under an

atmosphere of Argon atmosphere. Then the solvent was removed under reduced pressure and resultant crude material was purified by flash column chromatography (CH_2Cl_2: MeOH =10:1 v/v) to afford Compound C in 60 % yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.63(s, 3H), 1.84(s, 6H), 3.84(s, 3H), 5.15(s, 2H), 6.90-6.99 (m, 3H), 7.17-7.27(m, 2H), 7.47-7.65(m, 8H), 8.08-8.31(m, 4H)；^{13}C NMR(150 MHz, 300 MHz, $CDCl_3$): 14.2, 26.9, 29.6, 43.4, 52.2, 55.2, 111.8, 112.2, 113.9, 114.6, 122.6, 125.2, 126.5, 127.5, 128.4, 128.9, 129.6, 130.2, 131.4, 132.4, 132.8, 133.0, 140.3, 143.4, 144.2, 154.9, 160.1, 181.1. ESI-MS: $[M-Br]^+$ ($C_{29}H_{30}NO$): m/z Calcd 408.2322，Found 408.2313；

The synthesis of HCy-OMe-Br:



Bromine (0.40 g, 2.5 mmol) was added slowly to a solution of compound HCy-OMe (1.32 g, 2 mmol) in dry $CHCl_3$ (40 ml) at 0 °C, and the progress of the reaction was monitored by TLC. After 1 h, the solvent was concentrated on a rotary evaporator, and the residue was purified by short flash chromatography on silica gel (CH_2Cl_2: MeOH =8:1 v/v) to afford HCy-OMe-Br (1.04 g, 80 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 (s, 3H), 1.89 (s, 6H), 3.83(s, 3H), 4.89(s, 2H), 5.41(m, 2H), 6.94(d, *J* =6 Hz, 2H), 7.40-7.48(m, 2H), 7.62-7.70(m, 8H), 8.18(s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 179.4, 158.0, 143.1, 141.4, 138.1, 131.9, 131.0, 128.5, 128.2, 127.9, 127.4, 127.1, 127.0, 120.9, 114.1, 113.4, 111.9, 58.2, 53.5, 53.3, 43.1, 23.6, 13.0. ESI-MS：$[M-Br]^+$ ($C_{29}H_{30}Br_2NO$): m/z Calcd 568.0670，found 568.0743.

3. Fluorescence spectra of HCy-OMe-Br

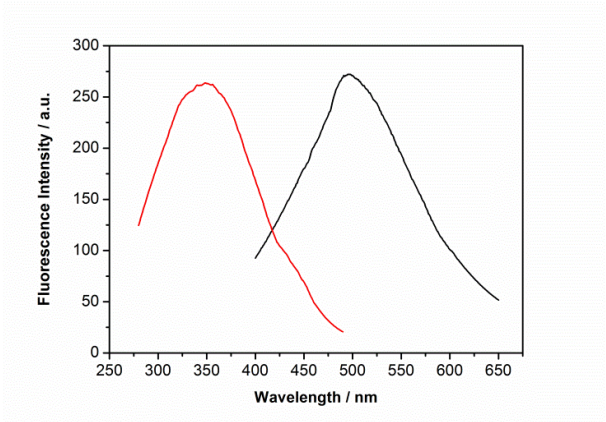


Figure S1 Excitation and emission spectra of HCy-OMe-Br (10 μM,)

4. Fluorescence spectra of HCy-OMe

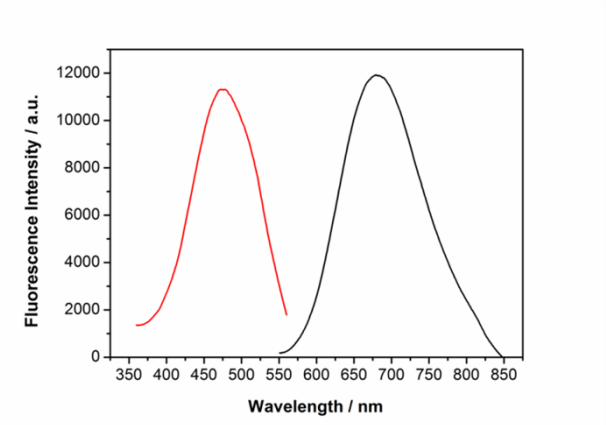


Figure S2 Excitation and emission spectra of HCy-OMe (10 μM)

5. Fluorescence spectra of HCy-OMe-Br treated by different concentrations of iodide

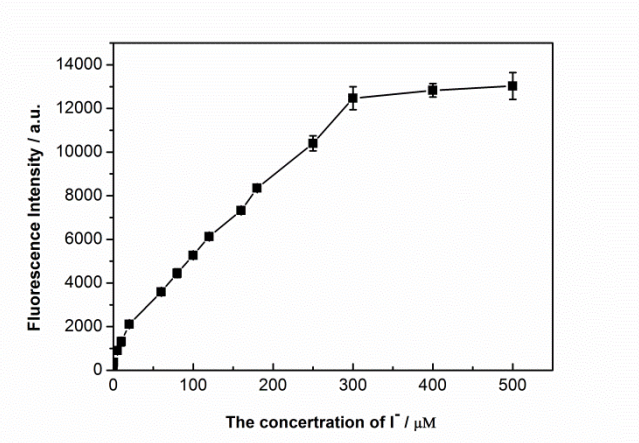


Figure S3. Fluorescence intensity at 418 nm for HCy-OMe-Br (10 μM) in a mixed solution of CH_3CN: HEPES buffer (6 : 4, 10 mM, pH 7.4) as a function of iodide

concentration.

6. Time dependent fluorescence changes of HCy-OMe-Br (10 μM) in the presence of various iodide concentrations

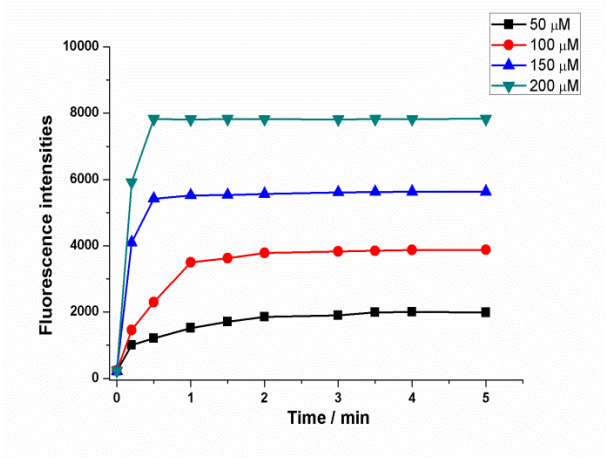


Figure S4. Time course for the change in the fluorescence intensities of HCy-OMe-Br (10 μM) in the presence of 5 equiv., 10 equiv., 15 equiv. and 20 equiv. of iodide in a mixed solution of CH_3CN: HEPES buffer (6 : 4, 10 mM, pH 7.4). The fluorescence intensities at 680 nm were monitored at time intervals.

7. Effect of pH

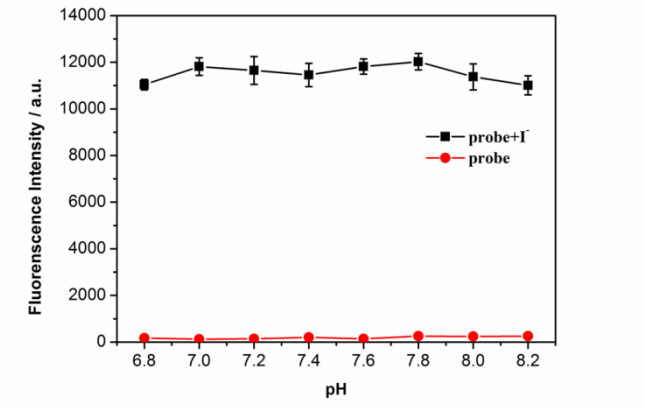


Figure S5. Variations in fluorescence intensities at 680 nm for HCy-OMe-Br and HCy-OMe-Br in the presence of iodide as a function of pH (in buffer solutions, λ_{ex} = 480nm)

8. References

[1] R. A.Velapoldi, and H. H.Tonnesen, *J. Fluoresc*., 2004, **14**, 465.

[2] C. E. Castro, *J. Am. Chem. Soc.* 1961, **83**, 3262.