Supporting Information

A highly selective turn-on fluorescent chemodosimeter for Cr(VI) and its application in living cell imaging

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General information.

All chemicals were obtained from commercial suppliers and used without further purification except for dichloromethane which were distilled from P_2O_5. Column chromatography was carried out over silica gel (BDH 200–300 mesh) and TLC was performed using silica gel GF254 plates. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance (III) 500MHz spectrometer (operating at 500 and 125 MHz, respectively) in DMSO. High-resolution mass spectra were recorded on HPLC-Q-Tof MS (Micro) spectrometer. Melting points were measured with a shanghai WRS-1B digital melting point apparatus and are uncorrected. The substrates **2** and **3** were prepared according to the literature.[S1]

Synthesis of compound 1.

Diaminomaleonitrile (221.2 mg, 2.0 mmol) and compound **3** (764 mg, 2 mmol) in 70 mL of absolute ethanol containing two drops of acetate acid as a catalyst were stirred for 12 hours at room temperature. The progress of the reaction was monitored by TLC (PE:EA = 3:1，v:v). When the raw material **3** disappeared, the reaction was terminated. The solvent was removed under reduced pressure, and the resulting residue was recrystallized from ethanol, and further purified by chromatography on silica using PE/EA (20:1, v:v) as eluent to give compound **1** (557.2 mg) in 56% yield, blank solid, m.p.= 228-230℃, ^{1}H NMR (500MHz, DMSO-d_6, ppm): δ = 8.21 (s, 1H), 7.30~7.28 (d, J = 5.0 Hz, 2H), 7.13~7.12 (d, J = 15.0 Hz, 2H), 6.33 (s, 1H), 3.82 (s, 3H), 3.29 (s, 3H), 2.71 (s, 3H), 1.62 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (125MHz, DMSO-d_6, ppm): δ = 160.80, 159.68, 155.79, 150.53, 146.33, 143.66, 141.38, 133.60, 130.97, 129.95, 126.27, 125.59, 125.12, 124.00, 115.48, 115.31, 114.27, 106.63, 55.96, 15.21, 14.52, 13.12. HRMS (ESI): m/z Calca for $C_{25}H_{23}BF_2N_6O$: 472.1994 found: 472.1998.

General Procedure for UV-Vis and Fluorescence Studies.

For absorption and emission spectra, the metal salts concentration (1×10^{-2} mol/L)were prepared by buffer solution pH = 6.8 and the compound **1** (1×10^{-5} mol/L) were prepared in solvent (H_2O:DMF = 3:7, v:v) as the mother liquor. Accurate draw 3 ml mother liquor, to which add 3 μL ions measuring their fluorescence intensity, selected response ion. Test the pH effect, interference ions on fluorescence probe(fig s8 and fig s9 ,The anions interference concentration is 1×10^{-4} mol/L and the concentration of cations interference is 1×10^{-5} mol/L). Both fluorescence titration and UV spectra titration test probe. All the tests only test one time and they are as follows S1 to S15.

Cell Culture Methods and Staining Procedure.

The compound **1** working solution for cell staining was prepared from a 5 mM aqueous stock solution (containing 10% DMF) of compound **1** by diluting with 1×PBS to a final concentration of 10 μM. Hela cells were maintained following protocols provided by the American type Tissue Culture Collection. Cells were seeded at a density of 1×10^6 cells mL^{-1} for confocal imaging in RPMI 1640 Medium supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2g/L), and 1% antibiotics (penicillin/streptomycin, 100 U/ml). Cultures were maintained at 37 ℃ under a humidified atmosphere containing 5% CO_2.Confocal fluorescence imaging studies were performed on a LSM 710 confocal laser-scanning microscope (Carl Zeiss Co. Ltd.). Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with PBS for three times.

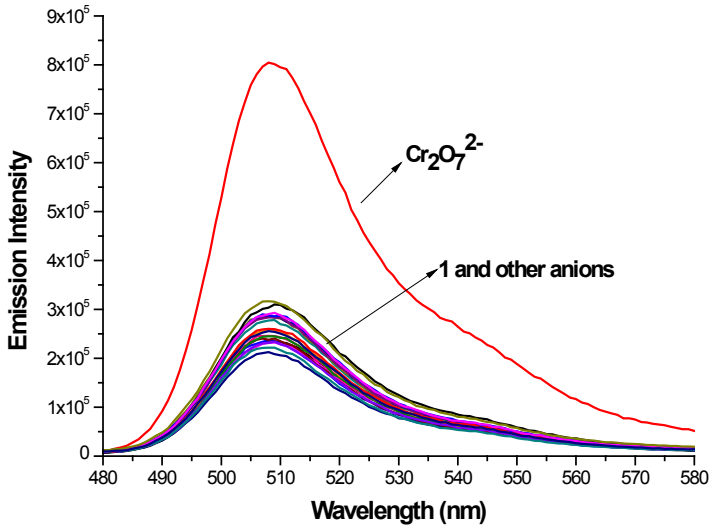

Fig. S1 Changes in the fluorescence spectra of probe **1** (1 μM) upon addition of various anions (1 equiv.) ($K_2Cr_2O_7$, $Na_2C_2O_4$,, $Mg(ClO_4)_2$, Na_2SO_3, NaClO, NaF, NaBr, $NaHSO_4$, $AgNO_3$, NaCl, $NaHCO_3$, Na_2CO_3, Na_2S, $NaNO_2$, NaI, Na_2SO_4, K_3PO_4, NaOAc, 1 μM) in PBS/DMF (3:7, v:v, pH = 6.8). Every drop of ion interval is 30s. λ_{ex} = 470 nm.

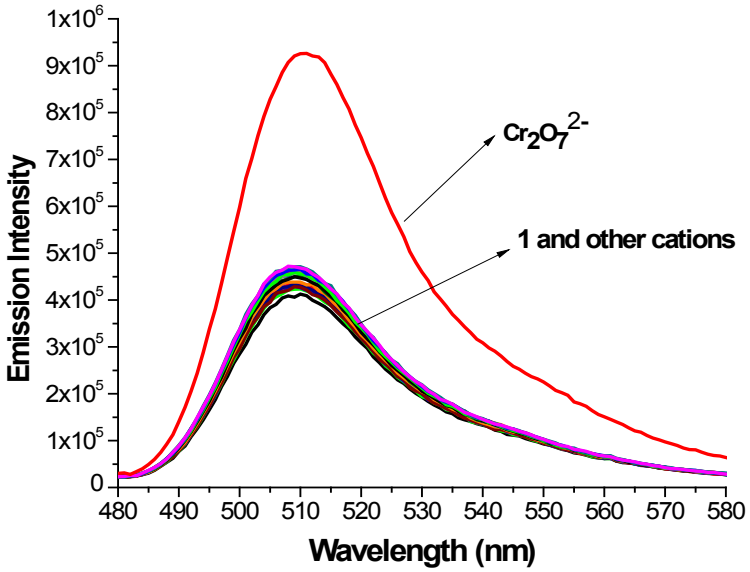

Fig. S2 Changes in the fluorescence spectra of probe **1** (1 μM) upon addition of various cations and H_2O_2 ($K_2Cr_2O_7$, LiCl, NaCl, KCl, $MgSO_4$, $CaCl_2$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $Pb(NO_3)_2$, $KMnO_4$, $FeCl_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $CuCl_2$, $Cr_2(SO_4)_3$, $AgNO_3$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, $(NH_4)_2Ce(NO_3)_6$, and H_2O_2, 1 μM) in PBS/DMF (3:7, v:v, pH = 6.8). Every drop of ion interval is 30s. λ_{ex} = 470 nm.

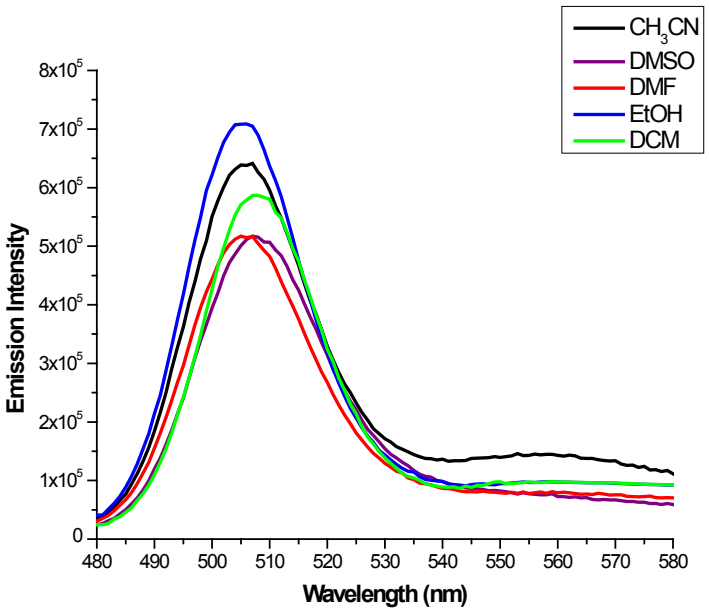

Fig. S3 The fluorescence spectra of probe **1** (1 μM) in different solvents, wait for 30s each test, λ_{ex} = 470 nm.

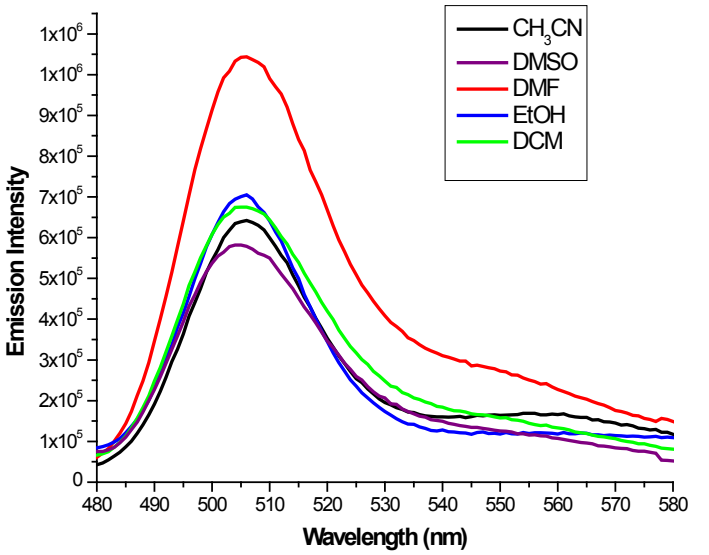

Fig. S4 The fluorescence spectra of probe **1** (1 μM) in the presence of Cr(VI) ($Cr_2O_7^{2-}$, 1 μM,) in different solvents, wait for 30s each test, λ_{ex} = 470 nm.

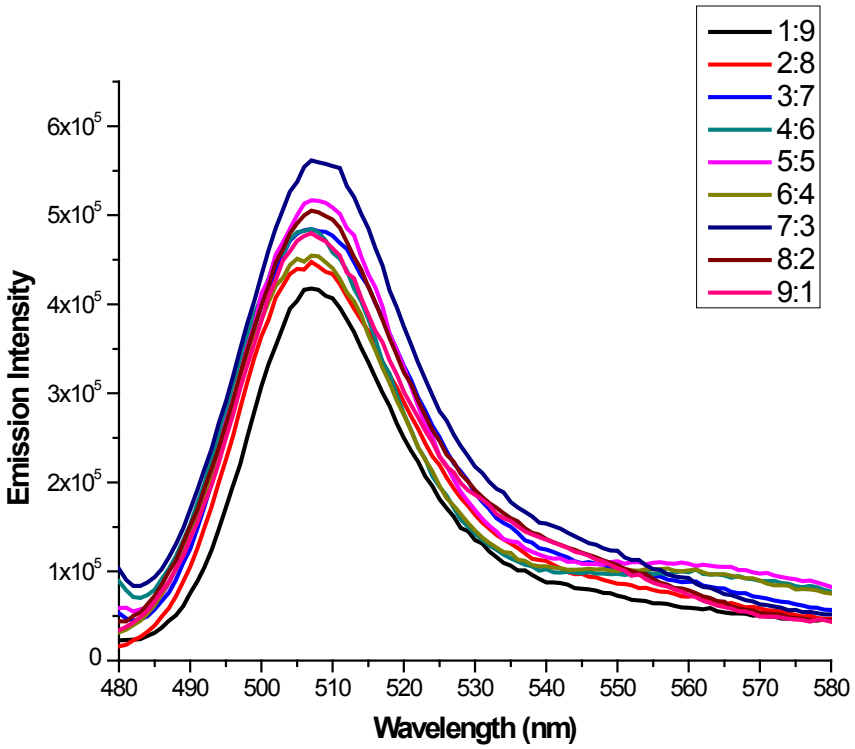

Fig. S5 The fluorescence spectra of probe **1** (1×10^{-5} M) in aqueous solutions with different ratios (H_2O:DMF), wait for 30s each test, λ_{ex} = 470 nm.

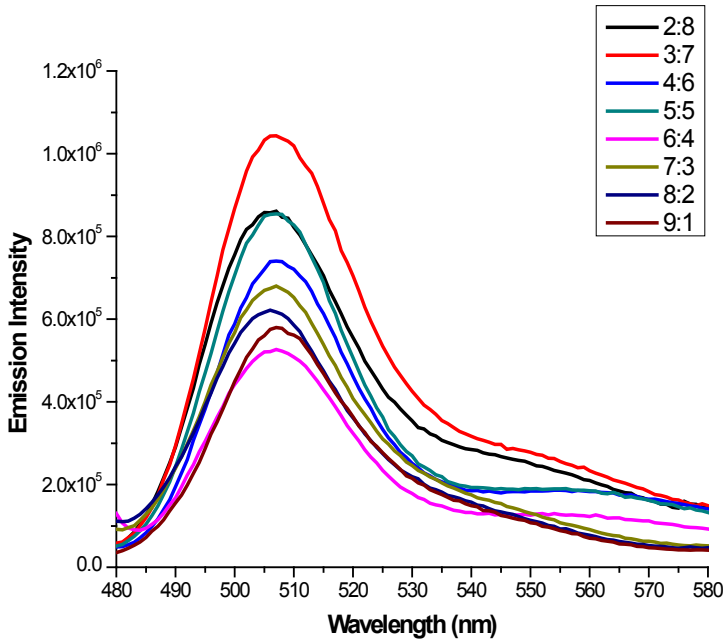

Fig. S6 The fluorescence spectra of compound **1** (1×10^{-5} M) upon addition of Cr(VI) ($Cr_2O_7^{2-}$, 1×10^{-5} M) in the aqueous solutions with different ratios (H_2O: DMF), wait for 30s each test, λ_{ex} = 470 nm.

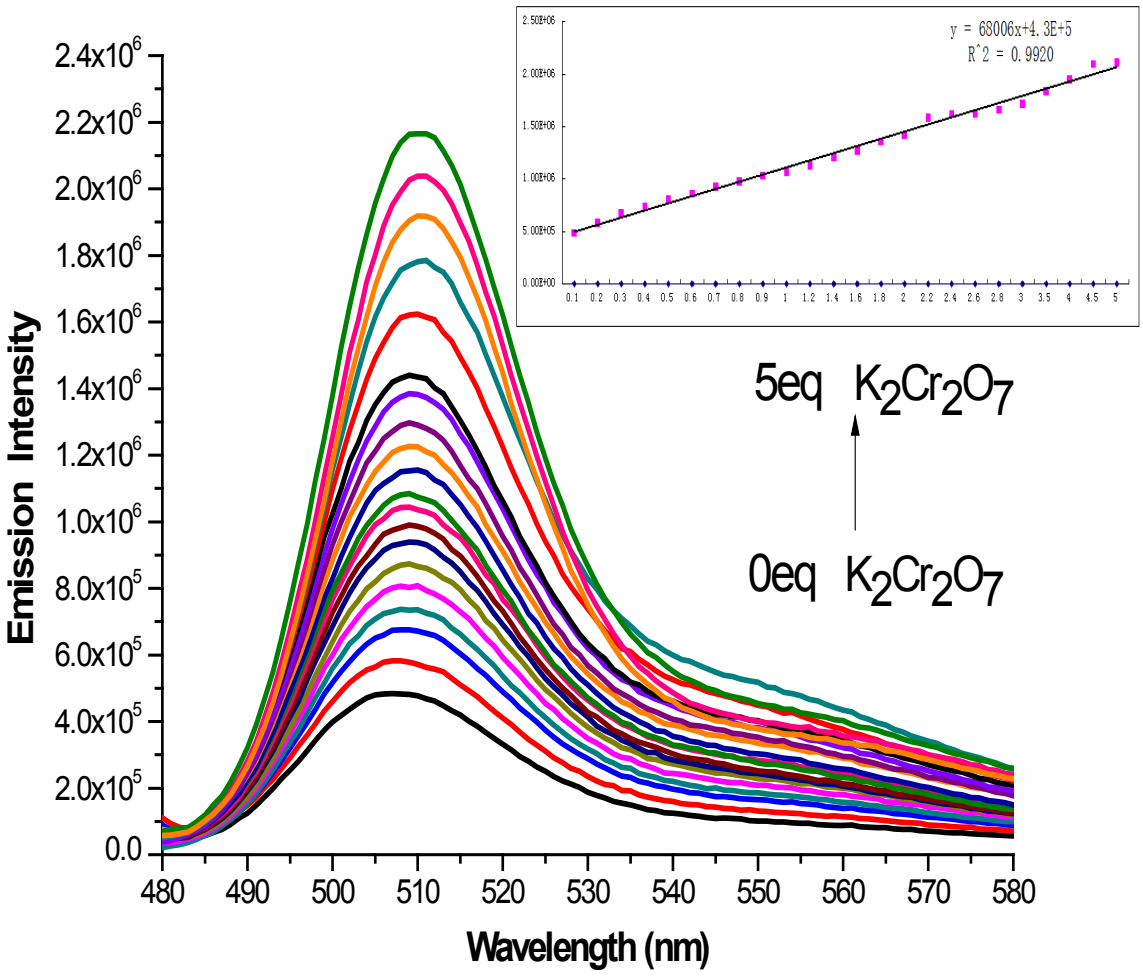

Fig. S7 The fluorescence spectra of compound **1** (1×10^{-5} M) upon addition of $Cr_2O_7^{2-}$ (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 equiv) in H_2O: DMF (3:7, v:v), wait for 30s each test, λ_{ex} = 470 nm. Inset: The linear relationship of fluorescence titration.

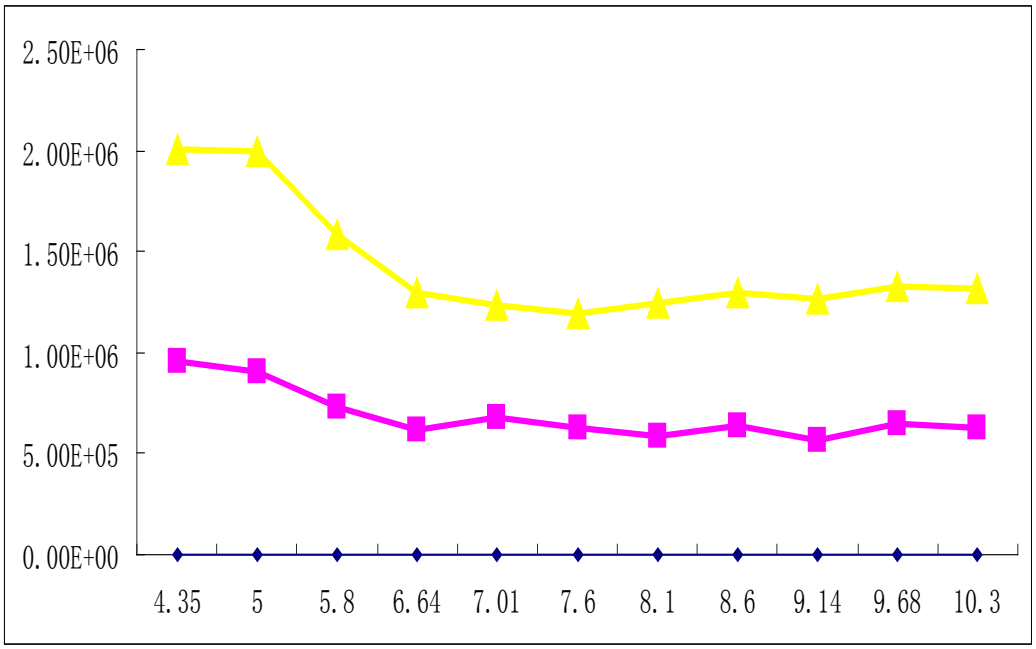

Fig. S8 The pH-titration of free **1** (1 μM, red) and **1**+ $Cr_2O_7^{2-}$ (5 μM, yellow) in H_2O: DMF (3:7, v:v) solution. The pH values of the solution were adjusted by very small amount of HCl and KOH aqueous solution. Wait for 30s each test, λ_{ex} = 470 nm.

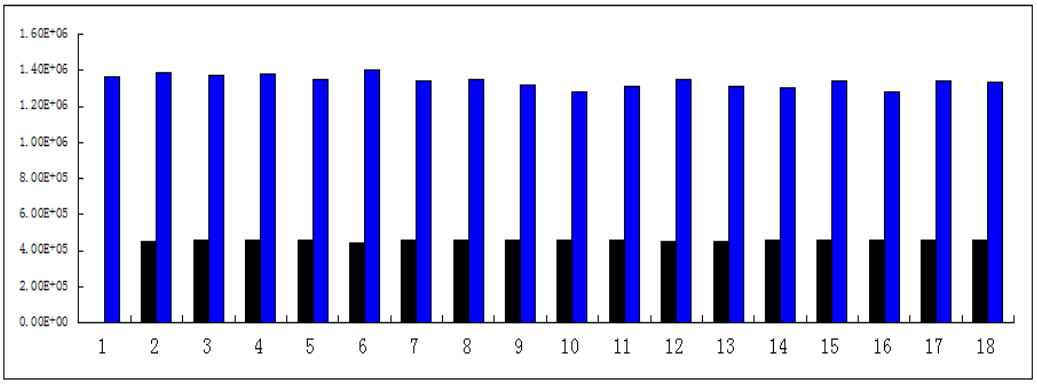

Fig. S9 Fluorescence responses of probe **1** (1 x 10^{-5} M) to various anions in H_2O: DMF (3:7, v:v), black bars represent addition of different anions (5 equiv.) to the solution. Blue bars represent the change of the emission that occurs upon the subsequent addition of Cr(VI) (5 equiv) to the above solutions. wait for 30s each test, λ_{ex} = 470 nm.

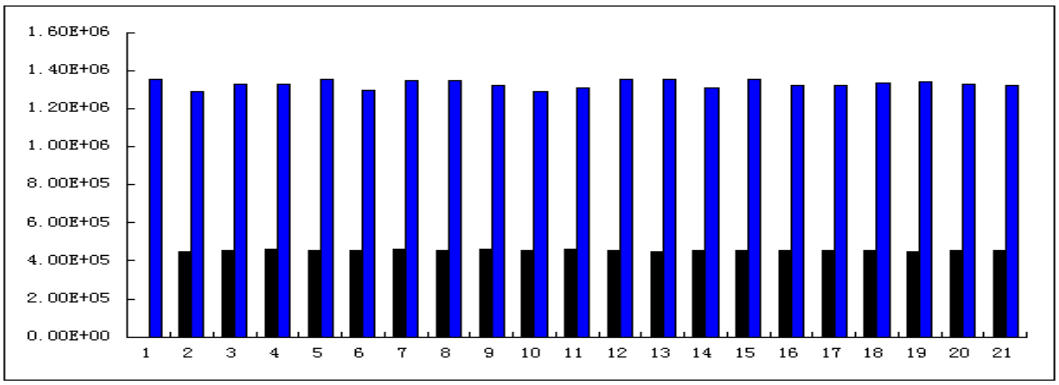

Fig. S10 Fluorescence responses of probe **1** (1×10^{-5} M) to various cations in H_2O: DMF (3:7, v:v), black bars represent addition of different cations (5 equiv.) to the solution. Blue bars represent the change of the emission that occurs upon the subsequent addition of Cr(VI) (5 equiv) to the above solutions. wait for 30s each test, λ_{ex} = 470 nm.

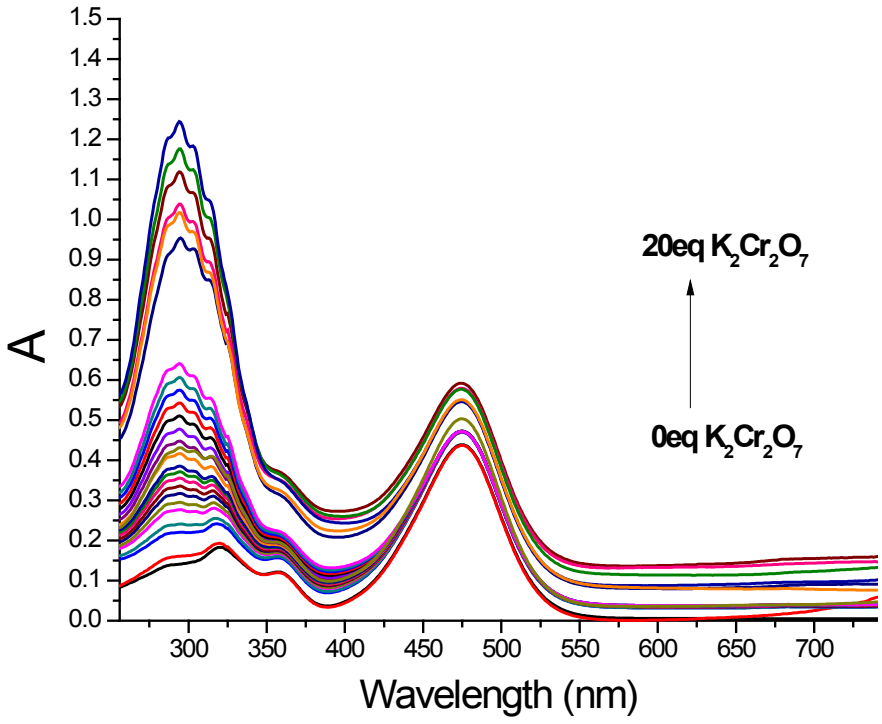

Fig. S11 The UV titration diagram of probe **1** (1×10^{-5} M) upon addition of Cr(VI) ($Cr_2O_7^{2-}$, 0 to 20 equiv) in H_2O: DMF (3:7, v:v) solution.

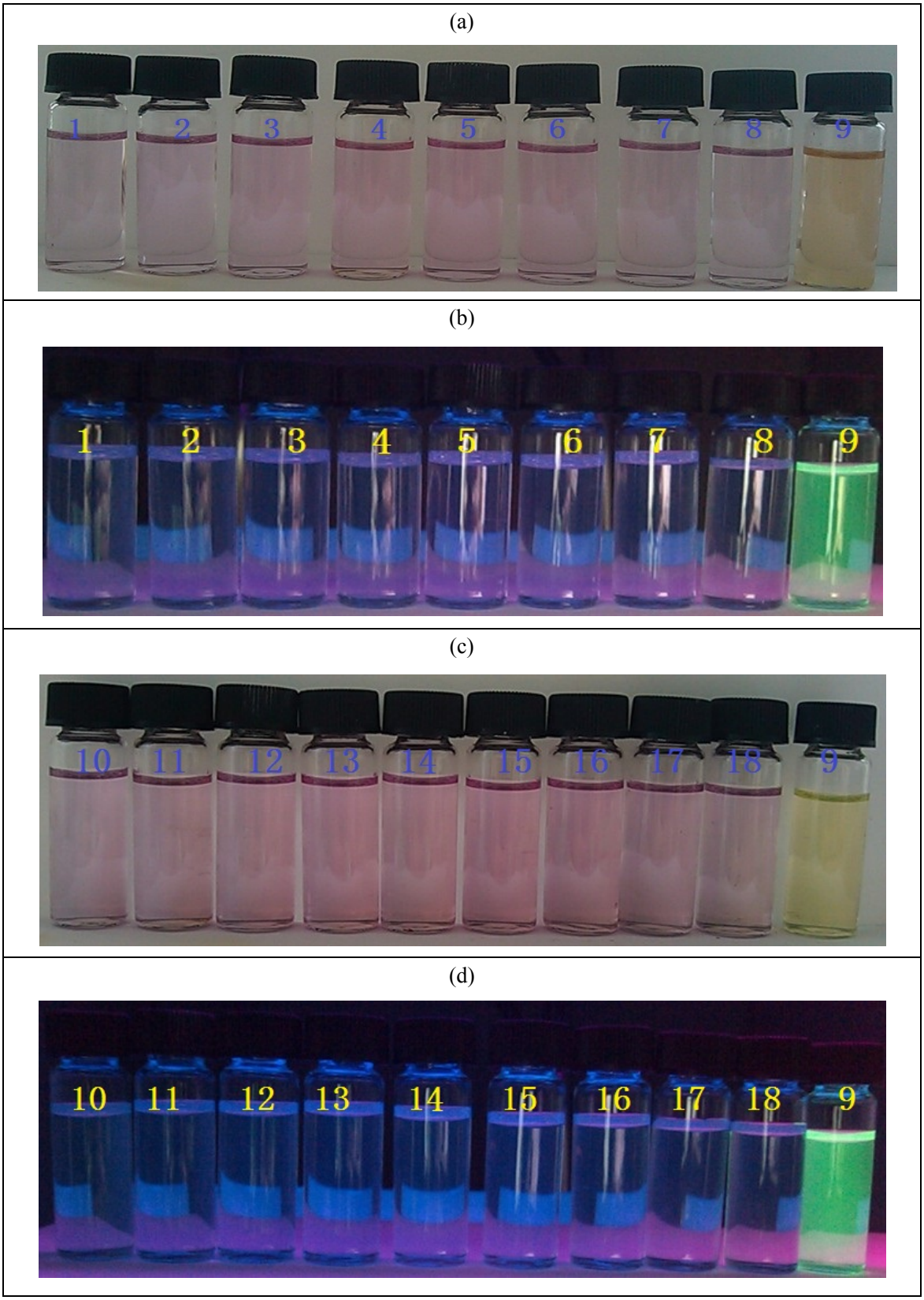

Fig. S12 (a, c) Color and (b, d) fluorescent (365 nm lamp) change of **1** (1×10^{-5} M) to various anions ($Na_2C_2O_4$, $Mg(ClO_4)_2$, Na_2SO_3, NaClO, NaF, NaBr, $NaHSO_4$, Na_2SO_4, $K_2Cr_2O_7$, $AgNO_3$, NaCl, $NaHCO_3$, Na_2CO_3, Na_2S, $NaNO_2$, NaI, K_3PO_4, NaOAc, 1×10^{-5} M) in H_2O: DMF (3:7, v:v) solution.

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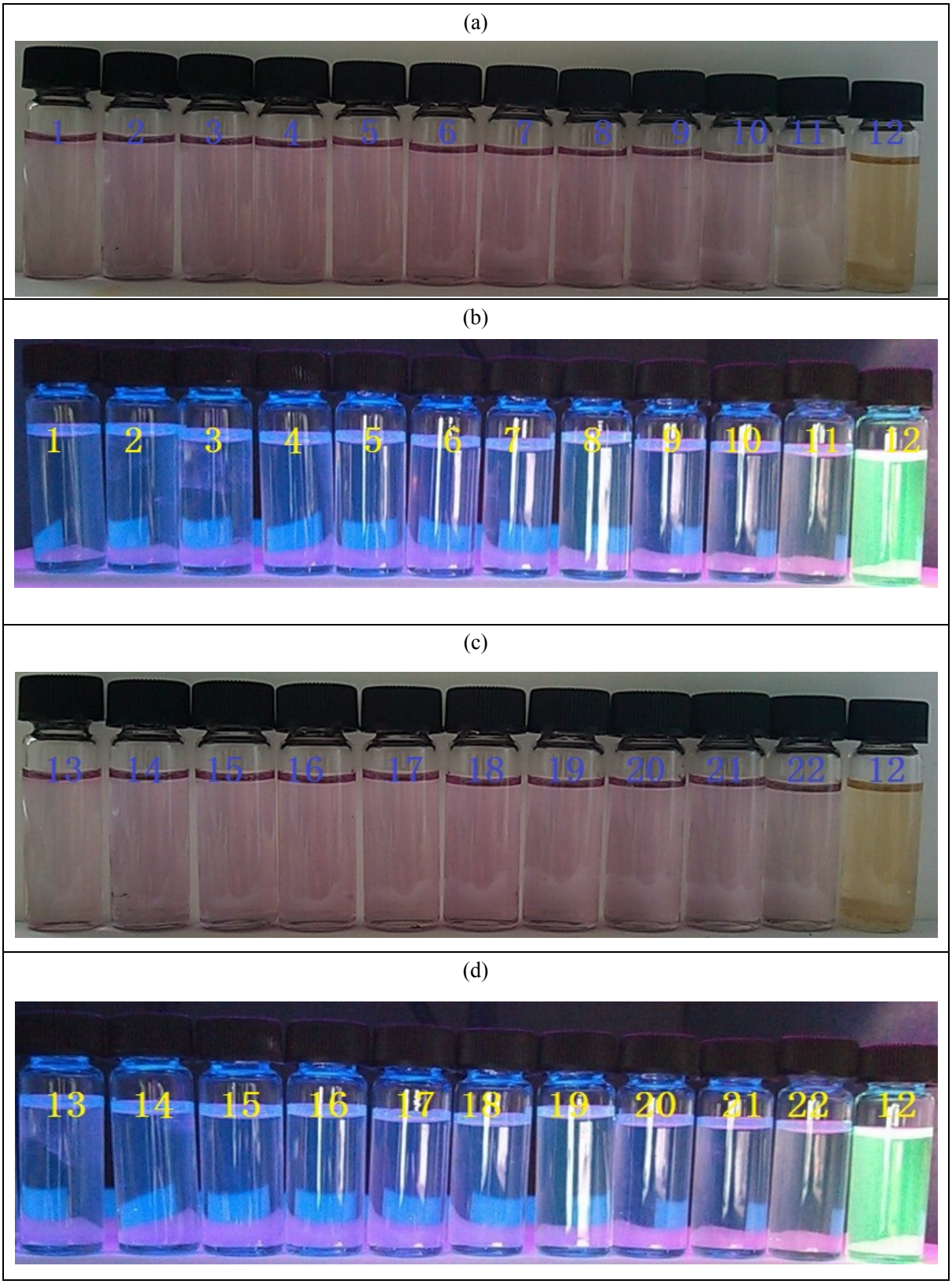
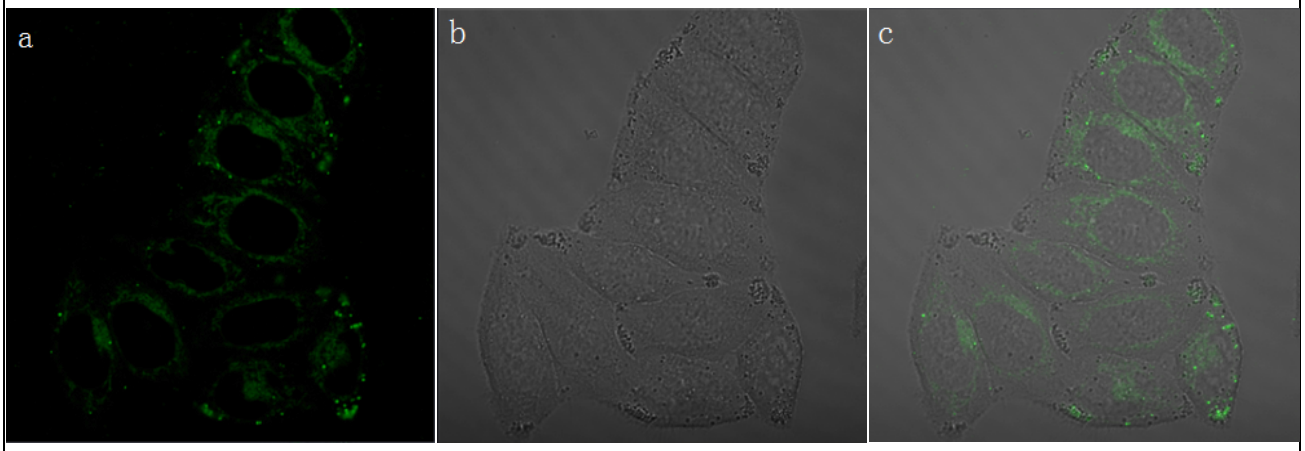


Fig. S13 (a, c) Color and (b, d) fluorescent (365 nm lamp) change of **1** (1×10^{-5} M) to various cations and H_2O_2 (blank, LiCl, NaCl, KCl, $MgSO_4$, $CaCl_2$, $Ba(NO_3)_2$, $Al(NO_3)_3$, $Pb(NO_3)_2$, $Cr_2(SO4)_3$, $KMnO_4$, $K_2Cr_2O_7$, $FeCl_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $CuCl_2$, $AgNO_3$, $Zn(OAc)_2$, $CdCl_2$, $HgCl_2$, $(NH_4)_2Ce(NO_3)_6$, and H_2O_2, 1×10^{-5} M) in H_2O: DMF (3:7, v:v) solution.

(1)



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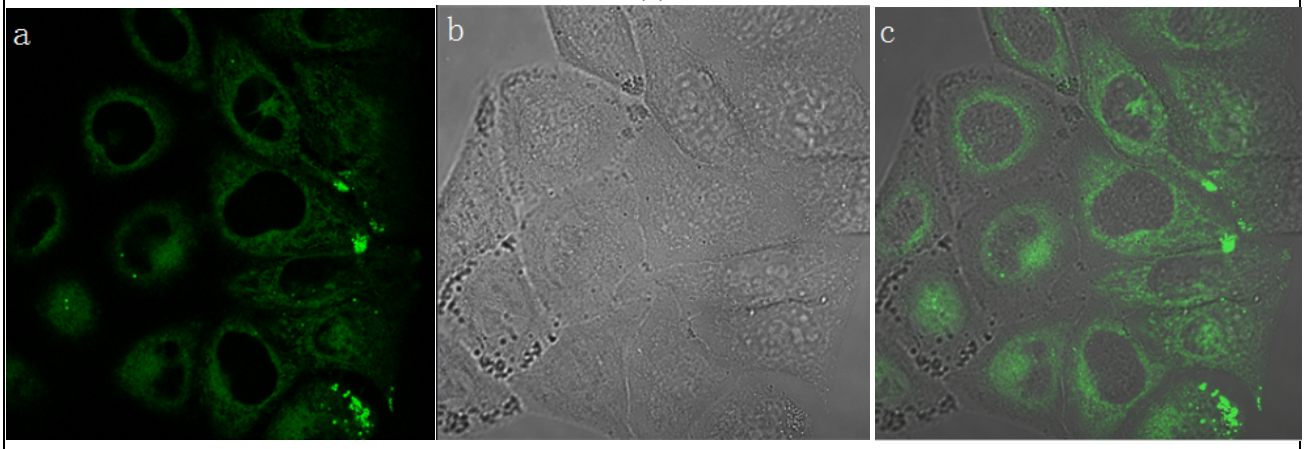

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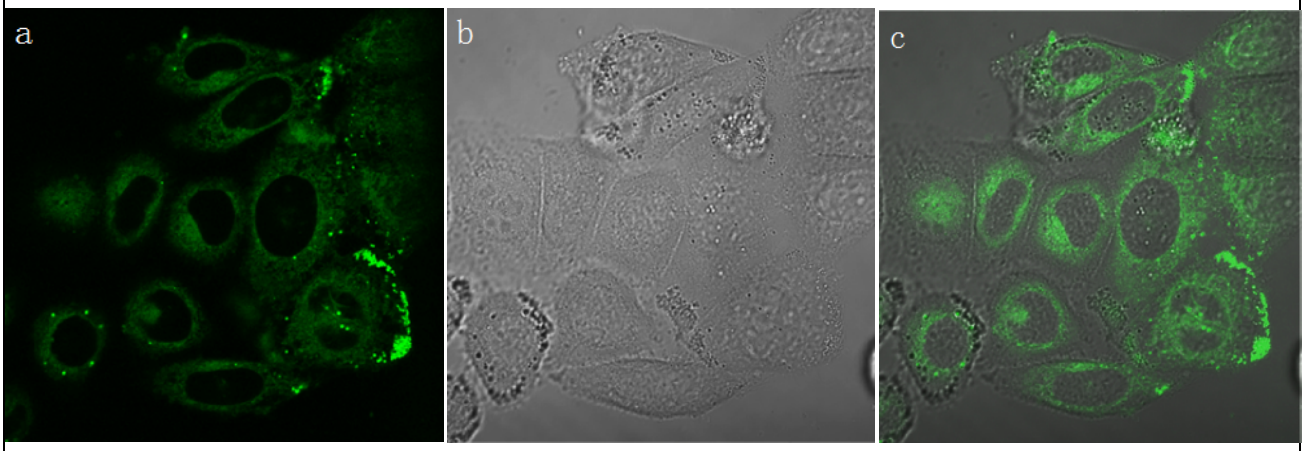


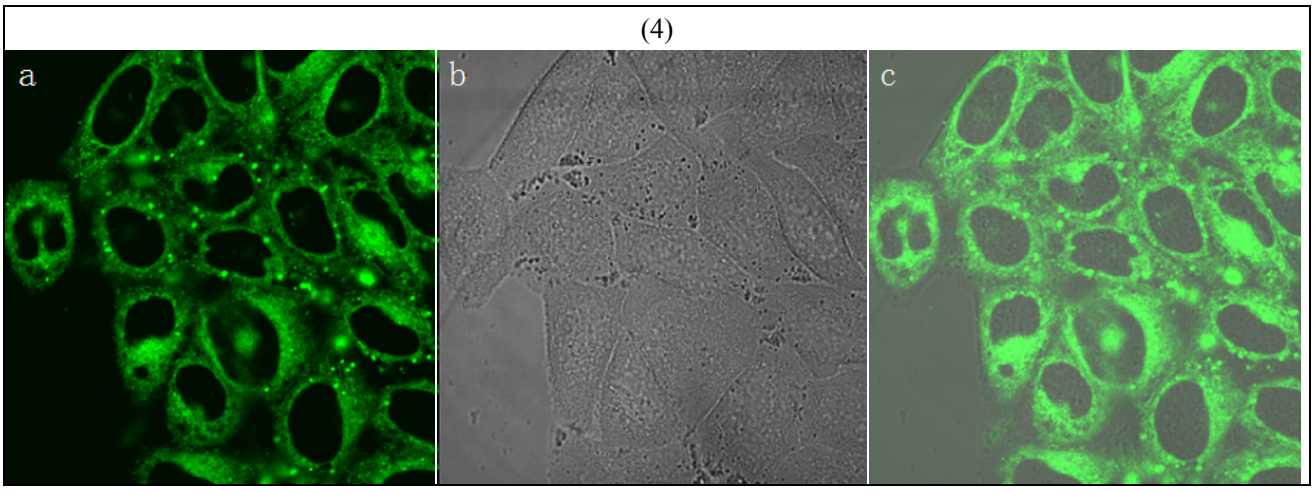


Fig. S14 Cell contrast diagram of probe molecules

(1) Confocal fluorescence images of living Hela cells: (a) Cells loaded with 10 μM probe at 25℃ for 1 h (λ_{ex} = 488 nm; band path, 490−650 nm);. (b) Bright field images. (c) Overlaid images of panels a and b.

(2) Confocal fluorescence images of living Hela cells: (a) Probe-loaded cells treated with 10 μM $Cr_2O_7^{2-}$ at 25℃ for 1 h (λ_{ex} = 488 nm; band path, 490−650 nm); (b) Bright field images. (c) Overlaid images of panels a and b.

(3) Confocal fluorescence images of living Hela cells: (a) Probe-loaded cells treated with 10 μM $Cr_2O_7^{2-}$ at 25℃ for 2 h (λ_{ex} = 488 nm; band path, 490−650 nm); (b) Bright field images. (c) Overlaid images of panels a and b.

(4)Confocal fluorescence images of living Hela cells: (a) Probe-loaded cells treated with 10μM $Cr_2O_7^{2-}$ at 25℃ for 4 h (λ_{ex} = 488 nm; band path, 490−650 nm); (b) Bright field images. (c) Overlaid images of panels a and b.

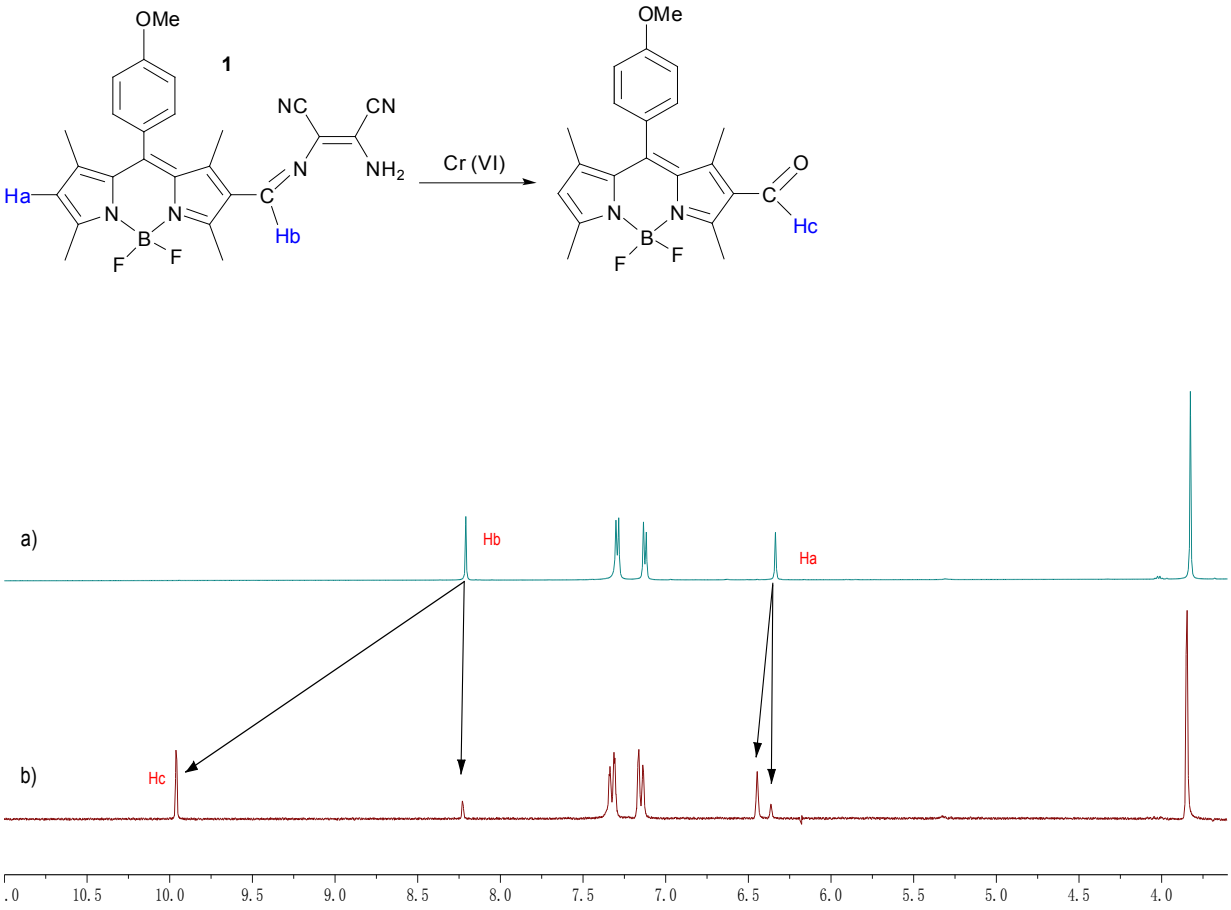
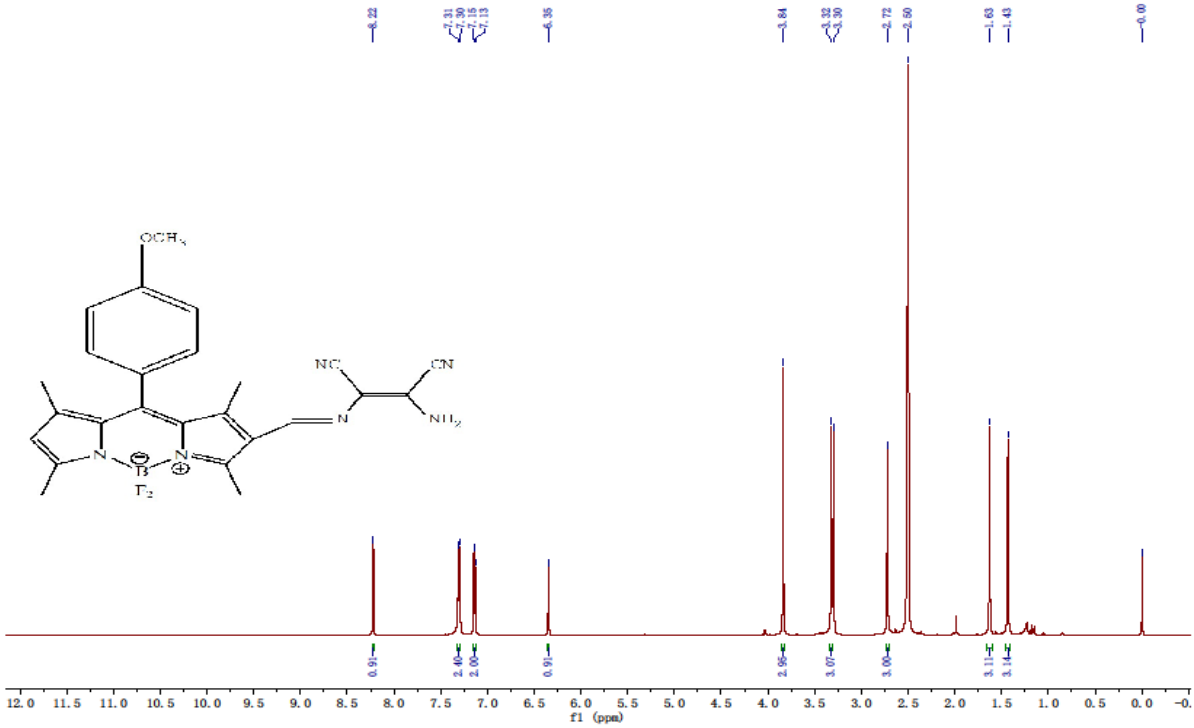


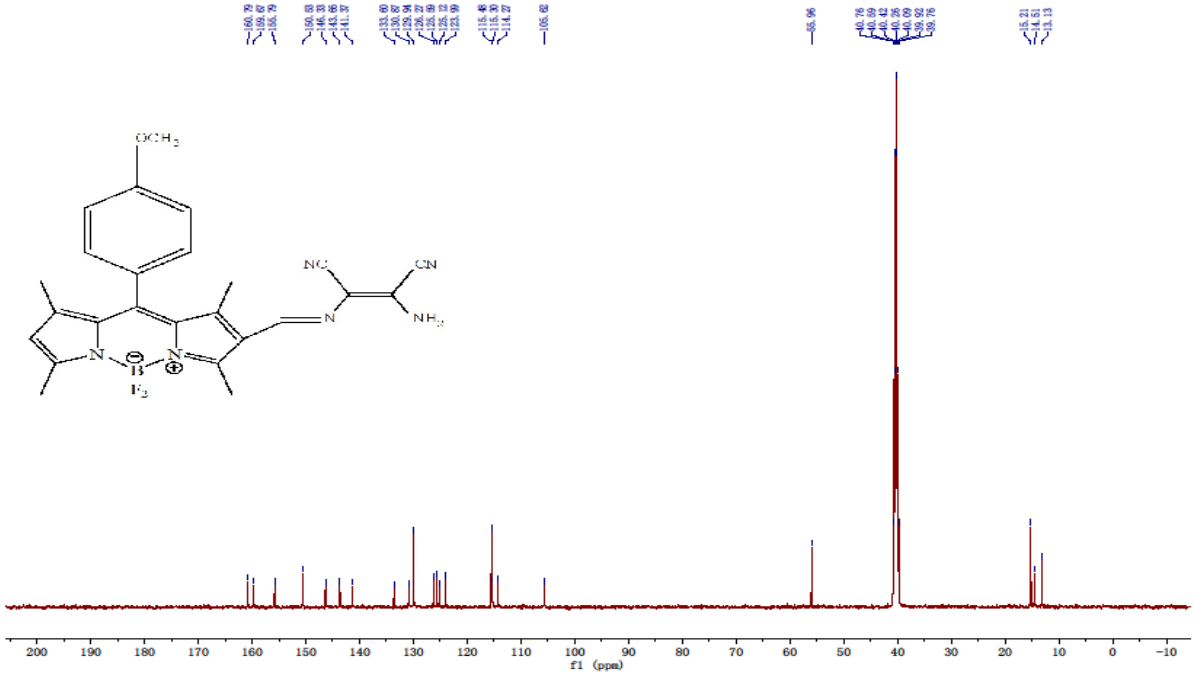
Fig. S15 Partial ^{1}H NMR spetra of probe **1** (5 μM) in DMSO-d_6 upon addition of (a) 0, (b) 1 equiv of $Cr_2O_7^{2-}$.

Reference

S1 L. Jiao, C. Yu, J. Li, Z. Wang, M. Wu and E. Hao, *J. Org. Chem.*, 2009, **74**, 7525.

Spectra





^{1}H NMR and ^{13}C NMR for compound **1** (DMSO-d_6)