Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A mitochondria-targeted fluorescent probe for ratiometric detection of endogenous sulfur dioxide derivatives in cancer cells

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Apparatus and chemicals

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance 300 spectrometer using $CDCl_3$, D_2O or DMSO-*d*6 as solvent and tetramethylsilane (TMS) as an internal standard. HR-MS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). IR spectra were recorded by use of the IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). Melting points were measured on an XD-4 digital micro-melting point apparatus. Thin-layer chromatography (TLC) was conducted on silica gel 60 F_{254} plates (Merck KGaA) and column chromatography was conducted over silica gel (mesh 200-300). Fluorescence measurements were recorded on a Perkin-Elmer LS-55 luminescence spectrophotometer, and UV–vis spectra were recorded on a U-4100 UV-Vis-NIR Spectrometer (Hitachi). Quartz cuvettes with a 1 cm path length and 3-mL volume were involved in fluorescence and UV-vis spectra measurements. The pH was measured by use of a PHS-3C digital pH-meter (YouKe, Shanghai). All reagents were purchased from J&K, Aladdin and Sinopharm Chemical Reagent Co. and used without further purification.

Preparation for UV-vis and fluorescence spectral measurements

Phosphate buffered saline (PBS, 10 mM) was used throughout the absorption and fluorescence determination. Probe HCy-D was dissolved in *N*,*N*-dimethylformamide (DMF) to get the stock solution (1×10^{-3} M). Twice-distilled water was used to prepare stock solution (1×10^{-3} M) of NaF, NaCl, NaBr, KI, $NaHCO_3$, KNO_3, NaClO, Na_2SO_4, KSCN, $Na_2S_2O_3$, Na_2S, Na_2SO_3, $NaHSO_3$, cysteine, homocysteine and glutathione. Stock solution of $NaHSO_3$ and Na_2SO_3 was freshly prepared each time before use. Test solution was prepared by placing 50 μL of the stock solution and an appropriate aliquot of each testing species solution into a 10-mL volumetric flask, and the solution was diluted to 10 mL with PBS buffer (10 mM, pH 7.4) containing 30% DMF (v/v).

Calculation of energy transfer efficiency

Energy transfer efficiency (*E*) was calculated using the following equation:

$$E = 1 - F_{DA}/F_D$$

Where, F_{DA} and F_D denote the donor fluorescence intensity with and without an acceptor, respectively.

2.5. Theoretical calculations

All the calculations were implemented with the Gaussian09 program package. The initial geometries of the compounds were generated by the Gauss View software. The ground state structures of Donor, Acceptor and Compound A were optimized using the density functional theory (DFT). The excited state related calculations (UV-vis absorption and fluorescence emission) were carried out with the time-dependent DFT (TD-DFT) with the optimized structures of the ground. The solvent effects were modeled with the polarizable continuum model (PCM).

Photostability study and cell imaging

Hela cells were cultured in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37℃. The probe HCy-D was dissolved in DMSO to get the stock solution (10 mM) and diluted to 1 μM before use. Hela cells were incubated with 1.0 μM HCy-D for 1 h and then treated with 0.05, 0.1, 0.5 or 1 mM $NaHSO_3$ for 0.5 h. Subsequently, excited at 405 nm, the

cells were imaged under a confocal microscope (LSM 700) and the images were collected at emission channels of 405-555 nm (green channel) and 560-700 nm (red channel), respectively.

Cell imaging of HepG2 cells and L-02 cells

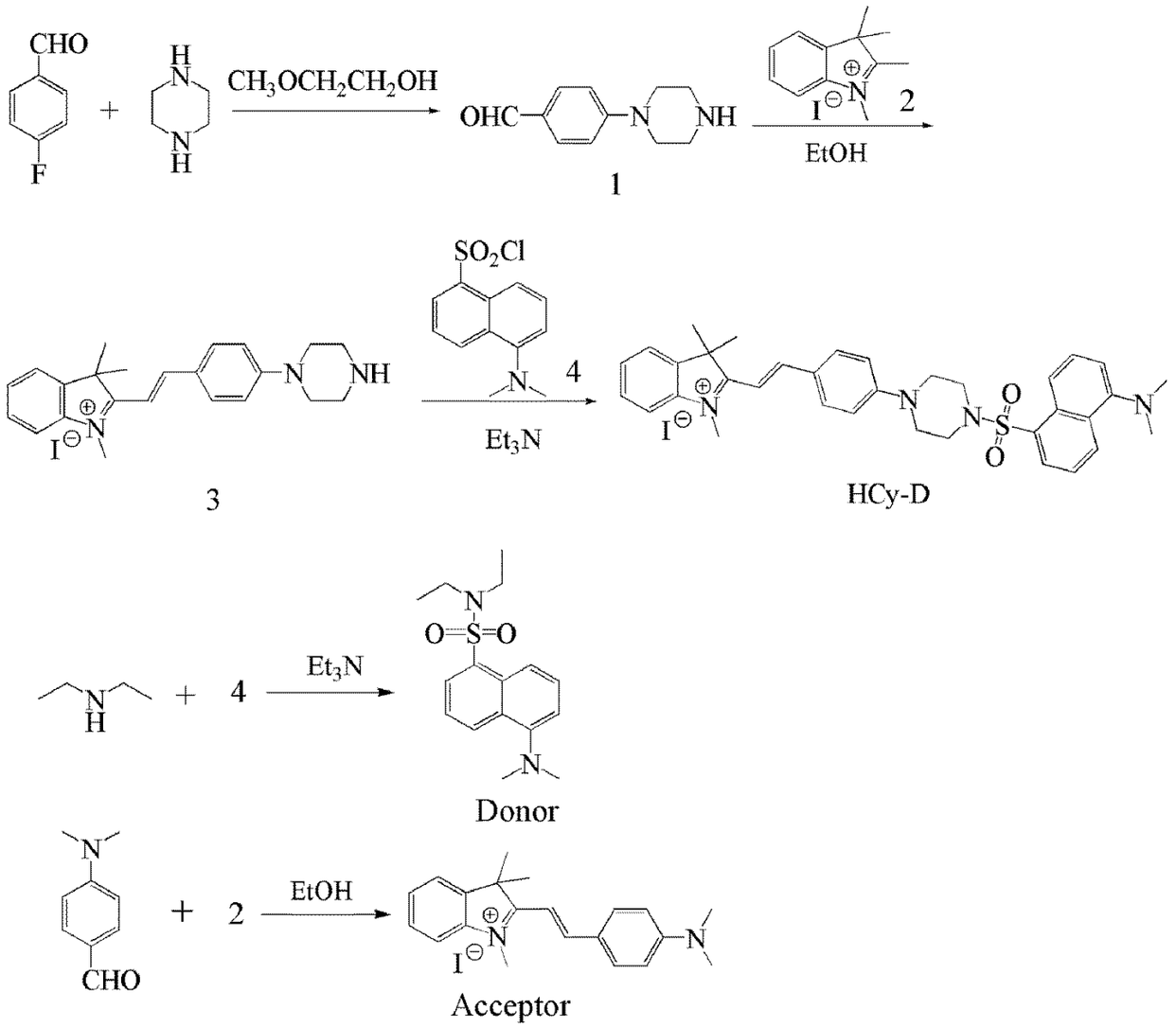
HepG2 cells or L-02 cells were cultured in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37℃. The probe HCy-D was dissolved in DMSO to get the stock solution (10 mM) and diluted to 1 μM before use. HepG2 cells or L-02 cells were incubated with 1.0 μM HCy-D for 1 h and then treated with a mixture of 500 μM GSH and 250 μM $Na_2S_2O_3$ for 0.5 h. For control experiments, HCy-D loaded HepG2 cells were pretreated with 10 mM TNBS for 0.5 h, and then treated with a mixture of 500 μM GSH and 250 μM $Na_2S_2O_3$ for another 0.5 h. Simultaneously, HCy-D loaded HepG2 cells were treated with 500 μM GSH only for 0.5h. Subsequently, excited at 405 nm, the cells were imaged under a confocal microscope (LSM 700) and the images were collected at emission channels of 405-555 nm (green channel) and 560-700 nm (red channel), respectively.

Colocalization imaging of cells

Hela cells were incubated with 1 μM HCy-D for 1 h at 37℃. Then, 1 μM Mito Tracker Deep Red was added and incubated for another 0.5 h and the confocal fluorescence images were captured.

Cytotoxicity Assay

HeLa Cells were cultured in DMEM supplemented with 10% FBS in an atmosphere of 5% CO_2 and 95% air at 37℃. The cells were placed in a 96-well plate, followed by addition of probe HCy-D with final concentrations of 1, 5, 10 μM, respectively. The cells were then incubated for 6 h, followed by SRB assays.



Scheme S1 Synthesis procedures of HCy-D, Donor and Acceptor.

Synthesis of compound 1

4-Fluorobenzaldehyde (0.57 g, 4.64 mmol) (dissolved in 5 mL 2-methoxyethanol) was added dropwise to piperazine (1.46 g, 17.4 mmol) dissolved in a mixture of H_2O (18 mL) and 2-methoxyethanol (25 mL) in 0.5 h. The mixture was then refluxed for 3 h. Yellow solid precipitated out at room temperature, and was washed thoroughly with water (100 mL). The solid was dissolved in 50 mL 10% hydrochloric acid and filtered to remove the residue, then 20% sodium hydroxide was added to the filtrate until the solution pH was about 10. The mixture was extracted with dichloromethane **(**CH_2Cl_2, 50 mL × 2) and the organic layer was separated, washed with brine (50 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a pale yellow solid (0.64g, 65.9%). Mp: 179-180℃.

Synthesis of compound 3

To a mixture of compound 1 (0.19 g, 1 mmol) and compound 2 (0.3 g, 1 mmol) was added 10 mL absolute ethanol. The mixture was reflux under N_2 atmosphere for 3.5 h, after which the solvent was removed under reduced

pressure to afford a deep red solid (0.4 g, 90%) without further purification.

Synthesis of HCy-D

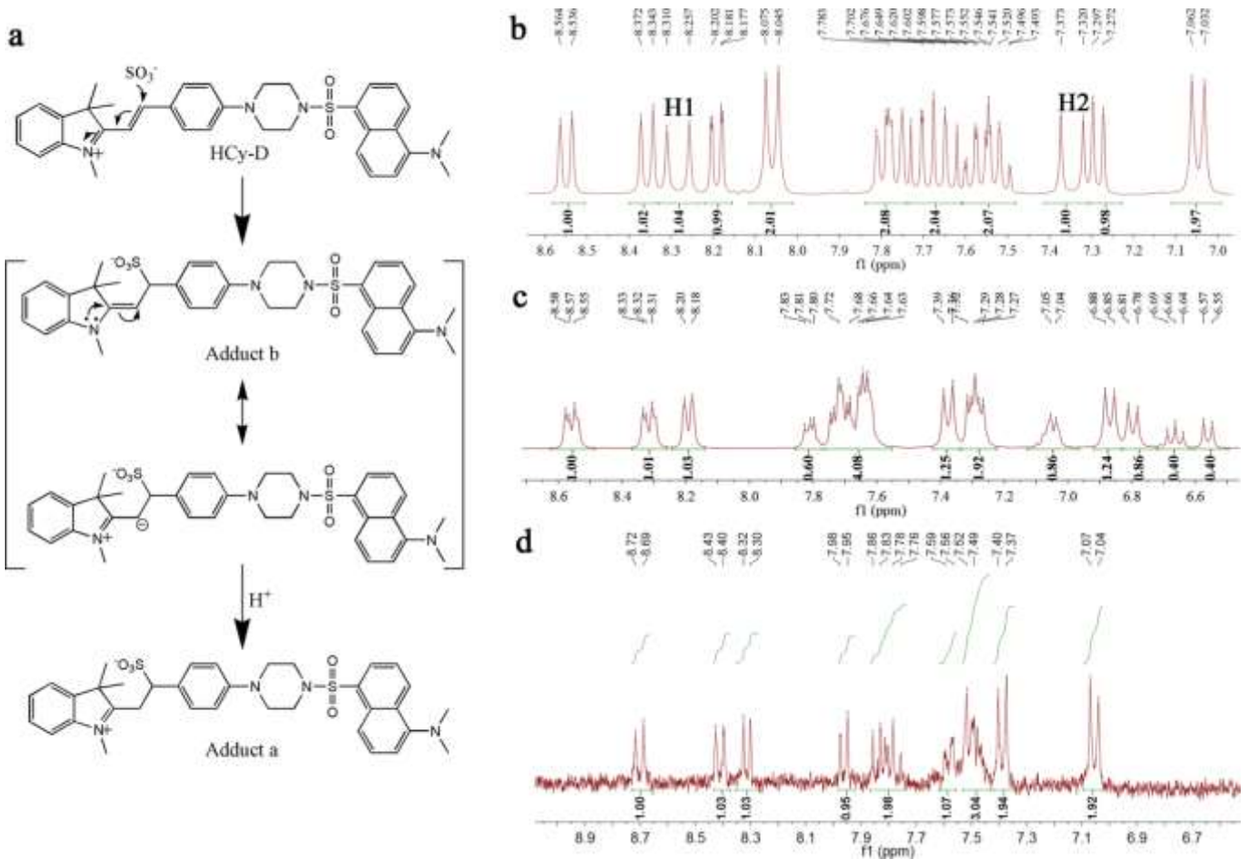
To a mixture of compound 3 (0.32 g, 0.67 mmol) and 0.2 mL distilled Et_3N in 5 mL distilled CH_2Cl_2 at 0℃, compound 4 (0.27 g, 1 mmol) dissolved in 3 mL distilled CH_2Cl_2 wad added dropwise over a period of 15 min. Then the mixture was stirred at room temperature until compound 3 was consumed (about 3 h). Then 10 mL water was added to the reaction mixture. The organic layer was separated and washed twice with water (20 mL× 2), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to afford a red solid (0.37 g, 75%). Mp: >300℃. ^{1}H NMR (DMSO-*d*6, 300 MHz) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.36 (d, *J* = 8.7 Hz, 1H), 8.28 (d, *J* = 15.9 Hz, 1H), 8.19 (dd, *J* = 7.5 Hz, 0.8 Hz, 1H), 8.06 (d, *J* = 9.0 Hz, 2H), 7.81-7.49 (m, 6H), 7.35 (d, *J* = 15.9 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 9.3 Hz, 2H), 4.02 (s, 3H), 3.58 (t, *J* = 2.4 Hz, 4H), 3.25 (t, *J* = 2.4 Hz, 4H), 2.83 (s, 6H), 1.74 (s, 6H); ^{13}C NMR (DMSO-*d*6, 75 MHz) δ 180.95, 154.10, 154.02, 151.95, 143.34, 142.41, 133.97, 132.74, 130.99, 130.69, 130.17, 129.70, 129.22, 128.82, 128.61, 124.62, 124.21, 123.13, 119.32, 115.83, 114.57, 114.43, 107.80, 51.74, 46.53, 46.26, 45.53, 34.05, 26.42; IR (KBr, cm^{-1}): 3465, 2921, 2856, 1634, 1576, 1526, 1451, 1384, 1299, 1240, 1192, 1113, 945, 620; HR-MS (ESI): m/z calculated for $C_{35}H_{39}N_4O_2S^+$ 579.2794, found 579.2698.

Synthesis of Donor

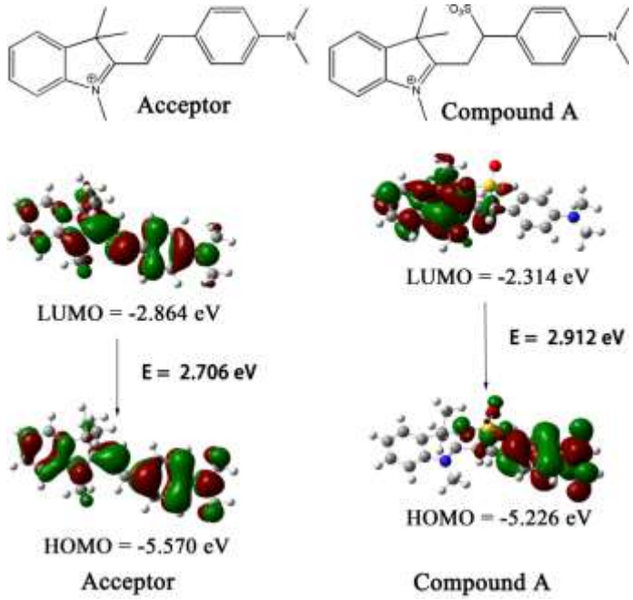
To a mixture of $NHEt_2$ (0.146 g, 2 mmol) and 0.14 mL distilled Et_3N in 5 mL distilled CH_2Cl_2 at 0℃, compound 4 (0.27 g, 1 mmol) dissolved in 10 mL distilled CH_2Cl_2 wad added dropwise over a period of 30 min. Then the mixture was stirred at room temperature for 1 h, after which the mixture was washed twice with water (50 × 2 mL). The organic layer was separated, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (petroleum ether : ethyl acetate = 5 : 1) to afford a greenish yellow solid (0.33 g, 95%). Mp: 106-107℃. ^{1}H NMR (DMSO-*d*6, 300 MHz) δ 8.54 (d, J = 8.7 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 7.5 Hz, 1H), 7.52 (q, J = 7.5 Hz, 2H), 7.18 (d, J = 7.2 Hz, 1H), 3.41-3.34 (q, J = 7.2 Hz, 4H), 2.89 (s, 6H), 1.12 (t, J = 7.2 Hz, 6H); ^{13}C NMR (DMSO-*d*6, 75 MHz) δ 135.69, 130.16, 130.04, 129.43, 127.81, 123.16, 119.81, 115.15, 45.46, 40.92, 13.77; HR-MS (ESI): m/z calculated for $C_{16}H_{22}N_2O_2S^+$ 306.1402, found 306.1422.

Synthesis of Acceptor

4-Dimethylaminobenzaldehyde (0.112 g, 0.75 mmol), compound 2 (0.15 g, 0.5 mmol) were mixed and dissolved in ethanol (10 mL). Then the mixture was refluxed for 4.5 h under nitrogen atmosphere. The solvent was then evaporated under reduced pressure, and the resulting residue was purified by flash column chromatography (DCM : MeOH = 20:1, V/V) to afford the product (0.194 g, 92%). Mp: 175-177℃. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 8.31 (d, J = 15.9 Hz, 1H), 8.07 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.58-7.45 (m, 2H), 7.26 (d, J = 15.9 Hz, 1H), 6.89 (d, J = 9 Hz, 2H), 3.97 (s, 3H), 3.16 (s, 6H), 1.75 (s, 6H); ^{13}C NMR (100 MHz, DMSO-*d*6): 206.93, 180.06, 154.90, 154.52, 143.02, 142.54, 129.15, 128.00, 123.08, 122.74, 114.04, 112.65, 105.66, 51.33, 33.57, 31.17, 26.71; HR-MS (ESI): m/z calculated for $C_{21}H_{25}N_2^+$: 305.2018, found: 305.2059.

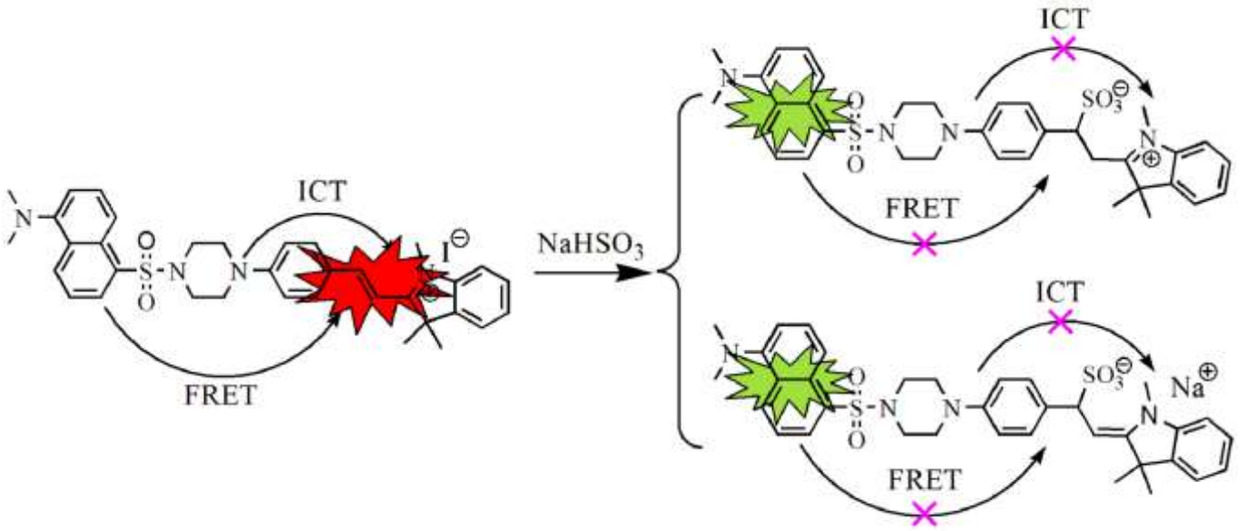


Scheme S2 (a) The proposed reaction mechanism of probe HCy-D with SO_2 derivatives. (b) Partial ^{1}H NMR spectra of HCy-D in DMSO-*d*6. (c) Partial ^{1}H NMR spectra of HCy-D in the presence of $NaHSO_3$ in DMSO-*d*6:D_2O = 4:1. (d) Partial ^{1}H NMR spectra of HCy-D in the presence of $NaHSO_3$ in DMSO-*d*6:D_2O = 1:4.



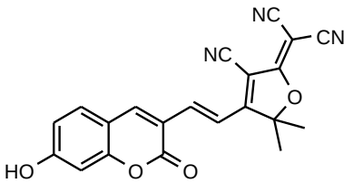
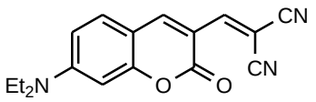
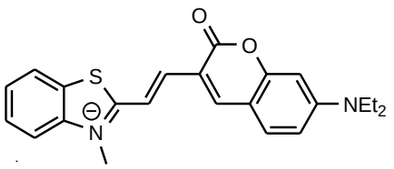
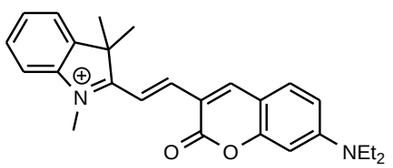
Scheme S3 Frontier molecular orbital plots of the Acceptor and Compound A in water (PCM model) involved in the

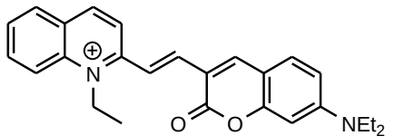
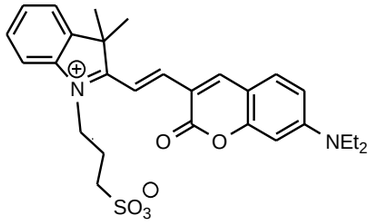
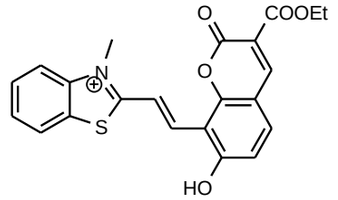
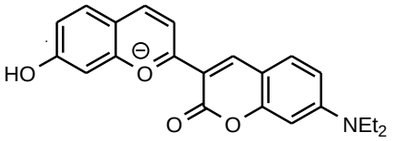
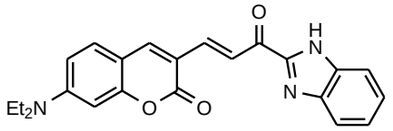
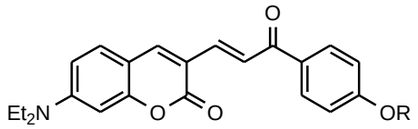
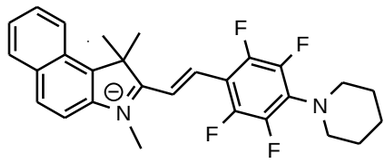
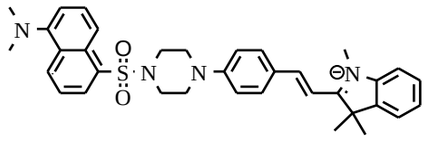
vertical excitation. Green and red shapes are corresponding to the different phases of the molecular wave functions for HOMO and LUMO orbitals.



Scheme S4 The inhibition of FRET-ICT process by addition of bisulfite.

Table S1. Comparison of ratiometric fluorescent probes for HSO_3^-/SO_3^{2-}.

Probe structures	λ_{ex}/nm [a]	λ_{em}/nm [a]	Response time	Interaction Mechanisms[b]	Cell imaging	Ref.
	466/580	523/663	90 s	ICT	U-2OS	5*o*
	446	480/578	30 s	ICT	--	5*g*
	445	478/633	5 min	ICT	HeLa	5*f*
	405	480/650	3 min	ICT	Mitochondria in HeLa cells	5*l*

	450	518/610	15 min	ICT	RAW 264.7 macrophage	5*i*
	450/550	485/667	--	ICT	HeLa	5*m*
	415/500	460/600	--	ICT	MCF-7	5*h*
	430/605	485/640	5 min	PET	HepG2	5*s*
	415	485/605	60 min	ICT	A549	5*p*
	410	465/592	$t_{1/2} \approx 5$ min	ICT	--	5*q*
	322/470	460/595	3 min	TICT	A549	5*t*
	410 nm	530/580	2 min	FRET-ICT	Mitochondria in HeLa cells; HepG2; L-02	This work

[a] "/" indicates two excitation wavelengths or two emission wavelengths for one probe. [b] FRET: Förster resonance energy transfer; PET: Photo-induced electron transfer; ICT: Intramolecular charge transfer; TICT: Twisted intramolecular charge transfer.

Table S2 Selected parameters for the vertical excitation (UV-vis absorbance) of Acceptor and Compound A based on the optimized ground state geometries.

Compound	Electronic Transitions	Excitation Energy (eV)[a]	f[b]	Composition	CI[c]
Acceptor	S_0 to S_1	2.6279 (471.81 nm)	1.5445	HOMO to LUMO	0.70619
Compound A	S_0 to S_1	3.8308 (323.65 nm)	0.3229	HOMO-2 to LUMO	0.63202
	S_0 to S_2	4.0416 (306.77 nm)	0.2267	HOMO to LUMO+1	0.56554

[a] Electronic excitation energies (eV), the numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] Coefficient of the wave function for each excitation. The CI coefficients are in absolute values.

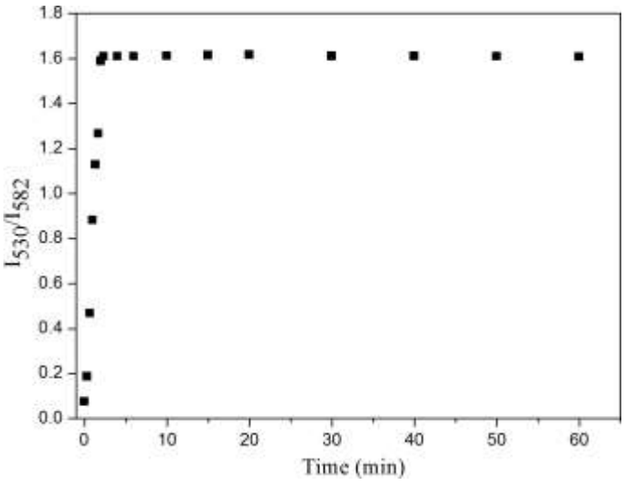


Fig. S1 Time-dependent fluorescence response of HCy-D (5 μM) to 6 equiv. of $NaHSO_3$ in PBS (pH = 7.4, 10 mM, containing 30% DMF). λex = 410 nm, slit: 10 nm/12 nm.

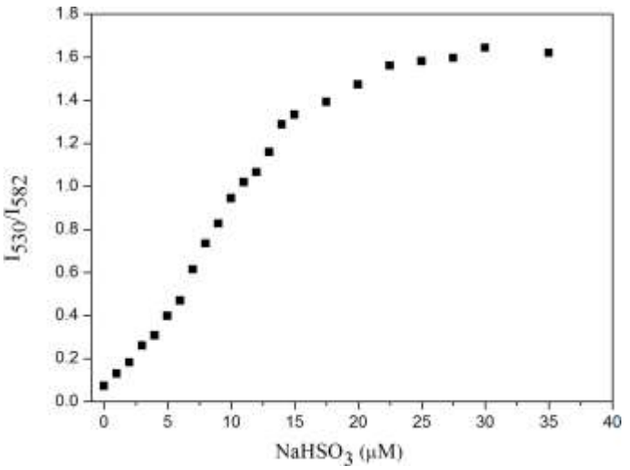


Fig. S2 Ratio (I_{530}/I_{582}) changes upon addition of $NaHSO_3$ (0-35 μM) in PBS (pH = 7.4, 10 mM, containing 30% DMF). [HCy-D] = 5 μM, λex = 410 nm, slit: 10 nm/12 nm.

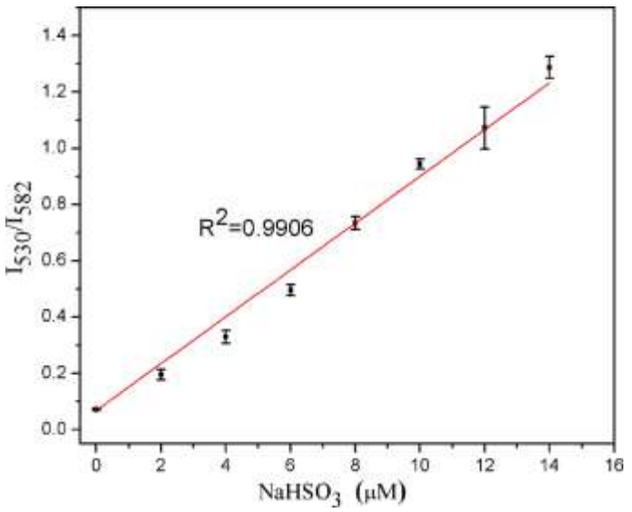


Fig. S3 The linear relationship between I_{530}/I_{582} and concentration of $NaHSO_3$ (0-15 μM).

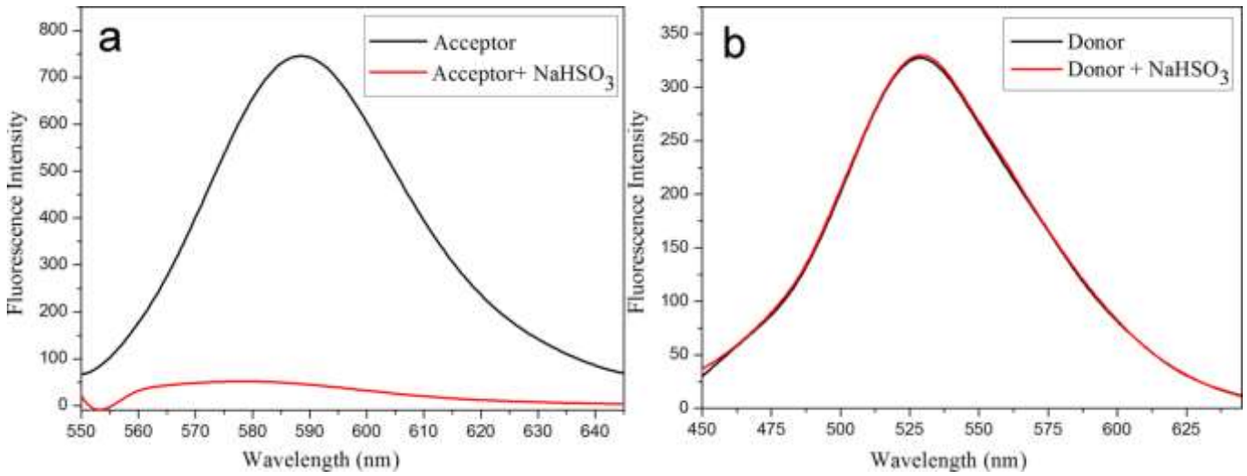


Fig. S4 Fluorescence emission of (a) Acceptor and (b) Donor in the absence and presence of 20 equiv. of $NaHSO_3$ in PBS (pH = 7.4, 10 mM, containing 30% DMF). λex = 410 nm for the Donor and λex = 530 nm for the Acceptor, slit: 10 nm/12 nm.

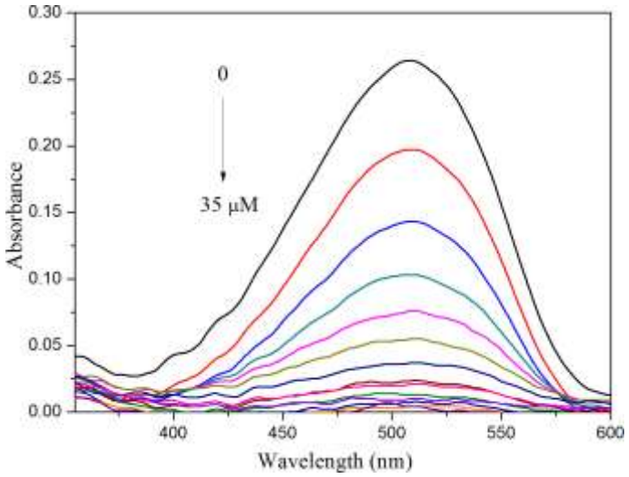


Fig. S5 UV-vis absorption spectra of HCy-D (5 μM) in the presence of different amounts of $NaHSO_3$ (0-35 μM) in PBS (pH = 7.4, 10 mM, containing 30% DMF).

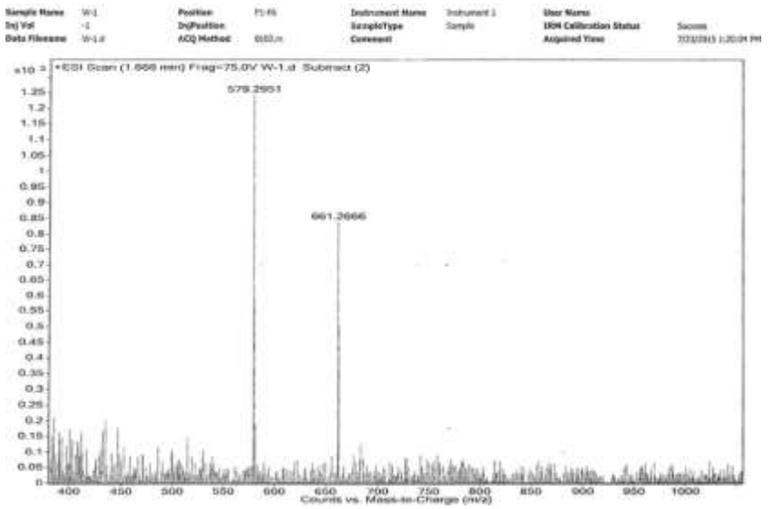


Fig. S6 HRMS spectrum of HCy-D in the presence of 6 equiv. of $NaHSO_3$ in DMSO/H_2O (4:1).

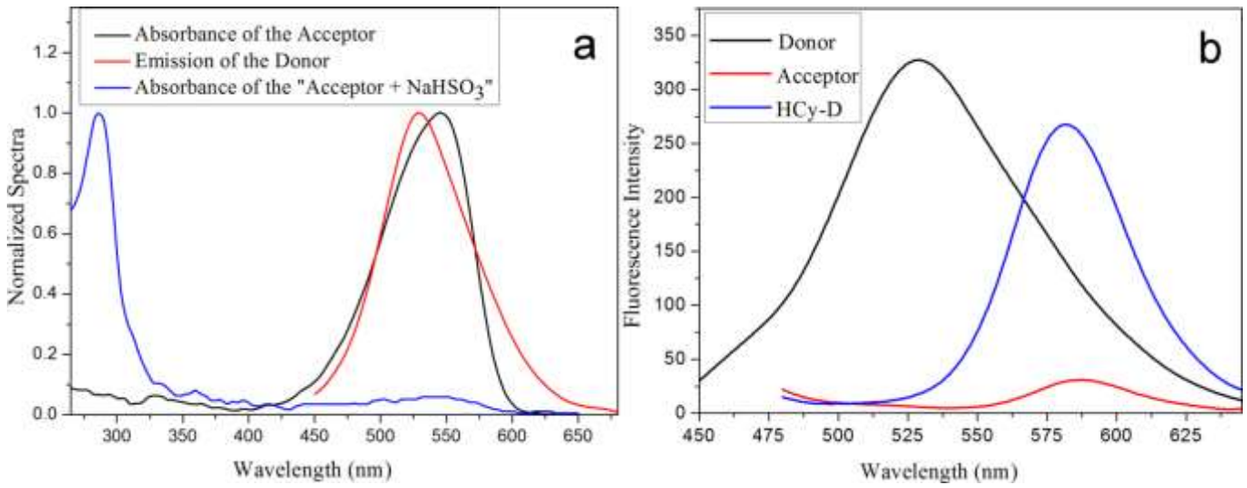


Fig. S7 (a) Normalized fluorescence emission spectrum of the Donor, and normalized UV-vis absorption spectrum of the Acceptor before and after addition of 20 equiv. of $NaHSO_3$. (b) The fluorescence emission of Donor, Acceptor and HCy-D in PBS (pH = 7.4, 10 mM, containing 30% DMF). λex = 410 nm, slit: 10 nm/12 nm. [Donor] = [Acceptor] = [HCy-D] = 5 μM.

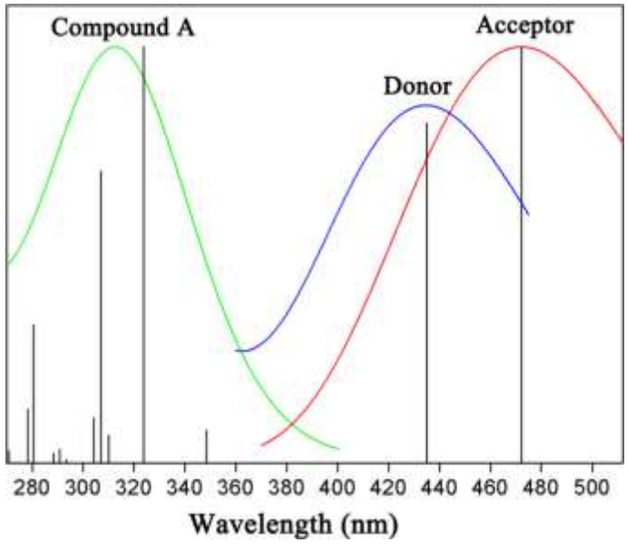


Fig. S8 Calculated absorption band of the Acceptor and Compound A, and calculated fluorescence emission band of the Donor.

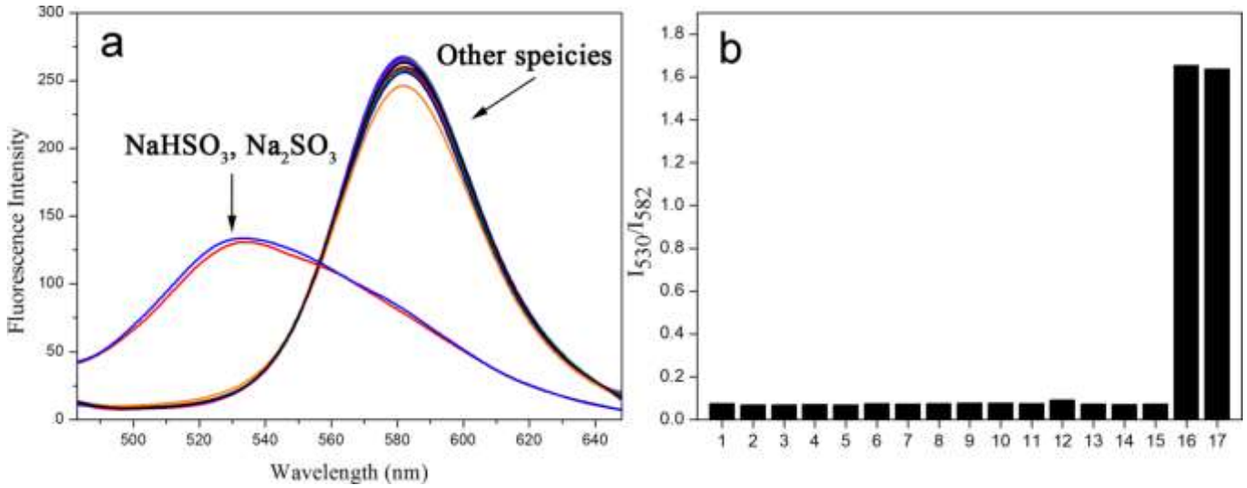


Fig. S9 (a) Fluorescence response of HCy-D (5 μM) to various anions in PBS (pH = 7.4, 10 mM, containing 30% DMF). 1. vacant; 2. F^-; 3. Cl^-; 4. Br^-; 5. I^-; 6. HCO_3^-; 7. NO_3^-; 8. ClO^-; 9. SO_4^{2-}; 10. SCN^-; 11. $S_2O_3^{2-}$; 12. S^{2-}; 13. Cys; 14. Hcy; 15. GSH; 16. HSO_3^-; 17. SO_3^{2-}. Final concentration for all the species was 50 μM except for 13-15 (1 mM) and 16-17 (30 μM). λex = 410 nm, slit: 10 nm/12 nm. (b) Response (I_{530}/I_{582}) of HCy-D (5 μM) to various anions in PBS (pH = 7.4, 10 mM, containing 30% DMF). 1. vacant; 2. F^-; 3. Cl^-; 4. Br^-; 5. I^-; 6. HCO_3^-; 7. NO_3^-; 8. ClO^-; 9. SO_4^{2-}; 10. SCN^-; 11. $S_2O_3^{2-}$; 12. S^{2-}; 13. Cys; 14. Hcy; 15. GSH; 16. HSO_3^-; 17. SO_3^{2-}. Final concentration for all the species was 50 μM except for 13-15 (1 mM) and 16-17 (30 μM).

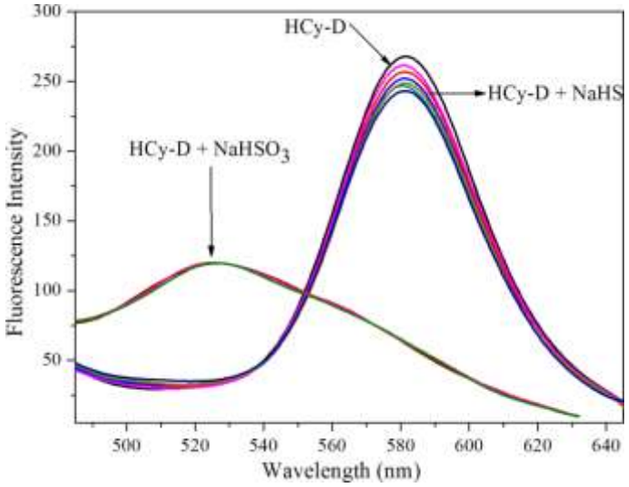


Fig. S10 Fluorescence response of HCy-D (5 μM) to $NaHSO_3$ (50 μM) and NaHS (50 μM, 100 μM) in PBS (pH = 7.4, 10 mM, containing 30% DMF). Data were acquired at 10, 20 and 30 min after $NaHSO_3$ (50 μM) or NaHS (50 μM, 100 μM) was added. λex = 410 nm, slit: 10 nm/12 nm.

Fig. S11 Response (I_{530}/I_{582}) of HCy-D (5 μM) to $NaHSO_3$ (30 μM) in the presence of various anions in PBS (pH = 7.4, 10 mM, containing 30% DMF). 1. vacant; 2. F^-; 3. Cl^-; 4. Br^-; 5. I^-; 6. HCO_3^-; 7. NO_3^-; 8. ClO^-; 9. SO_4^{2-}; 10. SCN^-; 11. $S_2O_3^{2-}$; 12. S^{2-}; 13. Cys; 14. Hcy; 15. GSH. Final concentration for all the potential competitive species was 50 μM except for 13-15 (1 mM).

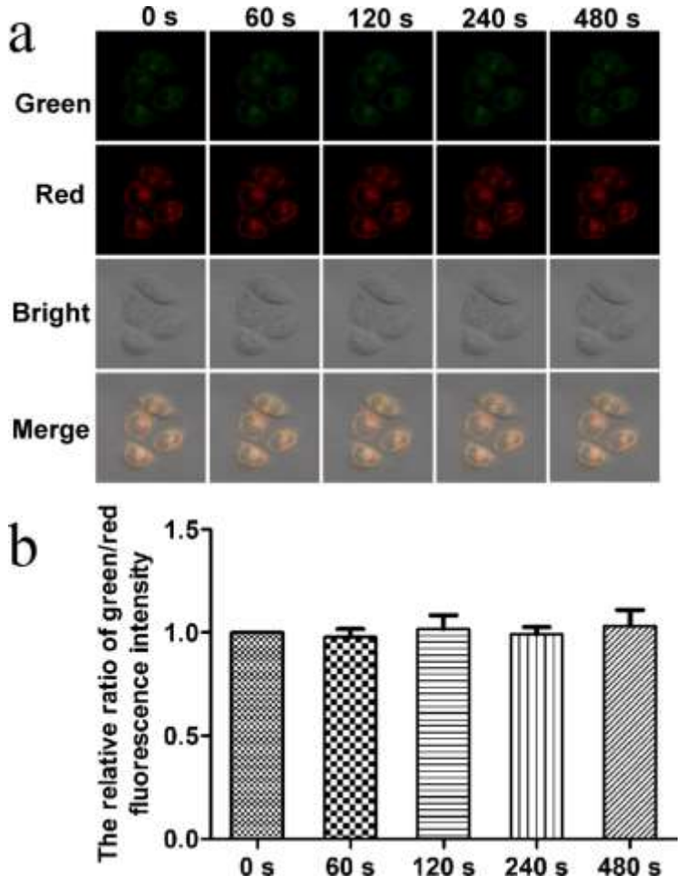


Fig. S12 (a) Photostability of HCy-D for fluorescence images of HeLa cells. The cells were incubated with HCy-D (1 μM) for 1 h beforehand. First line: fluorescence images from the green channel (405-555 nm); second line: fluorescence images at the red channel (560-615 nm); third line: bright field images; fourth line: overlay images of

the first, second and third lines. λex = 405 nm. (b) The relative ratio of green/red fluorescence intensity in correspondence with (a).

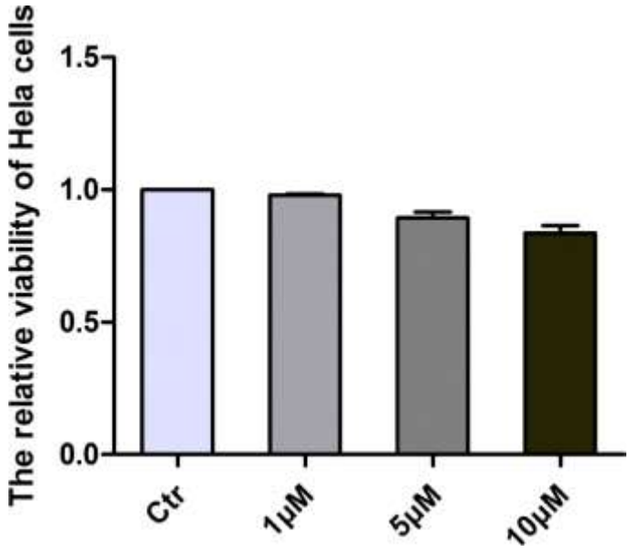


Fig. S13 .Cell viability by a standard SRB assay. HeLa cells were incubated with HCy-D (1, 5 and 10 μM) for 6 h.

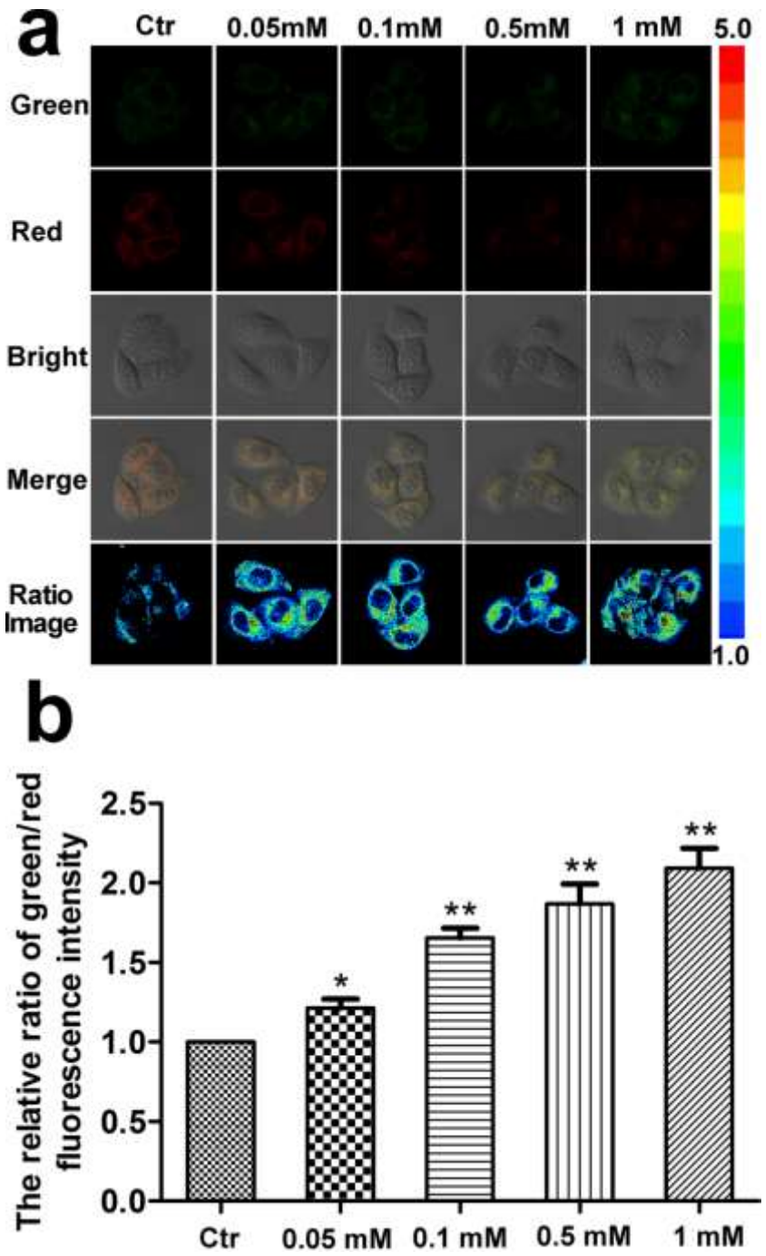


Fig. S14 (a) Fluorescence and bright field images of HeLa cells incubated with HCy-D (1 μM) for 1 h, then with $NaHSO_3$ (0, 0.05, 0.1, 0.5, 1 mM) for another 0.5 h. (b) The relative ratio of green/red fluorescence intensity. The ratio images were all obtained as F_{green}/F_{red}. Images were acquired from 405-555 nm for green fluorescence, and from 560-700 nm for red fluorescence. λex = 405 nm.

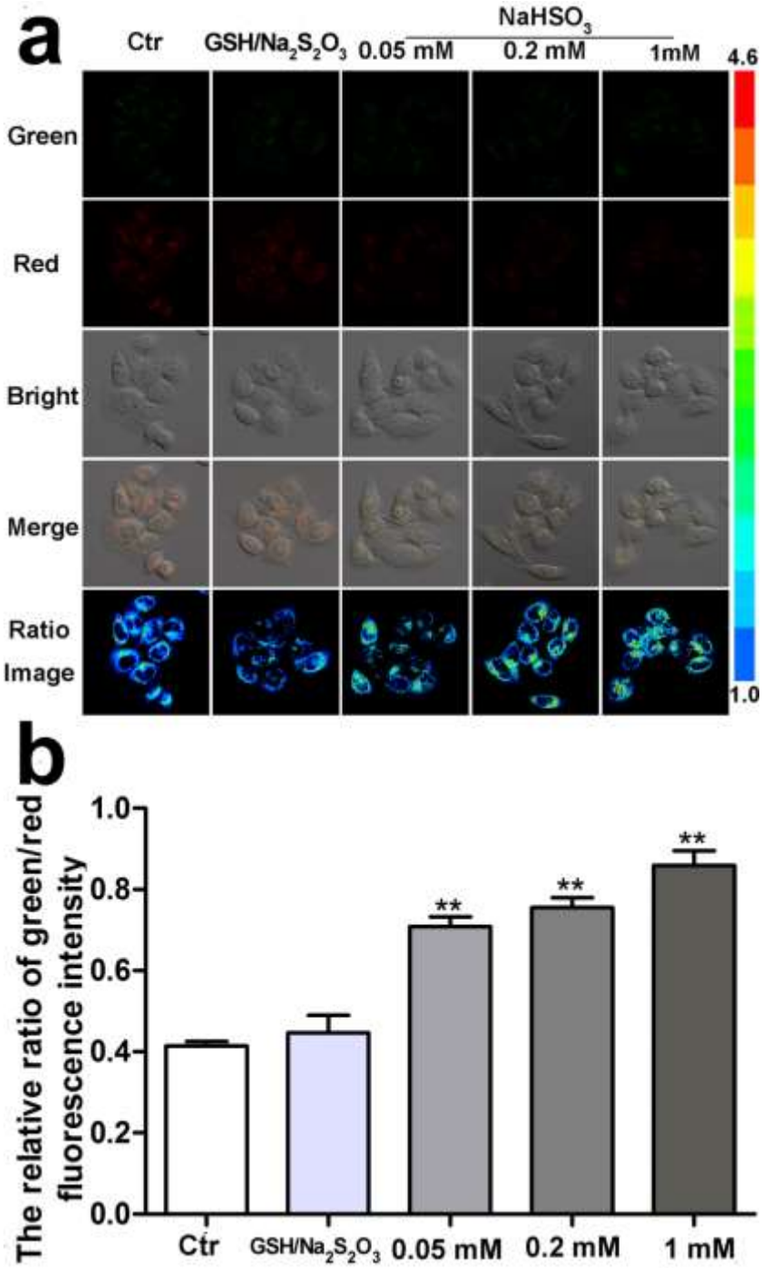


Fig. S15 (a) The first row (vertically): L-02 cells were incubated with HCy-D (1 μM) for 1 h; The second row: L-02 cells were incubated with HCy-D (1 μM) for 1 h, and then were incubated with 500 μM GSH and 250 μM $Na_2S_2O_3$ for another 0.5 h; The 3-5 row: L-02 cells were incubated with HCy-D (1 μM) for 1 h, and then with 0.05, 0.2 and 1 mM $NaHSO_3$ for another 0.5 h, respectively. (b) The relative ratio of green/red fluorescence intensity of row 1-5 in (a). The ratio images were all obtained as F_{green}/F_{red}. Images were acquired from 405-555 nm for green fluorescence, and from 560-700 nm for red fluorescence. λex = 405 nm.

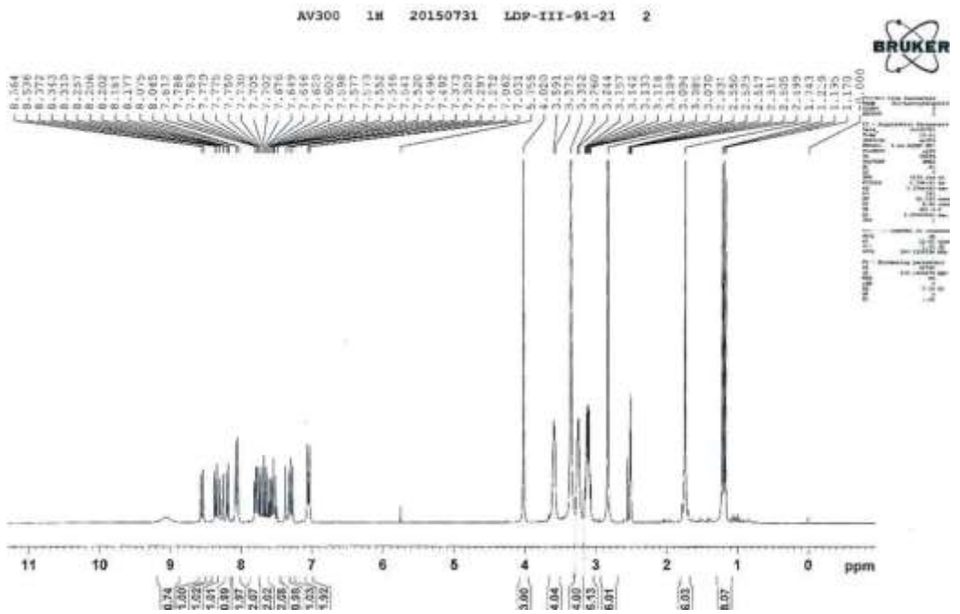


Fig. S16 ^{1}H NMR spectrum of probe HCy-D in DMSO-*d*6.

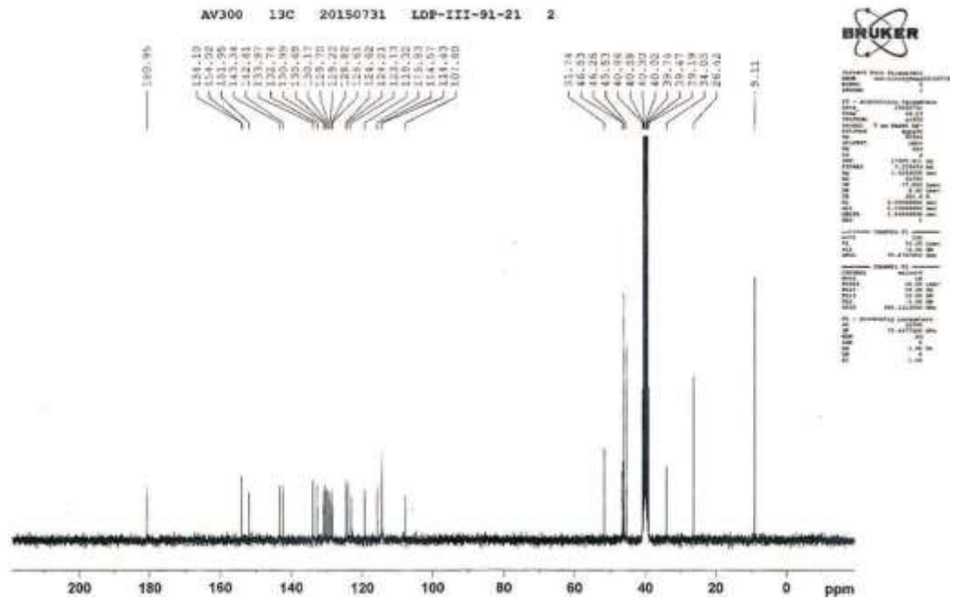


Fig. S17 ^{13}C NMR spectrum of probe HCy-D in DMSO-*d*6.

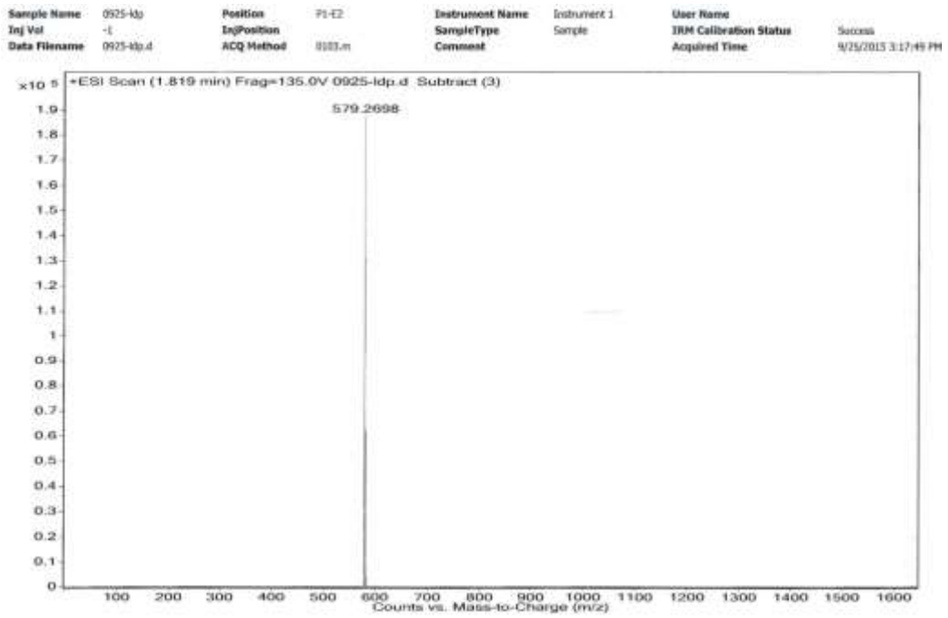


Fig. S18 HRMS spectrum of probe HCy-D.

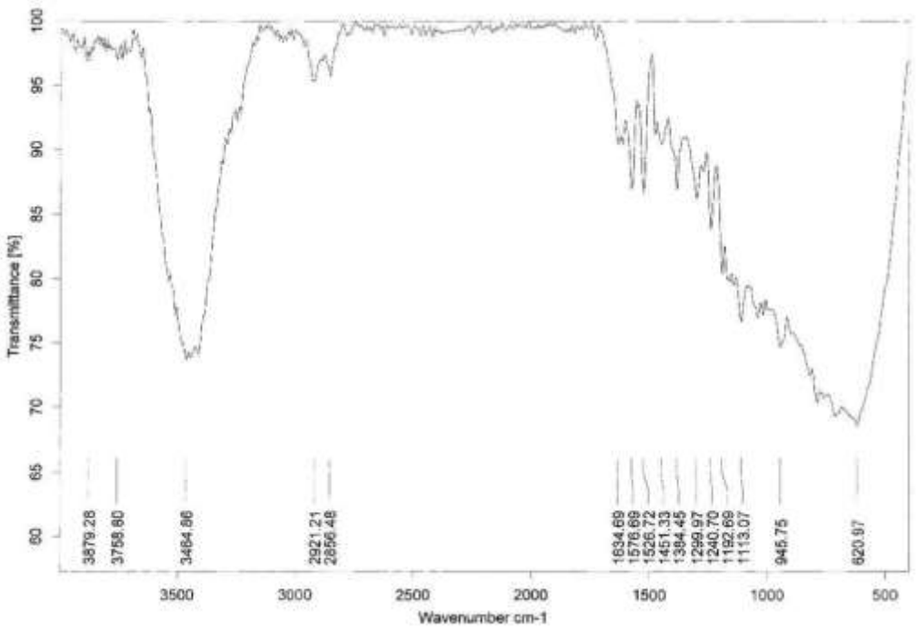


Fig. S19 FT-IR spectrum of probe HCy-D.

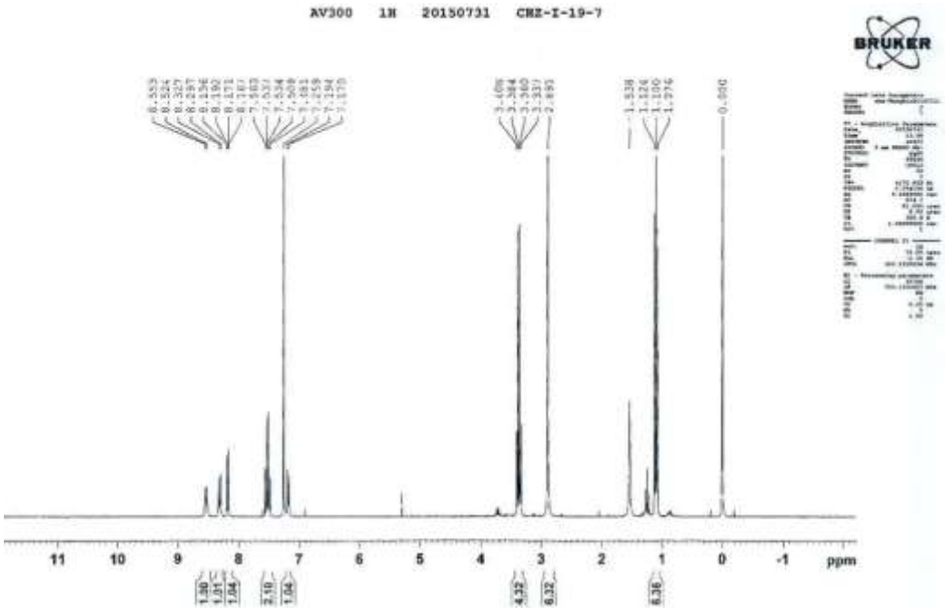


Fig. S20 ^{1}H NMR spectrum of Donor in $CDCl_3$.

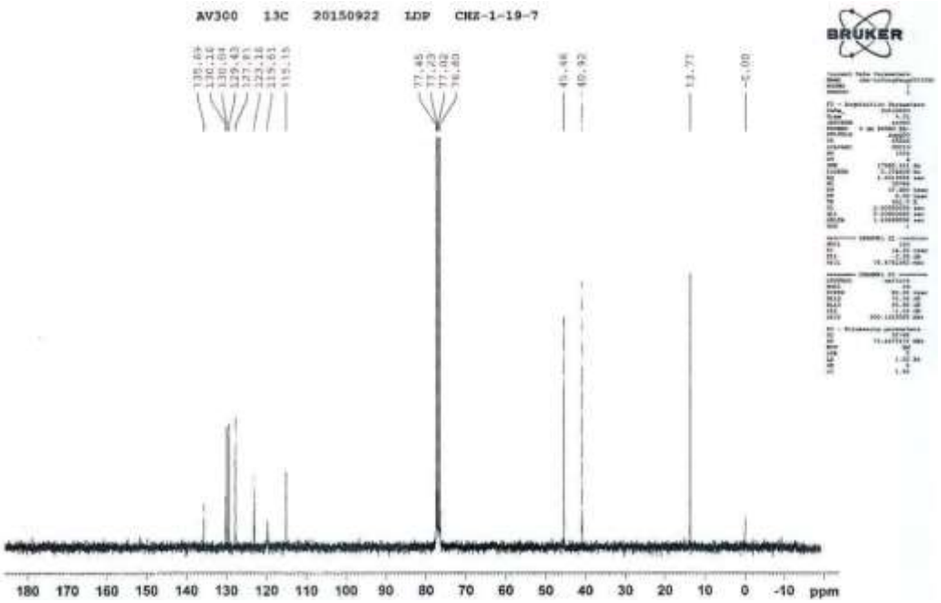


Fig. S21 ^{13}C NMR spectrum of Donor in $CDCl_3$.

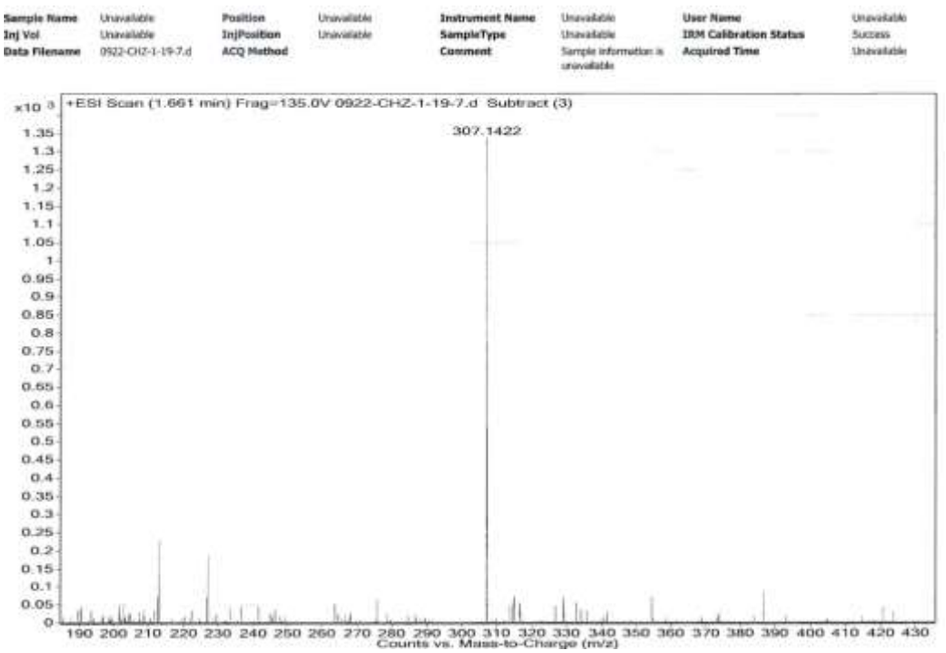


Fig. S22 HRMS spectrum of Donor.

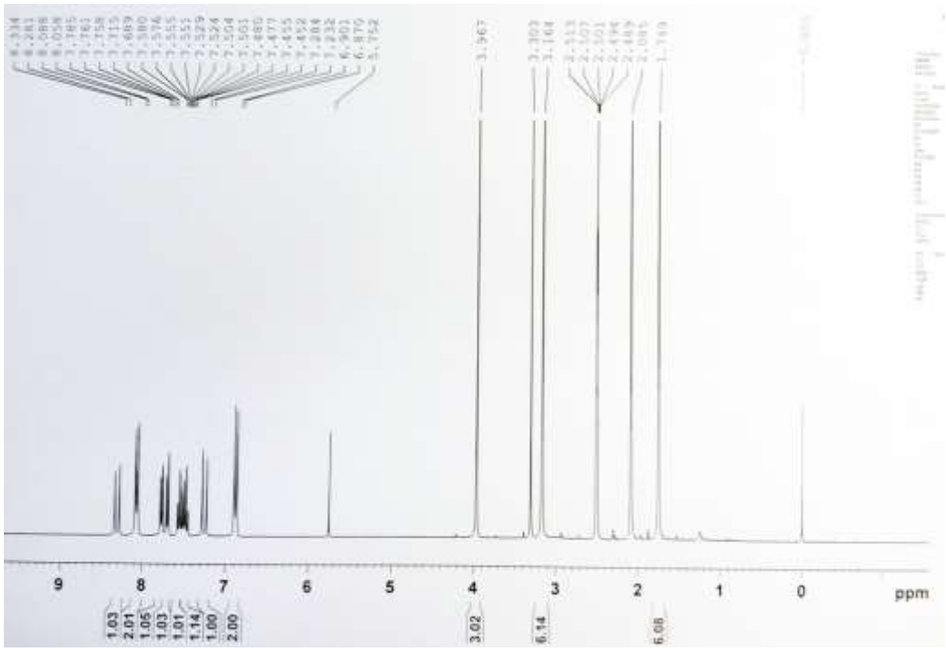


Fig. S23 ^{1}H NMR spectrum of Acceptor in DMSO-*d*6.

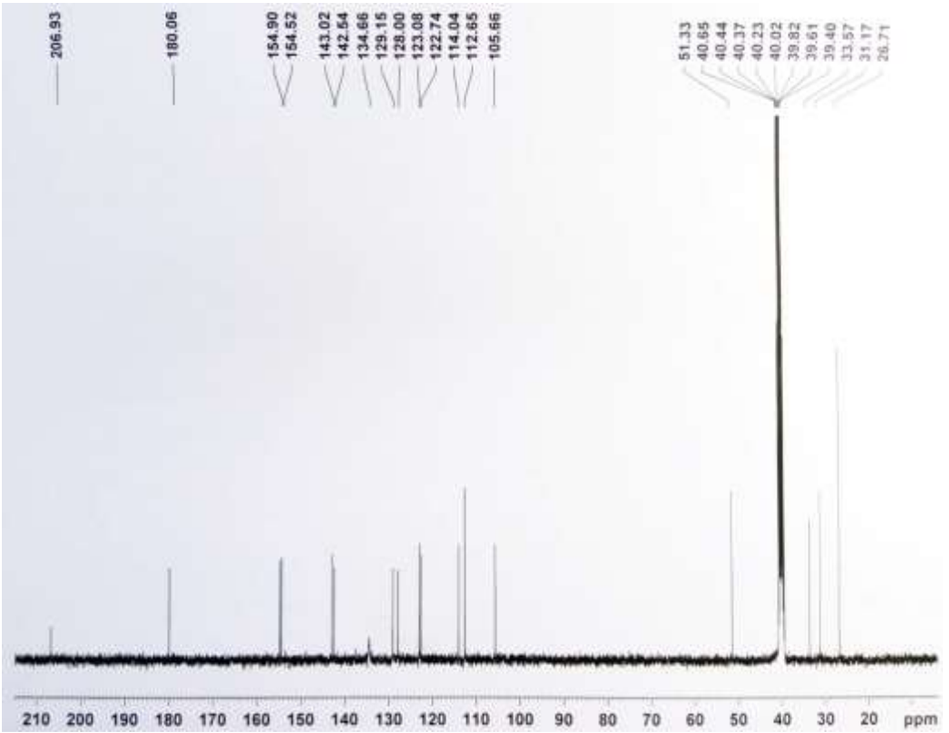


Fig. S24 ^{13}C NMR spectrum of Acceptor in DMSO-*d*6.

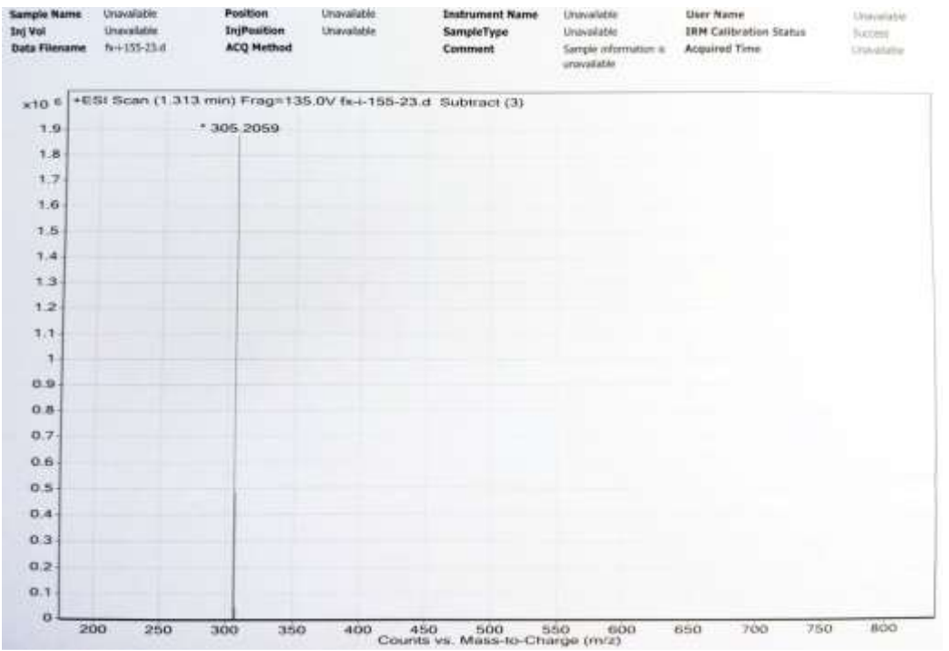


Fig. S25 HRMS spectrum of Acceptor.

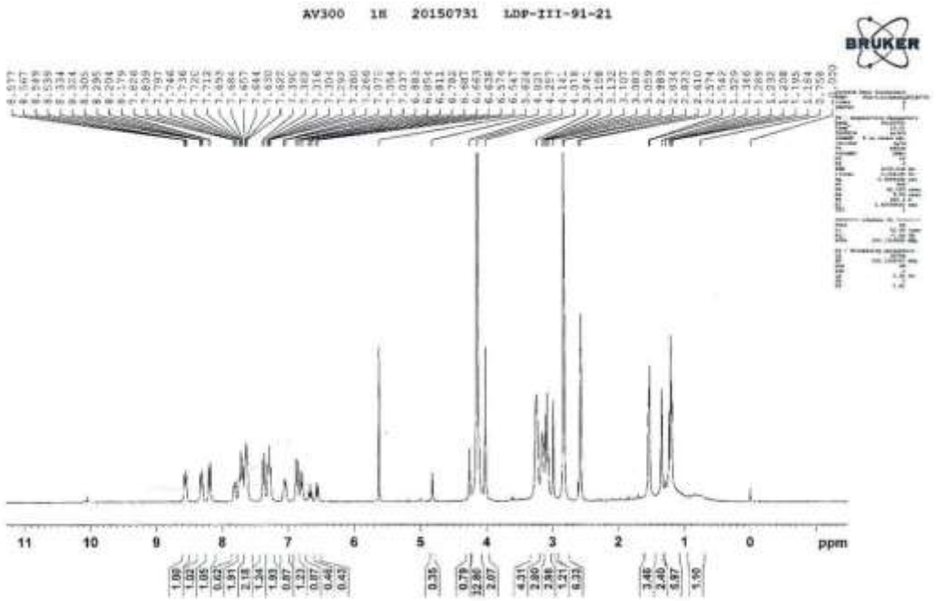


Fig. S26 ^{1}H NMR spectra of HCy-D in the presence of $NaHSO_3$ in DMSO-*d*6 (containing 20% D_2O).

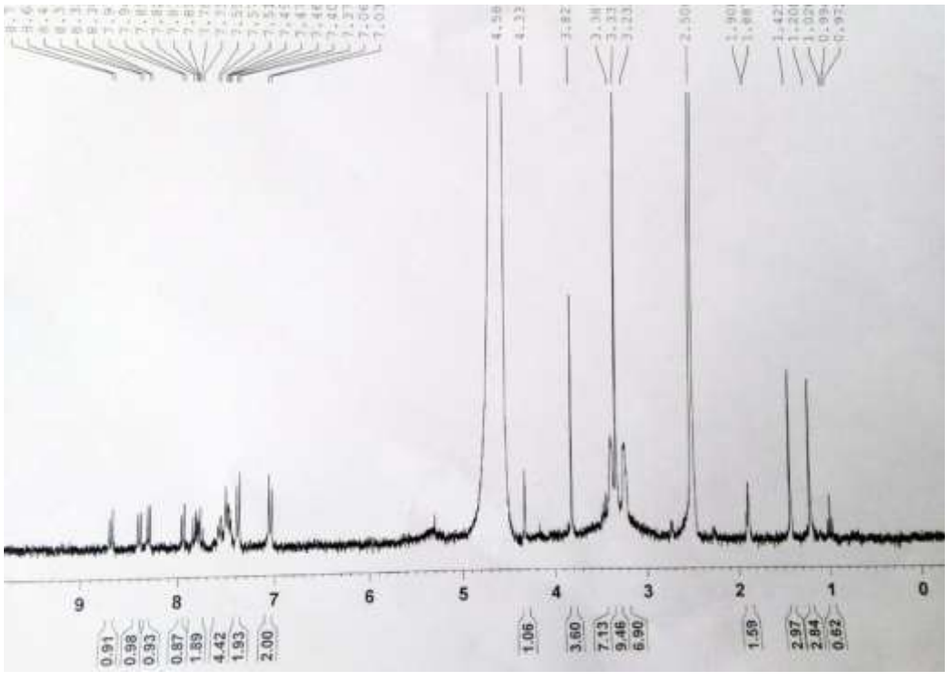


Fig. S26 ^{1}H NMR spectra of HCy-D in the presence of $NaHSO_3$ in DMSO-*d*6 (containing 80% D_2O).