Supporting Information

A tumor-specific and mitochondria-targeted fluorescent probe for real-time sensing of hypochlorite in living cells

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Content

1. Experimental Section

General information and methods.

All materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies. And all reactions were monitored by TLC analyses on silica gel GF 254. Column chromatographic purifications were carried out on silica gel (HG/T2354-92). NMR spectra were measured on a Bruker AV-400. The ^{1}H NMR (400 MHz) chemical shifts were given in ppm relative to the internal reference TMS (1H, 0.00 ppm). The ^{13}C NMR (100 MHz) chemical shifts were given using DMSO-d_6 as the internal standard. ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. Fluorescence excitation and emission spectra were obtained using FluoroMax-4 (JONIN-YVON, HORIBA) Spectrofluorophotometer. UV-Vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-Visible spectrophotometer.

Fluorescence analysis.

A stock solution of **BiTClO** (5 mM) was prepared in DMSO. All UV/Vis and fluorescence spectra experiments were performed using 10 μM **BiTClO** in PBS buffer solution (pH 7.4, 10 mM, containing 20% EtOH) at room temperature. Fluorescence emission spectra were obtained with a Xenon lamp and 1.0 cm quartz cells.

Determination of ROS.

The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 $M^{-1}cm^{-1}$) in PBS. The concentration of $ONOO^-$ was determined from the absorption at 302 nm (ε = 1670 $M^{-1}cm^{-1}$) in 0.1 N NaOH. The concentration of ClO^- was determined from the absorption at 292 nm (ε = 350 $M^{-1}cm^{-1}$) in PBS. $^{\bullet}OH$ was generated at 25 ^{o}C by means of the Fenton reaction with H_2O_2 and a 10-fold excess of $Fe(ClO_4)_2$.

Quantum yield.

The quantum yield was detected in PBS buffer solution and rhodamine B (Φ_S=0.69 in MeOH) was used as a reference. It was calculated according to the following formula:

$$\Phi_X = \Phi_S (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Φ: quantum yield; A: absorbance at the excitation wavelength; F: integral area of fluorescence spectra at the same excitation wavelength; n: the refractive index of solvents; S and X: the reference standard and unknown sample.

Detection limit.

The detection limit was calculated according to the following formula:

$$DL = 3\sigma / k$$

DL: detection limit; σ: the standard deviation of the fluorescence intensity of the probe scanning for 10 times; k: the slope of the line graph of fluorescence intensity and reactant concentration.

Confocal imaging of living cells.

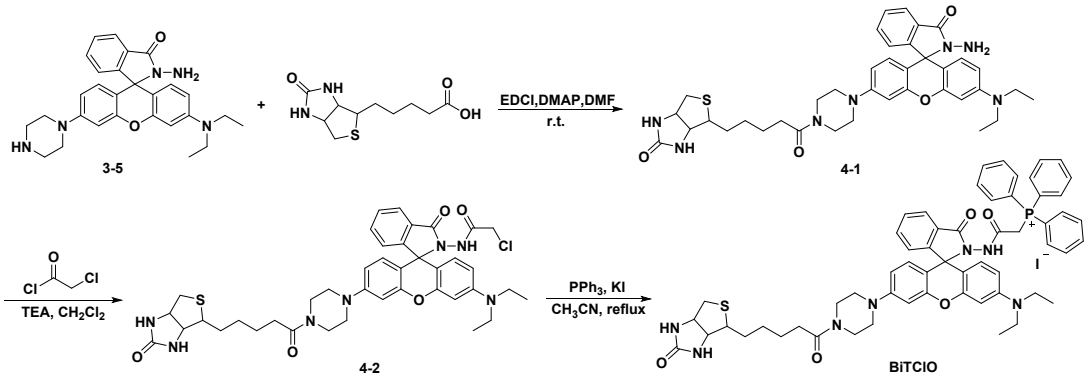
Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37^{o}C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3/well) were passed on confocal dishes and incubated for 24h. Immediately before the staining experiment, cells were washed three times with PBS (10 mM), and then incubated with 10 μM **BiTClO** for 30 min at 37^{o}C**.** Then the petri dish was washing with PBS for another three times, and incubating with 30 μM ClO^- for 15 min at 37^{o}C. Finally, wash

each dish with PBS (10 mM) for 3 times, and analyzed with a confocal fluorescence microscope.

Cytotoxicity assays.

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37 ^{o}C in a 5% CO_2/95% air incubator. Immediately before the experiment, the cells well placed in a 6-well plate, followed by addition of increasing concentrations of **BiTClO**. The final concentrations of the probe were kept from 0 to 40 μM. The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air for 24 h, followed by MTT assays (n= 5). Untreated assay with RPMI 1640 (n = 5) was also conducted under the same conditions.

2. Synthesis of probe BiTClO.



Scheme S1. Synthesis route of **BiTClO**.

Synthesis of compound 4-1: D-biotin (439 mg, 1.8 mmol) and 4-N,N-dimethylaminopyridine (DMAP, 220 mg, 1.8 mmol) were added to an ice bath cooled solution of compound **3-5** (560 mg, 1.2 mmol) in 5 mL anhydrous DMF under stirring. Then a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 345 mg, 1.8 mmol) of 5 mL dry DMF was added drop-wise to the mixture solution. The reaction mixture was heated to the room temperature and stirred until the reaction completed. The solvent was removed under reduced pressure and added 60 mL H_2O_2, extracted with CH_2Cl_2 (3*40 mL). The organic phase was washes by saturated brines (3*30 mL) and dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography over silica gel eluting with CH_2Cl_2/CH_3OH= 10: 1 to afford white solid as compound **4-1**. Yield: 780 mg, 93.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (dt, J = 7.0, 3.0 Hz, 1H), 7.56–7.41 (m, 2H), 7.05–6.92 (m, 1H), 6.72 (d, J = 2.2 Hz, 1H), 6.66 (dd, J = 8.8, 2.2 Hz, 1H), 6.46 (s, 1H), 6.42 (d, J = 8.8 Hz, 1H), 6.38–6.34 (m, 4H), 4.34 (s, 2H), 4.33–4.31 (m, 1H), 4.18–4.09 (m, 1H), 3.57 (t, J = 5.2 Hz, 4H), 3.35–3.25 (m, 4H), 3.25–3.07 (m, 5H), 2.82 (dd, J = 12.4, 5.0 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.35 (t, J = 7.4 Hz, 2H), 1.73–1.31 (m, 6H), 1.09 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.09, 165.88, 163.16, 153.32, 153.09, 152.13, 151.81, 148.64, 132.96, 129.96, 128.74, 128.09, 123.94, 122.73, 112.09, 110.21, 108.47, 105.69, 102.43, 97.80, 65.09, 61.49, 59.63, 55.95, 48.50, 48.09, 45.98, 45.07, 44.16, 41.14, 32.53, 28.68, 28.59, 25.29, 12.91. HRMS calcd for $C_{38}H_{45}N_7O_4S$ $[M + H]^+$: 696.3327, found: 696.3326.

Synthesis of compound 4-2: A solution of 2-chloroacetyl chloride (101 mg, 0.9 mmol) in 5 mL of dry $CHCl_3$ was added drop-wise to a solution of compound **4-1** (418 mg, 0.6 mmol) and TEA (97 mg, 0.96 mmol) in 10 mL of dry CH_2Cl_2 stirred in an ice bath. After the addition, the reaction mixture was warmed to the room temperature and stirred overnight. The solvent was removed under reduced pressure and the crude product was purified by column chromatography over silica gel eluting with CH_2Cl_2/CH_3OH= 30: 1 to afford white solid. Yield: 250 mg, 57.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.85 (dd, J = 6.4, 1.8 Hz, 1H), 7.62–7.51 (m, 2H), 7.03 (dd, J = 6.4, 1.4 Hz, 1H), 6.69–6.62 (m, 2H), 6.59 (d, J = 9.6 Hz, 1H), 6.50 (d, J = 8.8 Hz, 1H), 6.46 (s, 1H), 6.40–6.31 (m, 3H), 4.33–4.27 (m, 1H), 4.17–4.11 (m, 1H), 3.99 (s, 2H), 3.58 (m, 4H), 3.35–3.28 (m, 4H), 3.24–3.08 (m, 5H), 2.83 (dd, J = 12.4, 5.0 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.36 (t, J = 7.4 Hz, 2H), 1.67–1.35 (m, 6H), 1.09 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.10, 165.22, 163.89, 163.15, 153.32, 153.09, 152.05, 151.90, 148.92, 133.96, 129.66,129.55, 129.13, 128.63, 124.34, 123.23, 111.62, 108.31, 104.18, 101.73, 97.41, 65.31, 61.49, 59.63, 55.94, 55.39, 48.20, 47.79, 44.92, 44.10, 41.15, 32.53, 28.77, 28.59, 25.28, 12.90. HRMS calcd for $C_{40}H_{46}ClN_7O_5S$ $[M + H]^+$: 772.3042, found: 772.3043.

Synthesis of BiTClO: To a solution of **4-2** (117 mg, 0.15 mmol) dissolved in 10 mL of anhydrous CH_3CN, triphenylphosphine (398 mg, 1.5 mmol) and potassium iodide (116 mg, 1.0 mmol) were added in one portion and the reaction solution was heated to reflux for 24 h. Then, the solvent was removed under the reduced pressure and

the residue was further purified by column chromatography over silica gel eluting with CH_2Cl_2/CH_3OH = 20:1 to afford **BiTClO** as white solid. Yield: 141 mg, 83.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.89–7.81 (m, 4H), 7.74–7.65 (m, 6H), 7.58-7.53 (m, 8H), 6.99 (d, *J* = 7.4 Hz, 1H), 6.65–6.46 (m, 5H), 6.38–6.32 (m, 3H), 4.92 (d, *J* = 15.0 Hz, 2H), 4.34–4.29 (m, 1H), 4.17–4.12 (m, 1H), 3.67–3.53 (m, 4H), 3.35–3.32 (m, 4H), 3.24–3.09 (m, 5H), 2.82 (dd, *J* = 12.4, 5.0 Hz, 1H), 2.58 (d, *J* = 12.4 Hz, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.65–1.36 (m, 6H), 1.10 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.06, 165.01, 164.24, 161.67, 152.99, 152.81, 152.28, 151.76, 148.89, 135.14, 133.89, 133.78, 130.27, 130.14, 129.56, 129.09, 128.79, 128.34, 127.31, 123.87, 123.31, 118.11, 117.22, 112.28, 108.19, 103.29, 102.70, 97.68, 65.87, 61.81, 60.35, 58.27, 55.66, 49.07, 48.22, 45.59, 44.38, 41.24, 32.85, 28.39, 25.09, 12.76. HRMS calcd for $C_{58}H_{61}N_7O_5PS^+$ $[M]^+$: 998.4187, found: 998.4133.

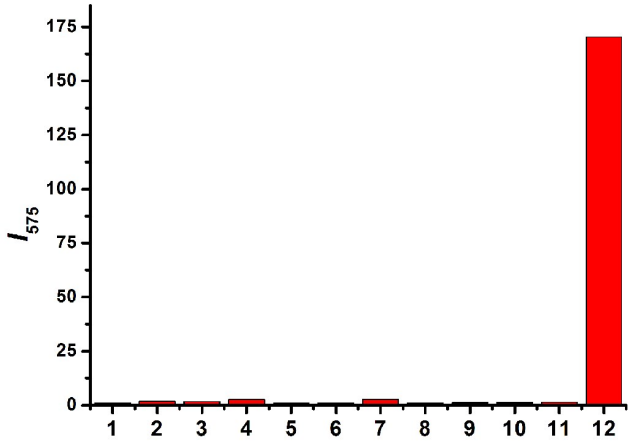


Figure S1. Fluorescence spectra of **BiTClO** (10 μM) in the presence of different kinds of metal ions (100 μM) in PBS solution (pH 7.4, 10 mM, containing 20% EtOH) within 10 s. 1: blank; 2: Na^{+}; 3: K^{+}; 4: Mg^{2+}; 5: Fe^{3+}; 6: Cr^{3+}; 7: Hg^{2+}; 8: Pb^{2+}; 9: Ca^{2+}; 10: Cu^{2+}; 11: Zn^{2+}; 12: ClO^{-}.

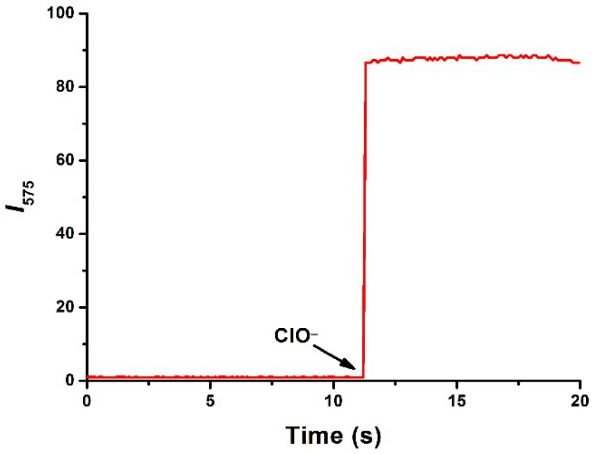


Figure S2. Temporal profile of fluorescence intensity of **BiTClO** (10 μM) after ClO^{-} (50 μM) was added.

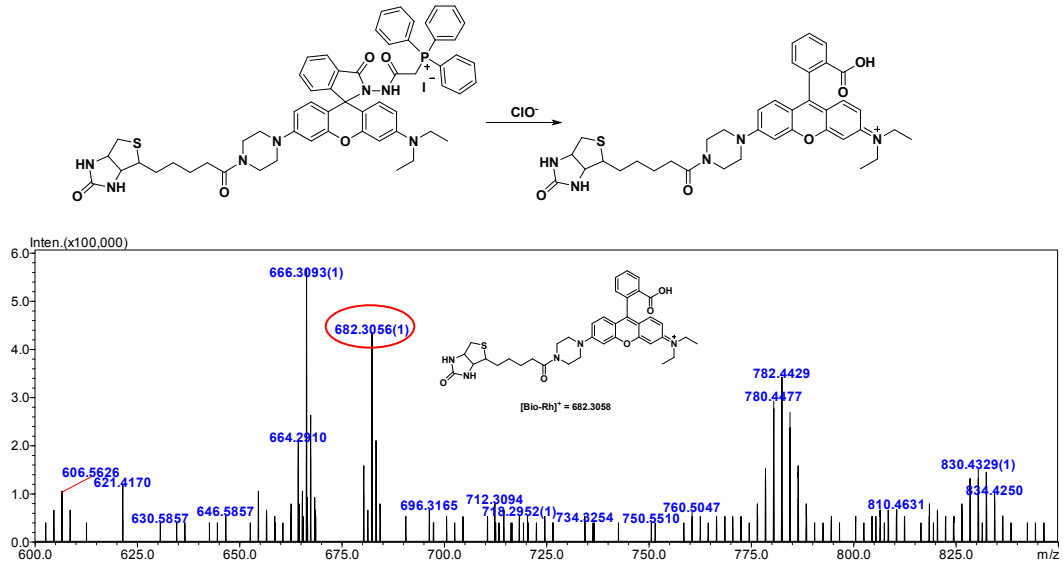


Figure S3. ESI spectra of **BiTClO** upon addition of ClO^{-}.

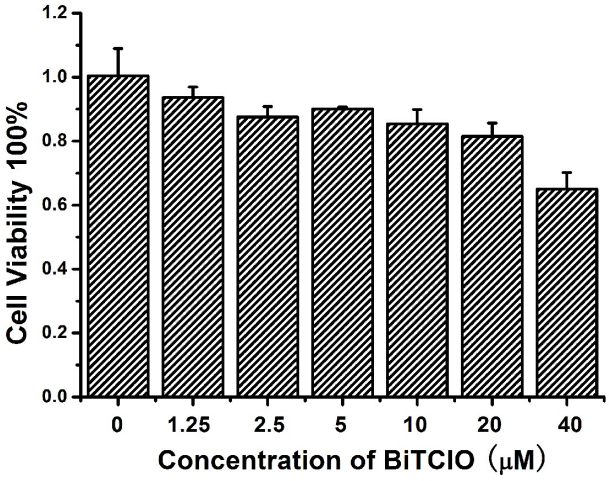
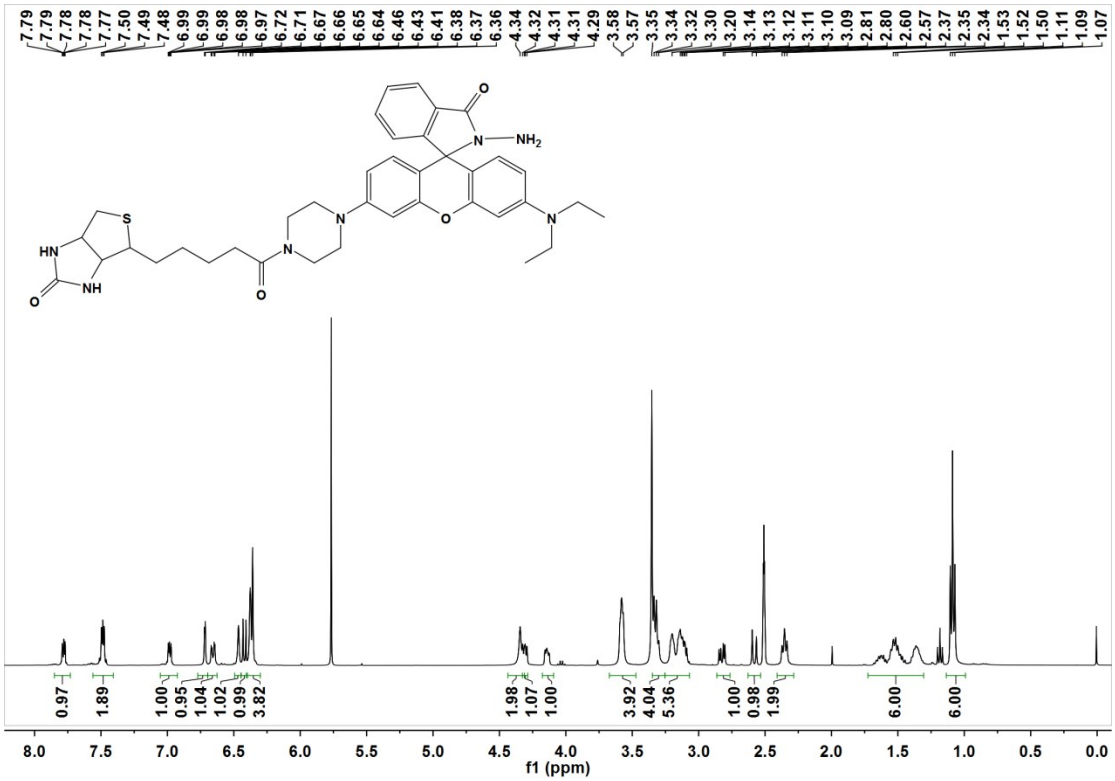
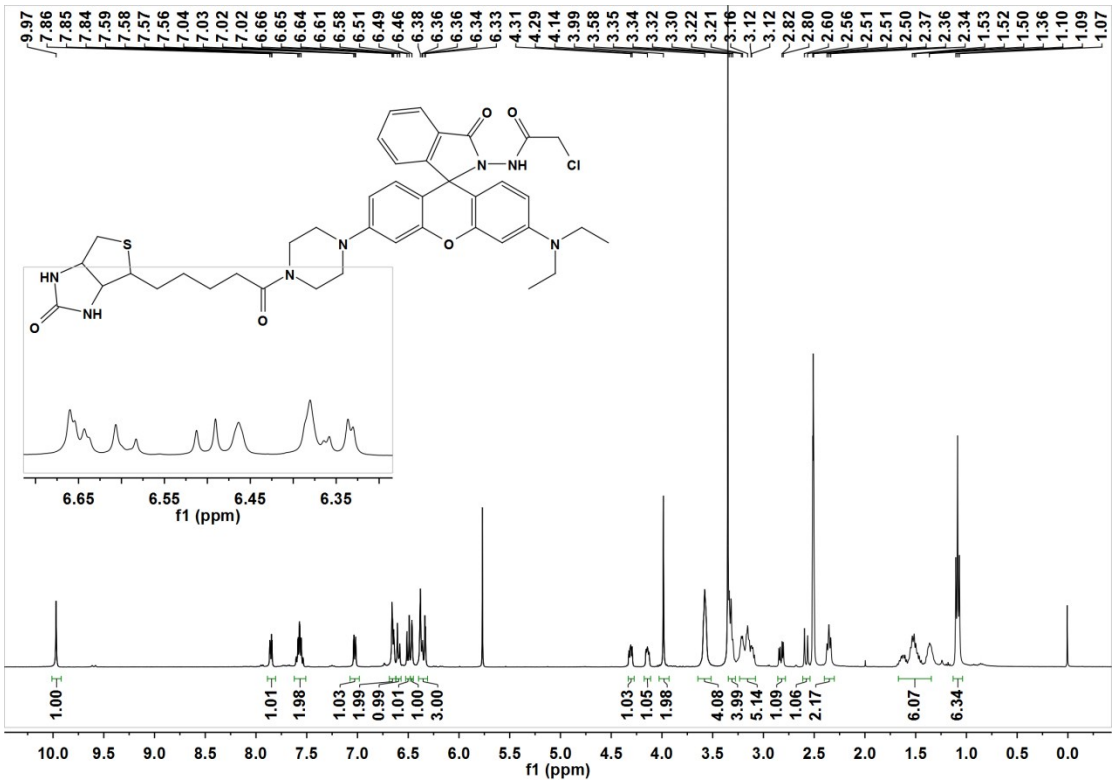


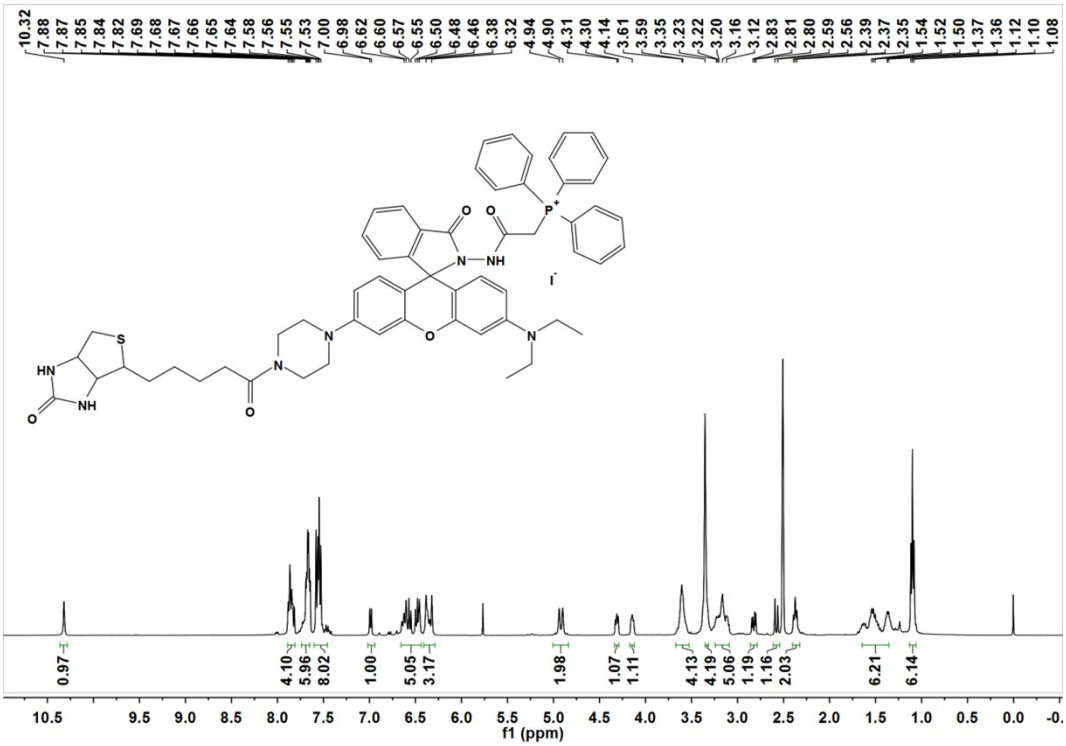
Figure S4. Effects of **BiTClO** at varied concentrations on the viability of HeLa cells. The results are the mean standard deviation of three separate measurements.



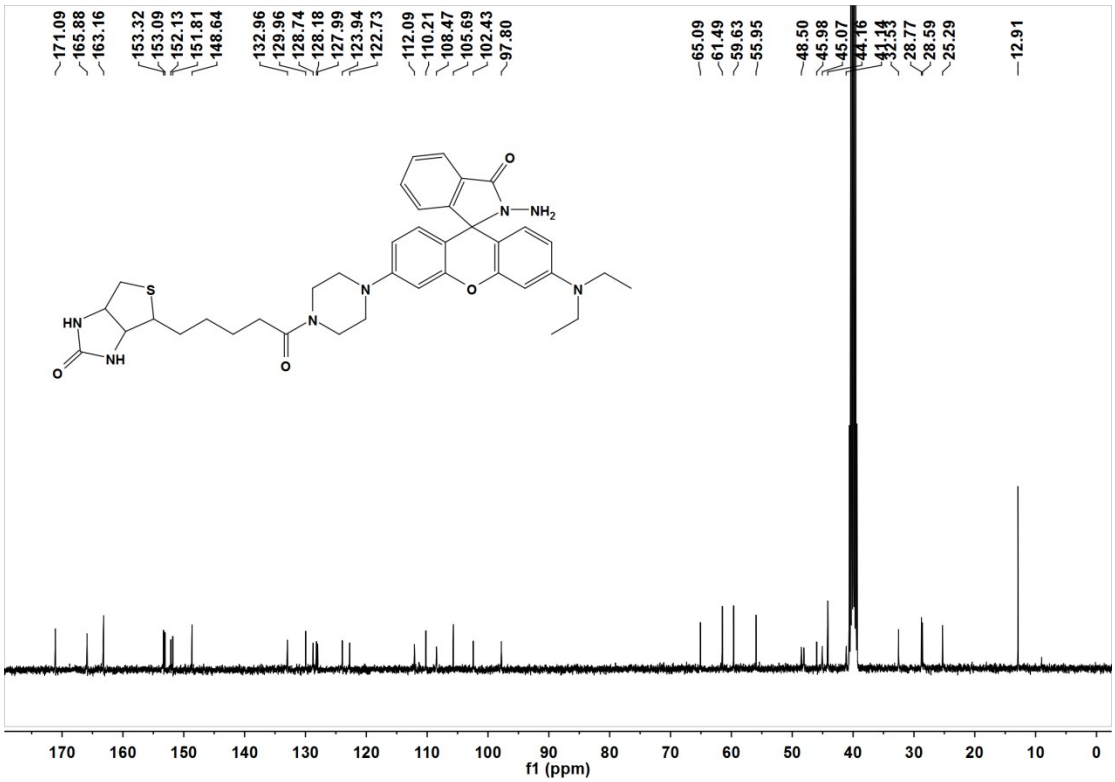
^{1}H-NMR Spectrum of **4-1** in DMSO-d6 (400 MHz)



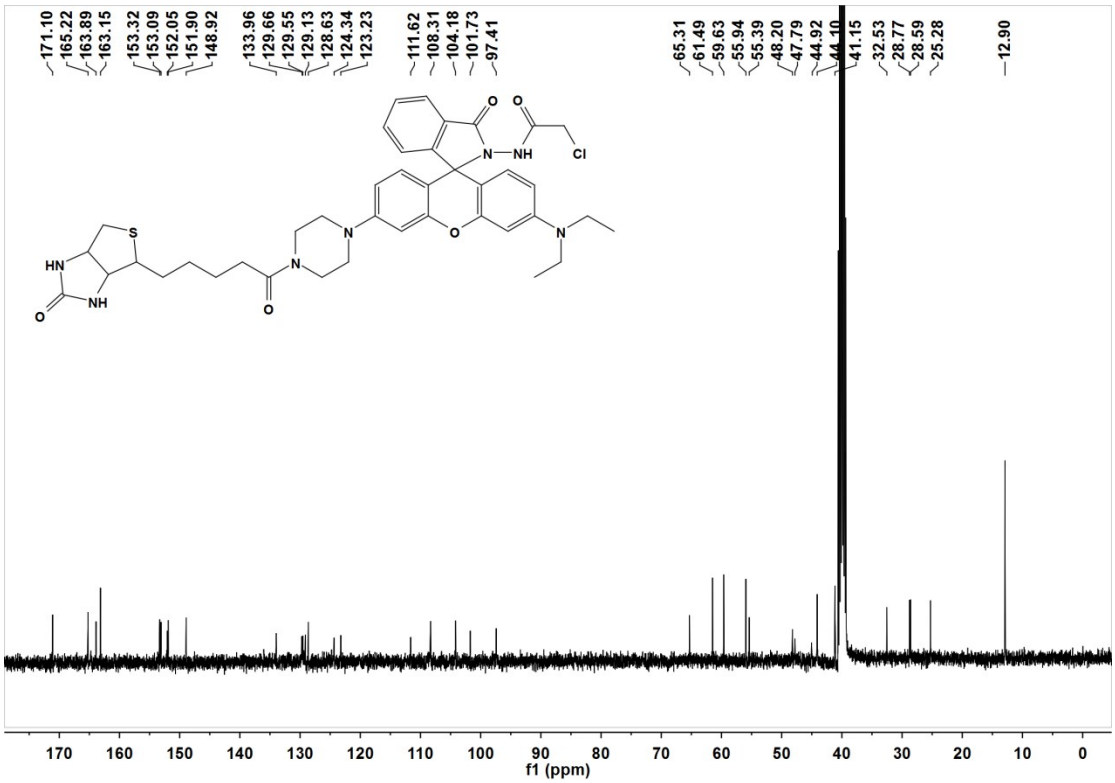
^{1}H-NMR Spectrum of **4-2** in DMSO-d6 (400 MHz)



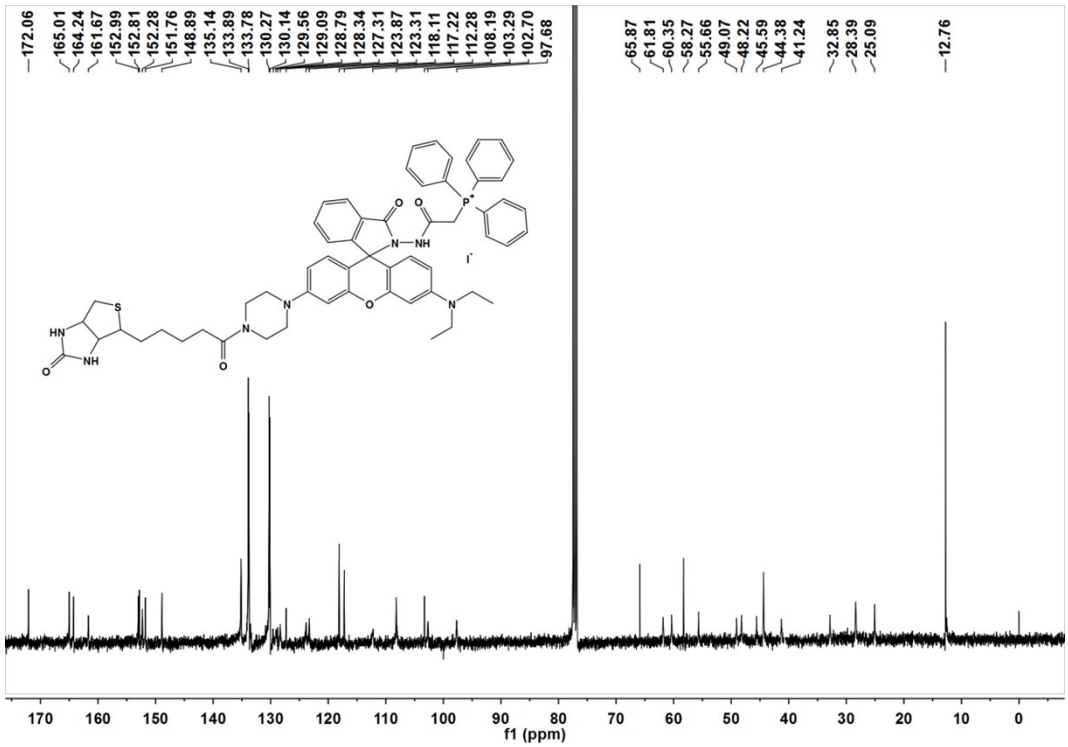
^{1}H-NMR Spectrum of **BiTClO** in DMSO-d6 (400 MHz)



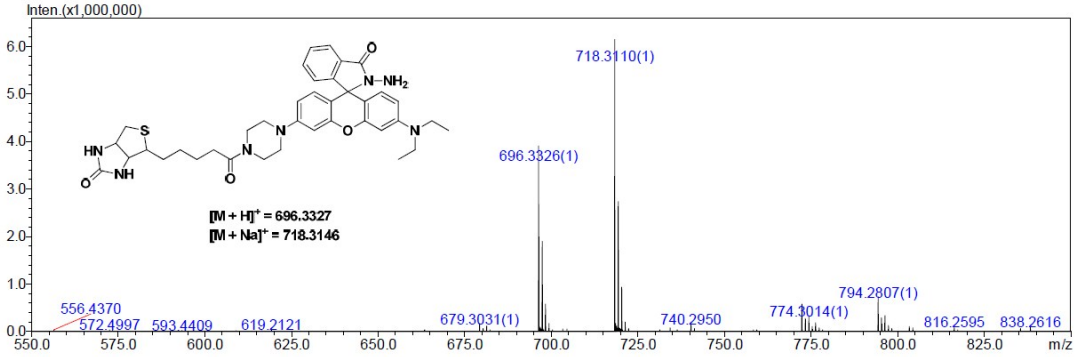
^{13}C-NMR Spectrum of **4-1** in DMSO-d6 (400 MHz)



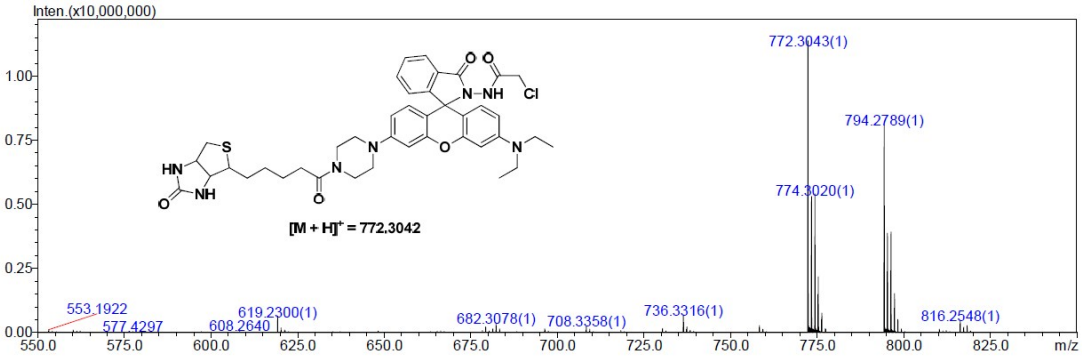
^{13}C-NMR Spectrum of **4-2** in DMSO-d6 (400 MHz)



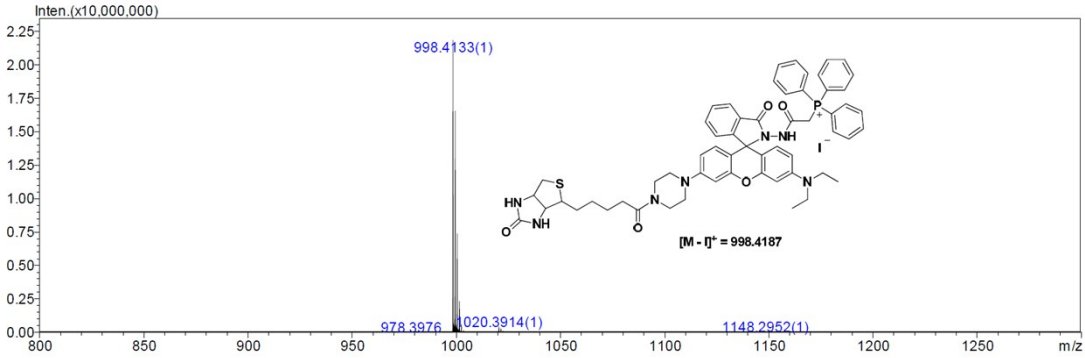
^{13}C-NMR Spectrum of **BiTClO** in DMSO-d6 (400 MHz)



HRMS spectra of **4-1**



HRMS spectra of **4-2**



HRMS spectra of **BiTClO**