A Coumarin-based Fluorescent Probe for Differential Identification of Sulfide and Sulfite in CTAB Micelle Solution

Haiyu Tian,[†] Junhong Qian,[*,†] Qian Sun,[†] Chenjia Jiang,[†] Runsheng Zhang,[‡] and Weibing Zhang[*,†]

[†]Shanghai Key Laboratory of Functional Materials Chemistry, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, China.

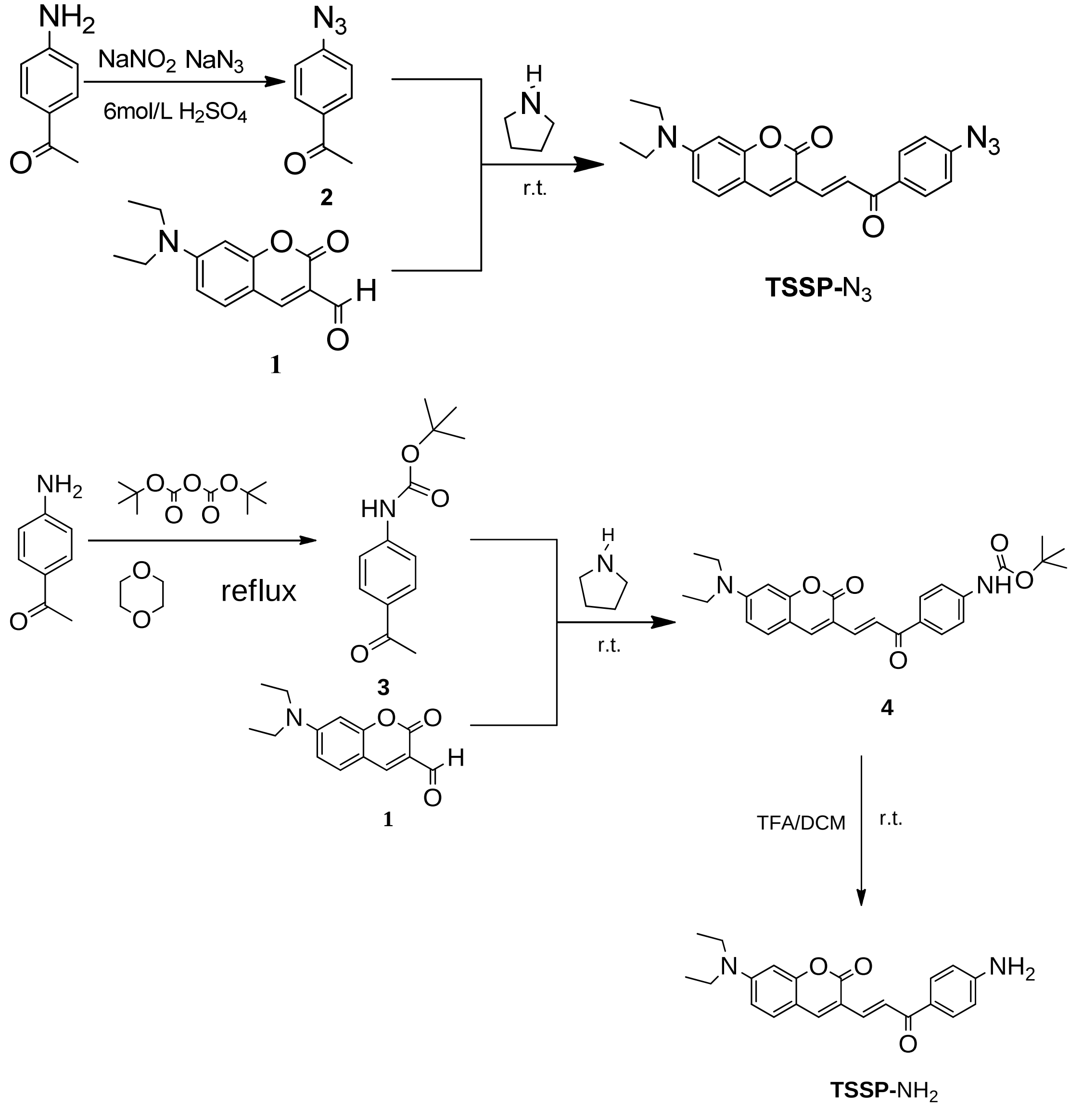
[‡]Shanghai Key Laboratory of Scene Material Evidence, Center of Material Evidence Identification, Shanghai Public Security Bureau, Shanghai, 200083, China.

junhongqian@ecust.edu.cn; weibingzhang@ecust.edu.cn

Supporting Information

Contents

Synthesis of the TSSP-N_3 and TSSP-NH_2. The synthesis procedures of **TSSP**-N_3 **and TSSP**-NH_2 are shown in Scheme 1. The experimental details are described as the following.



Scheme 1. Synthesis procedures of **TSSP**-N_3 and **TSSP**-NH_2.

Synthesis of Compound 2. At 0-5°C, $NaNO_2$ aqueous solution (8.0 mL, 7.4 mmol) was dropwise added to a solution of 4-aminoacetophenone (500 mg, 3.77 mmol) in 20 mL H_2SO_4 (6 mol/L); and the resulting solution was stirred for 30 min. 5 mL NaN_3 aqueous solution (7.4 mmol) was dropwise added to the above solution. After stirred for 12 hour at room temperature, the solvent was evaporated under vacuum, then the crude product was purified with column chromatography (PE / DCM = 6:1) to give **2** as faint yellow solid (yield: 94%).^{1}H NMR (400 MHz, $CDCl_3$): 7.97 (d, J = 8.47 Hz, 2H), 7.09 (d, J = 8.47 Hz, 2H), 2.59 (s, 3H).

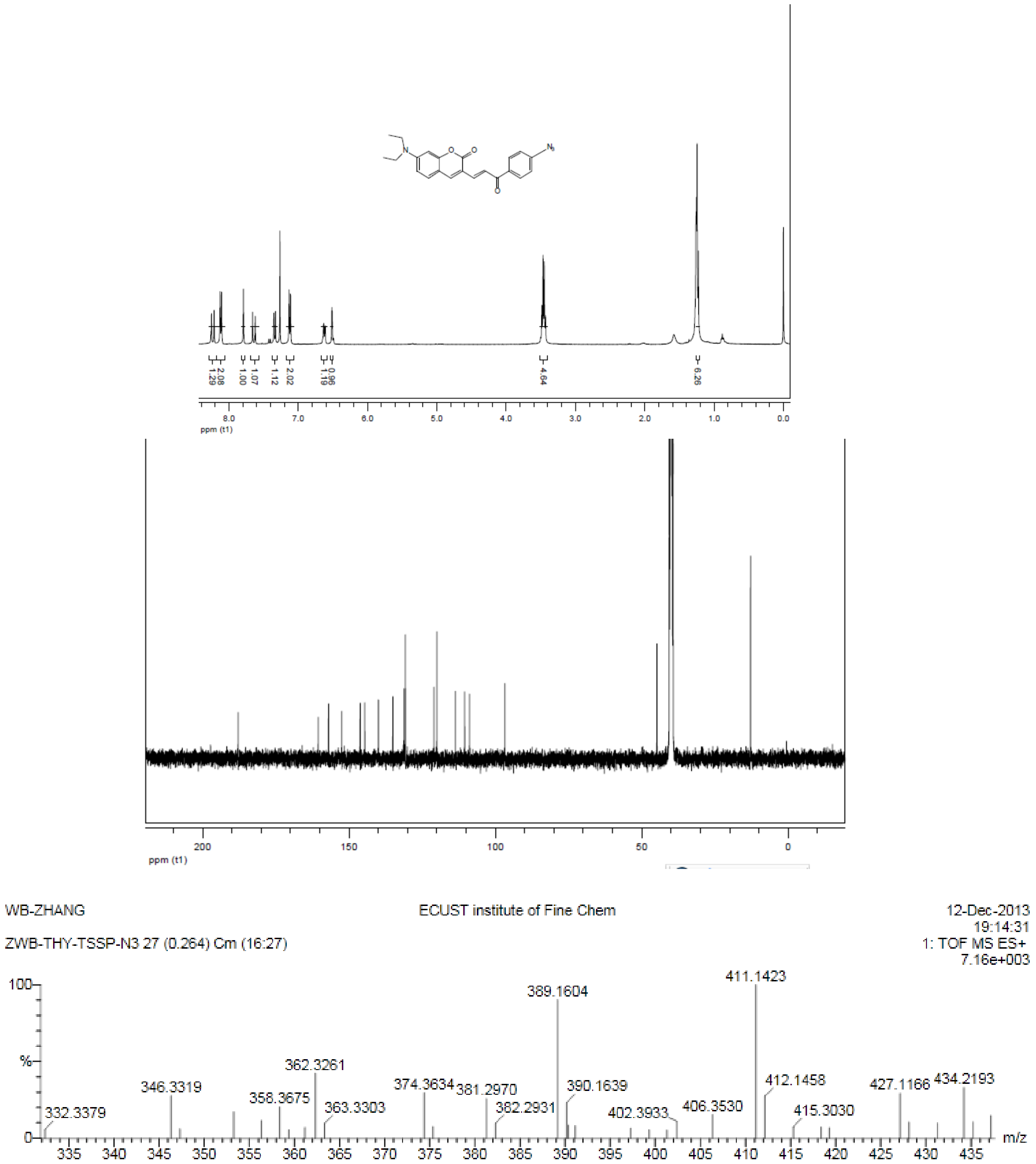
***TSSP-**N_3* was synthesized according to the reference[10]: 100 mg (0.4 mmol) of compound **1**, 0.72 mmol of compound **2** and five drops of pyrrolidine were added to 20 mL of EtOH/DCM (1:2, v/v). After stirred at room temperature for 48 hours, the solvent was evaporated under vacuum. The residual solid was purified with column chromatography (PE : DCM = 3:1) to afford **TSSP-**N_3 as salmon solid (yield: 45%).^{1}H NMR (400 MHz, $CDCl_3$): 8.23 (d, J = 15.20 Hz, 1H), 8.12 (d, J = 8.51 Hz, 2H), 7.79 (s, 1H), 7.64 (d, J = 15. 19 Hz, 1H), 7.34 (d, J = 8.89 Hz, 1H), 7.12 (d, J = 8.52 Hz, 2H), 6.62 (dd, J = 5.55 Hz, 1H), 6.51 (d, J = 1.93 Hz, 1H), 3.47 (q, J = 7.08 Hz, 4H), 1.24 (t, J = 7.09 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.8, 160.5, 157.0, 152.4, 146.2, 144.6, 139.9, 135.0, 131.2, 130.7, 120.9, 119.9, 113.6, 110.5, 108.8, 96.7, 44.8, 12.9; ESI (HR): *m/z* 411.1423 $(M+Na)^+$, calculated 411.1433 for $(M+Na)^+$.

Synthesis of Compound 3. The solution of 4-aminoacetophenone (900 mg, 6.66 mmol) and di-*tert*-butyl dicarbonate (1.89 g, 8.70mmol) in dioxane (20 mL) was refluxed for 12 hours and cooled to room temperature. After removal of the solvent, the residual solid was purified with column chromatography (PE / DCM = 4:1) to give **3** as white solid (yield: 91%).^{1}H NMR (400 MHz, $CDCl_3$): 7.92 (d, J = 8.69 Hz, 2H), 7.45 (d, J = 8.66 Hz, 2H), 6.70 (s, 1H), 2.57 (s, 3H), 1.53 (s, 9H).

Synthesis of Compound 4. 100 mg (0.4 mmol) of compound **1**, 0.72 mmol of compound **3** and five drops of pyrrolidine were added to 20 mL of EtOH/DCM (1:2, v/v). After stirred at room temperature for 48 hours, the solvent was evaporated under vacuum. The residual solid was purified with column chromatography (PE : DCM = 4:1) to give **4** as salmon solid (yield: 40%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.23 (d, J = 15.19 Hz, 1H), 8.07 (d, J = 8.65 Hz, 2H), 7.78 (s, 1H), 7.63 (d, J = 15. 20 Hz, 1H), 7.48 (d, J = 8.59 Hz, 2H), 7.34 (d, J = 8.92 Hz, 1H), 6.68 (s, 1H), 6.62 (dd, J = 6.63 Hz, 1H), 6.51 (d, J = 2.05 Hz, 1H), 3.47 (q, J = 7.03 Hz, 4H), 1.54 (s, 9H) , 1.23 (t, J =

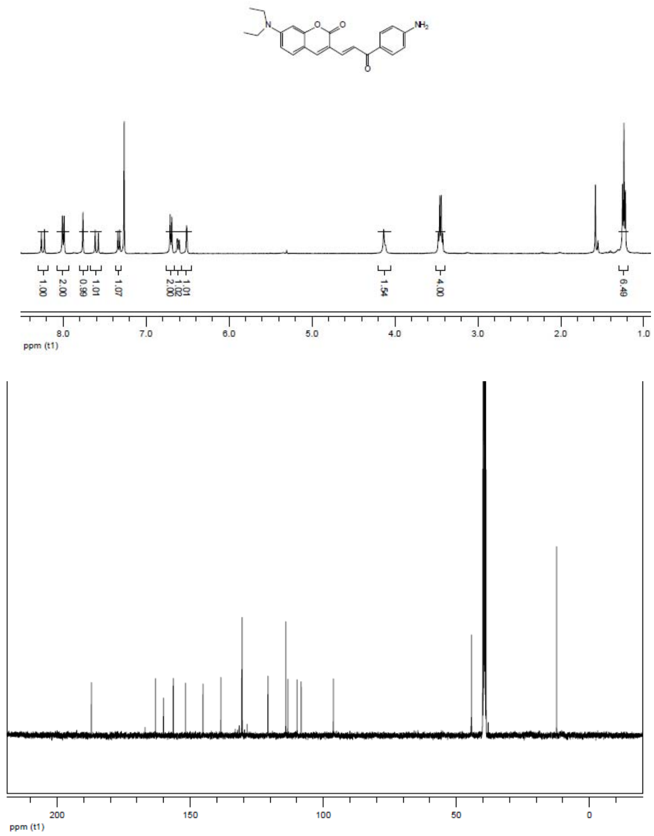
7.07 Hz, 6H).

*Synthesis of **TSSP**-NH$_2$.* Trifluoroacetic acid (10 µL) was added to a solution of **4** (30 mg, 65 µmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for one hour. The solution was concentrated to dryness and the residue was purified with column chromatography (PE : DCM = 2:1) to give **TSSP**-NH$_2$ as salmon solid (yield: 90%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.24 (d, J = 15.22 Hz, 1H), 8.00 (d, J = 8.43 Hz, 2H), 7.76 (s, 1H), 7.59 (d, J = 15. 26 Hz, 1H), 7.33 (d, J = 8.86 Hz, 1H), 6.70 (d, J = 8.43 Hz, 2H), 6.61 (dd, J = 5.45 Hz, 1H), 6.51 (d, J = 1.93 Hz, 1H), 4.13 (s, 2H), 3.47 (q, J = 7.08 Hz, 4H), 1.25 (t, J = 7.04 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.1, 163.1, 160.1, 156.4, 151.7, 145.2, 138.4, 130.6, 130.5, 130.4, 120.8, 114.1, 113.3, 109.9, 108.3, 96.2, 44.3, 12.3. ESI (HR): *m/z* 363.1702 $(M+H)^+$, calculated 363.1709 for $(M+H)^+$.

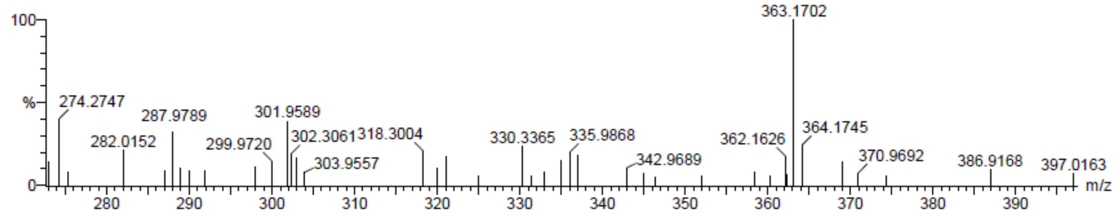


```
Minimum:                                       -1.5
Maximum:                 30.0      50.0       100.0

Mass      Calc. Mass     mDa       PPM        DBE        i-FIT      i-FIT (Norm) Formula

411.1423  411.1433       -1.0      -2.4       14.5       7.2        0.0          C22  H20  N4  O3  Na
```

Figure S1. ^{1}H NMR, ^{13}C NMR and HRMS spectra of **TSSP**-N$_3$.



Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 3 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-23   H: 0-50   N: 0-5   O: 0-3
WB-ZHANG   ECUST institute of Fine Chem   12-Dec-2013
19:25:39
ZWB-THY-TSSP-NH2 19 (0.205) Cm (12:24)   1: TOF MS ES+
9.20e+003



```
Minimum:                                 -1.5
Maximum:                 30.0     50.0   100.0

Mass        Calc. Mass   mDa      PPM    DBE      i-FIT     i-FIT (Norm) Formula

363.1702    363.1709     -0.7     -1.9   12.5     9.3       0.0          C22  H23  N2  O3
```

Figure S2. ^{1}H NMR, ^{13}C NMR and HRMS spectra of **TSSP**-NH_2.

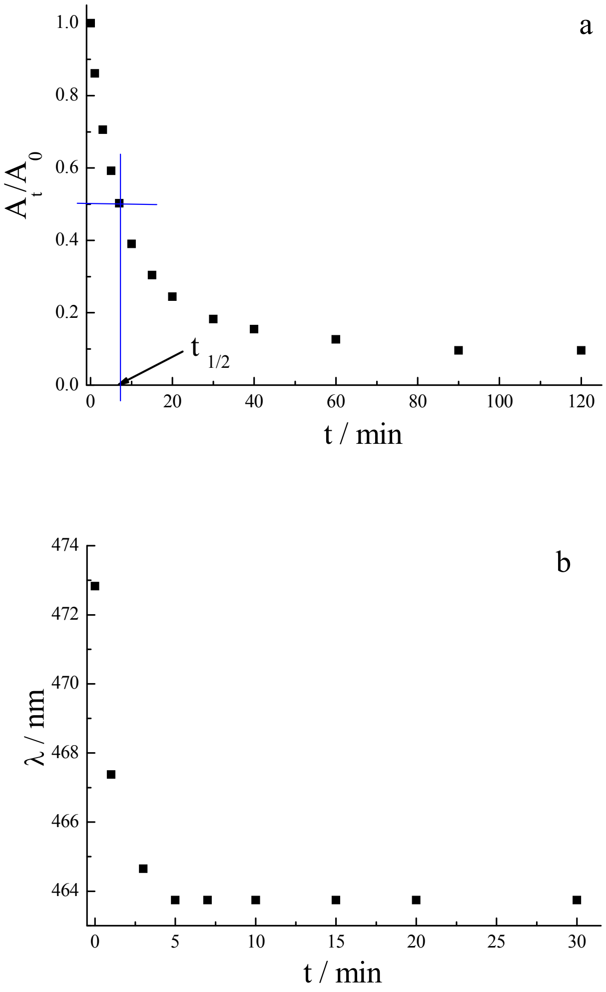
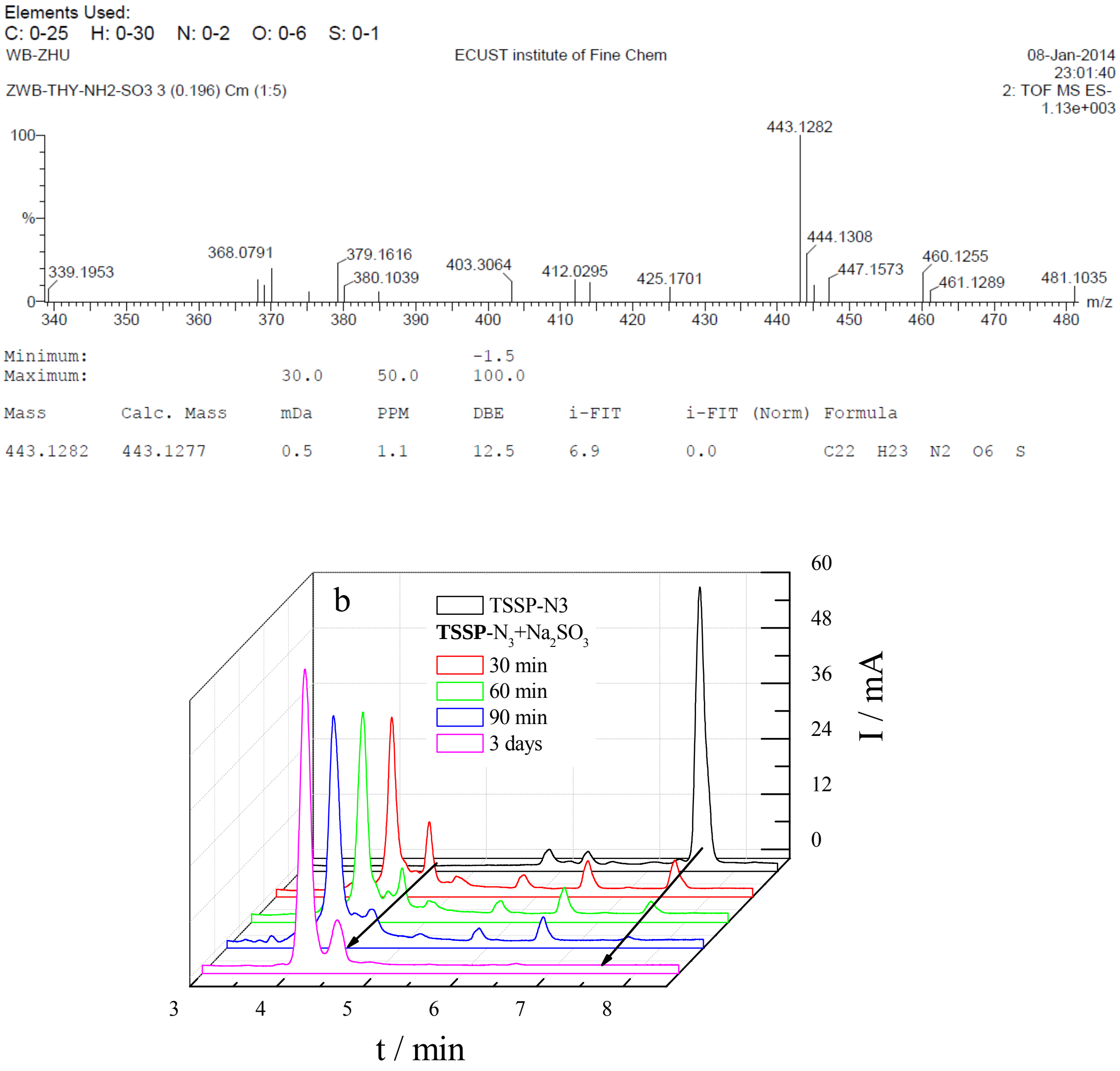


Figure S3. The A_t/A_0 plot of **TSSP**-N_3 vs time in the presence of 50 equiv. of sulfite (a) and the absorption wavelength change of **TSSP**-N_3 as a function of time in the presence of 10 equiv. of sulfide (b) in PBS-CTAB (1 mM) system. A_t and A_0 are the absorbance at 475 nm at t and 0 time, respectively. [**TSSP**-N_3**]=** 2 μM, [sulfite] = 100 μM, [sulfide] = 20 μM, 20 mM PBS, pH = 7.4, 25°C.



```
Elements Used:
C: 0-25    H: 0-30    N: 0-2    O: 0-6    S: 0-1
WB-ZHU                                   ECUST institute of Fine Chem                08-Jan-2014
                                                                                        23:01:40
ZWB-THY-NH2-SO3 3 (0.196) Cm (1:5)                                            2: TOF MS ES-
                                                                                   1.13e+003

Minimum:                                        -1.5
Maximum:                         30.0      50.0      100.0

Mass        Calc. Mass     mDa       PPM       DBE       i-FIT      i-FIT (Norm) Formula

443.1282    443.1277       0.5       1.1       12.5      6.9        0.0          C22  H23  N2  O6  S
```

Figure S4. HR-MS and HPLC spectra of the reaction solution between **TSSP**-N_3 and sulfite (A solution of 31 mg Na_2SO_3 in 2 mL H_2O was added dropwise to a solution of 10 mL EtOH containing 15 mg of **TSSP**-N3. The mixture was refluxed for three days), detected at 410 nm.

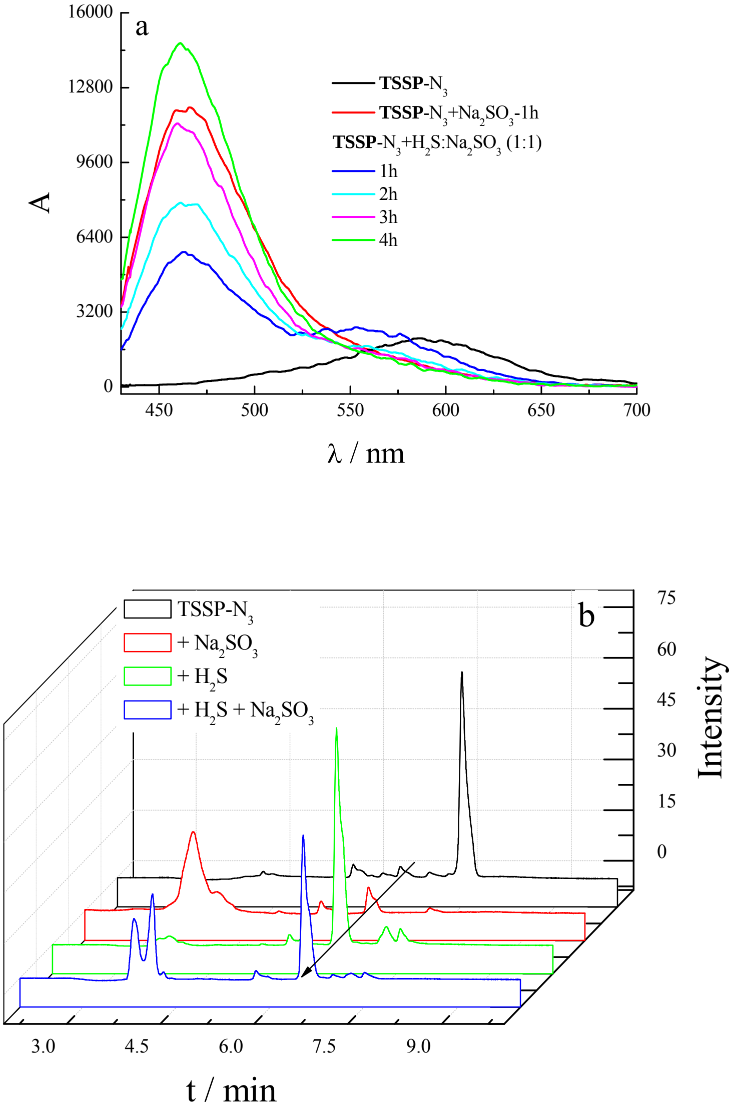


Figure S5. Time-dependent emission spectra of **TSSP**-N_3 coexisted with 1:1 sulfide:sulfite (a), and HPLC spectra (b) of the mixtures of **TSSP**-N_3 with sulfite (red), sulfide (green), sulfite:sulfide (1:1, blue) after 1h in PBS-CTAB (1 mM) system. [**TSSP**-N_3**]** = 15 μM, [sulfide]= [sulfite] = 750 μM. 20 mM PBS, pH = 7.4, detection wavelength = 410 nm, 25°C.

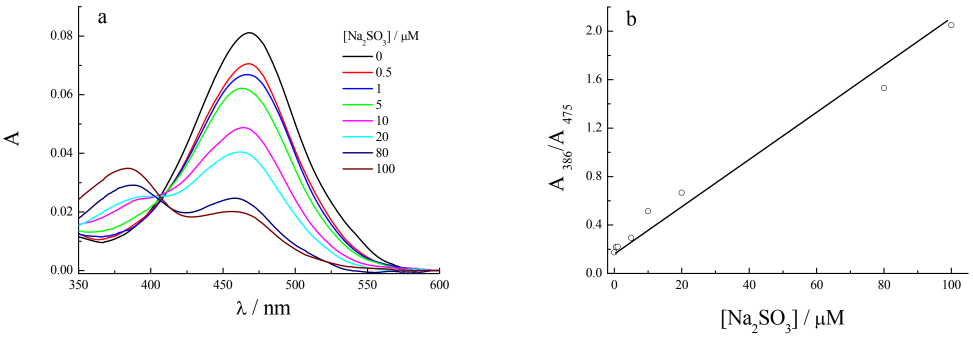


Figure S6. The absorption spectra(a) of **TSSP**-N_3 with different concentrations of sulfite after 1 h, and the ratio of absorbances at 386 nm and 475 nm (b, A_{386}/A_{475}) as a function of sulfite concentration. 20 mM PBS, pH 7.4, 25 °C, [CTAB] = 1 mM, [**TSSP**-N_3] = 2 μM.

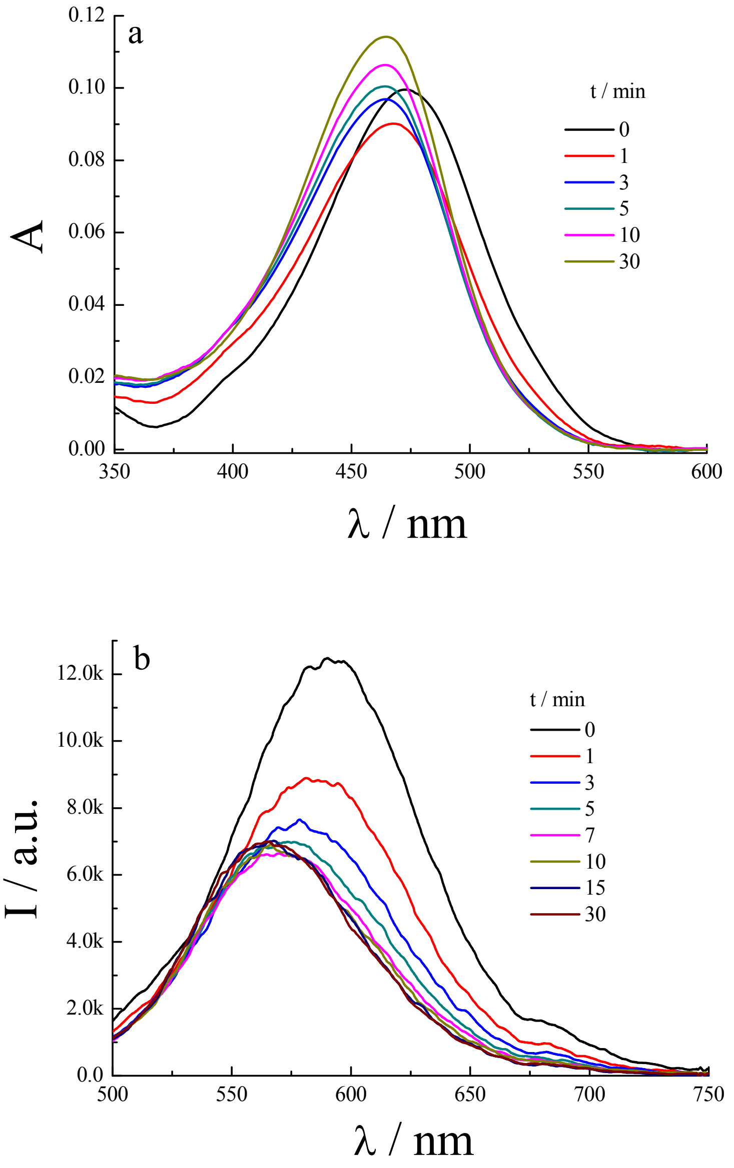


Figure S7. Time-dependent absorption and emission spectra of **TSSP**-N_3 in PBS-CTAB (1 mM) system. [**TSSP**-N_3] = 2 μM, [sulfide] = 20 μM, λ_{ex} = 410 nm, 20 mM PBS, pH = 7.4, 25°C.

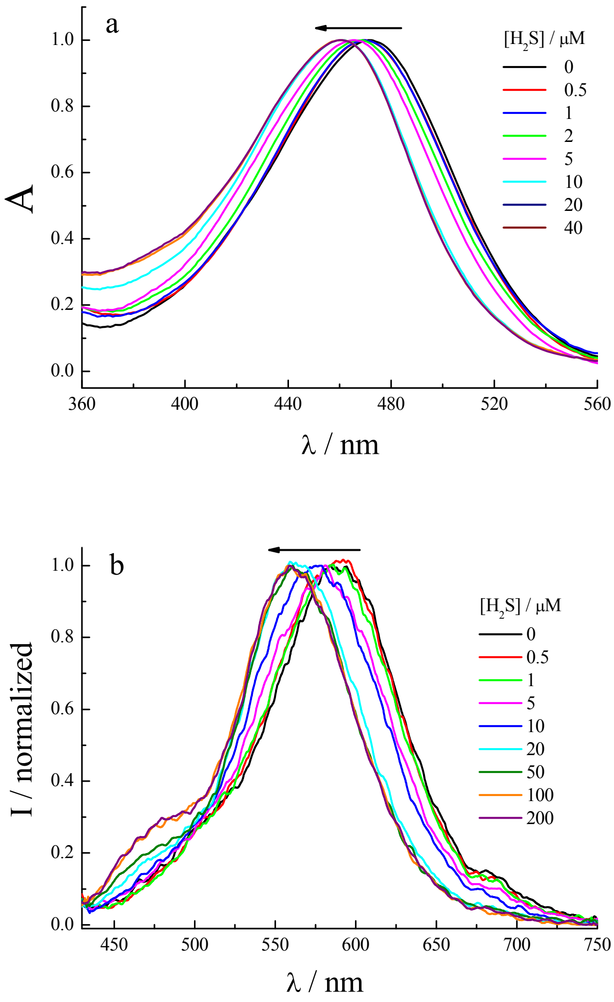


Figure S8. Normalized absorption (a) and emission (b) spectra of **TSSP**-N_3 (2 μM) in the presence of different concentrations of sulfide after 10 min in PBS-CTAB (1 mM) system. 20 mM PBS, pH 7.4, 25°C, excited at 410 nm.

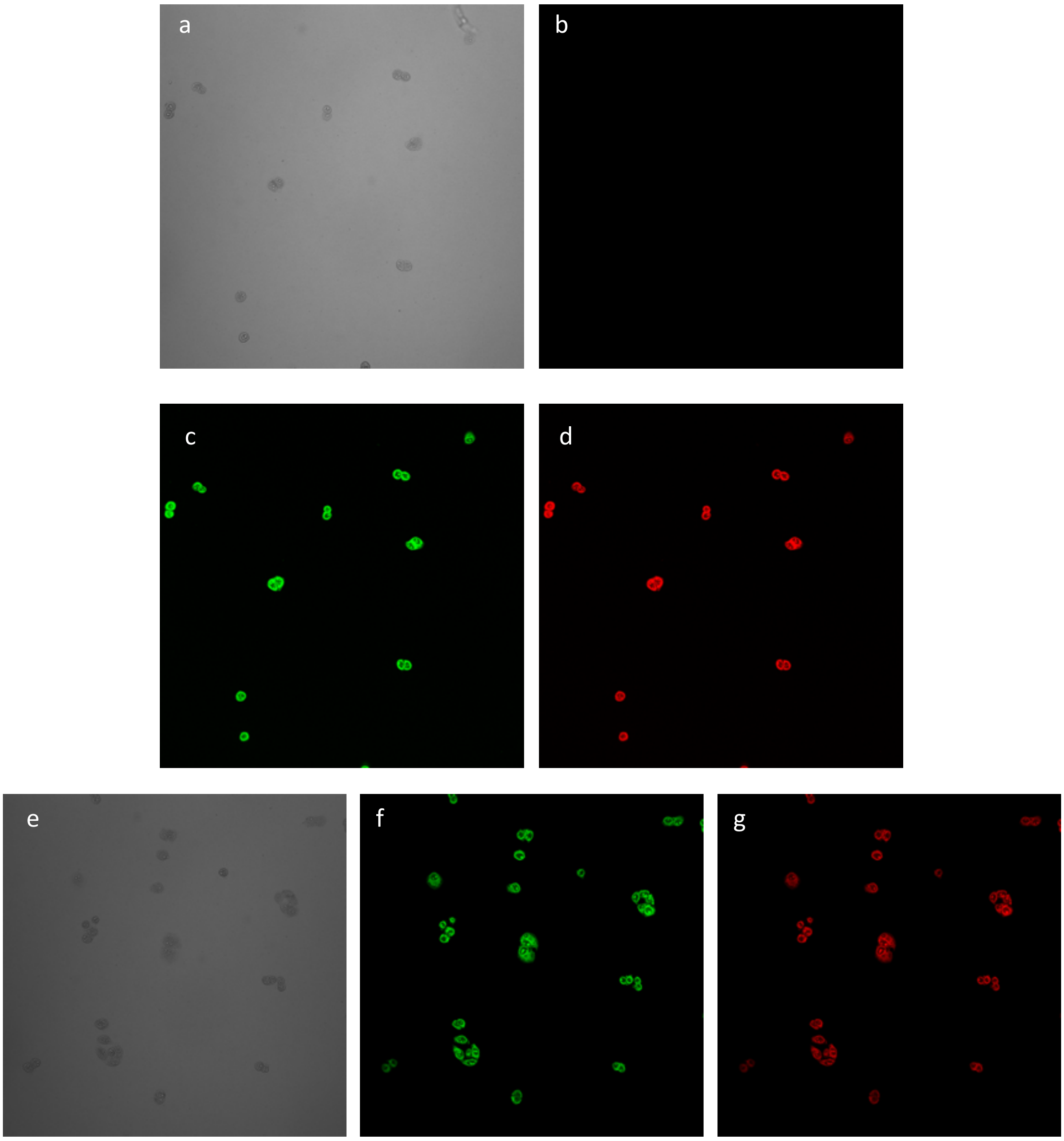


Figure S9. Confocal fluorescence (b-d, f-g) and bright-field (a, e) images of living HeLa cells incubated with **TSSP**-N_3 (5 μM) and CTAB (1 mM) for 60 min. (a-d): excited at 404 nm; (e-g): excited at 488 nm; (b): blue channel; (c, f): green channel; (d, g): red channel.

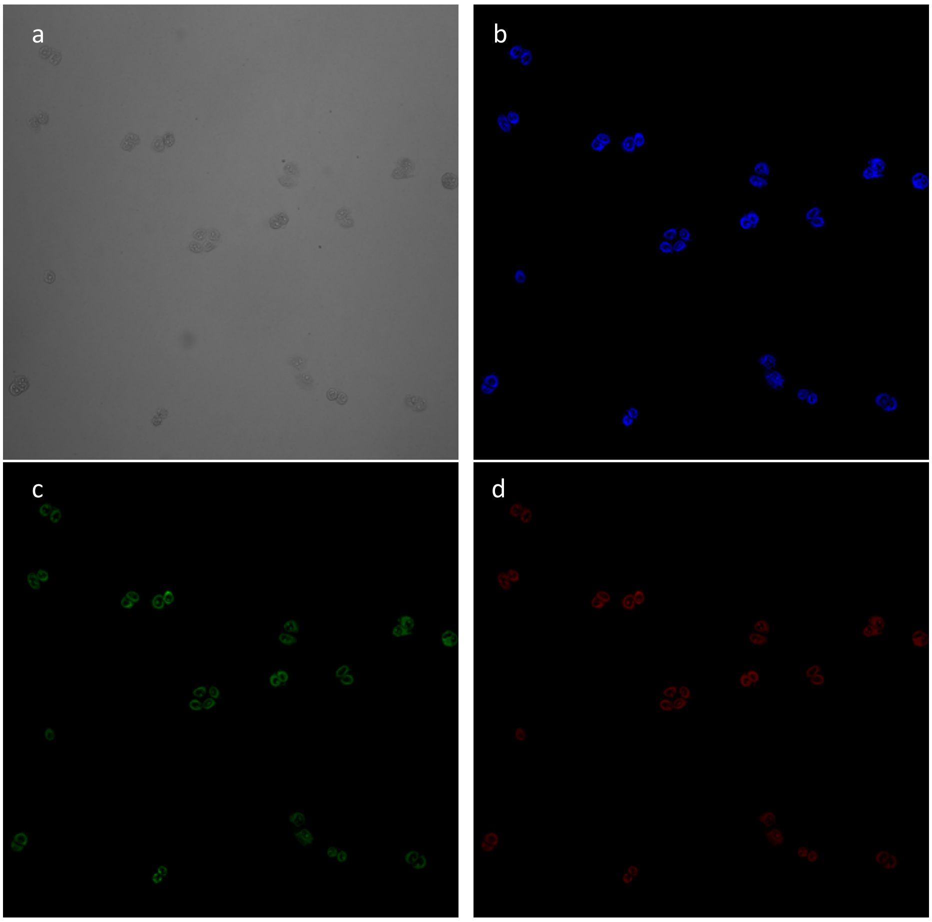


Figure S10. Confocal fluorescence (b-d) and bright-field (a) images of living HeLa cells preincubated with 100 μM sulfite followed by incubated with **TSSP**-N_3 (5 μM) and CTAB (1 mM) for 60 min. Excited at 404 nm; (b): blue channel; (c): green channel; (d): red channel.

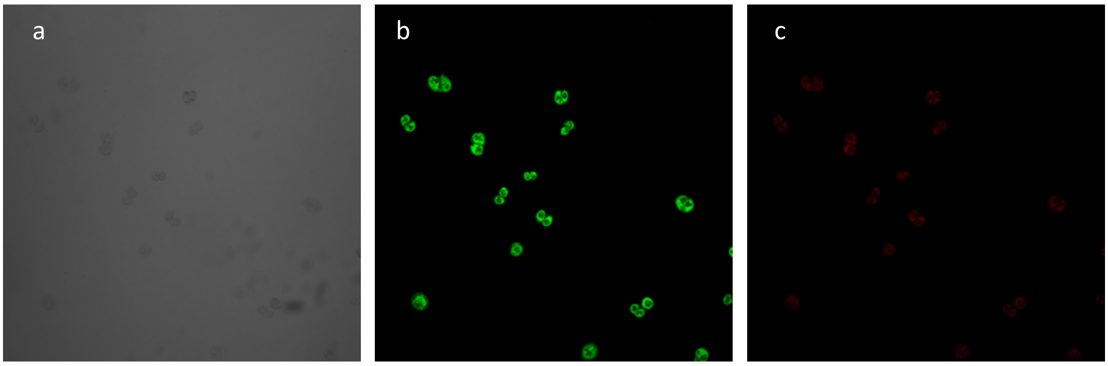


Figure S11. Confocal fluorescence (b-c) and bright-field (a) images of living HeLa cells incubated with **TSSP**-N_3 (5 μM) and CTAB (1 mM) for 60 min pretreated with 100 μM sulfide. Excited at 488 nm; (b): green channel; (c): red channel.