Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A ratiometric fluorescent dye for detection of Lys and Arg and its bioimaging in live cells and zebrafish larvae

Tongxin Zhang [a,b], Zhengfei Yang [c], Xiaoyan He [a], Luxin Guo [a], Jincheng Wang [a], Xuewen Jiang *[d], Ruling Shen *[c], Xiuhong Lu *[a,b]

a. Shanghai Key Laboratory of Molecular Imaging, Jiading District Central Hospital Affiliated Shanghai University of Medicine and Health Sciences, Shanghai 201318, China.

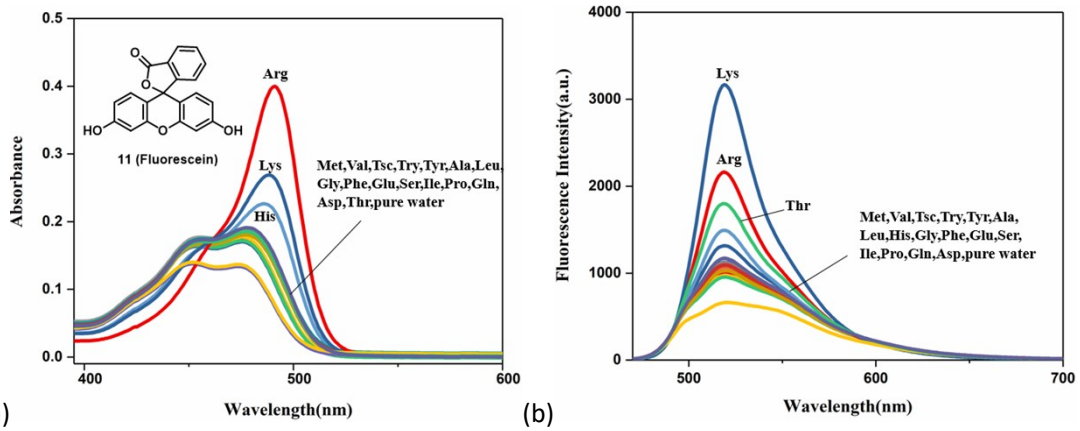
b. College of pharmacy, Shanghai University of Medicine and Health Sciences, Shanghai 201318, China.

c. Shanghai Laboratory Animal Research Center, Shanghai 201203, China.

d. Department of Nuclear Medicine, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Shandong, Yantai 264000, China.

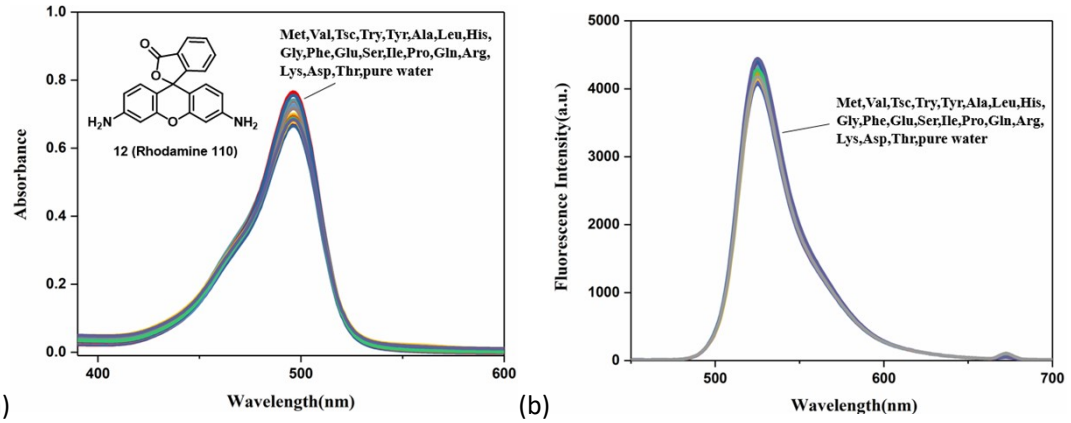
Contents:

1. Spectroscopic studies and cell images



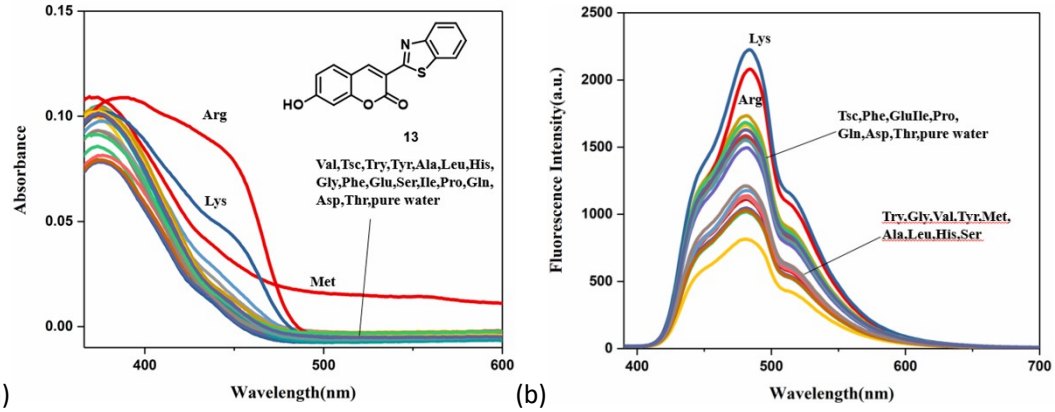
(a) (b)

***Figure S1*.** UV-vis and fluorescence spectra (λ_{ex} = 460 nm, slit: 5 nm/5 nm) of **11** (Fluorescein, 10 µmol) upon addition of different biological analytes (100 µmol) in DMSO/water (1/50, v/v) medium at room temperature. a) UV-vis spectra and b) Fluorescence spectra recorded after analytes were added immediately.



(a) (b)

***Figure S2*.** UV-vis and fluorescence spectra (λ_{ex} = 336 nm, slit: 5 nm/5 nm) of **12** (Rhodamine 110, 10 µmol) upon addition of different biological analytes (100 µmol) in DMSO/water (1/50, v/v) medium at room temperature. a) UV-vis spectra and b) Fluorescence spectra recorded after analytes were added immediately.



(a) (b)

***Figure S3*.** UV-vis and fluorescence spectra (λ_{ex} = 375 nm, slit: 5 nm/5 nm) of **13** (10 µmol) upon

addition of different biological analytes (100 μmol) in DMSO/water (1/50, v/v) medium at room temperature. a) UV-vis spectra and b) Fluorescence spectra recorded after analytes were added immediately.

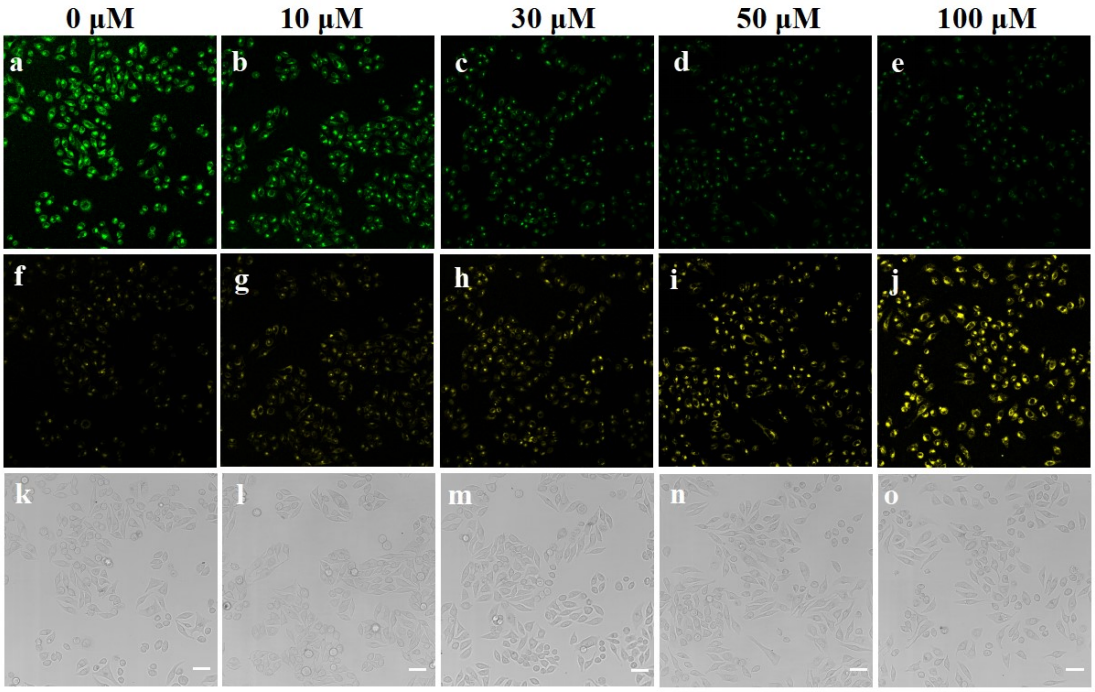


Figure S4. Confocal microscopy images of 10 μM **Dye IDE** in HepG2 cells at different concentrations of Arg from 0 μM to 100 μM. The green channel images were collected at 490-540 nm with excitation at 405 nm and yellow channel images were collected at 550-600 nm with excitation at 488 nm.

Table S1. pH values of test solution after different biological analytes (100 μM) were added.

biological analytes	pH	biological analytes	pH
Arg	8.63	Gly	6.16
Lys	7.57	Phe	6.06
His	6.60	Glu	4.31
Met	6.07	Ser	6.38
Val	6.20	Ile	5.97
Tsc	6.26	Pro	6.28
Try	6.11	Gln	6.60
Tyr	5.95	Asp	4.02
Ala	6.21	Thr	6.37
Leu	6.17	Pure water	6.25

Table S2. pH values of test solution after different concentration of basic amino acids were added.

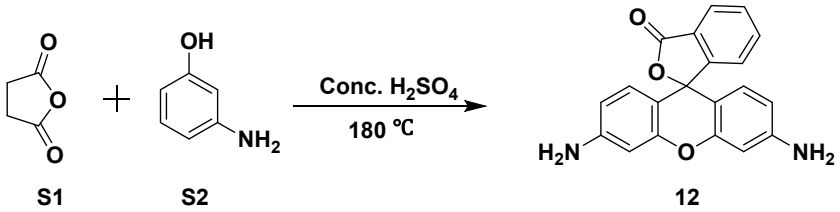
Concentration (μM)	pH (Lys)	pH (Arg)	pH (His)

0	6.28	6.23	6.24
10	6.30	6.29	6.26
30	6.70	6.51	6.31
50	6.98	7.52	6.38
100	7.57	8.63	6.60
200	8.71	9.20	6.82
300	8.98	9.38	6.94

2. Synthetic procedures of compounds 12 (Rhodamine 110), 13, 14 (Dye IDE)

2.1 Synthetic procedures of compound 12 (Rhodamine 110).

Compound **12** (Rhodamine B) was prepared by the modified literature method (Scheme S1). [1]

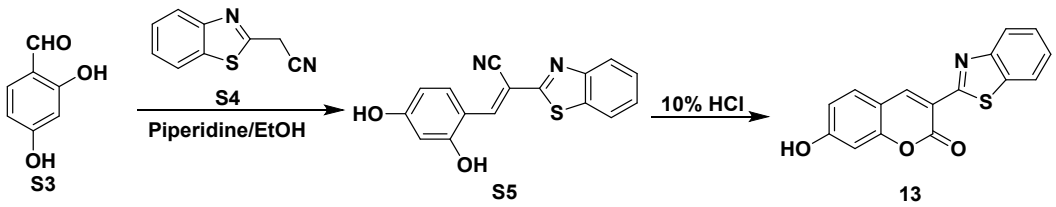


Scheme S1. The synthetic route of compound **12** (Rhodamine 110).

To a 250 mL three neck round bottom flask, Phthalic anhydride (S1, 5.00 g, 33.76 mmol, 1.0 equiv) and 3-aminophenol (S2, 6.25 g, 57.27 mmol, 1.7 equiv) were suspended in conc. sulfuric acid (98%, 25 mL) and heated to 180 °C for 5 hours. After cooling to room temperature, the sticky black liquid was carefully added to crushed ice (100 g) and divided to 4 centrifuge tubes, and centrifuged at 3000 rpm for 5 minutes. The brownish solid was washed with water (15 mL), then the solid was suspended by water (20 mL) and conc. ammonia solution (5 mL). This mixture was allowed to stand for 12 hours at 4 °C. After centrifugation the solid was combined and refluxed for 5 minutes in hydrochloric acid (0.3 M, 400 mL) and hot filtrated. The filtrate was allowed to stand for 48 hours at 4 °C, the brownish-red solid filtered and dried to yield rhodamine 110 (compound 12) (2.6 g, 7.87 mmol, 23%).
Brick red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.17 (dd, *J* = 6.4 Hz, 1H), 7.98 (br, 4H), 7.78 (dtd, *J* = 6.4, 7.5, 1.4 Hz, 2H), 7.40 (dd, *J* = 7.5, 1.3 Hz, 1H), 6.95 (d, *J* = 9.1 Hz, 2H), 6.83 – 6.71 (m, 4H). ESI-MS m/z calcd for $C_{20}H_{14}N_2O_3$ $[M+H]^+$, 331.3; found: 331.1.

2.2 Synthetic procedures of compound 13.

Compound **13** was prepared by the modified literature method (Scheme S2). [2]

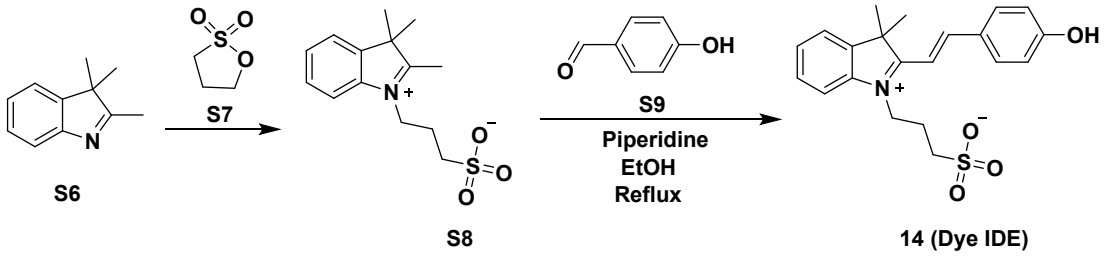


Scheme S2. The synthetic route of compound **13**.

To a 250 mL three neck round bottom flask, 2, 4-dihydroxybenzaldehyde (S3, 1.57 g, 11.4 mmol, 1.0 equiv.) and 2-(1,3-benzo-thiazol-2-yl)acetonitrile (S4, 2.0 g, 11.4 mmol, 1.0 equiv.) were dissolved in ethanol (50 mL) at room temperature, and 25 drops of piperidine were then added. The mixture was stirred at room temperature overnight. After filtration, the yellow solid was treated with hydrochloric acid (10%, 50 mL). The suspended solution was heated to 100 °C and stirred overnight. The resulting yellow residue was collected by filtration, washed with water, dried under reduced vacuum, and then purified by silica gel column chromatography (dichloromethane/ethanol = 5:1, V/V) to afford compound **13** (2.2 g, 65%).
Orange solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): DMSO): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.16 (s, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 8.05 (d, *J* = 8.1 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 6.94 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.88 (d, *J* = 2.2 Hz, 1H). ESI-MS m/z calcd for $C_{16}H_9NO_3S$ $[M+H]^+$ 296.3; found: 296.1.

2.3 Synthetic procedures of compound 14 (Dye IDE).

Compound **13** (**Dye IDE**) was prepared by the modified literature method (Scheme S3). [3]



Scheme S3. The synthetic route of compound 14 **Dye IDE**.

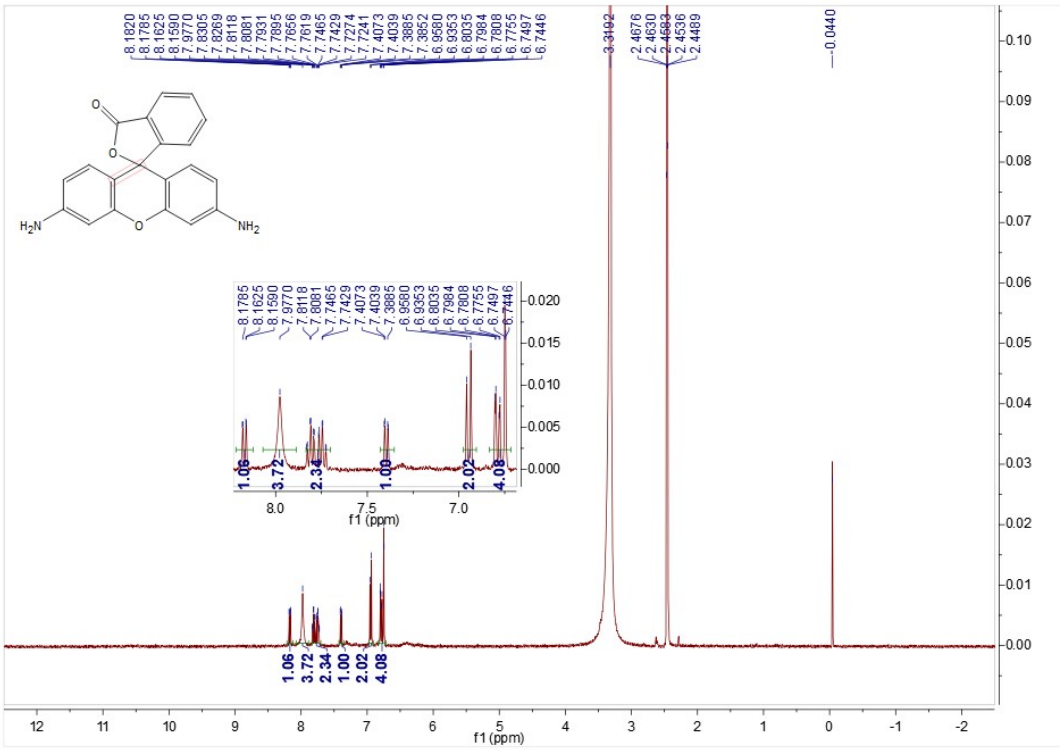
To a 250 mL three neck round bottom flask, 2,3,3-trimethylindolenine (S6, 5.0 g, 31 mmol, 1.0 equiv.) and 1,3-propane sultone (S7, 5.8 g, 47 mmol, 1.5 equiv.) were dissolved in toluene (50 mL), and the solution was heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature and the resulting dark red crystals were filtered and washed with acetone. The filtered product was recrystallized from a solution of MeOH and Et_2O. The crystals were collected and dried under vacuum. Then the crystals were added to 4-hydroxybenzaldehyde (S9, 3.8 g, 31 mmol, 1.0 equiv.) ethanol (50 mL) solution, and the solution was stirred at N_2 atmosphere for 15 minutes to remove the oxygen. Then, piperidine (3.2g, 37.2

mmol, 1.0 equiv.) was added into the solution. The reaction mixture was stirred at 80 °C under an N_2 atmosphere overnight, the solvent was removed under vacuum and the crude product was purified by silica gel chromatography (DCM/EtOH=9:1, v/v) to give compound **14** (4.9 g, 41%).

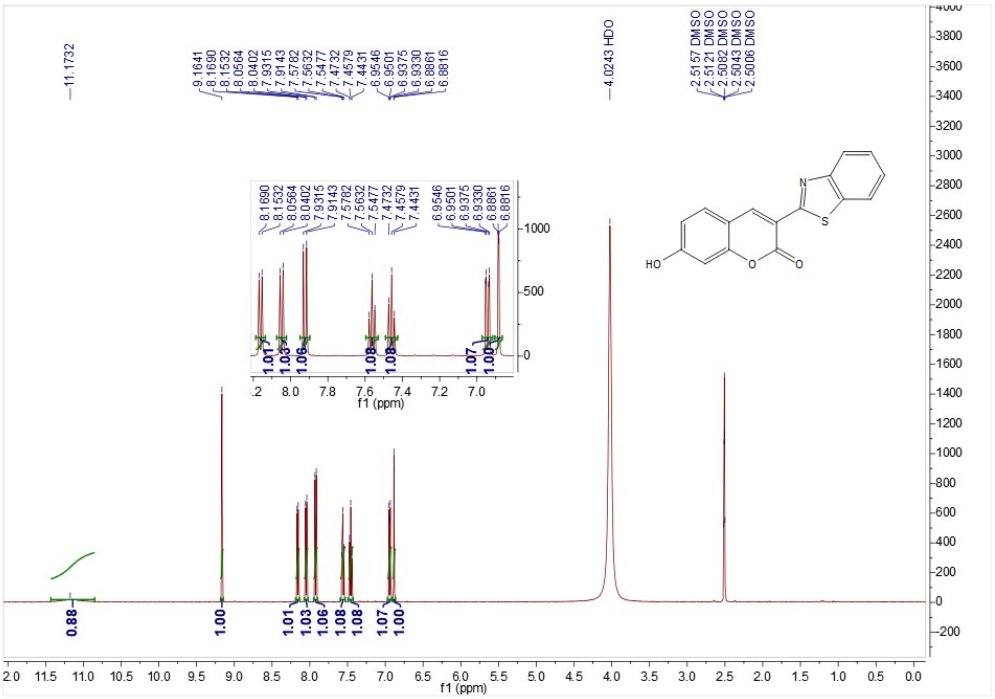
Dark red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.36 (d, *J* = 16.0 Hz, 1H), 8.21 – 8.12 (m, 2H), 7.91 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.83 – 7.76 (m, 1H), 7.65 (d, *J* = 16.1 Hz, 1H), 7.53 (pd, *J* = 7.5, 1.3 Hz, 2H), 6.93 – 6.86 (m, 2H), 4.74 (t, *J* = 7.9 Hz, 2H), 2.61 (t, *J* = 6.3 Hz, 2H), 2.10 (p, *J* = 6.6 Hz, 2H), 1.73 (s, 6H). ESI-MS m/z calcd for $C_{21}H_{23}NO_4S$ $[M+H]^+$, 386.5, found 386.1.

3. Copies of NMR Spectra

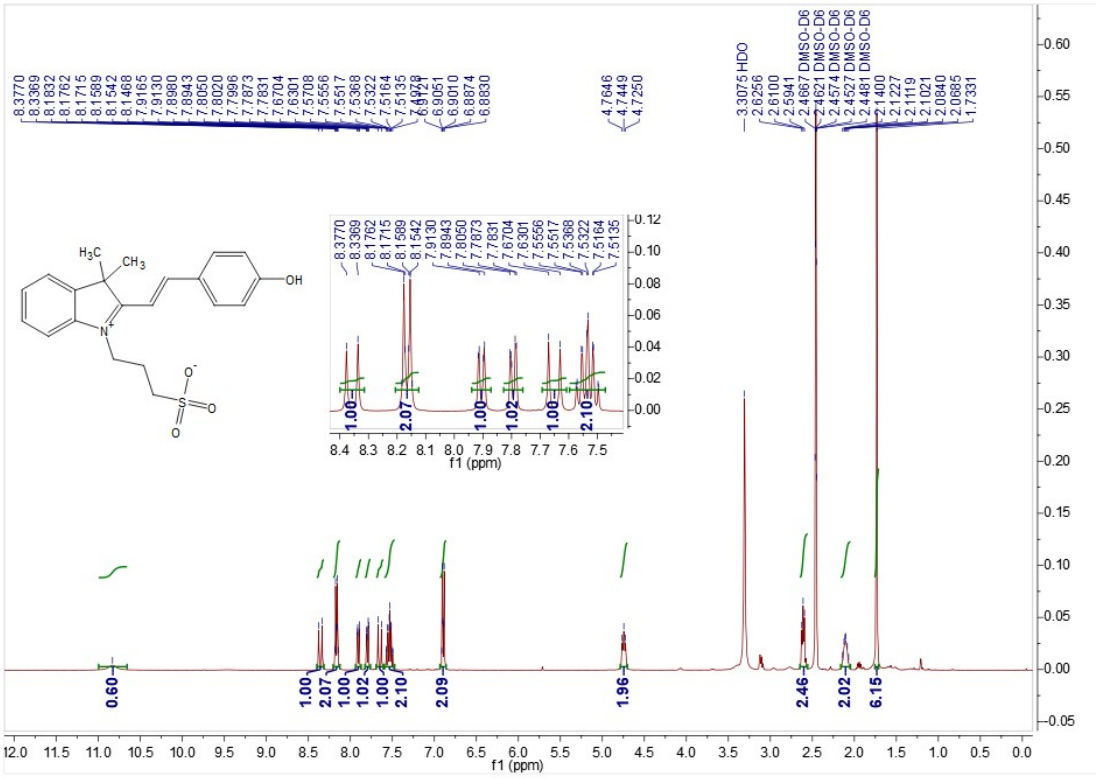
3.1 ^{1}H-NMR (400 MHz, DMSO-*d6*) Spectra of Compound 12 (Rhodamine B)



3.2 ^{1}H-NMR (500 MHz, DMSO-*d6*) Spectra of Compound 13.



3.3 ^{1}H-NMR (400 MHz, DMSO-*d6*) Spectra of compounds 14 (Dye IDE).



4. References

[1]: Hammler, D., Marx, A., Zumbusch, A. Fluorescence-Lifetime-Sensitive Probes for Monitoring ATP Cleavage. *Chem-A. Eur. J.*, 2018, 24(57), 15329-15335.

[2]: Lin, W., Long, L., Tan, W. A highly sensitive fluorescent probe for detection of benzenethiols in environmental samples and living cells. *Chem. Commun.*, 2010, 46, 1503-1505.

[3]: Zhang, Y., Guan, L., Yu, H., Yan, Y., Du, L., Liu, Y., Sun, M., Huang, D., Wang, S. Reversible Fluorescent Probe for Selective Detection and Cell Imaging of Oxidative Stress Indicator Bisulfite. *Anal Chem*. 2016, 88(8), 4426-4431.