

# Design and Synthesis of a Highly Selective Fluorescent Turn-on Probe for Thiol Bioimaging in Living Cells

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## Table of Contents:

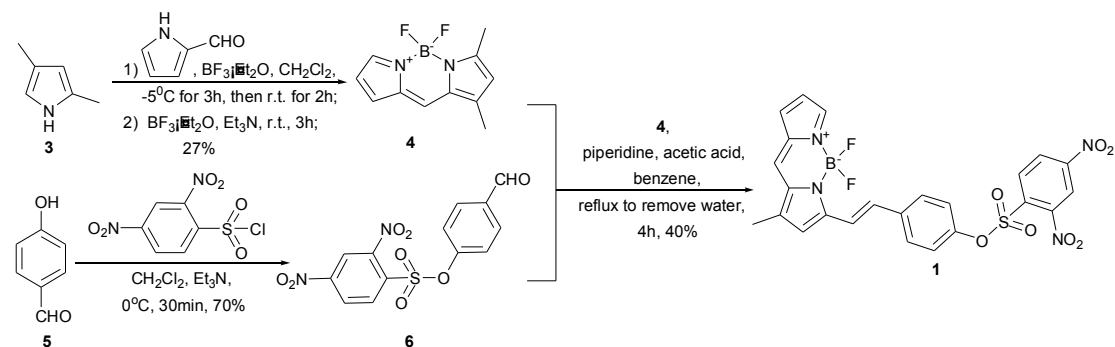
Synthesis and spectroscopic data of 1, 2, 4, 6	S2
Quantum Yield Measurements	S4
Stability of probe 1 at various pH and dependency of the present fluorometric assay on pH	S5
HPLC data of probe 1	S6
HPLC conditions and traces for probe 1	S6
NMR spectra of 4	S7- S8
NMR spectra of 6	S9- S10
NMR spectra of 1	S 11- S12
HRMS spectra of 1	S 13
NMR spectra of 2	S 14- S15

### Synthesis and spectroscopic data of 1, 2, 4, 6

General: Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Anhydrous  $\text{CH}_2\text{Cl}_2$  was obtained by being distilled from  $\text{GaH}_3$  and anhydrous benzene distilled from Na prior to use. Reactions were monitored by thin layer chromatography using TLC Silica gel 60 F254 supplied by Qingdao Puke Separation Material Corporation, Qingdao, P. R. Chin. Silica gel for column chromatography was 200-300 mesh and was supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. Characterization of intermediates and final compounds was done using IR, NMR spectroscopy and mass spectrometry, final purity of **1** was controlled using two different HPLC systems. Melting points were measured on BÜCHI B-540 and uncorrected. IR spectra were obtained on a Bruker Vector 22 spectrometer.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on Bruker DPX-400 or Bruker DPX-500 Fourier transform spectrometer or with  $d\text{-CHCl}_3$  or  $d_6\text{-DMSO}$  as solvents and tetramethylsilane (TMS) as the internal standard. All spectra were recorded at  $25^\circ\text{C}$  and chemical shifts were given in ppm and coupling constants ( $J$ ) in Hz. Low-resolution mass data were obtained on a Finnigan LCQ DECA XP plus LCMS spectrometer. High-resolution mass data were obtained on a Micromass Q-ToF Ultima<sup>TM</sup> spectrometer. HPLC chromatograms were recorded on an Agilent 1100 series LC system (Agilent ChemStation A.08.03) equipped with a VWD (G1314A) and C18 column ( $4.6 \times 200 \text{ mm} \times 5 \mu\text{m}$ , Diamonsil<sup>TM</sup>). Absorption spectra were acquired using a Hitachi U-3010 spectrophotometer. Fluorescence measurements were carried out on a PE LS45 fluorescence spectrometer.

#### Synthesis

##### Scheme S1. Synthesis of probe 1



**1,3-Dimethyl 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (4)**. Pyrrole 2-carboxyaldehyde (500 mg, 5.26 mmol) was dissolved in dry dichloromethane (30 mL) and cooled down to  $-5^\circ\text{C}$  under nitrogen atmosphere. 2,4-Dimethylpyrrole (**3**, 542  $\mu\text{L}$ , 5.26 mmol) was then added to the mixture slowly and stirred for 5 min, followed by dropwise addition of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (660  $\mu\text{L}$ , 5.26 mmol). After being stirred at  $-5^\circ\text{C}$  for 3 h, the reaction was allowed to stand at rt and stirred for 2 h. Then another portion of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (1.98 mL, 9.45 mmol) and  $\text{Et}_3\text{N}$  (2.19 mL, 15.8 mmol) were added subsequently at rt. After 3 h, the reaction mixture was quenched with  $\text{H}_2\text{O}$  (10 mL) and diluted with  $\text{CH}_2\text{Cl}_2$  (20 mL). The organic phase was then washed with  $\text{H}_2\text{O}$  ( $\times 3$ ), brine ( $\times 1$ ) and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was evaporated, and the residue was purified by silica gel column chromatography eluted with petrol ether (PE)/EtOAc (10:1) to yield 314 mg (1.43 mmol, 27%) of **4** as a dark red solid.

Mp (from PE/EtOAc = 10:1)  $136\text{--}138^\circ\text{C}$ .

IR (KBr):  $\nu_{\text{max}} = 3069, 2921, 1600, 1531, 1466, 1399, 1270, 985 \text{ cm}^{-1}$ .

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.64 (s, 1H), 7.20 (s, 1H), 6.92 (s, 1H), 6.43 (s, 1H), 6.16 (s, 1H), 2.58 (s, 3H), 2.27 (s, 3H).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  163.13, 145.79, 139.25, 136.51, 132.65, 126.51, 124.75, 121.26, 116.33, 15.18, 11.40.

ESI-MS  $m/z$  219.3 [M-H] $^-$ .

**4-Formylphenyl 2,4-dinitrobenzenesulfonate (6)**. To a stirred solution of 4-hydroxybenzaldehyde (**5**, 200 mg, 1.64 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (15 mL) was added  $\text{Et}_3\text{N}$  (250  $\mu\text{L}$ , 1.8 mmol). The mixture was then cooled to  $0^\circ\text{C}$  and a solution of 2,4-dinitrobenzenesulfonyl chloride (479 mg, 1.8 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (5 mL) was added dropwise. After being stirred at  $0^\circ\text{C}$  for 30 min, the starting materials were found to be totally consumed by TLC monitoring.  $\text{H}_2\text{O}$  was then added to quench the reaction. The mixture was transferred to separatory funnel and the organic phase was washed with  $\text{H}_2\text{O}$  ( $\times 3$ ) and brine ( $\times 3$ ) subsequently. After being dried over  $\text{Na}_2\text{SO}_4$ , the solvent was evaporated under reduced pressure to give the crude product, which was purified by recrystallization from  $\text{CH}_2\text{Cl}_2$  to give **6** as a colorless crystal (400 mg, 1.14 mmol, 70%).

Mp (from  $\text{CH}_2\text{Cl}_2$ ) 128-130 $^\circ\text{C}$ .

IR (KBr):  $\nu_{\text{max}}$  = 3099, 2873, 1694, 1595, 1540, 1390, 1347, 1194, 886, 843  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  10.02 (s, 1H), 8.70 (s, 1H), 8.55 (d,  $J$  = 8.6 Hz, 1H), 8.26 (d,  $J$  = 8.6 Hz, 1H), 7.95 (d,  $J$  = 8.3 Hz, 2H), 7.42 (d,  $J$  = 8.3 Hz, 2H).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  189.97, 152.35, 150.87, 148.75, 135.41, 133.54, 133.12, 131.45 (2C), 126.33, 122.54 (2C), 120.26.

ESI-MS  $m/z$  375.0 [M+Na] $^+$

**1-Methyl-3-(4-(2,4-dinitrophenylsulfonyloxy)styrenyl)-4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (1)**. Aldehyde **6** (80 mg, 0.23 mmol) was added to a solution of **4** (50 mg, 0.23 mmol) in dry benzene (45 mL) under nitrogen atmosphere. The mixture was cooled to  $0^\circ\text{C}$ , piperidine (0.28 mL) and AcOH (0.28 mL) were then added dropwise with stirring. After the addition, the reaction was refluxed for 4 h with continuous removal of water using a Dean-Stark water separator. The mixture was then cooled to r.t., transferred to a separatory funnel, washed with  $\text{H}_2\text{O}$  ( $\times 3$ ) and brine ( $\times 1$ ), and dried over  $\text{Na}_2\text{SO}_4$ . After evaporation of the solvent, the crude product was purified on a silica gel column eluted with PE/EtOAc (3:1) to yield **1** as a dark amaranthine solid (50 mg, 0.09 mmol, 40%).

Mp (from PE/EtOAc = 3:1) 235-236 $^\circ\text{C}$ .

IR (KBr):  $\nu_{\text{max}}$  = 2917, 1592, 1523, 1449, 1402, 1278, 1152, 964, 894, 817  $\text{cm}^{-1}$ .

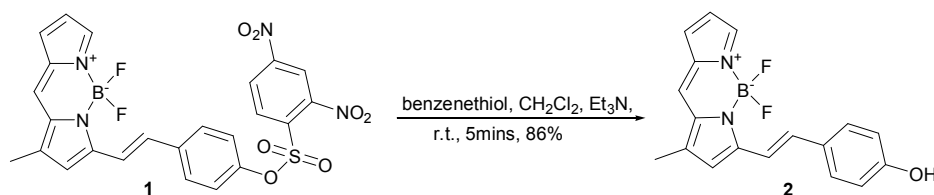
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.68 (s, 1H), 8.51 (d,  $J$  = 8.3 Hz, 1H), 8.18 (d,  $J$  = 8.8 Hz, 1H), 7.71 (s, 1H), 7.62-7.51 (m, 3H), 7.31 (d,  $J$  = 15.2 Hz, 1H), 7.24 (m, 3H), 7.00 (s, 1H), 6.73 (s, 1H), 6.49 (s, 1H), 2.34 (s, 3H).

$^{13}\text{C}$  NMR (125 MHz, DMSO):  $\delta$  170.59, 157.32, 151.82, 149.24, 148.34, 145.61, 139.99, 138.49, 137.41, 135.84, 133.94, 133.47, 130.73, 129.66 (2C), 127.73 (2C), 125.97, 123.14 (2C), 121.32, 119.05, 117.91, 117.30, 11.43.

HRMS  $m/z$  calc: 577.0777, found: 577.0768 [M + Na] $^+$ .

HPLC purity, system A: 96.5%; system B: 95.6%.

Scheme S2. Synthesis of free fluorophore **2**



**1-Methyl-3-(4-hydroxyl-styrenyl)-4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (2).** To a stirred solution of **1** (12 mg, 0.022 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added Et<sub>3</sub>N (2.7 μL, 0.02 mmol) and benzenethiol (3 μL, 0.03 mmol) subsequently. After the addition of benzenethiol, the reaction solution instantly turned from mauve to pink and TLC monitoring revealed the disappearance of **1** and the appearance of a fluorescent product. The mixture was then washed with H<sub>2</sub>O (×3) and brine (×1), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure and purified by silica gel column chromatography (PE/EtOAc = 2:1) to give **2** (6 mg, 0.018 mmol, 86%) as a black solid.

Mp (from PE/EtOAc = 2:1) 181-183°C.

IR (KBr):  $\nu_{\max}$  = 3447, 3108, 1596, 1543, 1460, 1394, 1281, 1140, 988, 829 cm<sup>-1</sup>.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.70 (s, 1H), 7.56 (m, 3H), 7.39 (d,  $J$  = 16.3 Hz, 1H), 7.19 (s, 1H), 6.95 (d,  $J$  = 3.7 Hz, 1H), 6.91 (d,  $J$  = 8.6 Hz, 2H), 6.79 (s, 1H), 6.49 (dd,  $J$  = 3.7, 2.1 Hz, 1H), 2.37 (s, 3H).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  159.44, 157.51, 144.60, 140.04, 138.29, 137.99, 132.97, 132.33, 129.91(2C), 128.84, 125.38, 122.49, 117.18, 116.46, 116.02 (2C), 11.50.

ESI-MS  $m/z$  323.3 [M-H].

#### Quantum Yield Measurements

Determination of the quantum yield is accomplished by comparison with standards of known quantum yield. For probe **1**, quinine sulfate dissolved in 0.1 M H<sub>2</sub>SO<sub>4</sub> ( $\Phi_F$  0.53,  $\lambda_{\text{ex}}$  360 nm, 22°C) was selected as the standard. While fluorescein dissolved in 0.1 M NaOH ( $\Phi_F$  0.95,  $\lambda_{\text{ex}}$  460 nm, 22°C) was the standard for **2**. Quantum yields were calculated by measuring the integrated emission area of the fluorescent spectra of **1** or **2** and comparing that value to the area measured for standards and were obtained with the following equation where  $\sum[F]$  was the integrated fluorescence intensity and Abs is absorbance at excitation wavelength.

$$\Phi_F^{\text{sample}} = \Phi_F^{\text{standard}} \cdot \text{Abs}^{\text{standard}} \cdot \sum[F^{\text{sample}}] / \text{Abs}^{\text{sample}} / \sum[F^{\text{standard}}]$$

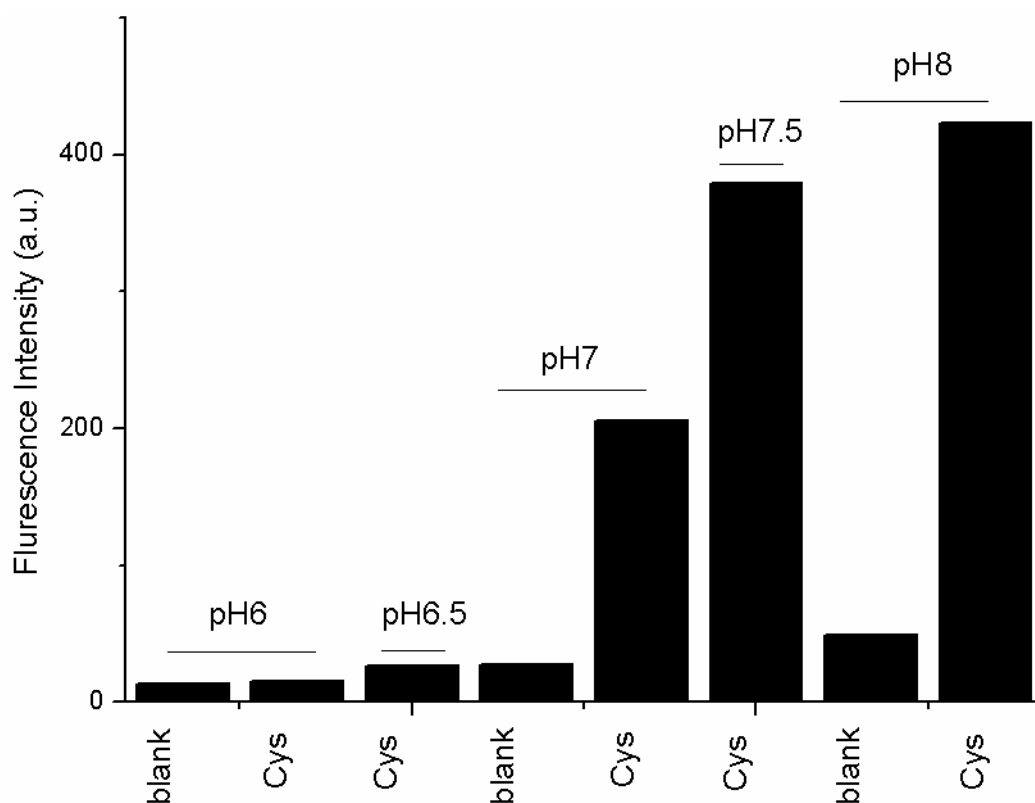


Figure S1. Stability of probe **1** at various pH and dependency of the present fluorometric assay on pH. Working solution of probe **1** in EtOH was diluted with aqueous PBS buffer (pH 6, 6.5, 7, 7.5, 8, respectively) to make a final concentration of 40  $\mu\text{M}$ . For the study of its stability, the solutions at pH 6, 7, 8 were diluted with equal volume of PBS (pH 6, 7, 8; 0.01M containing 1% EtOH). For the examination of dependency of the present fluorometric assay on pH, the solutions (pH 6, 6.5, 7, 7.5, 8) were added equal volume of PBS (pH 6, 6.5, 7, 7.5, 8; 0.01M containing 1% EtOH) containing 400 $\mu\text{M}$  of cysteine. After 1h of incubation at room temperature, the solutions were then sampled for fluorescence measurement at  $\lambda_{\text{ex}} = 527 \text{ nm}$  and the fluorescence intensity at  $\lambda_{\text{em}} = 570 \text{ nm}$  is plotted. (“blank” for dilution with PBS and “Cys” for dilution with cysteine solution).

### HPLC data of probe 1

Probe 1	mobile phase	purity
system A	CH <sub>3</sub> CN/H <sub>2</sub> O (80:20)	96.5%
system B	CH <sub>3</sub> OH/H <sub>2</sub> O (80:20)	95.6%

### HPLC conditions and traces for probe 1.

**General conditions:** C18 column (4.6 × 200 mm × 5 μm, Diamonsil™); ambient temperature; flow rate: 1.0 mL/min; injection volume: 15 μL; wavelength detection: 530 nm; mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O 80:20 for system A and CH<sub>3</sub>OH/H<sub>2</sub>O 80:20 for system B.

**HPLC traces of probe 1** (Figure S2 and S3).

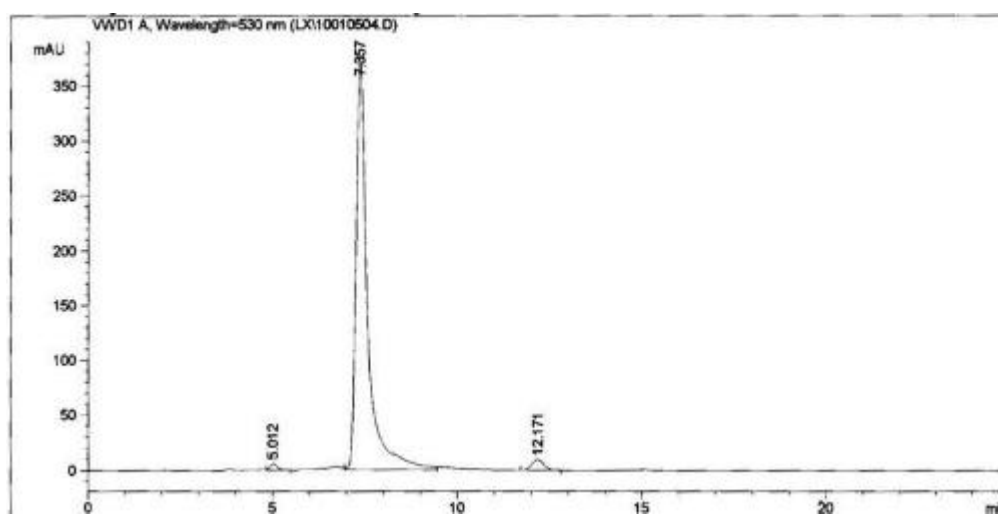


Figure S2. HPLC trace of **1** using the general conditions above and system A.

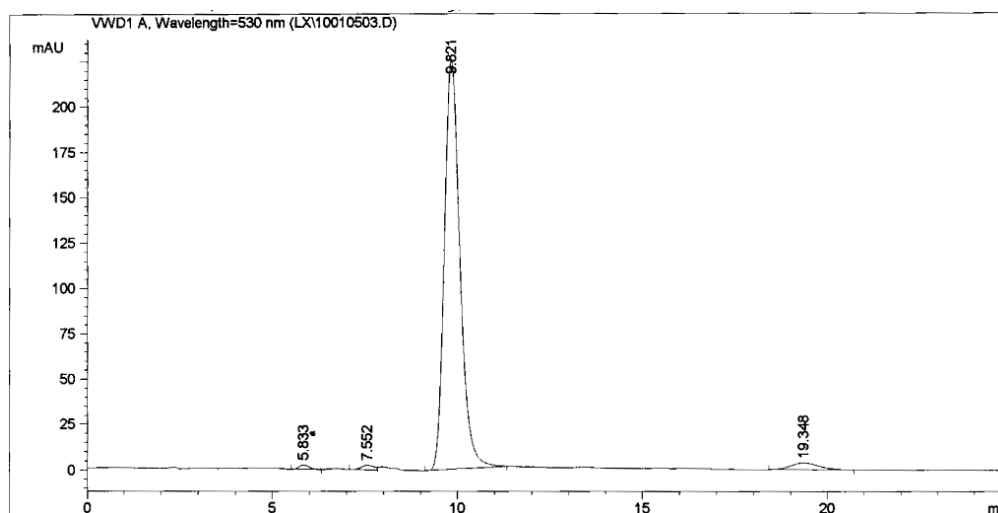


Figure S3. HPLC trace of **1** using the general conditions above and system B.

### Spectra of key compounds

