# **Supporting Information**

# Chemoselective Reduction and Self-immolation based FRET Probes for Detecting Hydrogen Sulfide in solution and in cells

Bifeng Chen, Peng Wang, Qingqing Jin, XinJing Tang\*

State Key Laboratory of Natural and Biomimetic Drugs School of Pharmaceutical Sciences, Peking University, Xue Yuan Road 38, Beijing 100191, China

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing,

China

\*Fax: (+86) 10 82805635; e-mail: xinjingt@bjmu.edu.cn.

#### Abbreviations

HCl, hydrochloric acid; H<sub>2</sub>SO<sub>4</sub>, sulphuric acid; NaNO<sub>2</sub>, sodium nitrite; HBr, hydrobromic acid; DCM or CH<sub>2</sub>Cl<sub>2</sub>,dichloromethane; AcOH, Acetic acid; Me<sub>2</sub>CO, Acetone: NaI. Sodium iodide;  $K_2CO_3$ , Potassium carbonate; DMF, DCC, N,N-dimethylformamide; TBS-Cl, tert-butyldimethylsilyl chloride; N,N'-dicyclohexylcarbodiimide; 4-dimethylamiopyridine; DMAP, Bu<sub>4</sub>NF, Tetrabutylammonium fluoride; THF, Tetrahydrofuran; PNP-Cl, 4-nitrophenyl chloroformate; CH<sub>3</sub>CN, acetonitrile; DIEA, N,N-Diisopropylethylamine; SiCl<sub>4</sub>, Silicon tetrachloride; PhOH, Phenol; FITC, fluorescein isothiocyanate; TEA, Triethylamine: NaCl, sodium chloride; NaHCO<sub>3</sub>, sodium bicarbonate; Na<sub>2</sub>SO<sub>4</sub>, sodium sulfate; NBS, N-bromosuccinimide; AIBN, azodiisobutyronitrile; CCl<sub>4</sub>, carbon tetrachloride; Ag<sub>2</sub>SO<sub>4</sub>, Silver sulfate; NaN<sub>3</sub>, sodium azide; KOH, Potassium hydroxide; MeOH, Methanol; HOBt, N-Hydroxybenzotriazole; CrO<sub>3</sub>, Chromium trioxide; (CH<sub>3</sub>)<sub>3</sub>SiCN, Trimethylsilyl cyanide; HCOONH<sub>4</sub>, ammonium formate; TMSN<sub>3</sub>, Azidotrimethylsilane; t-BuONO, tert-butyl nitrite: EDCI. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

### Materials

All solvents and chemicals were commercially available, they were purchased from Adamas, Sigma and Alfa Aesar.

#### Instruments

NMR spectra were recorded on a BRUKER AVANCE III 400 at 400 MHz for <sup>1</sup>H NMR and at 100 MHz for <sup>13</sup>C NMR, values are in ppm relative to tetramethylsilane. Mass spectra (MS) were measured with a BRUKER APEX IV FT-MS (7.0T) . UV-visible spectra were obtained on a Cary Eclipse ultraviolet spectrometer, Fluorescence spectra were tested on Cary Eclipse spectrofluorometer and Molecular Devices FlexStation III microplate reader. FTIR was tested on NEXUS-470.

# Synthesis of chemofluorophores (FRET-P1, FRET-P2, FRET-P3, FRET-P4 and FRET-P5)

#### **Synthesis of FRET-P1**



Scheme S1. Synthesis of FRET-P1. Reagents: (a) 5M HCl, H<sub>2</sub>SO<sub>4</sub>, NaNO<sub>2</sub>, (99%); (b) HBr, AcOH, DCM, (64%); (c) Me<sub>2</sub>CO, NaI; (d) K<sub>2</sub>CO<sub>3</sub>, DMF (84%); (e) TBS-Cl, Imidazole, DCM (74%); (f) DCC, DMAP, DCM (89%); (g) Bu<sub>4</sub>NF, THF, DCM (91%); (h) PNP-Cl, Py, CH<sub>3</sub>CN:DCM=1:1 (62%); (i) DIEA, THF (79%); (j) 1), SiCl<sub>4</sub>, PhOH, DCM; 2), FITC, TEA, DMF(65%).

4-Aminobenzylalcohol (1000 mg, 8.12 mmol) was dissolved in hydrochloric acid (5 mL 5 M). To this solution, sodium nitrite (840 mg, 12.18 mmol) dissolved in 20 mL of water was dropwise added within 30 min. The solution was vigorous stirred in ice-cold water. Sodium azide (2100 mg, 32.3 mmol) was batch added in. The resulting solution was stirred at room temperature overnight. The reaction was monitored by TLC. After the completion of reaction, the reaction solution was poured into saturated aqueous NaHCO<sub>3</sub> and extracted with ethyl acetate, The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether = 1:3) to obtain the pure product 1 as yellow oil (1450 mg, 99% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.25 (d, *J*=8.3 Hz, 2H), 6.92 (dd, *J*=8.7 Hz, 2.1 Hz, 2H), 4.55 (s, 2H), 2.02 ppm (s, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 139.37, 137.61, 128.53, 119.12, 64.63 ppm.



**1** (750 mg, 5 mmol) was dissolved in 10 mL CH<sub>2</sub>Cl<sub>2</sub>. And then it was cooled to 0°C temperature, To this solution, hydrogen bromide (1.6 mL, 6.5 mmol, 33 wt% in acetic acid) was added. The reaction mixture was then stirred at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into purified water and extracted with ethyl acetate, The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether =1:3) to obtain the pure product **2** as yellow oil (680 mg, 64% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.37 (d, *J*=8.3 Hz, 2H), 6.91 (d, *J*=8.7

Hz, 2H), 4.48 (s, 2H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 140.22, 134.51, 130.57, 119.38, 60.36 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+H]<sup>+</sup> 213.16, C<sub>7</sub>H<sub>6</sub>BrN<sub>3</sub> requires 210.97.\



A mixture of 2 (640 mg, 3 mmol) and NaI (480 mg, 3.2 mmol) in acetone was stirred at room temperature for 3 h, the reaction was monitored by TLC. After the reaction was done, the residue was filtered and washed with CH<sub>2</sub>Cl<sub>2</sub>, the filtrate was combined and concentrated, The crude product was used directly in next reaction without further purification. The crude product (424 mg, 1.6 mmol), 2,6-Bis(hydroxymethyl)-p-cresol (588 mg, 3.5 mmol) and K<sub>2</sub>CO<sub>3</sub> (483 mg, 3.5 mmol) were dissolved in 5 mL of DMF. The resulting solution was stirred at 50°C overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water and extracted with ethyl acetate, The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether = 1:5) to obtain the pure product **3** as white solid (412 mg, 84% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ = 7.42 (d, J=8.4 Hz, 2H), 7.14 (s, 2H), 7.04 (d, J=8.1 Hz, 2H), 4.90 (s, 2H), 4.65 (s, 4H), 2.32 (s, 3H), 2.04 ppm (m, 2H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 152.56, 140.24, 134.57, 133.86, 133.67, 129.76, 129.74, 119.27, 76.33, 61.07, 20.83, ppm. m/z MS  $(\text{ESI-TOF}^+)$  found  $[\text{M+Na}]^+$  322.30,  $C_{16}H_{17}N_3O_3$  requires 299.13.



**3** (924 mg, 3.1 mmol) was dissolved in 15 mL CH<sub>2</sub>Cl<sub>2</sub>, and cooled to 0 °C. Imidazole (212 mg, 3.1 mmol) and tert-Butyldimethylsilyl chloride (TBS-Cl, 452 mg, 3.0 mmol) were added and the mixture was stirred for 2.5 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then filtered and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate : petroleum ether = 1:5) to afford **4** as white solid (940 mg, 74% yield) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.33 (d, *J*=8.4 Hz, 2H), 7.13 (s, 1H), 7.00 (d, *J*=1.8 Hz, 1H), 6.96 (d, *J*=8.6 Hz, 2H), 4.81 (s, 2H), 4.62 (s, 2H), 4.54 (s, 2H), 2.24 (s, 3H), 0.83 (s, 9H), 0.00 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 151.94, 140.05, 134.28, 134.17, 134.10, 133.39, 129.58, 129.12, 128.87, 119.20, 61.33, 60.37, 25.96, 20.96, 18.41, -5.24 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup>436.39, C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>3</sub>Si requires 413.21.



**4** (922 mg, 2.24 mmol), 4-dimethylaminoazobenzene-4'-carboxylic acid (673mg, 2.5 mmol), dicyclohexylcarbodiimide (DCC, 515 mg, 2.5 mmol) and 4-dimethylaminopyridine (DMAP, 40 mg, 0.4 mmol) were dissolved in 20 mL DCM, The reaction mixture was cooled to  $0^{\circ}$ C and stirred under nitrogen overnight. The reaction was

monitored by TLC. After the completion of reaction, the residue was poured into deionized water and extracted with ethyl acetate, the combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether = 1:1, v:v) to obtain the pure product **5** as yellow solid (1.32 g, 89% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.07-7.99 (m, 2H), 7.84-7.70 (m, 4H), 7.31 (d, *J*=8.4 Hz, 2H), 7.19 (s, 1H), 7.13 (d, *J*=1.6 Hz, 1H), 6.96-6.88 (m, 2H), 6.65 (d, *J*=9.2 Hz, 2H), 5.29 (s, 2H), 4.84 (s, 2H), 4.64 (s, 2H), 3.00 (s, 6H), 2.26 (s, 3H), 0.83 (d, *J*=2.7 Hz, 9H), -0.00 ppm (d, *J*=3.2 Hz, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.11, 155.08, 151.92, 151.56, 142.74, 138.88, 133.48, 133.08, 133.05, 129.58, 129.17, 129.07, 128.83, 128.40, 127.63, 124.52, 120.98, 118.15, 110.46, 61.29, 59.34, 39.24, 24.96, 20.01, 17.40, -6.25 ppm.



**5** (520 mg, 0.78 mmol) was dissolved in 10 mL DCM. tetrabutylammonium fluoride solution (TBAF, 2.0 mL, 2 mmol, 1 M in THF) was added to the mixture and stirred for 4 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:3, v:v) as an eluent to afford **6** as pure yellow solid (390 mg, 91% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.05 (d, *J*=8.5 Hz, 2H), 7.80 (d, *J*=9.0 Hz, 2H), 7.78 (d, *J*=8.5 Hz, 2H), 7.74 (d, *J*=8.3 Hz, 2H), 7.34 (s, 1H), 7.18 (s, 1H), 6.96 (d, *J*=8.3 Hz, 2H), 6.68 (d, *J*=9.1 Hz, 2H), 5.33 (s, 2H), 4.90 (s, 2H), 4.61 (s, 2H), 3.02 (s, 6H), 2.27 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.11, 155.12, 152.26,

151.94, 142.71, 139.10, 133.51, 133.08, 132.63, 130.03, 129.59, 129.55, 128.92, 128.60, 128.21, 124.55, 121.01, 118.23, 110.46, 61.13, 60.10, 39.25, 19.85 ppm.



6 (110 mg, 0.2 mmol), 4-nitrophenyl chloroformate (PNP-Cl, 60 mg, 0.3 mmol) and pyridine (200 µL were dissolved in 10 mL mixed solvent of acetonitrile/ dichloromethane, 1:1, v: v). Then the reaction mixture was stirred under nitrogen at room temperature overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:3, v:v) as an eluent to afford 7 as yellow solid. Yield: 89 mg (62%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.24-8.15 (m, 2H), 8.07 (d, *J*=8.6 Hz, 2H), 7.85-7.77 (m, 4H), 7.38 (d, J=8.4 Hz, 2H), 7.32 (s, 1H), 7.31-7.25 (m, 2H), 7.23 (d, J=1.5 Hz, 1H), 6.96 (d, J=8.4 Hz, 2H), 6.69 (d, J=9.2 Hz, 2H), 5.37 (s, 2H), 5.25 (s, 2H), 4.95 (s, 2H), 3.04 (s, 6H), 2.31 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.05, 154.48, 153.06, 151.97, 151.42, 144.41, 142.68, 139.18, 133.67, 132.40, 131.70, 130.93, 129.60, 128.78, 128.73, 128.57, 126.85, 124.61, 124.27, 121.03, 120.71, 118.23, 110.48, 65.36, 60.93, 39.27, 19.84 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 738.65, C<sub>38</sub>H<sub>33</sub>N<sub>7</sub>O<sub>8</sub> requires 715.24.

$$N_3$$

7 (30 mg, 0.05 mmol), N,N-diisopropylethylamine (20 mg, 0.15 mmol) and N-tert-butylcarbonyl diamine (30 mg, 0.15 mmol) were dissolved in 5 mL THF, and the mixture was stirred for 4 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:1, v:v) as an eluent to afford 8 as yellow solid. Yield: 30 mg (79%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.15 (d, J=8.5 Hz, 2H), 7.93-7.73 (m, 4H), 7.37 (d, J=8.3 Hz, 2H), 7.22 (s, 1H), 7.17 (s, 1H), 6.95 (d, J=8.3 Hz, 2H), 6.68 (d, J=9.1 Hz, 2H), 5.32 (s, 2H), 5.10 (s, 2H), 4.88 (s, 2H), 4.79 (s, 1H), 4.52 (s, 1H), 3.13 (d, J=4.9 Hz, 2H), 3.02 (s, 6H), 2.27 (s, 3H), 1.62 (s, 2H), 1.43 (d, J=3.2 Hz, 2H), 1.35 ppm (s, 9H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta = 165.08$ , 155.35, 155.14, 152.64, 151.94, 142.72, 139.00, 133.36, 132.65, 130.60, 130.53, 129.58, 128.93, 128.61, 128.35, 124.54, 121.01, 118.19, 115.60, 110.45, 67.97, 61.12, 60.86, 39.24, 27.39, 26.39, 26.22, 25.61, 19.86 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 787.62, C<sub>41</sub>H<sub>48</sub>N<sub>8</sub>O<sub>7</sub> requires 764.36.

#### FRET-P1



A solution of 8 (60 mg, 0.08 mmol) in 2 mL DCM was added de-tertbutylcarbonyl protection based solution (500  $\mu$ L, 1 M SiCl<sub>4</sub> and 3 M PhOH in DCM). Then the reaction mixture was stirred for 10 min at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and directly used in the next reaction. The crude product in the previous step was redissolved in DMF (2 mL), N, N-diisopropylethylamine (20 mg, 0.15 mmol) and fluorescein isothiocyanate (FITC, 63 mg, 0.16 mmol) was then added. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether/methanol (10:5:1, v:v:v) and then acetone/methanol (10:1, v:v) as an eluent to afford **FRET-P1** as yellow solid. Yield: 55 mg (65%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 8.29 (s, 1H), 8.14 (d, J=8.6 Hz, 3H), 7.89 (dd, J=8.7 Hz, 7.7 Hz, 4H), 7.54 (d, J=8.5 Hz, 2H), 7.39 (s, 2H), 7.32 (s, 1H), 7.31-7.18 (m, 3H), 6.91 (d, J=9.3 Hz, 2H), 6.73 (d, J=2.2 Hz, 2H), 6.63 (dt, J=8.7 Hz, 5.4 Hz, 4H), 5.43 (s, 2H), 5.14 (s, 2H), 5.00 (s, 2H), 3.57 (s, 2H), 3.14 (s, 6H), 3.11 (d, J=6.2 Hz, 2H), 2.37 (s, 3H), 1.71-1.58 (m, 2H), 1.57-1.46 ppm (m, 2H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta = 208.87, 180.87, 169.97, 165.62, 156.50, 155.84, 153.53, 153.44,$ 143.15, 140.94, 139.64, 134.34, 134.11, 131.84, 131.39, 131.22, 131.03, 130.94, 130.52, 130.24, 129.86, 129.52, 125.84, 122.37, 119.61, 112.05, 111.03, 102.95, 76.28, 68.97, 62.36, 61.05, 56.30, 43.82, 32.58, 30.07, 27.41, 26.35, 20.93 ppm. m/z MS  $(\text{ESI-TOF}^+)$  found  $[\text{M}+\text{Na}]^+$  1077.61,  $C_{57}H_{51}N_9O_{10}S$  requires 1053.35.



Scheme S2. Synthesis of FRET-P2. Reagents: (a) NBS, AIBN, CCl<sub>4</sub>, (66%); (b) Ag<sub>2</sub>SO<sub>4</sub>, dioxane/H<sub>2</sub>O=1:1, (80%); (c) Zn, AcOH, DCM, (90%); (d) 5 M HCl, NaNO<sub>2</sub>, NaN<sub>3</sub>, (90%); (e) KOH, MeOH, reflux, (78%); (f) HOBt, DCC, CH<sub>3</sub>CN,rt, (77%) ; (g) DCC, DMAP, CH<sub>3</sub>CN,rt, (65%) ; (h) SiCl<sub>4</sub>, PhOH, DCM; (i) FITC, DMF, DIEA,(62%);

2-nitro-3-methyl benzoic acid methyl ester (5 g, 26 mmol), N-bromosuccinimide (NBS, 5.34 g, 30 mmol) and azodiisobutyronitrile (AIBN, 30 mg) were dissolved in

50 mL dry CCl<sub>4</sub> and the reaction mixture was refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:15, v:v) as an eluent to afford **9** as white solid. Yield: 4.9 g (66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.94 (d, *J*=1.2 Hz, 1H), 7.75 (dd, *J*=7.8 Hz, 1.1 Hz, 1H), 7.59 (dd, *J*=9.8 Hz, 5.8 Hz, 1H), 4.46 (s, 2H), 3.90 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 163.56, 149.37, 135.44, 131.25, 130.93, 130.42, 124.34, 53.22, 25.60 ppm.



A solution of **9** (1.33 g, 5 mmol) and silver sulfate (2.49 g, 8 mmol) in dioxane/ deionized water (1: 1, 60 mL) refluxed for 3 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:5, v:v) as an eluent to afford **10** as white solid. Yield: 844 mg (80%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.76-7.70 (m, 2H), 7.49 (d, *J*=6.0 Hz, 1H), 4.58 (s, 2H), 3.80 (s, 3H), 3.60 ppm (s, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 164.38, 148.27, 133.78, 132.87, 130.82, 129.85, 123.68, 59.98, 53.09 ppm.



**10** (422 mg, 2 mmol) and zinc powder (2.6 g, 40 mmol) were dissolved in 10 mL DCM, and acetic acid was added (2 mL) to the mixture with stirring, The reaction

continued for 5 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated NaHCO<sub>3</sub> solution and then was extracted with ethyl acetate. The organic layers were combined, washed with saturated NaHCO<sub>3</sub> solution and saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v: v) as an eluent to afford **11** as white solid. Yield: 326 mg (90%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.72 (dd, *J*=8.1 Hz, 1.5 Hz, 1H), 7.04 (dd, *J*=7.2 Hz, 1.2 Hz, 1H), 6.56-6.35 (m, 1H), 4.48 (s, 2H), 3.74 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 167.73, 149.13, 132.81, 130.41, 124.37, 114.29, 110.08, 62.99, 50.55 ppm. m/z MS (EI) found [M]<sup>+</sup> 181.10, C<sub>9</sub>H<sub>11</sub>NO<sub>3</sub> requires 181.19.



To a solution of **11** (189 mg, 1 mmol) in HCl solution (5 mL, 5M), sodium nitrite solution (117 mg, 2 mmol NaNO<sub>2</sub> was dissolved in 10 mL deionized water) was dropwise added within 30 min. The mixed solution was stirred in an ice-water bath, and then sodium azide (276 mg, 5 mmol) was added in batches. The reaction mixture was stirred for overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated NaHCO<sub>3</sub> solution, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as an eluent to afford **12** as pure white solid. Yield: 186 mg (90%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.74-7.62 (m, 1H), 7.56-7.39 (m, 1H), 7.09 (t, *J*=7.7 Hz, 1H), 4.58 (s, 2H), 3.85 (s, 3H), 3.28 ppm (s, 1H),. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.12, 136.61, 134.74, 131.13, 129.83, 124.25, 123.03, 60.21, 51.51 ppm. m/z MS (EI) found [M]<sup>+</sup> 207.10, C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> requires 207.19.



A solution of **12** (21 mg, 0.1 mmol) in 1 mL methanol was added to KOH methanol solution (11.2 mg, 0.2 mmol, 1 mL). The reaction mixture was refluxed for 5 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water at 0°C. Then 5 M HCl solution was added to the mixture and was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:2, v:v) with 1% acetic acid as an eluent to afford **13** as light yellow solid. Yield: 15 mg (78%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta = 13.53$  (s, 1H), 7.82 (dd, *J*=7.8 Hz, 1.5 Hz, 1H), 7.78-7.65 (m, 1H), 7.38 (t, *J*=7.7 Hz, 1H), 5.39 (s, 1H), 4.63 ppm (s, 2H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta = 166.79$ , 137.02, 136.07, 131.20, 129.72, 125.32, 125.03, 59.07 ppm. m/z MS (EI) found [M-N<sub>2</sub>]<sup>+</sup> 165.10, C<sub>8</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub> requires 193.16.



**13** (20 mg, 0.1 mmol), N-tert-butyl carbonyl diamine (28 mg, 0.15 mmol), 1-hydroxybenzotriazole (HOBT, 23 mg, 0.15 mmol) and dicyclohexylcarbodiimide (DCC, 31 mg, 0.15 mmol) were dissolved in 2 mL acetonitrile, and the mixture was stirred for overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate as an eluent to afford **14** as pure white solid. Yield: 28 mg (77%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta = 8.60$  (t, *J*=5.5 Hz, 1H), 7.60-7.46 (m, 1H), 7.31 (dd, *J*=7.6 Hz, 1.7 Hz, 1H), 7.24 (t, *J*=7.5 Hz, 1H), 6.81 (t, *J*=5.5 Hz, 1H), 5.30 (t, *J*=5.6 Hz, 1H), 4.53 (d, *J*=5.4 Hz, 2H), 3.21 (q, *J*=6.4 Hz, 2H), 2.92 (q, *J*=6.5 Hz, 2H), 1.59-1.25 ppm (m, 13H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 166.24, 155.56, 135.77, 133.16, 130.89, 128.85, 127.44, 124.89, 77.30, 58.99, 28.24, 26.97, 26.17 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 386.25, C<sub>17</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub> requires 363.41.



14 (272mg, 0.75 mmol), 4-amine azobenzene-dimethyl-4'-formic acid (269 mg, 1 mmol), dicyclohexylcarbodiimide (DCC, 206 mg, 1 mmol) and 4-dimethylamino -pyridine (DMAP, 25 mg, 0.2 mmol) were dissolved in mixed solvent of dichloromethane/acetonitrile (15 mL, 1:2, v:v). Then the mixture was cooled to 0 °C, and stirred under nitrogen for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/dichloromethane (1:1, v:v) as an eluent to afford 15 as yellow solid. Yield: 300 mg (65%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.10 (t, J=8.7 Hz, 2H), 7.93-7.76 (m, 4H), 7.51 (d, J=7.7 Hz, 2H), 7.18 (d, J=6.3 Hz, 2H), 6.71 (d, J=9.2 Hz, 2H), 6.52 (s, 1H), 5.39 (s, 2H), 4.56 (s, 1H), 3.47-3.42 (m, 2H), 3.19-2.93 (m, 8H), 1.72-1.45 (m, 4H), 1.35 ppm (s, 9H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta = 165.54$ , 164.91, 155.14, 155.12, 151.90, 142.60, 134.77, 130.72, 129.66, 129.38, 129.11, 128.57, 128.43, 124.55, 124.43, 121.02, 110.40, 61.74, 39.23, 38.72, 27.36, 26.63, 25.49 ppm.  $m/z MS (ESI-TOF^{+})$  found  $[M+Na]^{+} 637.27, C_{32}H_{38}N_8O_5$  requires 614.69.

#### **FRET-P2**



To a solution of 15 (56 mg, 0.09 mmol) in dichloromethane (2 mL), the methylene chloride solution of de-t-butoxycarbonyl protecting group (1 M SiCl<sub>4</sub> and 3 M PhOH in 1 mL) was added. The reaction mixture was stirred for 10 min at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and directly used in the next reaction. The crude product in the previous step was redissolved in N,N-dimethyl formamide (3 mL), N,N-diisopropylethylamine (175  $\mu$ L, 10.3 mmol) and fluorescein isothiocyanate (47 mg, 0.12 mmol) were then added. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether/methanol (10:5:1, v:v:v) and then acetone/ methanol (10:1, v:v) as an eluent to afford **FRET-P2** as yellow solid. Yield: 50 mg (62%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 8.88-8.59 (m, 2H), 8.47 (s, 1H), 8.12 (d, J=8.3 Hz, 2H), 7.95-7.81 (m, 4H), 7.63 (d, J=7.4 Hz, 1H), 7.50 (d, J=7.2 Hz, 1H), 7.38-7.21 (m, 1H), 7.16 (d, J=8.2 Hz, 1H), 6.84 (d, J=9.0 Hz, 1H), 6.74 (s, 3H), 6.59 (s, 5H), 5.42 (s, 2H), 4.59 (s, 1H), 3.53 (s, 2H), 3.41 (s, 2H), 3.07 (s, 6H), 1.64 ppm (s, 4H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 208.88, 180.98, 169.12, 166.50, 165.54, 160.07, 155.90, 153.54, 152.35, 143.15, 142.35, 135.37, 131.84, 131.53, 131.00, 129.82, 129.70, 129.41, 125.84, 125.76, 122.40, 113.10, 112.05, 110.18, 102.73, 68.97, 63.17, 56.27, 43.69, 32.56, 30.05, 29.47, 26.76, 26.40 ppm. m/z MS  $(\text{ESI-TOF}^+)$  found  $[\text{M}+\text{Na}]^+$  926.43,  $C_{48}H_{41}N_9O_8S$  requires 903.28.

# **Synthesis of FRET-P3**



Scheme S3. Synthesis of FRET-P3. Reagents: (a) H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>,(90%); (b) CrO<sub>3</sub>, AcOH, H<sub>2</sub>SO<sub>4</sub>,(99%); (c) MeOH, H<sub>2</sub>SO<sub>4</sub>, reflux, (91%); (d) NBS, AIBN, CCl<sub>4</sub>, (91%); (e) Ag<sub>2</sub>SO<sub>4</sub>, dioxane/H<sub>2</sub>O=1:1, (75%); (f) Zn, AcOH, DCM, (83%); (g) 5M HCl, NaNO<sub>2</sub>, NaN<sub>3</sub>, (85%); (h) TBS-Cl, Imidazole, DCM, (66%); (i) DCC, DMAP, DCM, (80%); (j) Bu<sub>4</sub>NF, THF, DCM,(80%); (k) PNP-Cl, Py, CH<sub>3</sub>CN:DCM=1:1, (69%); (l) DIEA, THF, (68%); (m) SiCl<sub>4</sub>, PhOH, DCM; (n) FITC, TEA, DMF, (53%).

NO<sub>2</sub>

To a solution of mesitylene (12 g, 0.1 mol) in cold concentrated sulfuric acid (9.6 mL, 0.18 mol) was added concentrated nitric acid (8.3 mL, 0.12 mmol), and the reaction mixture was stirred for 20 min. Then the solution was warmed to room temperature and stirred for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:15, v:v) as an eluent to afford **16** as yellow solid. Yield: 14.9 g (90%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 6.91 (s, 2H), 2.31 (s, 3H), 2.27 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 149.79, 140.29, 129.58, 129.43, 21.01, 17.51 ppm. m/z MS (EI) found [M]<sup>+</sup> 165.20, C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> requires 165.08.



**16** (9.2 g, 55.5 mmol) and chromium trioxide (16.7 g, 166.5 mmol) was added to acetic acid (50 mL), the reaction mixture was refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into ice cold deionized water. The white precipitate was filtered and washed with chloroform and 2 M NaOH solution. The aqueous layer was acidified with 5 M HCl solution, and then was extracted with chloroform. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate as an eluent to afford **17** as light yellow solid. Yield: 12 g (99%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.82 (s, 2H), 2.30 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 169.63, 153.98, 129.80, 128.99, 16.25 ppm. m/z MS (EI) found [M]<sup>+</sup> 195.20, C<sub>9</sub>H<sub>9</sub>NO<sub>4</sub> requires 195.05.



To a solution of **17** (600 mg, 3 mmol) in absolute methanol (30 mL) was added concentrated sulfuric acid (1 mL), and the reaction mixture was refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as an eluent to afford **18** as yellow solid. Yield: 14.9 g (91%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta = 7.62$  (s, 2H), 3.81 (s, 3H), 2.18 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta = 164.37$ , 153.23, 130.15, 129.02, 128.63, 51.39, 16.06 ppm. m/z MS (EI) found [M]<sup>+</sup> 209.20, C<sub>10</sub>H<sub>11</sub>NO<sub>4</sub> requires 209.07.



**18** (420 mg, 2 mmol), N-bromosuccinimide (NBS, 1.42 g, 8 mmol) and Azobis isobutyronitrile (AIBN, 30 mg) were dissolved in dry tetrachloromethane (10 mL), and the reaction mixture was refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:10, v:v) as an eluent to afford **19** as white solid. Yield: 670 mg (91%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.18 (s, 2H), 4.51 (s, 4H), 3.99 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ =164.23, 151.61, 132.88, 132.85, 131.37, 53.02, 25.77 ppm. m/z MS (EI) found [M-Br]<sup>+</sup> 288.20, C<sub>10</sub>H<sub>9</sub>Br<sub>2</sub>NO<sub>4</sub> requires 366.99.



**19** (3.33 g, 9.1 mmol) and silver sulfate (8.5 g, 27.3 mmol) were dissolved in 60 mL mixed solvent of dioxane and deionized water (1:1, v: v), and the reaction mixture was refluxed for 3 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:5, v:v) as an eluent to afford **20** as white solid. Yield: 1.65 g (75%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 8.13 (s, 2H), 5.75-5.62 (m, 2H), 4.59 (s, 2H), 4.57 (s, 2H), 3.92 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 164.93, 149.43, 135.20, 131.26, 128.14, 58.82, 52.65 ppm. m/z MS (EI) found [M-O]<sup>+</sup> 223.20, C<sub>10</sub>H<sub>11</sub>NO<sub>6</sub> requires 241.20.



Acetic acid (2 mL) was added to the mixture of **20** (1.0 g, 4.15 mmol) and zinc powder (5.4 g, 83 mmol) in 40 mL mixed solvent of DCM and CH<sub>3</sub>CN (1:1, v: v), the mixture was stirred for 5 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated sodium bicarbonate solution and saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as an eluent to afford **21** as white solid. Yield: 727 mg (83%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 7.69 (s, 2H), 5.52 (s, 2H), 5.22-5.12 (m, 2H), 4.45 (s, 2H), 4.43 (s, 2H), 3.76 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 167.15, 148.71,

128.43, 124.98, 115.97, 61.07, 51.65 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M-H]<sup>-</sup>210.15, C<sub>10</sub>H<sub>13</sub>NO<sub>4</sub> requires 211.21.



**21** (1.0 g, 4.74 mmol) in HCl solution (8 mL, 5M) was added sodium nitrite solution (690 mg, 10 mmol NaNO<sub>2</sub> was dissolved in 20 mL deionized water) within 30 min. The mixed solution was stirred and reacted vigorously in an ice-water bath, and then sodium azide (1.63 g, 25 mmol) was added in batches. The reaction mixture was stirred and refluxed for overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated sodium bicarbonate solution, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **22** as white solid. Yield: 955 mg (85%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 7.96 (s, 2H), 5.45 (s, 2H), 4.62 (s, 4H), 3.86 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 166.32, 138.81, 136.46, 128.39, 126.41, 59.71, 52.55 ppm. m/z MS (ESI-TOF<sup>-</sup>) found [M-N<sub>2</sub>]<sup>-</sup>209.10, C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub> requires 237.21.



**22** (370 mg, 1.56 mmol) was dissolved in DCM (15 mL) at 0°C. Imidazole (107 mg, 1.56 mmol) and tert-butyldimethylsilyl chloride (TBS-Cl, 234 mg, 1.55 mmol) were then added. The reaction mixture was stirred for 3 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by

silica gel column chromatography using ethyl acetate/petroleum ether (1:5, v:v) as an eluent to afford **23** as white solid. Yield: 361 mg (66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta = 8.00$  (d, *J*=1.8 Hz, 1H), 7.93 (d, *J*=1.9 Hz, 1H), 4.78 (s, 2H), 4.74 (s, 2H), 3.88 (s, 3H), 0.95 (s, 9H), 0.11 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ =166.57, 139.23, 135.00, 134.29, 129.26, 129.12, 126.72, 61.55, 61.39, 52.19, 25.88, 25.84, 18.30, -5.31 ppm. m/z MS (ESI-TOF<sup>-</sup>) found [M-N<sub>2</sub>]<sup>-</sup> 323.21, C<sub>16</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>Si requires 351.47.



To a solution of 23 (236 mg, 0.68 mmol in 10 mL DCM), 4-dimethylaminoazobenzene-4'-formic acid (269 mg, 1 mmol), dicyclohexylcarbodiimide (DCC, 206 mg, 1 mmol) and 4-dimethylaminopyridine (DMAP, 20 mg, 0.2 mmol) were added. The mixture was then stirred for overnight at  $0^{\circ}$ C under nitrogen atmosphere. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **24** as yellow solid. Yield: 327 mg (80%). <sup>1</sup>H NMR (400 MHz, CDCl3)  $\delta = 8.32-8.08$  (m, 4H), 8.08-7.83 (m, 4H), 6.79 (d, J=8.2 Hz, 2H), 5.51 (s, 2H), 4.88 (s, 2H), 4.8 2H), 3.95 (s, 3H), 3.14 (s, 6H), 0.98 (s, 9H), 0.15 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz,  $CDCl_3$ )  $\delta = 166.19, 165.89, 152.99, 143.68, 140.28, 135.72, 131.02, 130.75, 130.20, 130.75, 130.20, 143.68, 140.28, 135.72, 131.02, 130.75, 130.20, 130.20$ 129.64, 129.51, 127.24, 125.71, 122.04, 111.57, 63.10, 61.59, 52.29, 40.35, 25.88, 18.35, -5.25 ppm. m/z MS (ESI-TOF<sup>+</sup>) found  $[M+H]^+603.36$ ,  $C_{31}H_{38}N_6O_5Si$  requires 602.76.



To the solution of **24** (300 mg, 0.5 mmol) in DCM (10 mL), 2.0 mL tert-butyl ammonium fluoride (2 mmol, 1 M) in dichloromethane was added, The reaction mixture was stirred for 5 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as an eluent to afford **25** as yellow solid. Yield: 390 mg (80%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.30-8.04 (m, 4H), 7.88 (dd, *J*=8.0 Hz, 8.4 Hz, 4H), 6.75 (d, *J*=8.0 Hz, 2H), 5.48 (s, 2H), 4.85 (s, 2H), 3.92 (s, 3H), 3.11 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 166.05, 165.87, 162.62, 156.14, 152.96, 143.61, 140.93, 135.10, 131.56, 130.88, 129.73, 129.49, 127.22, 125.62, 122.04, 111.47, 63.02, 61.49, 52.33, 40.29 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+H]<sup>+</sup>489.26, C<sub>25</sub>H<sub>24</sub>N<sub>6</sub>O<sub>5</sub> requires 488.50.



To a solution of **25** (381 mg, 0.78 mmol) in acetonitrile/ dichloromethane (1:1, v:v, 10 mL), p-nitrophenyl chloroformate (PNP-Cl, 240 mg, 1.17 mmol) and pyridine (1 mL) were added. The reaction mixture was stirred for overnight at room temperature under nitrogen atmosphere. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water,

dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:1, v:v) as an eluent to afford **26** as yellow solid. Yield: 351 mg (69%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.14 (m, 6H), 7.81 (dd, *J*=8.4 Hz, 6.2 Hz, 4H), 7.34 (d, *J*=9.0 Hz, 2H), 6.68 (d, *J*=9.0 Hz, 2H), 5.47 (s, 2H), 5.37 (s, 2H), 3.87 (s, 3H), 3.04 ppm (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 164.77, 154.35, 151.17, 144.51, 142.66, 141.00, 132.50, 131.46, 129.74, 129.52, 128.24, 127.53, 126.67, 125.15, 124.60, 124.35, 121.09, 120.76, 114.64, 110.45, 65.79, 61.85, 51.50, 39.27 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+H<sup>+</sup>]<sup>+</sup>654.19, C<sub>32</sub>H<sub>27</sub>N<sub>7</sub>O<sub>9</sub> requires 653.60.



**26** (326 mg, 0.5 mmol), N,N-diisopropylethylamine (200 mg, 1.5 mmol) and N-tert-butyl carbonyl-diamine (300 mg, 1.5 mmol) were mixed in tetrahydrofuran (10 mL). The reaction mixture was stirred for 4 h at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **27** as yellow solid. Yield: 328 mg (68%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.21-8.07 (m, 4H), 7.96-7.82 (m, 4H), 6.76 (d, *J*=8.8 Hz, 2H), 5.50 (s, 2H), 5.25 (s, 2H), 5.02 (s, 1H), 4.67 (s, 1H), 3.93 (s, 3H), 3.36-2.98 (m, 10H), 1.66-1.34 ppm (m, 13H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.82, 156.25, 156.02, 155.79, 152.95, 143.70, 141.50, 132.15, 131.79, 131.16, 130.74, 130.20, 129.45, 127.49, 125.58, 122.08, 111.45, 62.95, 62.68, 52.38, 40.79, 40.27, 28.41, 27.35, 27.19 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup>725.59, C<sub>35</sub>H<sub>42</sub>N<sub>8</sub>O<sub>8</sub> requires 702.76.

#### FRET-P3



To the solution of 27 (59 mg, 0.085 mmol) in DCM (5 mL), the solution of 1 M SiCl<sub>4</sub> and 3 M PhOH in methylene chloride (500  $\mu$ L) was added. The reaction mixture was stirred for 10 min at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and was directly used in the next step reaction. The crude product in the previous step was redissolved in N, N-dimethylformamide (5 mL), triethylamine (1.5 mL) and fluorescein isothiocyanate (FITC, 38.9 mg, 0.1 mmol) were then added, and the reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether/methanol (10:5:1, v:v:v) and then acetone/methanol (10:1, v:v) as an eluent to afford **FRET-P3** as yellow solid. Yield: 45 mg (53%). <sup>1</sup>H NMR (400 MHz, DMSO-d6, ppm)  $\delta = 11.04$ (s, 1H), 9.21 (s, 1H), 8.14-8.08 (m, 3H), 8.04 (d, J=1.6 Hz, 1H), 7.93-7.74 (m, 5H), 6.85 (d, J=9.3 Hz, 2H), 6.70 (t, J=8.6 Hz, 4H), 6.33-6.20 (m, 6H), 5.55 (s, 2H), 5.21 (s, 2H), 3.87 (s, 3H), 3.13-2.97 (m, 10H), 1.60-1.44 ppm (m, 4H). <sup>13</sup>C NMR (101 MHz, DMSO-d6, ppm)  $\delta = 208.40, 180.51, 169.69, 165.16, 164.92, 155.60, 155.48,$ 155.32, 153.08, 142.65, 140.81, 140.44, 131.70, 130.79, 130.49, 130.15, 129.97, 129.08, 127.97, 126.43, 126.27, 125.38, 121.94, 111.57, 110.27, 102.45, 55.81, 52.35, 32.08, 29.57, 26.85, 25.81, 24.39 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 991.30, C<sub>51</sub>H<sub>45</sub>N<sub>9</sub>O<sub>11</sub>S requires 991.53.

## **Synthesis of FRET-P4**



Scheme S4. Synthesis of FRET-P4. Reagents: (a) CH<sub>3</sub>OH, cont. H<sub>2</sub>SO<sub>4</sub>, reflux, (91%); (b) AIBN, NBS, CCl<sub>4</sub>, reflux, (79%); (c) Ag<sub>2</sub>SO<sub>4</sub>, dioxane/H<sub>2</sub>O=1:1, (86%); (d) Zn, AcOH, DCM, (99%); (e) 5M HCl, NaNO<sub>2</sub>, NaN<sub>3</sub>, (81%); (f) HOBt, DCC, CH3CN, rt, (73%); (g) DCC, DMAP, CH<sub>3</sub>CN, rt, (54%); (h) SiCl<sub>4</sub>, PhOH, DCM; (i) FITC, DMF, DIEA, (60%).

O<sub>2</sub>N COOCH<sub>3</sub>

To a solution of 4-nitrobenzene acetic acid (9.05 g, 50 mmol) in absolute methanol (60 mL), concentrated sulfuric acid (500  $\mu$ L) was added. The reaction mixture was refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with

ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:15, v:v) as an eluent to afford **28** as yellow solid. Yield: 8.9 g (91%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.09 (dd, *J*=8.4 Hz, 2.2 Hz, 2H), 7.38 (dd, *J*=8.0 Hz, 1.8 Hz, 2H), 3.67 (s, 2H), 3.64 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 170.60, 147.18, 141.33, 130.32, 123.69, 52.34, 40.71 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M-1]<sup>-</sup> 194.15, C<sub>9</sub>H<sub>9</sub>NO<sub>4</sub> requires 195.17.



**28** (5 g, 25.6 mmol), N-bromosuccinimide (NBS, 4.8 g, 27 mmol) and Azodi -isobutyronitrile (AIBN, 492 mg, 3 mmol) was mixed in dry tetrachloromethane (50 mL). The reaction mixture was then refluxed for overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:10, v:v) as an eluent to afford **29** as white solid. Yield: 5.6 g (79%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.21 (d, *J*=8.1 Hz, 2H), 7.74 (d, *J*=8.6 Hz, 2H), 5.43 (s, 1H), 3.81 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 167.94, 148.14, 142.53, 129.87, 123.92, 53.78, 44.22 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M]<sup>-</sup> 274.04, C<sub>9</sub>H<sub>8</sub>BrNO<sub>4</sub> requires 274.07.



**29** (3.5 g, 12.8 mmol) and silver sulfate (4.67 g, 15 mmol) was dissolved in 60 mL mixed solvent of dioxane/deionized water (1:1). The reaction mixture was then refluxed for 3 h and free from light. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was

extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:5, v:v) as an eluent to afford **30** as white solid. Yield: 2.32 g (86%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.24 (d, *J*=8.7 Hz, 2H), 7.67 (d, *J*=8.5 Hz, 2H), 5.33 (s, 1H), 3.81 (s, 3H), 3.69 ppm (s, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 172.90, 147.96, 144.95, 127.47, 123.74, 72.01, 53.59 ppm.

Acetic acid (2 mL) was added to the 60 mL DCM solution of **30** (2.11 g, 10 mmol) and zinc powder (13 g, 200 mmol). The reaction mixture was refluxed for 5 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as an eluent to afford **31** as white solid. Yield: 1.8 g (99%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta = 7.02$  (d, *J*=7.9 Hz, 2H), 6.51 (d, *J*=7.8 Hz, 2H), 5.81-5.51 (m, 1H), 5.09 (s, 2H), 4.91 (s, 1H), 3.58 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta = 174.11$ , 148.98, 128.07, 127.08, 113.91, 72.79, 51.93 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+H]<sup>+</sup> 182.09, C<sub>9</sub>H<sub>11</sub>NO<sub>3</sub> requires 181.19.

To the solution of **31** (800 mg, 4.5 mmol) in 5 mL HCl solution (5M). Sodium nitrite solution (932 mg, 13.5 mmol in 10 mL deionized water) was slowly added within 30 min. The mixed solution was stirred vigorously in an ice-water bath, and then sodium azide (878 mg, 13.5 mmol) was added in batches. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated sodium

bicarbonate solution, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **32** as white solid. Yield: 755 mg (81%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 12.65 (s, 1H), 7.44 (d, *J*=8.3 Hz, 2H), 7.36-6.93 (m, 2H), 5.90 (s, 1H), 5.02 ppm (d, *J*=2.5 Hz, 1H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 174.38, 139.13, 137.66, 128.72, 119.30, 72.23 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M-N<sub>2</sub>]<sup>+</sup> 165.70, C<sub>8</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub> requires 193.05.



**32** (500 mg, 2.6 mmol), N- tert-butyl carbonyl diamine (564 mg, 3 mmol), 1-Hydroxy Benzotriazole (HOBT, 459 mg, 3 mmol) and dicyclohexylcarbodiimide (DCC, 618 mg, 3 mmol) were dissolved in acetonitrile (15 mL), and the reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate as an eluent to afford **33** as white solid. Yield: 690 mg (73%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 7.99 (t, *J*=5.6 Hz, 1H), 7.43 (d, *J*=8.3 Hz, 2H), 7.07 (d, *J*=8.4 Hz, 2H), 6.77 (s, 1H), 6.16 (d, *J*=4.7 Hz, 1H), 4.89 (d, *J*=4.6 Hz, 1H), 3.16-2.95 (m, 2H), 2.96-2.78 (m, 2H), 1.80-1.18 ppm (m, 13H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 172.22, 156.02, 138.99, 138.77, 128.58, 119.08, 77.77, 73.35, 33.81, 28.73, 27.39, 27.01 ppm. m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 386.36, C<sub>17</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub> requires 363.19.



33 (500 mg, 1.4 mmol), 4-amino azobenzene-dimethyl-4'-formic acid (538 mg, 2 mmol), dicyclohexylcarbodiimide (DCC, 412 mg, 2 mmol) and 4-dimethylamino -pyridine (DMAP, 25 mg, 0.2 mmol) were dissolved in acetonitrile (10 mL). The resulting solution was cooled to  $0^{\circ}$ C and the reaction was stirred overnight under nitrogen. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:2, v:v) as an eluent to afford **34** as yellow solid. Yield: 466 mg (54%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta = 8.20$  (d, J=7.8 Hz, 2H), 8.00-7.65 (m, 4H), 7.58 (d, J=7.2 Hz, 2H), 7.07 (d, J=8.1 Hz, 2H), 6.84-6.58 (m, 3H), 6.30 (s, 1H), 4.64 (s, 1H), 3.45-2.78 (m, 10H), 1.62-1.19 ppm (m, 13H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 168.29, 164.76, 156.49, 156.15, 153.06, 143.69, 140.76, 132.43, 130.83, 129.01, 125.69, 122.21, 119.37, 111.47, 79.21, 75.49, 40.27, 39.25, 33.94, 28.42, 27.59, 26.42 ppm. m/z MS  $(\text{ESI-TOF}^+)$  found  $[\text{M+Na}]^+$  637.52,  $C_{33}H_{42}N_8O_5$  requires 614.33.

#### **FRET-P4**



To the solution of **34** (204 mg, 0.33 mmol) dichloromethane (8 mL), 1 M SiCl<sub>4</sub> and 3 M PhOH in methylene chloride (600  $\mu$ L) were added. The reaction mixture was stirred for 10 min at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and directly used in the next reaction. The crude product in the previous step was redissolved in N, N-dimethylformamide (10 mL), triethylamine (1 mL) and fluorescein isothiocyanate (195 mg, 0.5 mmol) were then added. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether/methanol (10:5:1, v:v:v) and then acetone/methanol (10:1, v:v) as an eluent to afford **FRET-P4** as yellow solid. Yield: 181 mg (60%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  = 10.20 (s, 2H), 8.17 (t, J=8.2 Hz, 2H), 7.91-7.73 (m, 5H), 7.74-7.56 (m, 2H), 7.26-7.10 (m, 3H), 6.85 (d, J=9.2 Hz, 2H), 6.71 (s, 3H), 6.66-6.51 (m, 5H), 6.18-6.02 (m, 1H), 3.47 (s, 1H), 3.22-2.99 (m, 10H), 1.61-1.32 ppm (m, 4H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  = 208.85, 170.79, 169.13, 168.78, 168.07, 164.96, 160.07, 155.99, 153.56, 152.51, 152.34, 147.46, 143.16, 142.25, 140.24, 133.29, 131.27, 129.51, 125.87, 122.32, 119.76, 113.16, 112.06, 110.35, 102.73, 75.76, 68.97, 56.29, 26.91, 26.21, 25.92, 25.24 ppm. m/z MS  $(\text{ESI-TOF}^+)$  found  $[M]^+$  902.30,  $C_{48}H_{41}N_9O_8S$  requires 902.28.

## **Synthesis of FRET-P5**



Scheme S5. Synthesis of FRET-P5 Reagents: (a) cont HNO<sub>3</sub>, AcOH, 0°C, (31%); (b) CH<sub>3</sub>I, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, (87%); (c) (CH<sub>3</sub>)<sub>3</sub>SiCN, CH<sub>3</sub>CN, conc HCl,(94%); (d) CH<sub>3</sub>OH, conc H<sub>2</sub>SO<sub>4</sub>, reflux, (89%); (e) Pd-C, HCOOCH<sub>3</sub>,CH<sub>3</sub>OH, (76%); (f) TMSN<sub>3</sub>, t-BuONO, CH<sub>3</sub>CN, rt, (99%); (g) 2 M NaOH, MeOH, rt, (93%); (h) HOBt, EDCI, TEA, CH<sub>3</sub>CN, rt, (79%); (i) DCC, DMAP, CH<sub>3</sub>CN, rt, (72%); (j) 1)SiCl<sub>4</sub>, PhOH, DCM; 2) FITC, DMF, TEA, (55%).



To a solution of 3-Hydroxybenzaldehyde (10.0 g, 7.4 mmol) in acetic acid (60 mL), concentrated nitric acid (7.0 mL) in acetic acid (60 mL) was added. The reaction

mixture was cooled to 0°C for 3 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:10, v:v) as an eluent to afford **35** as yellow solid. Yield: 3.84 g (31%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.59 (s, 1H), 10.08 (s, 1H), 8.30 (d, *J* = 8.6 Hz, 1H), 7.68 (s, 1H), 7.52 (d, *J* = 9.4 Hz, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  190.08, 155.34, 142.20, 136.42, 126.17, 122.02, 119.33 m/z MS (EI) found [M]<sup>+</sup> 167.12, C<sub>7</sub>H<sub>5</sub>NO<sub>4</sub> requires 167.20.



**35** (500 mg, 2.99 mmol), methyl iodide (380 mL, 6.0 mmol) and potassium carbonate (420 mg, 3.0 mmol) were dissolved in DMF (6 mL). The resulting mixture solution was stirred for 5 h at 0 °C. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:5 v:v) as an eluent to afford **36** as yellow solid. Yield: 473 mg (87%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.08 (d, *J* = 1.5 Hz, 1H), 8.01-7.88 (m, 1H), 7.62 (s, 1H), 7.56 (dd, *J* = 5.8 Hz, 2.3 Hz, 1H), 4.06 (d, *J* = 1.4 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  190.21, 153.03, 143.38, 139.68, 125.92, 122.60, 112.55, 56.81 m/z MS (EI) found [M]<sup>+</sup> 181.20, C<sub>8</sub>H<sub>7</sub>NO<sub>4</sub> requires 181.15.



The mixture of 36 (292 mg, 1.60 mmol) and trimethylsilyl cyanide (446 mg, 4.50

mmol) in CH<sub>3</sub>CN (10 mL) was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and was directly used in the next reaction. The crude product in the previous step was redissolved in concentrated HCl (10 mL), and the resulting mixture solution was stirred for 2 h at 100°C. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate as an eluent to afford **37** as yellow oil. Yield: 341 mg (94%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  8.21-7.65 (m, 1H), 7.41-7.16 (m, 2H), 5.14 (s, 1H), 4.29-3.20 (m, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  173.55, 152.42, 147.86, 138.79, 125.46, 118.97, 112.47, 72.35, 57.05. m/z MS (EI) found [M]<sup>+</sup> 227.20, C<sub>9</sub>H<sub>9</sub>NO<sub>6</sub> requires 227.17.



To a solution of **37** (230 mg, 1 mmol) in absolute methanol (15 mL), concentrated sulfuric acid (200  $\mu$ L) was added. The reaction mixture was refluxed for 2 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **38** as yellow oil. Yield: 215 mg (89%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.86 (d, *J* = 8.3 Hz, 1H), 7.25 (s, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 5.26 (s, 1H), 4.00 (s, 3H), 3.82 (s, 3H), 3.73 (s, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  172.92, 153.16, 144.73, 139.31, 125.87, 118.45, 111.41, 72.08, 56.59, 53.54 m/z MS (EI) found [M]<sup>+</sup> 241.20, C<sub>10</sub>H<sub>11</sub>NO<sub>6</sub> requires 241.20.



The mixture of **38** (594 mg, 2.5 mmol), Pd-C (50 mg), ammonium formate (630 mg, 10 mmol) in CH<sub>3</sub>OH (10 mL) was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford **39** as white solid. Yield: 400 mg (76%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  6.84 (d, *J* = 1.5 Hz, 1H), 6.71 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 1H), 5.79 (s, 1H), 5.21 (s, 2H), 4.96 (s, 1H), 3.75 (s, 3H), 3.59 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6)  $\delta$  173.96, 146.87, 136.84, 128.72, 119.98, 114.28, 109.70, 72.93, 55.75, 51.99 m/z MS (EI) found [M]<sup>+</sup>211.30, C<sub>10</sub>H<sub>13</sub>NO<sub>4</sub> requires 211.21.



To the solution of **39** (105 mg, 0.5 mmol) in 3 mL CH<sub>3</sub>CN, azidotrimethylsilane (150 mg, 1.3 mmol) and t-butyl nitrite (130 mg, 1.3 mmol) were slowly added. The mixed solution was stirred vigorously in an ice-water bath for 2 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated sodium bicarbonate solution, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:3, v:v) as an eluent to afford **40** as colorless oil. Yield: 110 mg (99%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.99 (s, 3H), 5.15 (s, 1H), 3.89 (s, 3H), 3.78 (s, 3H). <sup>13</sup>C NMR

(101 MHz, CDCl<sub>3</sub>) δ 173.83, 152.04, 135.85, 128.56, 120.31, 119.61, 110.15, 72.47, 55.99, 53.13 m/z MS (EI) found [M]<sup>+</sup>237.20, C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub> requires 237.21.



To a solution of **40** (60 mg, 0.25 mmol) in 5 mL methanol, NaOH solution (160 mg, 4 mmol) in 2 mL deionized water was added. The reaction mixture was refluxed for 2 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water at 0°C. Then 5 M HCl solution was added to the mixture and was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) with 5% acetic acid as an eluent to afford **41** as light yellow oil. Yield: 52 mg (93%). <sup>1</sup>H NMR (400 MHz, DMSO-d6, ppm):  $\delta$  7.15 (s, 1H), 6.97 (dd, *J* = 7.3 Hz, 8.4 Hz 2H), 4.84 (s, 1H), 3.81 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-d6, ppm):  $\delta$  174.27, 151.99, 140.43, 126.26 120.49, 119.97, 111.41, 73.05, 56.39 m/z MS (EI) found [M-N<sub>2</sub>]<sup>+</sup> 165.10, C<sub>8</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub> requires 193.16.



**41** (208 mg, 0.9 mmol), N-tert-butyl carbonyl diamine (752 mg, 4 mmol), 1-hydroxy benzotriazole (HOBT, 612 mg, 4 mmol), TEA (2 mL) and 1-(3-Dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDCI, 768 mg, 4 mmol) were dissolved in acetonitrile (8 mL), The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by
silica gel column chromatography using ethyl acetate as an eluent to afford **42** as white solid. Yield: 260 mg (71%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 6.92 (s, 3H), 6.46 (s, 1H), 4.95 (s, 1H), 4.53 (s, 1H), 3.81 (s, 3H), 3.31-3.16 (m, 2H), 3.15-2.95 (m, 2H), 1.55-1.37 (m, 13H) <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  171.69, 155.28, 150.83, 137.02, 126.89, 119.08, 118.38, 109.44, 78.24, 72.52, 54.94, 37.92, 27.39, 26.40, 25.56 m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup>416.67, C<sub>18</sub>H<sub>27</sub>N<sub>5</sub>O<sub>5</sub> requires 393.44.



**42** (260 mg, 0.66 mmol), 4-amino azobenzene-dimethyl-4'-formic acid (269 mg, 1 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 192 mg, 1 mmol) and 4-dimethylamino-pyridine (DMAP, 25 mg, 0.2 mmol) were dissolved in DCM (10 mL). The resulting mixture solution was cooled to 0°C, and stirred overnight. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and then was extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/ petroleum ether (1:1, v:v) as an eluent to afford **43** as yellow solid. Yield: 181 mg (43%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.21 (d, *J* = 8.5 Hz, 2H), 7.93 (dd, *J* = 8.7 Hz, 5.8 Hz, 4H), 7.15 (d, *J* = 5.8 Hz, 2H), 7.03 (d, *J* = 8.6 Hz, 1H), 6.78 (d, *J* = 9.2 Hz, 2H), 6.60 (s, 1H), 6.28 (s, 1H), 4.63 (s, 1H), 3.93 (s, 3H), 3.49-3.27 (m, 2H), 3.14 (s, 8H), 1.76-1.32 (m, 13H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  167.23, 163.74, 155.54,

155.18, 152.05, 151.04, 142.69, 132.25, 129.82, 128.10, 127.80, 124.67, 121.23, 119.48, 118.94, 110.52, 110.45, 74.63, 55.07, 39.26, 38.27, 32.89, 28.68, 27.39, 26.63, 25.35 m/z MS (ESI-TOF<sup>+</sup>) found [M+Na]<sup>+</sup> 667.99, C<sub>33</sub>H<sub>40</sub>N<sub>8</sub>O<sub>6</sub> requires 644.72.



To the solution of 43 (181 mg, 0.28 mmol) in dichloromethane (8 mL), the solution of 1 M SiCl<sub>4</sub> and 3 M PhOH in methylene chloride (600  $\mu$ L) was added, The reaction mixture was stirred for 10 min at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was concentrated without further purification, and directly used in the next reaction. The crude product in the previous step was redissolved in N, N-dimethylformamide (5 mL), triethylamine (1 mL) and fluorescein isothiocyanate (137 mg, 0.35 mmol) were then added. The reaction mixture was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether/methanol (10:5:1, v:v:v) and then acetone/methanol (10:1, v:v) as an eluent to afford FRET-P5 as yellow solid. Yield: 133 mg (51%). <sup>1</sup>H NMR (400 MHz, DMSO-d6)  $\delta$  10.13 (s, 3H), 9.96 (s, 1H), 8.26 (s, 1H), 8.18 (d, J = 8.6 Hz, 2H), 7.86 (dd, J = 8.3 Hz, 8.8 Hz, 4H), 7.33 (d, J = 1.5 Hz, 1H), 7.20-7.17 (m, 1H), 7.11 (d, J = 8.1 Hz, 1H), 6.86 (d, J =9.3 Hz, 2H), 6.68 (d, J = 2.2 Hz, 4H), 6.63 -6.56 (m, 5H), 6.06 (s, 1H), 3.89 (s, 3H), 3.55-3.45 (m, 4H), 3.10 (s, 6H), 1.56-1.46 (m, 4H). <sup>13</sup>C NMR (151 MHz, DMSO-d6) δ 208.85, 169.02, 167.45, 166.05, 165.09, 164.99, 159.95, 159.88, 156.00, 155.94,

154.38, 153.56, 153.55, 153.28, 152.35, 152.27, 143.15, 143.10, 135.44, 135.37, 132.19, 132.05, 131.30, 131.25, 129.50, 129.13, 128.77, 128.74, 125.89, 125.55, 122.34, 121.97, 113.04, 112.06, 112.03, 110.20, 102.70, 67.86, 56.29, 49.07, 38.54, 31.15, 30.27, 30.07, 28.83. m/z MS (ESI-TOF<sup>+</sup>) found  $[M+H]^+$  935.23, C<sub>49</sub>H<sub>43</sub>N<sub>9</sub>O<sub>9</sub>S requires 933.99.



Scheme S6. Synthesis of 3-methyl-2-azide methylbenzoate. Reagents: (a) Zn, AcOH, DCM, (84%); (b) 5M HCl, NaNO<sub>2</sub>, NaN<sub>3</sub>, (81%).



Acetic acid (2 mL) was added to the mixture of 3-methyl-2-nitro methylbenzoate(500 mg, 2.6 mmol) and zinc powder (2.8 g, 50 mmol) in DCM (10 mL). The reaction mixture was refluxed for 5 h. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into deionized water, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:1, v:v) as an eluent to afford 3-methyl-2-amino methylbenzoate as white solid. Yield: 360 mg (84%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.66 (t, *J*=8.1 Hz, 0.9 Hz, 1H), 7.06 (dd, *J*=7.1 Hz, 0.4 Hz, 1H), 6.54-6.38 (m, 1H), 5.70 (s, 2H), 3.74 (s, 3H), 2.03 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 167.95, 147.96, 133.75, 128.09, 121.90, 114.53, 109.12, 50.41, 16.31 ppm. m/z MS (EI) found [M]<sup>+</sup> 165.20, C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> requires 165.19.



Sodium nitrite solution (117 mg, 2 mmol NaNO<sub>2</sub> was dissolved in 10 mL deionized water) was slowly added to HCl solution (5 mL, 5 M) of 3-methyl-2-amino methylbenzoate (140 mg, 0.85 mmol) within 30 min. The mixed solution was stirred vigorously in an ice-water bath, and sodium azide (1.63 g, 25 mmol) was then added in batches. The reaction mixture was refluxed for overnight at room temperature. The reaction was monitored by TLC. After the completion of reaction, the residue was poured into saturated sodium bicarbonate solution, and was then extracted with ethyl acetate. The organic layers were combined and washed with saturated salt water, dried over anhydrous sodium sulfate, then filtered and concentrated. The crude product was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1:3, v:v) as eluent to afford 3-methyl-2-azide methylbenzoate as white solid. Yield: 131 mg ( 81% ). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 7.73 (d, *J*=7.7 Hz, 1H), 7.35 (d, *J*=7.3 Hz, 1H), 7.13 (t, *J*=7.7 Hz, 1H), 3.97 (s, 3H), 2.36 ppm (s, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 166.33, 138.50, 134.64, 133.38, 129.33, 125.03, 124.61, 52.36, 18.20 ppm. m/z MS (EI) found [M-N<sub>2</sub>]<sup>+</sup> 163.10, C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> requires 191.19.

## **Procedure for cell studies**

HeLa cells were cultured in RPMI 1640 medium (Macgene, Beijing, China) supplemented with 10% (v/v) fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. For cellular experiment, cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells per well. After 24 h proliferation (for PMA treated, cells were incubated with PMA (1 µg ml<sup>-1</sup>) in media for 30min), the cells were incubated with FRET-P5 in PBS in the presence of CTAB (500 µM) for 20 min. After PBS washing, the cells were further incubated for 60 min or treated with NaHS (100 µM) or glutathione (250 µM) in media for 60 min. The cells were then washed, stained with Hoechst 33342 (10 mM). After washing out the staining reagents, the cells were then studied by high content analyzer (Operetta, Perkin Elmer).

**Figure S1.** Fluorescence response of 100  $\mu$ M **FRET-P1** to 100  $\mu$ M NaHS. Data were acquired at 37°C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4) with excitation at 488 nm. Emission was collected from 500 to 650 nm at 0, 5, 10, 20, 30, 40, 50 and 60 min after the addition of 100  $\mu$ M NaHS.



**Figure S2.** Fluorescence response of 100  $\mu$ M 3-methyl-2-azide methylbenzoate to 100  $\mu$ M NaHS. Data were acquired at 37 °C in 10 mM degassed solution (PBS:CH<sub>3</sub>CN=1:1, pH 7.4) with excitation at 327 nm. Emission was collected from 350 to 500 nm at 0, 1, 3, 5, 7, 10, 15, 20, 30, 40, 240 min after the addition of 100  $\mu$ M NaHS.



**Figure S3.** Fluorescence response of 100  $\mu$ M **FRET-P2** to 100  $\mu$ M NaHS. Data were acquired at 37 °C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4) with excitation at 488 nm. Emission was collected from 500 to 650 nm at 0, 5, 10, 15, 20, 30, 40, 50 and 60 min after the addition of 100  $\mu$ M NaHS.



**Figure S4.** Fluorescence response of 100  $\mu$ M **FRET-P3** to 100  $\mu$ M NaHS. Data were acquired at 37°C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4 ) with excitation at 488 nm. Emission was collected from 500 to 650 nm at 0, 5, 10, 15, 20, 30, 40, 50 and 60 min after the addition of 100  $\mu$ M NaHS.



**Figure S5.** Fluorescence response of 100  $\mu$ M **FRET-P4** to 100  $\mu$ M NaHS. Data were acquired at 37°C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4) with excitation at 488 nm. Emission was collected from 500 to 650 nm at 0, 5, 10, 15, 20, 30, 40, 50, 60 and 90 min after the addition of 100  $\mu$ M NaHS.



**Figure S6**. Reaction time profile of **FRET-P** series compounds and hydrogen sulfide. Fluorescence response of 100  $\mu$ M FRET-P series compounds to 100  $\mu$ M NaHS. Data were acquired at 37 °C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4) with excitation at 488 nm. Emission was collected from 500 to 650 nm at 2 h after the addition of 100  $\mu$ M NaHS.



**Figure S7.** Fluorescence responses of 100  $\mu$ M FRET-P5 to other relevant species other than RSS species. Bars represent the mean fluorescence responses at 1 h after the addition of 100  $\mu$ M NaSH or 1 mM all other common ions and relevant RNS and ROS species. All measurements were done in fluorospectro photometer. Data were acquired at 37 °C in 10 mM degassed solution (PBS:DMF, v:v=6:4, pH 7.4) with excitation at 488 nm.



Figure S8. HeLa Cell viability with different concentration of FRET-P5



Figure S9 MS spectra of [4'-(N,N'-dimethylamino)phenylazo] benzoyl acid after the reduction of azido and self-immolation reaction.



NMR of all the intermediates and probes















Compound 7 <sup>13</sup>C NMR





Compound 8 <sup>13</sup>C NMR



## FRET-P1 <sup>1</sup>H NMR



FRET-P1 <sup>13</sup>C NMR









Compound 10<sup>13</sup>C NMR





Compound 11 <sup>13</sup>C NMR





Compound 12<sup>13</sup>C NMR





Compound 13 <sup>13</sup>C NMR









FRET-P2<sup>13</sup>C NMR





Compound 16 <sup>13</sup>C NMR





Compound 17 <sup>13</sup>C NMR





Compound 18 <sup>13</sup>C NMR







ppm



Compound 21 <sup>13</sup>C NMR





Compound 22 <sup>13</sup>C NMR





Compound 23 <sup>13</sup>C NMR





Compound 24 <sup>13</sup>C NMR






Compound 26 <sup>13</sup>C NMR





Compound 27 <sup>13</sup>C NMR





FRET-P3 <sup>13</sup>C NMR









В

ÉR

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5





Compound 30

<sup>13</sup>C NMR





Compound 31 <sup>13</sup>C NMR





Compound 32 <sup>13</sup>C NMR





ppm















Compound 36 <sup>13</sup>C NMR









Compound 38 <sup>13</sup>C NMR





Compound 39 <sup>13</sup>C NMR







180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm



Compound 41 <sup>13</sup>C NMR





Compound 42 <sup>13</sup>C NMR





Compound 43 <sup>13</sup>C NMR







