Supporting information

A fluorogenic dye activated by S-nitrosothiols

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Materials and Methods: Reactions were carried out in oven or flame-dried glassware under an argon atmosphere, unless otherwise noted. All solvents were reagent grade. Diethyl ether (Et₂O) and tetrahydrofuran (THF) were freshly distilled from sodium / benzophenone under argon. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040 – 0.062mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ¹H NMR and chloroform (δ 7.76) ¹³C NMR. Absorption spectra were recorded on a Lambda 20 UV/Vis spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on QuantaMaster QM4 from Photon Technologies, Inc.

Salicaldehyde 7’. A solution of 8-hydroxyjulolidine (5.0 g, 26.4 mmol) in 5 mL DMF was added dropwise to a cold solution of POCl₃ (4.46g, 29.1 mmol, 2.66 mL) in 10 mL DMF which had been stirring for 15 min. After 30 min at rt, the solution was heated to 100 °C for 30 min and was then cooled to rt. 25 mL water was added with stirring and a blue-green solid formed slowly. The precipitate was filtered, washed with water, and dried in a vacuum. The solid was dissolved in benzene/5% EtOAc and filtered through silica gel. A yellowish oil (4.92g, 88%) was obtained after the solvent was removed: ¹H NMR (300 MHz, CDCl₃) δ 11.81 (s, 1H), 9.37 (s, 1H), 6.84 (s, 1H), 3.28 (dd, J=11.6, 4.1 Hz, 4H), 2.68 (t, J=6.3 Hz, 4H), 1.97-1.91 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 191.7, 159.5, 149.7, 131.3, 113.8, 110.8, 105.4, 50.5, 50.1, 27.4, 21.8, 20.7, 19.8; MS (MSI+) m/z 218.1 (M+H⁺).

Coumarin 8. A solution of salicaldehyde 7’ (2.2 g, 10 mmol), (2-methoxy-2-oxoethyl)-tri-phenylphosphonium bromide (5.0 g, 12 mmol), and DBU (1.8 mL,
1.8 g, 12 mmol) in DMSO (40 mL) was heated at reflux for 20 min. The solution was then cooled to rt, and poured into a mixture of crushed ice and water in a separatory funnel, then extracted four times with CH₂Cl₂. The combined CH₂Cl₂ extracts were washed twice with water and dried over Na₂SO₄. The product was purified by silica gel chromatography (toluene/EtOAc, 10:1). A brilliant yellow solid was obtained (2.25 g, 93%): ¹H NMR (300 MHz, CDCl₃) δ 7.46 (d, J=9.0 Hz, 1H), 6.84 (s, 1H), 5.99 (d, J=9.3 Hz, 1H), 3.26 (dd, J=11.1, 4.8 Hz, 4H), 2.88 (t, J=6.5 Hz, 2H), 2.75 (t, J=6.5 Hz, 2H), 2.01-1.92 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 163.0, 151.9, 146.2, 144.3, 125.2, 118.5, 108.4, 108.3, 106.8, 50.2, 49.8, 27.7, 21.7, 20.8, 20.5; MS (MSI+) m/z 242.5 (M+H⁺).

**3-Iodocoumarin 9.** A solution of NIS (2.52 g, 11.2 mmol) in THF (25 mL) was added dropwise to a vigorously stirred mixture of coumarin 8 (2.25 g, 9.3 mmol) and TsOH (0.18 g, 9.3 mmol) in 100 mL THF at rt. After stirring for 30 min following the addition the reaction was diluted with CH₂Cl₂, washed twice with 5% Na₂S₂O₃, once each with sat. NaHCO₃ and brine, then dried over Na₂SO₄. A yellow-orange solid (3.0 g, 87%) was obtained following purification over a silica column eluting with CH₂Cl₂: ¹H NMR (300 MHz, CDCl₃) δ 8.00 (s, 1H), 6.76 (s, 1H), 3.29-3.24 (m, 4H), 2.86 (t, J=6.6 Hz, 2H), 2.74 (t, J=6.2 Hz, 2H), 1.99-1.91 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 159.2, 152.6, 152.1, 146.6, 124.3, 118.9, 110.2, 106.5, 74.5, 50.2, 49.8, 27.7, 21.5, 20.6, 20.4; MS (MSI+) m/z 367.1 (M+H⁺).

**Phosphine 5.** A solution of 3-iodocoumarin 9 (1 g, 2.7 mmol), Pd(OAc)₂ (1.7 mg, 7.6 μmol), triethylamine (0.51 mL) in acetonitrile (50 mL) was cooled to -70 °C, degassed and filled with Ar. Diphenylphosphine (0.46 mL, 2.7 mmol) was added after the system warmed to rt. Then the solution was heated to reflux for 5 h. When cooled to rt, a solution of 300 mL water and 400mL ethyl acetate was used for partition followed by washing twice with brine and drying with Na₂SO₄. A brilliant yellow solid (1.04g, 90%) was obtained following purification over silica gel (hexane/EtOAc, 5:1 to 2:1): ¹H NMR (300 MHz, CDCl₃) δ 7.42-7.33 (m, 10H), 6.97 (d, J=3.6 Hz, 1H), 6.67 (s, 1H), 3.26 (t, J=5.4 Hz, 4H), 2.88 (t, J=6.5 Hz, 2H), 2.68 (t, J=6.2 Hz, 2H), 1.94 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 163.1, 162.8, 152.1, 148.5, 148.4, 146.4, 136.0, 135.8, 134.2, 134.1, 133.9, 129.3, 129.2, 128.9, 128.8, 125.4, 118.5, 117.4, 117.3, 109.1, 106.5, 50.3, 49.9, 27.6, 21.6, 20.7, 20.5; ³¹P NMR (121 MHz, CDCl₃) δ -14.4; MS (MSI+) m/z 426.1 (M+H⁺).
**Compound 6.** A solution of S-nitrosogluthathione (0.677 g, 2 mmol) in water (7 mL) was added dropwise to a solution of phospine 5 (0.0830 g, 0.2 mmol) in THF (21 mL), stirred at rt. for 2 hours. After extracted with dichloromethane (30 mL) for 3 times, the organic layer was washed twice with brine and dried with Na₂SO₄. A yellow-orange solid (0.0792 g, 92%) was obtained following purification over silica gel (hexane/EtOAc, 2:1 to 1:1): ¹H NMR (300 MHz, CDCl₃) δ 8.50 (d, J=13.5 Hz, 1H), 7.89-7.82 (m, 4H), 7.52-7.38 (m, 6H), 6.95 (s, 1H), 3.27 (q, J=5.7 Hz, 4H), 2.79 (t, J=6.5 Hz, 2H), 2.72 (t, J=6.3 Hz, 2H), 1.91 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 161.1, 160.9, 153.6, 153.5, 153.4, 148.3, 132.9, 132.2, 132.1, 132.0, 131.4, 128.5, 128.4, 126.9, 119.2, 109.3, 108.5, 108.4, 107.8, 106.0, 50.4, 50.0, 27.7, 21.4, 20.4, 20.2; ³¹P NMR (121 MHz, CDCl₃) δ 26.9; MS (MSI+) m/z 442.2 (M+H⁺).

**Determination of fluorescence quantum yields.** The fluorescence quantum yield (Φ) for the same compound has a relationship with several factors described in equation 1:

\[
I = 2.3ΦI_0εcl
\]  
(1)

In equation 1, \(I\) and \(I_0\) are fluorescent excitation and emission intensities in emission spectrum, \(ε\) is the molar absorption coefficient of the compound, \(c\) is the concentration of the compound and \(l\) is the width of the cuvette. For the determination of fluorescence quantum yields, it is convenient to use the same excitation wavelength for the emission detection of the samples for we can eliminate and avoid the detection of excitation intensity. Then do the same detection of a standard compound with known quantum yield (Φ₀), we can determine the unknown’s (Φ) by using equation 2:

\[
Φ = Φ_0 × (ε/ε_0) × (E/E_0)
\]  
(2)

Here, the molar absorption coefficient of the unknown (ε) was determined by plotting a series of absorption versus different concentrations (Fig. 1).

![Fig. 1](image.png)  
**Fig. 1** Determination of the molar absorption coefficients of compound 5 (♦) and 6 (■).
The area under the entire emission spectrum for both unknown (E) and the standard (E_0) which represent the emission intensity of each was determined by numeric integration using the computer program Origin 8.0. Quinine bisulfate (Φ = 0.55 in 1M H₂SO₄, ε₀ = 1205 cm⁻¹M⁻¹ at 380 nm) was used as a standard to determine the quantum yields of 5 and 6 in phosphate-buffered saline (PBS). Optically dilute solutions of the quinine bisulfate, 5 or 6 were exited at 380 nm and the areas under the emission spectra were determined by numerical integration (quinine bisulfate: 385-650 nm, 5,6: 450-600 nm).

Linear relationship between fluorescent emission intensity and the concentrations of S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP): A series of 0, 20, 40, 80, 160, 320 μL stock solution of GSNO in water (1.412 mM) were each mixed with a stock solution of phosphine 5 in DMSO (1.104 mM) and diluted to a 4240 μL PBS solution (pH 7.4). After 1 h, 20 μL of each were taken to 4000 μL PBS solution (pH 7.4), and detected by fluorescent emission spectrum (λ_ex = 437 nm, λ_em = 487 nm). The curve of the emission intensity at any concentration of GSNO was determined. To test its applicability for other RSNOs, a series of 0, 20, 40, 60, 80, 100 μL stock solution of SNAP in THF (2.664 mM) were each mixed with a stock solution of phosphine 5 in DMSO (1.104 mM). After 5 h, 20 μL of each were taken to 4000 μL PBS solution (pH 7.4), and detected by fluorescent emission spectrum (λ_ex = 437 nm, λ_em = 487 nm). All the data are combined and displayed in figure 2.

![Fig. 2 Determination of concentration of SNAP (■) using the linear relationship between the emission intensity and the concentration of GSNO (●) after the reaction with compound 5.](image)

The linear equation of the plot is \( y = 9.737 \times 10^{11} x + 1.512 \times 10^{4} \), is good in linearity (R²=0.9944), where 1.512×10^4 is the average background of compound 5.