Supporting Information for

A highly selective fluorescent probe for fast detection of nitric oxide in aqueous solution

Chang-Bo Huang^a, Junhai Huang^{b,*} and Lin Xu^{a,*}

^aShanghai Key Laboratory of Green Chemistry and Chemical Processes, Department of Chemistry, East China Normal University, 3663 N. Zhongshan Road, Shanghai, P.R. China. Email: lxu@chem.ecnu.edu.cn

^bZhangjiang Institute, China State Institute of Pharmaceutical Industry, 1599 Zhangheng Road, Shanghai 201203, P.R. China. Email: huangjunhai@sinopharm.com

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1.General information

In Fig. 1, the saturated NO aqueous solution ($c_{NO} = 1.9 \text{ mM}$, *ref. Chem. Rev.*, 2002, **102**, 1235) was prepared by bubbling NO into de-ionized water for 15 min. (*Ref. J. Am. Chem. Soc.*, 2010, **132**, 13114). The experiments in Figure 1 were conducted by adding various volumes of saturated NO aqueous solution ($c_{NO} = 1.9 \text{ mM}$) into the sodium phosphate buffer solution (50 mM, ethanol/water, 1:1, pH 7.4) of **NPA** (100 μ M) under aerobic conditions separately. The fluorescence intensities were detected at 490 nm with excitation at 400 nm.

In Fig. 2, the spectra were obtained by adding various of ROS/RNS such as NO, ${}^{1}O_{2}$, H₂O₂, KO₂, ClO⁻, NO₂⁻, NO₃⁻, ·OH, and ONO₂⁻ into the sodium phosphate buffer (50 mM, ethanol/water, 1:1, pH 7.4) of **NPA** (100 μ M) for 3 minutes.

In Fig. 3 and Fig. 4, the spectra were obtained by adding various amounts of NO into the sodium phosphate buffer (50 mM, ethanol/water, 1:1, pH 7.4) of **NPA** (100 μ M) for 3 minutes.

The detection limit (DL) of **NPA** for NO was determined based on the mathematical equation of $DL = 3 \cdot Sb \cdot m^{-1}$. (Sb: standard deviation, m: slope). (*Ref: Anal. Chim. Acta*, 2009, **651**, 227.).

2. Synthesis of compounds 2, 3, NPA, and NPT



Scheme S1 Synthesis of compound 2

Compound 2: In a dry flask under argon-atmosphere were placed **1** (500 mg, 1.5 mmol), anhydrous ethanol (15 mL), Pd(PPh₃)₂Cl₂ (52.8 mg, 0.075 mmol), CuI (14.3 mg, 0.075 mmol), and dry TEA (5 mL) followed by trimethylsilylacetylene (0.43 mL, 3.0 mmol). It was refluxed for 12 h. Then the reaction mixture was allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The crude product was then chromatographed on silica gel using dichloromethane/petroleum ether 1: 1 (v/v) as eluant to afford 413 mg (78.6%) **2** as beige solid. ¹H NMR (400 MHz, CDCl₃) δ 8.63 (d, *J* = 2.0 Hz, 1H), 8.61 (d, *J* = 0.8 Hz, 1H), 8.51 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.82 (t, *J* = 8.0 Hz, 1H), 4.17 (t, *J* = 8.0 Hz, 2H), 1.72 (dt, *J* = 21.0, 7.6 Hz, 2H), 1.45 (m, 2H), 0.98 (t, *J* = 7.6 Hz, 3H), 0.36 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz) δ : 164.15, 163.87, 132.53, 131.90, 131.70, 131.33, 130.37, 128.04, 127.64, 127.35, 123.06, 122.48, 105.36, 101.40, 40.48, 30.34, 20.54, 14.01, 0.002. EI-MS for C₂₁H₂₃NO₂Si [M⁺]: 349.



Scheme S2 Synthesis of compound 3

Compound 3: **2** (240 mg, 0.69 mmol) was dissolved in dry methanol (40 mL) and K_2CO_3 (759.6 mg, 5.50 mmol) were added. It was heated for 2 h at room temperature. The mixture was filtered, and the solvent was removed in vacuum to give a white solid. The crude product was then chromatographed on silica gel using dichloromethane/petroleum ether 2: 1 (v/v) as eluant to afford

153 mg (80.3 %) **3** as white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.70 – 8.61 (m, 2H), 8.54 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.83 (dd, J = 8.4, 7.6 Hz, 1H), 4.18 (t, J = 7.6 Hz, 2H), 3.73 (s, 1H), 1.71 (ddd, J = 12.6, 8.6, 6.6 Hz, 2H), 1.45 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ : 163.95, 163.67, 132.17, 131.94, 131.67, 131.65, 130.14, 127.90, 127.70, 126.16, 122.99, 122.84, 86.48, 80.34, 40.38, 30.20, 20.40, 13.88. EI-MS for C₁₈H₁₅NO₂ [M⁺]: 277.



Scheme S3 Synthesis of compound NPA

Compound NPA: In a dry flask under argon-atmosphere were placed **3** (200 mg, 0.72 mmol), THF (30 mL), Pd(PPh₃)₂Cl₂ (50.6 mg, 0.072 mmol), CuI (13.7 mg, 0.072 mmol), and dry TEA (30 mL) followed by 4-iodo-1, 2-phenylenediamine (168.7 mg, 0.72 mmol). It was refluxed for 12 h. Then the reaction mixture was allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The crude product was then chromatographed on silica gel using dichloromethane/methanol 50: 1 (v/v) as eluant to afford 231 mg (83.5%) **NPA** as reddish brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.68 (d, *J* = 8.0 Hz, 1H), 8.53 (d, *J* = 6.8 Hz, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 7.95 (t, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 6.88 (dd, *J* = 4.0, 2.4 Hz, 2H), 6.60 – 6.54 (m, 1H), 5.22 (s, 2H), 4.74 (s, 2H), 4.03 (t, *J* = 8.0 Hz, 2H), 1.64 – 1.57 (m, 2H), 1.41 – 1.29 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 163.23, 162.93, 137.98, 134.58, 131.90, 131.12, 130.56, 130.25, 129.51, 127.82, 127.48, 122.74, 122.55, 120.43, 116.87, 113.67, 108.03, 103.17, 83.96, 48.56, 29.60, 19.79, 13.71. HR-EI-MS for C₂₄H₂₁N₃O₂ [M⁺]: 383.1635. Elemental analysis for **NPA**: C, 73.64; N, 10.16; H, 5.69.



Scheme S4 Synthesis of compound NPT

Compound NPT: Sodium nitrite (NaNO₂, 89.7 mg, 1.3 mmol) was added dropwise to a mixture of **NPA** (50 mg, 0.13 mmol) in HCl (25 mL, aqueous, pH 2) solution over a period of 30 minutes at 0°C. The mixture was stirred for 1 h at room temperature. The crude product was extracted with CH₂Cl₂, washed with water in twice, and evaporated. The crude product was then chromatographed on silica gel using dichloromethane/methanol 100: 1 (v/v) as eluant to afford 31 mg (60.8%) **NPT** as yellow solid. ¹H NMR (400 MHz, CDCl₃ : CD₃OD = 3 : 2) δ : 8.51 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 7.96 (s, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.68 – 7.60 (m, 2H), 7.48 (dd, *J* = 8.6, 1.2 Hz, 1H), 3.90 (t, *J* = 8.0 Hz, 2H), 1.51 – 1.39 (m, 2H), 1.25 – 1.13 (m, 2H), 0.72 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃ : CD₃OD = 3 : 2) δ : 168.17, 167.88, 143.07, 142.16, 136.41, 135.64, 135.49, 134.85, 134.32, 133.52, 131.89, 131.59, 131.33, 126.65, 125.95, 123.66, 123.05, 118.79, 102.43, 90.42, 44.22, 34.00, 24.17, 17.44. HR-ESI-MS for C₂₄H₁₈N₄O₂ [M + H]⁺: 395.34.

3. The pH-titration of NPA



Fig. S1 The influence of pH on the absorption (a) and fluorescence (b, c) of NPA (10 μ M) in ethanol/water solution (1:1, v/v). Excitation was performed at 430 nm.



4. The nomalized absorption and fluorescence emission spectra of NPT

Fig. S2 The nomalized absorption (a) and fluorescence emission (b) spectra of NPA (100 μ M) in the presence of 2.5 equivalents NO solution (black line) and free NPT (100 μ M) (red line) in sodium phosphate buffer (50 mM, ethanol/water, 1:1, pH 7.4).