Electronic supplementary information

For

A Near Infrared Colorimetric and Fluorometric Probe for Organophosphorus Nerve Agent Mimic by Intramolecular Amidation

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Instruments and materials

NMR spectra were recorded on Bruker AV400 NMR spectrometers, and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, quint = quintet, m = multiplet, b = broad. High-resolution mass spectra (HR-MS) spectra were measured with a Bruker En Apex ultra 7.0T FT-MS mass spectrometer. Fluorescence spectra were performed on a Hitachi F7000 fluorescence spectrometer (Tokyo, Japan). Absorption spectra were recorded on a HITACHI U3900 spectrophotometer. The fluorescence quantum yield was determined on Horiba Jobin Yvon HJY-FM4P-TCSPC fluorescence spectrometer.

Diethyl chlorophosphate (DCP) and diethyl cyanophosphonate (DCNP) were purchased from Acros Organic. Dry DMF and 4-Aminobutyric acid were bought from J&K SCIENTIFIC LTD. All other reagents and solvents were purchased from commercial sources and used without further purification. Reactions were monitored by TLC with visual observation of the dye spots. Products were purified by column chromatography.

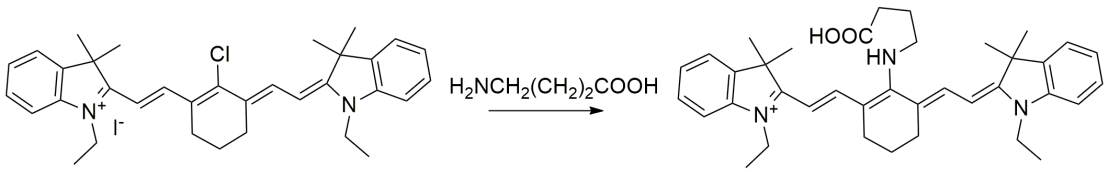
General detection procedures

Probe **1** was dissolved in dry DMF to make a 2.0×10^{-4} M stock solution. DMAP was dissolved in CH_2Cl_2 to make a 5.0×10^{-3} M stock solution. DCP, oxalyl chloride, thionyl chloride, and p-toluenesulfonic acid were dissolved in CH_2Cl_2 to make a 1.0×10^{-3} M stock solution, respectively.

HCl was prepared as 1.0×10^{-3} M stock solution in CH_3CN.

To totally 5 mL CH_3CN solution containing 50 μL of 5×10^{-3} M DMAP and different amounts of DCP, an aliquot of 25 μL of the stock solution of probe **1** was added by a pipette, then the solution was mixed well, and the absorption and fluorescence detection were run after 15 minutes.

Synthesis of probe 1



probe **1**

Heptamethine cyanine (63.9 mg, 0.1 mmol) and 4-Aminobutyric acid (41.2 mg, 0.4 mmol) were dissolved in dry N,N-dimethylformamide (30 mL). The mixture was stirred at 85 ℃ under an nitrogen atmosphere for 30 minutes. The saturated brine was added to the reaction solution, then extracted with methylene dichloride. The organic layer was collected and dried over sodium sulfate. The solution was filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane: methanol (60 ~ 10/1 = v / v) as an eluent to give the desired product in 70% yield.

^{1}H NMR (400 MHz, CDCl3) δ (ppm): δ 10.01 (bs, 1H, N**H**), 7.68 (d, J = 12.3 Hz, 2H), 7.30 – 7.25 (m, 4H), 7.05 (t, J = 7.4 Hz, 2H), 6.85 (d, J = 7.8 Hz, 2H), 5.57 (d, J = 12.3 Hz, 2H), 3.84 (m, 6H: -NHC**H**$_2$CH$_2$CH$_2$COOH + 2×CH$_3$C**H**$_2$N$^+$), 2.86 (m, 2H, -NHCH$_2$CH$_2$C**H**$_2$COOH), 2.52 (t, J = 6.1 Hz, 4H), 2.16 (m, 2H, -NHCH$_2$C**H**$_2$CH$_2$COOH), 1.85 (quint, J = 6.1 Hz, 2H), 1.67 (s,12H), 1.35 (t, J = 6.9 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 170.19, 165.94, 142.71, 140.31, 136.91, 127.98, 122.23, 122.08, 119.81, 107.79, 93.13, 53.47, 51.24, 47.47, 37.67, 35.25, 28.73, 26.18, 25.89, 21.24, 11.34.

HR-MS(ESI): Calcd. for $C_{38}H_{48}N_3O_2^+$: 578.37465(M^+); found: 578.37429.

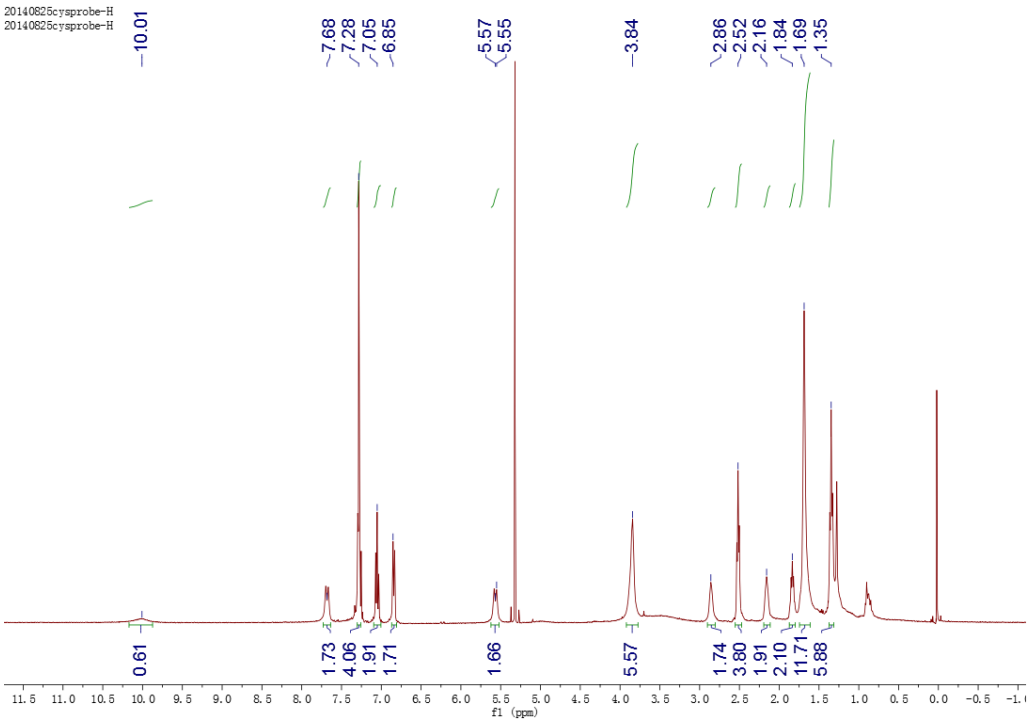


Fig. S1 ^{1}H NMR of probe **1** in $CDCl_3$.

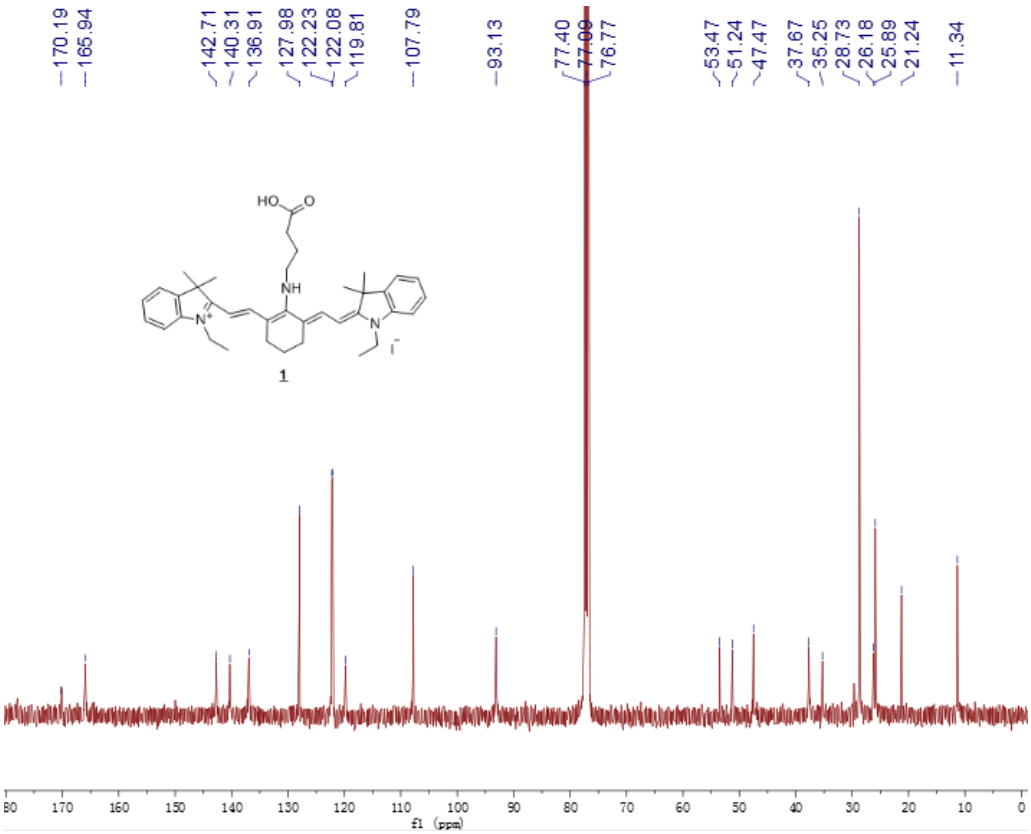


Fig. S2 ^{13}C NMR of probe **1** in $CDCl_3$.

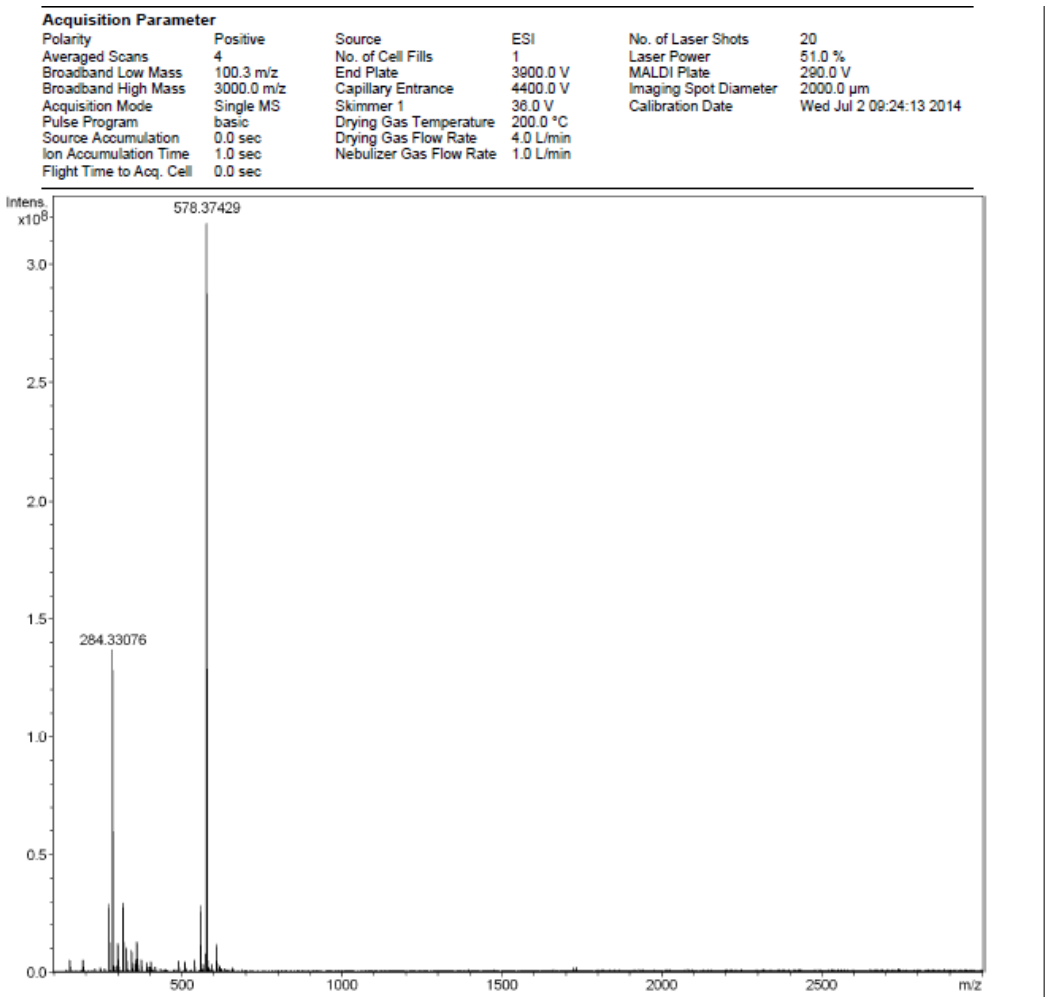


Fig. S3 HR-MS mass spectra (positive) of probe **1**.

Acquiring product 2

This experiment must be done in a well-ventilated place. Probe **1** (10.0 mg, 0.014 mmol) was dissolved in acetonitrile (10 mL) under the presence of DMAP (5.2 mg, 0.043 mmol), then DCP (3.7 mg, 0.021 mmol) was added. The mixture was stirred at room temperature for 60 minutes. The organic layer was evaporated under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane: methanol (10/1 = v / v) as an eluent to give the desired product in *ca.* 75% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 14.1 Hz, 2H), 7.43 – 7.33 (m, 4H), 7.23 (t, J = 7.4 Hz, 2H), 7.15 (d, J = 7.9 Hz, 2H), 6.20 (d, J = 14.0 Hz, 2H), 4.32-4.22 (m, 2H, 2×$CH_3C\underline{\mathbf{H}}^1H^2N^+$)，4.22-4.12 (m, 2H, 2×$CH_3CH^1\underline{\mathbf{H}}^2N^+$), 3.78 (t, J = 6.9 Hz, 2H, -NHC$\underline{\mathbf{H}}_2CH_2CH_2CO$-), 2.85-2.72 (m, 4H), 2.66-2.56 (m, 2H), 2.52-2.42 (m, 2H), 2.12-2.04 (m, 1H), 1.94 – 1.87 (m, 1H), 1.68 (s, 6H), 1.62 (s, 6H), 1.44 (t, J = 7.2 Hz, 6H).

HR-MS (ESI): Calcd. for $C_{38}H_{46}N_3O^+$: 560.36408(M^+); found: 560.36321.

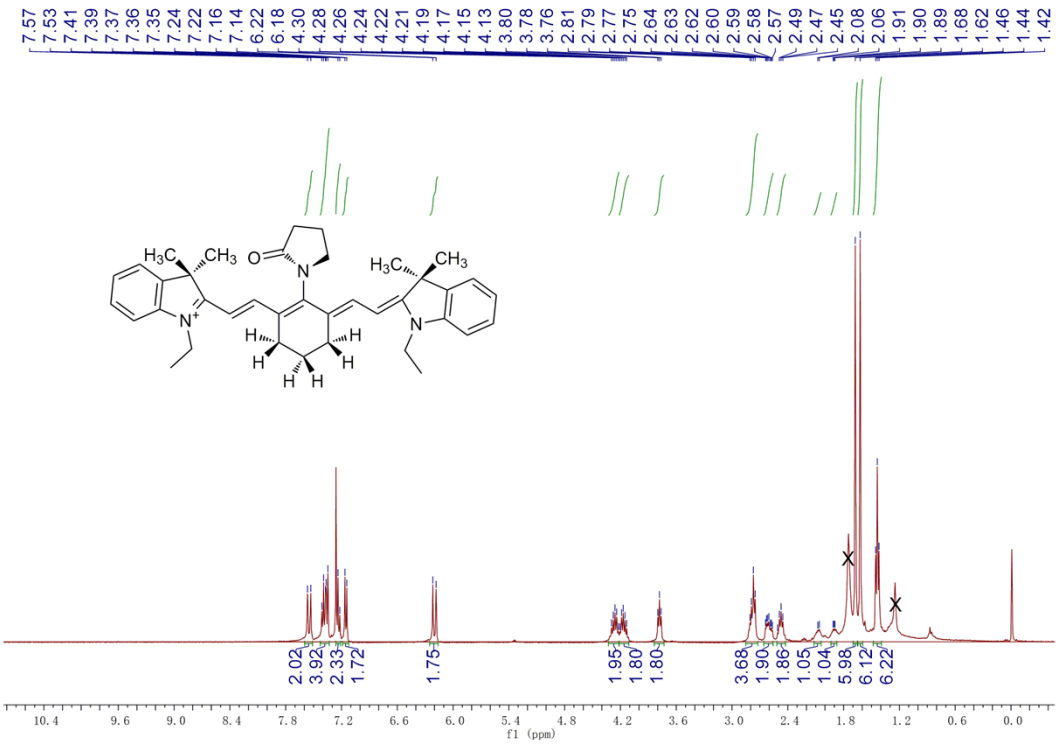


Fig. S4 ^{1}H NMR of product **2** in $CDCl_3$.

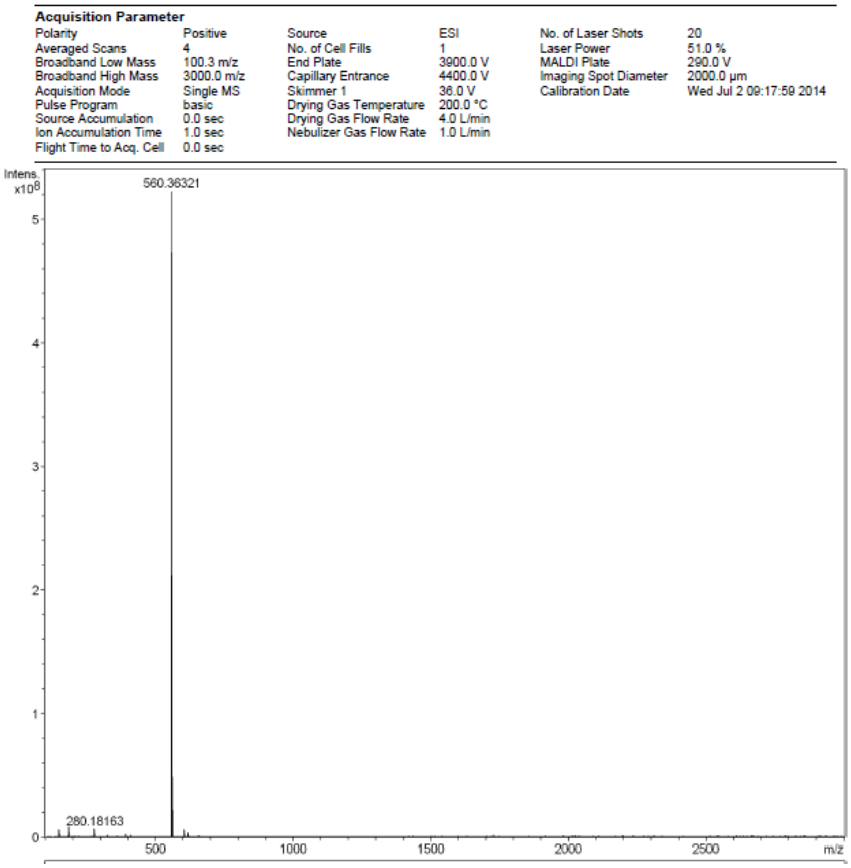


Fig. S5 HR-MS spectra (positive) of product **2**.

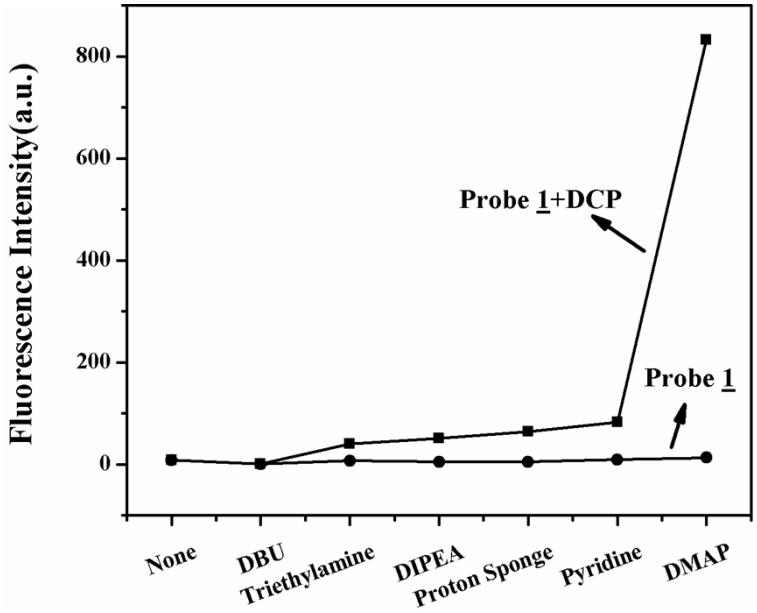


Fig. S6 Effect of various base (100.0 μM) on the fluorescent response of probe **1** (1.0 μM) to the DCP (10.0 μM). Base used: None, DBU, triethylamine, diisopropylethyl amine (DIPEA), proton sponge, pyridine and 4-dimethylaminopyridine (DMAP).

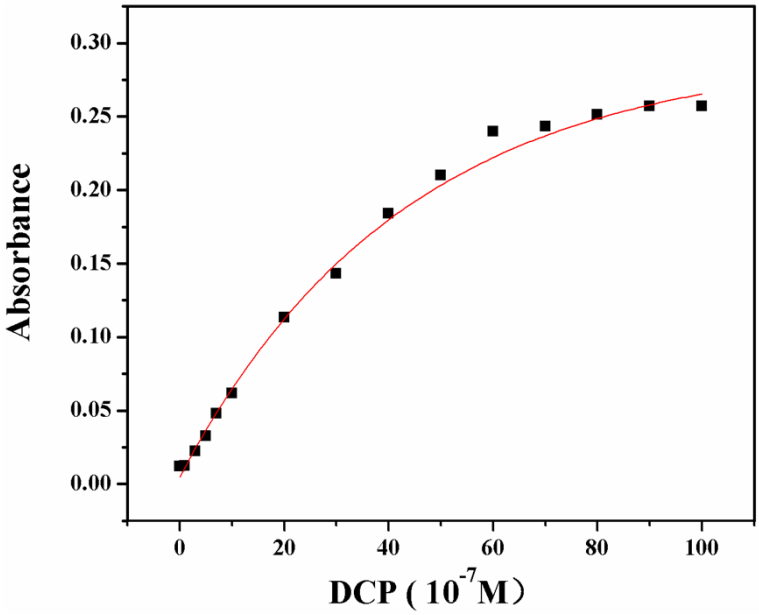


Fig. S7 Titration curve of the absorbance (784 nm) of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50 μM) *vs.* various concentrations of DCP. [DCP] = 0, 0.1, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0,7.0, 8.0, 9.0 ,10.0 μM, respectively.

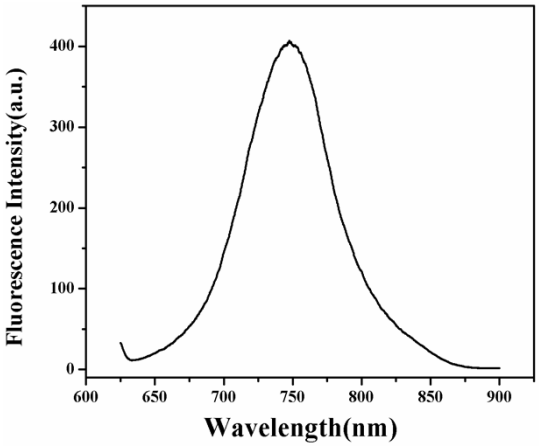


Fig. S8 Emission spectra of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50μM). λex = 620 nm.

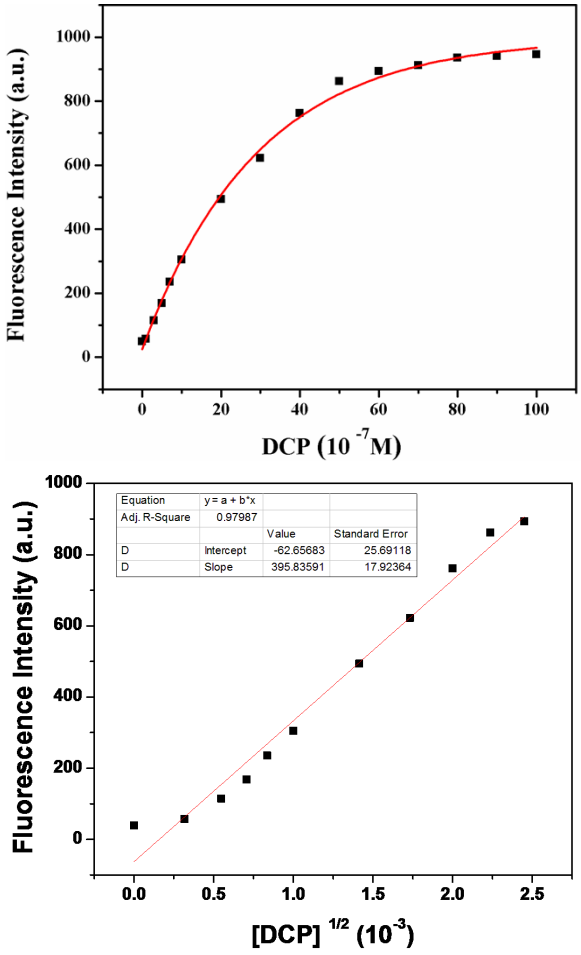


Fig. S9 (Upper) Titration curve of the NIR fluorescence (807 nm) enhancement of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50μM) *vs.* various concentrations of DCP. [DCP] = 0, 0.1, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM, respectively. λex = 760 nm. (Lower) LOD for probe **1** with DCP in acetonitrile at λex = 760 nm, λem = 807 nm. LOD is 0.136 nM between 0～6. 0μM of DCP (data fit to the nonlinear equation):

$$y = 395.84 \times \text{sqrt}(x) - 62.66, \qquad R^2 = 0.98$$

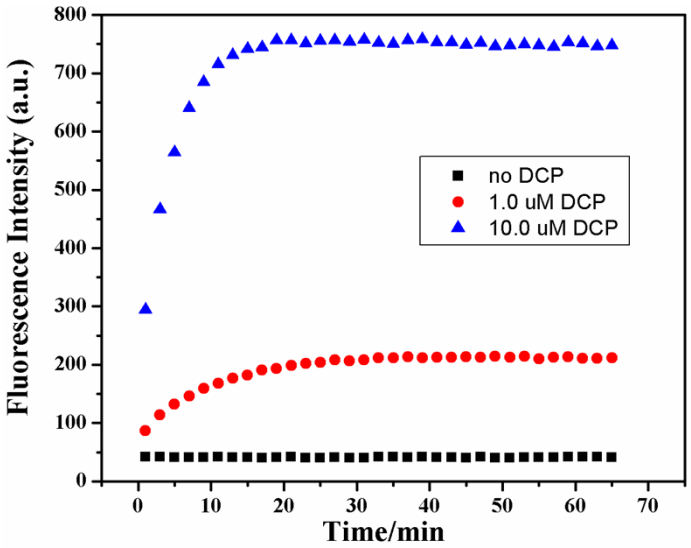


Fig. S10 Time course (0 – 3900 s) of fluorescence enhancement of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50.0μM) upon addition of two concentration of DCP. λex = 760 nm; λem = 807 nm.

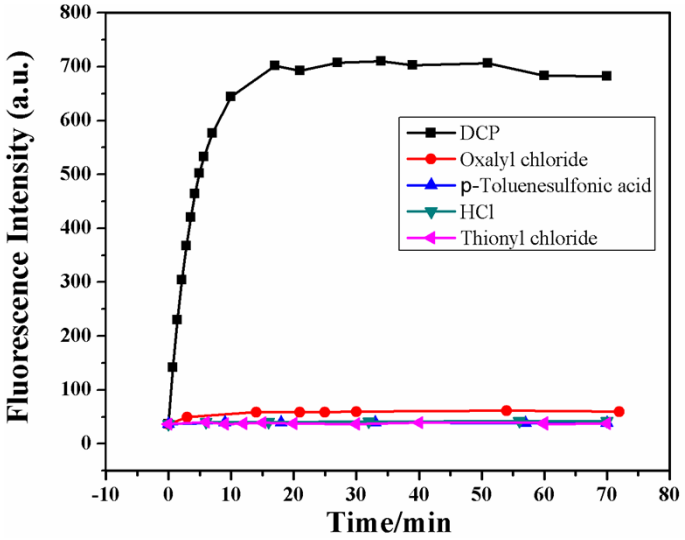


Fig. S11 Time course (0 – 4200 s) of fluorescence enhancement of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50.0μM) upon addition of various species (10.0 μM) of guests: DCP, thionyl chloride, oxalyl chloride, HCl and p-toluenesulfonic acid. λex = 760 nm; λem = 807 nm.

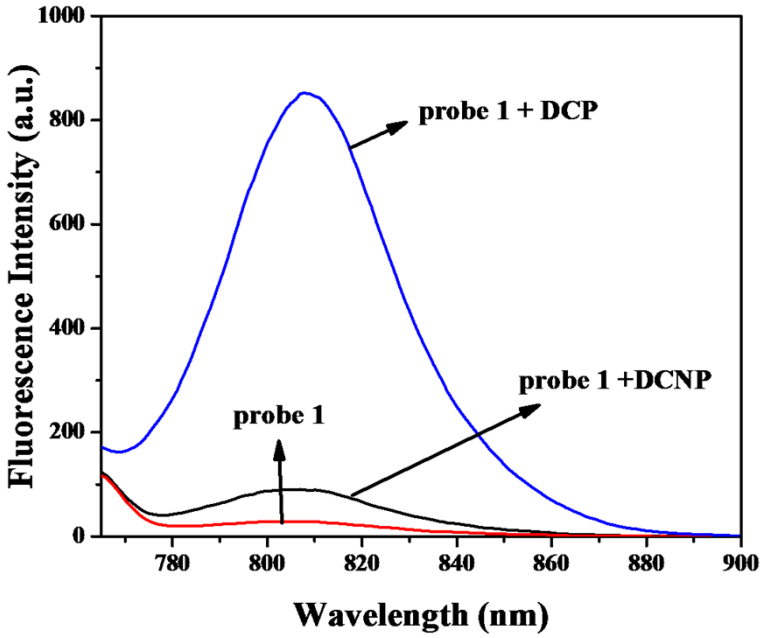


Fig. S12 NIR fluorescence enhancement of probe **1** (1.0 μM) in CH_3CN solution containing DMAP (50.0 μM) *vs.* DCP and DCNP, reapectively. [DCP] = [DCNP] = 10μM. λex = 760 nm, slit: 5nm/5nm.

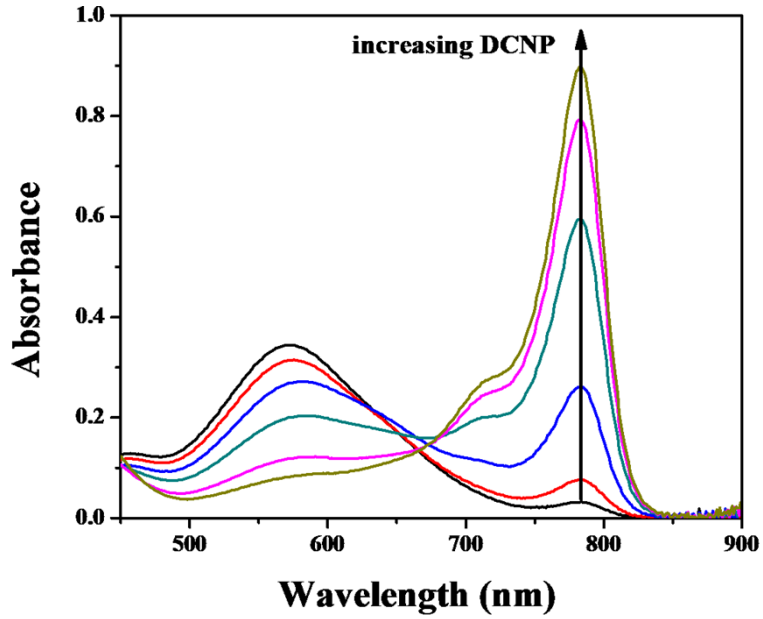


Fig. S13 The absorption spectra of probe **1** (10.0 μM) in CH_3CN solution containing DMAP (500.0μM) *vs.* concentrations of DCNP. [DCNP] = 0, 1.0, 10.0, 30.0, 70.0, 100μM, respectively.

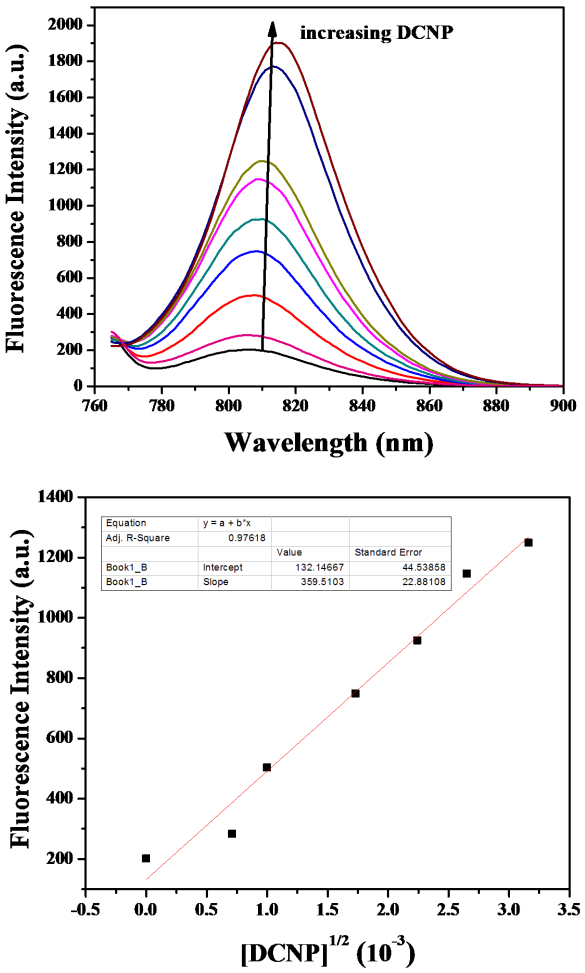


Fig. S14 (Upper) NIR fluorescence enhancement of probe **1** (10.0 μM) in CH_3CN solution containing DMAP (500.0 μM) *vs.* concentrations of DCNP. [DCNP] = 0, 0.5, 1.0, 3.0, 5.0, 7.0, 10, 30, 70μM, respectively. λex = 760 nm, slit: 5nm/5nm. (Lower) LOD for probe **1** with DCNP in acetonitrile at λex = 760 nm, λem = 807 nm. LOD is 2.23 nM between 0～10.0 μM of DCNP (data fit to the nonlinear equation):

$$y = 359.51 \times \text{sqrt}(x) + 132.15, \qquad R^2 = 0.98$$

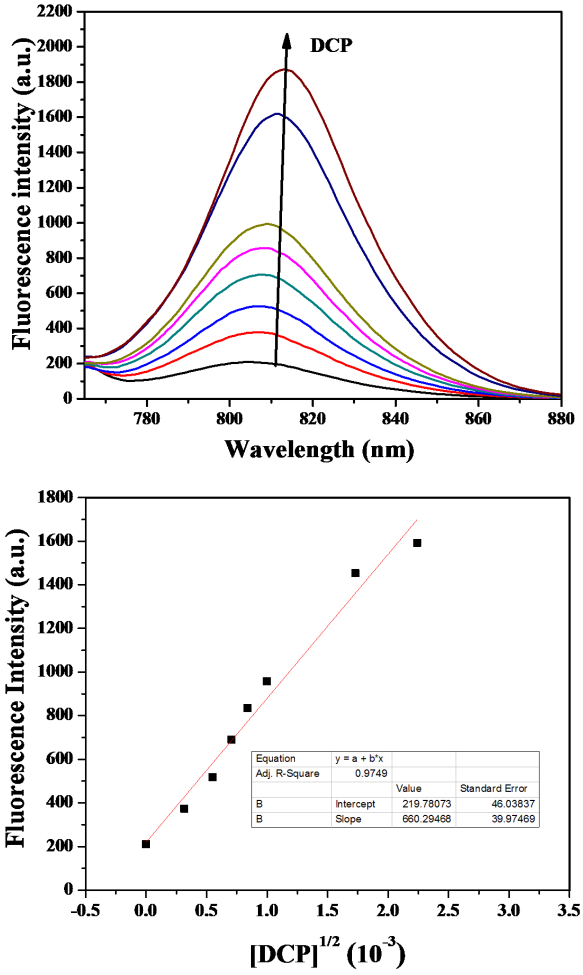


Fig. S15 (Upper) NIR fluorescence enhancement of probe **1** (10.0 μM) in CH_3CN solution containing DMAP (500.0 μM) *vs.* concentrations of DCP. [DCP] = 0, 0.1, 0.3, 0.5, 0.7, 1.0, 5.0, 7.0 μM, respectively. λex = 760 nm, slit: 5nm/5nm. (Lower) LOD is 0.661 nM between 0～5.0 μM of DCP (data fit to the nonlinear equation):

$$y = 660.29 \times \text{sqrt}(x) + 219.78, \qquad R^2 = 0.97$$

Fig.S16 The color of filter paper loaded with probe **1** in sealed vial containing air (left) and 15 ppm of DCP vapor (right).