Supporting information

A water-soluble boronate-based fluorescence probe for the selective detection of peroxynitrite and imaging in living cells

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Contents

Supporting information

1. General methods

All the chemicals were purchased from Sigma-Aldrich Chemical Co., and used as received.

1.1 Nitric oxide (NO) was prepared by treating sulfuric acid (3.6 M) solution with sodium nitrite solution (7.3 M) and its stock solution (2.0 mM) was prepared by bubbling NO into deoxygenated deionized water for 30 min**;**

1.2 ROO• was generated from 2, 2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride, 1 M) was added into deionizer water, and then stirred at 37 °C for 30 min;

1.3 Superoxide was generated from KO_2 with a saturated solution of KO_2 in DMSO (~1 mM);

1.4 Hydroxyl radical was generated by Fenton reaction. To prepare •OH solution, hydrogen peroxide (H_2O_2) was added in the presence of $Fe(ClO_4)_2$;[1]

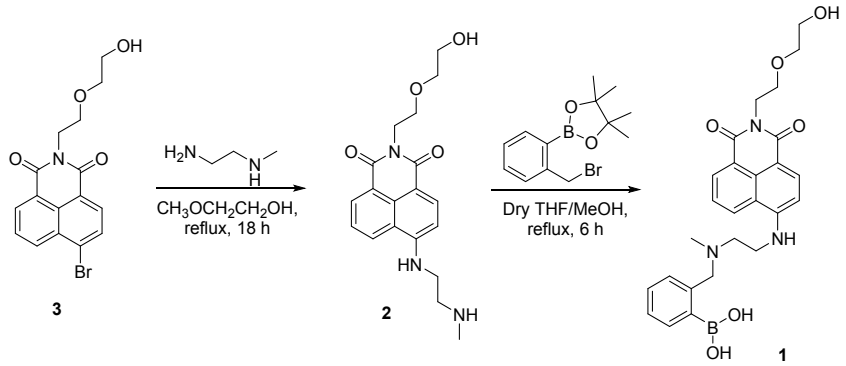
1.5 Peroxynitrite solution was synthesized as reported.[2] The concentration of peroxynitrite was estimated by using extinction co-efficient of 1670 cm^{-1} M^{-1} at 302 nm in 0.1 N sodium hydroxide aqueous solutions.

1.6 The concentration of ^{-}OCl was determined from the absorption at 292 nm (ε = 350 $M^{-1}cm^{-1}$).

1.7 The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 $M^{-1}cm^{-1}$).

All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise. The fluorescent titrations with peroxynitrite were carried out at 25 ^{o}C in pH 7.30 PBS buffer (KH_2PO_4, 1/15 M; Na_2HPO_4, 1/15 M) and pH 5.00 buffer (NaOAc/HOAc, 50mM).

2. Experimental Section



Scheme S1. Synthetic route for the target compound **1**.

2.1 Synthesis of compound 3[3]

Under a nitrogen atmosphere, to a solution of 4-bromo-1, 8-naphthalic anhydride (0.30 g, 1.08 mmol) in anhydrous ethanol (20 mL) was added 2-(2-aminoethoxy) ethanol (0.12 g, 1.19 mmol). The mixture was heated at reflux for 3 h and concentrated under reduced pressure. Purified by flash chromatography (CH_2Cl_2:CH_3OH, 50:1, v/v) afforded a yellow powder (0.35 g). Yield: 88%. Mp: 122 – 124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (dd, J_1 = 7.3 Hz, J_2 = 1.1 Hz, 1H), 8.53 (dd, J_1 = 8.5 Hz, J_2 = 1.1 Hz, 1H), 8.36 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.79 (dd, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 1H), 4.38 (t, J = 5.6 Hz, 2H), 3.79 (t, J = 5.6 Hz, 2H), 3.59 - 3.63 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 163.9, 163.8, 133.4, 132.2, 131.4, 131.1, 130.54, 130.52, 128.9, 128.1, 122.8, 121.9, 72.3, 68.3, 61.8, 39.6; HRMS (ES+) m/z calcd for $[M + Na]^+$, 386.0004; found, 386.0028.

2.2 Synthesis of compound 2

A solution of 2-bromoethylamine hydrobromide (1.5 g, 4.12 mmol) and *N*-methylethylene-diamine (0.322 g, 95% wt., 4.12 mmol) in 2-methoxyethanol was refluxed overnight under a nitrogen atmosphere. The mixture was concentrated under reduced pressure to obtain a purple oil product. Column chromatography on silica gel (CH_2Cl_2:CH_3OH, 3:1, v/v) was employed to purify the crude product as yellow solid (0.8 g). Yield: 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (dd, J_1 = 7.5 Hz, J_2 = 0.9 Hz 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.19 (dd, J_1 = 8.4 Hz, J_2 = 0.9 Hz 1H), 7.55 (dd, J_1 = 8.4 Hz, J_2 = 7.5 Hz, 1H), 6.59 (d, J = 8.4 Hz, 1H), 6.39 (br s, 1H), 4.41 (t, J = 5.4 Hz, 2H), 3.87 (t, J = 5.4 Hz, 2H), 3.68 - 3.72 (m, 4H), 3.48 (overlapped, 2H), 3.07 (t, J = 6.0 Hz, 2H), 2.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 165.0, 164.5, 150.0, 134.6, 131.2, 129.6, 126.9, 124.4, 122.3, 120.2, 109.4, 104.1, 72.4, 68.8, 61.7, 50.8, 42.1, 39.3, 35.9; HRMS (ES+) Calcd for $([M+H])^+$, 358.1766; Found, 358.1772.

2.3 Synthesis of compound 1

The product compound **1** was synthesised by refluxing compound **2** (0.20 g, 0.56 mmol) with 2-bromomethylphenylboronic acid pinacol ester (0.28 g, 0.94 mmol) in dry tetrahydrofuran (20 mL) for 6 hours. The product was purified on silica gel, using dichloromethane/methanol 5:1 to afford **1** as yellow solid (0.15 g). Yield: 50%. Mp: 119 – 121 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.60 (d, J = 8.1 Hz, 1H), 8.44 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 8.7 Hz, 1H), 7.58 (t, J = 7.8 Hz, 2H), 7.17 – 7.23 (m, 3H), 6.80 (d, J = 8.7 Hz, 1H), 4.32 (t, J = 6.3 Hz, 2H), 4.17 (s, 2H), 3.74 - 3.84 (m, 4H), 3.57 - 3.62 (m, 4H); 3.31 - 3.34 (overlapped, 2H), 2.59 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 166.6, 166.1, 152.4, 136.2, 134.6, 132.6, 131.5, 130.3, 128.8, 128.6, 128.5, 125.9, 123.6, 122.4, 110.5, 105.5, 73.8, 69.6, 64.7, 62.6, 56.3, 41.7, 40.5, 39.8, Note: the boron attached carbon is not visible; HRMS (ES+) m/z calcd for $[M + H]^+$, 492.2305; found, 492.2326.

3. Supplementary spectra

3.1 Non-linear relationship between probe 1-D-fructose and peroxynitrite

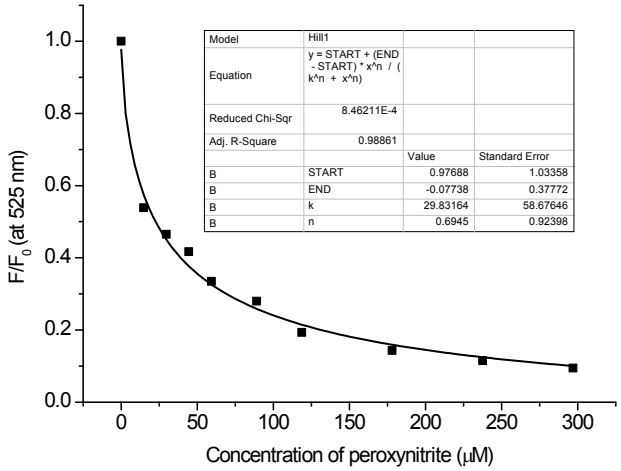


Figure S1. Non-linear relationship between fluorescence intensity ratio F/F_0 at 525 nm of probe **1**-D-fructose (probe **1**, 2 μM; D-fructose, 100 mM) and concentration of peroxynitrite (0 - 300 μM) at pH 7.30 buffer solution. The mixture was stirred for 5 min for each dose. The spectra were collected with excitation at 410 nm (Ex slit: 5.0, Em slit: 5.0).

3.2 UV-Vis absorption of probe 1, addition of D-fructose, addition of peroxynitrite

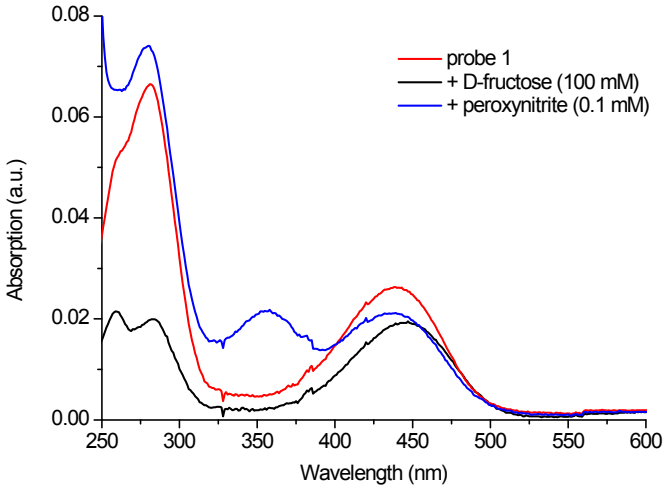


Figure S2. UV-Vis absorption of probe **1** (2 μM, red line), addition of D-fructose (100 mM, black line), addition of peroxynitrite (0.1 mM, blue line). The mixture was stirred for 10 min after addition of D-fructose. The spectra was collected after 5 min in the presence of peroxynitrite.

3.3 Time-course kinetic measurement between probe 1-D-fructose and peroxynitrite

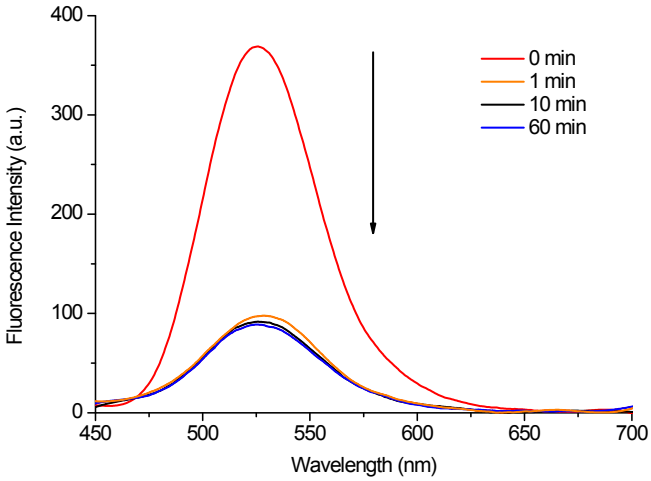


Figure S3. Time-course kinetic measurement of the fluorescence response of probe **1**-D-fructose (probe **1**, 2 μM; D-fructose, 100 mM) to peroxynitrite (100 μM) at pH 7.30 buffer solution. The spectra were collected with excitation at 410 nm (Ex slit: 5.0, Em slit: 5.0).

3.4 Fluorescence spectra of probe 1 towards hydrogen peroxide in PBS buffer

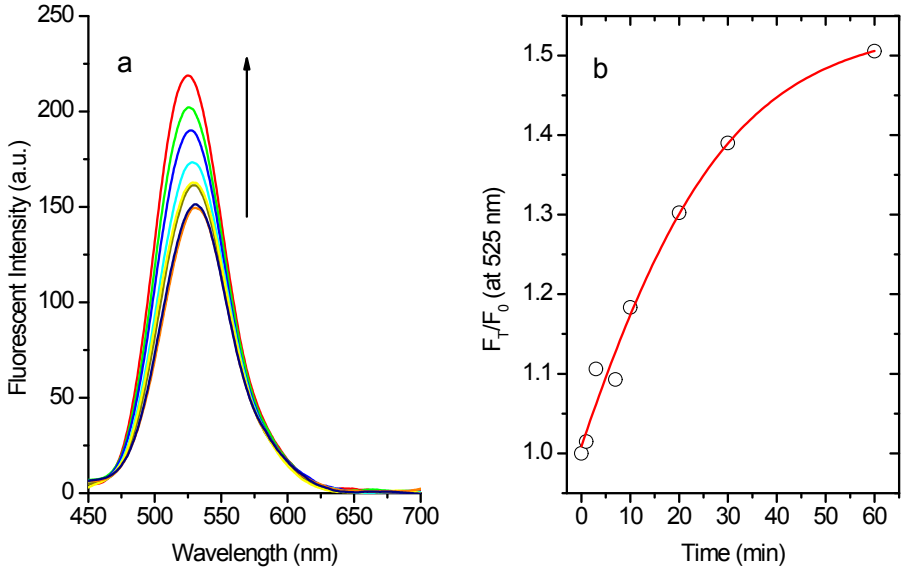


Figure S4. (a) Fluorescence spectra of probe **1** (2 μM) in the presence of hydrogen peroxide (100 μM) at pH 7.3 buffer solution; **(b)** Time curve of probe **1** (2 μM) with the fluorescence intensity ratio (F_T/F_0 at 525 nm). The data was collected in PBS buffer (1/15 M, pH 7.30) with excitation at 410 nm (Ex slit: 5 nm, Em slit: 5 nm).

3.5 ^{11}B NMR of probe 1 towards hydrogen peroxide in 20% D_2O/MeOD

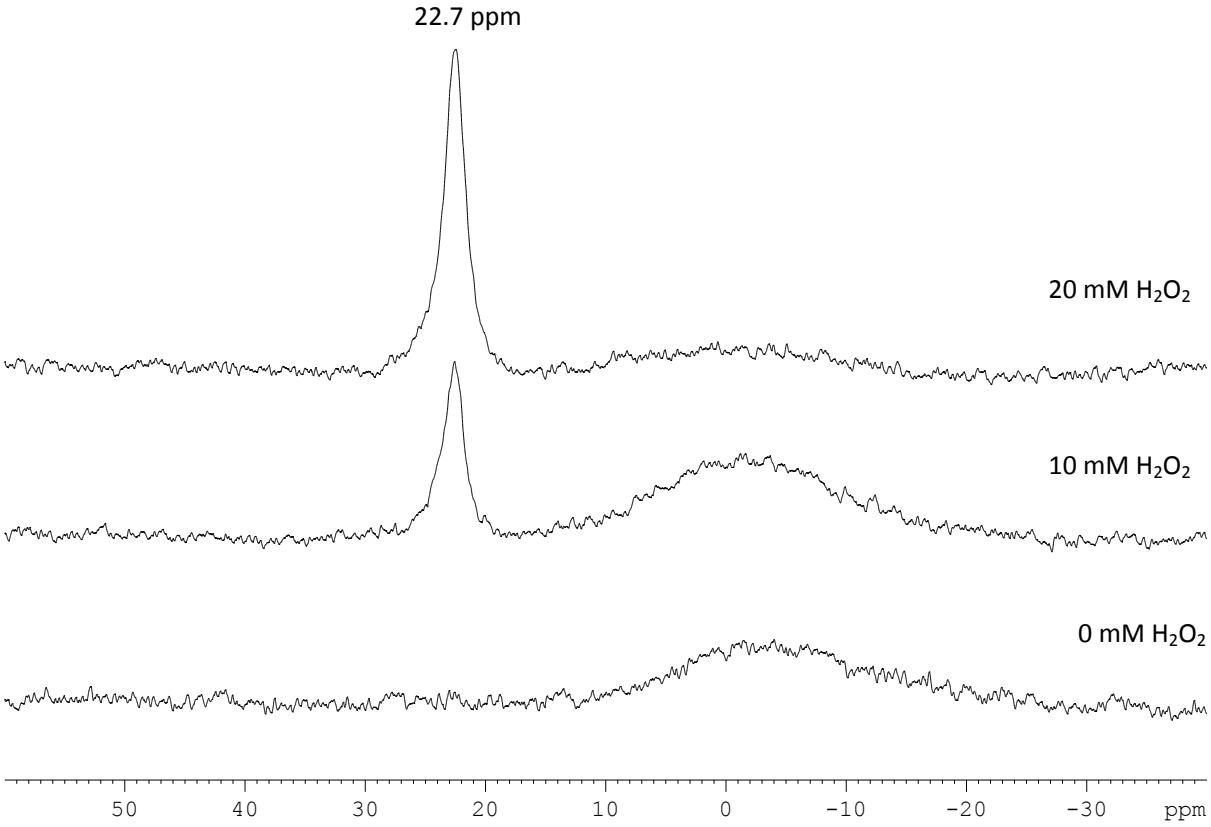
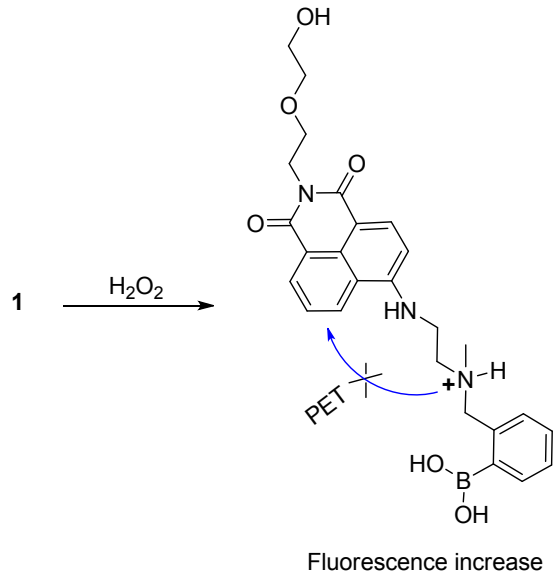


Figure S5. ^{11}B NMR of probe **1** (40 mM) in the presence of various concentrations of hydrogen peroxide (0 - 20 mM). The data was collected in 20% D_2O/MeOD.. Acquired an a Bruker AV300 spectrometer operating at 96.3 MHz for ^{11}B, using 128 scans for each spectrum. Spectra were acquired with no proton decoupling, a repetition time of 1.6 seconds, and using composite pulses to help suppress the background signal.



Scheme S2. Protonation of probe **1** with hydrogen peroxide causes fluorescence enhancement.

3.6 Half-life calculation and second-order reaction determination

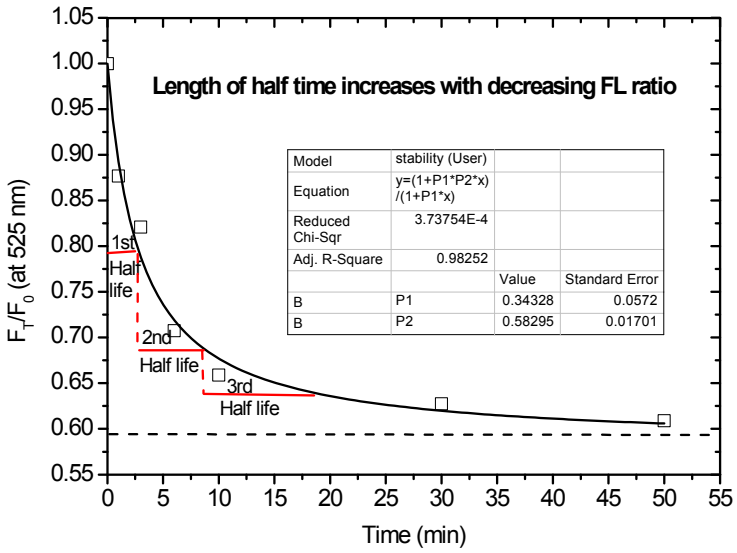


Figure S7. Time curve of **1**-D-fructose complex (probe **1**, 2 μM; D-fructose, 100 mM) in the presence of hydrogen peroxide (1 mM) with the fluorescence intensity ratio (F_T/F_0 at 525 nm). The data was collected in PBS buffer (1/15 M, pH 7.30) with excitation at 410 nm (Ex slit: 5 nm, Em slit: 5 nm).

Half-life calculation:

$$y = (1+P1*P2*x)/(1+P1*x) \qquad \textbf{Equation 1}$$

When $y = 0.79$

$$t_{1/2} = 2.95 \text{ min}$$

When $y = 0.6864$

$$t_{1/2} = 8.96 \text{ min}$$

When $y = 0.6346$

$$t_{1/2} = 20.5 \text{ min}$$

Therefore, it follows that the consumption of the probe is a second order process where:

$$A + B \rightarrow products, rate = k\,[A][B]$$

3.7 Selectivity test towards ROS/RNS

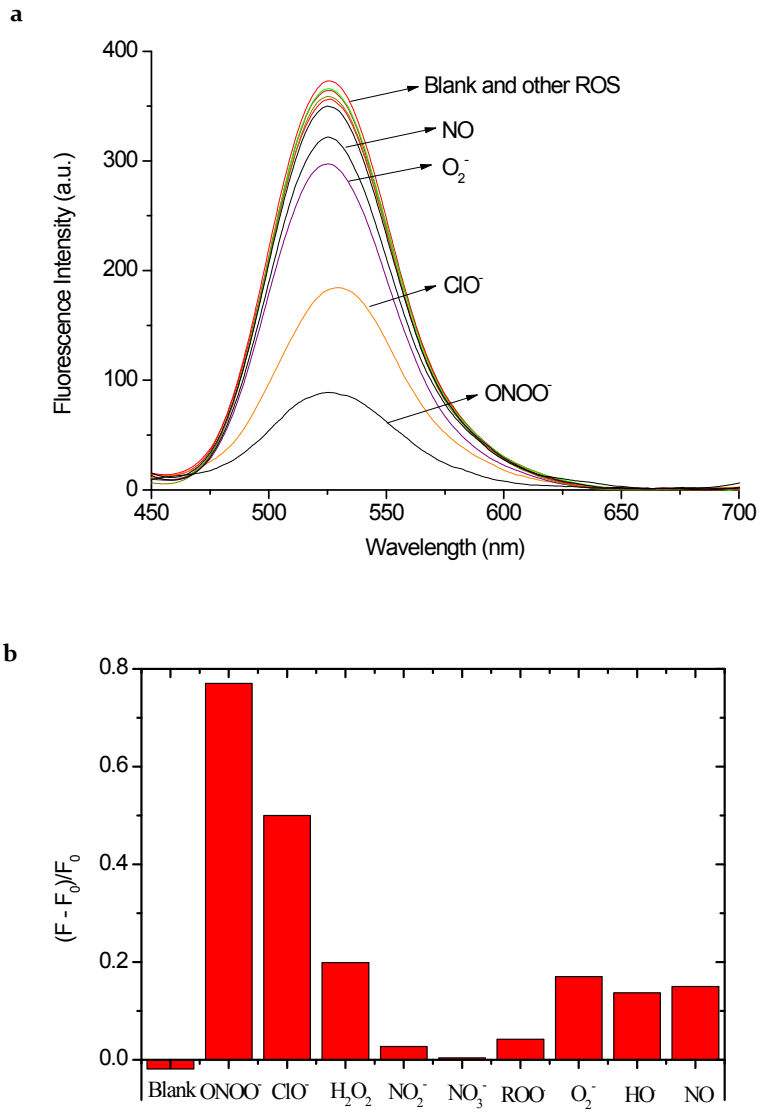


Figure S8. (a) Fluorescence spectra of probe 1-D-fructose (probe 1, 2 μM; D-fructose, 100 mM) in the presence of various ROS/RNS: $ONOO^-$ (100 μM, 5 min), ^-OCl (100 μM, 1 h), H_2O_2 (1 mM, 1 h), NO_2^- (1 mM, 1 h), NO_3^- (1 mM, 1 h), ROO• (1 mM, 1 h), $•O_2^-$ (100 μM, 1 h), •OH (100 μM, 1 h), NO (100 μM, 1 h) at pH 7.30 buffer solution; (b) Selectivity test of probe 1-D-fructose complex (probe 1, 2 μM; D-fructose, 100 mM) in the presence of various ROS/RNS at pH 7.3 buffer solution. The spectra were collected with excitation at 410 nm at 25 ˚C (Ex slit: 5.0, Em slit: 5.0).

3.8 Comparison for detection of $ONOO^-$ and H_2O_2 at pH 5.0

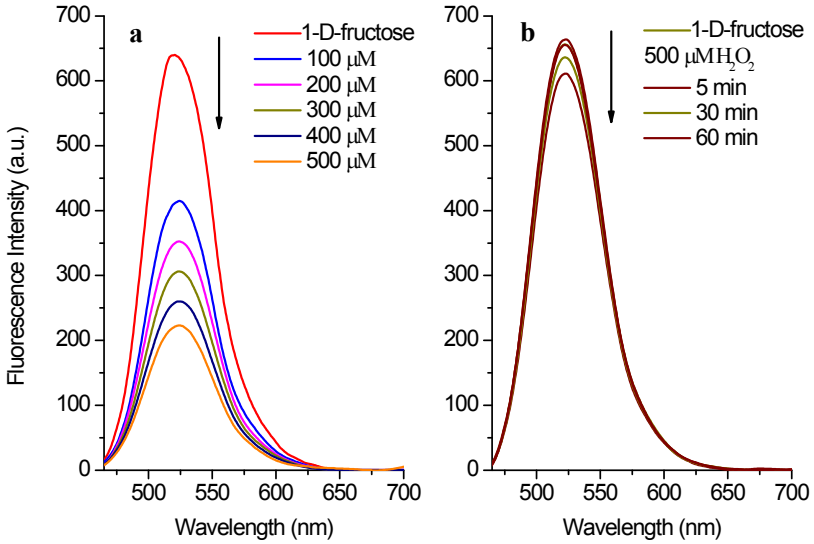


Figure S9. (**a**) Fluorescence spectra of probe **1**-D-fructose complex (probe **1**, 2 μM; D-fructose, 100 mM) in the presence of various concentrations of peroxynitrite at pH 5.0 buffer solution. The mixture was stirred for 5 min for each dose; (**b**) Time curve of probe **1**-D-fructose complex (probe **1**, 2 μM; D-fructose, 100 mM) in the presence of hydrogen peroxide (500 μM) at pH 5.0 buffer solution. The spectra were collected with excitation at 410 nm (Ex slit: 5.0, Em slit: 5.0).

4. Cell culture

HeLa cells (human epithelial adenocarcinoma) and RAW 264.7 cells (mouse macrophage cell) were obtained from Korean Cell Line Bank (Seoul, Korea). Cells were cultured in RPMI 1640 (Roswell Park Memorial Institute, without HEPES) supplemented with heat-inactivated 10 % fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin. All cells were kept in 5 % CO_2 at 37 ℃.

5. References

1. M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629-10637.
2. J. W. Reed, H. H. Ho and W. L. Jolly, *J. Am. Chem. Soc.*, 1974, **96**, 1248-1249.
3. G.-F. Zhang, M. P. Aldred, W.-L. Gong, C. Li and M.-Q. Zhu, *Chem. Commun.*, 2012, **48**, 7711-7713.

6. NMR spectra

Nuclear magnetic resonance (NMR) spectra were obtained in chloroform-D, and methanol-D. Where a Bruker AVANCE 300 was used, ^{1}H spectra were recorded at 300 MHz, ^{11}B spectra at 96 MHz and ^{13}C at 75 MHz. Chemical shifts (δ) are expressed in parts per million and are reported relative to the residual solvent peak as an internal standard in ^{1}H and ^{13}C spectra. The multiplicities and general assignments of the spectroscopic data are denoted as: singlet (s), doublet (d), triplet (t), double of doublets (dd), unresolved multiplet (m), and broad (br).

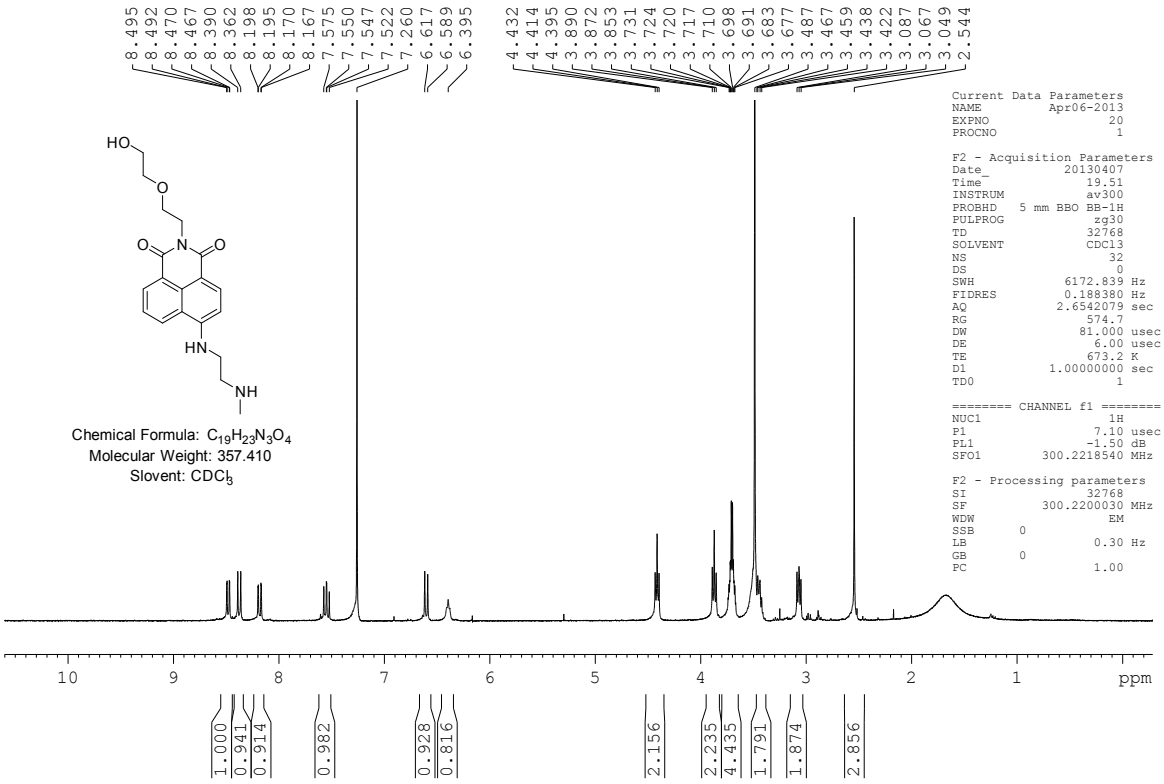


Figure S10. ^{1}H NMR of compound **2** in $CDCl_3$

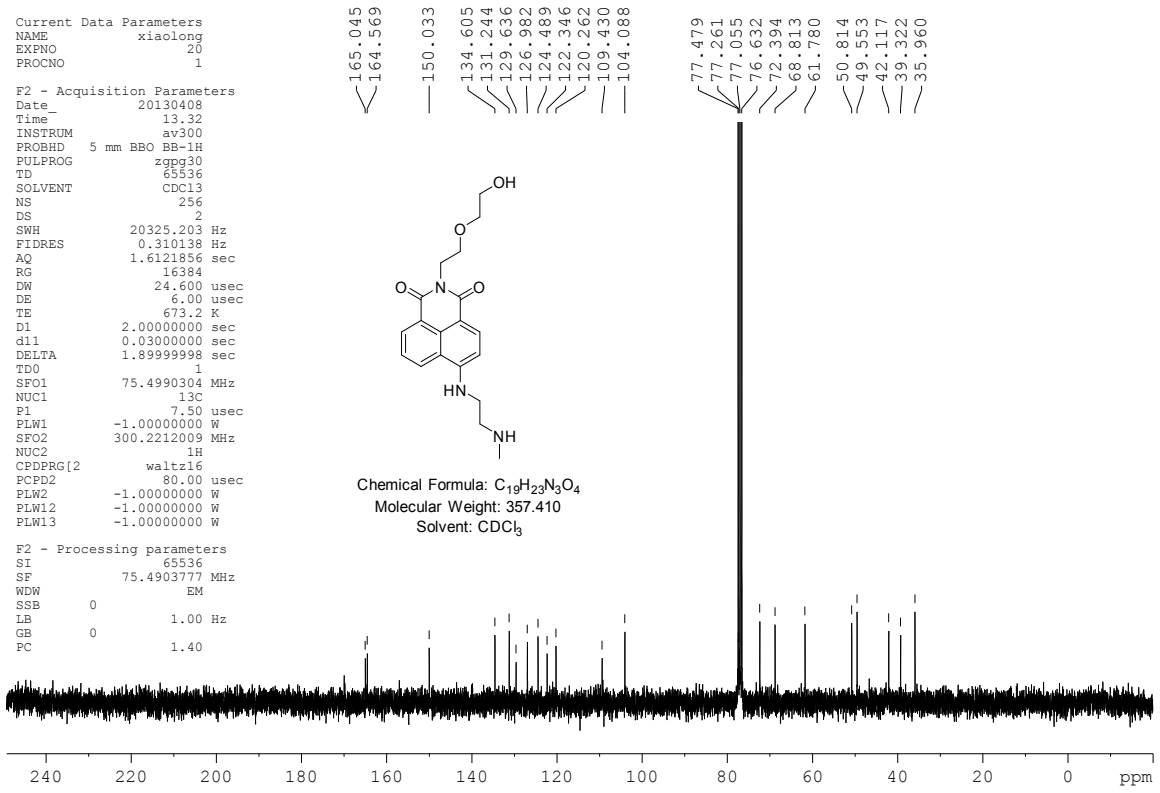


Figure S11. ^{13}C NMR of compound **2** in $CDCl_3$

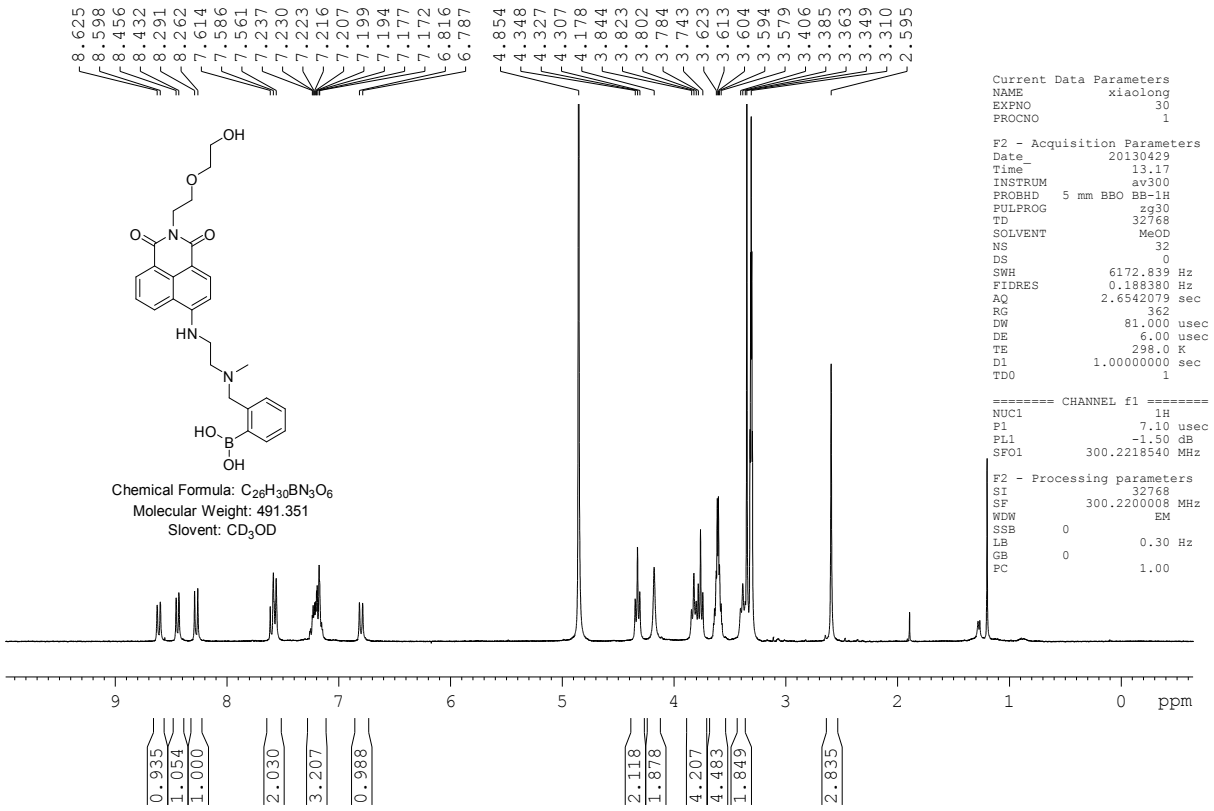


Figure S12. ^{1}H NMR of compound **1** in MeOD

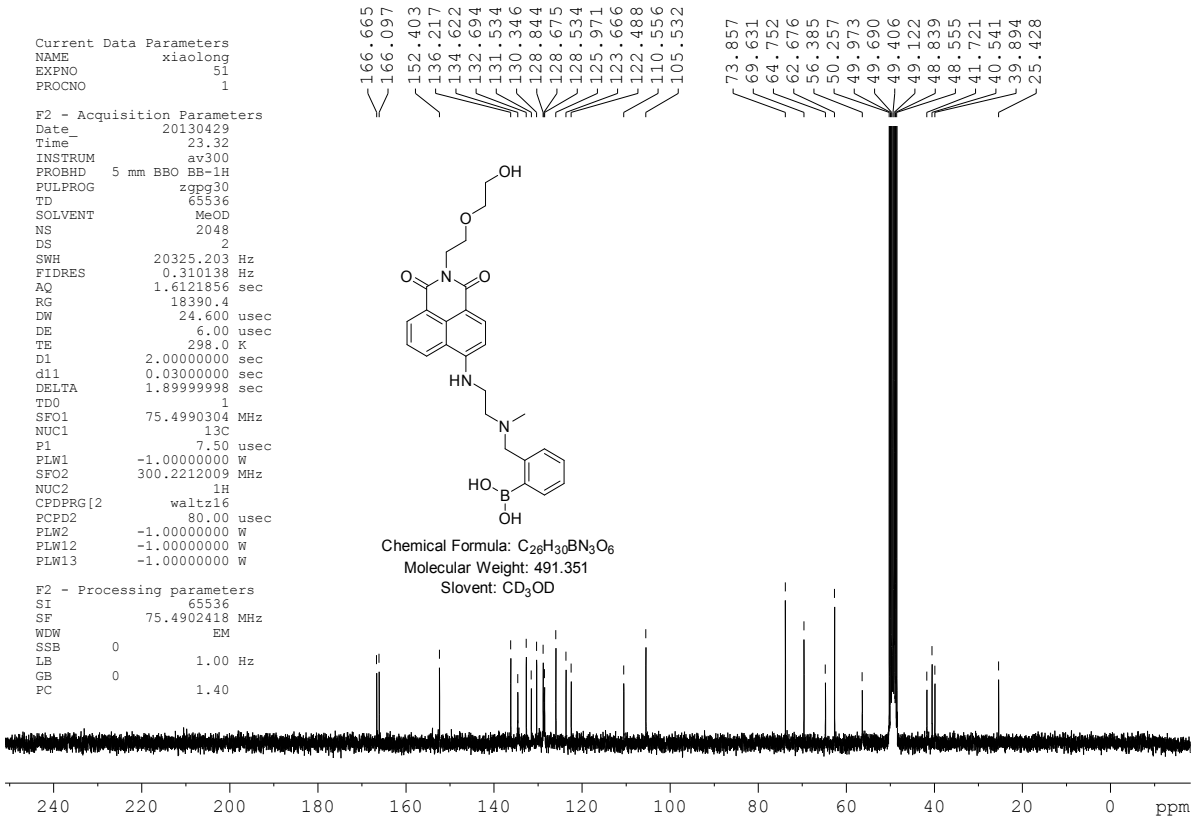


Figure S13. ^{13}C NMR of compound **1** in MeOD

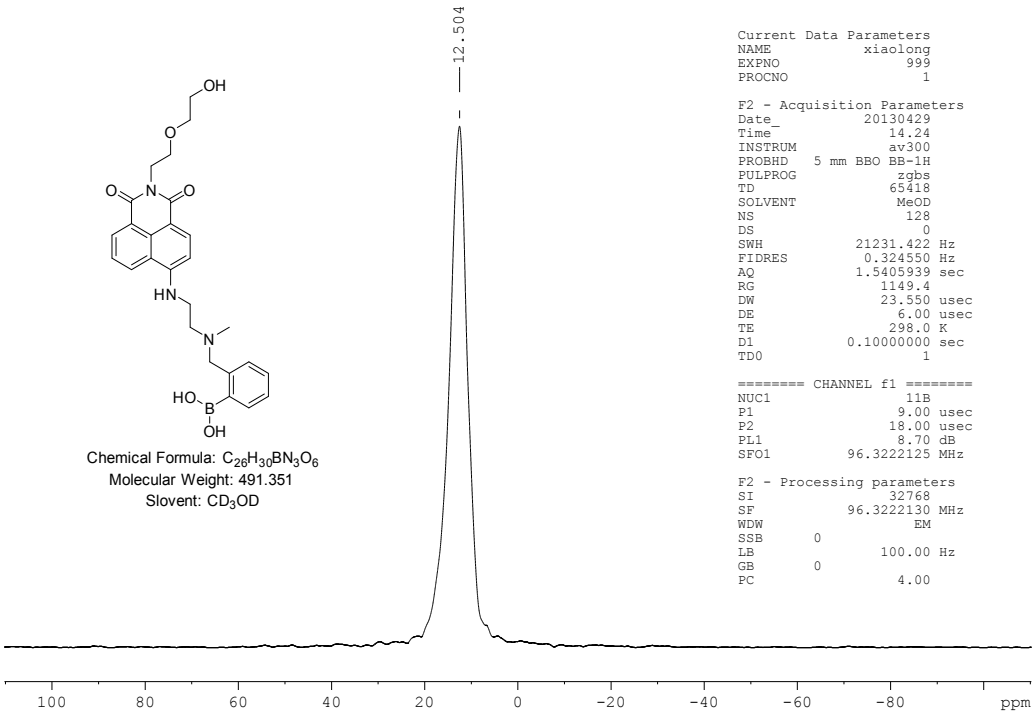


Figure S14. ^{11}B NMR of compound **1** in MeOD