Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Fast-response Fluorescent Probe for Hypochlorite Acid Detection and its Application of Exogenous and Endogenous HOCl Imaging in Living Cells

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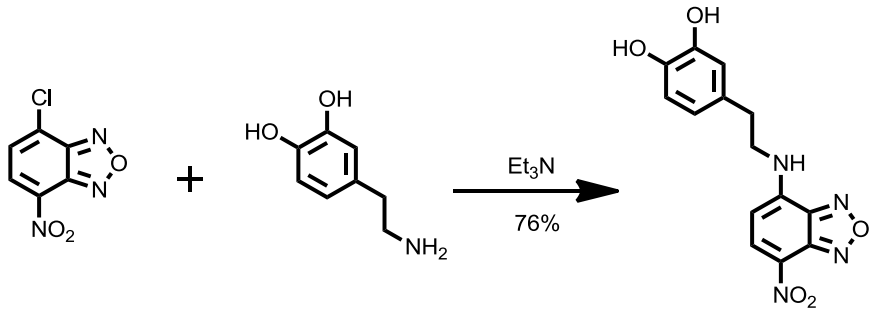
1. Experimental Section

1.1 Materials and chemicals

All chemicals used for synthesis were purchased from commercial suppliers and applied directly without purification. 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole (NBD-Cl) was purchased from J&K Chemical. Dopamine hydrochloride and propylamine were obtained from Sigma. Anhydrous dichloromethane (DCM) and ethanol were purchased from commercial suppliers and used in all reactions as solvent. Dulbecco's modified Eagle's medium (DMEM), PBS, fetal bovine serum (FBS), trypsin-EDTA and penicillin/streptomycin were purchased from Invitrogen. All reactions that utilize air- or moisture sensitive reagents were performed in dried glassware under dry N_2 atmosphere. Milli-Q water was used in all experiments. All spectroscopic measurements were performed in phosphate-buffered saline (PBS, 10 mM, pH 7.4). The progress of the reaction was monitored by thin-layer chromatography (TLC; Merck 60F-254). Merck silica gel 60 (70−200 mesh) was used for general column chromatography purification purpose. ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 400 MHz NMR spectrometer. For ^{1}H NMR, coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and bs (broad singlet). Mass spectra were obtained using PC Sciex API 150 EX ESI-MS system. High-resolution mass spectrometry was performed with ABI Qstar Elite Q-TOF. UV absorption spectra were obtained on Shimadzu 1700 UV/vis Spectrometer. Fluorescence spectra were acquired with a FluoroMax-4 fluorescence photometer. Fluorescence images were captured using a Leica TCS SP5 Confocal Scanning Microscope. pH value was recorded with a FiveEasy FE20 pH meter.

1.2 Synthesis and characterization

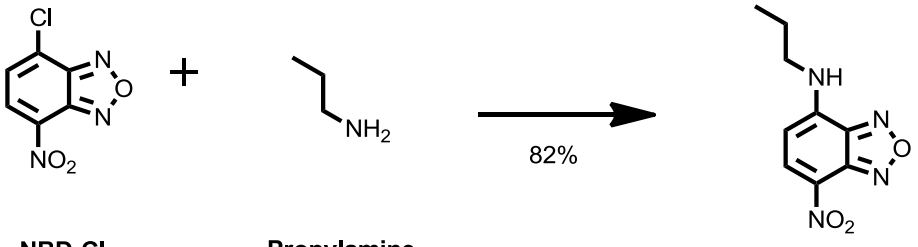
1.2.1 Synthesis of NBD-DOP



NBD-Cl **Dopamine** **NBD-DOP**

NBD-Cl (400 mg, 2.0 mmol), triethylamine (303 mg, 3 mmol) and dopamine hydrochloride (417 mg, 2.2 mmol) were dissolved in dry acetonitrile (15 mL) under nitrogen atmosphere. The mixture was stirred under 80 ^{o}C for 6h. Then, the solvent was evaporated under reduced pressure and adding water for extraction by ethyl acetate. After dryness with Na_2SO_4, the crude product was purified by silica gel column chromatography with hexane/ethyl acetate = 5/1 to obtain NBD-DOP as a red colour powder (486 mg, 77.2% yield); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.48 (d, *J* = 8 Hz, 1H), 6.68 (d, *J* = 8 Hz, 2H), 6.59 (dd, *J* = 8 Hz, 2 Hz, 1H), 6.27 (d, *J* = 8 Hz, 1H), 3.71 (bs, 2H), 2.90 (t, *J* = 8 Hz, 2H); ^{13}C NMR (100 MHz, *d*^{6}-DMSO): δ = 145.2, 145.0, 144.4, 144.1, 143.8, 137.9, 129.2, 120.6, 119.5, 116.2, 115.6, 99.3, 45.1, 33.1; ESI-MS: calcd. for $C_{14}H_{13}N_4O_5$ $[M+H]^+$ 317.1, found 317.3.

1.2.2 Synthesis of NBD-PRO

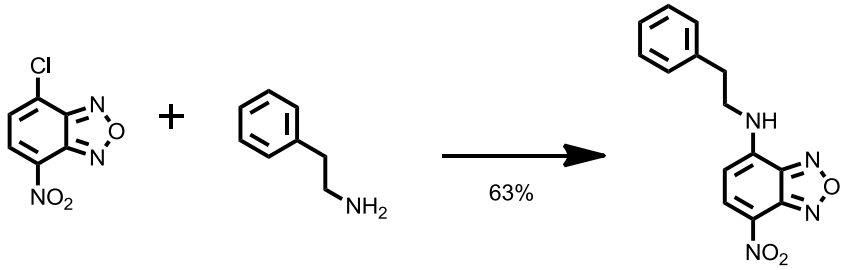


NBD-Cl **Propylamine** **NBD-PRO**

NBD-Cl (200 mg, 1.0 mmol) and n-propylamine (410 µL, 5.0 mmol) were dissolved in dry acetonitrile (5.0 mL) under nitrogen atmosphere. The mixture was stirred 60 ^{o}C for 5h. Then, the solvent was evaporated under reduced pressure and the crude compound was purified by silica gel column chromatography with hexane/dichloromethane = 10/1 to obtain NBD-PRO as a orange colour powder (182 mg, 82% yield); ^{1}H NMR (400 MHz, *d*^{6}-DMSO): δ = 9.58 (s, 1H), 8.51 (d, *J* = 8 Hz, 1H),

6.41 (d, *J* = 8 Hz, 1H), 3.44 (t, *J* = 6 Hz, 2H), 1.69 (dt, *J* = 7 Hz, 2H), 0.96 (t, *J* = 7 Hz, 2H); ^{13}C NMR (100 MHz, *d*^{6}-DMSO): δ = 145.2, 144.4, 144.1, 137.9, 120.5, 99.1, 45.0, 21.1, 11.3; ESI-MS: calcd. for $C_9H_{10}N_4O_3$ $[M+H]^+$ 223.1, found 223. 3.

1.2.3 Synthesis of NBD-PHE



NBD-Cl **2-phenylethanamine** **NBD-PHE**

NBD-Cl (200 mg, 1.0 mmol), 2-phenylethanamine (151 μ L, 1.2 mmol) and K_2CO_3 (690 mg, 5 mmol) were dissolved in dry acetonitrile (15 mL) under nitrogen atmosphere. The mixture was stirred under 80 °C for 8 h. After filtration, the solvent was evaporated under reduced pressure and adding water for extraction by ethyl acetate and dried with Na_2SO_4. The crude product was purified by silica gel column chromatography with hexane/ethyl acetate = 8/1 to obtain NBD-PHE (180 mg, 63.2% yield); ^{1}H NMR (400 MHz, *d*^{6}-DMSO): δ = 9.58 (s, 1H), 8.48 (d, *J* = 8.8 Hz, 1H), 7.30 (m, 6H),7.22 (s, 1H), 6.45 (d, *J* = 8.8 Hz, 2H), 3.72 (bs, 2H), 3.01 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, *d*^{6}-DMSO): δ = 144.8, 144.3, 144.0, 138.5, 137.7, 128.8, 128.4, 126.4, 120.7, 99.2, 44.7, 33.6; ESI-MS: calcd. for $C_{14}H_{12}N_4O_3$ $[M+H]^+$ 285.1, found 285.9.

1.3 Determination of detection limit

Detection limit or limit of detection was calculated based on the fluorescence titration curve of NBD-DOP in the presence of ClO^- and mean of the blank. In detailed, the fluorescence intensity of NBD-DOP was measured and the standard deviation of blank measurements was achieved and marked as σ using the following equation. σ is the standard deviation of the blank measurements; n is the tested number of blank measure (n= 11); $\bar{x}$ is the mean of the blank measures; X_i is the values of the blank measures.

$$\sigma = \sqrt{\frac{\Sigma(\bar{x}-x_i)^2}{n-1}}$$

In this manuscript, we use the following equation for the calculation of detection limit:

Detection limit = 3σ/s

s is the slope of fluorescence intensity versus ClO^- concentrations.

1.4 Absorption and fluorescence measurement

NBD-DOP was dissolved in an appropriate amount of DMSO to obtain 1 mM stock solution. NaOCl and other biological analytes were prepared as 1 mM or 10 mM stock solutions in PBS buffer. All the measurement was taken under room temperature. Firstly, NBD-DOP was diluted in PBS buffer (10 mM, pH 7.4) to afford a final concentration of 1 μM to a 10-mm quartz cuvette, then, different concentration of NaOCl or other analytes was added into the quartz cuvette for 1 min incubation. After that, the absorption or fluorescence was measured using Shimadzu 1700 UV/vis Spectrometer and FluoroMax-4 fluorescence photometer respectively. Specially, for the preparation of reactive oxygen species, such as HO•, $O_2^{\bullet -}$, •O^{t}Bu, the protocol from Chang's group was followed. [1]

[1] Aaron E. Albers, Voytek S. Okreglak, and Christopher J. Chang, *J. Am. Chem. Soc.*, **2006**, *128*, 9640–9641.

1.5 MTS assay

HeLa cells were seeded into 96-well plates at a density of 5×10^4 cells per well in 200 μL of DMEM supplemented with 10% heat-inactivated FBS (fetal bovine serum) and 1% penicillin/streptomycin. Cells were allowed to grow overnight under an atmosphere of 5% CO_2 and 95% air at 37 ^oC. The culture media was replaced by the new fresh medium with the various concentration of NBD-DOP (0, 1, 5, 10, 20 μM). The cells were incubated at the same condition for 24 h. Subsequently, 20 μL of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt (MTS, 2 mg/mL) was added and the cells were further cultured for another 2 h. Finally, measure the absorbance of treated cells using the plate reader at OD=490nm.

1.6 Cell culture and confocal fluorescence imaging

HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin). Approximately 10^5 cells were seeded in a confocal dish (35mm). After 24h, the cells were treated with NBD-DOP (5 μM) at 37 ^{o}C for 20 min and then incubated with NaOCl (50 μM) for another 20 min. Cells treated with NBD-DOP (5 μM) alone were used as a control. For endogenous HOCl test, the cells were treated with NBD-DOP (5 μM) at 37 ^{o}C for 20 min, then lipopolysaccharides (LPS, 2 mg/mL) and phorbol myristate acetate (PMA, 2 mg/mL) were added for another 2 h incubation. Fluorescence images were taken using a Leica TCS SP5 Confocal Scanning Microscope.

2. Supplemental figures

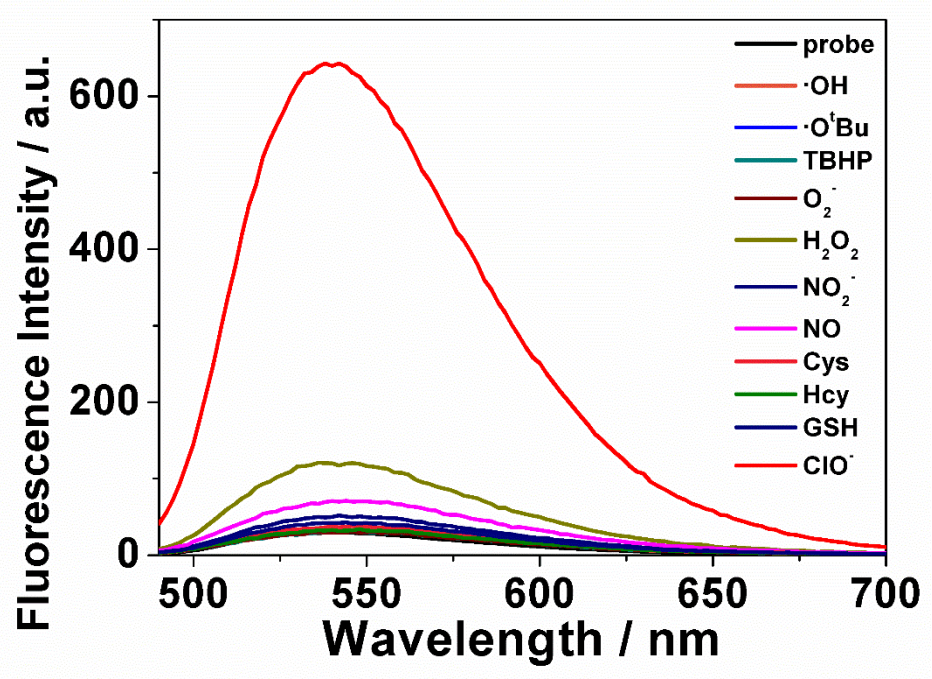


Fig. S1 Fluorescence spectrum of NBD-DOP (1 μM) toward NaOCl and various ROS, RNS and RSS at 540 nm in PBS buffer (10 mM, pH 7.4). OH• (100 μM), •O^{t}Bu (100 μM), TBHP (100 μM), $O_2^{\bullet-}$ (100 μM), H_2O_2 (100 μM), NO_2^- (100 μM), NO (100 μM), Cys (1 mM), Hcy (1 mM), GSH (1mM), NaOCl (20 μM). Ex: 470 nm.

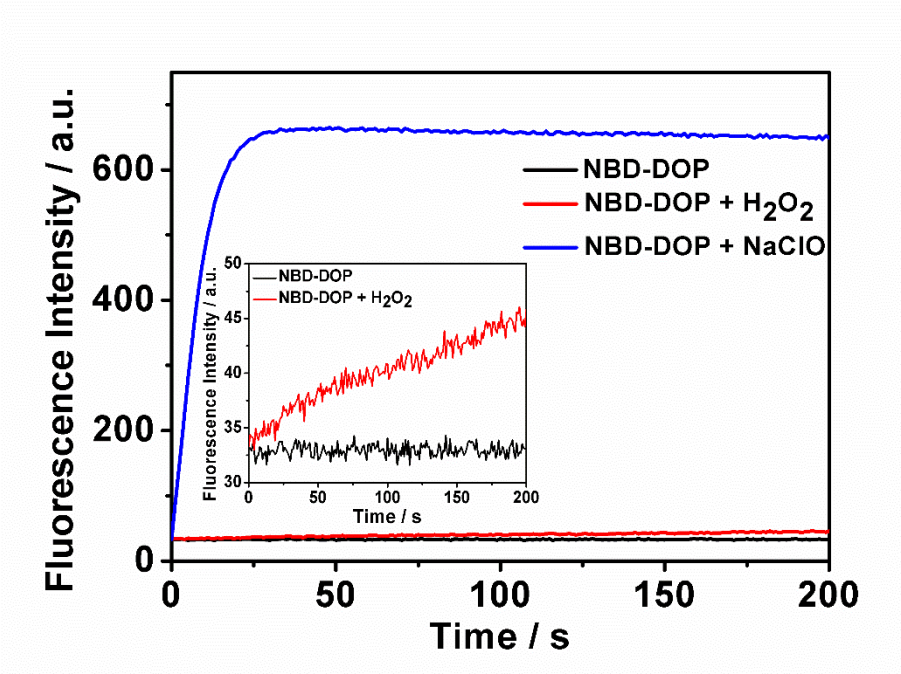


Fig. S2 Time-dependent fluorescence intensity change of the reaction between NBD-DOP (1 μM) and H_2O_2 or NaOCl (20 μM) in PBS buffer (10 mM, pH 7.4).

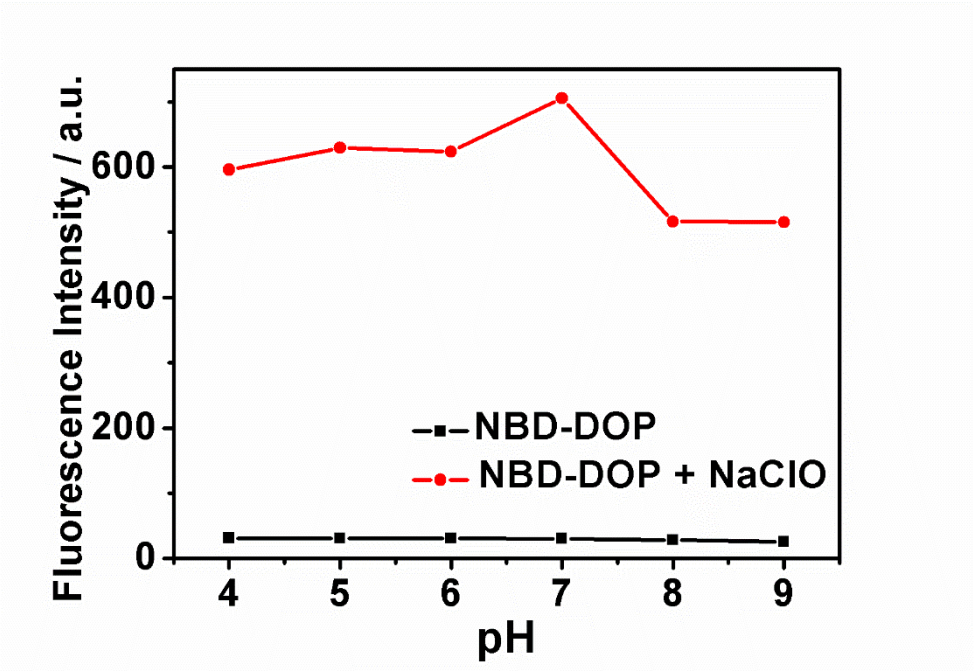


Fig. S3 Fluorescence response of NBD-DOP only (1 μM) and NBD-DOP (1 μM) with NaOCl (20 μM) under different pH values.

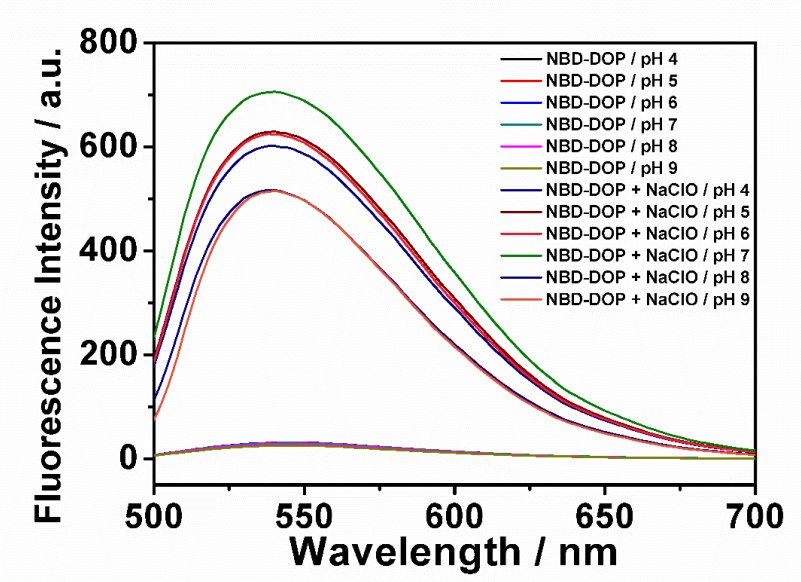


Fig. S4 Fluorescence spectrum of of NBD-DOP onyl (1 μM) and NBD-DOP (1 μM) with NaOCl (20 μM) under different pH values.

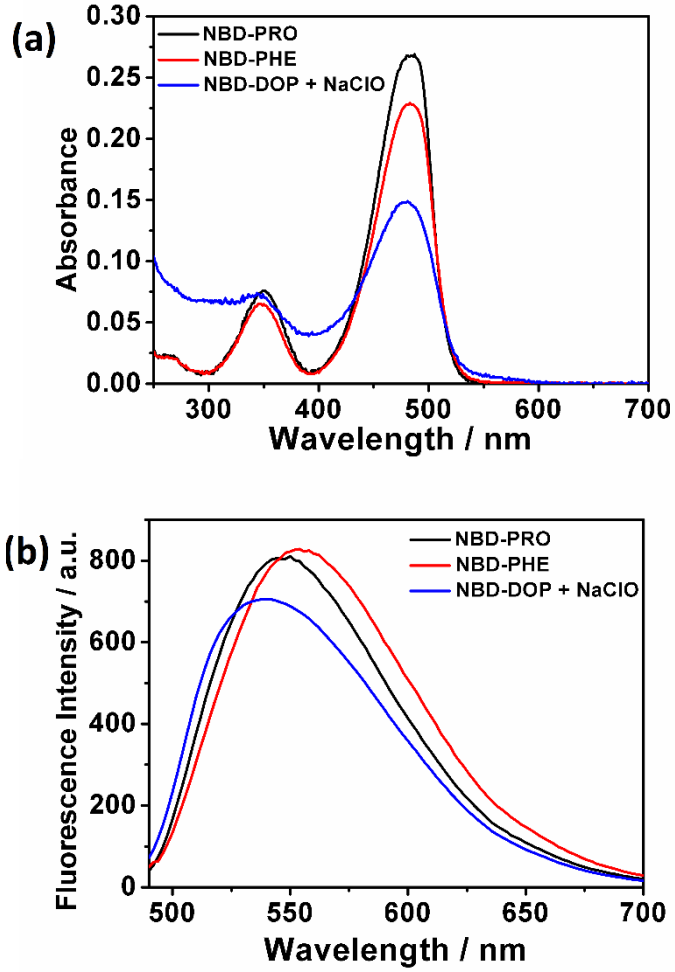


Fig. S5 Absorption (a) and emission spectrum (b) of NBD-PRO, NBD-PHE and the reaction of NBP-DOP with NaOCl.

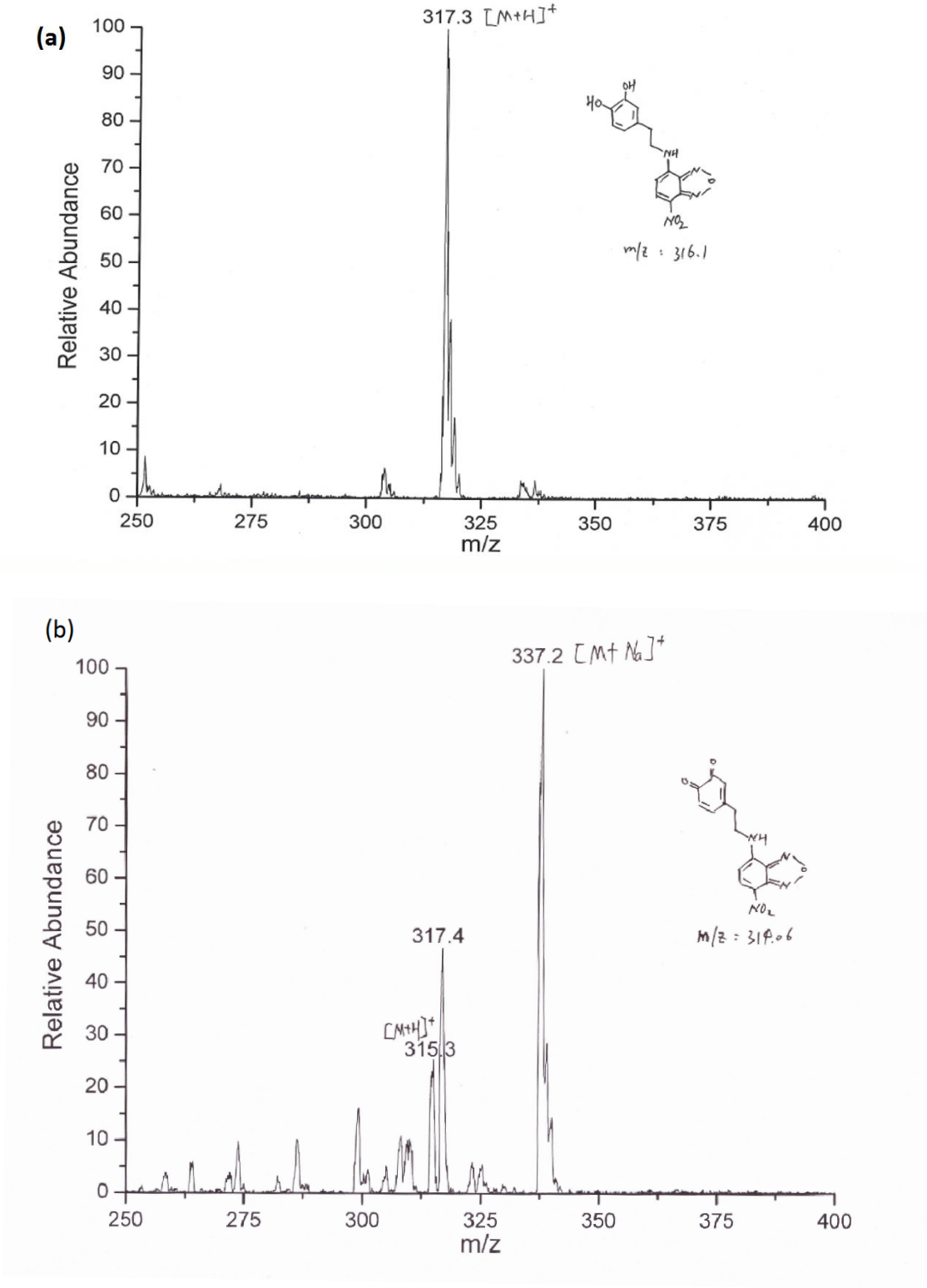


Fig. S6 Mass spectrum of NBD-DOP (a) and NBD-DOP with excessive NaOCl (b).

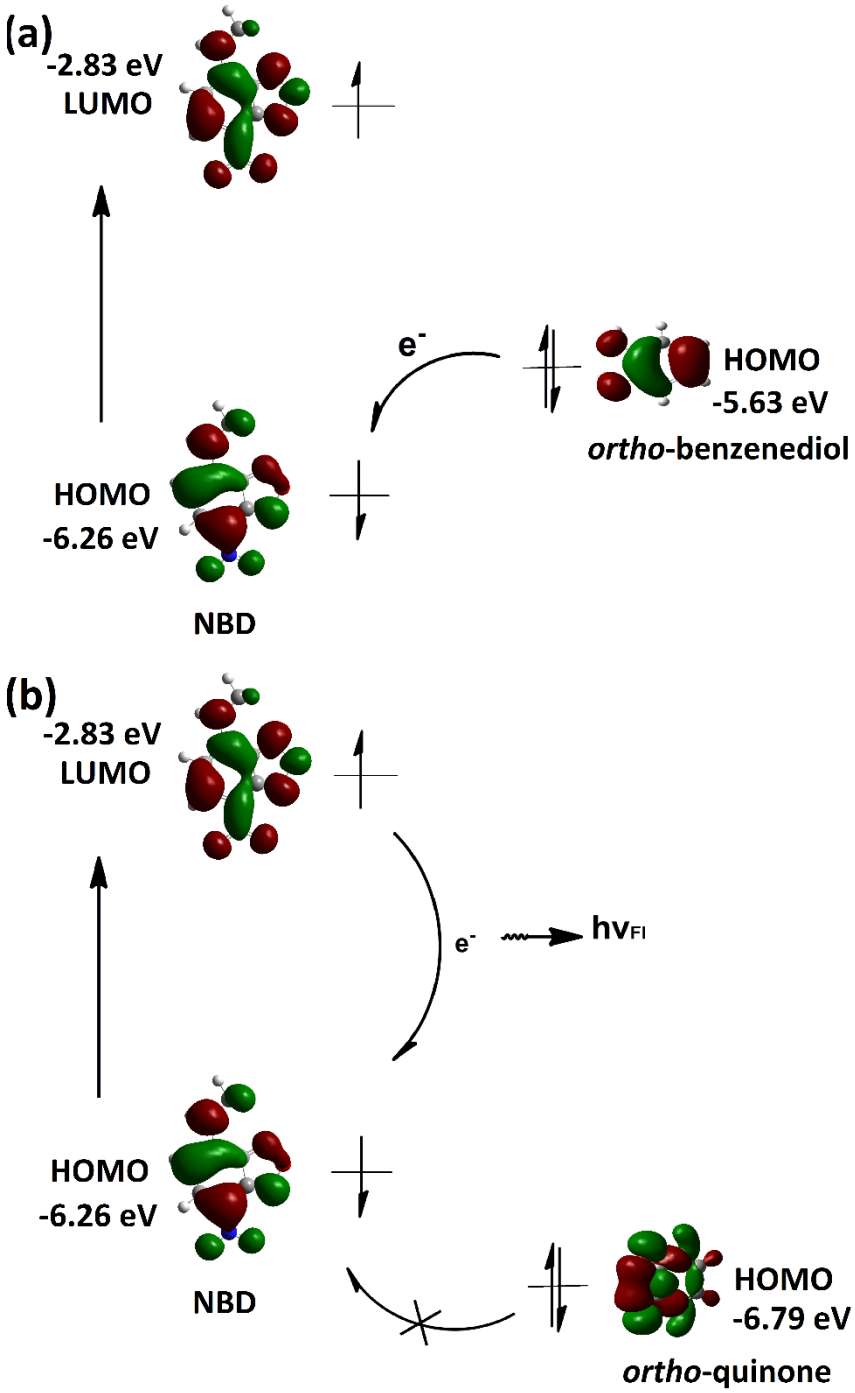


Fig. S7 Frontier orbital energy representation of the PET processes in NBD-DOP (a) and NBD-DOP-Q (b).

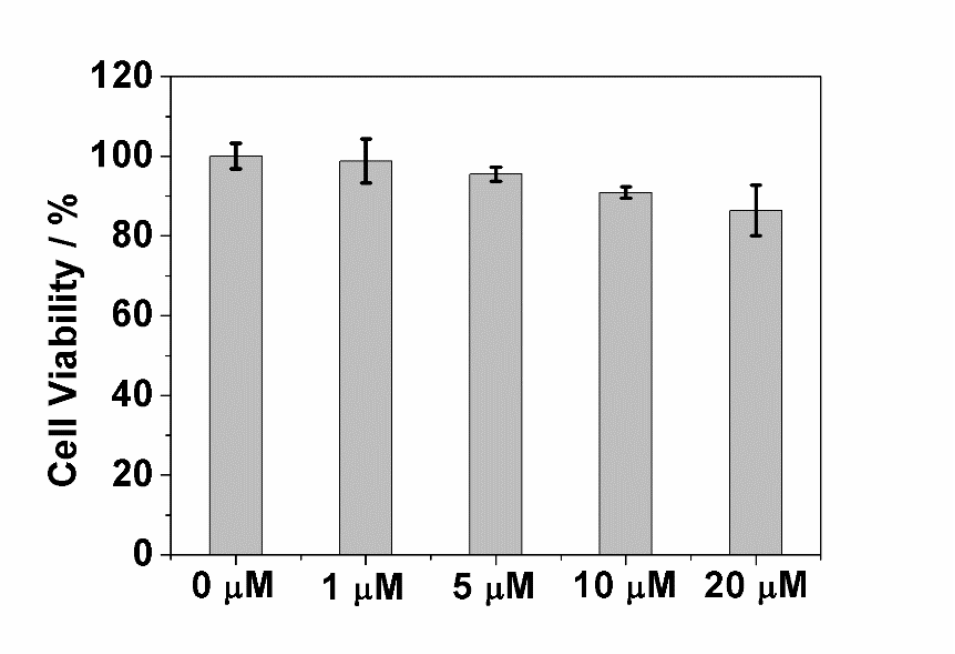


Fig. S8 MTS assay with different concentrations of NBD-DOP in HeLa cells after 24 h incubation.

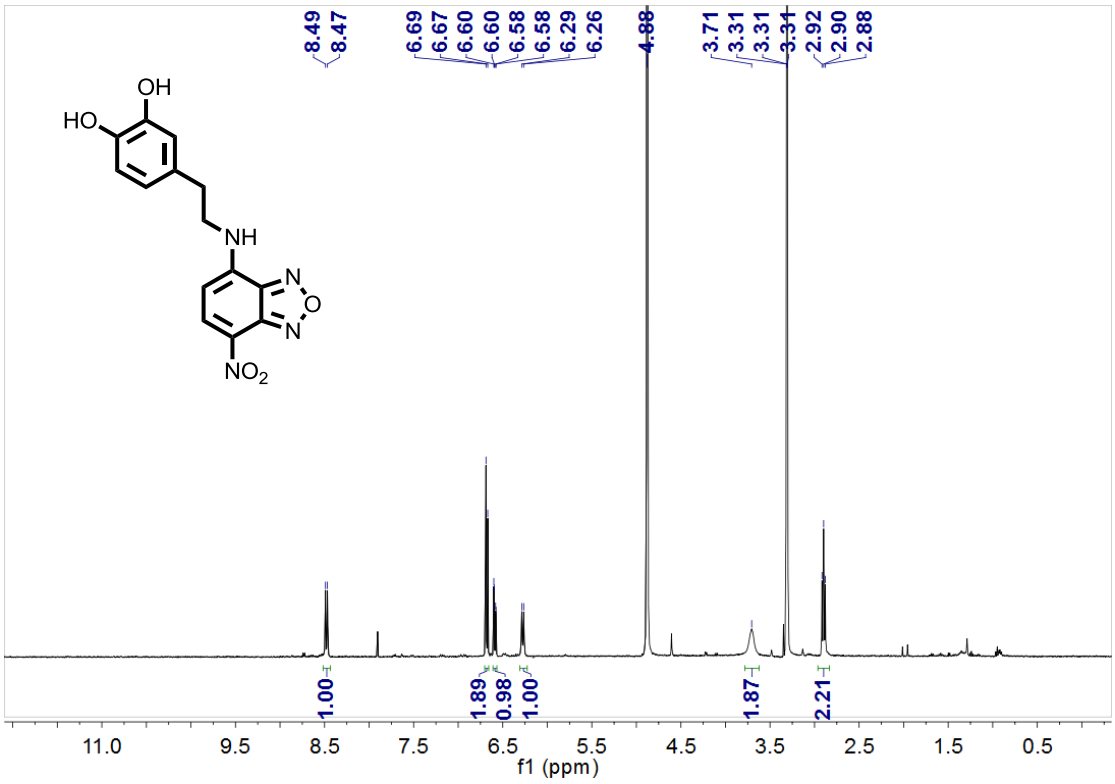


Fig. S9 ^{1}H NMR spectrum of NBD-DOP (CD_3OD).

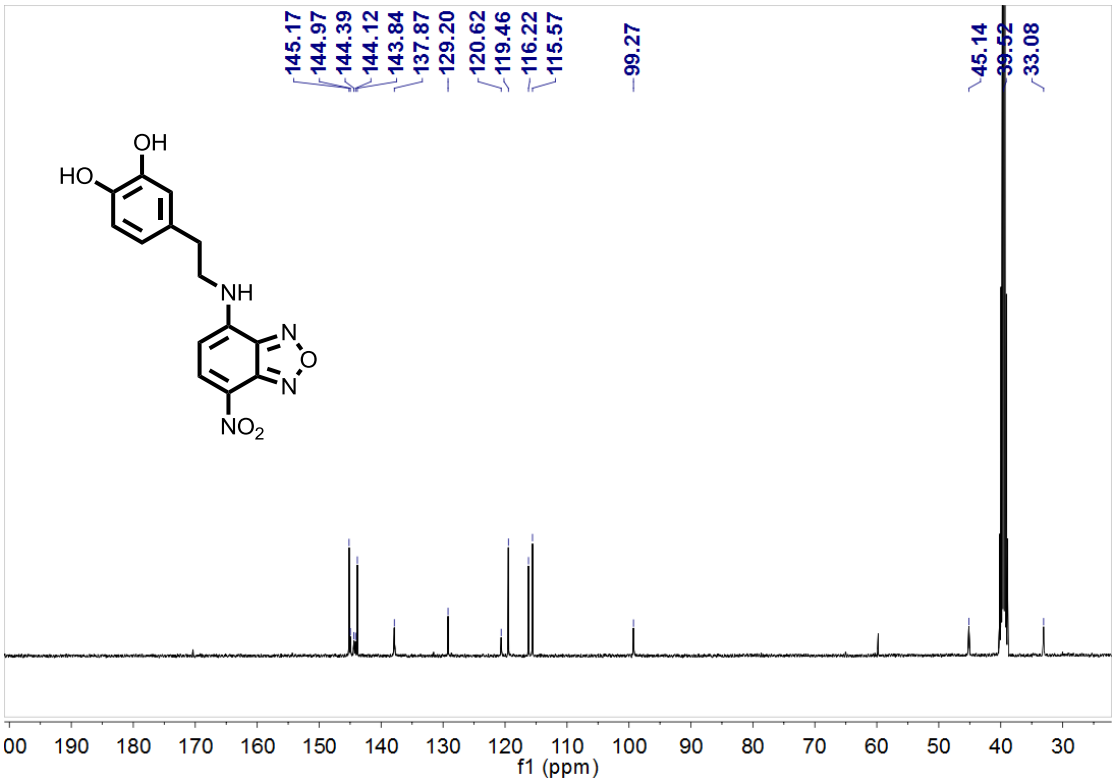


Fig. S10 ^{13}C NMR spectrum of NBD-DOP (*d*^{6}-DMSO).

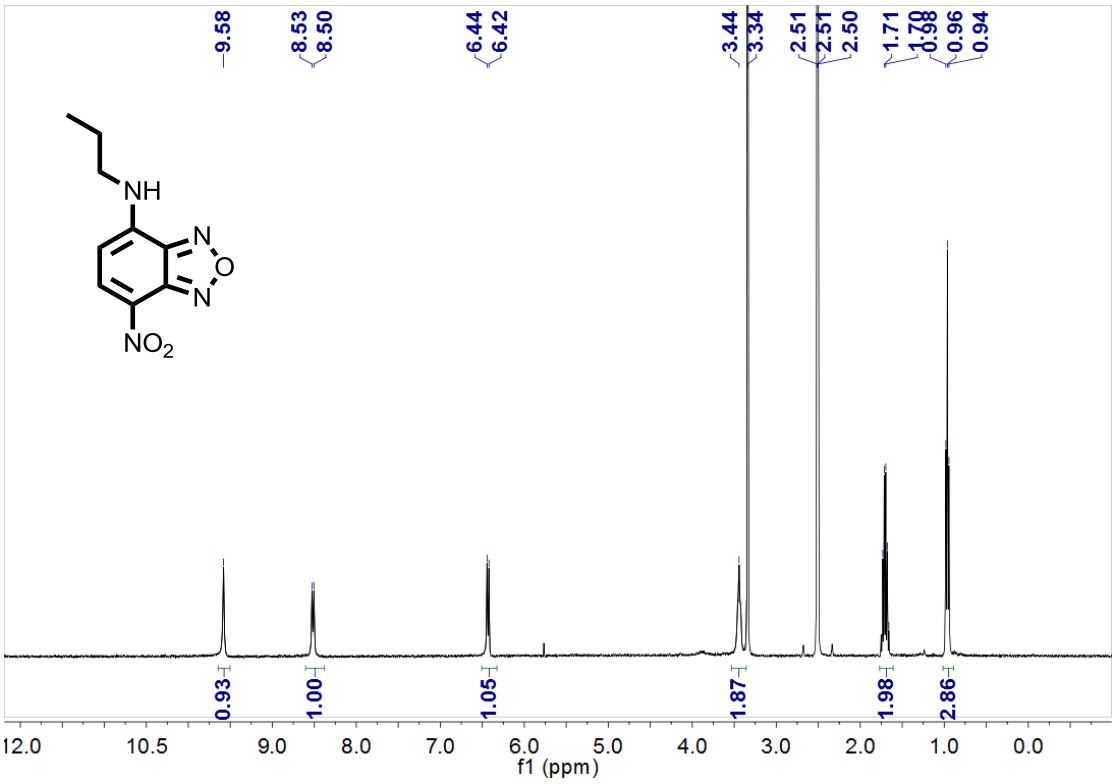


Fig. S11 ^{1}H NMR spectrum of NBD-PRO (*d*^{6}-DMSO).

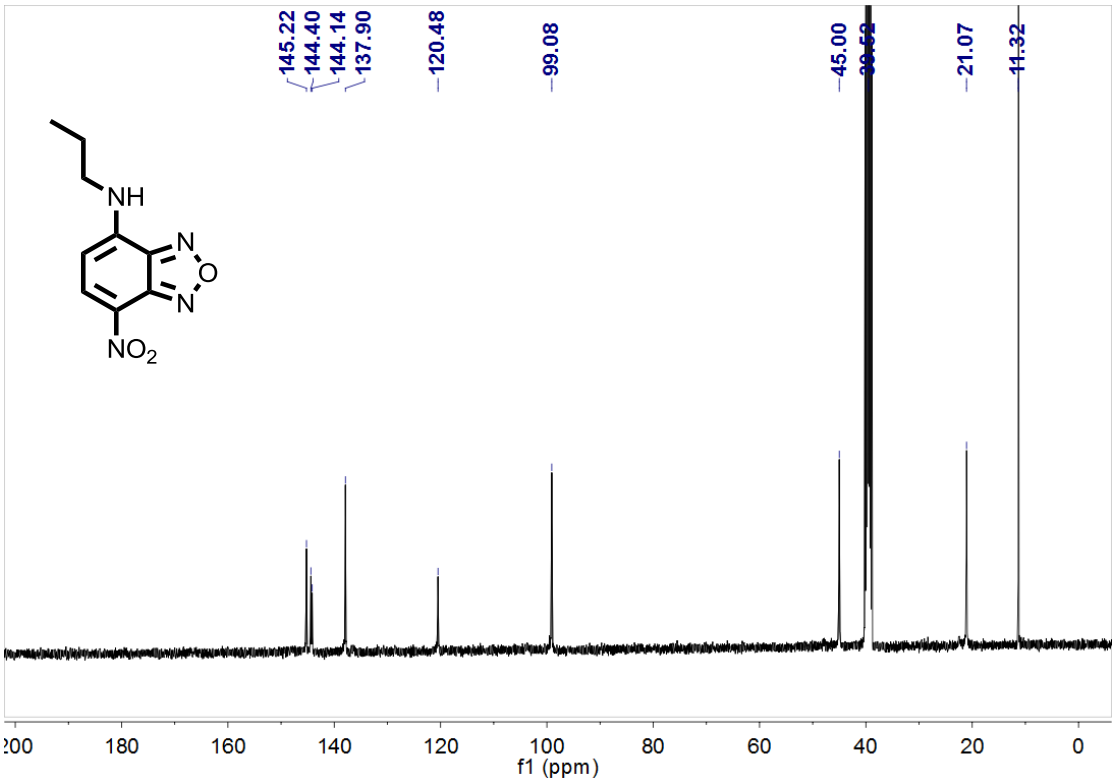


Fig. S12 ^{13}C NMR spectrum of NBD-PRO (d^6-DMSO).

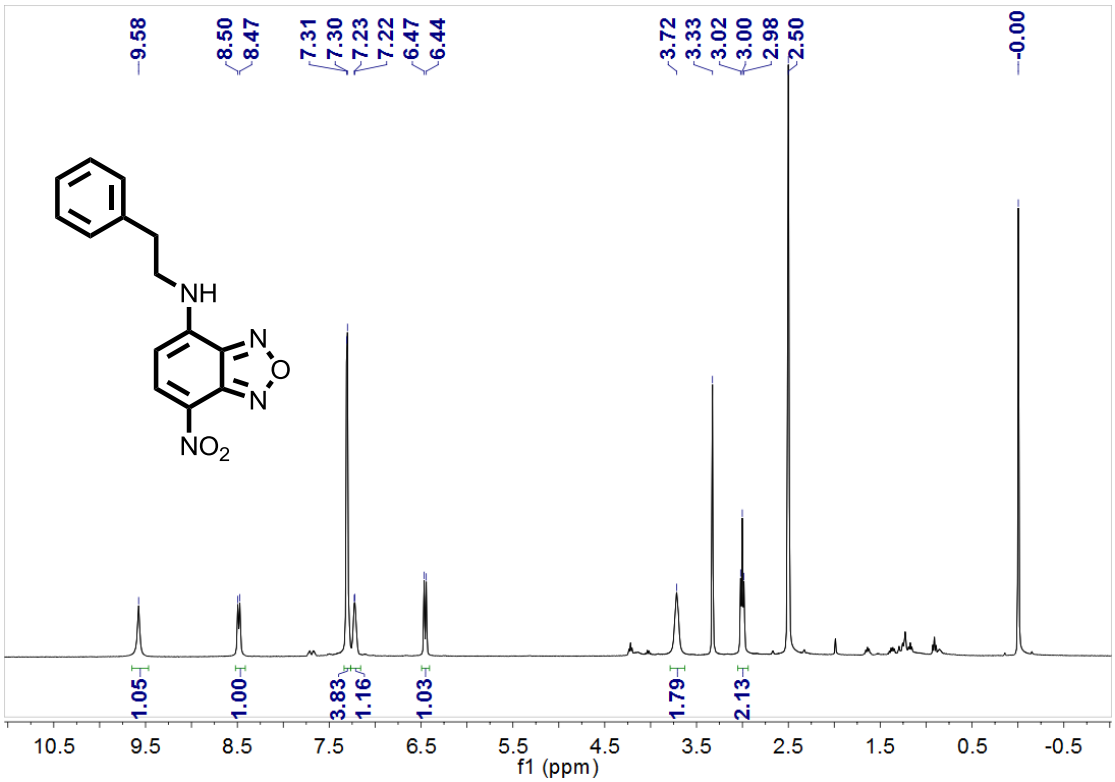


Fig. S13 ^{1}H NMR spectrum of NBD-PHE (d^6-DMSO).

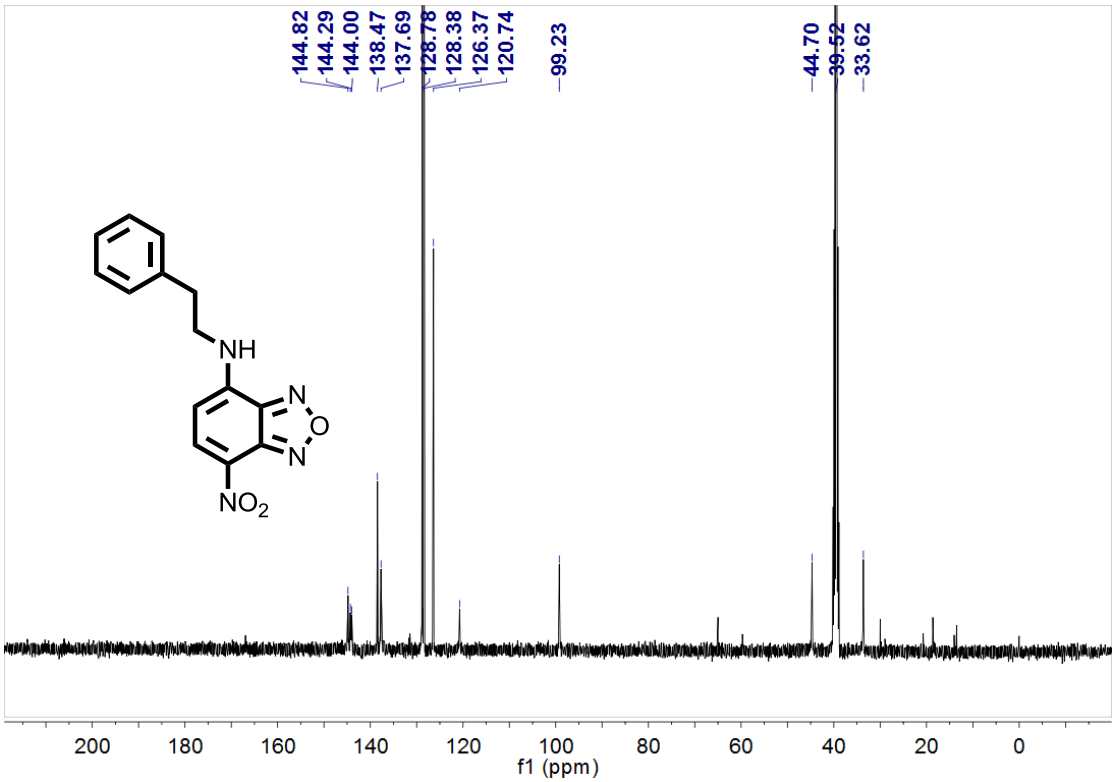


Fig. S14 ^{13}C NMR spectrum of NBD-PHE (*d*^{6}-DMSO).