Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

A Fluorescent Turn-on H_2S-responsive Probe: Design, Synthesis and Application

Yufeng Zhang,†[a] Haiyan Chen,†[b]* Dan Chen,[b] Di Wu,[a] Sheng Hua Liu*[a] and Jun Yin*[a]

[a] Key Laboratory of Pesticide and Chemical Biology of the Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, P. R. China.

[b] Department of Biomedical Engineering, School of Engineering, China Pharmaceutical University, 24 Tongjia Lane, Gulou District, Nanjing 210009, China.

[c] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemistry and Chemical Engineering, Nanjing Tech University, Nanjing 210009, China.

* E-mail: chenhaiyan@cpu.edu.cn; yinj@mail.ccnu.edu.cn

Contents

1 Solubility in HEPES buffer containing 5% DMSO

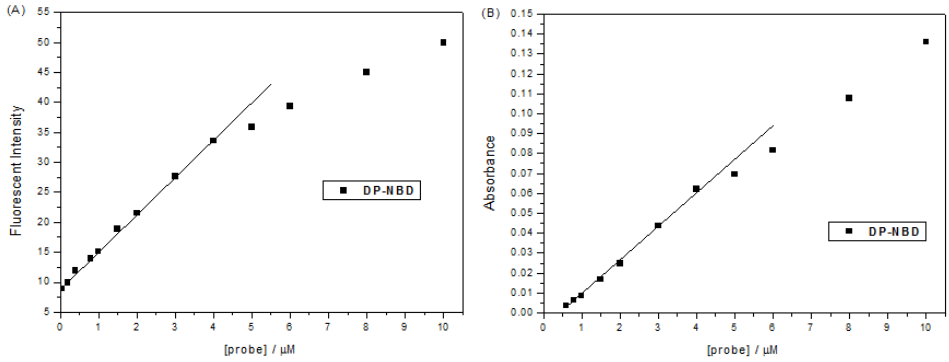


Figure S1 Plot of fluorescence intensity (A) and absorbance (B) against the total amount of **DP-NBD** in HEPES (pH=7.4, 0.02 M) containing 5% DMSO.

2 Spectra upon addition of H_2S depending on time

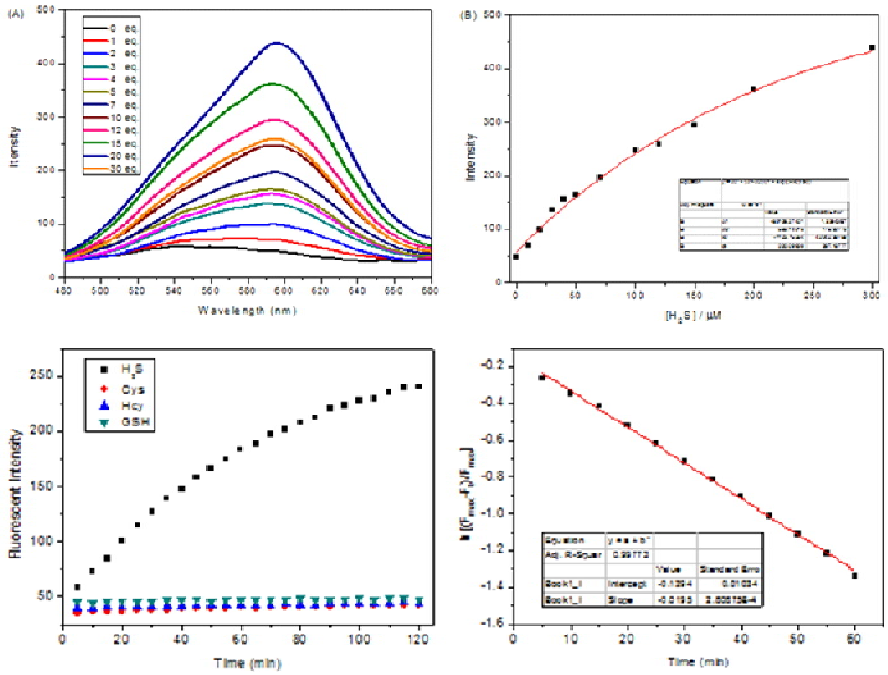


Figure S2 (A) Fluorescent spectra and (B) Absorption spectra of **DP-NBD** (10 uM) upon addition of H_2S (10 equiv) depending on time in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02M). (C) Time-dependent fluorescence changes of DP-NBD (10 μM) upon addition of H_2S , Cys, Hcy and GSH (10 equiv) within 120 min in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02 M). (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.). (D) The k_{obs} = 2.75 × 10^{-4} s^{-1} calculated from the slope of the plot of ln [(Fmax–Ft)/Fmax] (measured at 595 nm vs time using excitation wavelength 365 nm).

3 Fluorescent titration of DP-NBD upon addition of H_2S

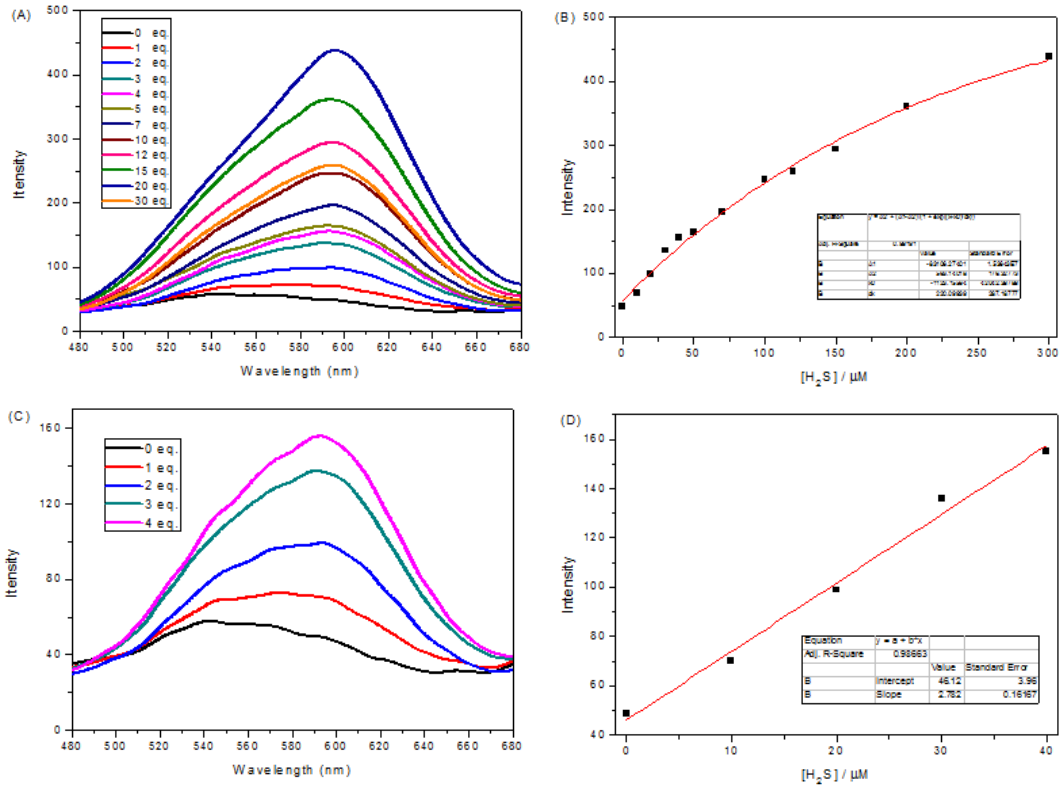


Figure S3 Fluorescence spectra of **DP-NBD** (10 μM) upon addition of H_2S (0 μM-300 μM for A and 0 μM-40 μM for C) in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02 M) and the corresponding relationship between the fluorescent intensity and H_2S concentration(0 μM-300 μM for B and 0 μM-40 μM for D). (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.)

4 Fluorescence response of DP-NBD upon addition of H_2S depending on *p*H

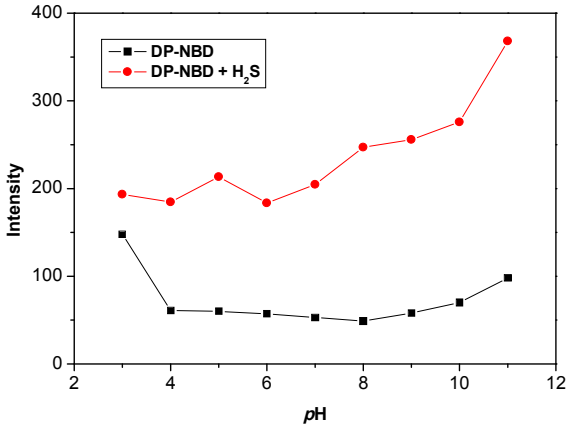


Figure S4 Fluorescence response of **DP-NBD** (10 μM) upon addition of H_2S (10 equiv) depending on *p*H(2-12) in a mixed solution of DMSO-HEPES(v/v=5:95, pH=7.4, 0.02M). Black line:only **DP-NBD**. Red bar: **DP-NBD**+ H_2S. (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.)

5 Cell viability assay

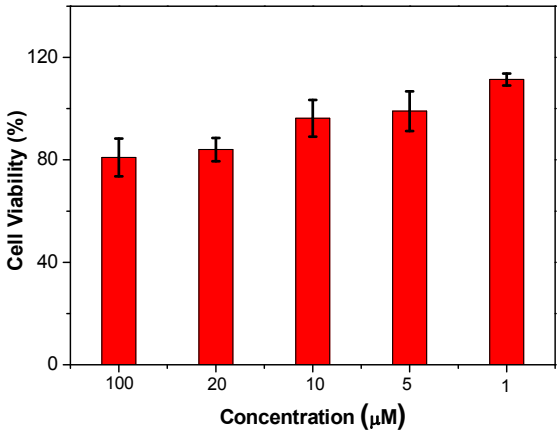


Figure S5 Cell viability of HeLa cells treated with probe **DP-NBD** at various concentrations

6 The MOLDI-TOF mass spectra

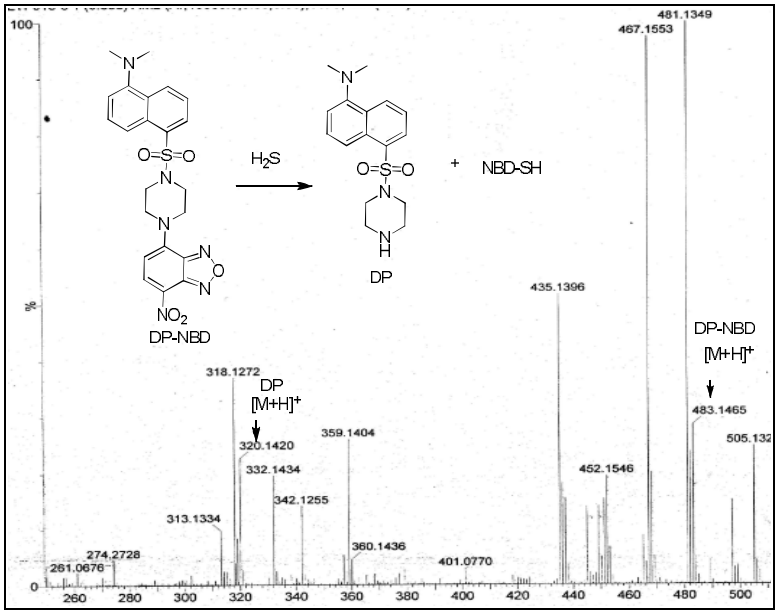


Figure S6 The MOLDI-TOF mass spectra after treating **DP-NBD** and H_2S

7 Fluorescent spectra of NBD-P and DP

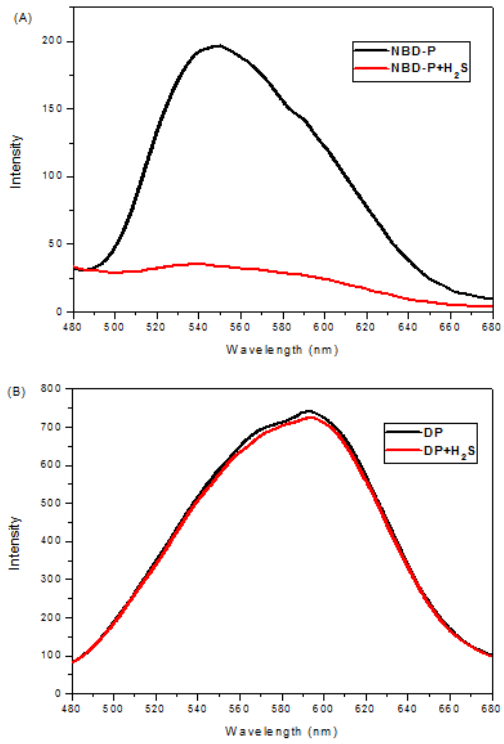


Figure S7 (A) The fluorescent spectra of NBD-P and (B) DP (10 uM) to H_2S (10 equiv) in a mixed solution of DMSO-HEPES(v/v=10:90, pH=7.4, 0.02M) (λex = 365 nm, λem = 595 nm, slit: 10/10 nm.)

8 TDDFT calculations of DP-NBD, DP and NBD-P

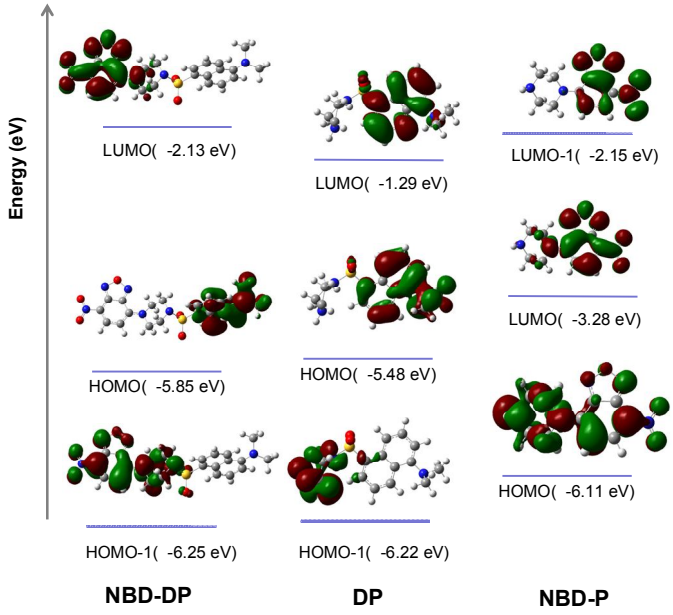


Figure S8 Frontier molecular orbital profiles of molecules based on TDDFT (B3LYP/6–31G*) calculations.

Table S1. Major electronic excitations for **DP-NBD, DP and NBD-P**.

Compound	Excited state	λ/nm [eV]	Osc. str (f)	Major contributions
DP-NBD	$S_0 \rightarrow S_2$	457.25 [2.71]	0.3238	HOMO-1→LUMO (69%)
	$S_0 \rightarrow S_{11}$	325.01 [3.81]	0.1680	HOMO-1→LUMO+2 (53%)
DP	$S_0 \rightarrow S_1$	344.04 [3.60]	0.0985	HOMO→LUMO (69%)
	$S_0 \rightarrow S_6$	248.38 [4.99]	0.0914	HOMO-2→LUMO+1 (45%)
	$S_0 \rightarrow S_8$	229.96 [5.39]	0.1008	HOMO→LUMO+2 (54%)
NBD-P	$S_0 \rightarrow S_1$	497.92 [2.49]	0.1675	HOMO→LUMO (69%)
	$S_0 \rightarrow S_4$	342.60 [3.62]	0.1737	HOMO→LUMO+1 (66%)
	$S_0 \rightarrow S_8$	295.12 [4.20]	0.0991	HOMO-1→LUMO+1 (69%)

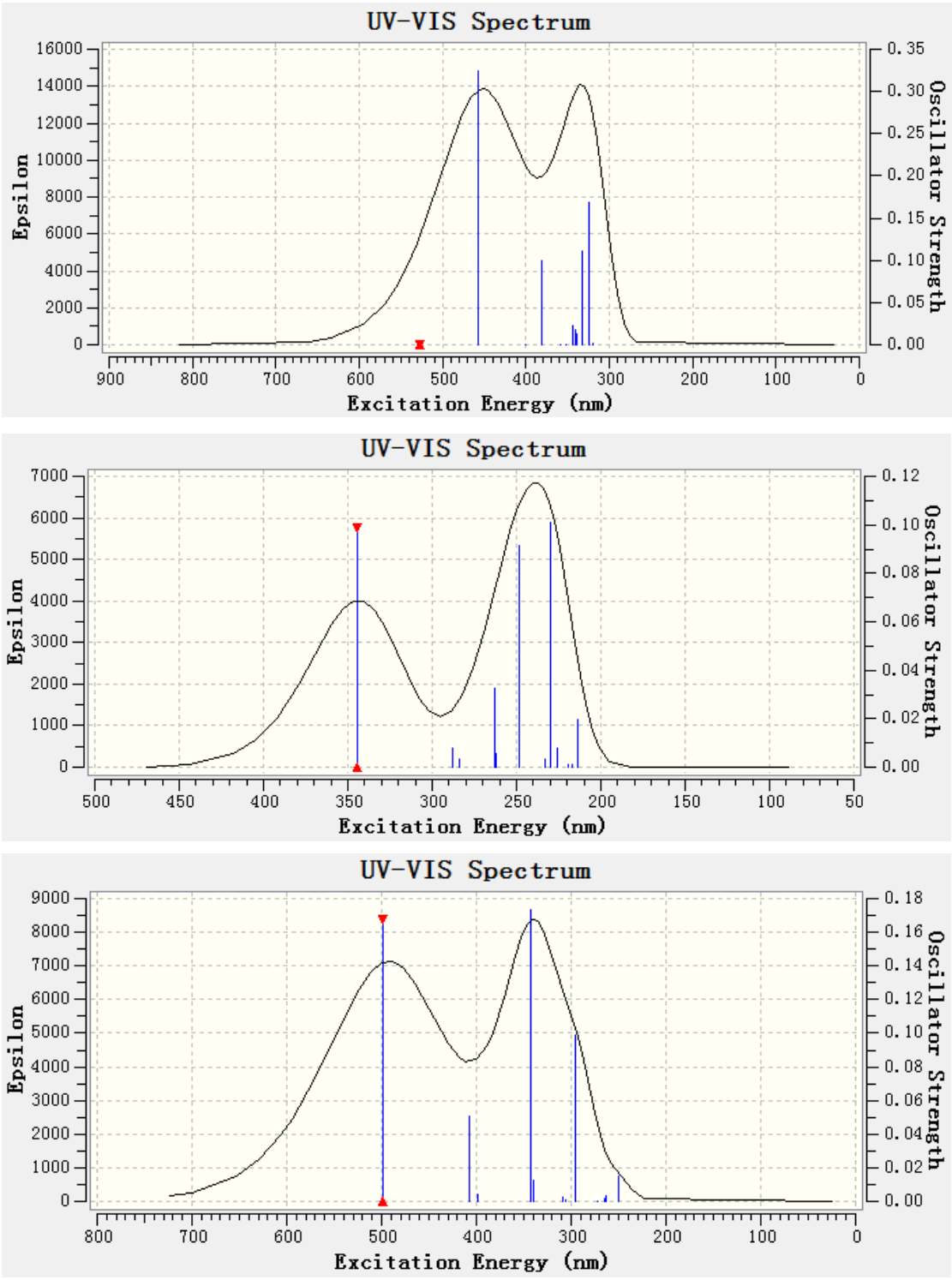
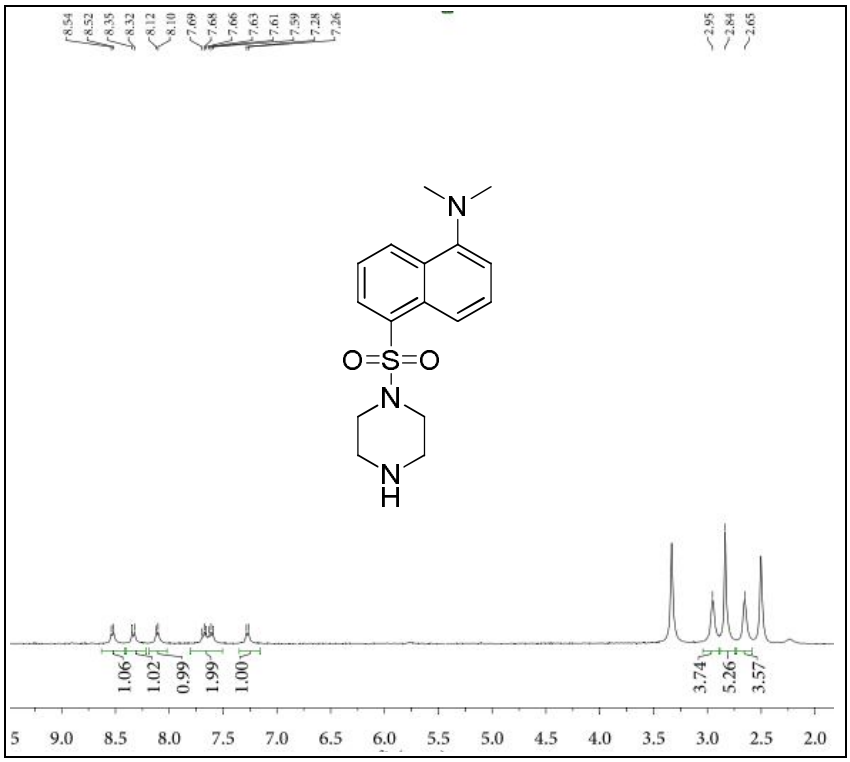
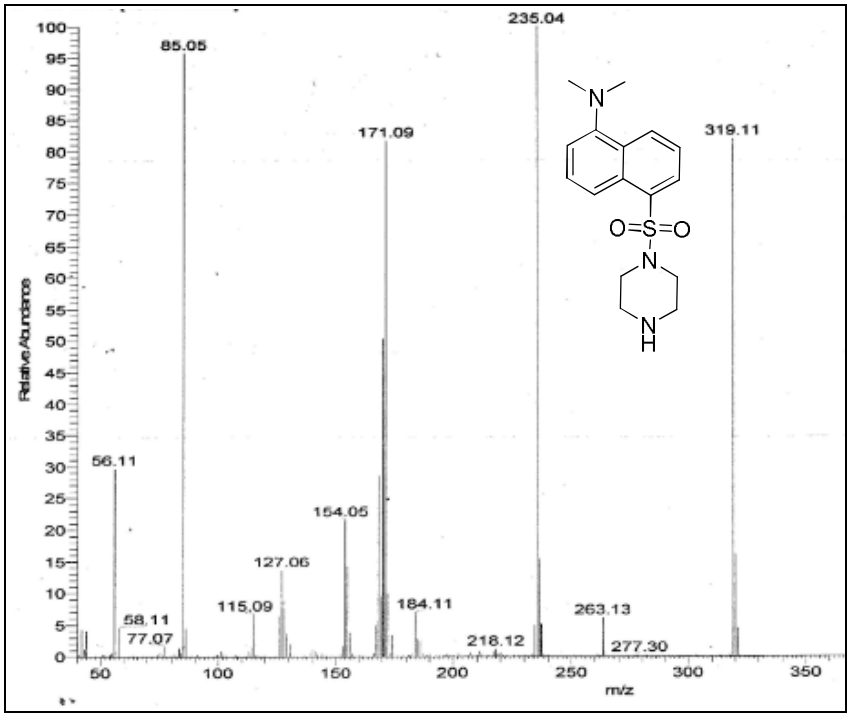
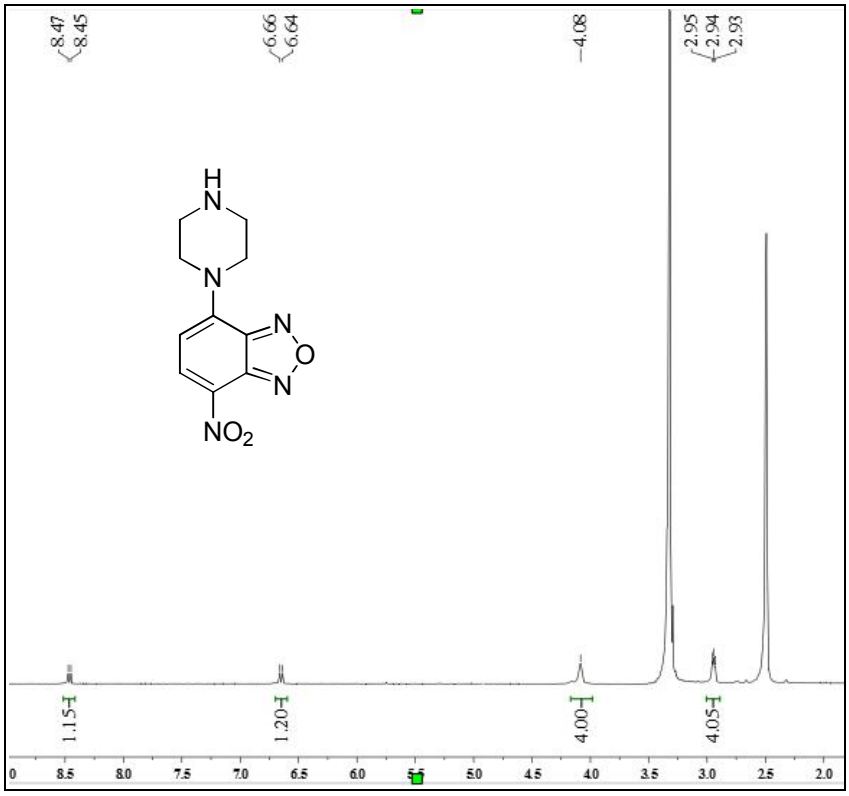


Figure S9. TD-DFT calculations of **DP-NBD, DP** and **NBD-P** at B3LYP/6-31G* level by using Gaussian 09 program.

9 Appendix: NMR and Mass spectra



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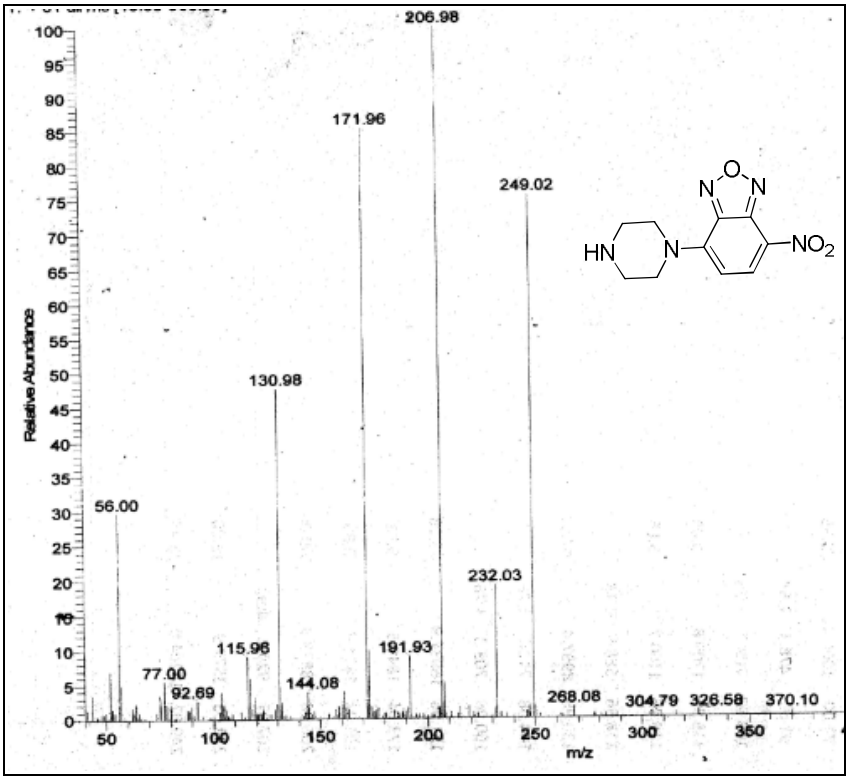
N
N
O
N
NO2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92.69
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326.58
370.10
Relative Abundance
m/z
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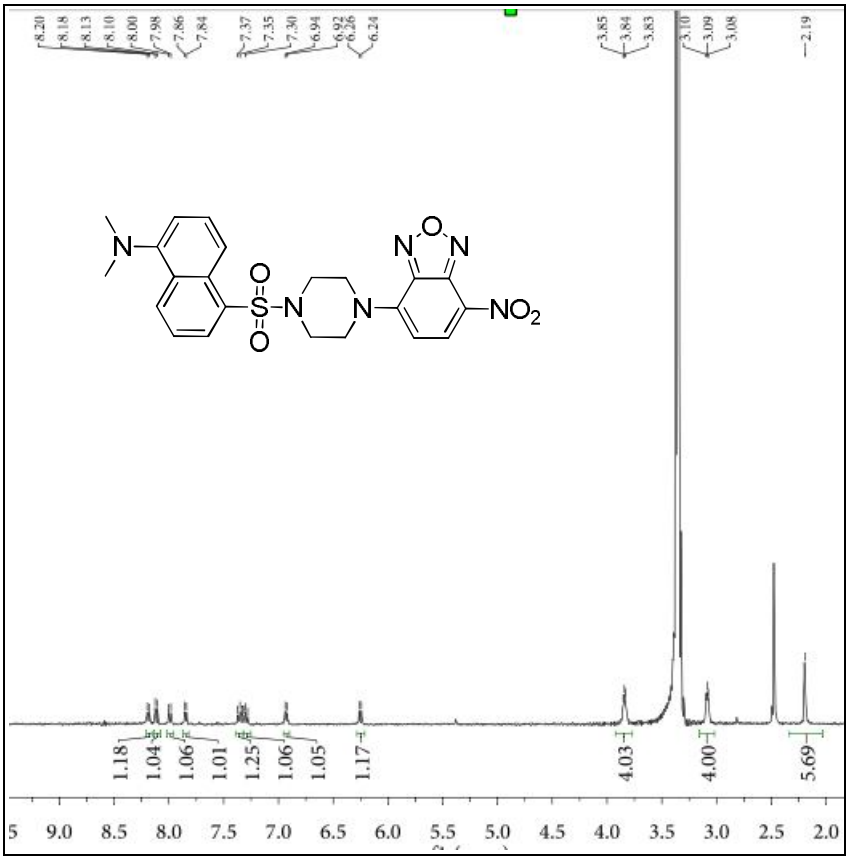
N
O
N
N
NO2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1.05
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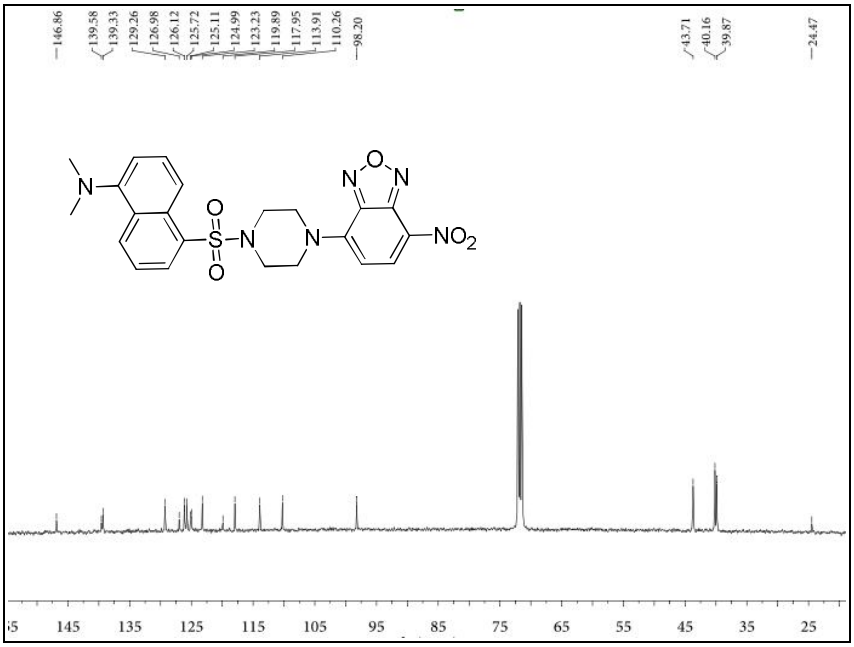
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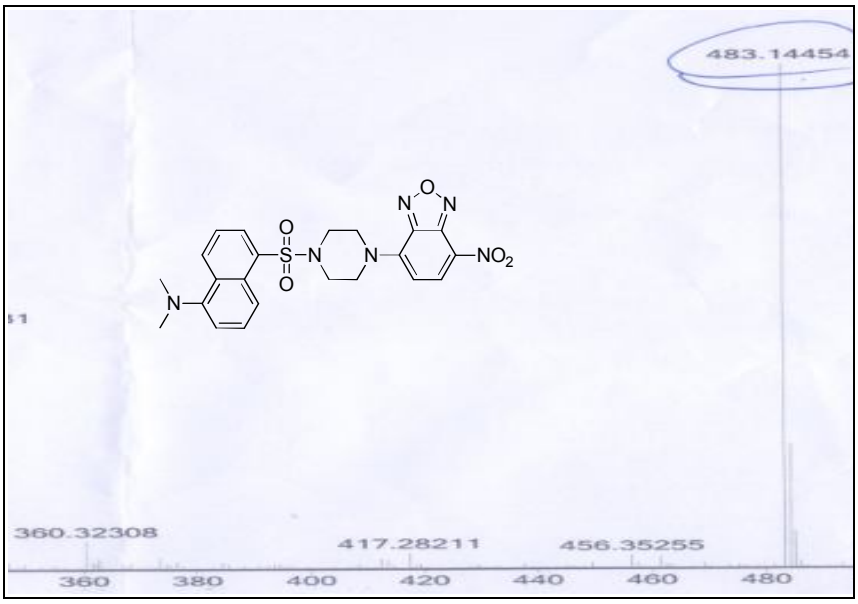
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