Supporting Information for

A Novel Two-photon Fluorescent Probe for Hydrogen Sulfide in Living Cells using an Acedan–NBD amine Dyad Based on FRET Process with High Selectivity and Sensitivity

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Additional spectra

Measurement of two-photon cross section. The two-photon cross section (σ) was determined by using a femtosecond (fs) fluorescence measurement technique. **L** was dissolved in EtOH-phosphate buffer solution (PBS, 25 mM, pH 7.4, containing 20% EtOH as co-solvent), at a concentration of 5.0×10^{-6} M and then the two-photon fluorescence intensity was measured at 720-800 nm by using Rhodamine B in MeOH as the standard, whose two-photon property has been well characterized in the literature.[1] The two-photon cross-section was calculated by using $\sigma = \sigma_r(F_t n_t^2 \Phi_r C_r) /(F_r n_r^2 \Phi_t C_s)$, where the subscripts t and r stand for the sample and reference molecules. F is the average fluorescence intensity integrated from TPE imaging, n is the refractive index of the solvent, C is the concentration, Φ is the quantum yield, and σ_r is the two-photon cross-section of the reference molecule.

[1] N. S. Makarov, M. Drobizhev, A. Rebane, *Opt. Exp.* **2008**, 16, 4029-4047.

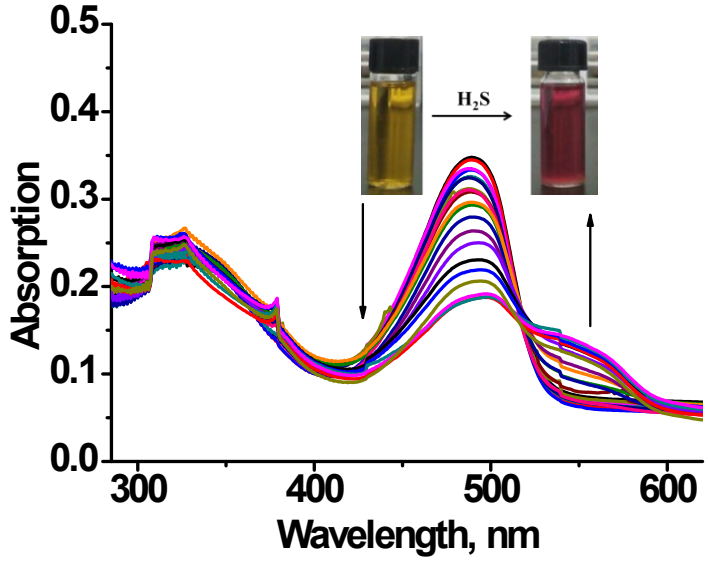


Fig. S1 Absorption spectra of **L** (5 μM) with H_2S (0-140.0 equiv.) in EtOH-phosphate buffer solution (PBS, 25 mM, pH 7.4, containing 20% EtOH as co-solvent) at 25 °C. Inset: visual color of the probe **L** in the absence (yellow) or presence (pink) of H_2S under visible light.

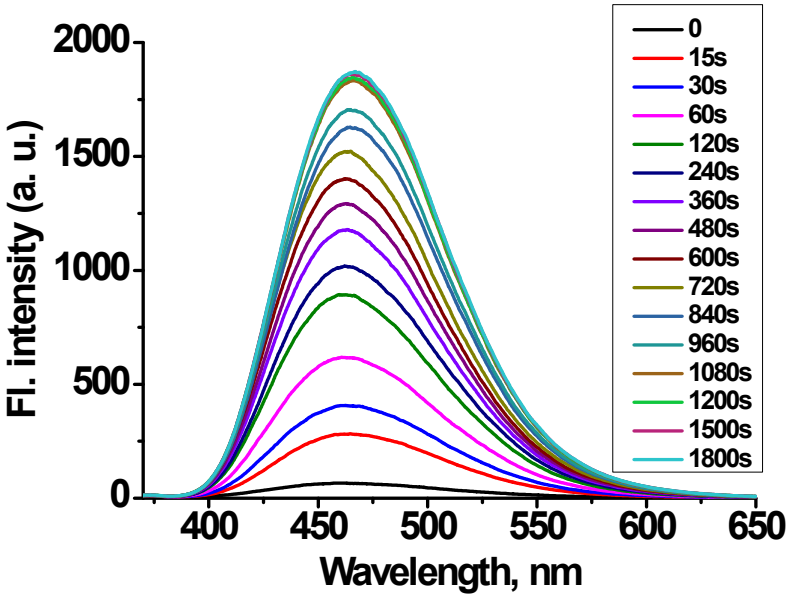


Fig. S2 Fluorescence spectra of probe **L** (5 μM) upon reaction with H_2S (700 μM) at 25 °C at different reaction times as shown inset.

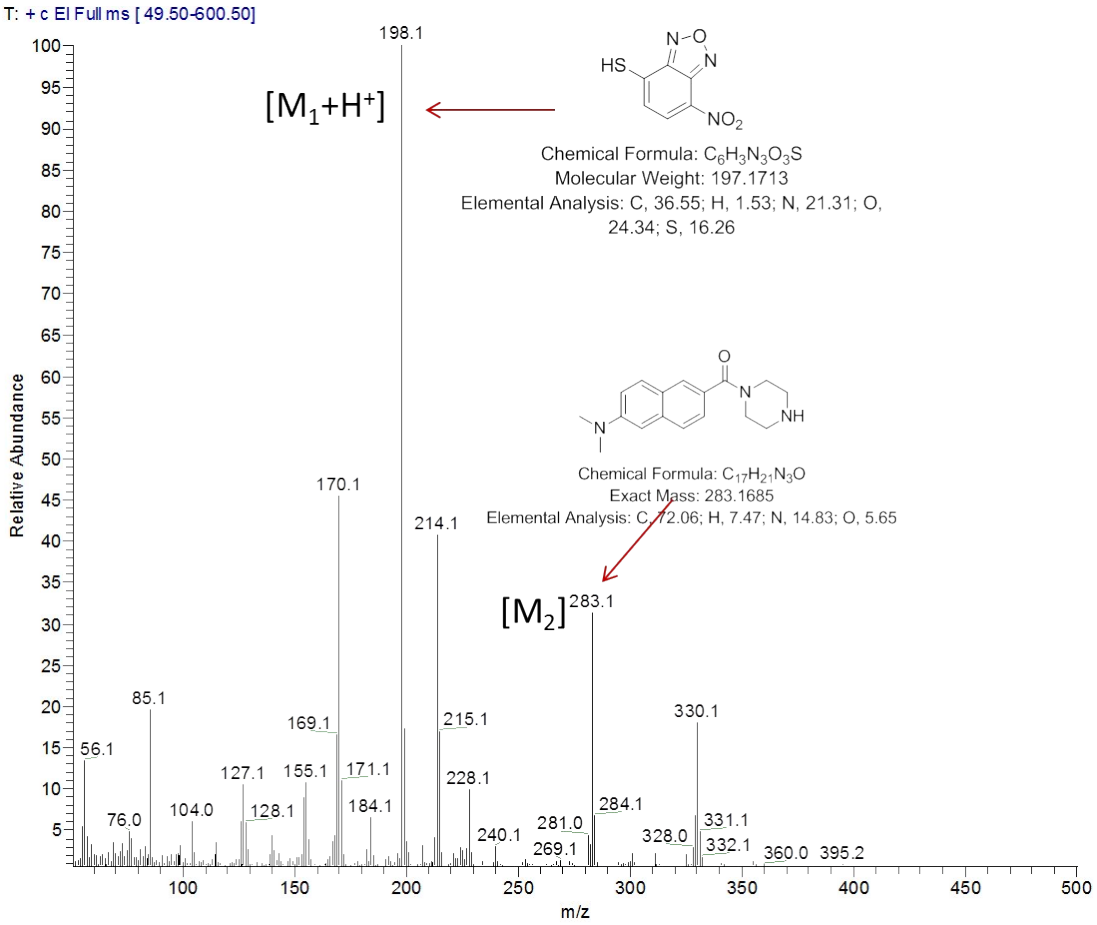


Fig. S3 EI-MS spectrum of the reaction of probe **L** with H_2S.

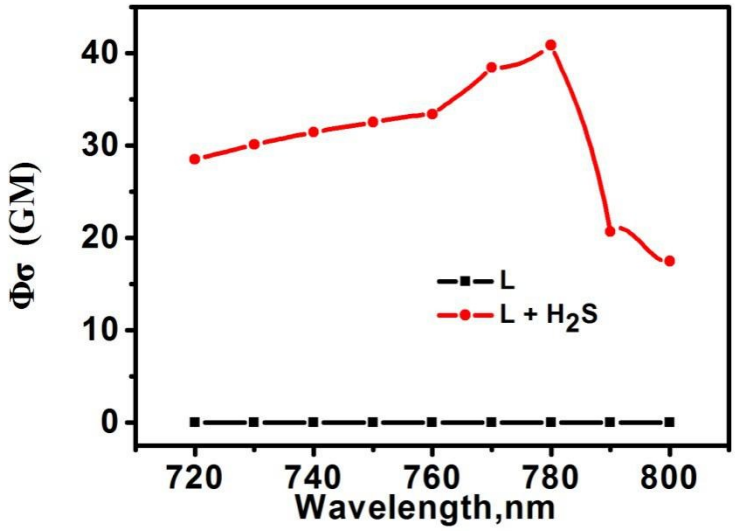


Fig. S4. Two-photon action spectra of Probe **L** (5 μM) and **L** with 250 μM H_2S in EtOH-phosphate buffer solution (PBS, 25 mM, pH 7.4, containing 20% EtOH as co-solvent).

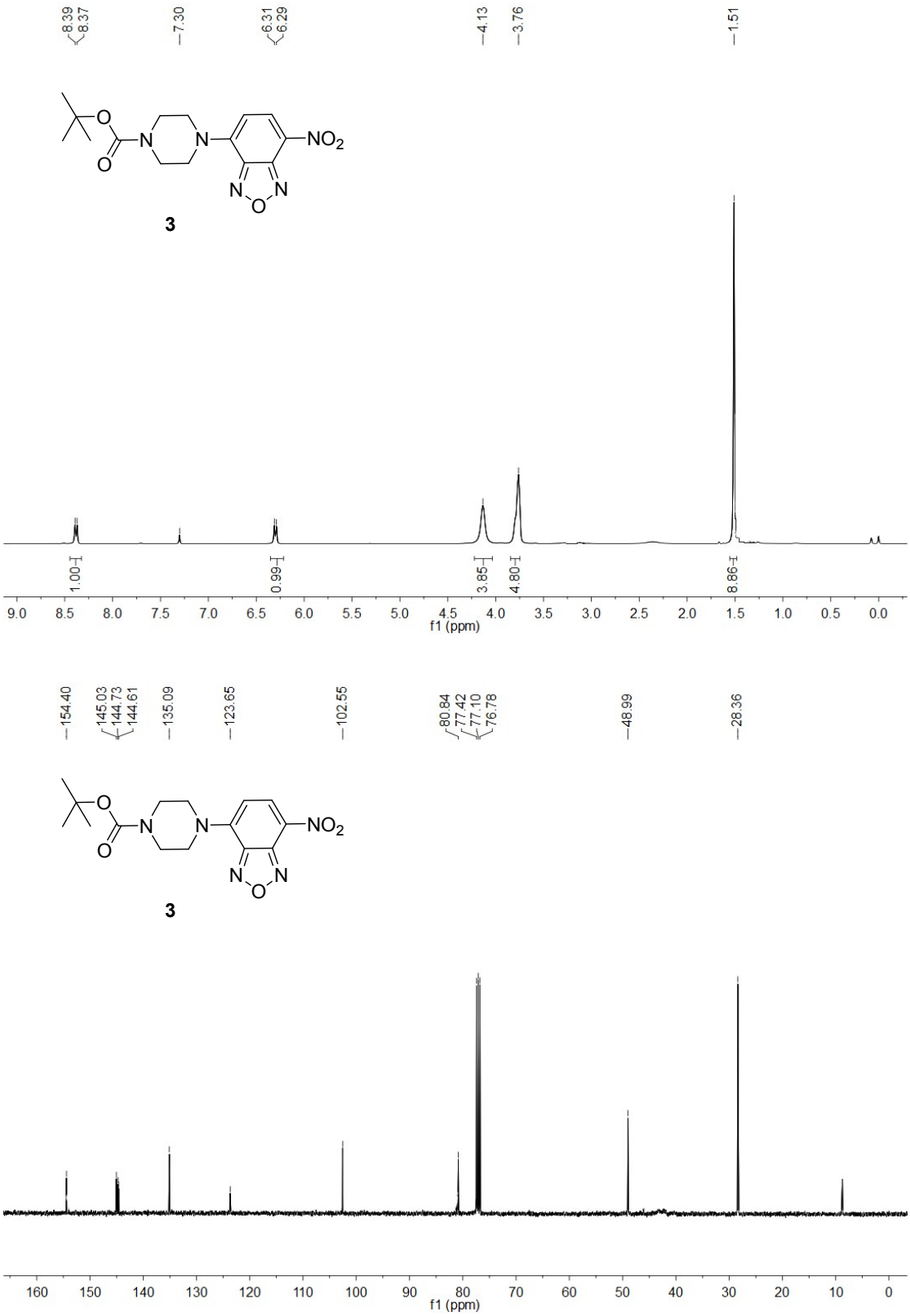
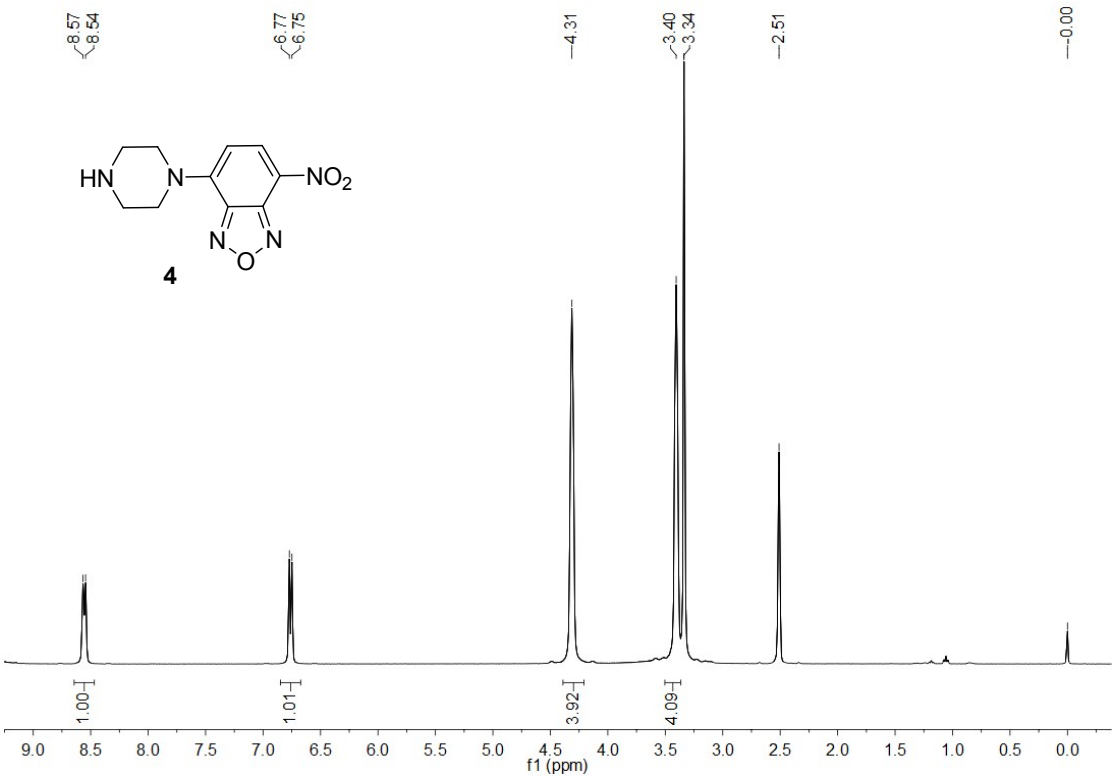


Fig. S5 The ^{1}H NMR and ^{13}C NMR spectrometry of **3**.



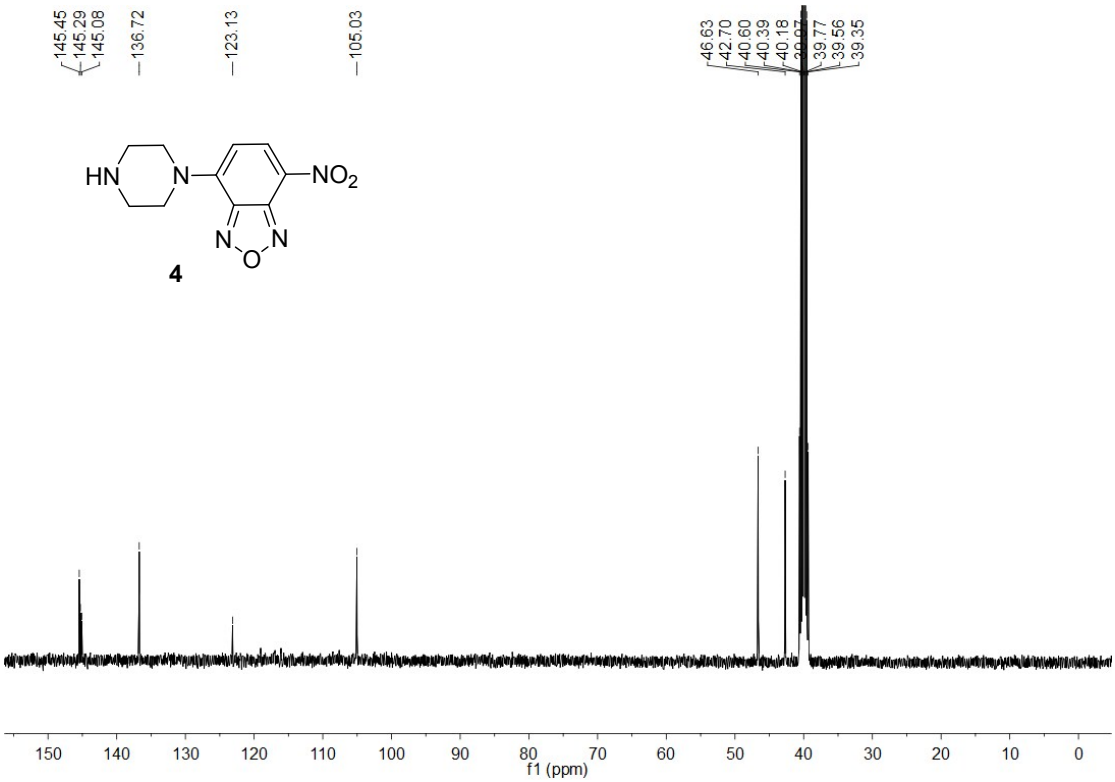


Fig. S6 The ^{1}H NMR and ^{13}C NMR spectrometry of **4**.

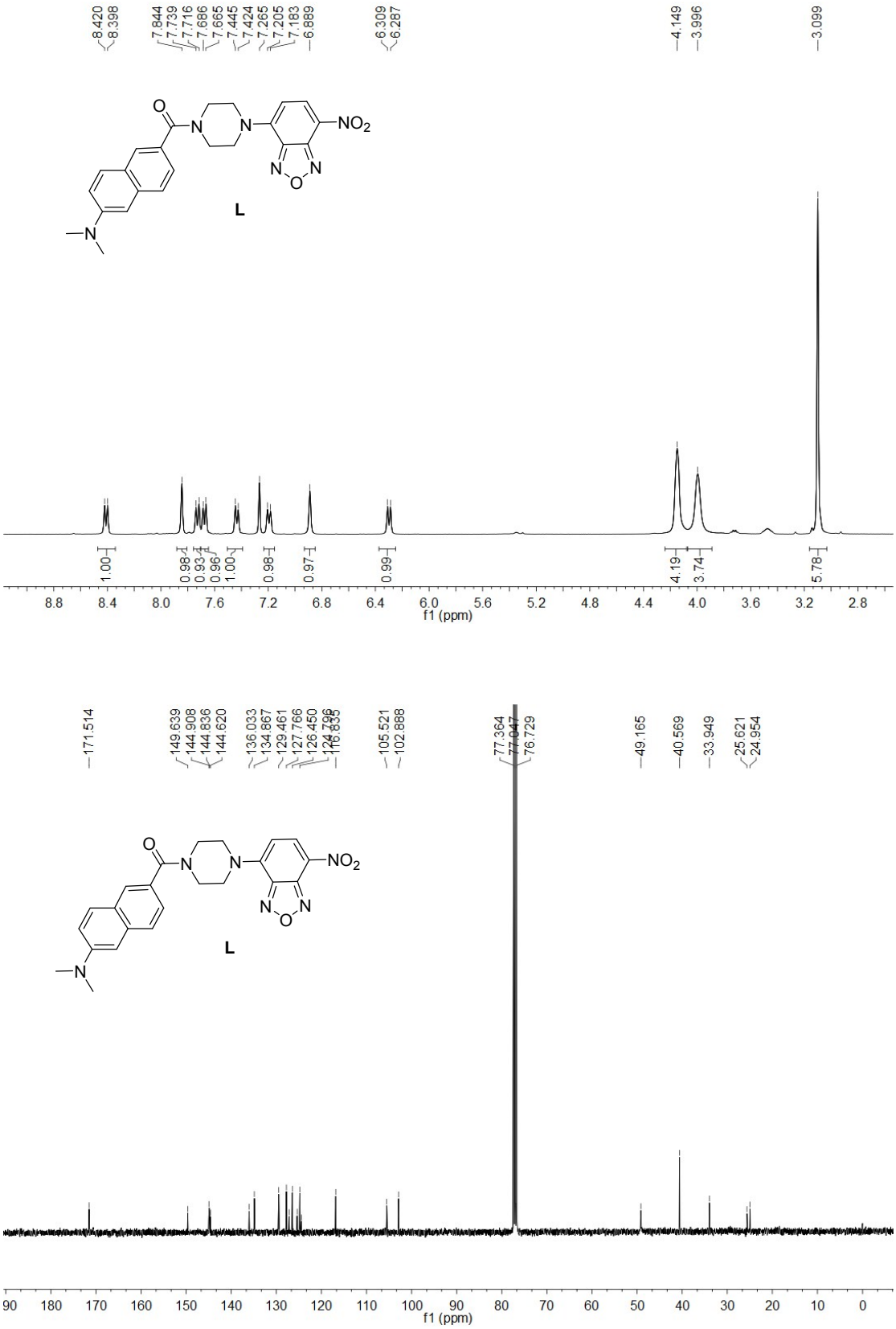
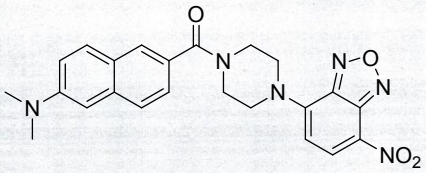


Fig. S7 The ^{1}H NMR and ^{13}C NMR spectrometry of probe **L**.

File : C:\Xcalibur\data\ty-170512-446-hr-av2.RAW
Full ms [410.500 - 467.500] - Range: 410.500 - 467.500
Scan No. 1 of 1

Mass	Relative Intensity	Theoretical Mass	Delta [ppm]	Delta [mmu]	Composition
446.171	100.0	446.1697	3.9	1.7	$C_{23}H_{22}O_4N_6$
		446.1737	-5.1	-2.3	$C_{28}H_{22}O_2N_4$
		446.1751	-8.1	-3.6	$C_{30}H_{24}O_3N_1$
		446.1665	11.0	4.9	$C_{34}H_{22}O_1$
		446.1652	14.0	6.3	$C_{32}H_{20}N_3$



Chemical Formula: $C_{23}H_{22}N_6O_4$
Exact Mass: 446.1703
Elemental Analysis: C, 61.87; H, 4.97; N, 18.82; O, 14.33

Fig. S8 The The HREI mass spectrometry of probe **L**.