Supporting Information

Hydrogen sulfide mediated cascade reaction forming iminocoumarin: Applications in fluorescent probe development and live-cell imaging

Pratryush Kumar Mishra,[a] Tanmoy Saha[a] and Pinaki Talukdar*[a]

[a] Department of Chemistry, Indian Institute of Science Education and Research, Pune 411008, India.

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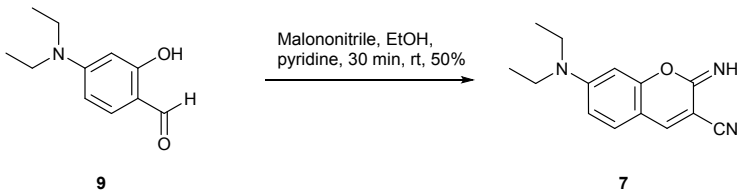
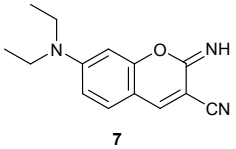
I. Synthesis.



Scheme S1: Synthesis of iminocoumarin **7**.[S1]

Synthesis of 7-(diethylamino)-2-imino-2*H*-chromene-3-carbonitrile 7 ($C_{14}H_{15}N_3O$): In a 25 mL round bottom flask 4-(diethylamino)salicylaldehyde **9** (100 mg, 0.52 mmol) and malononitrile (34.36 mg, 0.52 mmol) were added in ethanol (5 mL) with a drop of piperidine. The reaction mixture was stirred for 30 min at room temperature. After completion of the reaction, the reaction mixture was evaporated under reduced pressure to remove EtOH, obtained a



7

yellow residue which was purified by column chromatography over neutral alumina (*Eluent:* 2 % EtOAc in petroleum ether) to furnish the pure **7** (62 mg, 50%) as yellow solid. Obtained data was matched with the literature data.[S1]

II. Crystallographic Data.

Crystal Structure Parameters:[S2-S6]

The compound was crystallized from DMSO at room temperature. Single-crystal X-ray data of compound **5** was collected at 200 K on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data integration and reduction were processed with SAINT[S2] software. A multi-scan absorption correction was applied to the collected reflections. The structures were solved by the direct method using SHELXTL[S3] and were refined on F^2 by full-matrix least-squares technique using the SHELXL-97[S4] program package within the WINGX programme.[S5] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in successive difference Fourier maps and they were treated as riding atoms using SHELXL default parameters. The structures were examined using the *Adsym* subroutine of PLATON[S6] to assure that no additional symmetry could be applied to the models.

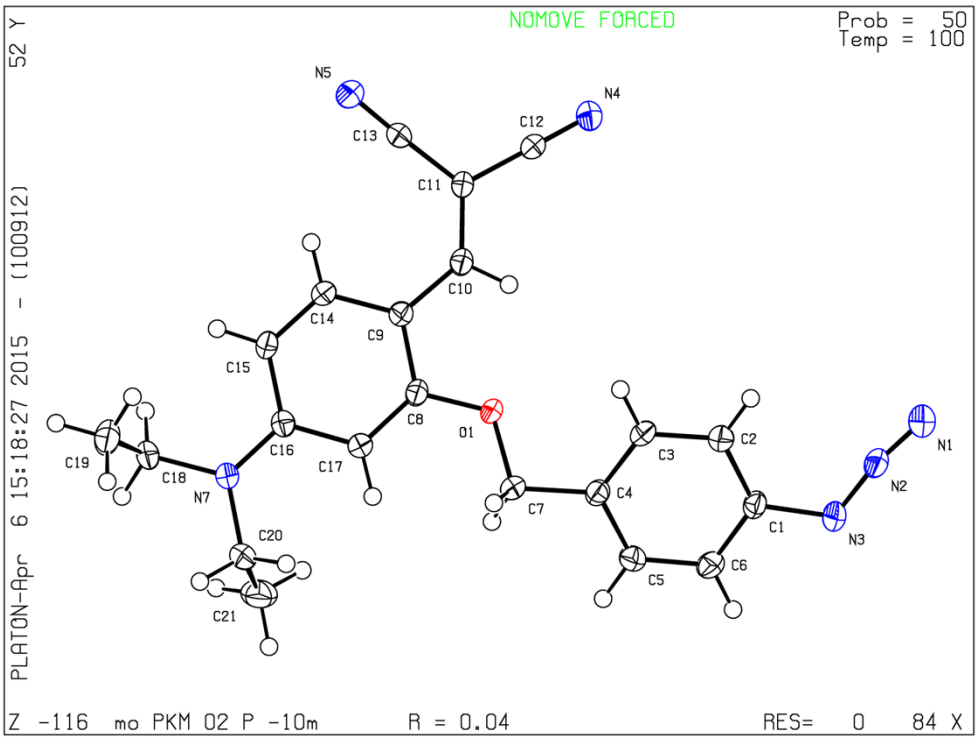


Fig. S1 ORTEP diagram of probe **5.**

III. Photophysical Properties.

Preparation of the medium: Deionized water was used throughout all experiments. All experiments were carried out in water (1 mM CTAB) / EtOH (9:1) solution with 1% DMSO (maximum).

Absorption and molar extinction coefficient of probe 5: Absorption spectra of probe **5** was recorded at concentrations c_5 = 1 - 11 μM (Fig. S2A). Subsequently, absorbance values at λ_{max} = 450 nm were noted and plotted against respective concentration values (Fig. S2B). The slope of the plot (using Beer-Lambert law) provided the molar extinction coefficient ε = 30020 M^{-1} cm^{-1}.

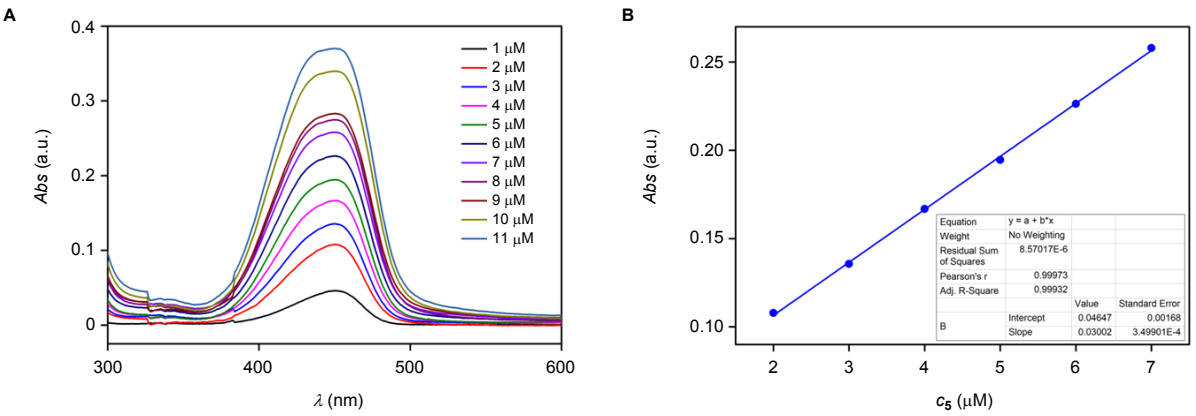


Fig. S2 UV-vis absorption spectra of probe **5** at concentrations c_5 = 1 - 11 μM in water (1 mM CTAB) / EtOH (9:1) (**A**). Absorbance A_{450} versus c_5 plot for **5** (**B**).

Absorption and molar extinction coefficient of probe 7: Absorption spectra of probe **7** was recorded at concentrations c_7 = 1 - 10 μM (Fig. S3A). Subsequently, absorbance values at λ_{max} = 438 nm were noted and plotted against respective concentration values (Fig. S3B). The slope of the plot (using Beer-Lambert law) provided the molar extinction coefficient ε = 36150 M^{-1} cm^{-1}.

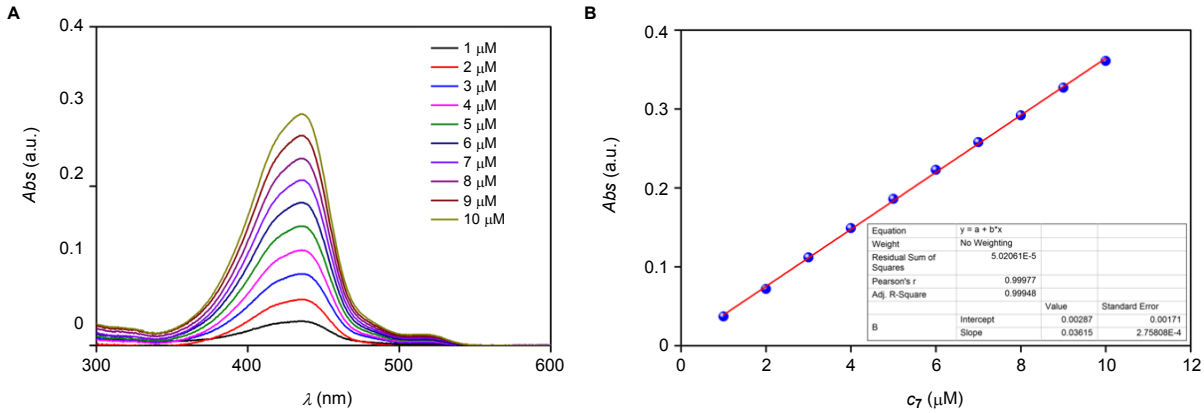


Fig. S3 UV-vis absorption spectra of iminocoumarin **7** at concentrations c_7 = 1 - 10 μM in water (1 mM CTAB) / EtOH (9:1) (**A**). Absorbance A_{438} versus c_7 plot for 7 (**B**).

Fluorescence spectra probe 5 and iminocoumarin 7: Fluorescence spectra of **5** (10 μM) and **7** (10 μM) were recorded in water (1 mM CTAB) / EtOH (9:1) with λ_{ex} = 440 nm (Fig. S4).

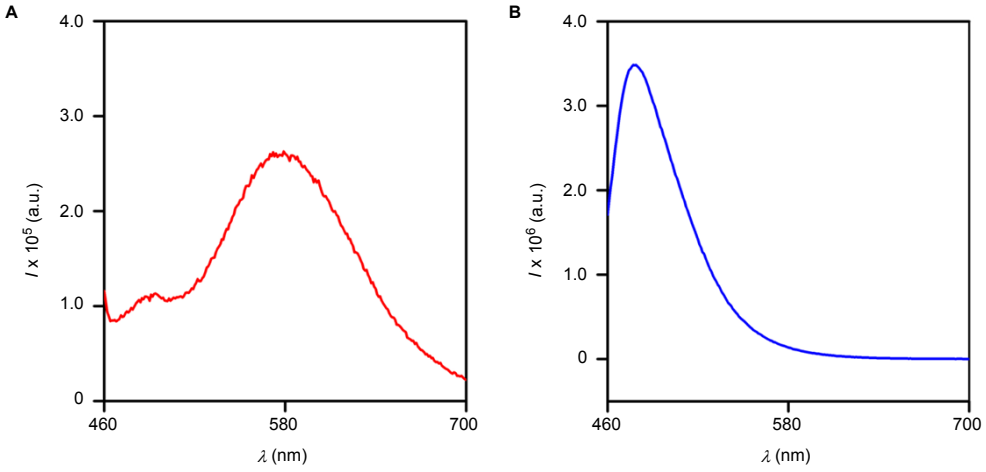


Fig. S4 Fluorescence spectra of probe **5** (A) and iminocoumarin **7** (B) at concentration 10 μM in water (1 mM CTAB) / EtOH (9:1).

Determination of quantum yields for 5 and 7: The quantum yield values of probe **5** and iminocoumarin **7** were determined according to the following Eq. S1:

$$\Phi_1 = \Phi_B \times \frac{I_1 \times A_B \times \lambda_{exB} \times (\eta_1)^2}{I_B \times A_1 \times \lambda_{ex1} \times (\eta_B)^2} \qquad \textbf{Eq. S1}$$

where, Φ is quantum yield; I is integrated area under the emission spectra; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts 1 and B refer to the unknown and the standard, respectively. Coumarin-153 was used as standard (in 1:1 EtOH/H_2O; $\Phi = 0.032$) for probe **5** and cumarin-153 was used as standard (in acetonitrile; $\Phi = 0.56$) for iminocoumarin **7**.

Table S1. Comparison of photophysical properties of probe **5** and iminocoumarin **7**.

Compound	λ_{max} **(nm)**	ε **(M^{-1} cm^{-1})**	λ_{em} **(nm)** [a]	Φ
5	450	30020	580	0.00134
7	438	36150	480	0.05750

[a] λ_{ex} = 440 nm.

IV. HPLC Analysis.

Conditions:

Column: Agilent Eclips plus 5μm
Flow: 1.0 mL/min
Method: Gradient
60 % Acetonitrile/water 0 min
60 % Acetonitrile 0 to 05 min
90 % Acetonitrile 05 to 25 min
Wavelength: 440 nm.

Fig. S5 HPLC chromatogram of pure probe **5** (100 μM).

Fig. S6 HPLC chromatogram of the reaction mixture containing probe **5** + Na_2S (5 equiv), recorded after 20 min.

Fig. S7 HPLC chromatogram of the reaction mixture containing probe **5** + Na_2S (10 equiv), recorded after 20 min.

Fig. S8 HPLC chromatogram of pure iminocoumarin **7** (100 μM).

V. Mass Spectrometric Analysis.

Reaction conditions: In a 10 mL round bottom flask, probe **5** (5 mg, 0.013 mmol) was dissolved in of ethanol (2 mL), and solid Na_2S (2 mg, 0.026 mmol) was added to it. Reaction mixture kept under stirring at room temperature for 5 min.

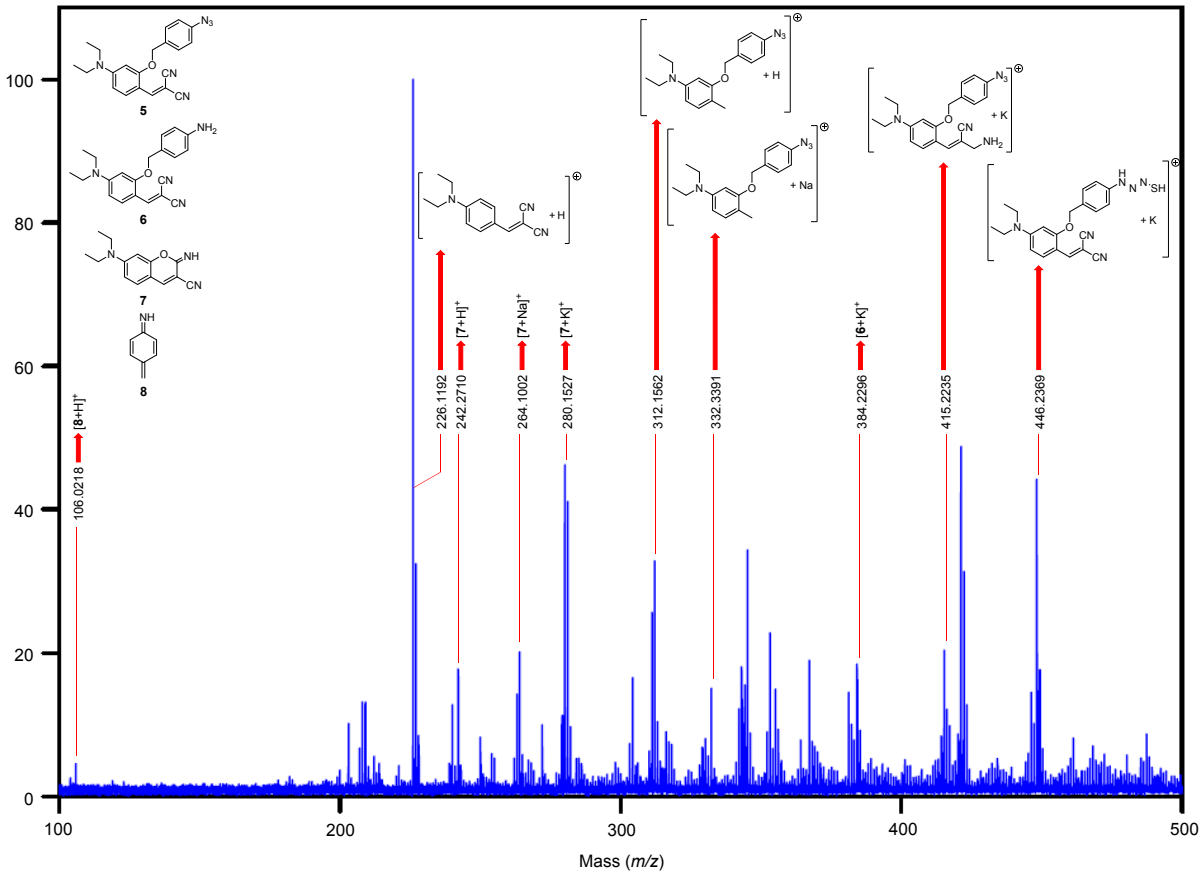


Fig. S9 MALDI Mass data of the reaction mixture containing probe **5** and Na_2S.

VI. FT-IR Analysis.

Reaction conditions: Na_2S (58 mg, 0.74 mmol) was dissolved in water (0.58 mL) to obtain a 1.27 M solution. In each of two 10 mL round bottom flask, probe **5** (2 mg, 0.0052 mmol) was dissolved in of ethanol (2 mL). 0.5 (2 µL) and 1.0 equivalent (4 µL) of the freshly prepared Na_2S solution were separately added to the solutions of **5**, and stirred at room temperature for 3 h. Subsequently, each reaction mixture was dried *under vacuo* and FT-IR spectrum (KBr pellet) was recorded.

FT-IR spectra (KBr pellets) of **5** and **7** were also recorded for comparison.

VII. Fluorescence Sensing.

Preparation of the solution of 5 and 7: Stock solution of **5** (2000 μM) and **7** (2000 μM) was prepared in DMSO. Final concentration for each of **5** and **7** during each assay was 10 μM with 1% DMSO (maximum).

Preparation of the solution of analytes: Appropriate amounts of Na_2S, cysteine (Cys), homocysteine (Hcy), glutathione (GSH), NaF, NaCl, NaBr, NaI, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, NaSCN, $NaNO_2$, $NaNO_3$, NaOH and H_2O_2 were dissolved separately in deionized water to provide stock solution of concentrations = 30 mM each. Calculated volume of an analyte was added from respective stock solutions to each fluorescence cuvette (2 mL) to provide final analyte concentration = 150 μM. Each fluorescence spectrum (λ_{ex} = 440 nm) was recorded after 30 min of an analyte addition. The excitation and emission slit width were 1 nm and 5 nm, respectively.

Reaction kinetics: Fluorescence spectra of a solution containing probe **5** (10 μM) and Na_2S (150 μM) were recorded at time t = 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 min (Fig. 5A). Subsequently, the fluorescence intensities at 480 nm (Fig. 5B) and 580 nm (Fig. 5B) were plotted with respect to time.

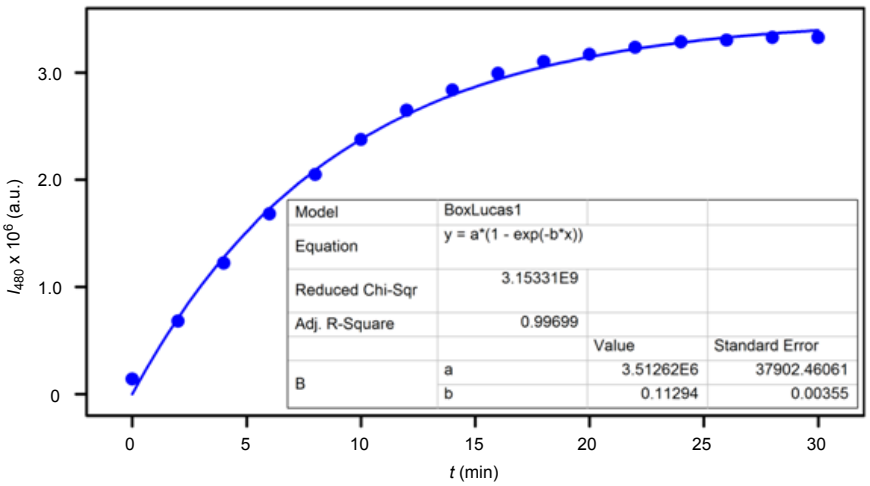


Fig. S10 Fluorescent intensities at 480 nm recorded at different time intervals during the reaction of **5** (10 μM) with Na_2S (150 μM).

From the I_{480} versus time plot, the pseudo first order rate constant k of the reaction was calculated based on the Eq. S1:

$$k = a \times (1 - e^{-kt}) \qquad \textbf{Eq. S1}$$

where, k = pseudo first order rate constant and a = arbitrary constant.

The time required for 50% completion of the reaction $t_{1/2}$ was calculation based on the Eq. S2:

$$t_{1/2} = 0.693/k \qquad \textbf{Eq. S2}$$

Table S2. Determination of k and $t_{1/2}$ values for the reaction kinetics of **5** with Na_2S.

k	$t_{1/2}$
0.11294 min^{-1}	6.136 min

Determination of the limit of H_2S detection by probe 5:

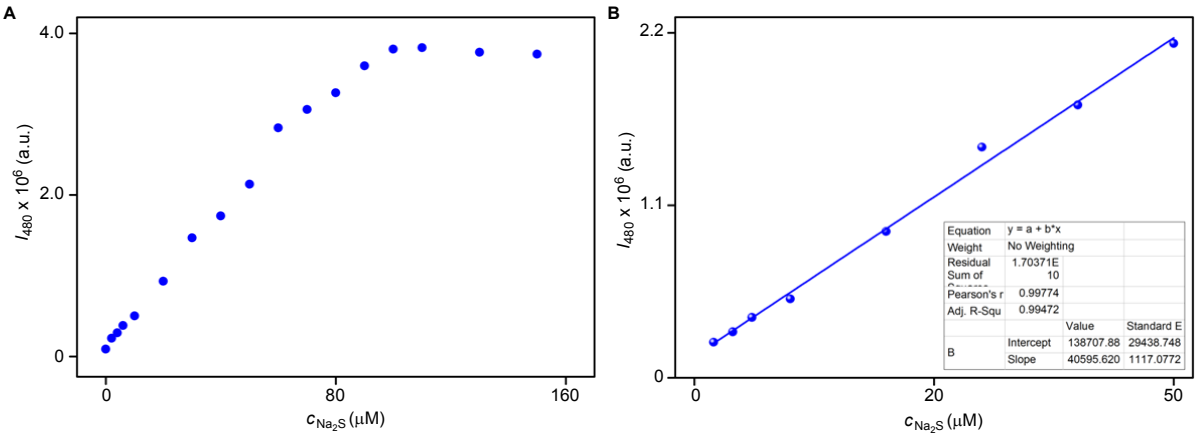


Fig. S11 Fluorescence intensity at 480 nm I_{480} versus concentration of Na_2S c_{Na2S} plot (**A**). Representation of the linear region of the plot and determination of slope (**B**).

The limit of detection (*LOD*) for H_2S sensing was determined following Eq. 3:

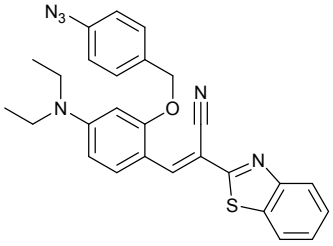
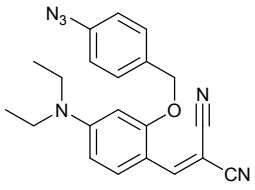
$$LOD = 3\sigma/m \qquad \textbf{Eq. 3}$$

where, σ = standard deviation of 8 blank measurements (*i.e.* fluorescence intensity of the probe **5** at λ = 480 nm), m = slope of the concentration profile obtained from **Fig. S11(B)**. The signal-to-noise ratio *S/N* for the measurement was considered as 3:1. [S7]

Table S3. Calculation of the limit of detection (*LOD*) of H_2S sensing:

S. No.	I_{480} of **5**	σ	m	$3\sigma/m$
1	90501.08523	2289.302106	40595.62 μM^{-1}	0.169178503 μM
2	92586.98528			or
3	91854.07632			169.178503 nM
4	88023.81078			
5	94085.16004			
6	93412.03142			
7	94977.22001			
8	90247.78741			

Table S4. Comparison of properties for reported and present probes working on similar sensing mechanism.

	Reported probe[S8]	Present probe **5**
Probe structure		
clogS	-7.577	-5.799
clogP	6.8517	4.8887
% of organic solvent in assays	50% DMF	10% EtOH
Pseudo first order rate constant	0.00085 s^{-1}	0.00185 s^{-1}
Response time	60 min	24 min
LOD	150 nm	169 nM
Increased fold	80	31
Incubation time for cell imaging	60 min	30 min

VIII. NMR Spectra.

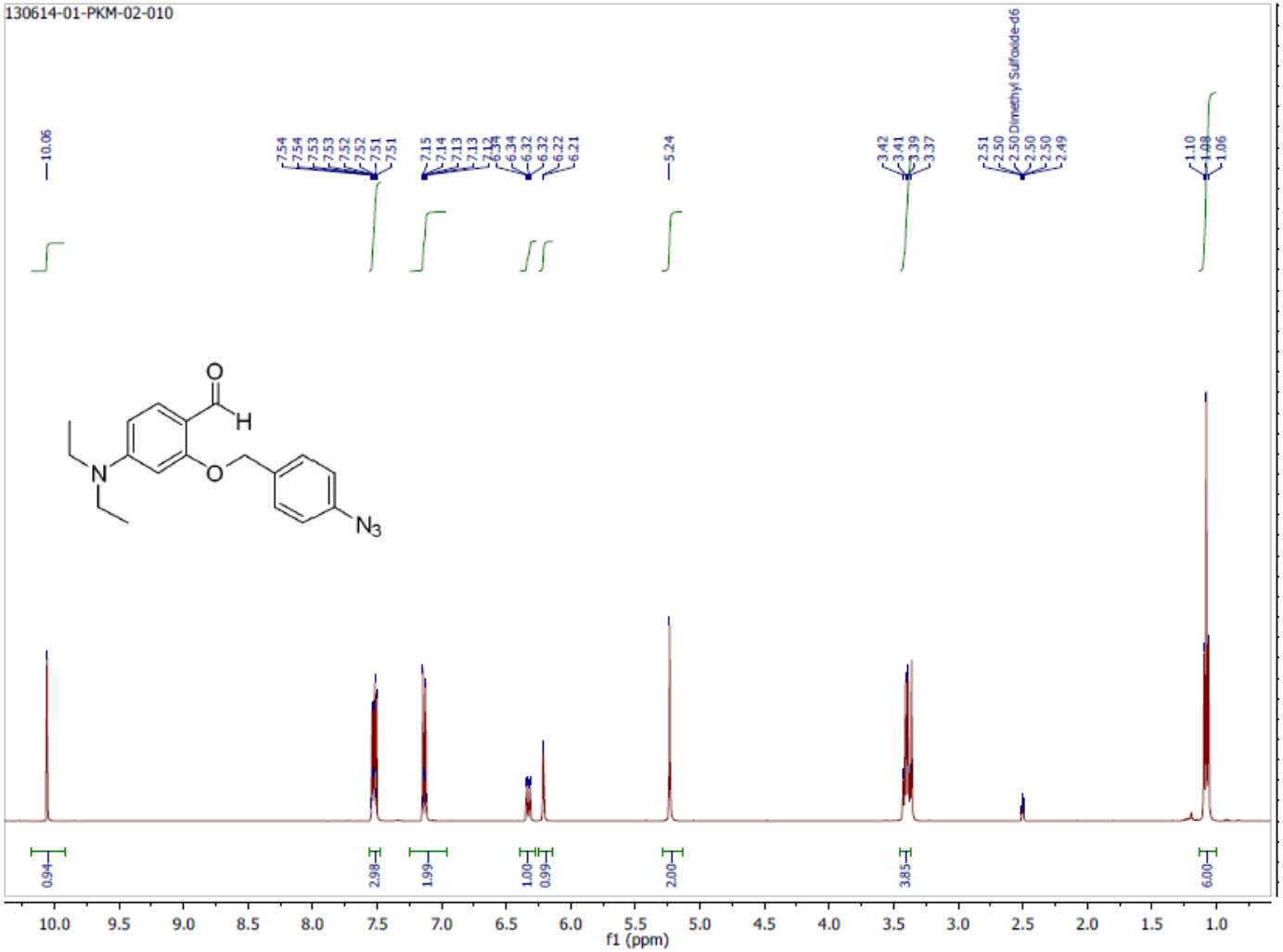


Fig. S12 400 MHz ^{1}H NMR spectrum of **11** in DMSO-d_6.

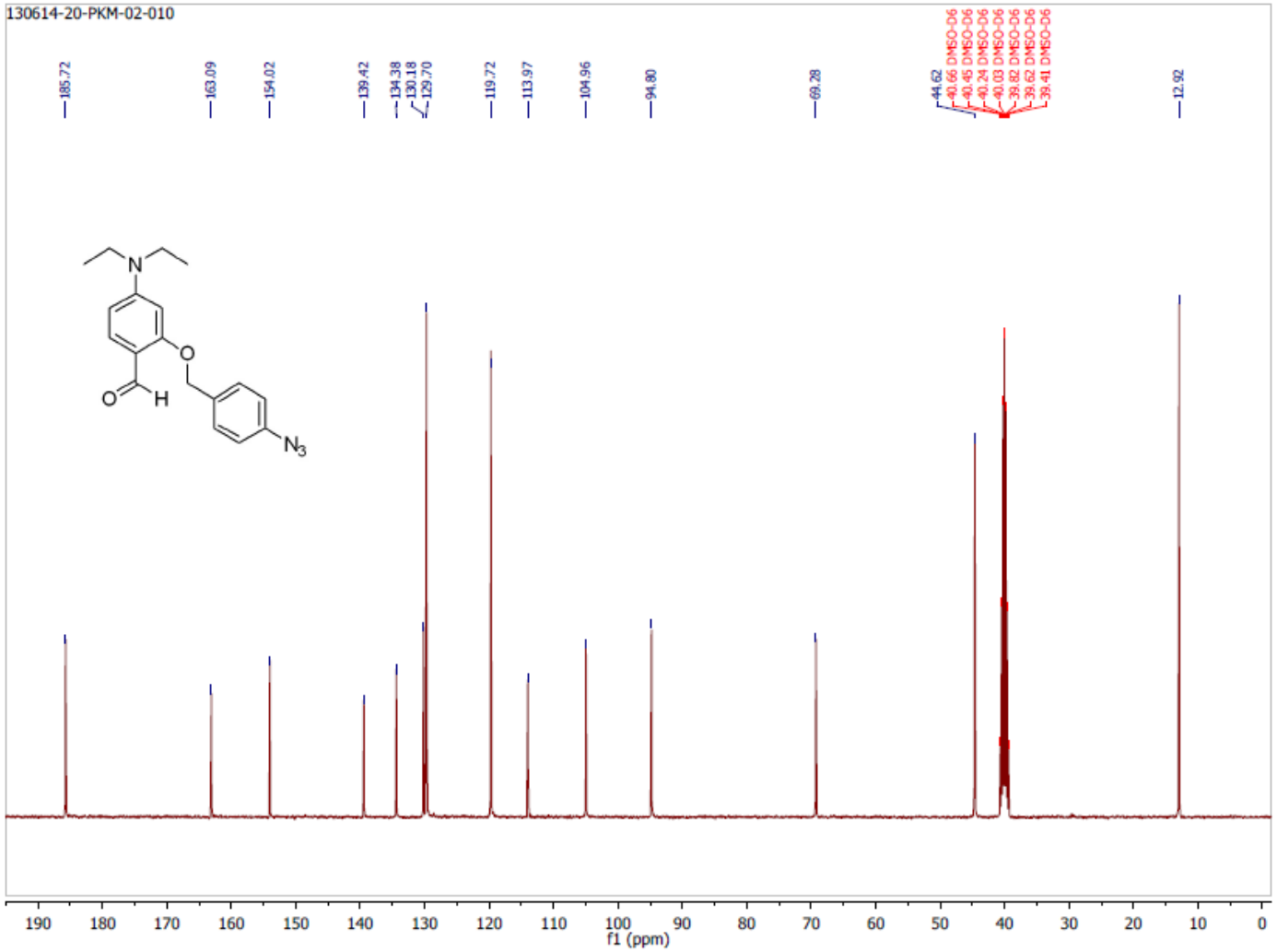


Fig. S13 100 MHz ^{13}C NMR spectrum of **11** in DMSO-d_6.

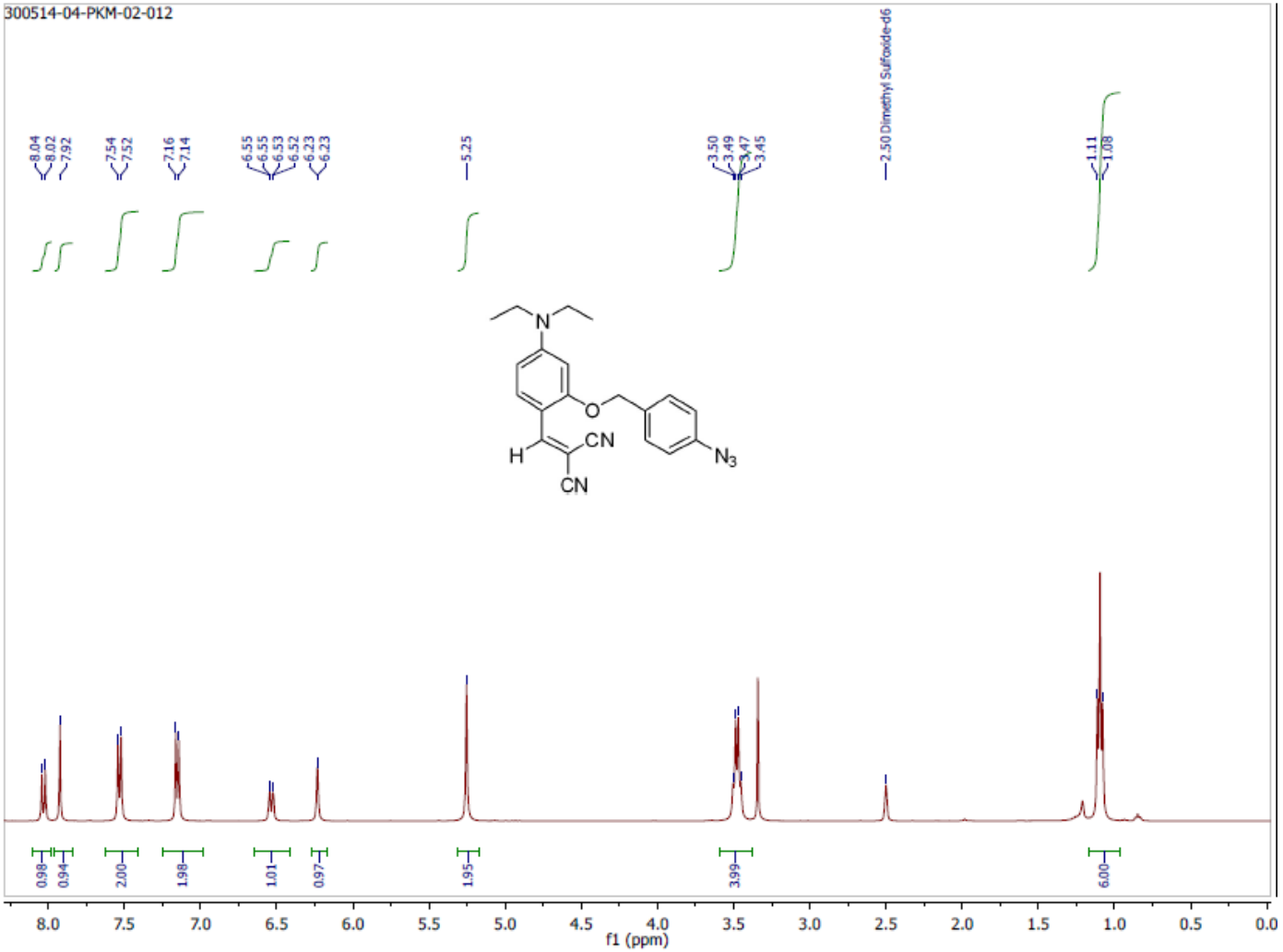


Fig. S14 400 MHz ^{1}H NMR spectrum of **5** in DMSO-d_6.

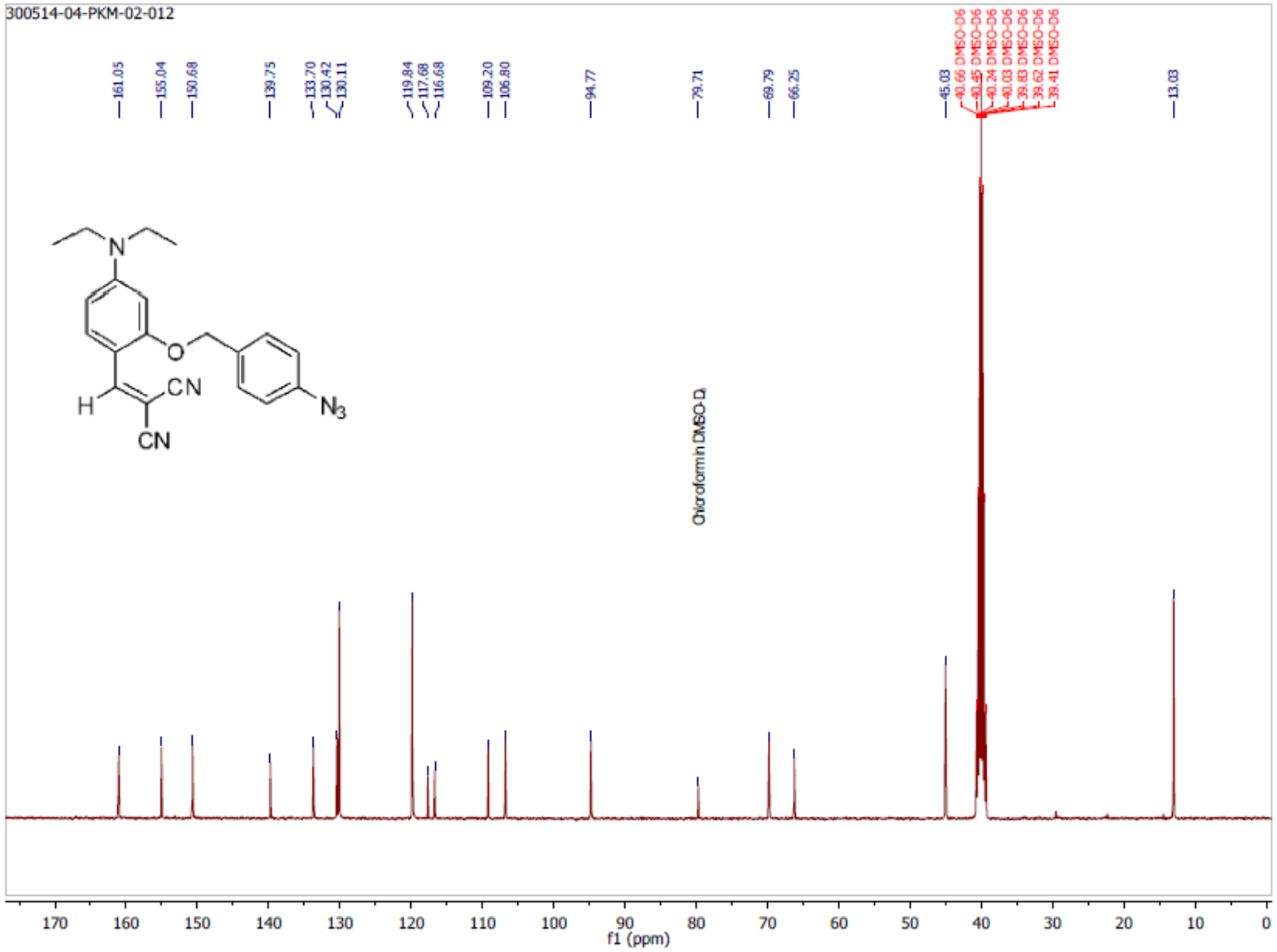


Fig. S15 100 MHz ^{13}C NMR spectrum of **5** in DMSO-d_6.

IX. References.

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