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Supporting Information

Sensitive Monitoring of NAD(P)H Levels within Cancer Cells Using Mitochondria-Targeted Near-Infrared Cyanine Dyes with Optimized Electron-Withdrawing Acceptors

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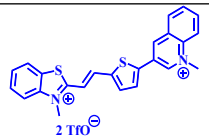
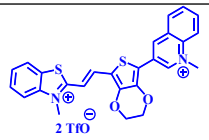
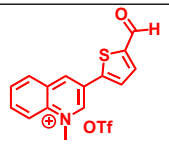
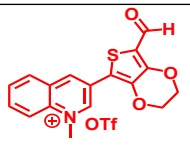
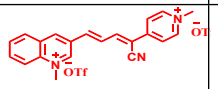
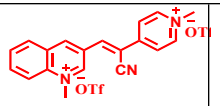
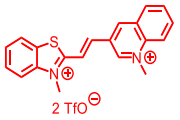
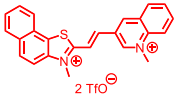
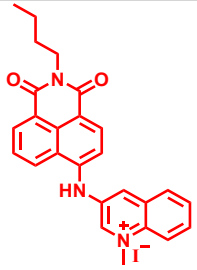
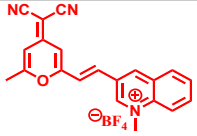
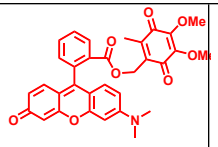
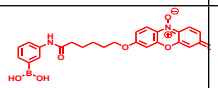
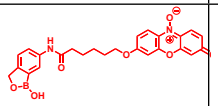
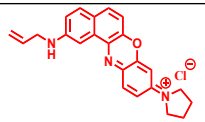
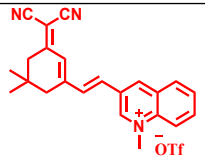
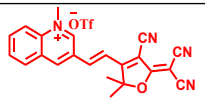
Table of Contents

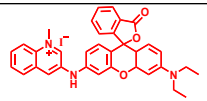
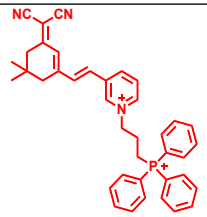
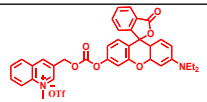
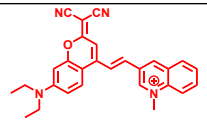
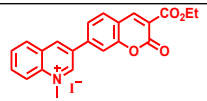
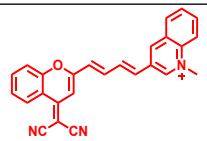
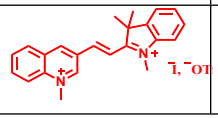
Contents

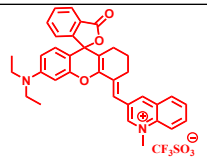
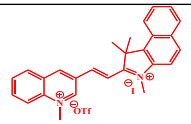
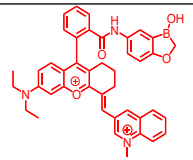
1. **Table S1. Summary of fluorescent probes for NADH detection**

S.N.	Probes	Naked-eye detection	Detection Medium	λ_{ex}/ λ_{em} (nm)	Stokes shifts (nm)	Fluorescence response	Response Time	Targeting ability	Biological Application	References
1		Yes	PBS buffer pH 7.4 37 °C	630/748	117	Turn ON	50 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	This Work
2		Yes	PBS buffer pH 7.4 37 °C	630/730	49	Turn ON	120 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	This Work
3		Yes	PBS buffer pH 7.4 37 °C	475/619	144	Turn ON	150 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	1
4		Yes	PBS buffer pH 7.4 37 °C	486/576	90	Turn ON	150 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	1
5		Yes	PBS buffer pH 7.4 37 °C	610/657	47	Turn ON	25 min	Mitochondria	Cells Mice	2

6		Yes	PBS buffer pH 7.4 37 °C	515/565	50	Turn ON	3 min	-	-	2
7		Yes	PBS buffer pH 7.4 37 °C	533/572	39	Turn ON	6 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	3
8		Yes	PBS buffer pH 7.4 37 °C	535/586	51	Turn ON	6 min	Mitochondria	Cells Drosophila melanogaster (Fruit flies)	3
9		No	PBS buffer pH 7.4 37 °C	390/460 450/550	70 100	Turn ON	50 min	Cytoplasm	Cells Tumor spheroids	4
10		Yes	PBS–EtOH (PBS, 10 mM, pH 7.4, 1: 1, v/v)	510(528)/624	96	Turn ON	60 min	-	Cells	5

11		No	PBS buffer pH 7.4	488/520	32	Turn OFF	10 min	-	Cells	6
12		No	PBS buffer pH 9.5, 37 °C	480/575	95	Turn ON	25 min	Cytoplasm	Cells	7
13		No	PBS buffer pH 7.4 37 °C	480/575	95	Turn ON	25 min	Cytoplasm	Cells	7
14		No	EtOH/ 0.1 mM PBS buffer solution (10/90, pH 7.4) 37 °C	560/650	90	Turn OFF	10 min	-	Cells	8
15		No	PBS buffer pH 7.4 37 °C	568/660	92	Turn ON	15 min	Mitochondria	Cells Mice	9
16		No	PBS buffer pH 7.4 37 °C	582/610	28	Turn ON	25 min	-	Cells Mice	10

17		No	PBS buffer pH 7.4 37 °C	510/548	38	Turn ON	4 h	Cytoplasm	Cells	11
18		Yes	25 mM PIPES, pH 7.4 37 °C	570/615	45	Turn ON	23 h	Mitochondria	Cells	12
19		Yes	PBS buffer pH 7.4 37 °C	510/552	42	Turn ON	40 min	Cytoplasm	Cells	13
20		No	PBS buffer pH 7.4 37 °C	590/640	50	Turn ON	80 min	-	Cells Tumor spheroids	14
21		Yes	PBS buffer pH 7.4 37 °C	390/460	70	Turn ON	40 min	Mitochondria	Cells Tumor tissues	15
22		No	1:1 mixed DMSO and PBS solution	595/670 400/550	75 150	Turn ON	20 min	Cytoplasm	Cells	16
23		Yes	25 mM PIPES, 101 mM	537/561	24	Turn ON	5 min	Cytoplasm	Cells Tumor spheroid	17

			NaCl pH 7.0 25 °C						Model	
24		No	PBS buffer pH 7.4 (5% DMSO)	670/740	70	Turn ON	120 min	Mitochondria	Cells	18
25		Yes	PBS buffer (10 mM)	553/584	31	Turn ON	3 min	Mitochondria	Influenza virus infected cells (MDCK and BHK-21)	19
26		NO	PBS buffer, pH 7.4	725/745	20	Turn ON	75 min	Mitochondria	Cells (NCM-460 and HCT-116b) Mice	20

2. Experimental Section

2.1 Instrumentation.

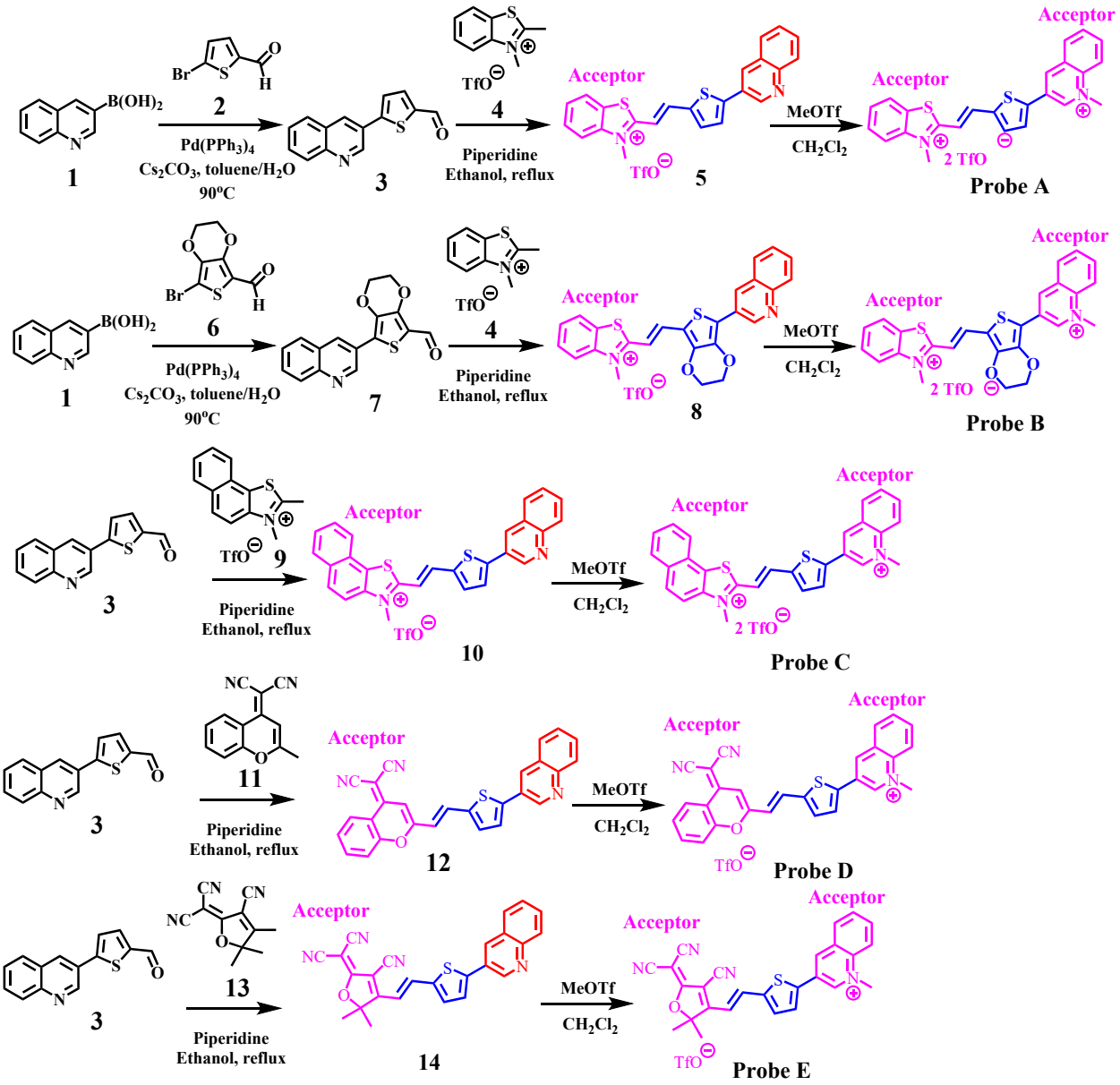
The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the fluorescent probes were acquired using a Bruker NMR Spectrophotometer at 500 MHz (Ascend 500) in $CDCl_3$ or DMSO-d_6 solution with concentration around 2.0×10^{-2} M. The absorption spectra of the probes were measured using a PerkinElmer Lambda 35 UV/vis spectrometer, while the fluorescence spectra were recorded using a Jobin Yvon Fluoromax-4 spectrofluorometer.

2.2. Reagents

All reagents including metal ions, biothiols, other chemicals and cyanine dye (IR-780) were obtained from commercial suppliers and utilized without additional purification. Intermediates 3 and 7 were synthesized and characterized according to our reported procedure.[1]

2.3. Synthesis of fluorescent probes.

The fluorescent probes were published according to Scheme 1



Scheme 1. *Synthetic approach to prepare fluorescent probes.*

2.3.1 Synthesis of compound **5**.

Compound **3**[1] (100.0 mg, 0.41 mmol) and compound **4** (130.89 mg, 0.41 mmol) were dissolved in 10 mL of ethanol, followed by the addition of a catalytic amount of piperidine (10 mol %). The resulting mixture was refluxed for 8 hours under a nitrogen atmosphere. Upon completion of the reaction (monitored by TLC), the reaction mixture was cooled down, and the solvent was evaporated under reduced pressure. Subsequently, 20 mL of cold water was added, and the mixture was subjected to extraction with dichloromethane, repeating the process 10 times. The combined organic layer was then dried over sodium sulfate. After evaporating the solvent, a crude product was obtained. To purify the crude product, column chromatography was performed using a mixture of dichloromethane and methanol as the eluent (9.5:0.5 ratio). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 4.34 (3H, s), 7.68 (1H, t, J = 7.5 Hz), 7.75 (3H, m), 7.87 (1H,

t, *J* = 7.5 Hz), 8.06 (3H, m), 8.09 (1H, d, *J* = 5.0 Hz), 8.24 (1H, d, *J* = 10.0 Hz), 8.42 (1H, d, *J* = 10.0 Hz), 8.48 (1H, d, *J* = 15.0 Hz), 8.76 (1H, s), 9.36 (1H, s).

2.3.2. Synthesis of probe **A**.

Compound **5** (100.0 mg, 0.19 mmol) was dissolved in 10 mL of dichloromethane, and methyl trifluorosulfonate (1.1 equivalents) was added at room temperature. The resulting mixture was stirred under a nitrogen atmosphere for 6 hours at room temperature. A red-colored precipitate formed, which was subsequently filtered, washed with dichloromethane, and air-dried. ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 4.37 (3H, s), 4.72 (3H, s), 7.81 (3H, m), 8.10 (3H, m), 8.28 (2H, m), 8.46 (1H, d, *J* = 5.0 Hz), 8.51 (3H, m), 9.56 (1H, s), 10.08 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6,) δ (ppm): 36.90, 46.10, 114.15, 117.43, 119.77, 119.92, 122.48, 124.86, 127.46, 128.55, 129.07, 129.66, 129.87, 130.07, 131.18, 135.84, 137.87, 140.18, 141.63, 141.79, 142.57, 143.10, 148.91, 171.43.

2.3.3. Synthesis of compound **8**:

To initiate the reaction, compound **7**[1] (100.0 mg, 0.33 mmol) and compound **4** (105.37 mg, 0.33 mmol) were dissolved in 10 mL of ethanol, followed by the addition of a catalytic amount of piperidine (10 mol %). The resulting mixture was subjected to reflux for 8 hours under a nitrogen atmosphere. Once the reaction was deemed complete (monitored by TLC), the reaction mixture was allowed to cool down, and the solvent was subsequently evaporated under reduced pressure. Next, 20 mL of cold water was introduced, and the resulting solution underwent extraction with dichloromethane, repeating the process 10 times. The combined organic layer was then dried using sodium sulfate, and the solvent was evaporated, resulting in a crude product. To purify the crude product, column chromatography was performed utilizing a mixture of dichloromethane and methanol as the eluent (in a 9.5:0.5 ratio). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 4.27 (3H, s), 4.58 (2H, t, *J* = 5.0 Hz), 4.63 (2H, t, *J* = 5.0 Hz), 7.53 (1H, d, *J* = 15.0 Hz), 7.67 (1H, t, *J* = 7.5 Hz), 7.75 (1H, t, *J* = 5.0 Hz), 7.80 (1H, m), 7.85 (1H, t, *J* = 5.0 Hz), 8.05 (1H, d, *J* = 5.0 Hz), 8.09 (1H, d, *J* = 5.0 Hz), 8.12 (1H, d, *J* = 15 Hz), 8.21 (1H, d, *J* = 5.0 Hz), 8.36 (1H, d, *J* = 5.0 Hz), 8.72 (1H, s), 9.28 (1H , s).

2.3.4. Synthesis of probe **B**:

In a reaction vessel, compound **8** (100.0 mg, 0.17 mmol) was dissolved in 10 mL of dichloromethane, followed by the addition of methyl trifluorosulfonate (1.1 equivalents) at room temperature. The resulting mixture was stirred under a nitrogen atmosphere for 6 hours at room temperature, resulting in the formation of a precipitate with a red color. The precipitate was isolated by filtration, thoroughly washed with dichloromethane, and subsequently dried in ambient air. ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 4.29 (3H, s), 4.62 (2H, t, *J* = 2.5 Hz), 4.66 (2H, t, *J* = 2.5 Hz), 4.74 (3H, s), 7.62 (1H, d, *J* = 10.0 Hz), 7.78 (1H, t, *J* = 7.5 Hz), 7.87 (1H, t, *J* = 7.5 Hz), 8.08 (1H, t, *J* = 7.5 Hz), 8.20 (1H, d, *J* = 15.0 Hz), 8.26 (2H, m), 8.41 (1H, d, *J* = 10.0 Hz), 8.53 (2H, m), 9.47 (1H, s), 9.81 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6,) δ (ppm): 36.54, 46.31, 65.77, 66.17, 111.75, 115.37, 116.81, 117.17, 119.72, 119.85, 122.41, 124.63, 124.97, 126.24, 128.19, 128.76, 129.57, 129.90, 131.07, 136.01, 136.68, 137.55, 141.46, 142.46, 146.55, 148.33, 171.32.

2.3.5. Synthesis of compound 10: In a 50 mL round-bottom flask, compound 3 (100.0 mg, 0.41 mmol) and Compound 9 (148.83 mg, 0.41 mmol) were combined with 10 mL of ethanol. A catalytic amount (10 mol %) of piperidine was introduced to the reaction mixture, which was subsequently refluxed for 8 hours under a nitrogen atmosphere. The progression of the reaction was tracked through TLC, and upon its completion, the reaction mixture was allowed to cool. A red-colored precipitate formed, which was then

separated by filtration and washed thrice with cold ethanol (5 mL each time). The resulting product was utilized in the subsequent step without the need for additional purification.

2.3.6. Synthesis of probe **C:** A 10 mL portion of dry dichloromethane was employed to dissolve compound **10** (100.0 mg, 0.17 mmol). Subsequently, methyl trifluoromethanesulfonate (1.1 equivalents) was introduced to the solution at room temperature under a nitrogen atmosphere, and the resulting mixture was stirred for 6 hours at room temperature. A dark-colored precipitate materialized, which was subsequently separated by filtration, washed with cold dichloromethane, and allowed to air-dry. The resulting compound was characterized by ^{1}H NMR (500 MHz, DMSO-d_6) as follows: δ (ppm): 4.74 (3H, s), 4.82 (3H, s), 7.89 (2H, m), 8.0 (1H, d, J = 15.0 Hz), 8.13 (1H, m), 8.28 (2H, d, J = 10.0 Hz), 8.32 (3H, m), 8.46 (4H, m), 9.01 (1H, d, J = 10.0 Hz), 9.56 (1H, s), 10.14 (1H, s).

2.3.7. Synthesis of compound **12**: Synthesis of compound **12** was carried out to combine Compound 3 and compound 11 together. First, a round-bottom flask was obtained, and ethanol was added to make a 10 milliliter solution. Next, 100.0 milligrams of Compound 31 and 85.30 milligrams of Compound 11 were carefully measured out and added to the flask, giving a 0.41 molar concentration of each compound in the ethanol solution. After the compounds dissolved, a catalyst called piperidine was added in a 10 mol% amount relative to the reactants. The flask was then connected to a condenser and heated to reflux temperature while nitrogen gas gently bubbled through the solution. The reaction mixture was left to reflux for 8 hours to allow the reaction to reach completion. When finished, the flask was disconnected from the heat and allowed to slowly return to room temperature. During cooling, a dark precipitate formed from the reaction mixture. This solid was collected by vacuum filtration and washed 3 times with 5 milliliters of cold ethanol. Finally, the precipitate was isolated and used in subsequent experiments without any further processing.

2.3.8. Synthesis of probe **D**: A quantity of 100.0 mg (0.23 mmol) of Compound **12** was dissolved in 10 mL of dry dichloromethane in a reaction vessel under a nitrogen atmosphere. Methyl trifluoromethanesulfonate (1.1 equivalents relative to compound **12**) was then added slowly at room temperature, and the reaction mixture was stirred for 6 hours. Over this time, a dark colored precipitate formed. The solid was collected by vacuum filtration, washed with cold dichloromethane, and allowed to dry under ambient conditions. The isolated product was characterized by ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.69 (3H, s), 7.07 (1H, s), 7.28 (1H, d, J = 15.0 Hz), 7.55 (1H, t, J = 7.5 Hz), 7.74 (2H, t, J = 5.0Hz), 7.89 (3H, m), 8.04 (1H, t, J = 7.5 Hz), 8.22 (1H, t, J = 7.5 Hz), 8.44 (2H, m), 8.64 (2H, d, J = 10.0 Hz), 9.37 (1H, s), 9.97 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.98, 61.20, 107.67, 116.13, 117.30, 117.46, 119.41, 119.52, 120.32, 124.90, 126.60, 127.61, 129.43, 129.53, 130.79, 130.88, 131.08, 132.99, 135.92, 137.41, 139.36, 14058, 143.27, 148.29, 152.25, 152.86, 157.55.

2.3.9. Synthesis of compound **14**: A reaction was carried out between compound **3** (100.0 mg, 0.41 mmol) and compound **13** (81.61 mg, 0.41 mmol) in a 50 mL round bottom flask containing 10 mL of ethanol. The two compounds were dissolved completely in the ethanol solvent before adding a catalytic quantity of piperidine (10 mol% relative to the reactants) to the flask. The reaction mixture was then fitted with a condenser and refluxed at the solvent's boiling point for 8 hours under a nitrogen atmosphere to avoid side reactions. After the allotted reaction time, the flask was removed from heat and allowed to cool to room temperature. During cooling, a dark colored precipitate formed which was collected via vacuum filtration. The solid was washed three times with 5 mL portions of cold ethanol and isolated. The purified product was carried on without additional processing for use in subsequent reactions.

2.3.10. Synthesis of probe **E**: A quantity of 100.0 mg (0.23 mmol) of compound **14** was dissolved in 10 mL of anhydrous dichloromethane within a reaction vessel under a nitrogen atmosphere. Methyl trifluoromethanesulfonate (equivalent to 1.1 times the molar amount of compound **14**) was gently added at room temperature, and the reaction mixture was stirred for a duration of 6 hours. During this period, a dark-colored precipitate materialized. The precipitate was isolated via vacuum filtration, washed with cold dichloromethane, and left to dry under ambient conditions. The resulting product was subjected to analysis by ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.86 (6H, s), 4.72 (3H, s), 9.93 (1H, d, J = 20.0 Hz), 8.0 (3H, m), 8.21 (2H, m), 8.48 (2H, m), 9.56 (1H, s), 10.17 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.84, 45.93, 55.00, 99.63, 100.78, 111.17, 112.35, 113.09, 115.52, 119.66, 119.83, 122.39, 127.21, 129.59, 129.87, 131.09, 136.07, 137.36, 137.80, 139.67, 141.68, 142.69, 142.79, 148.78, 174.56, 177.12.

2.4. NMR and Mass Spectra of Fluorescent Probes.

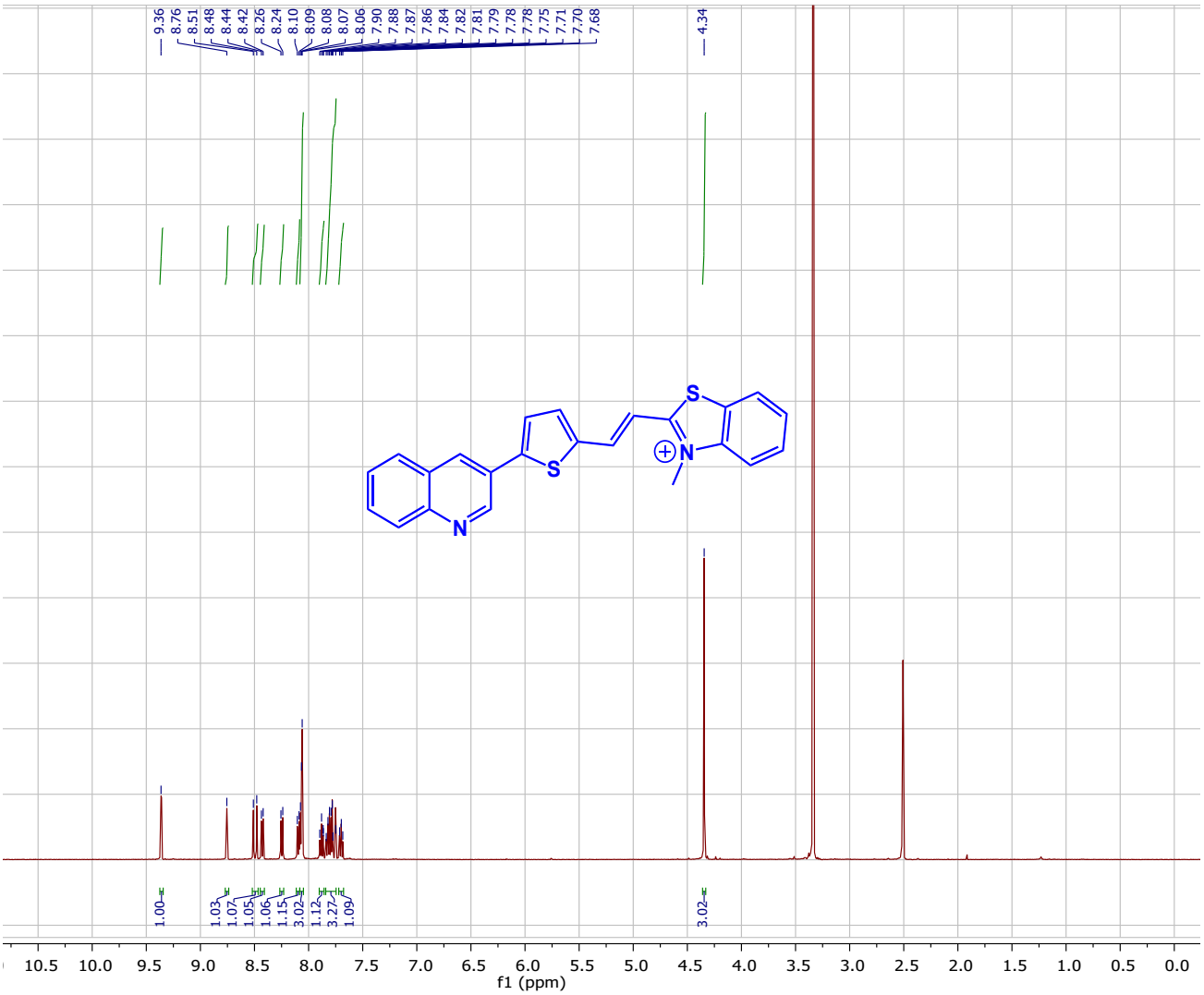


Figure S1: ^{1}H NMR spectrum of compound **5** in DMSO-d_6 solution.

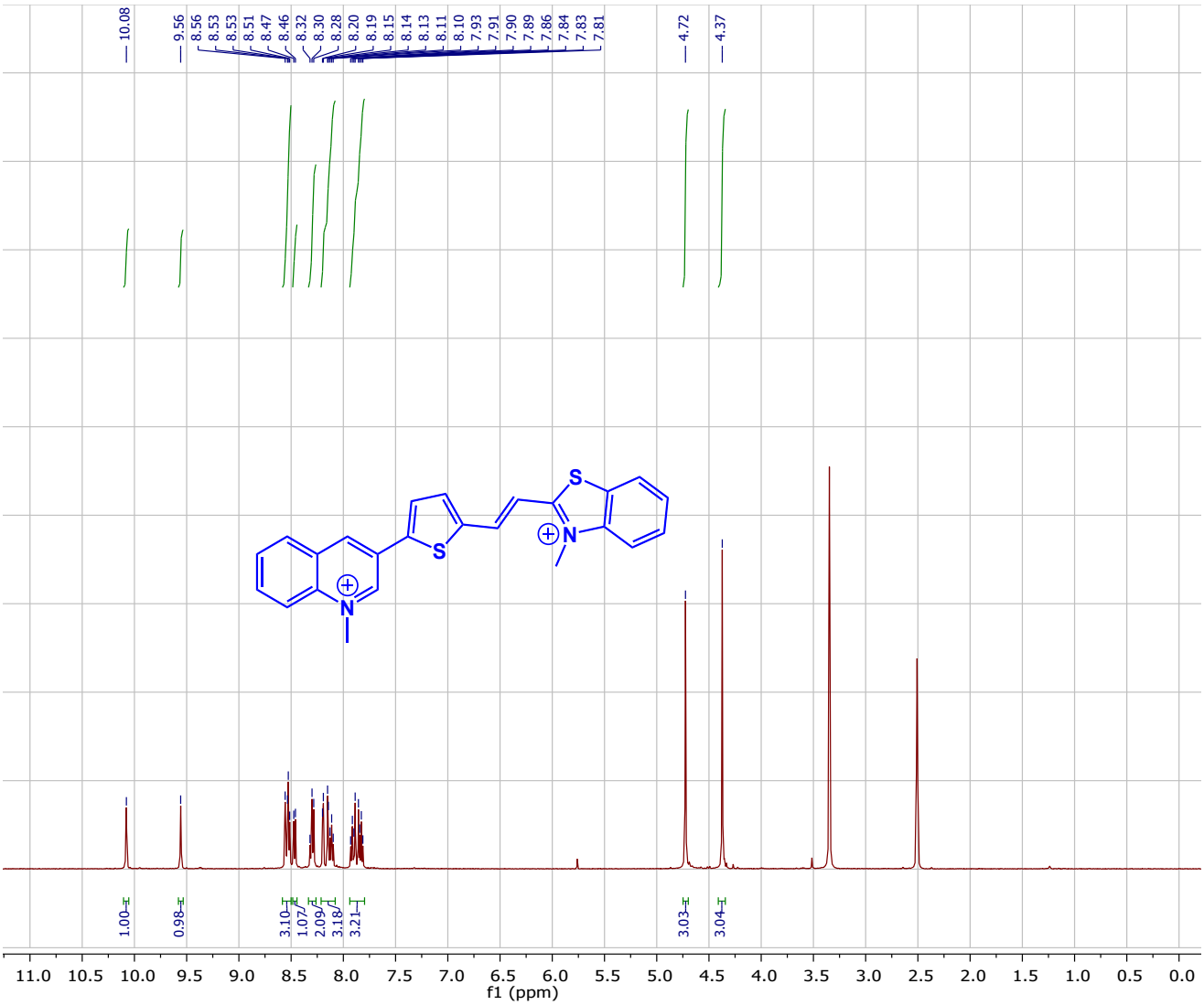


Figure S2: ^{1}H NMR spectrum of probe **A** in DMSO-d_6 solution.

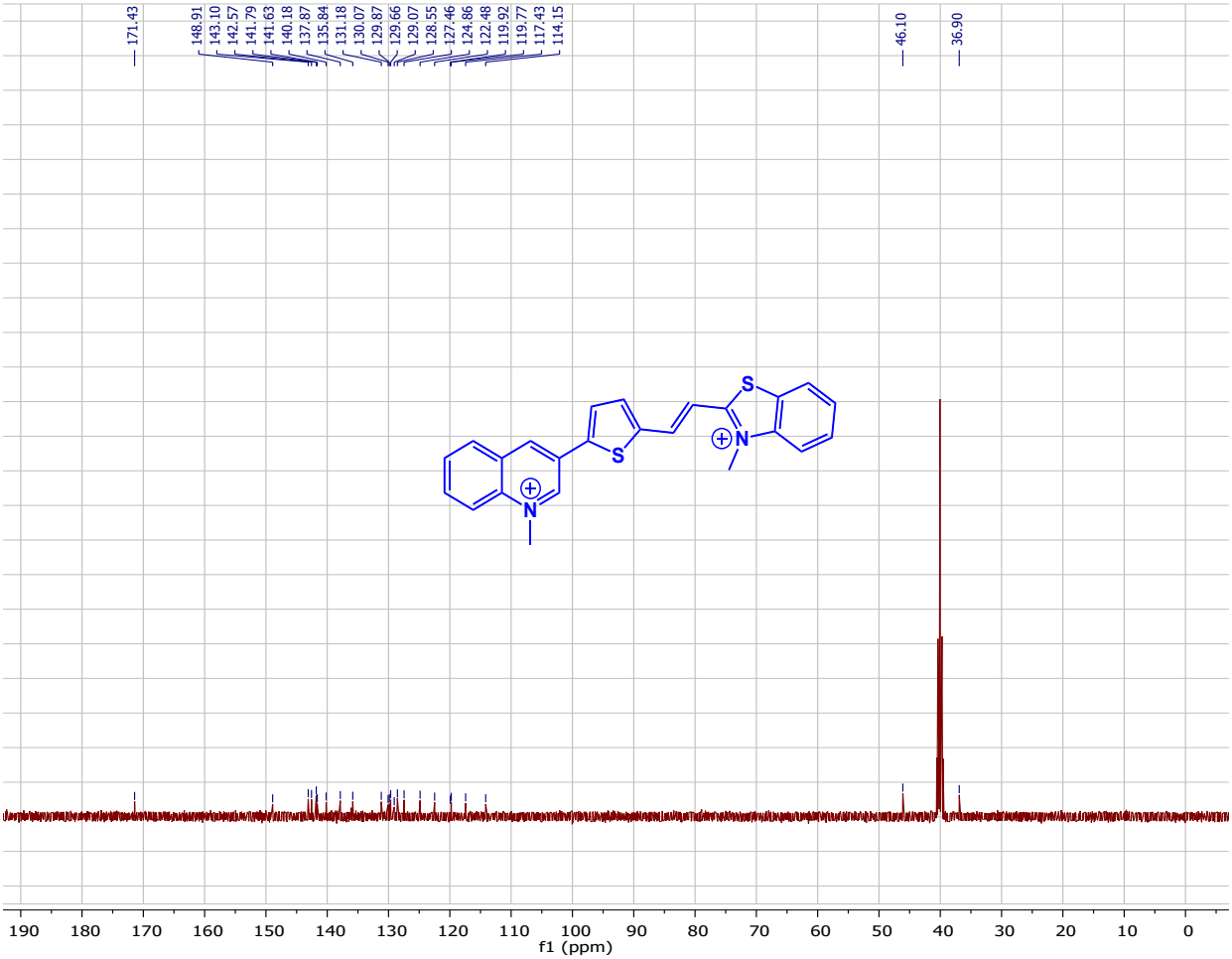


Figure S3: ^{13}C NMR spectrum of probe **A** in DMSO-d_6 solution.

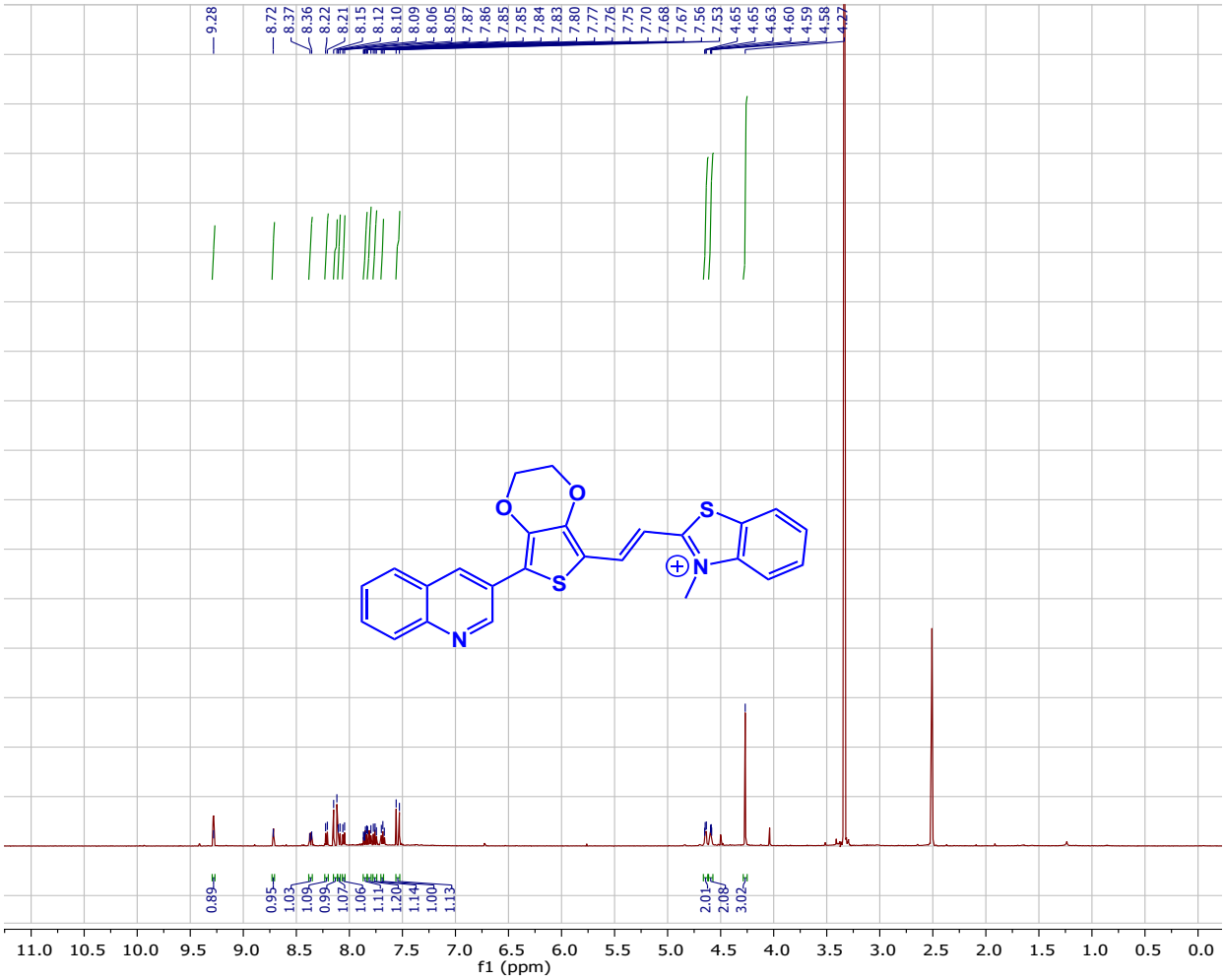


Figure S4: ^{1}H NMR spectrum of compound **8** in DMSO-d_6 solution

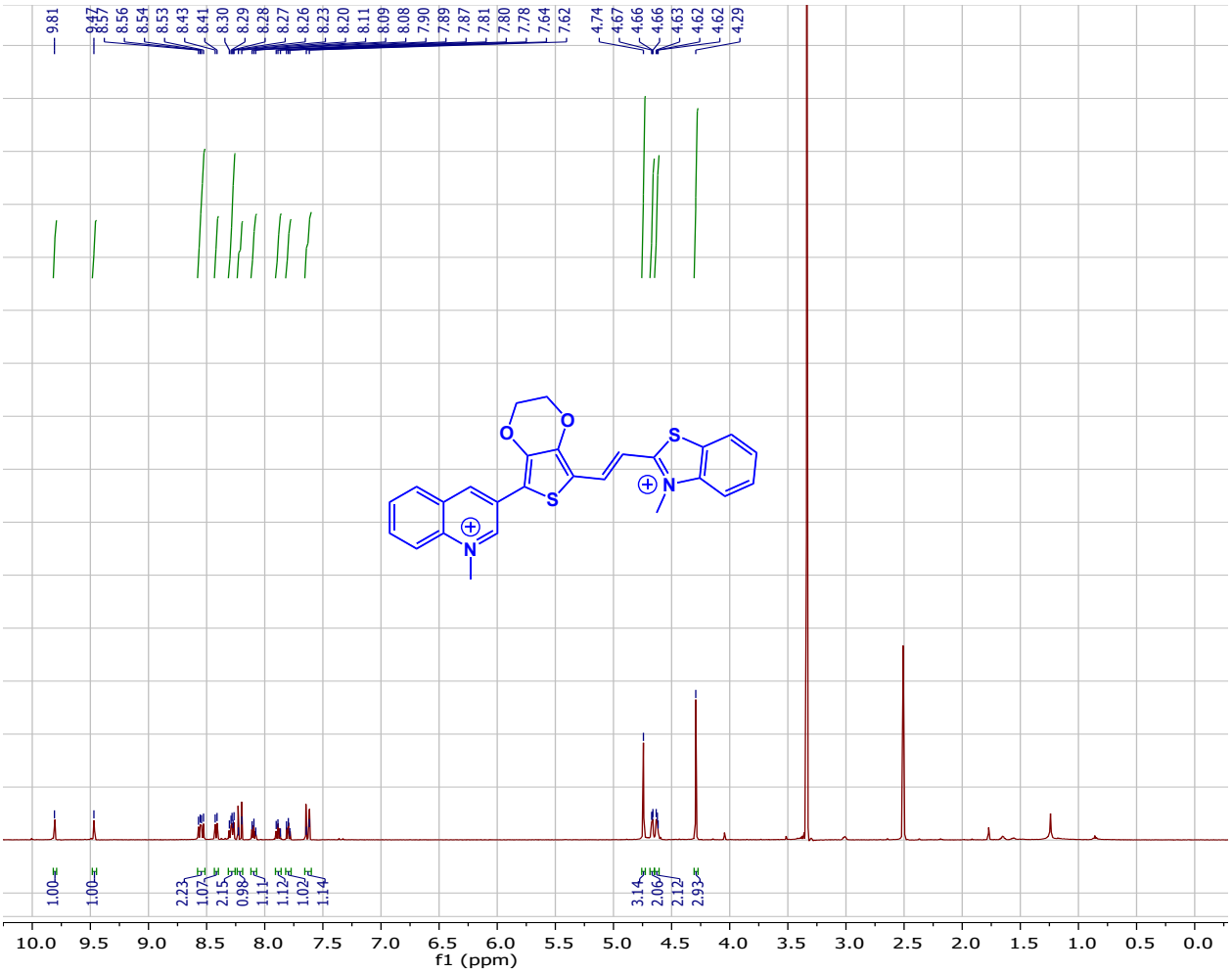


Figure S5: ^{13}C NMR spectrum of probe **B** in DMSO-d_6 solution.

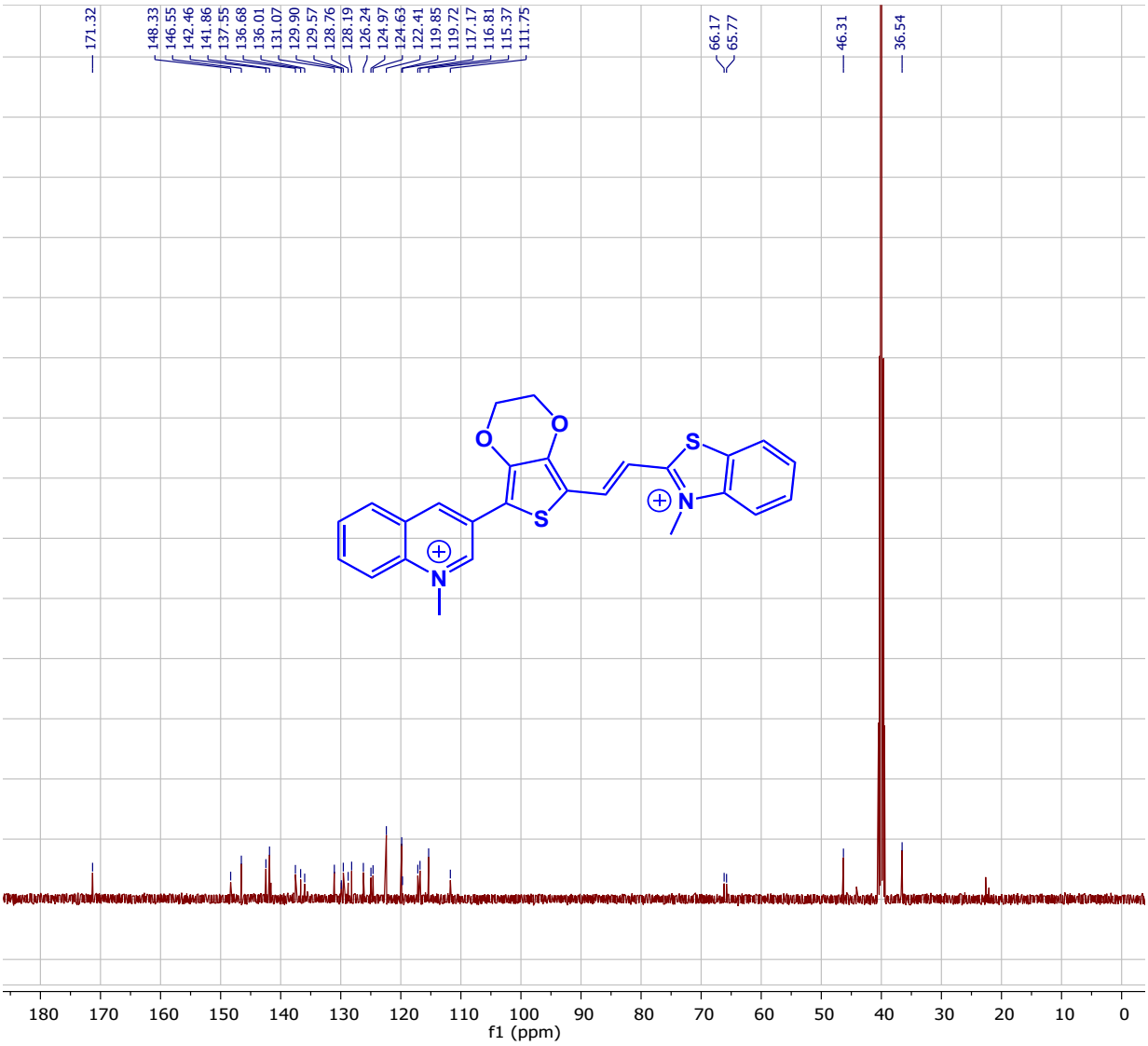
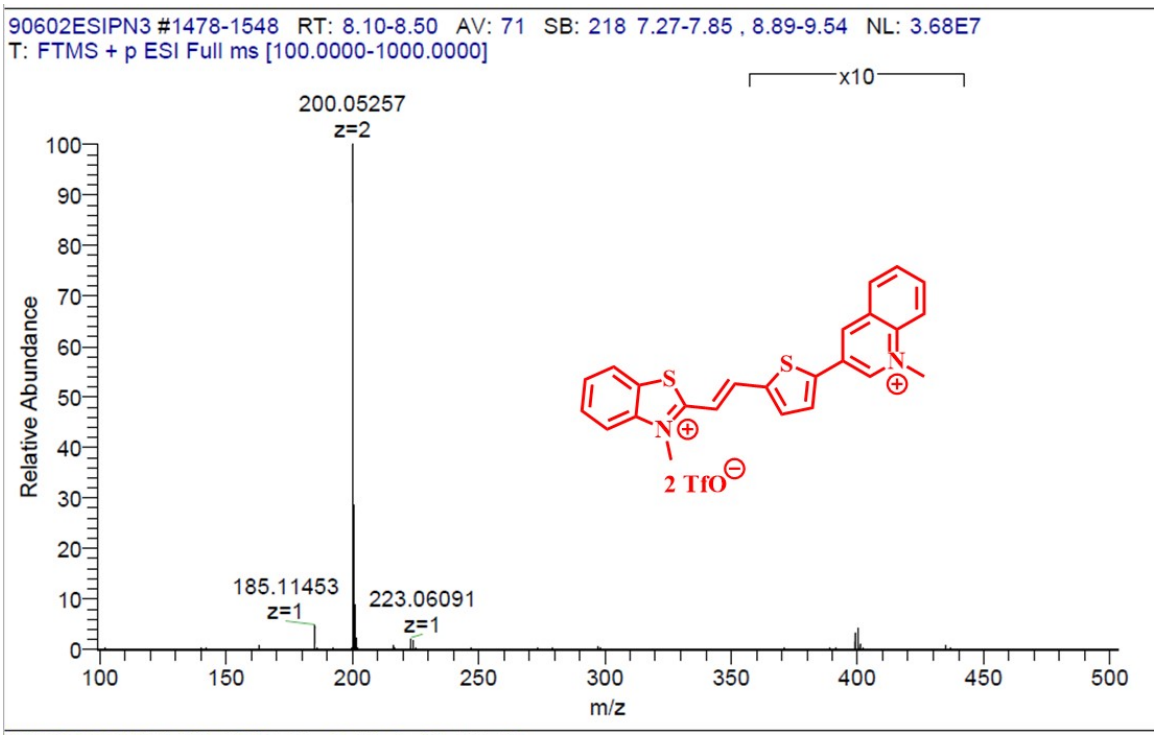


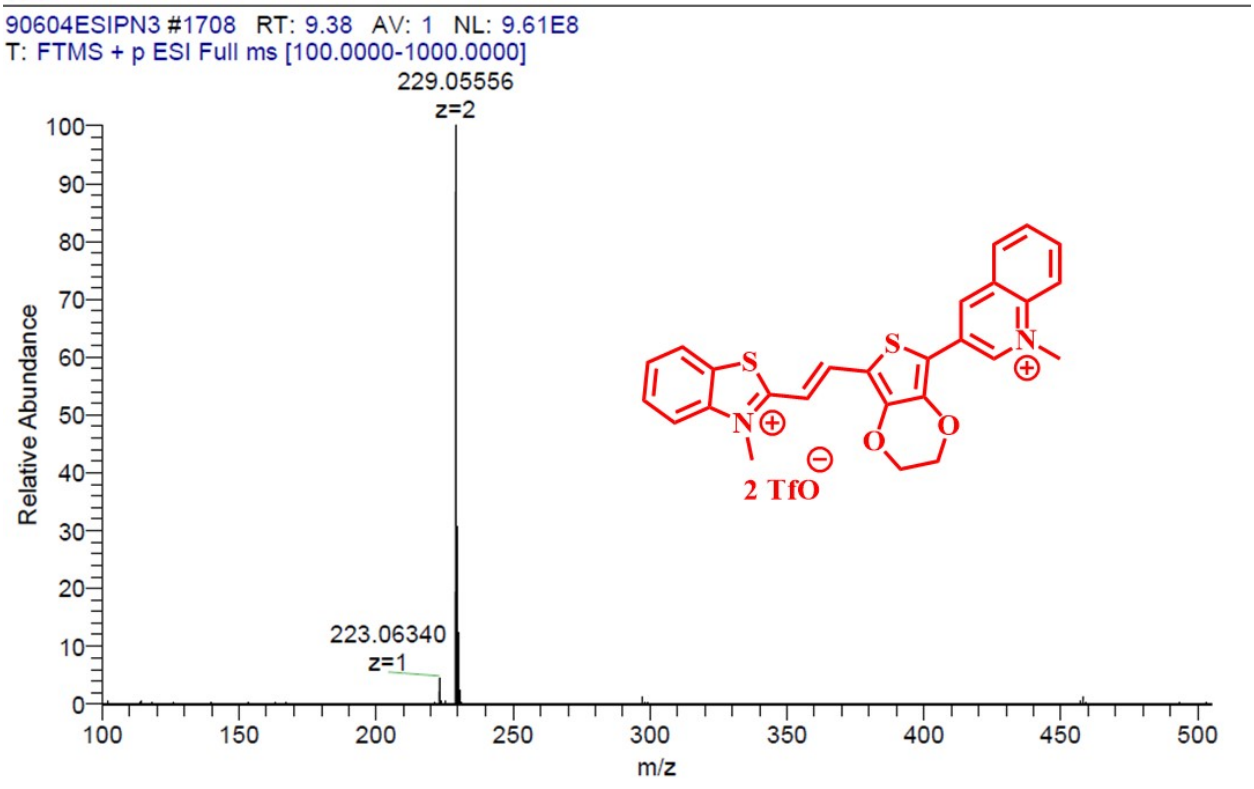
Figure S6: ^{13}C NMR spectrum of probe **B** in DMSO-d_6 solution.



90602ESIPN3#1478-1548 RT: 8.10-8.50 AV: 71
SB: 218 7.27-7.85 , 8.89-9.54
T: FTMS + p ESI Full ms [100.0000-1000.0000]
m/z= 100.00000-503.13587

m/z	Intensity	Relative	Charge	Theo. Mass	Delta (ppm)	Composition
200.05257	38098336.0	100.00	2.00	200.05285	-1.40	$C_{24}H_{20}N_2S_2$

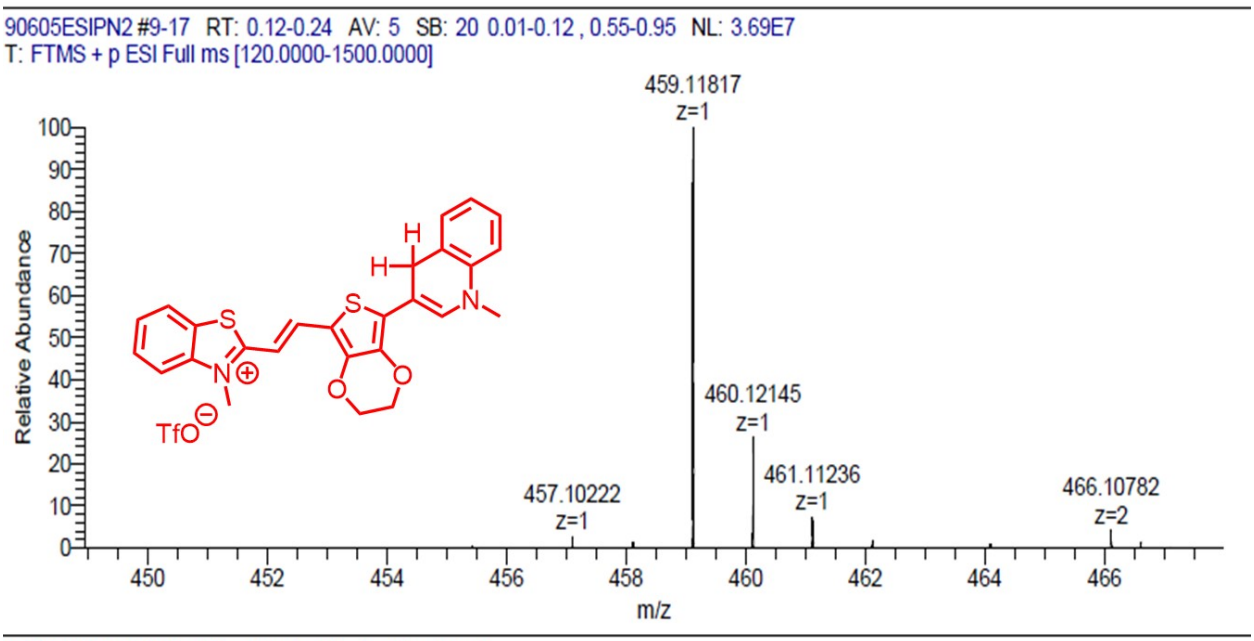
Figure S7: HRMS spectrum of probe **A**.



90604ESIPN3#1708 RT: 9.38
T: FTMS + p ESI Full ms [100.0000-1000.0000]
m/z= 100.00000-504.89345

m/z	Intensity	Relative	Charge	Theo. Mass	Delta (ppm)	Composition
229.05556	1023466752.0	100.00	2.00	229.05559	-0.13	$C_{26}H_{22}O_2N_2S_2$

Figure S8: HRMS spectrum of probe **B**.



90605ESIPN2#9-17 RT: 0.12-0.24 AV: 5
SB: 20 0.01-0.12 , 0.55-0.95
T: FTMS + p ESI Full ms [120.0000-1500.0000]
m/z= 448.94035-467.98057

m/z	Intensity	Relative	Charge	Theo. Mass	Delta (ppm)	Composition
459.11817	37055484.0	100.00	1.00	459.11955	-3.00	$C_{26}H_{23}O_2N_2S_2$

Figure S9: HRMS spectrum of probe **BH**.

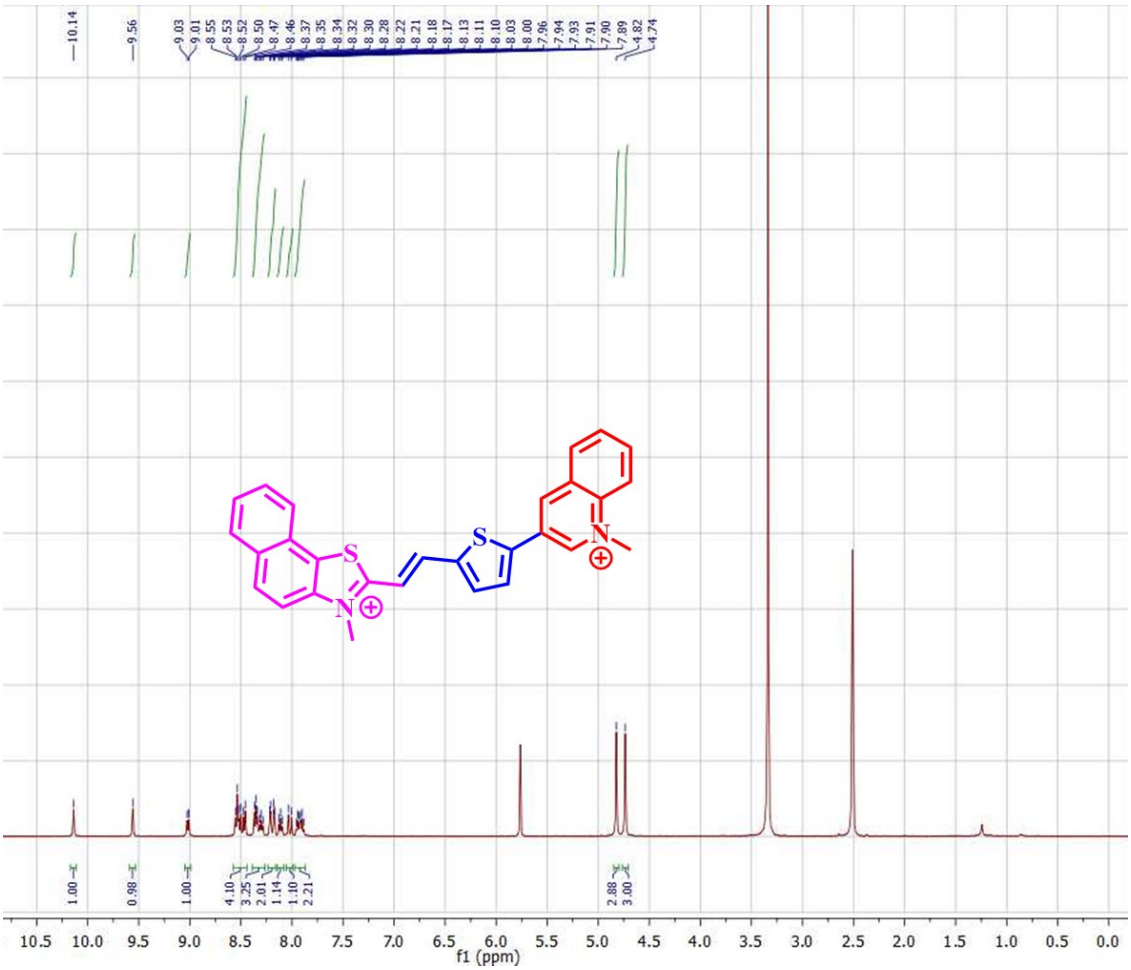


Figure S10: ^{1}H NMR spectrum of probe **C** in DMSO-d_6 solution.

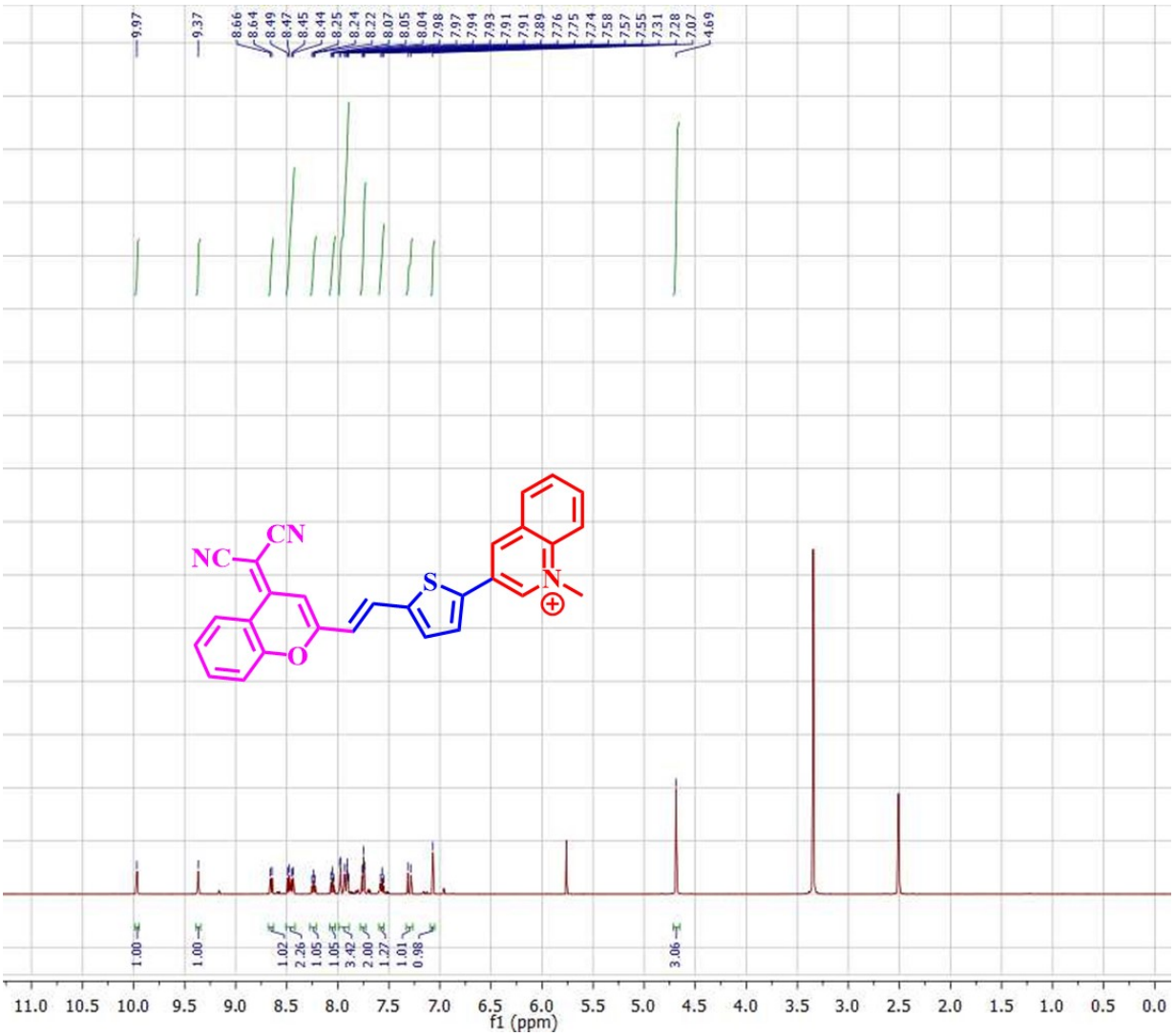


Figure S11: ^{1}H NMR spectrum of probe **D** in DMSO-d_6 solution.

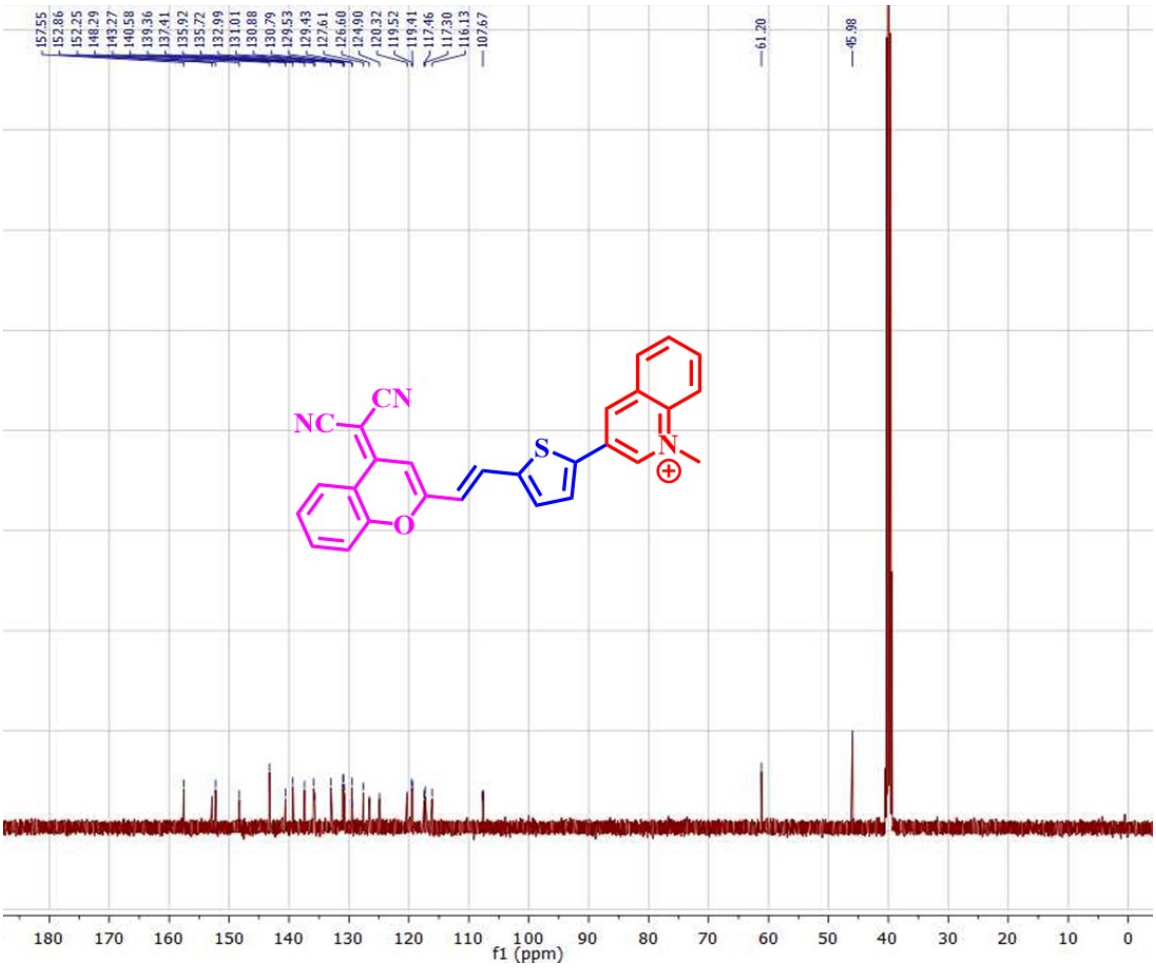


Figure S12: ^{13}C NMR spectrum of probe **D** in DMSO-d_6 solution.

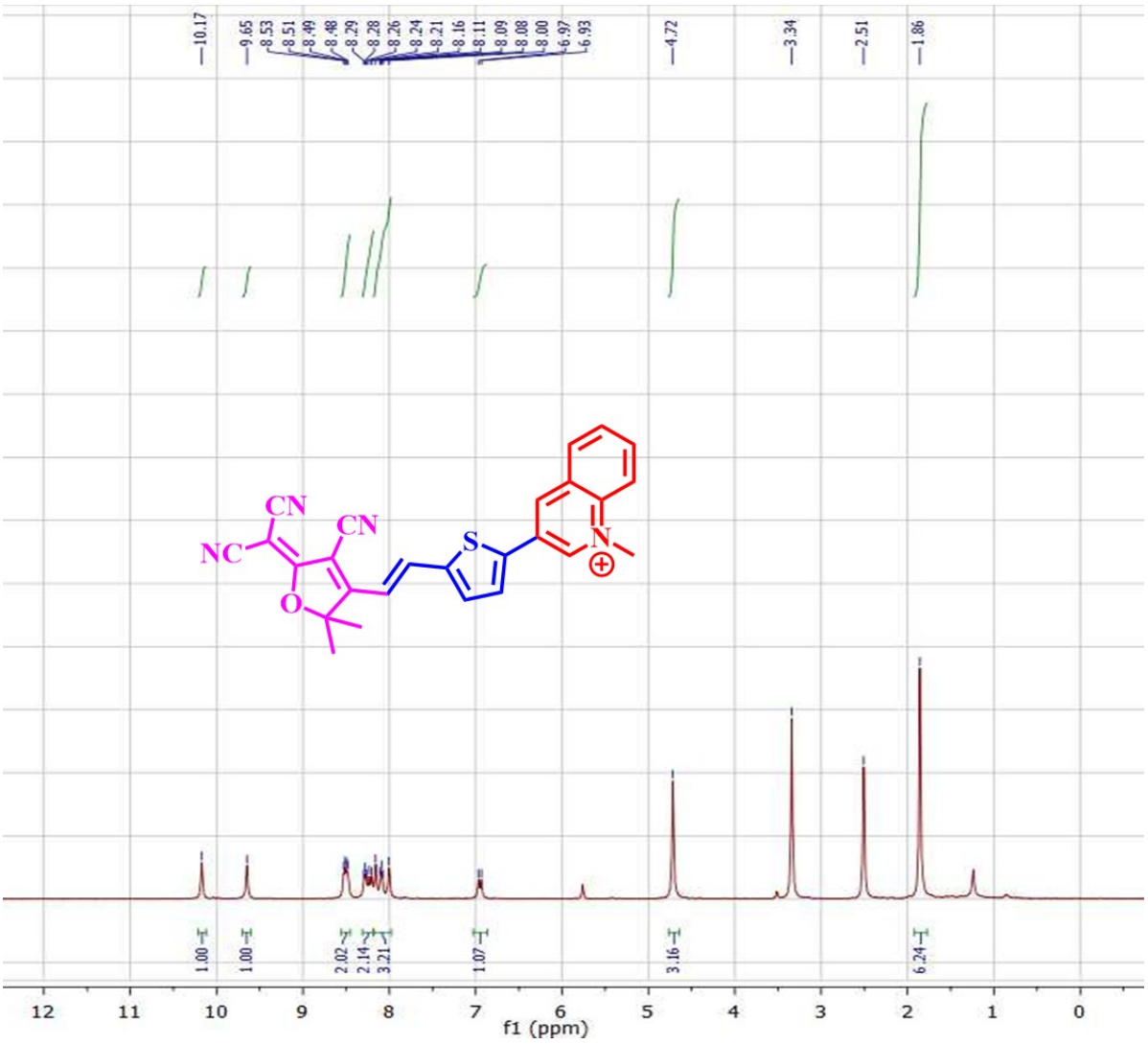


Figure S13: ^{1}H NMR spectrum of probe **E** in DMSO-d_6 solution.

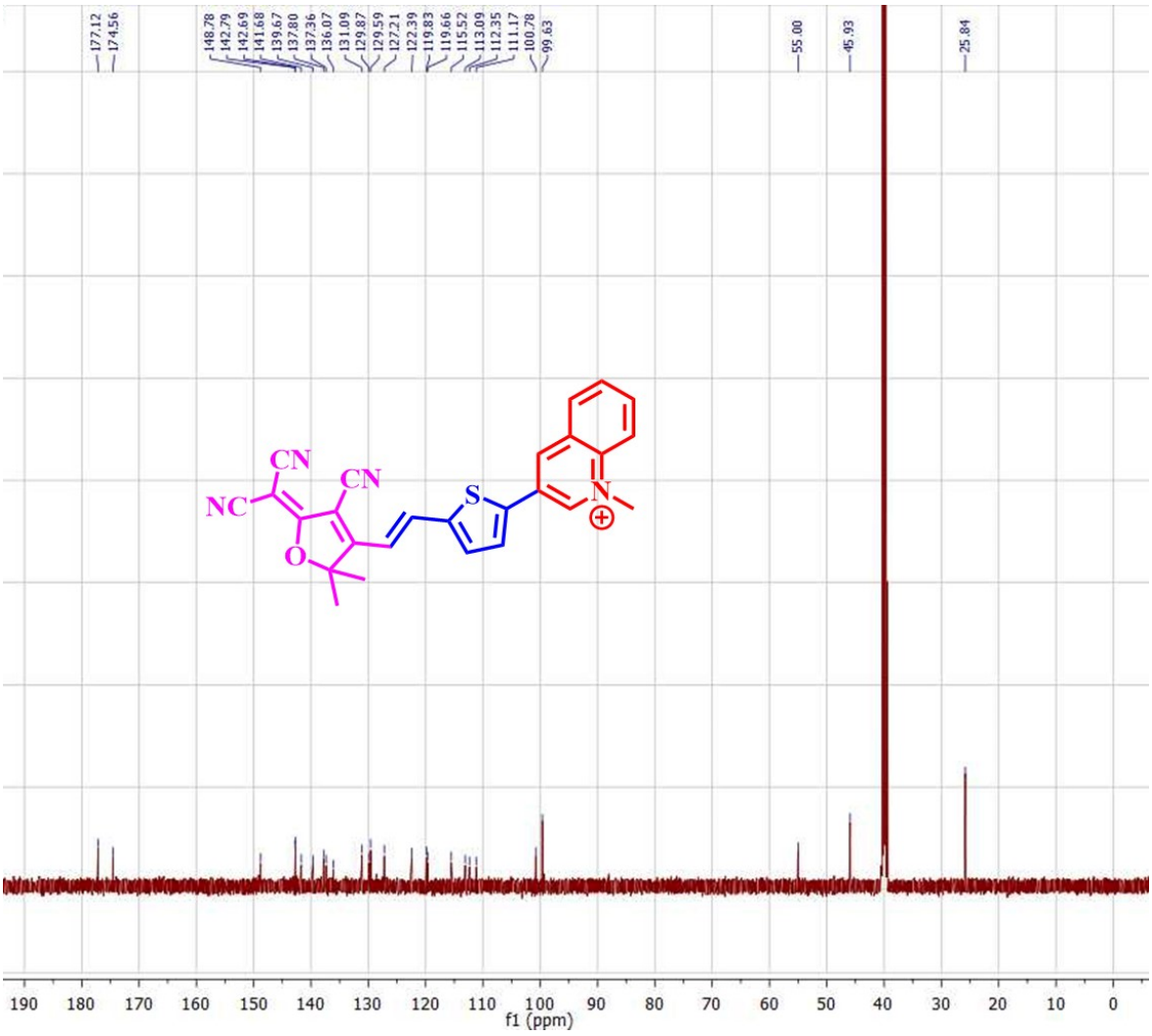
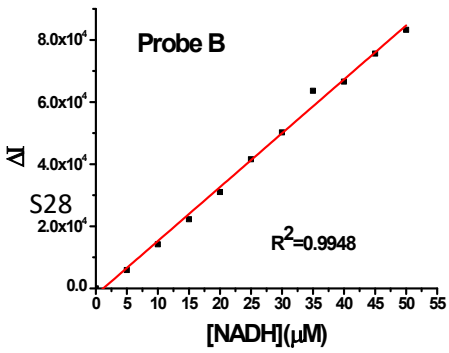
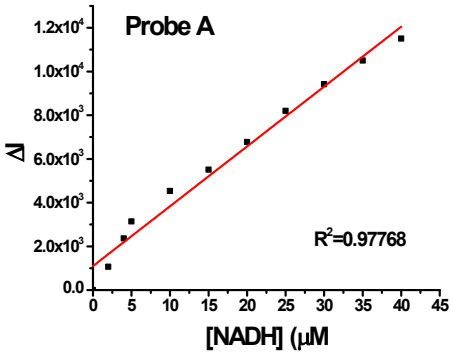


Figure S14: ^{13}C NMR spectrum of probe **E** in DMSO-d_6 solution.

2.5. Optical Properties of Fluorescent Probes.

2.5.1. The linear fluorescence responses of probes to NADH





LOD for probe A =0.151 µM **LOD for probe B = 0.073 µM**

Figure S15: Fluorescence intensity changes of probes **A** (left) and **B** (right) (10 µM) with different concentration of NADH (0-50 µM). Probes **A** and **B** possess a detection limit of 0.151 µM and 0.073 µM, respectively.

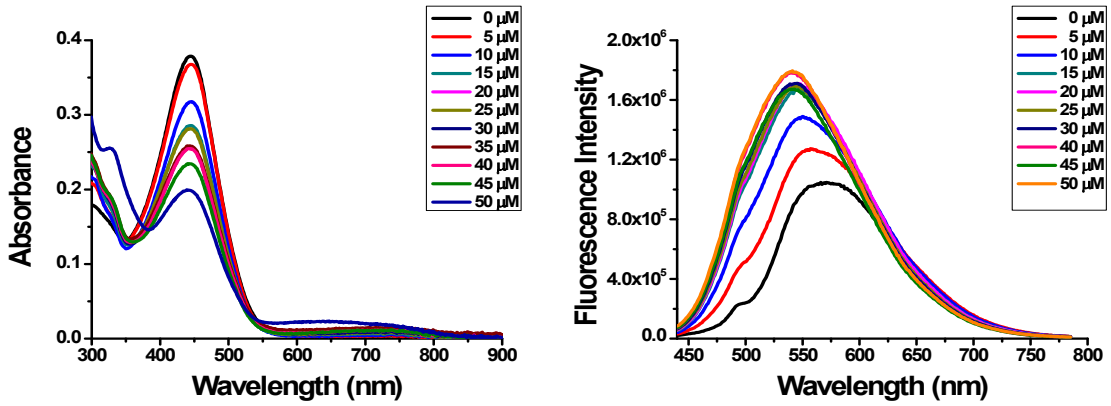


Figure S16: Absorbance and emission spectra of probe **C** (10 μM) upon exposure to varying NADH concentrations (0-50 μM) in PBS buffer (pH 7.4) with 10% DMSO during a 4-hour incubation period, observed under excitation at 420 nm.

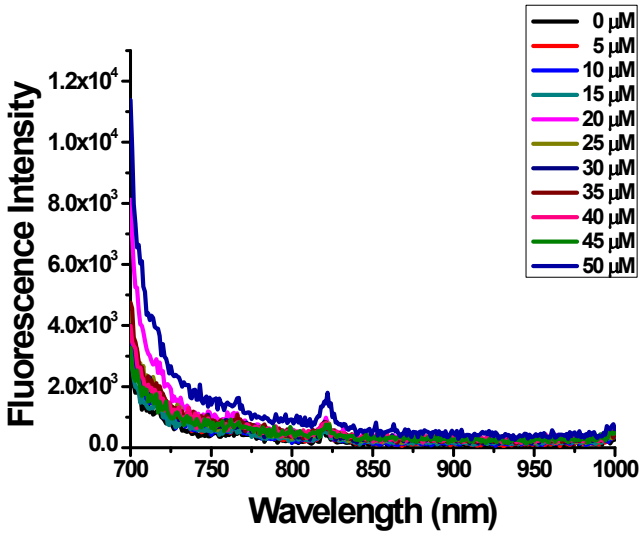


Figure S17: Absorbance and emission spectra of probe **C** (10 μM) in response to various NADH concentrations (0-50 μM) in PBS buffer (pH 7.4) with 10% DMSO during a 4-hour incubation period, acquired under excitation at 680 nm.

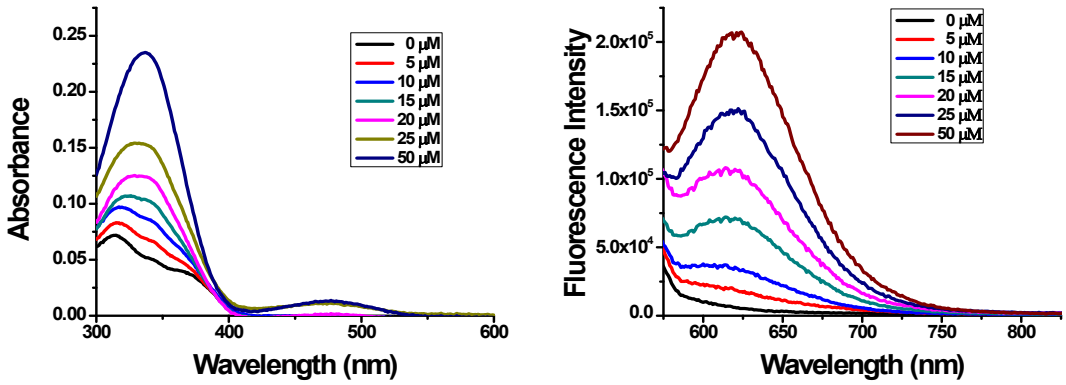


Figure S18: Absorbance and emission spectra of probe **D** (10 μM) in response to varying NADH concentrations (0-50 μM) in PBS buffer (pH 7.4) with 10% DMSO, recorded during a 4-hour incubation period and excited at 480 nm.

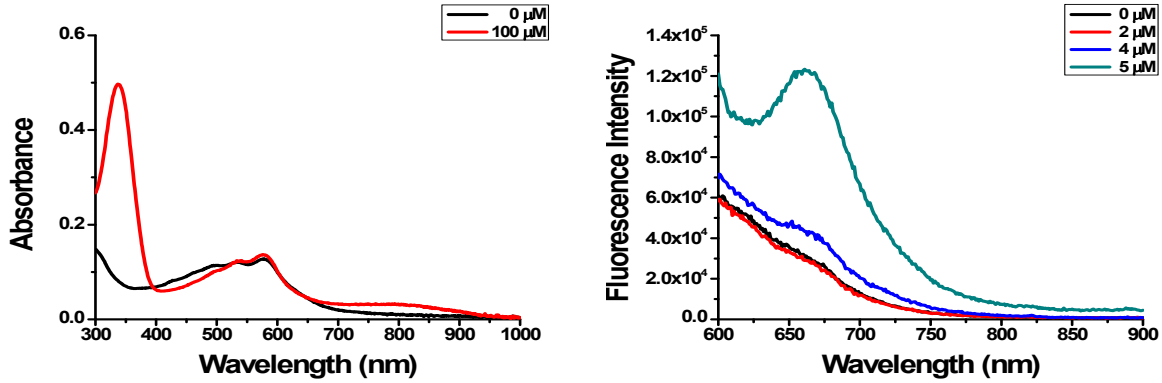


Figure S19: Absorbance and emission spectra of Probe **E** (10 μM) in response to different NADH concentrations (0, 2, 4, and 6 μM) in PBS buffer (pH 7.4) with 10% DMSO. These spectra were recorded during a 2-hour incubation period and excited at 480 nm.

2.5.2. The probe selectivity

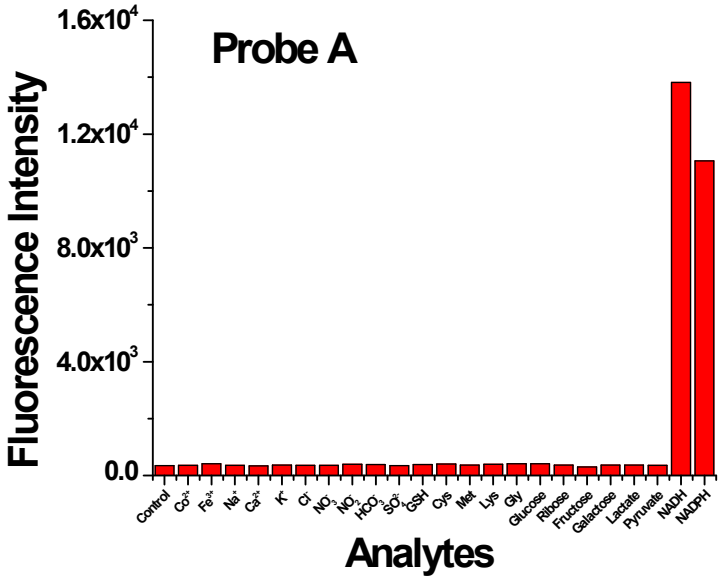


Figure S20: Fluorescence responses of 10 μM probe **A** to various biomolecules (100 μM), NADH (50 μM) and NADPH (50 μM) under excitation at 630 nm.

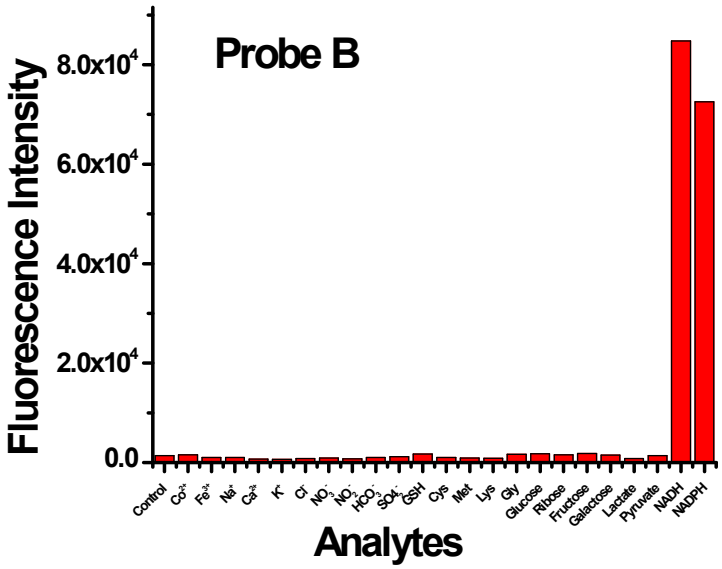


Figure S21: Fluorescence responses of 10 μM probe **B** to various biomolecules (100 μM), NADH (50 μM) and NADPH (50 μM) under excitation at 630 nm.

1.5.3. Photostability of the probes.

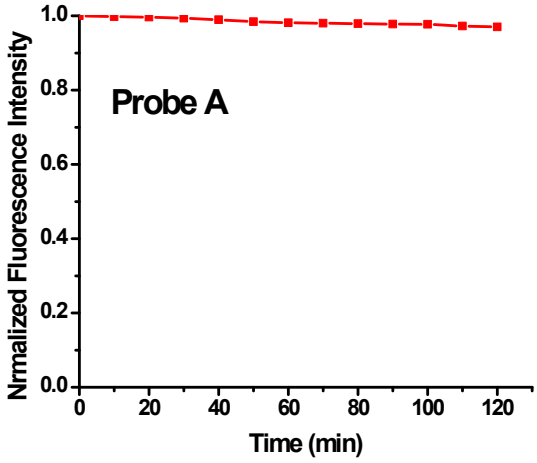


Figure S22: The fluorescence intensity of 10 µM probe **A** was measured over time when excited at 630 nm in the presence of 50 µM NADH.

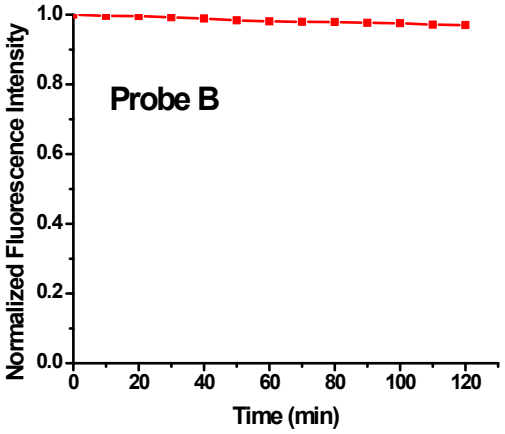


Figure S23: The fluorescence intensity of 10 µM probe **B** was measured over time when excited at 630 nm in the presence of 50 µM NADH.

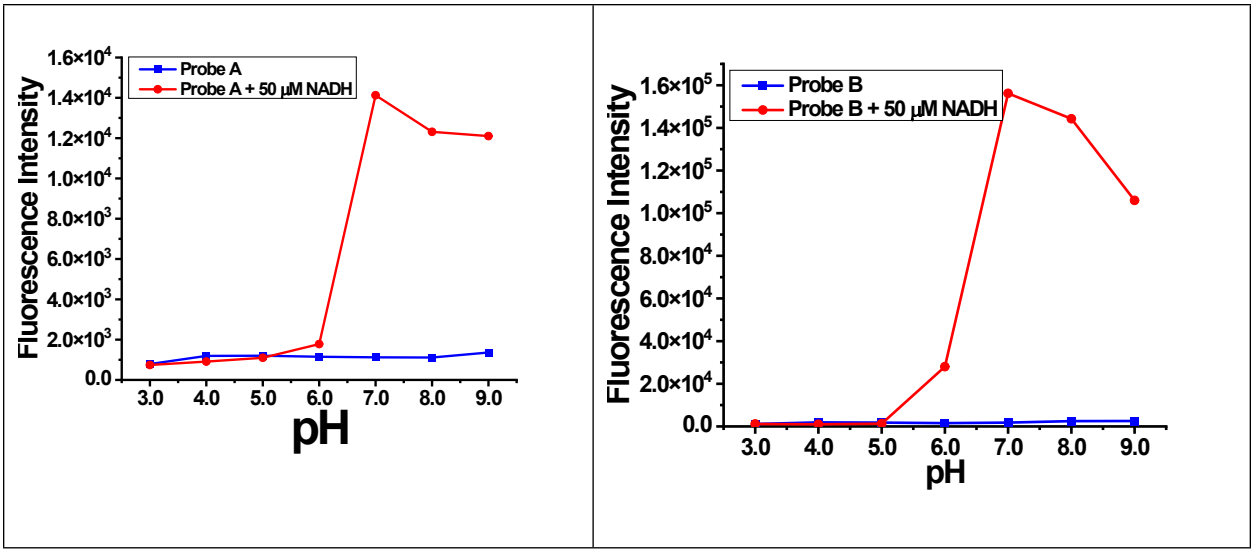


Figure S24: The impact of pH on fluorescence intensity is shown for Probes A (left) and B (right) within various pH PBS buffers, with 10% DMSO, both in the absence and presence of 50 μM NADH.

In the absence of NADH, the fluorescence probe demonstrated a consistent behavior, with no discernible pH dependency. This constancy stemmed from the presence of two electron-withdrawing acceptor groups within the probe's structure, as depicted in Figure S25. However, as the pH transitioned to neutral or slightly basic conditions, the probe exhibited a marked increase in fluorescence intensity in response to the presence of NADH. In contrast, at acidic pH levels, the probe's fluorescence response weakened. This diminished response was attributed to the protonation of the 1-methyl-1,4-dihydroquinoline electron donor moiety, as illustrated in Figure S25. This protonation event hindered the effective intramolecular charge transfer in the reduced form of the probe. Considering that we are detecting NAD(P)H levels in mitochondria, where the pH typically hovers around 8.0, any potential pH effect on the detected NAD(P)H levels within the mitochondria is expected to be minimal.

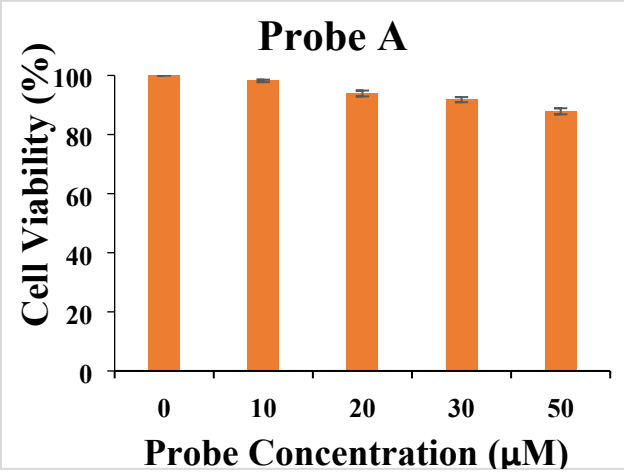
2.6. Cell culture and cell imaging.

2.6.1. Cell culture.

In all experimental procedures, A549 cells were treated with probe **A** at a concentration of 5 μM for a duration of 30 minutes. For the NADH-dependent study, A549 cells were initially incubated with various concentrations of NADH (5, 20, 50 μM) in DMEM medium for 30 minutes. Subsequently, the cells were further exposed to 5 μM of probe **A** for an additional 30 minutes. In the glucose-dependent study, A549 cells were pretreated with serum-free DMEM medium containing glucose concentrations of 0, 5, 10, and 20 mM for 30 minutes prior to the introduction of 5 μM of probe **A** for 30 minutes. As for the pyruvate/lactate-dependent studies, A549 cells were pre-treated with 10 mM lactate, 5 mM pyruvate, or a combination of 10 mM lactate and 5 mM pyruvate in serum-free DMEM medium for 30 minutes before being exposed to 5 μM of probe **A** in serum-free DMEM medium for an additional 30 minutes.

2.6.2. Assessing Cell Viability Using MTT Assay.

In this experiment, cells were seeded into individual wells of 96-well plates at a density of 5000 cells per well. Following an overnight incubation to allow for cell attachment and growth, fresh medium containing various concentrations of either probe **A** or probe **B** was added to each respective well. After 48 hours of treatment, 10 μL of MTT solution (5 mg/mL in phosphate buffer solution) was introduced to



each well, followed by a 4-hour incubation at 37°C. Subsequently, 100 µL of DMSO was added to each well and incubated at 37°C for 15 minutes to dissolve the formazan crystals. The absorbance of each well was then measured at 590 nm using a plate reader. This experiment was conducted in triplicate to ensure reliable results.

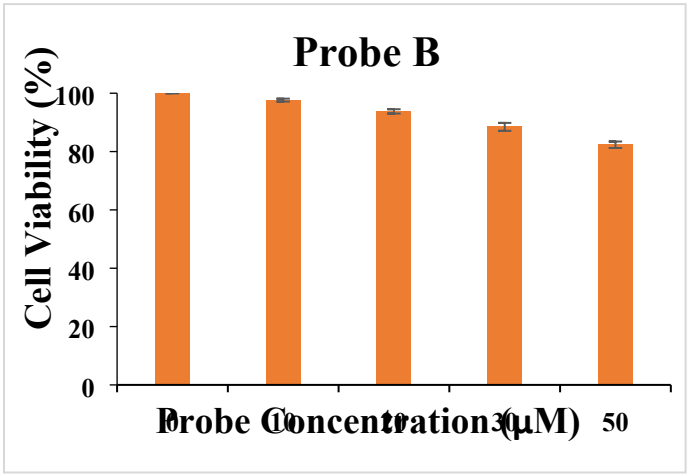


Figure S25: MTT assay for probe **A**(left) and probe **B** (right) with varying concentrations.

2.6.3. Drug treatment of A549 cells.

To assess the impact of cisplatin on NAD(P)H levels in A549 cells, the cells were exposed to various concentrations of cisplatin (5 µM, 10 µM, and 20 µM) in serum-free DMEM medium for a duration of two hours. Following this treatment, the cells were incubated with 5 µM of probe **A** in serum-free DMEM medium for thirty minutes to examine its effects.

To examine the influence of camptothecin on NAD(P)H levels in A549 cells, the cells were cultured in serum-free DMEM medium with different concentrations of gemcitabine (5 µM, 10 µM, and 20 µM) for a period of two hours. Subsequently, the cells were treated with 5 µM of probe **A** in serum-free DMEM medium for thirty minutes to investigate the impact.

In order to investigate the impact of gemcitabine on NAD(P)H levels in A549 cells, the cells were treated with various concentrations of gemcitabine (5 µM, 10 µM, and 20 µM) in DMEM medium without serum for a duration of two hours. Following this treatment, the cells were incubated with 5 µM of probe **A** in serum-free DMEM medium for thirty minutes to analyze its effects.

2.6.4. Colocalization Study.

To perform the colocalization analysis, A549 cells were initially exposed to 25 mM glucose for a duration of 30 minutes. Subsequently, the cells were incubated with 5 µM of probe **A** and a cyanine dye (IR-780) in serum-free DMEM medium for 30 minutes.

2.6.5. Hypoxia experiment.

In order to examine the NADH levels under hypoxic conditions, A549 cells were pre-treated with varying concentrations of $CoCl_2$ (50, 100, and 150 µM) for a period of 12 hours. Following this pre-treatment, the cells were incubated with 5 µM of probe **A** in serum-free DMEM medium for 30 minutes.

2.6.6. Cell culture conditions and cellular imaging for fibroblast cells.

Fibroblast cells were exposed to probe A at a concentration of 5 µM for a duration of 30 minutes. In the glucose-dependent study, fibroblast cells were initially treated with serum-free DMEM medium containing different glucose concentrations (0, 5, 10, and 20 mM) for 30 minutes before being exposed to 5 µM of probe **A** for an additional 30 minutes.

For the time-dependent study, fibroblast cells were pre-treated with 15 µM NADH in glucose-free DMEM medium for 30 minutes. Subsequently, they were incubated with 5 µM of probe **A** in glucose-free DMEM medium for various incubation times. Fluorescence signals from both sets of images were captured between 700 nm and 780 nm upon excitation at 633 nm.

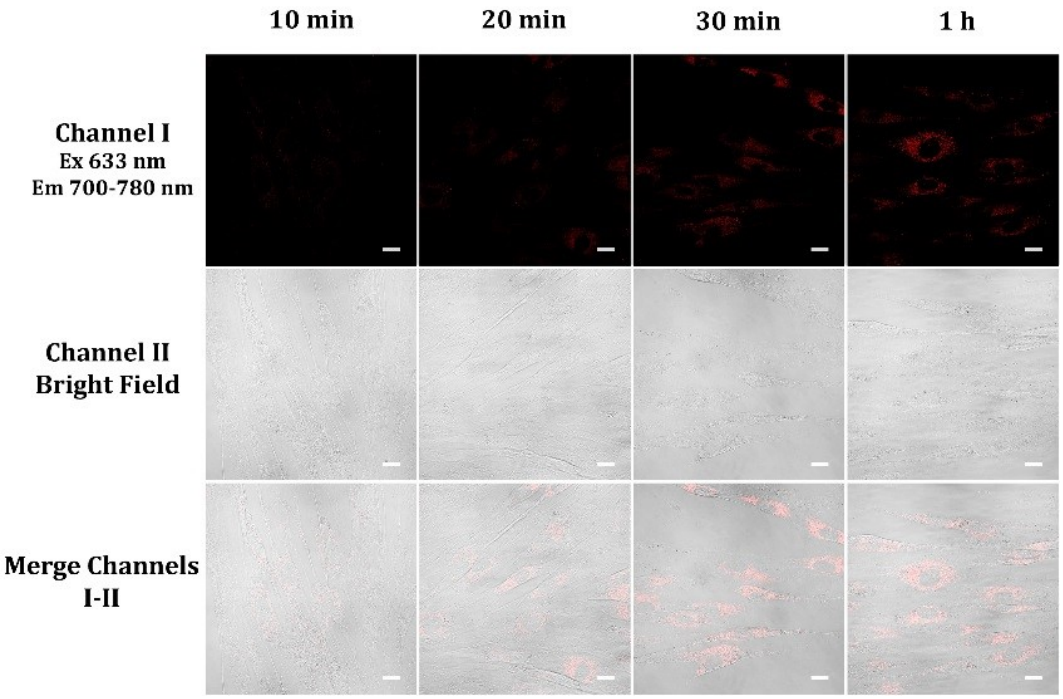


Figure S26: Cellular Fluorescence images of Fibroblast cells incubated with 20 µM NADH in glucose-free DMEM medium for 30 minutes and incubated with 5 µM probe **A** in glucose-free DMEM medium for varying incubation times. Image fluorescence signals were captured between 700 nm to 780 nm upon 633 nm excitation for both sets of images.

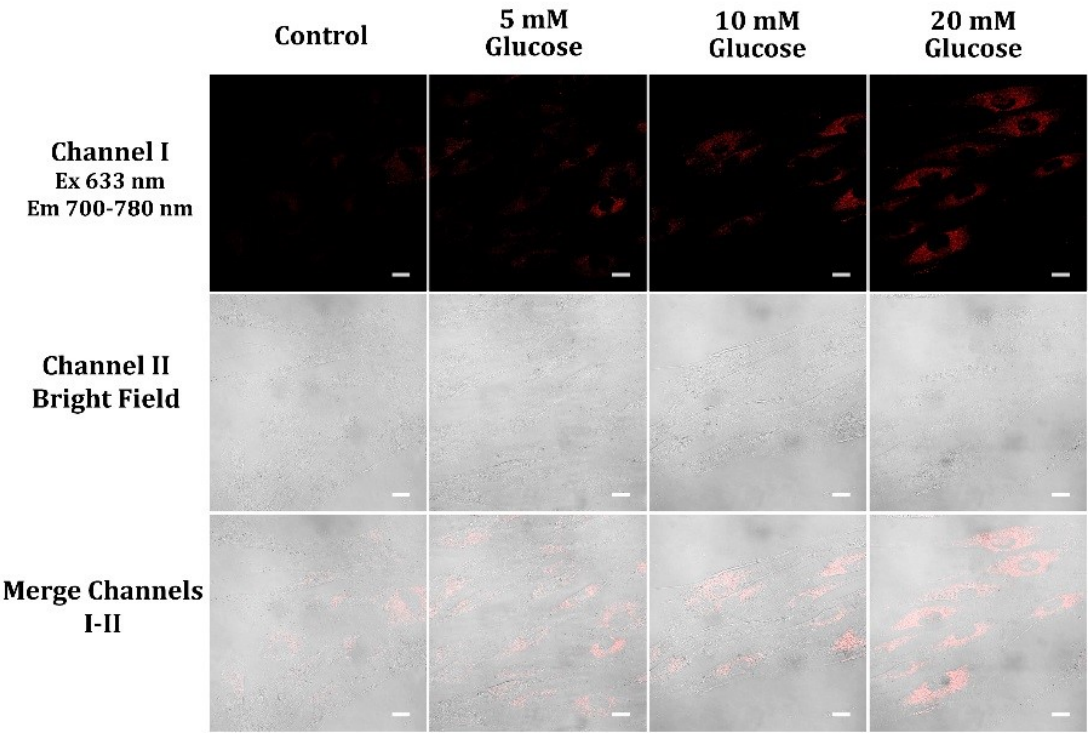


Figure S27: Cellular fluorescence images of Fibroblast cells cultivated in DMEM medium containing 0, 5, 10, and 20 mM glucose for 30 min and subsequently treated with 5 µM of probe **A** in glucose-deficient DMEM medium for 30 minutes. Image fluorescence signals were captured between 700 nm to 780 nm upon 633 nm excitation for both sets of images.

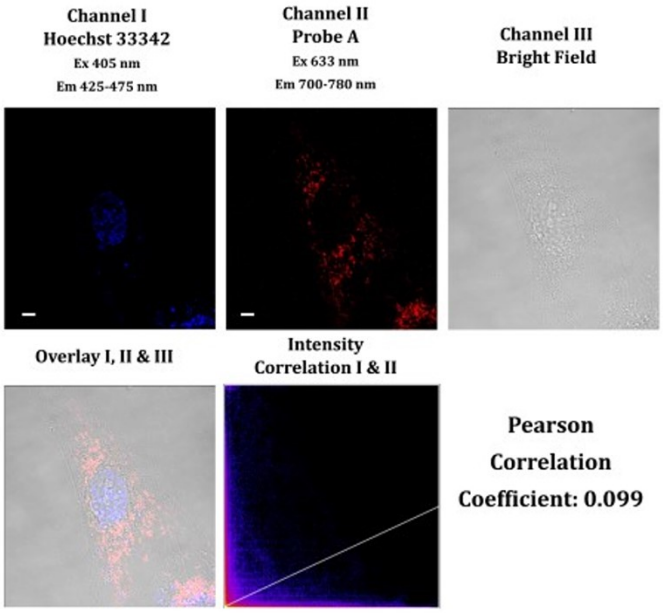


Figure S28: Cellular fluorescence images of Fibroblast cells following a 30-minute pre-treatment with 10 mM glucose in cell medium. Subsequently, the cells were co-incubated with 5 µM of the nucleus-specific stain Hoechst 33342, 5 µM of the lysosome-specific marker LysoSensor Green, and 5 µM of Probe A in the same cell medium for an additional 30 minutes. Fluorescence imaging was conducted within specific wavelength ranges: Hoechst 33342 images were acquired between 425 nm and 475 nm under excitation at 405 nm, LysoSensor Green images were captured between 500 nm and 550 nm under excitation at 488 nm, and probe **A** images were recorded in the range of 700 nm to 780 nm under excitation at 633 nm.

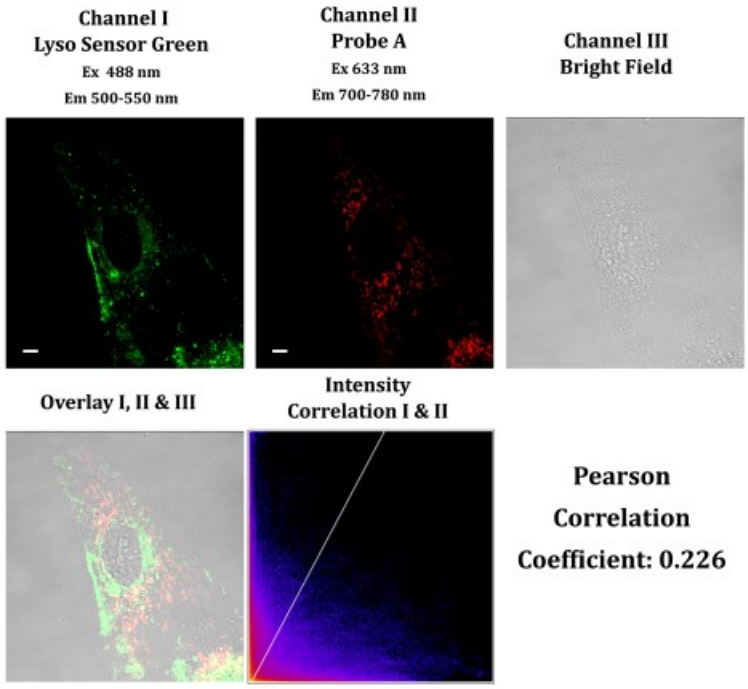


Figure S29: Fluorescence imaging of Fibroblast cells treated with glucose and stained with multiple dyes. Cells were pretreated for 30 minutes with medium containing 10 mM glucose. They were then coincubated for 30 additional minutes with 5 µM Hoechst 33342 to stain nuclei, 5 µM LysoSensor Green to label lysosomes, and 5 µM probe **A**. The cells were imaged using fluorescence microscopy with the following settings: Hoechst 33342 excitation 405 nm, emission 425-475 nm; LysoSensor Green excitation 488 nm, emission 500-550 nm; probe **A** excitation 633 nm, emission 700-780 nm. This allowed visualization of the nucleus (blue), lysosomes (green), and probe **A** signal (red).

2.7. *D. melanogaster* larval imaging

The study utilized the Canton-S strain of wild-type *Drosophila melanogaster* flies. Female flies were allowed to deposit their eggs on agar plates containing a mixture of sucrose and Baker's yeast. After an incubation period of 8 hours, the eggs were carefully collected, and the yeast was removed to facilitate the hatching of larvae. The hatched larvae were gently collected using a pin and rinsed twice with 1 mL of PBS.

To collect the eggs, young adult *Drosophila melanogaster* flies were placed in plastic egg-lay chambers containing sugar agar plates supplemented with live Baker's yeast. The chambers were maintained at room temperature under a 12:12-hour light/dark cycle. Eggs were collected in the early morning and late afternoon using a moist brush, followed by a 1-minute rinse with tap water in a filter basket. Subsequently, the eggs were transferred to small Petri dishes placed on moist paper towel layers. The small dishes were then placed inside larger dishes with water at the bottom to prevent desiccation. The eggs were incubated at 18°C until the larvae hatched, which typically occurred after 2 days. Upon hatching, the newly emerged larvae were delicately transferred with a fine brush to glass viewing dishes and immediately used for the staining experiments. Any larvae that perished during the experiment due to starvation were eliminated during the washing steps.

Freshly hatched starved fruit fly larvae were exposed to various concentrations of NADH, ranging from 0 (control) to 50 µM, in a pH 7.4 PBS buffer for 1 hour. Following the exposure, they underwent three washes with the PBS buffer before being immersed in a PBS buffer solution containing 10 µM of probe **A** for 2 hours. Fluorescence signals from the resulting images were captured within a wavelength range of 700-780 nm upon excitation at 633 nm.

Freshly hatched starved fruit fly larvae were exposed to different concentrations of cisplatin, ranging from 0 (control) to 20 µM, in a pH 7.4 PBS buffer for 2 hours. Subsequently, they were washed three times with the PBS buffer and immersed in a PBS buffer solution containing 10 µM of probe **A** for 2 hours. Fluorescence signals from the resulting images were acquired within a wavelength range of 700-780 nm upon excitation at 633 nm.

3. Theoretical calculations

Models of probes **A**, **AH**, **B**, and **BH** were generated through the use of Gaussian 16[21] and density functional theory (DFT), using the APFD functional and electron basis sets at the 6-311+g(d) for optimization of the geometry in a Polarizable Continuum Model (PCM) of water. Upon confirming the lack of imaginary frequencies, the CAM-B3LYP/6-311+g(d) basis set was also used in a TD-DFT calculation to calculate the absorption energies. Results were interpreted using GaussView 6[22] for all data and figures.

Table S2: Calculated atomic coordinates for probe **A(*cis*)** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-9.26072	-0.11565	-1.27285	28	C	8.91157	0.470575	-0.19302
2	C	-9.4422	0.954693	-0.37299	29	C	9.13926	1.74933	-0.64489
3	C	-8.39816	1.44582	0.378264	30	C	8.0567	2.609754	-0.92061
4	C	-7.12642	0.864673	0.244155	31	C	6.761954	2.182163	-0.7417
5	C	-6.9269	-0.21802	-0.65634	32	C	-6.20377	2.430148	1.907299
6	C	-8.02471	-0.69133	-1.41386	33	H	-10.103	-0.47923	-1.85177
7	N	-6.04049	1.314688	0.967165	34	H	-10.4242	1.404428	-0.26821
8	C	-4.84261	0.757628	0.843235	35	H	-8.57053	2.269704	1.058497
9	C	-4.58761	-0.30769	-0.03149	36	H	-7.86577	-1.51565	-2.10152
10	C	-5.64835	-0.78105	-0.7832	37	H	-4.06566	1.17055	1.473448
11	C	-3.25786	-0.89785	-0.1211	38	H	-5.49802	-1.58717	-1.49392
12	C	-2.95798	-2.21126	-0.40782	39	H	-3.71278	-2.97046	-0.57432
13	C	-1.57722	-2.4664	-0.41564	40	H	-1.1403	-3.43995	-0.60652
14	C	-0.8102	-1.35091	-0.13579	41	H	1.076251	-2.30002	-0.26385
15	S	-1.82322	0.037191	0.130957	42	H	0.938338	0.736297	0.244405
16	C	0.617831	-1.33465	-0.07031	43	H	5.913969	-2.816	1.003916
17	C	1.393858	-0.24662	0.156169	44	H	8.193764	-1.97358	0.659042
18	C	2.823449	-0.24914	0.184097	45	H	3.930114	-2.69858	2.005883
19	N	3.648435	-1.23178	0.560834	46	H	2.243217	-2.31325	1.660846
20	C	4.995923	-0.9332	0.399062	47	H	3.187363	-3.28668	0.497461
21	C	5.20325	0.355112	-0.06587	48	H	9.743682	-0.1945	0.020077
22	S	3.683785	1.146707	-0.32963	49	H	10.15584	2.101315	-0.79135
23	C	6.078206	-1.79921	0.667584	50	H	8.247294	3.617445	-1.27688
24	C	7.344681	-1.32481	0.466755	51	H	5.934848	2.853379	-0.95667
25	C	7.594326	-0.00198	0.003481	52	H	-6.93063	2.159359	2.671279
26	C	6.505614	0.874278	-0.27825	53	H	-5.24632	2.633043	2.376978
27	C	3.221821	-2.46581	1.213951	54	H	-6.53467	3.315227	1.365957

Table S3: Calculated atomic coordinates for probe **A(*trans*)** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-6.79039	2.996061	-2.8E-05	11	C	-2.19422	-1.13485	-1.1E-05
2	C	-7.83078	2.044715	0.000001	12	C	-1.79735	-2.45799	-8E-06
3	C	-7.56675	0.692949	0.00002	13	C	-0.40462	-2.61379	-0.00001
4	C	-6.23202	0.258006	0.00001	14	C	0.282304	-1.41242	-1.3E-05
5	C	-5.17095	1.203345	-1.8E-05	15	S	-0.82682	-0.07417	-1.6E-05
6	C	-5.48282	2.583854	-3.8E-05	16	C	1.699457	-1.27648	-1.2E-05
7	N	-5.90089	-1.08265	0.000028	17	C	2.396383	-0.11067	-1.1E-05
8	C	-4.63966	-1.48965	0.000019	18	C	3.818484	-0.0476	-7E-06
9	C	-3.55002	-0.6037	-7E-06	19	N	4.506233	1.100598	0.000013
10	C	-3.84436	0.749345	-2.6E-05	20	C	5.889423	0.943501	0.000013

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
21	C	6.262481	-0.40595	-7E-06	35	H	-2.4807	-3.29778	-5E-06
22	S	4.853214	-1.42722	-2.8E-05	36	H	0.098069	-3.5742	-8E-06
23	C	6.861712	1.944266	0.000031	37	H	2.233123	-2.22542	-9E-06
24	C	8.192713	1.559373	0.000028	38	H	1.866117	0.835727	-1.2E-05
25	C	8.561593	0.20768	0.000008	39	H	6.601094	2.995537	0.000046
26	C	7.600975	-0.79176	-0.00001	40	H	8.962006	2.324469	0.000042
27	C	3.848993	2.405066	0.000037	41	H	9.612337	-0.06304	0.000007
28	C	-6.96713	-2.0922	0.000058	42	H	7.87878	-1.83988	-2.6E-05
29	H	-7.0303	4.054011	-4.3E-05	43	H	3.237602	2.51264	0.895102
30	H	-8.86264	2.380456	0.000008	44	H	4.603583	3.183322	0.00006
31	H	-8.3878	-0.01198	0.000042	45	H	3.237615	2.512681	-0.89503
32	H	-4.66909	3.301974	-0.00006	46	H	-7.57752	-1.97493	-0.89385
33	H	-4.49914	-2.56101	0.000034	47	H	-6.51469	-3.07896	0.000076
34	H	-3.05147	1.4913	-4.8E-05	48	H	-7.57751	-1.97489	0.893973

Table S4: Calculated atomic coordinates for probe **AH(*cis*)** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	7.414916	3.435199	0.000469	16	C	-0.57403	-1.7978	0.000094
2	C	8.53334	2.60829	0.000155	17	C	-1.38057	-0.67837	0.000223
3	C	8.382084	1.226942	-0.00013	18	C	-2.78163	-0.57249	0.000206
4	C	7.103837	0.656064	-0.00011	19	N	-3.74604	-1.51794	0.00031
5	C	5.968866	1.482556	0.000204	20	C	-5.04476	-1.01398	0.00017
6	C	6.147061	2.862178	0.00049	21	C	-5.08393	0.369757	0.000016
7	N	6.944073	-0.73999	-0.00041	22	S	-3.47241	1.01981	0.000017
8	C	5.712013	-1.28931	-0.00036	23	C	-6.2412	-1.76448	0.000168
9	C	4.541713	-0.58291	-0.00005	24	C	-7.43499	-1.09474	0.000036
10	C	4.570633	0.920841	0.000232	25	C	-7.50508	0.324353	-0.00011
11	C	3.294294	-1.2654	-3.2E-05	26	C	-6.30288	1.090995	-0.00013
12	C	3.006062	-2.63977	-0.00016	27	C	-3.45535	-2.94238	0.00061
13	C	1.651056	-2.9191	-0.00012	28	C	8.110582	-1.60535	-0.00077
14	C	0.827752	-1.78712	0.000047	29	H	4.024321	1.308702	-0.87244
15	S	1.818414	-0.34299	0.000157	30	H	4.024485	1.30837	0.873151

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
31	C	-8.74484	1.004482	-0.00025	44	H	-6.23111	-2.84733	0.000248
32	C	-8.79325	2.378388	-0.0004	45	H	-8.36171	-1.66069	0.000032
33	C	-7.59956	3.130944	-0.00043	46	H	-4.38433	-3.50113	0.001082
34	C	-6.37716	2.501823	-0.0003	47	H	-2.89487	-3.20891	-0.89564
35	H	7.526635	4.515178	0.000696	48	H	-2.89431	-3.20838	0.896665
36	H	9.532519	3.033563	0.000134	49	H	7.781124	-2.64171	-0.00098
37	H	9.267469	0.602568	-0.00037	50	H	8.719691	-1.43486	-0.89134
38	H	5.265535	3.499004	0.000731	51	H	8.719932	-1.43529	0.88971
39	H	5.70892	-2.37183	-0.00061	52	H	-9.66167	0.421259	-0.00024
40	H	3.768925	-3.40851	-0.00025	53	H	-9.75112	2.889772	-0.00051
41	H	1.240886	-3.92363	-0.0002	54	H	-7.64765	4.215756	-0.00055
42	H	-0.99931	-2.79376	-2.8E-05	55	H	-5.46477	3.092178	-0.00032
43	H	-0.88187	0.288281	0.000274					

Table S5: Calculated atomic coordinates for probe **AH(***trans* in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-6.81551	3.001712	-3.8E-05	18	C	3.806557	-0.03762	-4E-06
2	C	-7.83273	2.053101	-1.7E-05	19	N	4.50819	1.115829	0.000016
3	C	-7.52517	0.698031	0.000006	20	C	5.890825	0.957184	0.000011
4	C	-6.19033	0.277118	0.00001	21	C	6.268628	-0.39052	-8E-06
5	C	-5.15677	1.226783	-0.00001	22	S	4.858989	-1.4225	-2.6E-05
6	C	-5.49061	2.577239	-3.5E-05	23	C	6.862945	1.956479	0.000023
7	N	-5.87265	-1.09297	0.000032	24	C	8.198663	1.576414	0.000017
8	C	-4.58859	-1.49909	0.000019	25	C	8.570701	0.228727	0
9	C	-3.50454	-0.6633	-1E-06	26	C	7.606316	-0.77139	-1.3E-05
10	C	-3.70419	0.827182	-3E-06	27	C	3.846251	2.411826	0.000051
11	C	-2.19086	-1.20045	-0.00001	28	C	-6.93487	-2.08442	0.00006
12	C	-1.75194	-2.53709	-1.4E-05	29	H	-3.20566	1.274219	0.872826
13	C	-0.37704	-2.66559	-1.6E-05	30	H	-3.20565	1.274219	-0.87283
14	C	0.316677	-1.447	-1.4E-05	31	H	-7.04952	4.061926	-5.7E-05
15	S	-0.82636	-0.12042	-1.1E-05	32	H	-8.87379	2.36189	-1.9E-05
16	C	1.703922	-1.29453	-1.1E-05	33	H	-8.33347	-0.02322	0.00002
17	C	2.407468	-0.10671	-8E-06	34	H	-4.68747	3.310412	-5.1E-05

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
35	H	-4.46224	-2.57422	0.000029	43	H	7.885674	-1.81953	-2.8E-05
36	H	-2.42567	-3.38485	-1.7E-05	44	H	3.229949	2.518037	-0.89327
37	H	0.14191	-3.61849	-1.8E-05	45	H	3.229888	2.517954	0.893344
38	H	2.255147	-2.23444	-8E-06	46	H	4.593698	3.197789	0.000116
39	H	1.864428	0.832584	-1.1E-05	47	H	-6.49124	-3.07723	0.000089
40	H	6.599907	3.007535	0.000034	48	H	-7.55875	-1.98271	0.890696
41	H	8.964459	2.345425	0.000026	49	H	-7.55875	-1.98276	-0.89058
42	H	9.621491	-0.04242	-4E-06					

Table S6: Calculated atomic coordinates for probe **B** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	8.594824	-1.81256	-0.16078	31	O	-2.19111	2.576183	0.199487
2	C	8.796163	-0.43322	-0.02504	32	H	9.449697	-2.47756	-0.22661
3	C	7.728755	0.446951	0.061778	33	H	9.806768	-0.04025	0.013574
4	C	6.437758	-0.079	0.01027	34	H	7.908822	1.509973	0.167131
5	C	6.241281	-1.4585	-0.12433	35	H	7.148646	-3.40854	-0.31682
6	C	7.31395	-2.34194	-0.21196	36	H	2.707698	1.424855	0.181054
7	N	5.232653	0.614235	0.078242	37	H	4.608824	2.486586	-0.6323
8	C	4.140117	-0.1593	0.004898	38	H	4.646343	2.321414	1.148152
9	C	2.819332	0.355901	0.057713	39	H	6.149003	2.480012	0.231626
10	C	5.147747	2.064803	0.215393	40	H	1.829098	-1.50495	-0.1651
11	C	1.708111	-0.42966	-0.04273	41	H	-4.11727	1.880237	-0.72407
12	C	0.372964	0.036685	-0.00578	42	H	-3.42992	-2.16016	0.574638
13	C	-0.10684	1.336957	0.098442	43	H	-8.40303	0.072298	-0.46236
14	C	-1.52198	1.410735	0.081902	44	H	-9.29943	-2.11746	0.126307
15	C	-2.12898	0.175192	-0.04392	45	H	-7.80204	-3.98491	0.782759
16	S	-0.95091	-1.08423	-0.14924	46	H	-5.35017	-3.63842	0.8481
17	C	-3.55143	-0.11037	-0.06404	47	H	-6.00841	2.675546	-1.1069
18	C	-4.45828	0.895193	-0.44118	48	H	-7.19845	1.536679	-1.76649
19	N	-5.7696	0.700341	-0.47555	49	H	-7.30813	2.068093	-0.06316
20	C	-6.33448	-0.51624	-0.14997	50	H	0.617259	4.431153	0.435249
21	C	-5.46319	-1.57533	0.225158	51	H	-0.14495	3.816745	-1.05352
22	C	-4.08018	-1.3471	0.265675	52	H	-1.90789	4.555197	0.550054
23	C	-7.72348	-0.7221	-0.18221	53	H	-1.24186	3.472714	1.798842
24	C	-8.22615	-1.9598	0.151625	54	S	4.540554	-1.83239	-0.15648
25	C	-7.37619	-3.02105	0.524878					
26	C	-6.01885	-2.83294	0.562085					
27	C	-6.63271	1.816964	-0.87951					
28	O	0.681183	2.414106	0.212843					
29	C	-0.02432	3.655176	0.021115					
30	C	-1.36343	3.628271	0.722832					

Table S7: Calculated atomic coordinates for probe **BH** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	8.555546	-1.94628	0.000854	29	C	0.026333	3.738331	-0.30329
2	C	8.801951	-0.57051	0.011236	30	C	-1.2987	3.78824	0.427172
3	C	7.760877	0.349354	0.014716	31	O	-2.14864	2.714107	0.009072
4	C	6.452198	-0.13078	0.007439	32	H	9.385719	-2.64523	-0.00155
5	C	6.211212	-1.50972	-0.00272	33	H	9.82471	-0.20736	0.016943
6	C	7.25283	-2.43067	-0.00619	34	H	7.977076	1.411068	0.023394
7	N	5.274744	0.609709	0.008818	35	H	7.050647	-3.49644	-0.014
8	C	4.139785	-0.12681	0.002415	36	H	2.7663	1.499214	0.021395
9	C	2.854856	0.421124	0.006283	37	H	4.712777	2.428397	-0.86905
10	C	5.235469	2.063428	0.015898	38	H	4.734288	2.421394	0.916166
11	C	1.703536	-0.35113	--0.00854	39	H	6.24821	2.452	0.004518
12	C	0.396106	0.120419	-0.0073	40	H	1.812084	-1.4355	-0.02229
13	C	-0.09225	1.440671	-0.01395	41	H	-4.17866	1.996212	-0.07343
14	C	-1.485	1.529097	-0.01127	42	H	-3.50805	-1.97313	0.899567
15	C	-2.13504	0.28508	-0.02073	43	H	-8.38982	0.035909	-0.03393
16	S	-0.95709	-0.99175	-0.02259	44	H	-9.27124	-2.24489	0.022358
17	C	-3.51749	-0.03166	-0.01734	45	H	-7.71372	-4.19165	0.076128
18	C	-4.46752	0.955314	-0.04681	46	H	-5.26744	-3.79084	0.071355
19	N	-5.79882	0.734922	-0.0456	47	H	-6.11799	2.78661	-0.10123
20	C	-6.3129	-0.57161	-0.01354	48	H	-7.33453	1.839103	-0.96955
21	C	-5.42906	-1.66184	0.016156	49	H	-7.33858	1.88694	0.81076
22	C	-3.93393	-1.47779	0.014581	50	H	0.685699	4.540835	0.024394
23	C	-7.69501	-0.79504	-0.01105	51	H	-0.12149	3.807605	-1.3849
24	C	-8.1963	-2.0907	0.020832	52	H	-1.8317	4.709717	0.19638
25	C	-7.3281	-3.17701	0.050779	53	H	-1.14812	3.71752	1.509343
26	C	-5.95557	-2.94904	0.048002	54	S	4.493629	-1.8332	-0.00874
27	C	-6.70367	1.870563	-0.0782	55	H	-3.50555	-2.01272	-0.84628
28	O	0.720152	2.511642	-0.02037					

Table S8: Calculated atomic coordinates for probe **C** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-7.66048	0.228722	0.092092	28	C	6.556277	2.934455	-1.01646
2	C	-7.67543	-1.18124	-0.09971	29	H	5.392181	-1.9309	0.76935
3	C	-6.52645	-1.9133	-0.21049	30	H	-8.63559	-1.68337	-0.15926
4	C	-5.29074	-1.23382	-0.12995	31	H	-6.57297	-2.98477	-0.3564
5	C	-5.24454	0.138506	0.057785	32	H	-1.42066	-2.32763	-0.29149
6	C	-6.41886	0.923219	0.175765	33	H	-3.23828	-3.37791	-1.35091
7	N	-4.02331	-1.79448	-0.22094	34	H	-3.2333	-3.62827	0.423183
8	C	-3.01912	-0.91433	-0.11186	35	H	-4.7421	-3.73357	-0.49185
9	S	-3.60027	0.690344	0.12002	36	H	-0.87135	0.684169	0.015512
10	C	-1.64324	-1.27385	-0.17541	37	H	4.284366	1.845396	-0.95556
11	C	-3.78794	-3.2233	-0.42296	38	H	8.887335	2.226515	-0.37668
12	C	-0.63202	-0.37164	-0.09582	39	H	10.63108	0.745622	0.468355
13	C	0.756561	-0.69852	-0.14571	40	H	10.09602	-1.53516	1.284002
14	C	1.379816	-1.92779	-0.28709	41	H	7.752811	-2.34231	1.248023
15	C	2.776777	-1.83972	-0.28626	42	H	5.628621	3.388906	-1.35182
16	C	3.240533	-0.54525	-0.14488	43	H	6.980622	3.528706	-0.20796
17	S	1.933577	0.568773	-0.00375	44	H	7.253819	2.873903	-1.85093
18	C	4.633057	-0.11898	-0.09493	45	C	-8.82911	2.330704	0.388047
19	C	5.009979	1.152595	-0.54952	46	C	-7.59628	3.010462	0.470035
20	N	6.265228	1.581507	-0.5236	47	C	-6.41089	2.321677	0.366226
21	C	7.292136	0.787763	-0.05533	48	C	-8.85922	0.968464	0.203086
22	C	6.969024	-0.51747	0.407864	49	H	-9.75594	2.888733	0.471719
23	C	5.63333	-0.94513	0.386087	50	H	-7.58395	4.085595	0.616041
24	C	8.622821	1.236846	-0.02922	51	H	-5.46651	2.853826	0.430425
25	C	9.603848	0.397982	0.449053	52	H	-9.80596	0.44077	0.13946
26	C	9.300613	-0.89855	0.91294	53	H	0.84135	-2.86044	-0.39919
27	C	8.00623	-1.34855	0.894815	54	H	3.431951	-2.69273	-0.41187

Table S9: Calculated atomic coordinates for probe **CH** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-7.71272	0.198587	0.002581	29	H	5.502539	-1.79266	0.870509
2	C	-7.71692	-1.22264	-0.01032	30	H	5.520464	-1.7918	-0.87829
3	C	-6.5593	-1.95268	-0.01551	31	H	-8.67243	-1.7373	-0.01624
4	C	-5.32798	-1.26188	-0.00772	32	H	-6.59724	-3.03459	-0.02541
5	C	-5.2942	0.121619	0.004792	33	H	-1.4476	-2.3355	-0.00945
6	C	-6.47343	0.904529	0.010457	34	H	-3.25152	-3.53216	-0.92275
7	N	-4.05767	-1.82456	-0.01129	35	H	-3.25084	-3.54947	0.866246
8	C	-3.04767	-0.93082	-0.00255	36	H	-4.75657	-3.78836	-0.02989
9	S	-3.64972	0.695776	0.012726	37	H	-0.9344	0.70476	-0.00184
10	C	-1.68573	-1.27874	-0.00506	38	H	4.212484	2.038608	-0.04441
11	C	-3.80875	-3.26088	-0.02531	39	H	8.841581	2.315704	-0.00638
12	C	-0.66995	-0.3518	-0.00354	40	H	10.70298	0.727901	0.044902
13	C	0.704295	-0.64339	-0.00472	41	H	10.25769	-1.72486	0.078189
14	C	1.361501	-1.87357	-0.00515	42	H	7.915815	-2.53443	0.057423
15	C	2.745304	-1.76904	-0.00675	43	H	5.535069	3.655812	-0.07824
16	C	3.219506	-0.44894	-0.00888	44	H	7.036732	3.442358	0.836631
17	S	1.877141	0.657889	-0.00538	45	H	7.059475	3.408325	-0.94501
18	C	4.590803	-0.06081	-0.01247	46	C	-8.89492	2.315229	0.020397
19	C	4.959052	1.252392	-0.03016	47	C	-7.66489	3.006782	0.028119
20	N	6.234159	1.700679	-0.03232	48	C	-6.47581	2.317336	0.023248
21	C	7.305402	0.794053	-0.0046	49	C	-8.9162	0.940878	0.007909
22	C	7.044659	-0.58614	0.013141	50	H	-9.82503	2.874207	0.024391
23	C	5.641437	-1.13988	-0.00204	51	H	-7.65885	4.091956	0.037936
24	C	8.627509	1.254296	0.006998	52	H	-5.53439	2.859065	0.029282
25	C	9.68462	0.352722	0.036373	53	H	-9.85996	0.403754	0.00196
26	C	9.436952	-1.01548	0.054944	54	H	0.835568	-2.82091	-0.00461
27	C	8.120706	-1.46714	0.043385	55	H	3.407758	-2.62485	-0.00805
28	C	6.487793	3.130898	-0.05576					

Table S10: Calculated atomic coordinates for probe D in water.

Row	Symbol	X	Y	Z	26	C	-6.30381	-2.69609	0.392705
1	C	-3.32914	-0.75179	0.067849	27	C	-8.27112	-1.31543	0.253257
2	C	-1.95984	-1.19289	0.100529	28	C	-7.67944	-2.57407	0.407818
3	C	-0.89016	-0.37157	0.031337	29	C	-5.16743	0.846443	-0.11259
4	C	0.477264	-0.80766	0.05754	30	C	-4.53478	3.178475	-0.44967
5	C	1.008358	-2.08089	0.148622	31	N	-3.72058	3.993747	-0.57987
6	C	2.411466	-2.09637	0.138379	32	C	-6.84428	2.716786	-0.33866
7	C	2.969104	-0.8373	0.040894	33	N	-7.88779	3.221644	-0.38819
8	S	1.743594	0.373266	-0.04703	34	H	-1.83178	-2.26691	0.193643
9	C	4.381844	-0.48771	0.009545	35	H	-1.04712	0.701135	-0.04828
10	C	5.300264	-1.40752	-0.5185	36	H	0.402888	-2.97489	0.229206
11	N	6.604447	-1.17198	-0.57102	37	H	2.998081	-3.00238	0.230636
12	C	7.145864	0.014368	-0.11862	38	H	9.214782	-0.46253	-0.57855
13	C	6.259488	0.991544	0.411202	39	H	10.07182	1.668068	0.24387
14	C	4.884714	0.715995	0.469832	40	H	8.549251	3.389589	1.17824
15	C	8.526299	0.268643	-0.17629	41	H	6.111657	2.957299	1.285197
16	C	9.005923	1.472356	0.288381	42	H	6.872436	-3.05139	-1.43818
17	C	8.141193	2.451288	0.819253	43	H	7.993541	-1.79835	-2.01522
18	C	6.792185	2.215948	0.880327	44	H	8.203744	-2.51865	-0.39117
19	C	7.481581	-2.20212	-1.14269	45	H	-7.9732	0.765838	-0.03273
20	C	-7.4868	-0.19135	0.084221	46	H	-5.81057	-3.6544	0.510035
21	C	-3.78831	0.523933	-0.1002	47	H	-9.35107	-1.21758	0.265483
22	C	-6.08132	-0.27338	0.06226	48	H	-8.29689	-3.45612	0.540076
23	C	-5.52253	-1.55641	0.222392	49	H	4.221523	1.461676	0.89696
24	O	-4.18247	-1.77786	0.223384	50	H	4.974631	-2.35524	-0.92527
25	C	-5.53571	2.188286	-0.29125	51	H	-3.05947	1.312369	-0.23224

Table S11: Calculated atomic coordinates for probe **DH** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-3.34936	-0.79884	0.000759	27	C	-8.31749	-1.24604	-0.00268
2	C	-2.00696	-1.26715	0.001373	28	C	-7.76129	-2.52872	-0.00101
3	C	-0.90781	-0.45661	0.000164	29	C	-5.14903	0.86085	-0.0003
4	C	0.437209	-0.89342	0.000722	30	C	-4.46946	3.206441	-0.00066
5	C	0.968804	-2.18188	0.002987	31	N	-3.6316	4.009791	-0.0013
6	C	2.357386	-2.22053	0.002924	32	C	-6.78605	2.789854	0.001833
7	C	2.958326	-0.9557	0.000499	33	N	-7.81874	3.321987	0.003464
8	S	1.735248	0.273611	-0.00159	34	H	-1.89621	-2.34698	0.002737
9	C	4.339768	-0.5846	-0.00053	35	H	-1.0578	0.620817	-0.00146
10	C	5.312219	-1.53695	-0.00056	36	H	0.350121	-3.07162	0.004814
11	N	6.645307	-1.28368	-0.0012	37	H	2.921426	-3.14467	0.004814
12	C	7.117307	0.035228	-0.00055	38	H	5.062449	-2.59033	-0.00012
13	C	6.200942	1.100806	-0.00068	39	H	9.210052	-0.50903	0.000506
14	C	4.710819	0.873061	-0.00234	40	H	10.02407	1.798481	0.002082
15	C	8.492488	0.302026	0.000423	41	H	8.409752	3.69854	0.002181
16	C	8.954706	1.612487	0.001347	42	H	5.976851	3.223517	0.000394
17	C	8.055008	2.673093	0.001402	43	H	7.018528	-3.3258	-0.00212
18	C	6.689902	2.403113	0.000417	44	H	8.214388	-2.36992	-0.89199
19	C	7.580658	-2.39419	-0.00139	45	H	8.213726	-2.37089	0.889727
20	C	-7.49965	-0.13133	-0.00253	46	H	-7.95886	0.846273	-0.004
21	C	-3.78832	0.507218	-0.00013	47	H	-5.92121	-3.6628	0.001565
22	C	-6.09759	-0.24777	-0.00071	48	H	-9.39466	-1.11976	-0.00415
23	C	-5.57395	-1.55458	0.000467	49	H	-8.40231	-3.40398	-0.00104
24	O	-4.24037	-1.8116	0.001492	50	H	4.266086	1.374831	0.870259
25	C	-5.49259	2.230051	-8E-06	51	H	4.268399	1.371969	-0.8778
26	C	-6.3878	-2.68404	0.000493	52	H	-3.03971	1.28837	-0.00012

Table S12: Calculated atomic coordinates for probe **E** in water.

Row	Symbol	X	Y	Z	Row	Symbol	X	Y	Z
1	C	-3.67558	-0.73132	-0.09075	27	C	-6.53469	2.605304	0.344514
2	C	-2.31621	-1.13237	-0.13714	28	N	-6.41679	3.746136	0.503587
3	C	-1.24273	-0.3017	-0.02132	29	C	-8.07163	0.761796	0.070659
4	C	0.116851	-0.72702	-0.0808	30	N	-9.16747	0.393743	0.006248
5	C	0.650843	-1.99071	-0.28273	31	C	-3.62563	1.737085	0.255048
6	C	2.04974	-2.00367	-0.27809	32	N	-3.06022	2.735629	0.400313
7	C	2.604096	-0.75232	-0.07419	33	H	-2.14341	-2.19431	-0.27971
8	S	1.382958	0.443713	0.121992	34	H	-1.40667	0.761165	0.126448
9	C	4.017715	-0.4066	-0.02089	35	H	0.047009	-2.87498	-0.44271
10	C	4.939445	-1.37084	0.413366	36	H	2.63878	-2.89582	-0.45108
11	N	6.243774	-1.13991	0.474802	37	H	4.61805	-2.35216	0.735261
12	C	6.780997	0.083059	0.125943	38	H	3.848554	1.615105	-0.72838
13	C	5.890404	1.103944	-0.3058	39	H	8.854261	-0.43372	0.516246
14	C	4.51526	0.834446	-0.37553	40	H	9.703497	1.763447	-0.11707
15	C	8.16176	0.33117	0.191216	41	H	8.17313	3.562502	-0.87507
16	C	8.637005	1.572521	-0.16754	42	H	5.733978	3.140348	-0.99718
17	C	7.767991	2.595503	-0.59857	43	H	6.519761	-3.09008	1.164664
18	C	6.418368	2.366088	-0.66755	44	H	7.836057	-2.46711	0.148107
19	C	7.12713	-2.21896	0.937009	45	H	7.654071	-1.89672	1.834111
20	C	-4.27555	0.498277	0.075162	46	H	-3.85805	-3.02588	-1.73271
21	C	-5.70029	0.311623	0.035424	47	H	-5.62871	-3.06962	-1.71328
22	O	-5.99155	-0.96303	-0.14461	48	H	-4.77993	-1.66661	-2.4083
23	C	-4.76923	-1.75829	-0.24351	49	H	-3.88345	-3.38765	0.858361
24	C	-4.7576	-2.41847	-1.61705	50	H	-4.81792	-2.26074	1.863841
25	C	-4.7812	-2.76807	0.897841	51	H	-5.65335	-3.41724	0.797784
26	C	-6.73489	1.220475	0.150428					

Table S13: Calculated atomic coordinates for probe **EH** in water.

Row	Symbol	X	Y	Z
1	C	-3.68266	-0.75792	0.000017
2	C	-2.35573	-1.1748	-2.5E-05
3	C	-1.24615	-0.33935	-0.0005
4	C	0.080791	-0.76547	-0.00035
5	C	0.616746	-2.06616	0.000512
6	C	1.992506	-2.10731	0.000463
7	C	2.600763	-0.83241	-0.00044
8	S	1.386819	0.402366	-0.00124
9	C	3.972718	-0.47729	-0.00079
10	C	4.936419	-1.451	-0.00065
11	N	6.261506	-1.21744	-0.00078
12	C	6.758865	0.098379	-5.9E-05
13	C	5.860731	1.176925	-0.0002
14	C	4.368424	0.972794	-0.00196
15	C	8.137792	0.336782	0.000992
16	C	8.623067	1.63854	0.002038
17	C	7.741288	2.714071	0.002115
18	C	6.371674	2.470711	0.001008
19	C	7.182551	-2.3431	-0.00074
20	C	-4.28333	0.509176	-0.00016
21	C	-5.69308	0.335997	0.000056
22	O	-6.01237	-0.95039	0.000289
23	C	-4.80354	-1.77322	0.00049
24	C	-4.824	-2.61787	-1.26687
25	C	-4.82373	-2.61686	1.268536
26	C	-6.73559	1.258874	0.000125
27	C	-6.53601	2.654422	-0.00046
28	N	-6.4179	3.807829	-0.00093
29	C	-8.07028	0.796986	0.000689
30	N	-9.16933	0.4275	0.001182
31	C	-3.622	1.749726	-0.00051
32	N	-3.04272	2.753662	-0.00066
33	H	-2.19185	-2.24804	0.000271
34	H	-1.40975	0.73433	-0.00109
35	H	-0.00403	-2.95398	0.001103
36	H	2.556021	-3.0313	0.001104
37	H	8.840546	-0.48684	0.001034
38	H	9.695219	1.80614	0.002839
39	H	8.114518	3.732868	0.003016
40	H	5.67392	3.303806	0.000994
41	H	6.607189	-3.26619	-0.00138
42	H	7.81427	-2.3232	-0.89188
43	H	7.813455	-2.32389	0.890995
44	H	-3.93561	-3.25135	-1.30573
45	H	-5.70784	-3.2597	-1.26787
46	H	-4.84004	-1.98186	-2.15439
47	H	-3.93522	-3.25015	1.307829
48	H	-4.83976	-1.98012	2.155535
49	H	-5.70746	-3.25883	1.270164
50	H	3.932396	1.480457	0.871119
51	H	3.93473	1.478279	-0.8775
52	H	4.66888	-2.4997	-0.00042

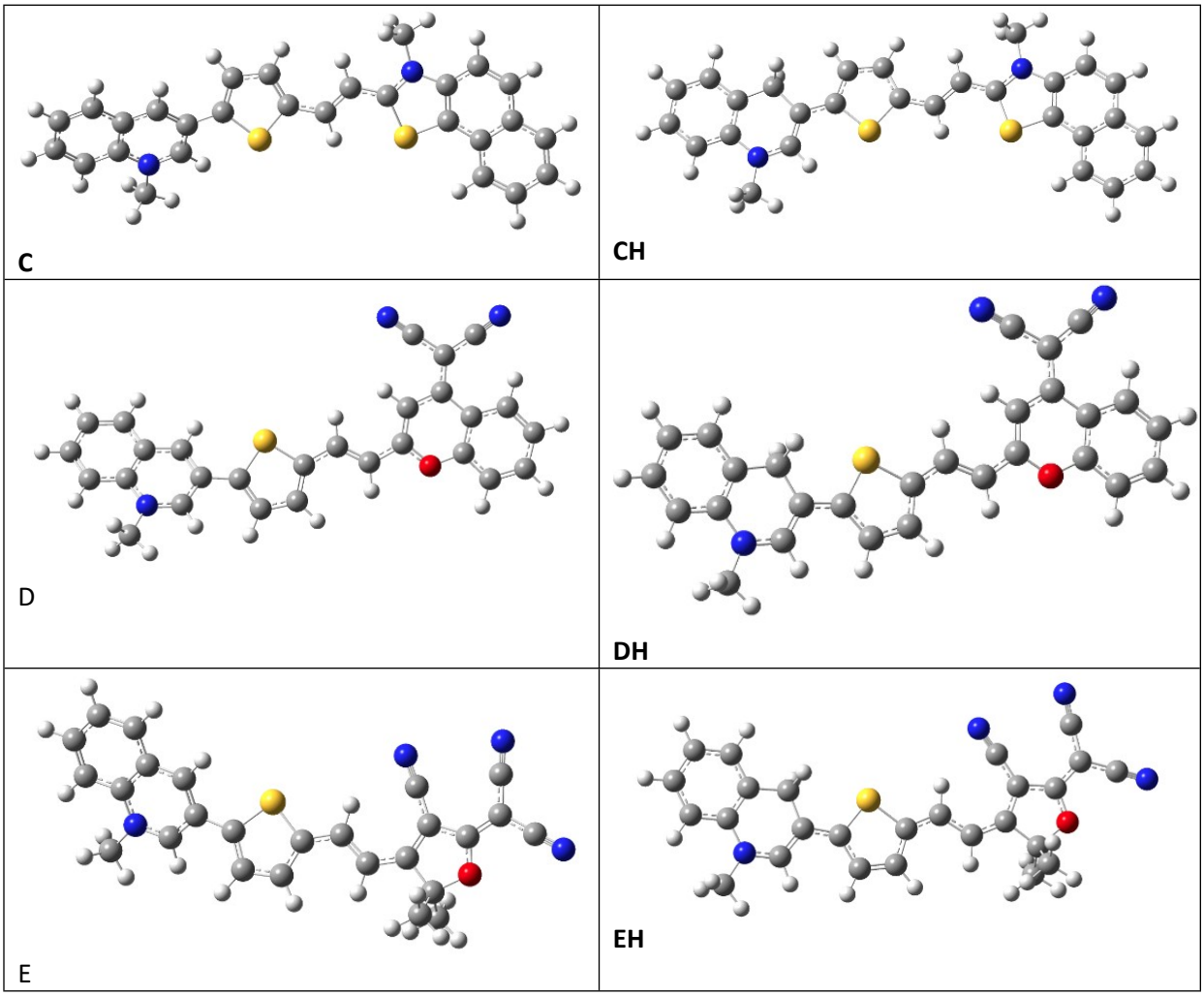


Figure S28. Drawings of the molecules **C**, **D**, **E, CH**, **DH**, and **EH** listed in Table 1 using GausView.[22]

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