

Electronic Supporting Information

Deciphering Micro-polarity Inside the Endoplasmic Reticulum Using Two-photon Active Solvatofluorochromic Probe

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General

THF was distilled/dried using standard sodium/benzophenone ketyl protocol. All other chemicals were used as received unless otherwise stated. Thin layer chromatography was performed using pre-coated plates from Merck and visualized using UV irradiation ($\lambda=254/365\text{nm}$). Silica gel from Merck (particle size 100-200 mesh) was used for column chromatography. ^1H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometers with operating frequencies of 100 MHz for ^{13}C . Chemical shifts (δ) are reported in ppm relative to the residual solvent signal ($\delta = 7.26$ for ^1H and $\delta = 77.0$ for ^{13}C NMR). High-resolution mass spectrometry (HRMS) data were recorded on MicrOTOF-Q-II mass spectrometer using methanol as the solvent.

Instrumentation and methodology

Steady-state absorption and fluorescence measurement

Steady-state absorption was done using Cary 5000 Spectrophotometer from Agilent Technologies quartz cuvette. All solvent and temperature-dependent steady-state fluorescence measurements were carried out using HORIBA Jobin Yvon Fluorolog fluorimeter. A dilute solution of the sample was taken for all the measurements to keep the absorption value at such that we can avoid inner filter effect. Fluorescence spectra were recorded using 1 cm path length quartz cuvette and with excitation and emission slit were adjusted accordingly. All the experiments were carried out at ambient temperature (298 K) otherwise mentioned.

Time-resolved measurement

Time-resolved fluorescence measurements were performed using the time-correlated single photon counting (TCSPC) setup from Horiba. The instrument response function (IRF) was measured before and after fluorescence lifetime measurement using a dilute suspension of Ludox (from Sigma) colloidal silica. The emission polarizer was positioned at magic angle (54.7°) polarization with respect to excitation polarizer. Single and bi-exponential fitting function was employed by iterative deconvolution method using supplied software DAS v6.2. The quality of the fitted data was judged from the reduced chi-squared value (χ^2), calculated using the IBH software provided with the instrument.

Two-photon microscopy Two-photon fluorescence imaging experiments were performed using Olympus laser-scanning microscope (LSM) attached to a Mai Tai eHP Spectra physics femtosecond (fs) laser as an excitation source, pulse width ≤ 70 fs, tuning wavelength range 690-

1040 nm, average power >2.5 mW, repetition rate 80 MHz ± 1 MHz was used to acquire the two photon fluorescence image.

Two Photon Induced Fluorescence

Two-photon absorption cross section was determined by using two photon induced fluorescence technique.¹⁻² For this, we used MIRA 900 F oscillator which is a commercially available Ti: Sapphire oscillator pumped by Verdi-5. This laser source uses Kerr lens mode locking phenomenon to produce femtosecond pulses. It produces transform limited 120-200 fs pulses at 76 MHz repetition rate. The output of the laser is around 500 mW. All the samples were prepared in Acetonitrile having 10⁻³ M concentration whereas the reference Rh6G was prepared in methanol at the same concentration (10⁻³ M)

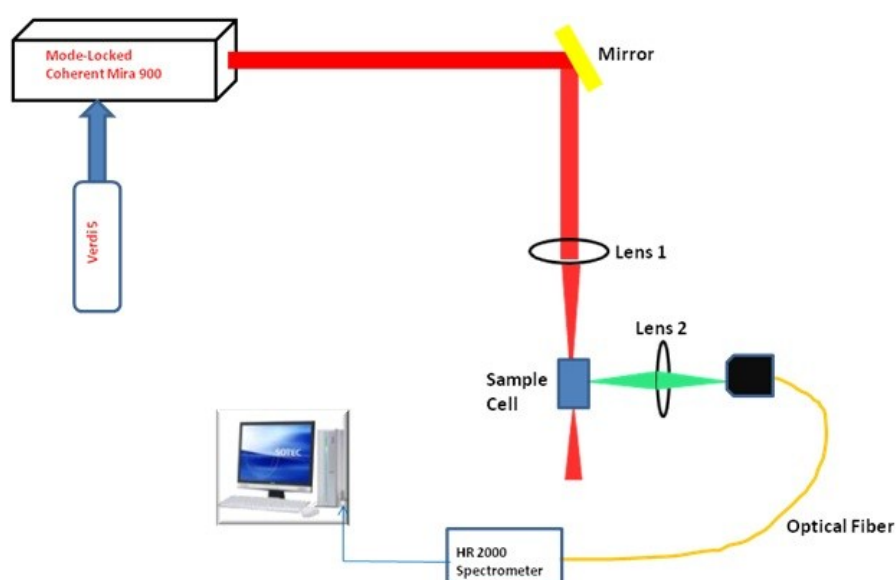


Fig. S1: Schematic diagram of the two photon induced optical setup.

We used the following equation to determine the Two-photon absorption cross section of the samples:³

$$\sigma = \sigma_{ref} \frac{F\Phi_{ref} C_{ref} n_{ref}}{F_{ref} \Phi C n}$$

where “ref” subscript stands for reference while the ones without any are for the sample.

σ : Two photon absorption cross section, F: Integrated area of Two photon Induced fluorescence spectra, Φ : Fluorescence quantum yield, C: Concentration in moles/Litre, n: Refractive Index of the solvents used.

We used 780 nm as excitation wavelength with pulse duration of 149 fs (can be calculated as

$$\Delta t = \frac{0.441\lambda^2}{c|\Delta\lambda|}, \text{ where } \lambda = 780 \text{ nm and } \Delta\lambda = 6 \text{ nm and also } c \text{ is the speed of light in vacuum)}$$

for power dependent study for each sample by keeping the integration time as 1000 ms.

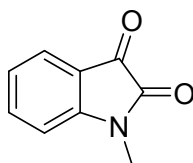
Co-localisation microscopy and fluorescence spectral scanning experiment

Glass bottom culture dish was seeded with MDA-MB-231 cells and were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. The cells were washed thrice with PBS (pH 7.4 with 5 mM MgCl₂). Now for colocalization experiment, cells were stained with 2.0 μ M of **10b** and 1.0 μ M of ER-Tracker red for 30 min at 37 °C. Subsequently washed thrice with aforementioned buffer and imaged under microscope. For **10b** and ER-Tracker red blue and red channel were used respectively. For the spectral scanning experiment, cells were only stained with 2.0 μ M of **10b** and scanning was carried out with a array of detector. To obtain the spectrum unmixing was done with associated software.

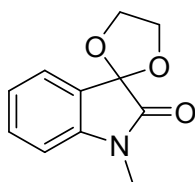
MTT assay:

Around 5000-10000 cell/well were seeded in a 96 well plate and grown for 24 hours and the treatment of **10b** with various concentrations was given for 24 hours. Then 5 mg/mL in PBS MTT solution (20 μ L in each well) was added and kept for 4 hours. After that media was removed completely and add 200 μ L DMSO in each well to dissolve the crystals and finally the absorption was recorded at 570 nm.

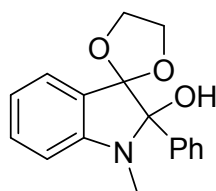
Synthetic procedure:



1-methylindoline-2,3-dione (1): Indoline-2,3-dione (5 g, 33.98 mmol) was dissolved in anhydrous DMF (35 ml), and the resultant solution was cooled to 0 °C and stirred for 5 min, whereupon NaH (60% dispersion in oil, 1.67 g, 40.7 mmol) was added in portions and stirred for 20 minutes. Iodomethane (2.53 ml, 40.7 mmol) was added and the reaction was stirred for 30 min. The reaction mixture was then poured into saturated aqueous NH₄Cl and extracted with EtOAc (4 × 30 ml). The combined organic layers were washed with water (3 × 15 ml) and brine (20 ml), then dried over anhydrous Na₂SO₄, filtered, and concentrated to give the crude product which was subjected to column chromatography in silica (100-200 mesh) with solvent EtOAc/ Hexane (1:3). R_f = 0.55 (50 % EtOAc/hexane) to obtain pure N-methyl isatin as deep red colored solid (96 % yield). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.72 – 7.45 (m, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 7.9 Hz, 1H), 3.24 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ_{ppm} 183.36, 158.24, 151.47, 138.44, 125.26, 123.84, 117.44, 109.95, 26.22.

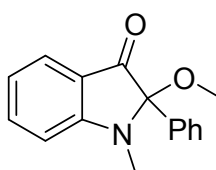


1'-methylspiro[[1,3]dioxolane-2,3'-indolin]-2'-one (2): A mixture of **1** (4.9 g, 30.41 mmol), ethylene glycol (17.04 ml, 18.91 g, 304.98 mmol) and p-toluene sulfonic acid (0.578 g, 3.041 mmol) was dissolved in dry toluene (150 ml) and set to reflux in dean-stark apparatus for overnight. The reaction progress was checked with TLC. After completion of the reaction, solid Na₂CO₃ (12.0 g) was added to the reaction mixture to quench the reaction. Then water was added and the product was extracted with DCM. The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated in rotary evaporator. The product was purified by column chromatography in silica gel in EtOAc/hexane (1:10) to obtain a pale yellow colored product (**2**, 87% yield). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.37 (t, *J* = 7.3 Hz, 2H), 7.06 (dd, *J* = 25.2, 17.6 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 4.70 – 4.43 (m, 2H), 4.39 – 4.19 (m, 2H), 3.13 (s, 3H).



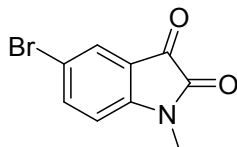
1'-methyl-2'-phenylspiro[[1,3]dioxolane-2,3'-indolin]-2'-ol (3): Mg turnings (0.915 g, 37.644 mmol) (kept in hot air oven at 120° C overnight prior to use) were taken in a three-necked round bottom flask and decorated with condenser and septum and the inside atmosphere were made of N₂. Then 40 ml dry THF was added to it followed by a pinch of iodine crystal. Then Phenyl bromide (4.0 ml, 6.0 g, 38.219 mmol) was added drop-wise. The color of the reaction mixture should be faint within few minutes and gradually tends to blackish color. The reaction should be self-sustainable but water bath should be kept nearby to control the reaction if needed. No particles of Mg turnings were left, indicates the completion of the reaction (40-50 mins) *i.e.*, the Grignard reagent was ready for use.

2 (5.15 g, 25.096 mmol) was taken in a round bottom flask and dissolved in 100 ml dry THF using N₂ as inner atmosphere. Then the freshly prepared PhMgBr was added to the second RB dropwise with a syringe at room temperature and stirred for 18 hrs. The reaction progress was monitored by TLC. After the consumption of the reactant, completely saturated NH₄Cl solution (40 mL) was added to reaction mixture to quench the reaction. Then 50 ml water was added and the crude was extracted with EtOAc. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated in vacuum. Column chromatography was done in silica gel with the solvent system EtOAc/Hex (1:10) to obtain a pale yellow colored product (**3**, 85% yield).

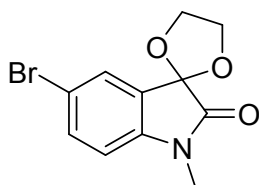


2-methoxy-1-methyl-2-phenylindolin-2-one (4): **3** (6.1 g, 21.53 mmol) was dissolved in 40 ml Methanol and freshly prepared 1.923 ml 8(N) H₂SO₄ solution was added to it. The solution becomes deep green colored almost instantly and then the reaction mixture was stirred at room temperature for 30 min. The progress of the reaction was monitored by TLC. Column chromatography was done in silica (100- 200 mesh) with 5%- 30% EtOAc/Hex solvent systems to obtain the pure bright yellow colored product with 95% yield. ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.59 – 7.49 (m, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.29 (m, 3H), 6.81

(d, $J = 8.4$ Hz, 1H), 6.75 (t, $J = 7.4$ Hz, 1H), 3.27 (s, 3H), 2.84 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ_{ppm} 199.08, 161.49, 138.84, 135.39, 128.88, 126.29, 125.51, 118.64, 117.90, 107.45, 95.49, 51.70, 27.33, Mass calculated $[\text{M}+\text{Na}]^+=276.0995$, obtained=276.0994.

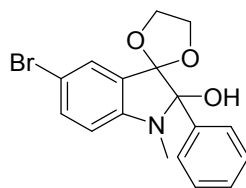


5-bromo-1-methylindoline-2,3-dione (1'):⁴ A mixture of 1-methylindoline-2,3-dione (**1**) (4.9 g, 30.43 mmol) and NH_4OAc (0.264 g, 3.43 mmol) was dissolved in MeCN (150 ml) and NBS (5.69 g, 31.95 mmol) was added to it. The reaction mixture was stirred at room temperature for 2.5 hrs. The reaction could not be monitored by TLC as the R_f values of the reactant and the product are same. The reaction mixture was concentrated under vacuum and extracted with EtOAc (3×20 ml) and washed with water (3×30 ml). The combined organic layer was washed with brine, dried over anhydride Na_2SO_4 and concentrated under vacuum to obtain a bright red colored solid (**1'**) which was used without further purification (97 % yield). ^1H NMR (400 MHz, CDCl_3) δ_{ppm} 7.73 (d, $J = 2.0$ Hz, 1H), 7.71 (s, 1H), 6.81 (d, $J = 8.9$ Hz, 1H), 3.25 (s, 3H) ^{13}C NMR (100 MHz, CDCl_3): δ_{ppm} 182.08, 157.28, 149.82, 140.70, 127.77, 118.65, 116.61, 111.82, 26.36.



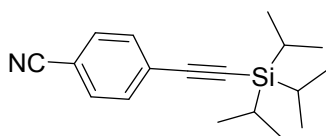
5'-bromo-1'-methylspiro[[1,3]dioxolane-2,3'-indoline]2'-one (5): A mixture of **1'** (9.9 g, 41.24 mmol), ethylene glycol (23.13 ml, 25.67 g, 413.53 mmol) and *p*-toluene sulfonic acid (0.784 g, 4.124 mmol) was dissolved in ml dry toluene (180 ml) and set to reflux in deanstark apparatus for overnight. The reaction progress was checked with TLC. After completion of the reaction, solid Na_2CO_3 (15 g) was added to the reaction mixture to quench the reaction. Then water was added and the product was extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuum. The product was purified by column chromatography in silica gel with EtOAc/Hexane (1:10) as eluent to obtain a pale yellow colored product with 82 % yield. ^1H NMR (400 MHz, CDCl_3): δ_{ppm} 7.49-7.45 (m, 2H), 6.66 (d, $J = 8.14$ Hz, 1H), 4.55 (m, 2H), 4.30 (m, 2H), 3.10 (s, 3H). ^{13}C

NMR (100 MHz, CDCl₃): δ_{ppm} 172.71, 143.72, 134.37, 128.12, 125.95, 116.98, 115.87, 110.14, 101.75, 65.98, 25.92.

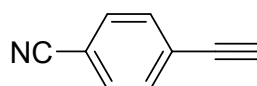


5'-bromo-1'-methyl-2'-phenylspiro[[1,3]dioxolane-2,3'-indoline]2'-ol (6): Mg turnings (0.204 g, 8.51 mmol) (kept in hot air oven at 120° C overnight prior to use) were taken in a three-necked round bottom flask and attached with condenser and septum and the inside atmosphere were made of N₂. Then 25 ml dry THF was added to it followed by a pinch of I₂ crystal. Then PhBr (0.906 ml, 1.36 g, 8.68 mmol) was added drop-wise with syringe. The color of the reaction mixture should become faint within few minutes and gradually to blackish color. The reaction is self-sustainable but water bath should be kept nearby to control the reaction if needed. No remaining of Mg turnings indicating the completion of the reaction (40-50 min) *i.e.*, now the Grignard reagent is ready to use.

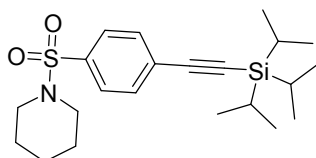
5 (2.2 g, 7.74 mmol) was taken in another round bottom flask and dissolved in 25 ml dry THF and the inner atmosphere of the RB was made of N₂. Then the freshly prepared phenyl magnesium bromide (PhMgBr) was added to the second RB dropwise with syringe at room temperature and stirred for 18 hrs. The reaction progress was monitored by TLC. After the consumption the reactant completely saturated NH₄Cl solution (25 mL) was added to reaction mixture to quench the reaction. Then 50 ml water was added and the crude was extracted with EtOAc. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated in vacuum. Column chromatography was done in silica (100-200 mesh) with the solvent system EtOAc/Hex (1:10) to obtain a pale yellow colored product (**6**, 82%). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.53 – 7.46 (m, 2H), 7.38 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.36 – 7.33 (m, 3H), 7.31 (d, *J* = 2.0 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 4.10 (dd, *J* = 14.4, 7.4 Hz, 2H), 4.06 – 4.00 (m, 1H), 3.96 – 3.89 (m, 1H), 3.88 (s, 1H), 3.20 (dd, *J* = 14.9, 7.6 Hz, 1H), 2.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ_{ppm} 150.56, 137.10, 134.30, 128.36, 127.91, 127.60, 127.43, 126.75, 113.09, 109.51, 108.44, 97.11, 66.20, 65.24, 28.43.



4-((triisopropylsilyl)ethynyl)benzonitrile (8a): In a clean and dry Schlenk tube, *p*-iodobenzonitrile (*p*-CN-C₆H₄-I) (500 mg, 2.18 mmol) was dissolved in 8.7 ml piperidine and degassed thoroughly by deep freezing the solution in liquid nitrogen and vacuum. Then Pd(PPh₃)₄ (0.252 g, 0.218 mmol), CuI (42 mg, 0.218 mmol) and (Triisopropylsilyl) acetylene (0.980 ml, 0.796 g, 4.37 mmol) were added sequentially and the reaction mixture was stirred for 20 min. The progress of the reaction was checked by TLC. After completion of the reaction, the reaction mixture was washed with EtOAc and concentrated in vacuum. Then column chromatography was done in silica gel with EtOAc/Hexane (5-10%) to obtain the pure liquid product (**8a** with 99% yield). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.57 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 1.11 (d, *J* = 2.7 Hz, 21H).

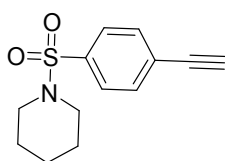


4-ethynylbenzonitrile (9a): In a round bottom flask **8a** (0.577 g, 2.034 mmol) was dissolved in dry THF (30 ml) and the inner atmosphere was made of N₂. After cooling the solution to 0° C, tetrabutylammonium fluoride (2.39 ml, 2.39 mmol, 1M solution in THF) was added drop-wise to this solution and stirred for 5 min. The progress of the reaction was checked by TLC. After the reaction was over ml water was added and the crude was extracted with DCM (3x20 ml). The combined organic layer was washed with water three times and dried over anhydrous Na₂SO₄. After evaporation of the solvent purify the product by column chromatography in silica gel with EtOAc/hexane (1:20) to obtain the pure solid product with quantitative yield (**9a**). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.62 (d, *J* = 8.4 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 3.30 (s, 1H).

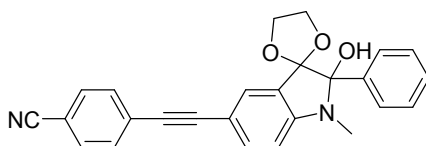


1-((4-((triisopropylsilyl)ethynyl)phenyl)sulfonyl)piperidine (8b): In a dry Schlenk tube, 4-iodobenzene-1-sulfonyl chloride (500 mg, 1.653 mmol) was dissolved in 7.62 ml piperidine

and degassed thoroughly by deep freezing the solution in liquid nitrogen and vacuum. Then Pd(PPh₃)₄ (0.191 g, 0.165 mmol), CuI (32 mg, 0.165 mmol) and (Triisopropylsilyl)acetylene (0.749 ml, 0.603 g, 3.306 mmol) were added sequentially and the reaction mixture was stirred for 20 mins. The progress of the reaction was checked by TLC. After completion of the reaction, the reaction mixture was washed with EtOAc and concentrated in vacuum. Then column chromatography was done in silica (100- 200 mesh) with EtOAc/Hex (10%) to obtain the pure liquid product with 99% yield (**8b**). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.68 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 3.05 – 2.86 (m, 4H), 1.62 (dd, *J* = 11.0, 5.6 Hz, 5H), 1.41 (d, *J* = 4.1 Hz, 2H), 1.13 (d, *J* = 2.3 Hz, 21H).

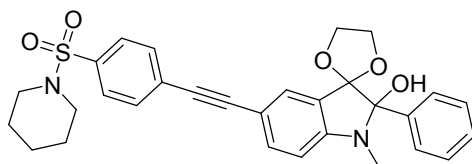


1-((4-ethynylphenyl)sulfonyl)piperidine (9b): In a round bottom flask **8b** (0.5812 g, 1.63 mmol) was dissolved in dry THF (30 ml) and the inner atmosphere was made of N₂. After cooling the solution to 0° C, tetrabutylammonium fluoride (TBAF) (2.45 ml, 2.45 mmol, 1M solution in THF) was added dropwise to this solution and stirred for 5 min. The progress of the reaction was checked by TLC. After the reaction water was added and the crude was extracted with DCM (3x20 ml). The combined organic layer was washed with water three times and dried over anhydrous Na₂SO₄. After evaporation of the solvent purified the product by column chromatography in silica (100-200 mesh) with ethyl acetate/hexane (1:10) to obtain the pure solid product with quantitative yield (**9b**). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.71 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 3.25 (s, 1H), 3.02 – 2.96 (m, 4H), 1.69 – 1.60 (m, 4H), 1.43 (dt, *J* = 11.6, 5.9 Hz, 2H).



4-((2'-hydroxy-1'-methyl-2'-phenylspiro[[1,3]dioxolane-2,3'-indolin]-5'-yl)ethynyl)benzotrile (10a):⁵ In a microwave reaction vessel, **5** (272 mg, 0.750 mmol), **9a** (100 mg, 0.787 mmol), trimethylamine (0.45 ml) and dry toluene (2.25 ml) was mixed well and degassed thoroughly by deep freezing in liquid N₂ and vacuum and. When the mixture was solid due to exposure to liquid N₂, Pd₂(dba)₃ (69 mg, 0.075 mmol) and *p*-(*o*-tolyl)₃ (149 mg, 0.488 mmol)

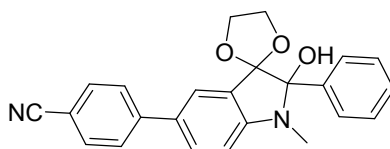
was added and then the vessel was sealed with cap decorated with septum and the inner atmosphere was made of N₂. Then the reaction vessel was entered to microwave chamber and the reaction mixture was exposed to microwave for 10 minutes at 60 °C. Progress of the reaction was checked by TLC. After the reaction was over, the reaction mixture was washed with DCM and concentrated in vacuum. Column chromatography was done in silica (100-200 mesh) with 10%-30% EtOAc/Hexane solvent system to obtain the pure product with 90% yield (**10a**). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.69 – 7.60 (m, 4H), 7.58 (d, *J* = 13.3 Hz, 1H), 7.53 (d, *J* = 14.3 Hz, 2H), 7.49 (s, 1H), 7.34 (s, 3H), 6.62 (d, *J* = 8.3 Hz, 1H), 4.20 – 4.06 (m, 2H), 4.01 – 3.92 (m, 2H), 3.23 (dd, *J* = 15.1, 7.5 Hz, 1H), 2.66 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ_{ppm} 152.00, 136.90, 136.11, 131.99, 131.62, 129.05, 128.41, 128.35, 127.97, 127.39, 125.00, 118.82, 113.00, 111.05, 110.55, 106.52, 97.07, 95.30, 86.24, 66.18, 65.32, 28.20, Mass calculated [M+H]⁺=409.1547, obtained=409.1547.



1'-methyl-2'-phenyl-5'-((4-(piperidine-1-ylsulfonyl)phenyl)ethynyl)spiro[[1,3]

dioxolane-2,3'-indolin]-2'-ol (10b) In a microwave reaction vessel, **5** (378 mg, 1.044 mmol), **9b** (220 mg, 1.096 mmol), trimethylamine (0.627 ml) and dry toluene (3.132 ml) was mixed well and degassed thoroughly by deep freezing in liquid N₂ and vacuum and. When the mixture was solid due to exposure to liquid N₂, Pd₂(dba)₃ (95 mg, 0.104 mmol) and *p*-(*o*-tolyl)₃ (207 mg, 0.679 mmol) was added and then the vessel was sealed with cap connected with septum and the inner atmosphere was made of N₂. Then the reaction vessel was entered to microwave chamber and the reaction mixture was exposed to microwave for 10 mins at 60°C. Progress of the reaction was checked by TLC. After the reaction was over, the reaction mixture was washed with DCM and concentrated in vacuum. Column chromatography was done in silica (100-200 mesh) with 10%- 30% EtOAc/hexane solvent to obtain the pure product with 82% yield (**10b**) ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.79 – 7.69 (m, 3H), 7.63 – 7.58 (m, 2H), 7.55 – 7.48 (m, 3H), 7.44 (d, *J* = 1.8 Hz, 1H), 7.40 – 7.34 (m, 3H), 6.55 (d, *J* = 8.2 Hz, 1H), 4.20 – 4.13 (m, 2H), 4.10 (t, *J* = 6.5 Hz, 2H), 3.99 (d, *J* = 5.8 Hz, 1H), 3.32 (q, *J* = 7.5 Hz, 6H), 3.02 (t, *J* = 5.4 Hz, 3H), 2.69 (s, 1H), 1.51 – 1.40 (m, 4H). ¹³C NMR (101 MHz, CDCl₃) δ_{ppm} 136.77, 135.65, 134.80, 131.52, 128.72, 128.42, 128.27, 127.97, 127.59,

127.49, 125.03, 112.82, 110.86, 106.29, 97.01, 67.30, 66.24, 46.95, 28.38, 25.43, 23.54, Mass calculated $[M+H]^+=531.1948$, obtained=531.1967.



4-(2'-hydroxy-1'-methyl-2'-phenylspiro[[1,3]dioxolane-2,3'-indolin]-5'-yl)benzonitrile

(7): To a mixture of **6** (190 mg, 0.525 mmol), 4-cyanophenylboronic acid (78 mg, 0.53 mmol), Pd(OAc)₂ (0.6 mg, 0.0027 mmol) and K₂CO₃ (256 mg, 1.86 mmol); PEG-400 (1.59 ml) and H₂O (1.59 ml) was added and stirred at room temperature for 2 hrs. The progress of the reaction was checked by TLC. The reaction was incomplete even after 2 hrs. The reaction mixture was extracted with Et₂O (3 × 20 ml), dried over anhydrous Na₂SO₄ and evaporated under vacuum. Column chromatography was done in silica (100- 200 mesh) with 10 % EtOAc/hexane solvent system to get the pure product (**7**, 97% yield). ¹H NMR (400 MHz, CDCl₃) δ_{ppm} 7.70 – 7.61 (m, 4H), 7.58 (d, *J* = 8.3 Hz, 1H), 7.55 – 7.51 (m, 2H), 7.49 (s, 1H), 7.39 – 7.32 (m, 3H), 6.64 (d, *J* = 8.3 Hz, 1H), 4.16 (q, *J* = 7.3 Hz, 2H), 4.09 (td, *J* = 7.1, 4.5 Hz, 1H), 3.98 (dd, *J* = 7.4, 4.8 Hz, 1H), 3.95 (d, *J* = 1.1 Hz, 1H), 3.26 (q, *J* = 7.5 Hz, 1H), 2.69 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ_{ppm} 152.08, 145.64, 137.07, 132.47, 131.05, 129.18, 128.43, 128.01, 127.60, 126.70, 125.31, 123.44, 119.29, 113.49, 109.40, 107.19, 97.16, 66.24, 65.11, 28.37, Mass calculated $[M+H]^+=385.1547$, obtained=385.1522.

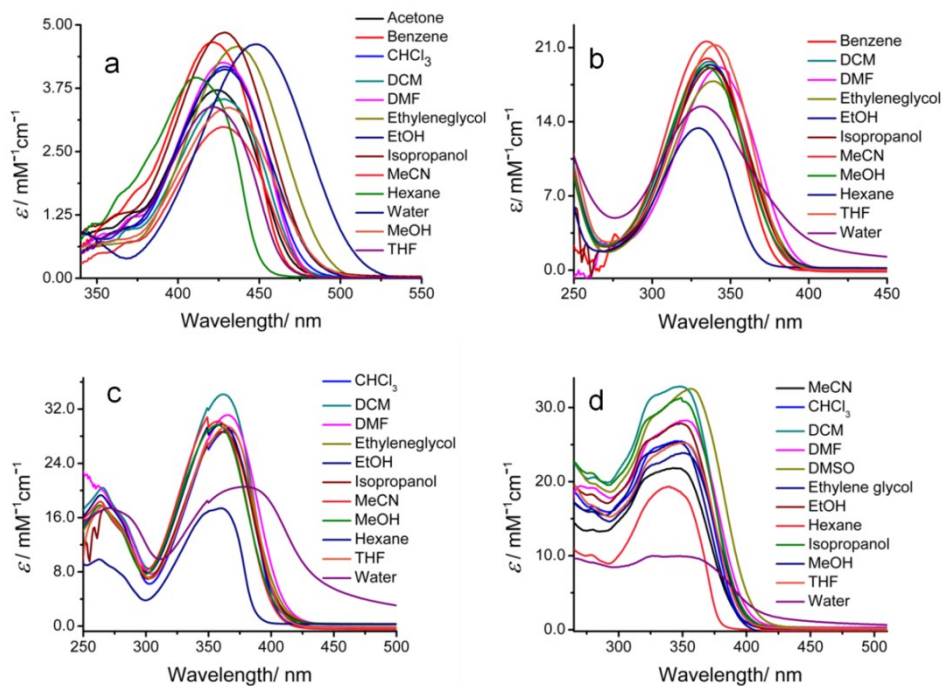


Fig. S1. Solvent polarity-dependent UV-Vis. spectra of **4** (a), **7** (b), **10a** (c) and **10b** (d) showing minimal effect of solvent polarity.

Table S1. Solvent-dependent photophysical properties of multicolor probes

Solvent	Dye	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	$E_T(30)^a$	Stokes shift (cm^{-1})	Φ^b (%)	τ (ns)
Acetone	4	424	504	42.2	3744	23.2	12.5
	7	338	434		6545	27.5	2.0
	10a	360	501		7818	24.6	2.1
	10b	357	481		7221	32.5	1.54
Benzene	4	421	489	34.3	3303	20.3	10.1
	7	335	403		5037	80.5	1.3
	10a	363	426		4074	60.5	1.5
	10b	344	416		5032	99.0	1.44
CHCl_3	4	428	514	39.1	3909	25.3	15.6
	7	339	420		5689	70.0	1.4
	10a	366	456		5392	60.5	1.9

	10b	346	442		6278	62.0	2.23
DCM	4	428	506	40.7	3601	32.2	15.9
	7	339	426		6025	45.2	1.8
	10a	362	472		6438	40.4	2.5
DMF	10b	349	461		6961	44.0	2.45
	4	428	506	43.2	3601	22.6	14.2
	7	343	442		6531	25.5	2.1
	10a	365	520		8166	22.0	1.5
	10b	355	503		8288	15.2	1.55
Ethylene glycol	4	437	560	56.3	5026	4.3	4.08
	7	340	456		7482	15.0	2.2
	10a	362	506		7861	14.6	2.0
	10b	357	495		7809	13.3	0.66
EtOH	4	429	546	51.9	4995	10.1	7.9
	7	337	443		7101	25.6	2.1
	10a	360	502		7857	10.5	0.5
	10b	351	471		7259	7.2	0.78
Hexane	4	410	467	31.0	2977	10.0	5.0
	7	330	385		4329	35.0	1.0
	10a	361	400		2701	18.5	0.7
	10b	339	385		3525	20.0	5.1
<i>i</i> PrOH	4	429	538	48.6	4722	12.5	11.0
	7	338	440		6859	15.8	2.0
	10a	361	490		7293	14.0	1.4
	10b	351	467		7077	9.0	1.3
MeCN	4	427	514	46.0	3964	31.0	15.1
	7	335	440		7124	38.6	2.1
	10a	357	521		8817	28.0	1.4
	10b	343	501		9194	13.0	1.4
MeOH	4	432	550	55.5	4966	8.4	5.3
	7	335	448		7530	15.9	2.2
	10a	357	515		8594	10.2	1.0
	10b	355	489		7719	3.6	1.16

THF	4	421	501	37.4	3793	29.5	10.2
	7	340	421		5659	80.3	1.7
	10a	365	467		5984	55.5	2.5
	10b	353	455		6351	60.0	2.5
Water (pH ~6.0)	4	449	581	63.0	5060	2.0	13.5
	7	332	470		8843	25.0	1.4
	10a	378	481		5665	15.6	1.2
	10b	360	470		6501	11.5	5.5

^a $E_T(30)$ values were taken from reference⁶, ^b with respect to Fluorescein dye in 0.1(N) NaOH,⁷ ^c measured at absorption maxima.

Table S2. Two-photon absorption crosssection of **10b** in Methanol

S. no.	Excitation Wavelength/ nm	TPACS/ GM
1	770 nm	58.15
2	775 nm	46.52
3	780 nm	30.23
4	785 nm	33.85
5	790 nm	33.62

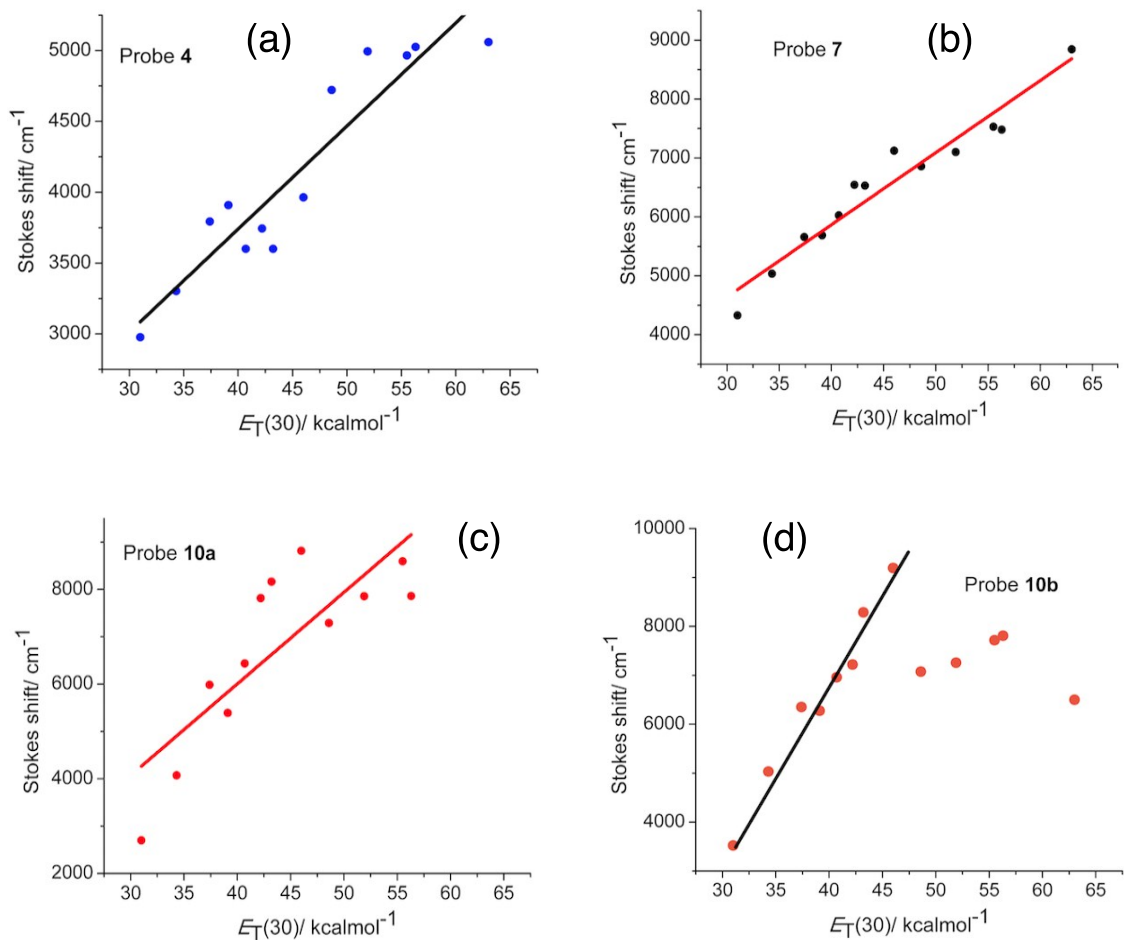


Fig. S2. Plot of Stokes shift against the solvent polarity parameter $E_T(30)$ for (a) **4**, (b) **7**, (c) **10a**, and (d) **10b**.

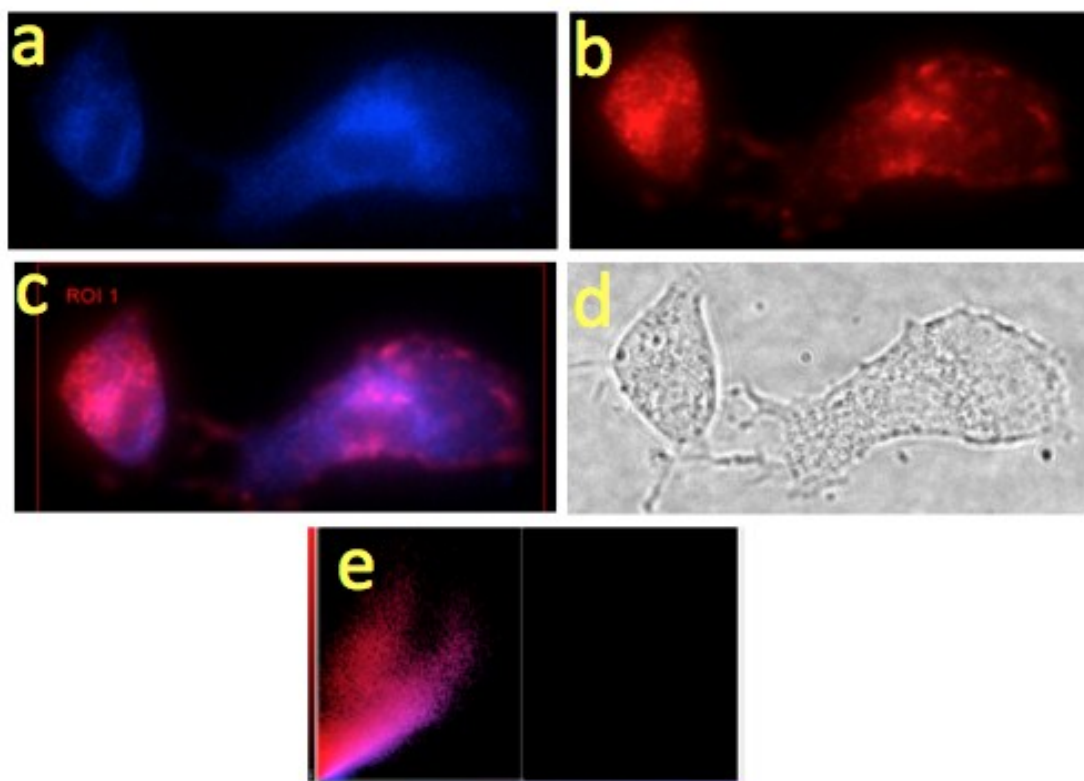


Fig. S3. Co-localization of **10b** and LysoTracker Red inside live cancer cell line B16f10 incubated with (a) $5 \mu\text{M}$ **10b**, (b) $1 \mu\text{M}$ LysoTracker Red, (c) merge images, (d) bright field, and (e) showing scatter plot between blue and red channel Pearson's coefficient = (0.57 ± 0.05)

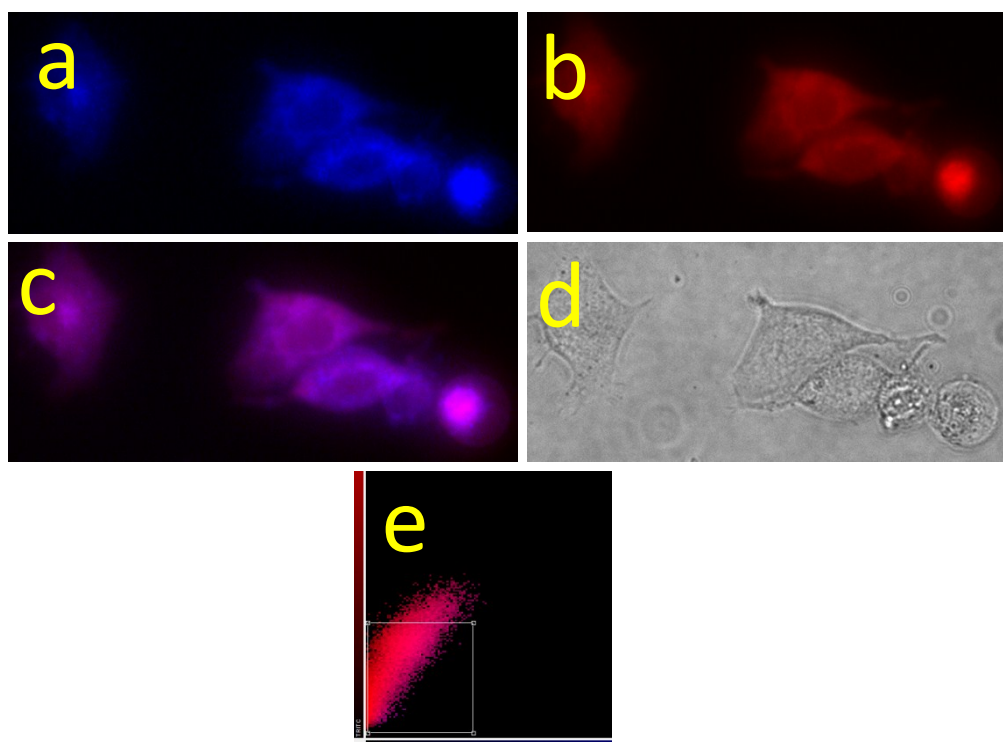


Fig. S4. Co-localization of **10b** and MitoTracker Red inside live cancer cell line B16f10 incubated with (a) $5 \mu\text{M}$ **10b**, (b) $1 \mu\text{M}$ MitoTracker Red, (c) merge images, (d) bright field, and (e) showing scatter plot between blue and red channel Pearson's coefficient = (0.73 ± 0.06)

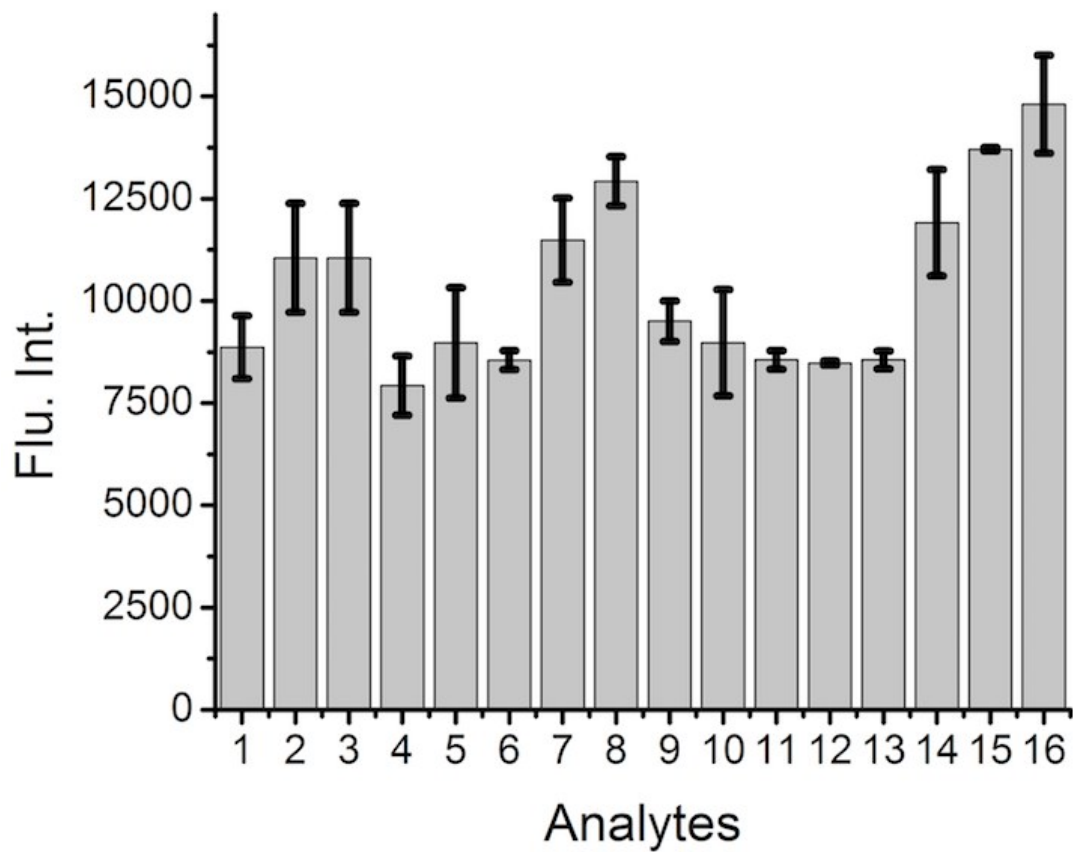


Fig. S5. Effect of different biologically relevant analytes (0.5 mM) on the fluorescence of 10 μ M **10b** 1. Only **10b** 2. Na⁺ 3. HPO₄²⁻ 4. Zn²⁺ 5. Cl⁻ 6. Ca²⁺ 7. HCO₃⁻ 8. Mg²⁺ 9. Cu²⁺ 10. K⁺ 11. Fe²⁺ 12. Fe³⁺ 13. SO₄²⁻ 14. Homocysteine 15. Glutathione 16. Cysteine.

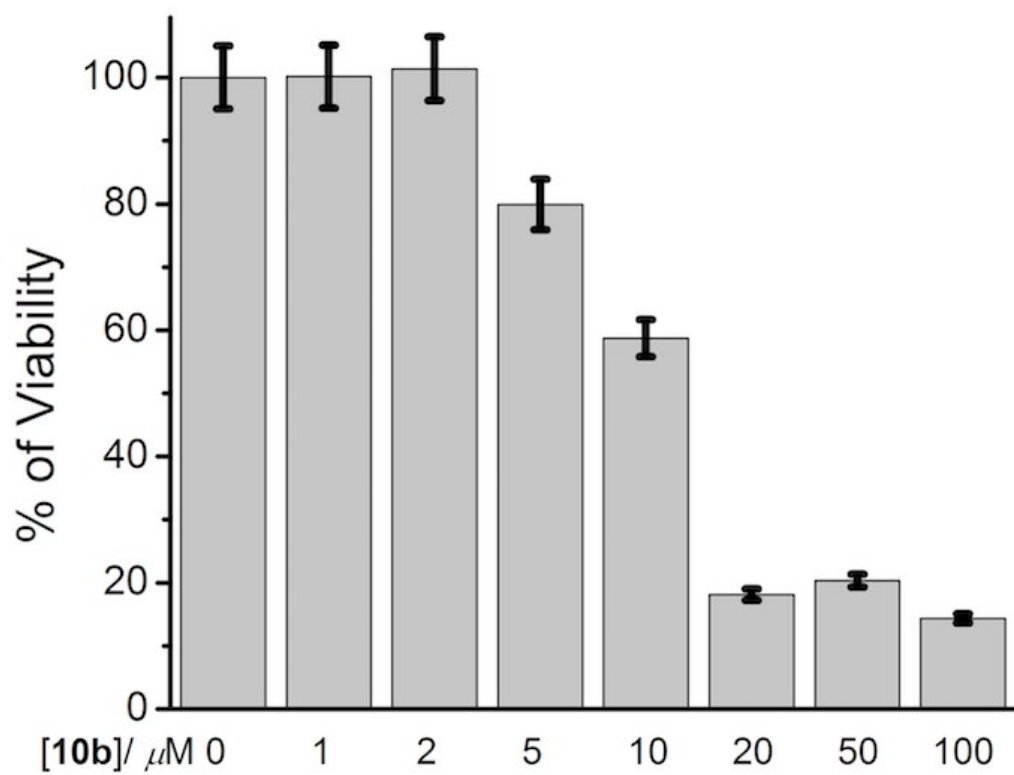


Fig. S6. Cellular viability against various concentration of **10b**, MTT assay showing the IC_{50} value is more than $10 \mu\text{M}$

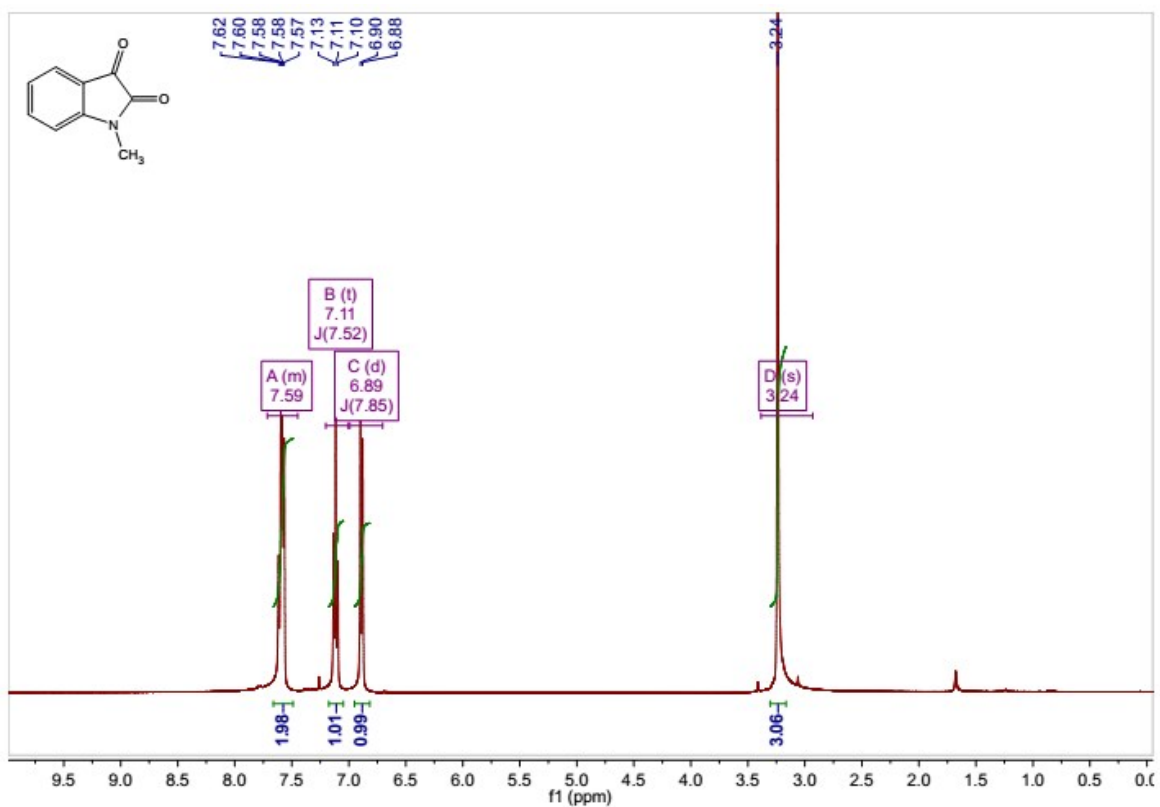


Fig. S7: ¹H NMR spectrum of **1**.

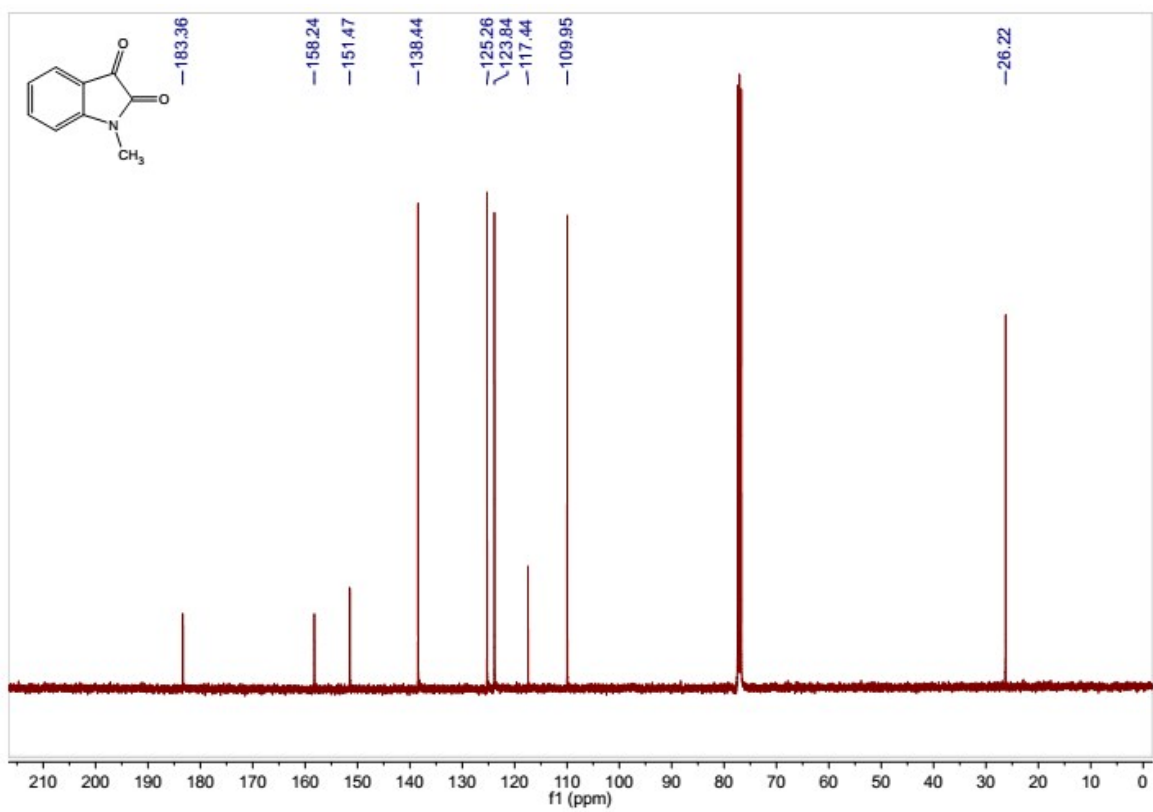


Fig. S8: ¹³C NMR spectrum of **1**.

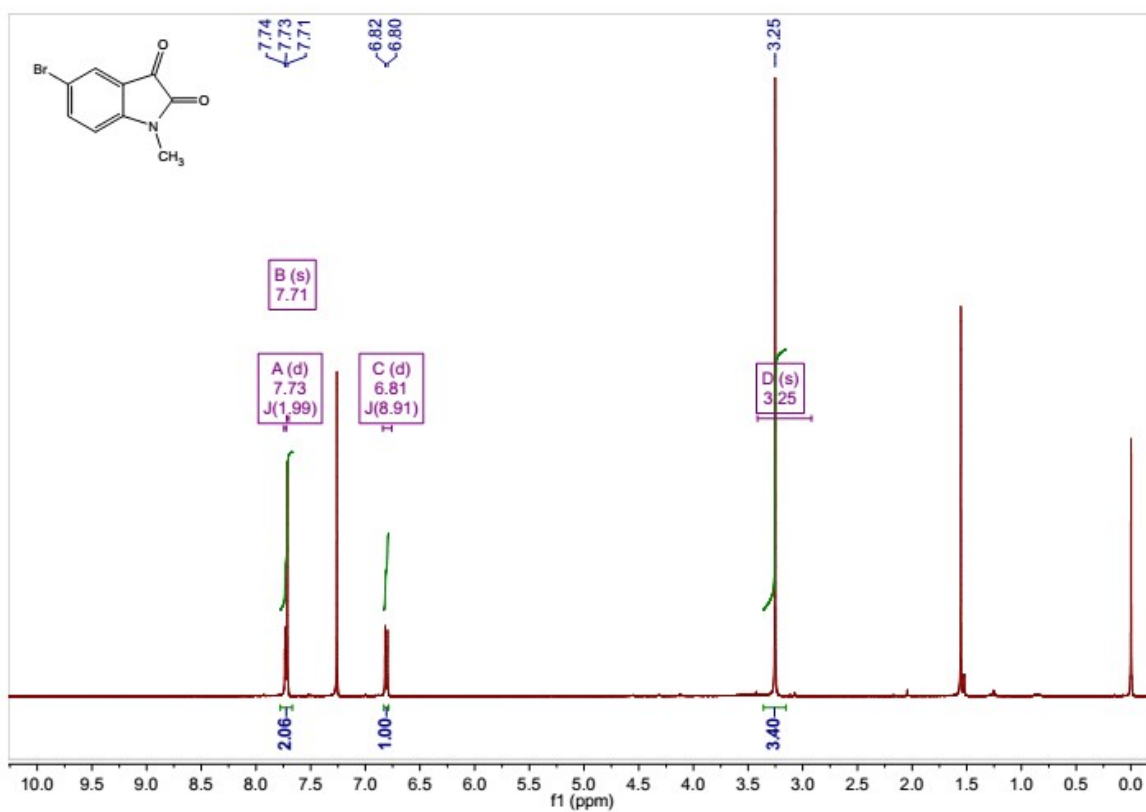


Fig. S9: ¹H NMR spectrum of 1'.

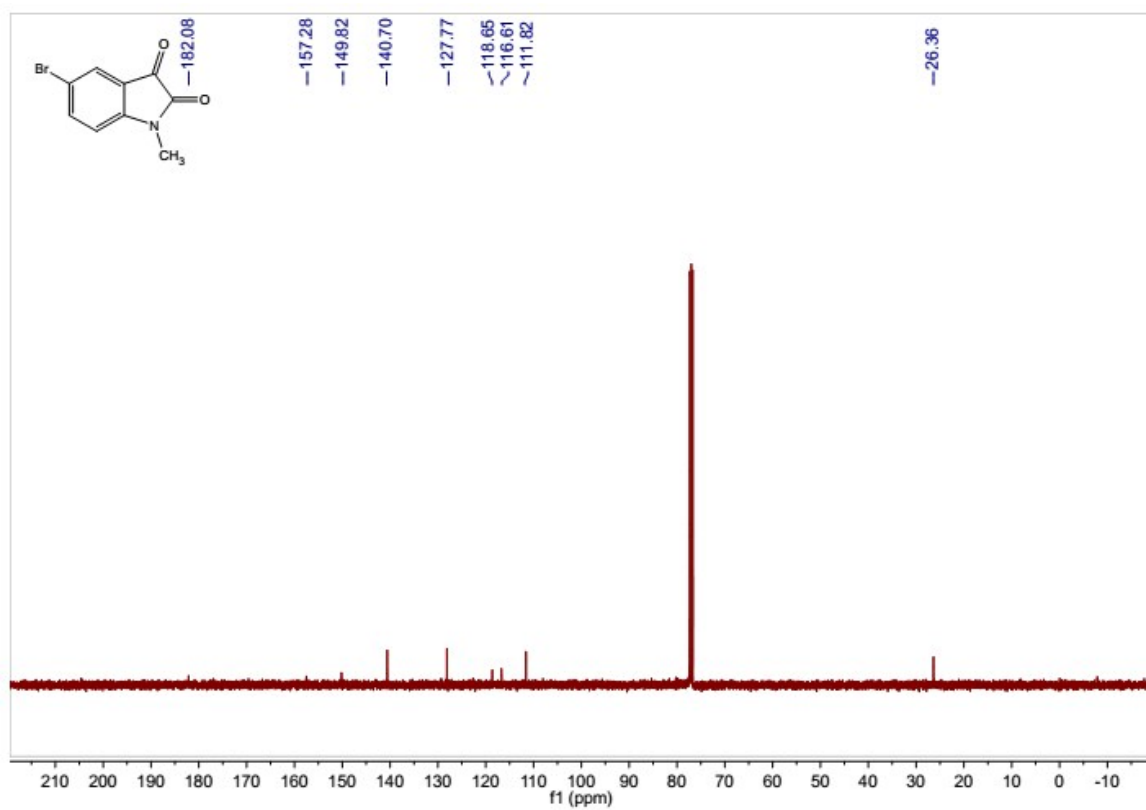


Fig. S10: ¹³C NMR spectrum of 1'.

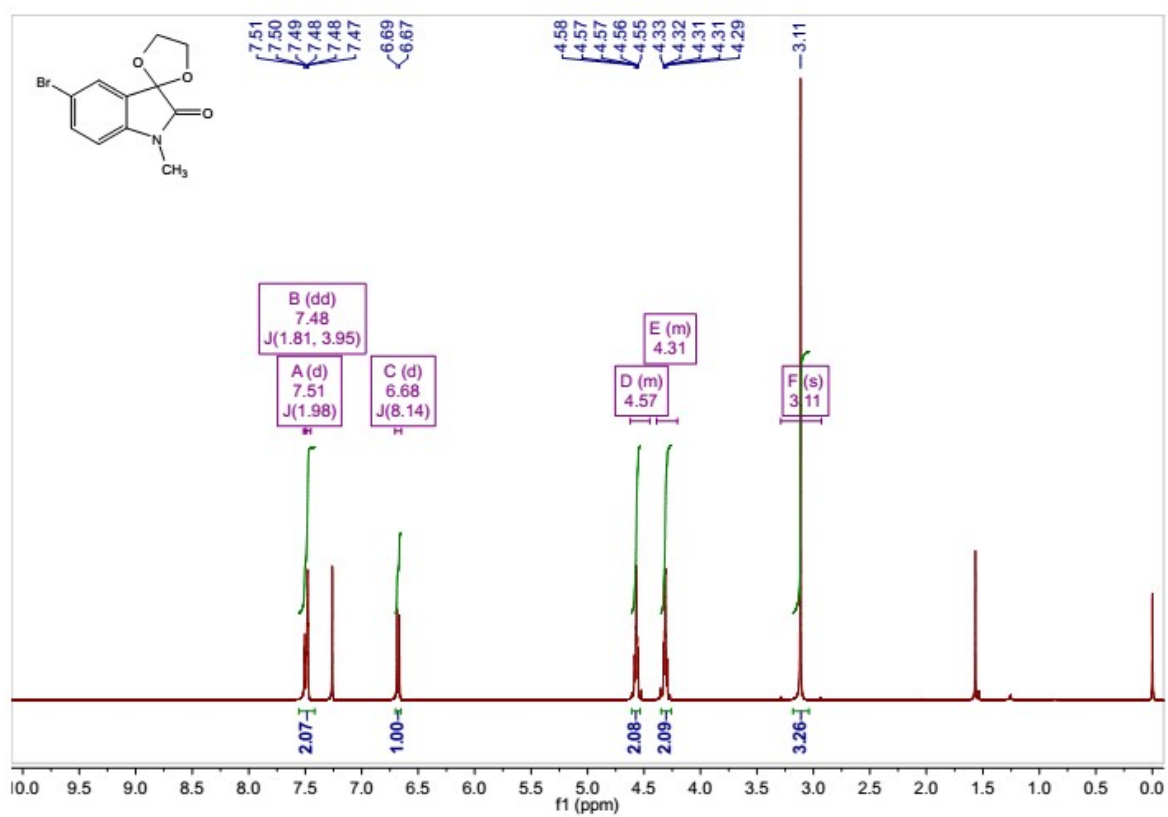


Fig. S11: ^1H NMR spectrum of **5**.

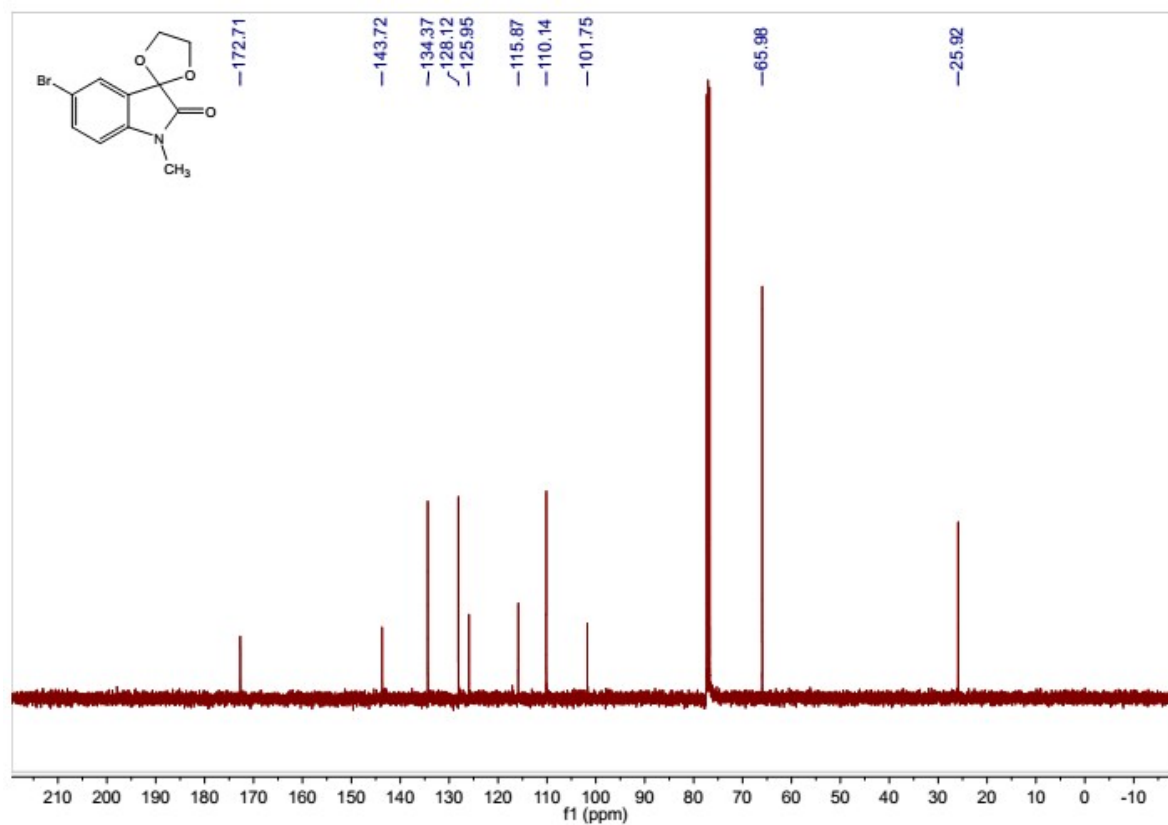


Fig. S12: ^{13}C NMR spectrum of **5**.

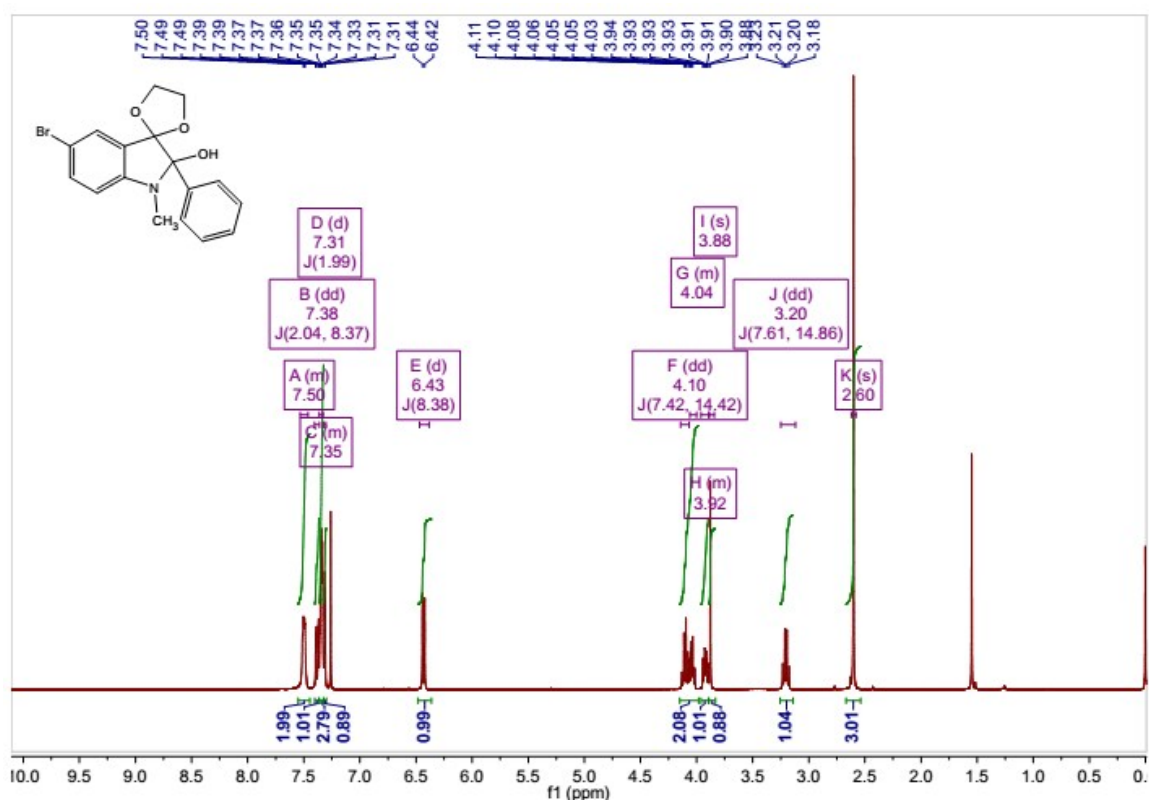


Fig. S13: ¹H NMR spectrum of 6.

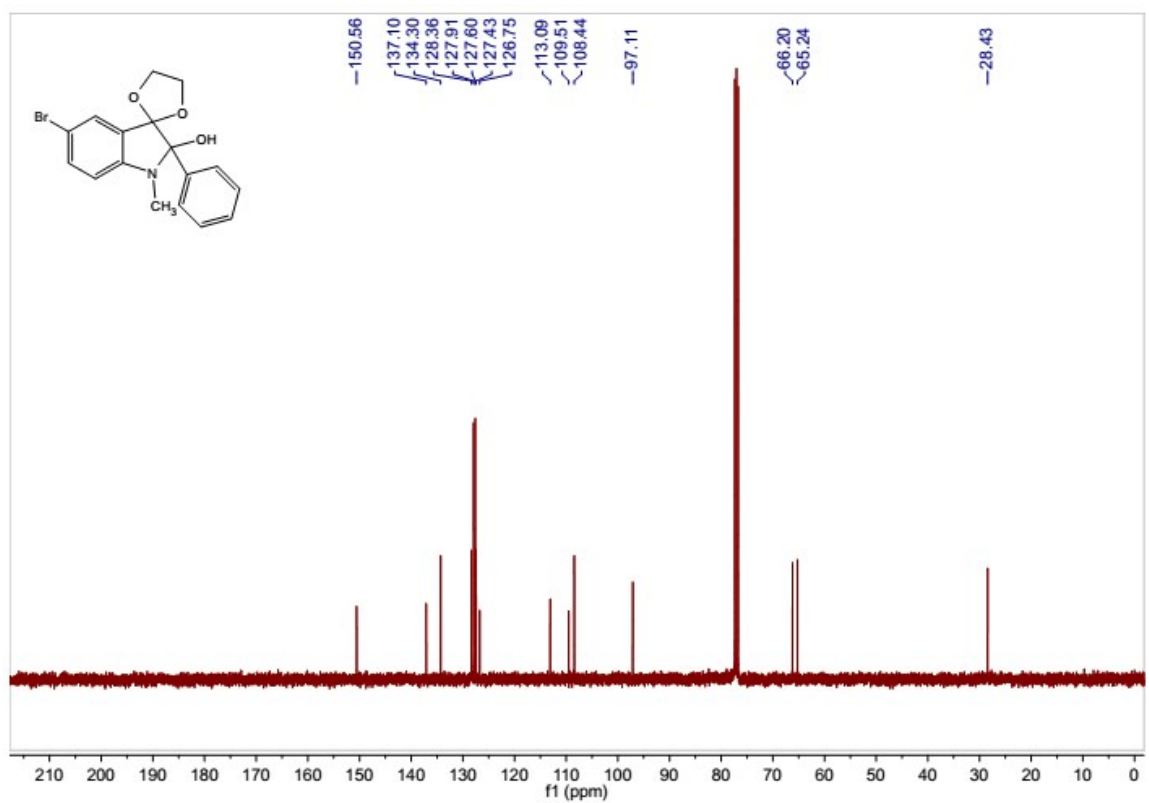


Fig. S14: ¹³C NMR spectrum of 6.

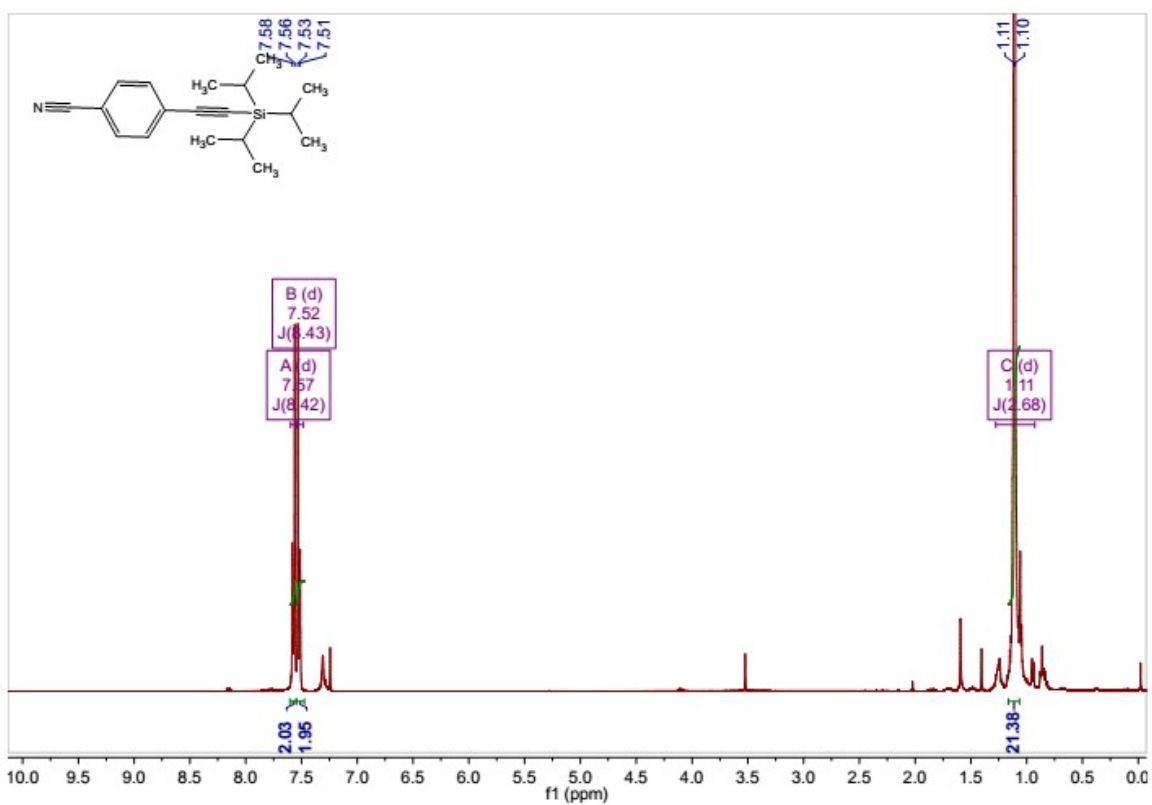


Fig. S15: ¹H NMR spectrum of **8a**.

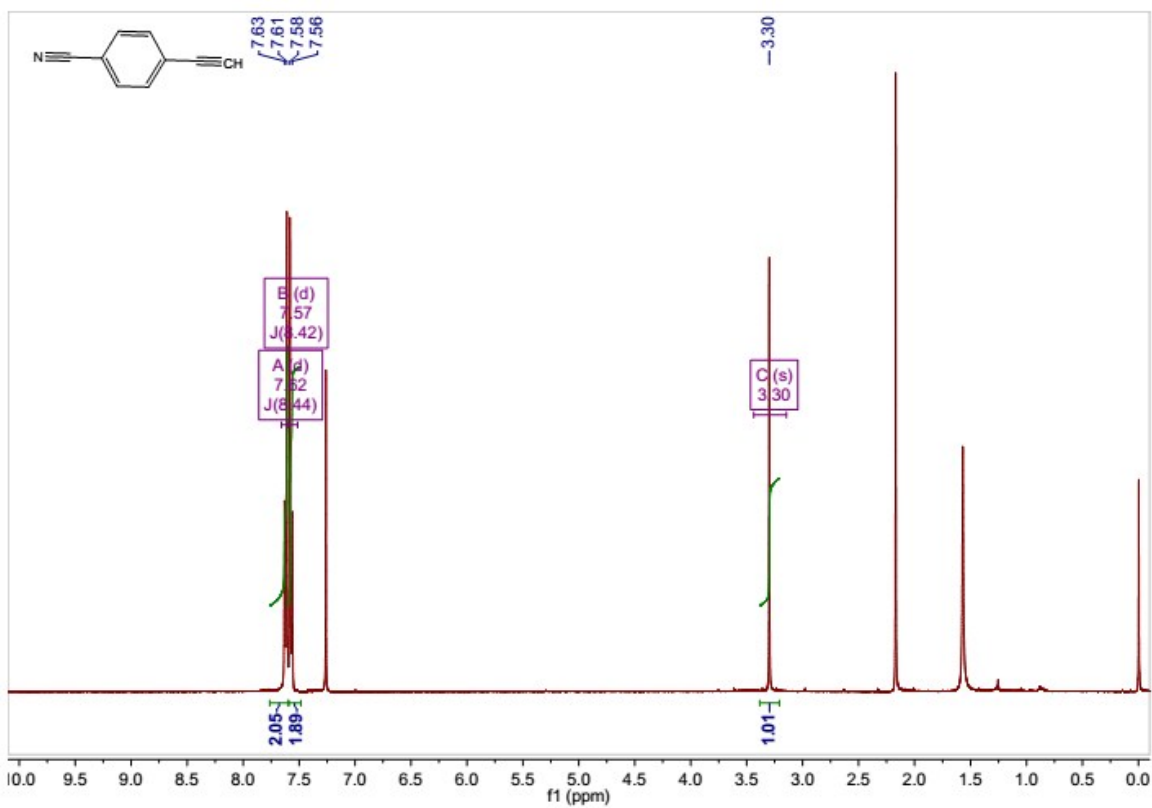


Fig. S16: ¹H NMR spectrum of **9a**.

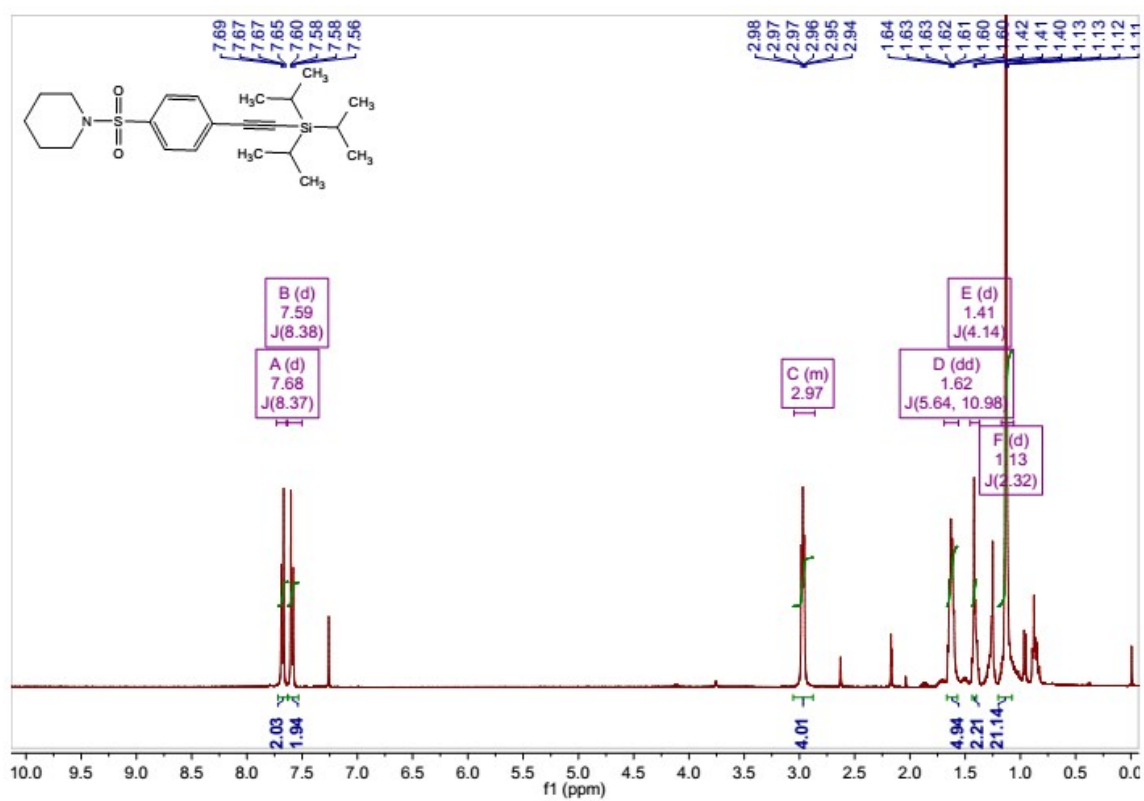


Fig. S17: ¹H NMR spectrum of **8b**.

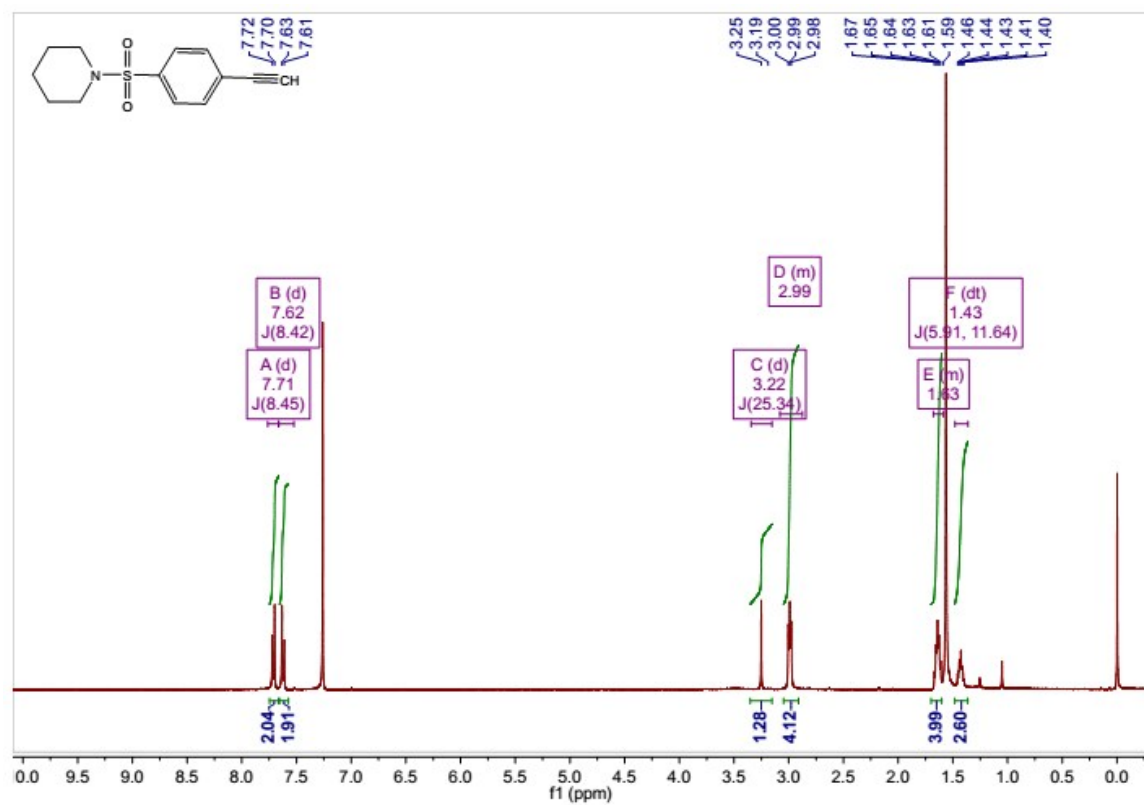


Fig. S18: ¹H NMR spectrum of **9b**.

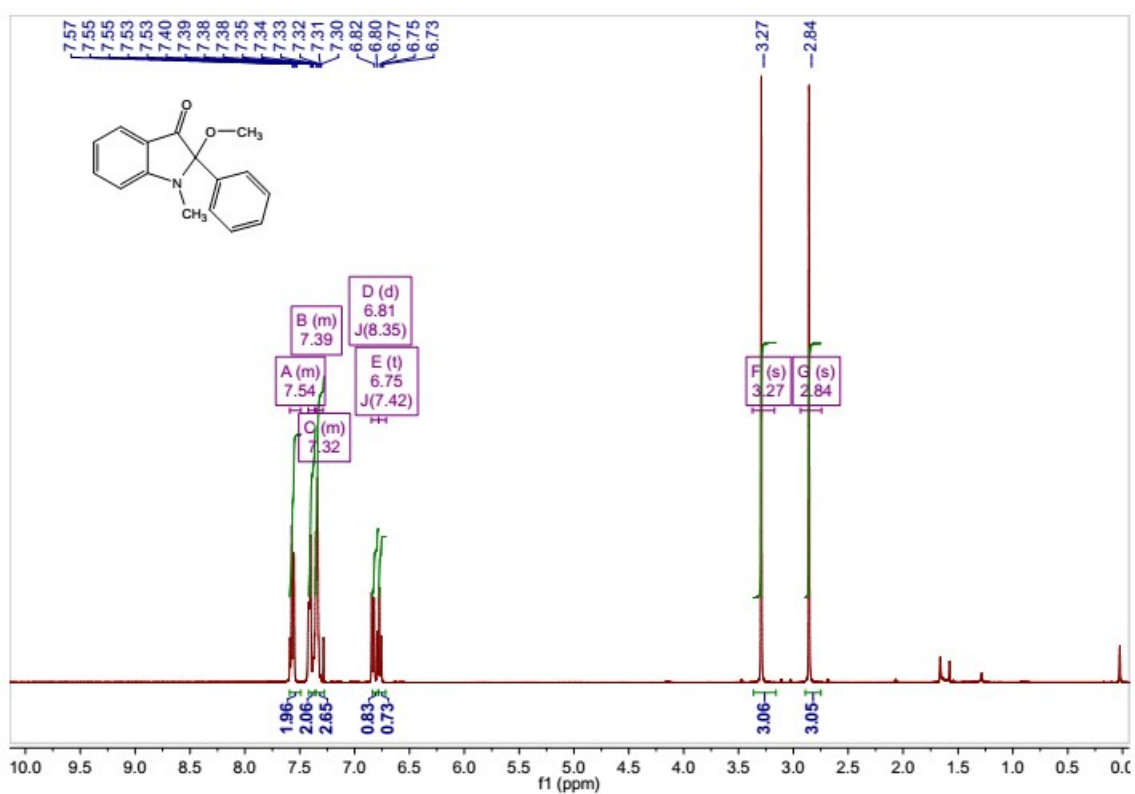


Fig. S19: ^1H NMR spectrum of 4.

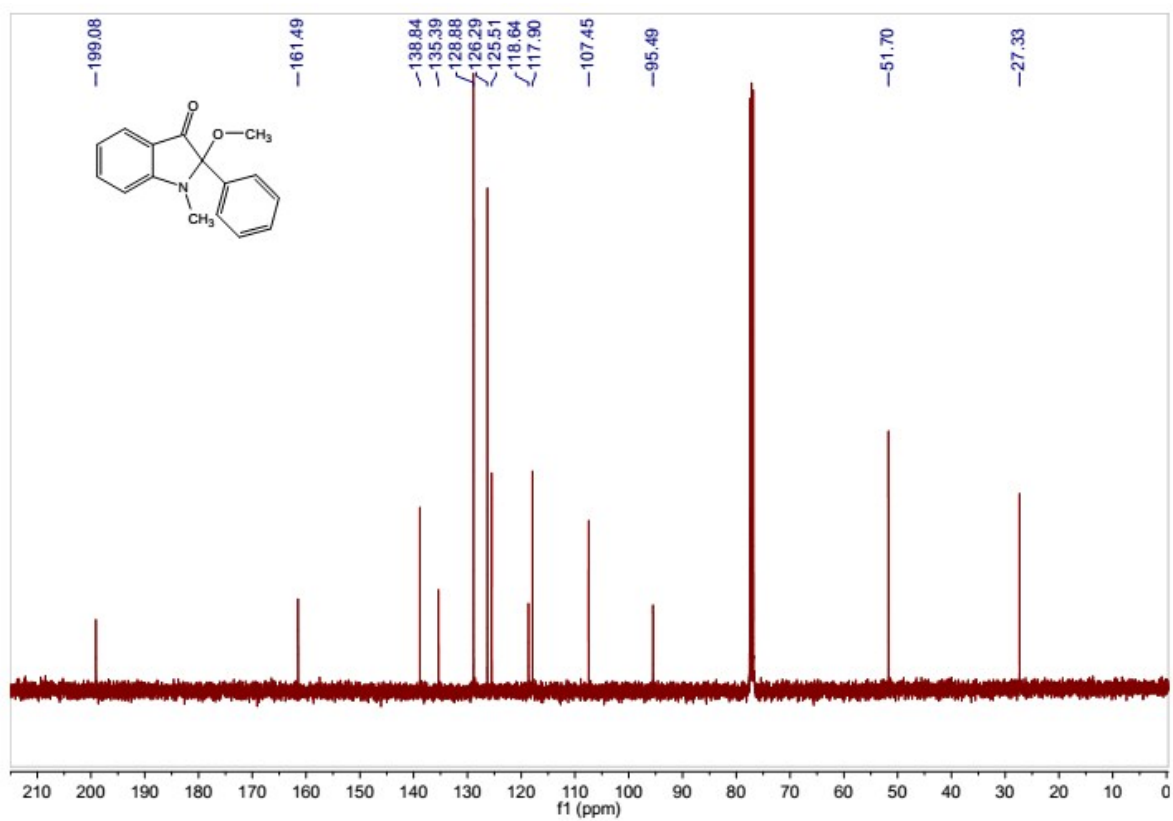


Fig. S20: ^{13}C NMR spectrum of 4.

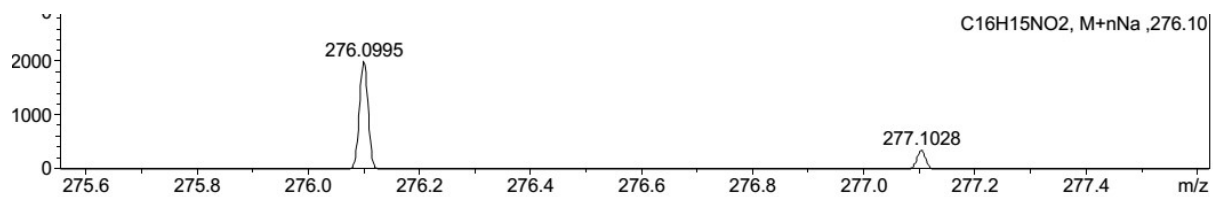


Fig. S21: Mass spectrum of 4.

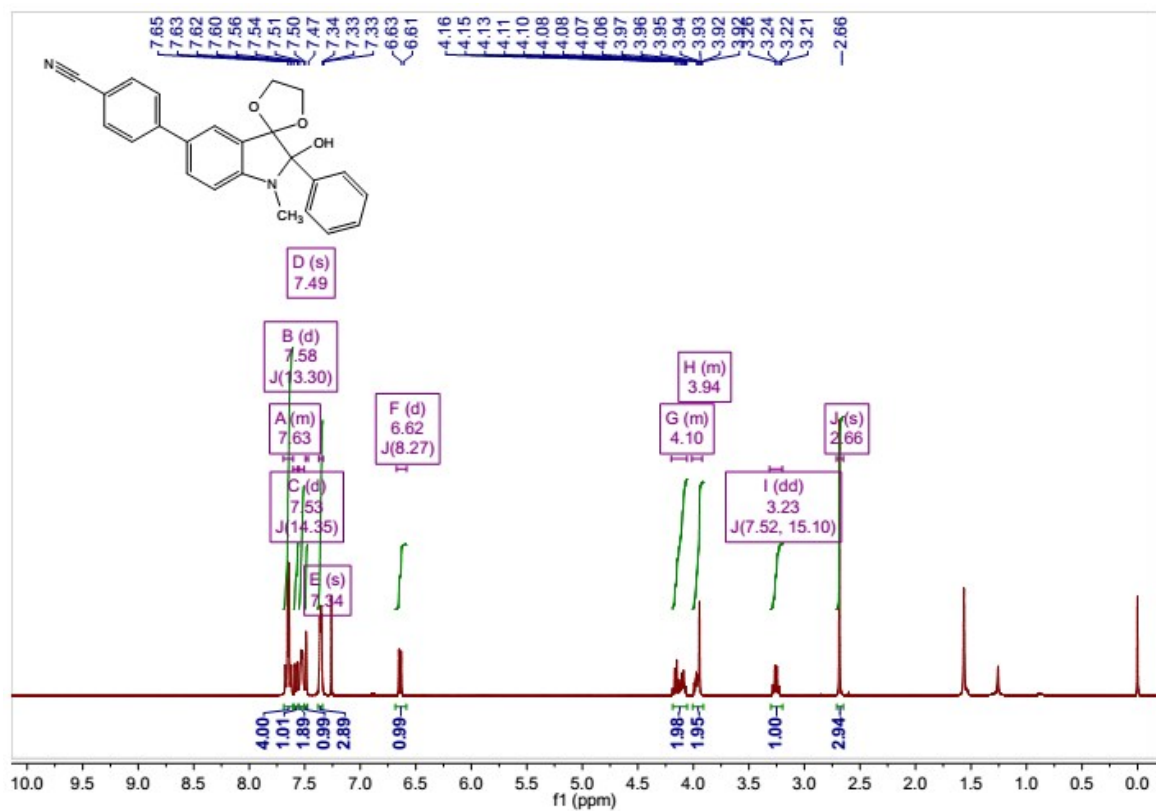


Fig. S22: ¹H NMR spectrum of 7.

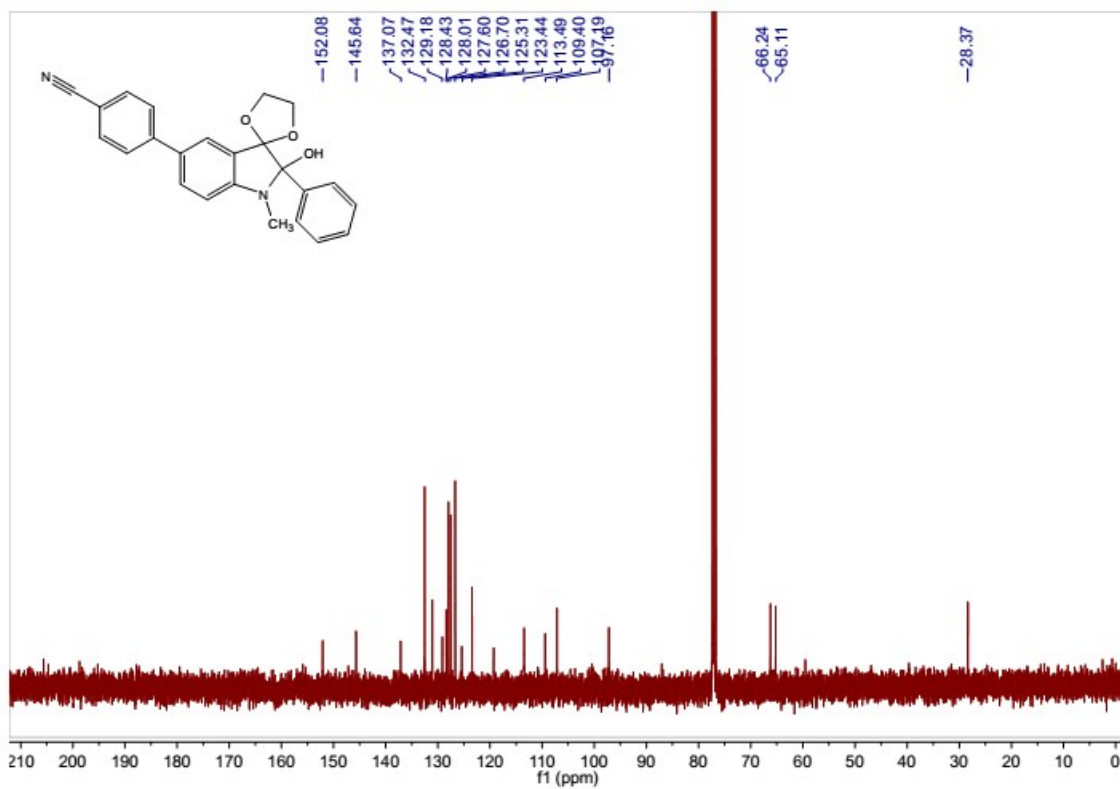


Fig. S23: ^{13}C NMR spectrum of 7.

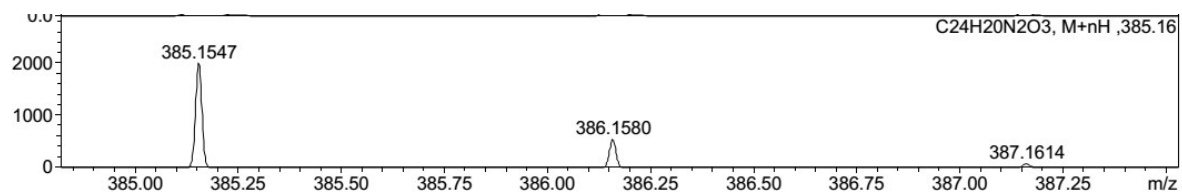


Fig.S24: Mass spectrum of 7.

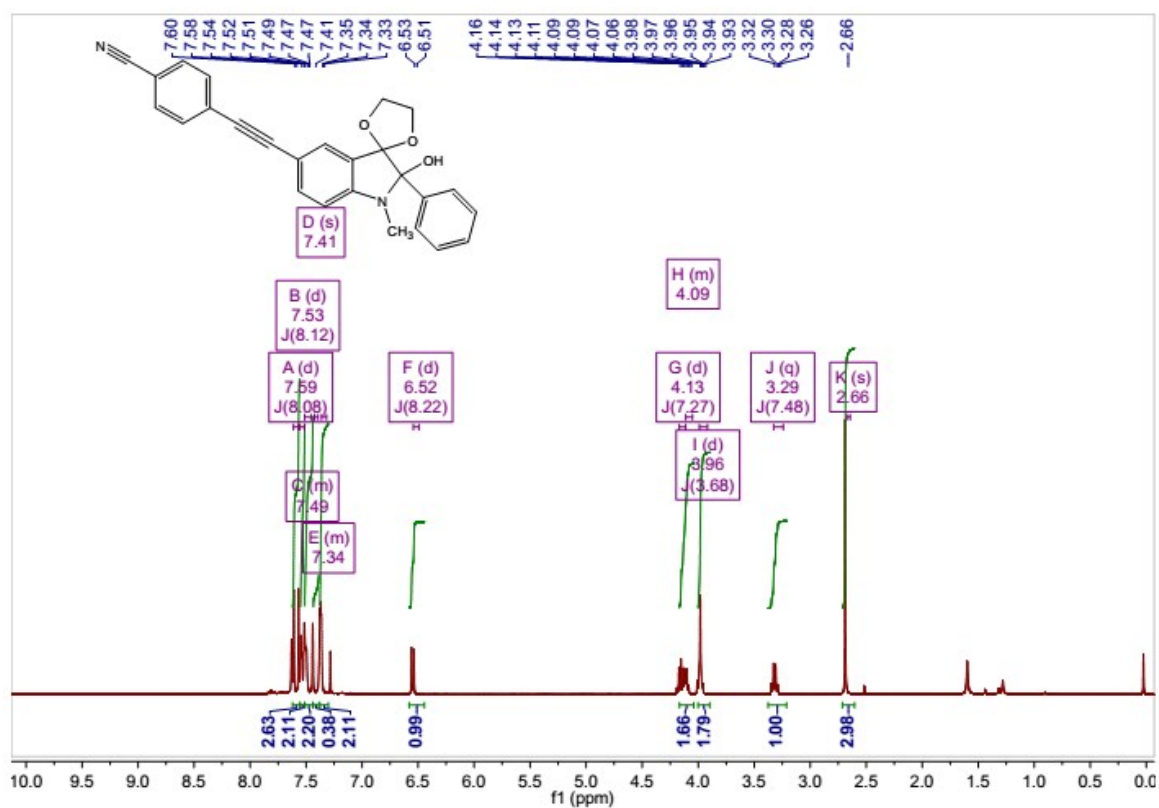


Fig. S25: ¹H NMR spectrum of 10a.

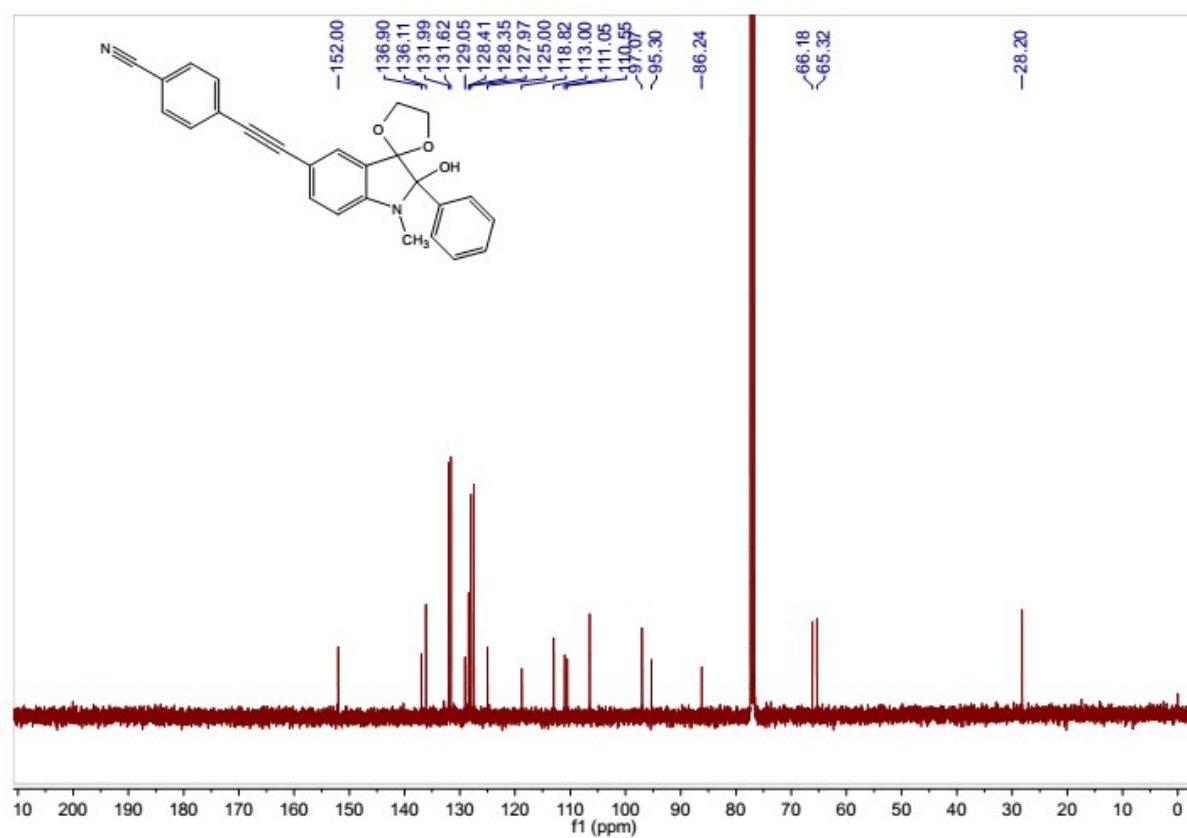


Fig. S26: ¹³C NMR spectrum of 10a.

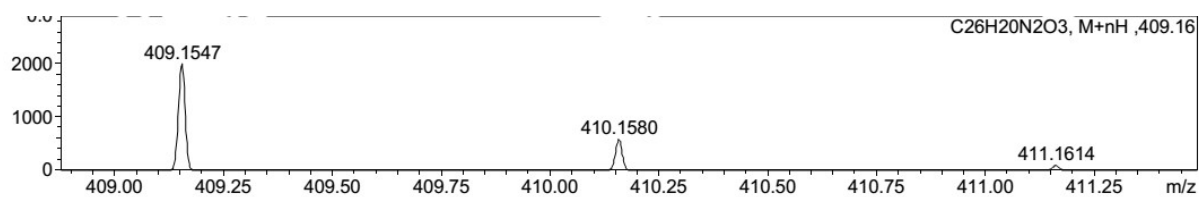


Fig. S27: Mass spectrum of 10a.

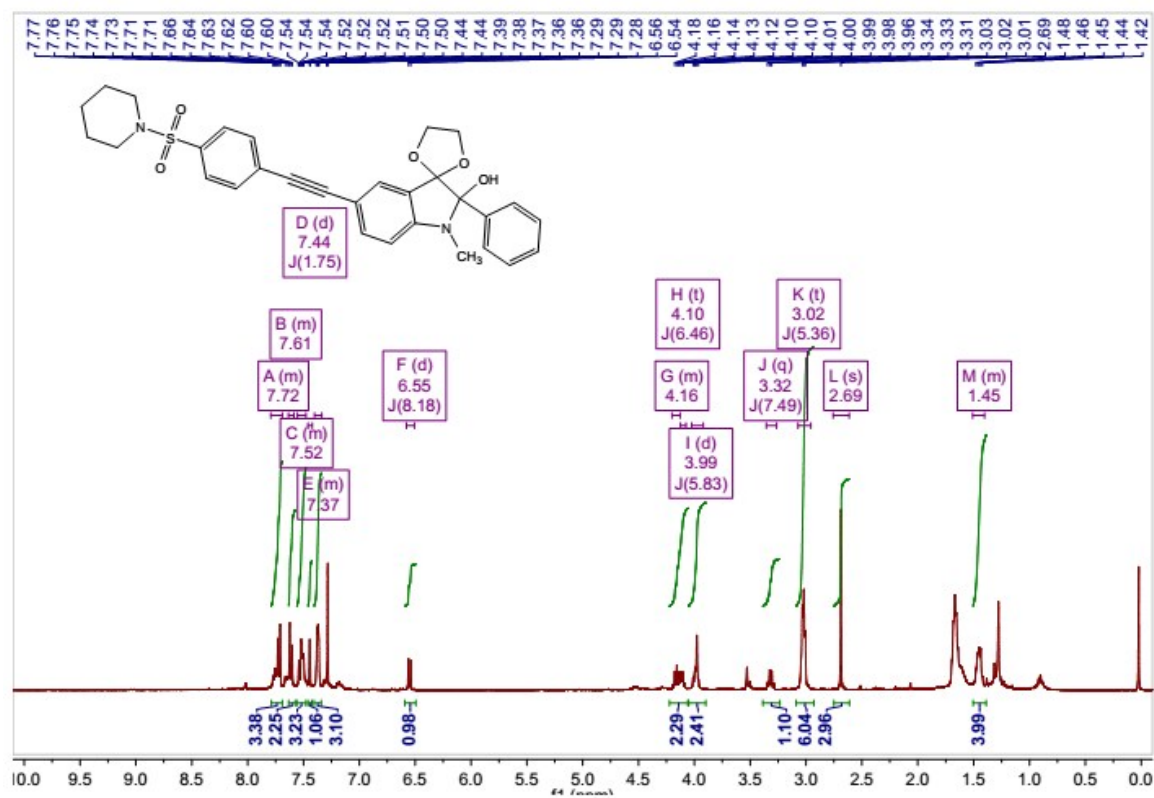


Fig. S28: ¹H NMR spectrum of 10b.

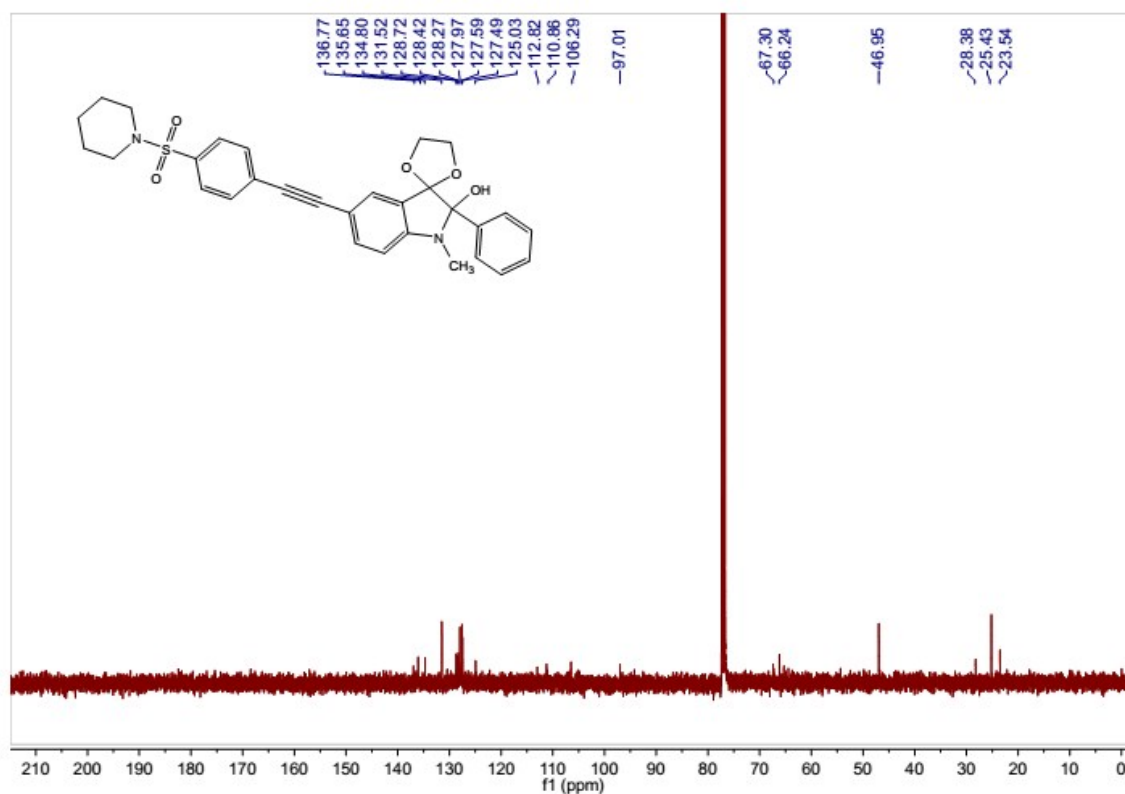


Fig. S29: ^{13}C NMR spectrum of **10b**.

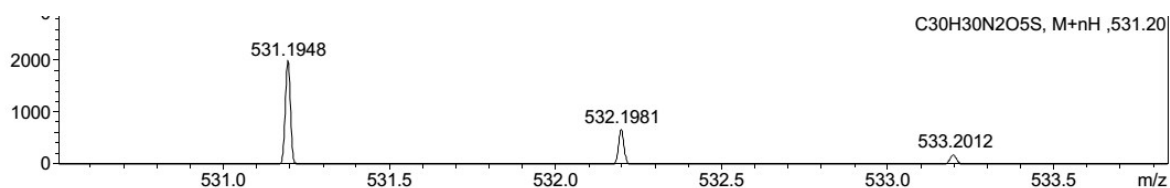


Fig. S30: Mass spectrum of **10b**.

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