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Supplementary Material

The synthesis and application of a colour-switch β-arylethenesulfonyl fluoride fluorescent probe in the detection of serum albumin

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1. Methods

1.1 General Chemical Experimental Techniques and Procedures

Unless otherwise stated, all reactions were performed with reagent-grade solvents. All reagents and solvents were obtained from commercial sources and used without further purification. Reactions were monitored using thin-layer chromatography (TLC) and visualised using UV light and stained using a basic $KMnO_4$ (potassium permanganate) solution. Flash column chromatography was performed using a Biotage® Isolera TM on Biotage® KP-Sil SNAP cartridges. Petrol refers to petroleum spirit (b.p. 40-60 °C). NMR spectra (^1H, ^{13}C, and ^{19}F) were recorded on a Bruker Ascend TM 400 (400 MHz) spectrometer as dilute solutions in the stipulated solvent. All chemical shifts (δ) are reported in parts per million (ppm) with ^1H and ^{13}C NMR referenced to solvent signals [^1H NMR: $CDCl_3$ (7.26), ^{13}C NMR: $CDCl_3$ (77.16)]. Coupling constants (J) are reported in Hertz (Hz) and recorded after averaging. The multiplicity of the ^1H NMR signals are designated by one of the following abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, appt s=apparent singlet, appt d=apparent doublet, appt t=apparent triplet signal. Absorbance and fluorescence spectra were obtained on a Cary 300 UV-Vis spectrometer and Cary Eclipse fluorimeter (Agilent Technologies Inc., Santa Clara, CA, USA), respectively. Data were plotted using Origin 2018 (OriginLab Corp., Northampton, MA, USA). CPV-ESF was stored as a solid in the dark at room temperature and remained stable for over 1 year.

DFT calculations were carried out using B3LYP/6-31+g with Gaussian16, using the University of Melbourne HPC system, Spartan, for all calculations. Visualisation of structures were done using Avogadro 1.20[1] and GaussView 5.0. No negative frequencies for optimised structures were observed.

1.2 General Biological Experimental Techniques and Procedures

HeLa cells were cultured in DMEM (Life Technologies, Catalog Number: 11965118) supplemented with 10% fetal bovine serum (Sigma, Australia origin, Catalog Number: F9423) at 37 °C in 5% CO_2 air with humidification.

Cell Staining:

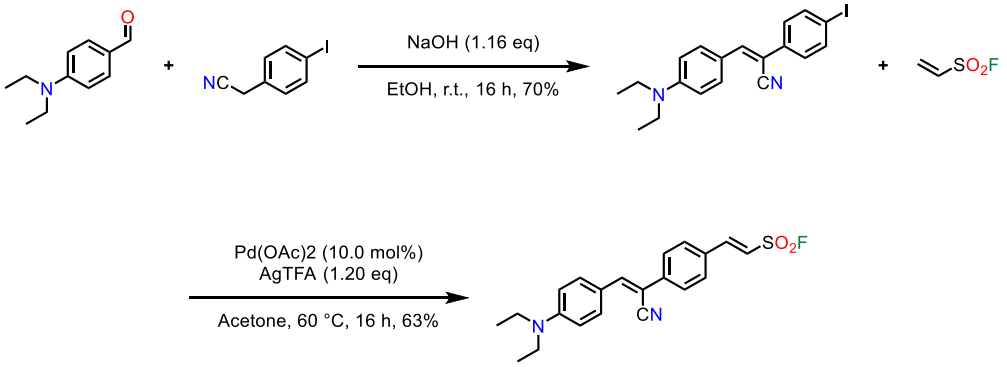
CPV-ESF were dissolved in DMSO at 1 mM stock concentration. Stock solution of dye were kept at -20 °C in the dark. HeLa cells (1.4×10^4) were plated on an ibidi µSlide 8 Well, ibiTreat (ibidi, Catalog number: 80826) 24 h prior to dye application. Plated cells were treated with freshly diluted dye (10

μM in DMEM) for 30 min at 37 °C. Cells were rinsed with PBS and then fixed on plate with 4% (w/v) paraformaldehyde (PFA) in PBS for 10 min at room temperature.

Confocal Laser Scanning Microscopy:

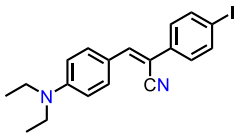
After staining, HeLa cells were fixed with 4% (*w/v*) paraformaldehyde in PBS for 10 min at room temperature. Images were acquired on a Zeiss Elyra LSM880 microscope using a 63× oil immersion objective 1.4 NA, with a pixel frame size set at 512 × 512 and a pixel dwell time of 32.67 μs. For CPV-ESF (excitation: 405 nm; emission: 450 - 550 and 550 - 650 nm, 405 nm dichroic), laser power for 405 nm excitation source was set at 1.5%.

2. Procedures of Synthesis of CPV-ESF



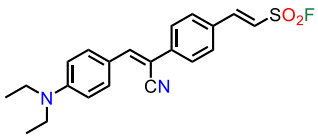
Scheme S1. Synthetic scheme for probe **CPV-ESF**

(*Z*)-3-(4-(diethylamino)phenyl)-2-(4-iodophenyl)acrylonitrile (1)



To a solution of 4-diethylaminobenzaldehyde (177 mg, 1.00 mmol, 1.00 eq) and 2-(4-iodophenyl)acetonitrile (243 mg, 1.00 mmol, 1.00 eq) in EtOH (10.0 mL) was added NaOH (46.4 mg, 1.16 mmol, 1.16 eq) and the solution stirred at room temperature for 16 h. The resultant precipitate collected by filtration and dried under vacuum to give the title compound (2) as a yellow solid (281 mg, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.86 (appt d, *J* = 9.0 Hz, 2H), 7.74 (appt d, *J* = 8.7 Hz, 2H), 7.40 – 7.36 (m, 3H), 6.71 (appt d, *J* = 9.1 Hz, 2H), 3.45 (appt q, *J* = 7.1 Hz, 4H), 1.24 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.7, 142.9, 138.0, 135.5, 132.0, 127.2, 120.7, 119.4, 111.3, 102.6, 93.2, 44.7, 12.7.

CPV-ESF



To a solution of (*Z*)-3-(4-(diethylamino)phenyl)-2-(4-iodophenyl)acrylonitrile (1) (200 mg, 500 μmol, 1.00 eq), $Pd(OAc)_2$ (6.00 mg, 50.0 μmol, 0.10 eq) and AgTFA (132 mg, 600 μmol, 1.20 eq) in acetone (3.00 mL) was added ethenesulfonyl fluoride (84.0 μL, 1.00 mmol, 2.00 eq). The resultant mixture was refluxed at 60 °C for 16 h then cooled to room temperature, filtered through a pad of Celite® and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (10-60% EtOAc in petrol) to give the title compound (**CPV-ESF**) as an orange solid (121 mg, 63%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.89 (appt d, *J* = 9.0 Hz, 1H), 7.80 (d, *J* = 15.5 Hz, 1H), 7.73

(appt d, *J* = 8.4 Hz, 2H), 7.58 (appt d, *J* = 8.4 Hz, 2H), 7.50 (s, 1H), 6.87 (dd, *J* = 15.5, 2.2 Hz, 1H), 6.74 (appt br s, 1H), 3.45 (q, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 148.9, 147.9 (d, *J* = 2.5 Hz), 143.8, 139.9, 132.4, 129.8, 126.2, 122.2, 119.0, 117.9 (d, *J* = 28.2 Hz), 112.8, 112.3 (d, *J* = 48.0 Hz), 103.2, 45.8, 12.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ 62.7. **HRMS** (ESI^+): calculated for $C21H21FN_2O_2S$ [$M+H^+$]: m/z = 385.1381, m/z found 385.1387.

3. Experimental operations of BSA detection

Preparation of **CPV-ESF:BSA** complex:

CPV-ESF was dissolved in DMSO solution to give a 1 mM stock solution. BSA was dissolved in PBS buffer to give a 100 μM stock solution. 50 μL of the CPV-ESF stock solution was added to 100 μL of BSA stock solution and 850 μL of the stipulated buffer added to give [**CPV-ESF**] = 100 μM and [BSA] = 10 μM. The conjugate stock solution was heated to 37 °C and shaken at 1000 rpm for 1 h. The fluorescence intensity of the sample was then measured using a Cary Eclipse fluorimeter at λ_{ex} = 440 nm.

4. Photophysical Studies

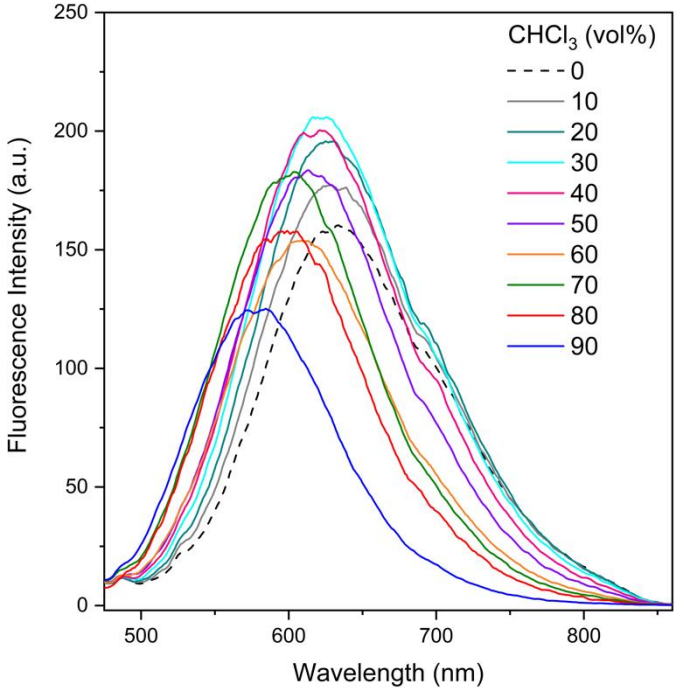


Figure S1. Fluorescence emission spectra of CPV-ESF in DMSO/$CHCl_3$ mixtures, with increasing fraction of $CHCl_3$ from 0 to 90%; λ_{ex} = 440 nm.

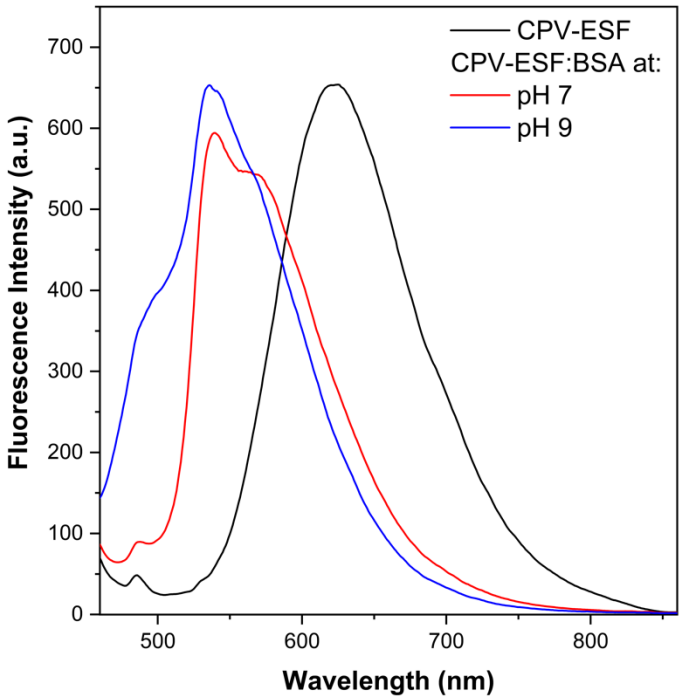


Figure S2. Investigations in the formation of the CPV-ESF:BSA complex at pH 7 and 9.; λ_{ex} = 440 nm.

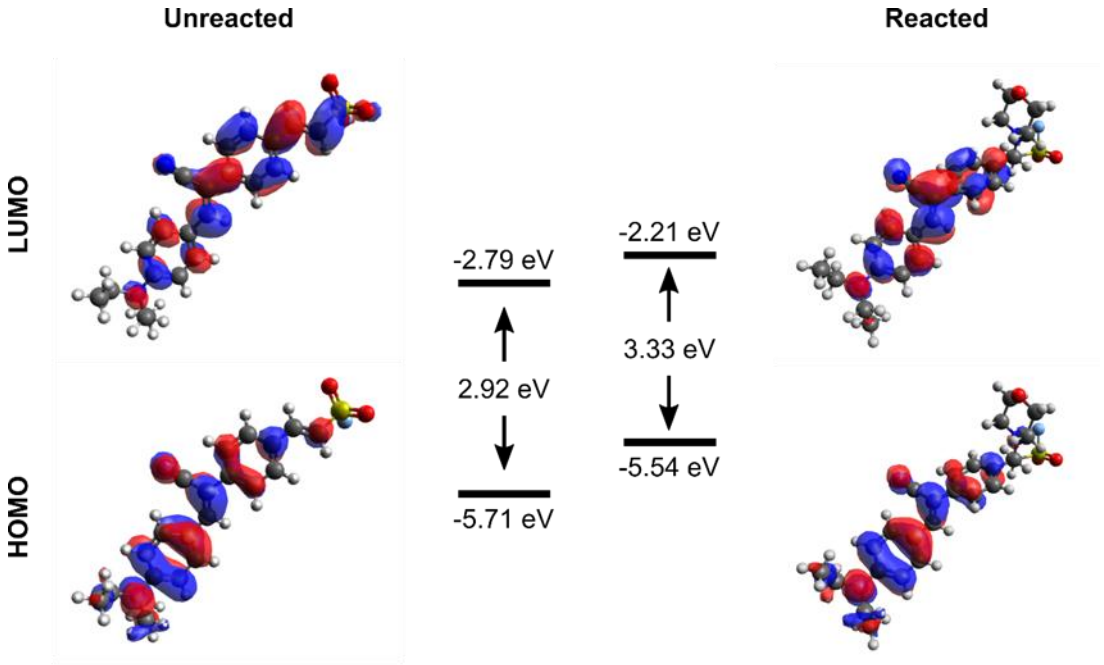


Figure S3. DFT calculations for CPV-ESF showing HOMO and LUMO orbitals and a comparison of HOMO, LUMO and energy gaps before and after reaction.

5. CPV-ESF:BSA Complex with Trypsin

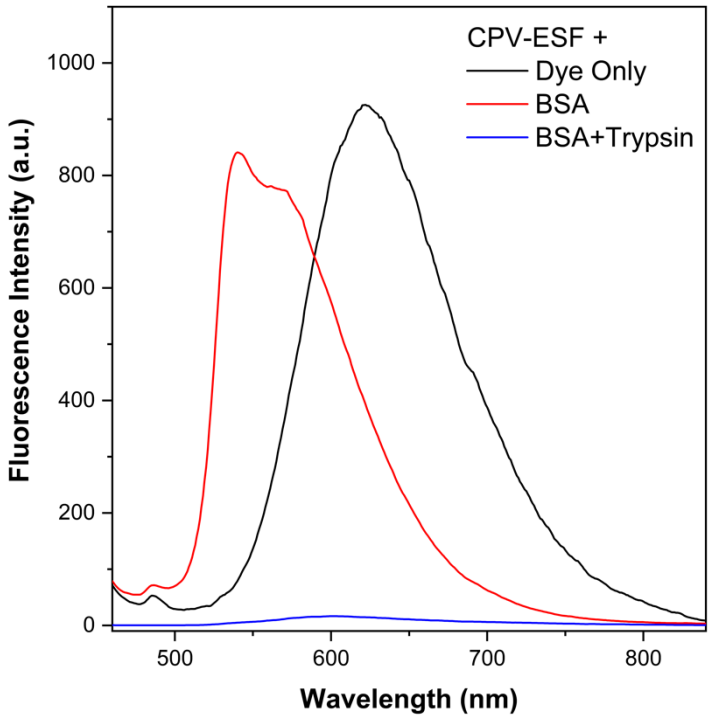


Figure S4. Fluorescence emission spectra of CPV-ESF:BSA complex (100 µM:10 µM) in the presence of trypsin (10 µM) in PBS buffer, t = 1 h; λ_{ex} = 440 nm.

6. Confocal Microscopy

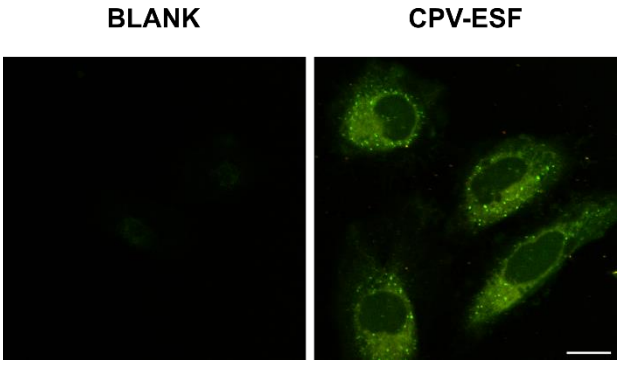


Figure S5. Background experiments. Confocal images of intracellular distribution of CPV-ESF stained HeLa cells. HeLa cells were stained with 10 µM concentration of CPV-ESF for 0.5 h before fixation. Scale bar, 20 µm.

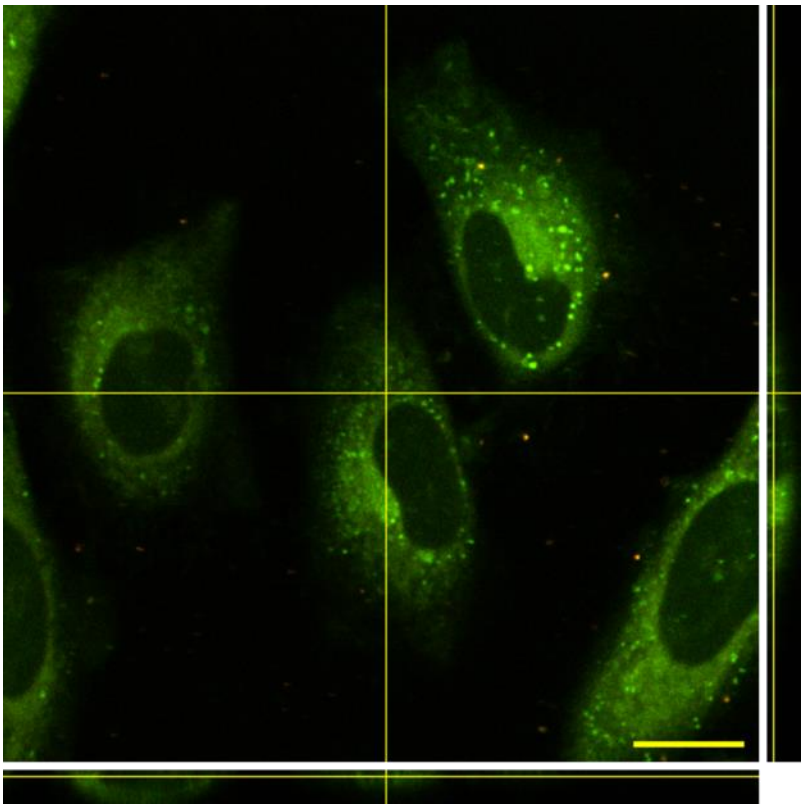
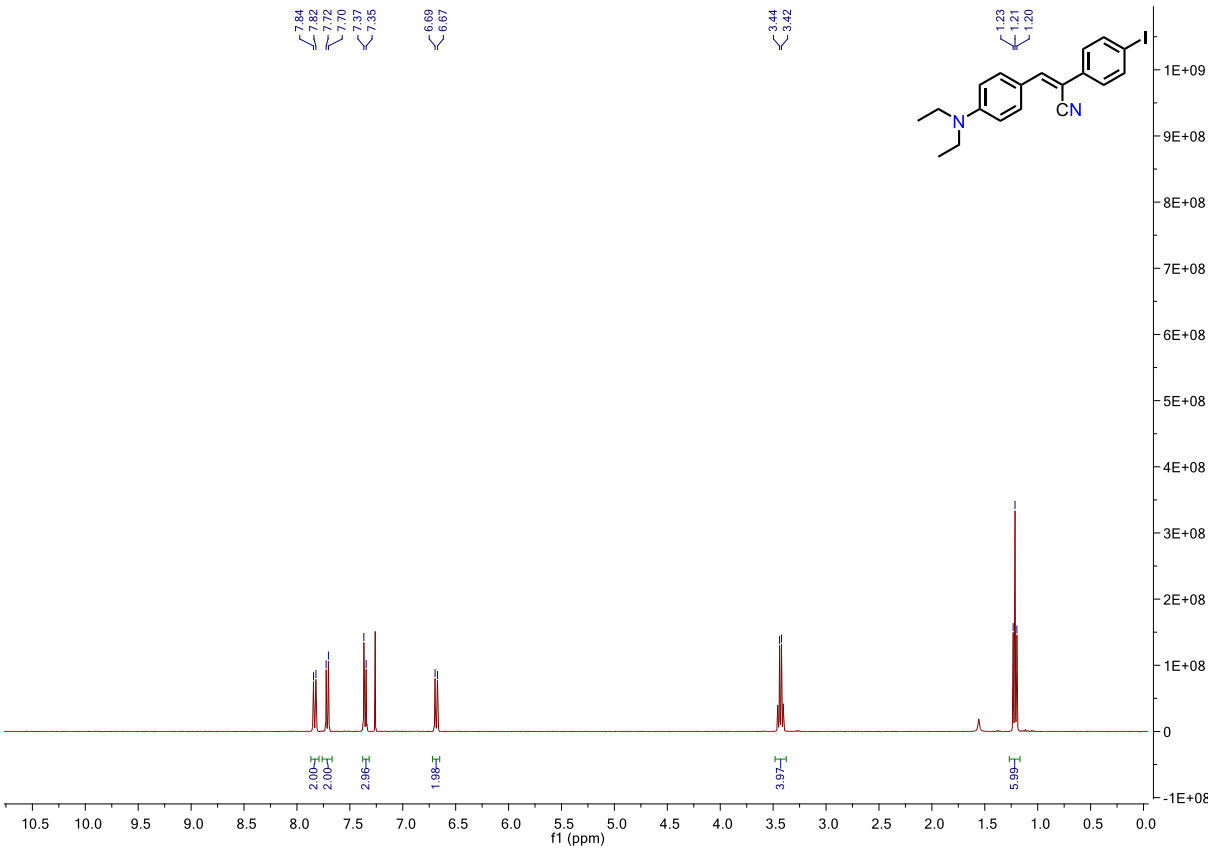


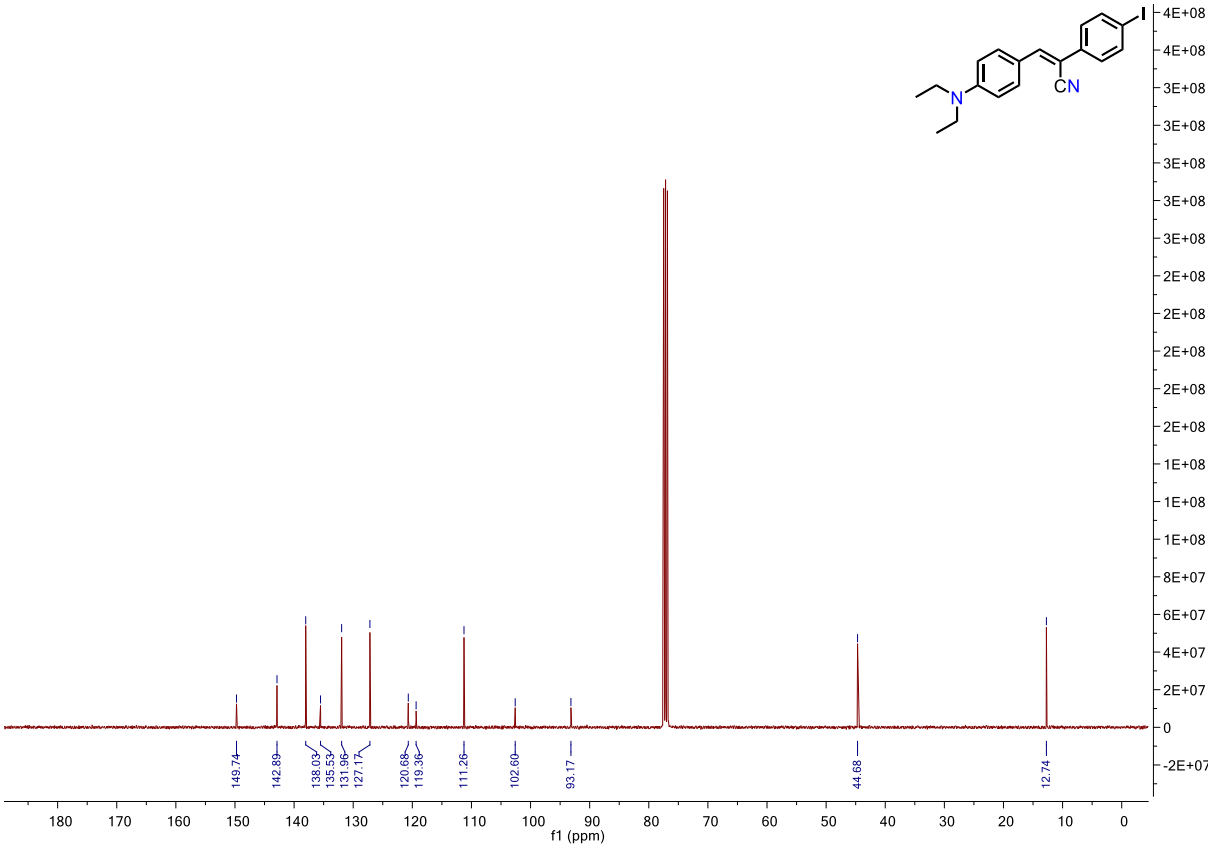
Figure S6. Z-stack image for confirmation of localization of CSV-ESF.10 µM of CSV-ESF was used. Scale bar, 20 µm

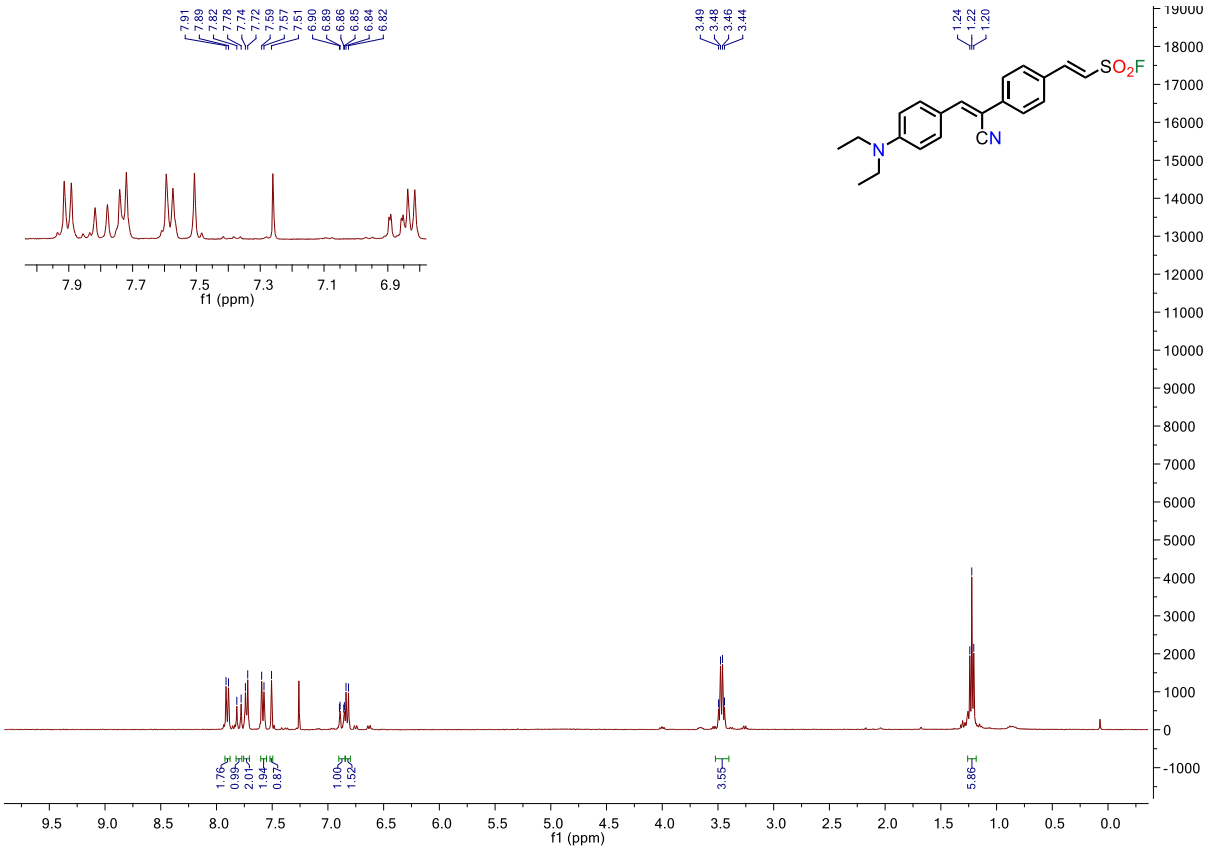
7. References

1. M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek, G. R. Hutchison, J. Cheminformatics 2012, 4, 17.

8. NMR Spectra



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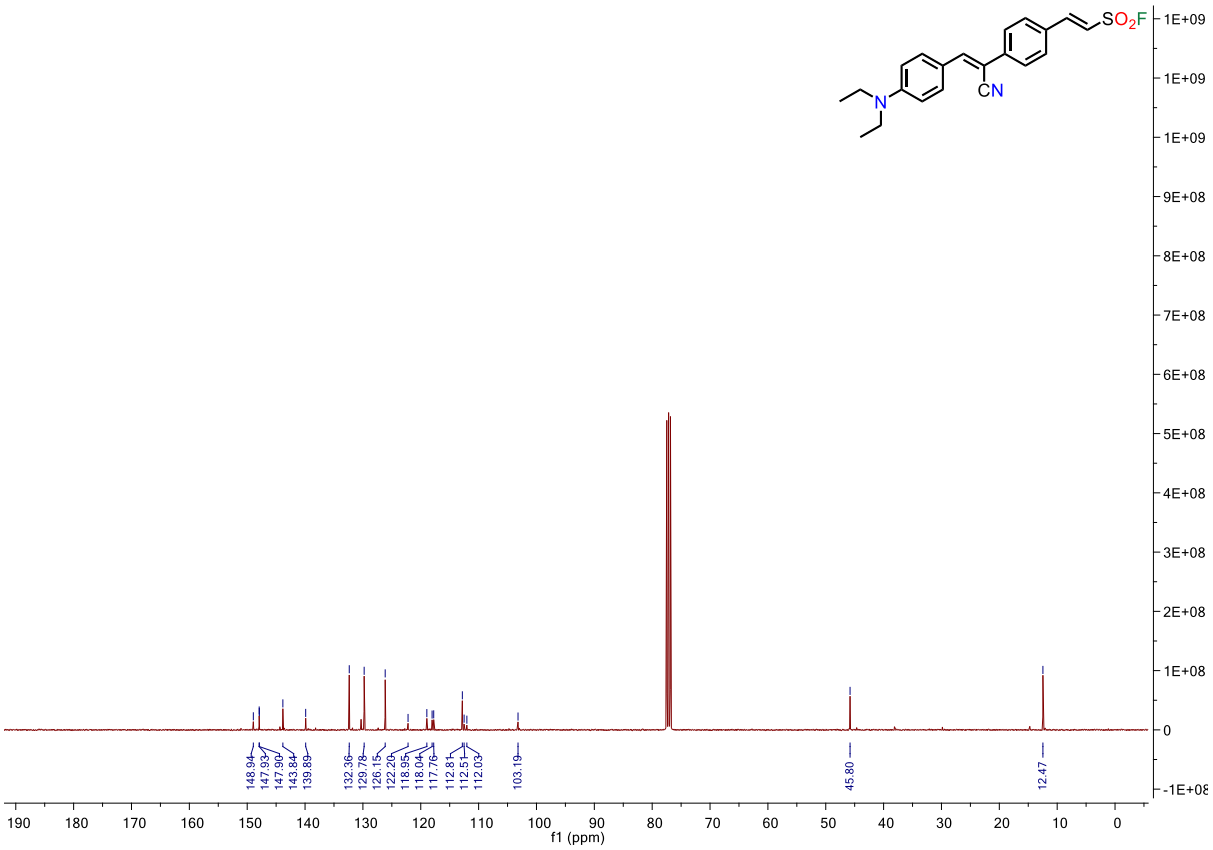
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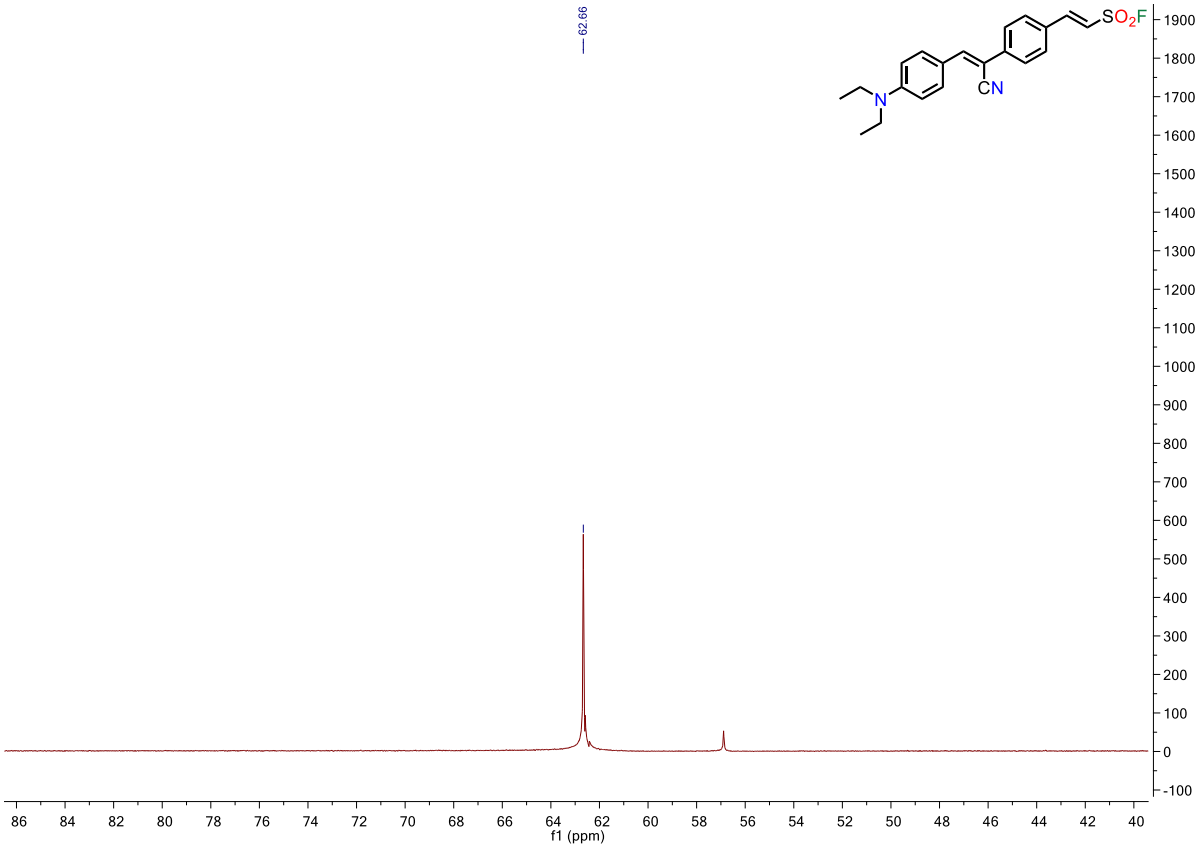
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f1 (ppm)