Design and Synthesis of Photostable Triphenylamine Based Neutral AIE Nano Luminogens: Specific and Long-Term Tracking of Mitochondria in Cells

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Electronic Supporting Information

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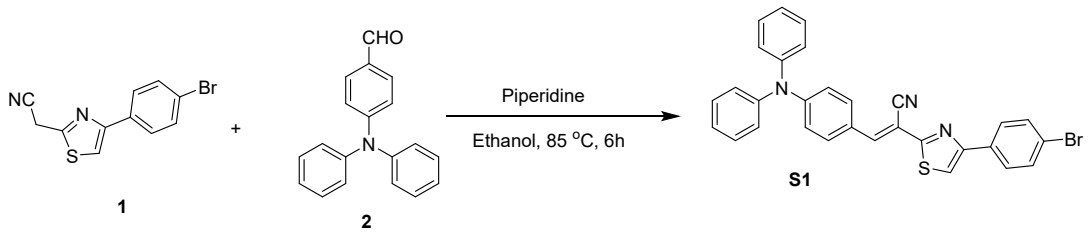
1. Materials and Methods

All the chemicals were purchased from commercial sources especially from Sigma Aldrich and used without any further purification. The synthetic strategy to obtain the targeted PI fluorophores was carried out by condensation reaction product reacted with relevant boronic acid by Suzuki coupling reaction. The synthetic strategy to obtain the target PI fluorophores has been provided in Scheme. The compound characterized for ^{1}H NMR, ^{13}C NMR and DEPT spectra were performed 400 MHz and 100 MHz Bruker spectrometer. The Chemical shifts values in parts per million (ppm) with TMS (0 ppm) and $CDCl_3$ as standards for ^{1}H NMR and ^{13}C NMR spectra. Infrared spectroscopy of all the compound were measured using Bruker-Alpha-p instrument. All the TLC analyses were carried out using Merck silica gel 60 F254 plates. UV-vis absorption spectra of all compounds were recorded in THF on a Bruker UV–visible spectrophotometer. Emission spectra were taken in a Bruker fluorescence spectrophotometer. The excitation and emission slits were 3 nm wide for the emission measurements. All the measurements were done at 298 K. Column chromatography was performed on Merck silica gel (100–200 mesh).

2. Synthesis

2.1 Synthesis of Precursors:

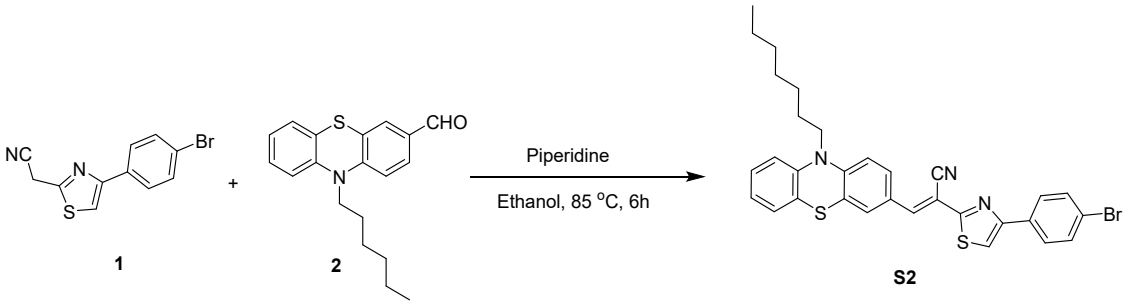
2.1.1 Experimental procedures for the Synthesis of (E)-2-(4-(4-bromophenyl)thiazol-2-yl)-3-(4-(diphenylamino)phenyl)acrylonitrile (S-1)



An oven dried 100 ml round bottom kept reflux condenser added 2-(4-(4-bromophenyl)thiazol-2-yl)acetonitrile **1** (500 mg, 1.0 mmol) and piperidine (0.35 ml, 2.0 mmol) were successively added to anhydrous EtOH (50 mL), which was stirred at room temperature for 10 min. Then 4-(diphenylamino)benzaldehyde **2** (489 mg, 1.0 mmol) was added to the reaction solution, and the mixture was heated and refluxed at 85 °C for 6 h. After cooling to ambient temperature, the solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography using hexane/ethyl acetate mixture as eluent to give **S1** as an 840 mg of yellow

colour solid in 85% of yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.11 (s, 1H), 7.89 – 7.80 (m, 4H), 7.59 – 7.54 (m, 2H), 7.49 (s, 1H), 7.38 – 7.31 (m, 4H), 7.22 – 7.13 (m, 6H), 7.06 – 7.00 (m, 2H) ppm; **^{13}C NMR (100 MHz, $CDCl_3$)** δ 163.96, 155.53, 151.34, 146.27, 144.14, 133.06, 132.07, 131.96, 129.85, 129.79, 128.20, 126.31, 126.11, 125.16, 124.85, 122.68, 120.12, 117.84, 113.59, 100.52 ppm.

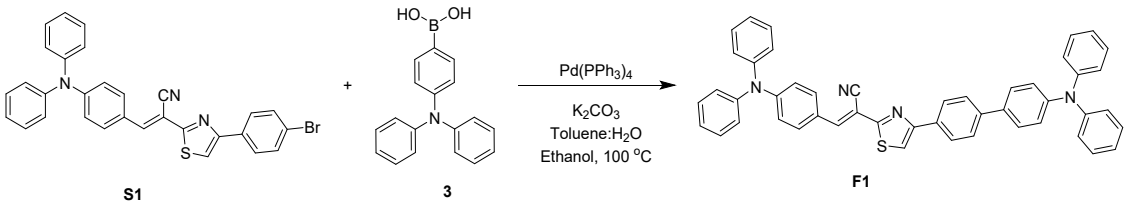
2.1.2 Experimental procedures for the Synthesis of (E)-2-(4-(4-bromophenyl)thiazol-2-yl)-3-(10-heptyl-10H-phenothiazin-3-yl)acrylonitrile (S-2)



An oven dried 100 ml round bottom kept reflux condenser added 2-(4-(4-bromophenyl)thiazol-2-yl)acetonitrile **1** (500 mg, 1.0 mmol) and piperidine (0.35 ml, 2.0 mmol) were successively added to anhydrous EtOH (50 mL), which was stirred at room temperature for 10 min. Then 10-hexyl-10H-phenothiazine-3-carbaldehyde **2** (557 mg, 1.0 mmol) was added to the reaction solution, and the mixture was heated and refluxed at 85 ℃ for 6 h. After cooling to ambient temperature, the solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography using hexane/ethyl acetate mixture as eluent to give **S2** as an 825 mg red colour solid in 78% of yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.04 (s, 1H), 7.87 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.84 – 7.79 (m, 2H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.49 (s, 1H), 7.16 (ddd, *J* = 8.2, 7.3, 1.6 Hz, 1H), 7.10 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.95 (td, *J* = 7.5, 1.1 Hz, 1H), 6.90 – 6.84 (m, 2H), 3.91 – 3.81 (m, 2H), 1.87 – 1.76 (m, 2H), 1.45 (dq, *J* = 14.6, 7.0 Hz, 2H), 1.32 (dq, *J* = 7.2, 3.7 Hz, 4H), 0.94 – 0.84 (m, 3H) ppm; **^{13}C NMR (100 MHz, $CDCl_3$)** δ 163.49, 155.59, 148.50, 143.56, 143.21, 132.95, 132.07, 129.98, 129.34, 128.19, 127.67, 126.76, 124.93, 123.61, 123.53, 122.73, 117.44, 115.84, 115.23, 113.88, 101.49, 48.07, 31.54, 26.86, 26.69, 22.73, 14.12 ppm.

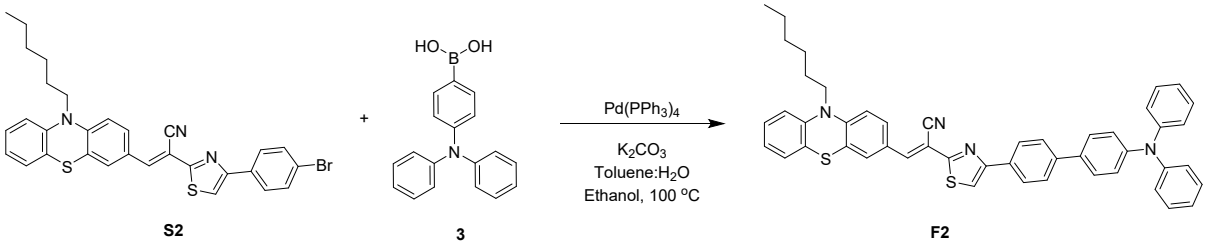
2.2 Synthesis of F1 and F2

2.2.1 Procedure for the synthesis of F1 using Suzuki coupling reaction



A mixture of compound **S1** (0.374 mg, 0.7 mmol), K_2CO_3 (276 mg, 2 mmol), $Pd(PPh_3)_4$ (25 mg, 0.3 mmol) and (4-(diphenylamino)phenyl)boronic acid **3** (204 mg, 1 mmol) in THF/H_2O (18 mL/2 mL) was refluxed at 80 °C for 24 h under nitrogen atmosphere. After cooling to ambient temperature, diluted with 50 mL of DCM, filtered through a celite pad, and washed with 20-40 mL of DCM. The combined organic phases were dried anhydrous Na_2SO_4 and concentrated under reduced pressure, the residue was purified by silica gel chromatography using hexane/ethyl acetate mixture as eluent to give compound **F1** as a 395 mg of orange colour solid 80% of yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.02 – 7.99 (m, 1H), 7.86 (dd, *J* = 9.2, 2.6 Hz, 2H), 7.67 – 7.59 (m, 3H), 7.54 – 7.49 (m, 3H), 7.36 – 7.28 (m, 7H), 7.20 – 7.13 (m, 13H), 7.06 – 7.02 (m, 4H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.63, 160.09, 156.49, 151.22, 147.75, 147.57, 146.49, 146.33, 143.91, 140.77, 134.44, 132.78, 132.62, 131.91, 129.83, 129.76, 129.45, 127.77, 127.74, 127.06, 127.04, 126.28, 126.07, 125.09, 124.66, 124.64, 123.95, 123.17, 120.21, 120.00, 117.95, 114.79, 113.10, 100.74 ppm; HRMS m/z (ESI): calculated for $C_{48}H_{35}N_4S$ $[M+H]^+$ 699.2582, found 700.2569.

2.2.2 Procedure for the synthesis of F2 using Suzuki coupling reaction



A mixture of compound **S2** (351 mg, 0.6 mmol), K_2CO_3 (276 mg, 2.0 mmol), $Pd(PPh_3)_4$ (20 mg, 0.03 mmol) and (4-(diphenylamino)phenyl)boronic acid **3** (174 mg, 1 mmol) in THF/H_2O (18 mL/2 mL) was refluxed at 80 °C for 24 h under nitrogen atmosphere. After cooling to ambient temperature, diluted with 50 mL of DCM, filtered through a celite pad, and washed

with 20-40 mL of DCM. The combined organic phases were dried anhydrous Na_2SO_4 and concentrated under reduced pressure, the residue was purified by silica gel chromatography using Hexane/ethyl acetate mixture as eluent to give compound **F2** as a 365 mg of red colour solid 82% in of yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 8.02 – 7.96 (m, 2H), 7.87 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.68 – 7.60 (m, 3H), 7.55 – 7.47 (m, 3H), 7.31 – 7.22 (m, 5H), 7.14 (dt, *J* = 6.8, 1.5 Hz, 7H), 7.06 – 7.01 (m, 2H), 6.97 – 6.91 (m, 1H), 6.88 – 6.83 (m, 2H), 3.85 (t, *J* = 7.3 Hz, 2H), 1.81 (p, *J* = 7.6 Hz, 2H), 1.43 (p, *J* = 5.7, 4.1 Hz, 2H), 1.32 (dd, *J* = 7.2, 3.5 Hz, 4H), 0.88 (d, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.14, 156.51, 148.35, 147.71, 147.53, 143.58, 142.96, 140.75, 134.36, 132.47, 129.92, 129.43, 129.29, 128.94, 127.75, 127.65, 127.63, 127.03, 127.00, 126.86, 126.64, 124.85, 124.61, 123.91, 123.60, 123.47, 123.16, 117.55, 115.79, 115.21, 113.37, 101.63, 48.02, 31.53, 26.83, 26.68, 22.72, 14.13 ppm; HRMS m/z (ESI): calculated for $C_{48}H_{41}N_4S_2$ $[M+H]^+$ 737.2773, found 737.2734.

3.Copies of NMR spectrum of synthesized compounds (S1-S2)

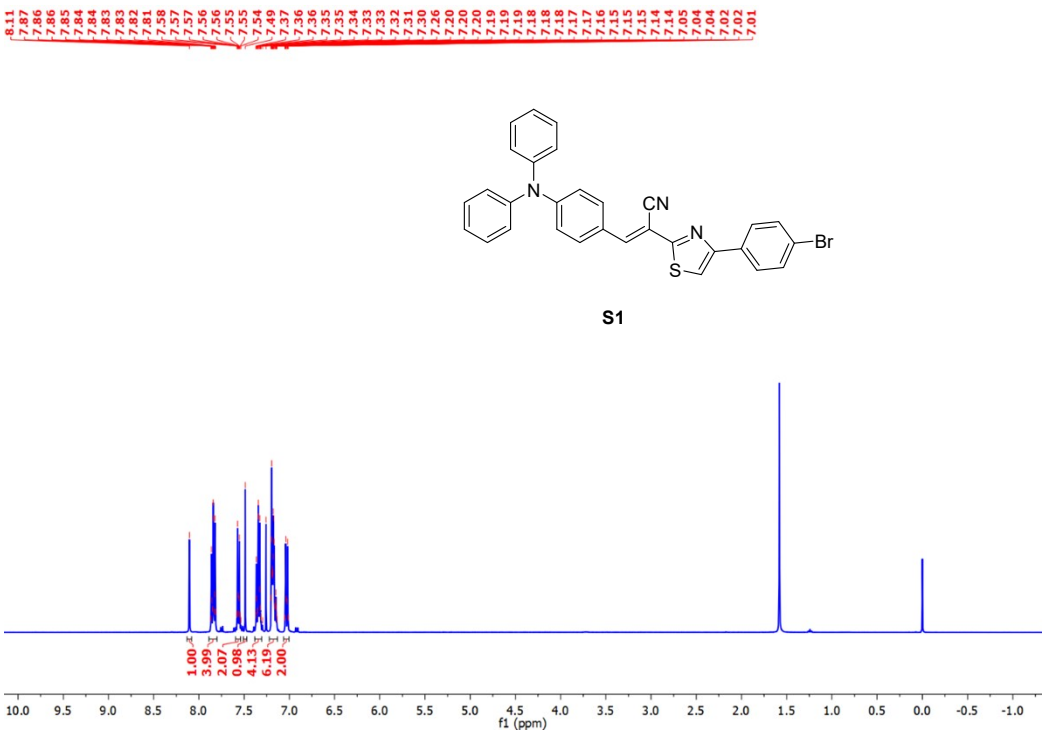


Figure 1. ^{1}H NMR spectrum of compound **S1** in $CDCl_3$

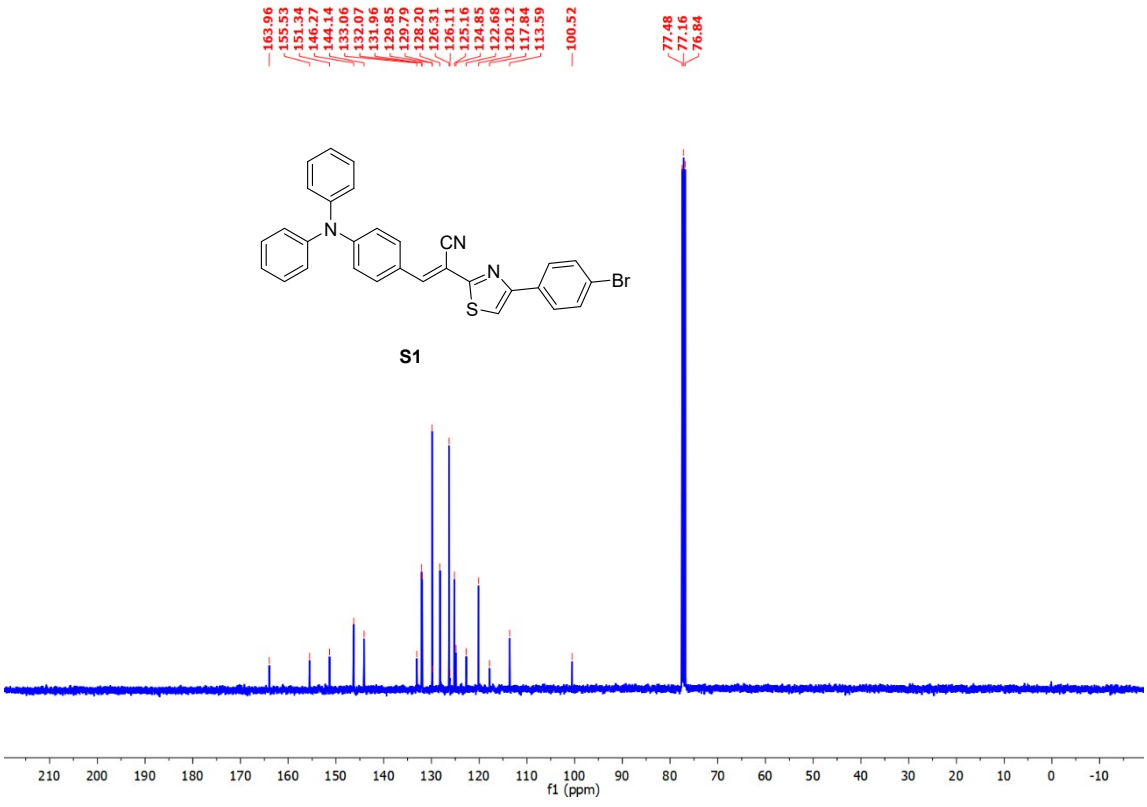


Figure 2. ^{13}C NMR spectrum of compound **S1** in $CDCl_3$

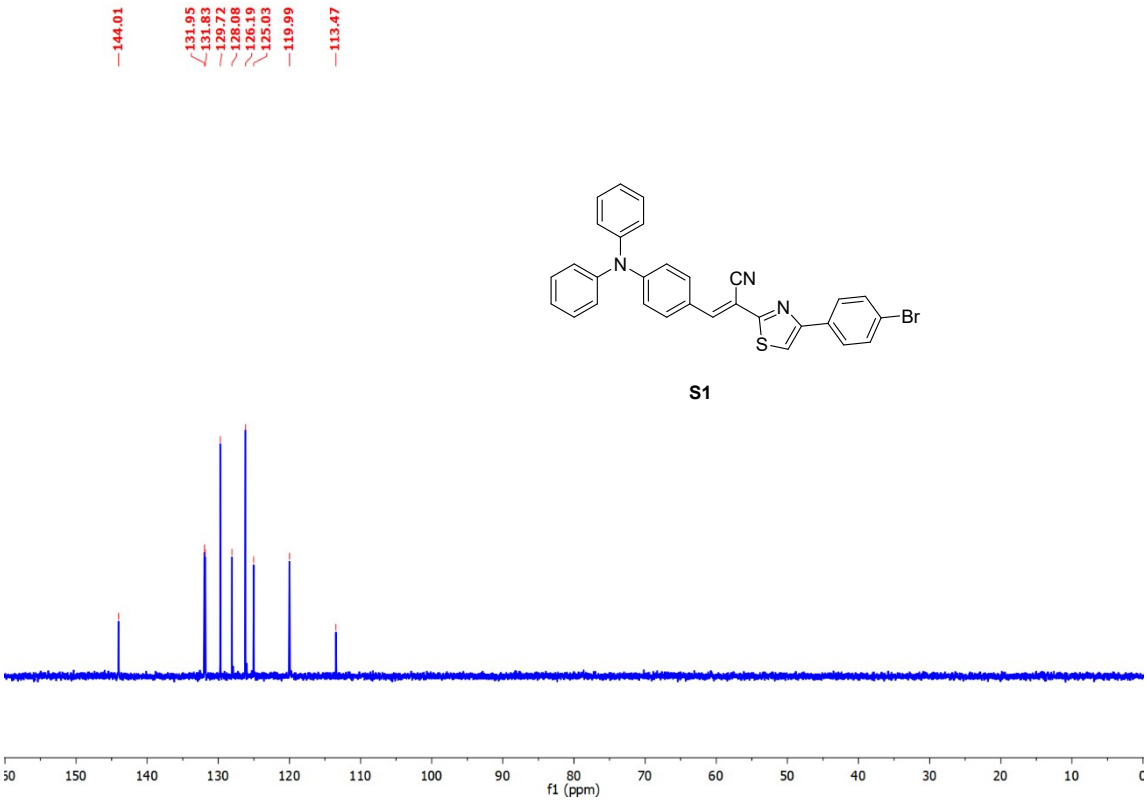


Figure 3. DEPT-135 NMR spectrum of compound **S1** in $CDCl_3$

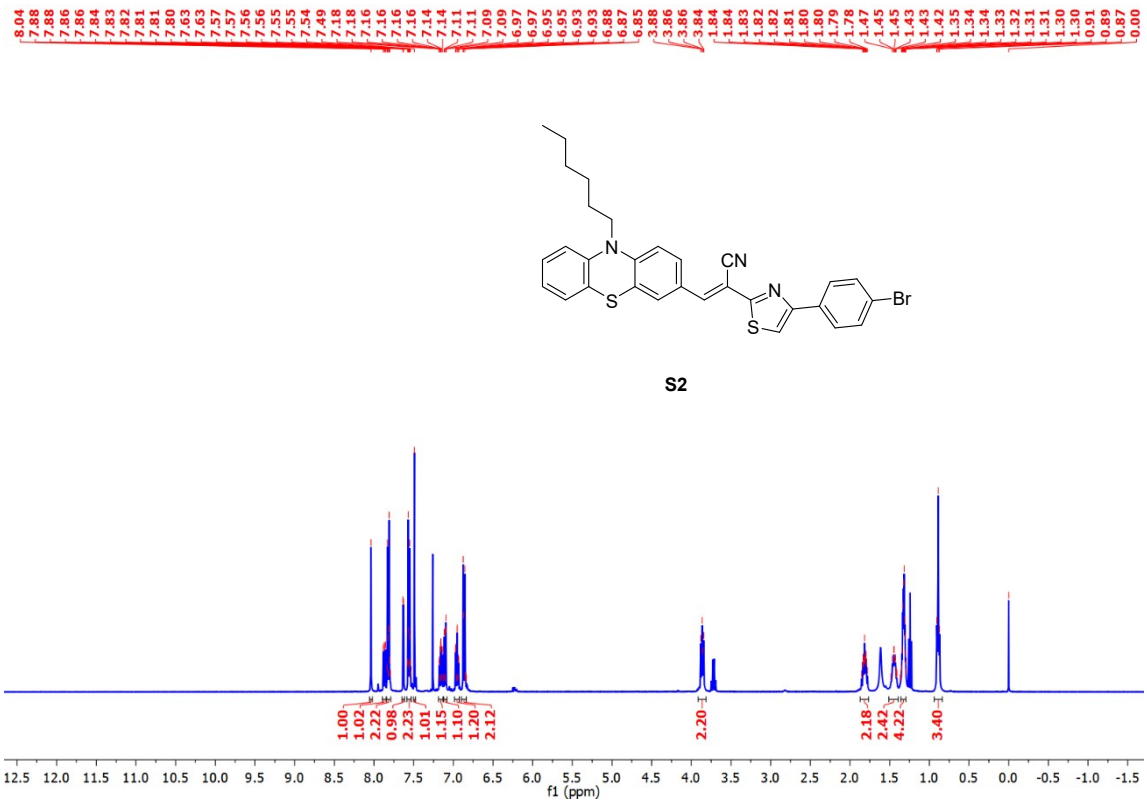


Figure 4. ^1H NMR spectrum of compound **S2** in $CDCl_3$

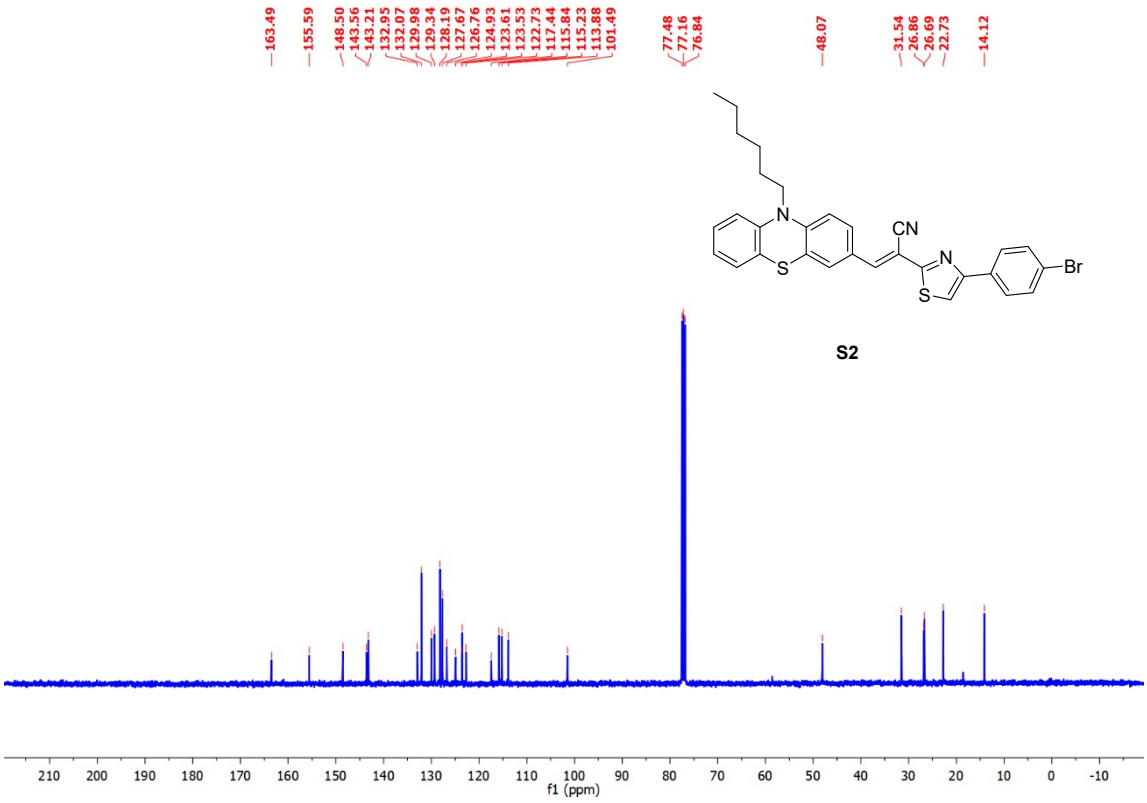


Figure 5. ^{13}C NMR spectrum of compound **S2** in $CDCl_3$

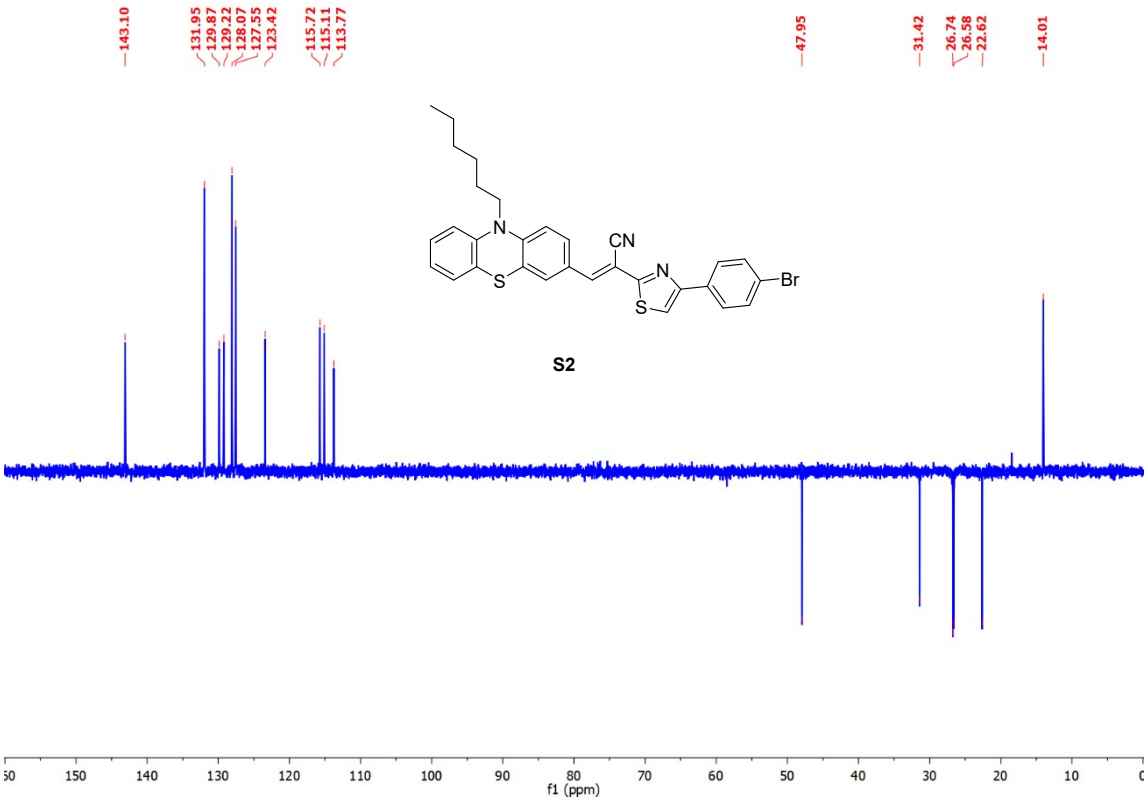


Figure 6. DEPT-135 NMR spectrum of compound **S2** in $CDCl_3$

Copies of ^{1}H, ^{13}C NMR and Mass spectrum of synthesized compounds (F1-F2)

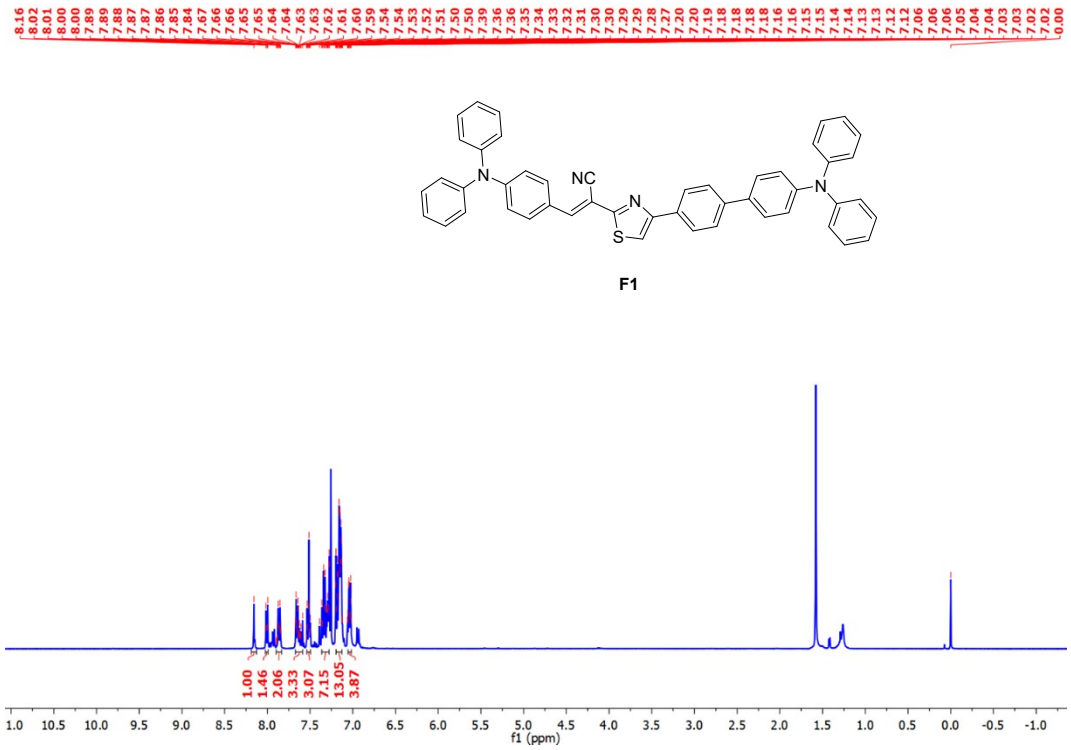


Figure 7. ^{1}H NMR spectrum of compound **F1** in $CDCl_3$

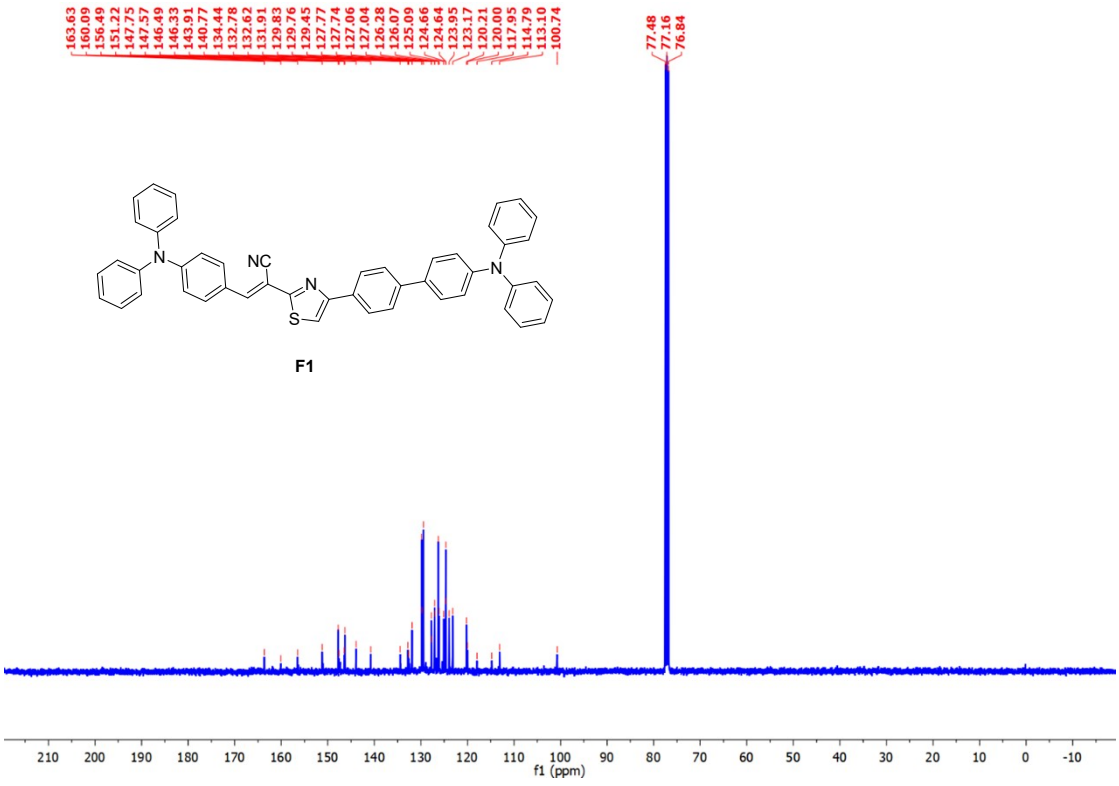


Figure 8. ^{13}C NMR spectrum of compound **F1** in $CDCl_3$

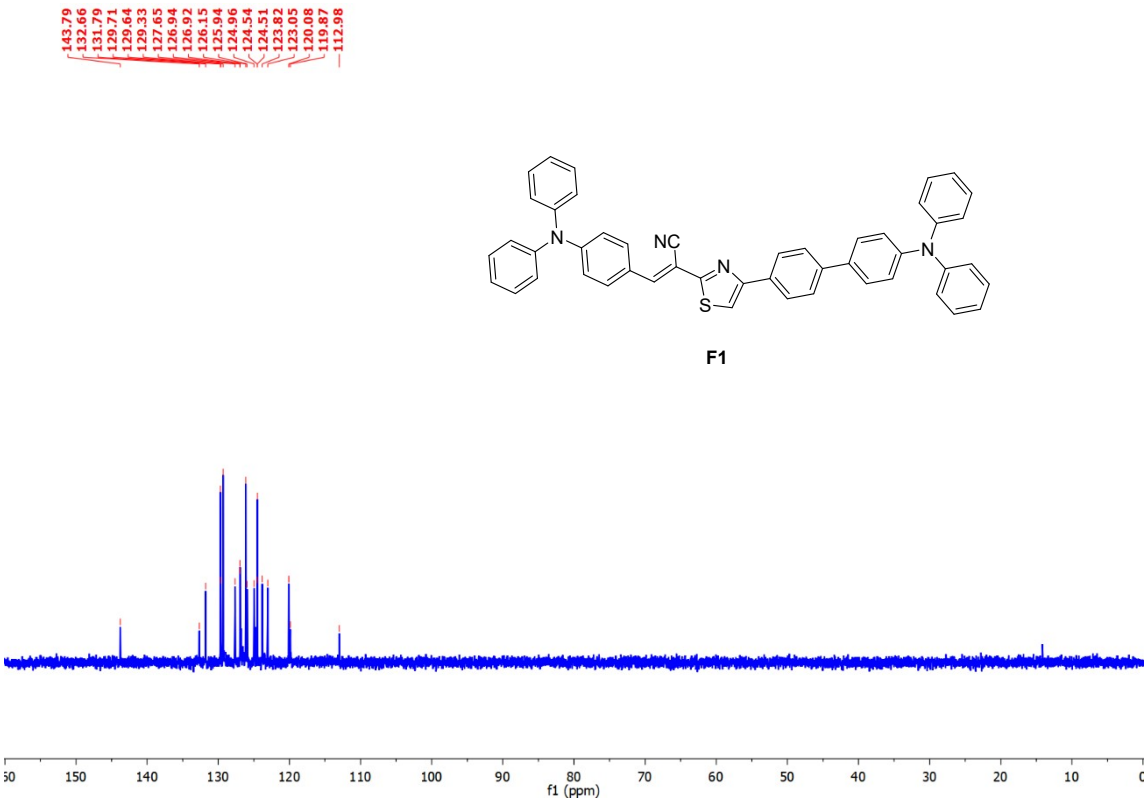


Figure 9. DEPT-135 NMR spectrum of compound **F1** in $CDCl_3$

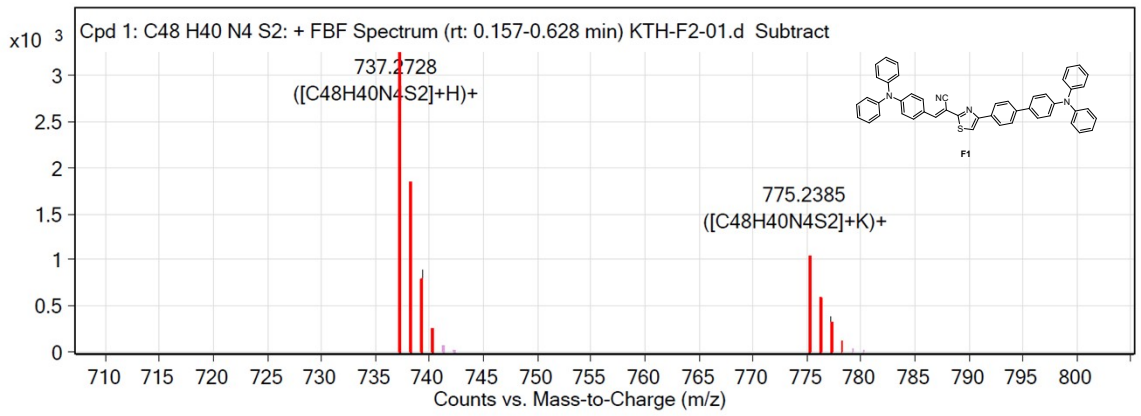


Figure 10. HRMS spectrum of compound **F1**

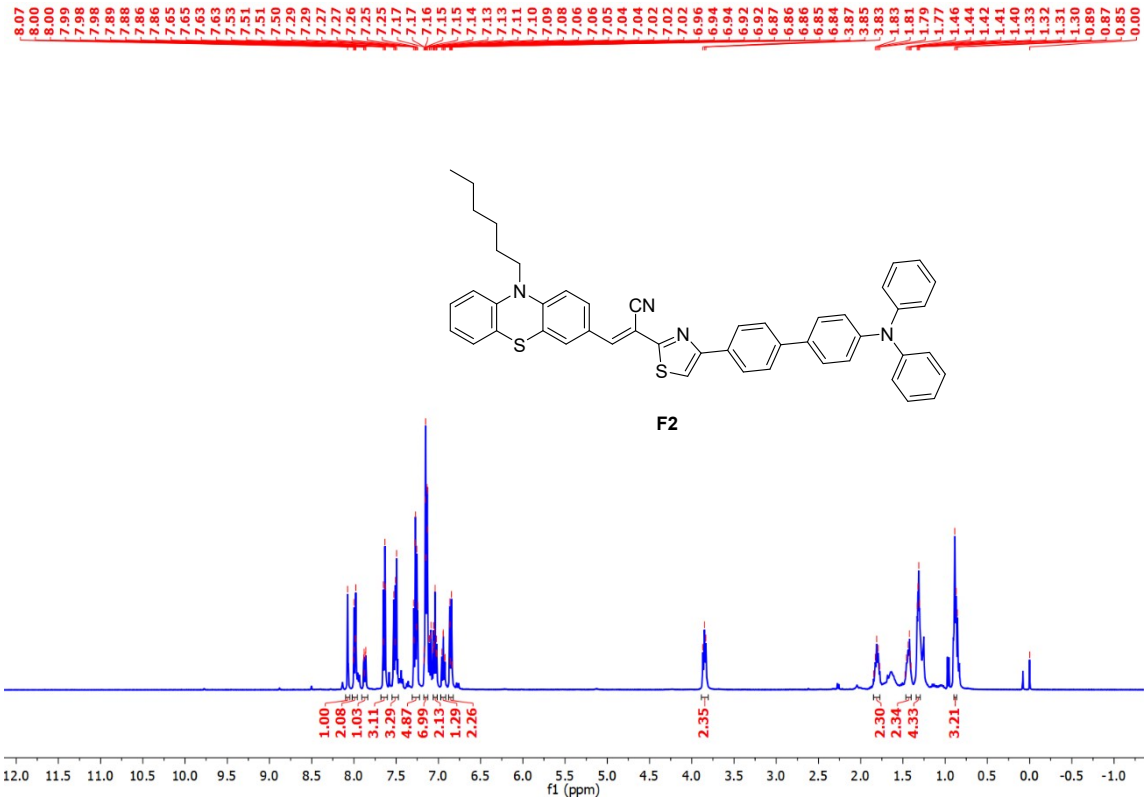


Figure 11. ^{1}H NMR spectrum of compound **F2** in $CDCl_3$

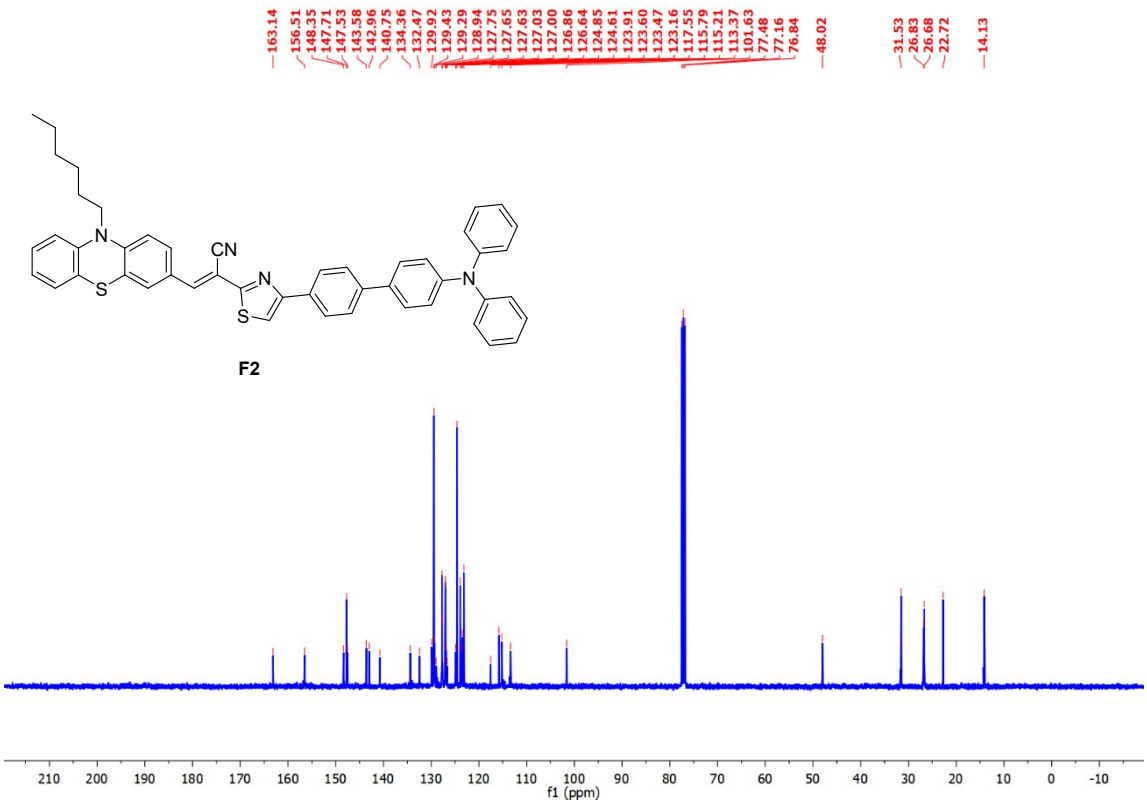


Figure 12. ^{13}C NMR spectrum of compound **F2** in $CDCl_3$

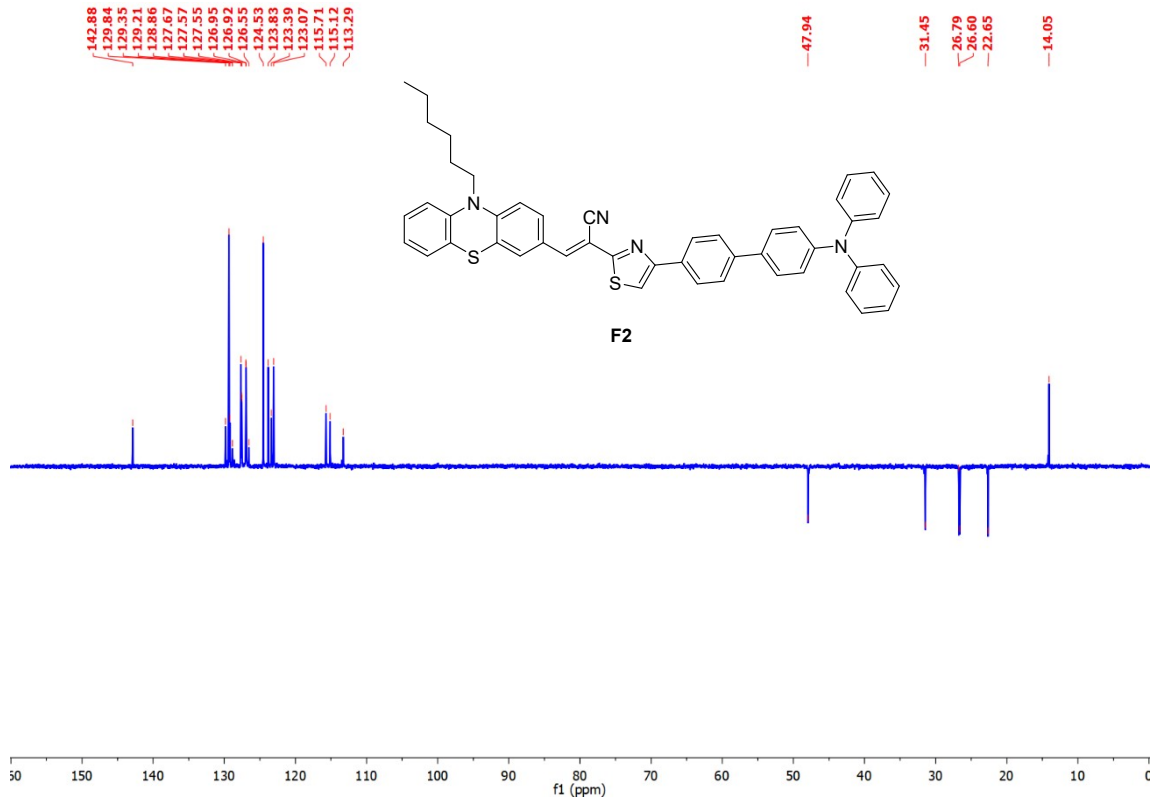


Figure 13. DEPT-135 NMR spectrum of compound **F2** in $CDCl_3$

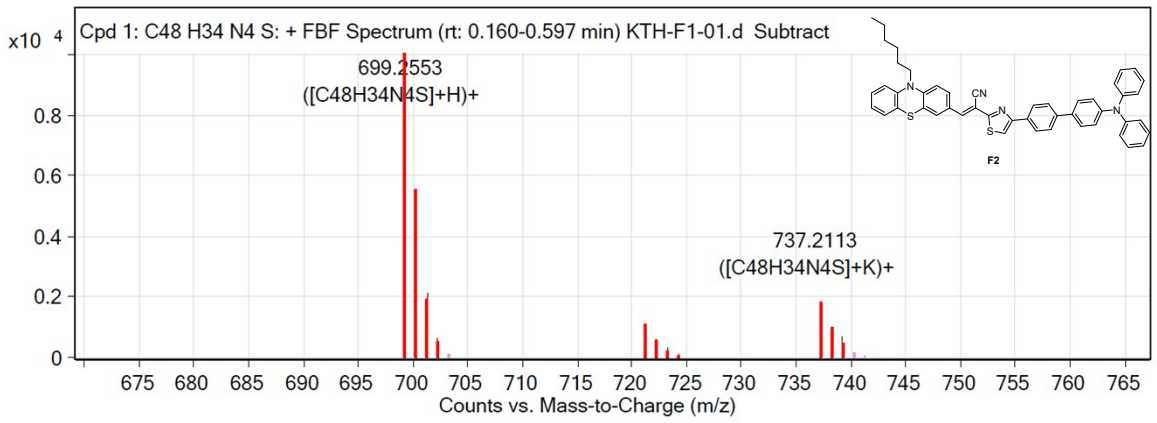


Figure 14. HRMS spectrum of compound **F2**

4. Thermal Properties

Thermogravimetric analysis (TGA) was carried out using a TA SDTQ-600 instrument. The findings of the TGA of all five luminogens were studied under a nitrogen environment at a heating rate of 5 °C min^{-1} from 30 to 1000 °C. Differential scanning calorimetry (DSC) was performed on a TA SDTQ – 200 instrument and was carried out at a heating rate of 10 °C from 30 to 500 °C under a nitrogen environment.

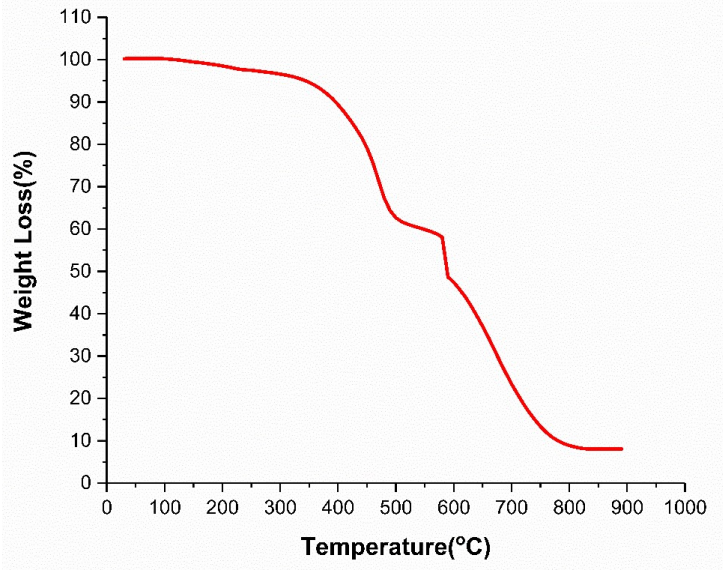


Figure 15: Thermogravimetric analysis (TGA) curves of **F1**.

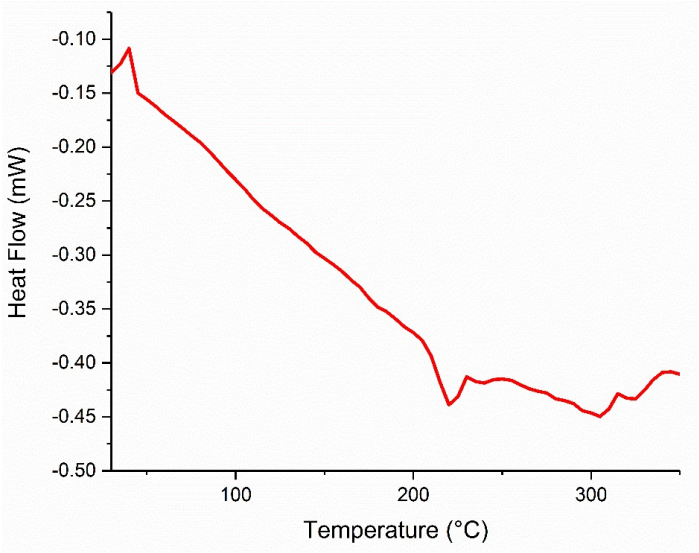


Figure 16: Differential scanning calorimetry (DSC) curves of **F1**.

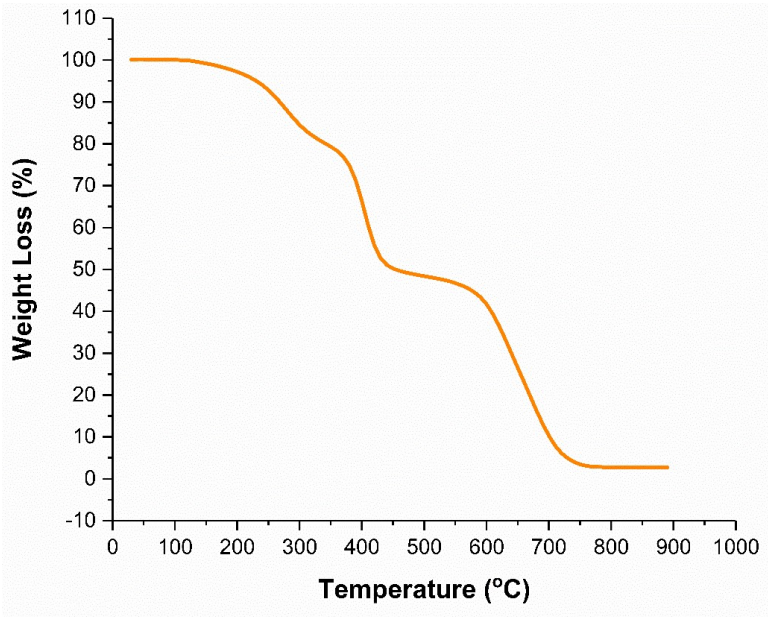


Figure 17: Thermogravimetric analysis (TGA) curves of **F2**.

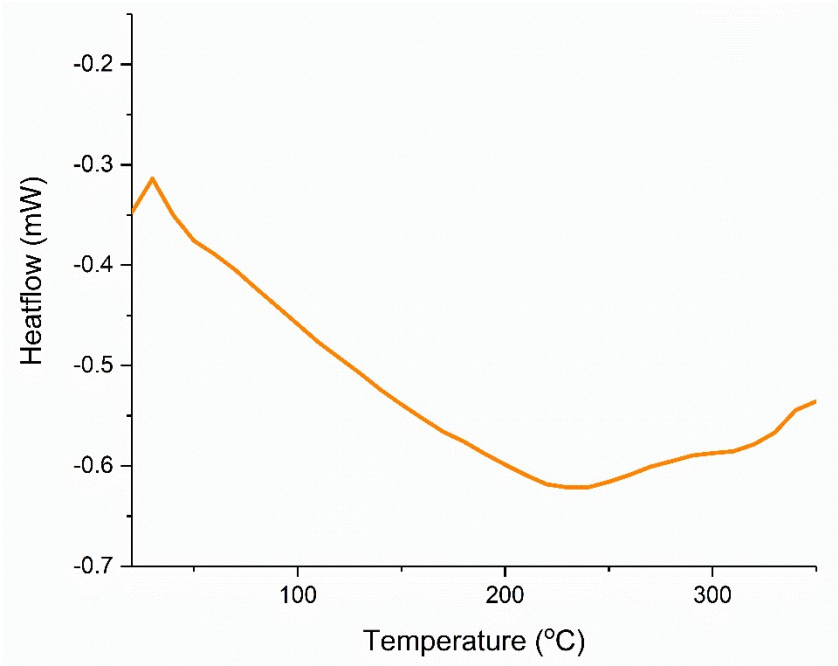


Figure 18: Differential scanning calorimetry (DSC) curves of **F2**.

5.DFT Calculations

Density functional theory (DFT) calculation of the compounds from F1 and F2 were carried out using Gaussian 09 program package. The ground state geometries of these luminogens were optimized using DFT-B3LYP-6-311G basis set. To obtain the information on absorption properties, Time-dependent density functional theory (TDDFT) calculations were performed using the optimized geometries.

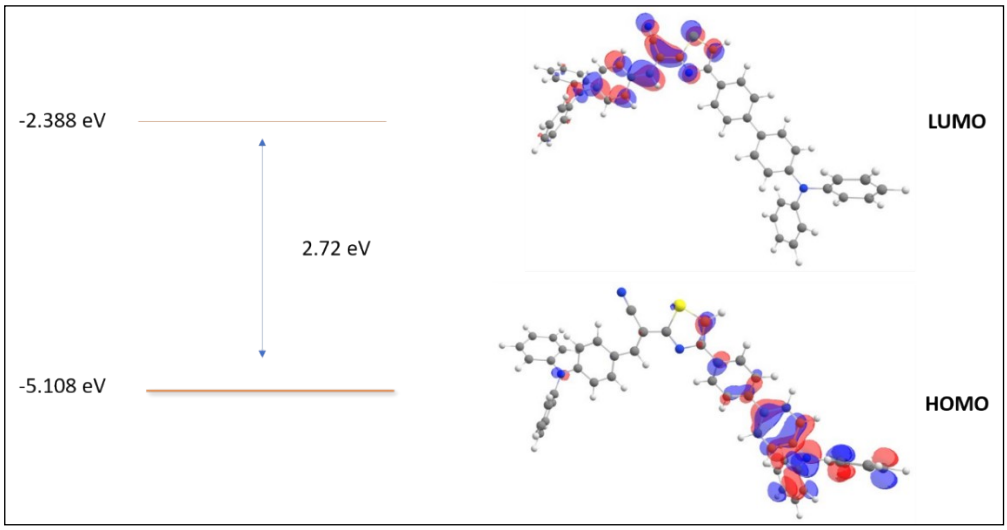


Figure 19: HOMO and LUMO levels of F1

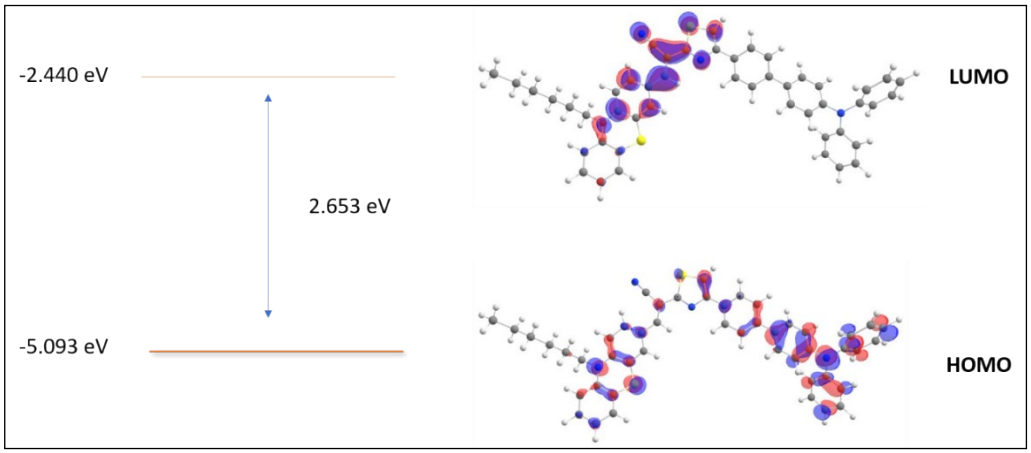


Figure 20: HOMO and LUMO levels of F2

6.Photoluminescence Studies

6.1 Absorption and Fluorescent spectra of synthesised compounds

The steady state and time resolved photoluminescence properties of 2 using fluorescence spectrometer from Edinburgh Instruments (FLS 1000) was investigated. For steady state luminescence measurement, the sample was exited using 380 and 420 nm collimated beam from the excitation monochromator of the spectrometer, which is pumped using a 450 W Xe2 continuous xenon lamp. The emission spectrum after passing through emission monochromator is scanned and detected in high-gain red sensitive photomultiplier (PMT) detector with spectral coverage from 400 nm to ~ 800 nm. The luminescence lifetime was measured using standard time-correlated single photon counting (TCSPC) technique using picosecond pulsed diode laser of wavelength 405 nm from Edinburgh Instruments (EPL-405) with repetition rate 200 KHz. The decay is fitted using reconvolution fit analysis using the IRF to extract the life-time parameters from the whole time resolved measurement.

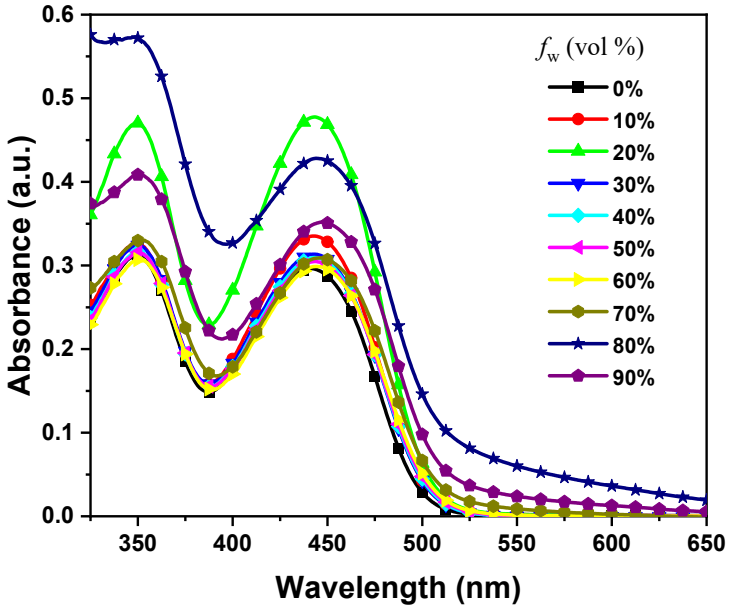


Figure 21: Full absorption spectrum of **F1** in THF/Water mixtures with varying water fraction(*fw*); Con-10µM; Excitation wavelength-380 nm.

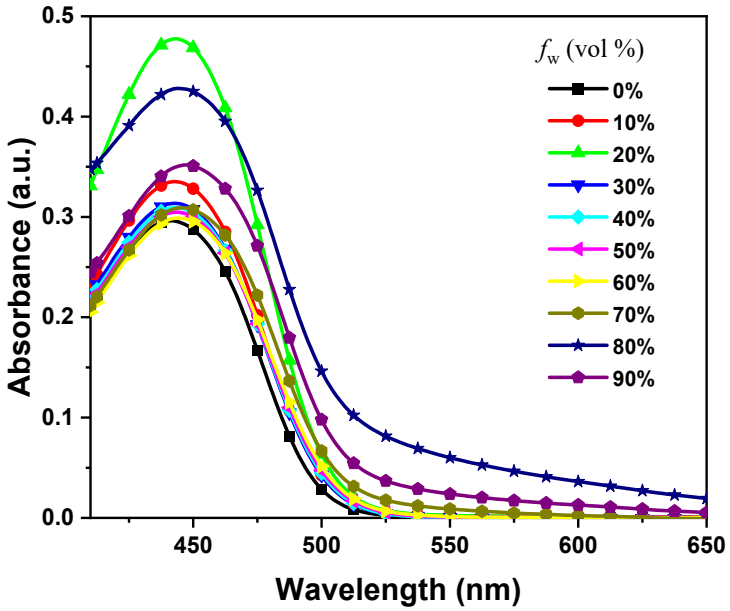


Figure 22: Absorption spectrum of **F1** in THF/Water mixtures with varying water fraction(*fw*); Con-10μM; Excitation wavelength-380 nm.

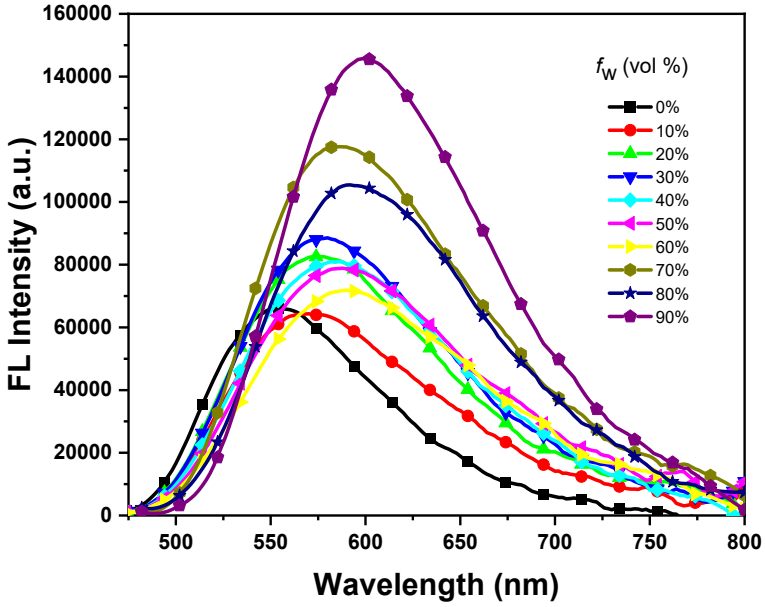


Figure 23: PL spectrum of **F1** in THF/Water mixtures with varying water fraction(*fw*); Con-10μM; Excitation wavelength-380 nm.

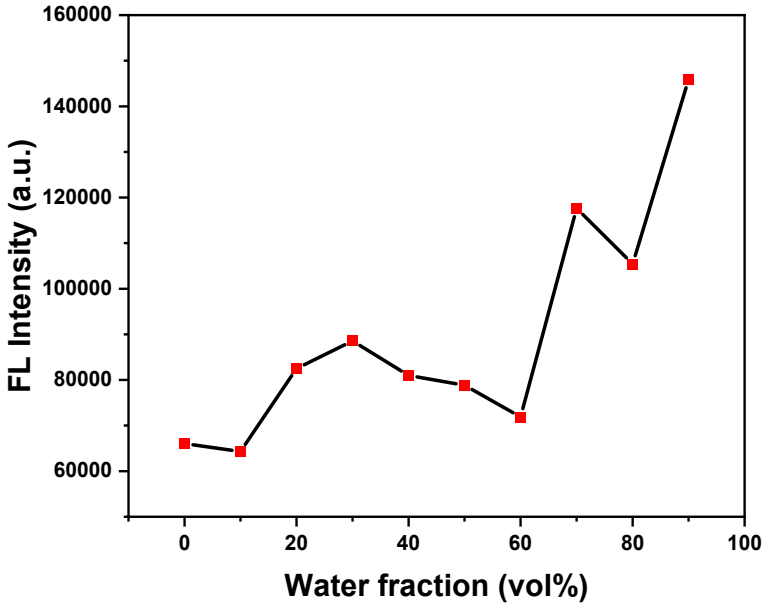


Figure 24. varying water fraction(*fw*)

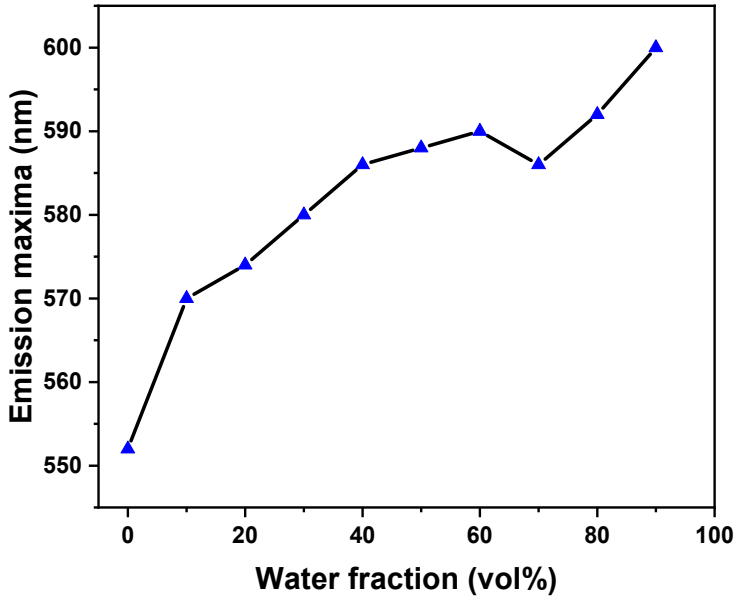


Figure 25. varying water fraction(*fw*)

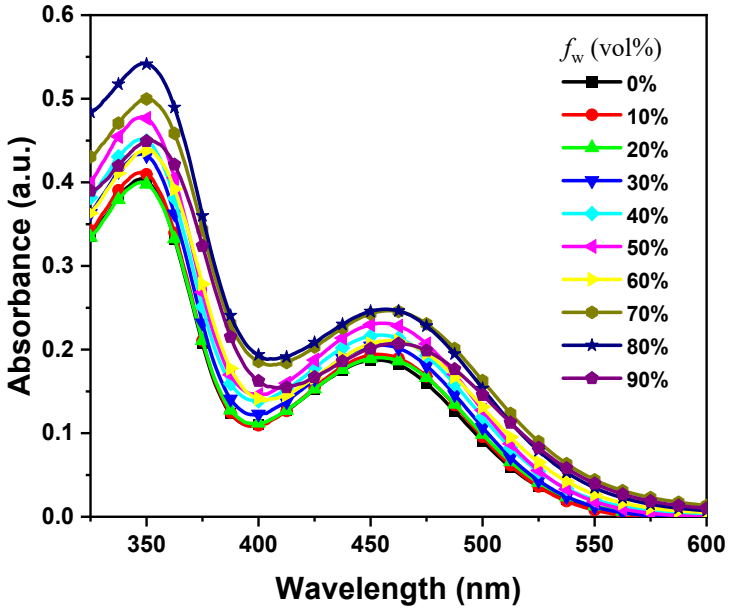


Figure 26: Full absorption spectrum of **F2** in THF/Water mixtures with varying water fraction(*fw*); Con-10μM; Excitation wavelength-380 nm.

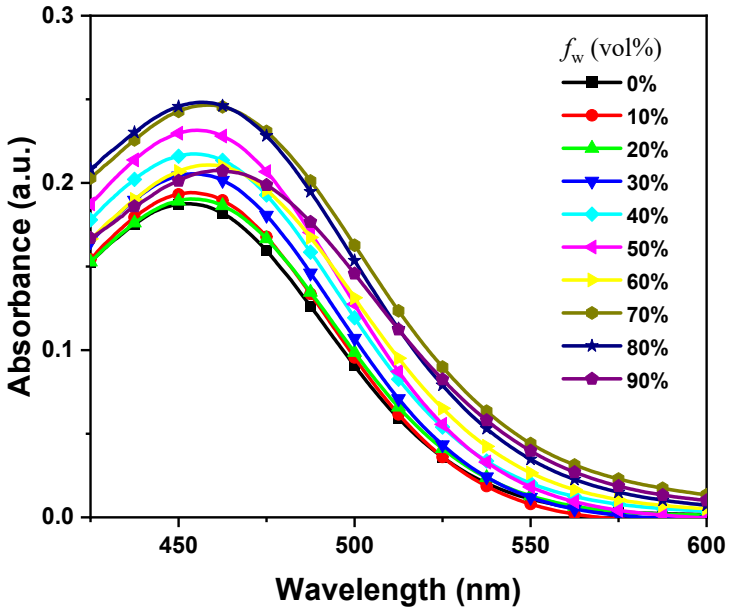


Figure 27: Absorption spectrum of **F2** in THF/Water mixtures with varying water fraction(*fw*); Con-10μM; Excitation wavelength-380 nm.

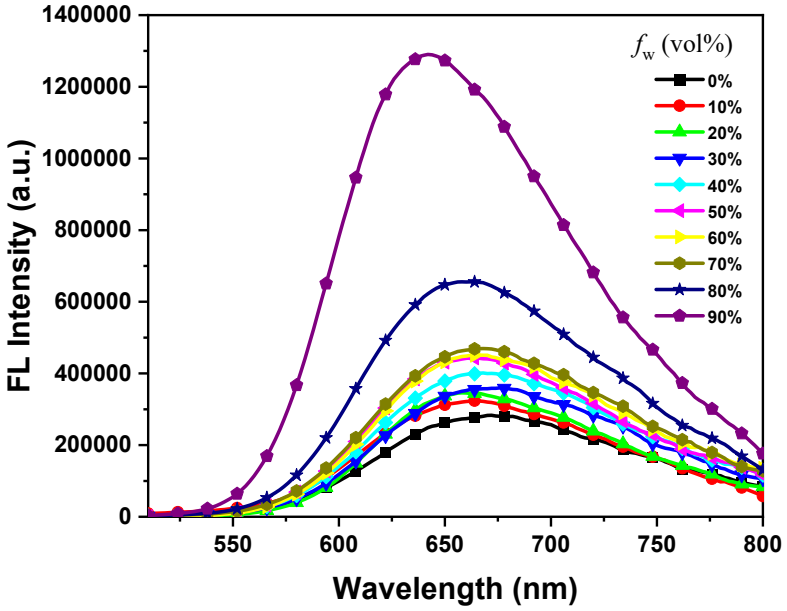


Figure 28: PL spectrum of **F1** in THF/Water mixtures with varying water fraction(*fw*); Con-10μM; Excitation wavelength-380 nm.

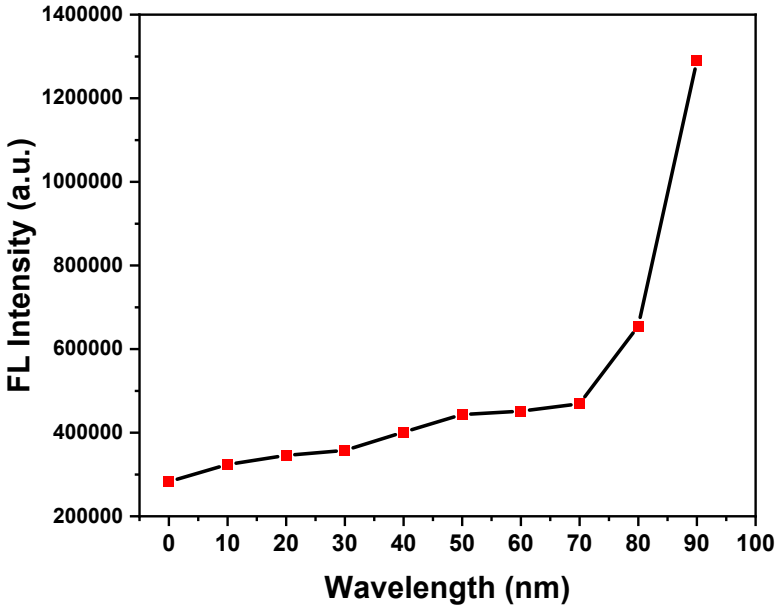


Figure 29. varying water fraction(*fw*)

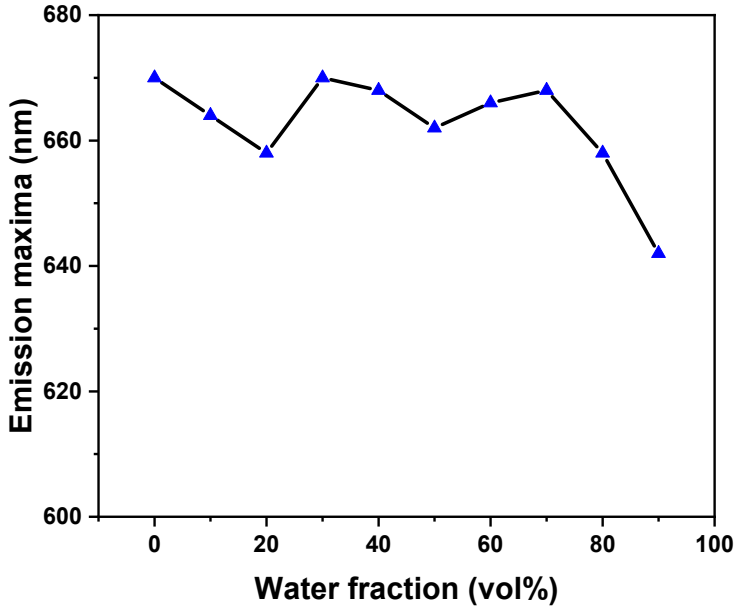
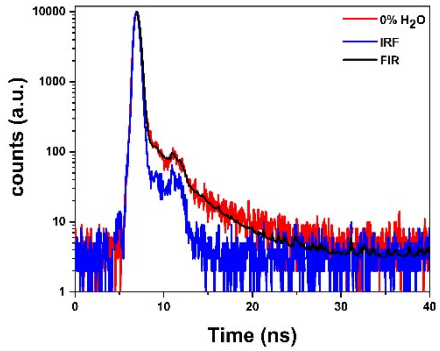


Figure 30. varying water fraction(*fw*)

6.2 Tail Fitting of Time Resolved Decay (Solid Thin Film Samples)

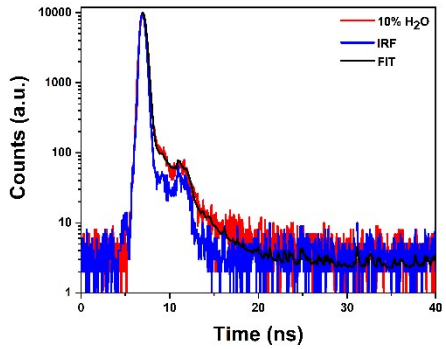
Compound F1:



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1248	0.00396	B_1	☐	0.421	0.0100	94.48
τ_2	☐	3.8121	0.08197	B_2	☐	0.001	0.0000	5.52
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0699	0.0072	A	☐	3.104		

χ^2 : 1.348

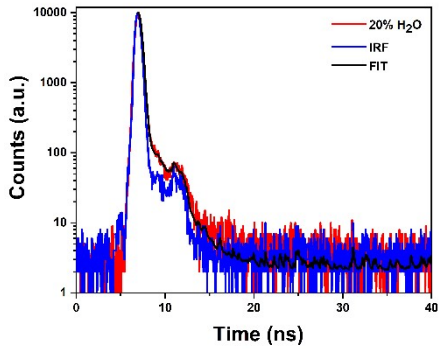
Figure 31: Lifetime studies of the Luminogen **F1** at *f*w 0%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1424	0.00316	B_1	☐	0.373	0.0064	96.43
τ_2	☐	2.7549	0.08118	B_2	☐	0.001	0.0000	3.57
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0628	0.0055	A	☐	2.287		

χ^2 : 1.302

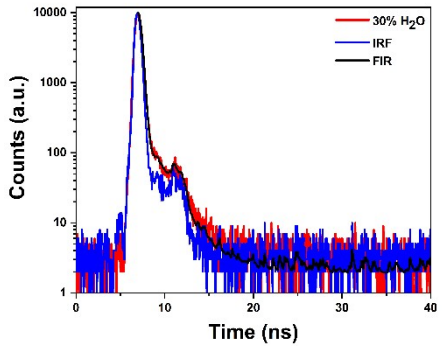
Figure 32: Lifetime studies of the Luminogen **F1** at *f*w 10%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1292	0.00376	B_1	☐	0.400	0.0097	96.54
τ_2	☐	2.0903	0.06814	B_2	☐	0.001	0.0001	3.46
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0347	0.0055	A	☐	2.154		

χ^2 : 1.324

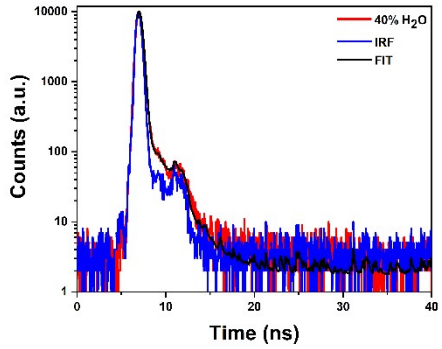
Figure 33: Lifetime studies of the Luminogen **F1** at *f*w 20%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1512	0.00331	B_1	☐	0.357	0.0053	97.28
τ_2	☐	2.3406	0.08743	B_2	☐	0.001	0.0000	2.72
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0581	0.0068	A	☐	1.941		

χ^2 : 1.179

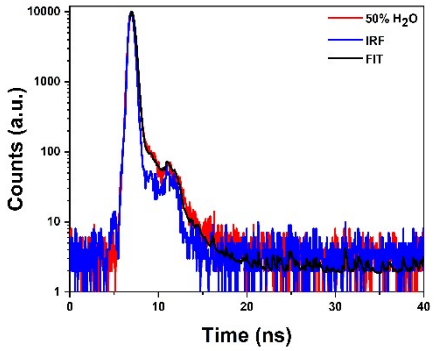
Figure 34: Lifetime studies of the Luminogen **F1** at *f*w 30%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1490	0.00242	B_1	☐	0.362	0.0050	96.79
τ_2	☐	2.2066	0.07147	B_2	☐	0.001	0.0000	3.21
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0492	0.0035	A	☐	1.803		

χ^2 : 1.150

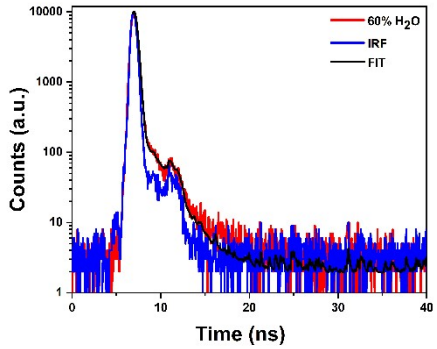
Figure 35: Lifetime studies of the Luminogen **F1** at *f*w 40%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1490	0.00244	B_1	☐	0.358	0.0050	96.75
τ_2	☐	2.2723	0.07448	B_2	☐	0.001	0.0000	3.25
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0493	0.0035	A	☐	1.870		

χ^2 : 1.191

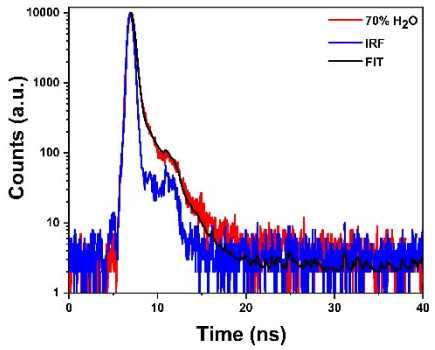
Figure 36: Lifetime studies of the Luminogen **F1** at *f*w 50%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.1413	0.00343	B_1	☐	0.378	0.0079	96.25
τ_2	☐	2.2977	0.06654	B_2	☐	0.001	0.0000	3.75
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.0432	0.0047	A	☐	1.930		

χ^2 : 1.295

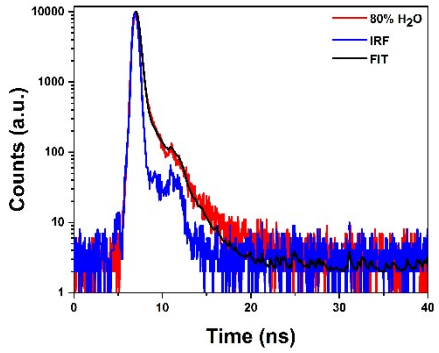
Figure 37: Lifetime studies of the Luminogen **F1** at *f*w 60%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.2239	0.00332	B_1	☐	0.255	0.0026	90.39
τ_2	☐	1.8327	0.03252	B_2	☐	0.003	0.0001	9.61
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.1080	0.0054	A	☐	1.998		

χ^2 : 1.575

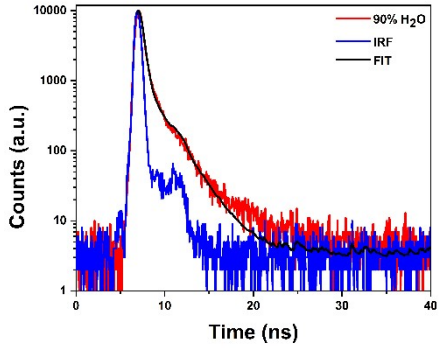
Figure 38: Lifetime studies of the Luminogen **F1** at *f*w 70%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.2375	0.00342	B_1	☐	0.247	0.0023	89.99
τ_2	☐	1.9368	0.03240	B_2	☐	0.003	0.0001	10.01
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.1071	0.0061	A	☐	1.948		

χ^2 : 1.688

Figure 39: Lifetime studies of the Luminogen **F1** at *f*w 80%**.**

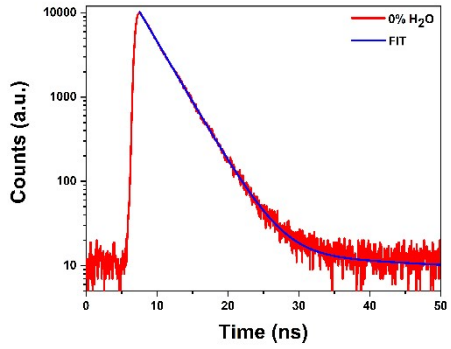


	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.3442	0.00373	B_1	☐	0.181	0.0013	80.25
τ_2	☐	2.1910	0.02318	B_2	☐	0.007	0.0002	19.75
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
δt	☐	0.1224	0.0042	A	☐	3.026		

χ^2 : 2.076

Figure 40: Lifetime studies of the Luminogen **F1** at *f*w 90%**.**

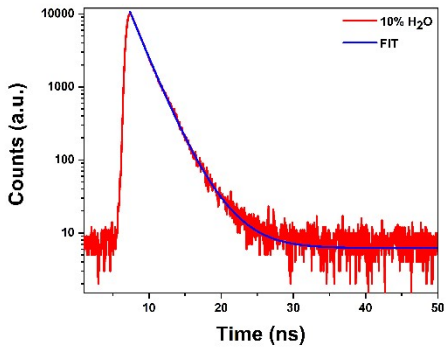
Compound F2:



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	3.0277	0.00502	B_1	☐	10365.397	19.2898	93.02
τ_2	☐	129.1813	403.67880	B_2	☐	18.226	35.7946	6.98
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	-2.884		

χ^2 : 1.190

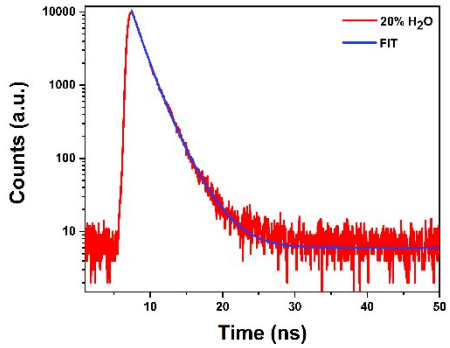
Figure 41: Lifetime studies of the Luminogen **F2** at *f*w 0%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	1.7306	0.01596	B_1	☐	10276.019	139.6283	87.23
τ_2	☐	3.3605	0.15691	B_2	☐	774.488	152.2152	12.77
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	6.176		

χ^2 : 1.540

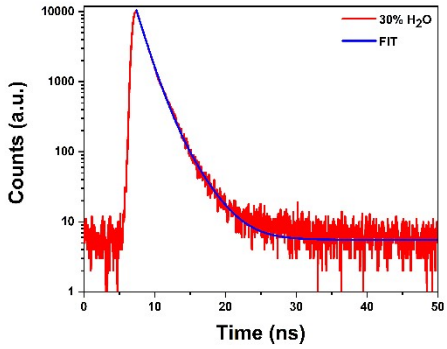
Figure 42: Lifetime studies of the Luminogen **F2** at *f*w 10%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	1.3941	0.01748	B_1	☐	9293.639	182.1912	75.67
τ_2	☐	2.6643	0.07793	B_2	☐	1563.581	196.9660	24.33
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	6.036		

χ^2 : 1.435

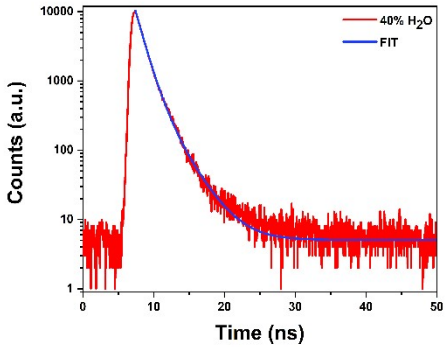
Figure 43: Lifetime studies of the Luminogen **F2** at *f*w 20%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	1.2426	0.01215	B_1	☐	9600.274	105.0708	77.68
τ_2	☐	2.6844	0.06526	B_2	☐	1276.866	117.1099	22.32
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	5.539		

χ^2 : 1.407

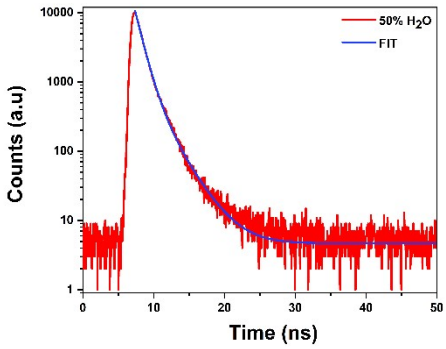
Figure 44: Lifetime studies of the Luminogen **F2** at *f*w 30%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	1.1147	0.00967	B_1	☐	9597.613	73.5401	77.20
τ_2	☐	2.6720	0.05449	B_2	☐	1182.632	82.2575	22.80
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	5.071		

χ^2 : 1.278

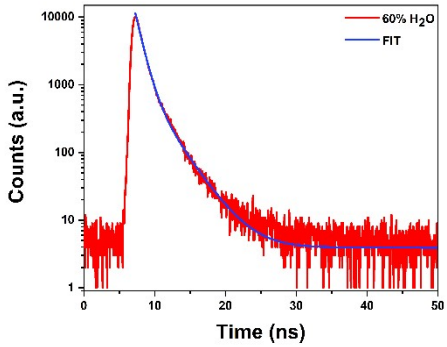
Figure 45: Lifetime studies of the Luminogen **F2** at *f*w 40%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.9879	0.00767	B_1	☐	10021.526	58.1558	77.17
τ_2	☐	2.6150	0.04623	B_2	☐	1119.962	62.2654	22.83
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	4.663		

χ^2 : 1.399

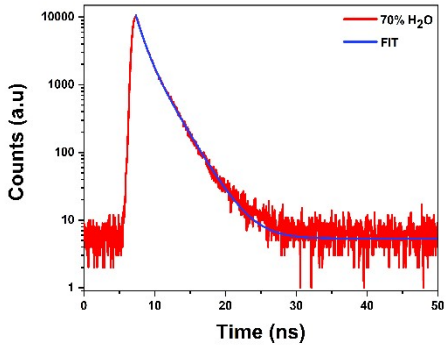
Figure 46: Lifetime studies of the Luminogen **F2** at *f*w 50%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.8960	0.00582	B_1	☐	10800.046	46.6010	74.17
τ_2	☐	2.8453	0.03445	B_2	☐	1184.679	39.6815	25.83
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	3.915		

χ^2 : 1.974

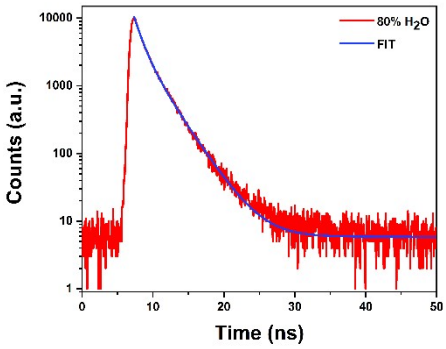
Figure 47: Lifetime studies of the Luminogen **F2** at *f*w 60%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.9826	0.01501	B_1	☐	7071.668	94.4725	40.75
τ_2	☐	2.4727	0.01965	B_2	☐	4085.621	104.8806	59.25
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	5.327		

χ^2 : 1.407

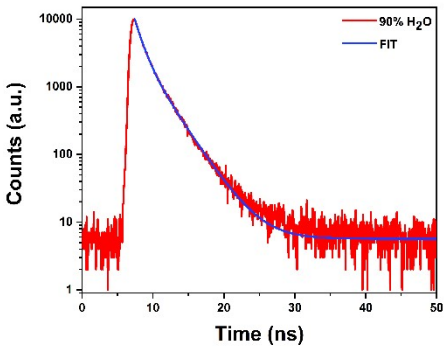
Figure 48: Lifetime studies of the Luminogen **F2** at *f*w 80%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	1.0444	0.01497	B_1	☐	6934.769	80.8451	39.79
τ_2	☐	2.7551	0.02005	B_2	☐	3977.703	89.2460	60.21
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	5.880		

χ^2 : 1.573

Figure 49 : Lifetime studies of the Luminogen **F2** at *f*w 80%**.**



	Fix	Value / ns	Std. Dev / ns		Fix	Value	Std. Dev	Rel %
τ_1	☐	0.9965	0.01350	B_1	☐	6846.469	69.5667	41.26
τ_2	☐	2.7656	0.02017	B_2	☐	3511.462	75.7398	58.74
τ_3	☐			B_3	☐			
τ_4	☐			B_4	☐			
				A	☐	5.686		

χ^2 : 1.453

Figure 50 : Lifetime studies of the Luminogen **F2** at *f*w 90%**.**

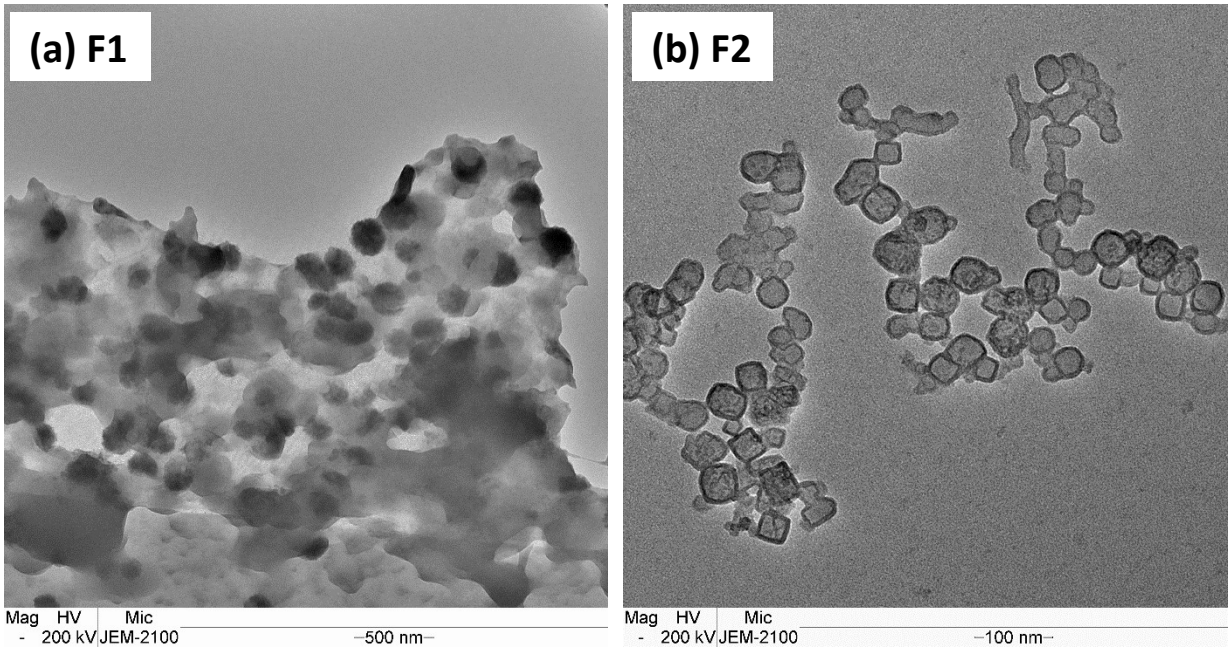
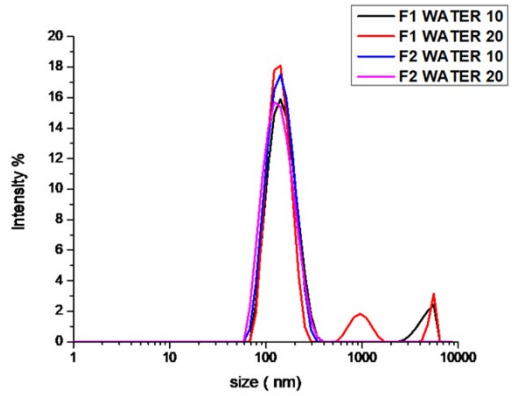


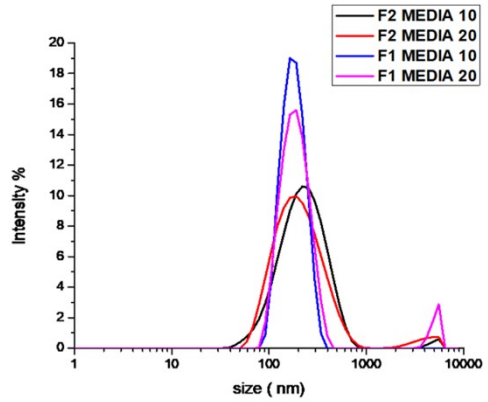
Figure 51: Transmission electron micrographs of (a) F1 dye in *fw* 90% and (b) F2 dye in *fw* 70%. Scale bar is 500 nm for (a) and 100 nm for (b).

(a) **Water**



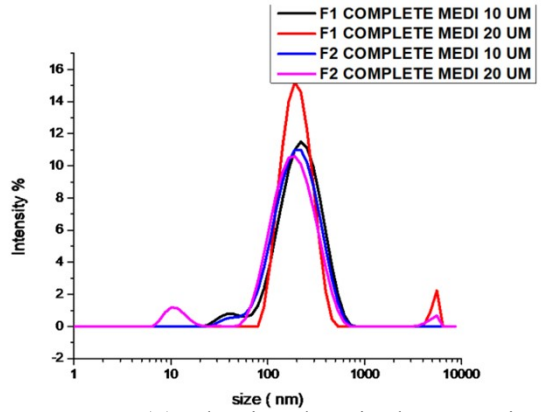
Name	Size(nm)	PDI	Zeta potential
F1 water 10 μM	177	0.361	-46
F1 water 20 μM	198	0.321	-37
F2 water 10 μM	130	0.211	-49
F2 water 20 μM	134	0.276	-45
Only water(25°C)	25	0.875	-0.0054

(b) **Serum free medium**



Name	Size(nm)	PDI	Zeta potential
F1 only media 10 μM	195	0.278	1.56
F1 only media 20 μM	199	0.380	2.300
F2 only media 10 μM	198	0.411	2.311
F2 only media 20 μM	179	0.250	1
Only water	25	0.875	+1

(c) **Serum (10%) containing medium**



Name	Size(nm)	PDI	Zeta potential
F1 10% FBS-media 10 μM	193	0.378	2.56
F1 10% FBS-media 20 μM	172	0.480	2.45
F2 10% FBS-media 10 μM	145	0.475	0.078
F2 10% FBS-media 20 μM	136	0.350	0.0567
Only water	25	0.875	+3

Figure 52: (a) Physicochemical properties of aggregated dye in water:DMSO. Formation of F1 and F2 dyes in fw 90% followed by table showing size, PDI and zeta potential for two concentrations (10 and 20 μM) study in water. The stability of such aggregated dye probed in different cell culture media. (b) Size, PDI and zeta potential of F1 and F2 nano-aggregates incubated for two concentrations (10 and 20 μM) aggregated in water:DMSO and then incubated in serum free biological medium for 24 h. (c) Size, PDI and zeta potential for two concentrations (10 and 20 μM) aggregated dye in water:DMSO and then incubated in 10 % serum (FBS) containing biological medium for 24 h.