Lutidine Derivatives for Live-Cell Imaging of Mitochondria and Endoplasmic Reticulum

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Supporting information

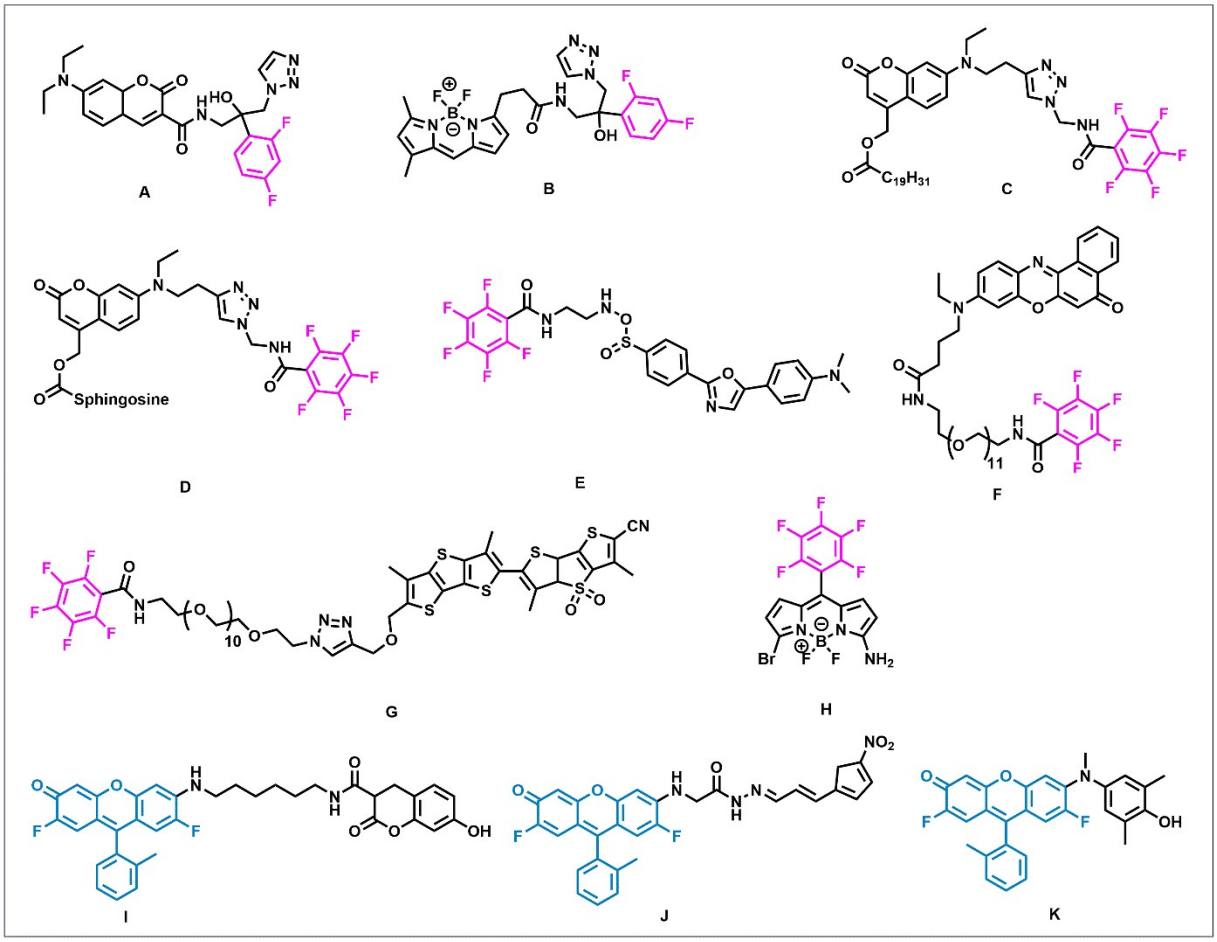


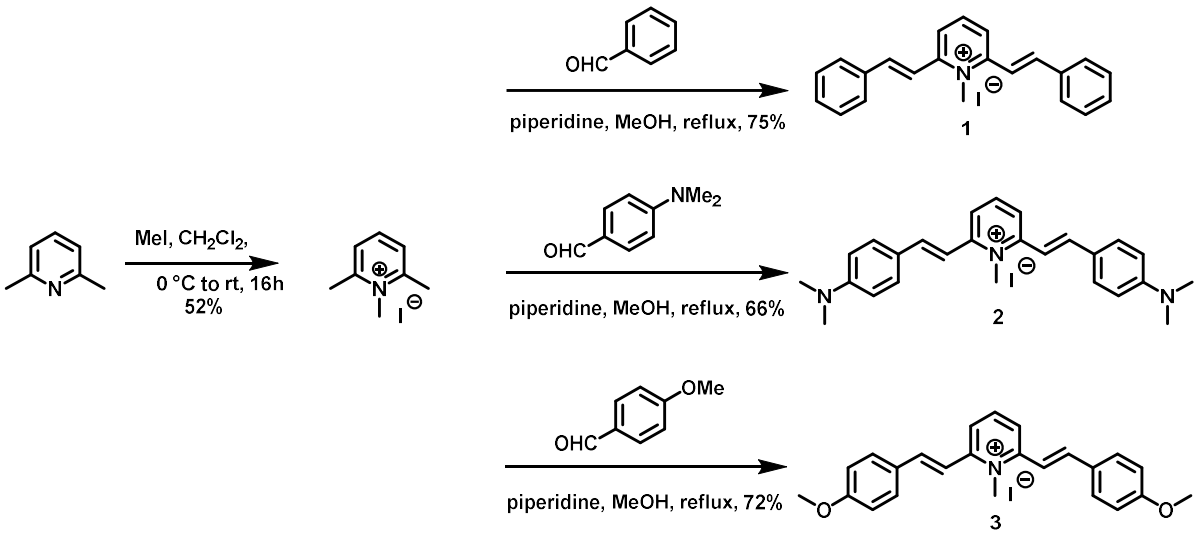
Fig S1. Molecules bearing aryl fluoro moieties known in literature for targeting ER.

Table- S1 Previous studies done on ER using poly fluorinated compounds

SNo.	Probe	ER Imaging (type of cells)	Other Biological Investigations if any	references
1	A-B	Fungal cells	Live fungal cell imaging	1
2	C-D	Live cells (HeLa cells)	Live cell imaging	2
3	E	Live cells and fixed cells	Live cell imaging (Commercial dye)	3
4	F	Live cells (KB cells)	Lipid order study	4
5	G	Live cells (Hela Kyoto cells)	Membrane tension	5
6	H	Live cells (HeLa cells)	Live cell imaging	3
7	I	Live cells (HeLa cells)	·OH detection	6
8	J	Live cells (HeLa cells)	Small molecule delivery	7
9	K	Live cells (RAW 264.7 cells)	NO_3^- detection	8

Scheme 1: Synthetic schemes and procedures

Synthesis:



Synthesis of 1-3: To 2,6 -lutidine (1 equiv) dissolved in dichloromethane, methyl iodide (1 equiv) was added and stirred at room temperature for around 18h. A White colored solid precipitate formed is filtered and washed with hexanes. The obtained solid was dissolved in methanol, piperidine was added and the 2.5 equivalents of the desired aldehydes: benzaldehyde, 4-dimethylaminobenzaldehyde, 4-methoxybenzaldehyde to obtain **1**,**2** and **3** respectively. The precipitate obtained was filtered out and washed with cold diethyl ether and hexane.

^{1}H and ^{13}C for 1

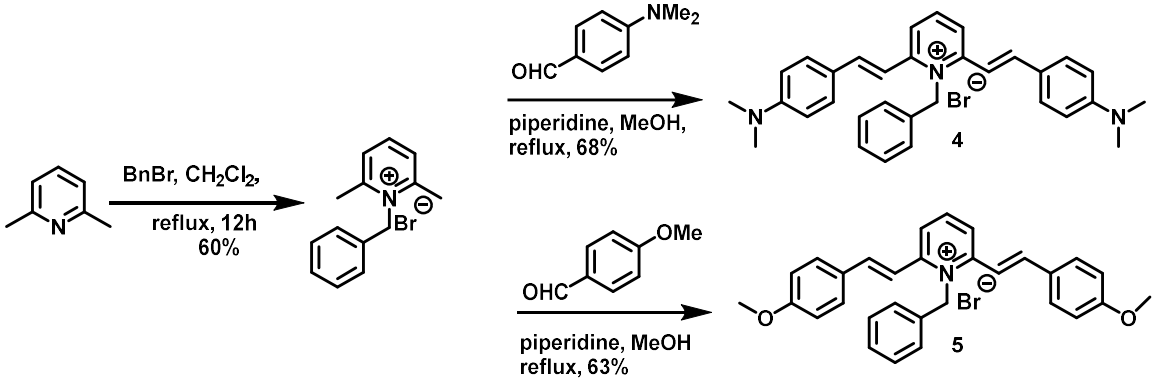
^{1}H NMR (500MHz, DMSO-D6) δ 8.47 (t, *J* = 8.1 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 2H), 7.88 (d, *J* = 8.8 Hz, 4H), 7.78 (d, *J* = 15.7 Hz, 2H), 7.71 (d, *J* = 15.7 Hz, 2H), 7.51 (m, 6H), 4.33 (s, 3H). **^{13}C NMR** (126 MHz, DMSO=D6) δ 153.78, 143.70, 142.86, 135.50, 130.94, 129.47, 128.96, 124.56, 119.70, 42.33. **HRMS (ESI) (**m/z): [M]+ calculated 298.1590, obtained 298.1548.

^{1}H and ^{13}C for 2

^{1}H NMR (500 MHz, DMSO-D6) δ 8.21 (t, J = 8.0 Hz, 1H), 8.09 (d, J = 8.1 Hz, 2H), 7.73 – 7.69 (m, 4H), 7.70 (dd, J = 12.2, 7.9 Hz, 6H), 7.32 (d, J = 15.7 Hz, 2H), 6.79 (d, J = 8.9 Hz, 4H), 4.20 (s, 3H), 3.03 (s, 12H). **^{13}C NMR** (126 MHz, DMSO-D6) δ 154.12, 152.27, 143.27, 141.87, 130.79, 123.08, 121.62, 113.31, 112.25, 41.43, 40.59. **HRMS (ESI) (m/z):** [M]+ calculated 384.2434, obtained 384.2409.

^{1}H and ^{13}C for 3

^{1}H NMR (500 MHz, DMSO-D6) δ 8.38 (t, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 2H), 7.84 (d, *J* = 8.9 Hz, 4H), 7.74 (dd, *J* = 15.7Hz, 2H), 7.53 (d, *J* = 15.7 Hz, 2H), 7.07 (d, *J* = 8.9 Hz, 4H), 4.27 (s, 3H), 3.84 (s, 6H). **^{13}C NMR** (126 MHz, DMSO-D6) δ 161.69, 153.97, 143.07, 142.58, 130.82, 128.25, 123.43, 117.01, 114.96, 55.93, 41.97. **HRMS (ESI) (m/z**): [M]+ calculated 358.1802, obtained 358.1768.



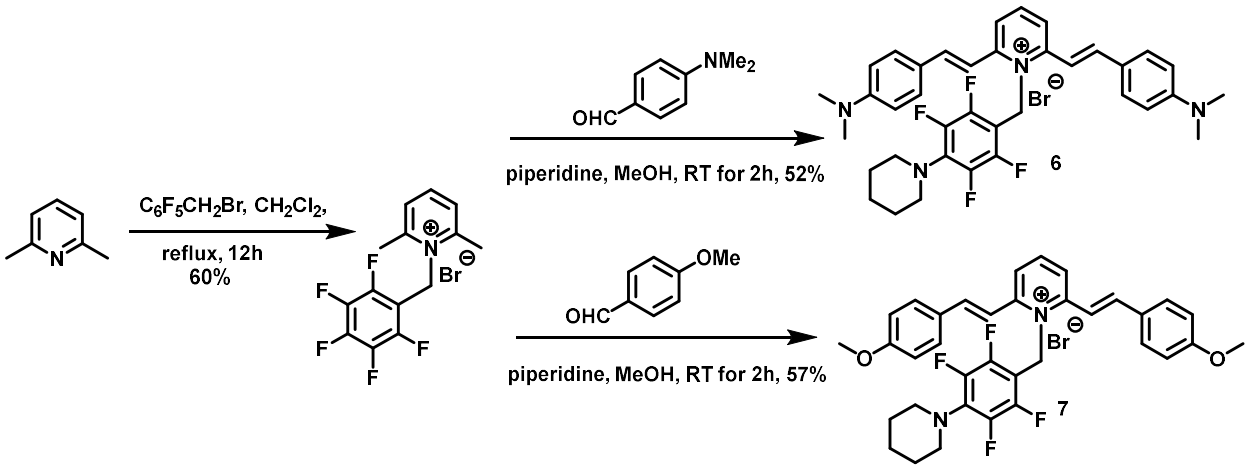
Synthesis of 4 and 5: 2,6 -lutidine (1 equiv) was dissolved in Dichloromethane and added benzylbromide (1 equiv) and refluxed for around 12h. The pale white coloured precipitate formed, was filtered and washed with hexane. The solid obtained was dissolved in methanol, piperidine was added to it, followed by the addition of 2.5 equivalents of 4-dimethylaminobenzaldehyde and 4-methoxybenzaldehyde to obtain **4** and **5** respectively. Product was purified by column chromatography by dichloromethane: methanol (90:10).

^{1}H and ^{13}C for 4

^{1}H NMR (500 MHz, DMSO) δ 8.32(m, 1H), 8.21 (d, *J* = 8.1 Hz, 2H), 7.75 (d, *J* = 15.5 Hz, 2H), 7.55 (d, *J* = 8.9 Hz, 4H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.24 (d, 2H), 7.22 (d, *J* = 15.3 Hz, 2H), 6.74 (d, *J* = 8.9 Hz, 4H), 6.15 (s, 2H), 3.00 (s, 12H). **^{13}C NMR** (126 MHz, DMSO) δ 154.11, 152.29, 143.81, 142.48, 135.29, 130.94, 129.65, 128.70 – 128.25, 126.31, 122.92, 121.90, 112.62, 112.11, 40.59. **HRMS (ESI)** (m/z): [M]+ calculated 460.2747, obtained 460.2734.

^{1}H and ^{13}C for 5

^{1}H NMR (500 MHz, DMSO) δ 8.49 (t, *J* = 8.1 Hz, 1H), 8.36 (d, *J* = 8.1 Hz, 2H), 7.80 (d, *J* = 15.7 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 4H), 7.49 (d, *J* = 15.7 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.23 (d, *J* = 7.5 Hz, 2H), 7.03 (d, *J* = 8.8 Hz, 4H), 6.27 (s, 2H), 3.81 (s, 6H). **^{13}C NMR** (500 MHz, DMSO) δ 161.76, 154.05, 143.91, 143.33, 135.11, 130.76, 129.60, 128.63, 128.12, 126.69, 124.09, 116.55, 114.97, 55.91, 49.06. ESI mass profile: HRMS (ESI) (m/z): [M]+ calculated 434.2115, obtained 434.2101.



Synthesis of 6 & 7: 2,6-lutidine (1equiv) dissolved in dichloromethane and refluxed for 16h after the addition of pentafluoro benzylbromide (1.5 equiv). The pale white precipitate formed was filtered and washed with hexane. This precipitate is then dissolved in methanol, followed by the addition of the piperidine and 2.5 equivalents of 4-dimethylaminobenzaldehyde and 4-methoxybenzaldehyde to obtain **6** and **7** respectively. The desired product was purified by column chromatography by Dichloromethane: methanol (90:10).

^{1}H, ^{13}C and ^{19}F for 6

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (t, J = 8.1 Hz, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.9 Hz, 4H), 7.34 (d, J = 15.5 Hz, 2H), 7.23 (d, J = 15.5 Hz, 2H), 6.67 (d, J = 8.9 Hz, 4H), 6.15 (s, 2H), 3.05 (s, 4H), 3.02 (s, 12H), 1.70 (s, 6H). **^{13}C NMR** (126 MHz, DMSO) δ 154.62 (s), 152.38 (s), 143.96 (s), 143.26, 142.05, 130.69 (s), 122.79 (s), 122.46 (s), 112.74 (s), 112.50, 112.74, 51.99 (s), 26.21 (s), 23.82 (s). **^{19}F NMR** (470 MHz, $CDCl_3$) δ -143.37(d,2F), -150.82(d, 2F). ESI mass profile: HRMS (ESI) (m/z): [M]+ calculated 615.3105, obtained 615.3119.

^{1}H, ^{13}C and ^{19}F for 7

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (t, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.7 Hz, 3H), 7.50 (d, J = 15.8 Hz, 2H), 7.37 (d, J = 15.7 Hz, 2H), 6.93 (d, J = 8.6 Hz, 4H), 6.28 (s, 2H), 6.28 (s, 2H), 3.83 (s, 6H), 3.04 (s, 4H), 1.54 (s, 7H). **^{13}C NMR** (126 MHz, CDCl3) δ 160.84 (s), 153.79 (s), 142.94 (s), 141.81 (s), 129.39 (s), 126.17 (s), 123.32 (s), 115.39 (s), 113.66 (s), 54.38 (s), 50.72 (s), 25.15 (s), 22.75 (s). **^{19}F NMR** (470 MHz, $CDCl_3$) δ-143.32(d,2F), -150.72(d,2F). **HRMS (ESI) (m/z**): [M]+ calculated 589.2473, obtained 589.2477, difference 0.0004

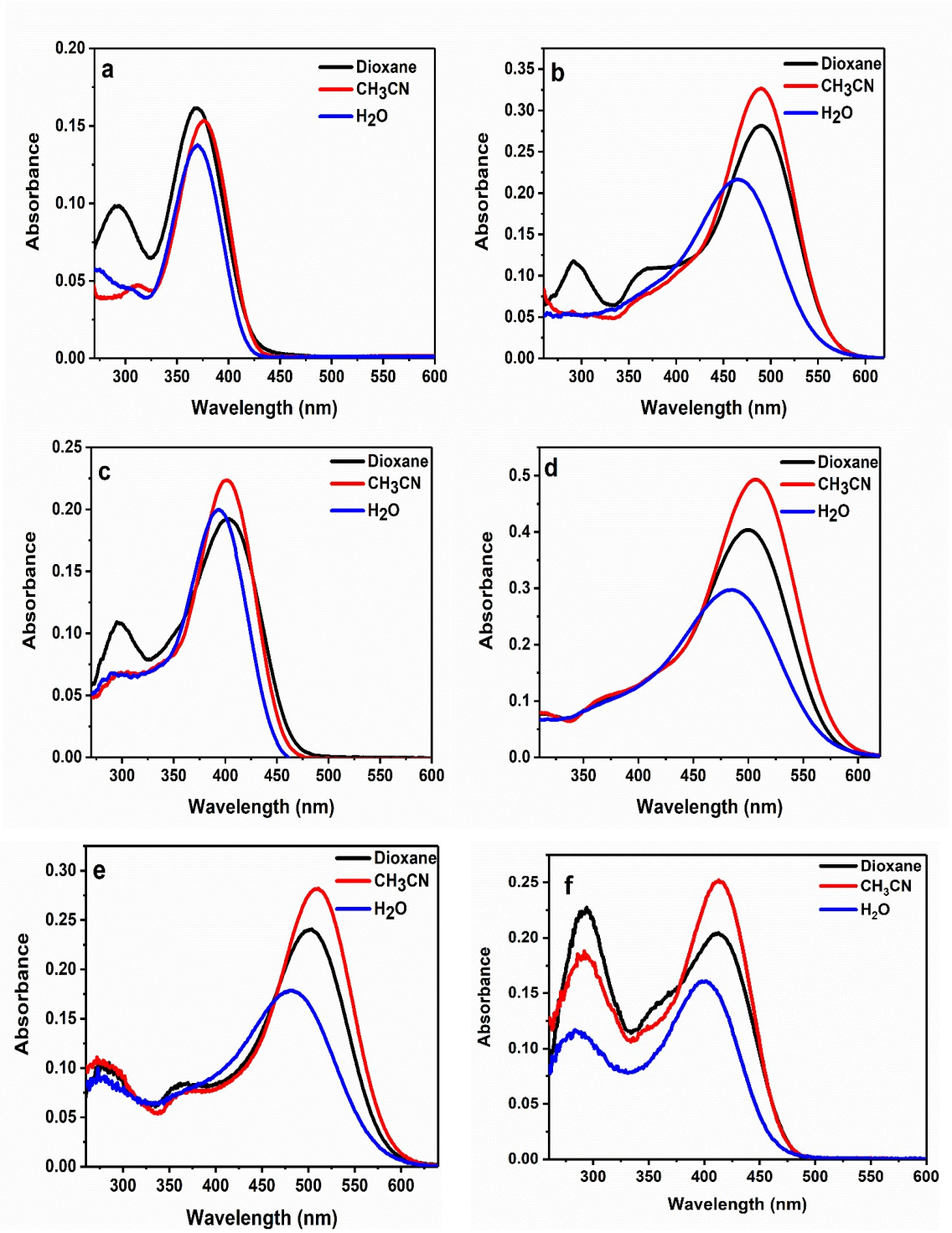


Fig S2: The spectra a-f represents the absorption spectra for molecules **1**-**4**,**6** and **7** respectively in different solvents.

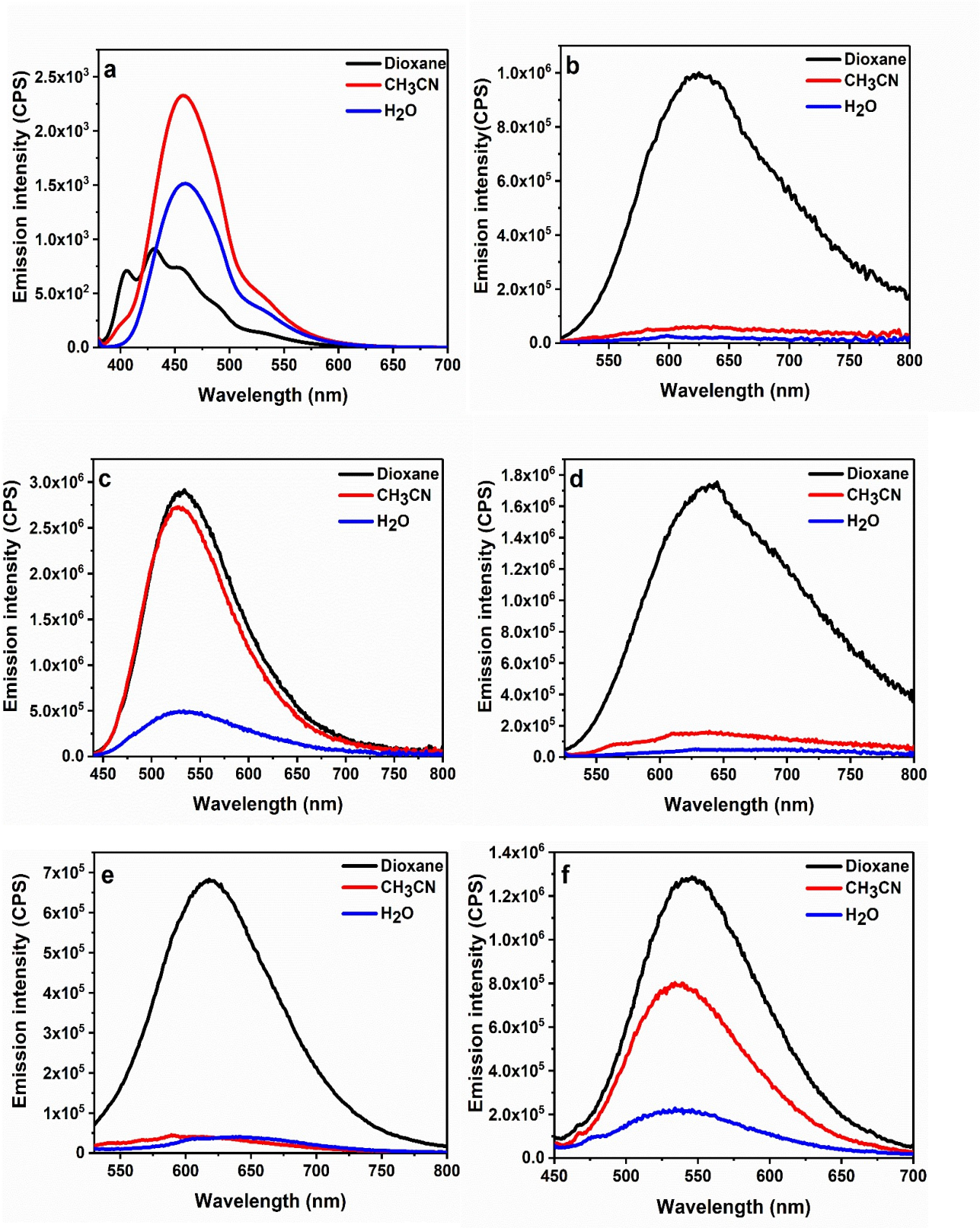


Fig S3: Spectra a-f represents emission spectra for molecules **1**-**4**,**6** and **7** respectively in different solvents**.**

Table S2: Absorption maxima, emission maxima and quantum yield for **1**-**7** in different solvents.

Probe	**Solvents**	**λ_{Abs} max (nm)**	**$\lambda_{Em.}$ Max (nm)**	**Stokes shift (nm)**	**% Q.Y.**
1	Dioxane	370	433	63	0.8
	Acetonitrile	368	458	90	1.5
	Water	370	458	88	2.7
2	Dioxane	490	625	135	0.1
	Acetonitrile	490	625	135	0.03
	Water	466	610	144	0.04
3	Dioxane	400	530	130	0.4
	Acetonitrile	400	530	130	1.9
	Water	395	530	135	0.5
4	Dioxane	500	640	140	0.2
	Acetonitrile	505	635	130	0.03
	Water	487	635	148	0.05
5	Dioxane	416	545	129	0.6
	Acetonitrile	412	535	123	2.2
	Water	404	540	136	0.6
6	Dioxane	500	618	118	0.2
	Acetonitrile	500	600	100	0.03
	Water	485	625	140	0.07
7	Dioxane	410	540	130	1.1
	Acetonitrile	410	530	120	0.8
	Water	400	525	125	0.6

Effect of viscosity: emission in glycerol-water mixture

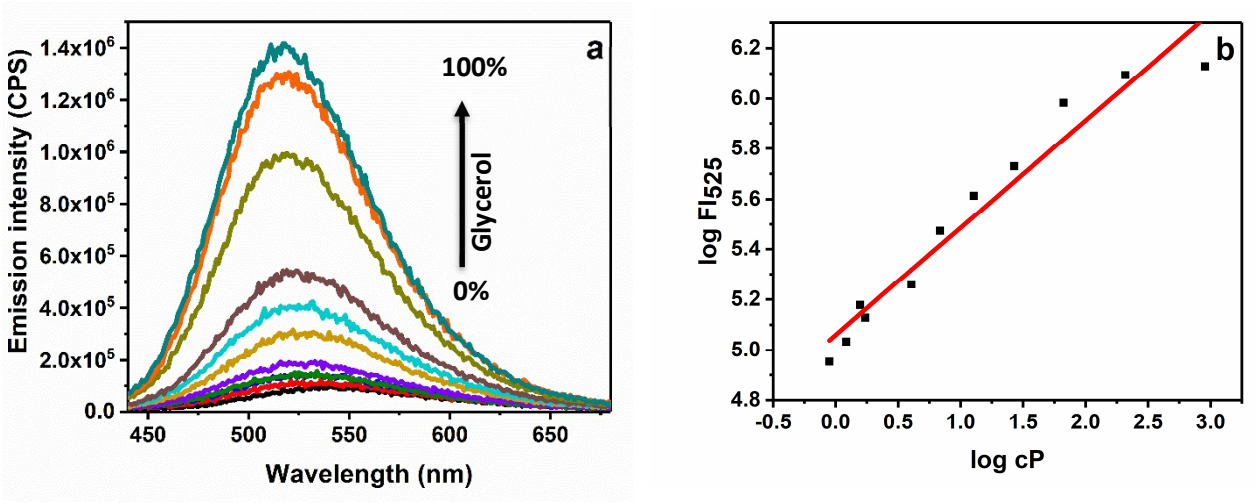


Fig S4: (a)- Fluorescence emission spectral changes of **7** with increasing (0-100%) glycerol concentrations in water. (b)- Linear relationship between log FI_{525} and log cP.

Sub-Cellular Imaging

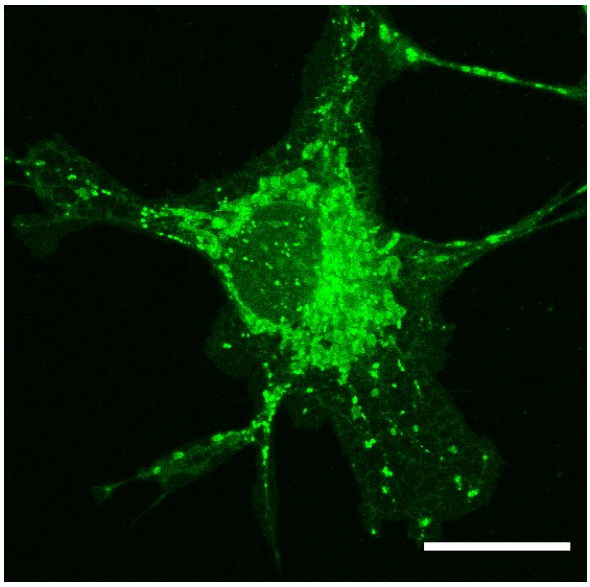


Fig S5: COS-7 cells incubated with **4** [400 nM] showing mesh-like structures in some cells indicating possible localization in ER also. The scale bar is 10 µm.

CCCP treatment of COS-7 cells with 4

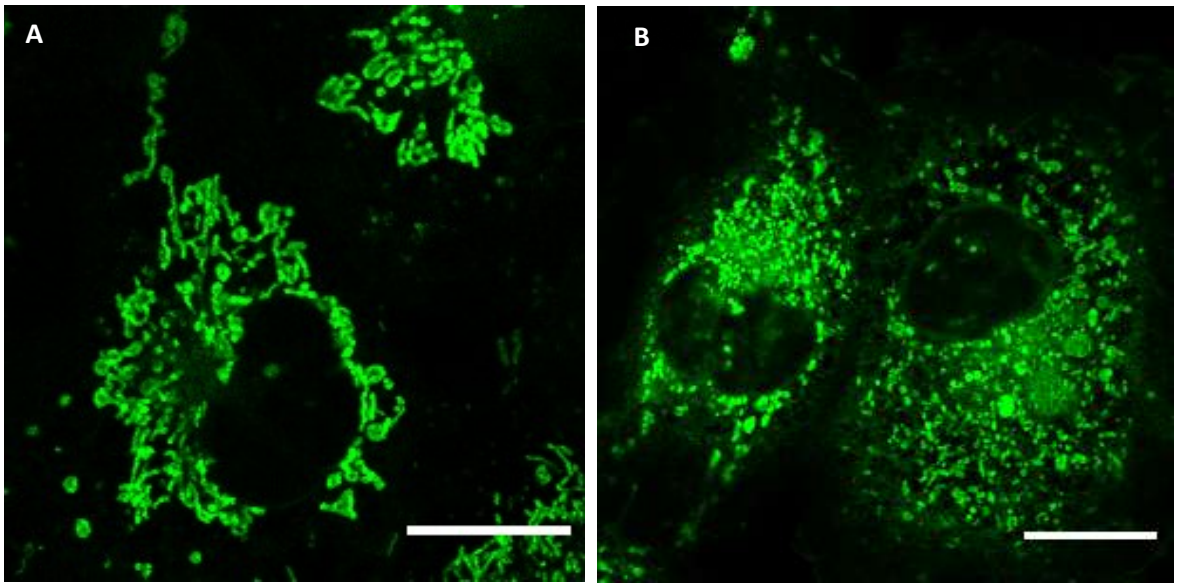


Fig S6: (A) COS-7 cells incubated with **4** at 400 nM concentration. (B) Cells treated with 10 µM CCCP for 90 minutes followed by the addition of the fluorophore. Scale bar is 10 µm.

MTT assay data:

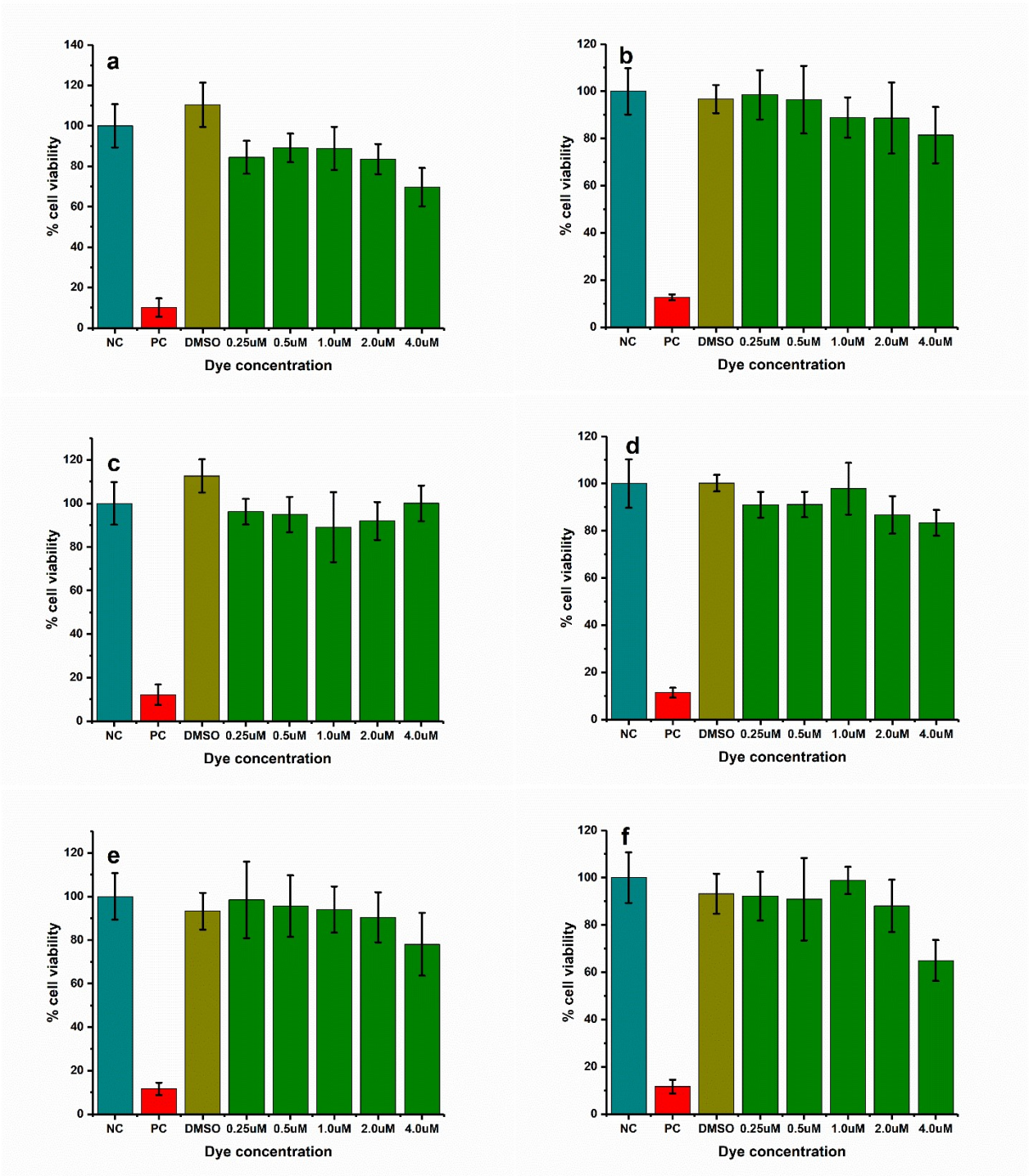
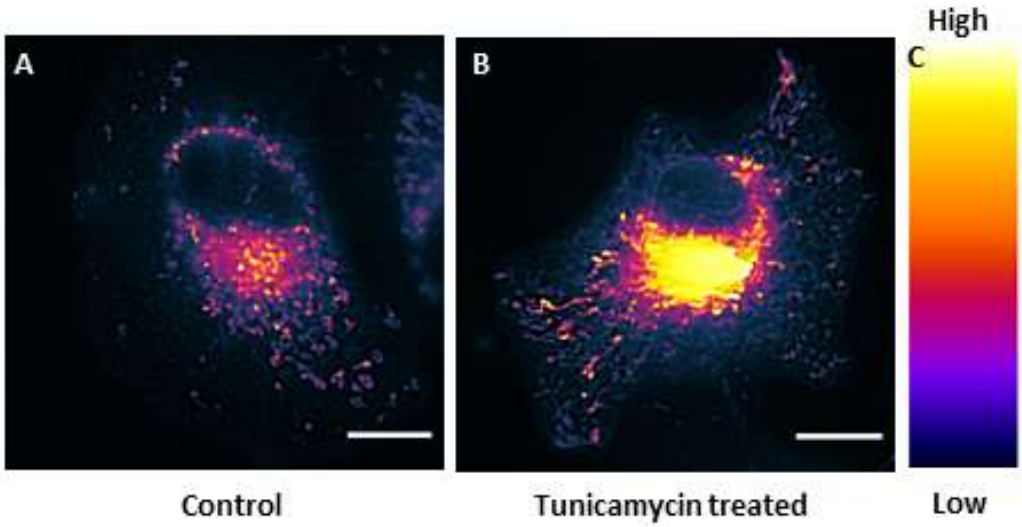


Fig S7: Bar graphs a-f represents MTT assay data for probes 2, 3, 4,5,6 and 7 respectively. The molecules are non-toxic at the imaging concentrations. PC is positive control and NC is neutral control.

Viscosity detection with 7 in COS-7 cells:



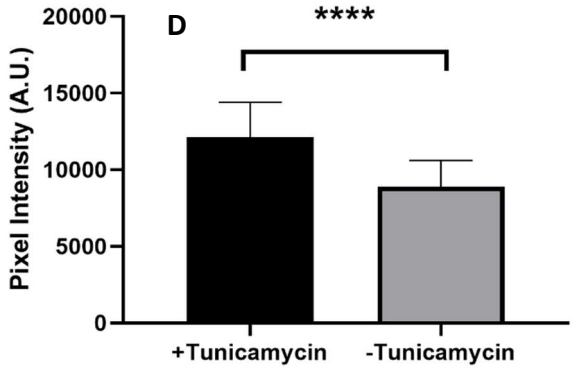
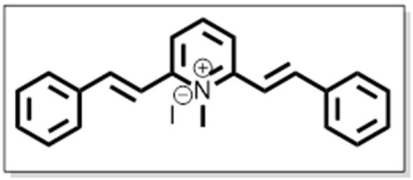
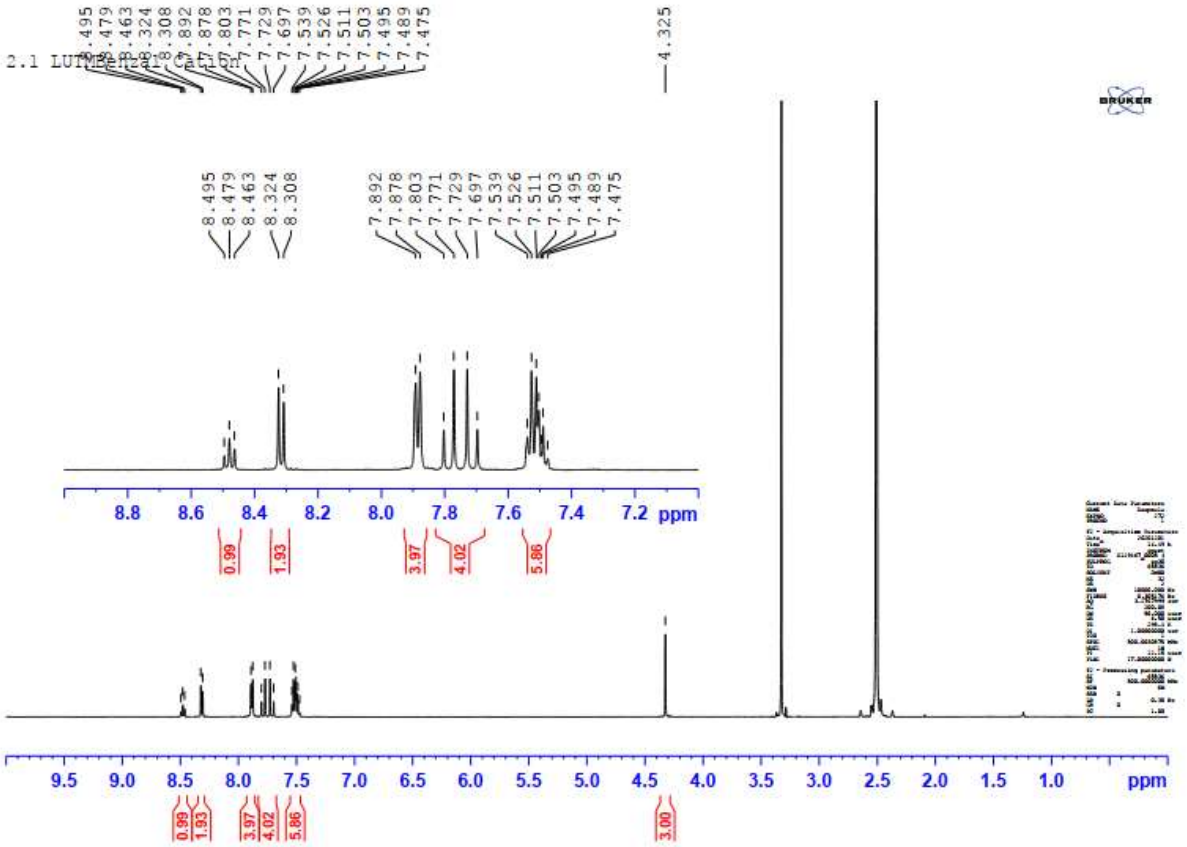


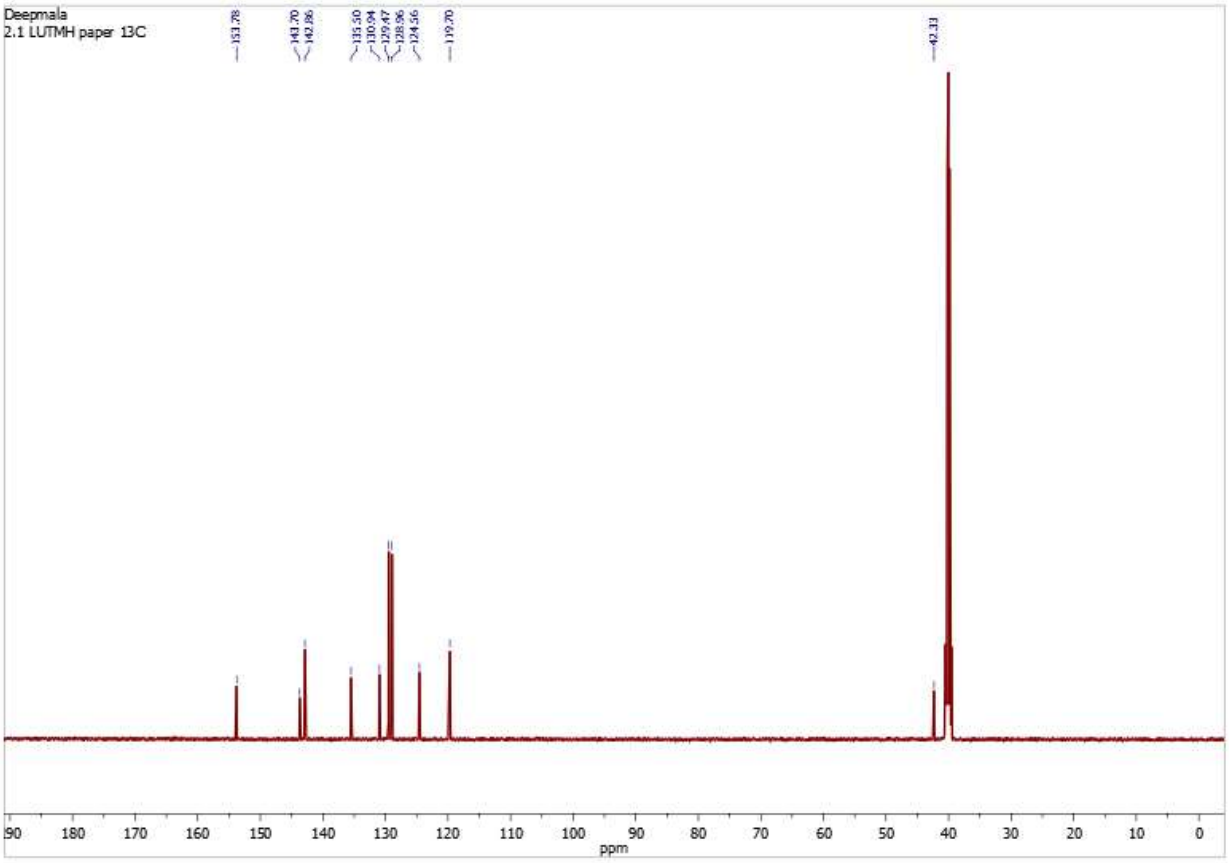
Fig S8: Viscosity detection in COS-7 cells. A is the control cell images treated with only **7** (800 nM); B represents cells treated with Tunicamycin (Tm) at 20 µg/ml concentration and **7** (800nM). C shows color bar and D pixel intensity graph for **7**. scale bar is 10 µm. 30 cells were used for each set of probes for quantification. P values were calculated using two-sample t-tests: ****$p < 0.05$ A total of 30 cells were taken into account for this calculation for each molecule. The color bar indicates bright yellow to the highest pixel intensity and black to lowest pixel intensity.

^{1}H, ^{13}C and ^{19}F NMR spectra



^{1}H and ^{13}C spectra for 1





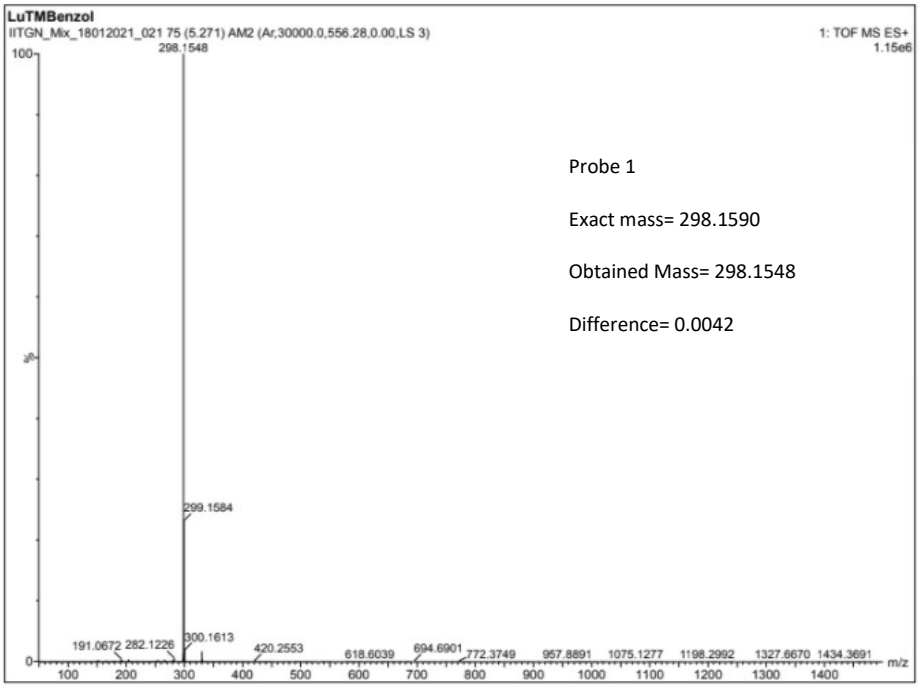
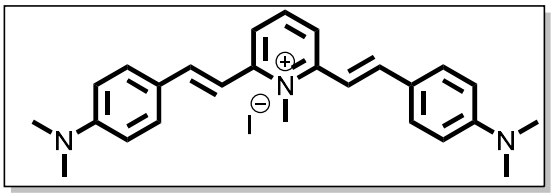
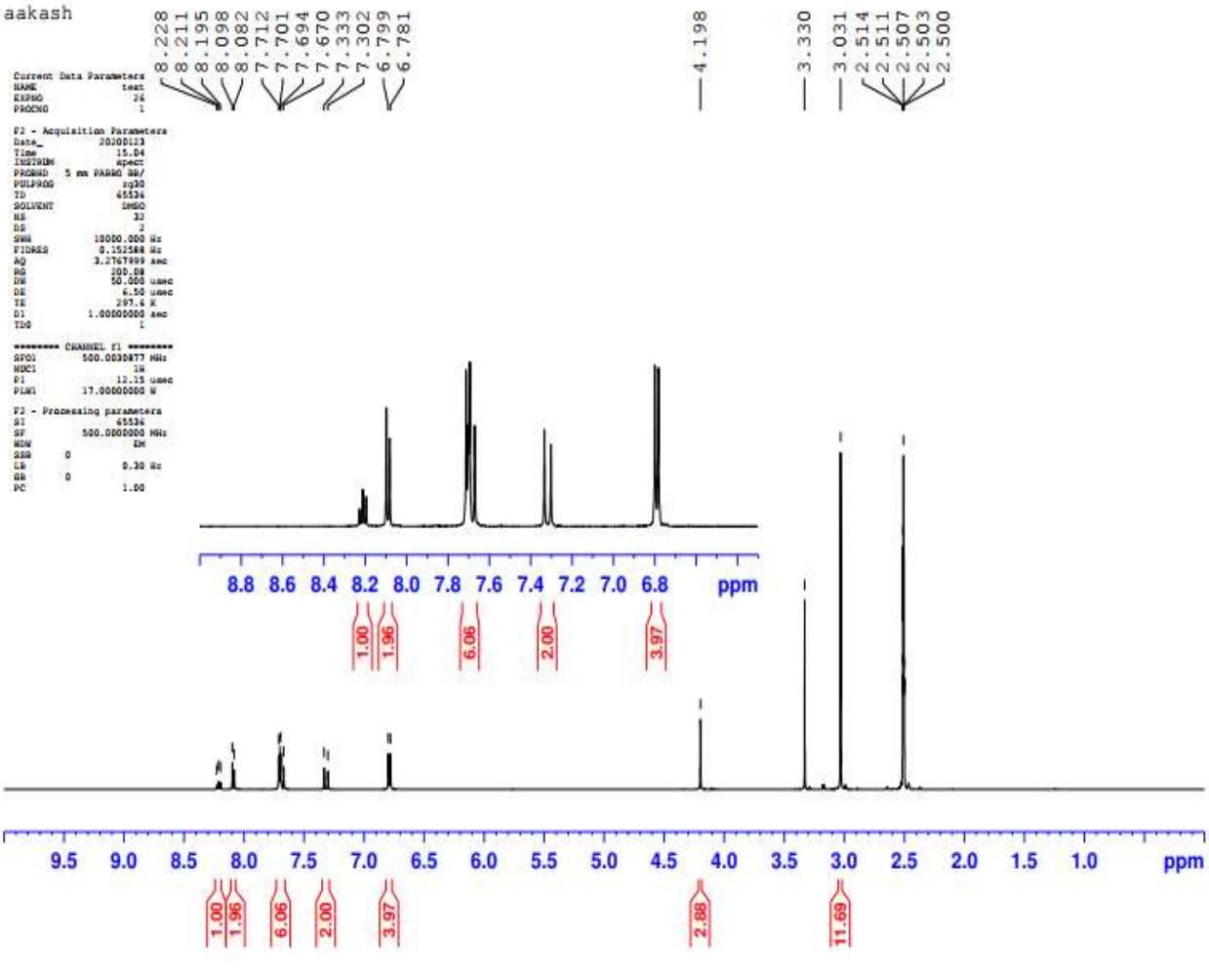


Fig S9: ^{1}H, ^{13}C and HRMS spectra for 1

^{1}H and ^{13}C spectra for 2





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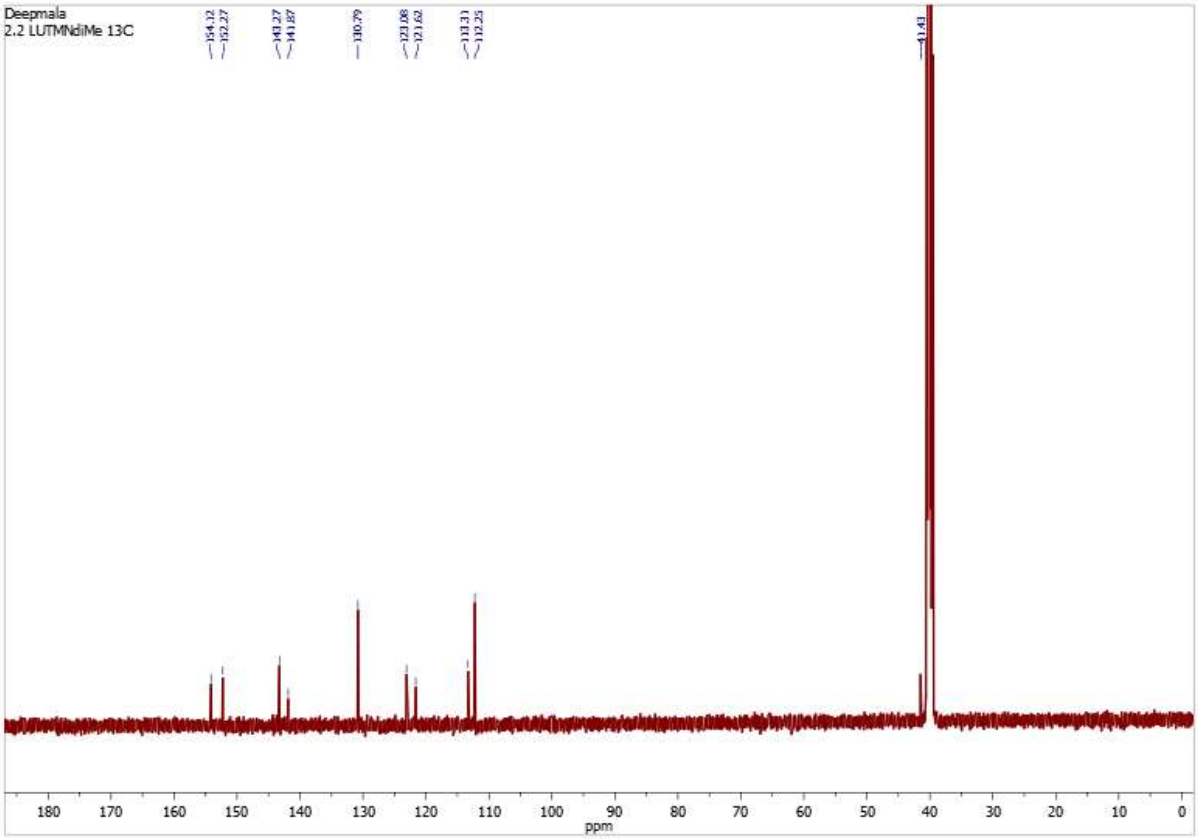


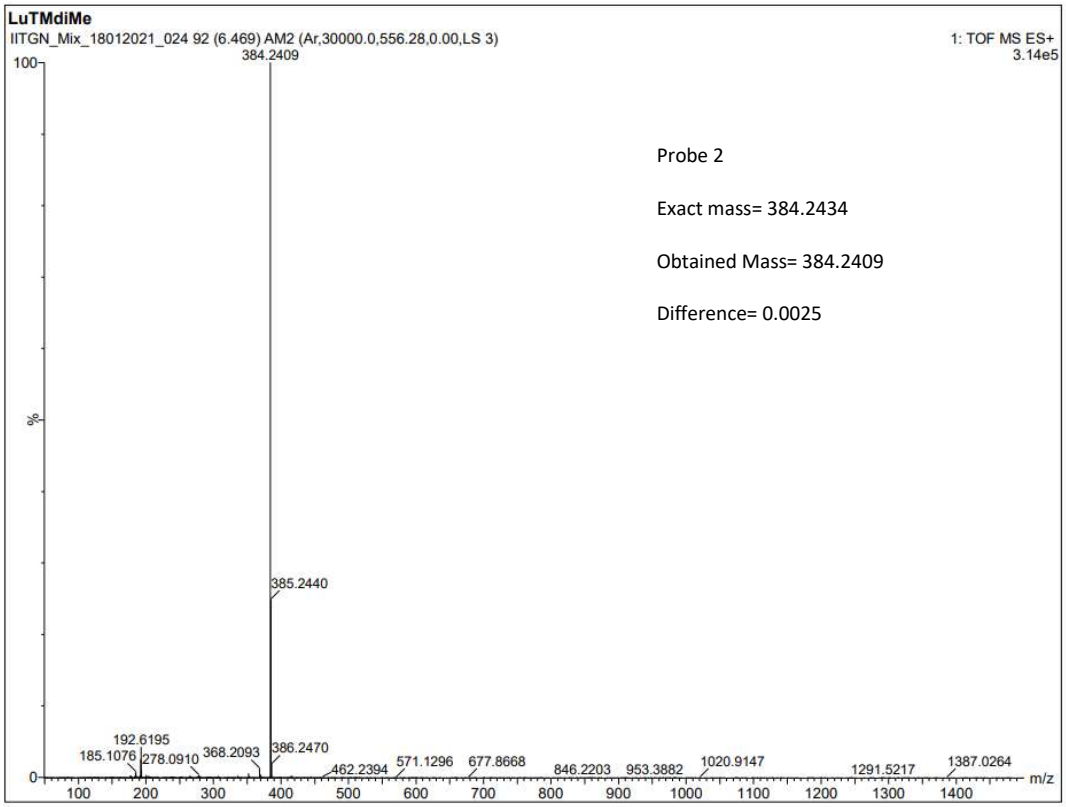
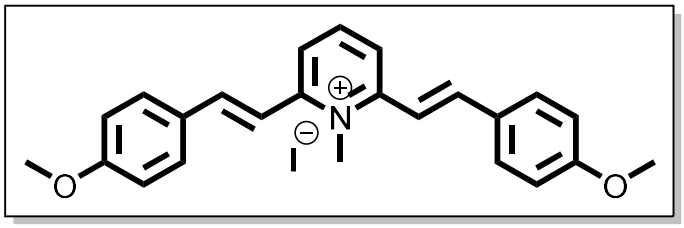
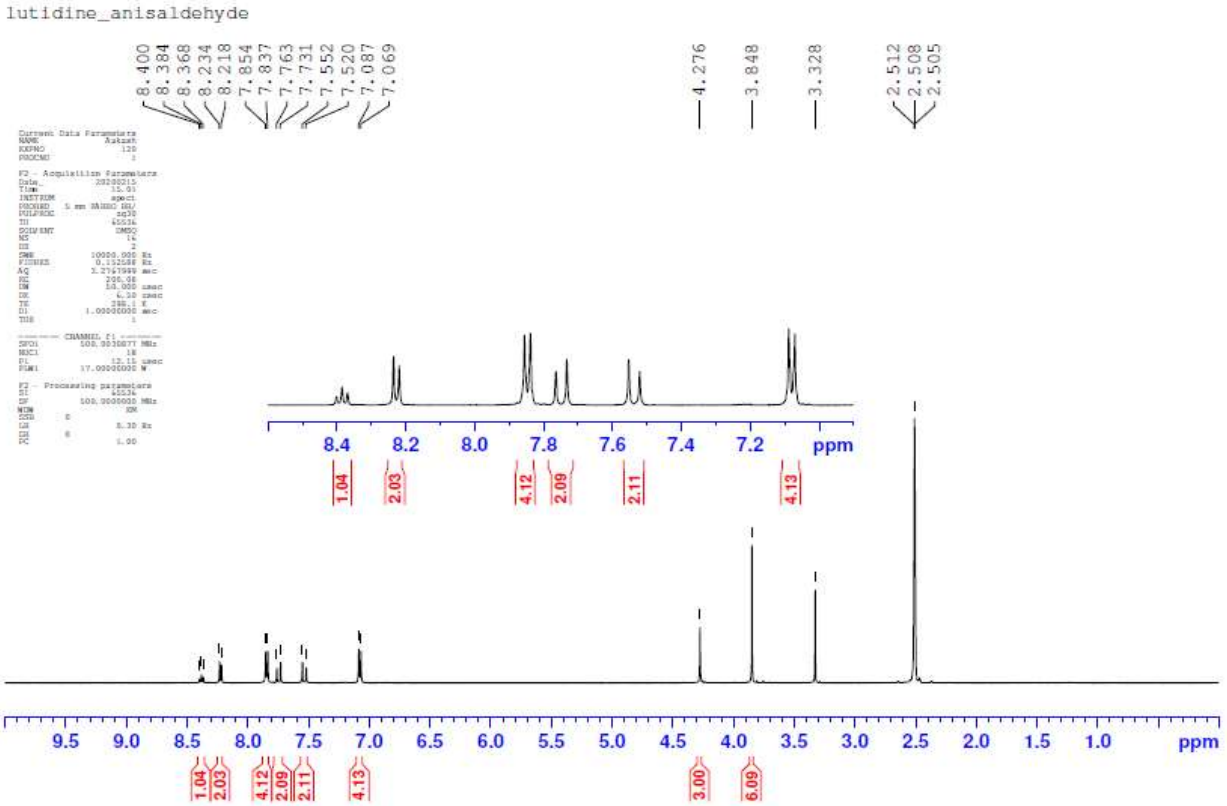
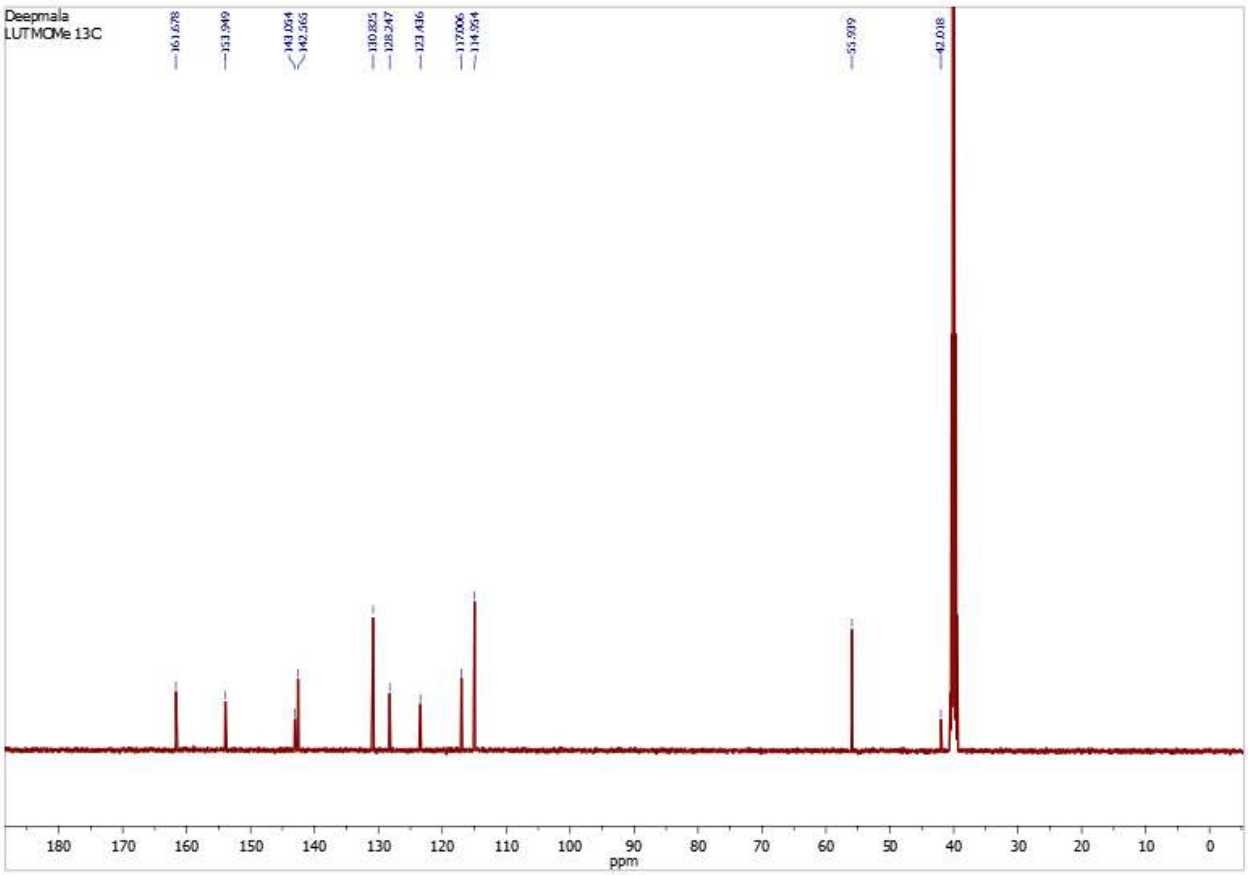


Fig S10: ^{1}H, ^{13}C and HRMS spectra for 2

^{1}H and ^{13}C spectra for 3







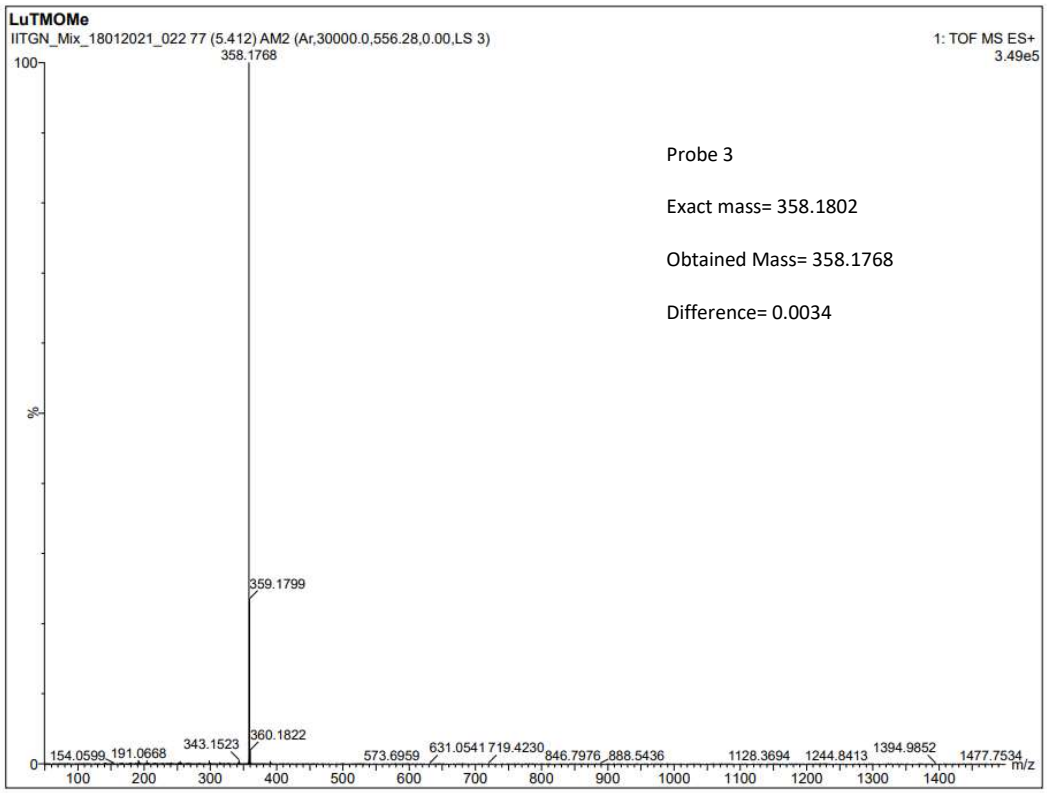
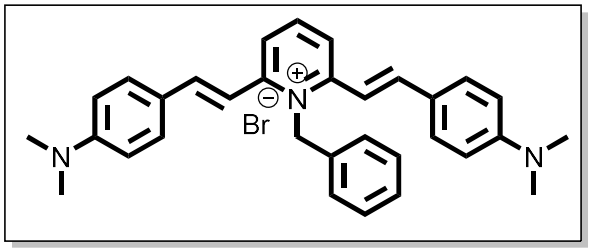
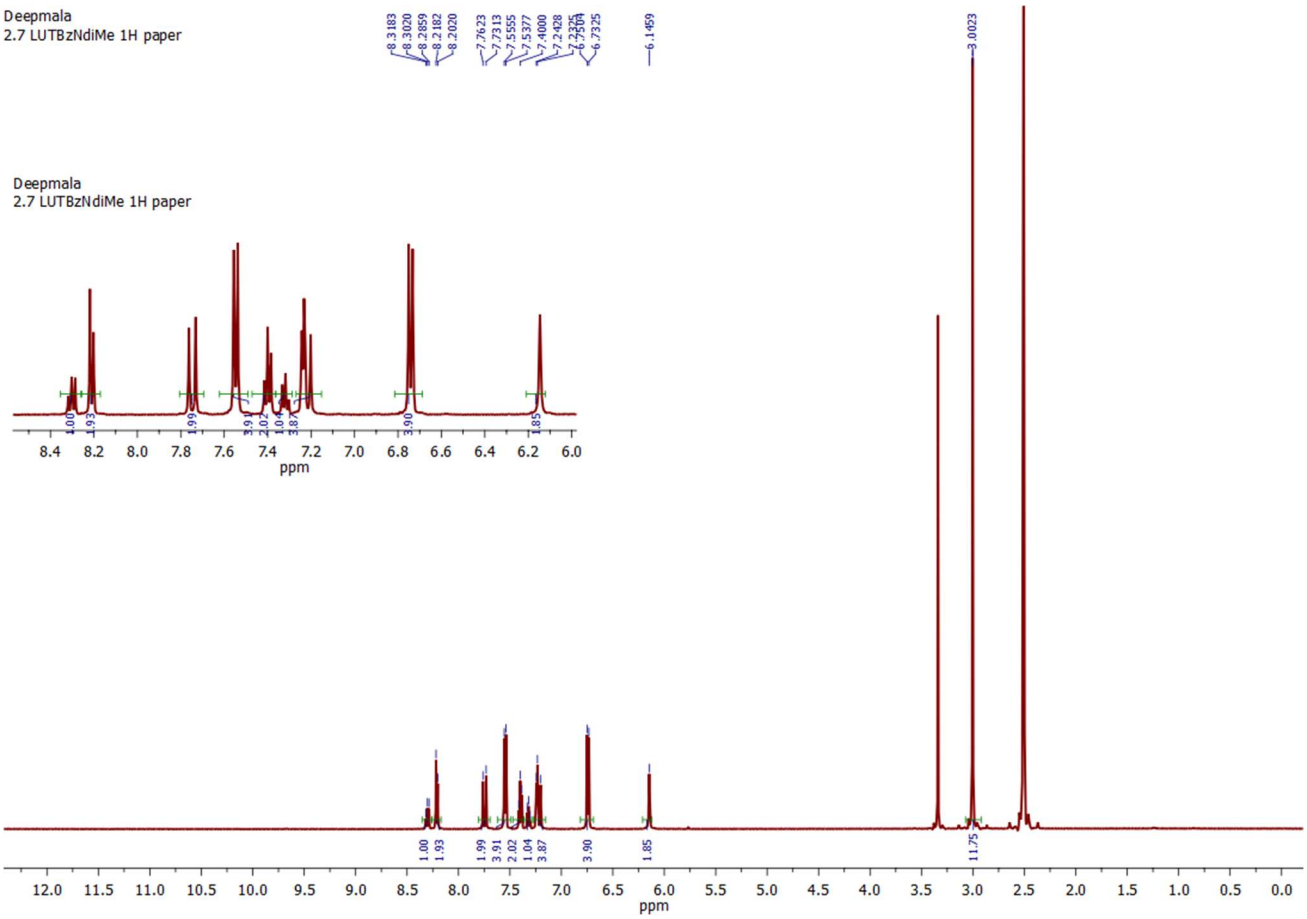
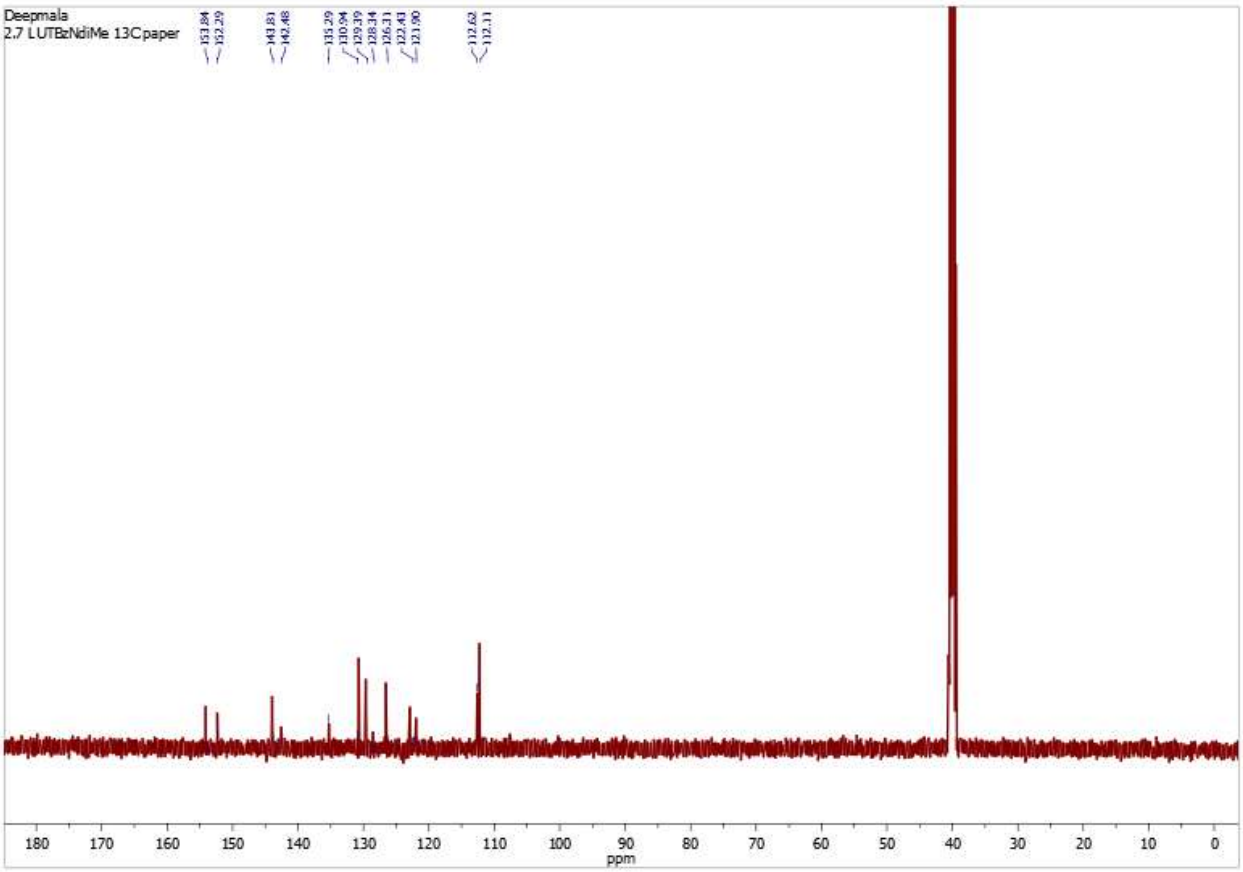


Fig S11: ^{1}H, ^{13}C and HRMS spectra for 3

^{1}H and ^{13}C for **4**







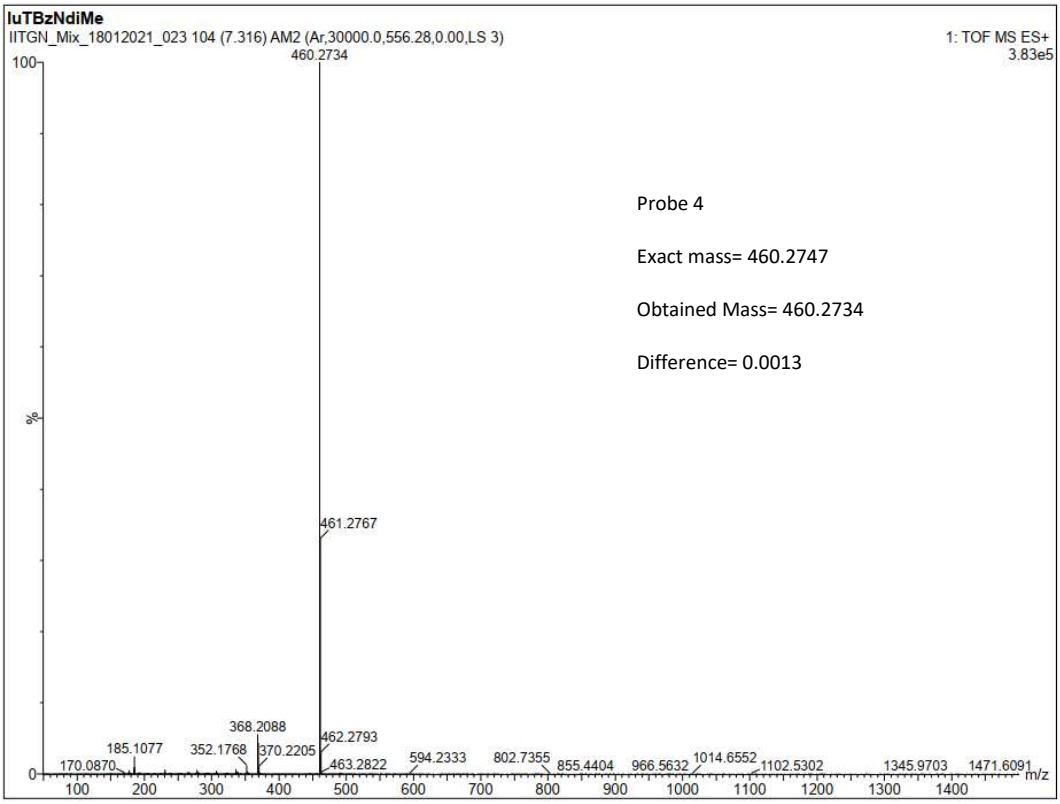
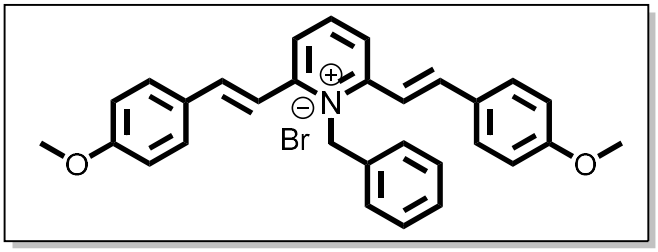
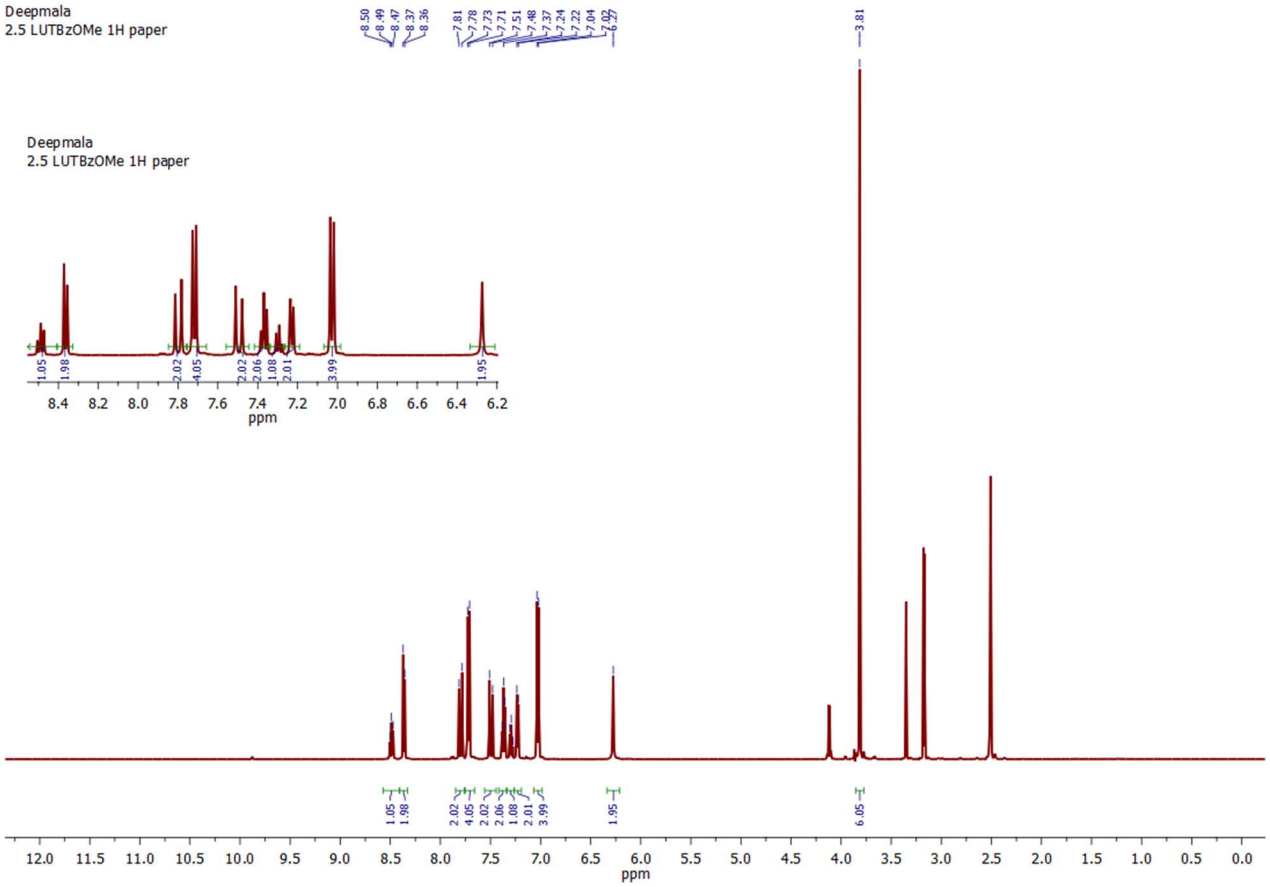
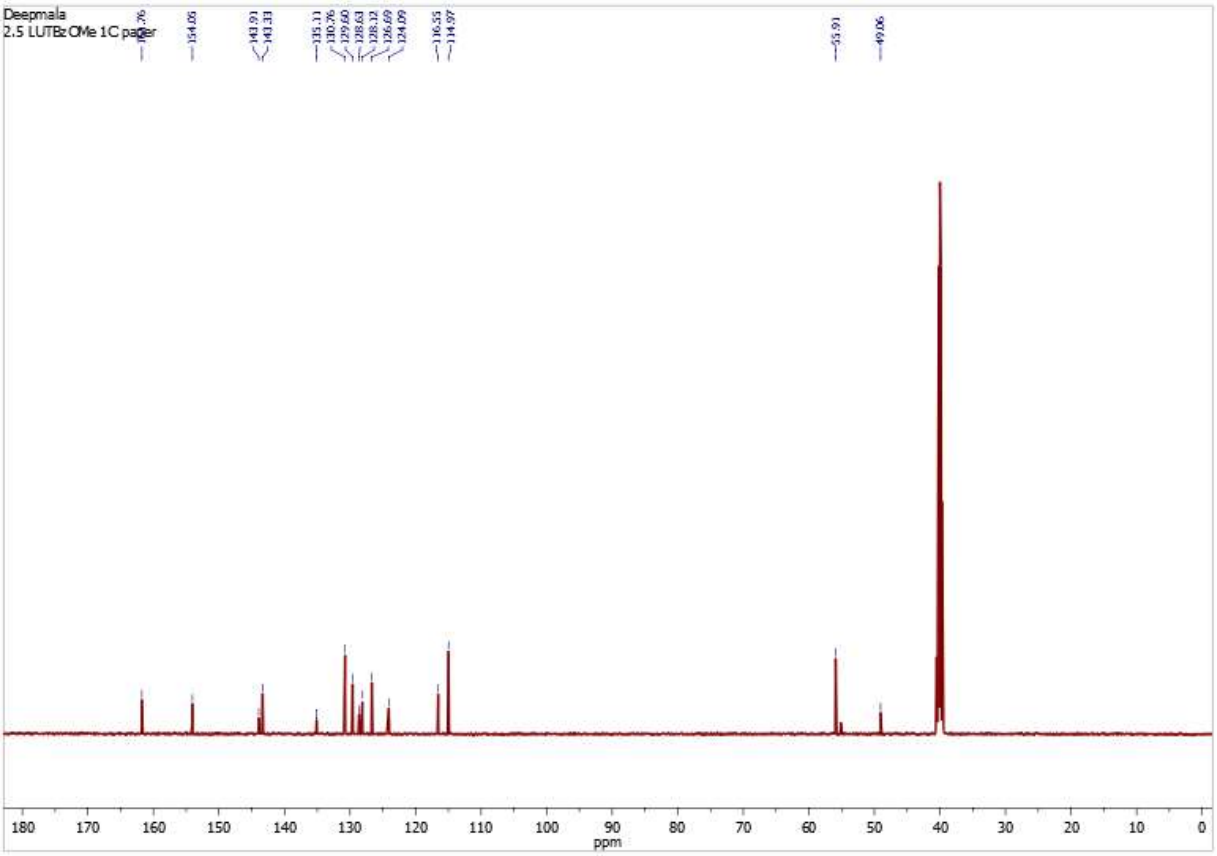


Fig S12: ^{1}H, ^{13}C and HRMS spectra for 4

^{1}H and ^{13}C NMR spectra for 5







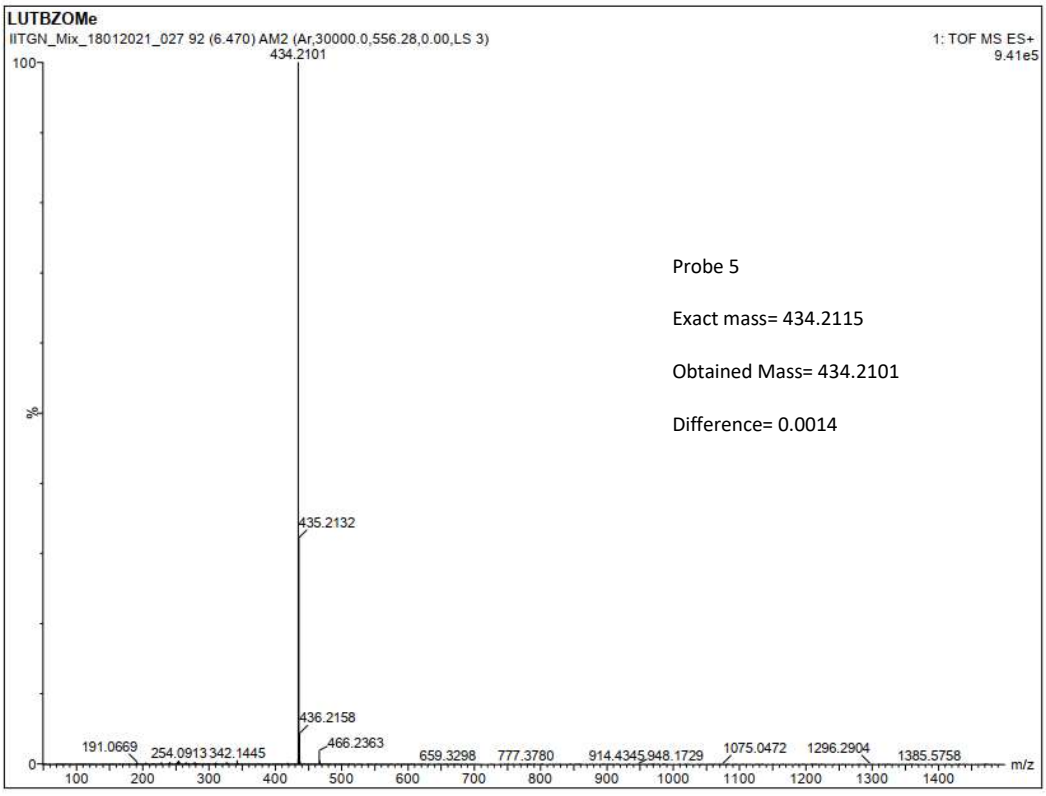
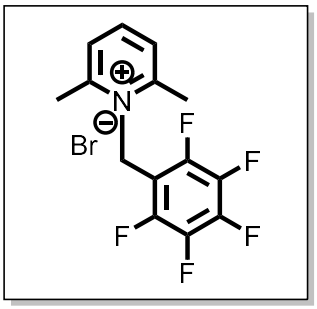
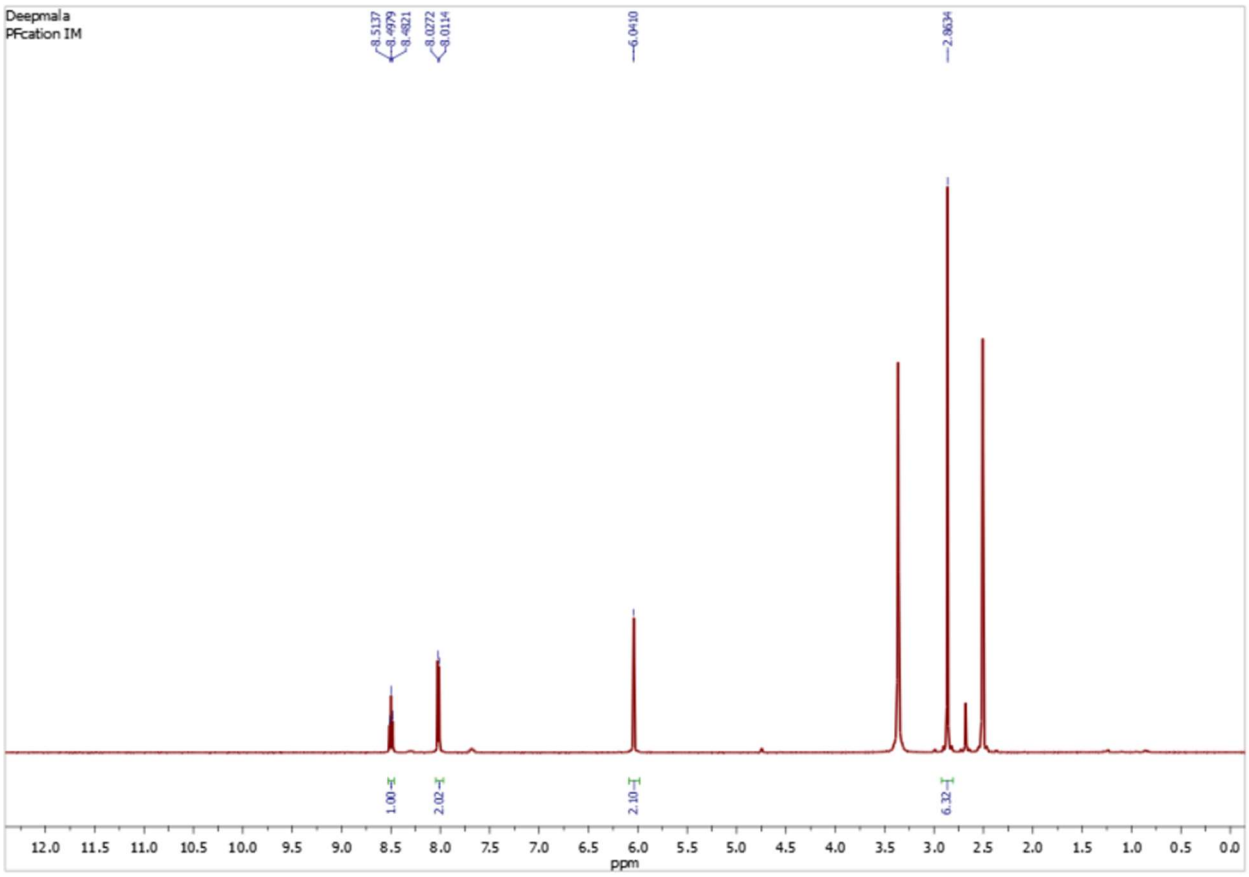


Fig S13: ^{1}H, ^{13}C and HRMS spectra for **5**

2,6-dimethyl-1-((perfluorophenyl)methyl) pyridin-1-ium (intermediate for the preparation of **6** and **7**)

^{1}H and ^{19}F (The molecule is known previously in the literature[9]





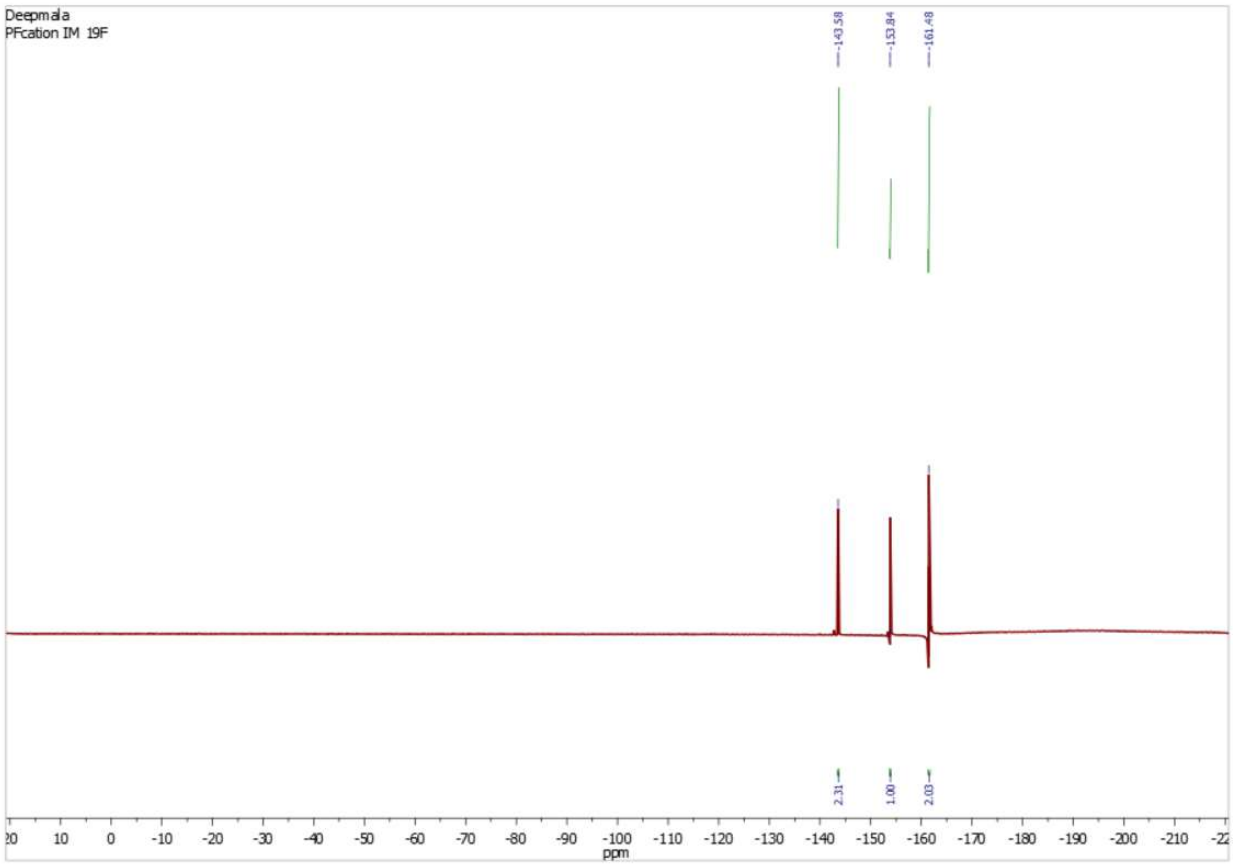
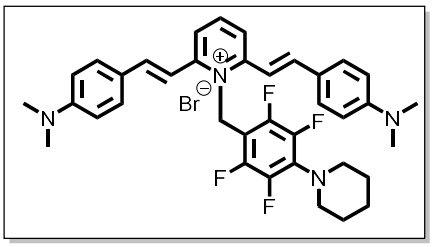
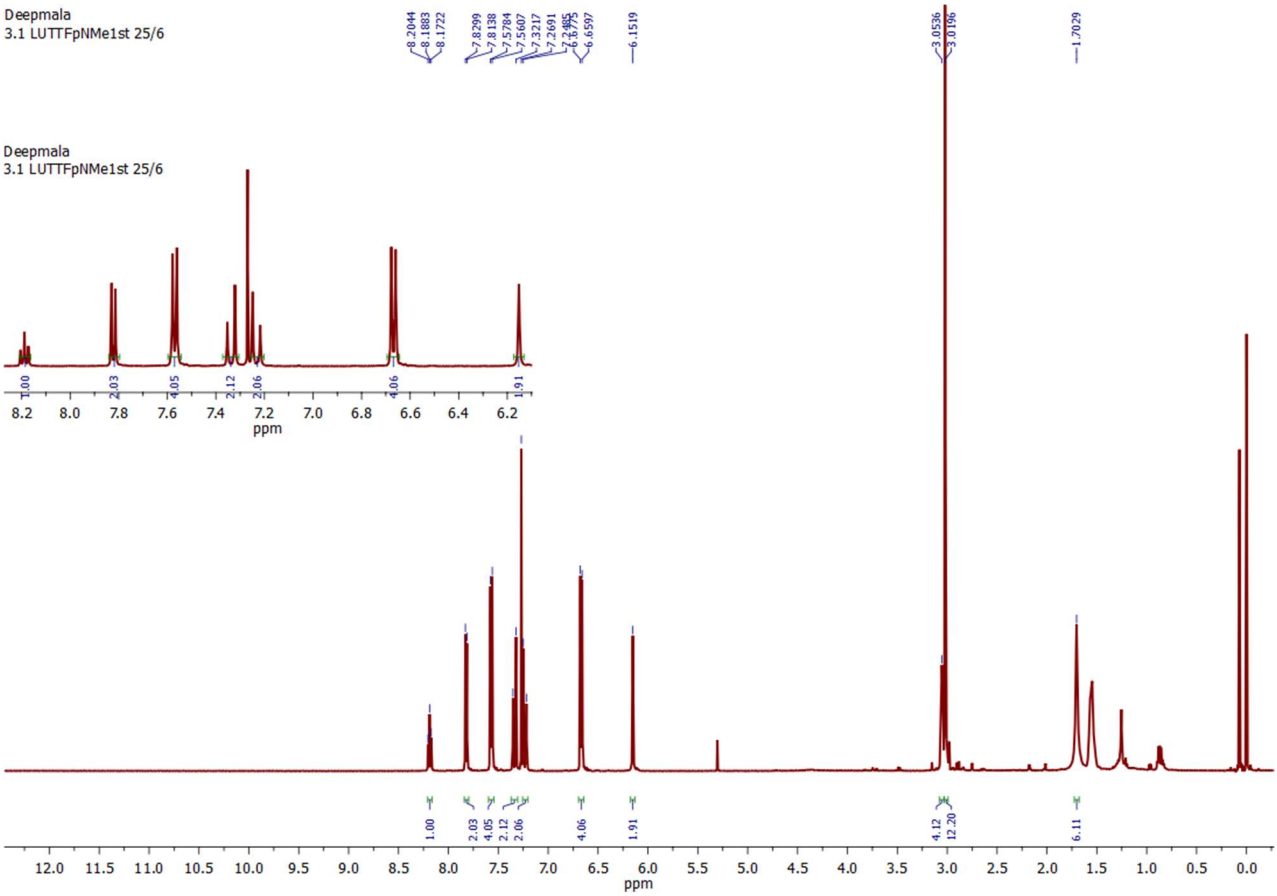
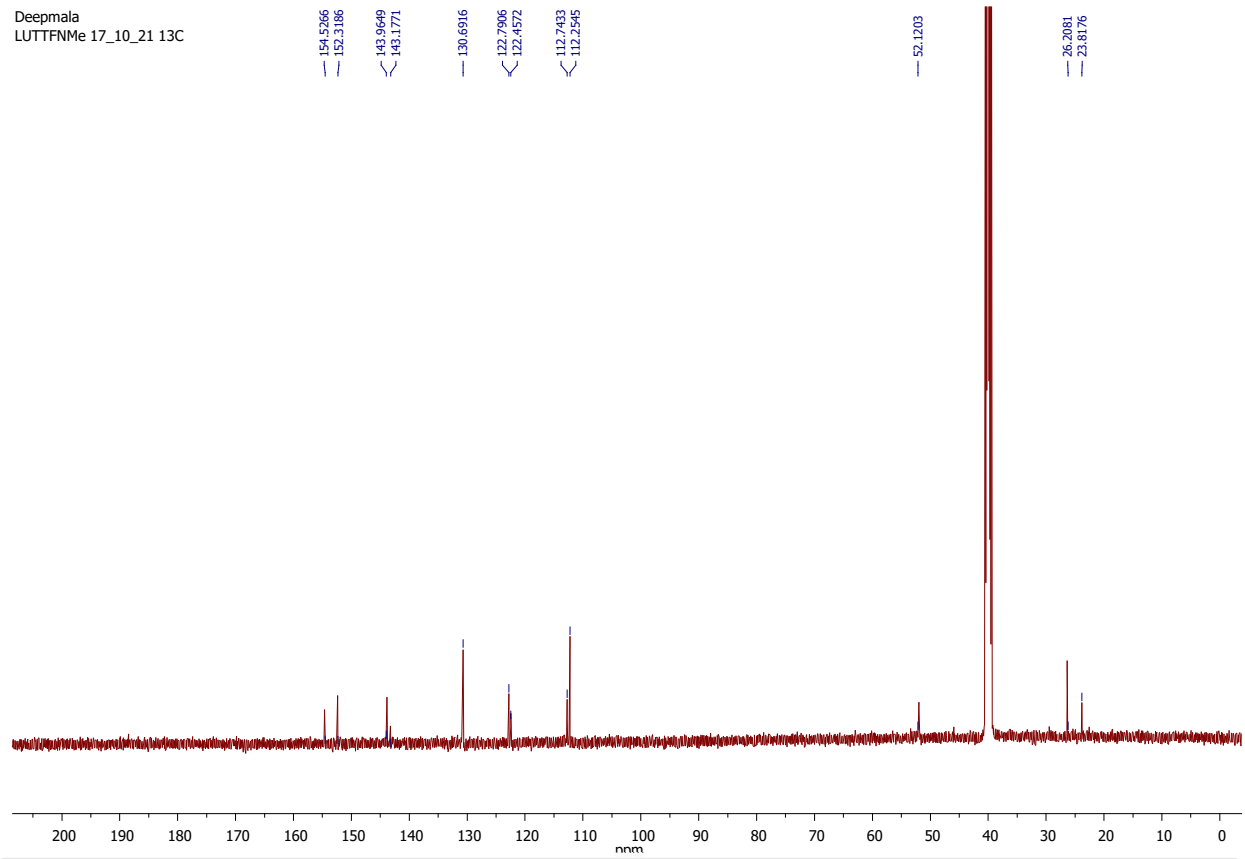


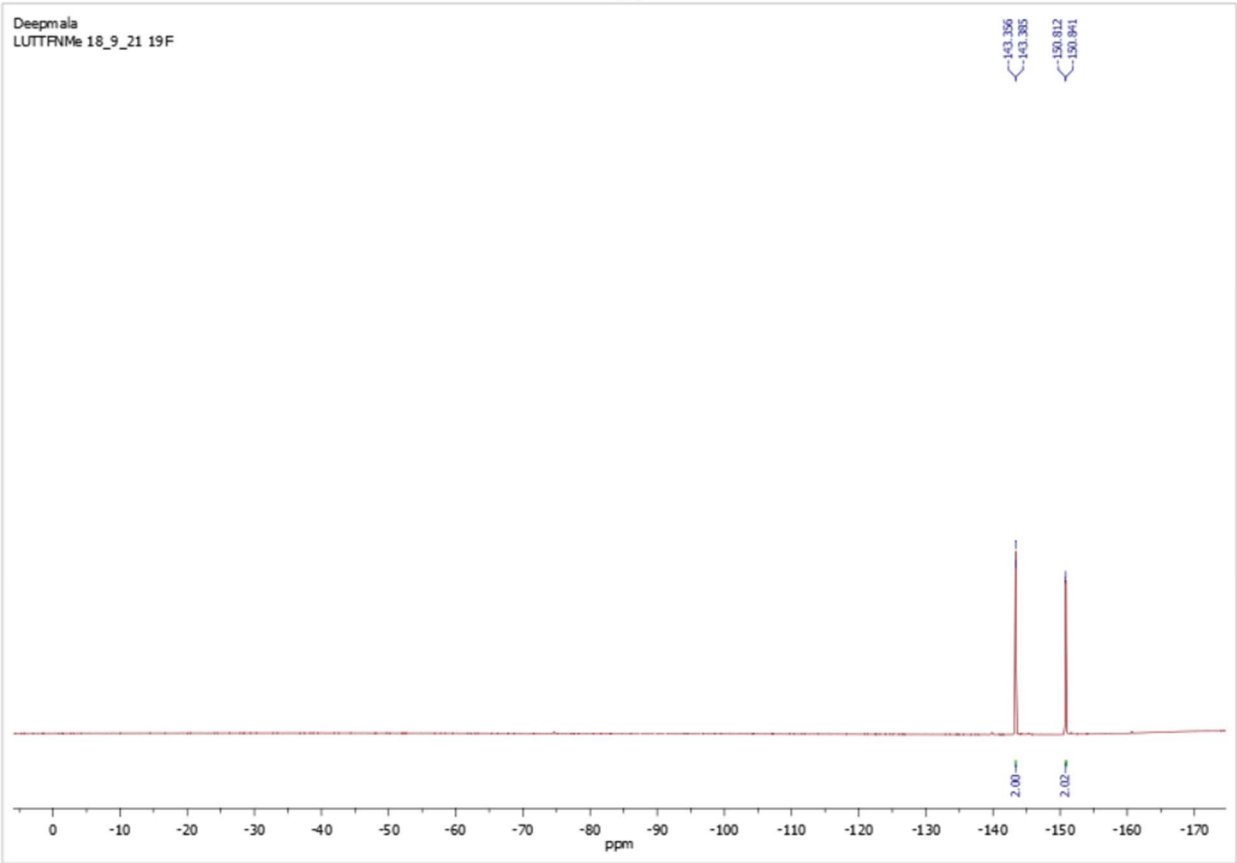
Fig S14: ^{1}H and ^{19}F spectra for 2,6-dimethyl-1-((perfluorophenyl)methyl) pyridin-1-ium

^{1}H and ^{13}C and ^{19}F NMR spectra for **6**






Deepmala
LUTTFNMe 17_10_21 13C
154.5266
152.3186
143.9649
143.1771
130.6916
122.7906
122.4572
112.7433
112.2545
52.1203
26.2081
23.8176
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


Deepmala
LUTTFNMe 18_9_21 19F
143.356
143.305
150.812
150.841
2.00
2.02
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170
ppm

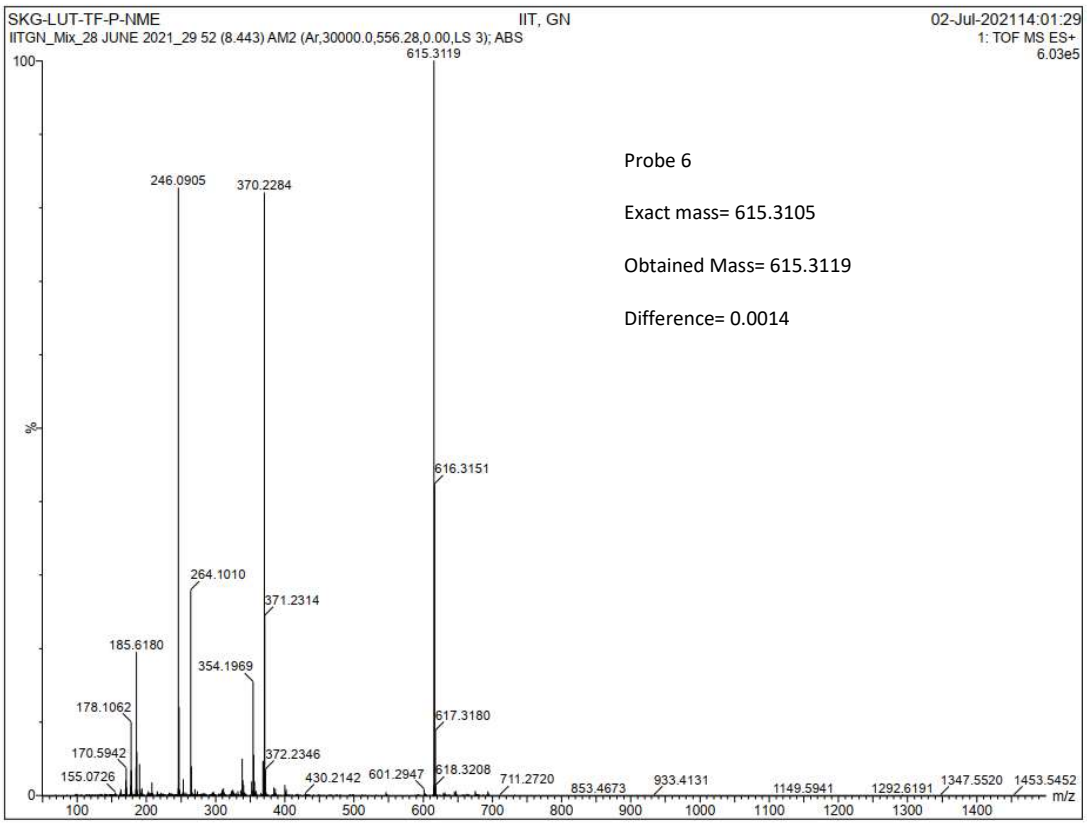
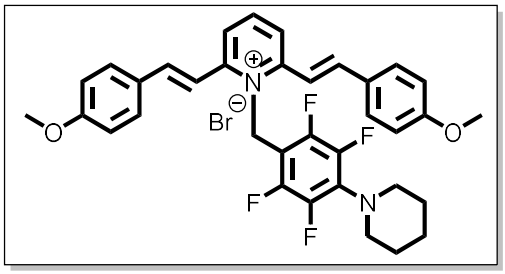
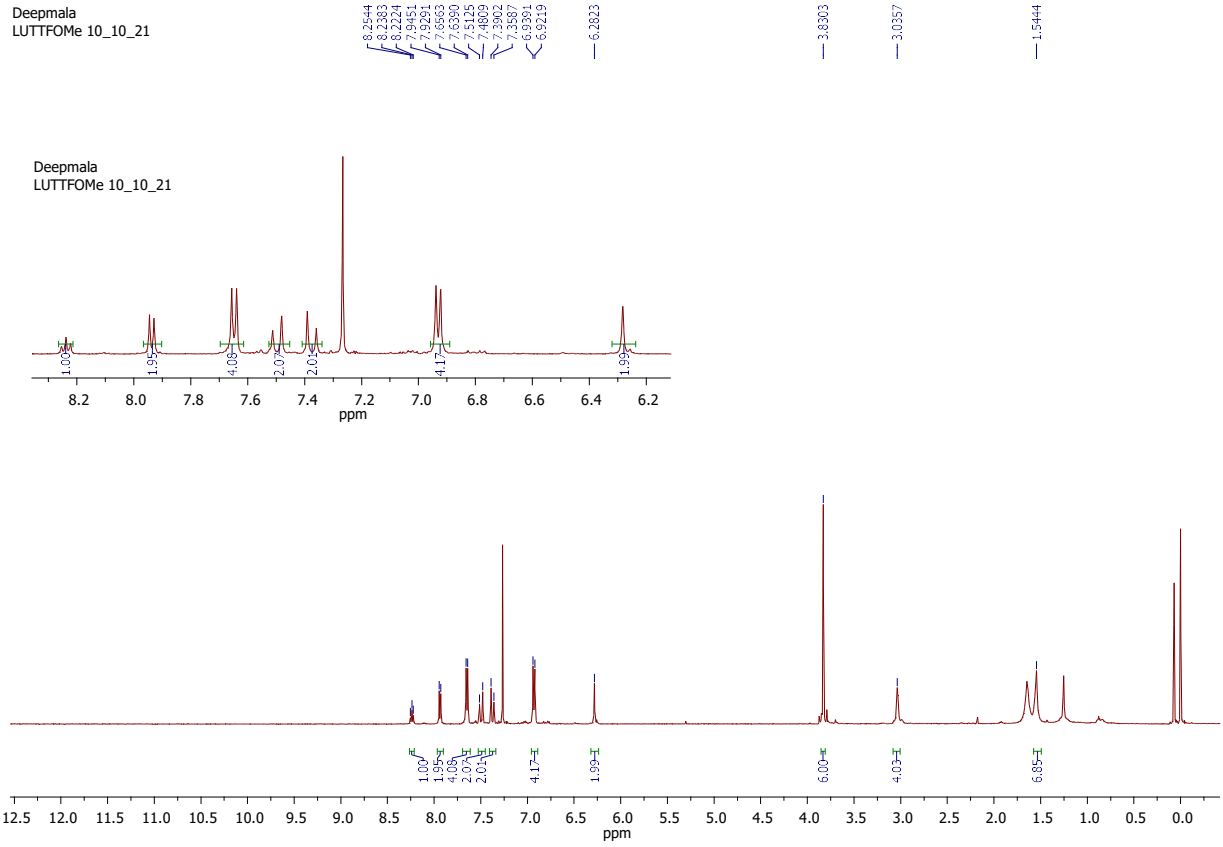
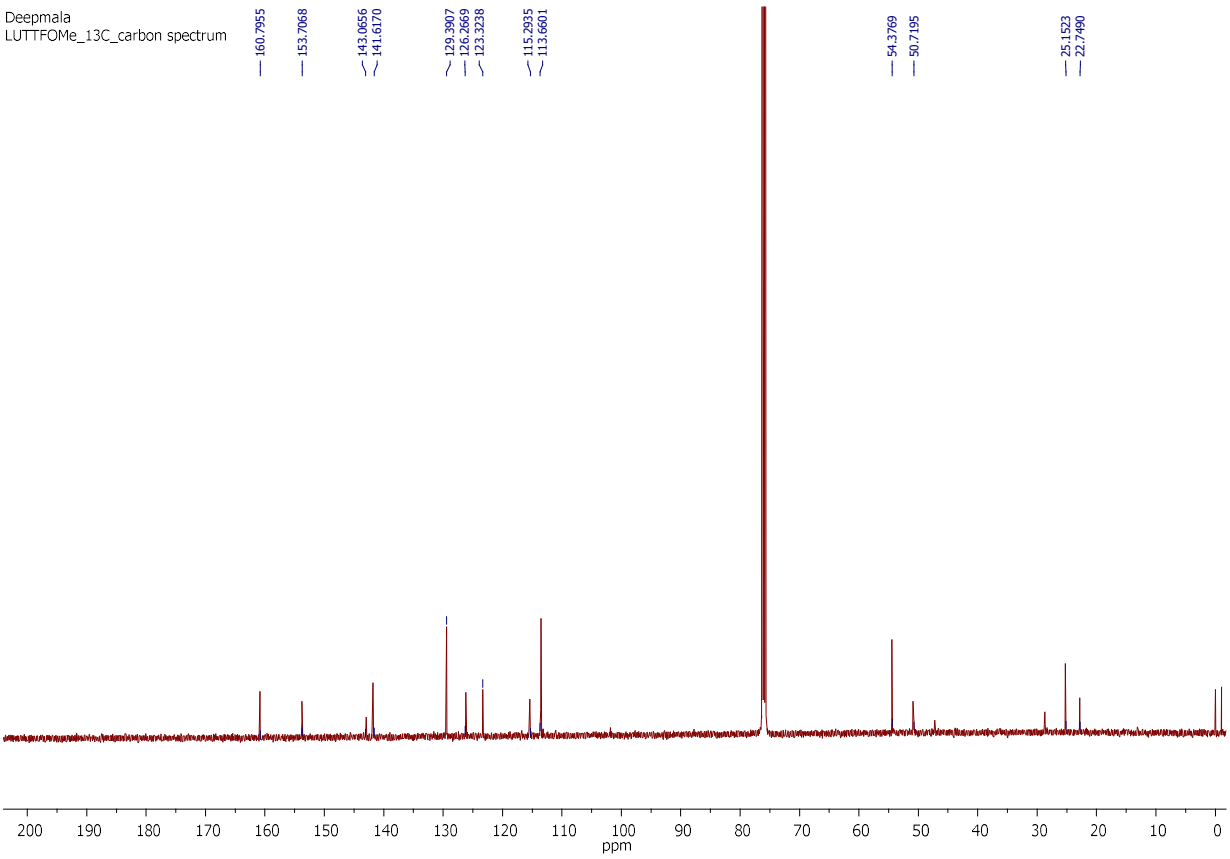


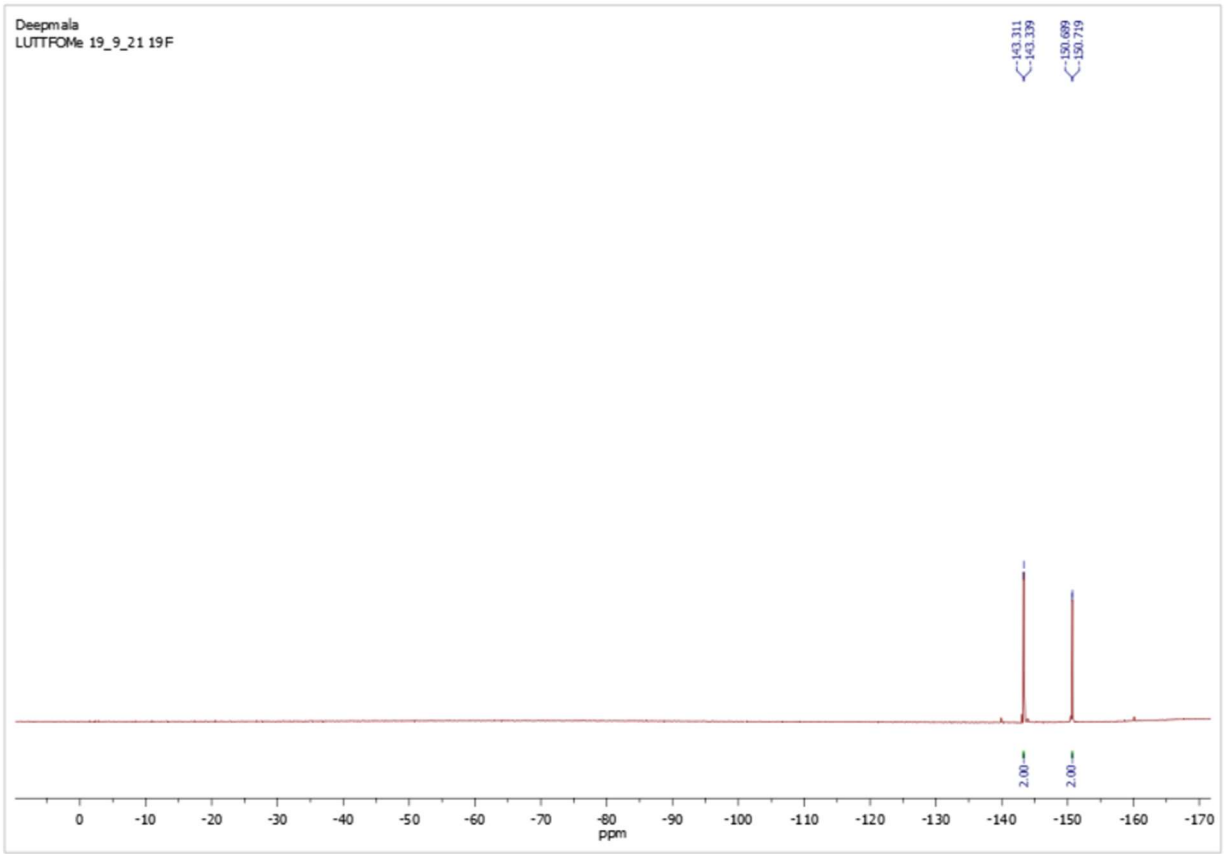
Fig S15: ^{1}H, ^{13}C, ^{19}F and HRMS spectra for **6**

^{1}H and ^{13}C and ^{19}F NMR for **7**






Deepmala
LUTTFOMe_13C_carbon spectrum
160.7955
153.7068
143.0656
141.6170
129.3907
126.2669
123.3238
115.2935
113.6601
54.3769
50.7195
25.1523
22.7490
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


Deepmala
LUTTFOMe 19_9_21 19F
143.311
143.339
150.689
150.719
2.00
2.00
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170
ppm

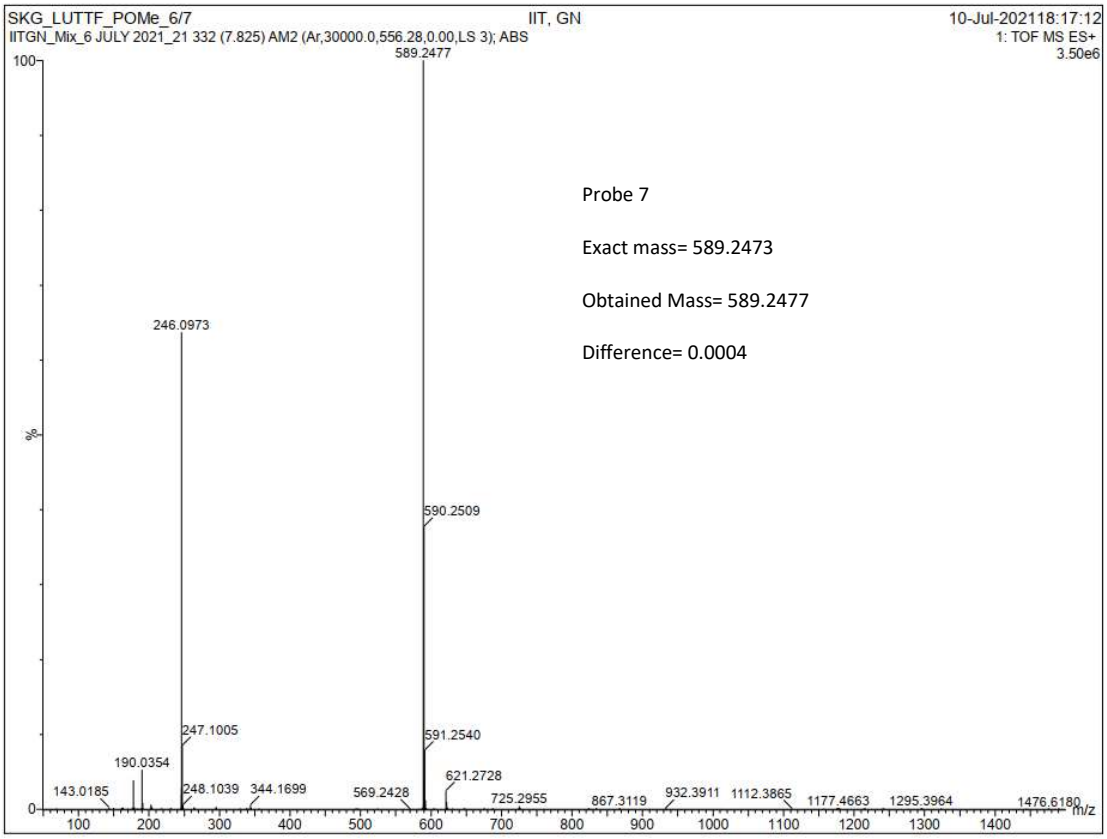


Fig S16: ^{1}H, ^{13}C, ^{19}F and HRMS spectra for 7

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