Supporting information

Mitochondria specific highly cytoselective Iridium (III)-Cp* dipyridophenazine (dppz) complexes as cancer cell imaging agents†

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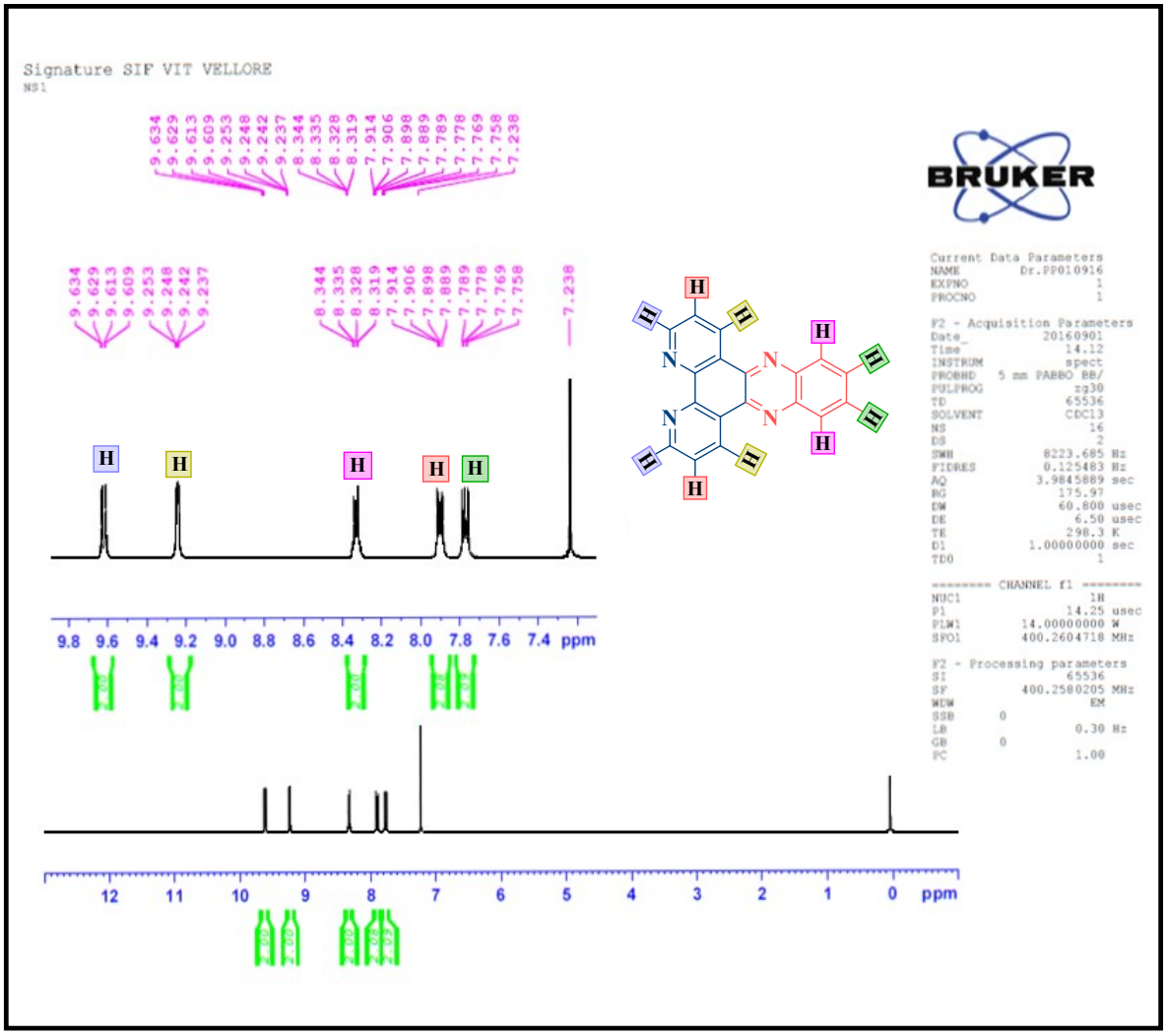
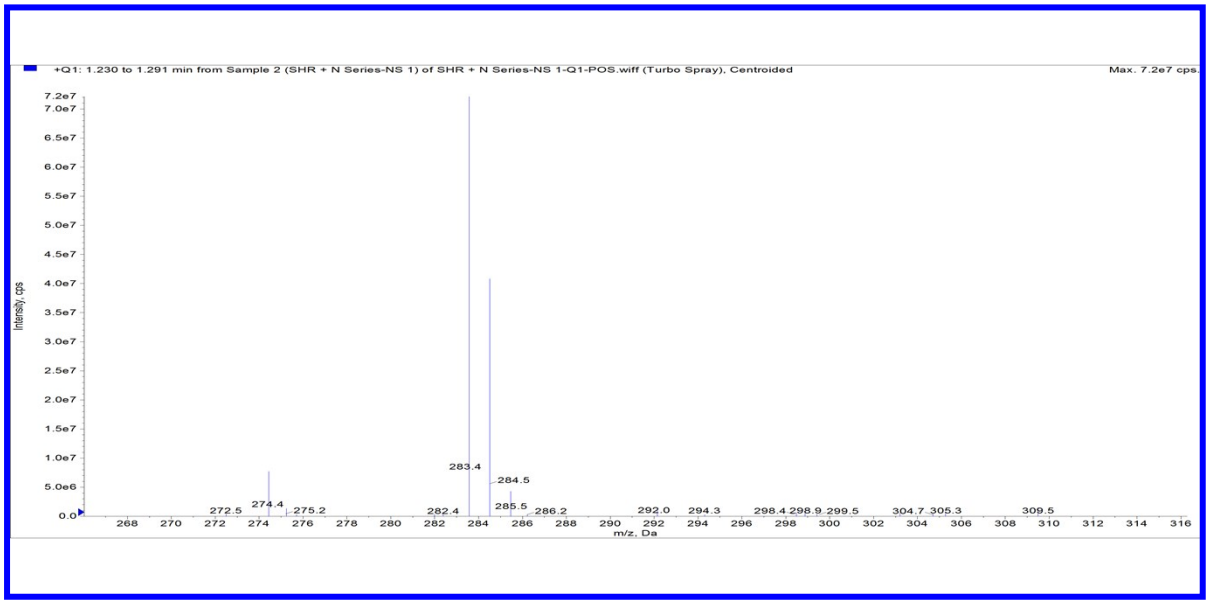
Email :Bipasha.bose@yenepoya.edu.in
[c]School of Bioscience of Technology, Vellore Institute of Technology

Page No

^{1}H NMR spectrum of Ligand L1



ESI-MS of ligand L1



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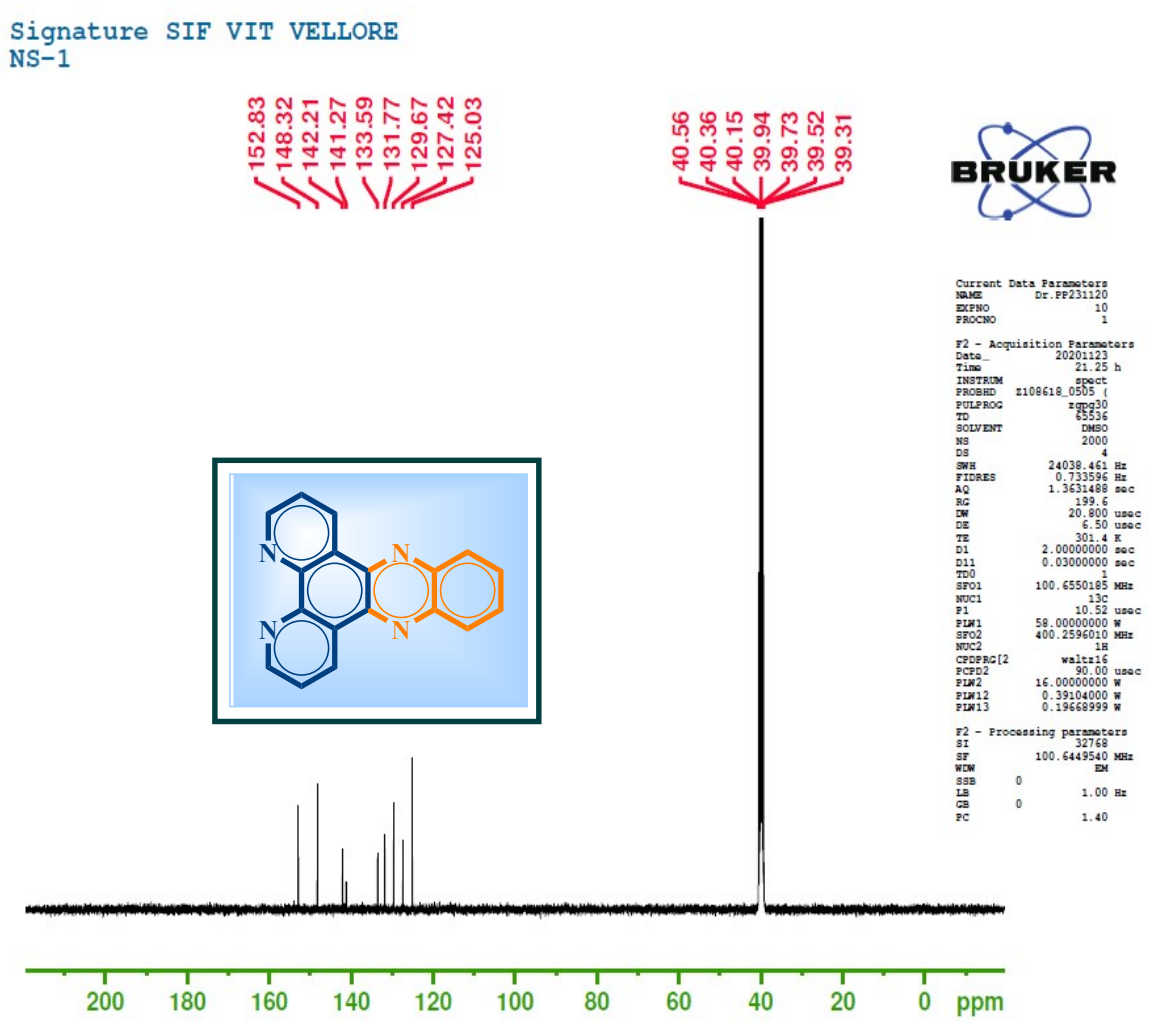

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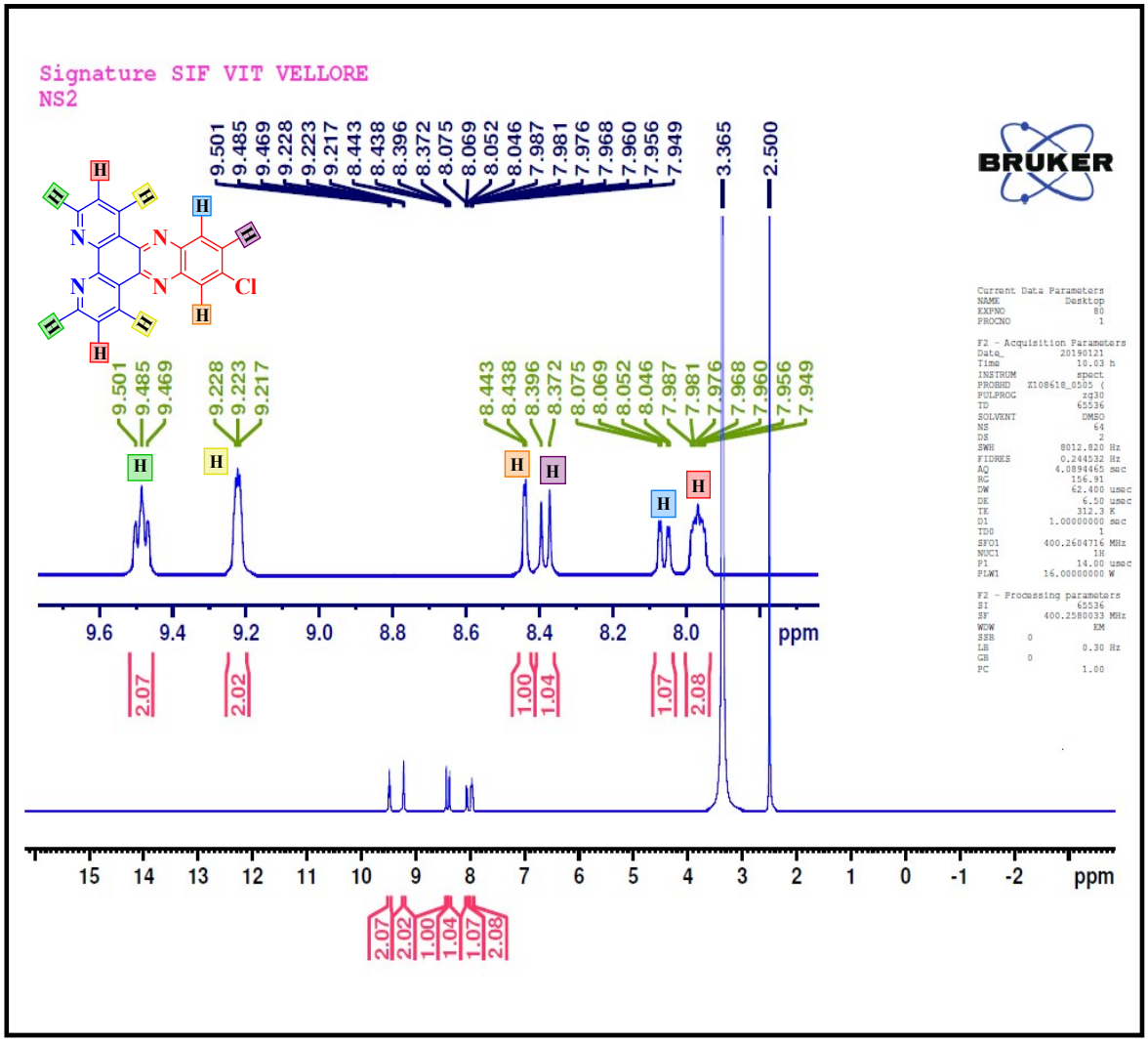

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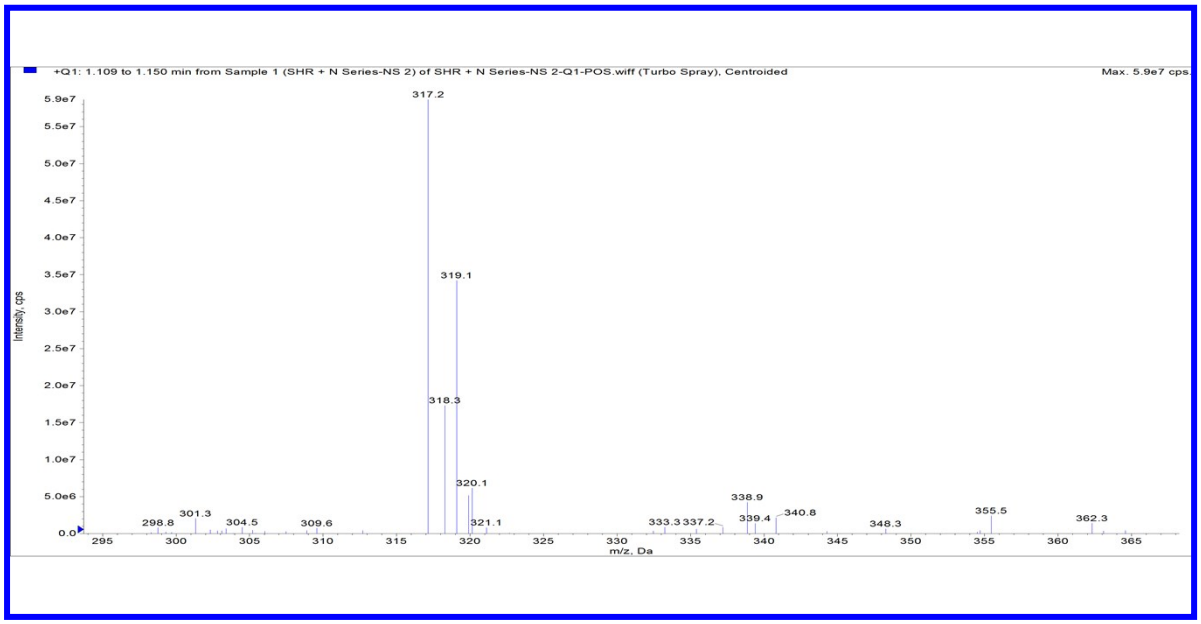

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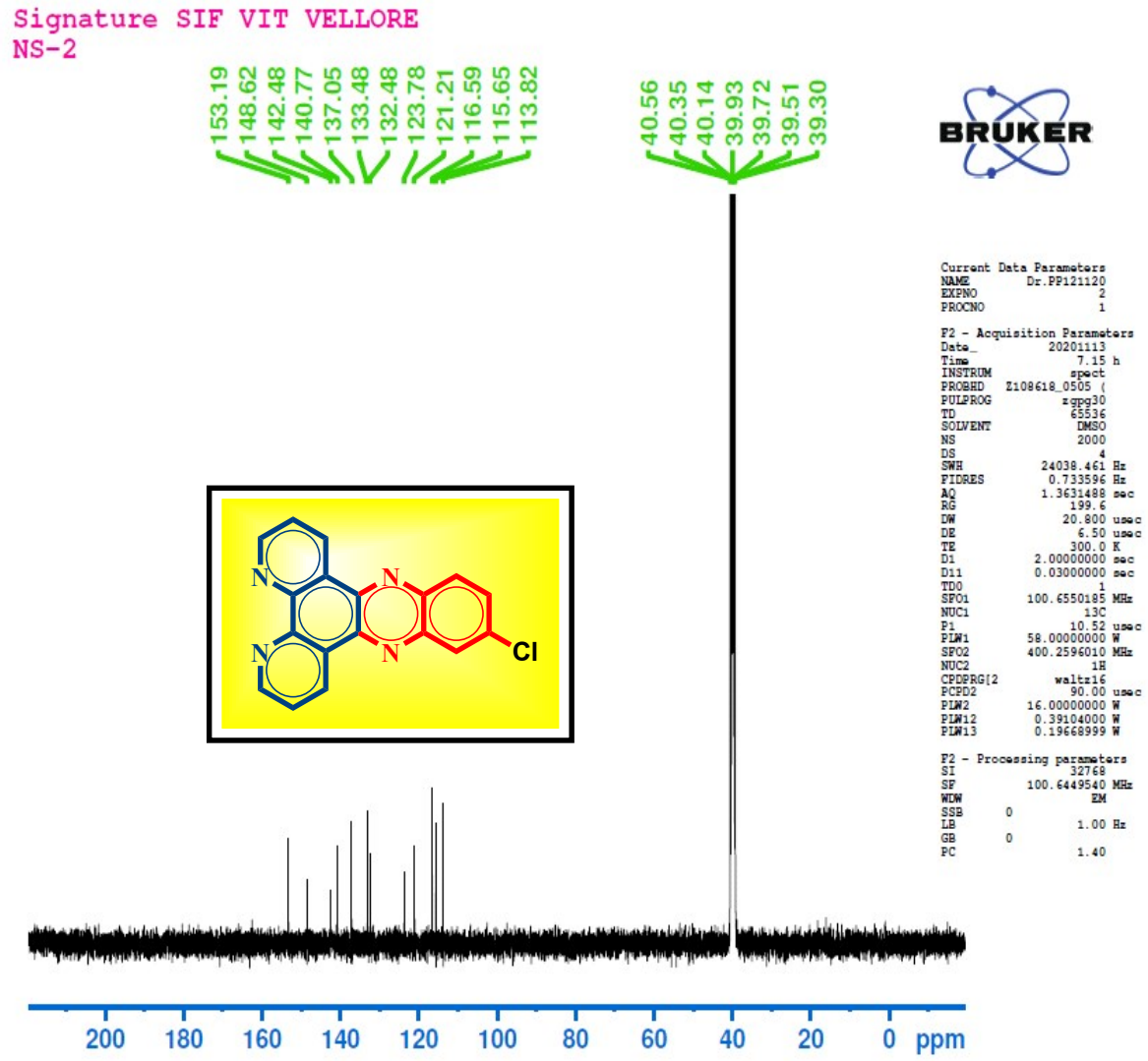

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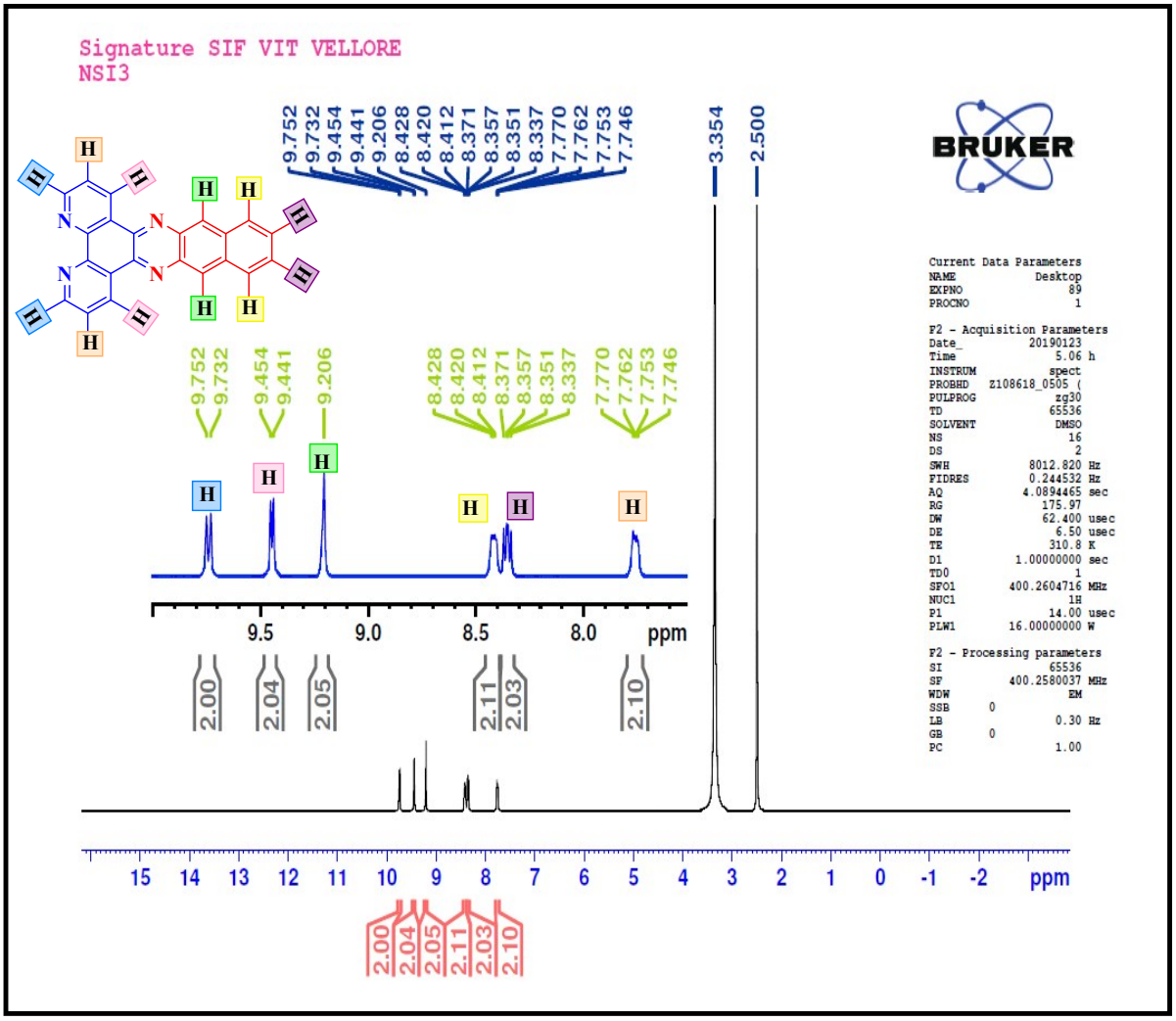

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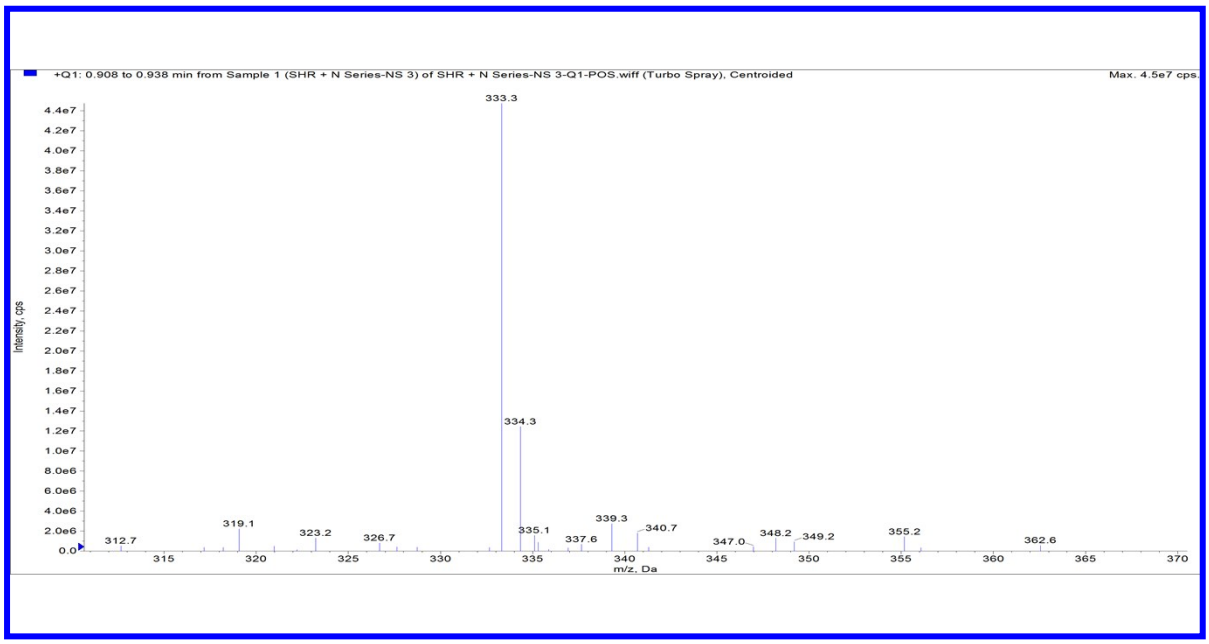

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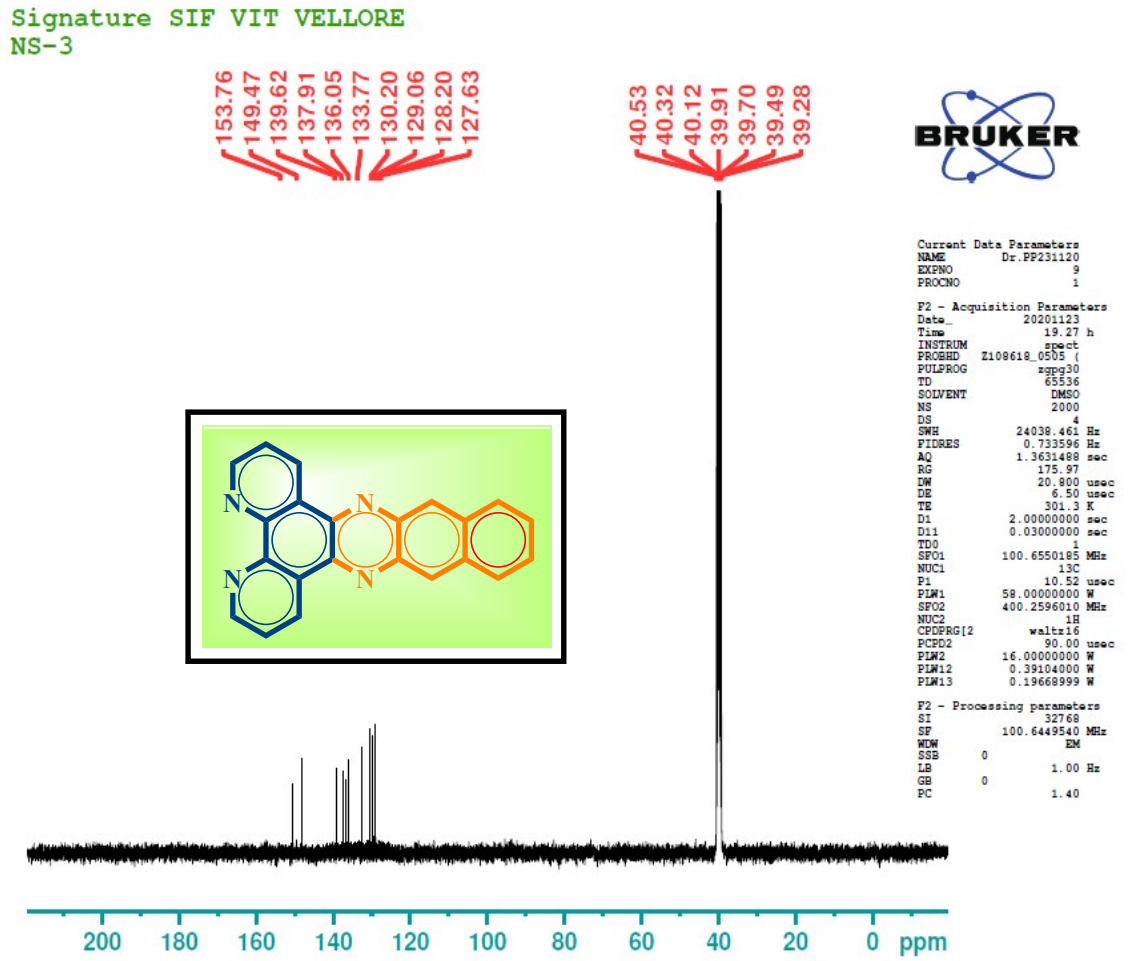

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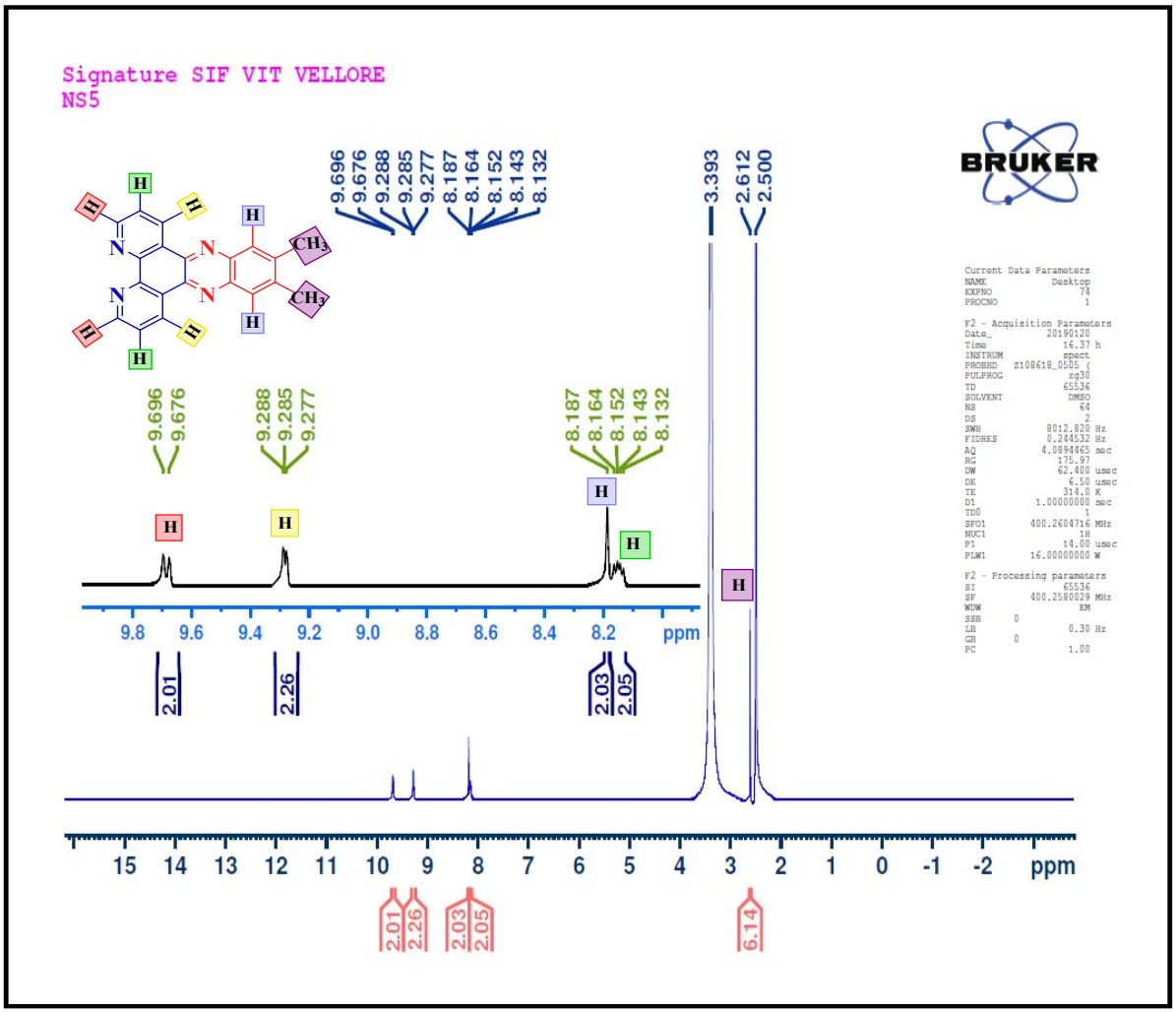

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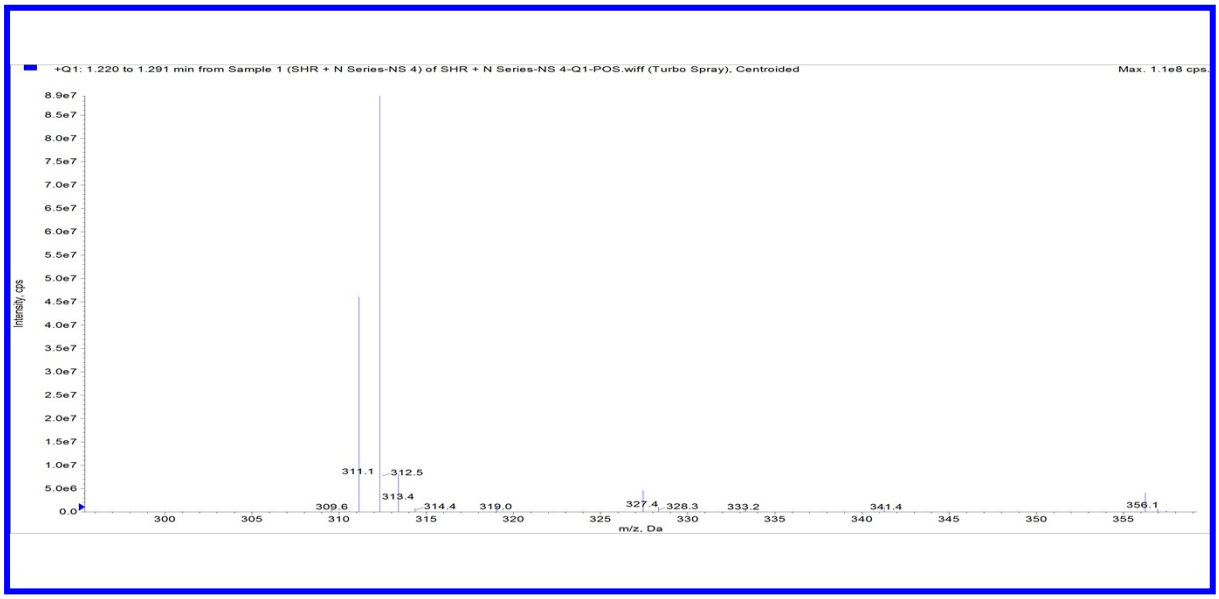

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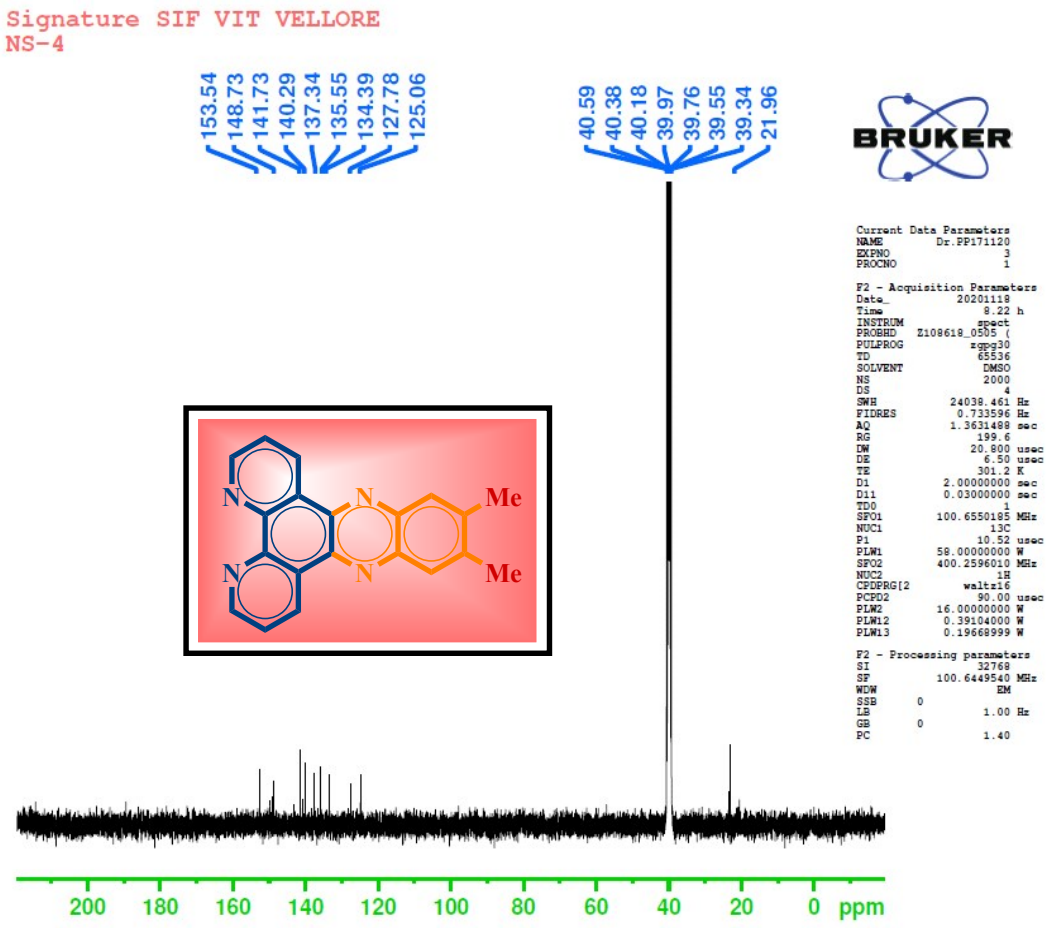

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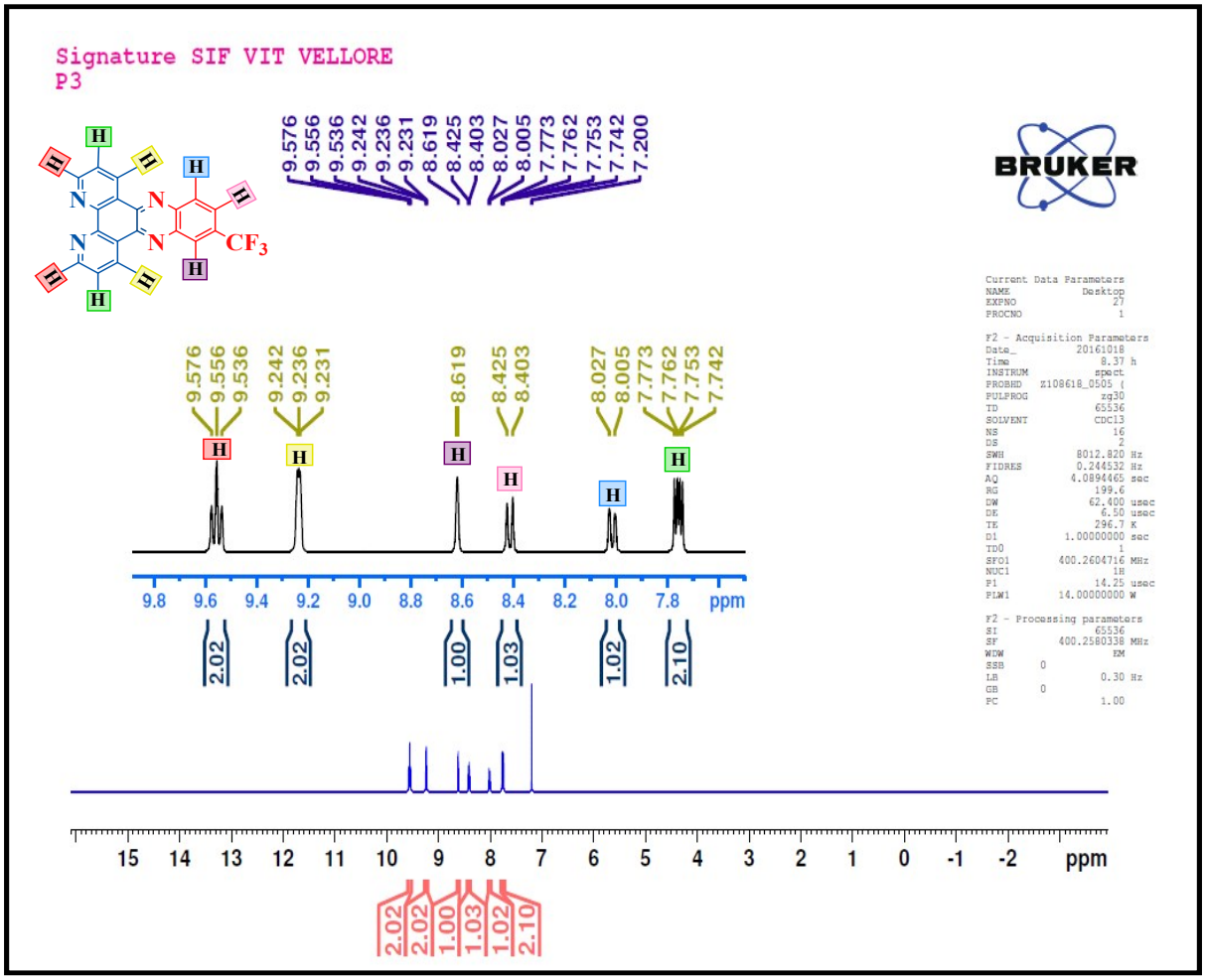

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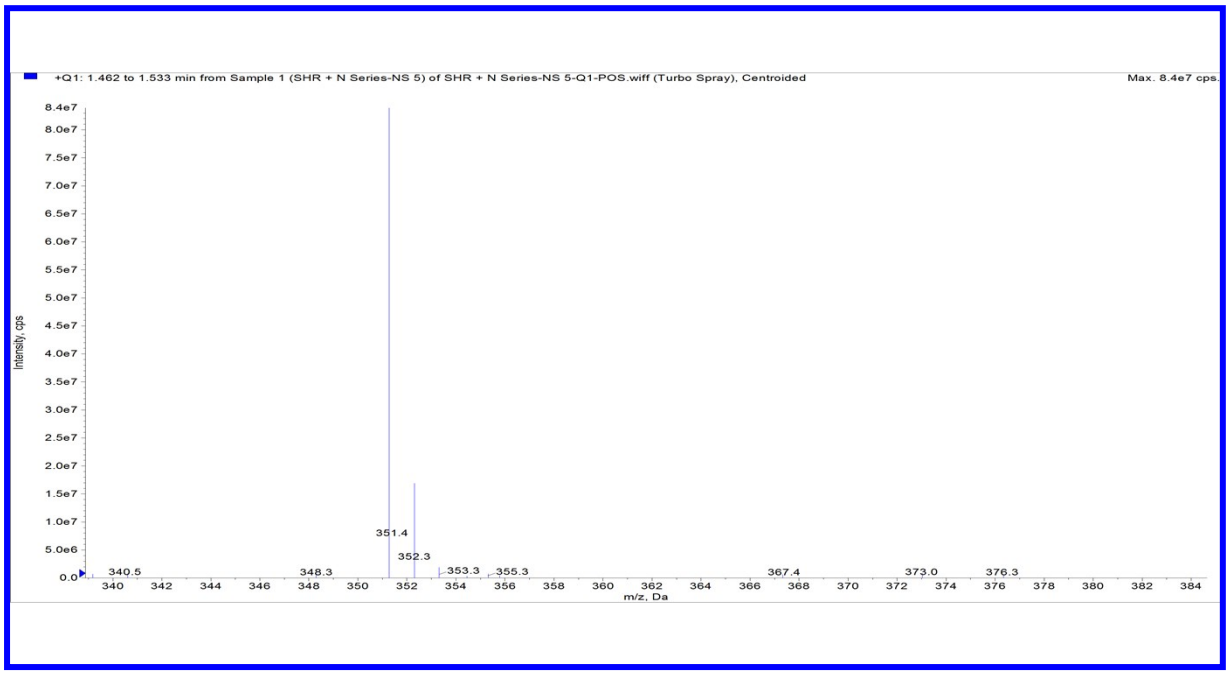
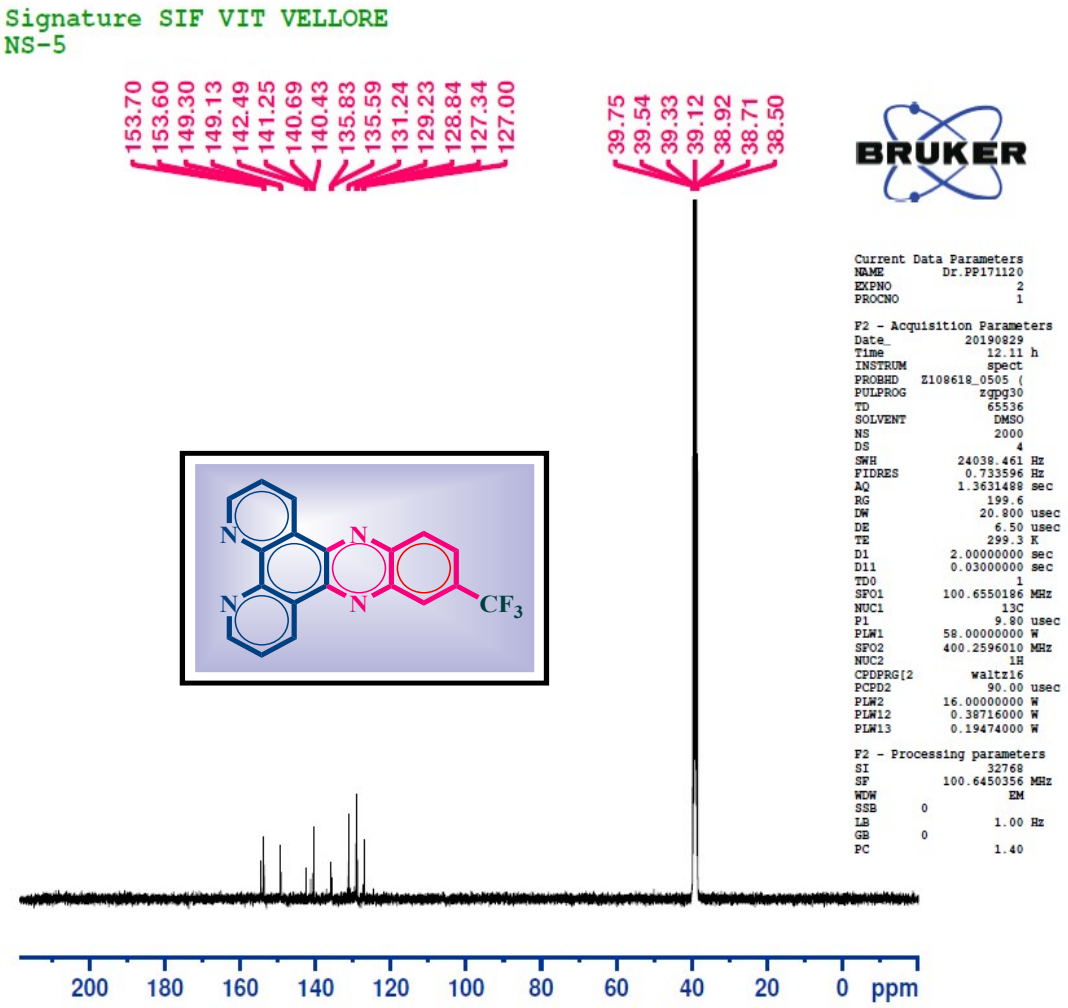


^{13}C NMR spectrum of Ligand L5

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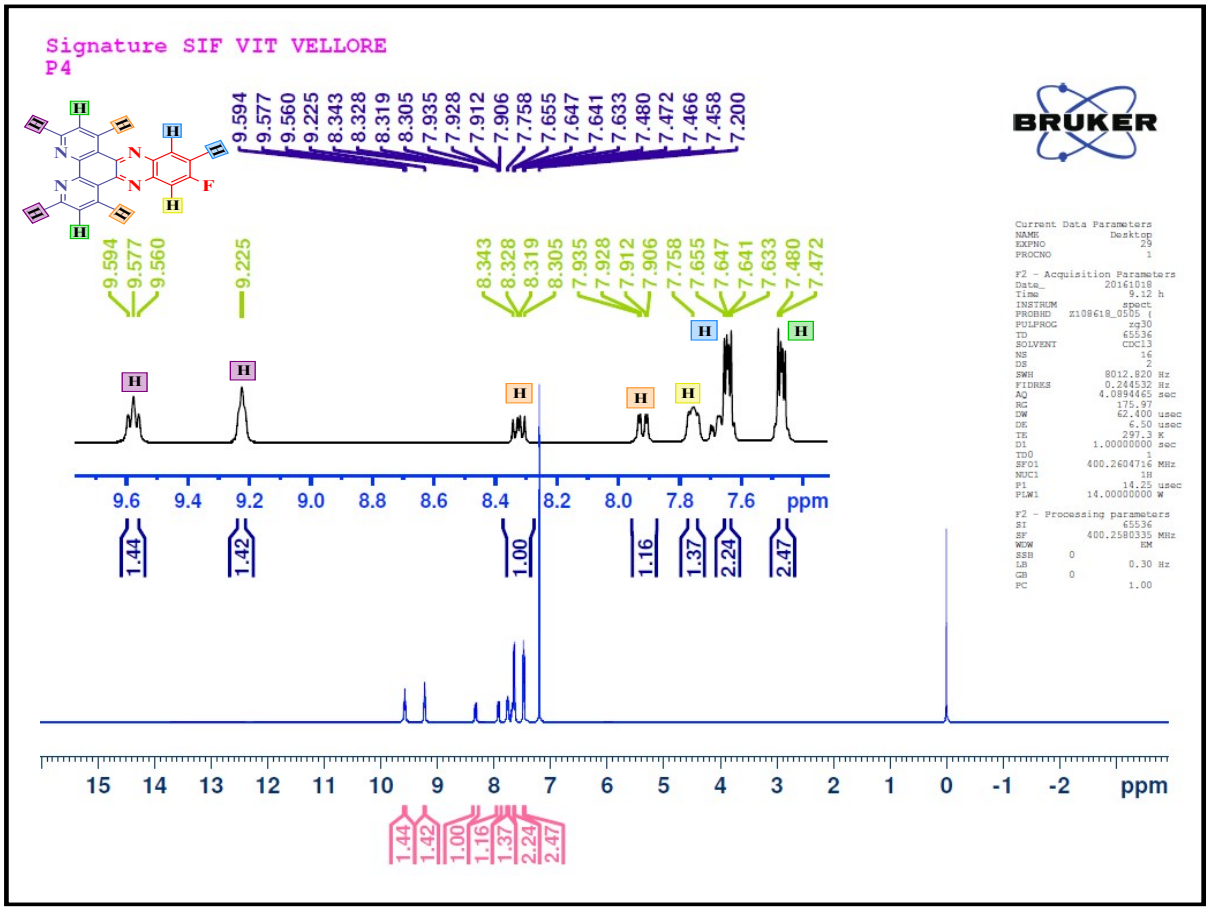

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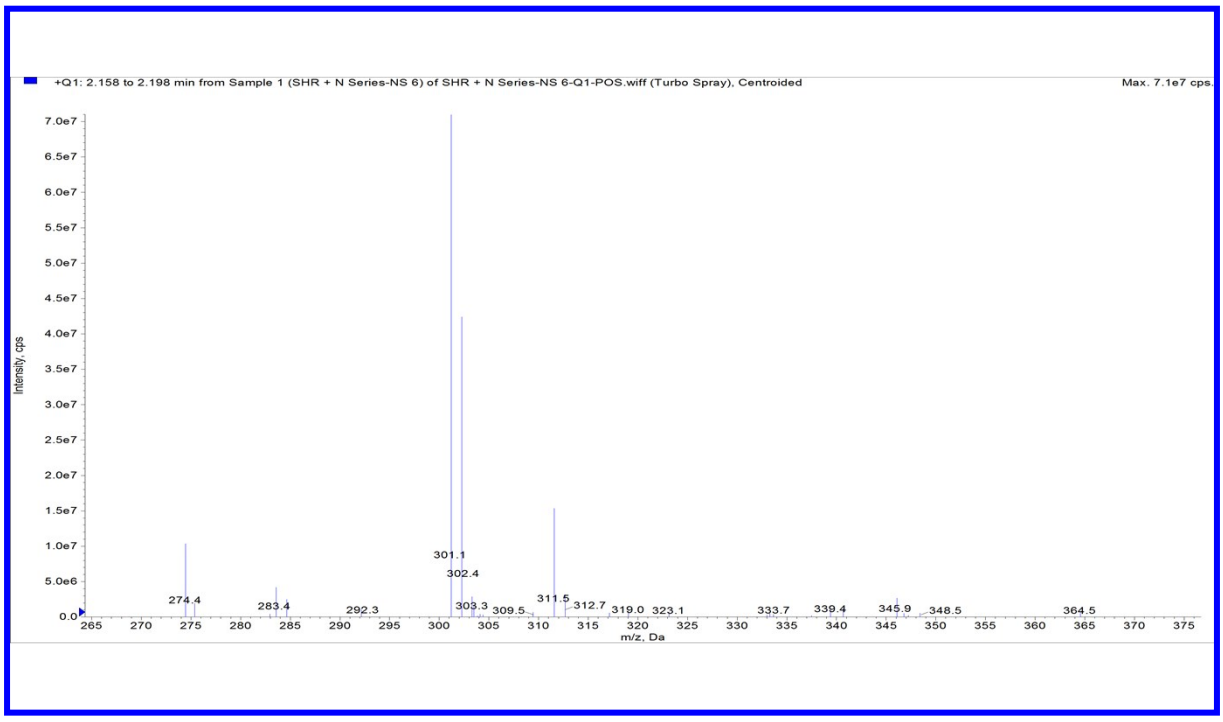

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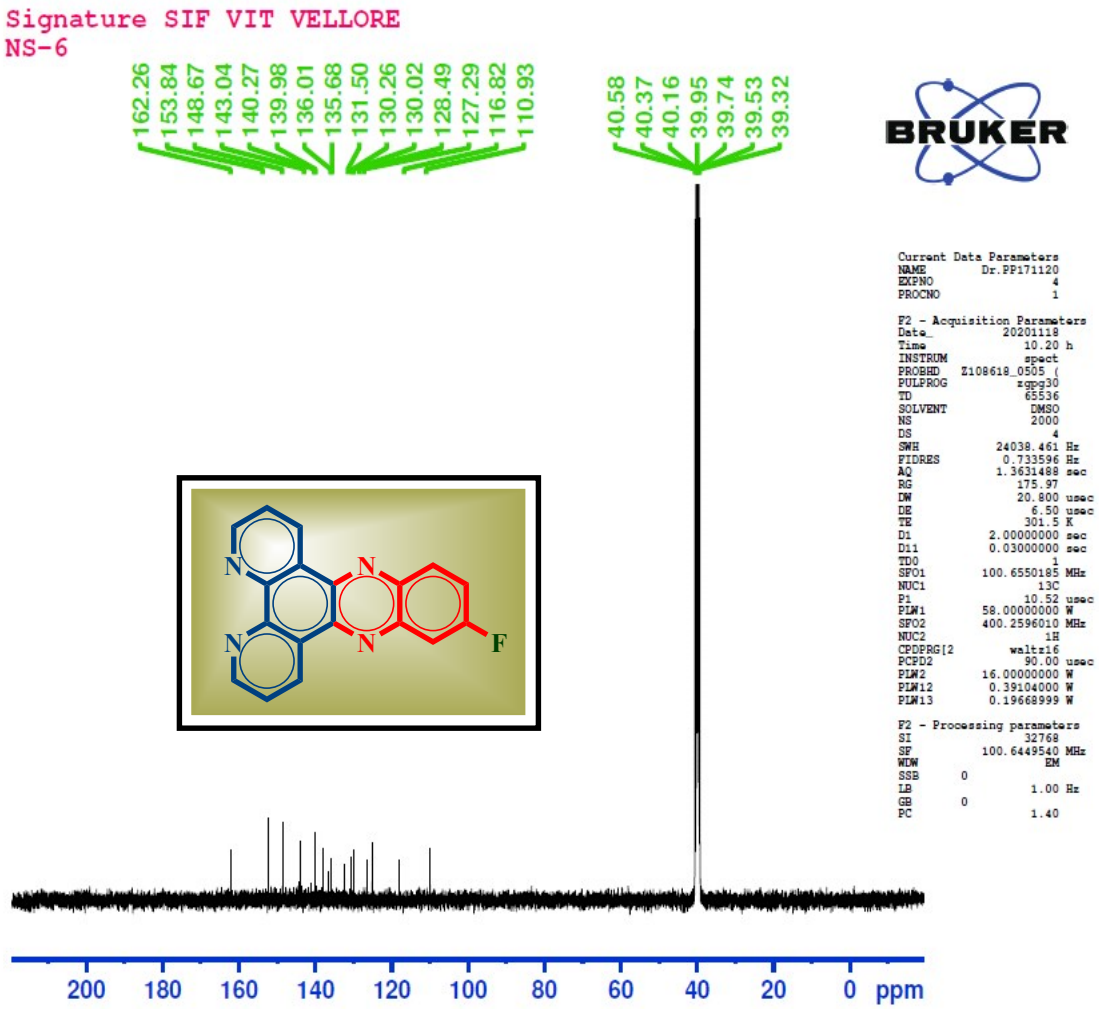

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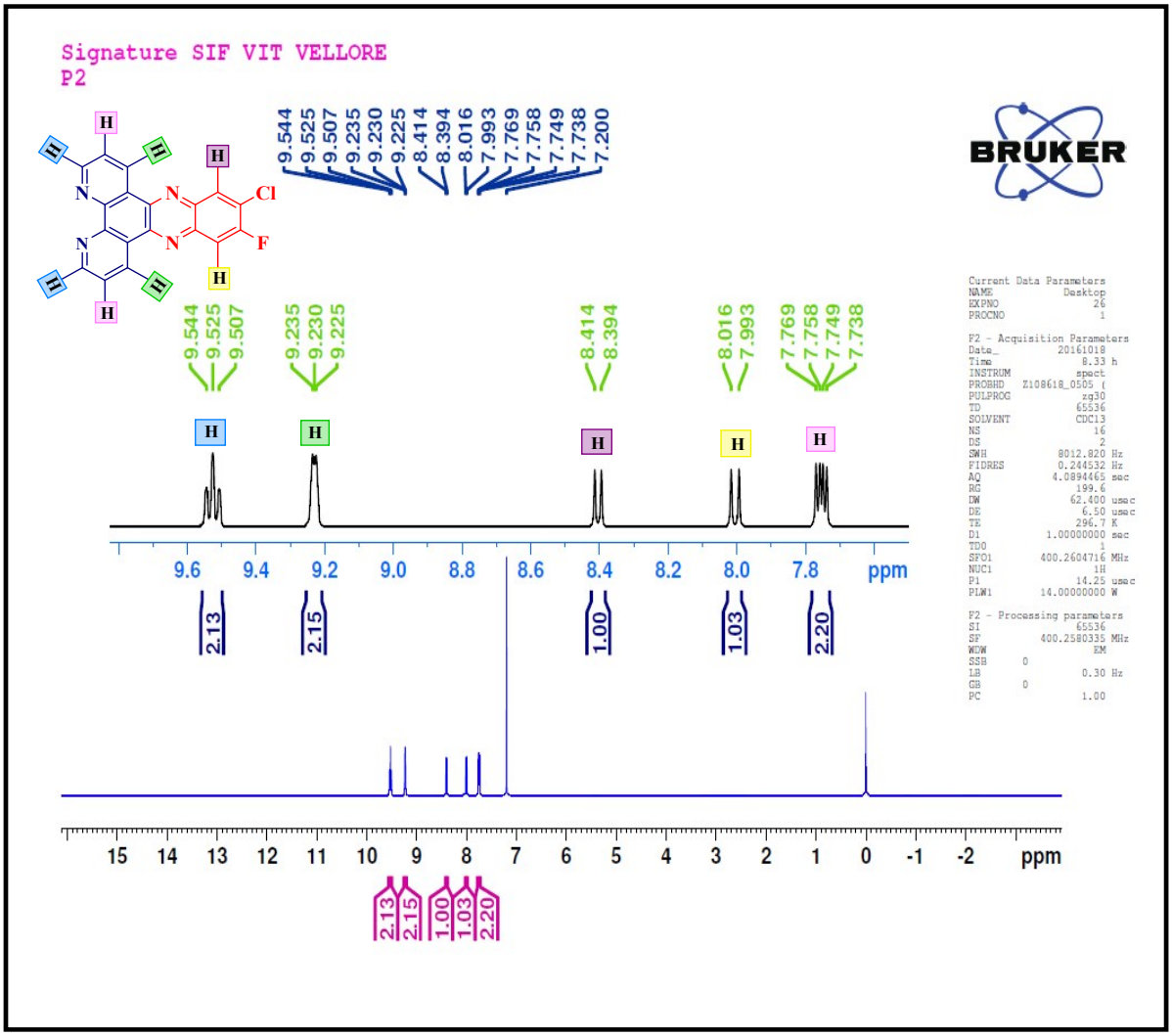

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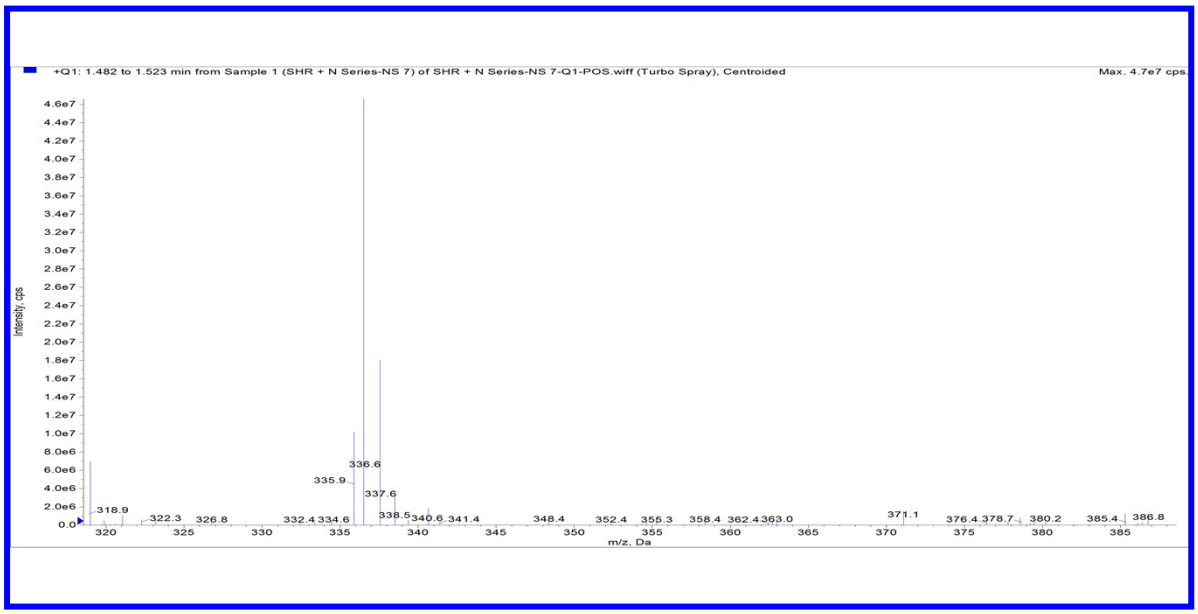
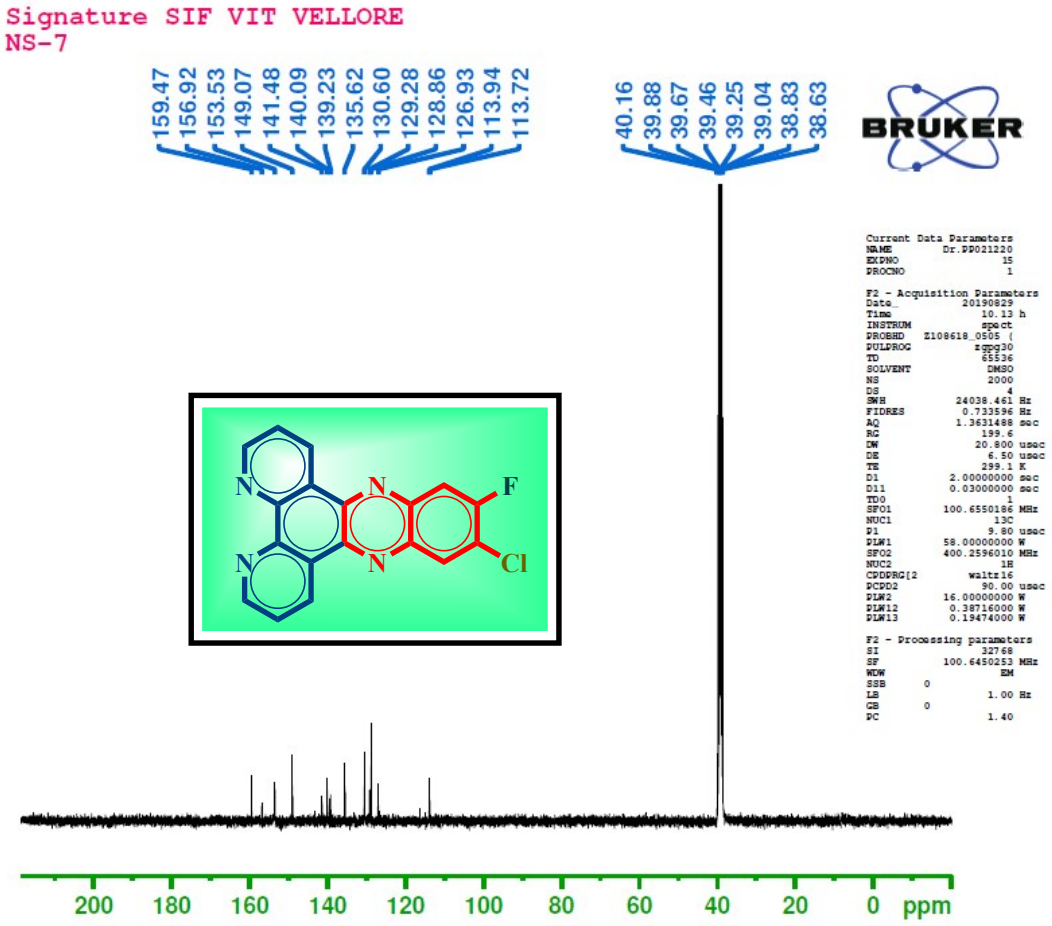


^{13}C NMR spectrum of Ligand L7



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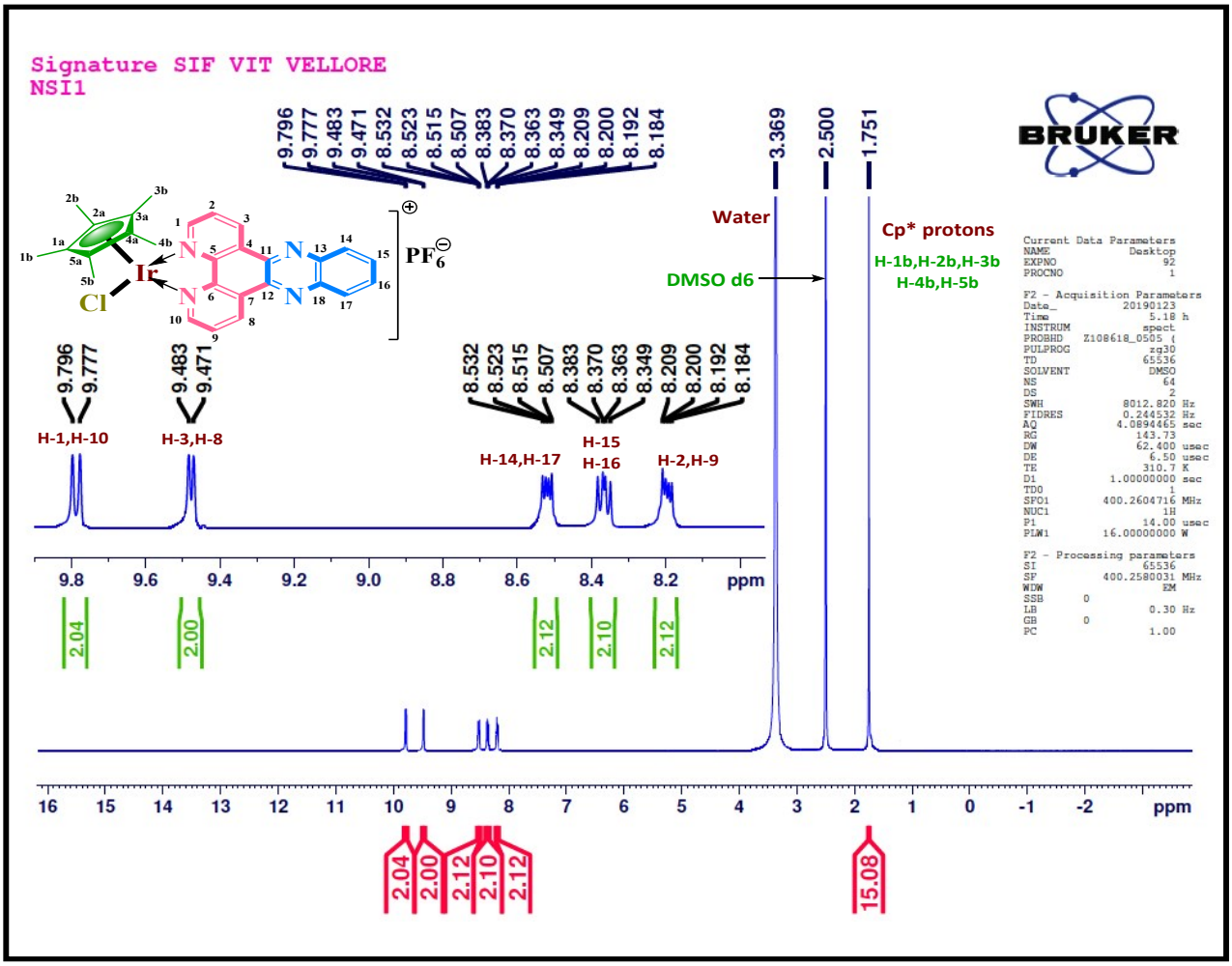
^{1}H NMR spectrum of Complex IrL1



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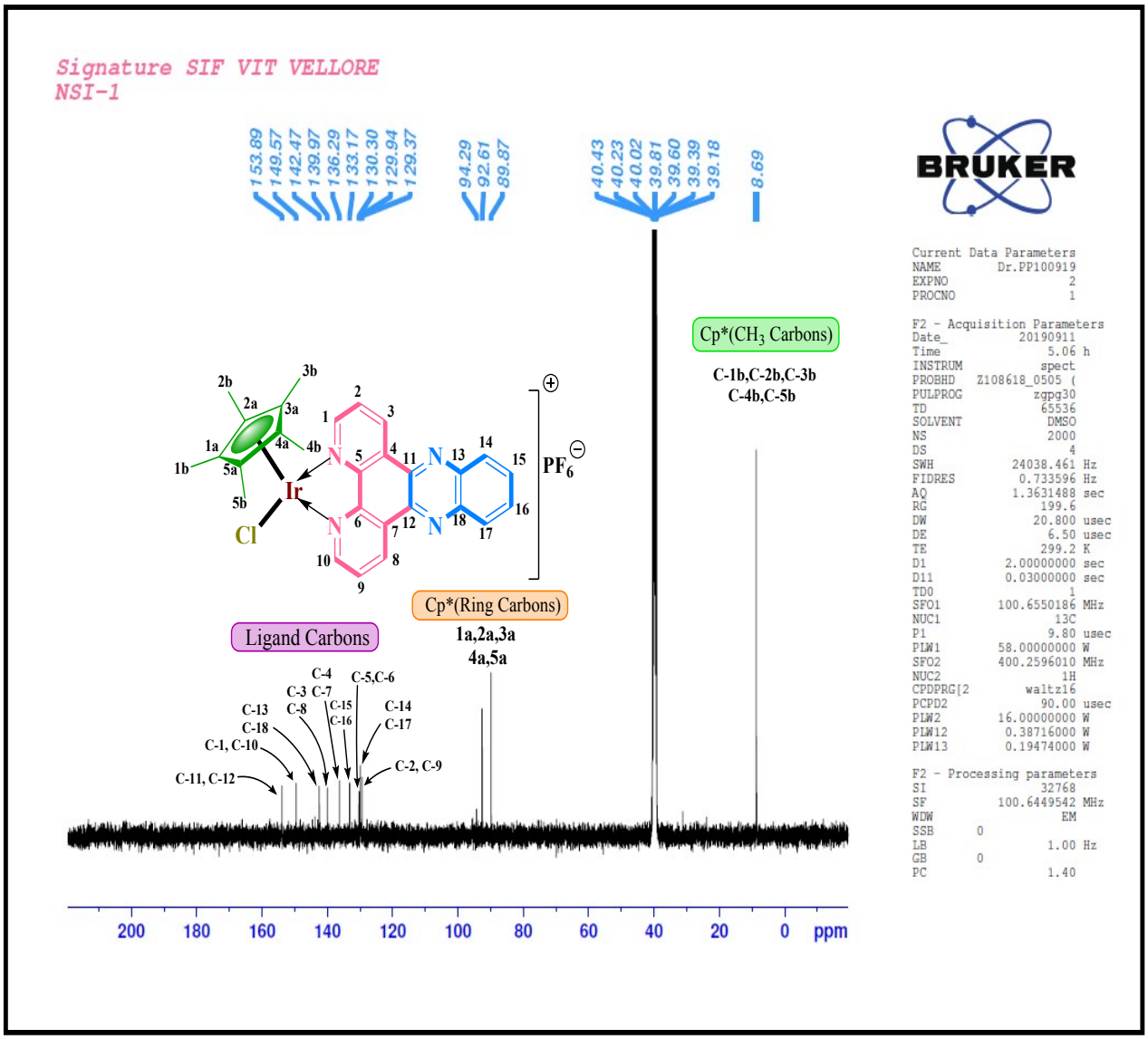

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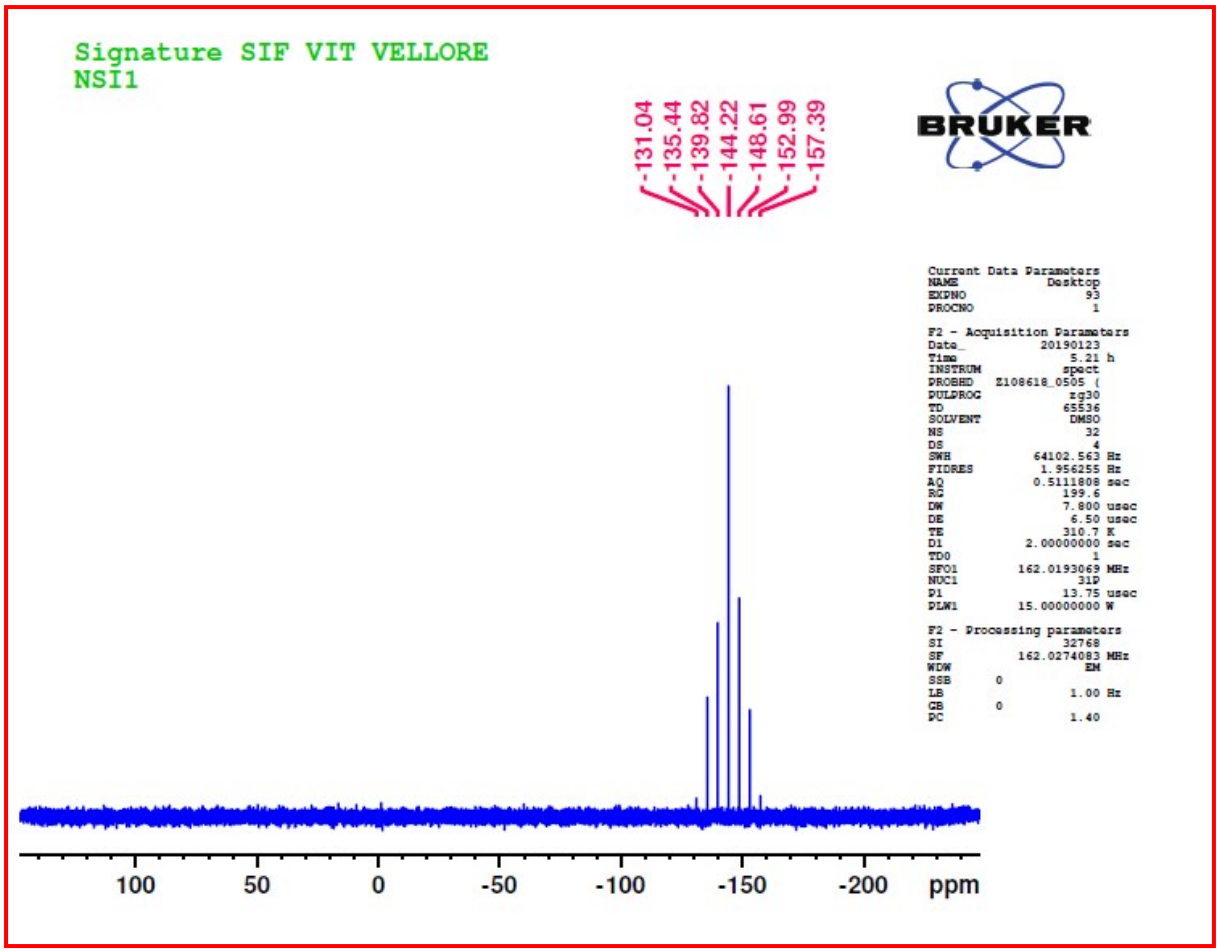

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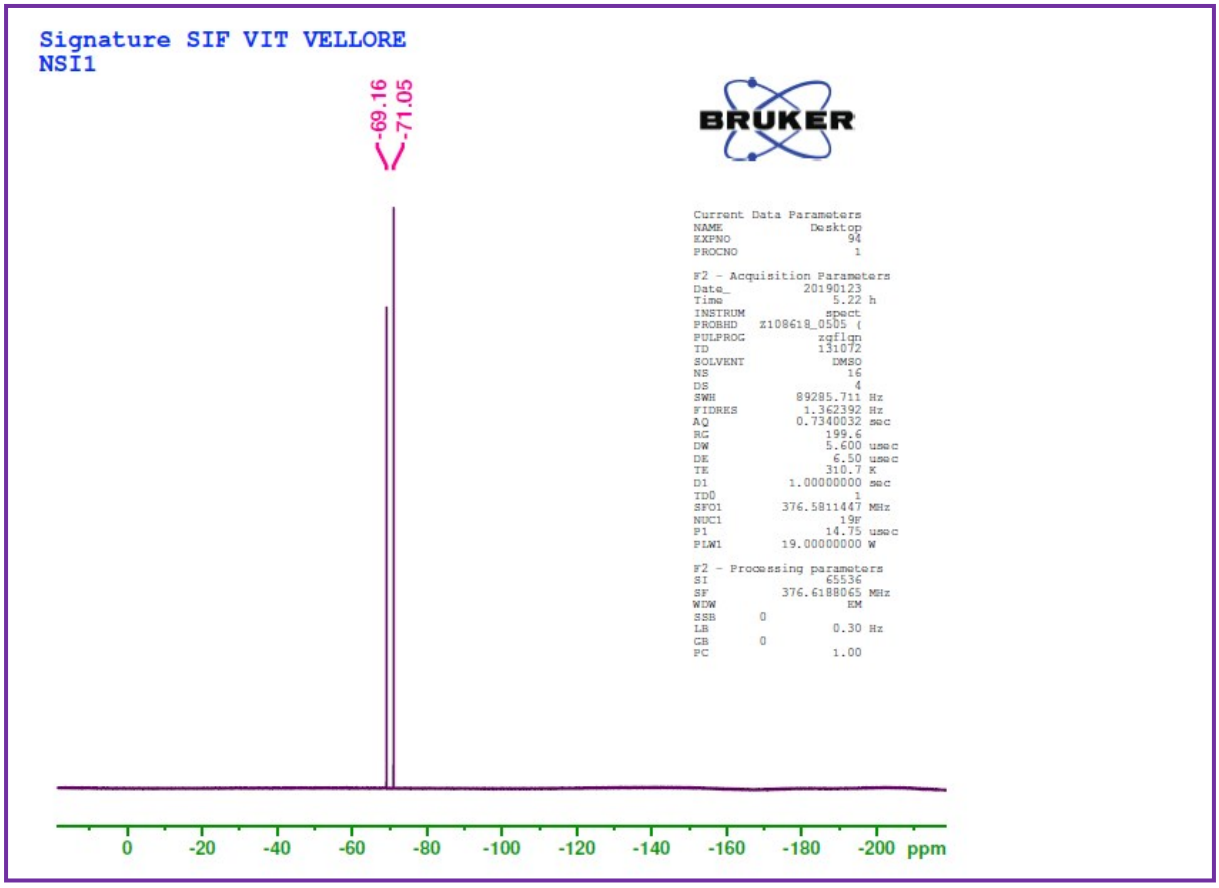

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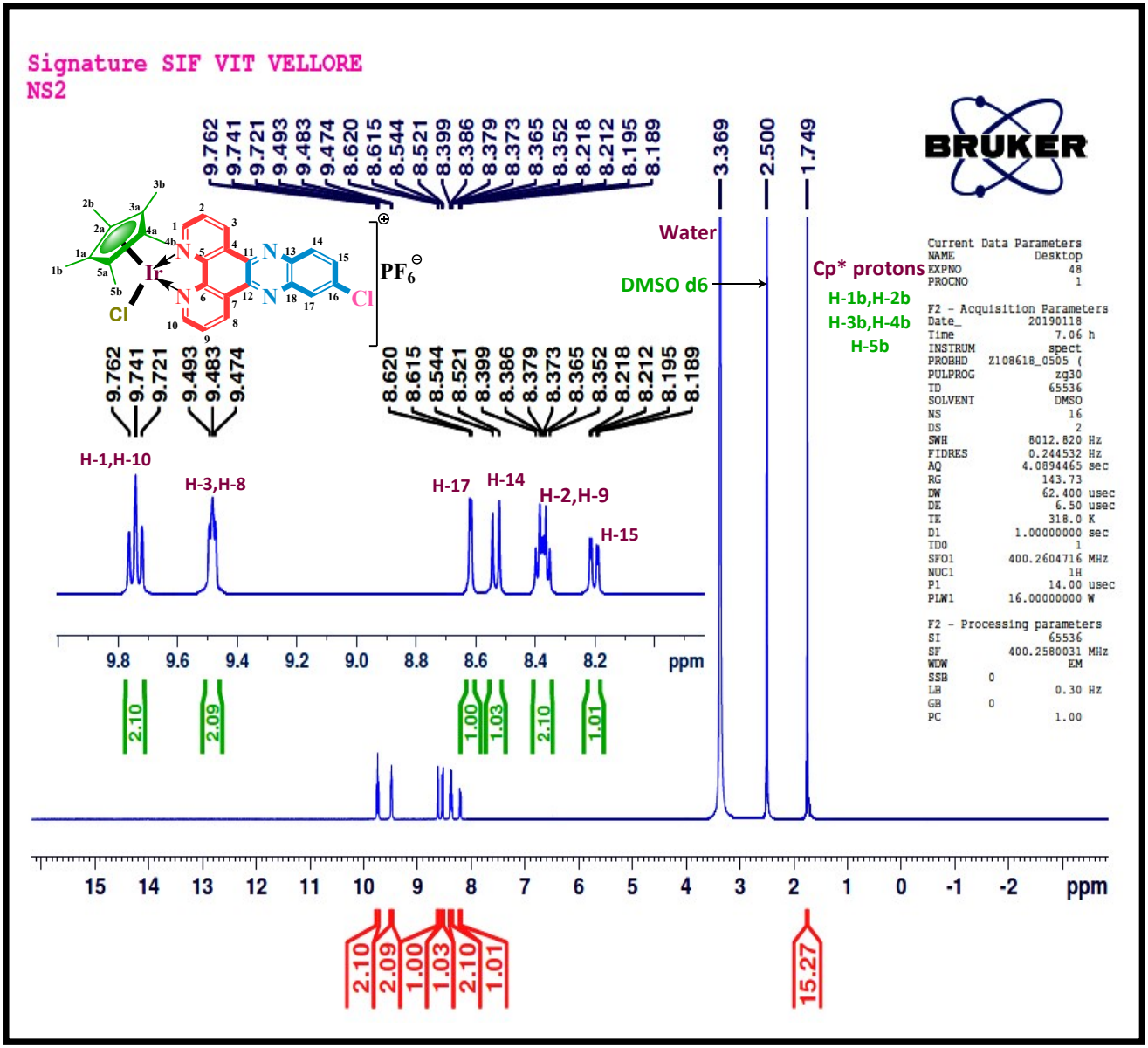

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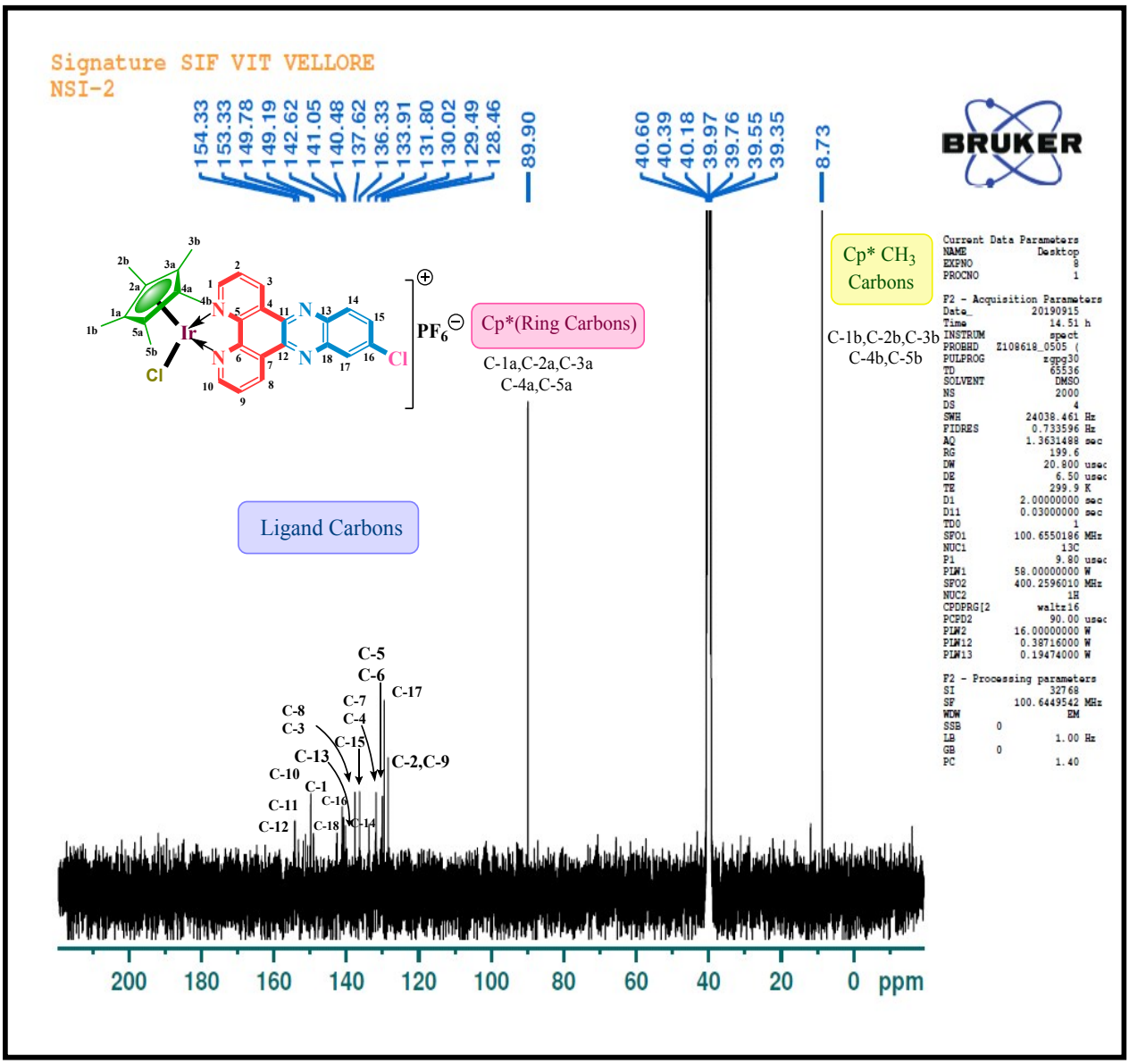

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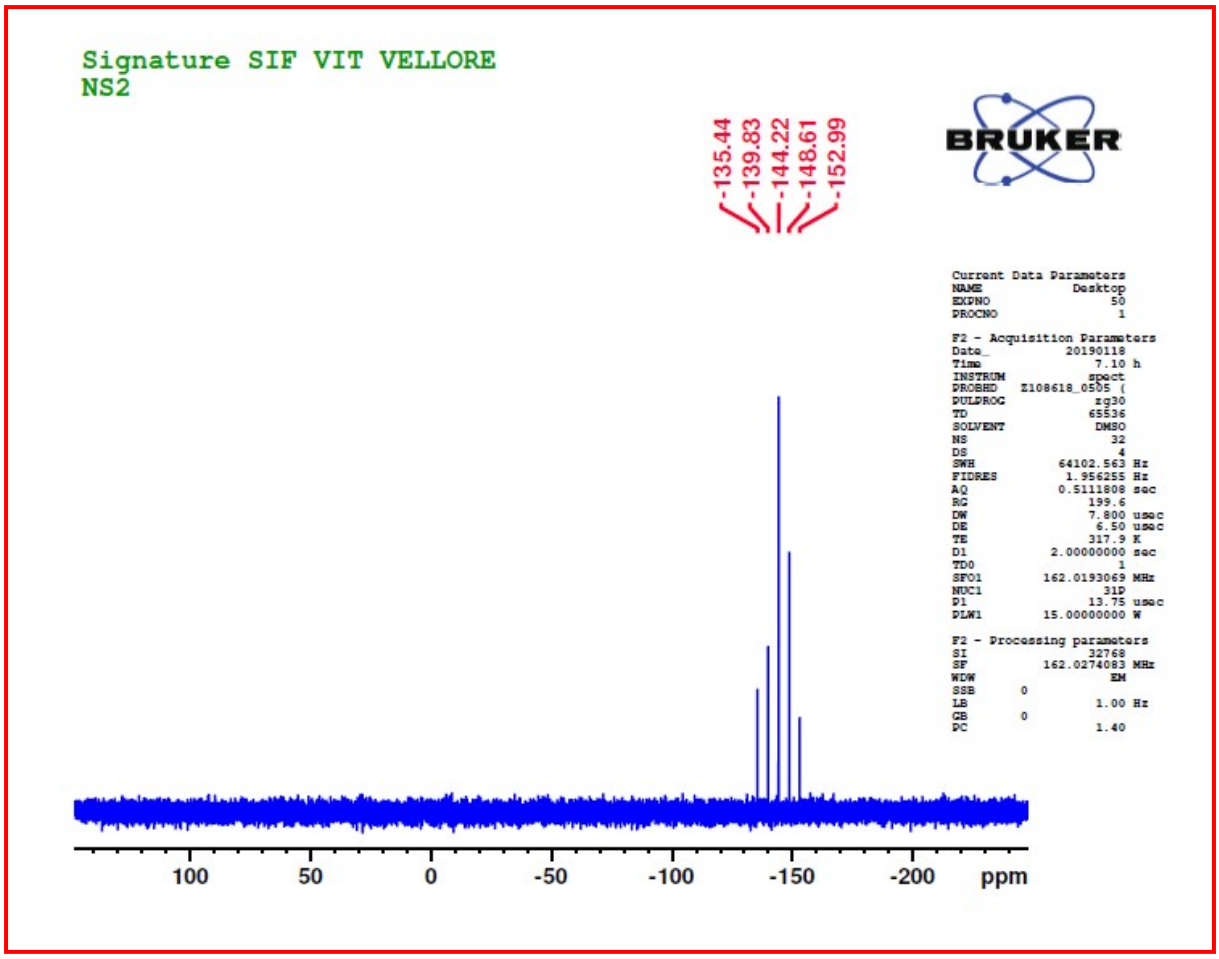
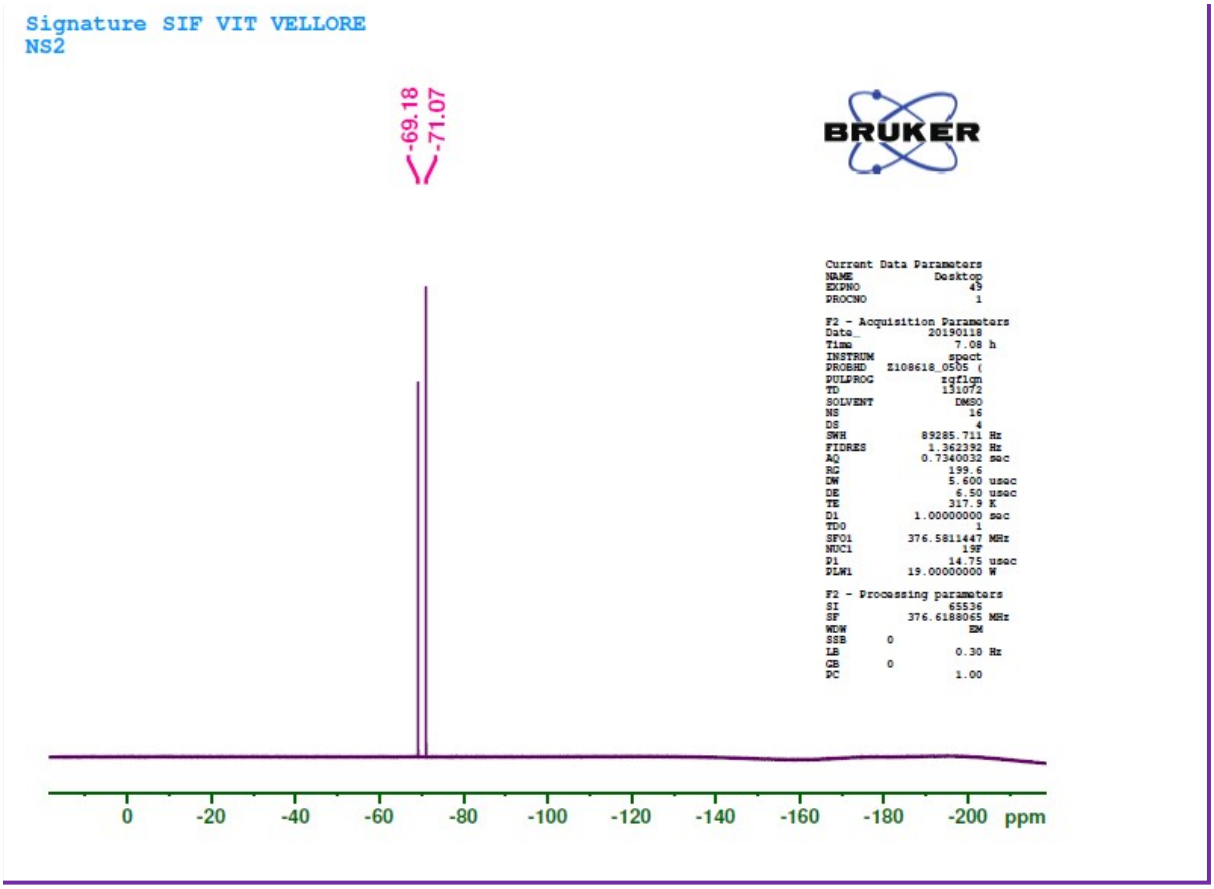


^{19}F NMR Spectrum of Complex IrL2



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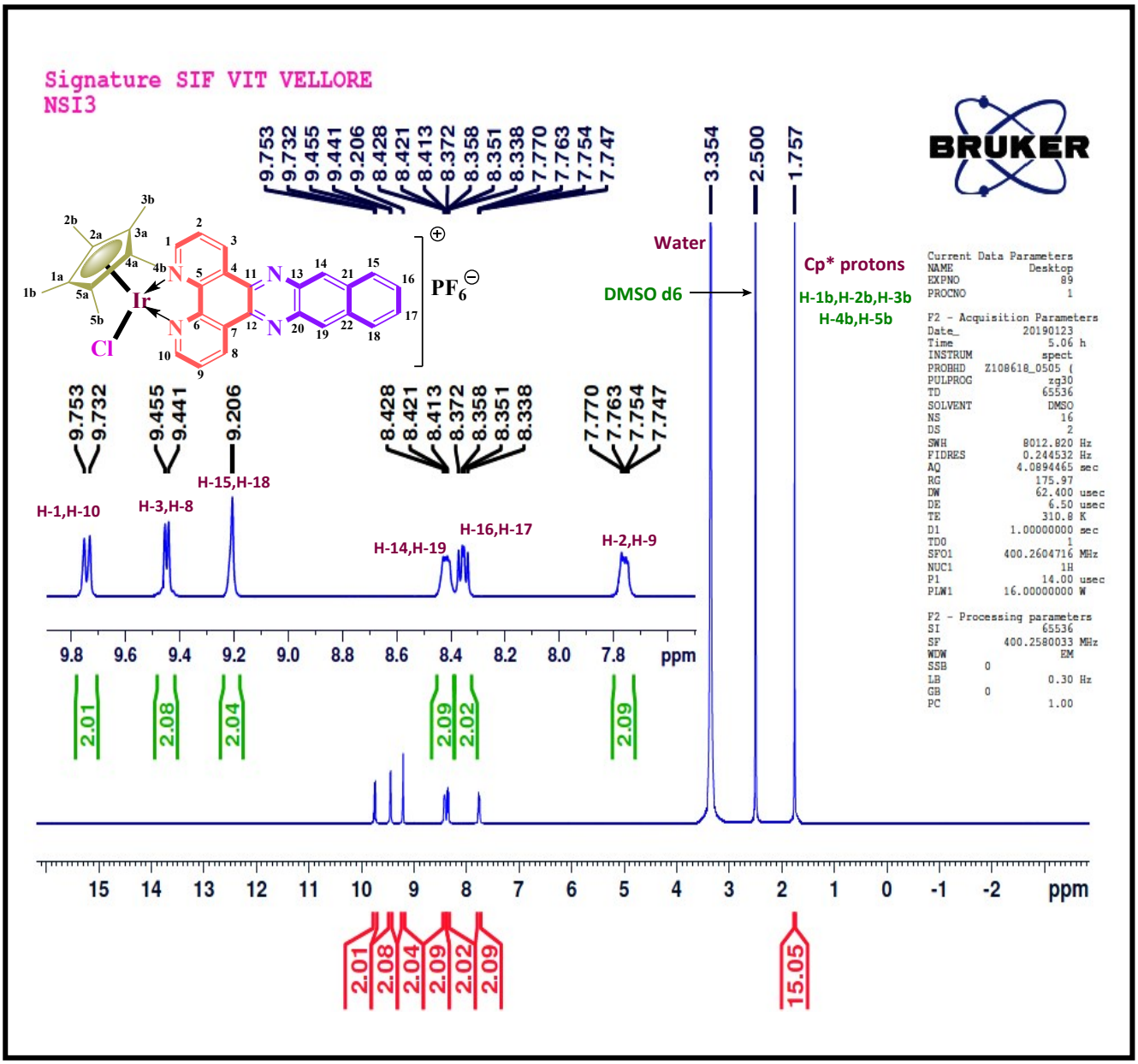

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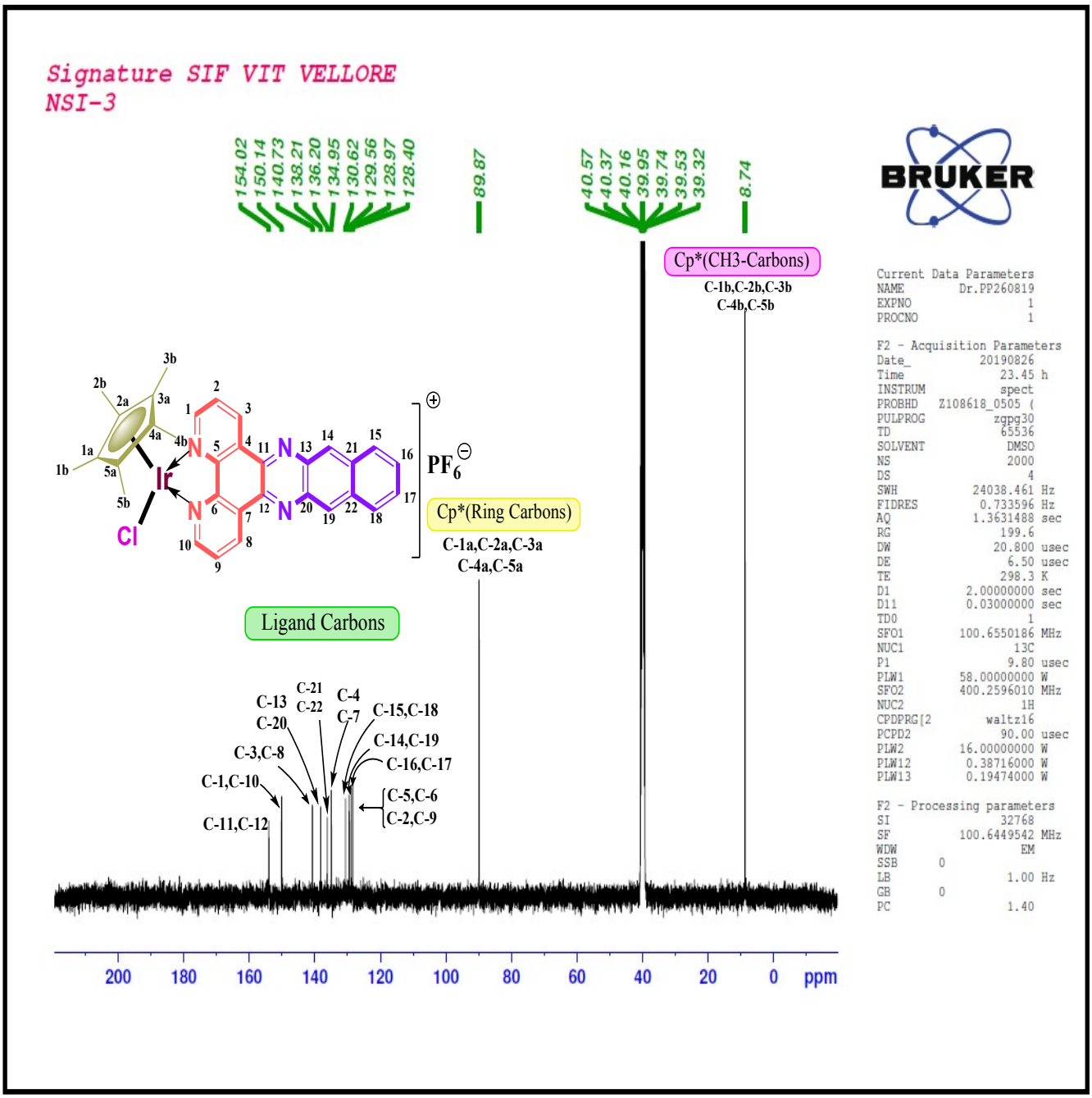

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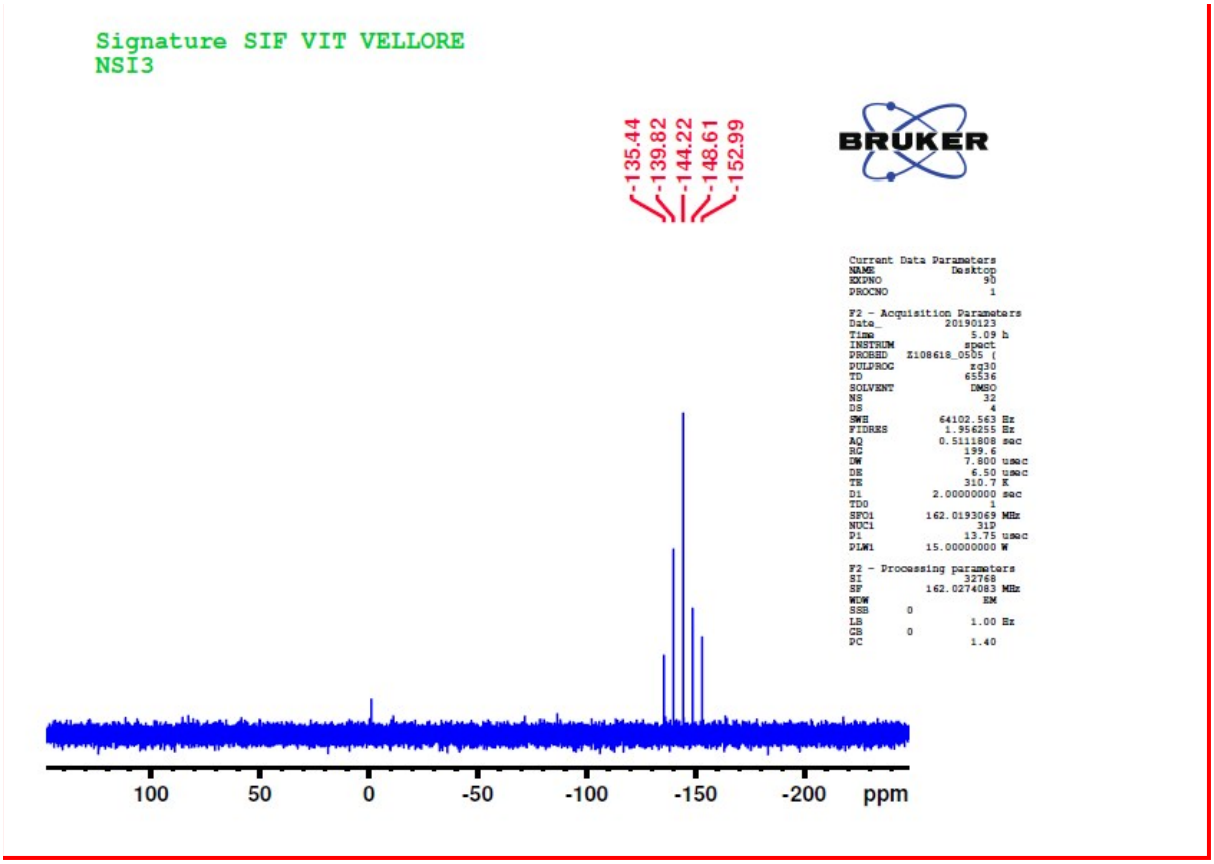

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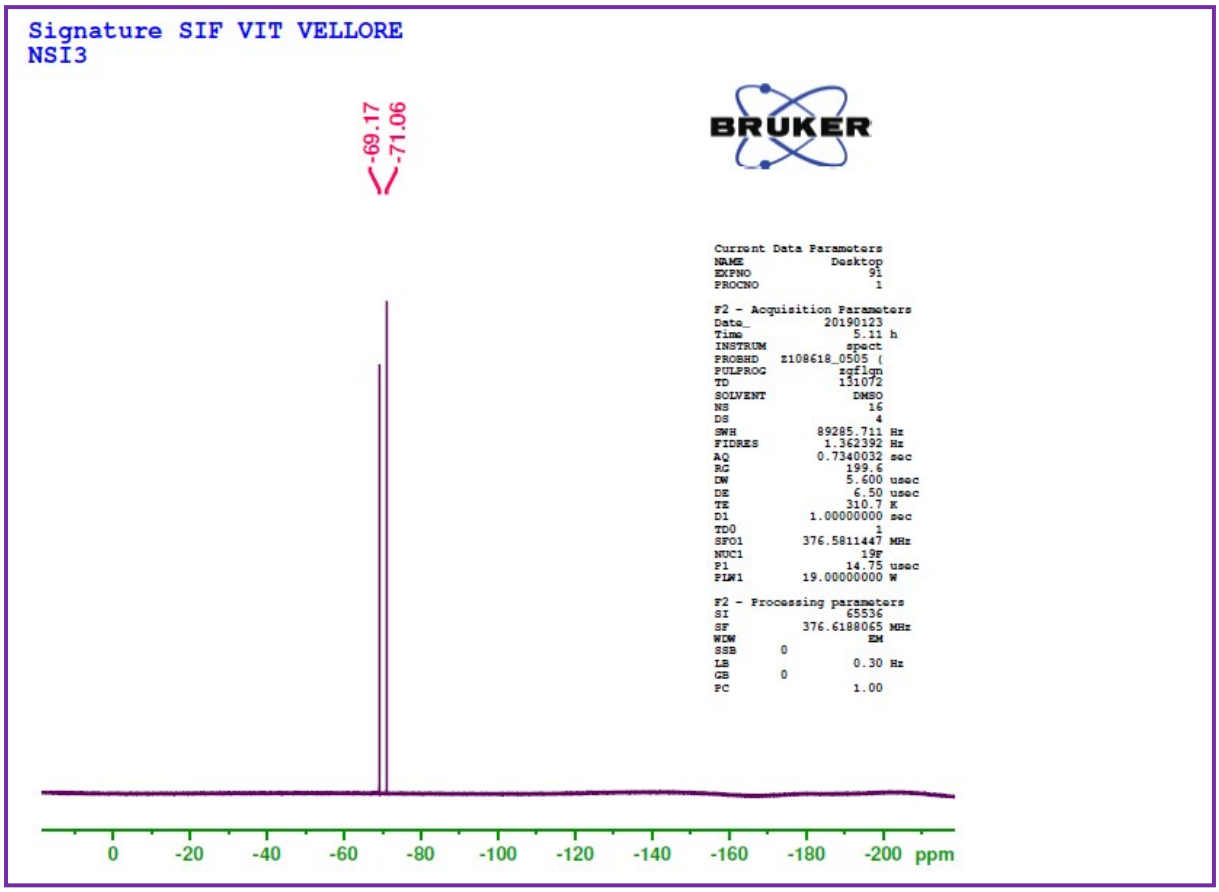

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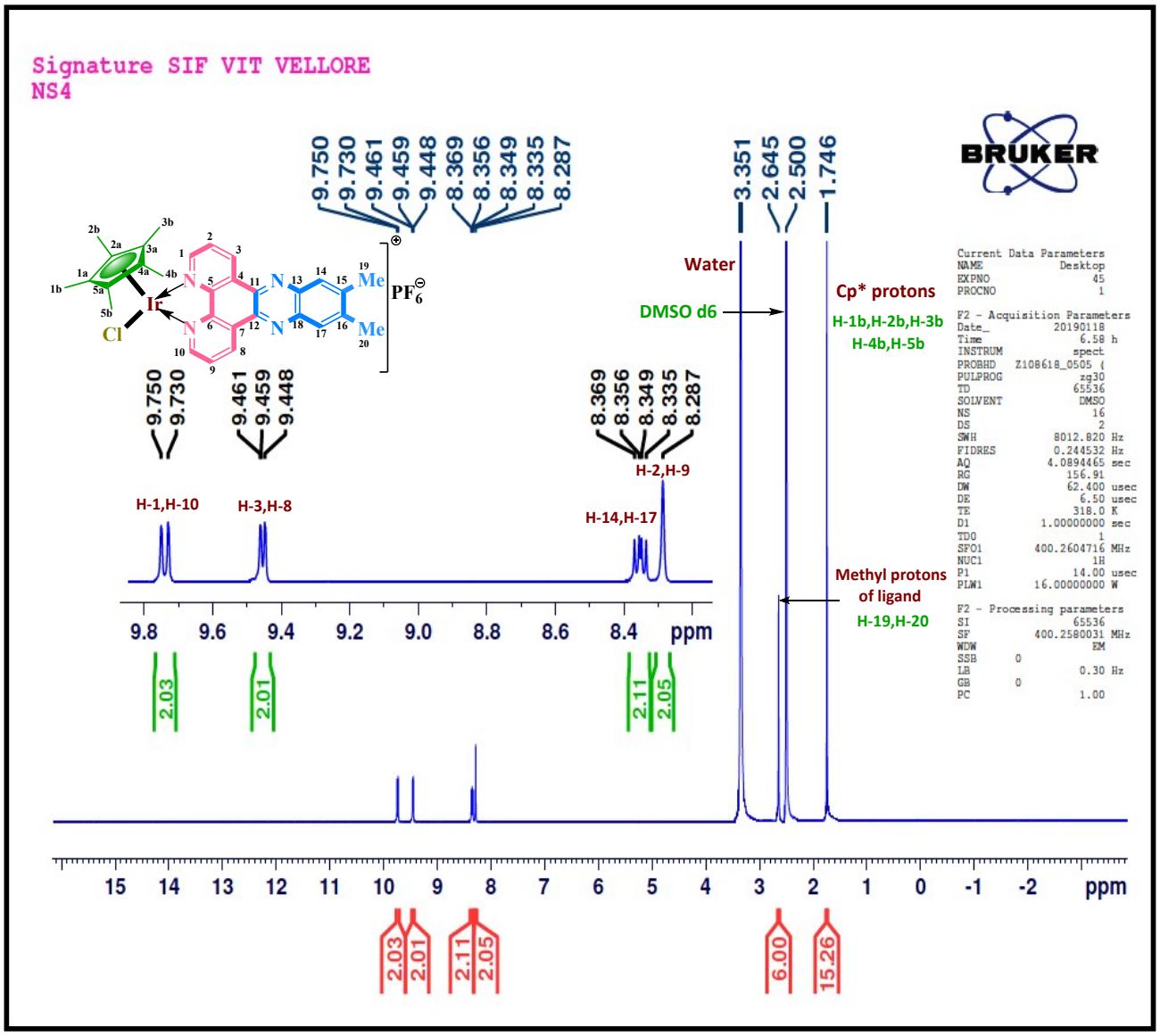

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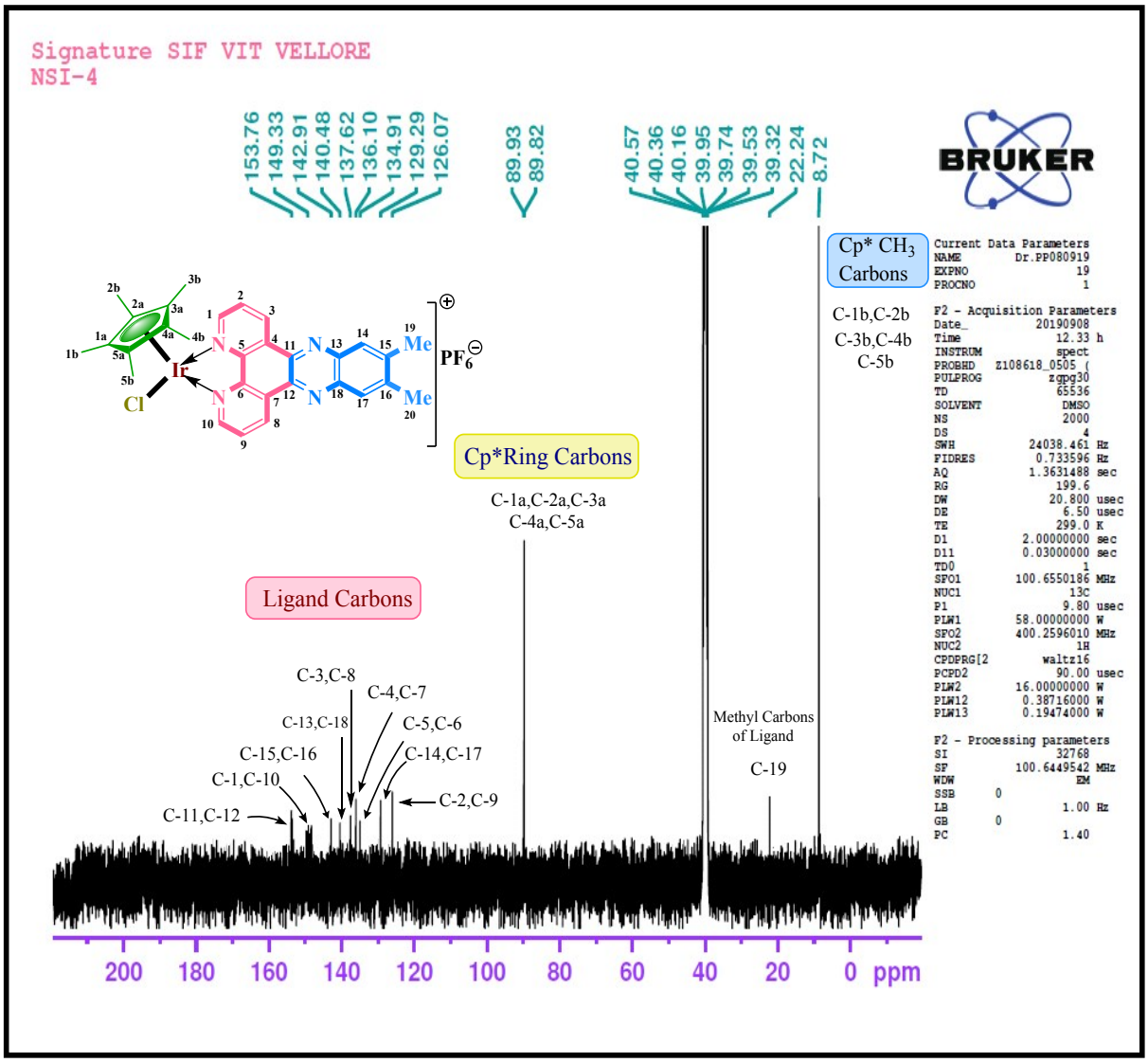

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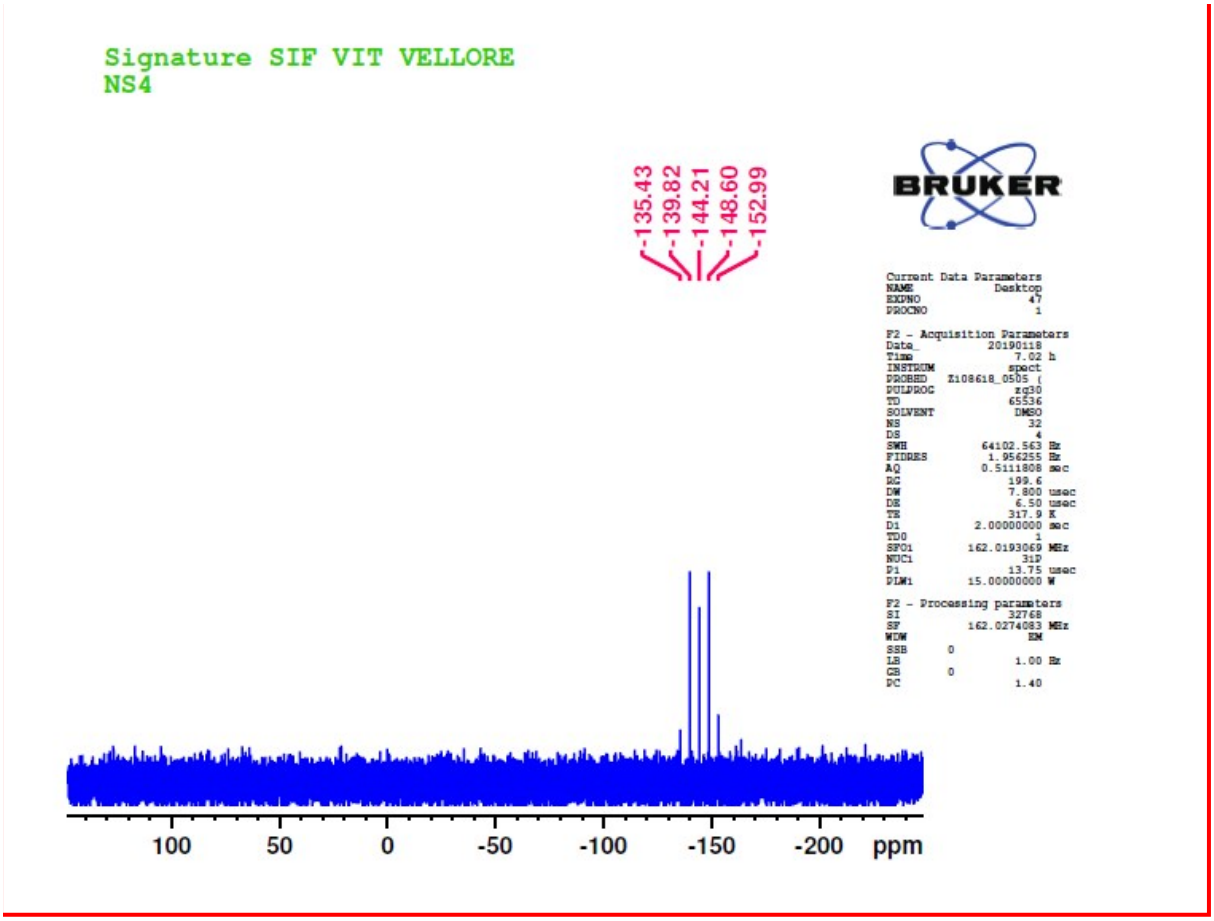

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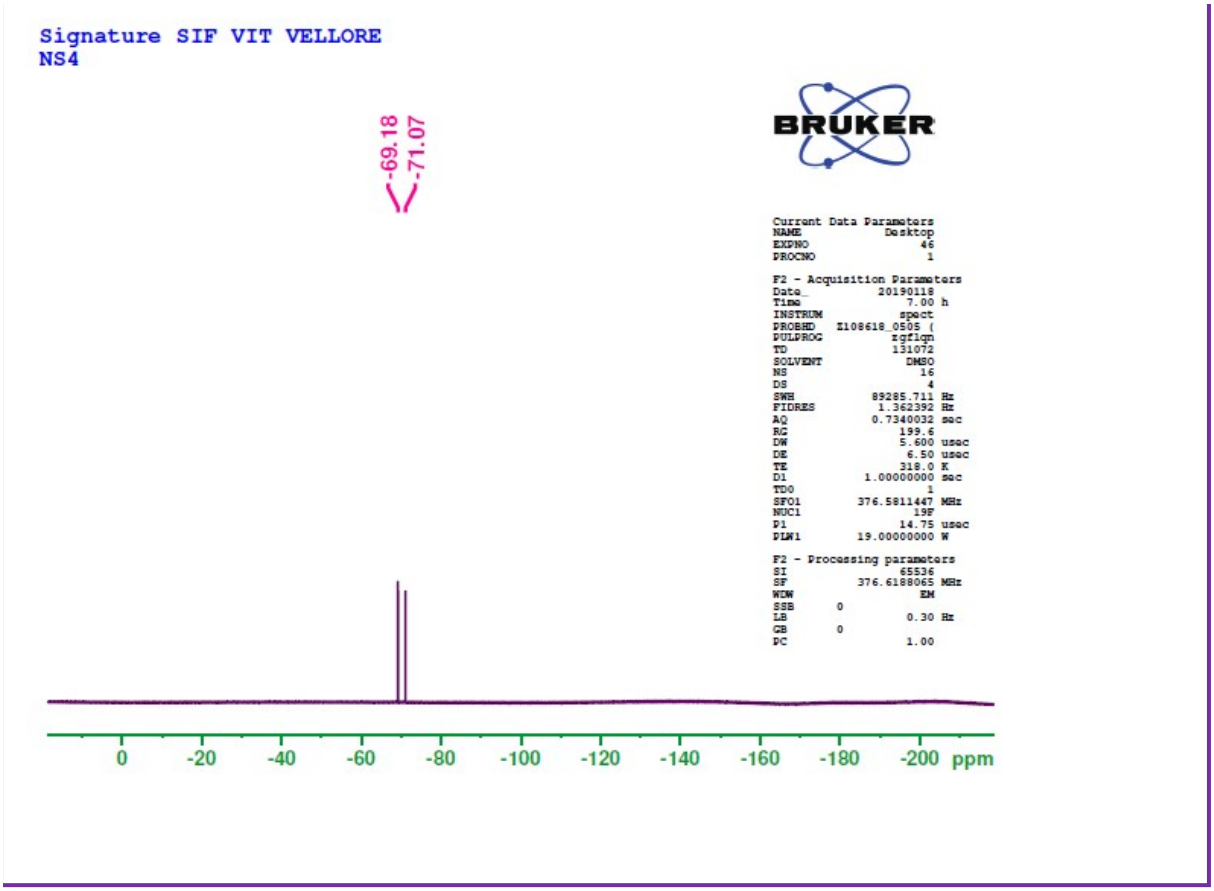

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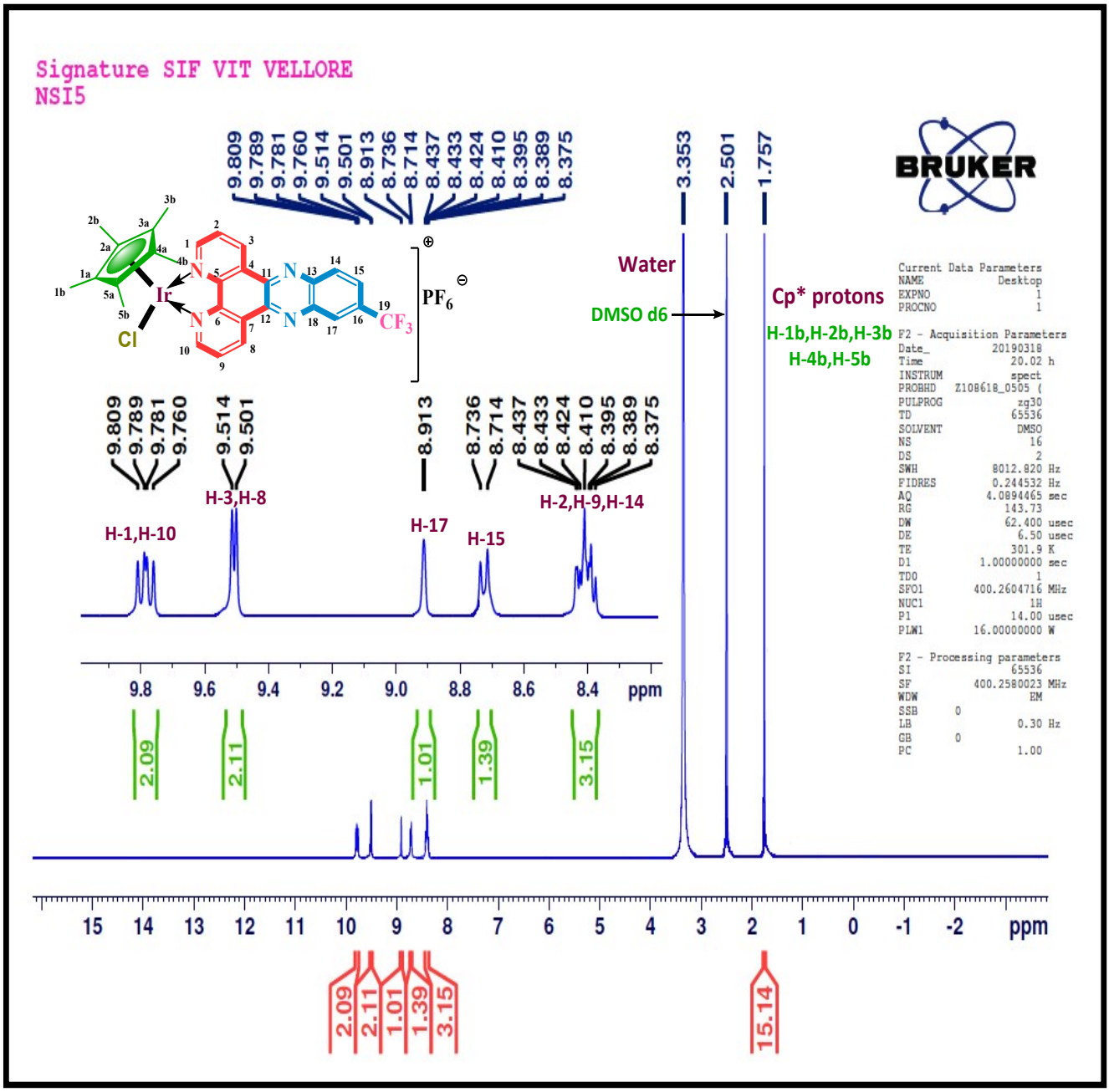

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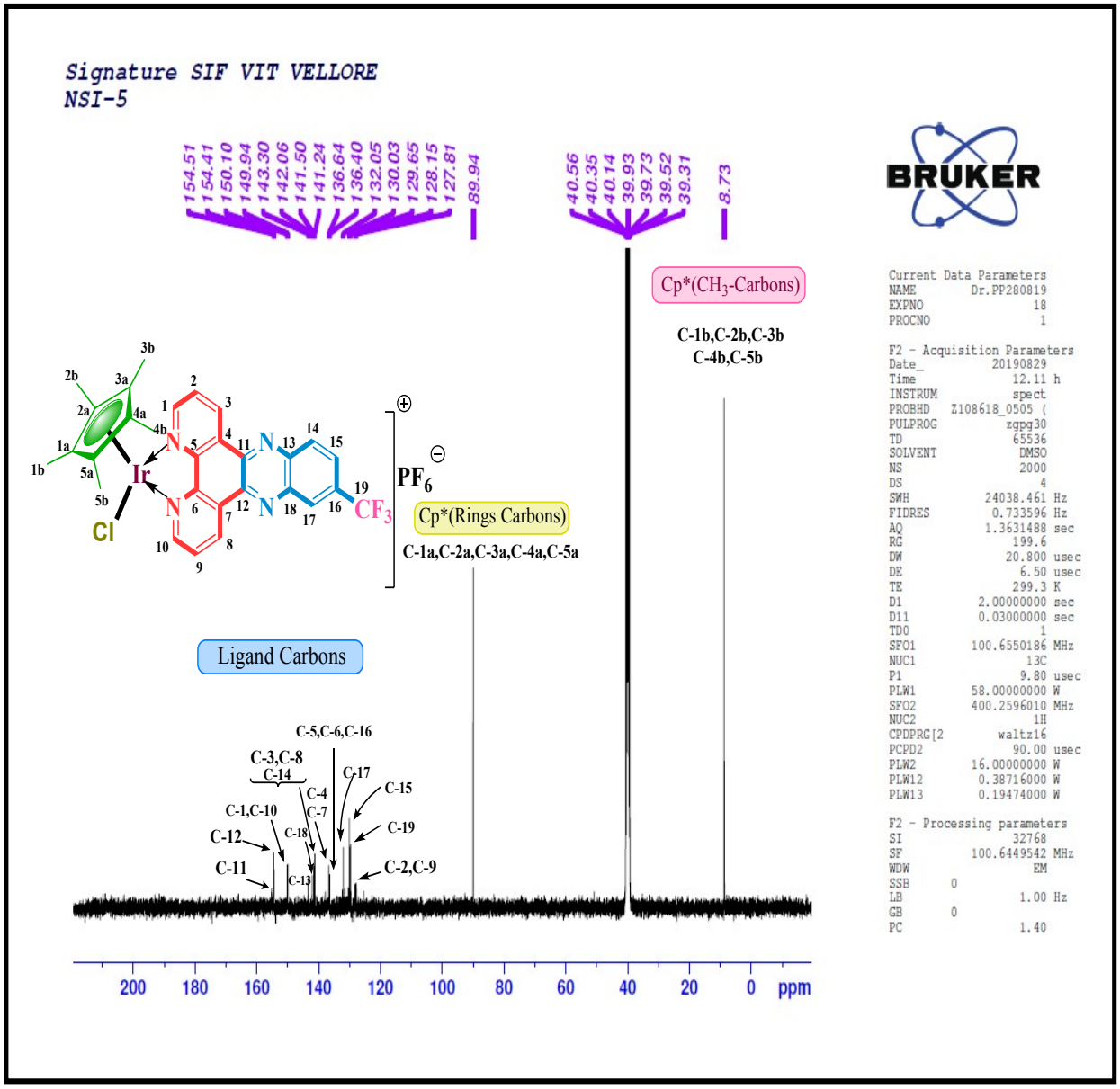

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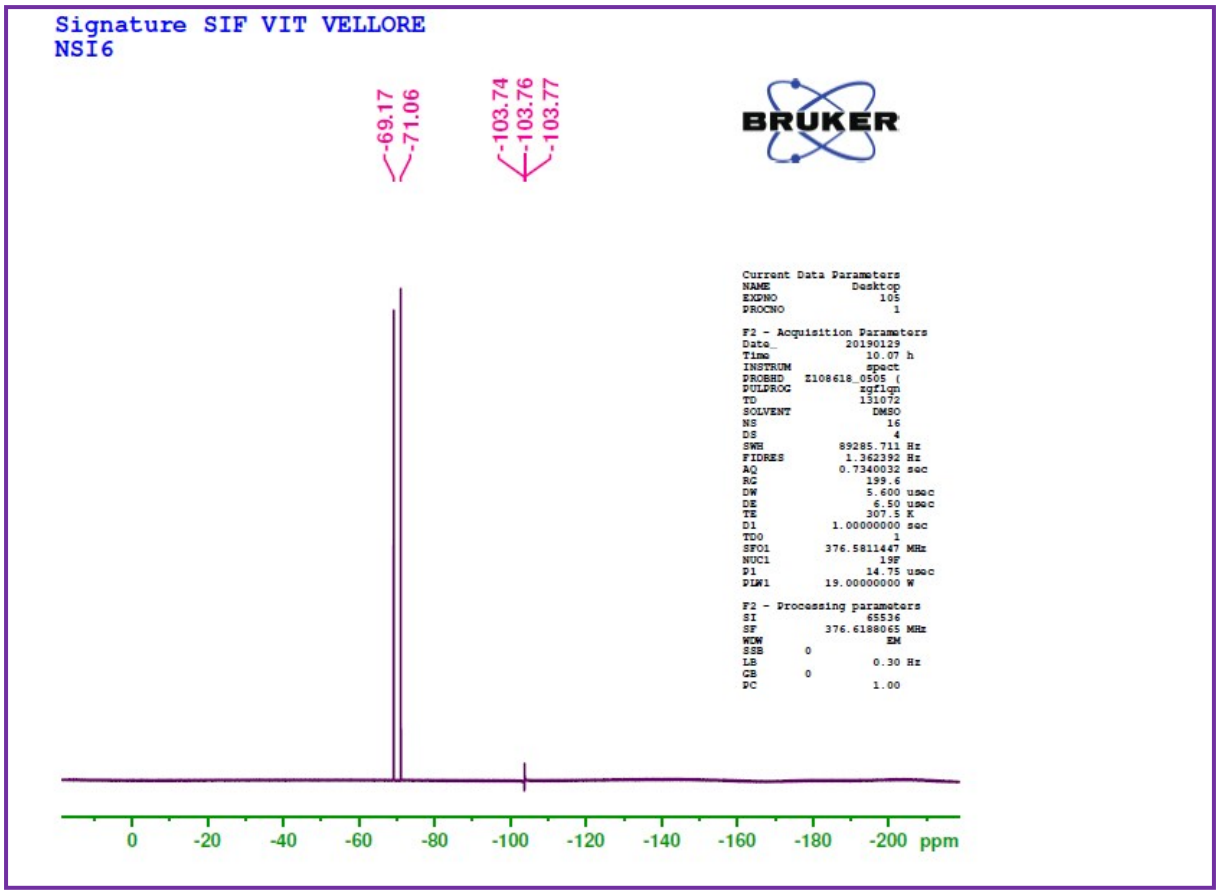

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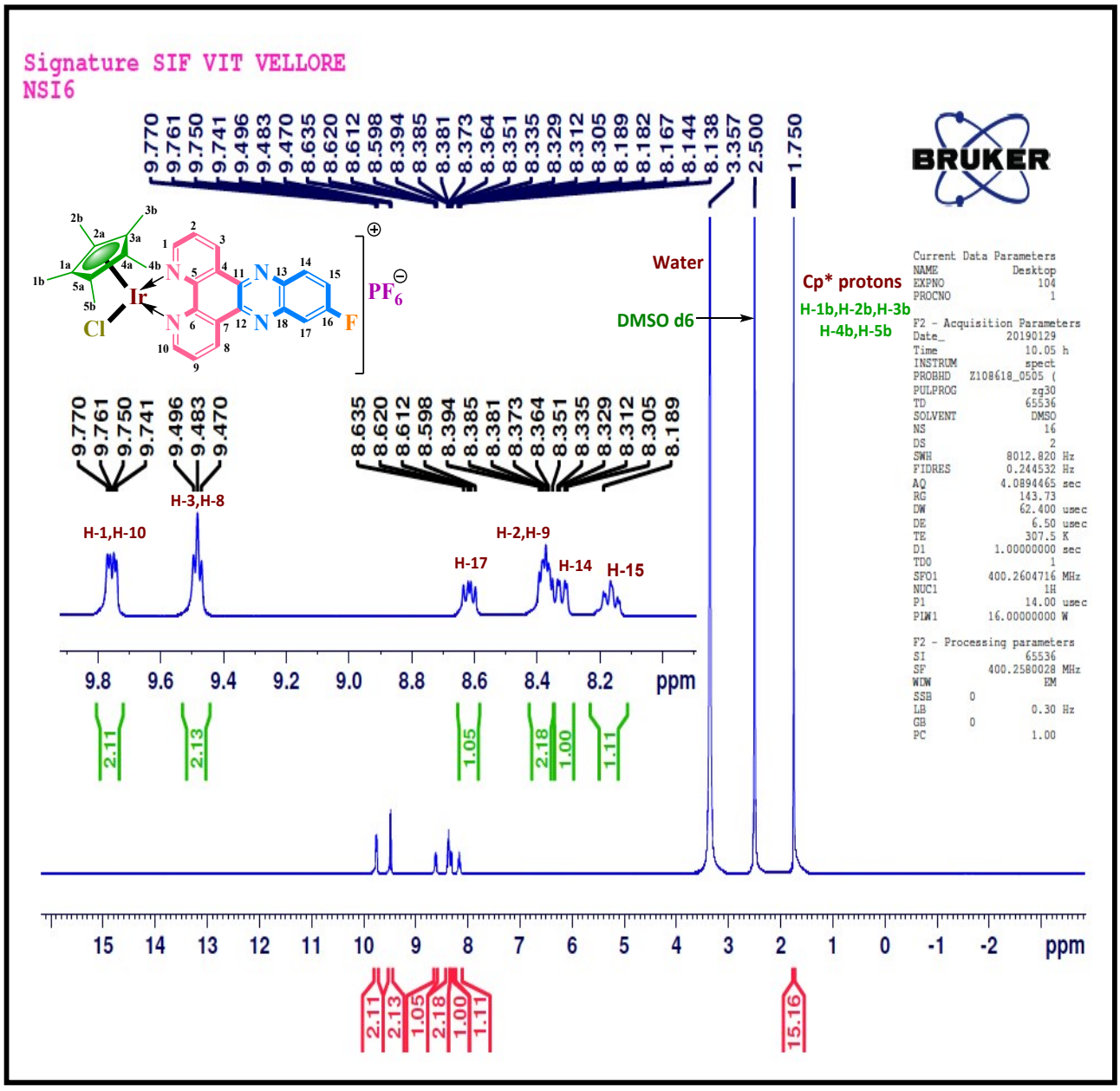
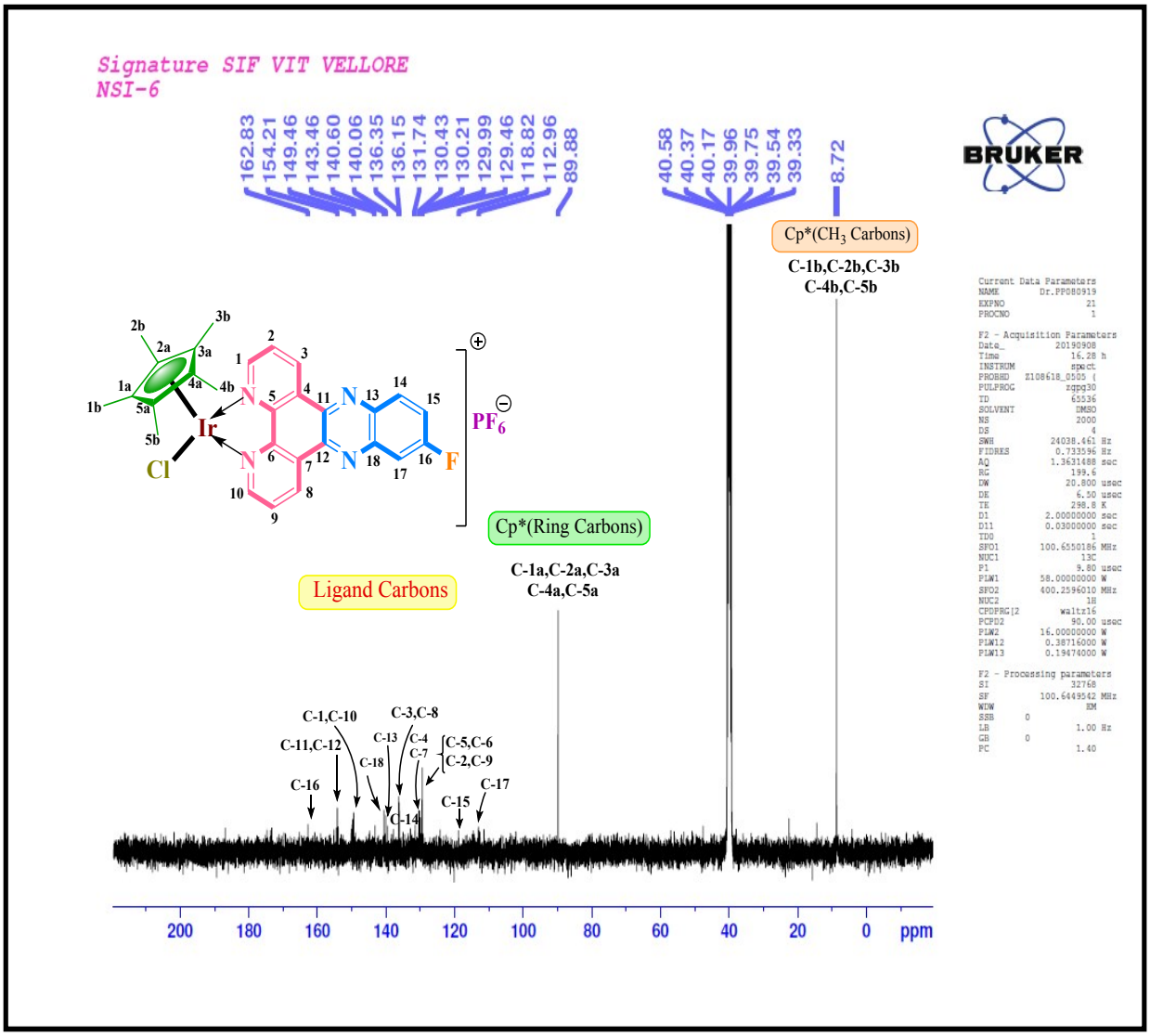
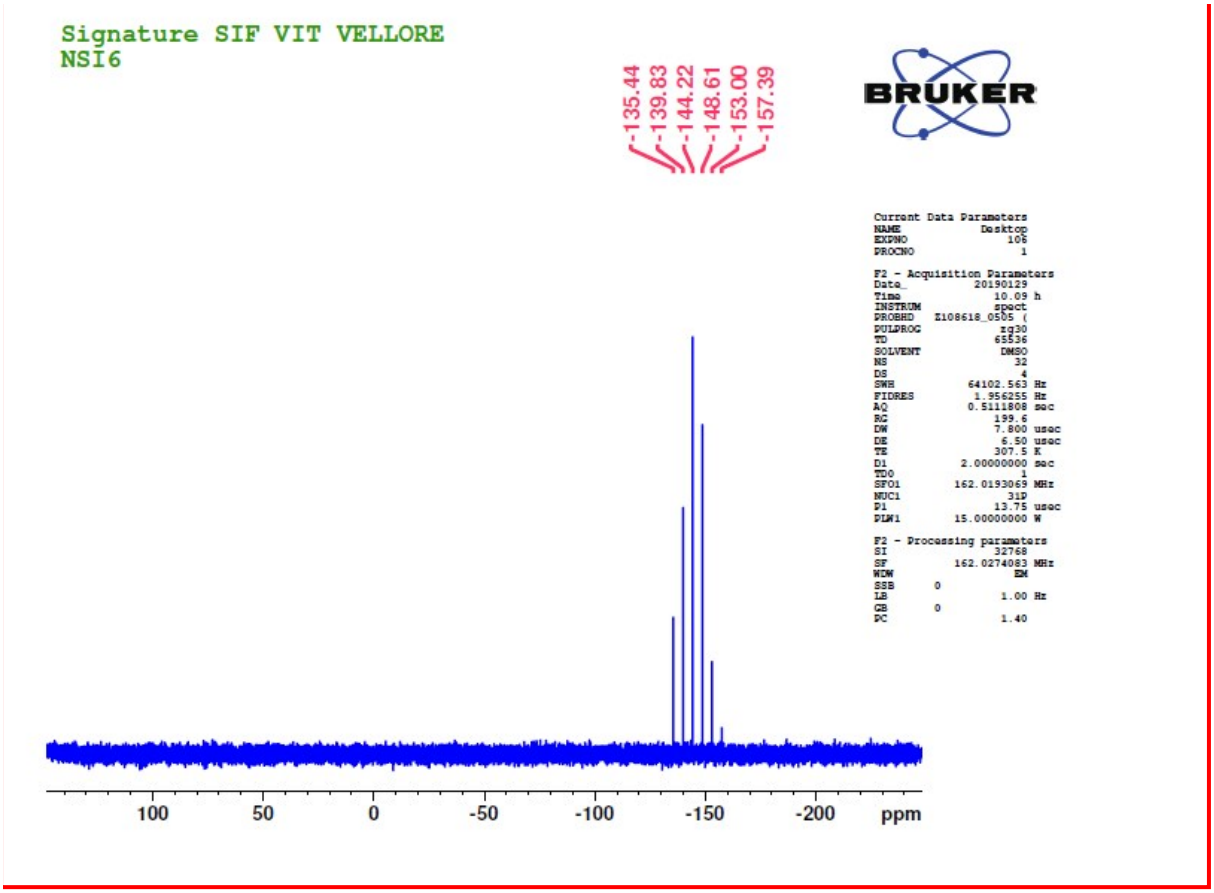


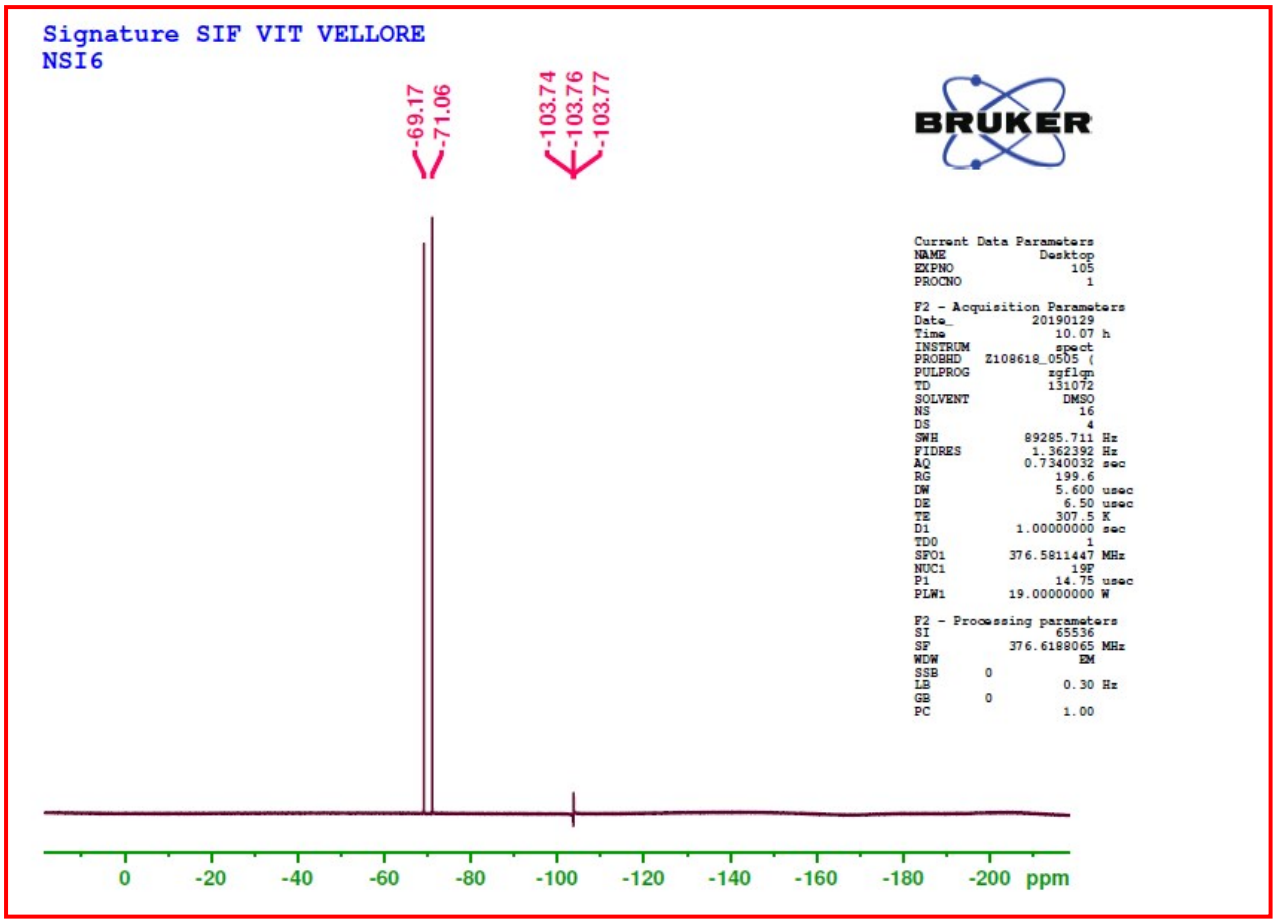
^{13}C NMR spectrum of Complex IrL6



^{31}P NMR Spectrum of Complex IrL6



^{19}F NMR Spectrum of Complex IrL6



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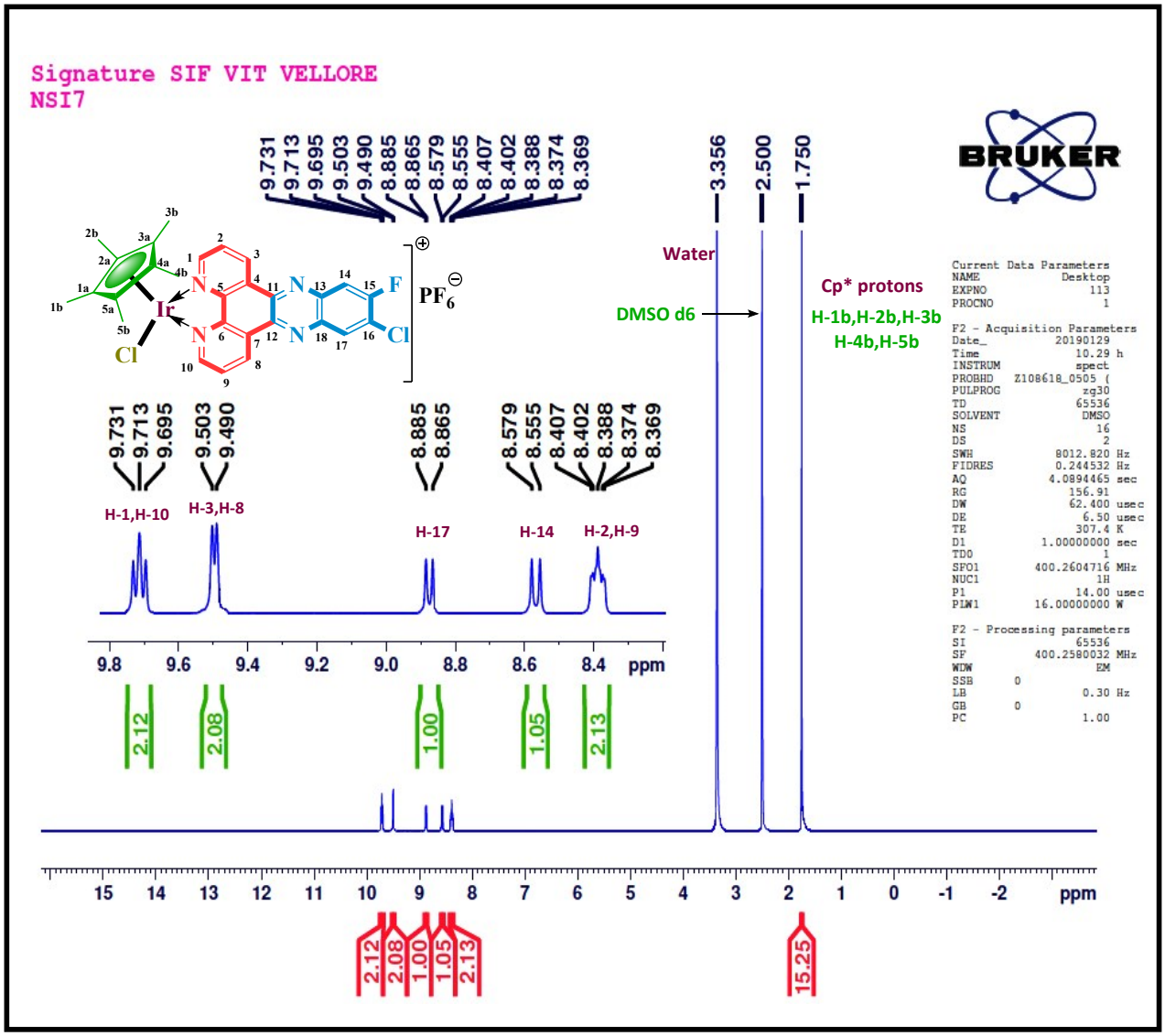
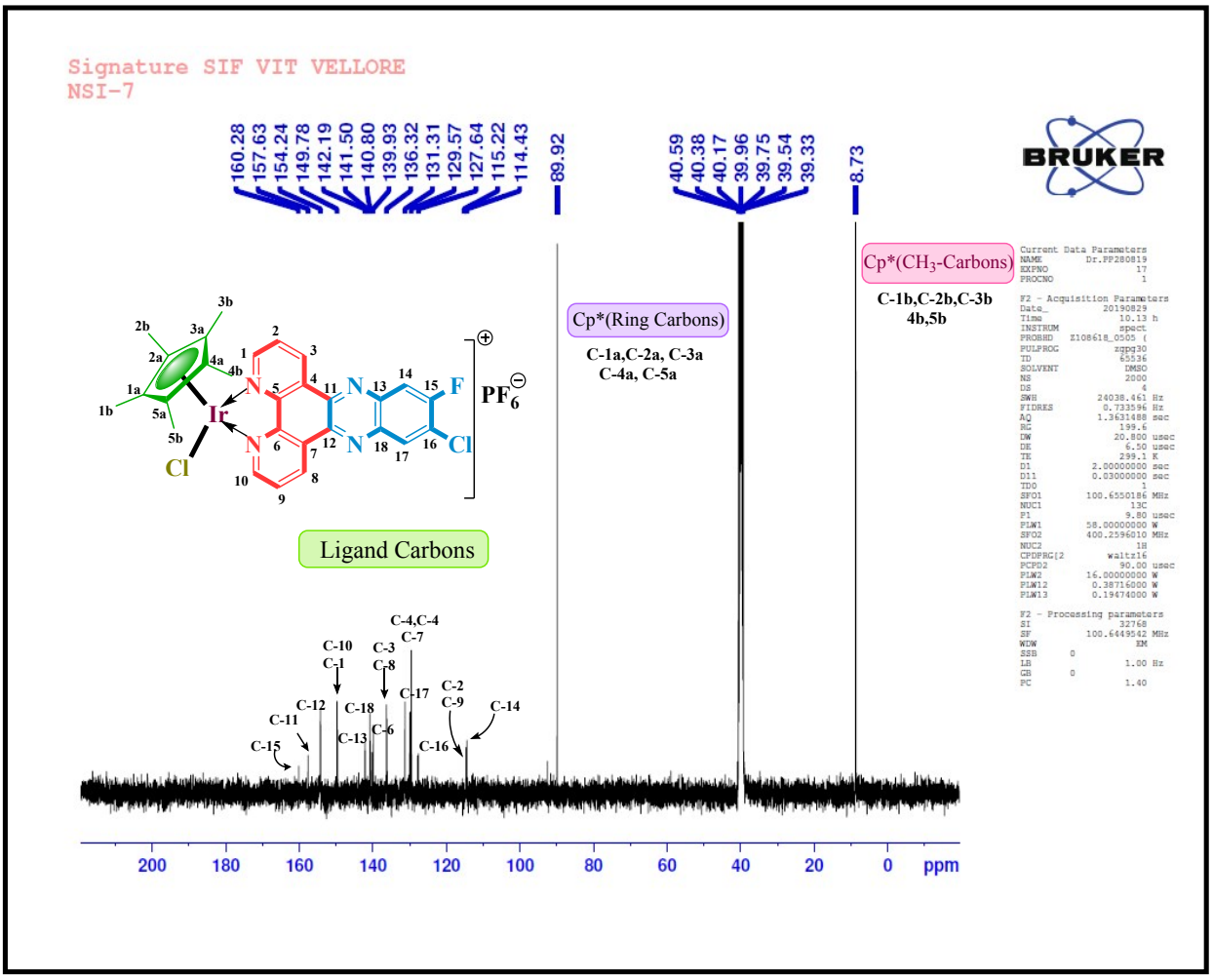


^{13}C NMR spectrum of Complex IrL7



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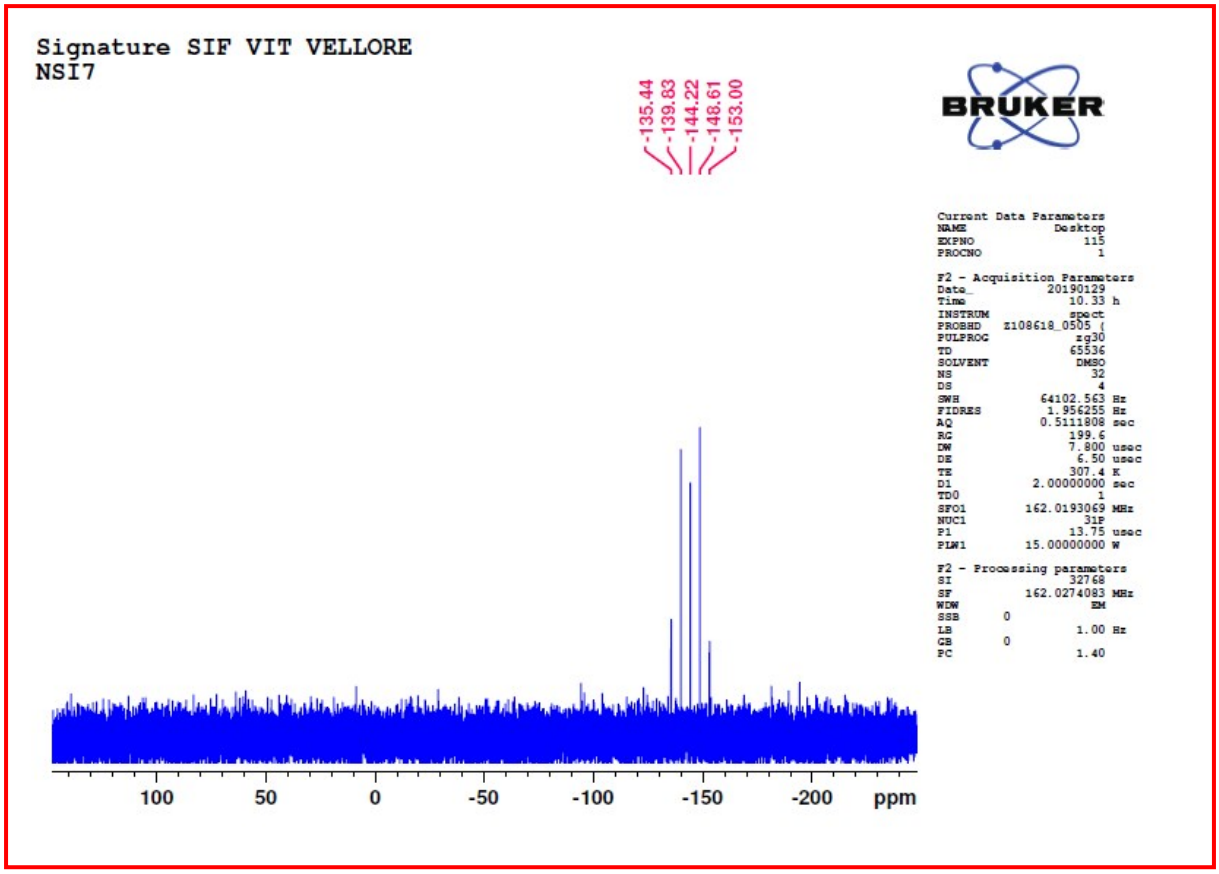
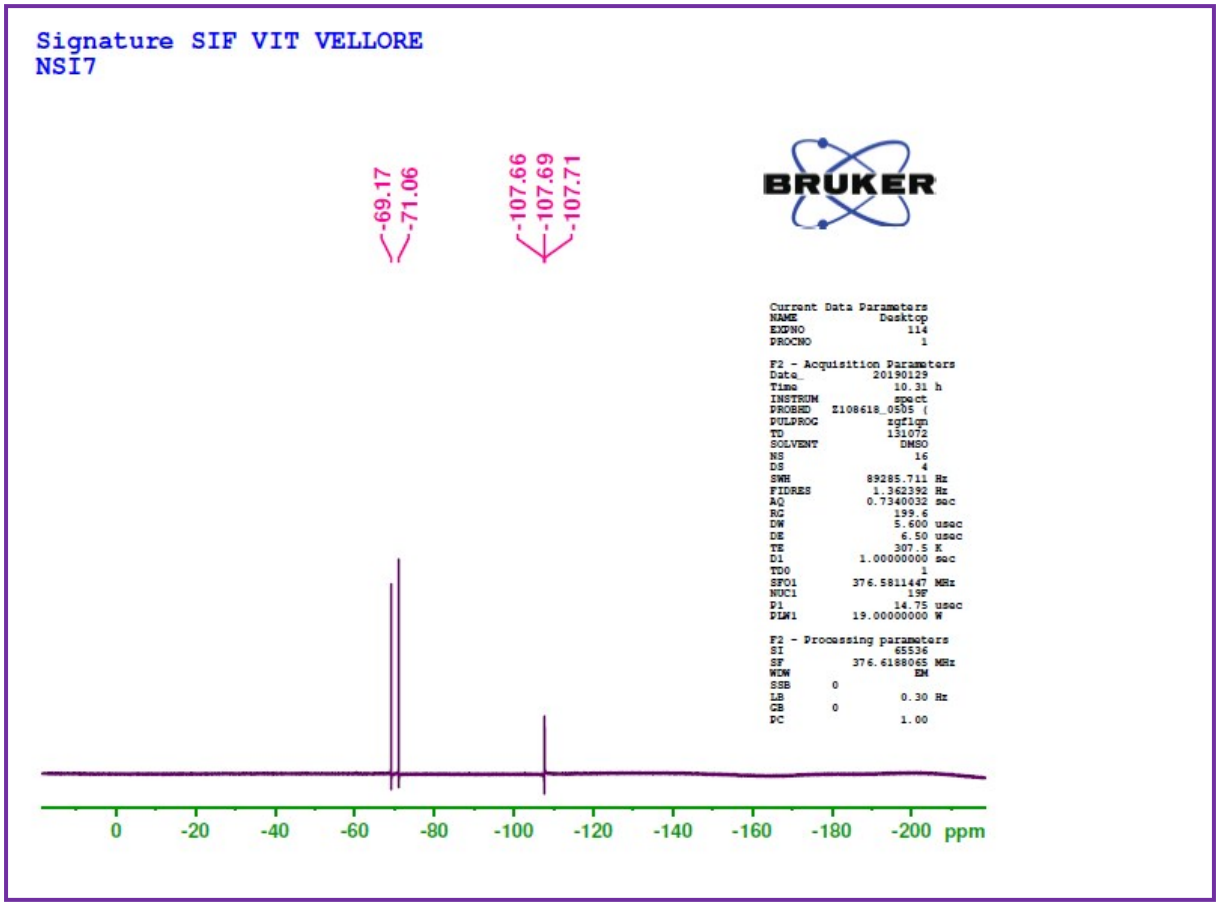
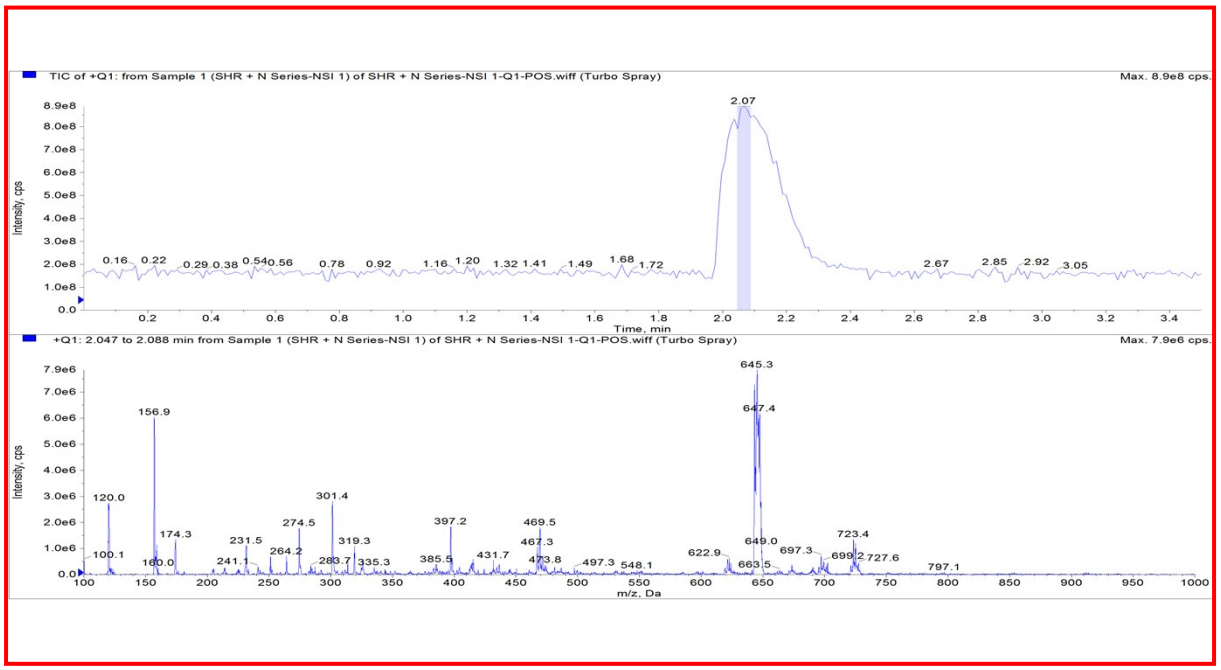


^{19}F NMR Spectrum of Complex IrL7



ESI-MS of IrL1



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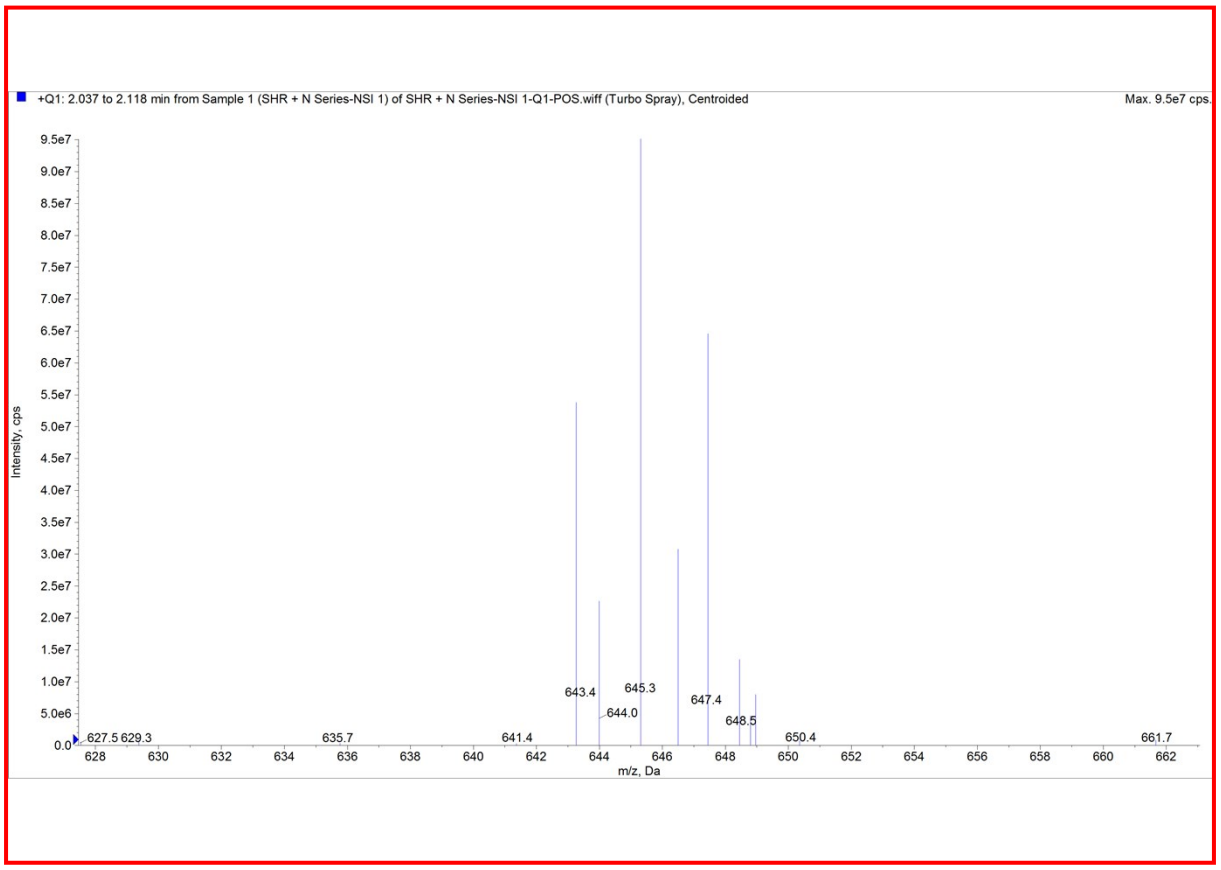
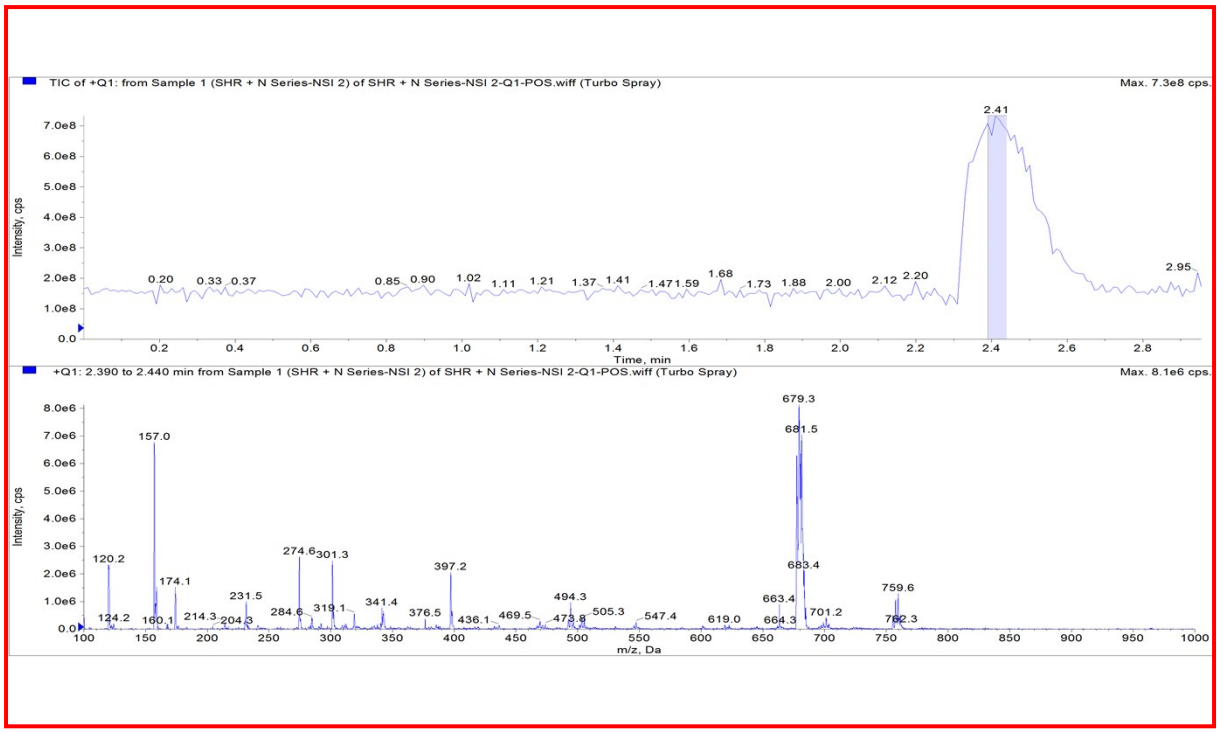
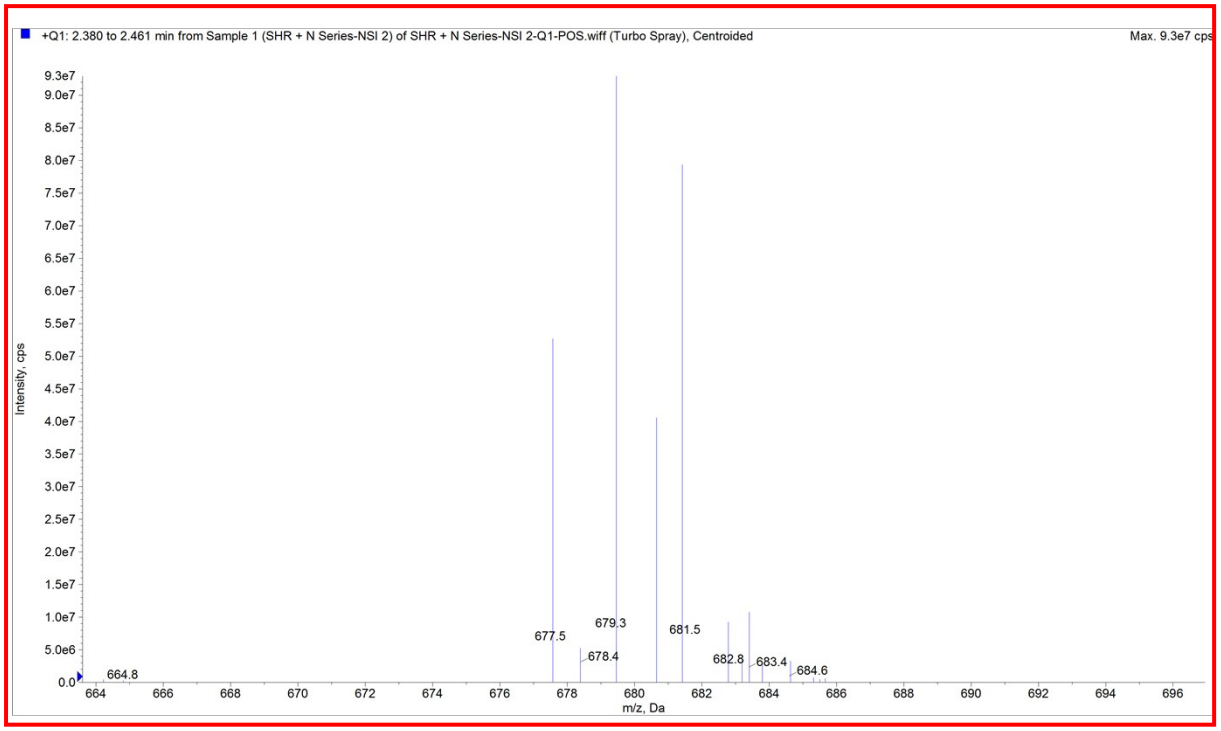


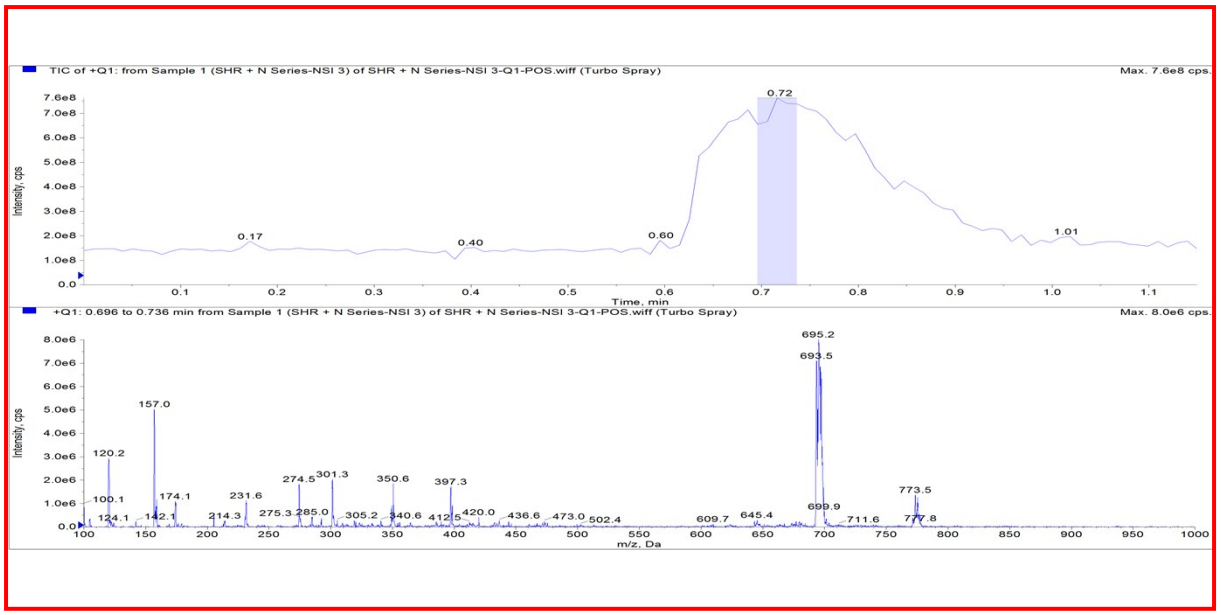
ESI-MS of IrL2



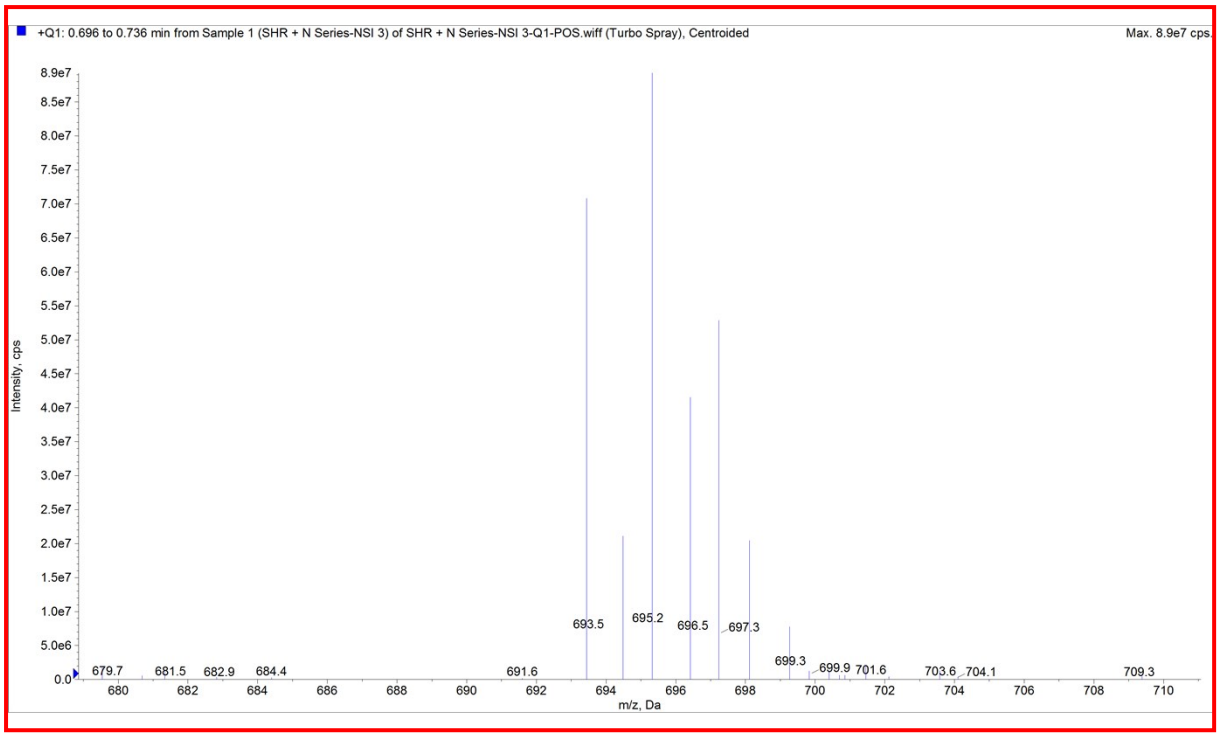
Zoom in Scan



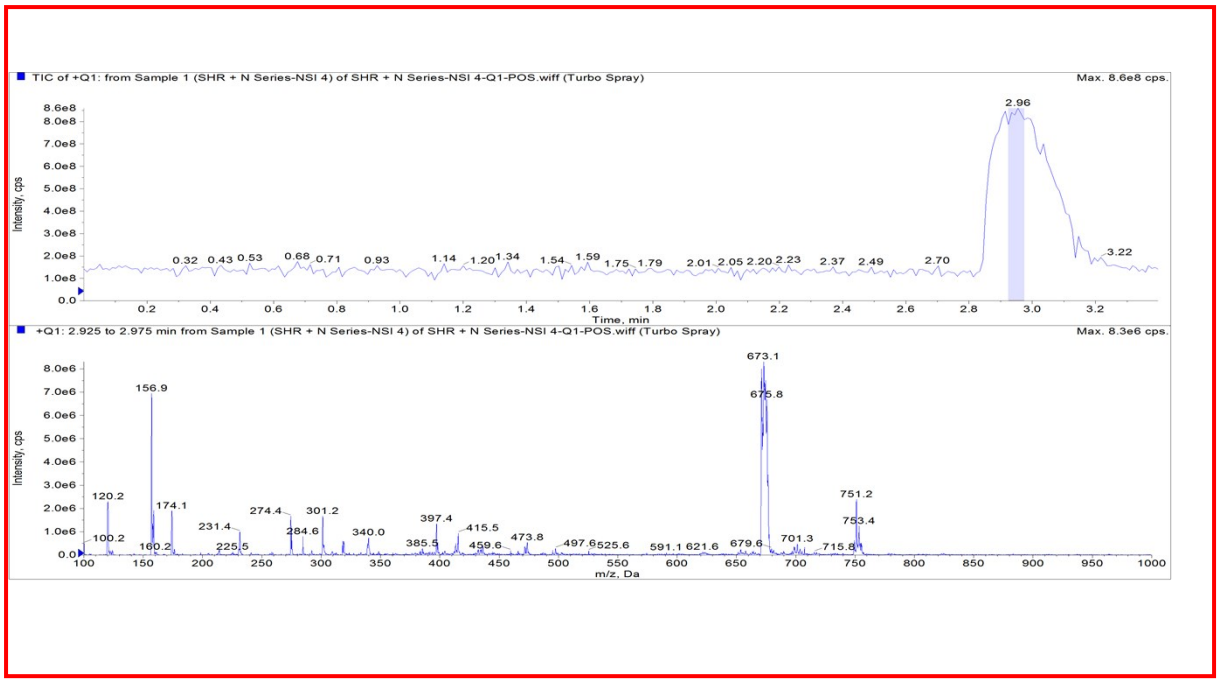
ESI-MS of IrL3



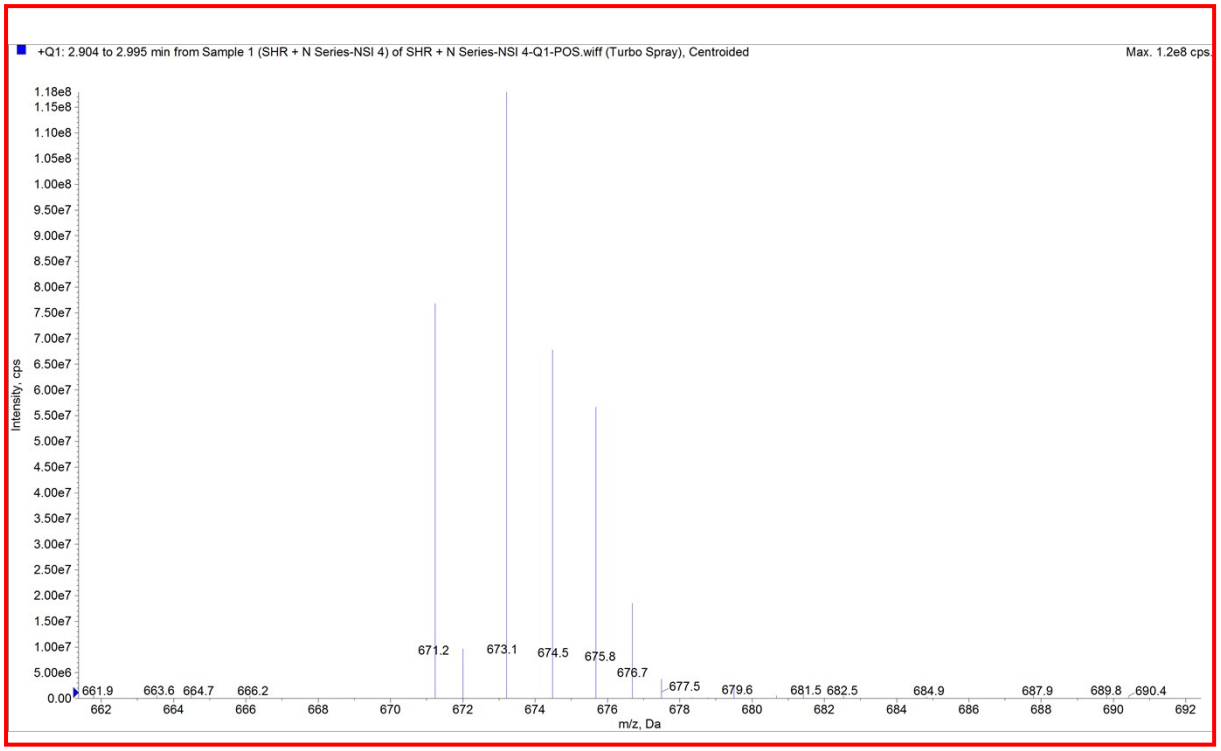
Zoom in Scan



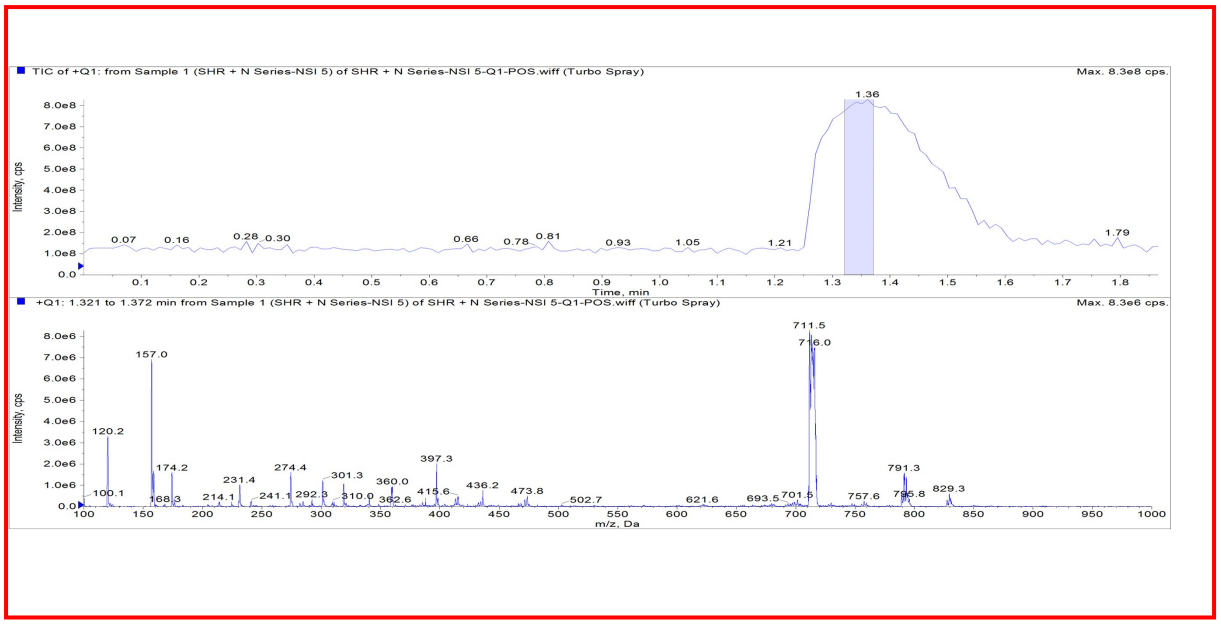
ESI-MS of IrL4



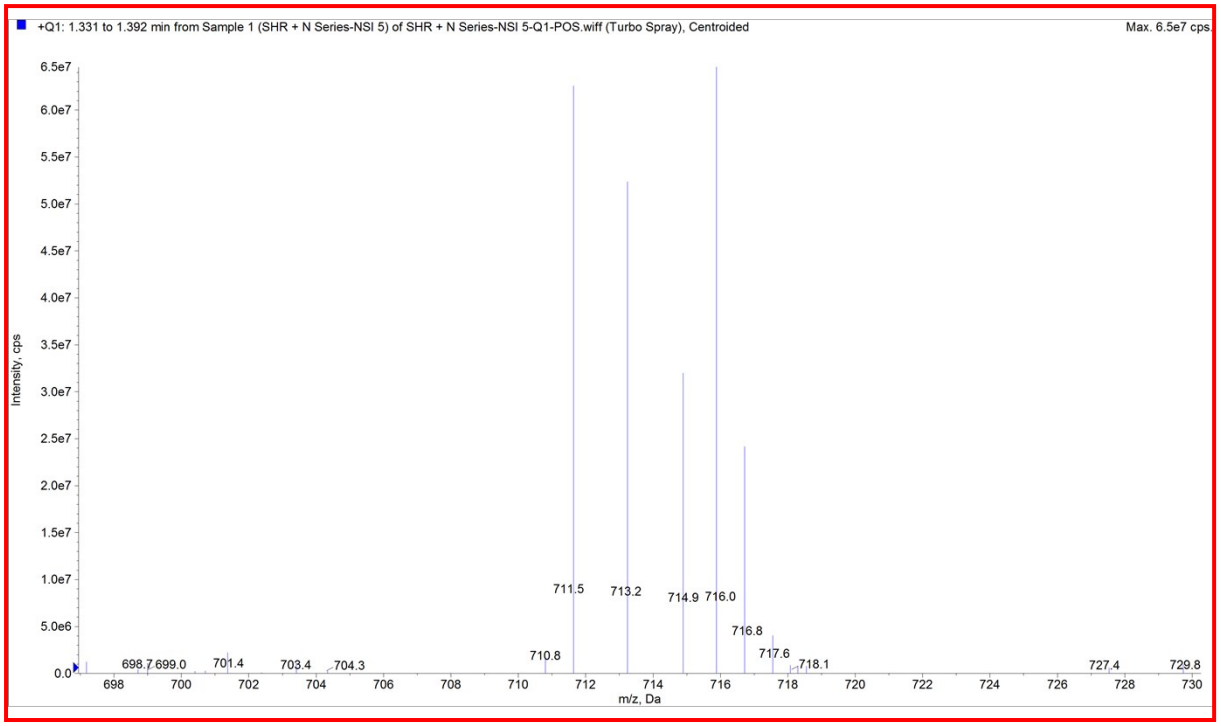
Zoom in Scan



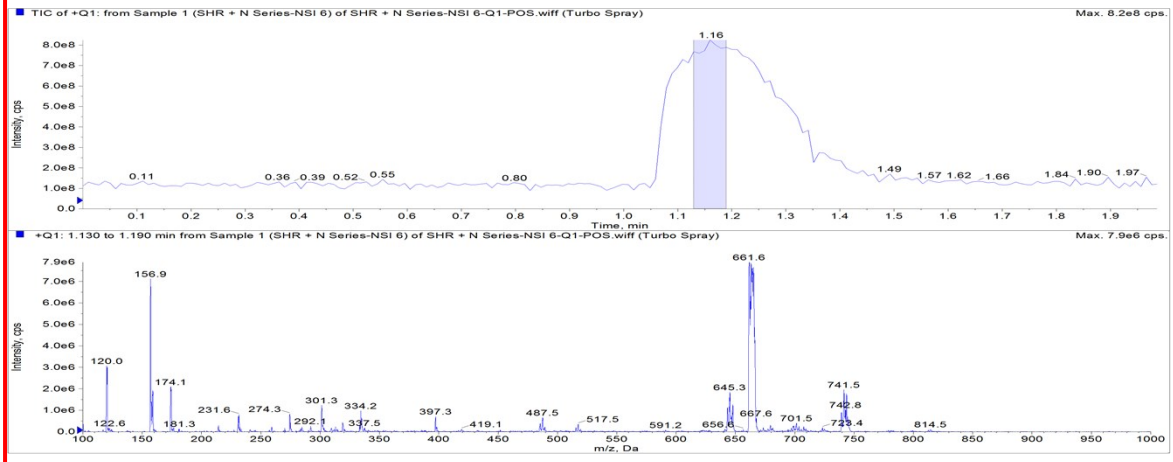
ESI-MS of IrL5



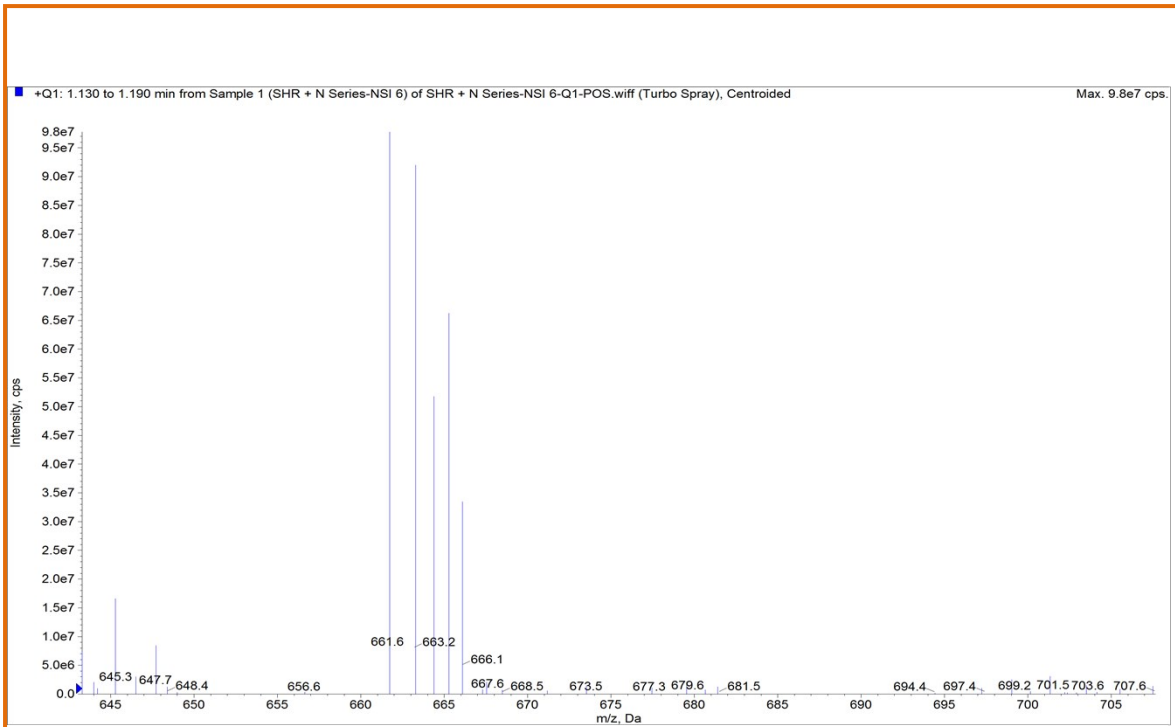
Zoom in Scan



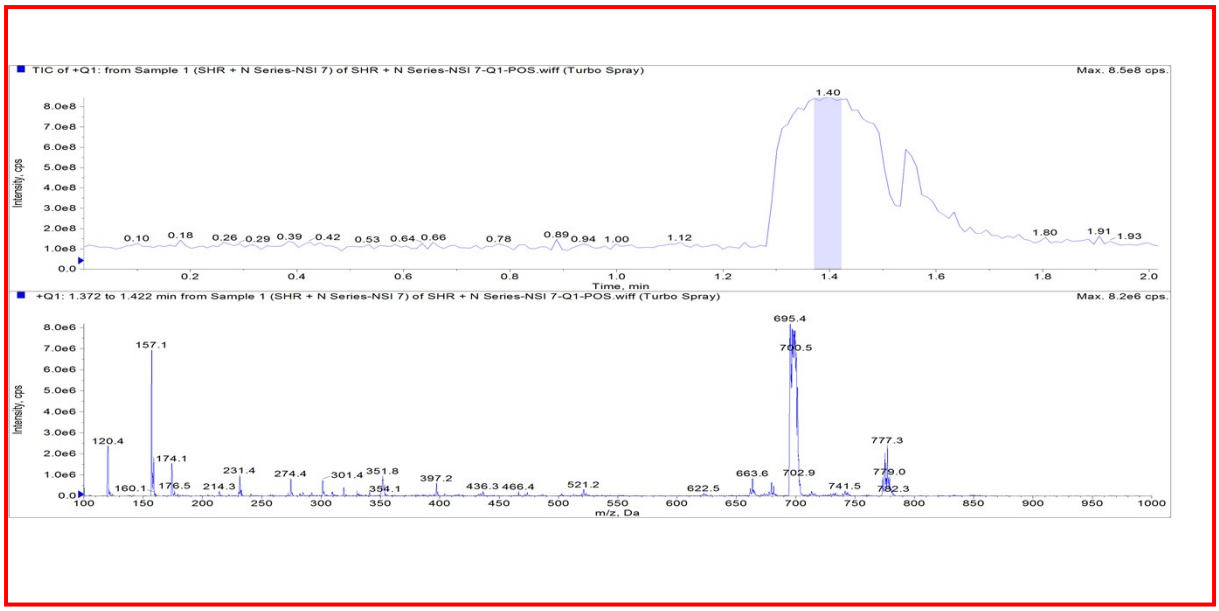
ESI-MS of IrL6



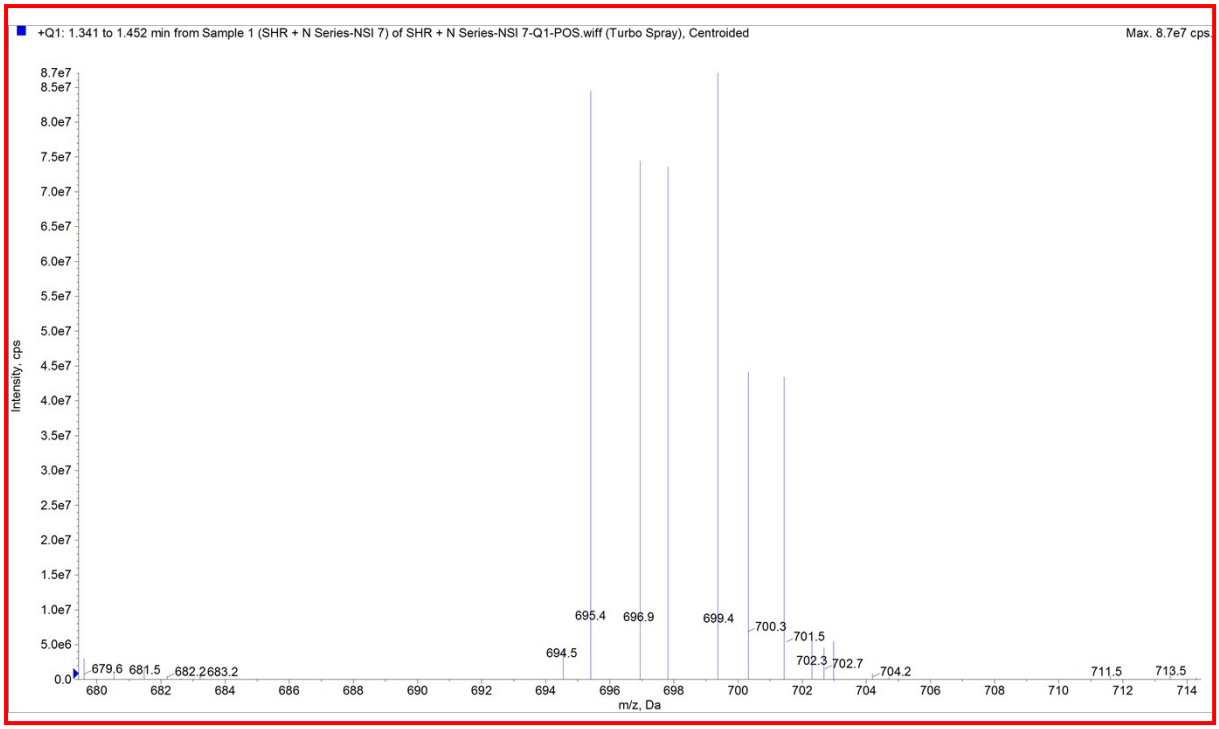
Zoom in Scan



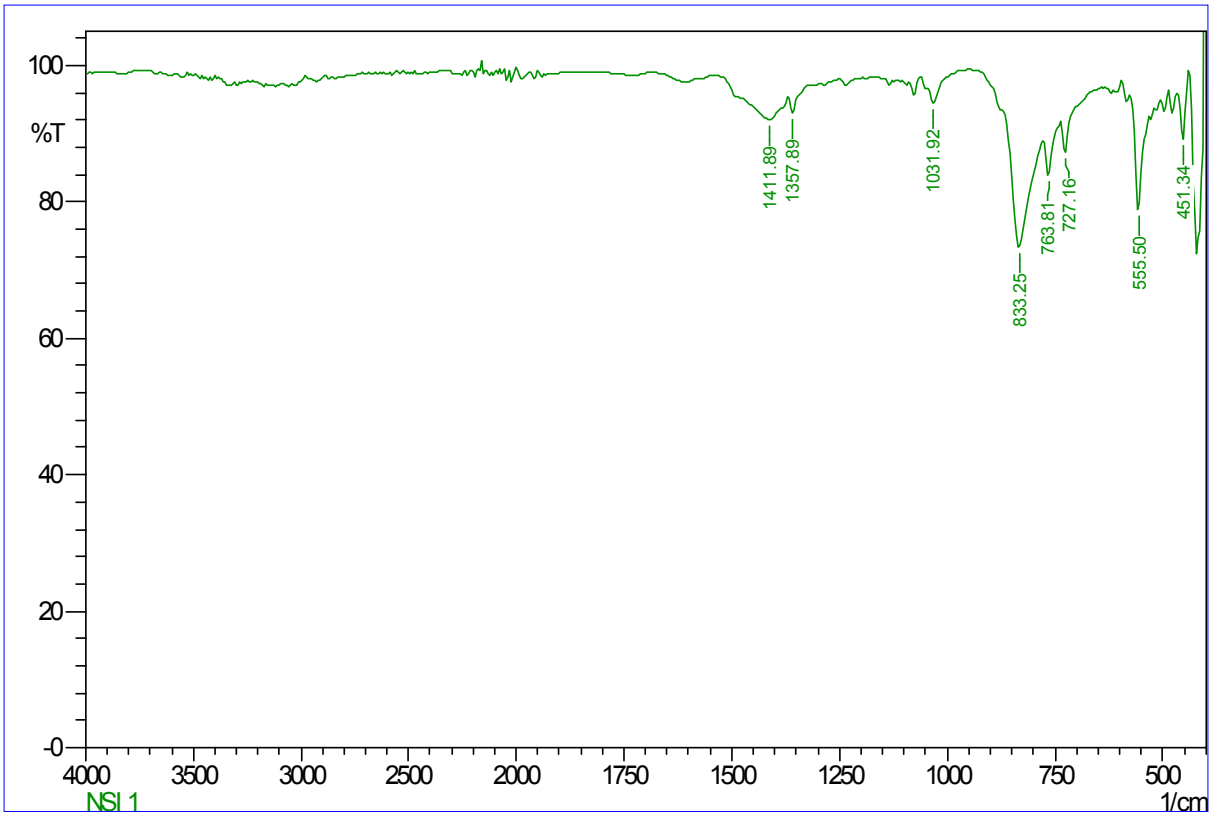
ESI-MS of IrL7



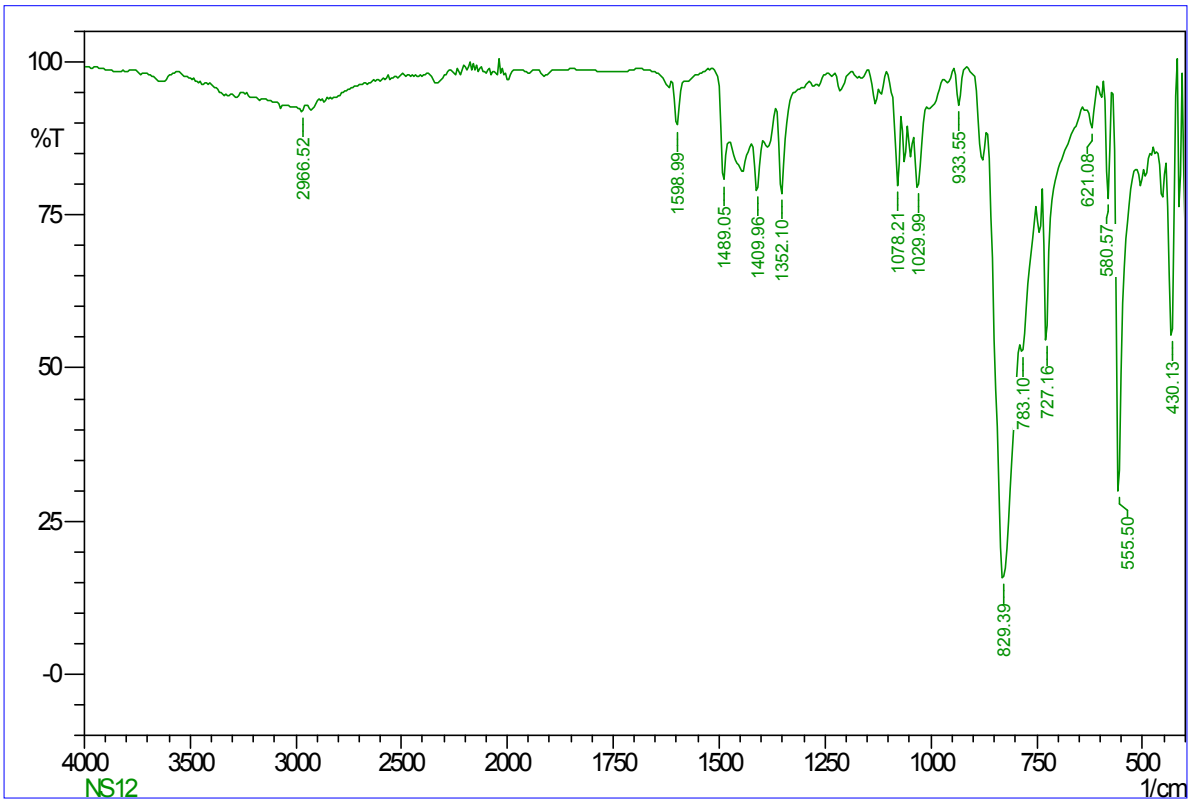
Zoom in Scan



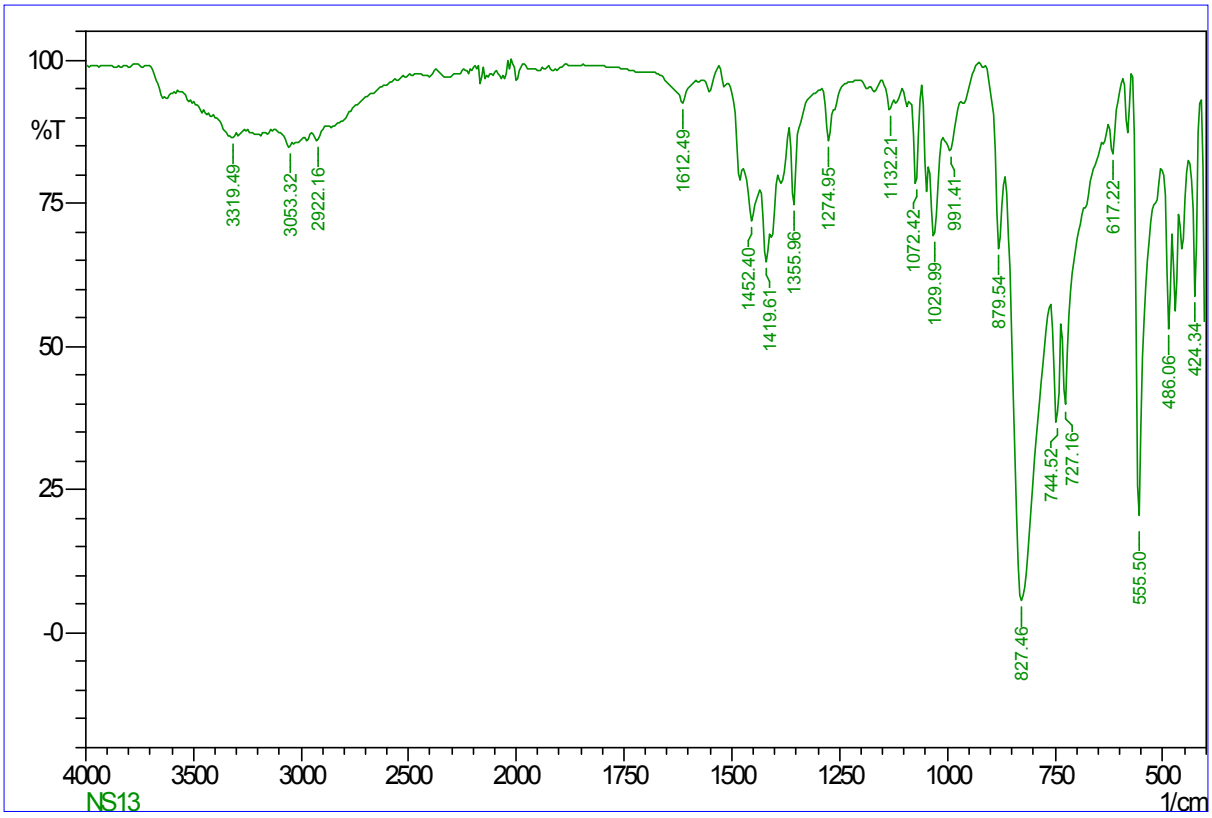
IR spectrum of IrL1



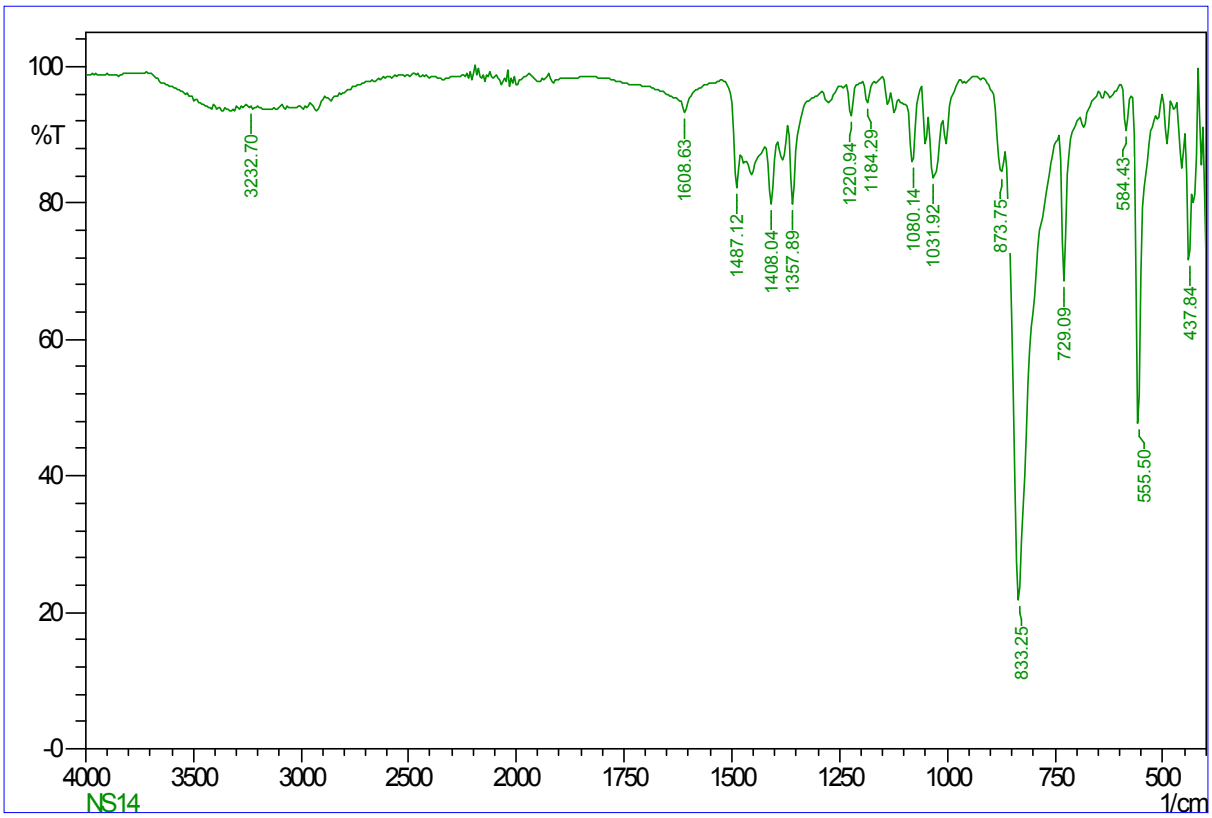
IR spectrum of IrL2



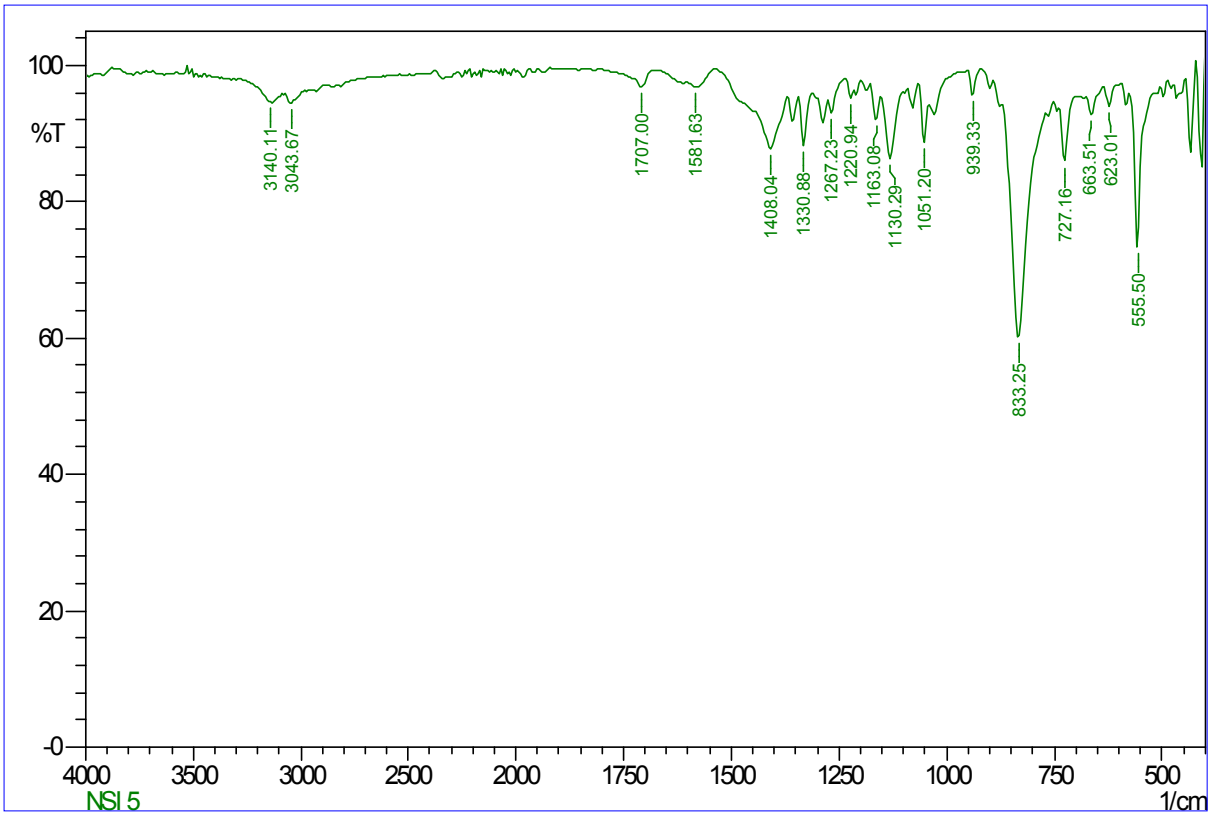
IR spectrum of IrL3



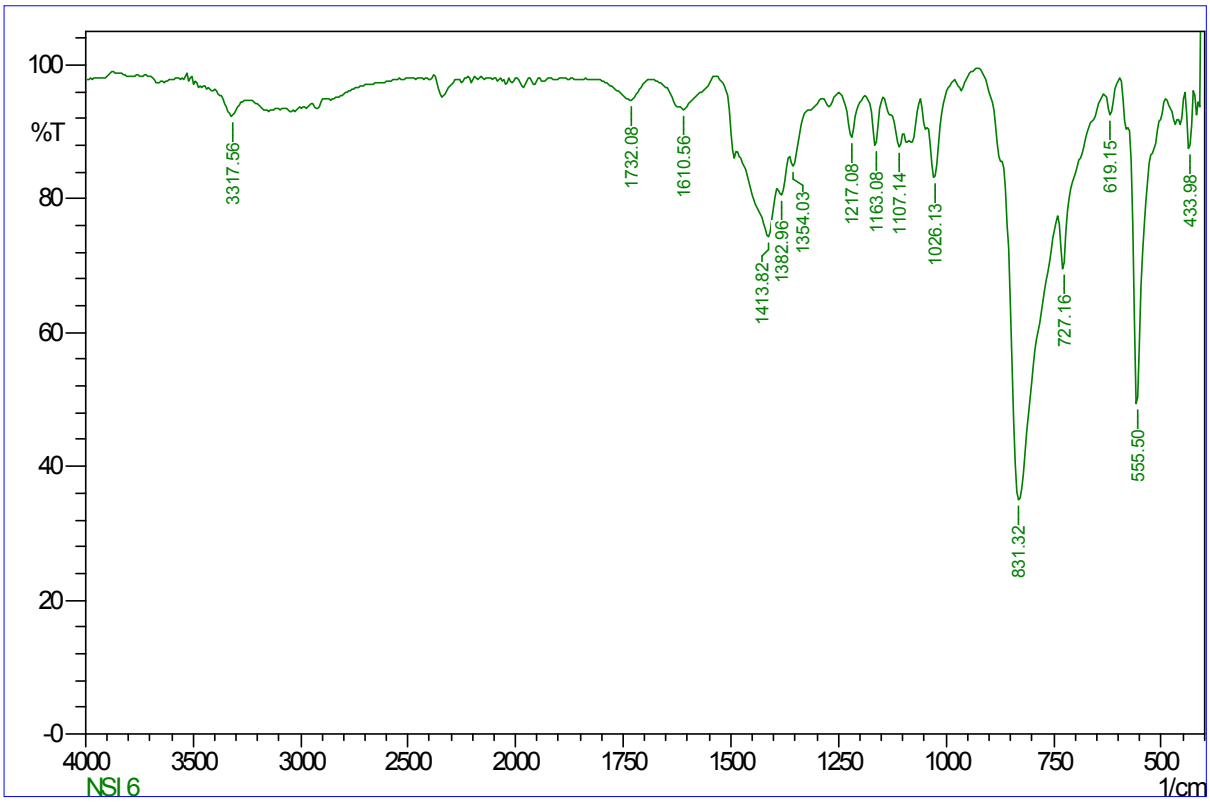
IR spectrum of IrL4



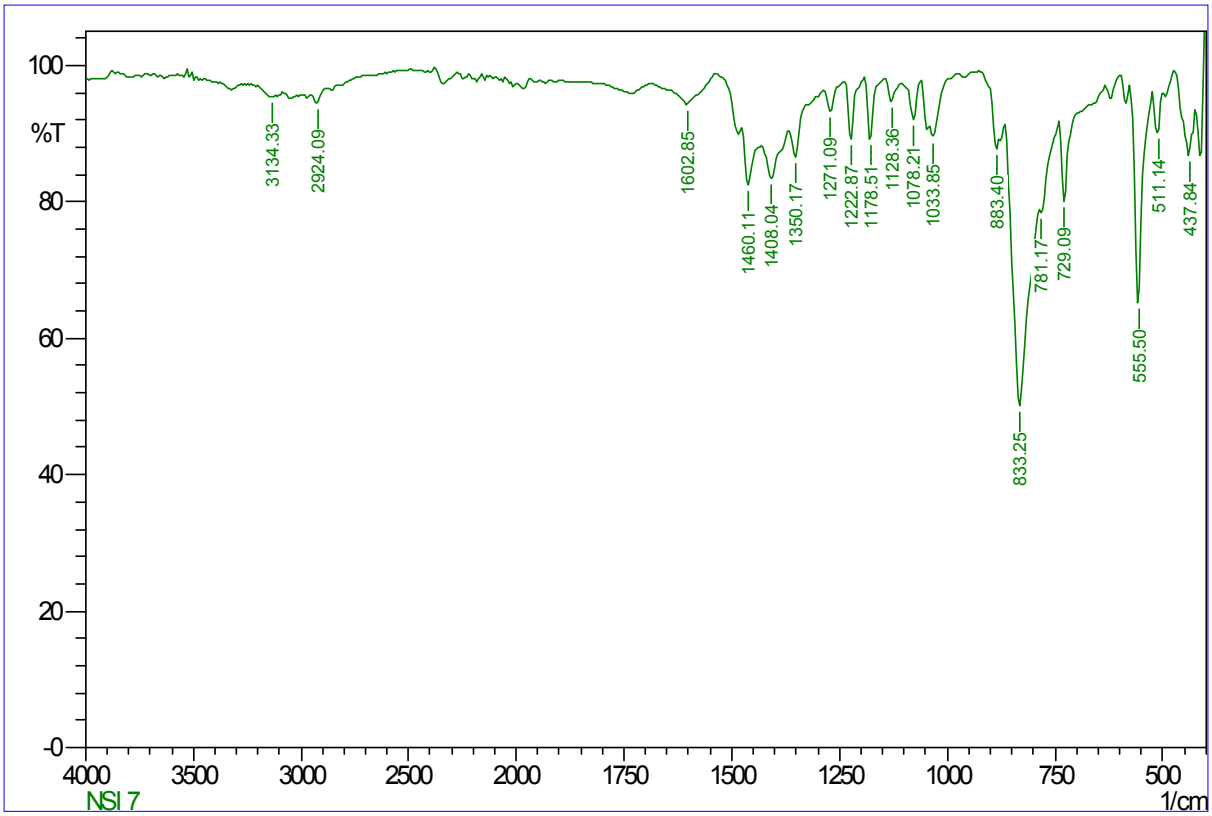
IR spectrum of IrL5



IR spectrum of IrL6



IR spectrum of IrL7



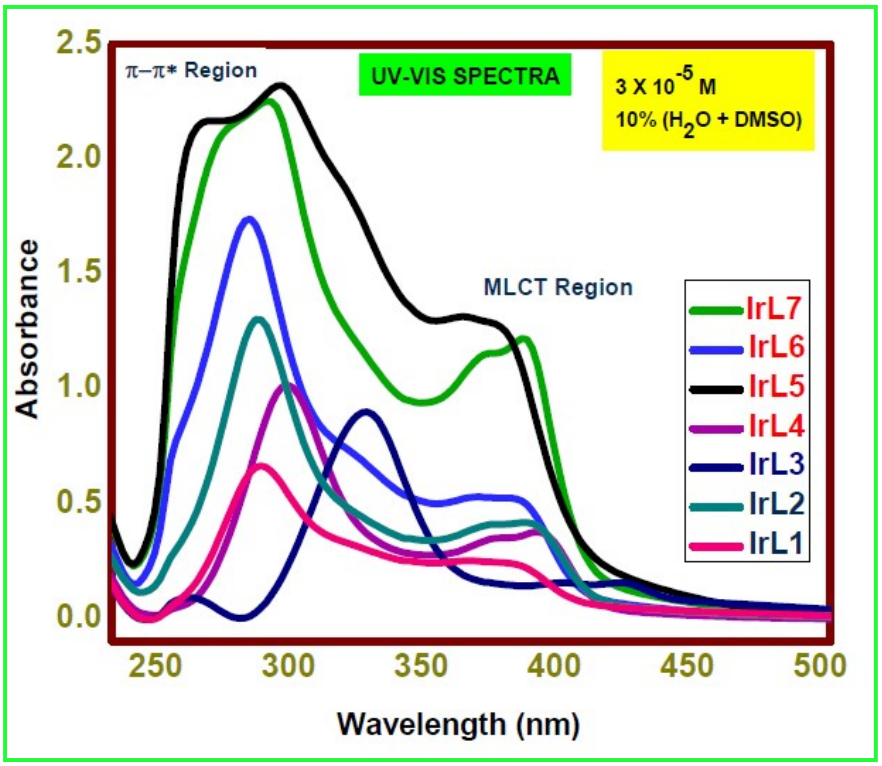


Fig. S1 UV-Vis spectra of complex IrL1-IrL7

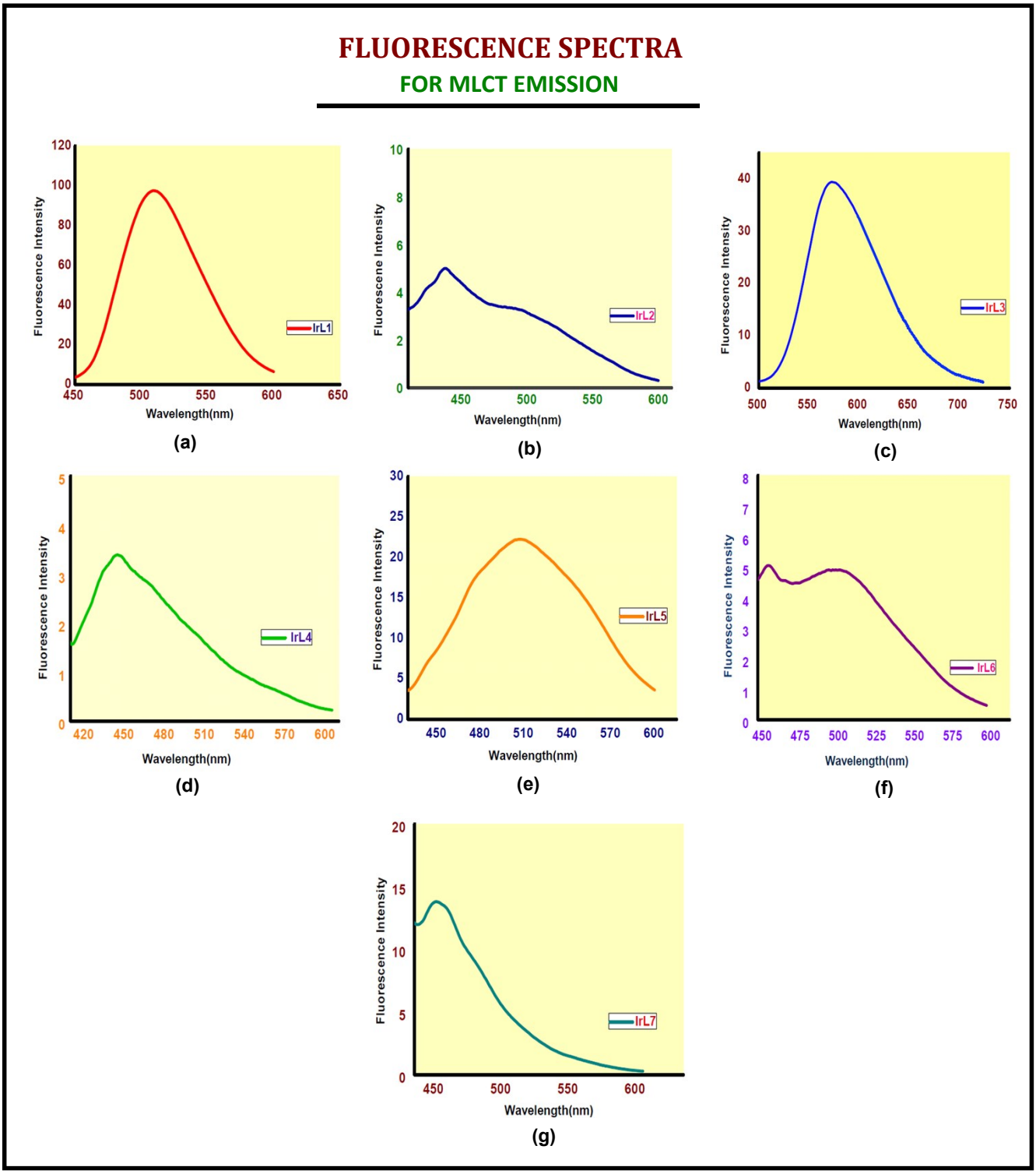


Fig. S2 emission spectra of complexes IrL1–IrL7 in 10% DMSO-water solvent system at room temperature, λex = 400-420 nm.

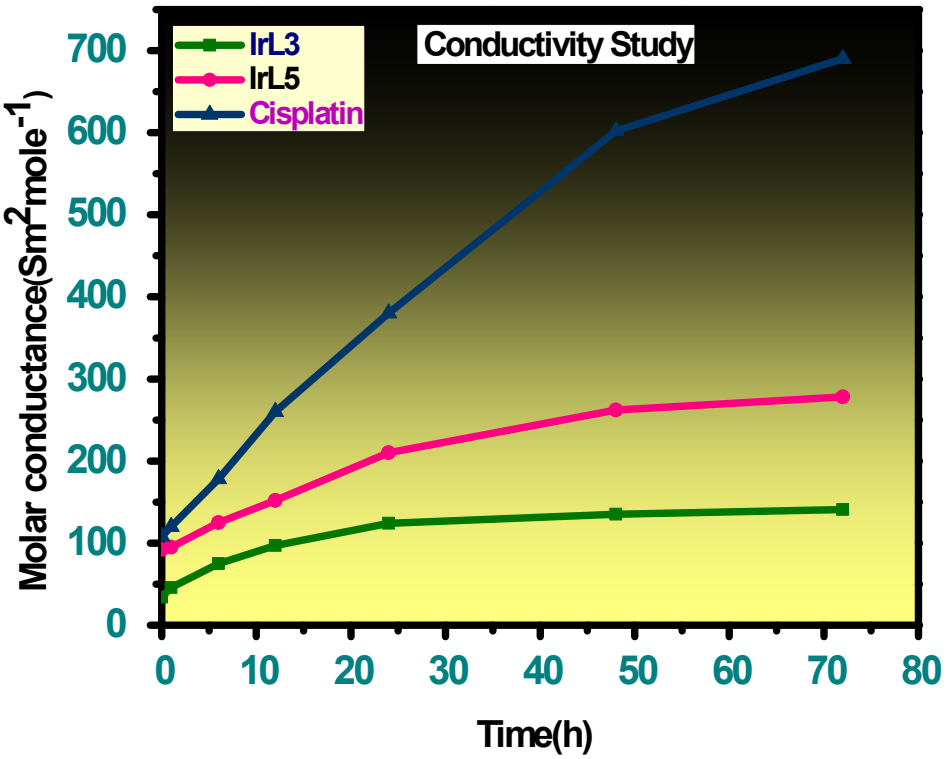


Fig. S3 Time dependent molar conductivity of compound IrL3 and IrL5 in 10% DMSO

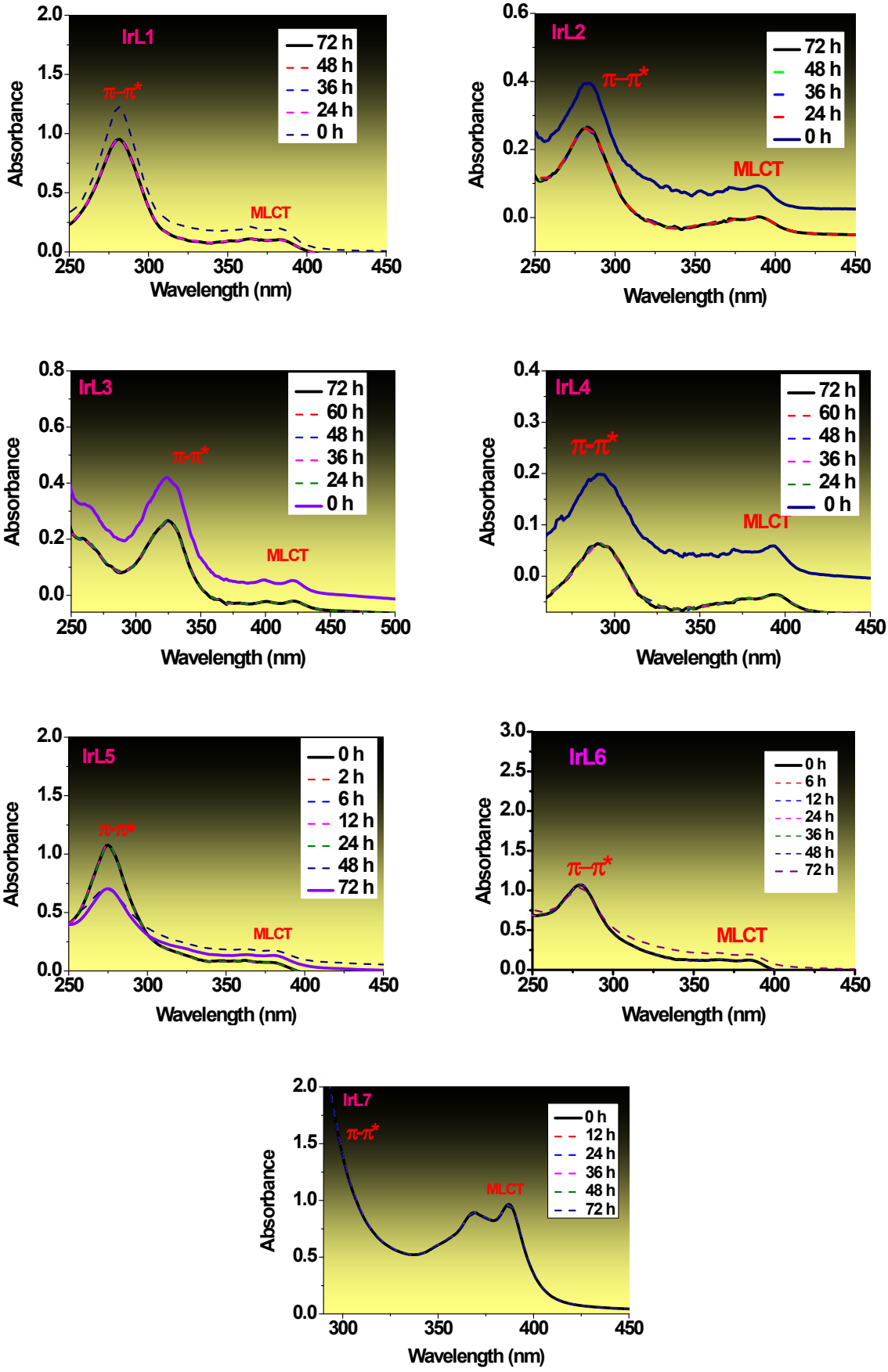


Fig. S4 Stability study of all the Ir(III) complexes in 10 % DMSO media

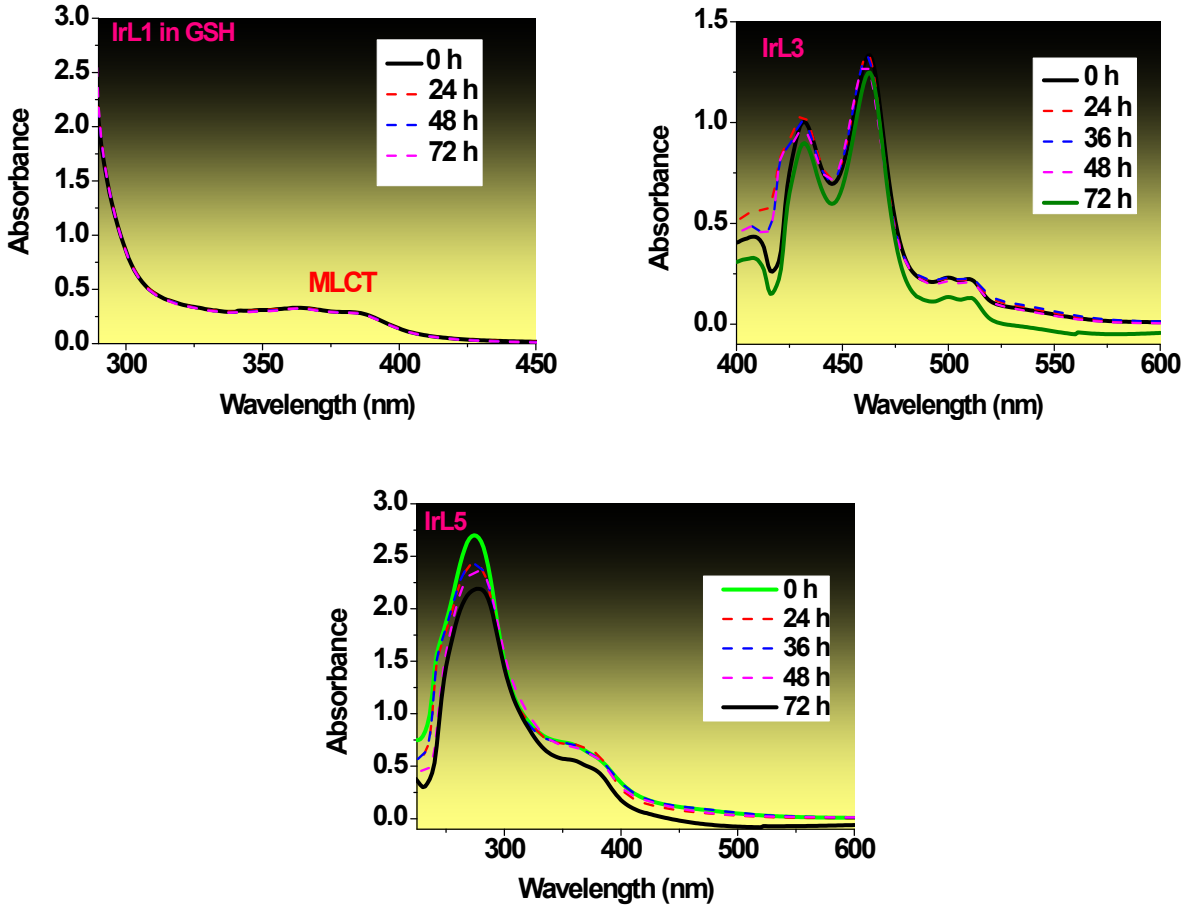


Fig. S5 Stability of selected Ir(III) complexes (IrL1, IrL3 and IrL5) in aqueous GSH media

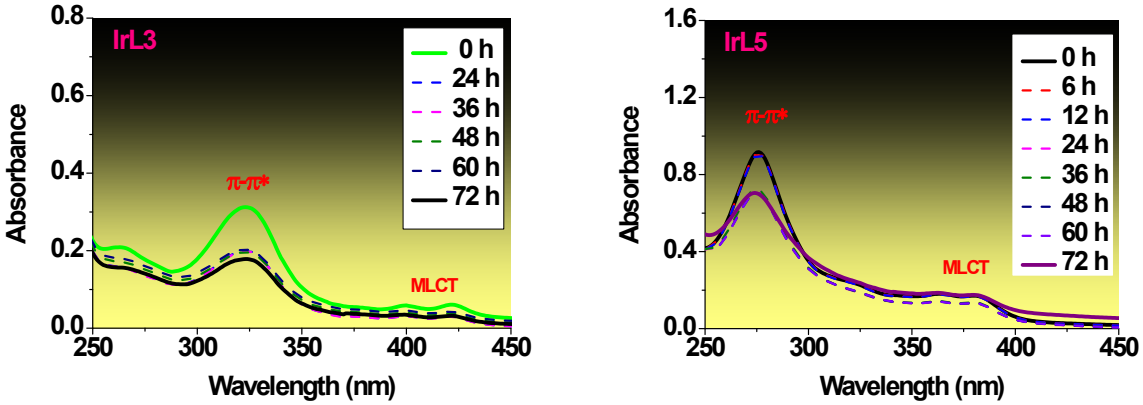
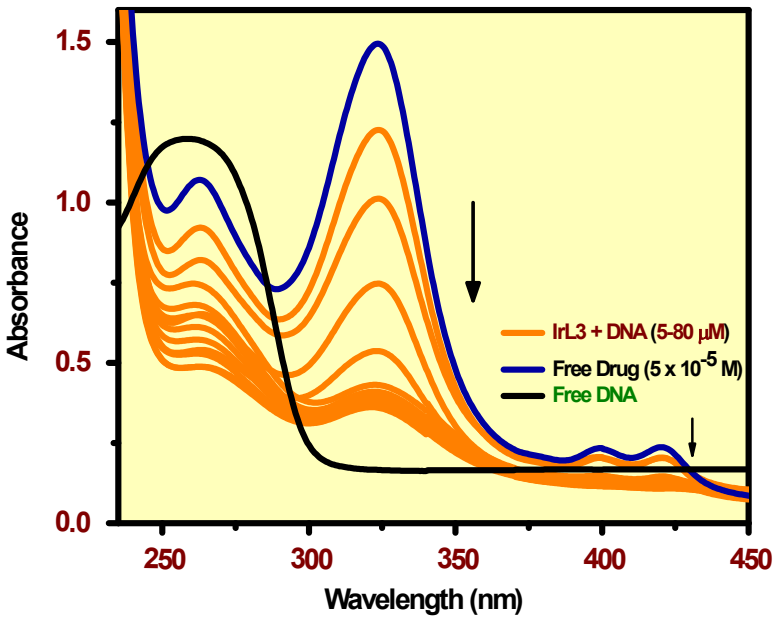
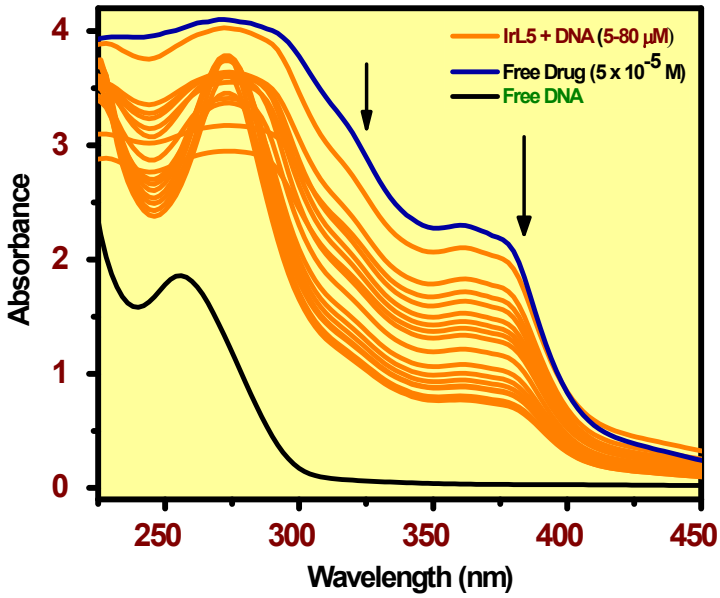


Fig. S6 Stability of IrL3 and IrL5 in PBS buffer media

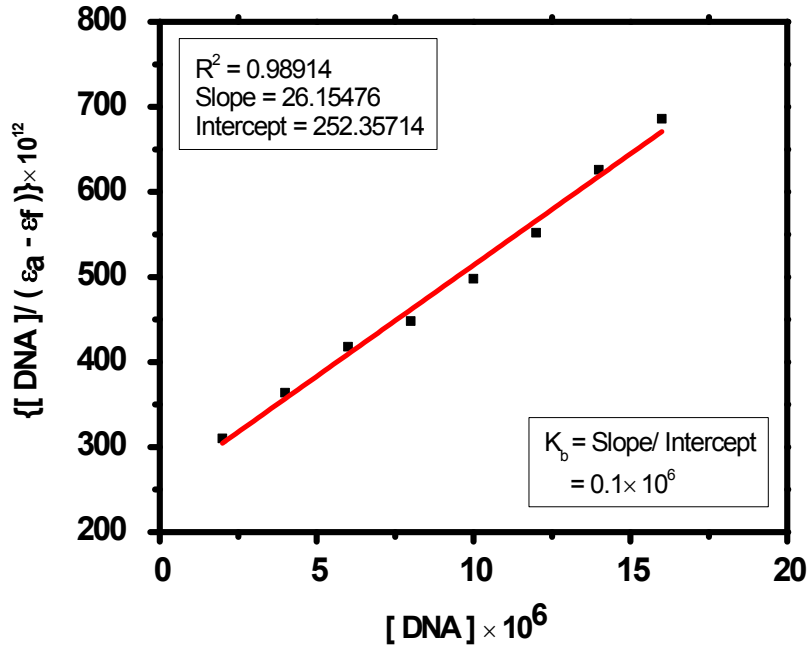


(a)

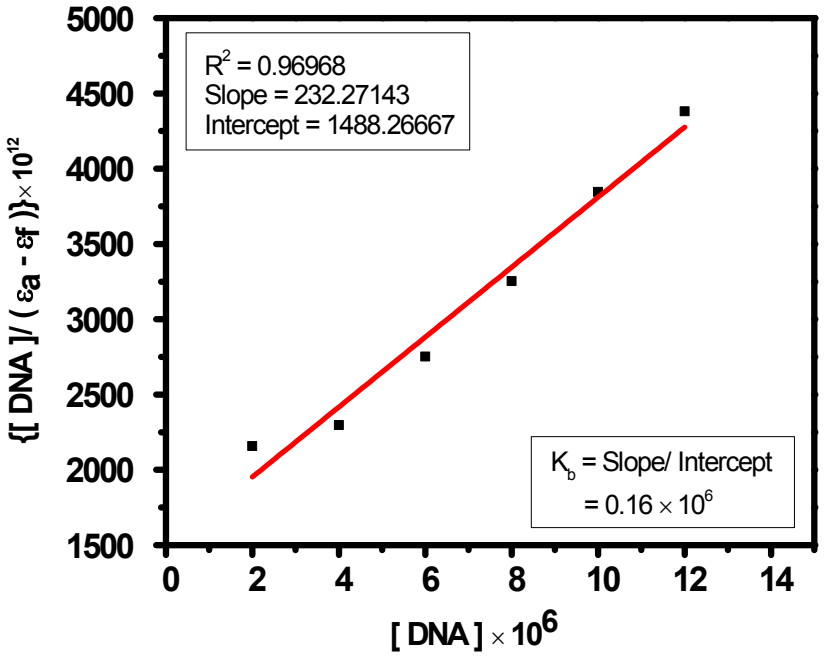


(b)

Fig. S7: DNA binding plots of (a) complex IrL3 and (b) complex IrL5

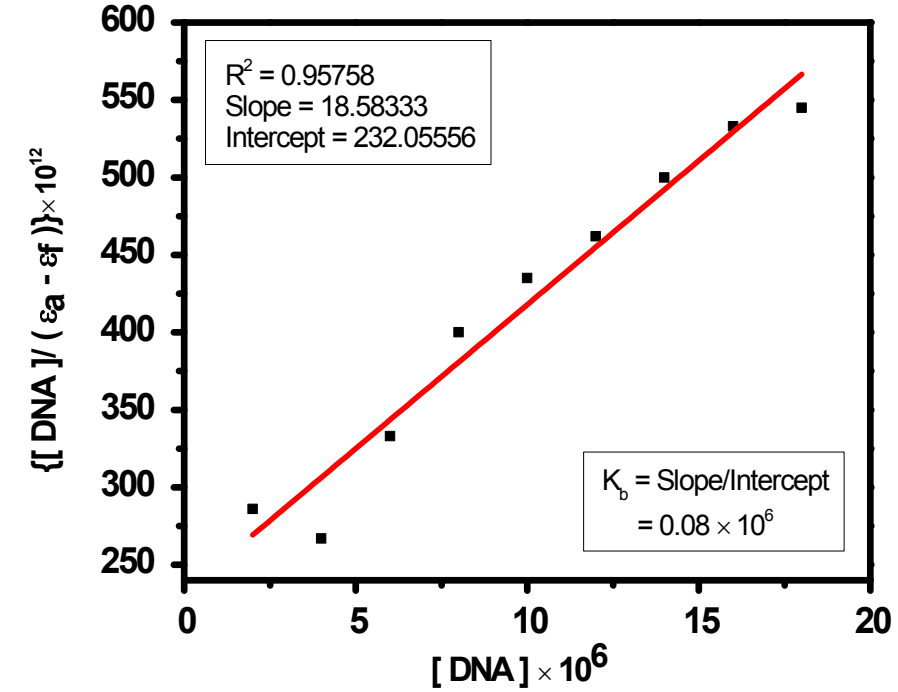


(a)

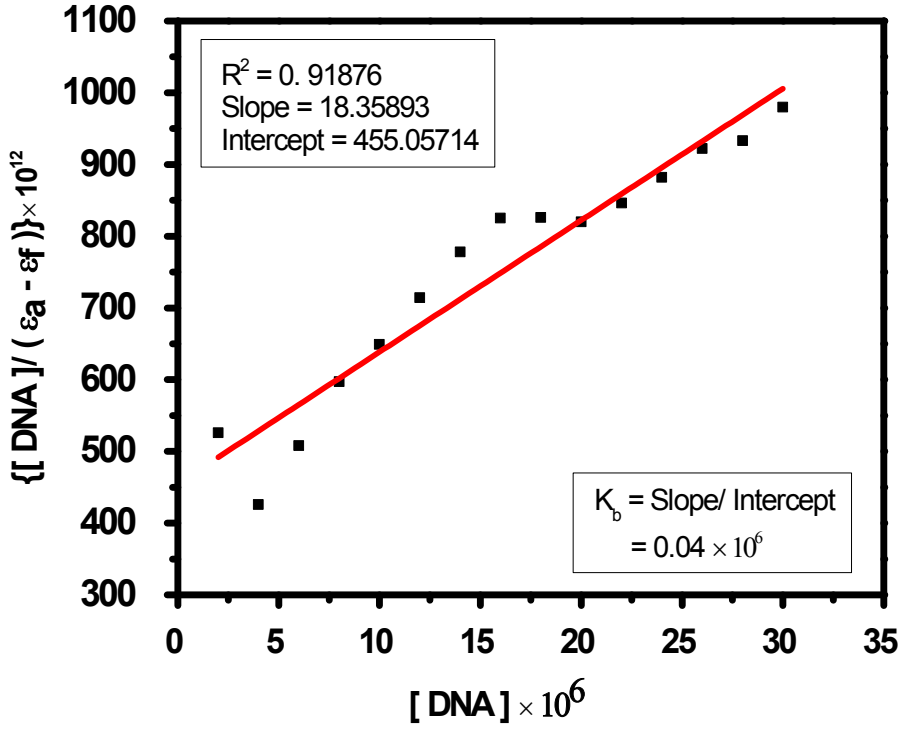


(b)

Fig. S8 [DNA]/(ε_a-ε_f) vs. [DNA] linear plots of complex IrL3 at (a) λ_{max} = 325 nm (π-π*) (b) λ_{max} = 425 nm (MLCT)



(a)



(b)

Fig. S9 [DNA]/(ε_a-ε_f) vs. [DNA] linear plots of complex IrL5 at (a) λ_{max} = 325 nm (π-π*) (b) λ_{max} = 380 nm (MLCT)

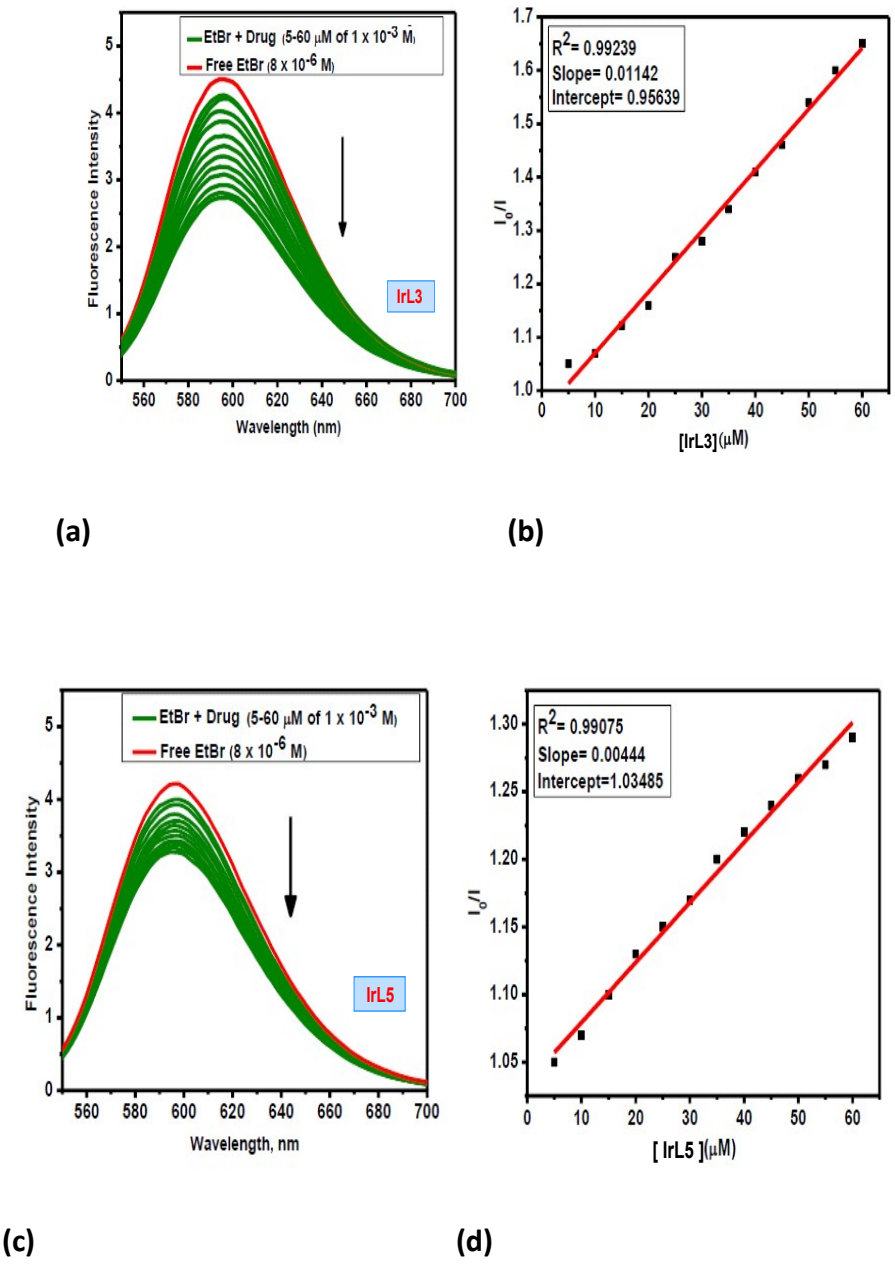


Fig. S10 EtBr quenching plots of complex **(a)IrL3**and **(c)IrL5**. Stern-Volmer plot of I_0/I vs [complex] **(b)IrL3** and **(d)IrL5**

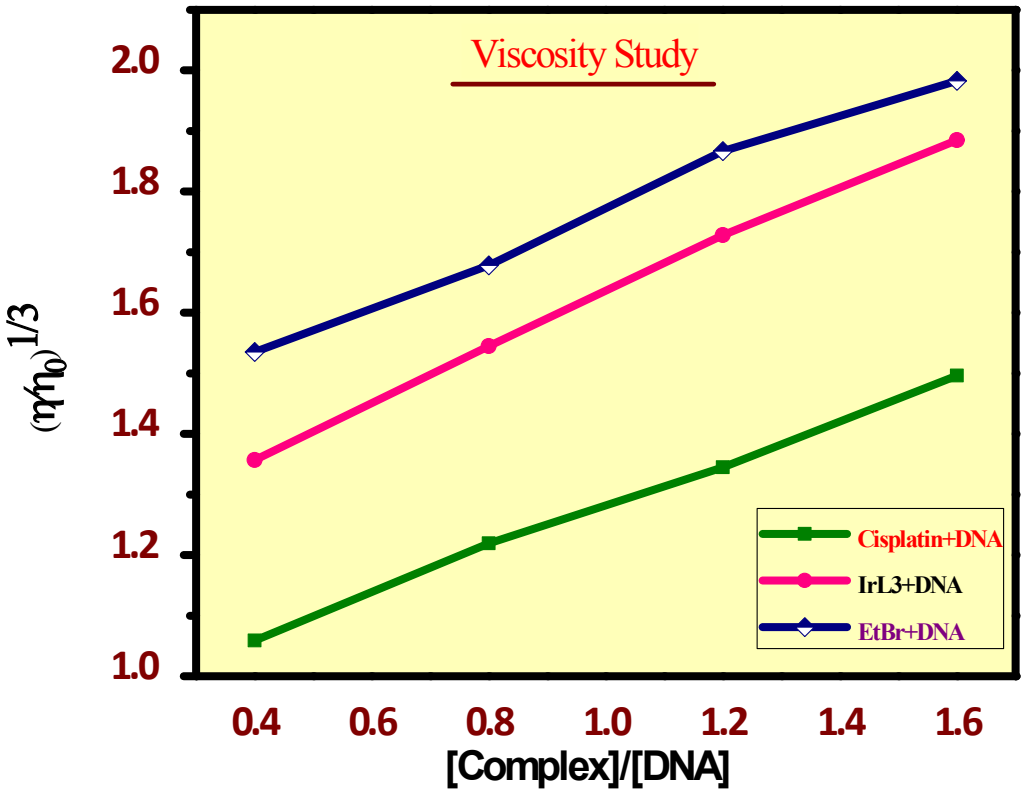


Fig. S11: Viscosity plot of complexes IrL3 and EtBr with Ct-DNA

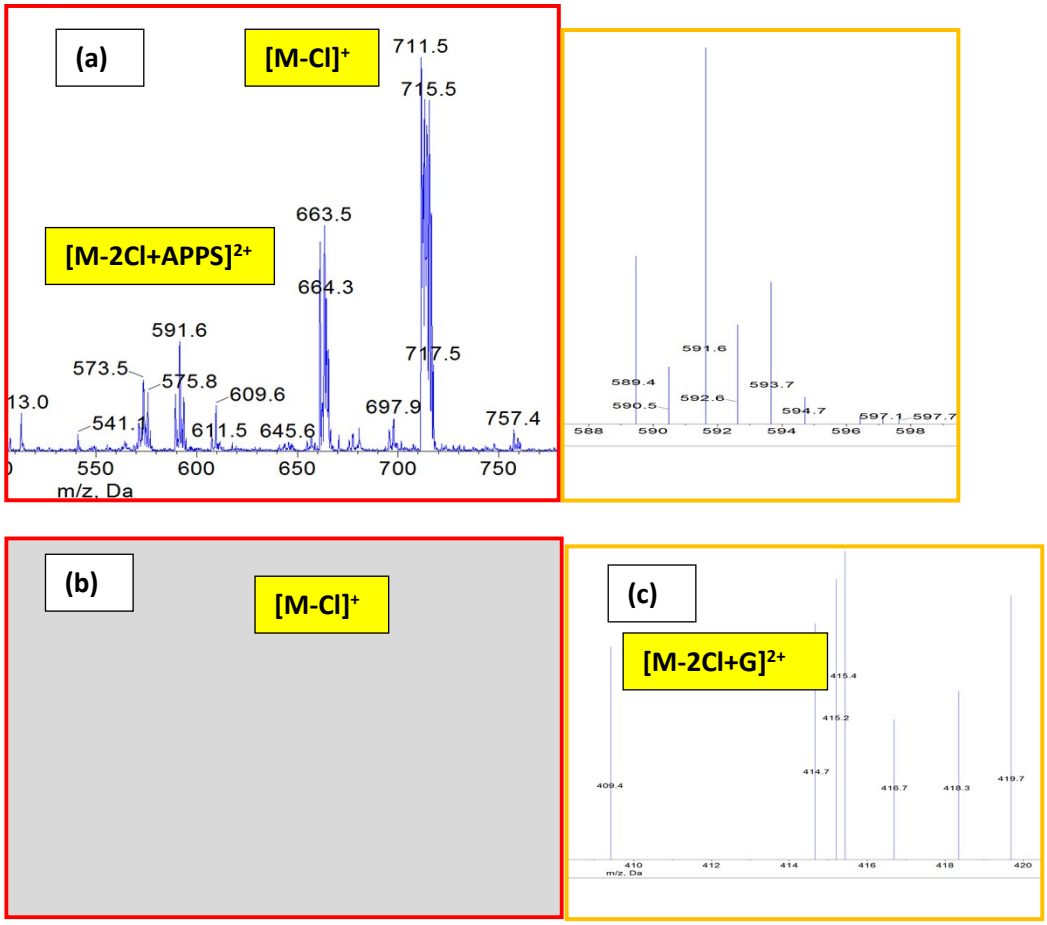
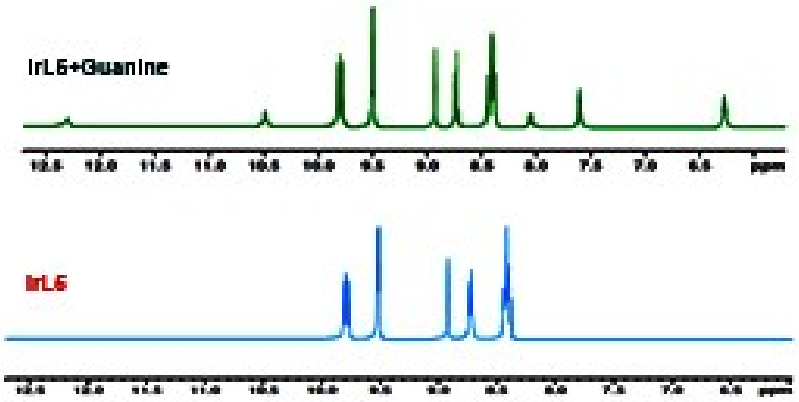


Fig. S12 ESI-MS of IrL5 treated with APPS (Adenosine-3'-phosphate 5'-phosphosulfate Lithium salt hydrate) and Guanine [(a) IrL5+APPS; (b) IrL5; (c) IrL5+G]

(a)



(b)

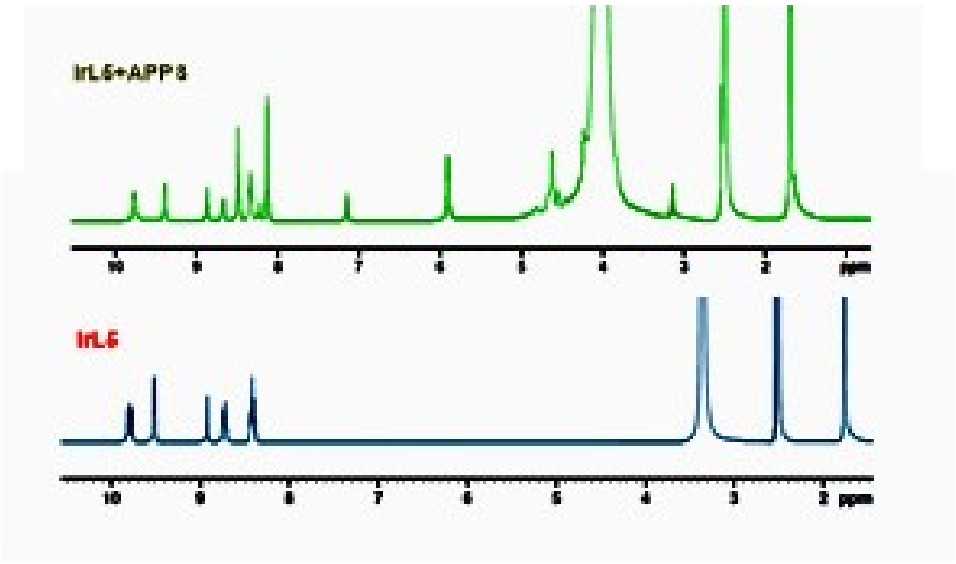
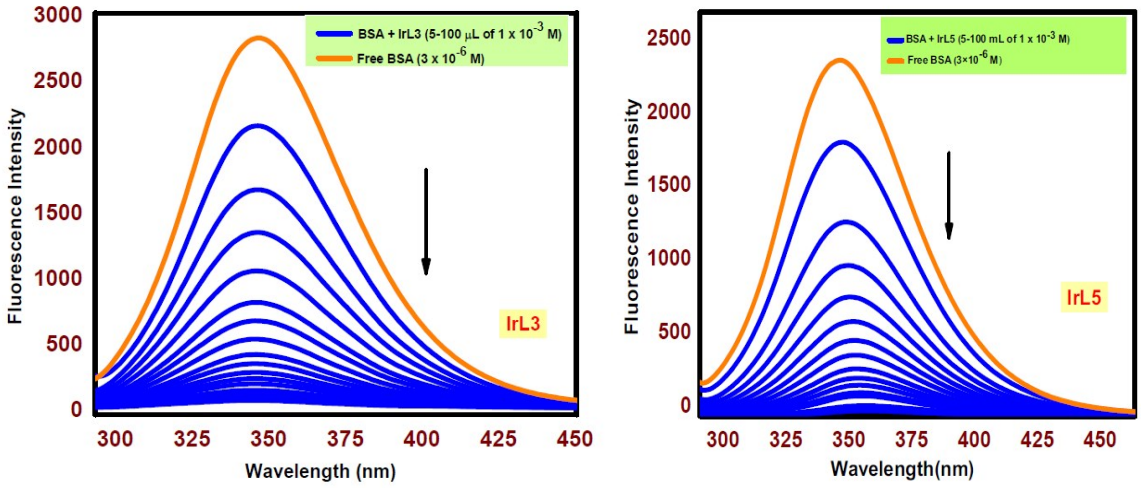
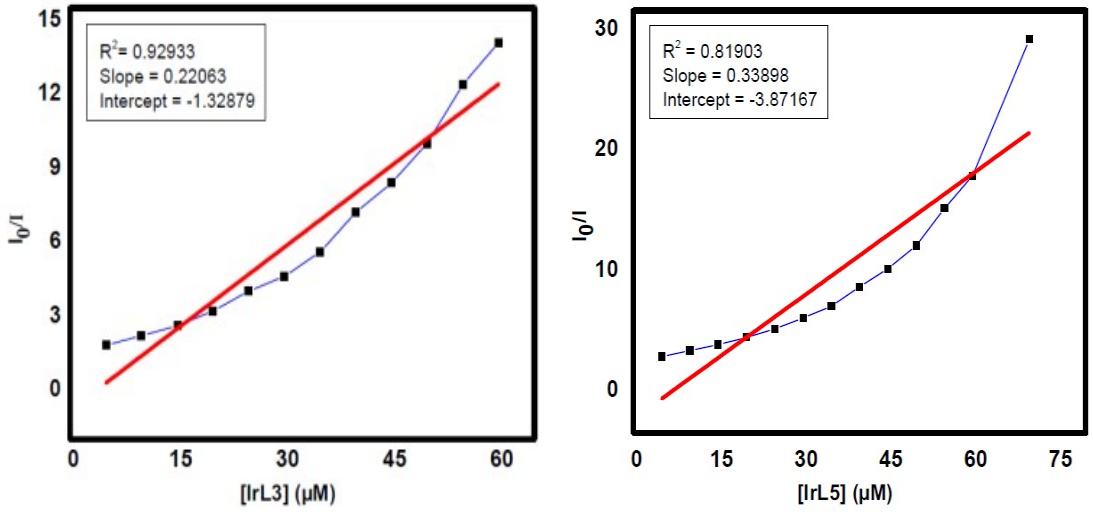


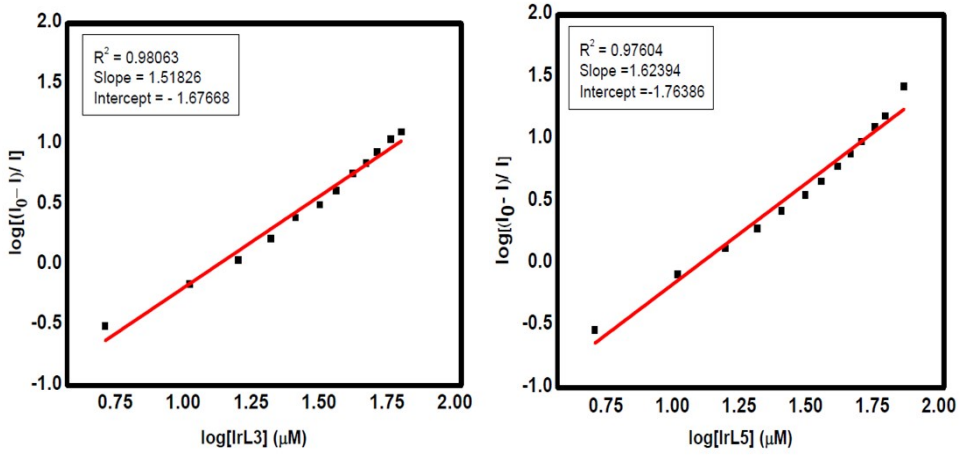
Fig.S13 ^{1}H NMR spectra recorded for the reaction of IrL5 with 1 eq. of (a) G and (b) APPS.



(a) **(b)**



(c) **(d)**



(e) **(f)**

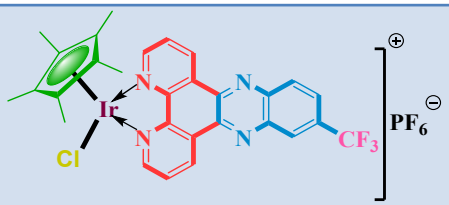
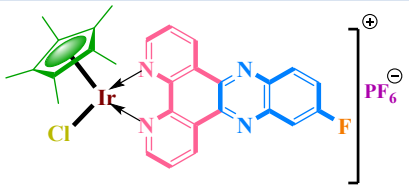
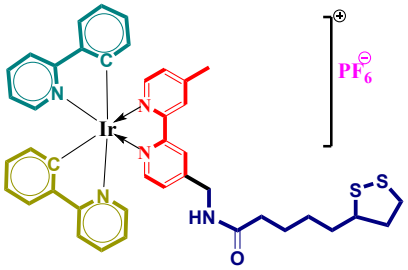
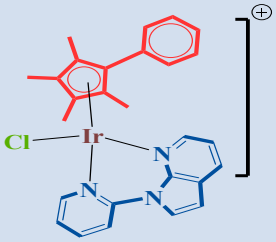
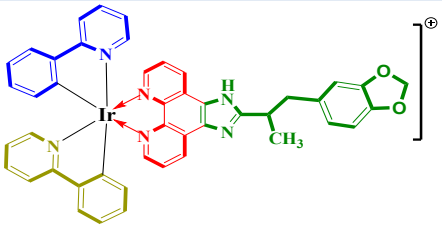
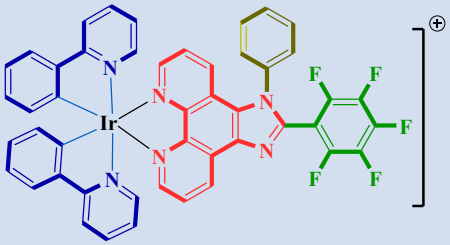
Fig. 14 Interaction of complexes **(a)IrL3** and **(b)IrL5** with BSA. Stern-Volmer Plot of I_0/I vs. concentration of complex **(c)IrL3(d) IrL5**. Scatchard Plot of $\log([I_0\text{-}I]/I)$ vs. log[Complex] for BSA in the presence of for complex **(e) IrL3 (f) IrL5**.

Table S1 Photophysical characterisation at MLCT region, solubility, lipophilicity and conductivity

Samples		λ_f (nm)[b]	Stoke's shift	O.D[c]	ε (M^{-1} cm^{-1})[d]	(φ_f)[e]	solubility (M)[f]	log P[g]	Λ_M[h](μs) DMSO	10% DMSO
IrL1	400	510	110	0.26	8667	0.057	0.025	0.49± 0.01	21	32
IrL2	400	438	38	0.43	14333	0.001	0.021	0.52± 0.03	42	51
IrL3	423	598	175	0.16	5333	0.088	0.026	0.78 ± 0.10	23	34
IrL4	400	440	40	0.38	12667	0.002	0.018	0.69±0.05	18	21
IrL5	400	507	107	1.32	44000	0.004	0.029	1.1±0.12	71	92
IrL6	400	504	104	0.54	18000	0.002	0.022	0.97± 0.06	62	76
IrL7	400	447	47	1.23	41000	0.002	0.017	0.91±0.05	65	81
Quinine Sulphate	350	452	102	0.24	8000	0.546	-	-	-	

[a]Absorption maxima at MLCT. [b]Emission wavelength. [c]Optical density. [d]Extinction coefficient. [e]Quantum yield. [f]DMSO-10% DMEM medium (1 : 99 v/v,comparable to cell media). [g]n-Octanol/water partition coefficient. [h]Conductance in DMSO and 10% aqueous DMSO.

Table S2 IC_{50} comparison table of the synthesized complexes with the reported complexes

Potent Complexes	Structures	IC_{50} HeLa Cell	Reference Numbers
IrL_5		2.47± 0.68	
IrL_6		1.70± 0.49	
Reference Complexes			
Complex1		6.02± 0.16	1
Complex2		10.5± 0.7	2
Complex3		4.2± 0.9	3
Complex4		0.5± 0.1	4

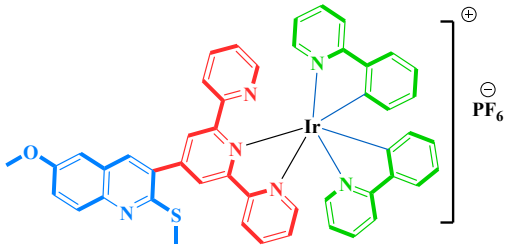
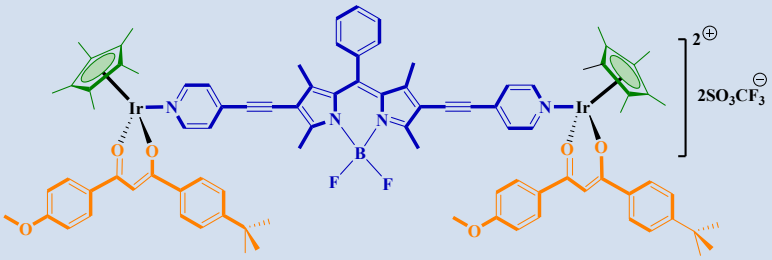
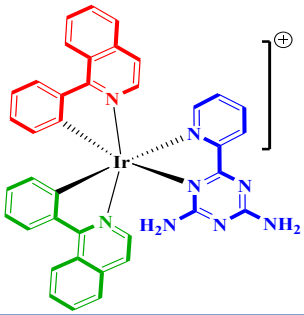
Complex5		3.46± 0.62	**5**
Complex6		2.0± 0.3	**6**
Complex7		29.2± 4.5	**7**

Table S3 DNA binding parameters for complexes **IrL3** and **IrL5** with ct-DNA

Complex	λ_{max} [nm]	Change in absorbance intensity	[a]Δε (%)	[b]K_b($\times 10^6 M^{-1}$)	[c]K_{SV}(x10^6 M^{-1})	[d]K_{app}($\times 10^6$ M^{-1})
IrL3	324	Hypochromism	83	0.1	0.0114	1.33
	420	Hypochromism	60	0.16		
IrL5	320	Hypochromism	66	0.08	0.0044	1.45
	362	Hypochromism	60	0.04		

[a]Δε, % of change in hypochromism. [b]k_b, intrinsic DNA binding constant from UV-Vis absorption titration. [c]K_{SV}, Stern-Volmer quenching constant. [d]K_{app}, apparent DNA binding constant from competitive displacement.

Table S4 Binding parameters for interaction of complexes **IrL3** and **IrL5** with BSA.

Complex	**K_{BSA} [M^{-1}][a]**	**k_q[$M^{-1}s^{-1}$][b]**	**K [M^{-1}][c]**	**n[d]**
IrL3	0.22 x10^6	2.2 x10^{13}	2.1 x 10^4	1.5
IrL5	0.34 x10^6	3.4 x10^{13}	1.7 x 10^4	1.6

[a]K_{BSA}, Stern Volmer quenching constant; [b]K_q, quenching rate constant; [c]K, binding constant with BSA; [d]n, number of binding sites.

References:

1. M. -M. Wang, X. -L. Xue, X. -X. Sheng, Y. Su, Y. -Q. Kong, Y. Qian, J. -C. Bao, Z. Su, and H-K. Liu, *RSC Adv.*, 2020, **10**, 5392–5398.

2. P. Starha, Z. Travnícek, H. Crlíkova, J. Vanco, J. Kasparkova, and Z. Dvorak, *Organometallics*, 2018, **37**, 2749−2759.

3. Q. -Y. Yi, D. Wan, B. Tang, Y-J. Wang , W-Y. Zhang , F. Du, M. He, Y-J. Liu, *European Journal of Medicinal Chemistry*, 2018, **145**, 338-349.

4. M. Ouyang, L. Zeng, H. Huang, C. Jin, J. Liu, Y. Chen, L. Ji and H. Chao, *Dalton Trans.*, 2017, **46**, 6734–6744.

5. S. Mukhopadhyay, R. S. Singh, R. P. Paitandi, G. Sharma, B. Koch and D. S. Pandey, *Dalton Trans.*, 2017, **46**, 8572- 8585.

6. G. Guptaa, S. Cherukommub, G. Srinivasb, S. W. Leea, S. H. Munc, J. Jungc, N. Nagesh, and C. Y. Leea, *Journal of Inorganic Biochemistry*, 2018, **189**, 17–29.

7. Q.Y. Y, W. Y. Zhang, M. He, F. Du, X. Z. Wang, Y. J. Wang, Y. Y. Gu, L. Bai, and Y. J. Liu, *Journal of Biological Inorganic Chemistry*, 2019, **24**, 151–169.