

Supporting information

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

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Vellore-632014, Tamilnadu (India), E-mail: priyanka.paira@vit.ac.in

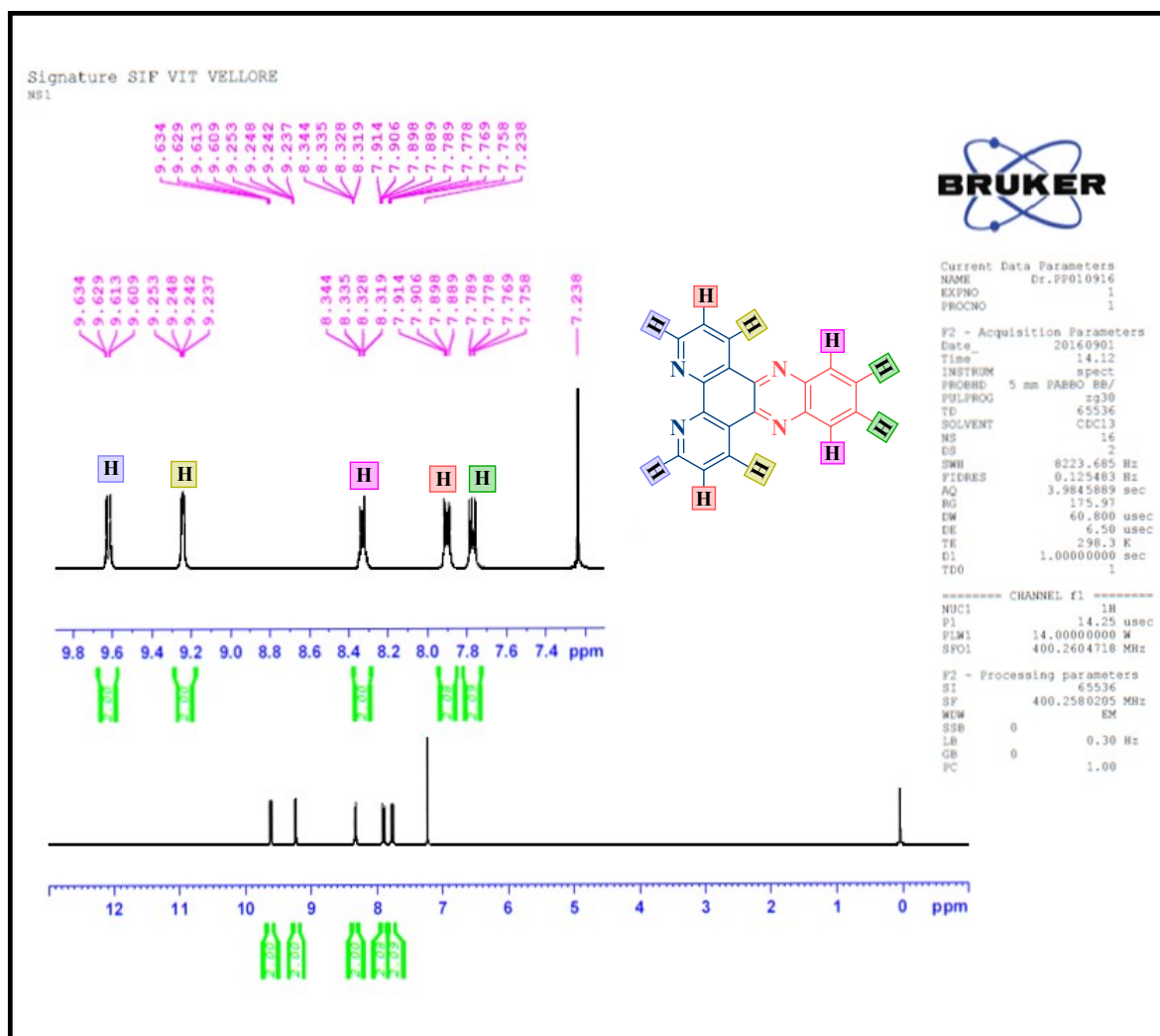
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Yenepoya University, University Road, Derlakatte, Mangalore 575018, Karnataka, India
Email : Bipasha.bose@yenepoya.edu.in

^cSchool of Bioscience of Technology, Vellore Institute of Technology

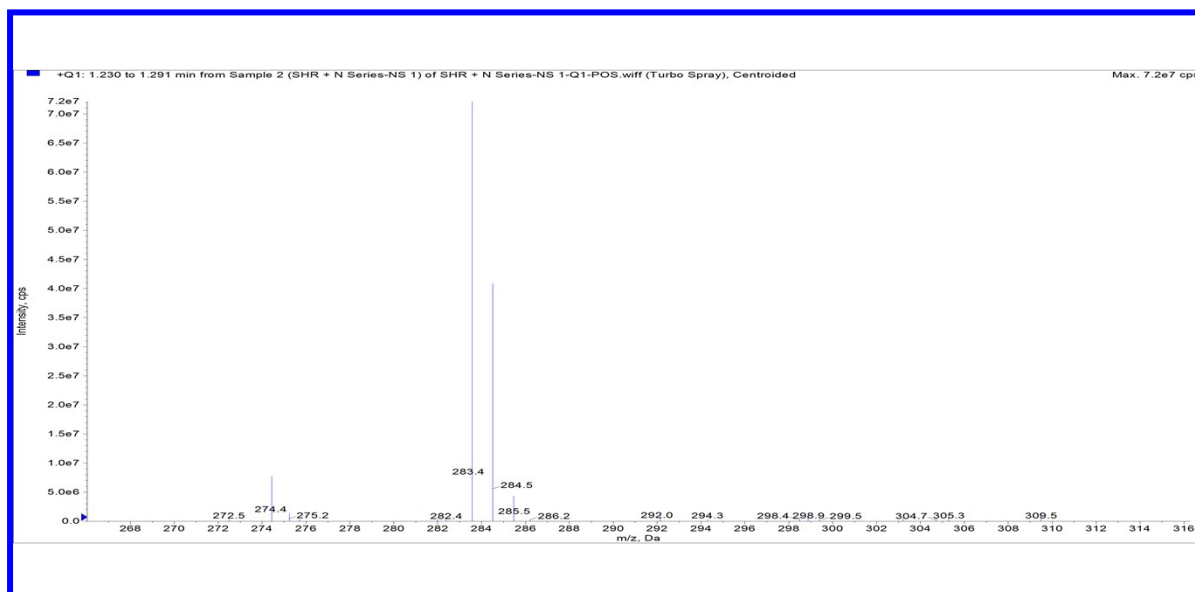
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¹H NMR spectrum of Ligand L1

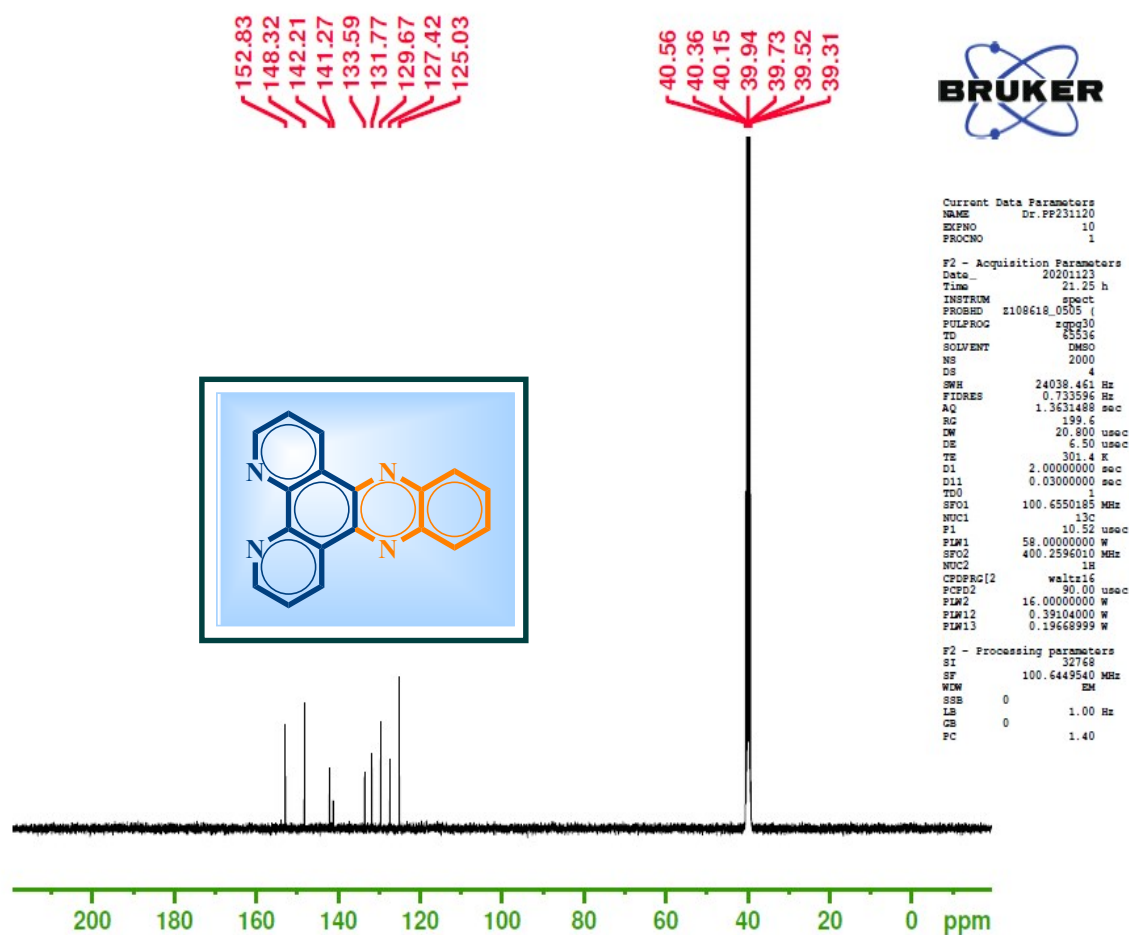


ESI-MS of ligand L1

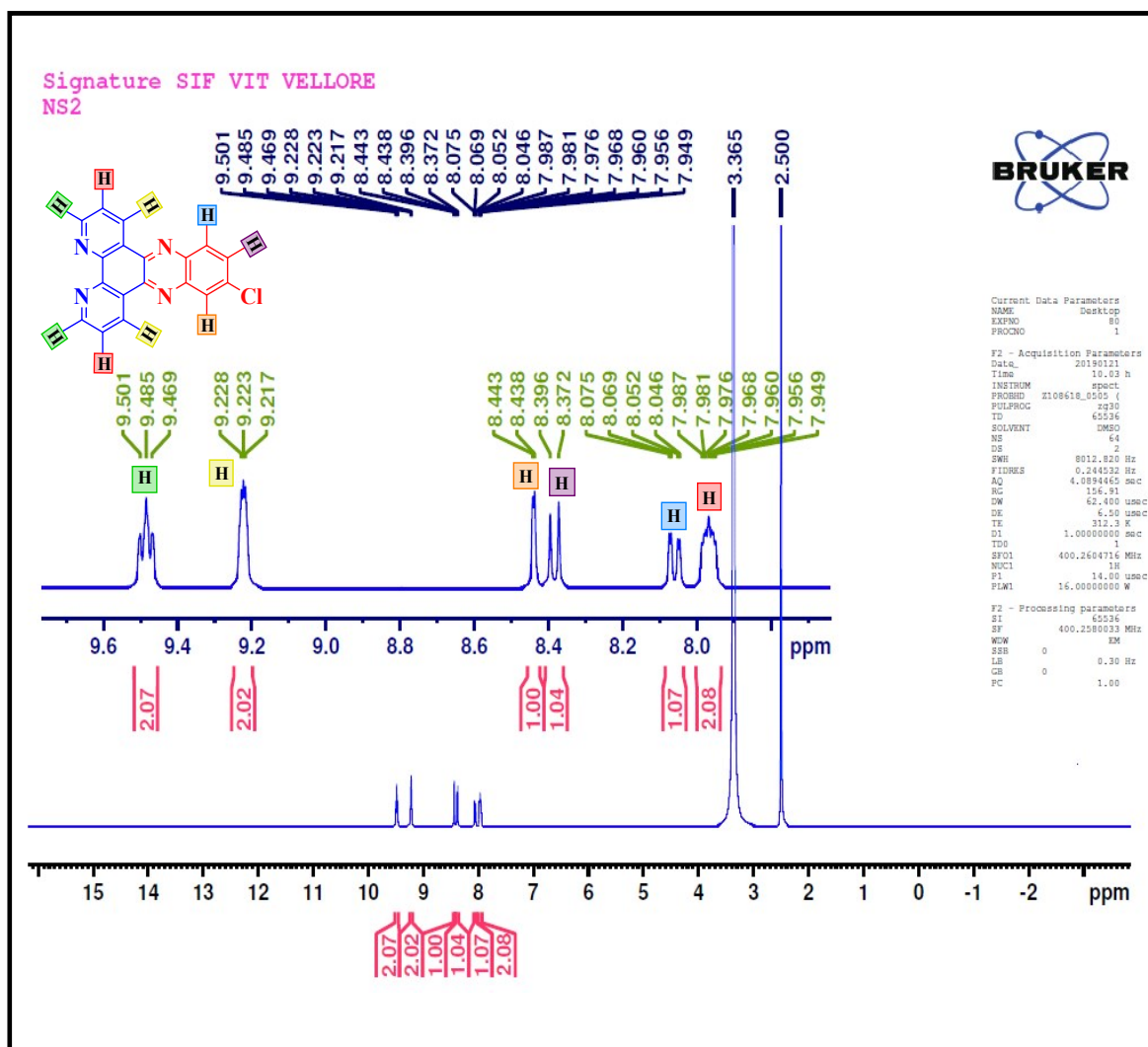


¹H NMR spectrum of Ligand L1

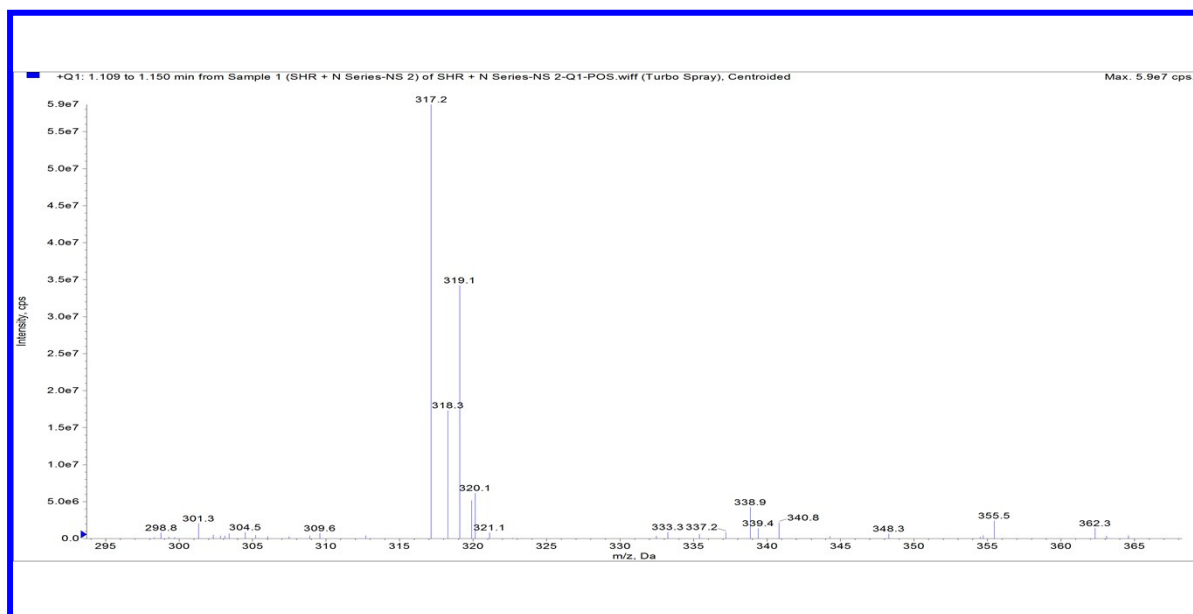
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¹H NMR spectrum of Ligand L2

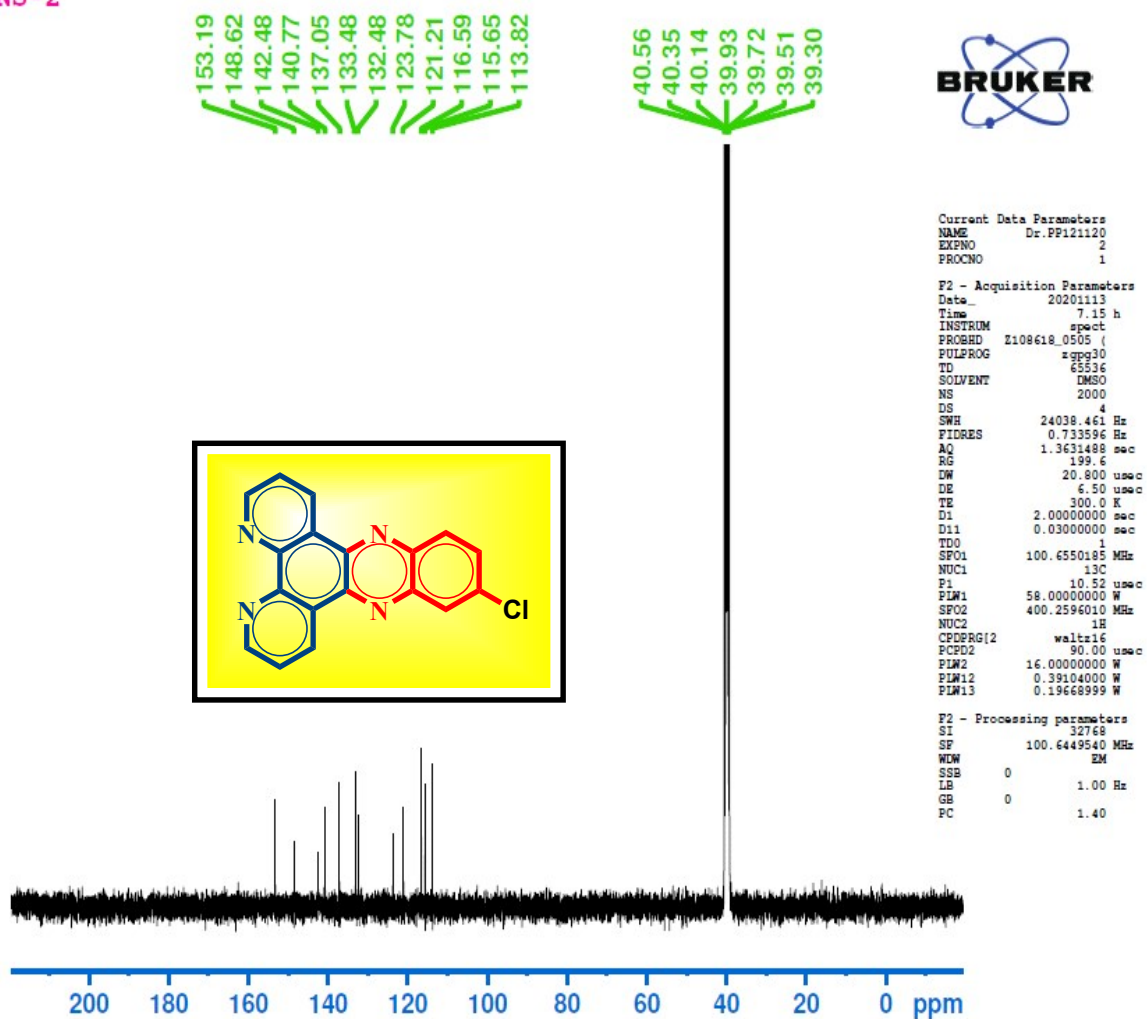


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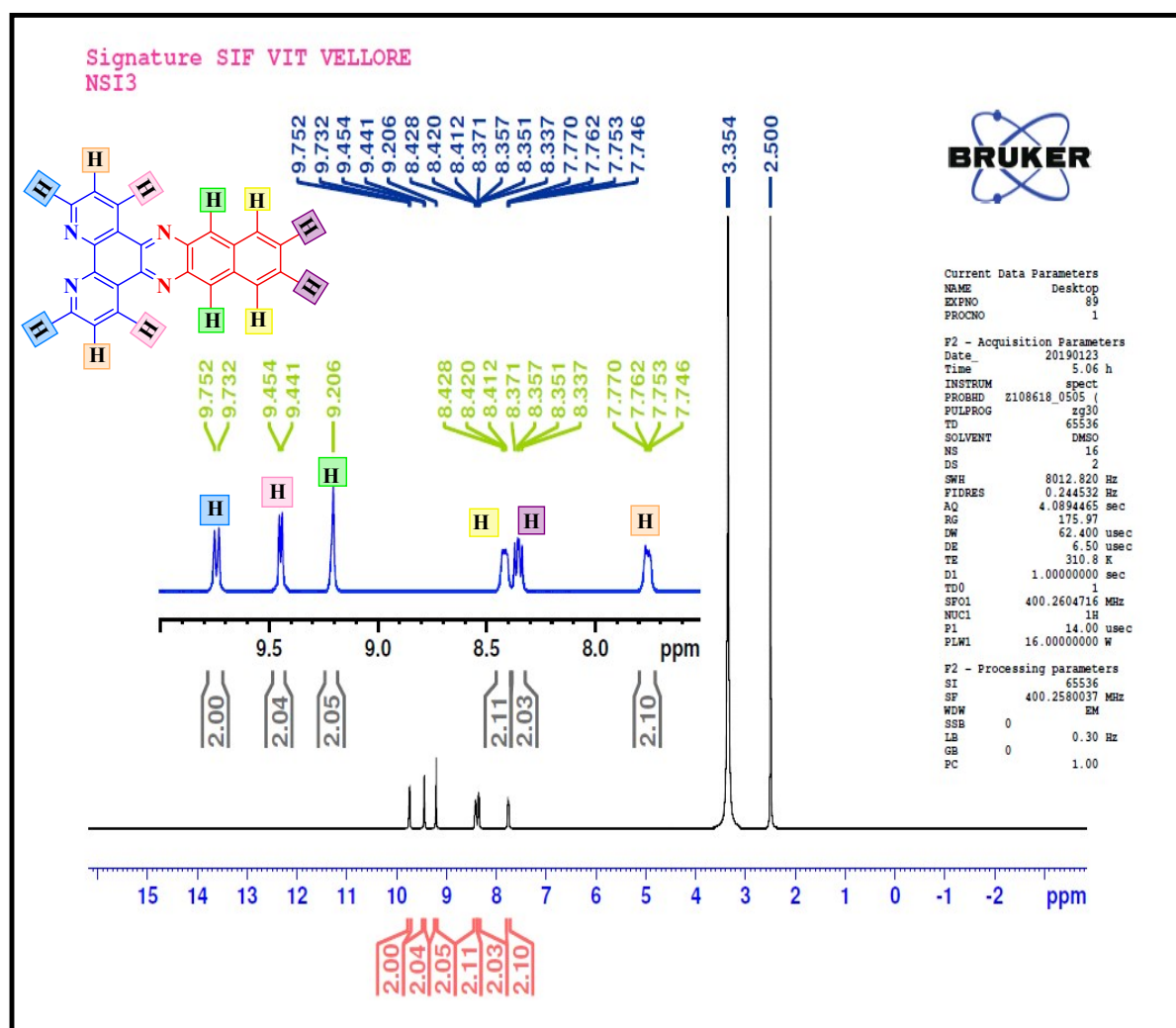


¹³C NMR spectrum of Ligand L2

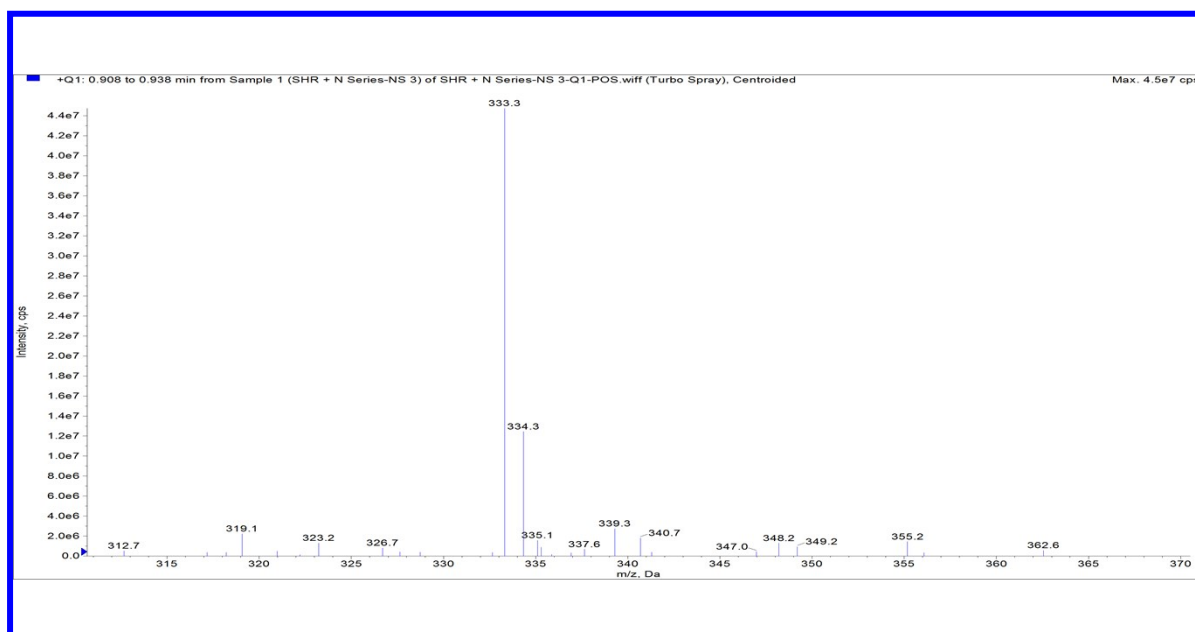
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NS-2



¹H NMR spectrum of Ligand L3

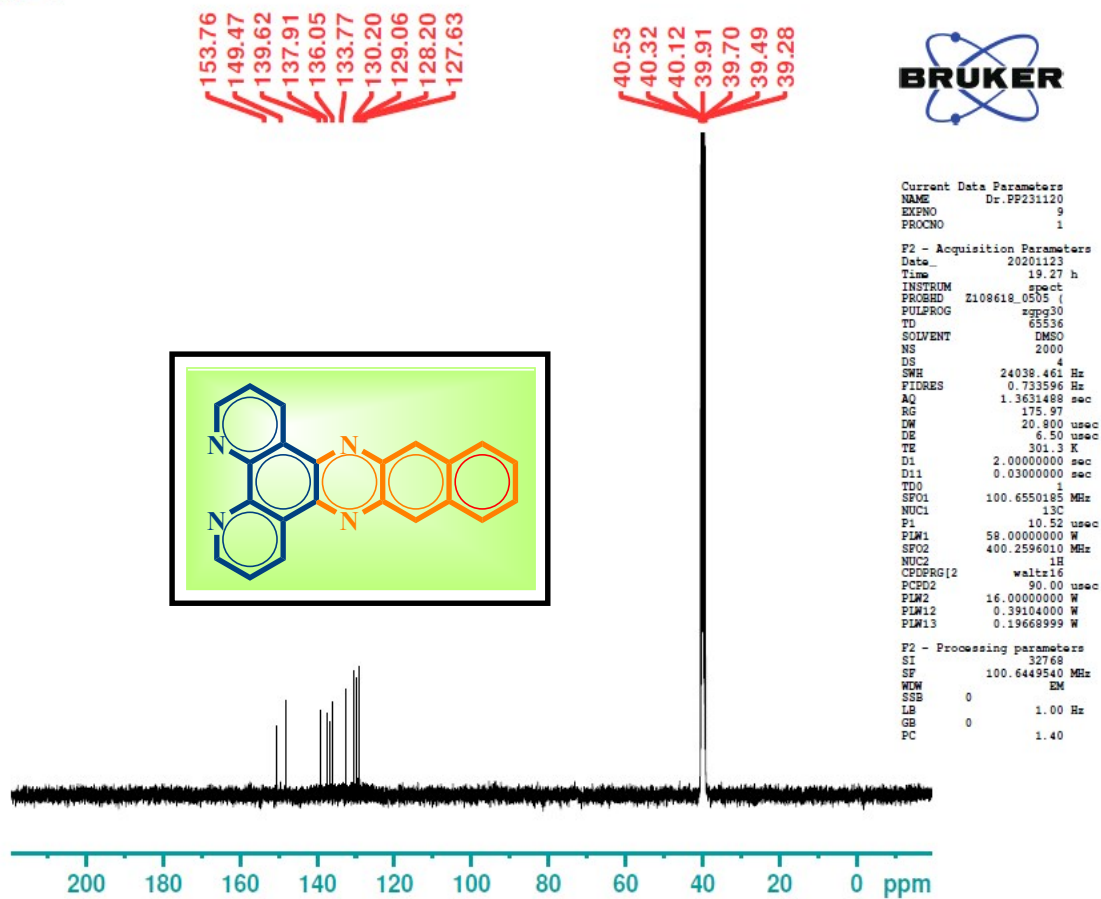


ESI-MS of ligand L3

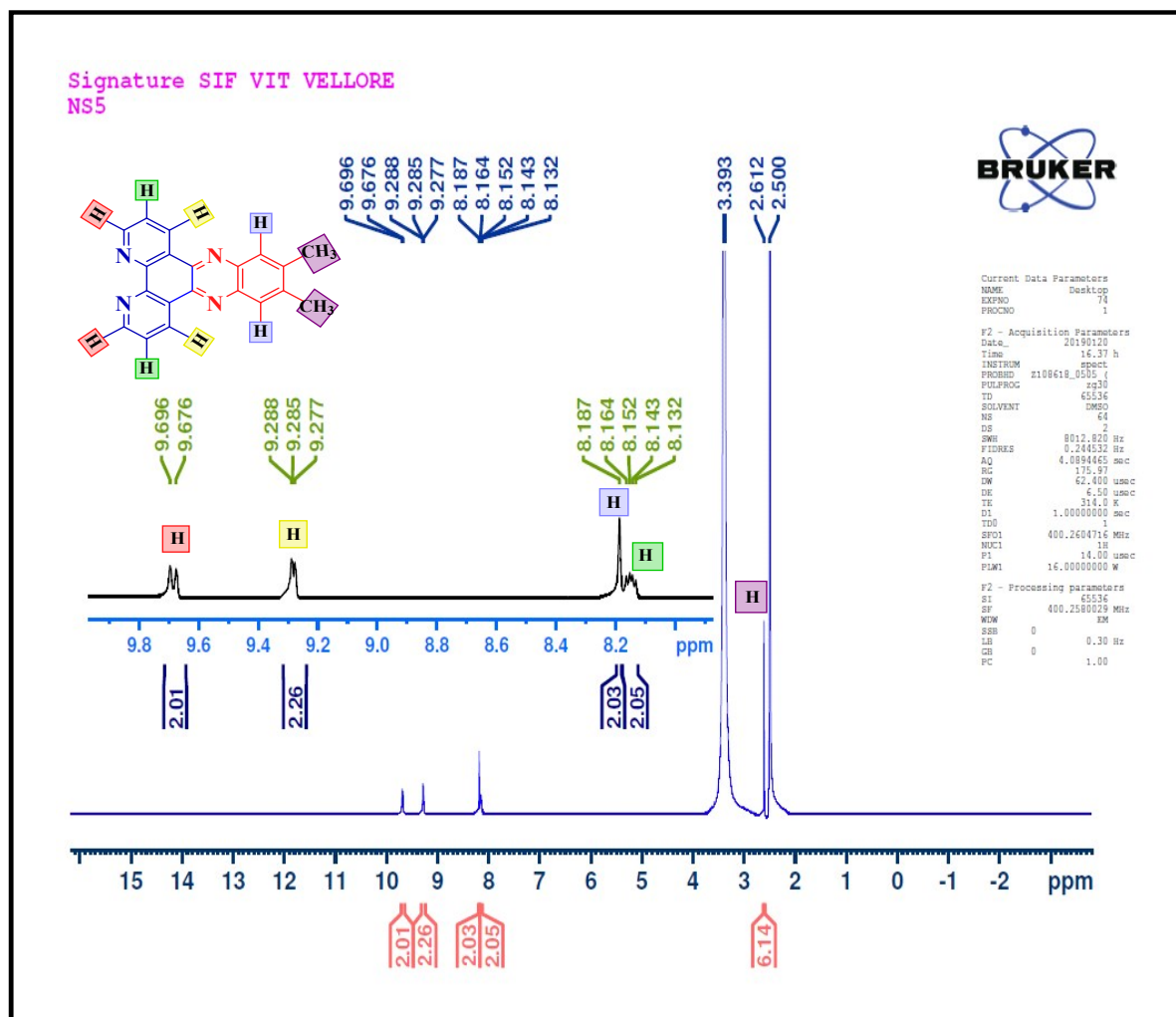


¹³C NMR spectrum of Ligand L3

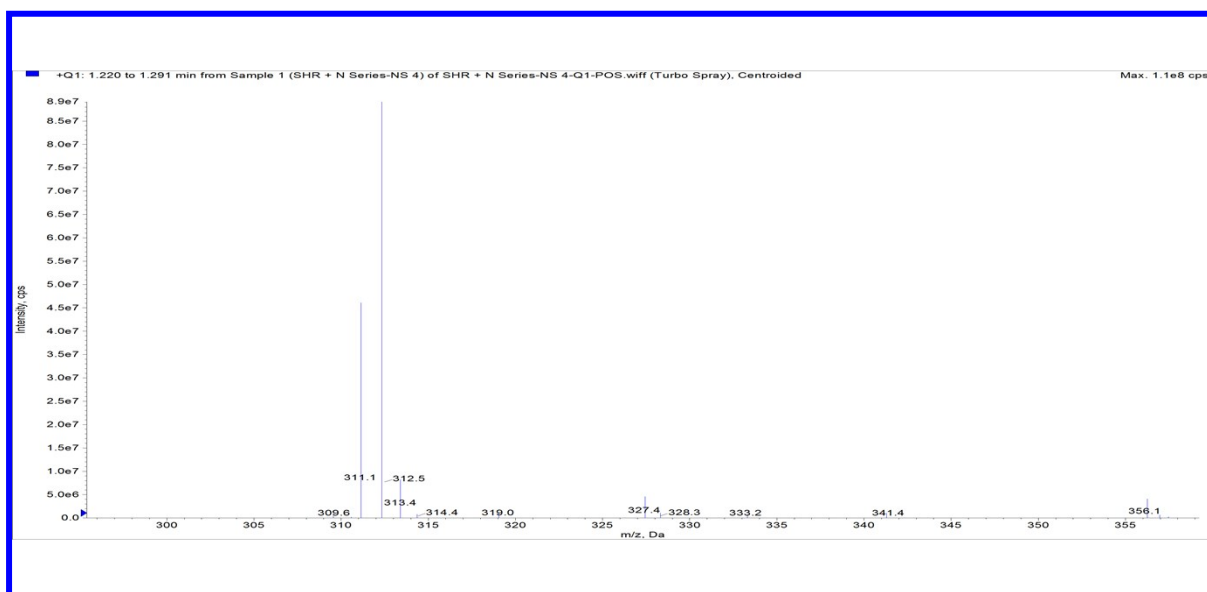
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NS-3



¹H NMR spectrum of Ligand L4

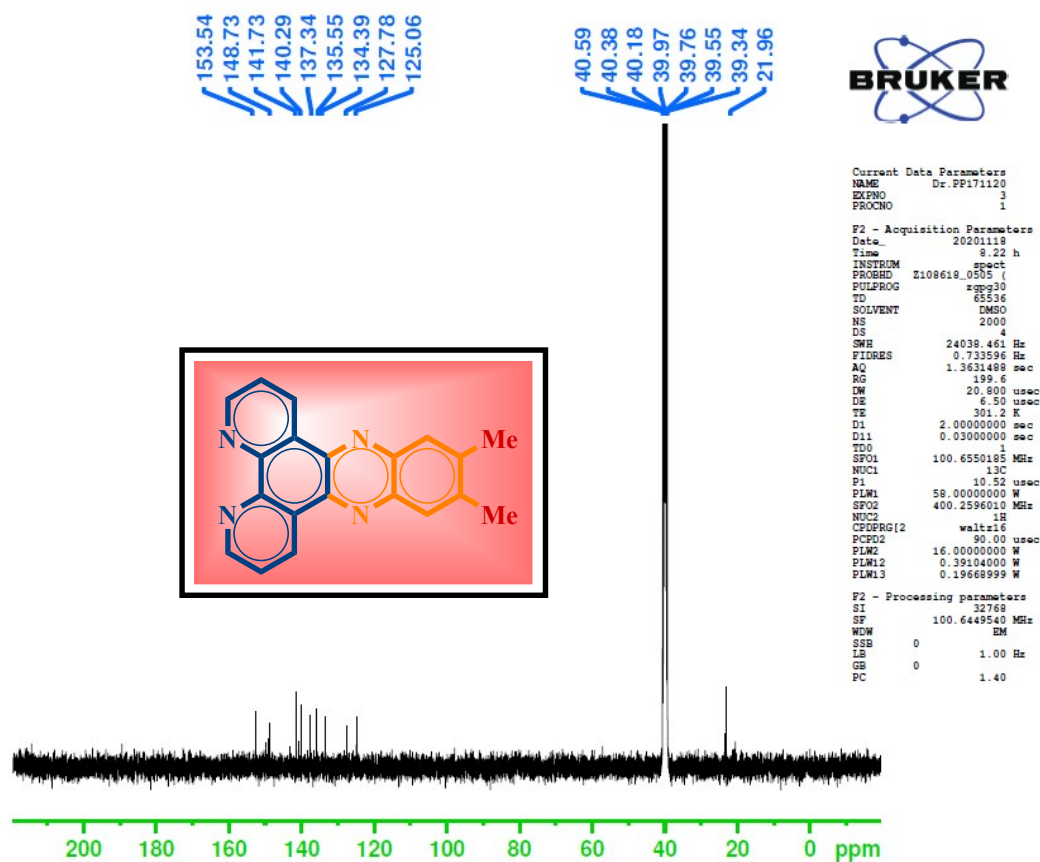


ESI-MS of ligand L4

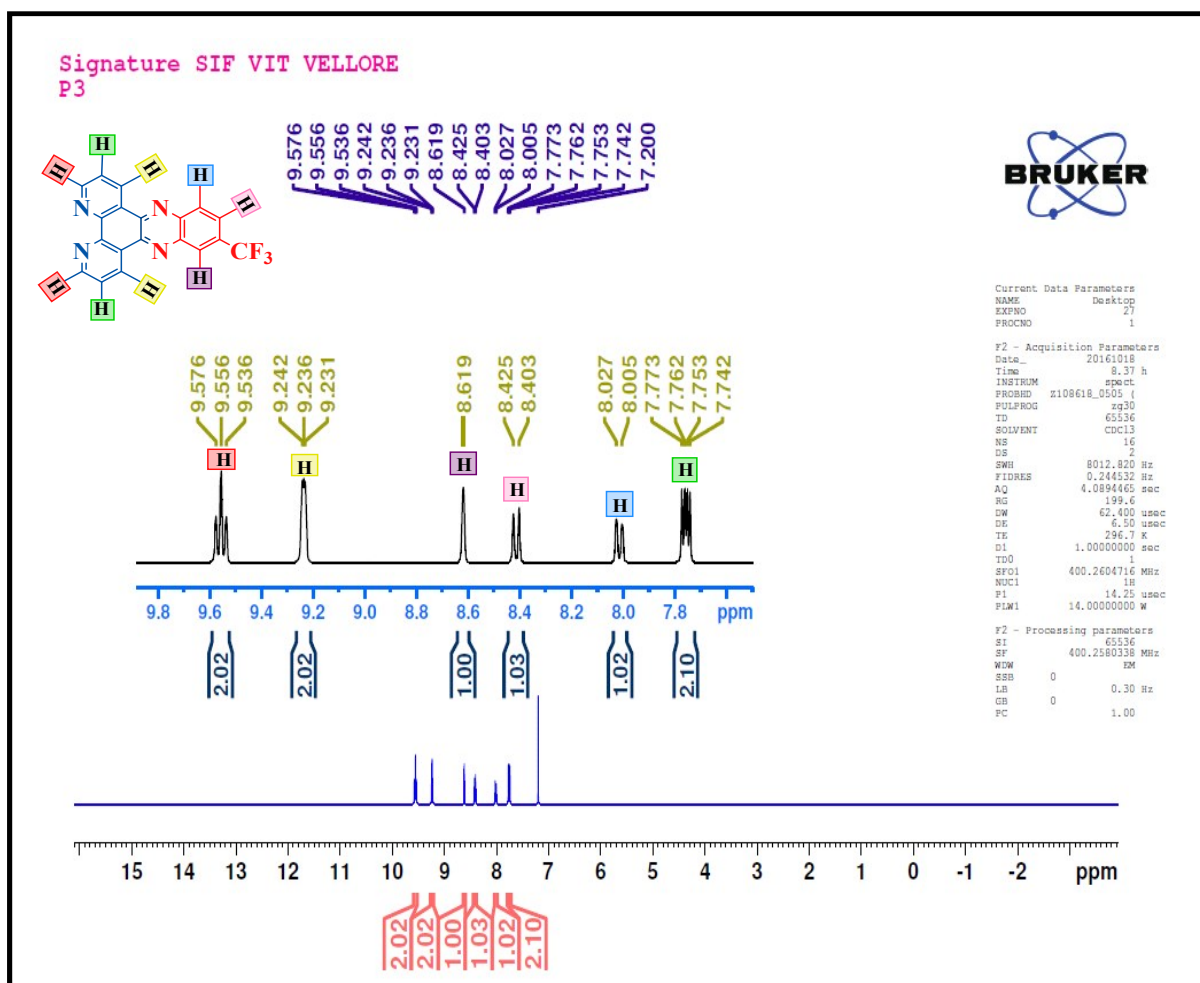


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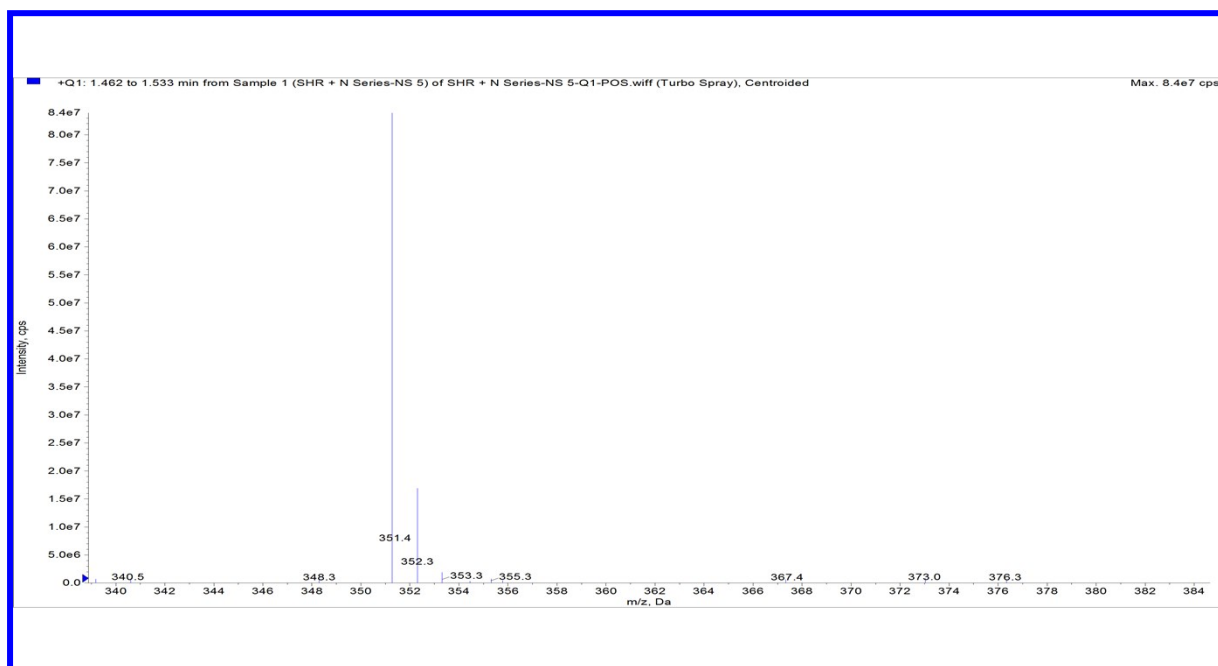
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¹H NMR spectrum of Ligand L5

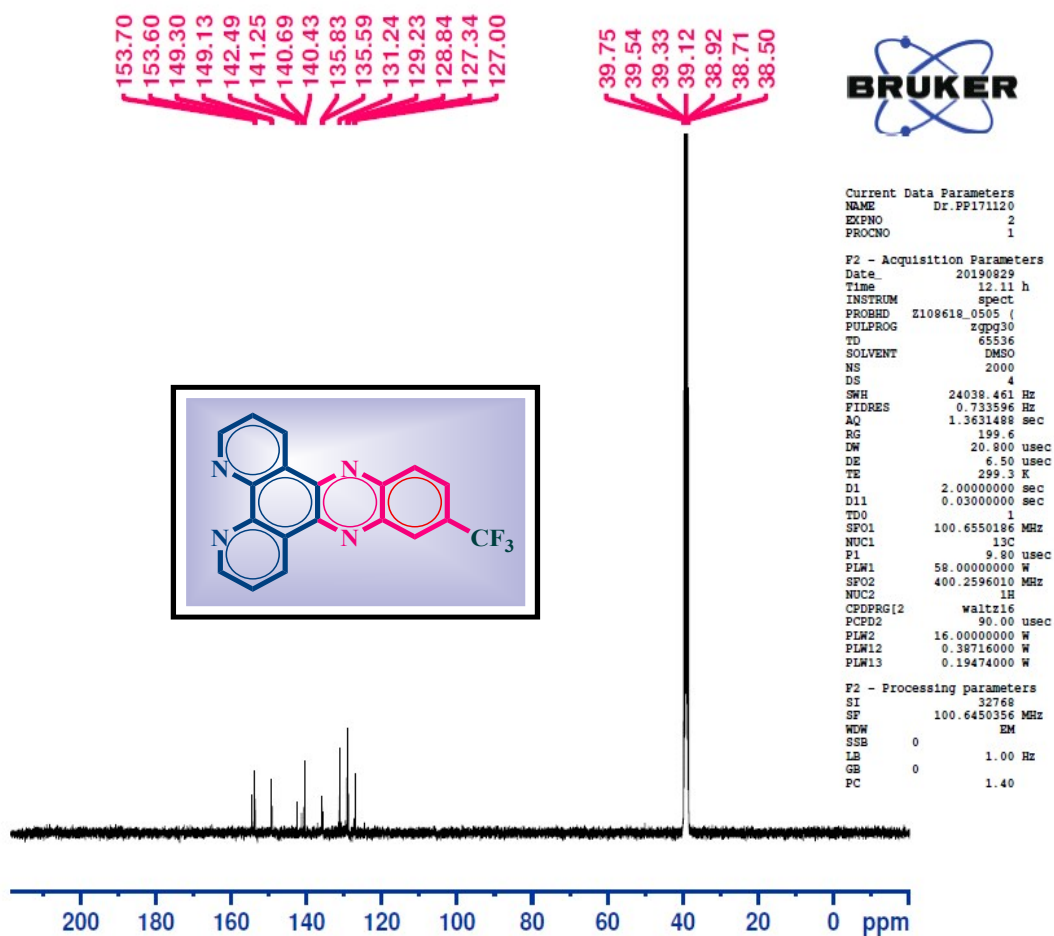


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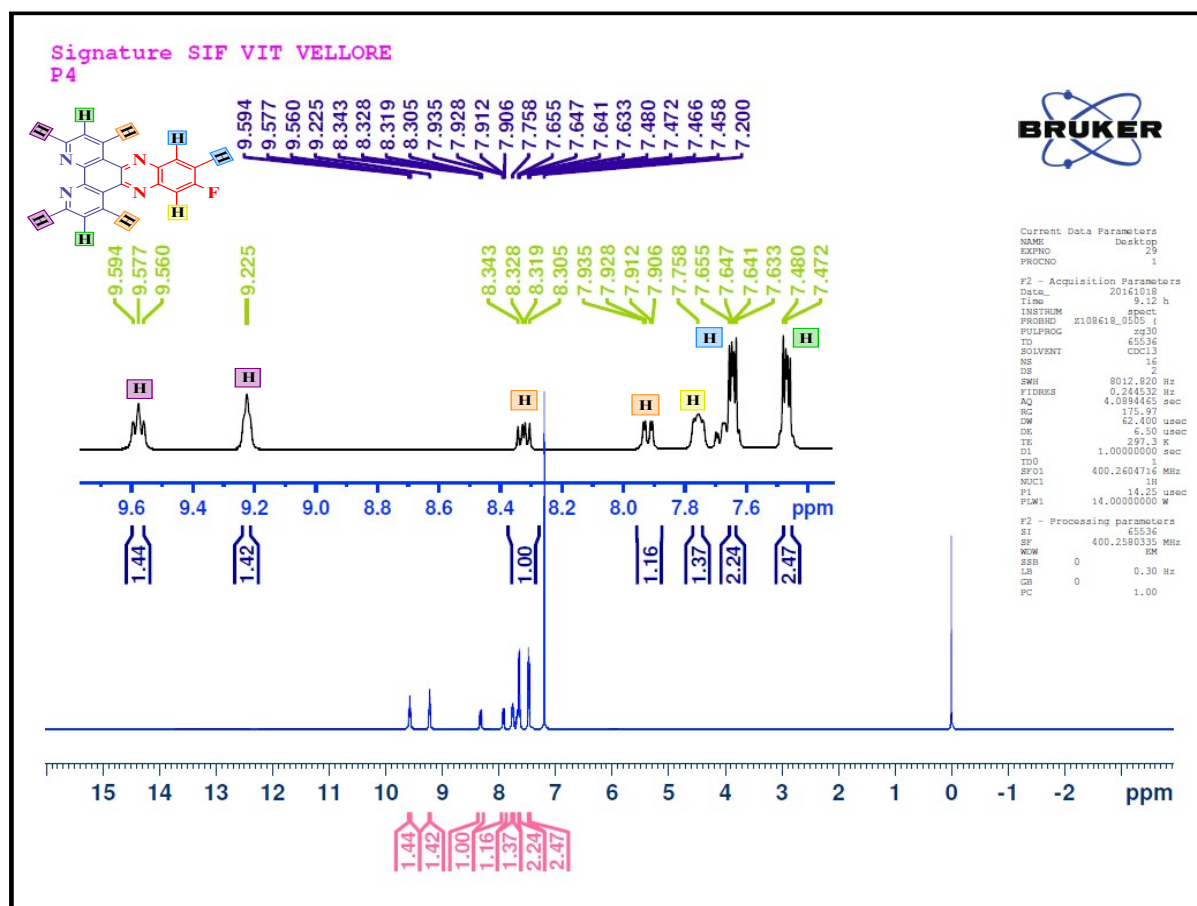


¹³C NMR spectrum of Ligand L5

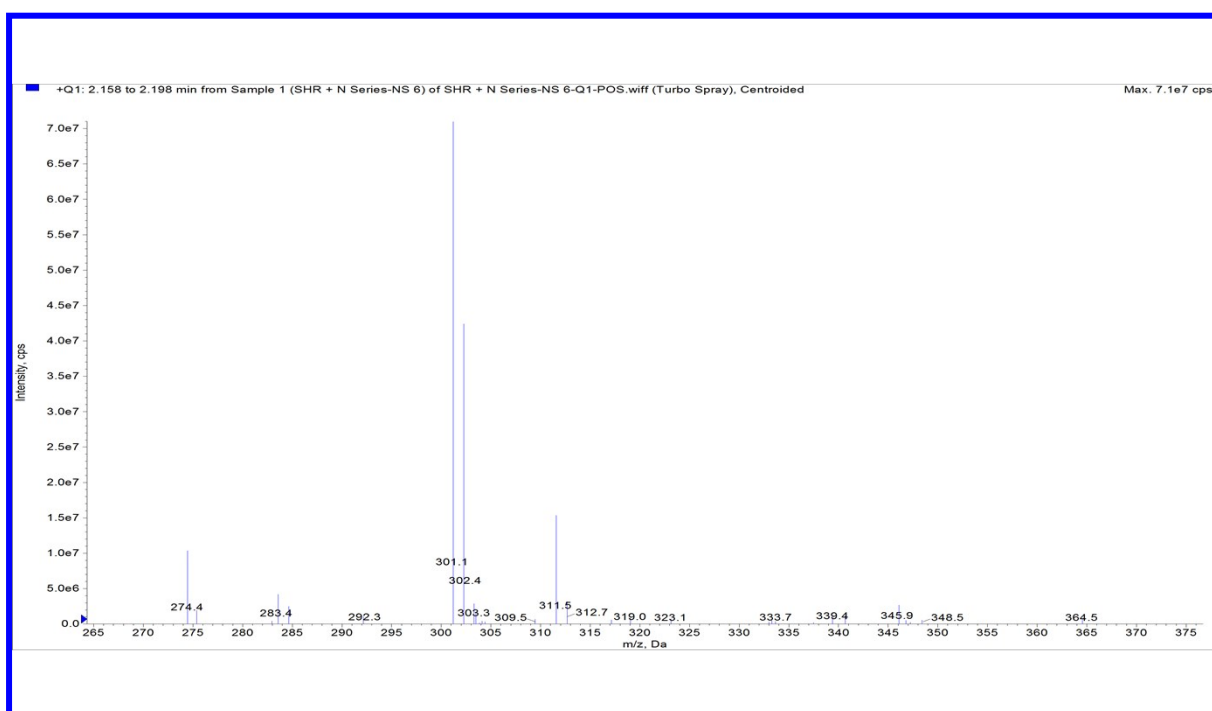
Signature SIF VIT VELLORE
NS-5



¹H NMR spectrum of Ligand L6



ESI-MS of ligand L6

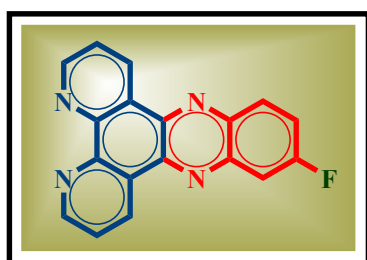


¹³C NMR spectrum of Ligand L6

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NS-6

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153.84
148.67
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139.98
136.01
135.68
131.50
130.26
130.02
128.49
127.29
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110.93

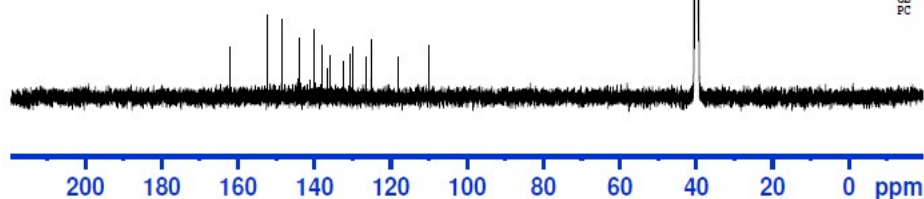
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40.16
39.95
39.74
39.53
39.32



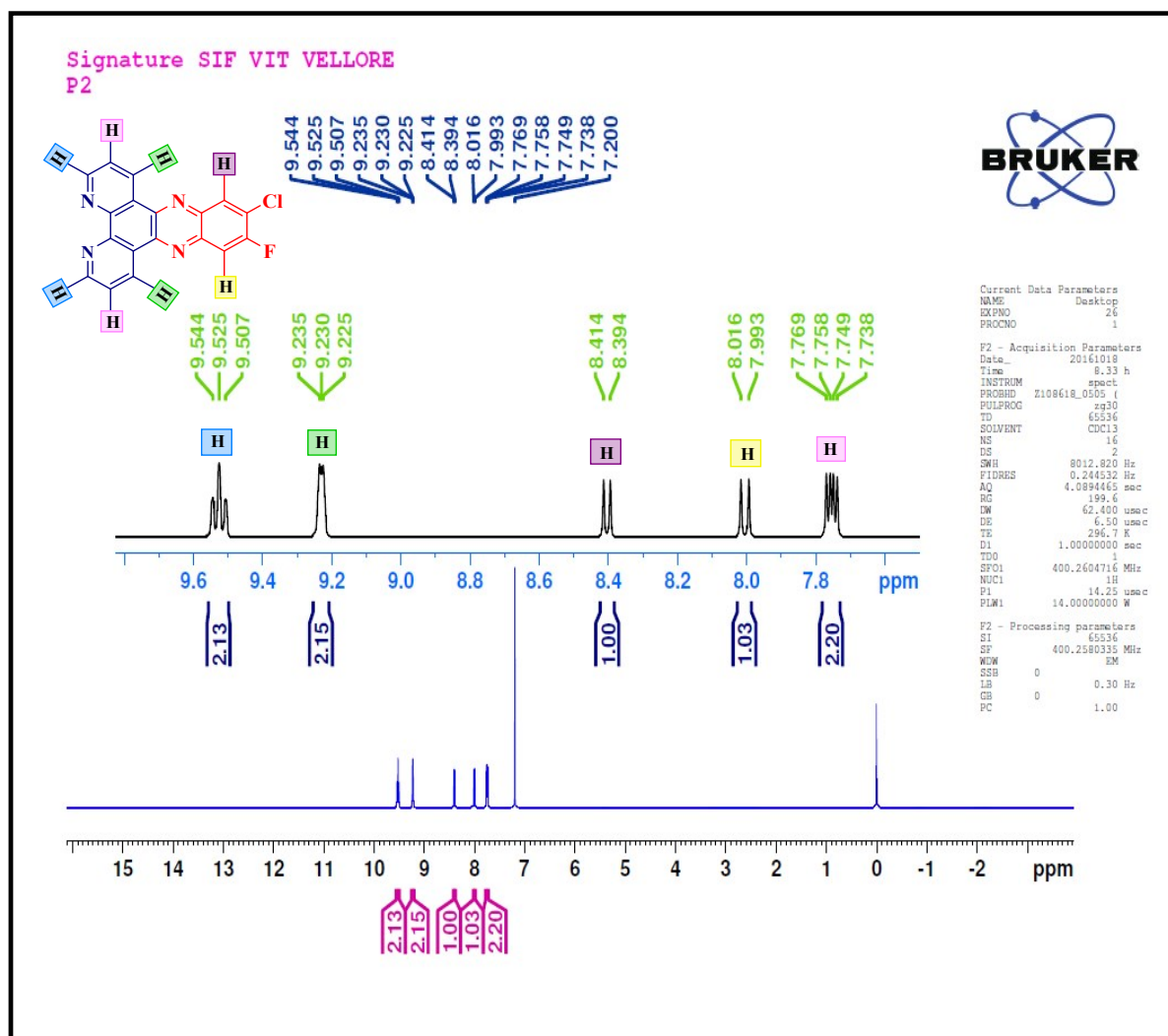
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DE 6.50 usec
TE 301.5 K
D1 2.0000000 sec
D11 0.0300000 sec
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SFO1 100.6550185 MHz
NUC1 13C
F1 10.52 usec
PLW1 58.0000000 W
SFO2 400.2596010 MHz
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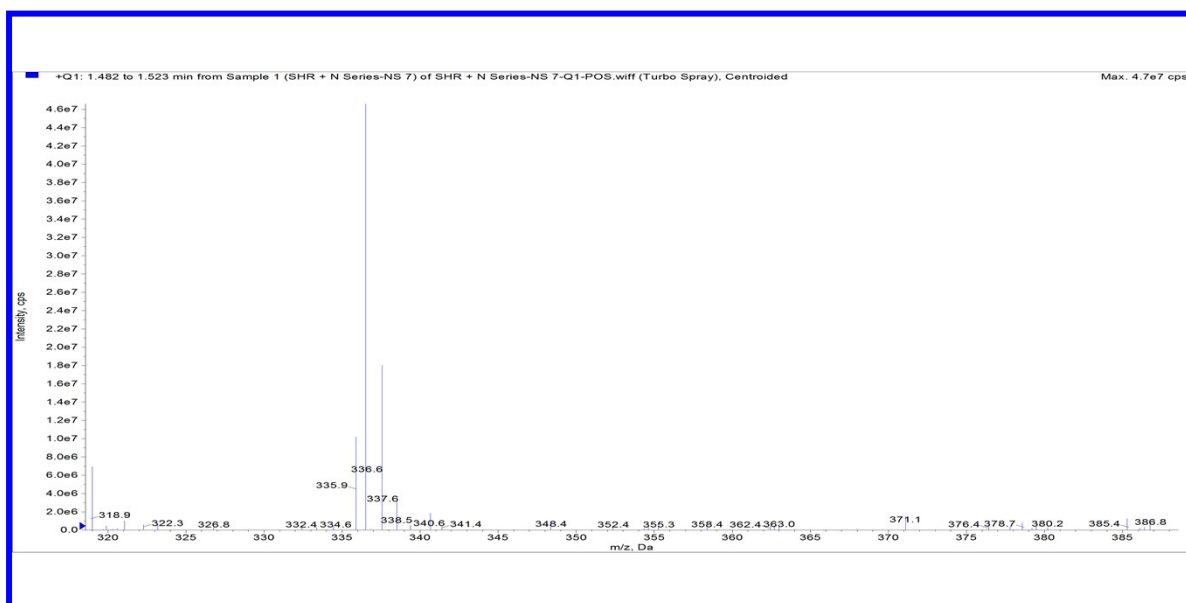
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¹H NMR spectrum of Ligand L7

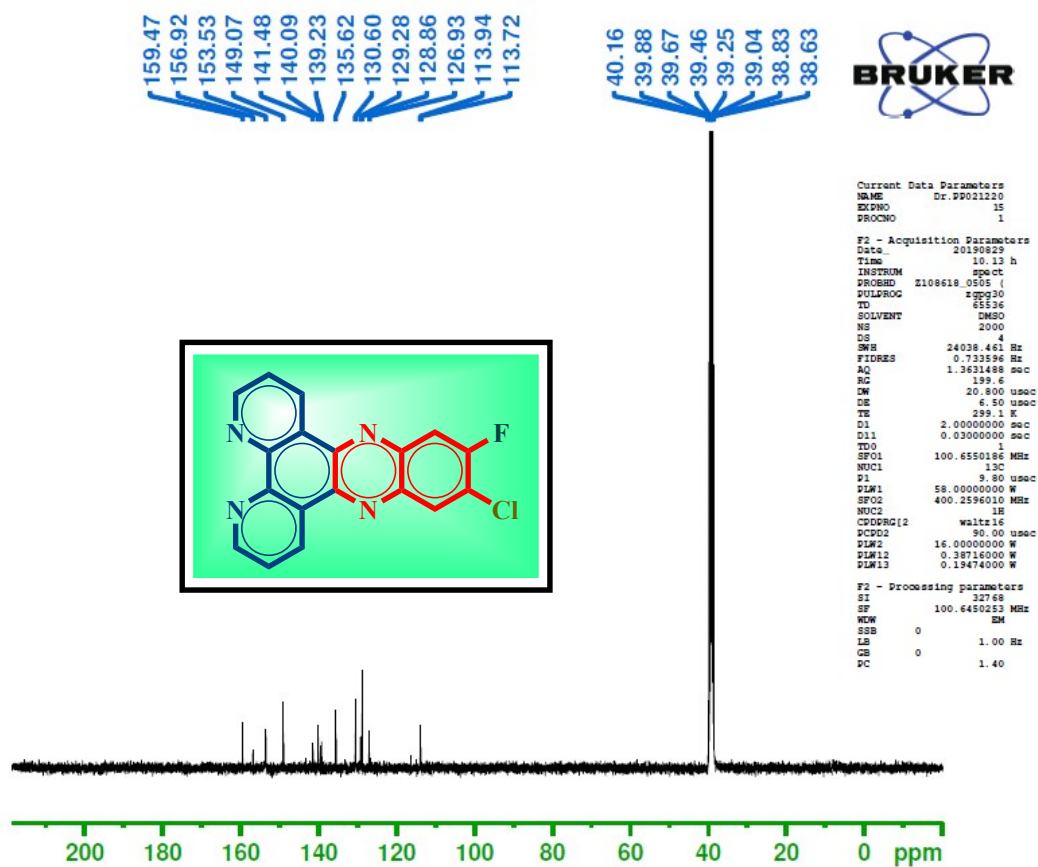


ESI-MS of ligand L7

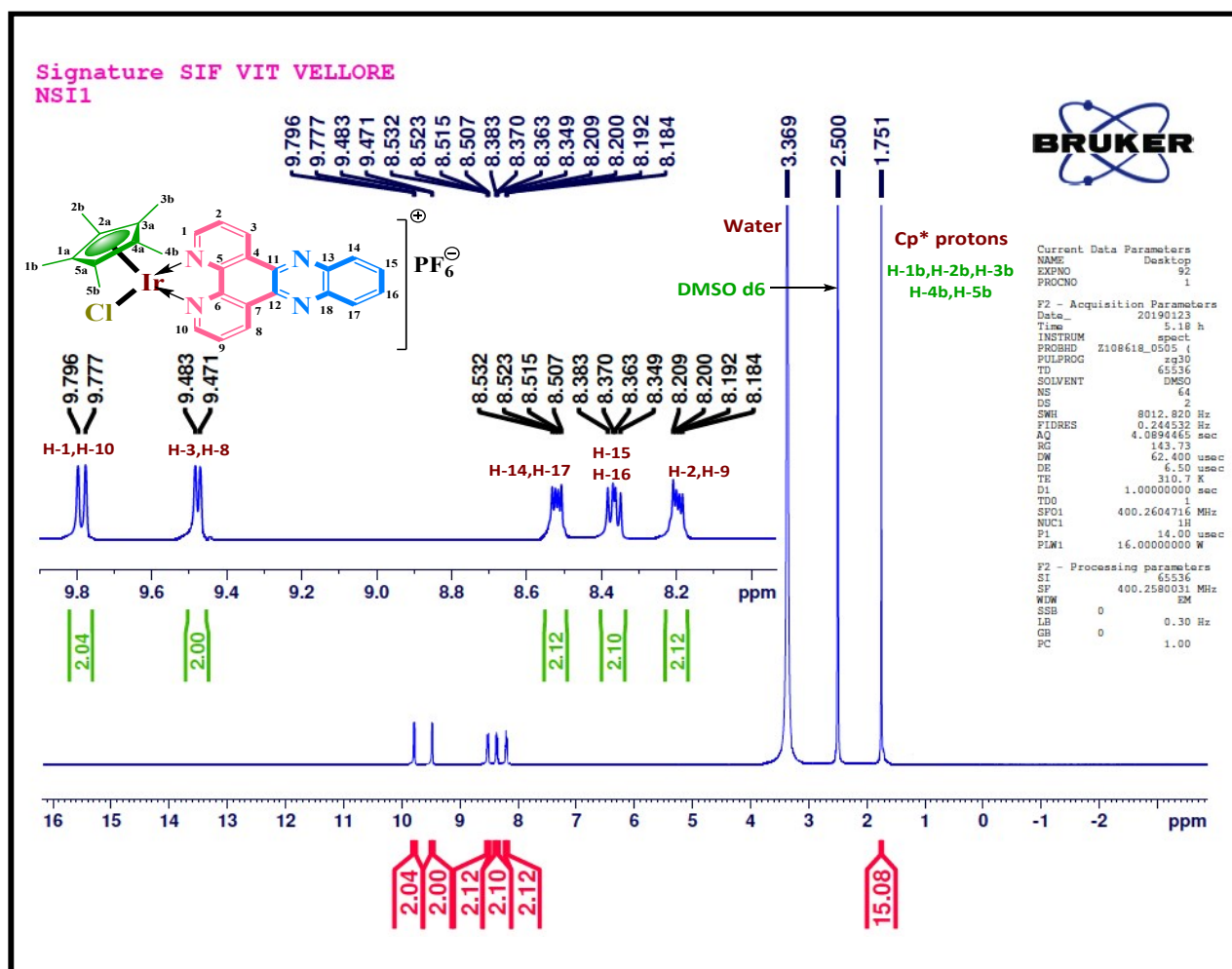


¹³C NMR spectrum of Ligand L7

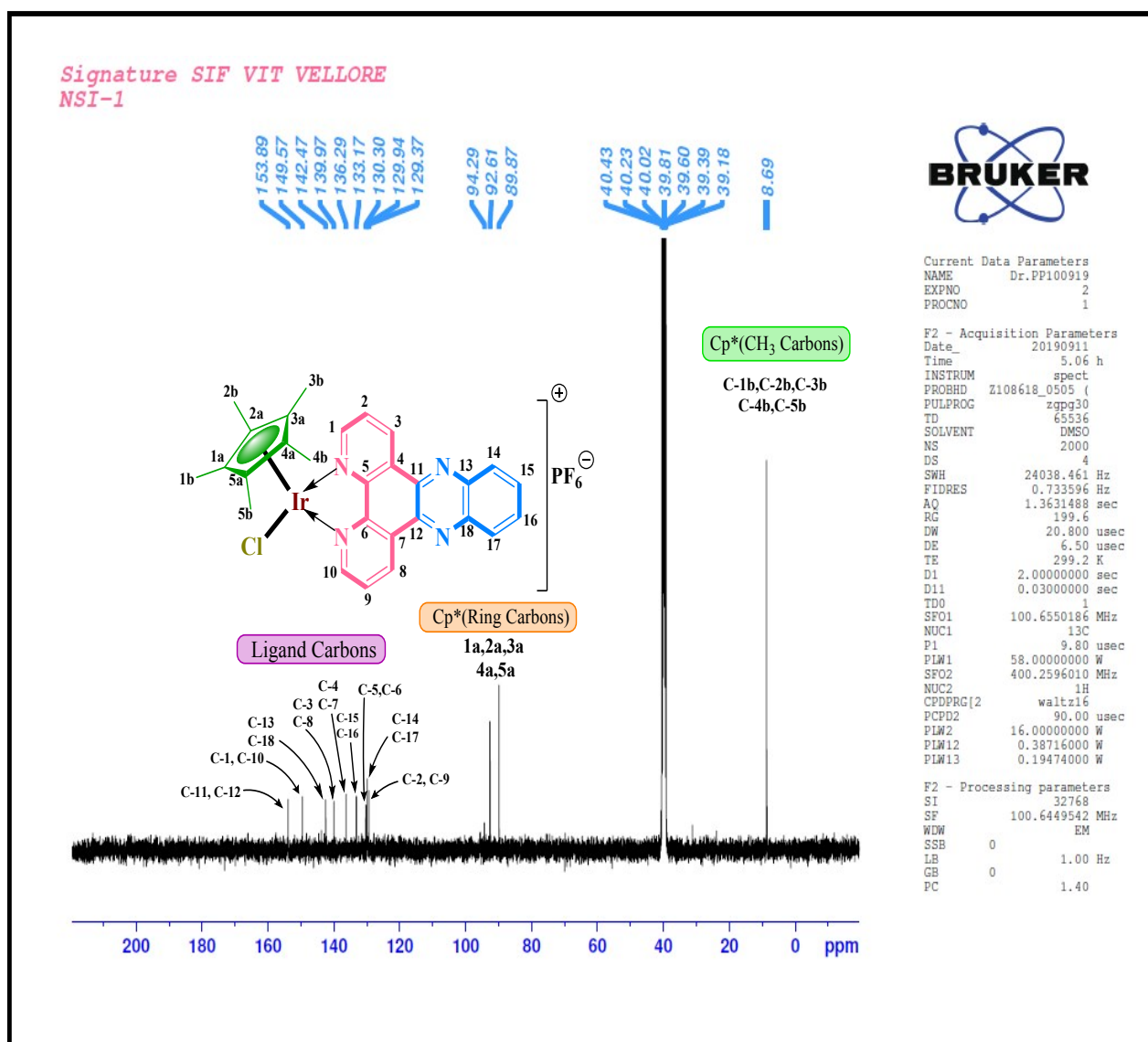
Signature SIF VIT VELLORE
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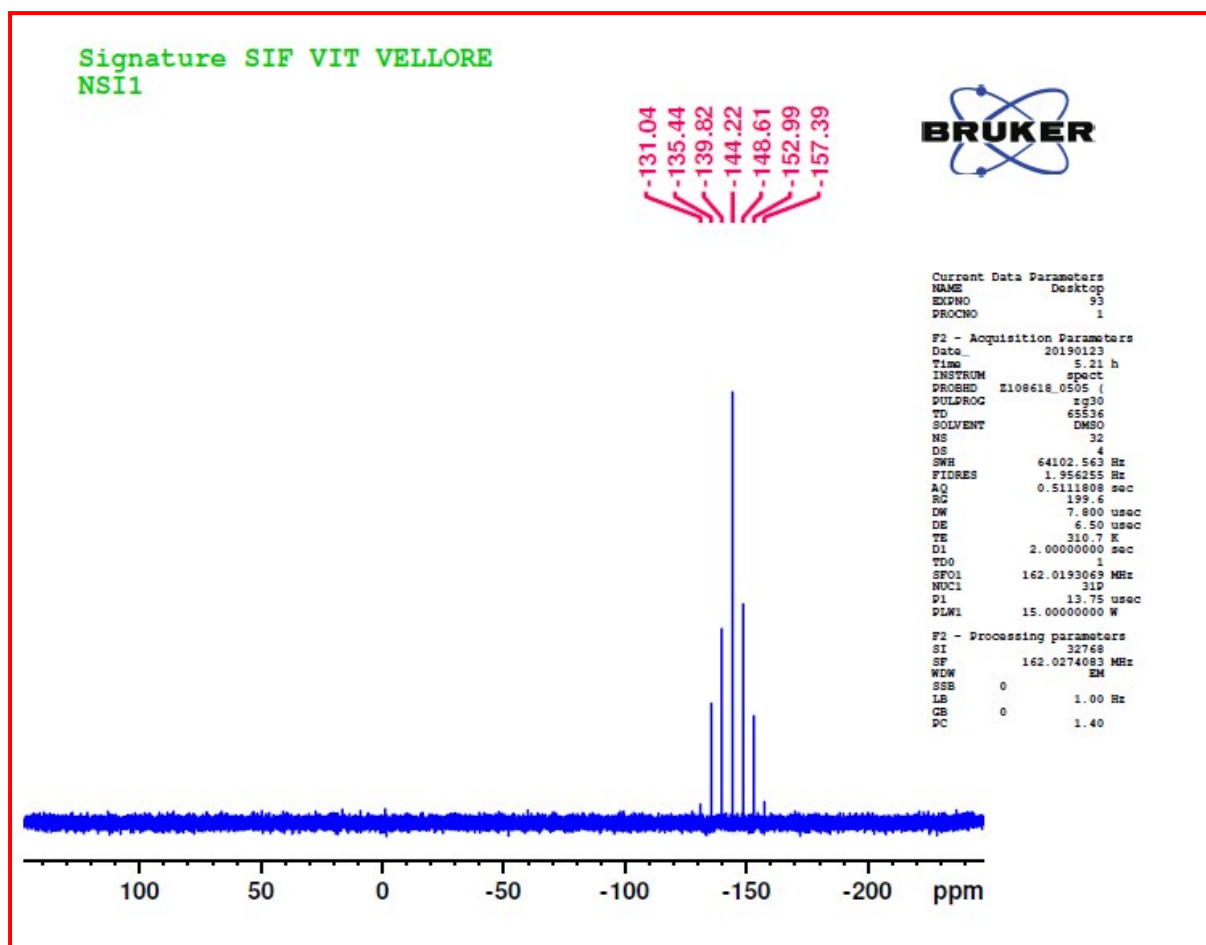
¹H NMR spectrum of Complex IrL1



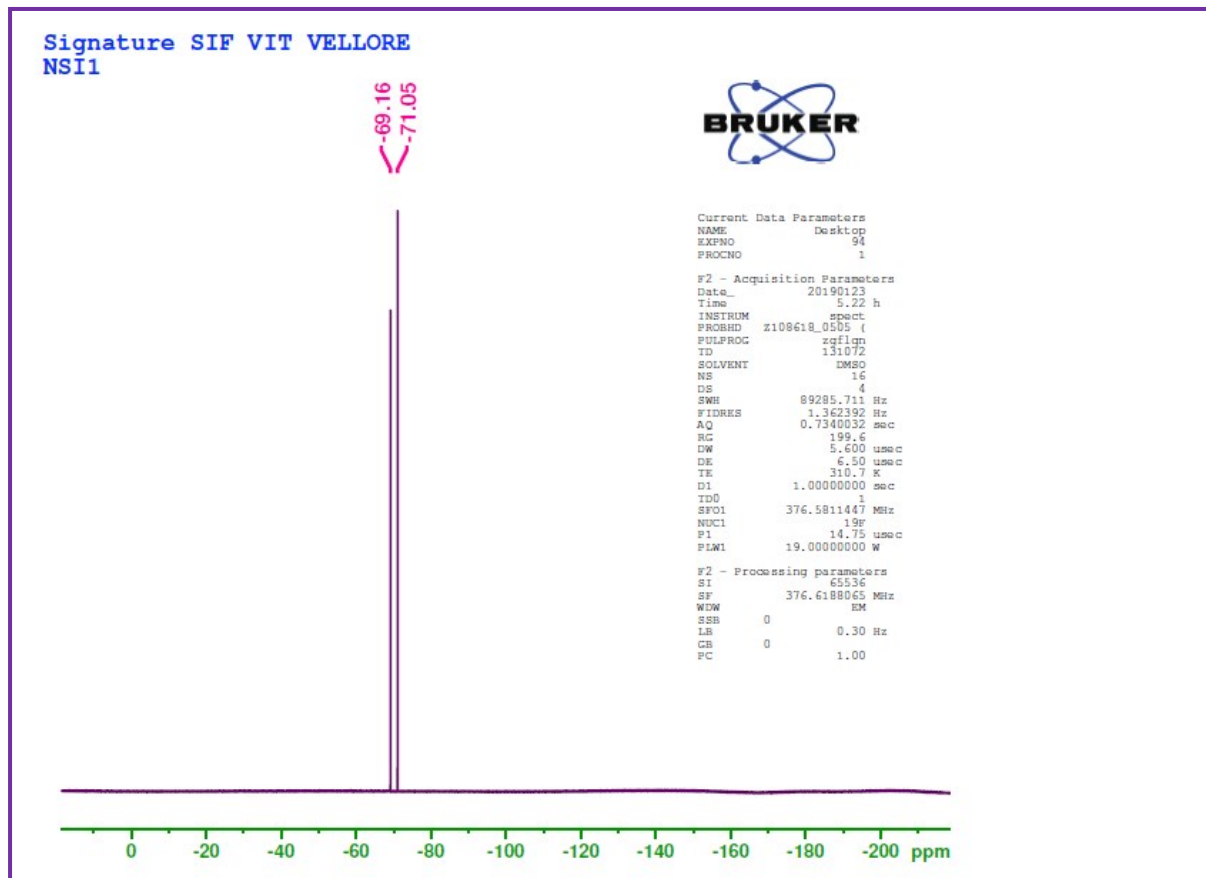
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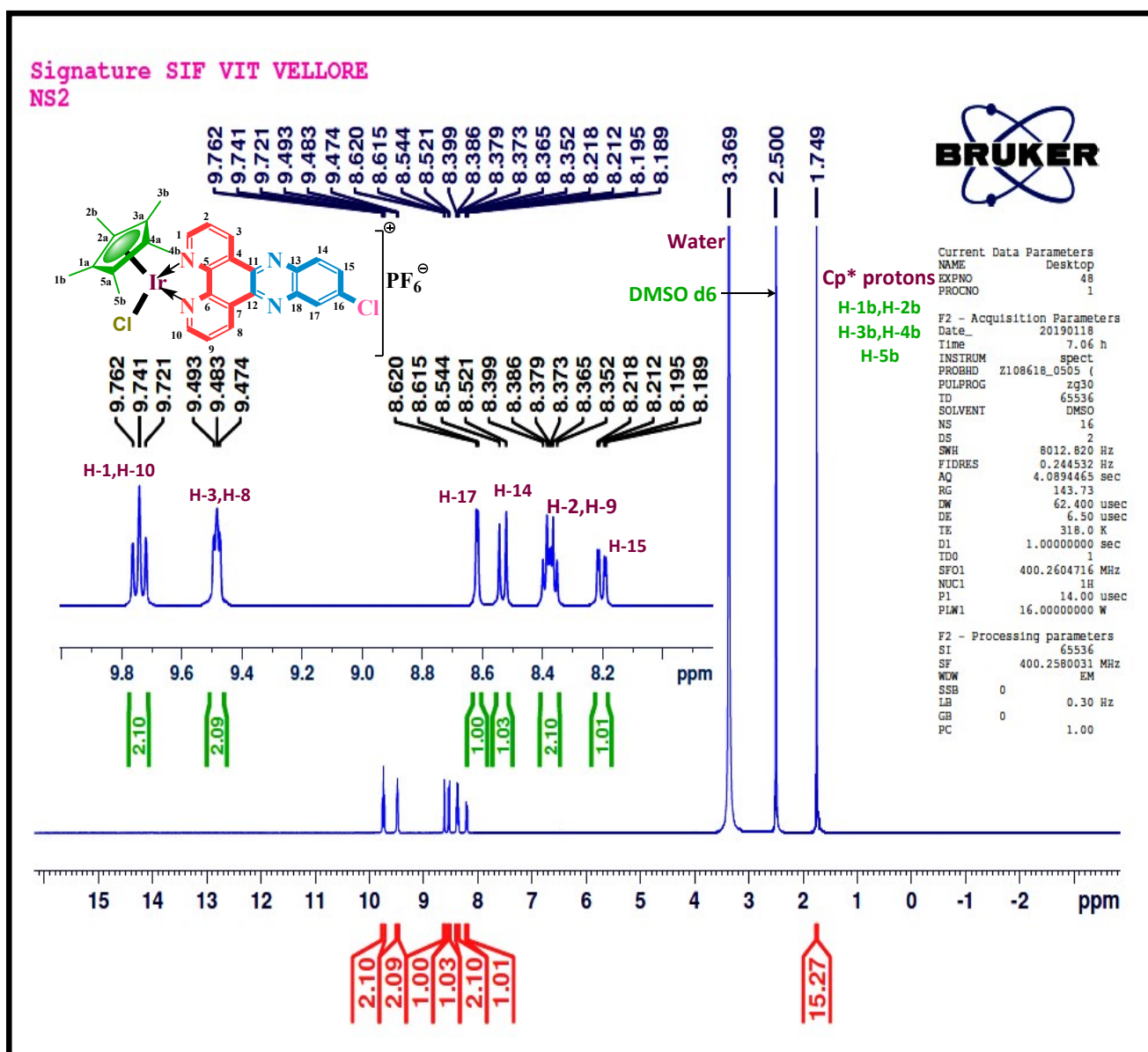
^{31}P NMR Spectrum of Complex IrL1



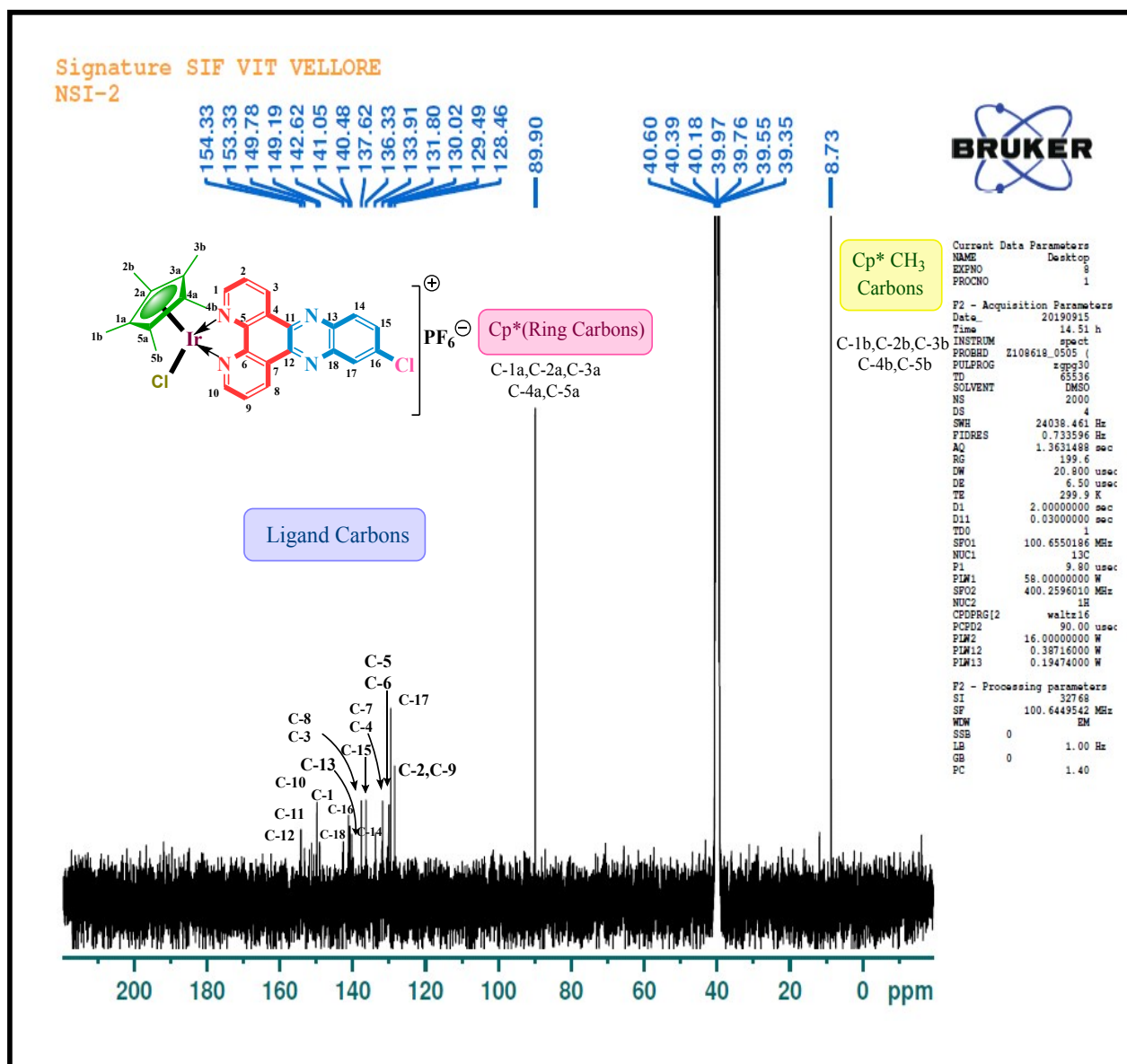
¹⁹F NMR Spectrum of Complex IrL1



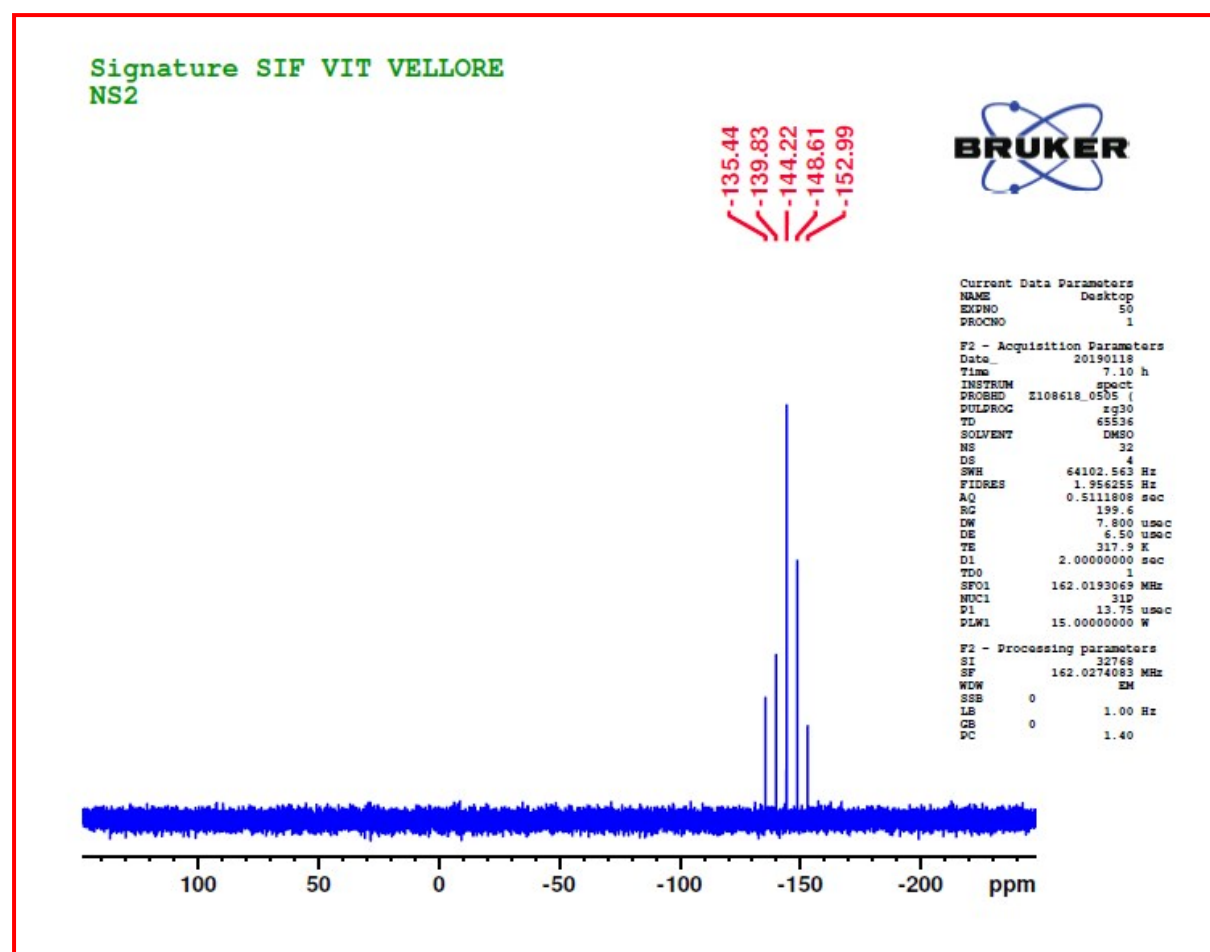
¹H NMR spectrum of Complex IrL2



¹³C NMR spectrum of Complex IrL2

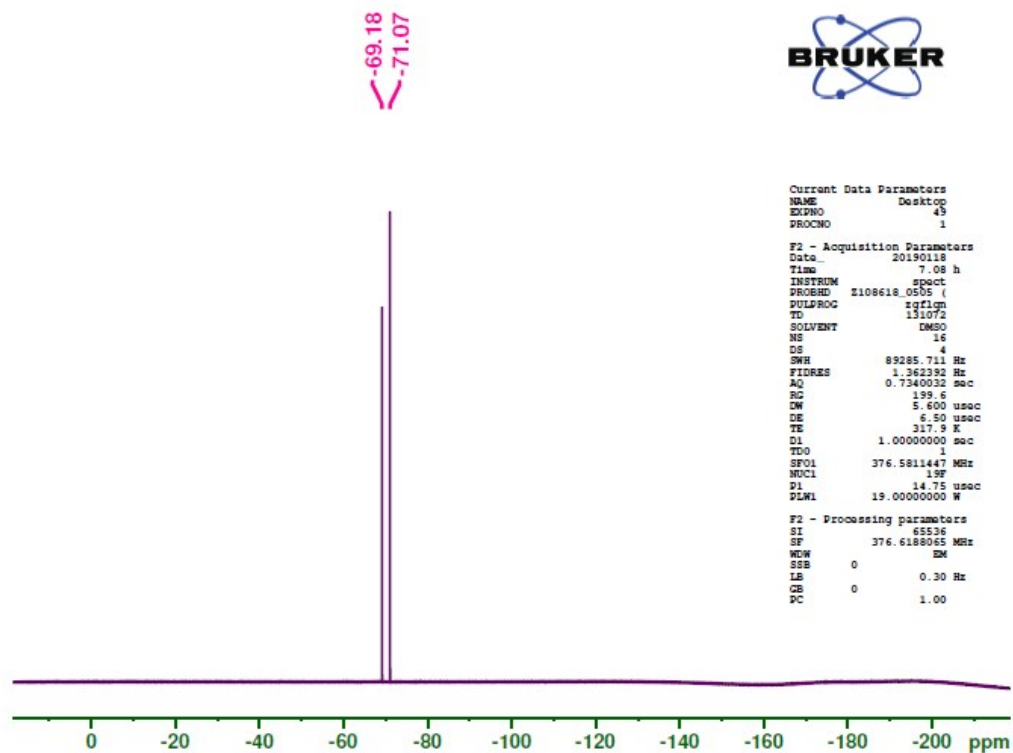


³¹P NMR Spectrum of Complex IrL2

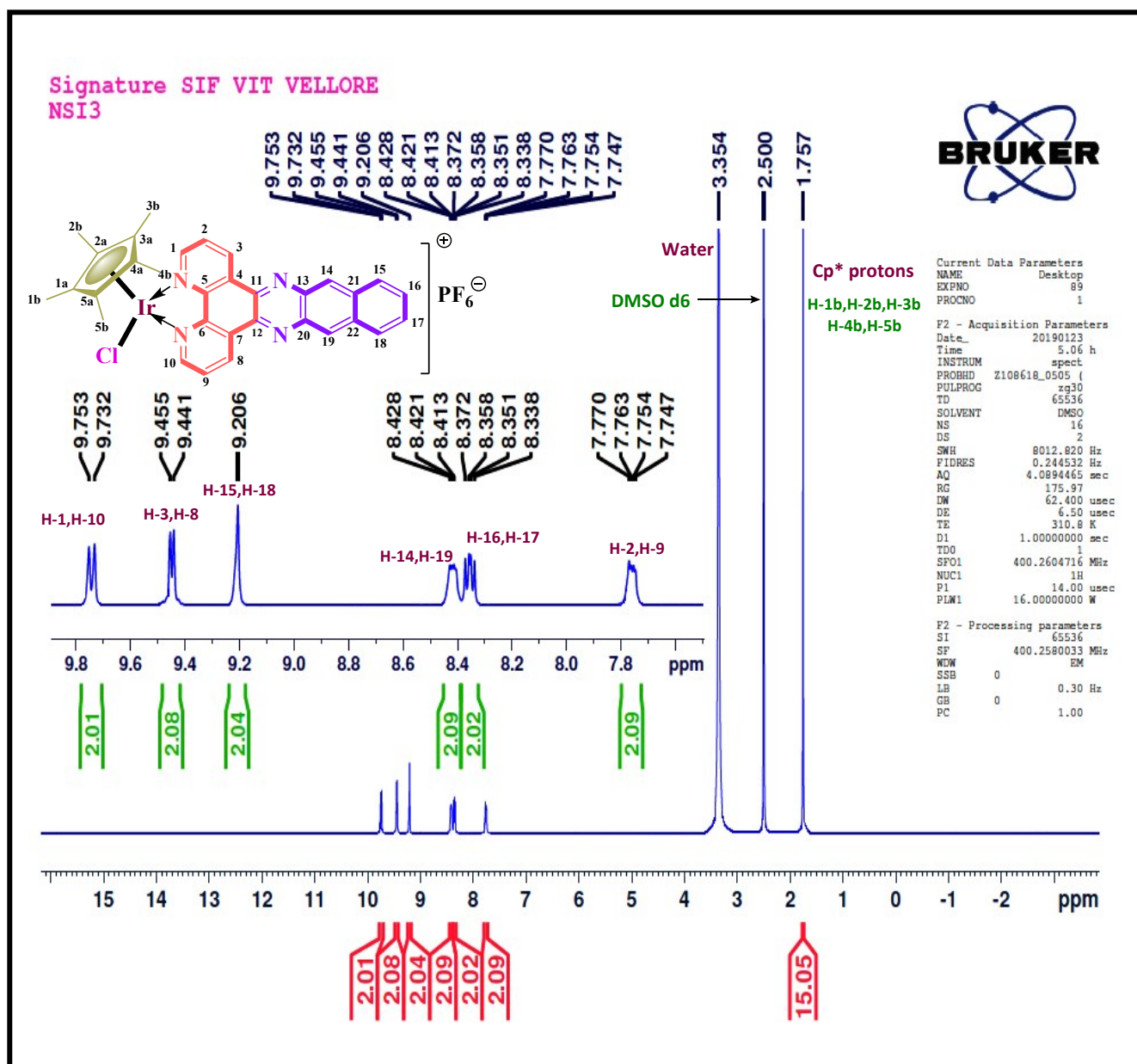


¹⁹F NMR Spectrum of Complex IrL2

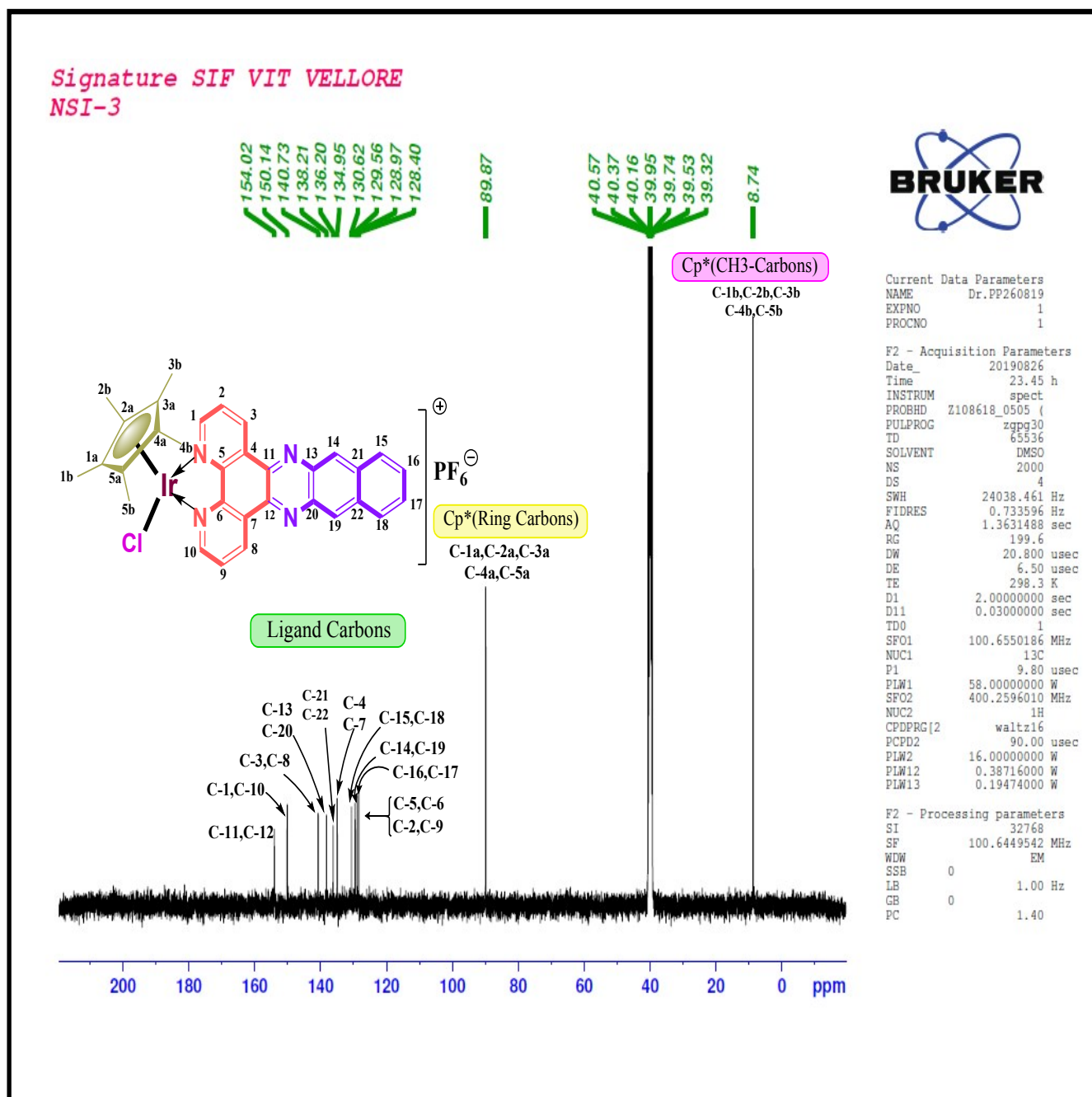
Signature SIF VIT VELLORE
NS2



¹H NMR spectrum of Complex IrL3



¹³C NMR spectrum of Complex IrL3



^{31}P NMR Spectrum of Complex IrL3

Signature SIF VIT VELLORE
NSI3

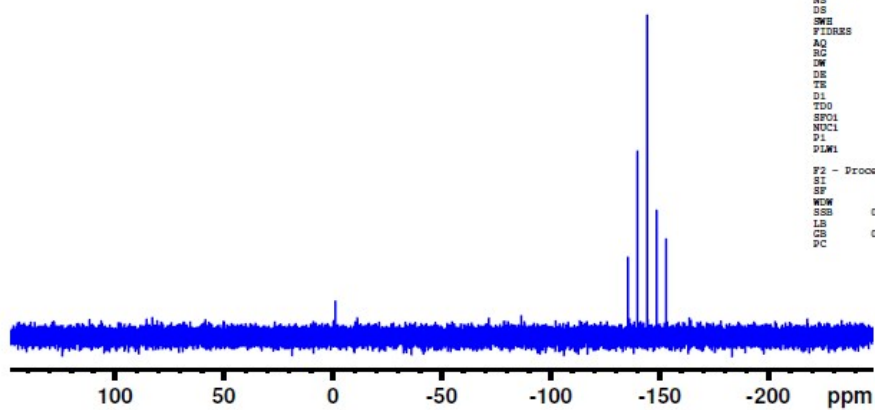
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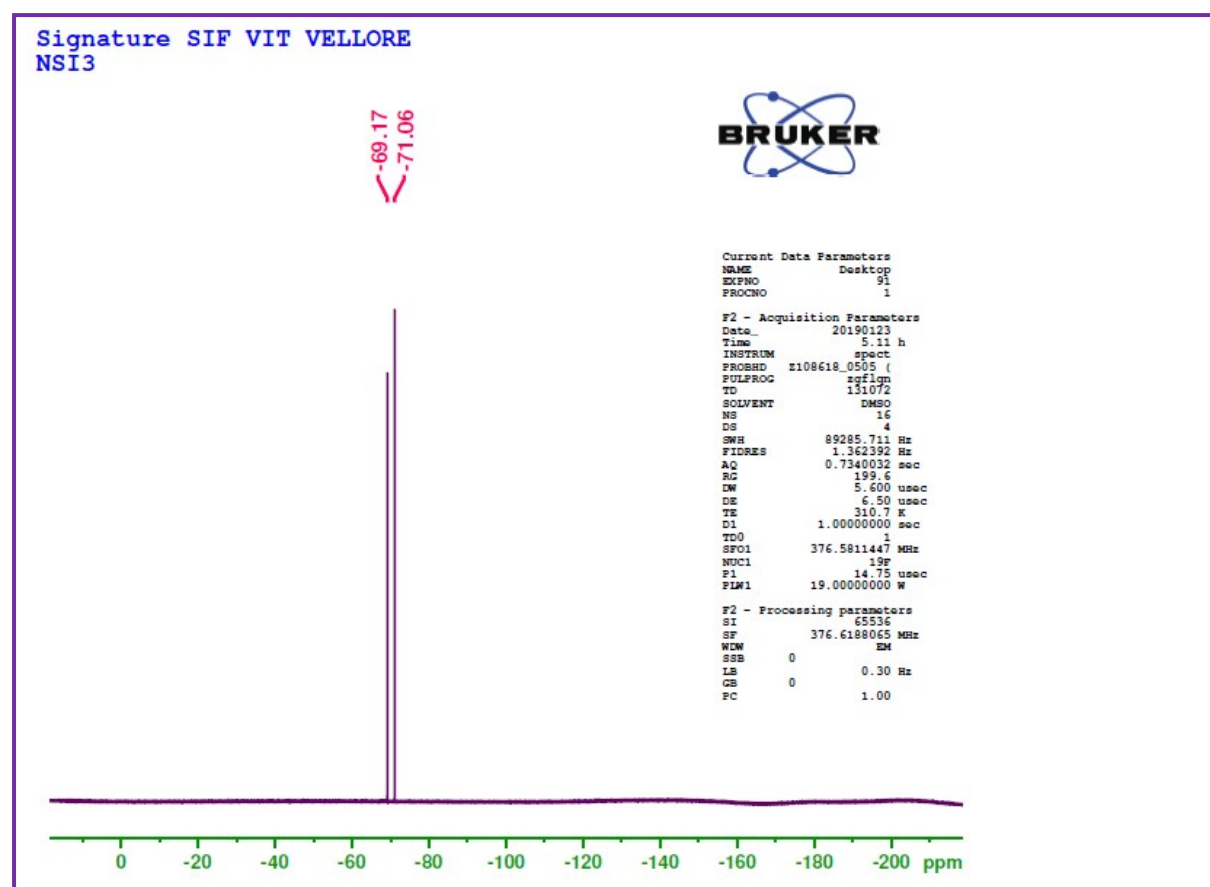
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RG 399.6
DW 7.800 usec
DE 6.50 usec
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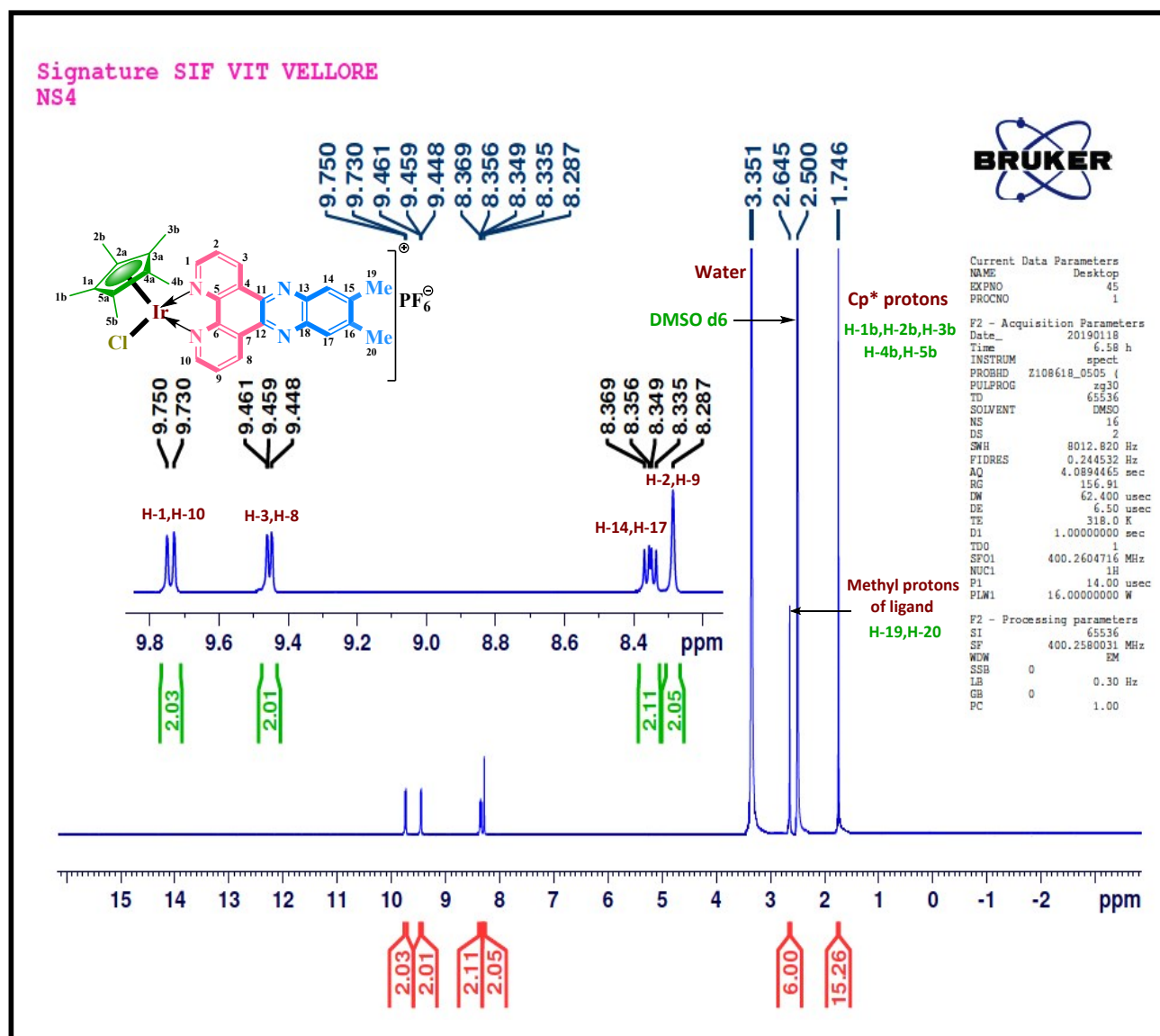
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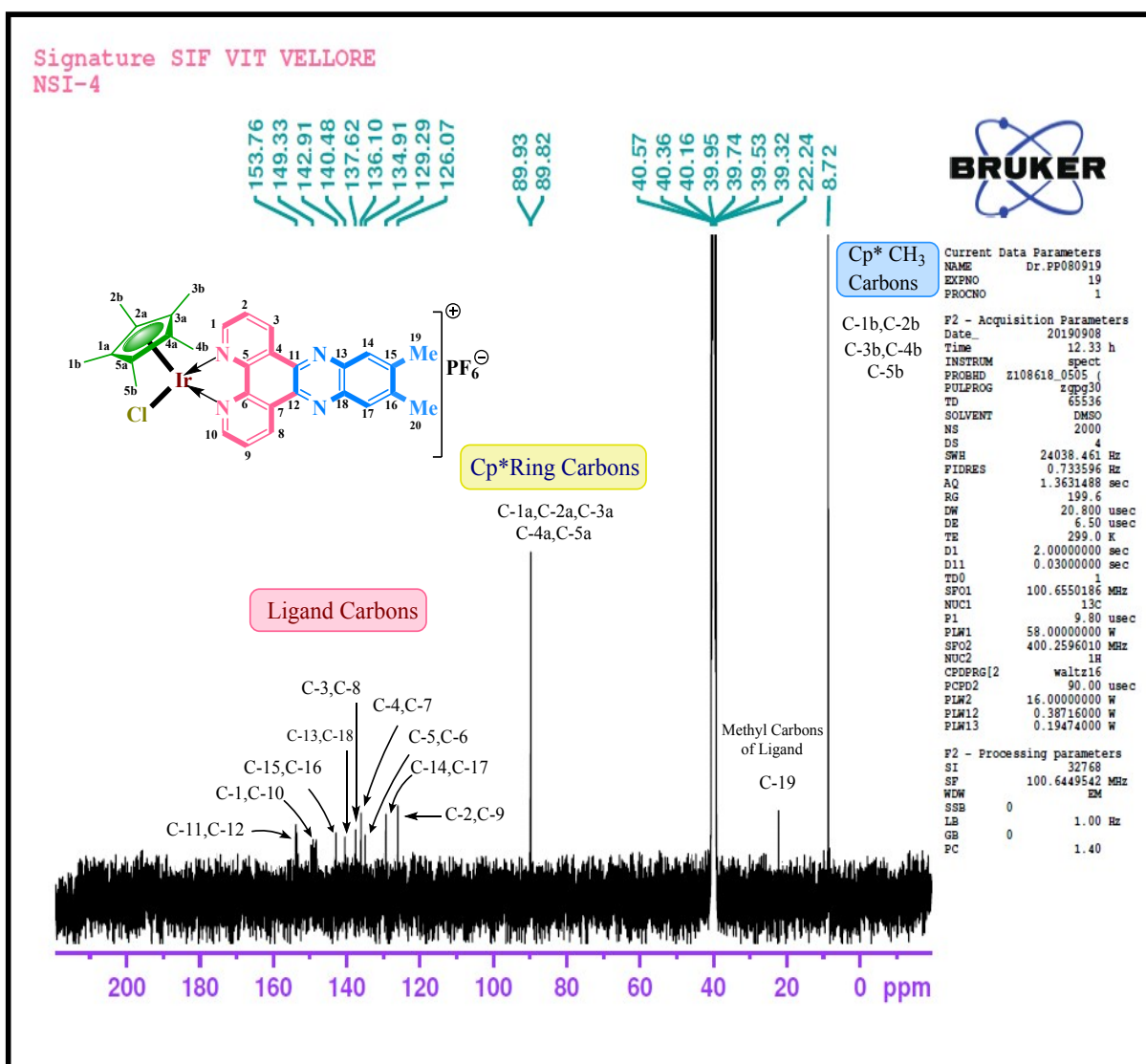
¹⁹F NMR Spectrum of Complex IrL3



¹H NMR spectrum of Complex IrL4



¹³C NMR spectrum of Complex IrL4



^{31}P NMR Spectrum of Complex IrL4

Signature SIF VIT VELLORE
NS4

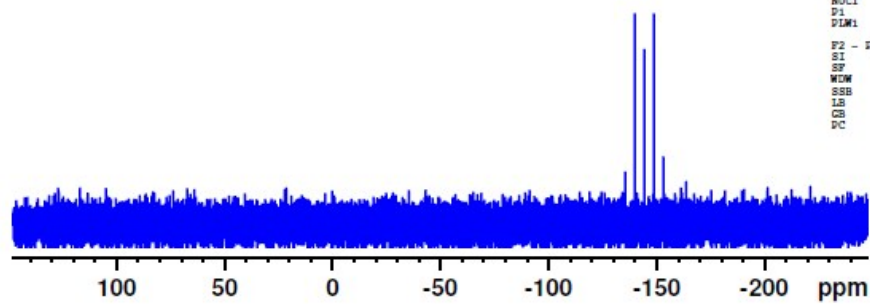
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SOLVENT DMSO
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FIDRES 1.956255 Hz
AQ 0.5111808 sec
RG 199.6
RW 7.800 usec
DE 6.50 usec
TE 317.9 K
D1 2.00000000 sec
TD0 1
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F2 - Processing parameters
SI 32768
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¹⁹F NMR Spectrum of Complex IrL4

Signature SIF VIT VELLORE
NS4

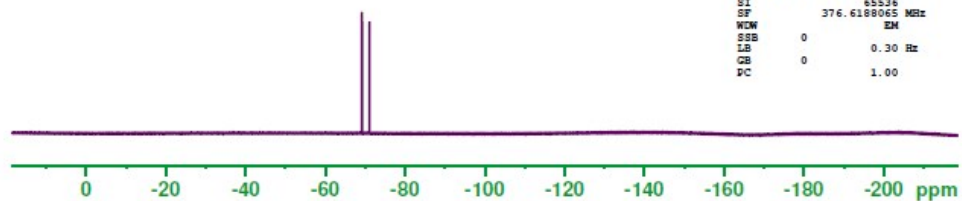
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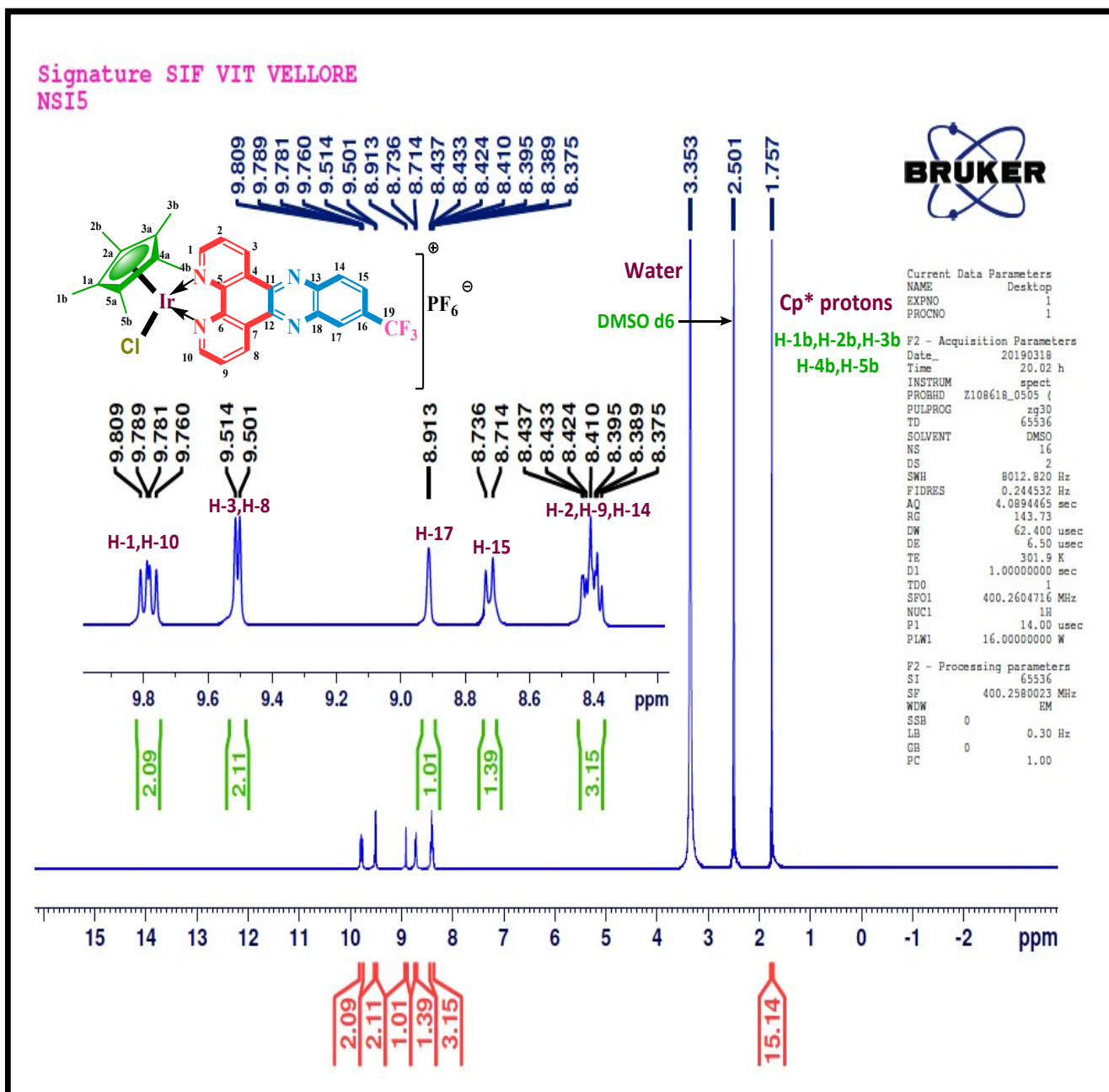
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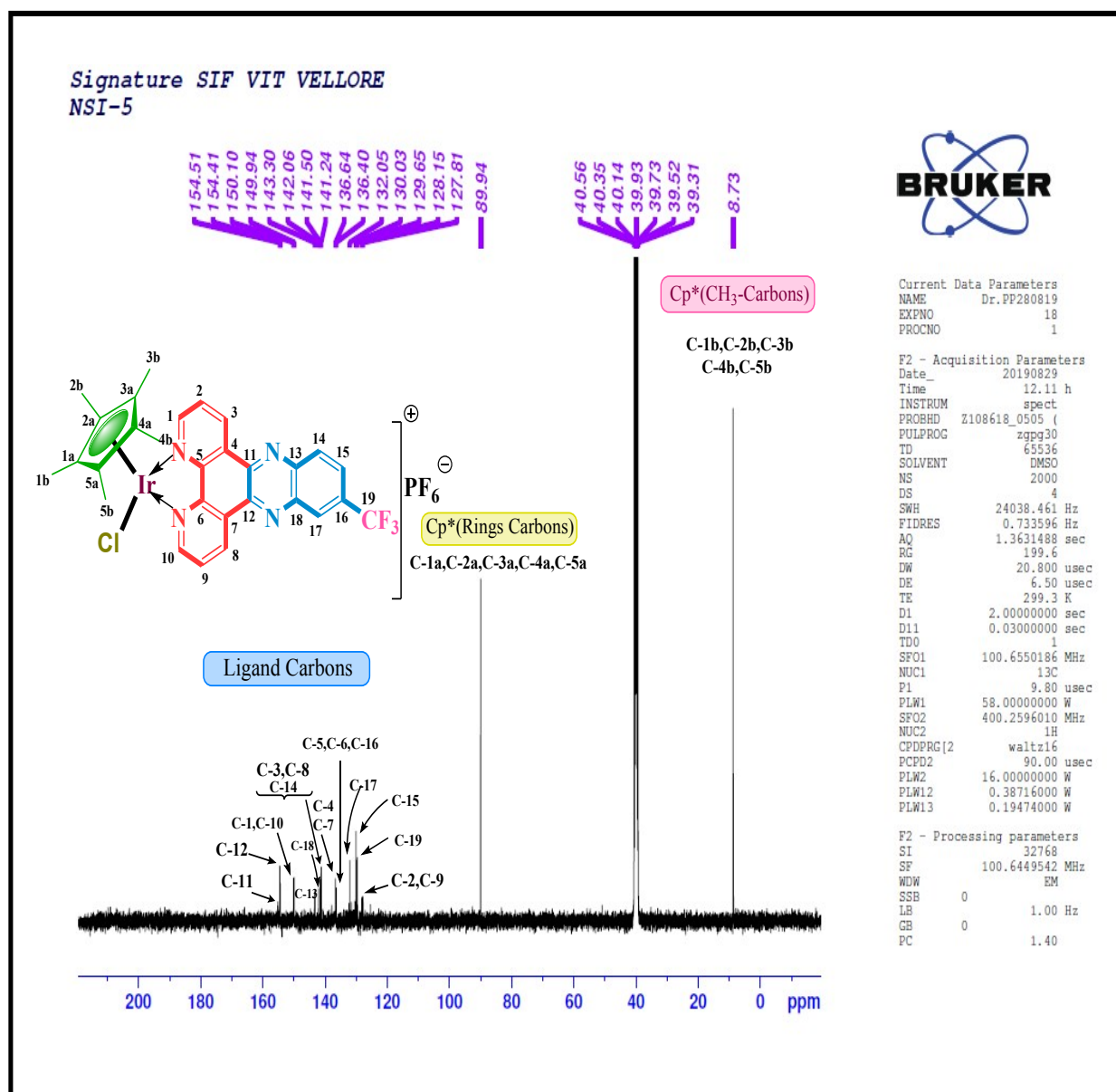
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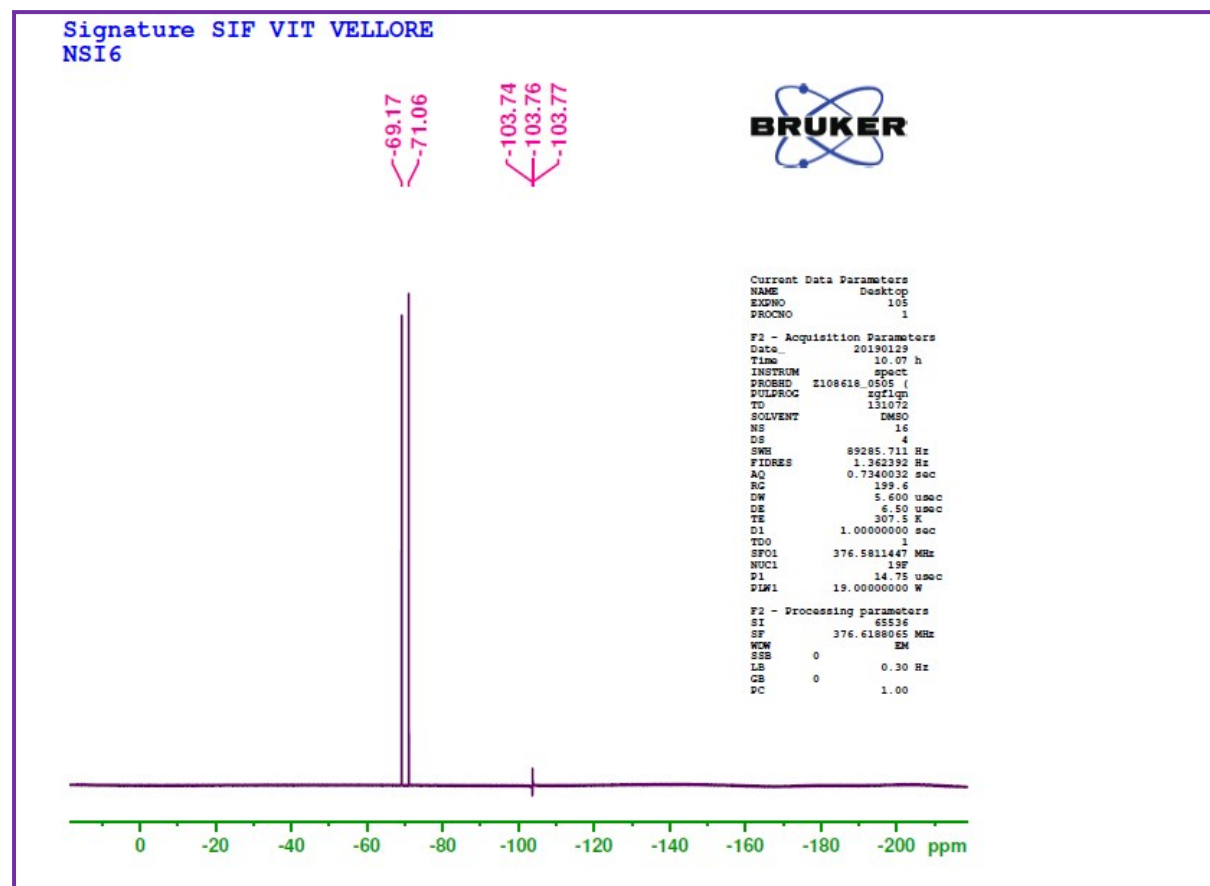
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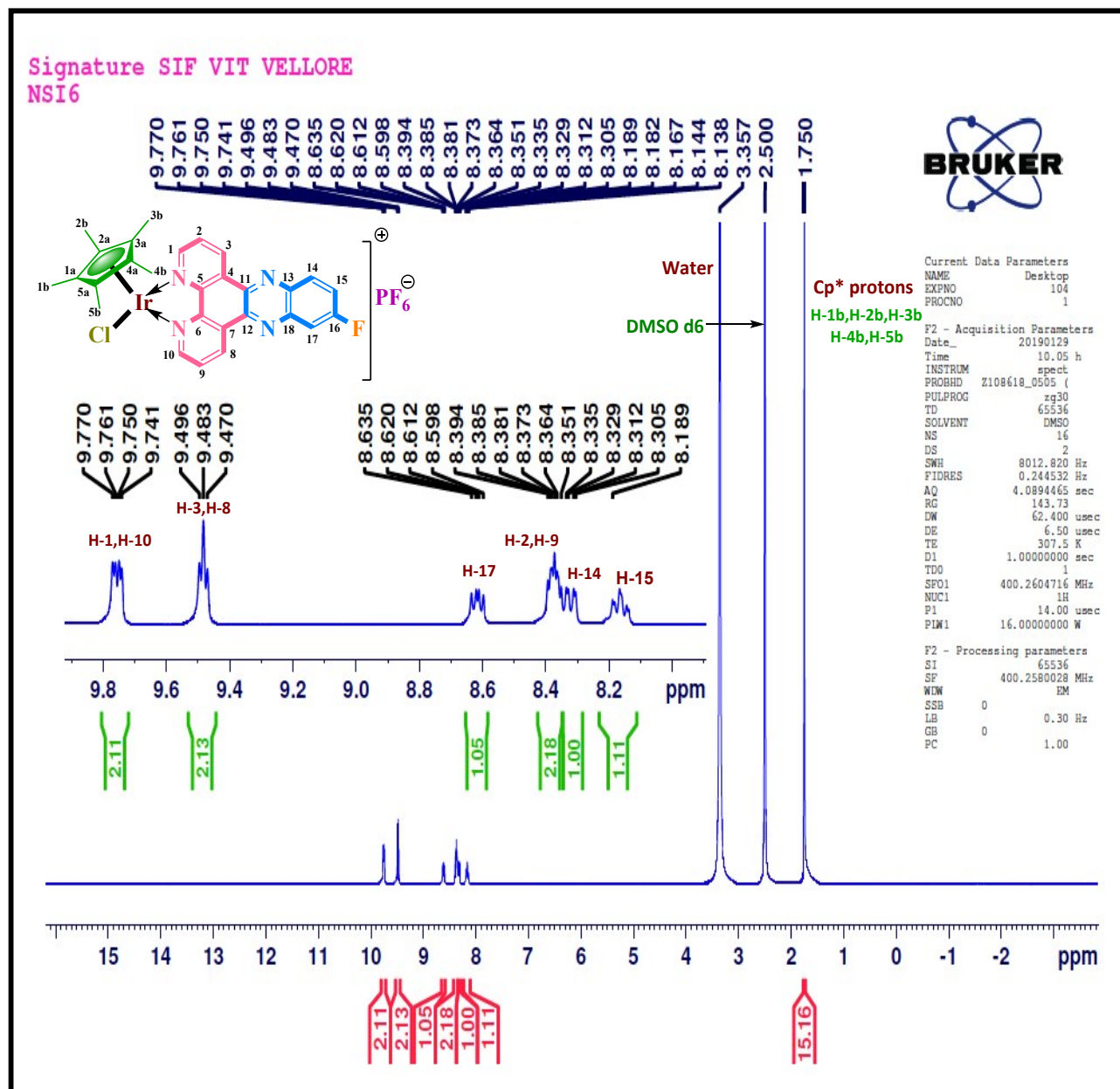
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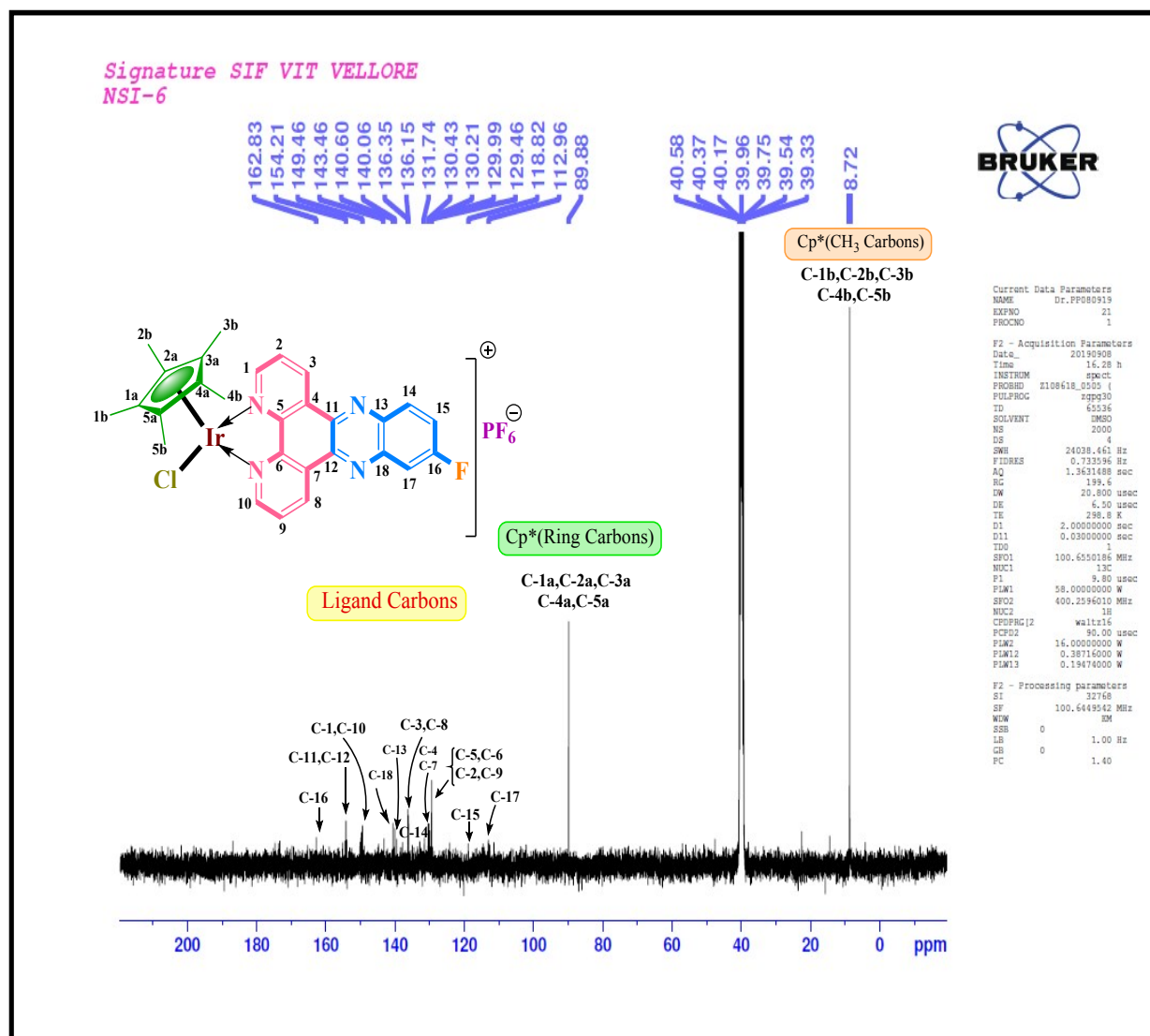
¹⁹F NMR Spectrum of Complex IrL5



¹H NMR spectrum of Complex IrL6



¹³C NMR spectrum of Complex IrL6



^{31}P NMR Spectrum of Complex IrL6

Signature SIF VIT VELLORE
NSI6

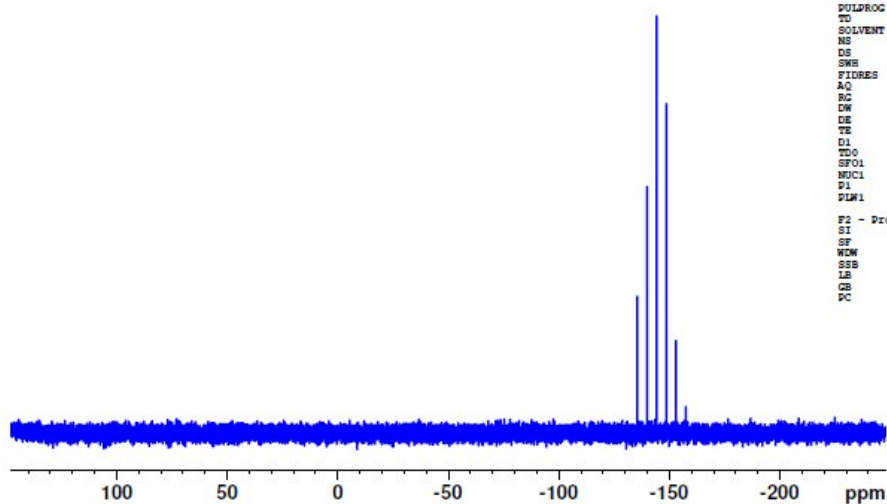
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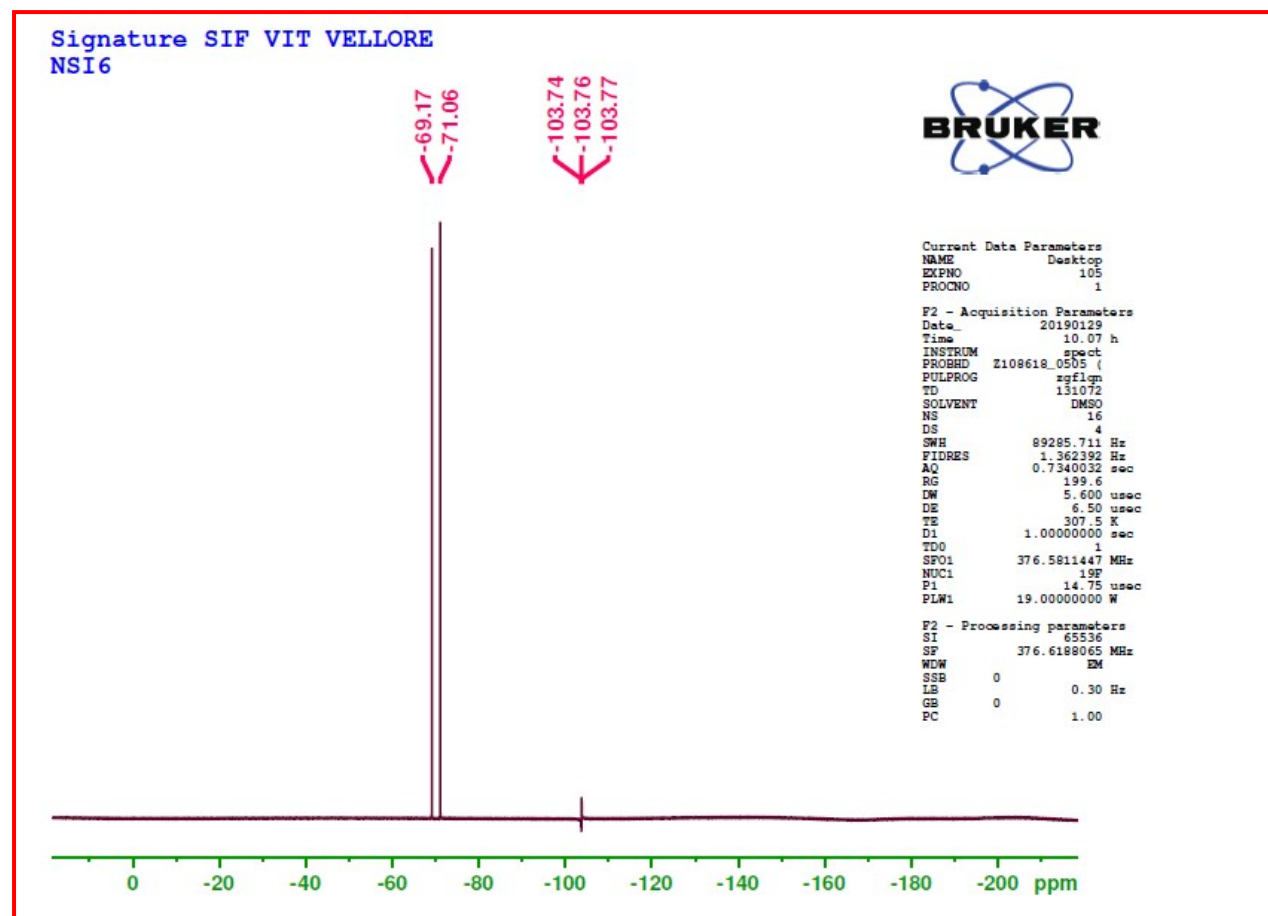
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FIDRES 1.956255 Hz
AQ 0.5111808 sec
RG 199.6
DW 7.800 usec
DE 6.50 usec
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TDO 1
SFO1 162.0193069 MHz
NUC1 ^{31}P
P1 13.75 usec
PLW1 15.00000000 W

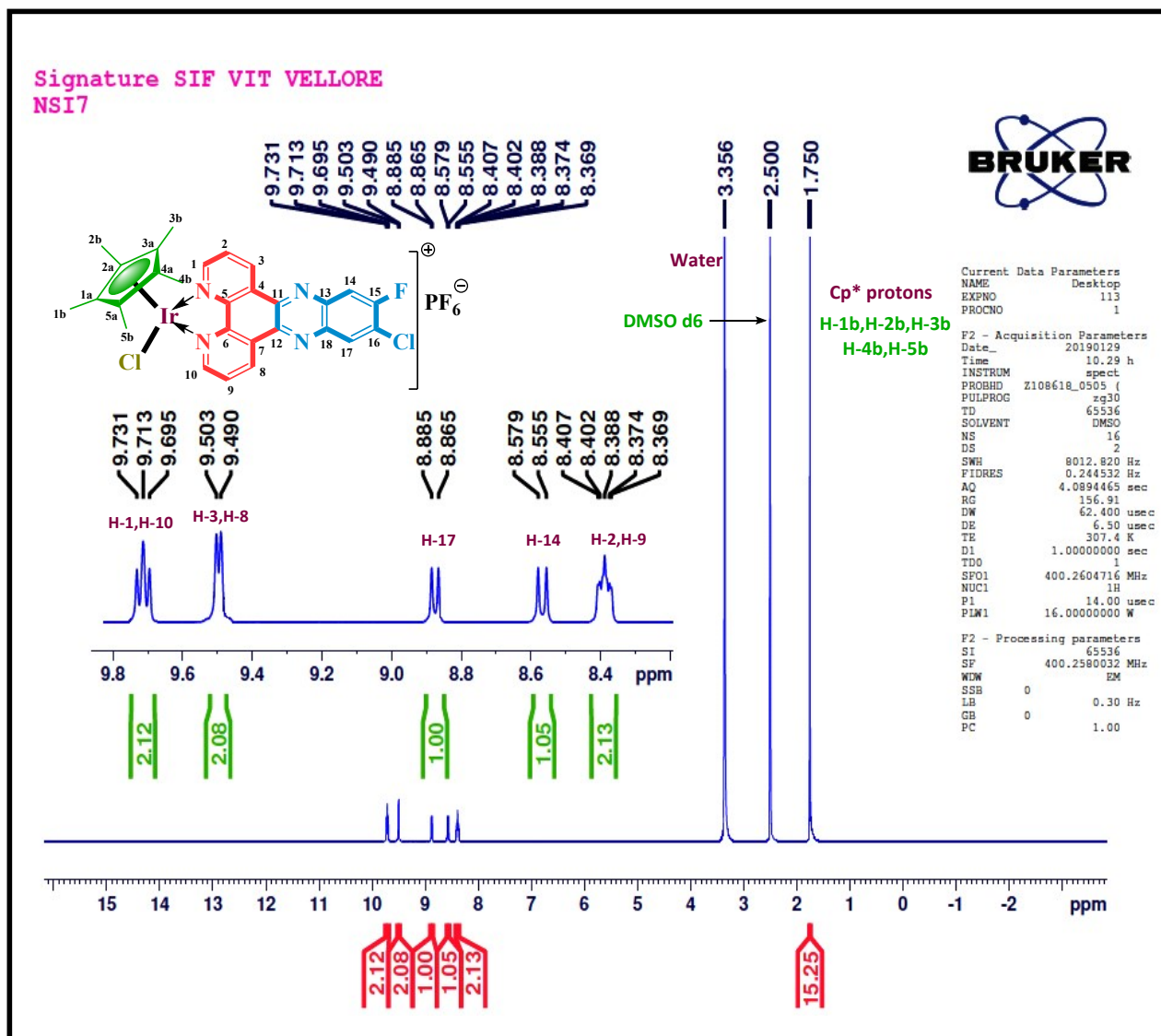
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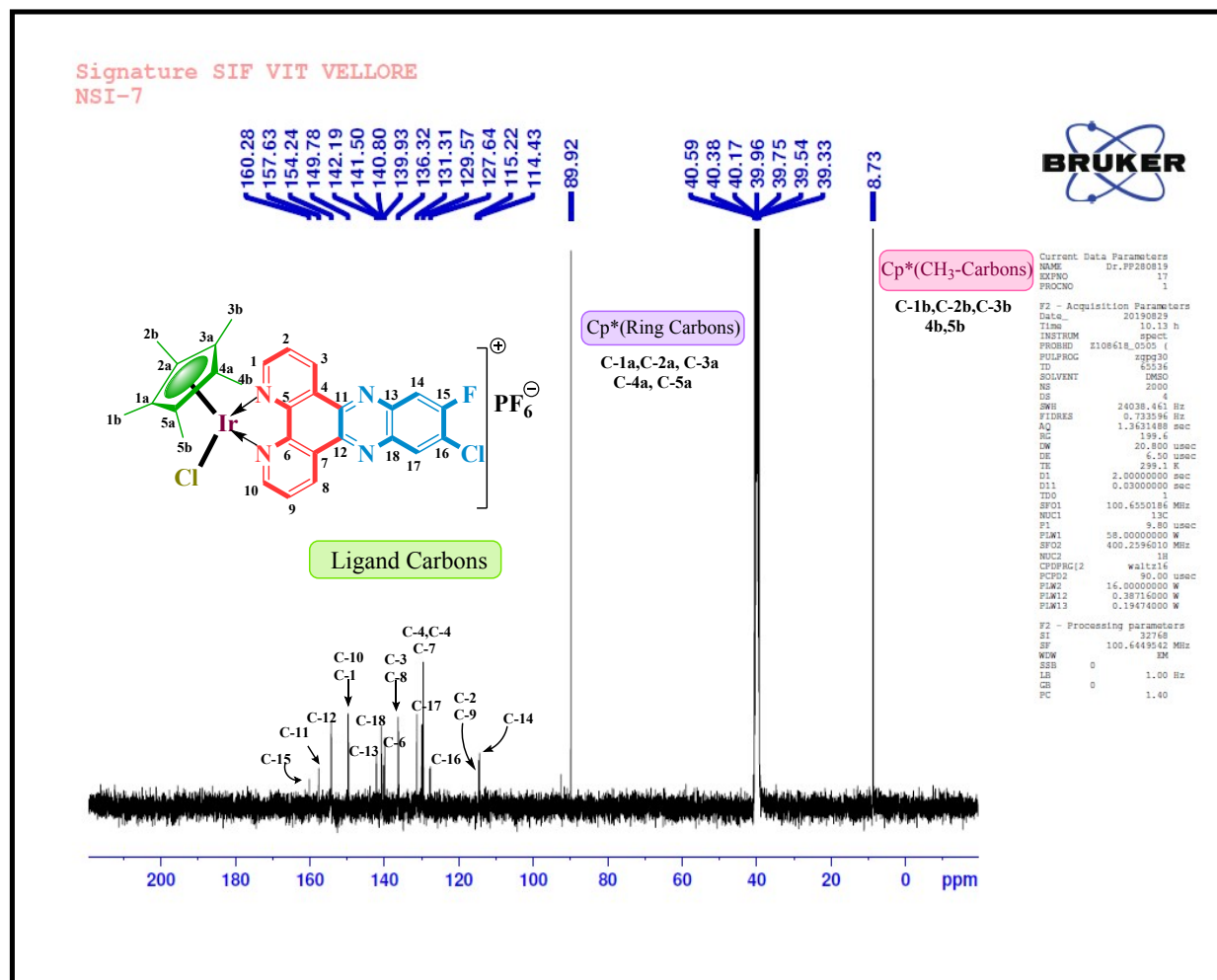
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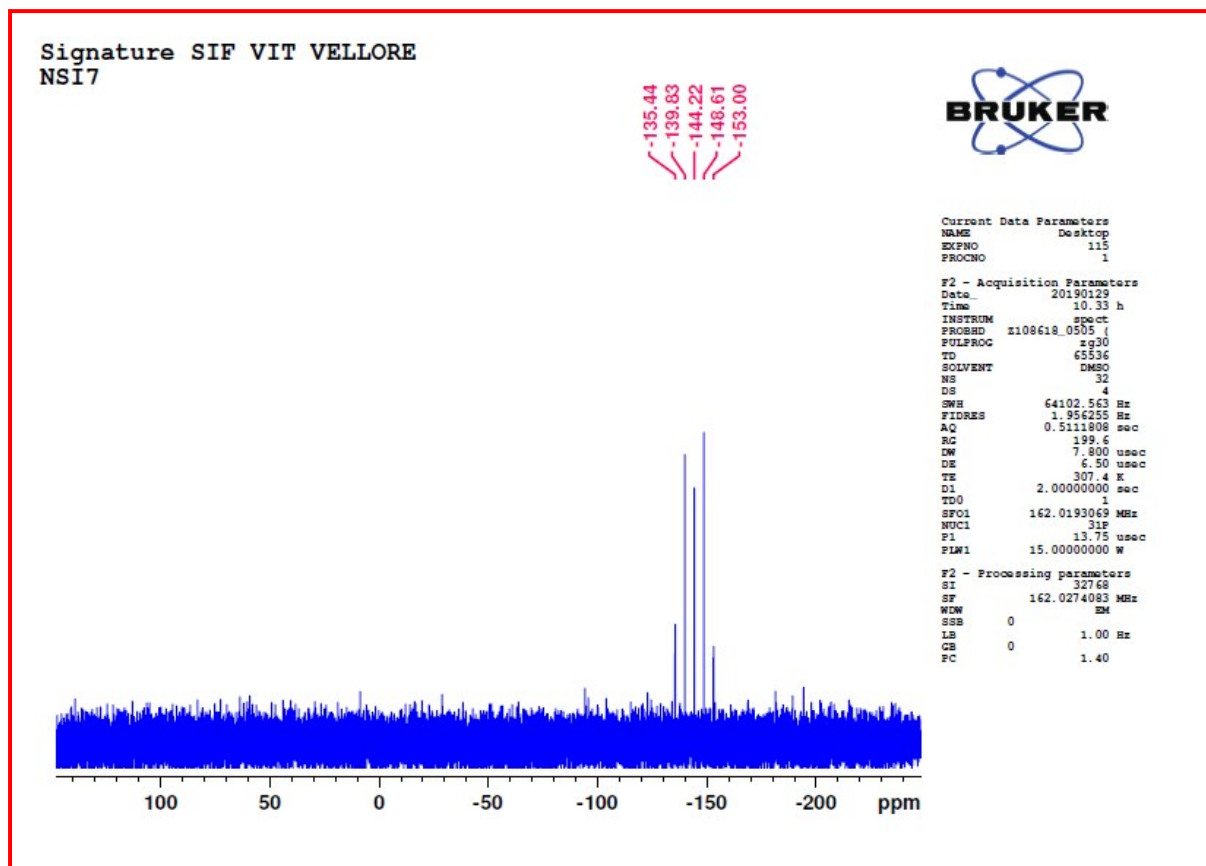
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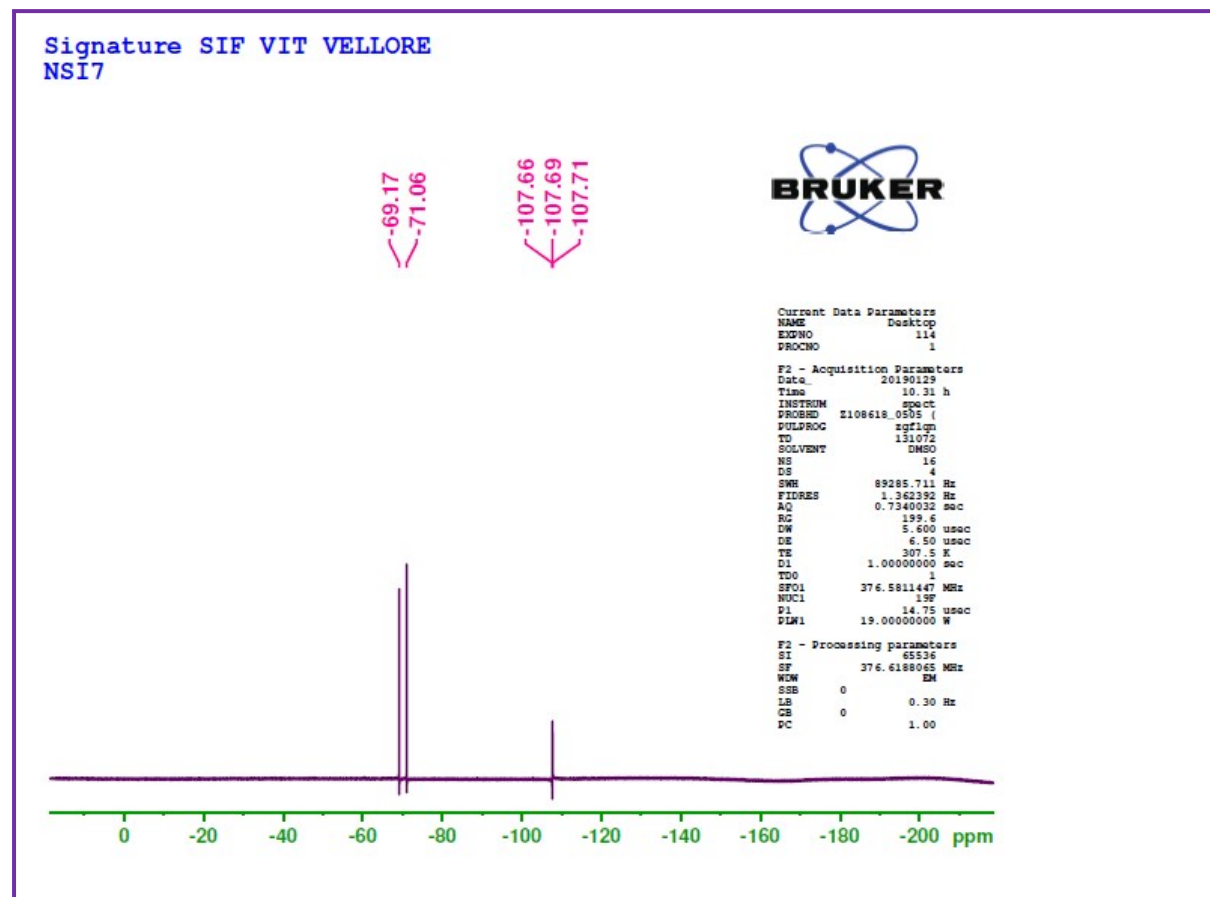
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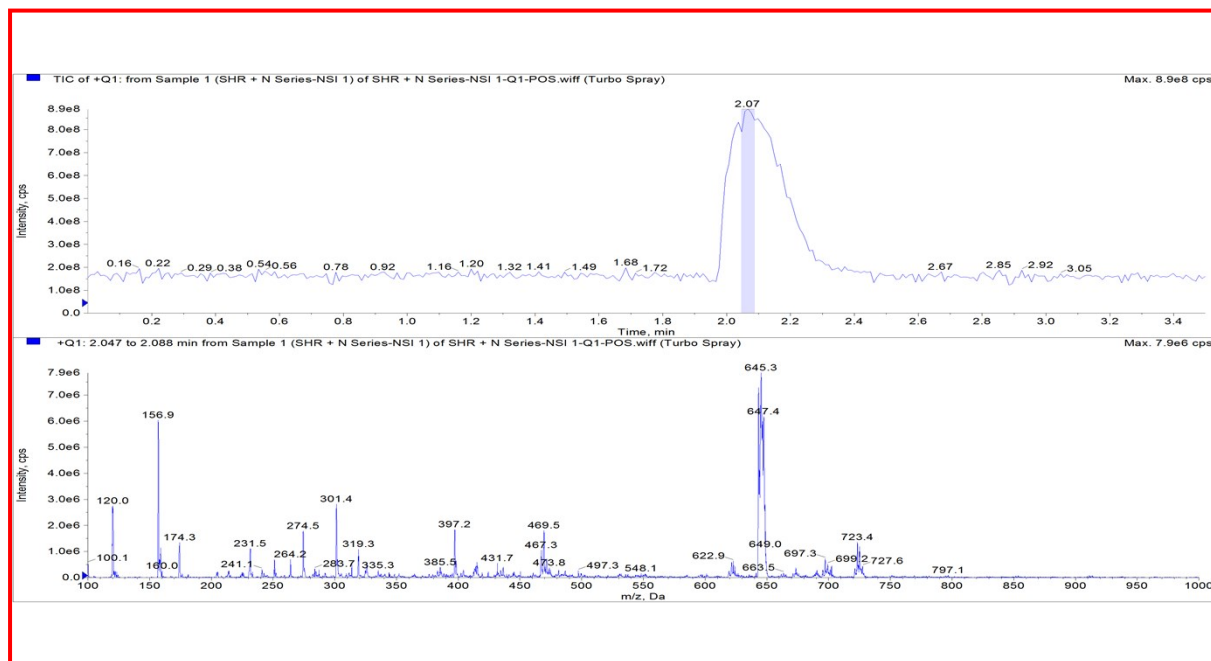
³¹P NMR Spectrum of Complex IrL7



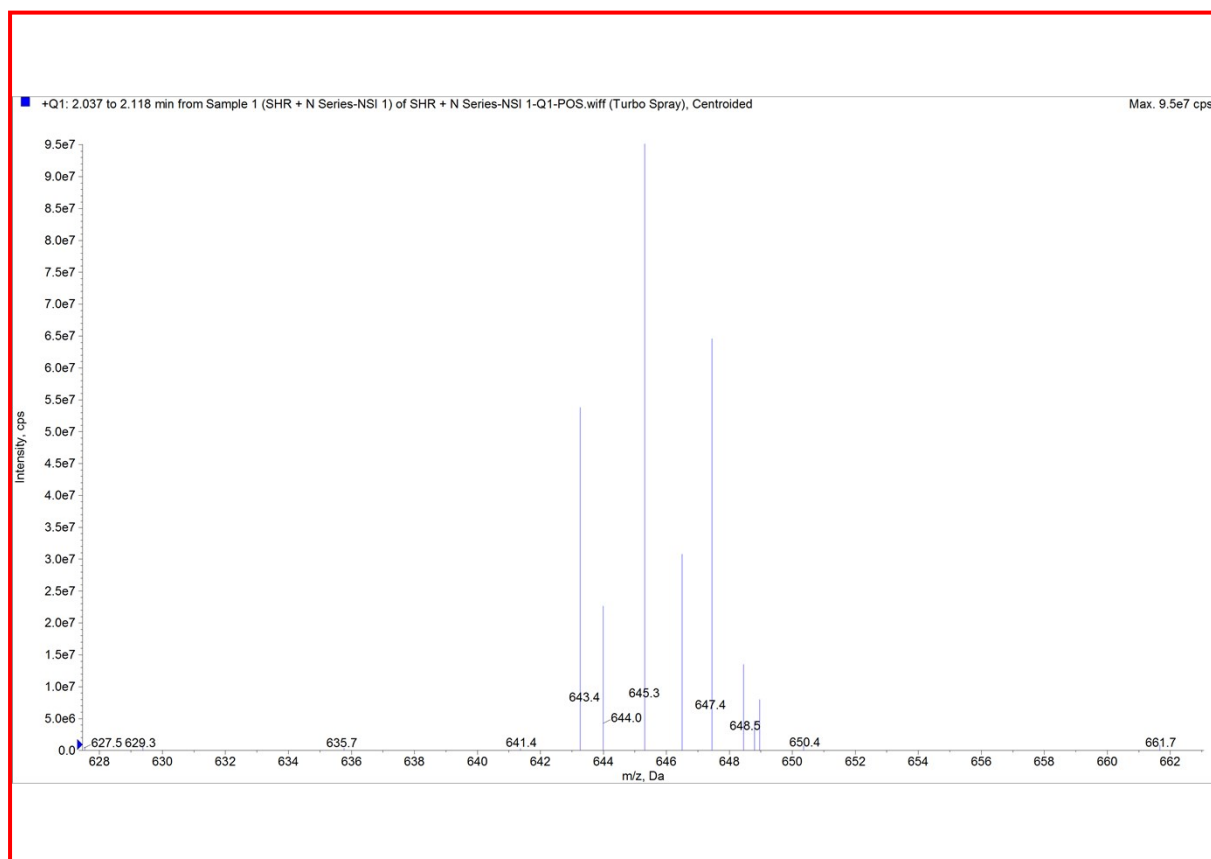
¹⁹F NMR Spectrum of Complex IrL7



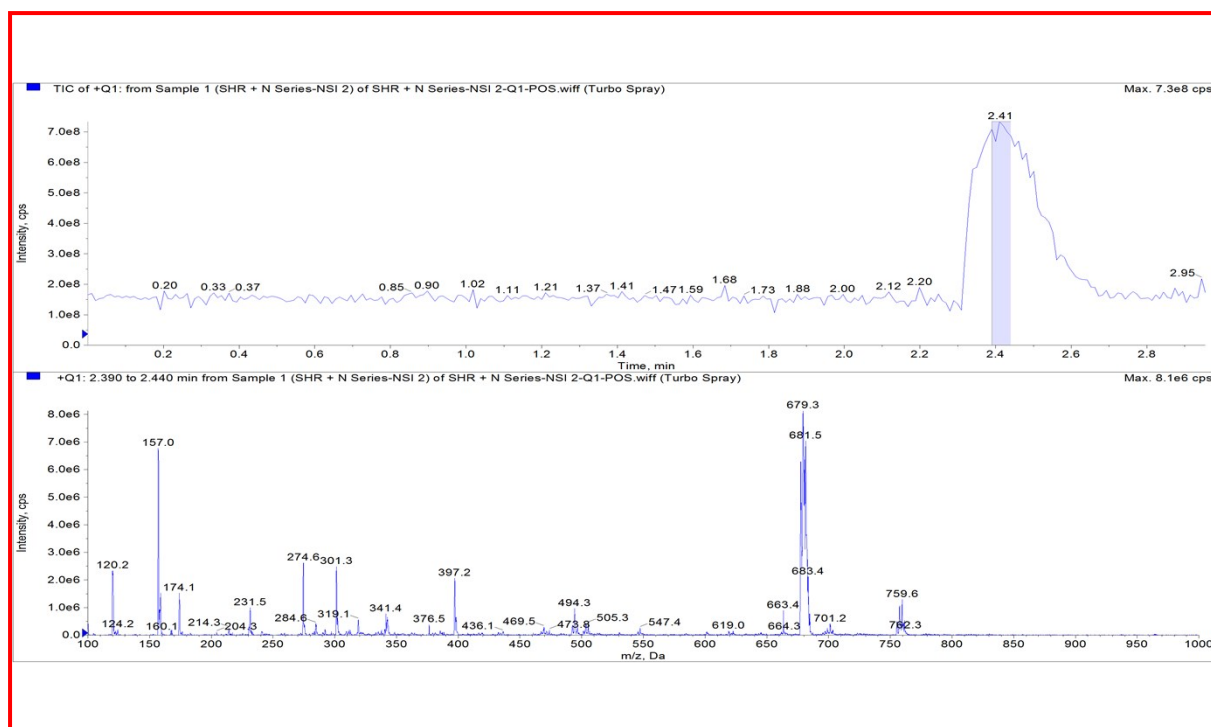
ESI-MS of IrL1



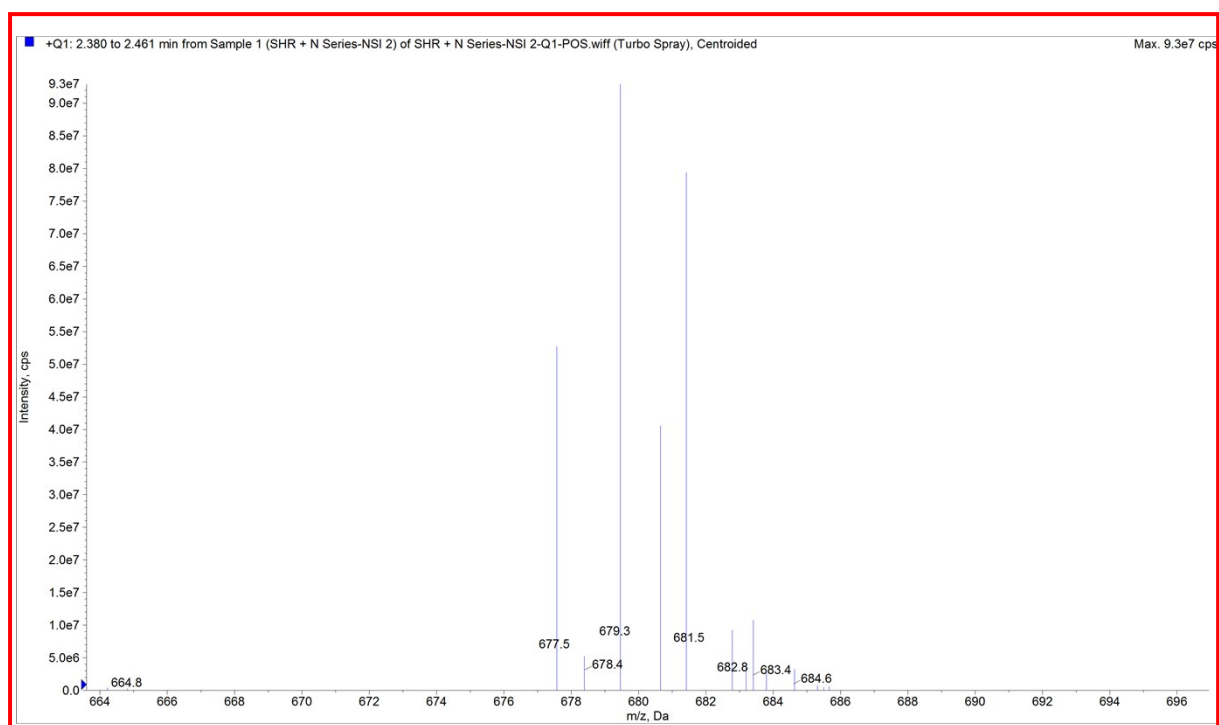
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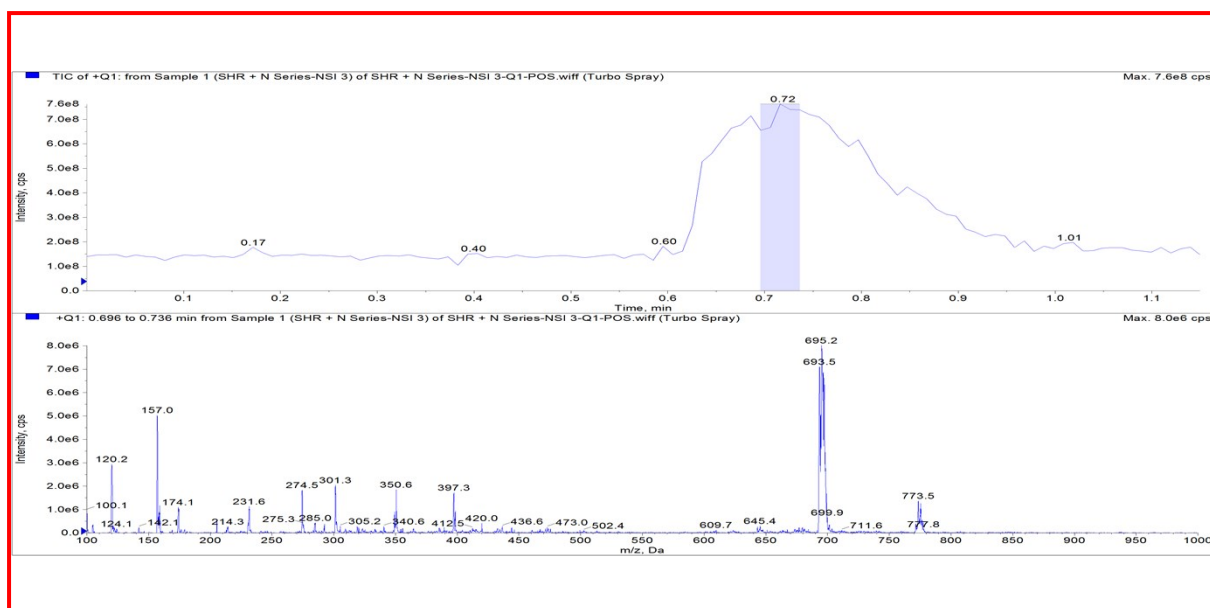
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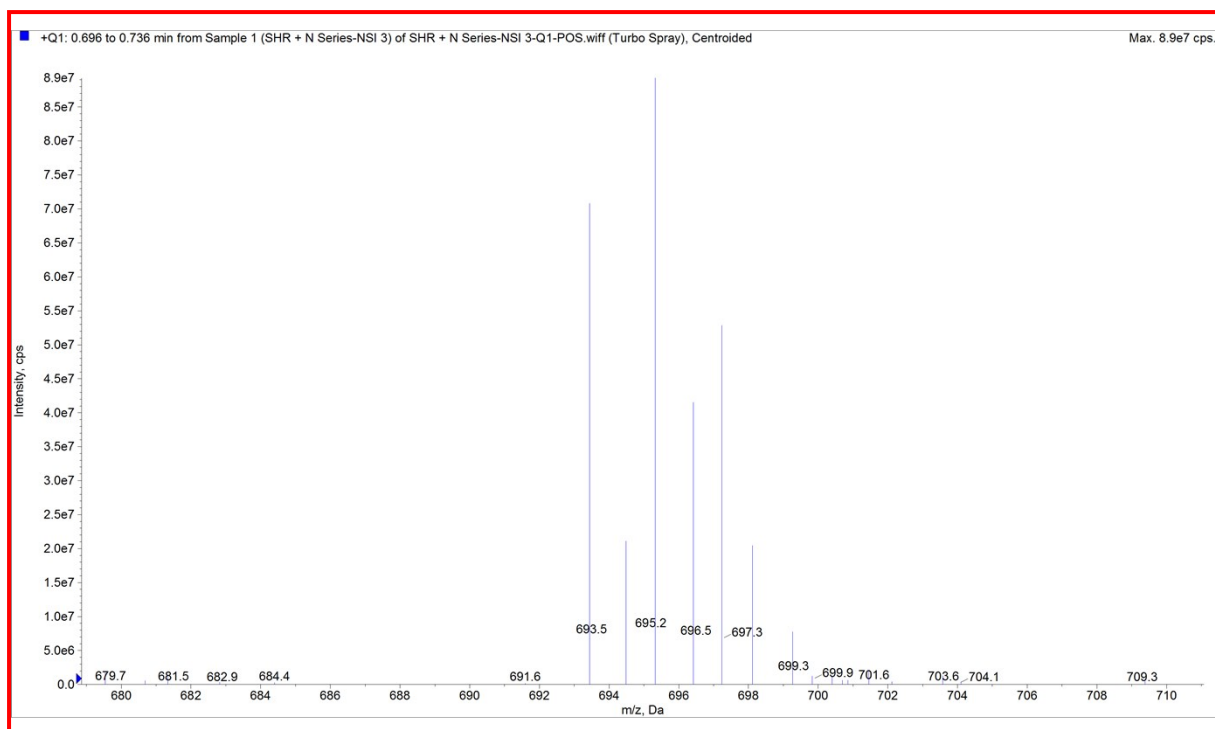
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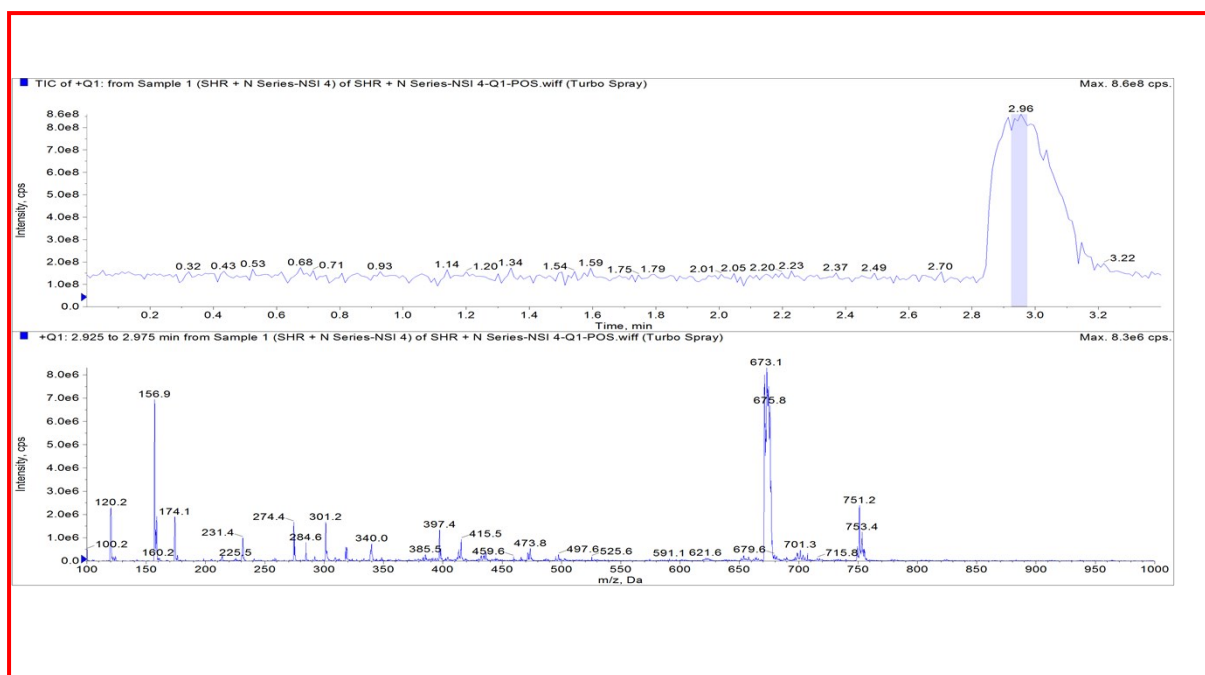
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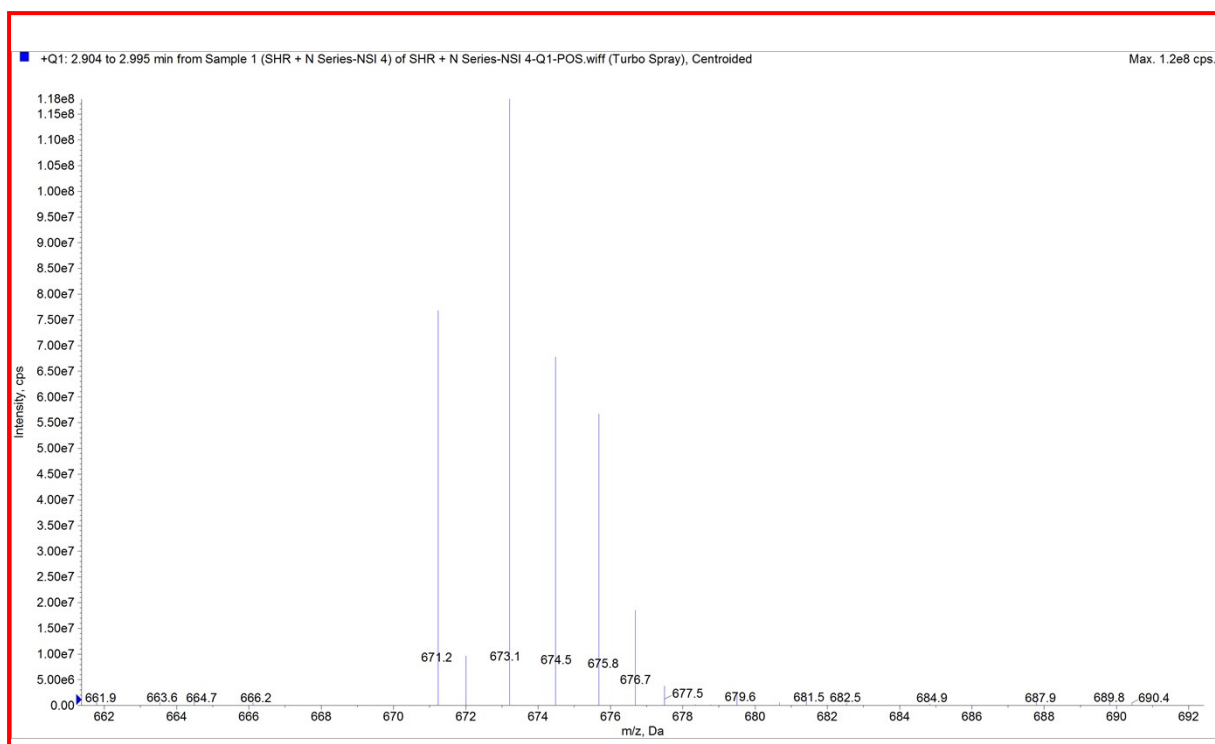
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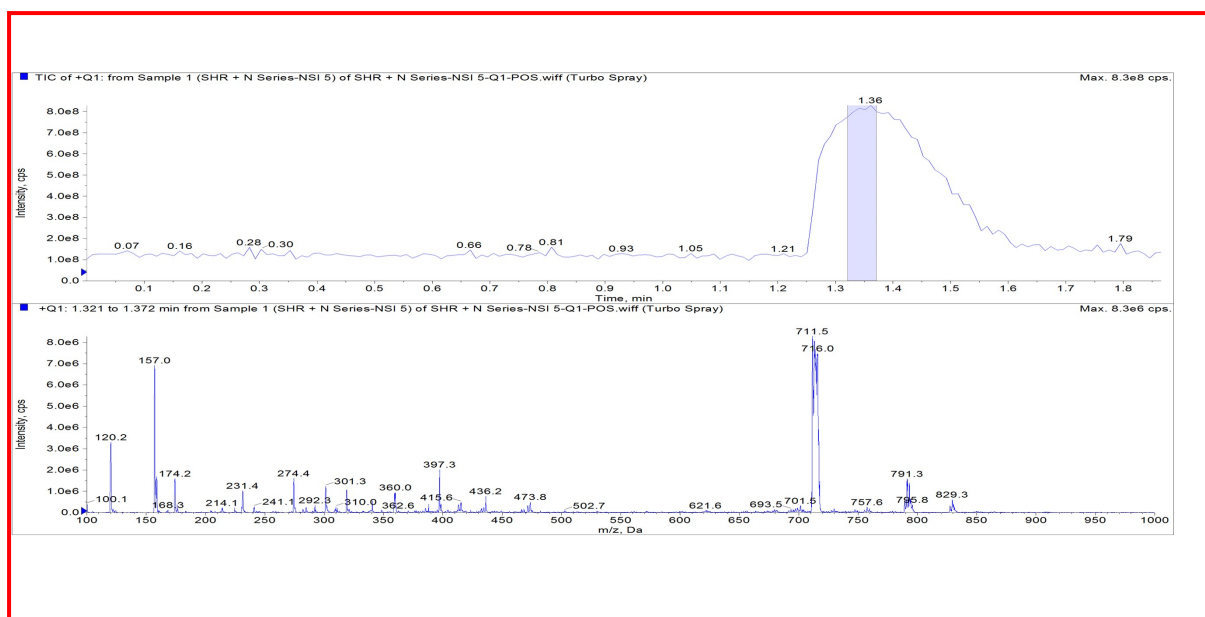
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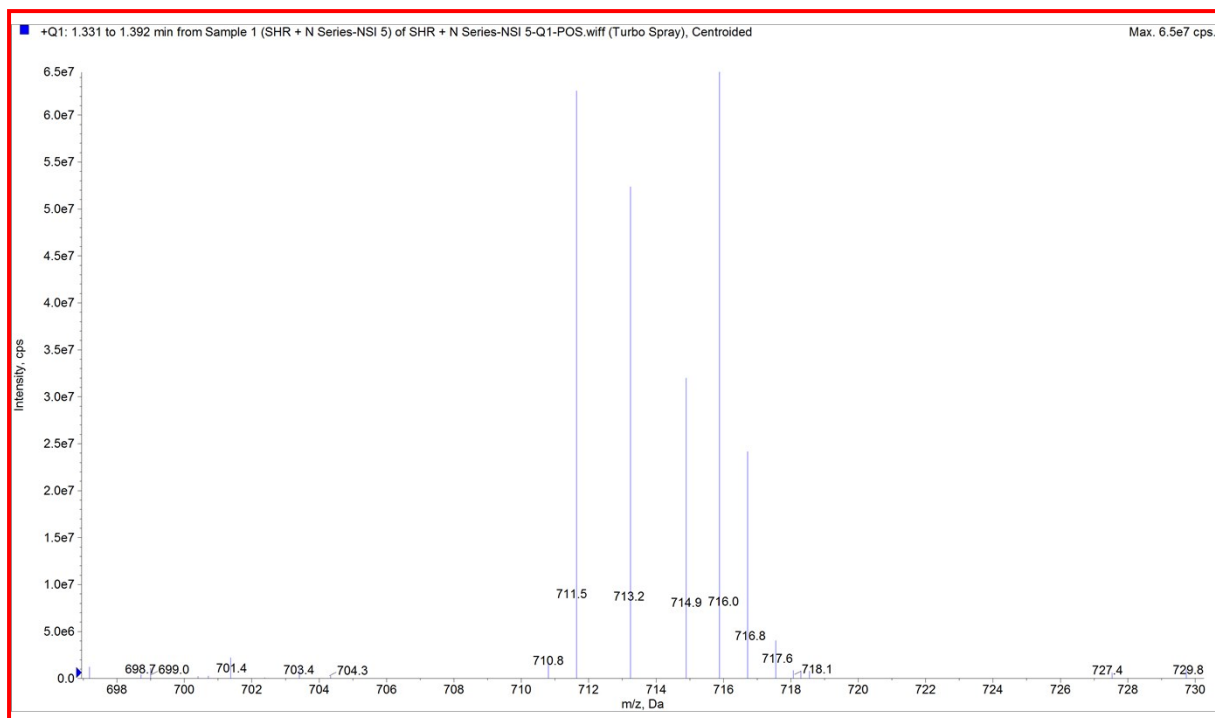
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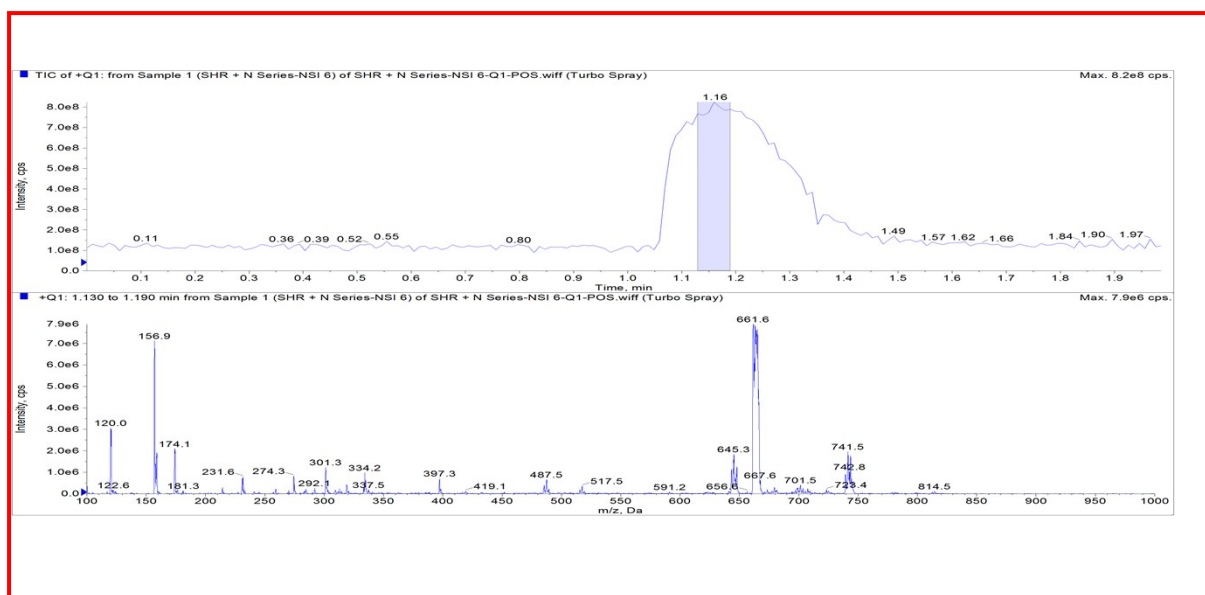
ESI-MS of IrL5



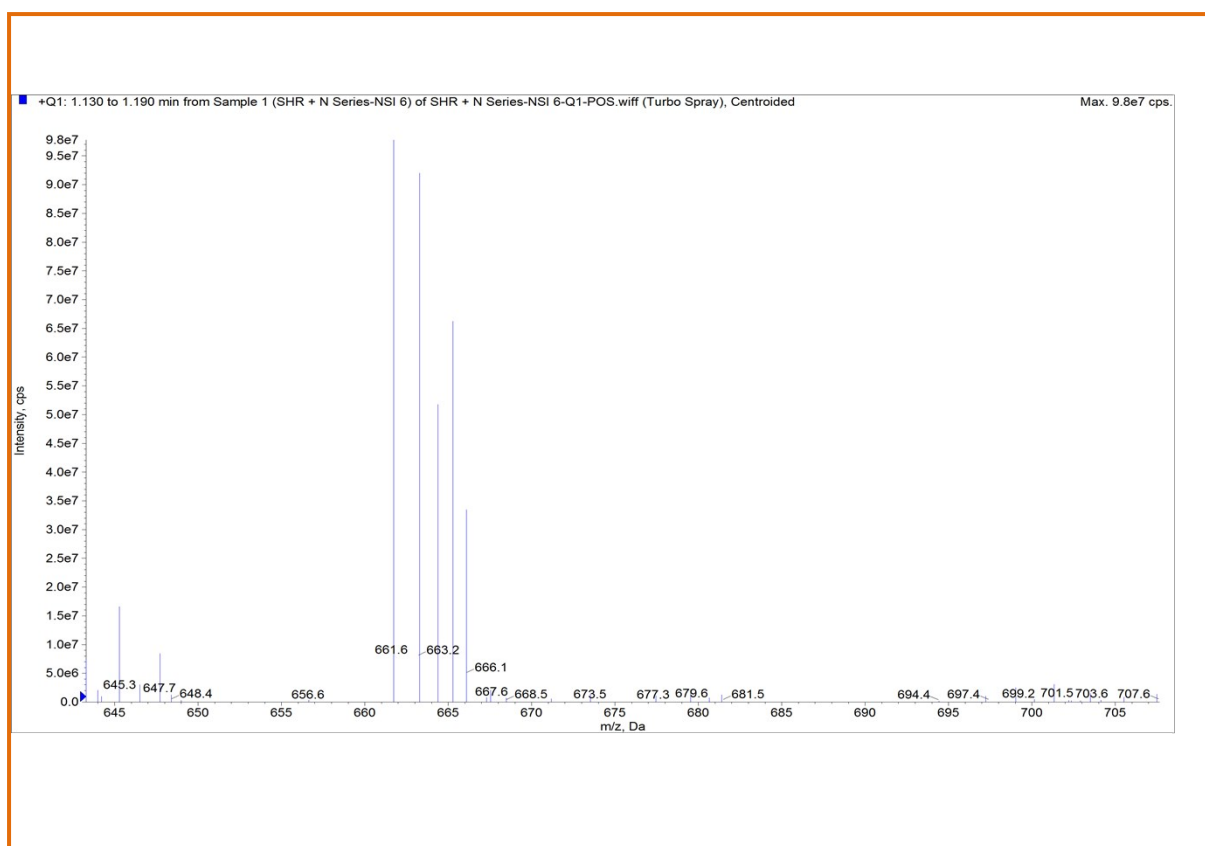
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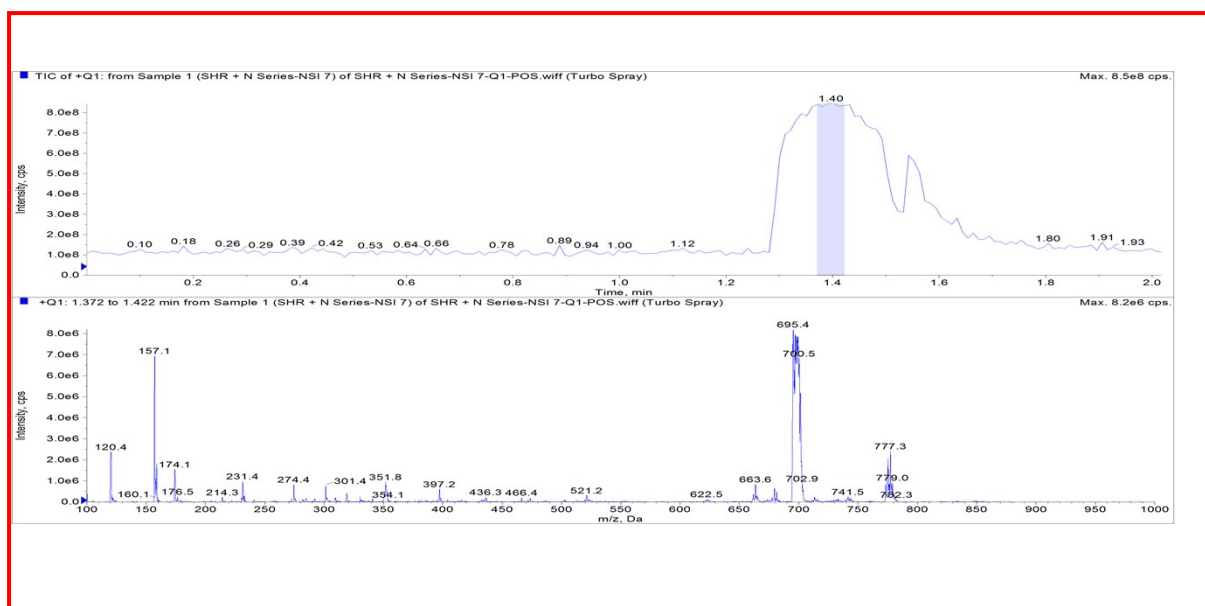
ESI-MS of IrL6



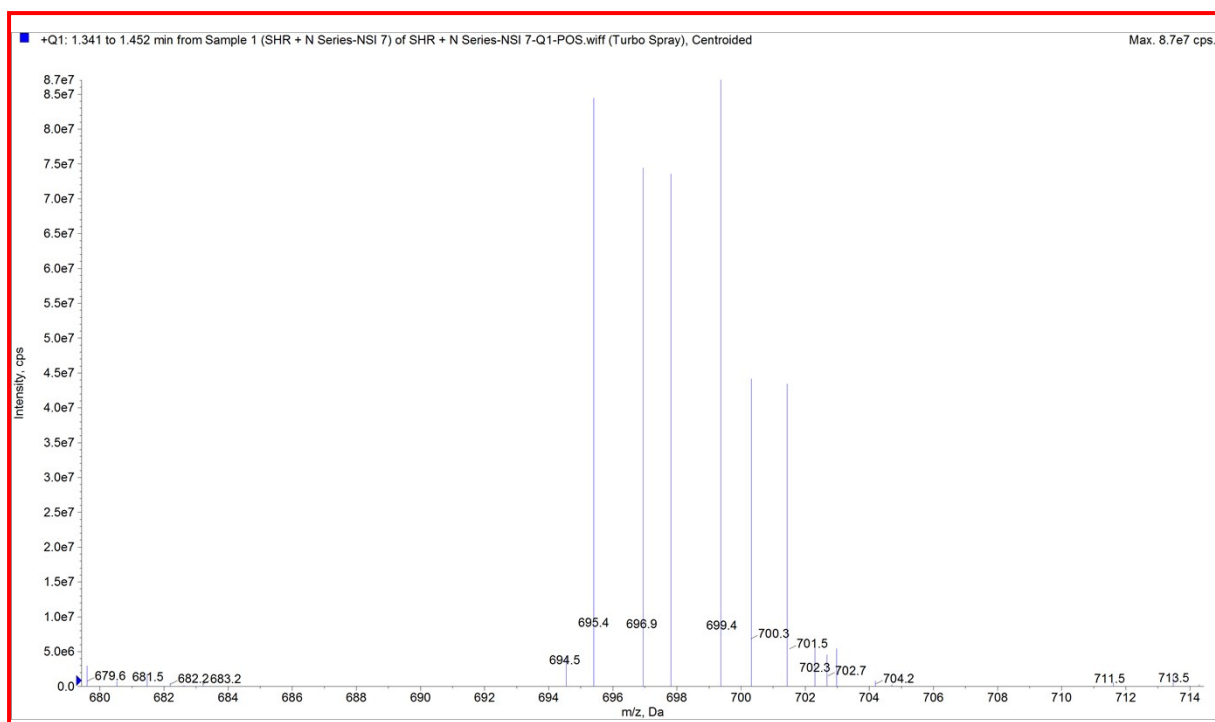
Zoom in Scan



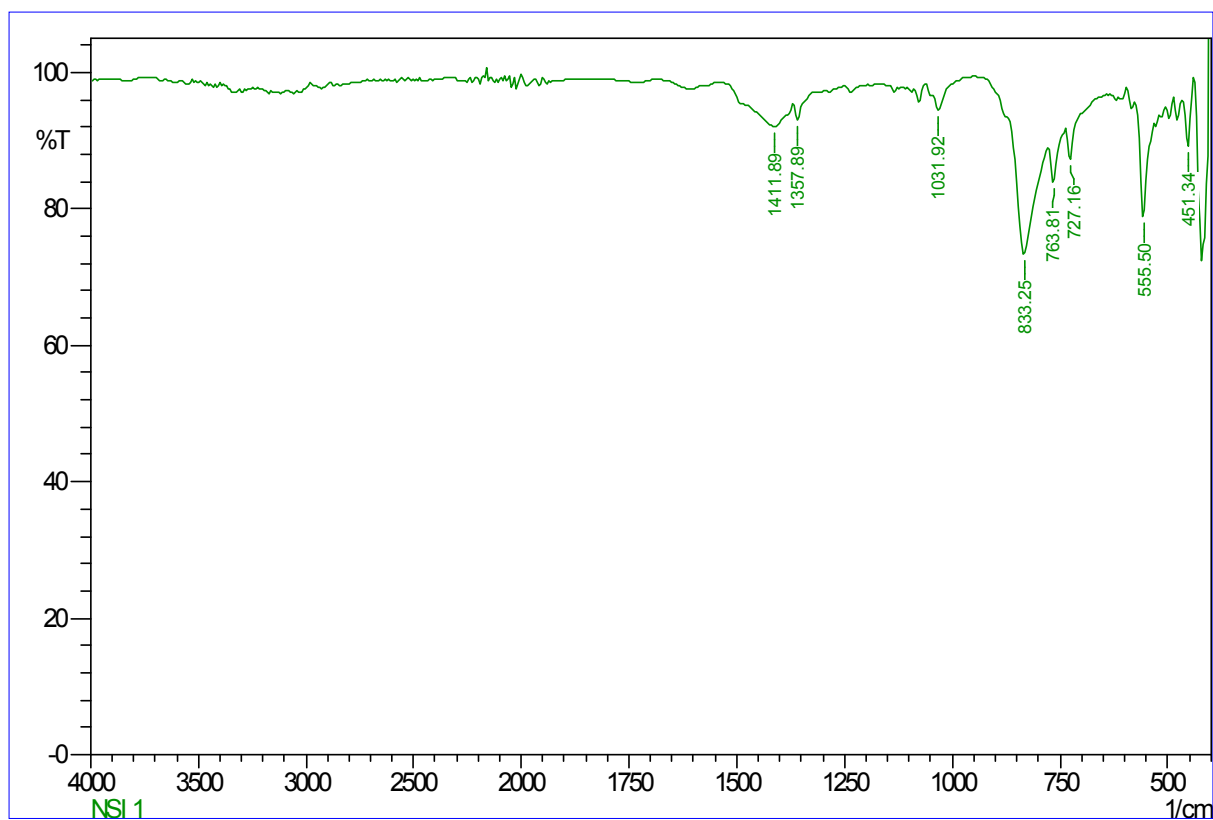
ESI-MS of IrL7



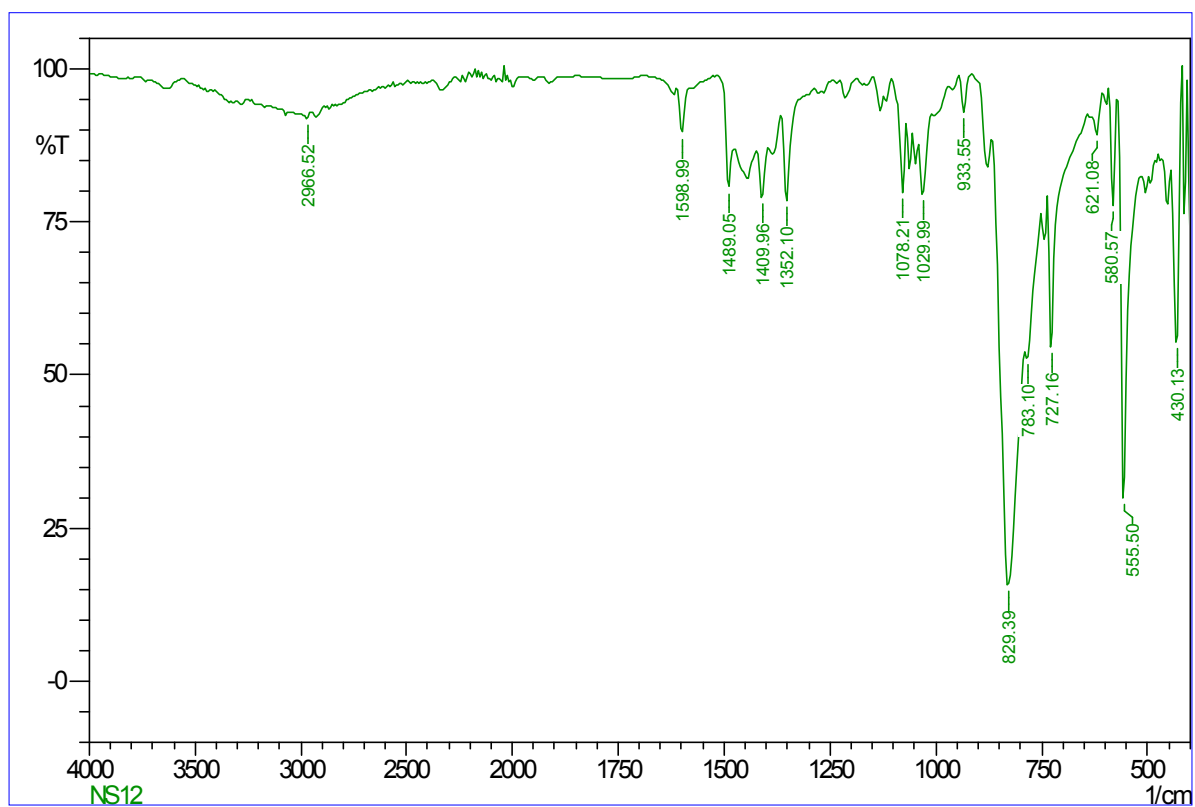
Zoom in Scan



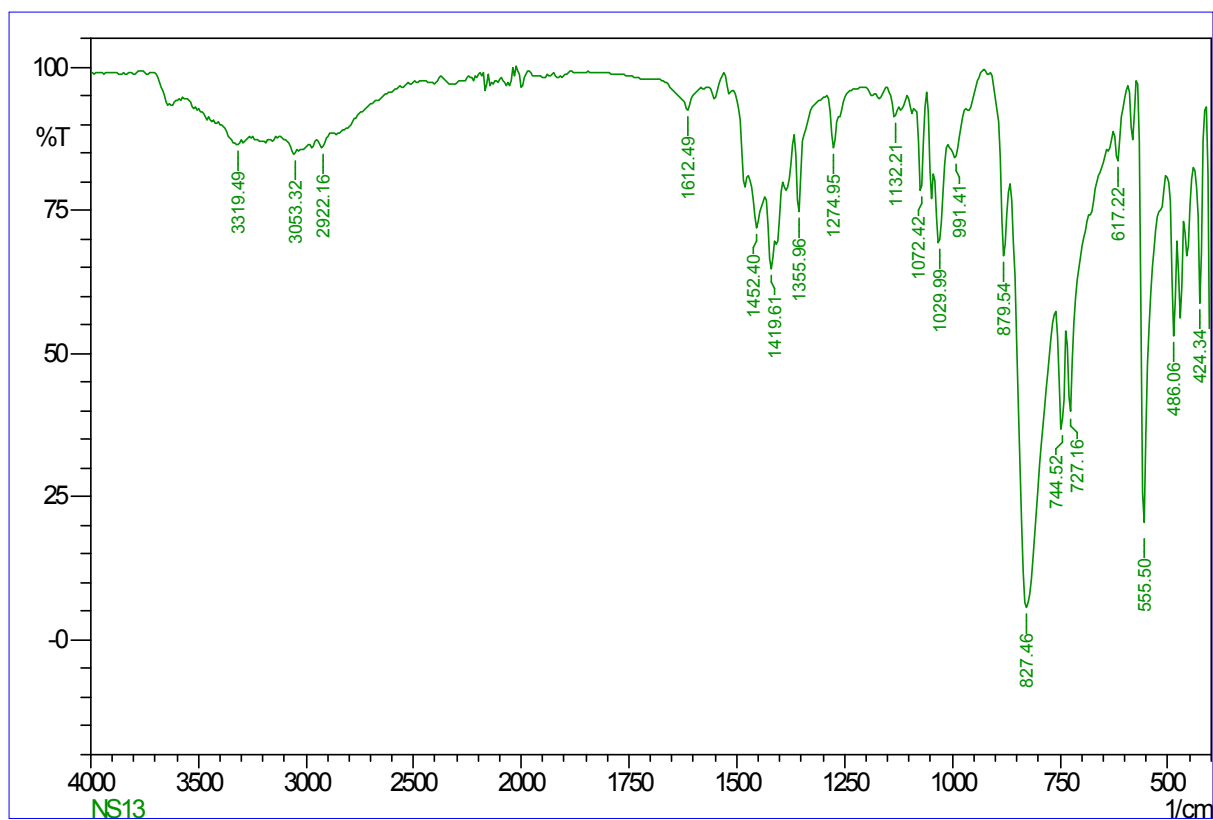
IR spectrum of IrL1



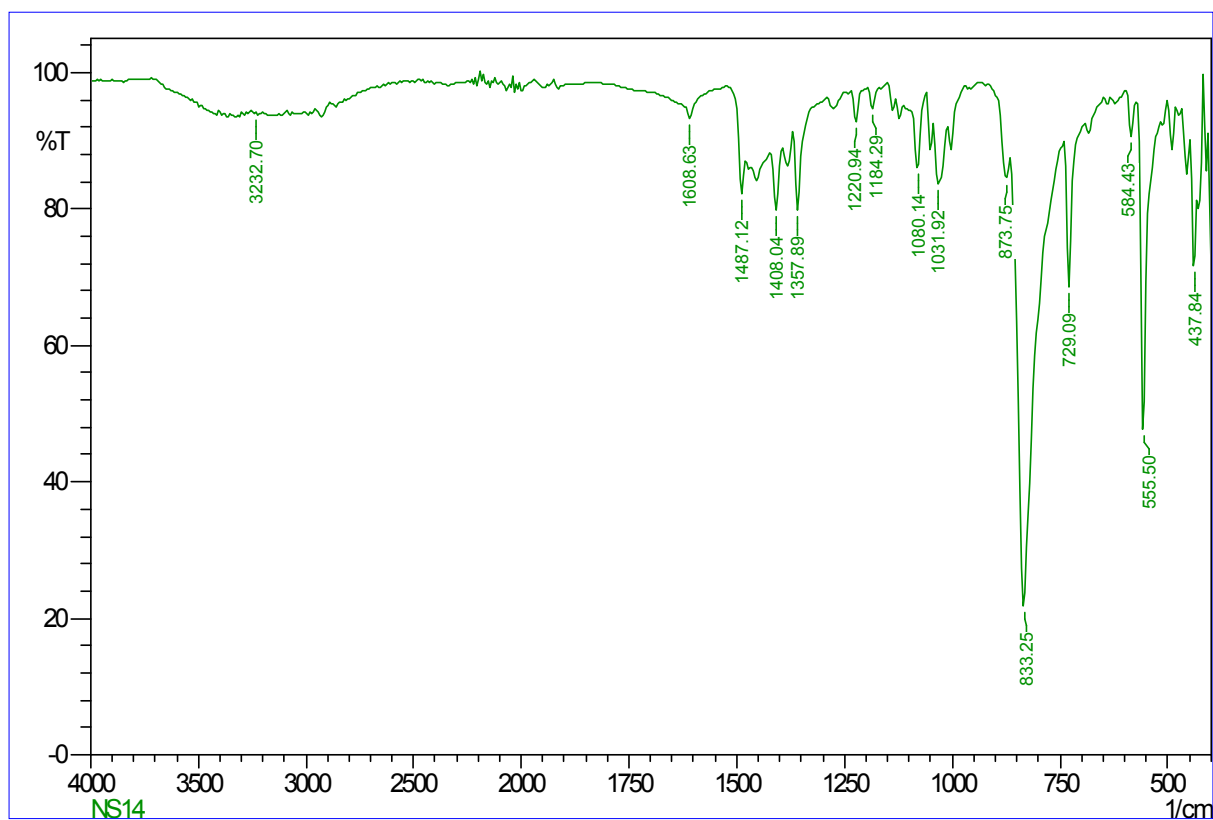
IR spectrum of IrL2



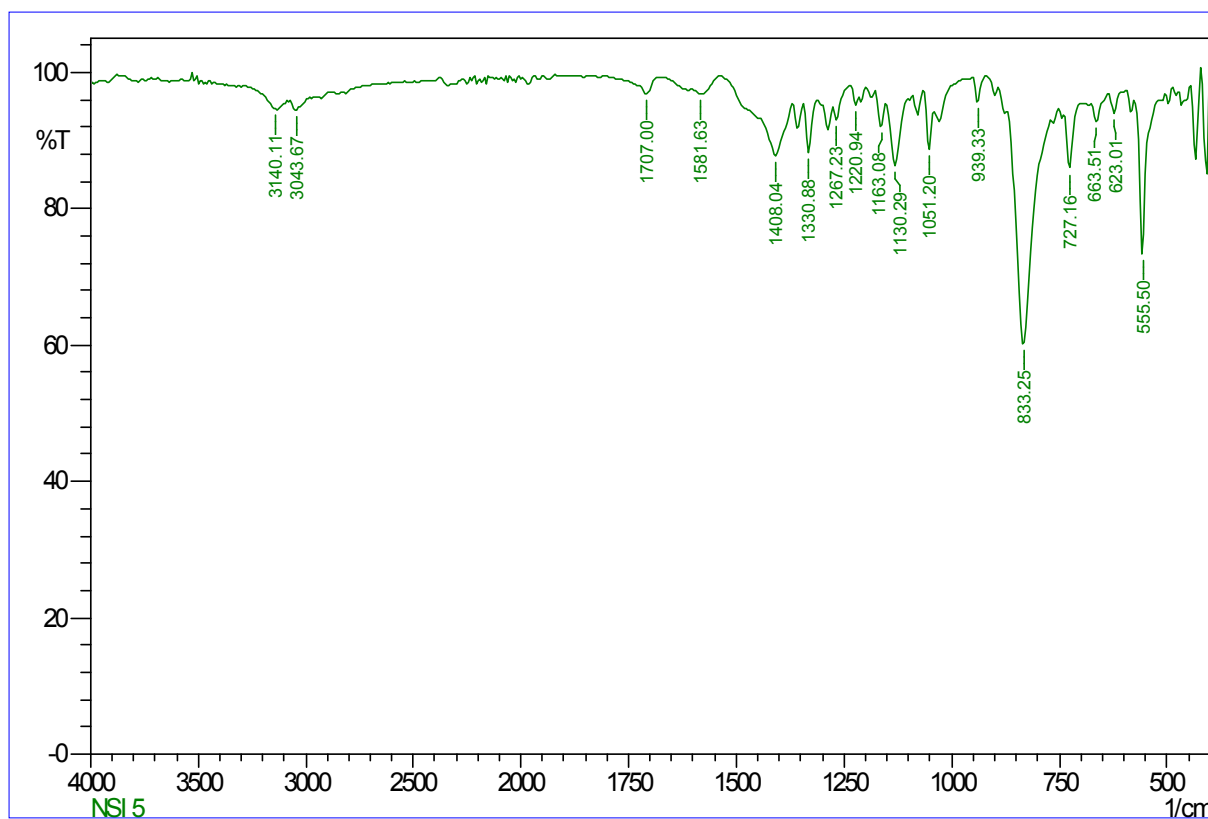
IR spectrum of IrL3



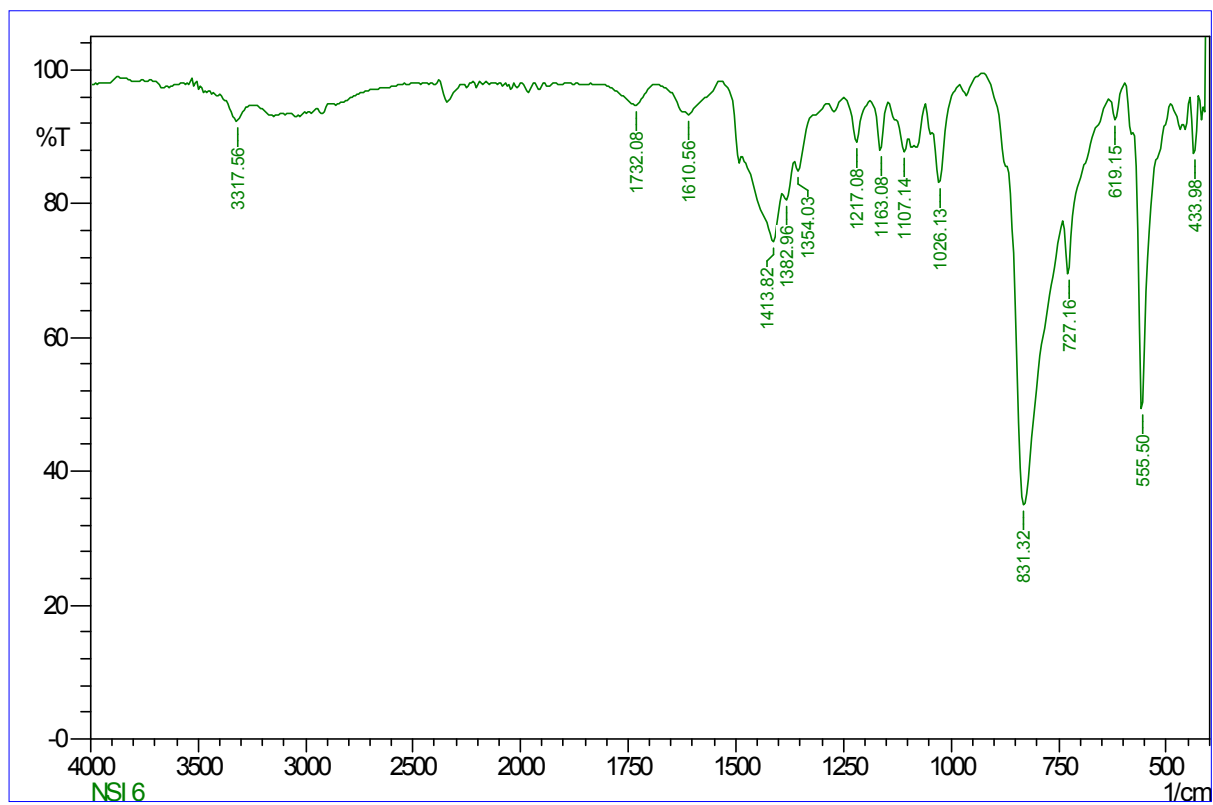
IR spectrum of IrL4



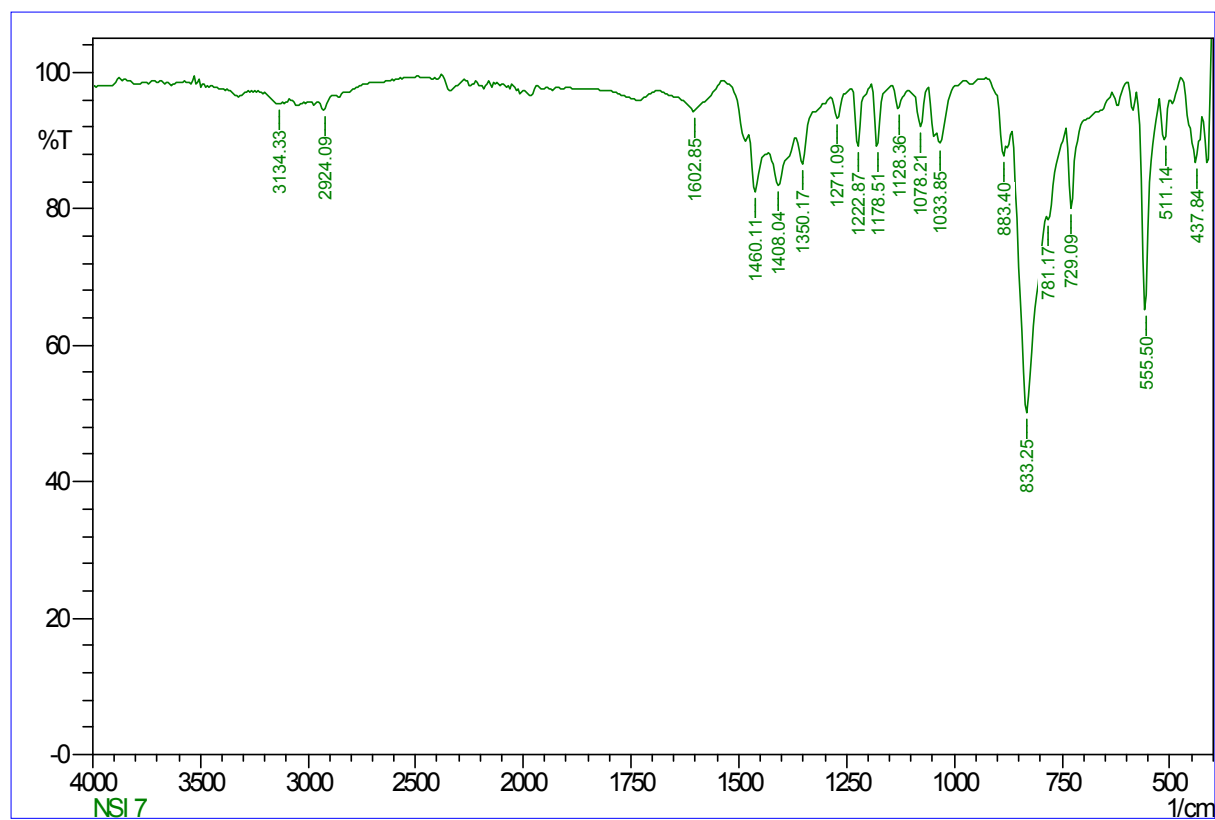
IR spectrum of IrL5



IR spectrum of IrL6



IR spectrum of IrL7



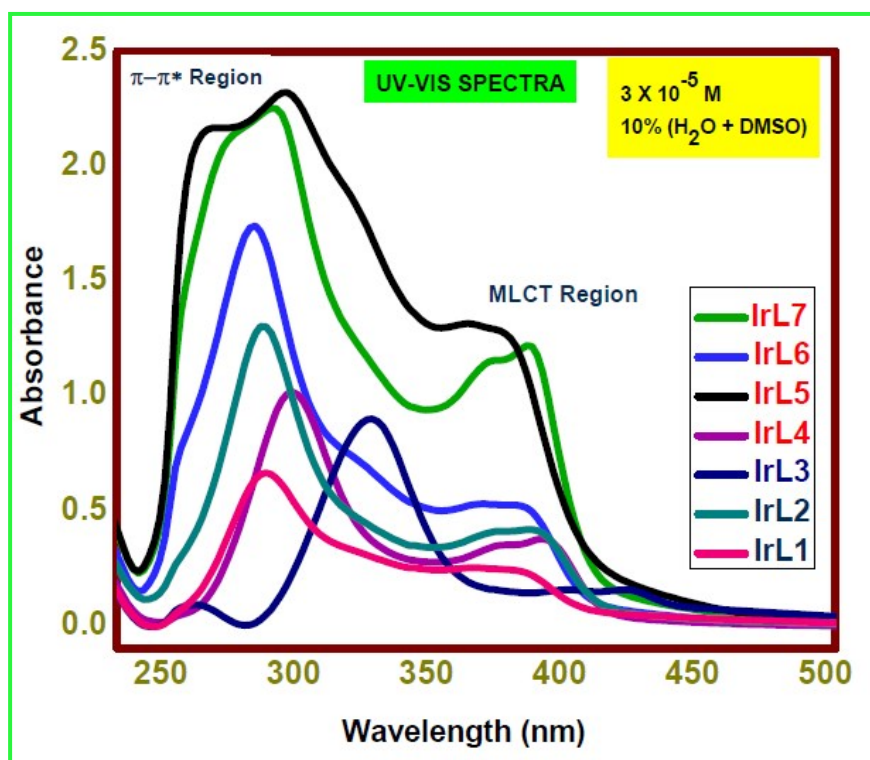


Fig. S1 UV-Vis spectra of complex IrL1-IrL7

FLUORESCENCE SPECTRA FOR MLCT EMISSION

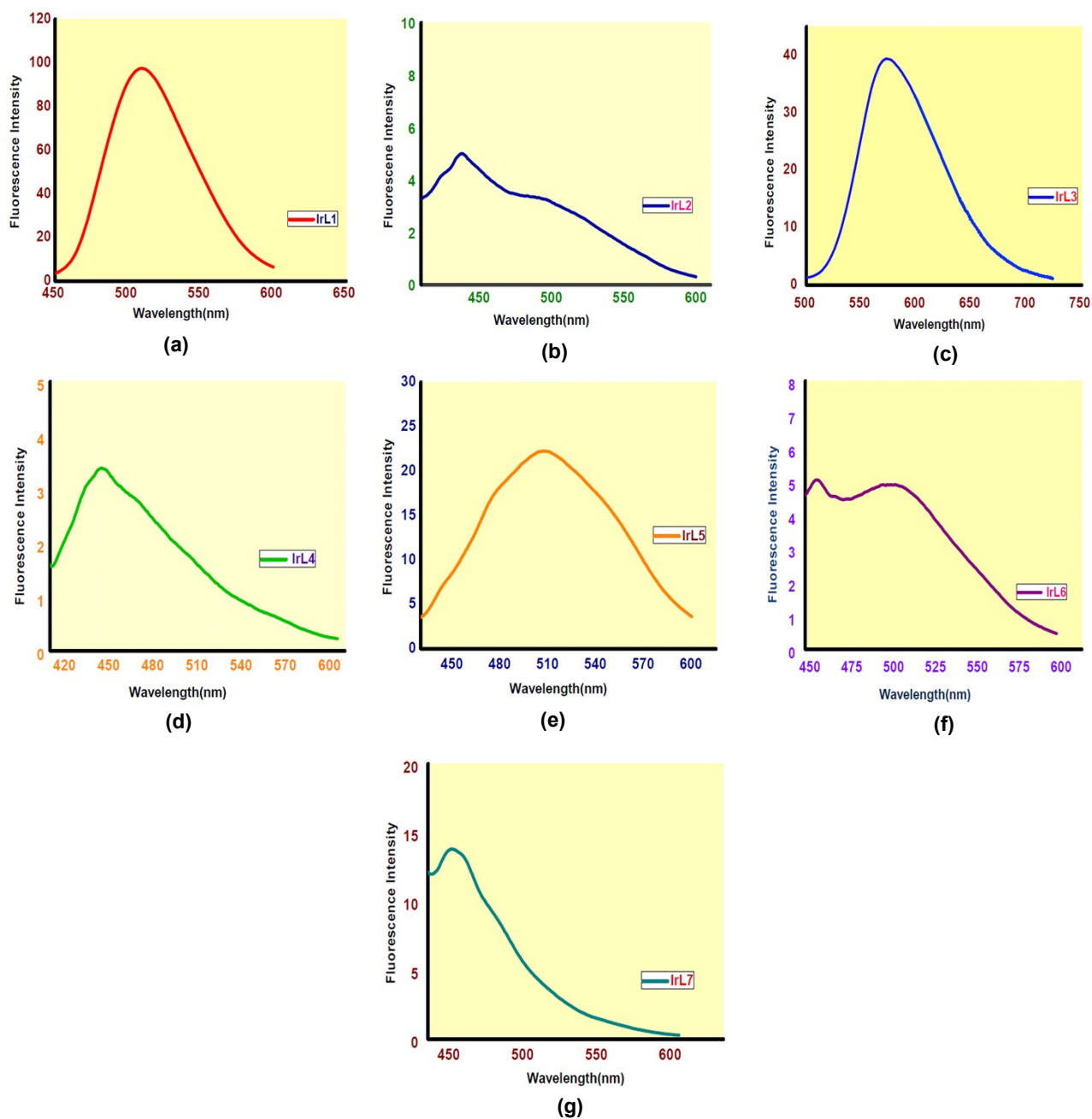


Fig. S2 emission spectra of complexes IrL1–IrL7 in 10% DMSO-water solvent system at room temperature, $\lambda_{\text{ex}} = 400\text{--}420\text{ nm}$.

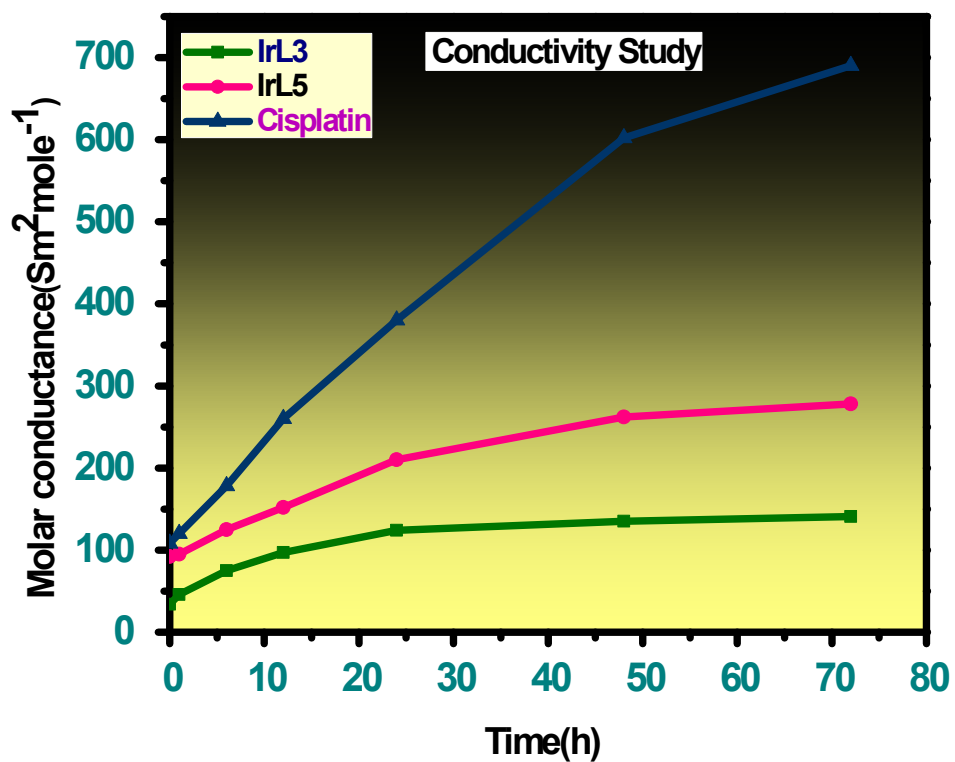


Fig. S3 Time dependent molar conductivity of compound IrL3 and IrL5 in 10% DMSO

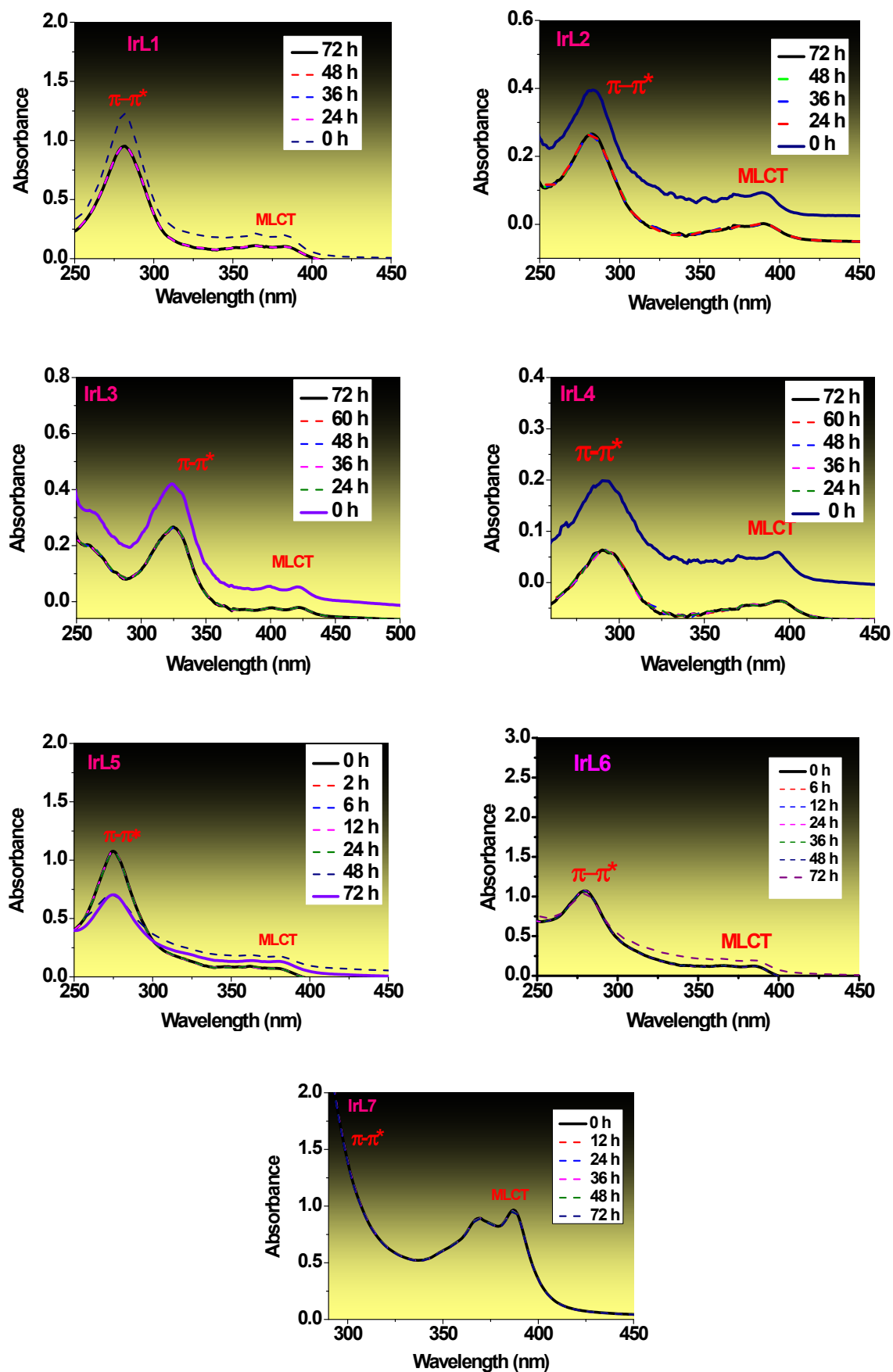


Fig. S4 Stability study of all the Ir(III) complexes in 10 % DMSO media

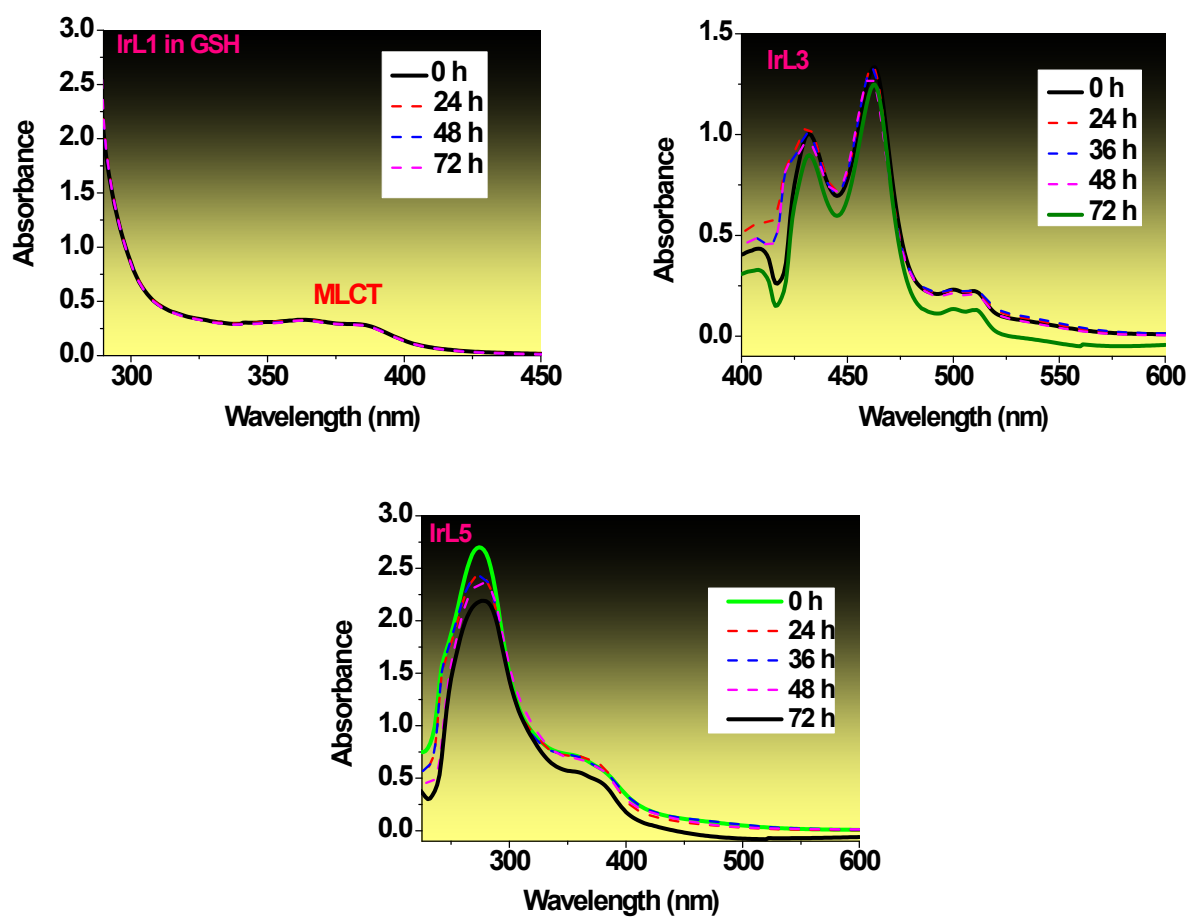


Fig. S5 Stability of selected Ir(III) complexes (IrL1, IrL3 and IrL5) in aqueous GSH media

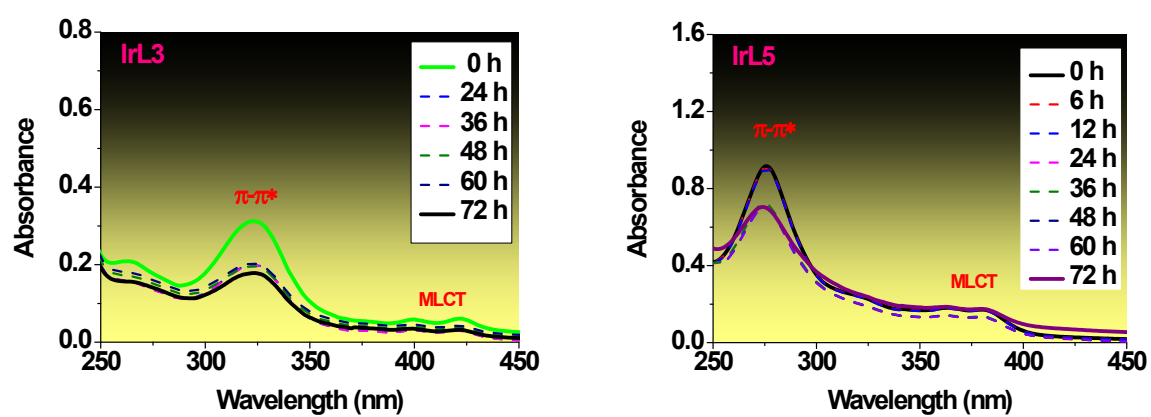
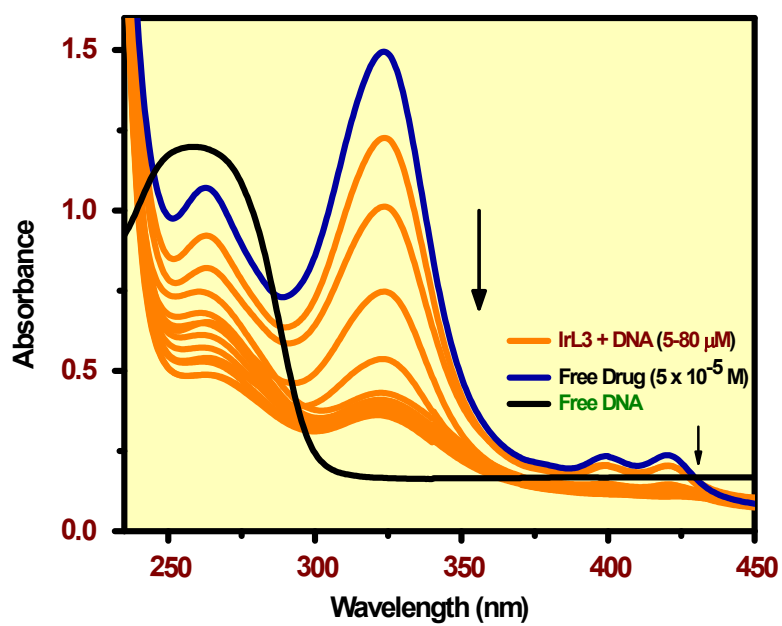
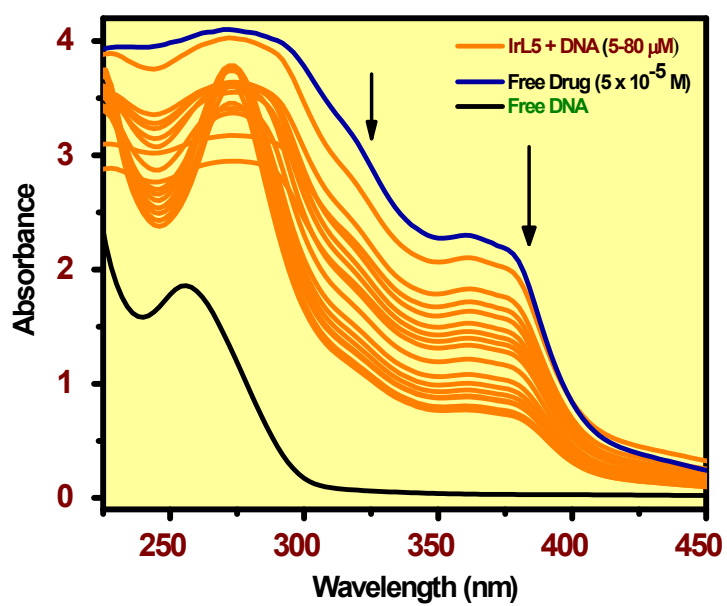


Fig. S6 Stability of IrL3 and IrL5 in PBS buffer media

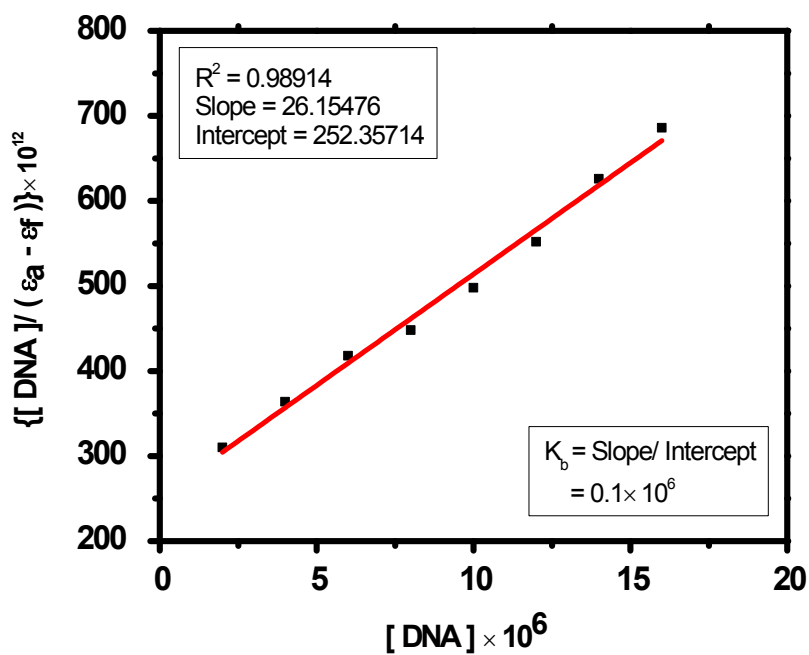


(a)

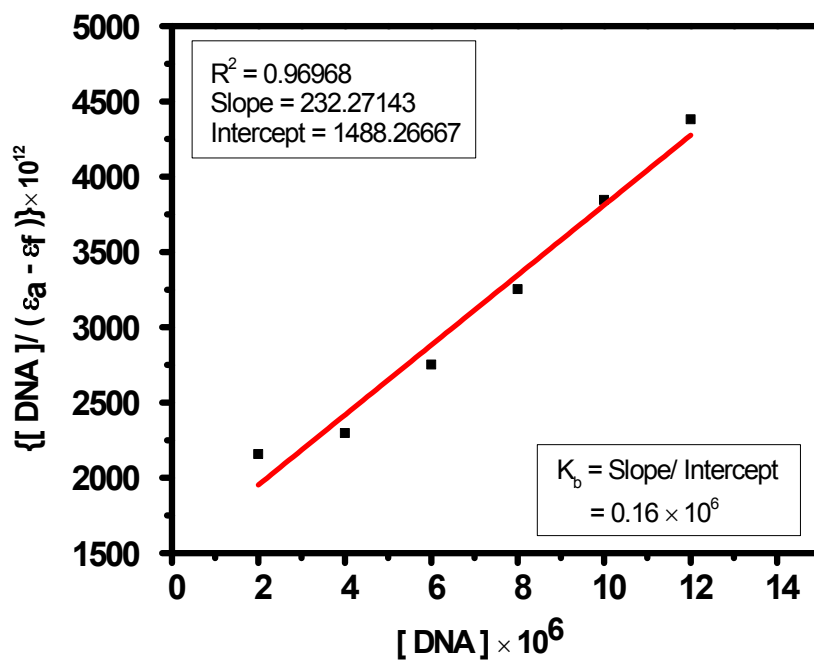


(b)

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

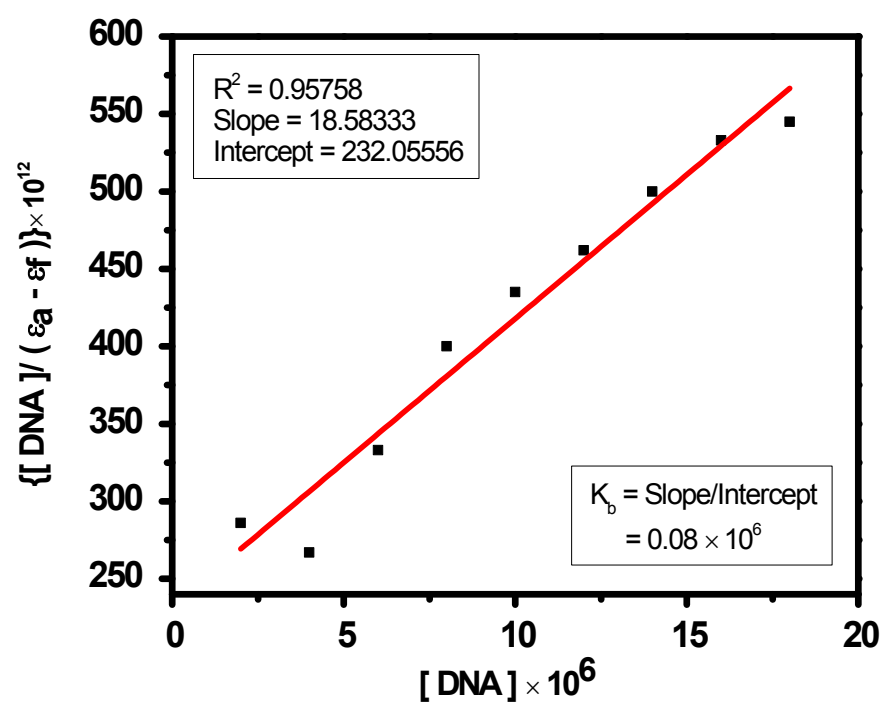


(a)

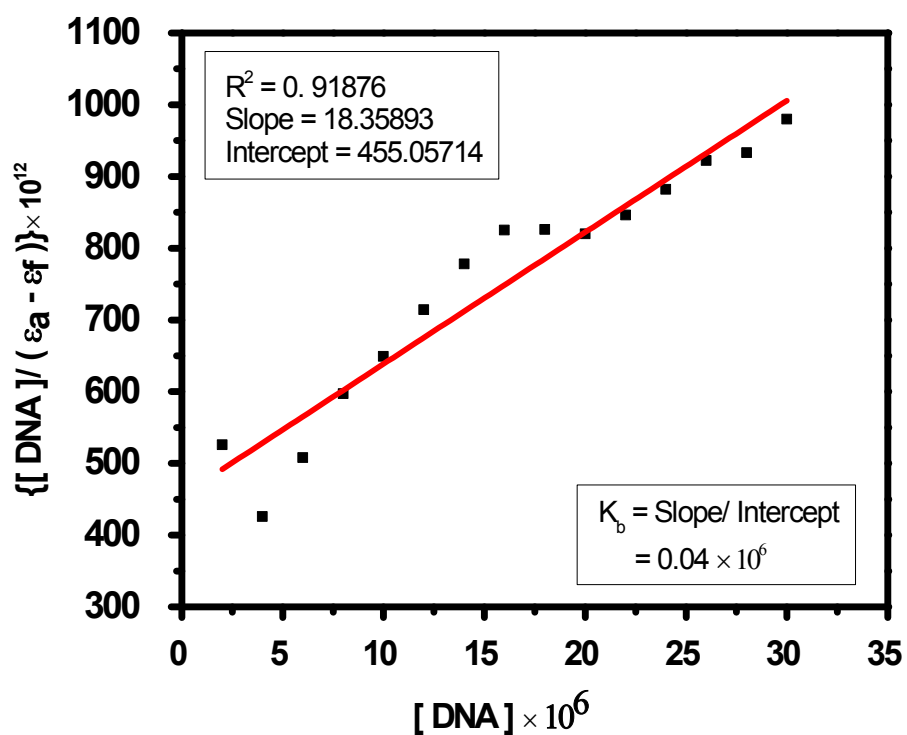


(b)

Fig. S8 $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$ linear plots of complex IrL3 at (a) $\lambda_{\text{max}} = 325 \text{ nm}$ (π - π^*) (b) $\lambda_{\text{max}} = 425 \text{ nm}$ (MLCT)

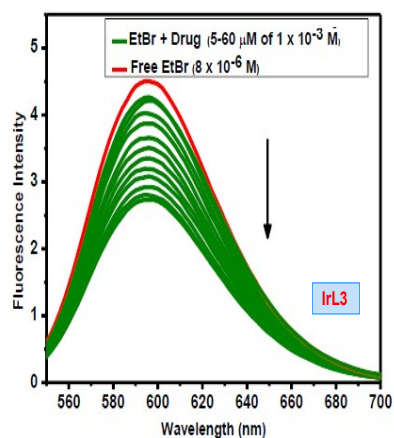


(a)

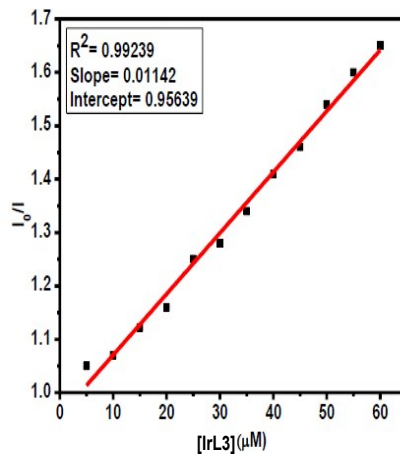


(b)

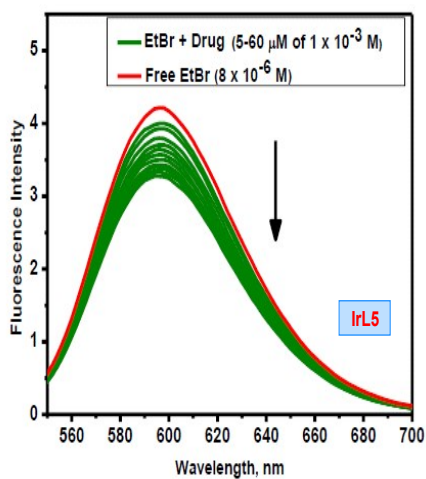
Fig. S9 $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$ linear plots of complex IrL5 at (a) $\lambda_{\text{max}} = 325 \text{ nm}$ (π - π^*) (b) $\lambda_{\text{max}} = 380 \text{ nm}$ (MLCT)



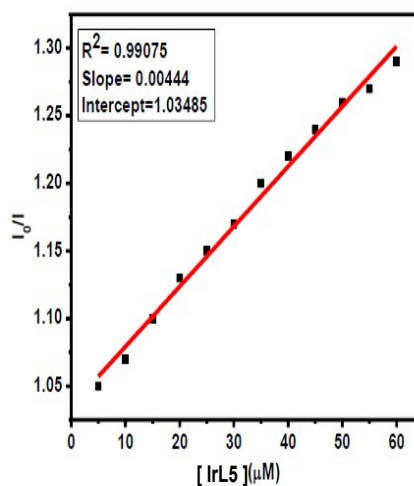
(a)



(b)



(c)



(d)

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**

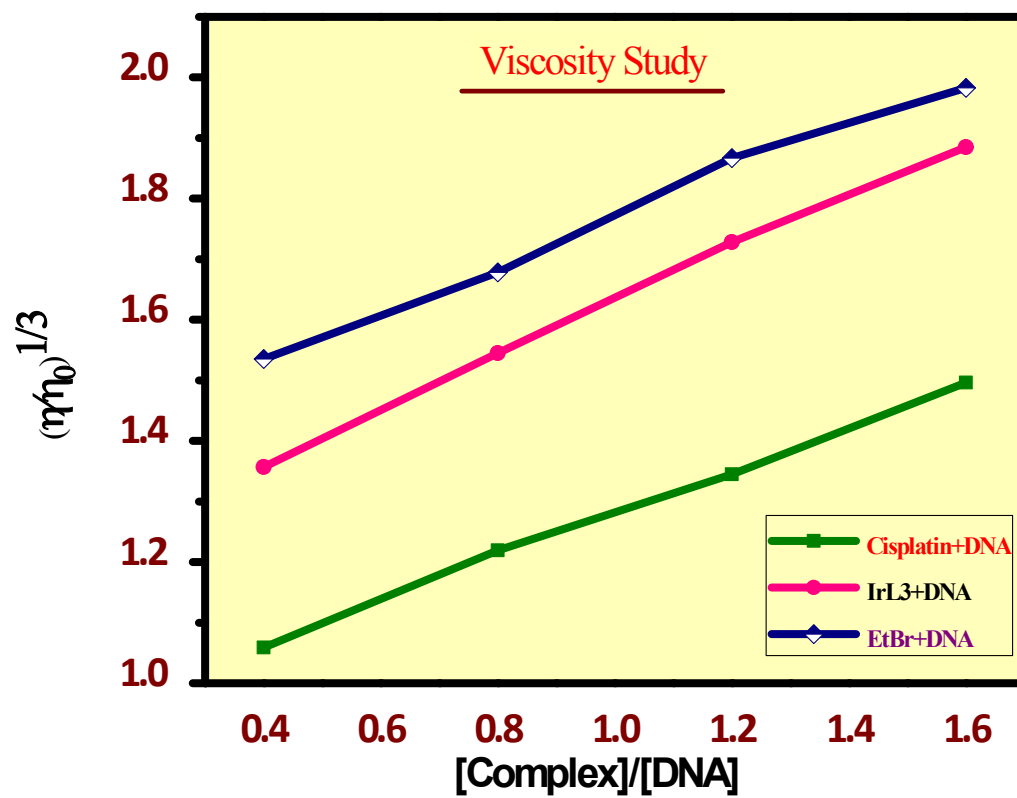


Fig. S11: Viscosity plot of complexes IrL3 and EtBr with Ct-DNA

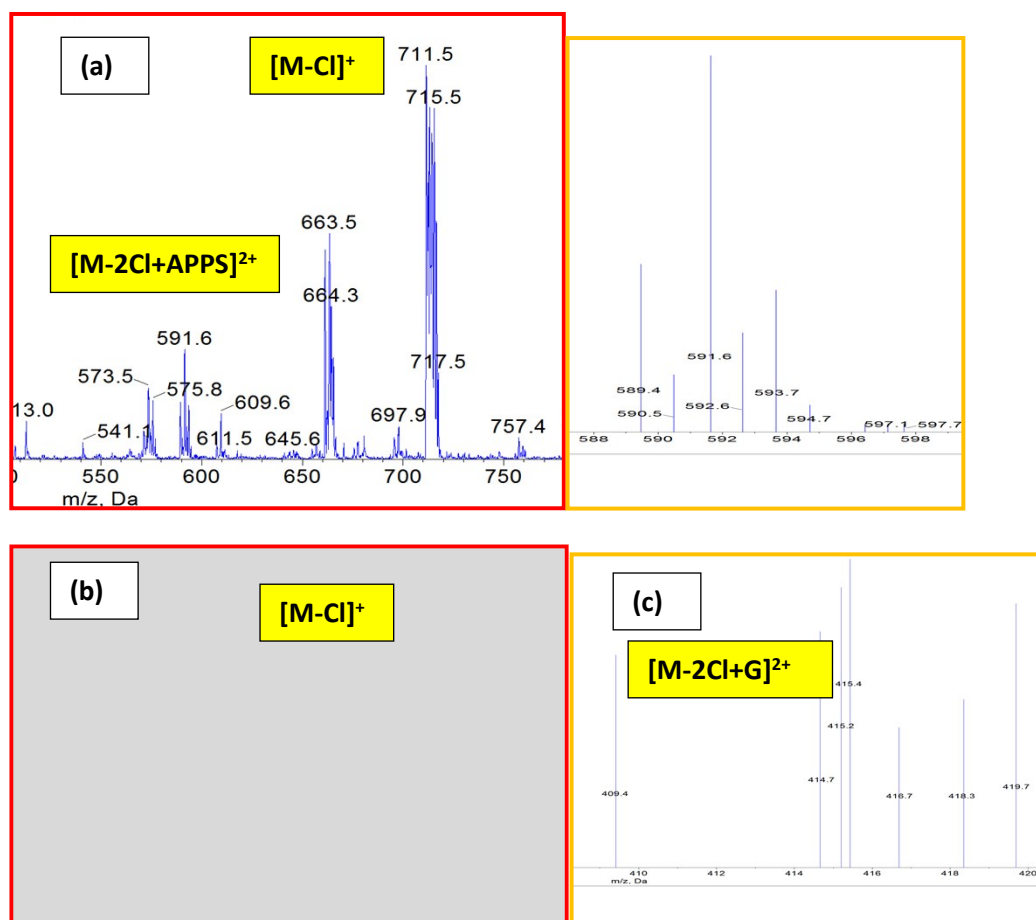
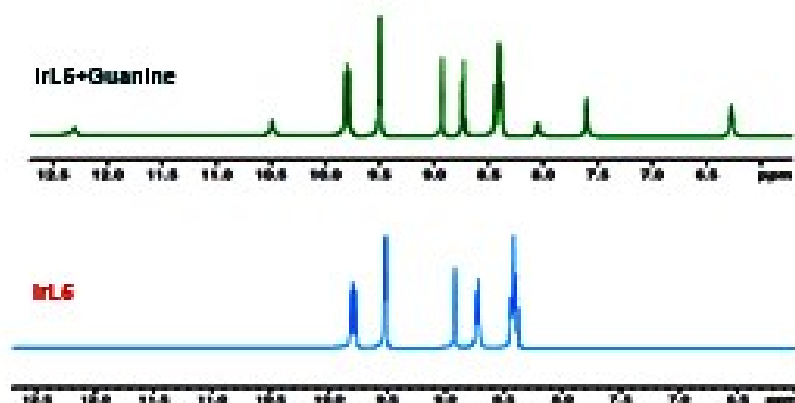


Fig. S12 ESI-MS of IrL5 treated with APPS (Adenosine-3'-phosphate 5'-phosphosulfate Lithium salt hydrate) and Guanine [(a) IrL5+APPS; (b) IrL5; (c) IrL5+G]

(a)



(b)

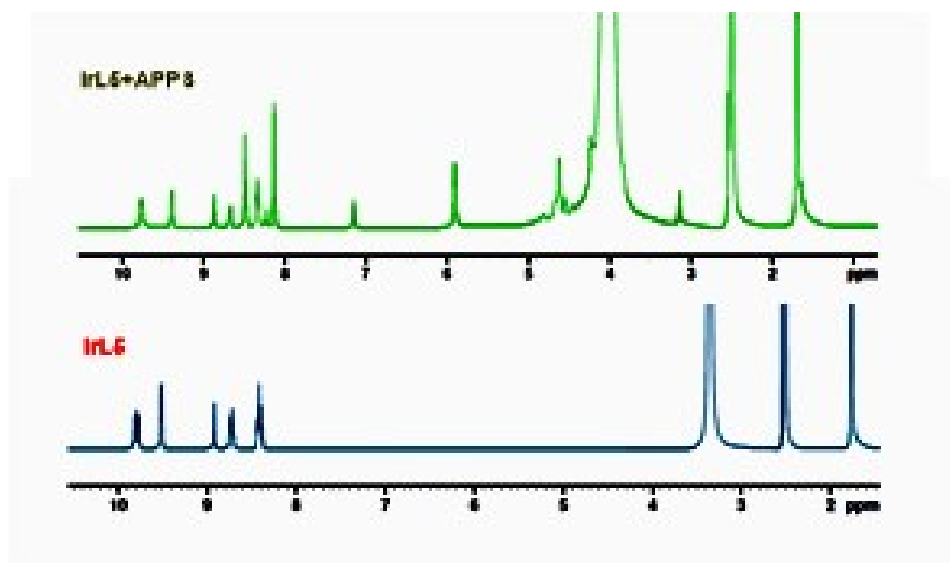


Fig.S13 ^1H NMR spectra recorded for the reaction of IrL5 with 1 eq. of (a) G and (b) APPS.

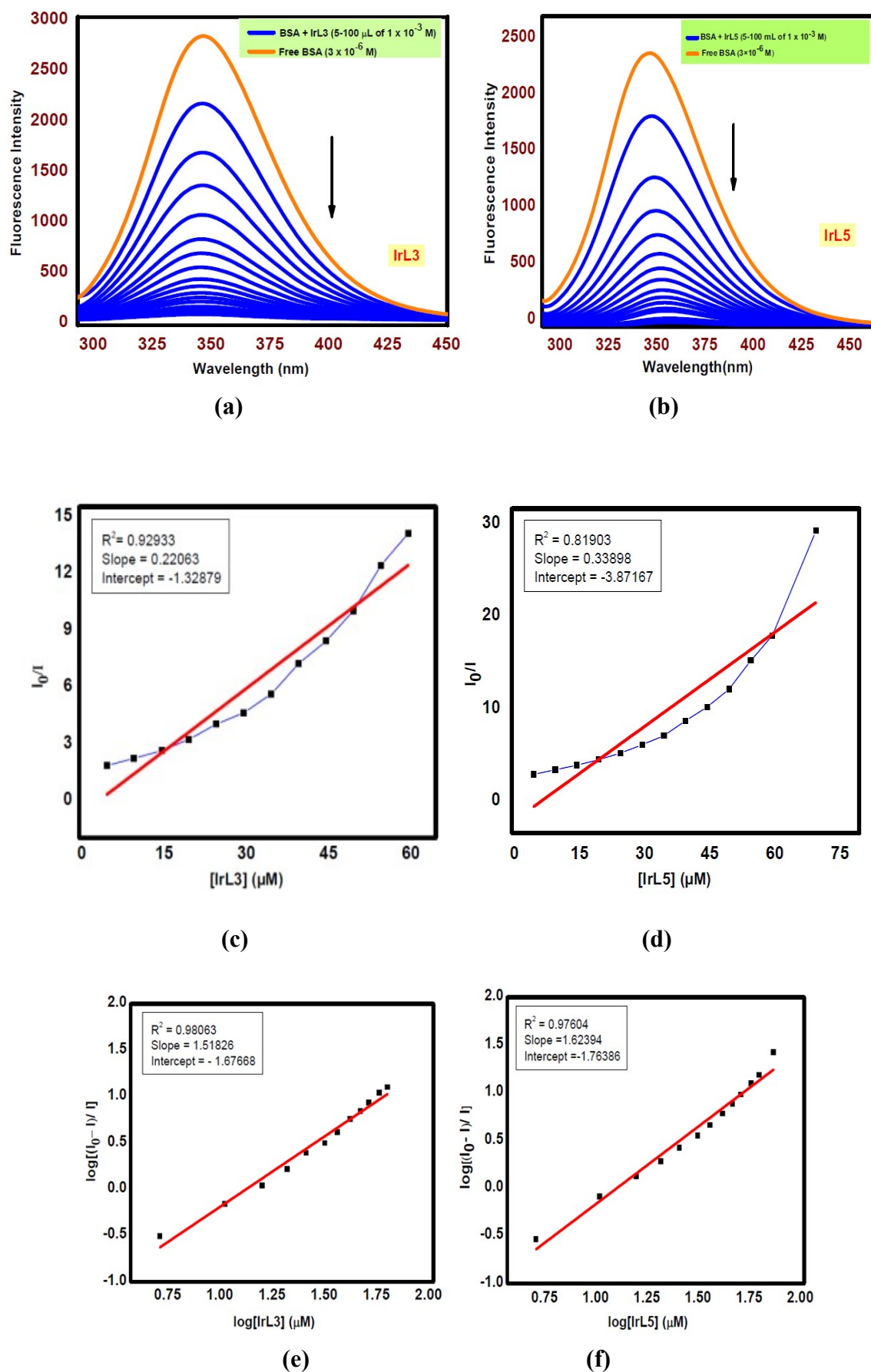


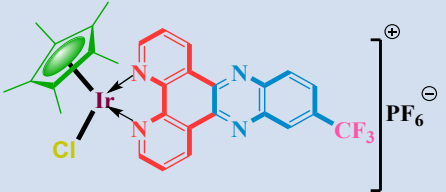
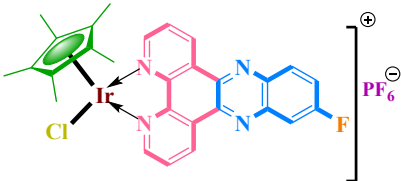
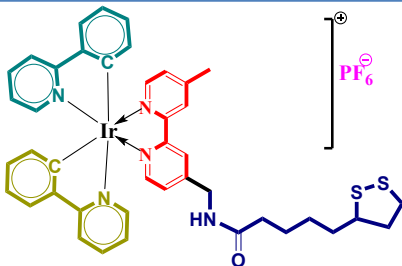
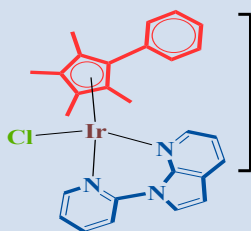
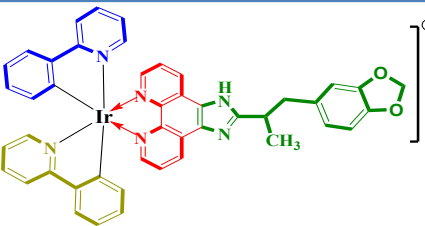
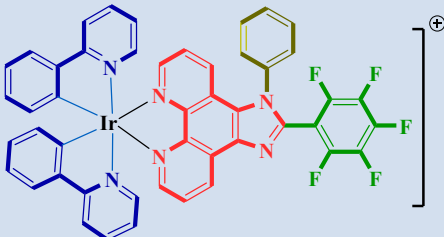
Fig. 14 Interaction of complexes (a)IrL3 and (b)IrL5 with BSA. Stern-Volmer Plot of I_0/I vs. concentration of complex (c)IrL3(d) IrL5. Scatchard Plot of $\log[(I_0 - I)/I]$ vs. $\log[\text{Complex}]$ for BSA in the presence of for complex (e) IrL3 (f) IrL5.

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 (μ s) DMSO 10% DMSO	
IrL1	400	510	110	0.26	8667	0.057	0.025	0.49 $\pm$ 0.01	21	32
IrL2	400	438	38	0.43	14333	0.001	0.021	0.52 $\pm$ 0.03	42	51
IrL3	423	598	175	0.16	5333	0.088	0.026	0.78 $\pm$ 0.10	23	34
IrL4	400	440	40	0.38	12667	0.002	0.018	0.69 $\pm$ 0.05	18	21
IrL5	400	507	107	1.32	44000	0.004	0.029	1.1 $\pm$ 0.12	71	92
IrL6	400	504	104	0.54	18000	0.002	0.022	0.97 $\pm$ 0.06	62	76
IrL7	400	447	47	1.23	41000	0.002	0.017	0.91 $\pm$ 0.05	65	81
Quinine Sulphate	350	452	102	0.24	8000	0.546	-	-	-	

^aAbsorption maxima at MLCT. ^bEmission wavelength. ^cOptical density. ^dExtinction coefficient. ^eQuantum yield. ^fDMSO-10% DMEM medium (1 : 99 v/v, comparable to cell media). ^gn-Octanol/water partition coefficient. ^hConductance in DMSO and 10% aqueous DMSO.

Table S2 IC₅₀ comparison table of the synthesized complexes with the reported complexes

Potent Complexes	Structures	IC ₅₀	Reference Numbers
		HeLa Cell	
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

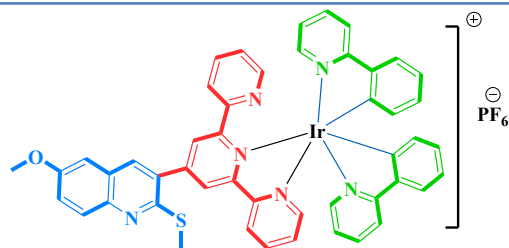
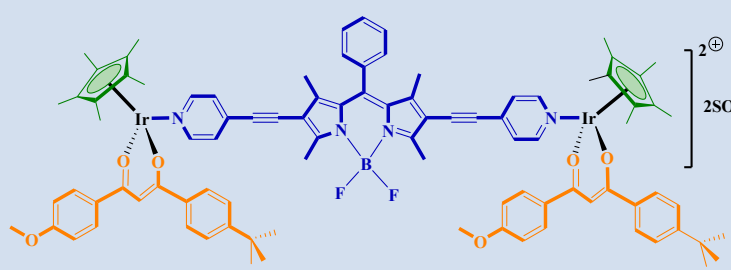
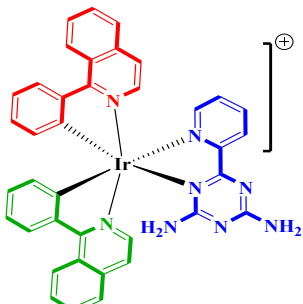
Complex5		3.46± 0.62	5
Complex6		2.0± 0.3	6
Complex7		29.2± 4.5	7

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

Complex	$\lambda_{\max}$ [nm]	Change in absorbance intensity	$^a\Delta\epsilon$ (%)	$^bK_b(\times 10^6 M^{-1})$	$^cK_{SV}(\times 10^6 M^{-1})$	$^dK_{app}(\times 10^6 M^{-1})$
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		

$^a\Delta\epsilon$, % of change in hypochromism. bK_b , intrinsic DNA binding constant from UV-Vis absorption titration. $^cK_{SV}$, Stern-Volmer quenching constant. $^dK_{app}$, 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	0.22×10^6	2.2×10^{13}	2.1×10^4	1.5
IrL5	0.34×10^6	3.4×10^{13}	1.7×10^4	1.6

$^aK_{BSA}$, Stern Volmer quenching constant; bK_q , quenching rate constant; cK , binding constant with BSA; dn , number of binding sites.

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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.
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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.