Supplementary material

Non-mutagenic Ru(II) complexes: cytotoxicity, Topoisomerase IB inhibition, DNA and HSA binding

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Table 1S. Crystal data and structure refinement parameters obtained for the complexes 1, 3 and 5.

	1	**3**	**5**
Empirical formula	$[RuC_{41}H_{40}N_3P_2S2]ClO_4$	$[RuC_{42}H_{41}N_6P_2S]PF_6$	$[RuC_{46}H_{41}N_4OP_2S]PF_6.2H_2O$
Formula weight	901.34	969.85	1041.90
Crystal system	Orthorhombic	Orthorhombic	Monoclinic
Space group	Pbca	Pbca	$P2_1/c$
Unit cell dimensions			
a (Å)	18.669(5)	18.3678(8)	10.0164(4)
b (Å)	19.702(5)	20.3902(5)	31.8519(14)
c (Å)	21.195(5)	22.2064(9)	14.9197(7)
β (°)	90	90	100.055
Volume ($Å^3$)	7796(3)	8316.8(5)	4686.9(4)
Z	8	8	4
Density calculated (Mg/m^3)	1.536	1.549	1.477
μ (mm^{-1})	0.707	0.610	0.550
F(000)	3696	3952	2128
Crystal size (mm^3)	0.184 x 0.056 x 0.051	0.105 x 0.092 x 0.085	0.090 x 0.040 x 0.040
θrange (°)	3.006 to 25.682	2.93 to 25.59	1.279 to 26.381
Index ranges	-20≤h≤22, -24≤k≤ 21, -25≤l≤ 23	-18≤h≤22, -24≤k≤24, -26≤l≤24	-11<=h<=12,-39<=k<=39, -18<=l<=18
Reflections collected	35026	26718	76595
Independent reflections	7375 [R(int) = 0.0737]	7787 [R(int) = 0.0875]	9602 [R(int) = 0.1752]
Completeness to θ (%)	99.7	99.6	100
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	7375 / 0 / 487	7787 / 0 / 484	9602 / 0 / 566
Goodness-of-fit on F^2	1.022	1.015	0.931
Final R indices [I>2sigma(I)]	R1 = 0.0454, wR2 = 0.1059	R1 = 0.0542, wR2 = 0.1222	R1 = 0.0604, wR2 = 0.1657
R indices (all data)	R1 = 0.0766, wR2 = 0.1170	R1 = 0.0997, wR2 = 0.1436	R1 = 0.1200, wR2 = 0.1870
$\Delta\rho_{max.}$and$\Delta\rho_{min.}$ ($e.Å^{-3}$)	0.621 and -0.457	0.507 and -0.538	1.52 d -0.908

Table 2S. Bond and angle lengths (Å, °) for the complexes 1, 3 and 5.

Complex 1	
Ru(1)-N(2)	2.116(3)
Ru(1)-N(3)	2.139(3)
Ru(1)-N(1)	2.151(3)
Ru(1)-P(1)	2.3095(11)
Ru(1)-P(2)	2.3296(11)
Ru(1)-S(1)	2.4375(11)
N(2)-Ru(1)-N(3)	77.28(12)
N(2)-Ru(1)-N(1)	90.60(13)
N(3)-Ru(1)-N(1)	82.96(12)
N(2)-Ru(1)-P(1)	107.35(9)
N(3)-Ru(1)-P(1)	91.70(9)
N(1)-Ru(1)-P(1)	159.72(10)
N(2)-Ru(1)-P(2)	100.43(9)
N(3)-Ru(1)-P(2)	173.83(9)
N(1)-Ru(1)-P(2)	91.37(9)
P(1)-Ru(1)-P(2)	94.46(4)
N(2)-Ru(1)-S(1)	156.34(9)
N(3)-Ru(1)-S(1)	90.93(9)
N(1)-Ru(1)-S(1)	67.39(10)
P(1)-Ru(1)-S(1)	93.26(4)
P(2)-Ru(1)-S(1)	89.13(4)
Complex 3	
Ru-N(21)	2.121(4)
Ru-N(11)	2.134(4)
Ru-N(1)	2.184(4)
Ru-P(1)	2.3038(13)
Ru-P(2)	2.3360(13)
Ru-S(1)	2.3939(12)
N(21)-Ru-N(11)	77.28(14)
N(21)-Ru-N(1)	93.82(14)
N(11)-Ru-N(1)	82.72(13)
N(21)-Ru-P(2)	106.13(10)
N(11)-Ru-P(2)	92.16(10)
N(1)-Ru-P(2)	157.82(11)
N(21)-Ru-P(1)	100.08(11)
N(11)-Ru-P(1)	172.84(10)
N(1)-Ru-P(1)	90.87(10)
P(2)-Ru-P(1)	94.97(5)
N(21)-Ru-S(1)	159.53(10)
N(11)-Ru-S(1)	91.53(10)
N(1)-Ru-S(1)	67.51(11)
P(2)-Ru-S(1)	91.19(5)
P(1)-Ru-S(1)	88.94(4)
Complex 5	
Ru(1)-N(3)	2.095(4)
Ru(1)-N(4)	2.134(4)
Ru(1)-N(2)	2.157(5)
Ru(1)-P(1)	2.2938(16)
Ru(1)-P(2)	2.3372(15)
Ru(1)-S(1)	2.4346(16)
N(3)-Ru(1)-N(4)	77.33(18)
N(3)-Ru(1)-N(2)	97.78(17)
N(4)-Ru(1)-N(2)	83.10(17)
N(3)-Ru(1)-P(1)	89.64(13)
N(4)-Ru(1)-P(1)	92.17(13)
N(2)-Ru(1)-P(1)	170.08(12)
N(3)-Ru(1)-P(2)	101.43(13)
N(4)-Ru(1)-P(2)	174.01(13)
N(2)-Ru(1)-P(2)	91.30(12)
P(1)-Ru(1)-P(2)	93.68(6)
N(3)-Ru(1)-S(1)	161.62(13)
N(4)-Ru(1)-S(1)	89.85(14)
N(2)-Ru(1)-S(1)	67.27(12)
P(1)-Ru(1)-S(1)	104.14(6)
P(2)-Ru(1)-S(1)	89.94(6)

Table 3S. Mutagenic activity expressed as the mean and standard deviation of the number of revertants and mutagenic index (MI) (in brackets) in strains TA98, TA100, TA102 and TA97a exposed to compounds 1, 2 and 3 at various doses, with (+S9) or without (-S9) metabolic activation.

Treatments	Number of revertants (M ± SD)/plate and MI							
µg/per plate	TA 98		TA 100		TA 102		TA 97a	
(1)	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
0.00[a]	17 ± 3	32 ± 3	111 ± 7	133 ± 7	343 ± 20	336± 20	106 ± 13	149± 8
0.78	16 ± 5 (0,9)	35 ± 4 (1,0)	113 ± 10 (1,0)	136 ± 11 (1,0)	338 ± 21 (1.0)	341 ± 17 (1.0)	94 ± 11 (0,9)	141 ± 11 (0,9)
1.56	15 ± 4 (0,9)	36 ± 2 (1.1)	102 ± 9 (0,9)	131 ± 10 (0,9)	325 ± 19 (0,9)	329 ± 19 (1,0)	88 ± 8 (0,8)	137 ± 15 (1,0)
3.12	17 ± 4 (1,0)	36 ± 5 (1,1)	100 ± 8 (0,9)	132 ± 9 (1,0)	321 ± 27 (0,9)	338 ± 24 (1,0)	90 ± 7 (0,8)	140 ± 11 (1,0)
6.25	18 ± 3 (1,0)	32 ± 3 (1,0)	98 ± 11 (0,9)	126 ± 14 (0,9)	317± 29 (0,9)	347 ± 31 (1,0)	91 ± 10 (0,8)	136 ± 16 (1,1)
9.37	15 ± 5 (0,9)	34 ± 4 (1,0)	94 ± 7 (0,8)	119 ± 12 (0,9)	319 ± 28 (0,9)	327 ± 29 (1,0)	85 ± 9 (0,8)	122 ± 21 (1,1)
C +	830 ± 37[b]	1227 ± 89[e]	1001 ± 48[c]	1340 ± 73[e]	1207 ± 44[d]	1411 ± 41[f]	1663 ± 58[b]	1012±92[e]
(2)								
0.00[a]	18 ± 2	23 ± 4	102 ± 9	144 ± 10	344 ± 16	321 ± 17	129 ± 10	133 ± 14
0.78	17 ± 4 (0,9)	22 ± 3 (0,9)	105 ± 5 (1,0)	141 ± 15 (0,9)	347 ± 5 (1,0)	332 ± 18 (1,0)	120 ±17 (0,9)	145 ± 10 (1,0)
1.56	21 ± 3 (1,1)	24 ± 2 (1,1)	109 ± 7 (1,0)	137 ± 9 (0,9)	330 ± 15 (0,9)	338 ± 9 (1,0)	110 ± 18 (0,8)	147 ± 12 (1,1)
3.12	19 ± 3 (1,0)	21 ± 5 (0,8)	99 ± 9 (1,0)	131± 13 (0,9)	318 ± 19 (0,9)	353 ± 21 (1,1)	109 ± 13 (0,8)	139 ± 9 (1,0)
6.25	20 ± 2 (1,1)	22 ± 5 (1,0)	101 ± 9 (1,0)	128 ± 11 (0,9)	303 ± 22 (0,9)	342 ± 27 (1,0)	103 ± 16 (0,8)	148 ± 4 (1,1)
9.37	19 ± 3 (1,0)	20 ± 4 (0,8)	90 ± 10 (0,9)	137 ± 10 (0,9)	306 ± 21 (0,9)	335 ± 24 (1,0)	106 ± 19 (0,8)	138 ± 11 (1,0)
C +	913 ± 47[b]	2216 ± 88[e]	1613 ± 49[c]	1761 ± 82[e]	1255 ± 56[d]	1308 ± 64[f]	1032± 93[b]	17083 ± 110[e]
(3)								
0.00[a]	22 ± 3	25 ± 3	109 ± 8	139 ± 7	349 ± 20	327 ± 18	99 ± 12	129 ± 11
0.78	19 ± 3 (0,8)	21 ± 2 (0,8)	117 ± 11 (1,0)	144 ± 12 (1,0)	353 ± 21 (1,0)	321 ± 16 (1,0)	95 ± 7 (0,9)	141 ± 9 (1,0)
1.56	20 ± 2 (0,9)	22 ± 3 (0,9)	107 ± 9 (0,9)	136 ± 7 (0,9)	341 ± 19 (1,0)	336 ± 11 (1,0)	88 ± 9 (0,9)	146 ± 13 (1,1)
3.12	20 ± 4 (0,9)	24 ± 4 (0,9)	103 ± 7 (0,9)	134± 11 (0,9)	321 ± 21 (0,9)	346 ± 19 (1,0)	96 ± 5 (0,9)	133 ± 8 (1,0)
6.25	21 ± 2 (0,9)	27 ± 6 (1,1)	98 ± 10 (0,9)	127 ± 10 (0,9)	307± 25 (0,9)	339 ± 25 (1,0)	90 ± 7 (0,9)	145 ± 7 (1,1)
9.37	18 ± 4 (0,8)	22 ± 3 (0,9)	107 ± 8 (0,8)	132 ± 11 (0,9)	310 ± 27 (0,9)	331 ± 22 (1,0)	83 ± 11 (0,8)	139 ± 10 (1,0)
C +	953 ± 44[b]	2187 ± 81[e]	1201 ± 51[c]	1801 ± 75[e]	1293 ± 39[d]	1327 ± 60[f]	1451 ± 61[b]	1712 ± 91[e]

[a] Negative control: dimethylsulfoxide (DMSO) – 100 mL per plate; C+ = Positive control. [b] 4-Nitro-o-phenylenediamine (NOPD) – 10.0 mg per plate –positive control in the absence of S9 for TA98 and TA97a. [c] Sodium azide (1.25 mg per plate) – positive control in the absence of S9 for TA100.[d]Mitomycin (0.5 mg per plate) – positive control in the absence of S9 for TA102. [e] 2-Anthramine (1.25 mg per plate) – positive control in the presence of S9 for TA97a, TA98, and TA100. [f] 2-Aminofluorene (10.0 mg per plate) – positive control in the presence of S9 for TA102.

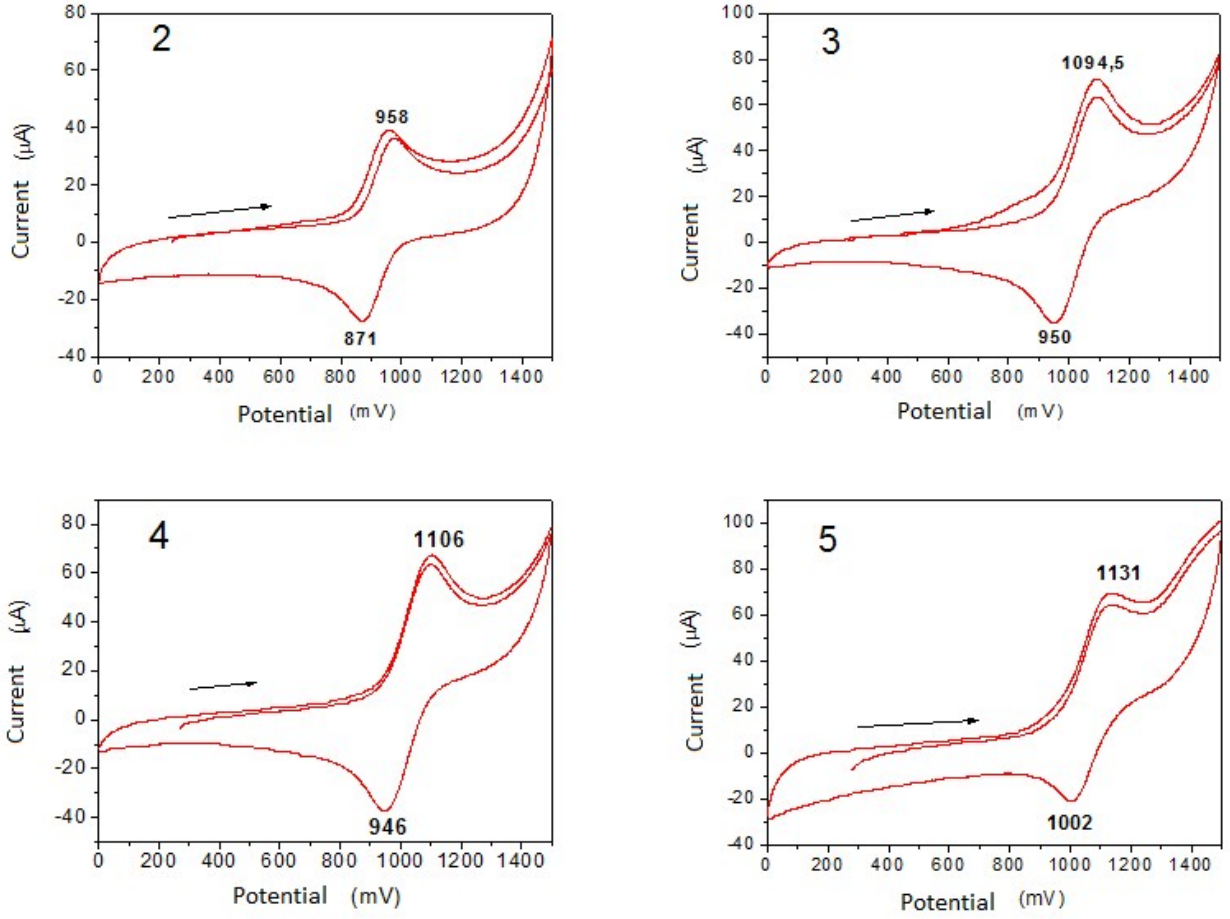
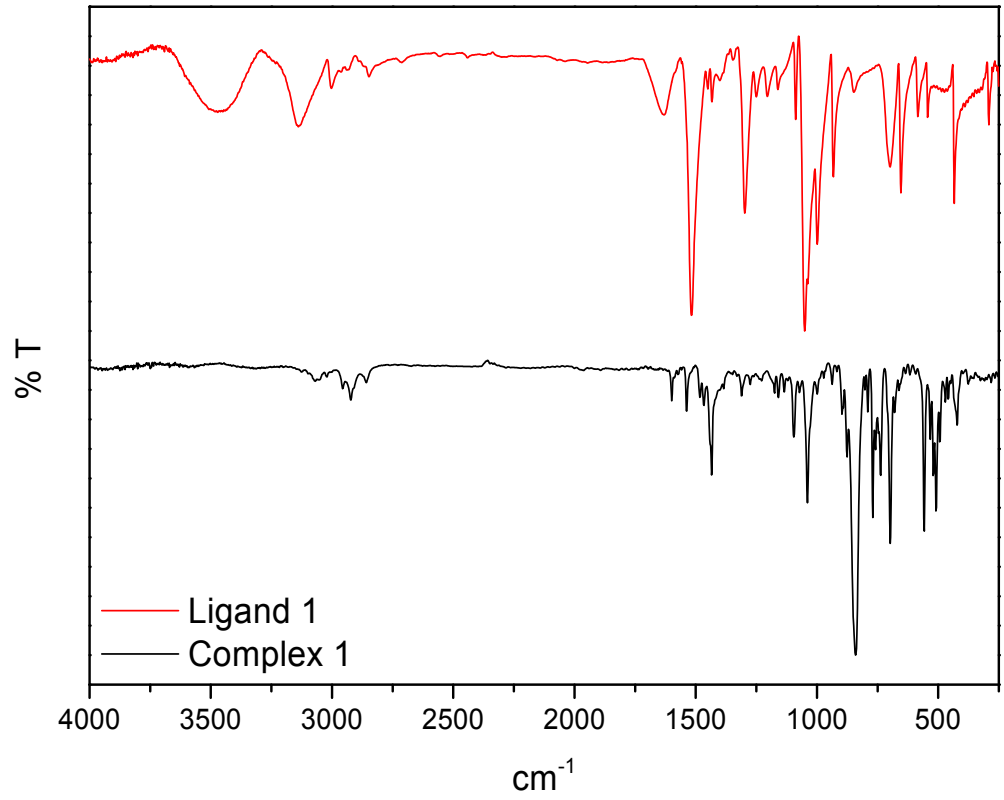


Figure 1S. Cyclic voltammograms of complexes 2-5 in CH_2Cl_2, scan rate 100 mV/s.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Complex 1
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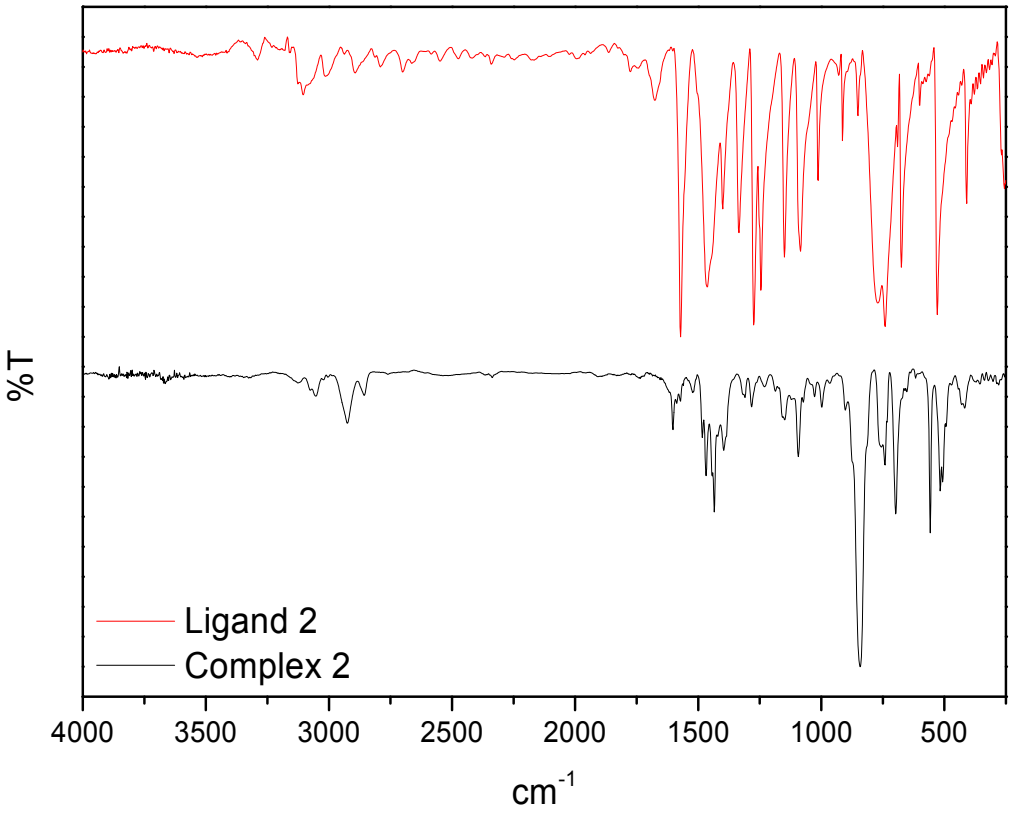
3500
3000
2500
2000
1500
1000
500
cm-1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Complex 2
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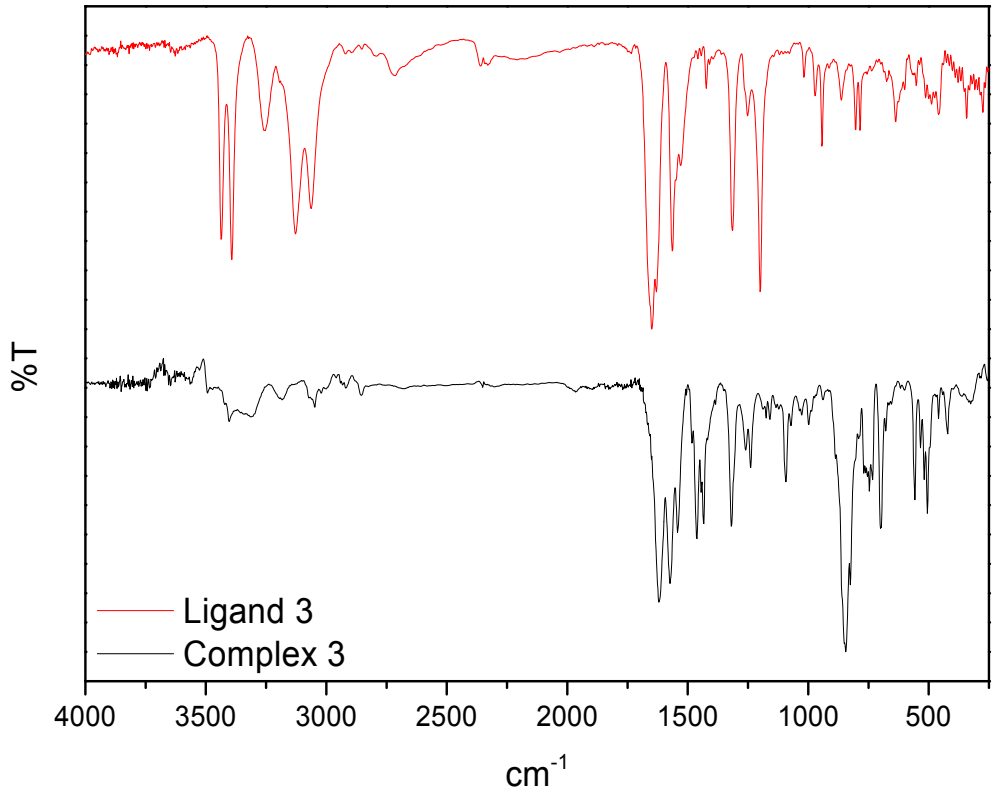
3500
3000
2500
2000
1500
1000
500
cm-1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Complex 3
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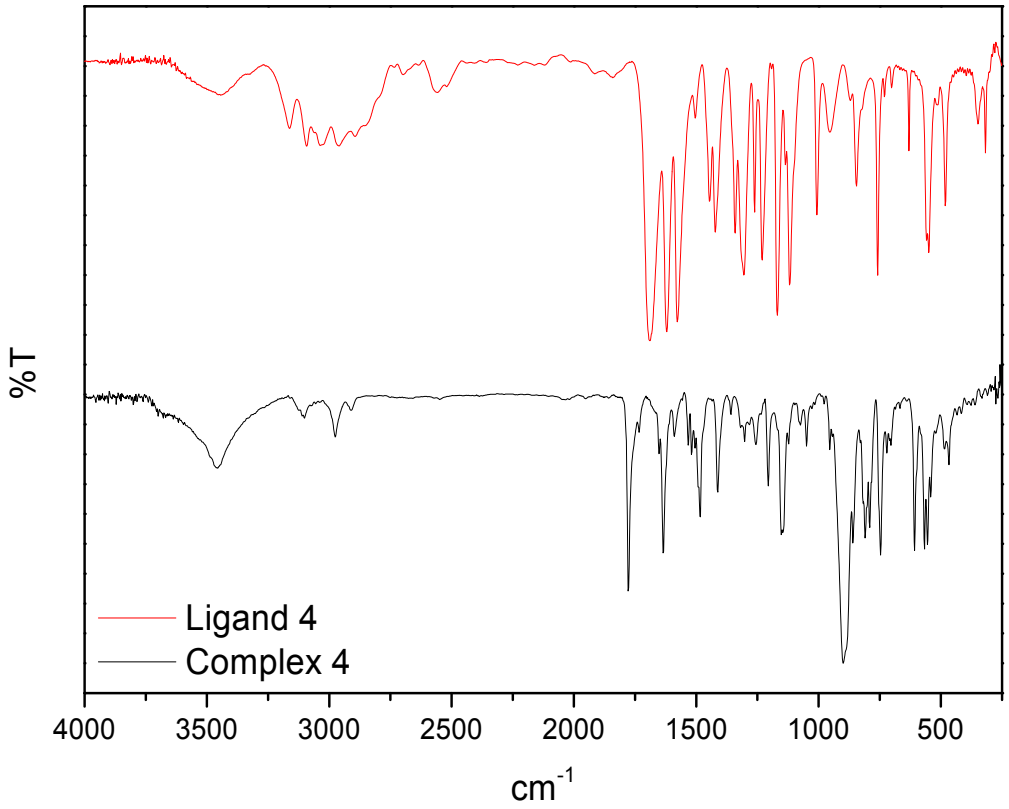
3500
3000
2500
2000
1500
1000
500
cm-1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Complex 4
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3500
3000
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1500
1000
500
cm-1

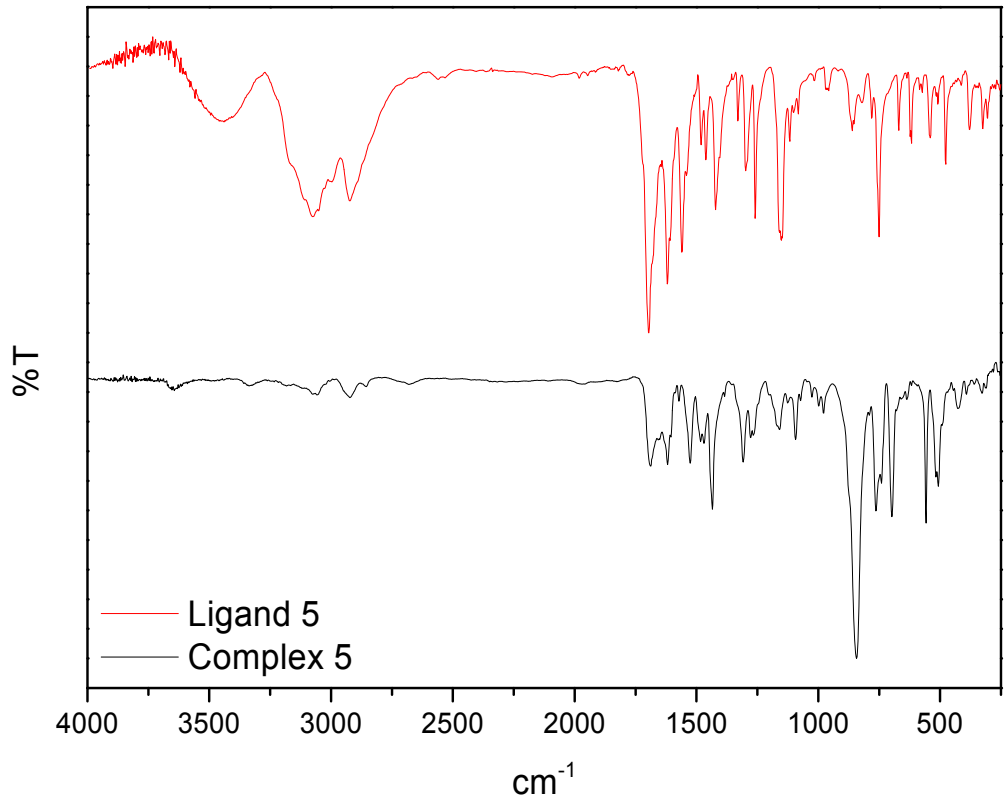
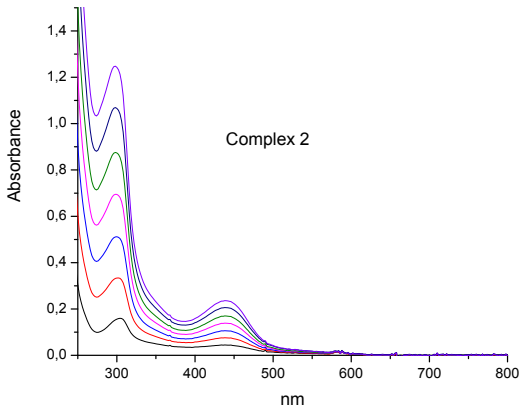
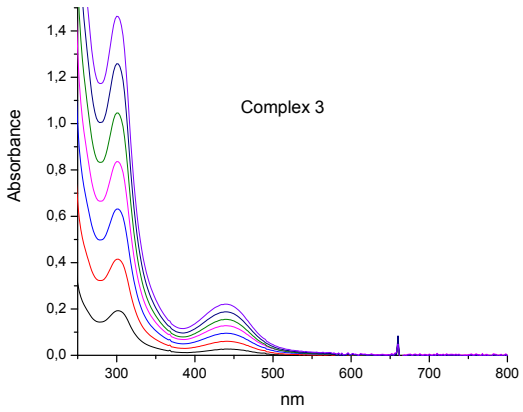


Figure 2S. FT-Infrared spectra of the ligads (1-5) and complexes 1-5





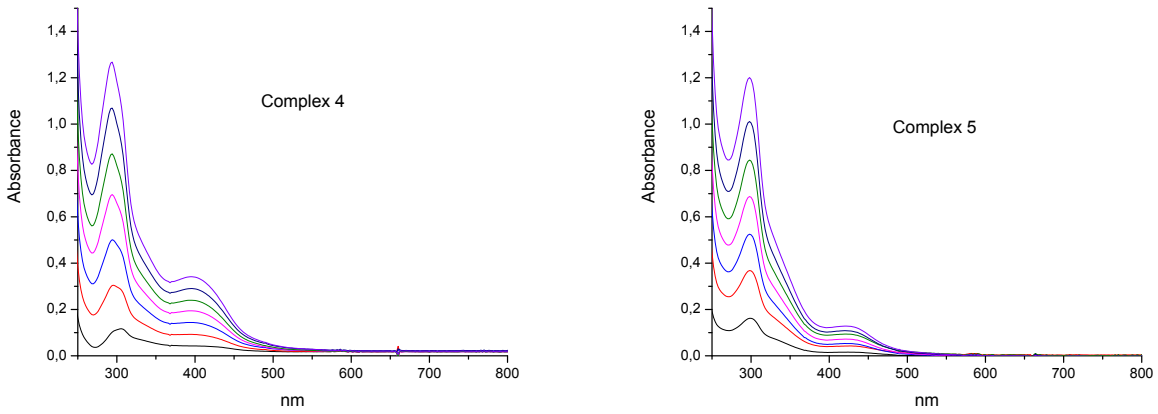
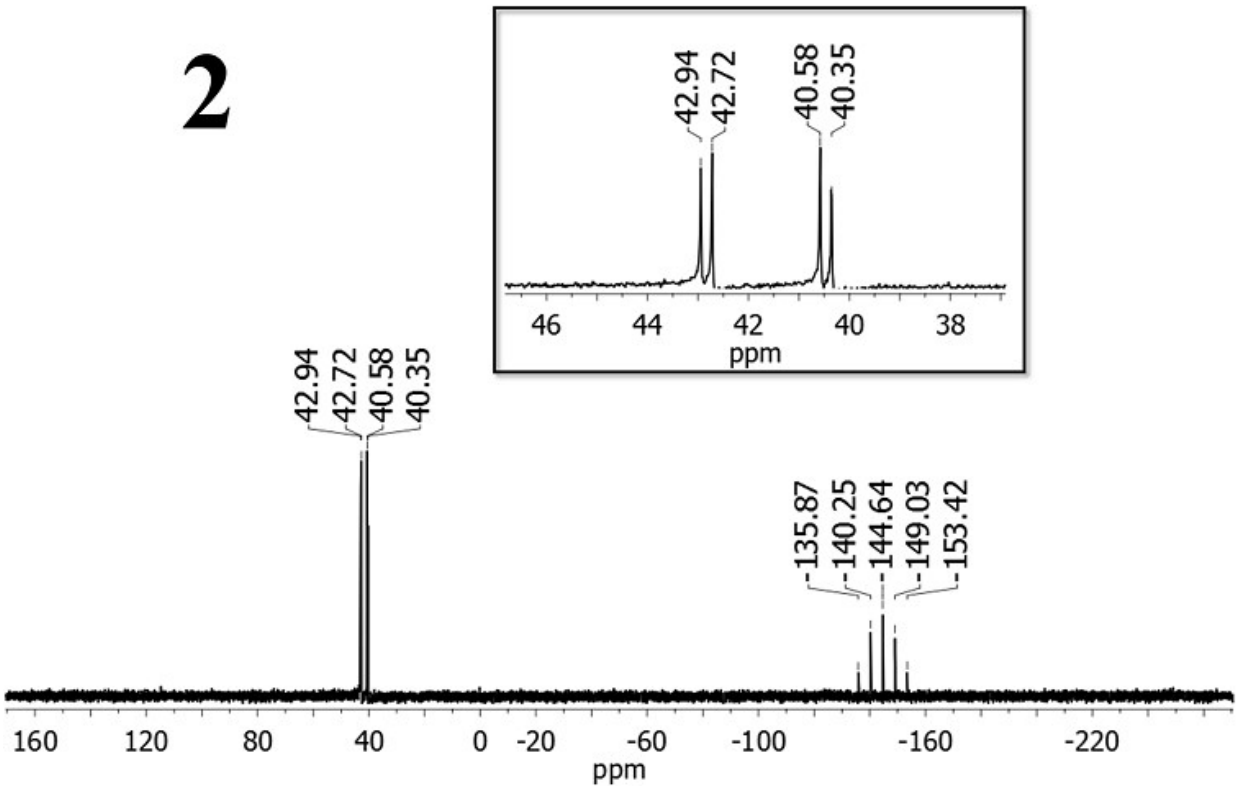


Figure 3S. Uv/Vis spectra of complexes 2-5, in $CHCl_2$.



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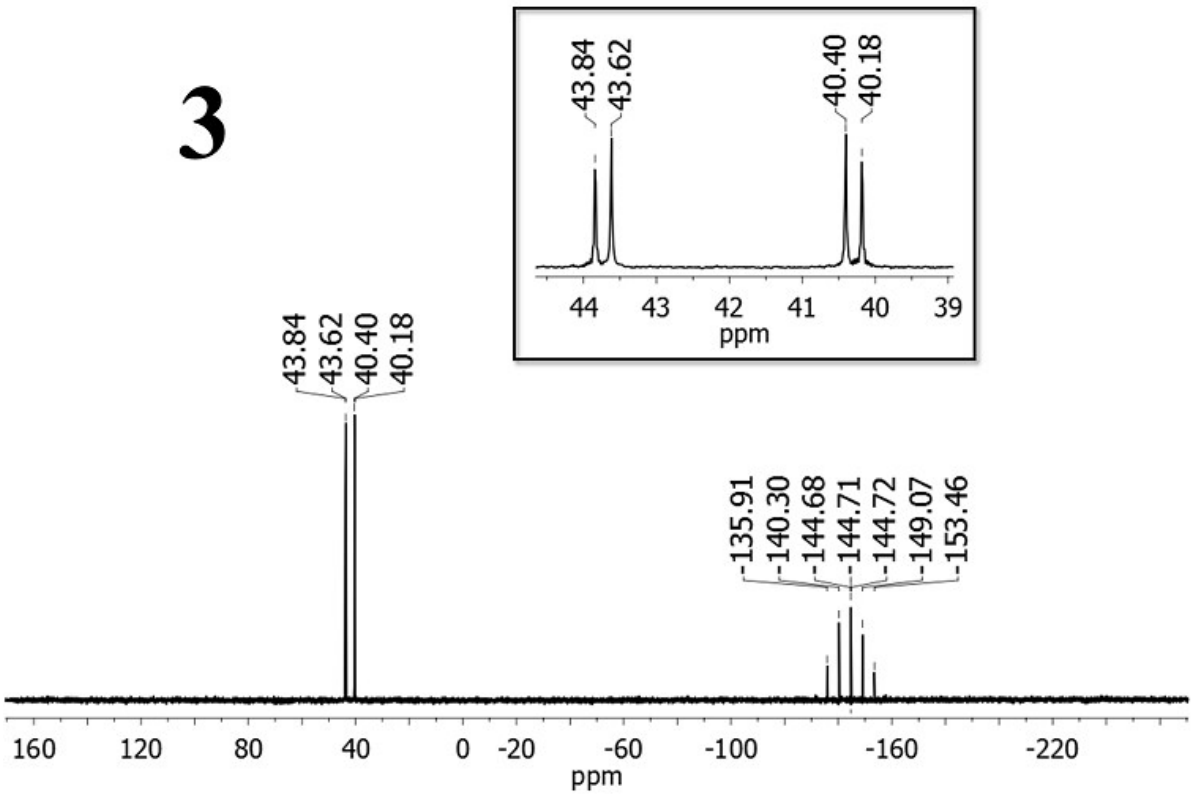
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43
42
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40
39
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43.62
40.40
40.18
135.91
140.30
144.68
144.71
144.72
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153.46
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120
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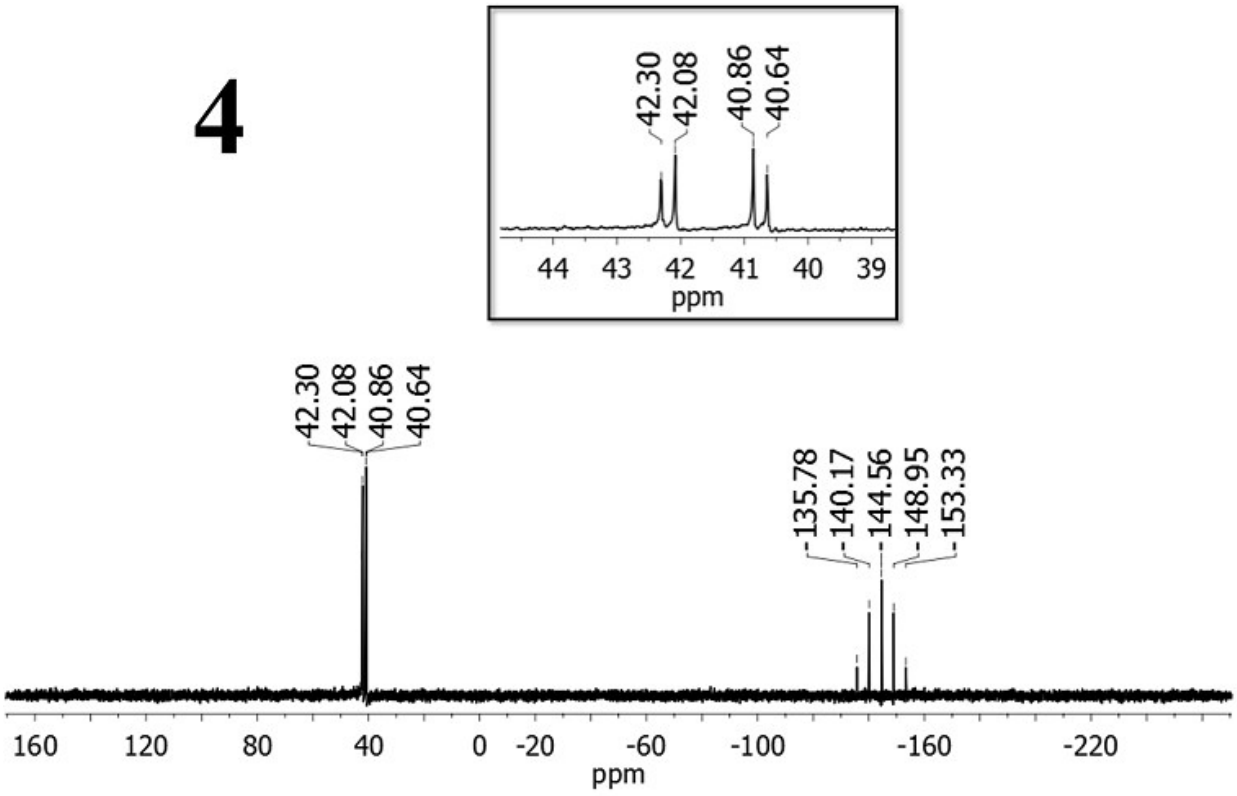
-20
-60
-100
-160
-220
ppm

4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42
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144.56
148.95
153.33
160
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0
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-220
ppm

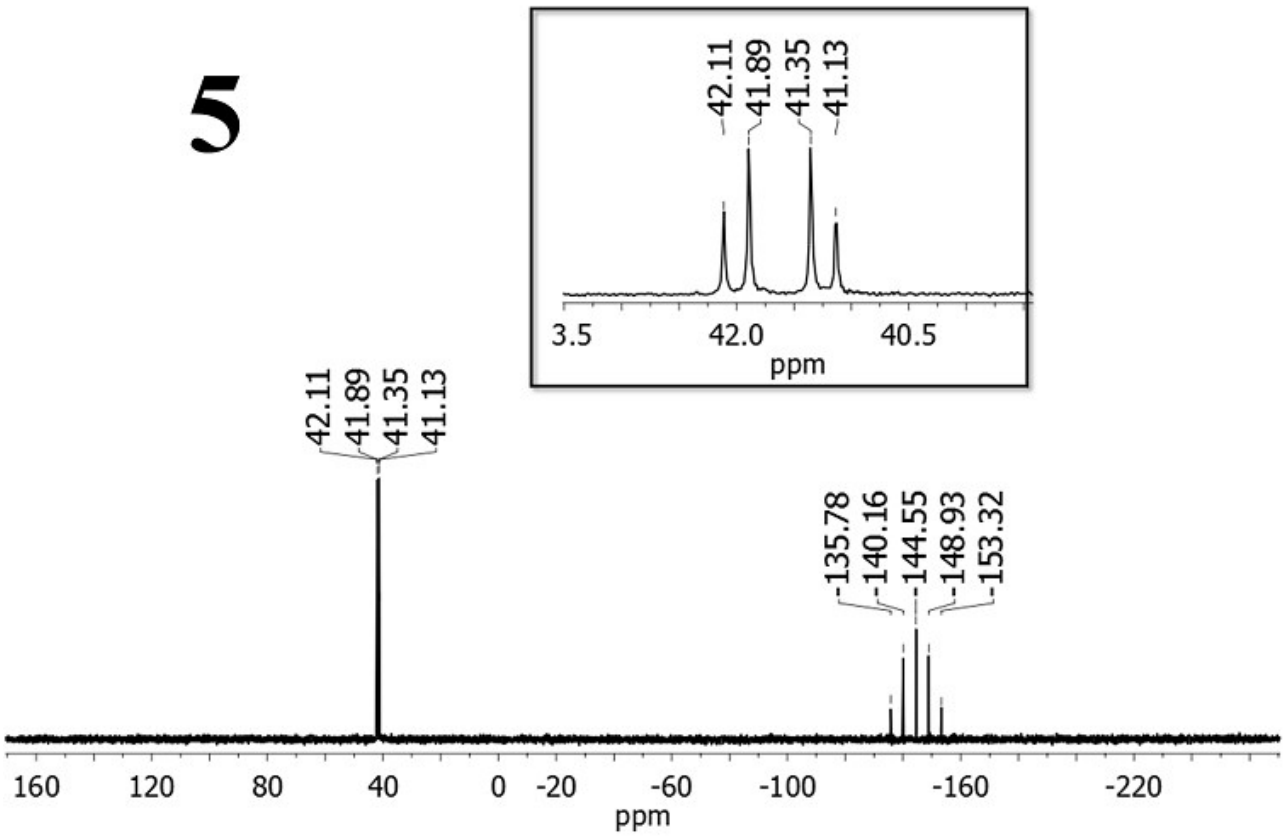
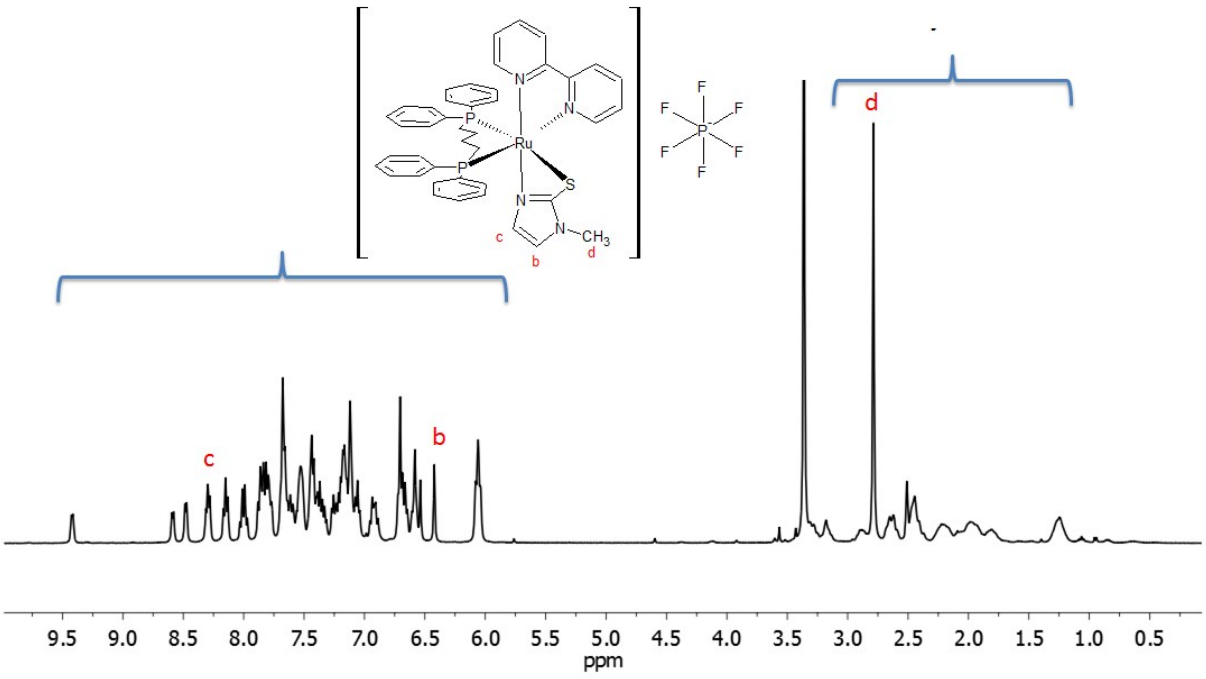


Figure 4S. $^{31}P\{^{1}H\}$ NMR spectra for complexes 2-5 in CH_2Cl_2/D_2O.



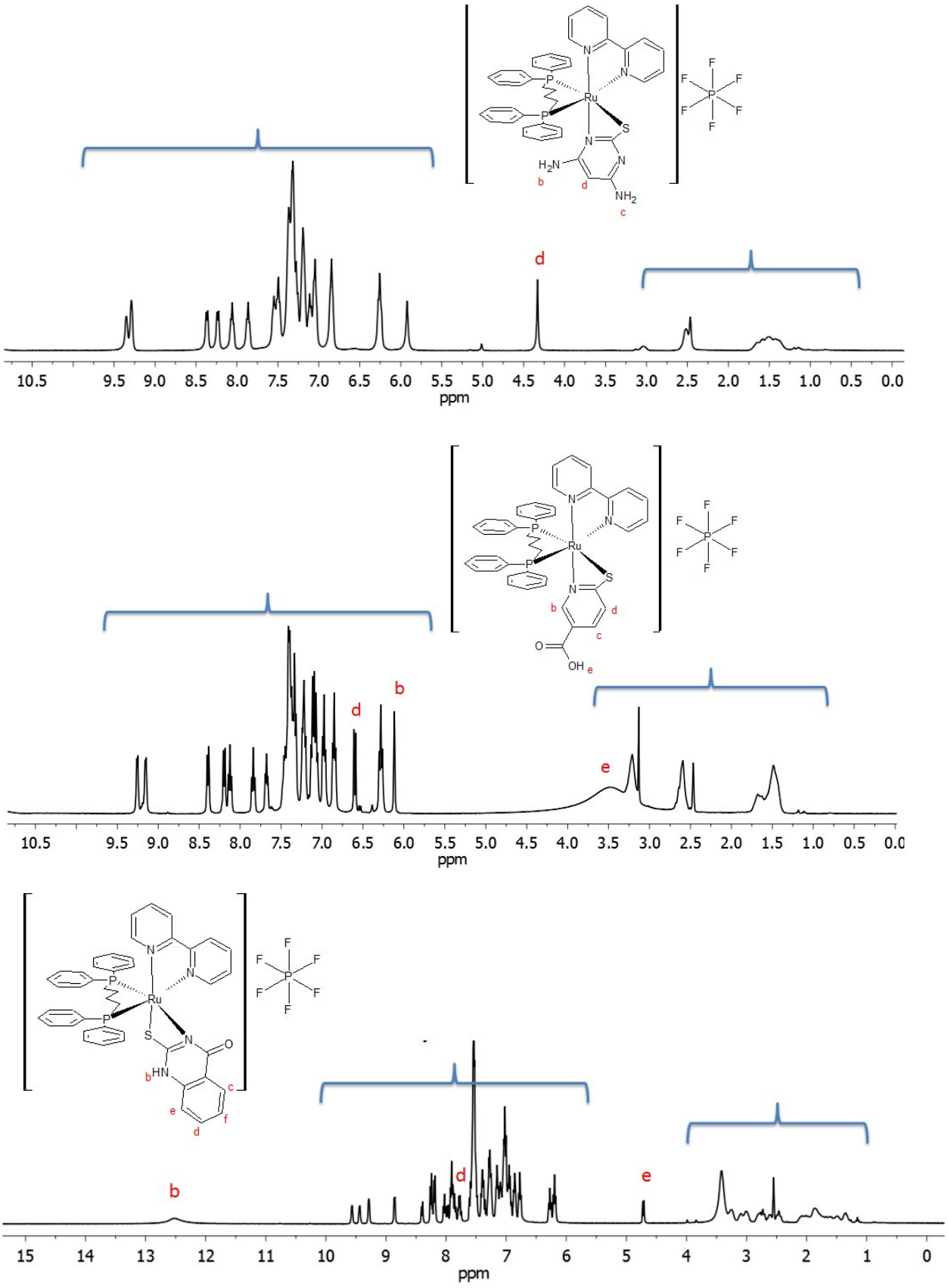
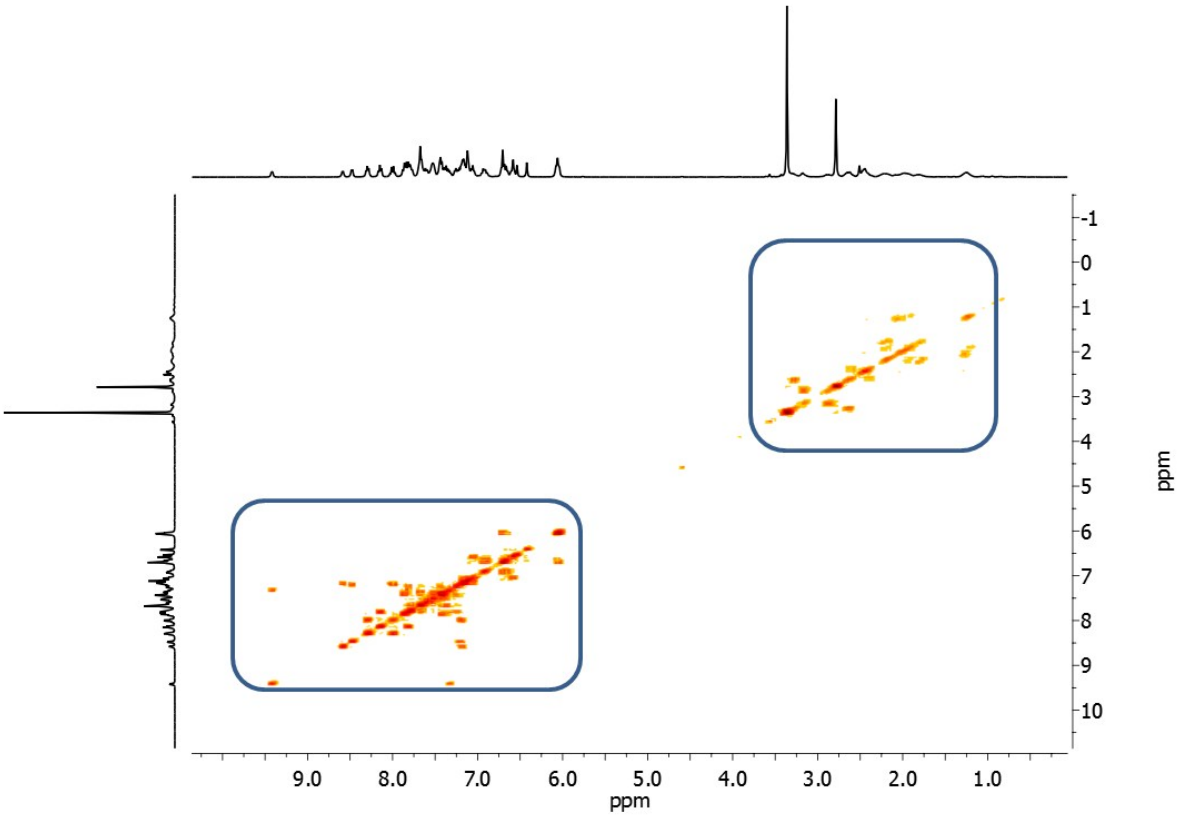


Figure 5S: {^{1}H} NMR spectra for complexes 2-5 in DMSO-d_6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0
1
2
3
4
5
6
7
8
9
10
ppm
9.0
8.0
7.0
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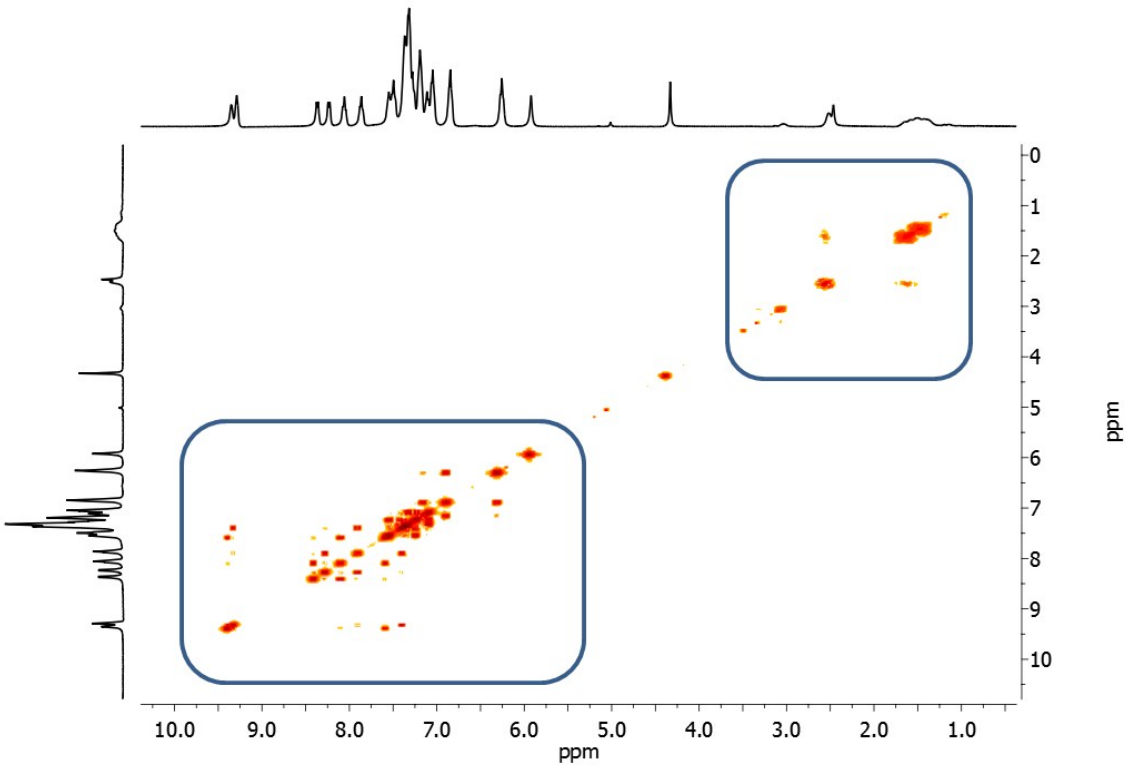
5.0
4.0
3.0
2.0
1.0
ppm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2
3
4
5
6
7
8
9
10
ppm
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4.0
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2.0
1.0
ppm

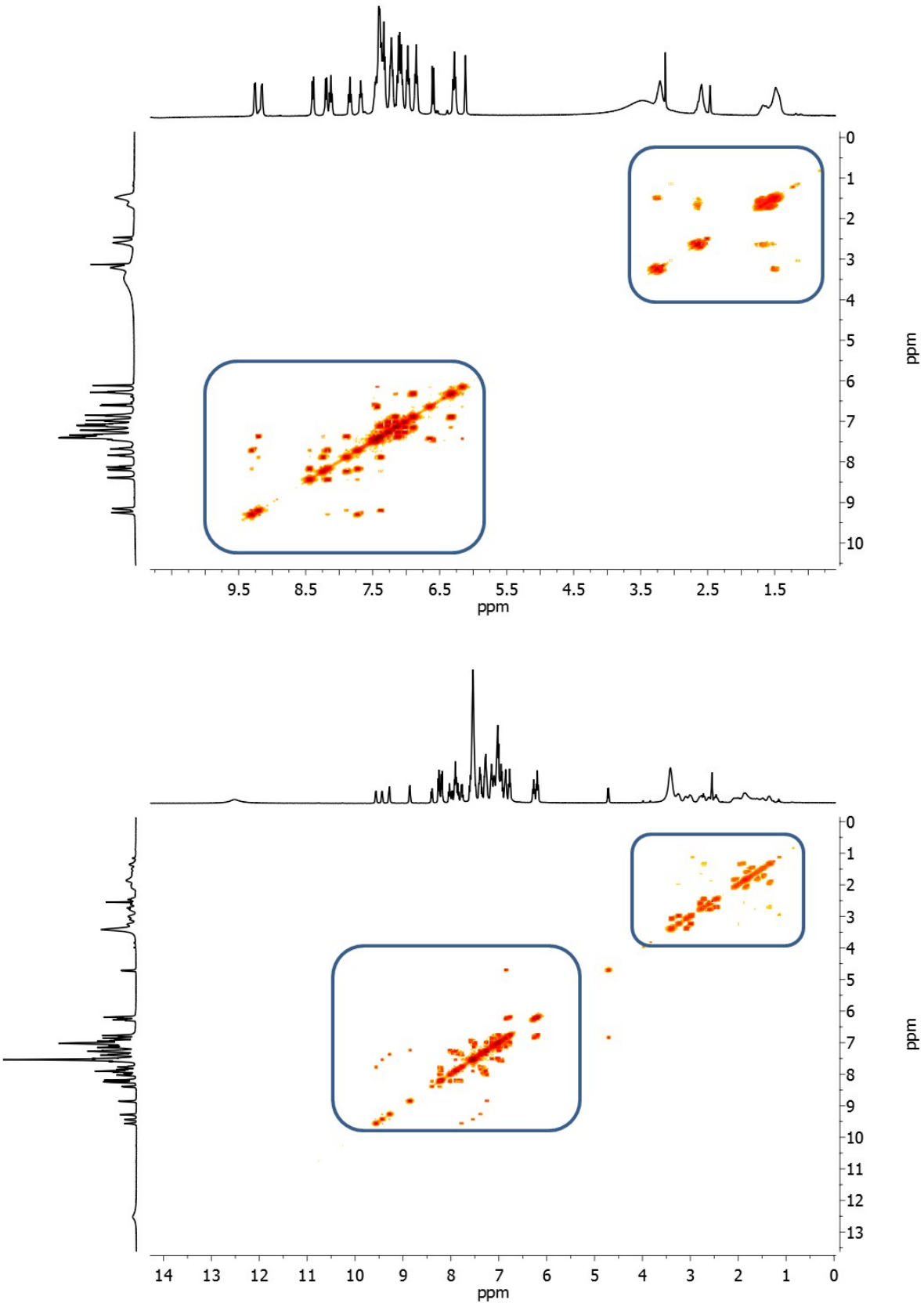
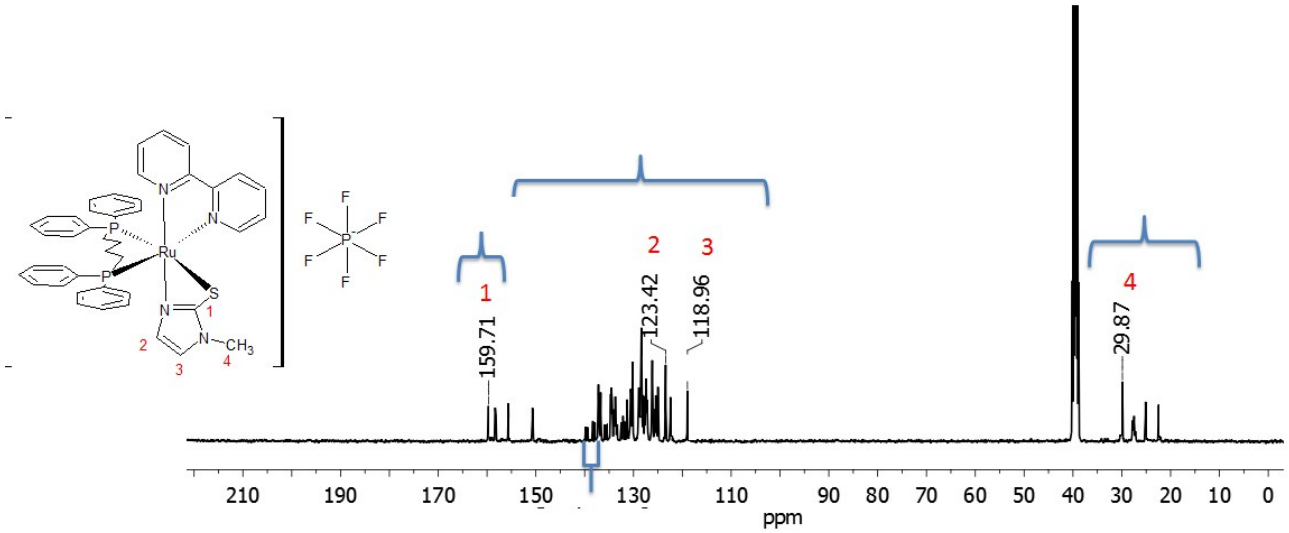


Figure 6S: COSY (^{1}H-^{1}H) NMR spectra for complexes 2-5 in DMSO-d_6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4
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118.96
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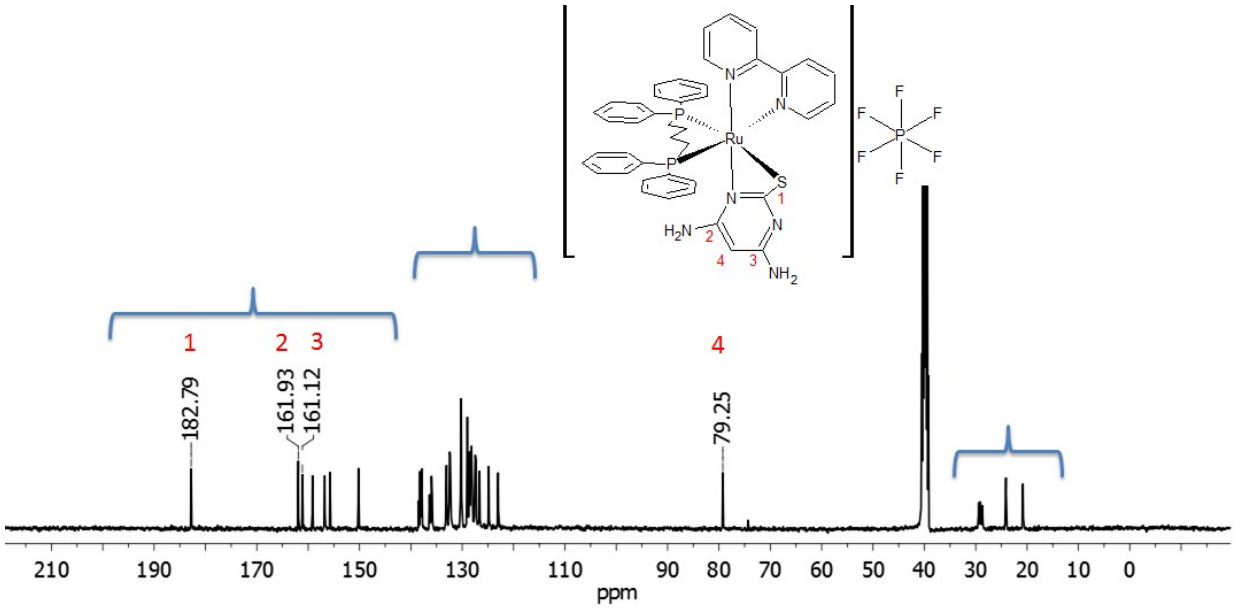
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2
3
4
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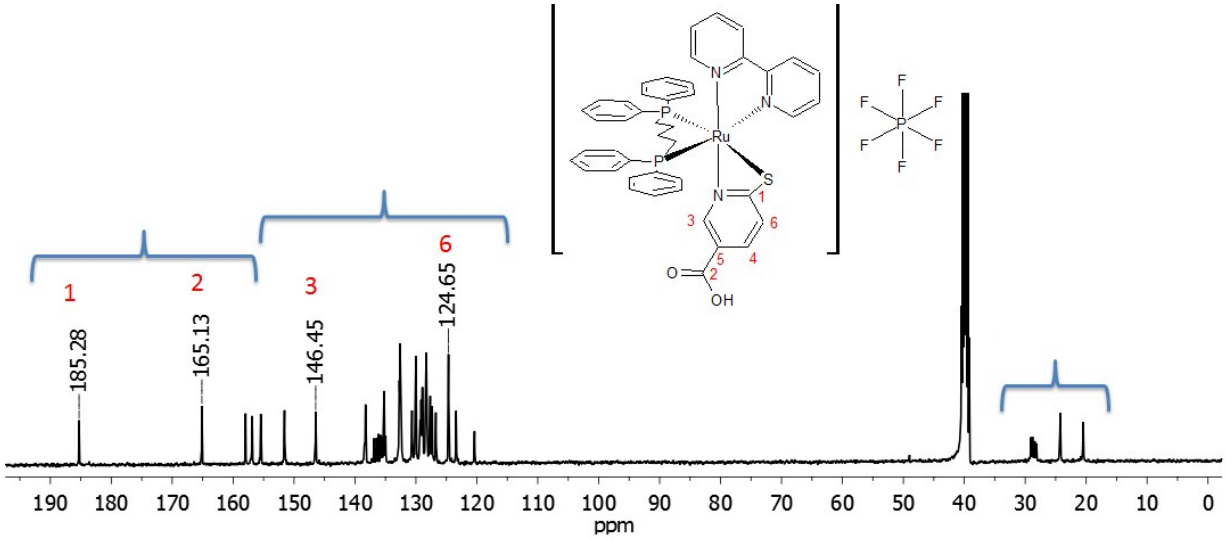
161.93
161.12
79.25
ppm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2
3
6
185.28
165.13
146.45
124.65
ppm

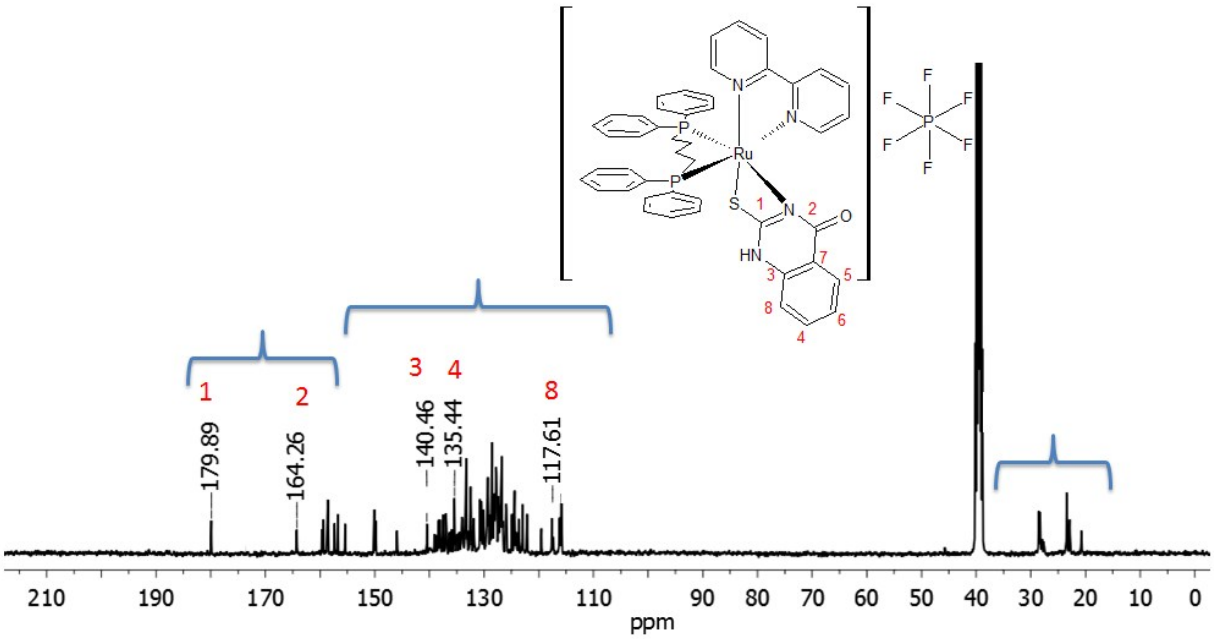
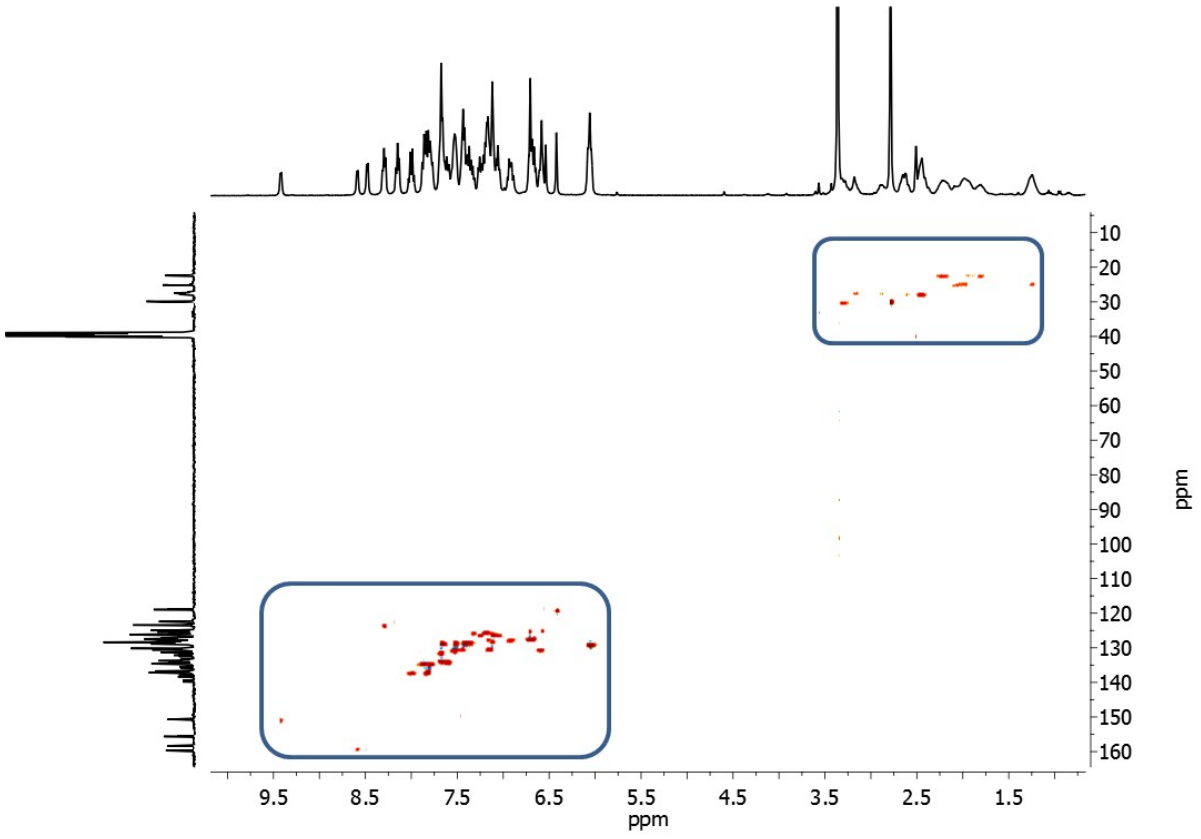
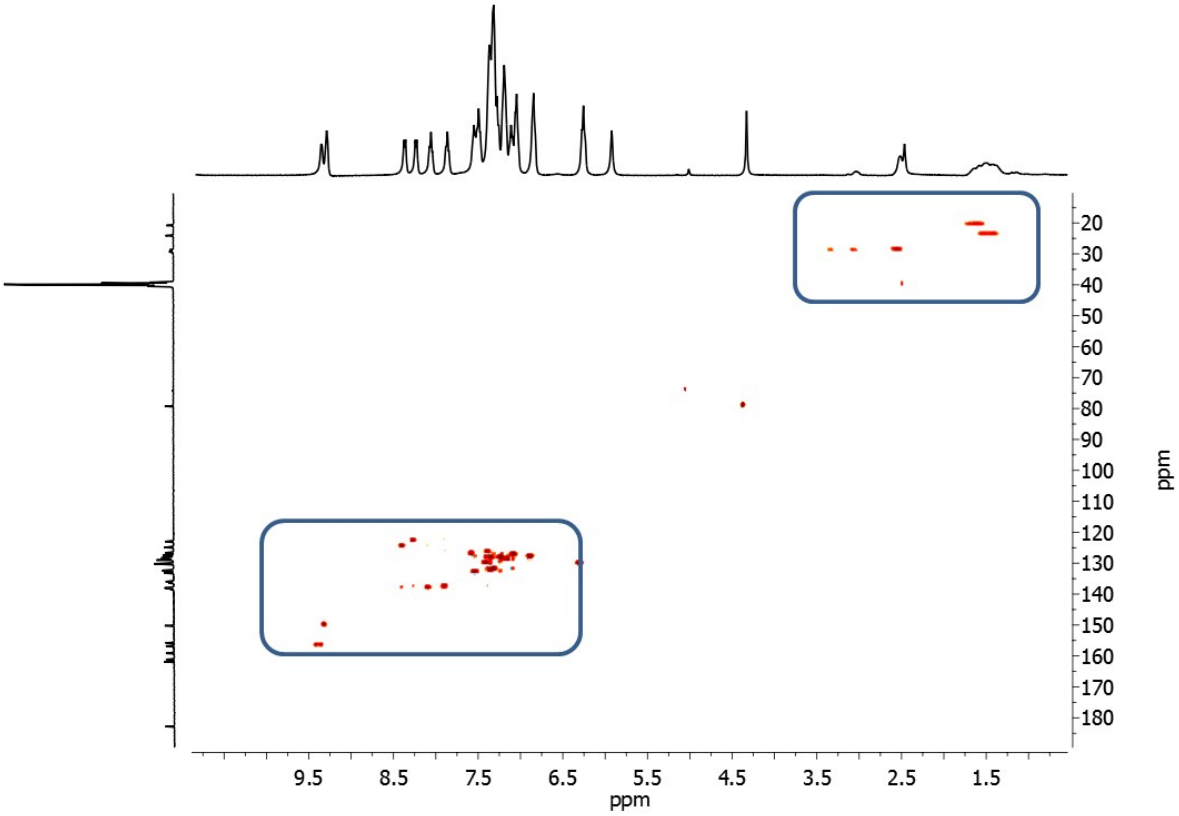
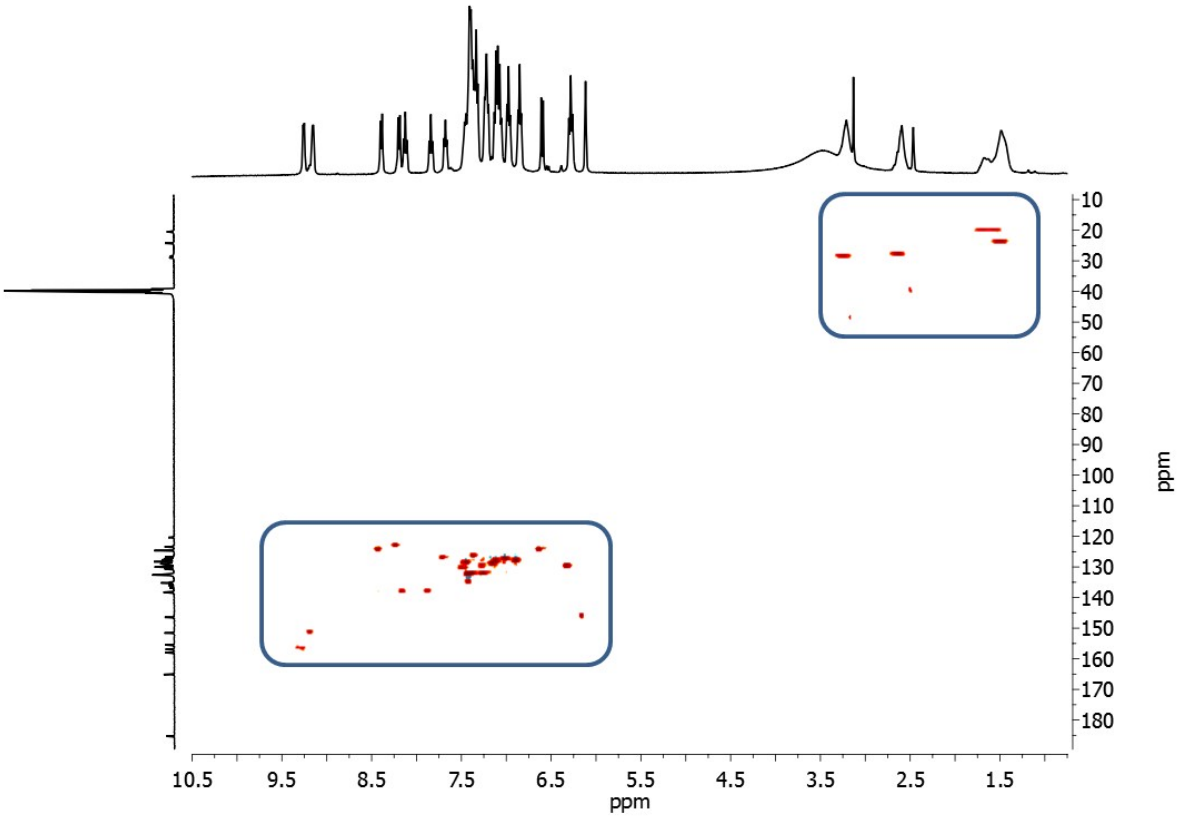


Figure 7S: $^{13}C\{^{1}H\}$ NMR spectra for complexes 2-5 in DMSO-d_6




20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
ppm
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
ppm


10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
ppm
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
ppm

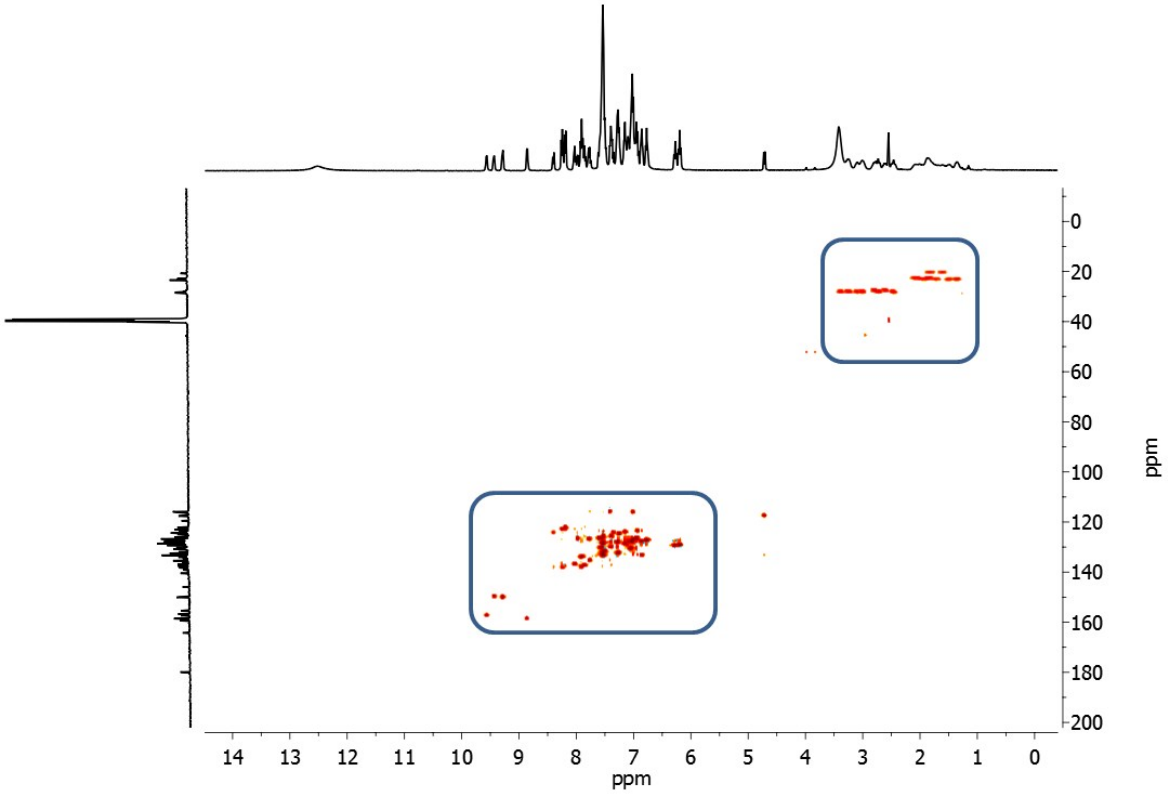
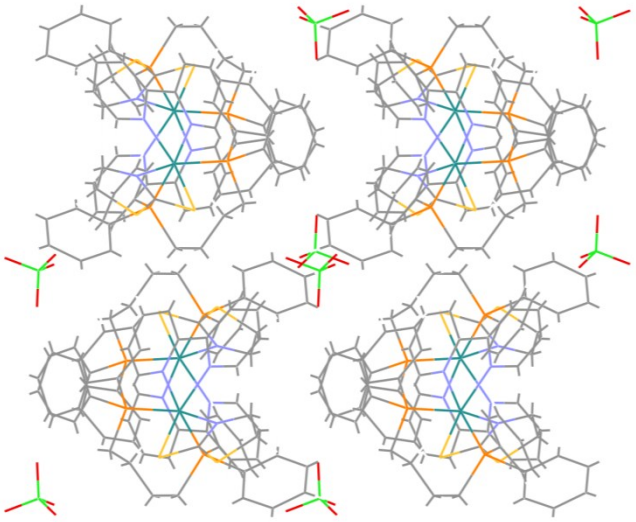


Figure 8S: HSQC (^{1}H-^{13}C) NMR spectra for complexes 2-5 in DMSO-d_6

(A)



(B)

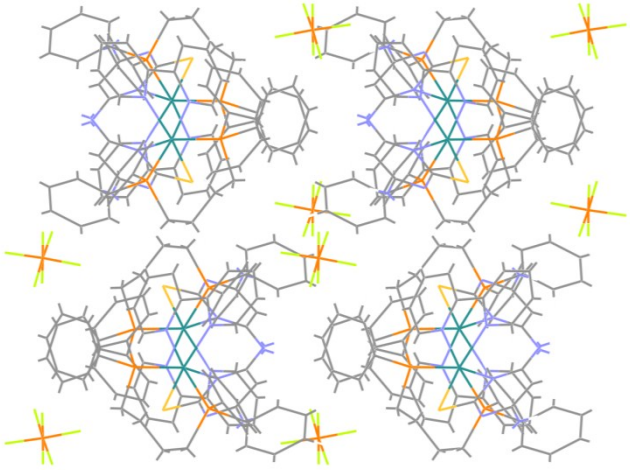
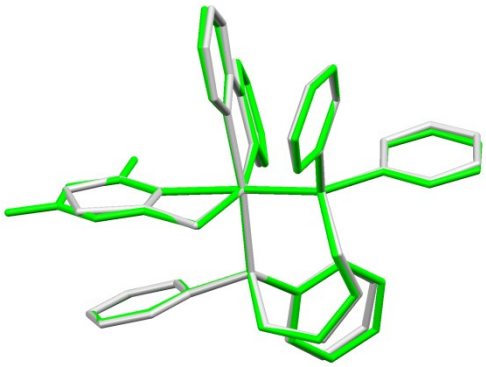
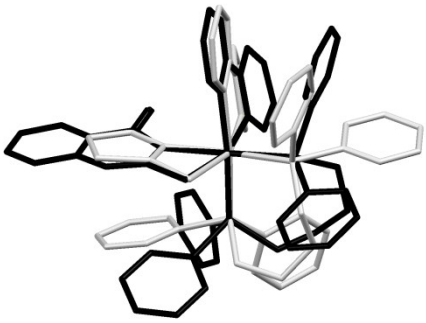


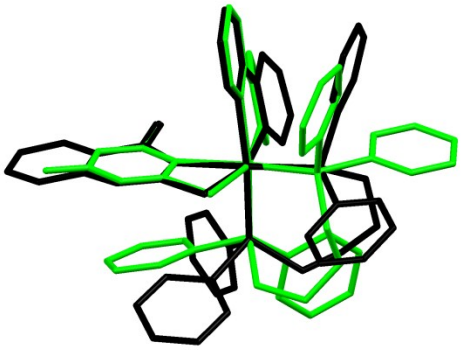
Figure 9S. Representation of the crystal packing of the complex 1.ClO_4 (A) and the complex 3 (B), showing the same supramolecular pattern.



Overlap of 1 (light gray) and 3 (green).



Overlap of 1 (light gray) and 5 (black).



Overlap of 3 (green) and 5 (black).

Figure 10S. Overlap between the molecules of the complexes 3 and 5.

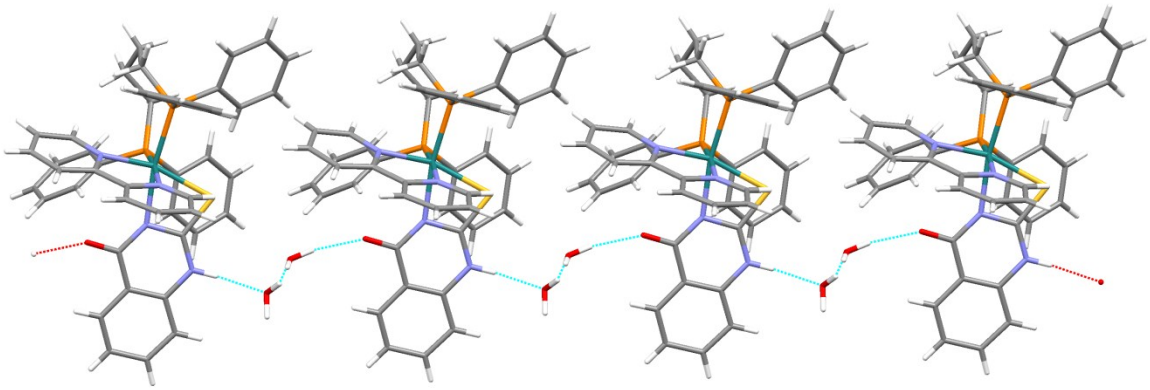


Figure 11S. H-bonding of 5 along *a*-axis.

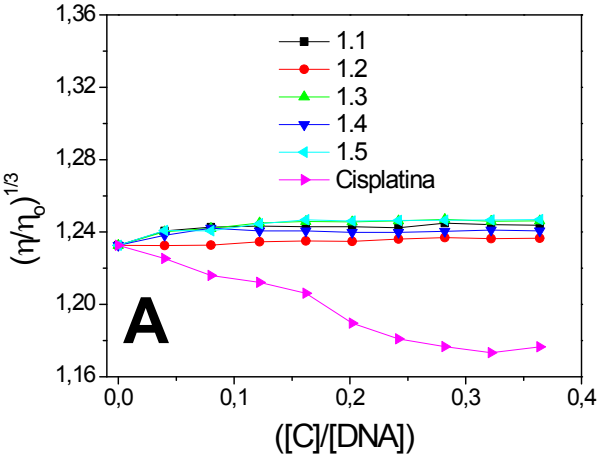


Figure 12S**:** Viscosity of *ct*-DNA $(\eta/\eta_o)^{1/3}$, increasing complexes **1–5** concentrations. Experiments were carried out at 298 K, in a Tris-HCl buffer, pH 7.4.

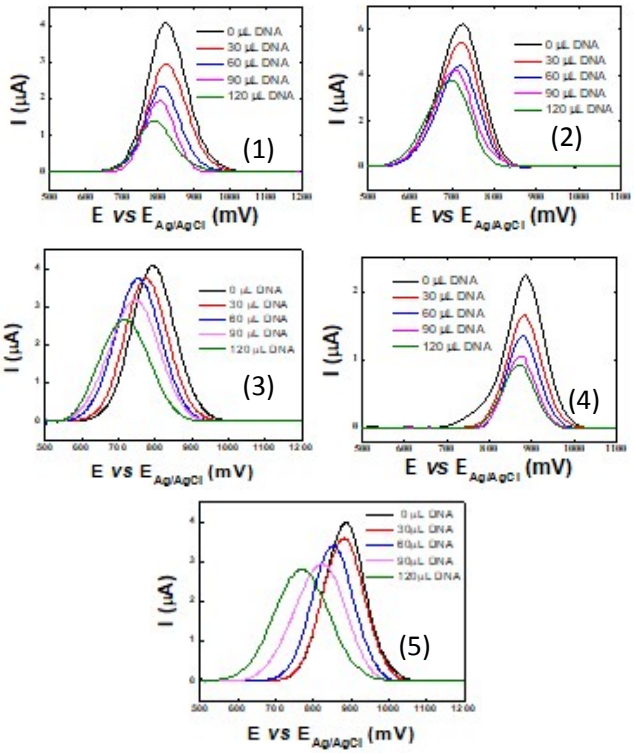


Figure 13S. Square-wave voltammogram of 1.0 mM of complexes 1-5 at the GC electrode in Tris-HCl buffer (pH 7.4), 30% DMSO, was used as a supporting electrolyte, *ct*-DNA 4.2 µM. Frequency = 50 Hz, pulse height = 75 mV and potential increment = 2 mV.

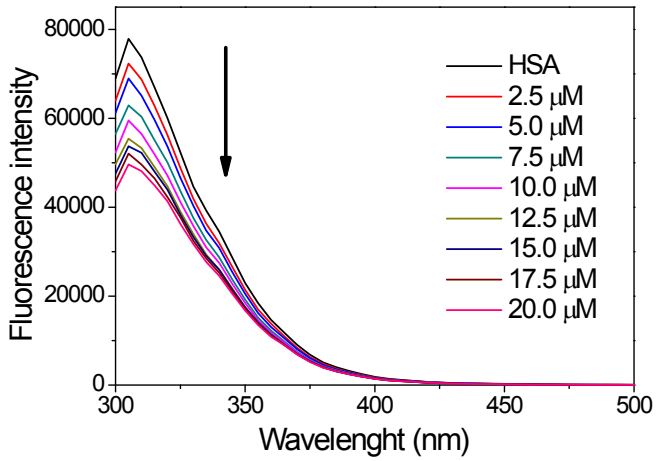


Figure 14S **.** Fluorescence quenching spectra of HAS, at different concentration of complex **1** with the excitation wavelength at 305 nm, at 310 K, in a Trizma buffer, pH 7.4.