UNIVERSITÀ DEGLI STUDI DI PISA

Dipartimento di Chimica e Chimica Industriale

CORSO DI LAUREA MAGISTRALE IN

CHIMICA

"Design, synthesis and characterization of Iridium compounds with potential anticancer activity."

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Anno Accademico 2014/2015

Summary

ABSTRACT

Chemotherapy is widely used in treatments for cancers, and Platinum compounds play an important role in this respect. New generation of metal-based anticancer drugs may offer a solution against platinum resistance and may help to extend the range of treatable cancer diseases. Recently reported Iridium(III) complexes have shown promising anticancer activity. The mode of action of these complexes may involve both the attack to DNA and some modification of the redox balance inside cells, e.g. by promoting the oxidation of NADH to NAD^+, giving an increased production of reactive oxygen species. In this thesis, the synthesis and the characterization of new Iridium complexes designed as anticancer compounds will be presented and discussed.

CHAPTER 1

1.INTRODUCTION

The World Health Organization defines cancer as “the uncontrolled growth and spread of cells”. Six hallmarks accompanying the tumour growth can be highlighted: 1) the sustenance of proliferative signalling; 2) the evasion from growth suppressor; 3) the activation of metastasis and dissemination; 4) the activation of replicative immortality; 5) the induction of angiogenesis; 6) the resistance to cell death.[1] The half-maximal inhibitory concentration (IC_{50}) can be used to measure the effectiveness of a substance to inhibit a biological function. For anticancer drugs, it represents the concentration at which 50% of cell growth is inhibited.

Chemotherapy, along with surgery and radiotherapy, is widely used in treatments for cancers, and metal-based compounds play an important role in this context. In 1965 Rosenberg et al. discovered that cis-diamminedichloridoplatinum(II), cis-[$PtCl_2(NH_3)_2$] or Cisplatin, could arrest cell division of *Escherichia coli,*[2] giving rise to a new perspective for platinum chemistry and its medical application (Figure 1).[3]

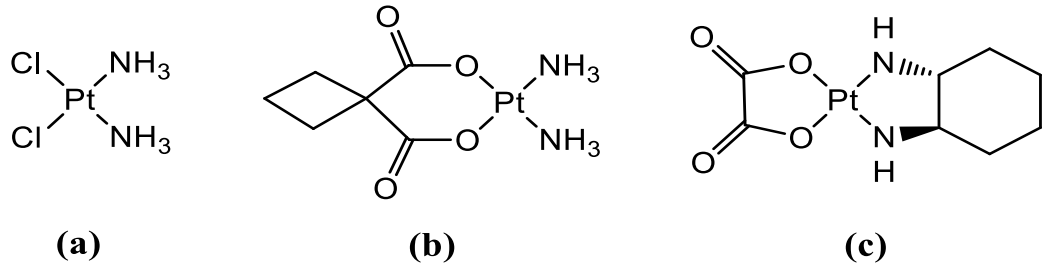


Figure 1. Structures of cisplatin (a); carboplatin (b); oxaliplatin (c).

Cisplatin was the first platinum-based drug with anti-neoplastic activity approved by the U.S. Food and Drug Administration (FDA; 1978). It can be used alone or in combination with other chemotherapeutic drugs against cancer diseases that cannot be treated with surgery or radiotherapy, such as bladder and advanced cervical cancer, non-small cell lung or ovarian cancer that are locally advanced or have metastasized. It is also used to treat

[1] Hanahan, D; Weinberg, R.A.; *Cell*, **2011**, 144, 646-674.
[2] a) Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature,* **1965**, 200, 698− 699. b) Rosenberg, B.; Van Camp, L.; Grimley, E. B.; Thomson, J. *J. Biol. Chem.,* **1967,** 242, 1347−1352.
[3] Fricker, S. P. *Dalton Trans*., **2007**, 4903−4917.

malignant mesothelioma, squamous cell carcinoma of the head and neck, and testicular cancer.[4]

Unfortunately, the use of cisplatin in therapy implies several side effects, which range from nausea and vomiting to acute renal failure. Another significant problem associated with the use of cisplatin is represented by the high incidence of resistance to this kind of chemotherapeutic agent, which reduces the range of treatable tumours. Therefore, research efforts have been addressed in the direction of overcoming such limitations, and to improve the pharmacological performances of the drug.

Cisplatin manifests its cytotoxic action by forming linkages with DNA, after removal of one or two chloride ligands in the cell environment. It is injected directly into the bloodstream and requires hospitalization for treatment. It is now widely accepted that cisplatin is a prodrug, converting into a more active form once inside the tumour cell. This occurs most likely in the nucleus, as a consequence of the low chloride concentration (4mM). Otherwise, the concentration of the chloride ion is higher in the cytoplasm (20mM) and much higher in the bloody plasma (100 mM).[5] Hydrated forms of Cisplatin can bind the DNA, by forming monofunctional adducts that can evolve into cross-links, especially intra-strand. Resistance can arise mainly from three mechanisms: (1) impaired cellular accumulation as a consequence of reduced cellular uptake or increased cellular efflux, (2) deactivation by binding to sulphur containing proteins and (3) increased repair of DNA lesions.[6] It should be remarked that only 1% of intravenously administered Cisplatin reaches the cell nucleus, and the remaining percentage may be responsible for collateral effects. Moreover, Cisplatin may alter mitochondrial functions by interfering with redox homeostasis, and this feature is strictly linked with side effects like nephrotoxicity[7] and hepatic malfunction.[8]

Currently, two other cis-diamineplatinum complexes have achieved FDA approval, i.e. Carboplatin and Oxaliplatin (Figure 1). Carboplatin is used against non-small cell lung cancer and ovarian cancer, while Oxaliplatin is active against colorectal cancers.[3] Even if these second-generation platinum compounds have given a significant contribution to

[4] Institute, N. C., National Cancer Institute, www.cancer.gov.

[5] Kelland, L. Nat. Rev. *Cancer*, **2007**, 7, 573−584.

[6] a) Sadler, P. J.; Guo, Z., *Pure Appl. Chem*., **1998**, 70, 863−871. b) Hall, M. D.; Okabe, M.; Shen, D. W.; Liang, X. X.; Gottesman, M,. *M. Annu. Rev. Pharmacol. Toxicol.,***2008**, 48, 495−535. c) Gately, D. P.; Howell, S. B. Br. J. Cancer 1993, 67, 1171−1176.

[7] Sultana, S.; Verma, K.; Khan, R.*, J. Pharm. Pharmacol.* **2012**, 64, 872−881.

[8] Martins, N. M.; Santos, N. A. G.; Curti, C.; Bianchi, M. L. P.; Santos, A. C., *J. Appl. Toxicol.* **2008**, 28, 337−344.

chemotherapy, they suffer from analogous drawbacks as those of Cisplatin and Cisplatin remains the most widely used metal-based anticancer drug.

On the other hand, the clinical use of platinum anticancer drugs has stimulated the search for complexes based on different metals, exhibiting some biological activity. Organometallic complexes offer versatility given by different, possible oxidation states and a vast range of coordination numbers and geometries. Besides, the ligands can play an important role in the reactivity and, then, the biological activity of the complexes. New metal-based anticancer drugs may provide restrained side effects, higher activity and the possibility of overcoming platinum resistance.

Actually several metal-complexes have been studied up to date, showing different targets and modes of action.

The biological activity of gold complexes to treat rheumatoid arthritis has been known since the discovery of Auranofin.[9] Starting from its anti-inflammatory and immunosuppressive activity, Auranofin was positively tested as anticancer.[10] The activity of Gold(I) seems to be related to the inhibition of the Thioredoxin reductase enzyme, causing mitochondrial alteration and disturbing the redox homeostasis of the cell. Also Gold(III) dithiocarbamato complexes trigger cell death, probably by a similar mechanism. Interestingly, in vivo studies on [$AuBr_2$(ESDT)] (ESDT = ethylsarcosinedithiocarbamateto) toward murine tumour models show up to 80% inhibition of tumour growth with a significant decrease of side effects (Figure 2).[11]

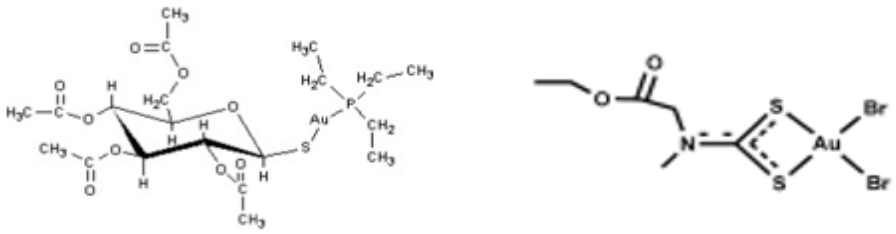


Figure 2. Structures of Gold complexes: Auranofin (Au^{+1}) and [$AuBr_2$(ESDT)] (Au^{+3}).

[9] Capell, H. A., Cole, D. S., Manghani, K. K., and Morris, R. W. (eds.). Auranofin, Proceedings of a Smith Kline and French International Symposium. Amster dam: Excerpta Medica, **1983**.
[10] Mirabelli, C.K.; Johnson, R.K.; Sung, C.M.; Faucette, L.; Muirhead, K.; Crooke,S.T.; *Cancer Research*; 1985; 45.
[11] Romero-Canelon, I.; Sadler, P.J.; *Inorg. Chem*.; **2013**, 52, 12276-12291.

Bis(η5-cyclopentadienyl)titanium dichloride, Titanocene dichloride, $Ti(\eta^5\text{-}C_5H_5)_2Cl_2$, shows a remarkable anticancer activity, whose assumed target is DNA. This complex was not approved for clinical use because it does not present a significant advantage over existing clinically approved drugs, such as Cisplatin. Besides, the lower water solubility and the poor hydrolytic stability of $Ti(\eta^5\text{-}C_5H_5)_2Cl_2$ complicate the picture. To increase both the aqueous solubility and the stability of titanocenes, a variety of titanium complexes containing hard chelating ligands have been developed, such as [1,2-di(cyclopentadienyl)-1,2-di-(4- N,N-dimethylaminophenyl)-ethanediyl] titanium dichloride and [1,2-di(cyclopentadienyl)- 1,2-bis(m-dimethoxyphenyl)ethanediyl] titanium dichloride (Figure 3).[12] Also the p-methoxybenzyl-substituted titanocene complex is highly active against several tumour cells lines (Figure 3).[13]

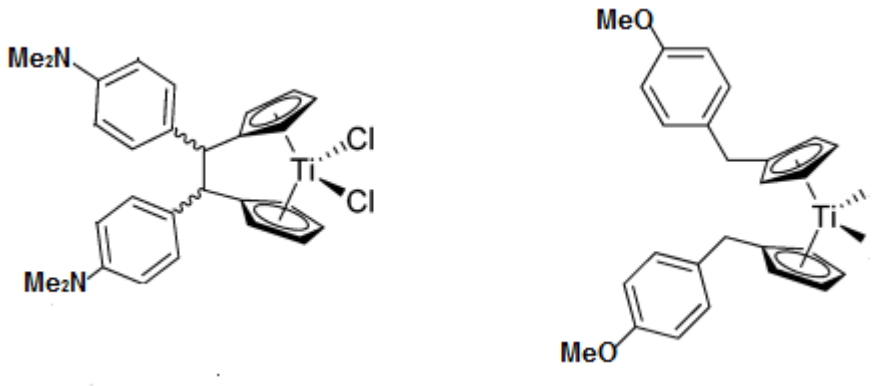


Figure 3. Structures of *Ansa*-bridged titanocenes.

The ruthenium(III) compounds NAMI-A and KP1019 (Figure 4) are effective anti-metastatic and anticancer agents currently under clinical trials.[14] The presumable mechanism of action, which involves preliminary reduction to Ru(II) species,[15] has aroused interest in the anticancer potentiality of ruthenium(II) compounds.[16] In particular, much work has focused

[12] a) Glasner, H., Tshuva, E.Y.; *Inorg. Chem*. **2014**, 53, 3170 – 3176 b) Edit Y. Tshuva,E. Y., Ashenhurst, J. A. *Eur. J. Inorg. Chem*., **2009**, 2203–2218

[13]Hartinger, C.G.; Metzler-Nolte, N; Dyson, P.J.; *Organometallics*; **2012**, 31, 5677-5685.

[14] (a) Hartinger,C.G.; Zorbas-Seifried, S.; Jakupec,M.A.; Kynast, B.; Zorbas, H.; Keppler,B.K.; *J. Inorg. Biochem.*, **2006**, *100*, 891-904. (b) Rademaker-Lakhai,J.M.; Van den Bongard, D.; Pluim, D.; Beijnen, J. H.; Schellens, J.H.; *Clin. Cancer Res.*, **2004**, *10*, 3717-3727.

[15] (a)Antonarakis, E.S.; Emadi,A.; *Cancer Chemoth. Pharm.*, **2010**, *66*, 1-9. (b) Clarke, M.J.; Zhu, F.; Frasca, D.R.; *Chem. Rev.*, **1999**, *99*, 2511-2534.

[16] a) Singh, A.K.; Pandey, D.S.; Xu, Q.; Braunstein,P.; *Coord. Chem. Rev.*, **2014**, *270–271*, 31–56. (b) Dougan, S.J.; Habtemariam, A.; McHale, S.E.; Parsons, S.; Sadler, P.J.; *Proc. Natl. Acad. Sci. USA*, **2008**, *105*, 11628-11633. (c) Meggers, E.; Atilla-Gokcumen,G.E.; Bregman, H.; Maksimoska, J.; Mulcahy, S.P.; Pagano, N.; Williams, D.S.; *Synlett*, **2007**, 1177-

on half-sandwich Ru(II) complexes of the type $Ru[(\eta^6\text{-arene})(YZ)(X)]^{0/+}$ (YZ = bidentate ligand, X = good leaving group; see figure 4): some of them display very promising *in vitro* and *in vivo* cytotoxicity, even against cisplatin-resistant tumours. DNA is considered to be their primary target.[17] These complexes are comprised of three blocks: an arene ligand, which stabilizes the oxidation state of the metal and increases the hydrophobicity of the overall compound, a monodentate ligand Z, which can be readily replaced by water thus supplying a free coordination site, and a bidentate ligand XY.[18] Although complexes with labile Ru-Z bond can attack DNA, this seems not to be the only way of action and other targets may be involved.[19] Their biological activity may be related to the interaction with the proteins kinase, carbonic anhydrases and topoisomerases.[20] Ruthenium polypyridyl complexes such as Δ-$[Ru(BiPy)_2(UIP)]^{2+}$ [where UIP = 2-(5-uracil)-1H-imidazo- [4,5-f][1,10]phenanthroline] can interact with DNA causing intercalation,[21] but they can also induce mitochondria-mediated and caspase dependent apoptosis.[22]

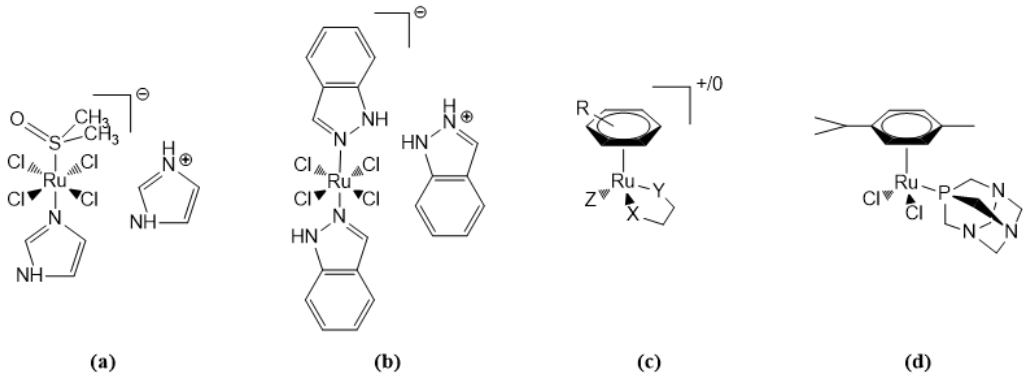


Figure 3. Structures of NAMI-A (a); KP1019 (b); general Ru(II)-arene compounds (c); RAPTA-C (d)

1189. (d) Hotze,A.C.; Caspers,S.E.;de Vos, D.; Kooijman, H.; Spek,A.L.; Flamigni,A.; Bacac,M.; Sava,G.; Haasnoot, J.G.; Reedijk,J.; *J. Biol. Inorg. Chem*., **2004**, *9*, 354-364. (e) Aird,R.E.; Cummings,J.; Ritchie,A.A.; Muir,M.; Morris,R.E.; Chen,H.; Sadler,P.J.; Jodrell,D.I.; *Brit. J. Cancer*, **2002**, *86*, 1652-1657.

[17] Chen,H.; Parkinson,J.A.; Novakova, O.; Bella,J.; Wang, F.; Dawson,A.; Gould,R.; Parsons,S.; Brabec,V.; Sadler,P.J.; *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 14623-14628.

[18] Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J., *Chem. Commun.* **2005,** 4764−4776. b) Sü ss-Fink, G. Dalton Trans. **2010**, 39, 1673−1688.

[19] Casini, A.; Hartinger, C. G.; Nazarov, A. A.; Dyson, P. J., *Top. Organomet. Chem.* **2010**, 32, 57−80.

[20] a)Loughrey, B. T.; Williams, M. L.; Healy, P. C.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Parsons, P. G.; Poulsen, S. A.; *J. Biol. Inorg. Chem.*, **2009**, 14, 935−945. b) Gao, F.; Chao, H.; Wang, J. Q.; Yuan, Y. X.; Sun, B.; Wei, Y. F.; Peng, B.; Ji, L. N. J.; *Inorg. Chem.*, **2007**, 12, 1015−1027. c) Du, K. J.; Wang, J. Q.; Kou, J. F.; Li, G. Y.; Wang, L. L.; Chao, H.; Ji, L. N. N.; *Eur. J. Med. Chem.*, **2011**, 46, 1056−1065. d) Vashisht Gopal, Y. N.; Kondapi, A. K.; *J. Biosci*., **2001**, 26, 271− 276.

[21] a) Corral, E.; Hotze, A. C. G.; Den Dulk, H.; Leczkowska, A.; Rodger, A.; Hannon, M. J.; Reedijk, J.; *J. Biol. Inorg. Chem.,* **2009**, 14, 439−448. b) Chen, X.; Gao, F.; Yang, W. Y.; Sun, J.; Zhou, Z. X.; Ji, L. N., *Inorg. Chim. Acta*, **2011**, 378, 140−147.

[22] a) Ali Ezadyar, S.; Kumbhar, A. S.; Kumbhar, A.; Khan, A., *Polyhedron*, **2012**, 36, 45−55. b) Chen, T.; Liu, Y.; Zheng, W. J.; Liu, J.; Wong, Y. S.; *Inorg. Chem*., **2010**, 49, 6366−6368.

The modulation of ligand substitution can improve the selectivity of the complex to the target. Usually, the first step in this kind of processes is the hydrolysis of the metal complex, therefore at least one ligand is sacrificed being readily substituted by water in physiological solution. On the other hand, water inertness might also be advantageous, allowing the complex to reach the desired target.

Inertness increases from the first to second to third row of transitions metals; Iridium is a third-row transition metal: it is a relatively rare element, which was discovered in 1803 as an impurity of native Platinum. Iridium, in the metallic form, is white but with a slight yellowish cast. It is hard, brittle and inert. It is the most corrosion resistant metal known, and was used in making the standard metre bar of Paris, which is a 90% platinum-10% iridium alloy. Iridium possesses several stable oxidation states, especially +1, +2 and +3, and it forms complexes with different geometries and different coordination numbers (prevalently 4 and 6)[23] (Table 1). It is used for electrical and electrochemical applications and in catalysis.[24] The dinuclear catalyst [{Ir(Cp*)-(Cl)}$_2$(thbpym)]Cl$_2$ (thbpym = 4,4′ ,6,6′ -tetrahydroxy-2,2′ -bipyrimidine) provides a way to store hydrogen as an aqueous solution of formic acid.[25] The Cativa process is one of the largest scale metal catalysed carbonylation reaction using a “precious metal” of the platinum-group. In addition, an iridium catalyst is used for the carbonylation of methanol to produce acetic acid. In contrast with the great use of Iridium in catalysis, Iridium-based pharmaceuticals are still poorly investigated.

[23] a)Sunley, G. J.; Watson, D.; *J. Catal. Today,* **2000**, 58, 293−307.b) Mak, K. H. G.; Chan, P. K.; Fan, W. Y.; Ganguly, R.; Leong, W. K.; *Organometallics*, **2013**, 32,1053−1059.c) Brewster, T. P.; Blakemore, J. D.; Schley, N. D.; Incarvito, C. D.;Hazari, N.; Brudvig, G. W.; Crabtree, R. H.; *Organometallics*, **2011**, 30, 965−973.d) Gilbert, T. M.; Bergman, R. G.; *Organometallics,* **1983**, 2, 1458−1460.

[24] Jollie, D.; *Platinum,* **2008**; Johnson Matthey: Hertfordshire, U.K.;2008; pp 42−43;

[25] Hull, J. F.; Himeda, Y.; Wang, W.-H.; Hashiguchi, B.; Periana, R.;Szalda, D. J.; Muckerman, J. T.; Fujita, E.; *Nat. Chem.,* **2012**, 4, 383−388.

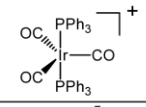
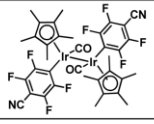
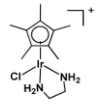
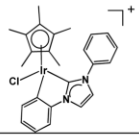
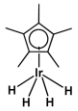
Oxidation state	Example	Geometry	Ref.
-1	$H[Ir(CO)_4]$	tetrahedral	
0	$Ir(CO)_4$	tetrahedral	
+1	$[Ir(CO)_2I_2]^-$	square-planar	2
		trigonal-bipyramidal	
+2		Pseudo-octahedral	3
+3		Pseudo-octahedral	
+4		Pseudo-octahedral	4
+5			5

Table 1. Oxidation States and Geometries of Organoiridium Compounds.[26]

Studies regarding the biological activity of Iridium complexes are relatively scarce. Early studies were performed on non-organometallic Ir(I) and Ir(III) complexes,[27] while more recently reports dealing with Ir(III) organometallic complexes have appeared in the literature.[28] Ir(III) analogues of the Ru(III) anticancer drugs NAMI-A and the imidazole analogue of the indazole complex KP1019, respectively trans-$[IrCl_4(DMSO)(Im)][ImH]$ and

[26] Liu,Z.; Sadler, P.J.;,*Acc. Chem. Res.,* **2014**, 47, 1174−1185.

[27] (a) Cleare, M. J.; *Coord. Chem. Rev.,* **1974**, 12, 349–405. (b) Sava, G.;Giraldi, T.; Mestroni, G.; Zassinovich, G.; *Chem.-Biol. Interact.*, **1983**, 45, 1–6. (c) Sava, G.; Zorzet, S.; Perissin,L.; Mestroni, G.; Zassinovich, G.; Bontempi, A.; *Inorg. Chim. Acta,* **1987**,137, 69–71. (d) Giraldi, T.; Sava, G.; Mestroni, G.; Zassinovich, G.;Stolfa, D.; *Chem.-Biol. Interact.,* **1978**, 22, 231–238. (e) K€opf-Maier, P.; *Eur. J. Clin.Pharmacol*., **1994**, 47, 1–16.

[28] (a) Casini, A.; Edafe, F.; Erlandsson, M.; Gonsalvi, L.; Ciancetta,A.; Re, N.; Ienco, A.; Messori, L.; Peruzzini, M.; Dyson, P. J.; *Dalton Trans.,* **2010**, 39, 5556–5563. (b) Amouri, H.; Moussa, J.; Renfrew, A. K.;Dyson, P. J.; Rager, M. N.; Chamoreau, L.-M.; *Int. Ed. Engl.* **2010**, 49, 7530–7533. (c) Ali Nazif, M.;Bangert, J.-A.; Ott, I.; Gust, R.; Stoll, R.; Sheldrick, W. S. J.; *Inorg. Biochem.,* **2009**, 103, 1405–1414. (d) Wirth, S.; Rohbogner, C.; Cieslak, M.;Kazmierczak-Baranska, J.; Donevski, S.; Nawrot, B.; Lorenz, I.-P.J.; *Biol. Inorg. Chem.,* **2010**, 15, 429–440. (e) Gras, M.; Therrien,B.; S€uss-Fink, G.; Casini, A.; Edafe, F.; Dyson, P. J. *J. Organomet. Chem.***2010**, 695, 1119–1125. (f) Sch€afer, S.; Sheldrick, W. S. *J. Organomet. Chem.* **2007**, 692, 1300–1309. (g) Kokoschka,M.; Bangert, J. A.; Stoll, R.; Sheldrick, W. S. *Eur.J. Inorg. Chem.* **2010**, 1507–1515. (h) Hartinger, C. G.*Angew. Chem.*, Int.Ed. Engl**. 2010**, 49, 8304–8305. (i) Leung, S.-K.; Kwok, K. Y.; Zhang, K. Y.;Lo, K. K.-W. *Inorg. Chem.* **2010,** 49, 4984–4995. (j) Shao, F.; Barton, J. K.*J. Am. Chem. Soc.* **2007**,129, 14733–14738.

trans-[$IrCl_4(Im)_2$][ImH],[29] are biologically inactive probably due to the inertness of the metal centre.

Carbon-bound π-bonded arenes and cyclopentadiene ligands can affect the cell uptake and targeting, through a fine tuning of the complex hydrophilicity. Organometallic Ru(II) and Os(II) arene anticancer complexes of the type (Ru/Os)[(η^6-arene)(NN)Cl]$^+$, where NN is a chelating diamine ligand, can be activated by hydrolysis of the Ru-Cl bond and then bind DNA. The arene ligand is important because it can cause distortions in DNA, in particular when it bears an extended planar system, which is able to intercalate between DNA's bases.[30]

Arene ligands do not stabilize Ir(III). On the other hand, negatively charged pentamethylcyclopentadienyl (Cp*) is an excellent stabilizing ligand for Ir(III). The low spin d^6 metal ion Ir(III) is described as very inert, thus water exchange in $[Ir(H_2O)6]_3^+$ is extremely slow. Nevertheless, it is also known that water exchange in $[Ir(\eta^5\text{-Cp*})(H_2O)_3]^{2+}$ is ca. 10^{14} times faster.[31] The hydrolysis equilibrium is strongly influenced by the nature of ligands, and hydrolysis of Cp*Ir(III) complexes is even faster than low spin d^6 arene Ru(II) and Os(II) complexes. Although the hydrolysis of Ruthenium(II) arene complexes can represent an important prerequisite for anticancer activity, other related complexes may manifest antiproliferative activity without undergoing hydrolysis. Piano-stool complexes with azopyridine ligand (strong π-acceptors) have shown antiproliferative activity against A2780 ovarian cancer and A549 lung cancer, in spite of being inert toward hydrolysis.[32] These complexes seem to oxidize glutathione, GSH, to the oxidized glutathione, GSSG, under physiological conditions. GSH is mainly involved in the deactivation of reactive oxygen species (ROS), such as hydroxyl radicals $OH^{\bullet}$ to produce water, and superoxide $O_2^{\bullet-}$ to generate H_2O_2, which can be subsequently transformed to H_2O.[33] The inhibition of GSH results in an increased level of ROS. The mechanism of formation of these ROS is still

[29] a)Messori, L.; Marcon, G.; Orioli, P.; Fontani, M.; Zanello, P.;Bergamo, A.; Sava, G.; Mura, P. *J. Inorg. Biochem.* **2003**, 95, 37–46. b) Marcon, G.; Casini, A.; Mura, P.; Messori, L.; Bergamo, A.;Orioli, P. *Metal-Based Drugs* **2000**, 7, 195–200.
[30] Blakemore, J. D.; Schley, N. D.; Olack, G. W.; Incarvito, C. D.;Brudvig, G. W.; Crabtree, R. H. *Chem.Sci.* **2011**, 2, 94−98.
[31] Cayemittes, S.; Poth, T.; Fernandez, M. J.; Lye, P. G.; Becker,M.; Elias, H.; Merbach, A. E.; *Inorg. Chem.* **1999**, 38, 4309–4316.
[32] Dougan, S. J.; Melchart, M.; Habtemariam, A.; Parsons, S.;Sadler, P. J.; *Inorg. Chem.* **2006**, 45, 10882−10894.
[33] Halliwell, B.; Gutteridge, J. M. C. Free Radicals in Biology and Medicine, 4th ed.; Oxford University Press: Oxford, U.K., 2007.

poorly understood, but may involve ligand-based reduction and it is presumed to be catalytic.[34]

In the light of this preamble, Prof. Sadler's group has investigated low spin $5d^6$ iridium(III) complexes. Complexes of the type Ir $[(\eta^5\text{-Cp*})\ (LL')(Cl)]^{0/+}$ have been prepared (Figure 5).

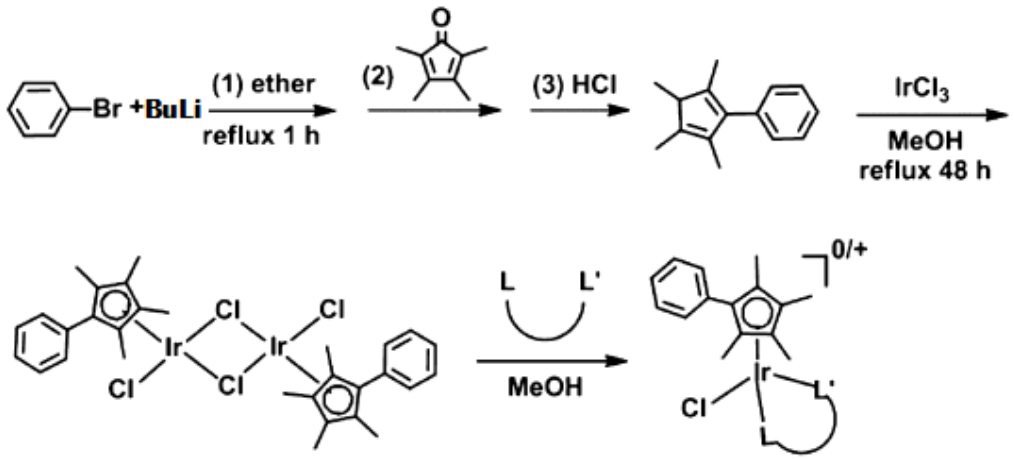


Figure 5. Synthesis of Ligands Cp*PhH and its Half-Sandwich Ir(III) Complex. L-L' = N,N- or C,N- ligand, e.g. bipyridine or phenylpyridine.[35]

In general, the redox activity of this kind of complexes can be related to hydride formation and to their influence on the NAD^+/NADH ratio inside the cells (Figure 6). In fact, $[Ir(\eta^5\text{-}C_5Me_4C_6H_5)(phpy)Cl]PF_6$ and $[Ir(\eta^5\text{-}C_5Me_4)(phpy)Cl][PF_6]$ (phpy = 2 – phenylpyridine; bipy= 2,2'bipirydine) can catalytically oxidize NADH to NAD+. Also the tetramethyl(biphenyl)cyclopentadienyl complex is capable of almost doubling the NAD^+/NADH ratio in A2780 ovarian cancer cells, possibly through the transfer of hydride from NADH to other biological substrates. This reactivity has a strong impact on the redox balance inside the cells.[36]

[34] Dougan, S. J.; Habtemariam, A.; McHale, S. E.; Parsons, S.;Sadler, P. J.; Proc. Natl. Acad. Sci. U.S.A. **2008**, 105, 11628−11633.
[35] Liu,Z.; Habtemariam, A.; Pizarro,A.M.; Fletcher, S. A.; Kisova, A.; Vrana, O.; Salassa,L.; Bruijnincx, P.; Clarkson,G. J.; Brabec,V.; Sadler, P.J.; *J. Med. Chem.* **2011**, 54, 3011–3026.
[36] Betanzos-Lara, S.; Liu, Z.; Habtemariam, A.; Pizarro, A. M.;Qamar, B.; Sadler, P. J.;*Angew. Chem., Int. Ed.* **2012**, 51, 3897−3900.

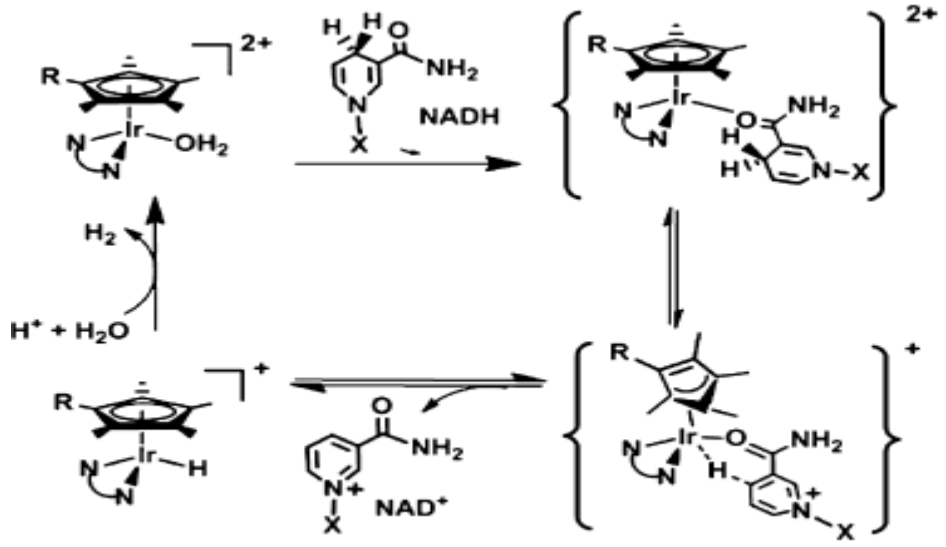


Figure 6. Interaction between Cp*Iridium-type compounds and NADH/NAD$^+$

High anticancer activity can be achieved by introducing little changes around the metal centre. For instance, the substitution of one methyl group (Cp*) with a phenyl (Cp°Ph) or a biphenyl group (Cp°BiPh). The biological activity increases along the series Cp* < Cp°Ph < Cp°BiPh (Cp° = C_5Me_4H).[28]

In vitro tests have shown that after exposition to Cp°BiPh, tumour cells developed small vacuoles throughout the cytoplasm, representing the swelled mitochondria, characterized by their double-layer membrane. The mitochondria appeared lacking of structure inside the inner membrane, while the outer membrane could remain unchanged.[37] The mitochondria are semi-autonomous organelles with their own genome and protein synthetic system. They are important for their role in metabolism, the generation of free radicals and they are also involved in cell death.[38] In addition, mitochondria can offer a selective target for anticancer drugs, because they present differences in cancer cells respect to the situation found in normal cells.[39]

The membrane potential of mitochondria in cancer cells is relatively high (i.e. more negative), thus allowing accumulation of cationic lipophilic compounds along an

[37] Dindo, D., Dahm, F., Szulc, Z., Bielawska, A., Obeid, L. M., Hannun, Y. A., Graf, R., and Clavien, P.-A.;*Mol. Cancer Ther*. **2000**,5, 1520−1529.
[38] Carew, J. S., and Huang, P.*Mol. Cancer*, **2002,** 1-9.
[39] Constantini, P., Jacotot, E., Decaudin, D., and Kroemer, G. *J. Natl. Cancer Inst.*, **2002**, 92, 1042−1053.

electrochemical gradient. The complex Cp°BiPh is the most hydrophobic one, and it could be readily taken up by mitochondria.[40]

One way for cell death is apoptosis. Apoptosis is the process of programmed cell death as a result of particular stimuli.[41] In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's lifecycle. After pro-apoptotic signal, a phase follows in which the machinery for cell death is activated. Later on, a degradation phase can start, and here apoptosis takes place.[42] Apoptosis can be activated by anticancer agents or by radiotherapy. It is characterized by the activation of intrinsic pathways, in which the cell kills itself because it senses cell stress, or extrinsic pathway, in which the cell kills itself as a result of signals from other cells. Both pathways present caspase enzymes as effector molecules. Caspase enzymes are enzymes involved in proteins degradation. The two pathways both activate initiator caspases, which then activate executioner caspases, which in turn kill the cell by degrading proteins indiscriminately. The first step of apoptosis is characterized by condensation of nuclear chromatin into delineated mass, which is localized on the nuclear membrane. This fact results in nuclear destabilization caused by DNAase enzymes, which are able to cleave DNA at internuclear linker sites. Besides, small vacuoles generated in the nucleus and in the nuclear membrane may become visible. This morphological alteration might produce abnormalities in chromatid packing and destabilization of the genetic material. Some of the vacuoles can arise from H_2 release, and the Iridium complex can react with coenzyme NADH to catalytically generate Iridium hydride and H_2. Apoptosis causes changes in cells outline, becoming convoluted and forming extensions, called 'budding'. These extensions separate from the cell and form "apoptotic bodies".[43] Usually, ordinarily cellular organelles, such as mitochondria, would remain preserved until the apoptotic bodies are phagocytised or are degraded by secondary necrosis.[44] After exposure to Cp°BiPh, the mitochondria inside the apoptotic cells are not preserved, and this suggests that something different from the normal process is going on. One way of resistance to apoptosis for cancer cells is up-regulating pro-

[40] Dindo, D., Dahm, F., Szulc, Z., Bielawska, A., Obeid, L. M., Hannun, Y. A., Graf, R., and Clavien, P.-A.; *Mol. Cancer Ther.,* **2006**, 5, 1520−1529

[41] Huo, H., Margro, P. G., Pietsch, E. C., Patel, B. B., and W, S. K.; *Cancer Res.;***2010,** 70, 8726−8735

[42] Lazebnik, Y. A., Cole, S., Cooke, C. A., Nelson, W. G., and Earnshaw, W. C.; *J. Cell. Biol.*, **1993**, 123, 7−22

[43] Betanzos-Lara, S., Liu, Z., Habtemariam, A., Pizarro, A. M., Qamar, B., and Sadler, P. J. *;.Angew. Chem., Int. Ed.,* **2012**, 51, 3897−3900.

[44] Kerr, J. F., Winterford, C. M., and Harmon, B. V.; *Cancer*, **1994**, 73, 2013−2026.

survival factors, using inhibitors of apoptosis proteins (IAPs). IAPs are able to block caspase cascades, which are required for apoptosis. However, Iridium complexes can affect mitochondria even causing energy deficiency. If IAPs block the caspase cascade successfully, ATP deficiency can still inhibit cell growth, giving rise to cytostasis.[45]

Swelling of mitochondria is linked to calcium imbalanced inside the cells, due to drug-induced oxidative stress. Induction of apoptosis is also strictly connected to time of exposure and concentration of administered compounds.[46]

Reactive oxygen species play important roles in regulating cell proliferation, death and signalling. For this reason, they can also play a significant role in the mechanism of action of anticancer drugs.

Different activity is also shown by isoelectronic complexes such as $[Ir(\eta^5\text{-}C_5Me_5)(BiPy)Cl]^+$ and $[Ir(\eta^5\text{-}C_5Me_5)(PhPy)Cl]$, which contain 2-2'-bipyridine(BiPy) and 2-phenylpyridine(PhPy) as N,N- and C,N- chelating ligands, respectively (Figure 7).[47]

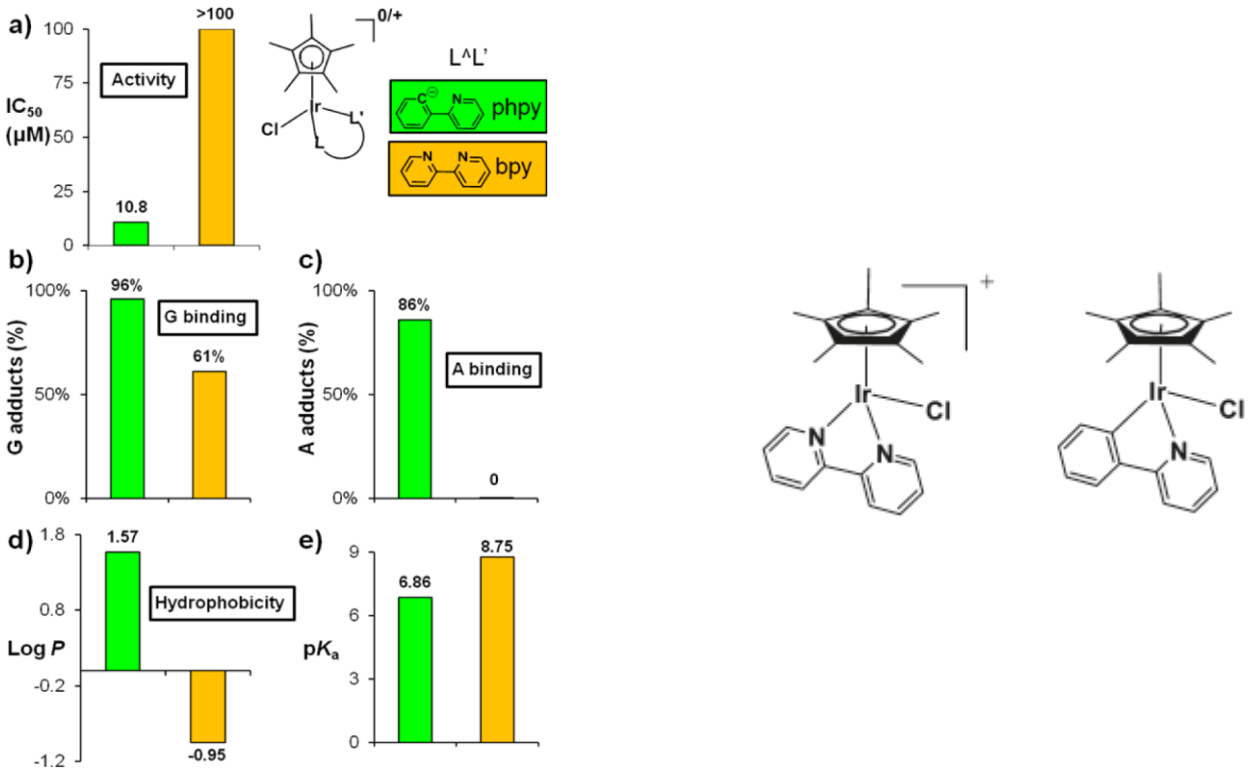


Figure 7. Influence of chelated ligands on the behaviour of complexes Ir $[(\eta^5\text{-Cp*})(BiPy)Cl]^+$ (1) and Ir $[(\eta^5\text{-Cp*})(PhPy)Cl]$ (2).[48]

[45] Dai, Y., Lawrence, T. S., and Xu, L. B.; *Am. J. Transl. Res.,* **2009,** 1, 1−15.

[46] (a) Rubbiani, R.; Can, S.; Kitanovic, I.; Alborzinia, H.;Stefanopoulou, M.; Kokoschka, M.; Monchgesang, S.; Sheldrick, W.S.; Wolfl, S.; Ott, I.; *J. Med. Chem.* **2011**, 54, 8646−8657. (b) Kowol,C.; Heffeter, P.; Miklos, W.; Gille, L.; Trondl, R.; Cappellacci, L.;Berger, W.; Keppler, B.; *J. Biol. Inorg. Chem.,* **2012**, 17, 409−423

[47] Liu,Z.; Habtemariam, A.; Pizarro,A.M.; Salassa,L.; Clarkson,G. J.;Sadler, P.J. *Inorg. Chem.* **2011**, 50, 5777–5783.

There is a change in the overall charge of the complex, from +1 for complex **1** to 0 for complex **2**. There is not any difference in the hydrolysis rates of complexes, which is very fast in any case. The fast hydrolysis could be ascribable to the presence of the five methyl groups on the Cp ring acting as strong electron donors, thus increasing the electronic density on the Iridium centre and assisting the chloride displacement. The cytotoxic activity is often related to the ability of anticancer drugs to bind DNA.[49] Complex **1** was found to form an adduct only with 9-ethylguanine (9-EtG) and not with 9-methyladenine (9-MeA) after 24h of exposure. In contrast, complex **2** can bind significantly either 9-EtG or 9-MeA, showing stronger bindings with both guanine and adenine bases. According to DFT calculations, there is a p-orbital interaction between N (NH_2 of adenine) and C1 and C2 (PhPy), explaining the formation of the adenine adduct (Figure 8). The negatively charged carbon on the phenyl ring appeared to be favoured compared to the analogous atoms in the pyridine ring. Although complex **2** can form 9-MeA adducts in MeOD-d_4 and D_2O, the adducts are not stable and readily dissociate in $CDCl_3$, acetone-d_6 and DMSO-d_6. Even hydrophobicity can concur with the different activity of the two compounds. Complex **2** shows higher hydrophobicity and is cytotoxic. Complex **1** is less hydrophobic and less active. This difference in hydrophobicity is likely to result in different degrees of cancer cell uptake. Complex **2** is relatively effective against A2780 ovarian cancer cells with a IC_{50} value of 10.8mM comparable to that of Carboplatin,[50] while **1** is poorly active with a IC_{50} >100mM. Complex **2** is the first reported Ir(III) anticancer complex with both Cp* and C,N-chelating ligands. Some square planar Au(III) complexes containing C,N-chelating ligands have been reported to be more active in vitro against MOLT-4 human leukaemia, however the mechanism of action is still unknown.[51]

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[48] Liu,Z.; Sadler, P.J.;,*Acc. Chem. Res.,* **2014**, 47, 1174−1185.

[49] (a) Zhang, C. X.; Lippard, S.; *J. Curr. Opin. Chem. Biol*. **2003**, 7, 481–489. (b) Deubel, D. V.; Lau, J. K.-C. *Chem. Commun*. **2006**, 2451–2453.

[50] Wang, F.; Habtemariam, A.; van der Geer, E. P. L.; Fernandez, R.; Melchart, M.; Deeth, R. J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D. I.; Parsons, S.; Sadler, P. J. Proc. Natl. Acad. Sci. U.S.A. **2005**, 102, 18269–18274.

[51] Fan, D.; Yang, C.-T.; Ranford, J. D.; Lee, P. F.; Vittal, J. J.; *Dalton Trans.,* **2003**, 2680–2685.

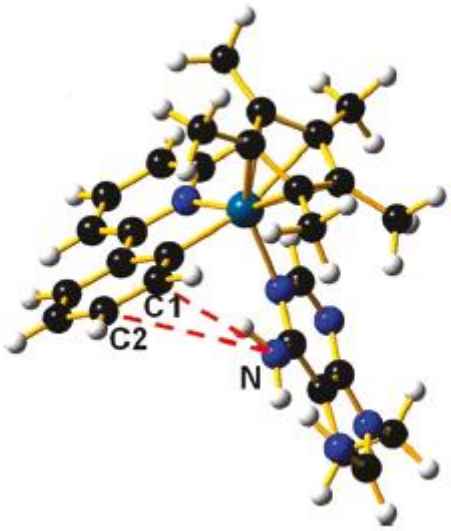


Figure 8. DFT optimized structure of complex [Ir(η^5-C_5Me_5)(phpy)(9-EtA-N1)]$^+$.

Regarding Cp Ir C^N compounds, the substitution of one chloride with the pyridine ligand results in a decrease of the hydrolysis rate, otherwise it does not show loss of anticancer activity. Surprisingly, the pyridine complex is highly effective, ca. 10 times and 6 times more active than CDDP and the chloride analogue, respectively.[52]

Recently, the complex [Ir(η^5- $C_5Me_4C_6H_5$)(PhPy)(Py)][PF_6] has been prepared, and it has been shown that the pyridine ligand is responsible for the block of hydrolysis. Surprisingly, the IC_{50} value exhibited by [Ir(η^5- $C_5Me_4C_6H_5$)(PhPy)(Py)][PF_6] towards A2780 ovarian cancer cells is lower respect to what observed for other complexes previously studied. Notwithstanding, this complex manifests an interesting activity: after 1h of drug exposure, there is a dramatic increase in ROS production. The effect of the pyridine ligand and its derivatives was investigated (Figure 9). The IC_{50} values for all these Ir(III) complexes are comparable or lower compared to that of cisplatin, suggesting that the former compounds are highly active (Figure 10). The presence of electron-withdrawing groups on the pyridine ring determines a lower activity of the complex in comparison with those complexes bearing electron-donating substituents.

[52] Liu, Z.; Romero-Canelón, I.; Qamar, B.; Hearn, J. M.;Habtemariam, A.; Barry, N. P. E.; Pizarro, A. M.; Clarkson, G. J.; Sadler, P. J.; *Angew. Chem., Int. Ed.* **2014**, 53, 3941−3946.

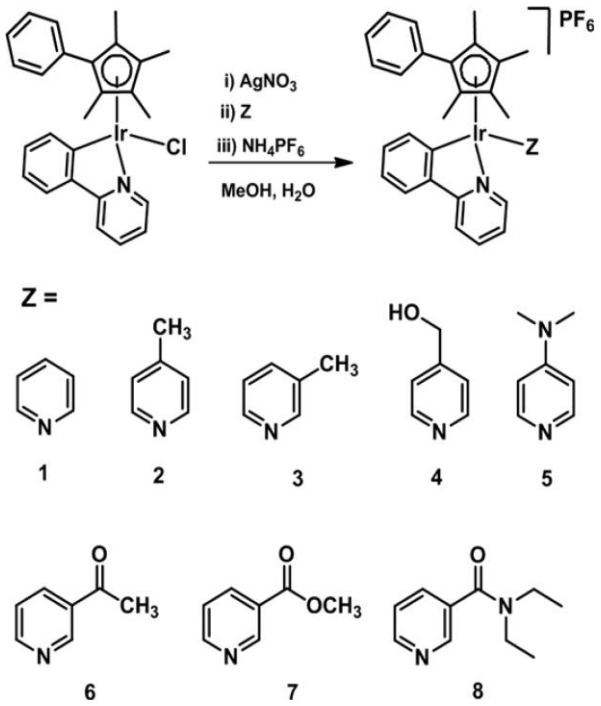


Figure 9. Synthesis of Iridium(III) complexes with pyridine ligand and its derivatives.[46]

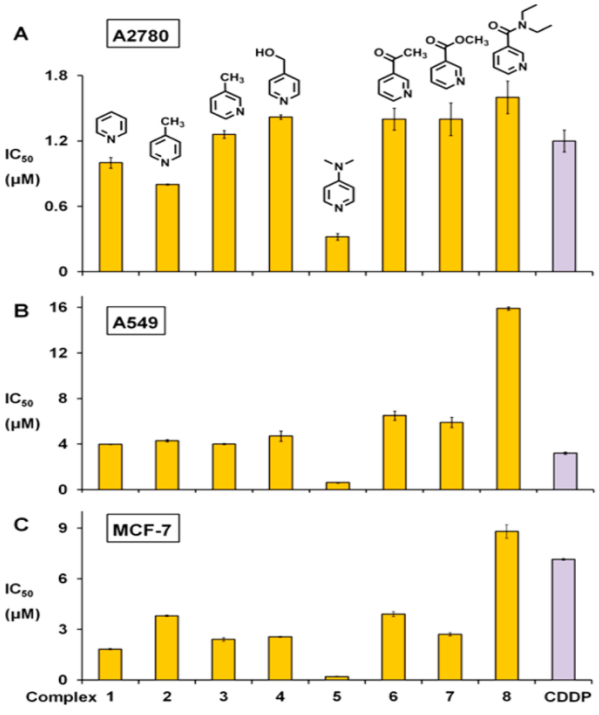


Figure 10.Inhibition of growth of (A) A2780 human ovarian cancer.[46]

All the complexes reacted with 9-EtG, but no 9-ethyladenine (9-EtA) adduct was observed. Interestingly, these complexes induce a dramatic increase in ROS levels and in superoxide levels inside cells, especially the pyridine complex **1** and the 3-N,N-dimethylpyrine complex **5** ROS were detected in more than 97% of A2780 cells.[53] Besides, these complexes induced significant changes in mitochondrial membrane potential, which is an important pre-requisite for the anticancer activity.

According to these previous results, the effect of the pyridine ligand is quite interesting and still little understood. The aims of my thesis's project were to design and synthesize new Iridium(III) complexes, containing the Cp* ligand or its simple derivatives. I spent a period in Prof. Sadler's group, during which I prepared two new ligands: 2,3,4,5-Tetramethyl-1-(8-quinolyl)Cyclopentadiene and 2-(2,3,4,5-tetramethylcyclopenta-1,3-dien-1-yl)pyridine, and the relevant Iridium complexes [η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl]bipyridil Iridium(III)][PF_6]$_2$, [η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl]phenylpyridil Iridium(III)][PF_6], [η^5–2,3,4,5-tetramethylcyclopentadienyl]-1-pyridine phenylpyriyl Iridium(III)][PF6] and [η^5–2,3,4,5-tetramethylcyclopentadienyl]-1-pyridine bipyridyl Iridium(III)][PF_6]$_2$.

[53] Liu, Z.; Romero-Canelon, I.;Habtemariam, A.;Clarkson, G. J.;Sadler, P. J.; *Organometallics,* **2014**, 33, 5324−5333.

In the course of my staying in the Dipartimento di Chimica e Chimica Industriale of the University of Pisa, I worked on the synthesis of an Ir complex containing a phenol-substituted Cp* ligand, i.e. bis(4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)phenol) diiridium(III)tetrachloride, with the aim of providing useful features to the drug, e.g. and enhanced water solubility.

CHAPTER 2

2.RESULTS AND DISCUSSION

The cyclopentadienyl ligand, Cp^-, is an aromatic fragment acting as a significant σ-donor towards metal centres. Although alternative coordination fashions have been observed (Figure 11), it usually coordinates via the η^5 mode, i.e. by all the five ring carbon atoms thus donating six electrons. In order to generate the anion Cp^-, deprotonation of cyclopentadiene, C_5H_6, is required and this is normally achieved by using strong bases. Cyclopentadiene can be preliminarily recovered by cracking reaction from its dimeric form, and it has to be readily used.

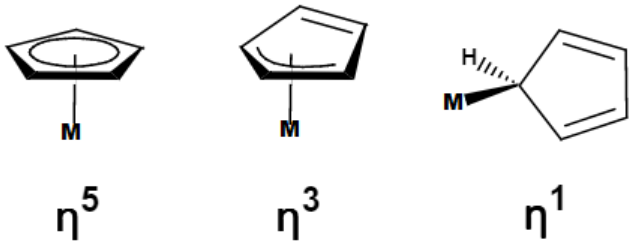


Figure 11. Modes of coordination of the cyclopentadienyl ligand

The pentamethylated cyclopentadienyl ligand, usually abbreviated as Cp*, seems to be one of the most important Cp-derivatives. Due to the introduction of five methyl substituents, Cp* is a much stronger σ-donor than Cp, moreover it presents a higher steric hindrance. In general, Cp* is able to confer enhanced solubility and tendency to crystallize, to the related metal complexes.

In comparison with arene ligands, both Cp and Cp* tend to bind the metal centres in a stronger way, due to the electrostatic attraction between the formal negative charge belonging to the ligand and the positive charge on the metal. Because of this, complete dissociation of a Cp anion from a metal has been rarely observed. In addition, the Cp*-metal bond survives both acidic and basic conditions, and is not commonly affected by the presence of either reducing or oxidizing agents.

The Iridium-chloro complex $[Ir(\eta^5\text{-}C_5Me_5)Cl_2]_2$ containing the pentamethylcyclopentadienyl ligand, are thermally stable and can be stored in air at room temperature for years. They are sparingly soluble in acetone and alcohols and readily soluble in chlorinated solvents. This metal compound are commercially available and, however, it can be prepared in the laboratory by a simple synthetic procedure (Figure 12).[54]

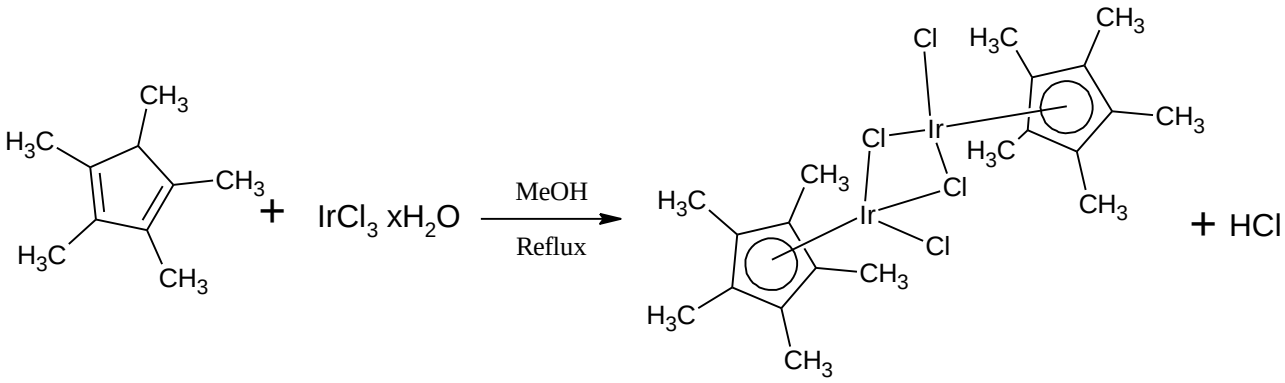


Figure 12. Synthesis of Cp*-Ir complexes.

On the other hand, the synthesis of analogous Ir compounds containing variously modified Cp* ligands, i.e. C_5Me_4R (R = H, alkyl or aryl group), is a more complicated task.[55] Indeed such synthetic strategy is required in order to tune the biological activity of the related Ir complexes. The preliminary preparation of the C_5Me_4HR precursor is needed, and the way of coordination to the metal could be influenced by the introduced functionalization on the ring.

Probably, the most convenient synthetic protocol to access C_5Me_4HR molecules is based on the use of 2,3,4,5-tetrametylcyclopenten-2-one as a precursor for the cyclopentadienyl moiety. 2,3,4,5-tetrametylcyclopenten-2-one is commercially available (Apollo Sci.) as a colourless liquid[56] (d = 0.917 g mL^{-1}), containing a mixture of cis and trans isomers. This ketone smoothly reacts, among the others, with lithiated aliphatic and aromatic compounds (R-Li). This reaction affords, after hydrolysis in acidic conditions, a R-substituted 2,3,4,5-tetramethyl cyclopentadiene (Figure 13).

[54] White, C. Y., A.; Maitlis, P. M.; *Inorg. Chem*.; **1992**, 29, 228–234.
[55] a)Macomber,D.W.; Hart;W.P.; Rausch, M.D.; *Adv. Organomet. Chem*., **1982**, 21,1;b) Jutzi, P.; Heiderman, T.; Neumann, B.; Stammler, H.G.; *Synthesis*, **1992**, 1096-1098.
[56] http://www.apolloscientific.co.uk/display_item.php?id=15749

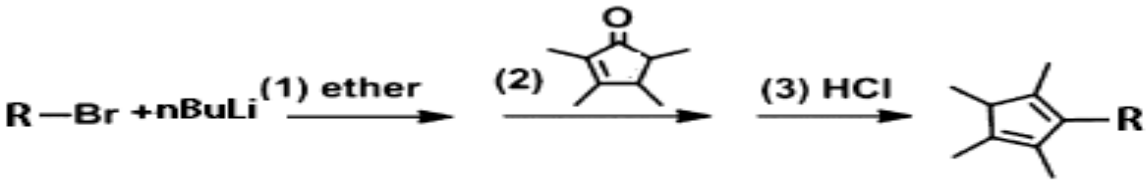


Figure 13. Synthesis of HCp*-derivatives (R = arene).

It is interesting to design functionalized C_5Me_4R ligands whose R group can reversibly bind the metal centre, thus obeying the conditions of the so called hemi-labile ligands.[57] This kind of compounds are of great importance, since they find application as catalytic precursors in stereoselective synthesis.[58] In particular, amine groups can be used as donor units within the pendant R substituent; the coordination strength improves when aromatic amine groups are employed. Typically, high valent metals are more strongly bound by the donor atom, instead a weaker or even no coordination may be observed with metals in a low oxidation state. To ensure good chelating properties, a C_2 or C_3 linker between the nitrogen atom and the Cp* ring atom is optimal. If the spacer is an alkyl chain, the ligand can achieve several conformations; in case the nitrogen does not interact with the metal centre, the complexes are often liquids, or non crystalline solids.

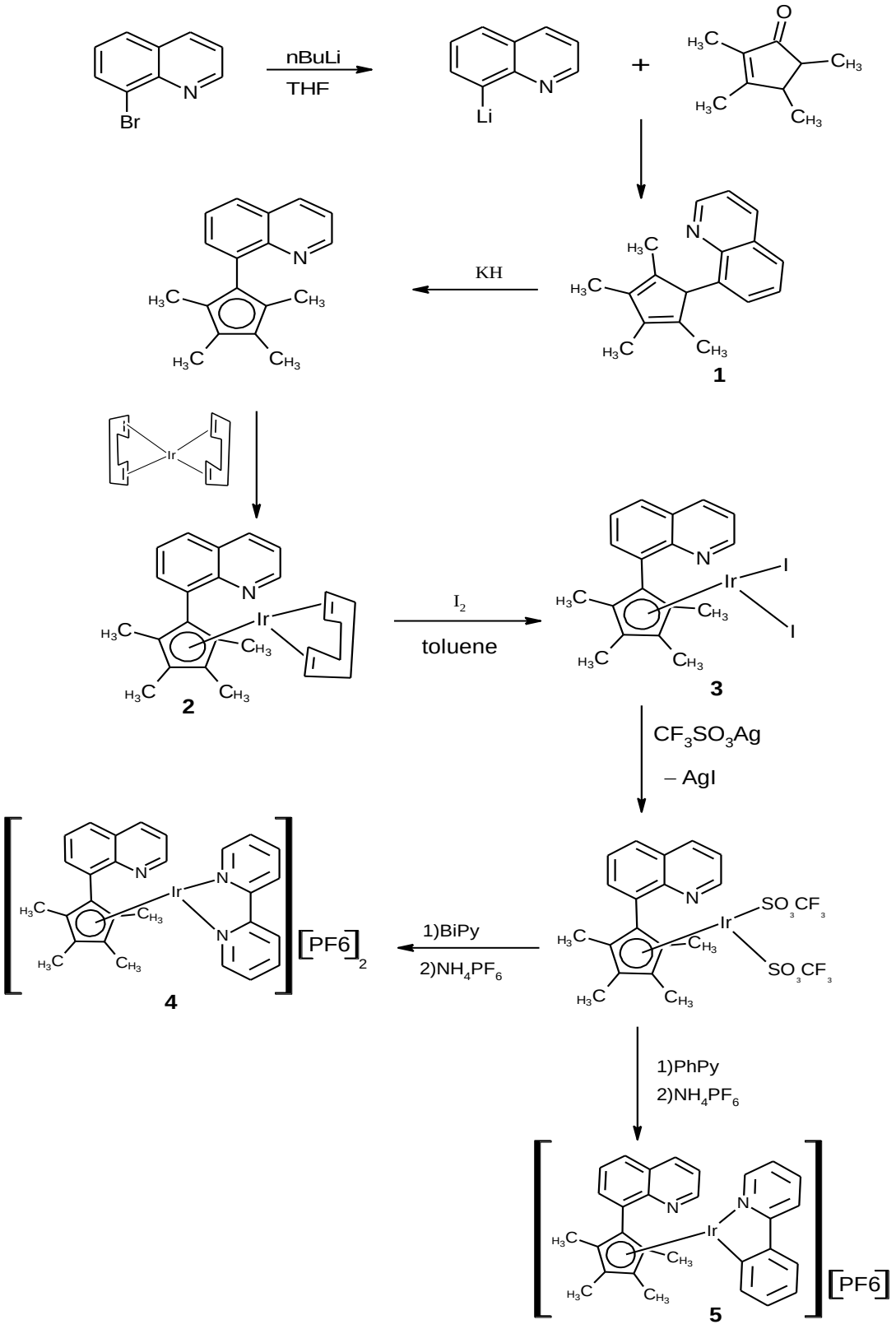
In the present work, the synthetic way depicted in Figure 3 has been adopted to prepare three C_5Me_4HR compounds, i.e. 2,3,4,5-Tetramethyl-1-(8-quinolyl)Cyclopentadiene **1**, 2-(2,3,4,5-tetramethylcyclopenta-1,3-dien-1-yl)pyridine **6** and 4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)phenol **11**. Besides, the iridium complexes of these three ligands were synthesized.

Although these syntheses were available in the literature, they had to be optimized in order to obtain the products with satisfying yields and purities; the details will be provided in the following paragraph.

[57] Enders, M.; Rudolph, R.; Pritkow, H.; Chem Ber. 1996, 129, 459-463.

[58] Erker, G; Aul, R.; *Organometallics*, **1988**,7,2070-2072. Christoffers, J.; Bergman, R.G.; *Angew.Chem*., **1995**, 107, 2423-242.

2.1 Synthesis of Tetramethylcyclopentadienylquinoline compounds.



Scheme 1-Synthesis of tetramethylcyclopentadienlilquinoline Iridium complexes.

The first C_5Me_4HR compound which was prepared comprises a quinoline moiety as R group, the C8 carbon of the quinoline being connected to the tetramethyl-cyclopentadienyl unit. In this system, the C_2 spacer between the nitrogen atom and the 5-membered ring belongs to the planar heterocycle, and it is permitted to rotate only around the single carbon-carbon bond.

The synthesis was performed as follows (Scheme 1): a cooled solution of 8-Bromoquinoline in tetrahydrofuran was treated with a solution of n-butyl lithium in hexane, to give 8-Li-quinoline. Then 2,3,4,5,-tetramethylcyclopenten-2-one was added, and the so formed lithium enolate was converted into the corresponding cyclopentadiene by hydrolysis and subsequent dehydration under acidic conditions.

According to NMR studies, two of the three possible isomers (Figure 14) were formed. These isomers differ in the site of addition of the quinoline moiety to the five-membered ring.

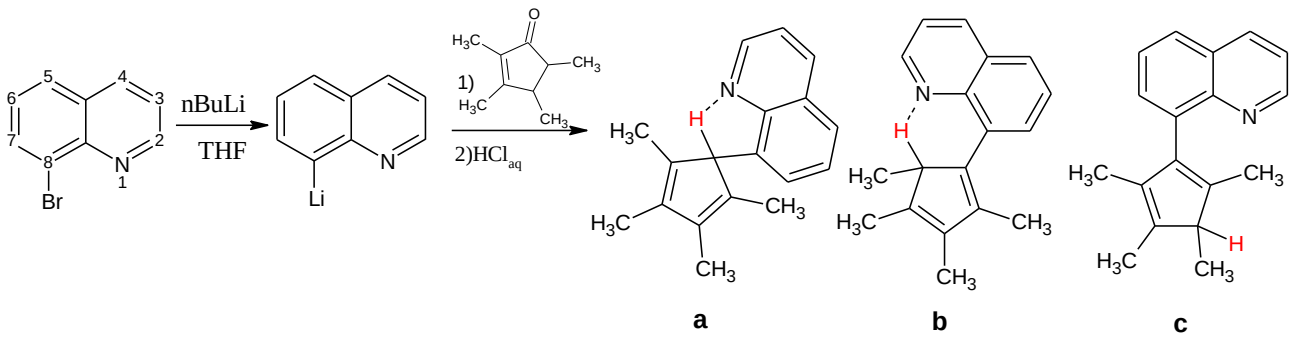
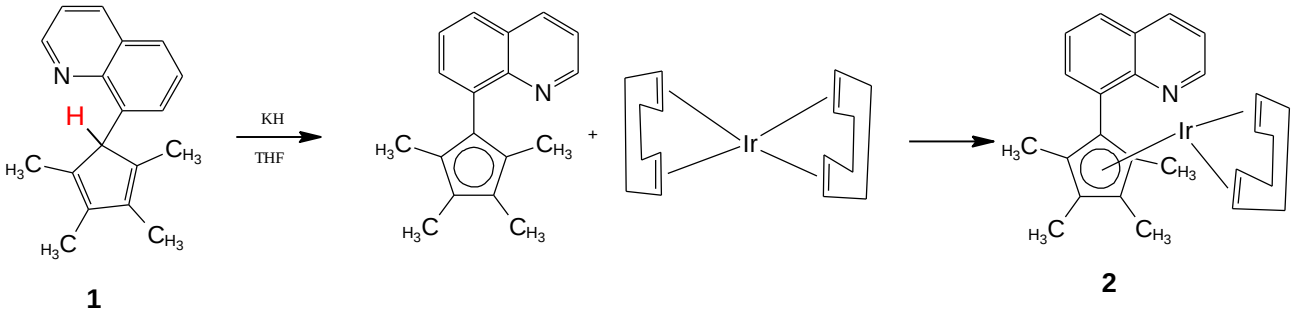


Figure 14. Synthesis of tetramethyl-cyclopentadienyl quinoline.

Indeed, it was possible to distinguish the isomers from the values of chemical shifts of the acidic Cp proton. Thus isomer **a** showed a singlet at 5.41 ppm ($CDCl_3$ solution), while isomer **b** showed a quartet at 4.00 ppm. These relatively low field resonances suggest the occurrence of an intramolecular hydrogen bond involving the cited proton and the quinoline nitrogen atom. The hypothetical isomer **c** was not detected. A possible explanation is that, in this case, the acidic Cp proton is far away from the quinoline nitrogen, thus preventing the possibility of establishing the stabilizing hydrogen bond interaction.

The cyclopentadiene-derivative **1** was deprotonated with potassium hydride to give a violet solution of the potassium salt in THF (Figure 15). Since potassium hydride is pyrophoric and must be stored under paraffin, the mixture of potassium hydride and paraffin was preliminarily washed with hexane and then suspended in dry THF, under rigorous nitrogen

atmosphere. Afterwards, a cold solution of $[Ir(COD)Cl]_2$ (COD= 1,5-Cyclooctadiene) in toluene was added. After stirring overnight, the volatile materials were evaporated and a brown oil was obtained.



1 **2**

Figure 15- Synthesis of compound 2.

The product **2** was purified by alumina chromatography, and the formation of this complex was unambiguously indicated by NMR spectroscopy. As a matter of fact, the presence of four distinct singlets, in the ^{1}H NMR spectrum, suggests that the four methyl groups are all chemically different from each other. This could imply the absence of interaction between the nitrogen atom and the Iridium(I). In fact, the presence of nitrogen-metal interaction should confer a blocked conformation of the complex with a mirror plane, and only two resonances for methyl groups should be seen.

The iridium(I) complex **2** was oxidized with elemental iodine, thus affording [η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl]diiodoIridium(III) **3** (see Scheme 1).[59] The ^{1}H NMR spectrum of **3** shows an almost clean product, in which two resonances accounting for the four methyl groups are detectable. This feature suggests that the complex possesses a mirror plane orthogonal to the Cp ring. The low field portion of the spectrum contains only the aromatic signals of the complex.

It should be remarked that the position of the ^{1}H NMR resonance due to the hydrogen adjacent to the quinoline nitrogen (Figure 16) is diagnostic for the possible metal coordination of the nitrogen atom. While in free quinoline this resonance falls at 8.80 ppm, in **3** it appears at 9.50 ppm (solvent: $CDCl_3$). The observed low field shift indicates that the nitrogen atom is coordinated to the Iridium(III) centre.

[59] G.Kohl, H.Pritkow, M.Enders, *Eur.J. Inorg. Chem.*, **2008**, 4230-4235.

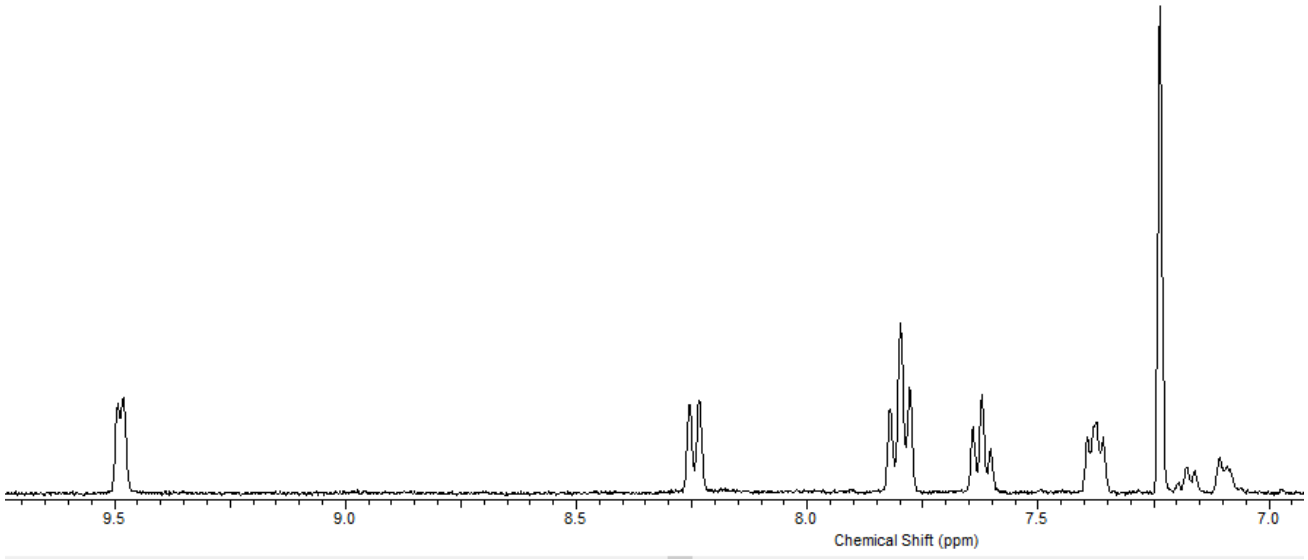


Figure 16. A particular of ^{1}H NMR spectrum of complex 3.

Compound **3** was also used as a starting material for the preparation of other two metal complexes.

Iridium complexes bearing isoelectronic arene-substituted Cp ligands have proved to exhibit potent anticancer activity. In particular, complexes like $[Ir(\eta^5\text{-}Cp^*)Ir(BiPy)Py]^{2+}$ and $[Ir(\eta^5\text{-}Cp^*)(PhPy)Py]^{+}$ (bipy= 2,2-bipyridine, phpy= Phenylpyridine, Figure 17) have shown a significant anticancer activity in vitro.[60] Thus similar complexes were synthesized, starting from **3**, in which Cp* is replaced by the deprotonated form of **1**.[61]

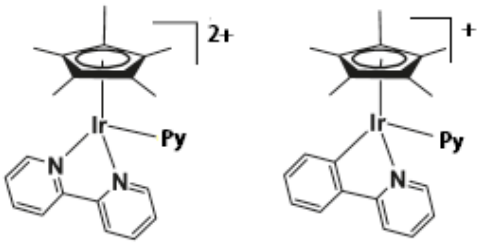


Figure 17. $[(\eta^5\text{-}Cp^*)Ir(BiPy)Py]^{2+}$ and $[(\eta^5\text{-}Cp^*)Ir(PhPy)Py]^{+}$ structures.

[60] Liu,Z.; Sadler, P.J.;,*Acc. Chem. Res.,* **2014**, 47, 1174−1185

[61] Liu,Z.; Habtemariam, A.; Pizarro,A.M.; Fletcher, S. A.; Kisova, A.; Vrana, O.; Salassa,L.; Bruijnincx, P.; Clarkson,G. J.; Brabec,V.; Sadler, P.J.; *J. Med. Chem.* **2011**, 54, 3011–3026.

To displace the iodide, an excess of silver triflate was added, forming the insoluble silver iodide which was easily removed from the solution. Therefore 2-phenylpiridine and 2,2'-bipyridine were added to obtain the cationic species $[Ir(\eta^5\text{-Cp°quino})\ (PhPy)]^+$ and $[Ir(\eta^5\text{-Cp°quino})\ (bipy)]^{2+}$ (Cp°quino=2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl), respectively. Both these complexes were precipitated as salt after the addition of ammonium hexafluorophosphate (complexes **4** and **5** respectively, Figure 18).

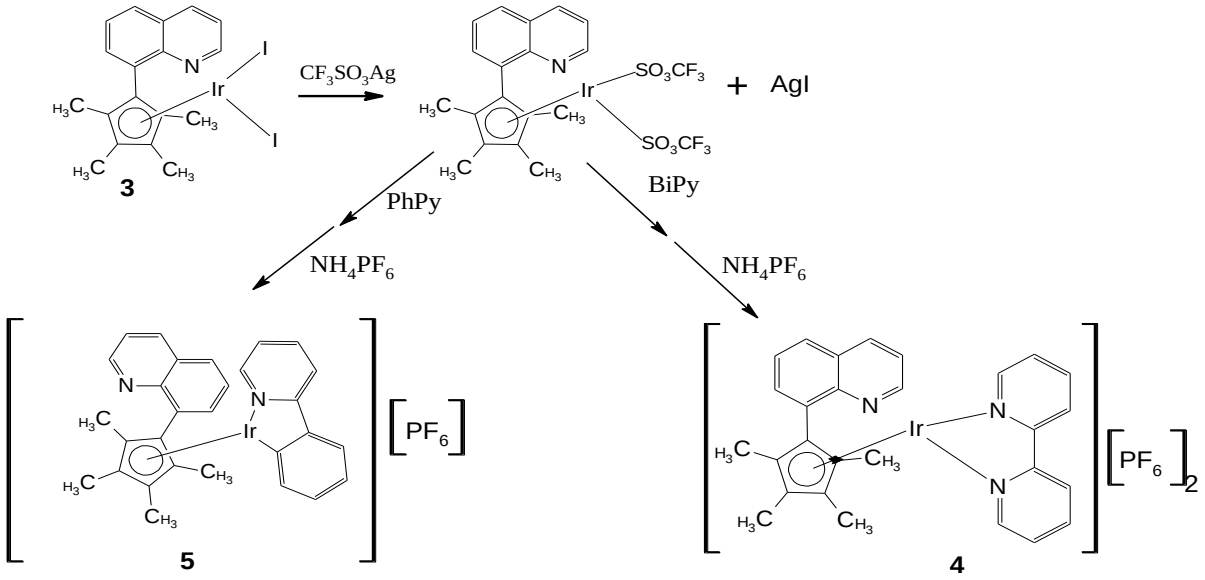


Figure 18. Synthesis of complexes 4 and 5.

The nature of **4** and **5** was confirmed by MS spectrometry and ^{1}H NMR analysis.

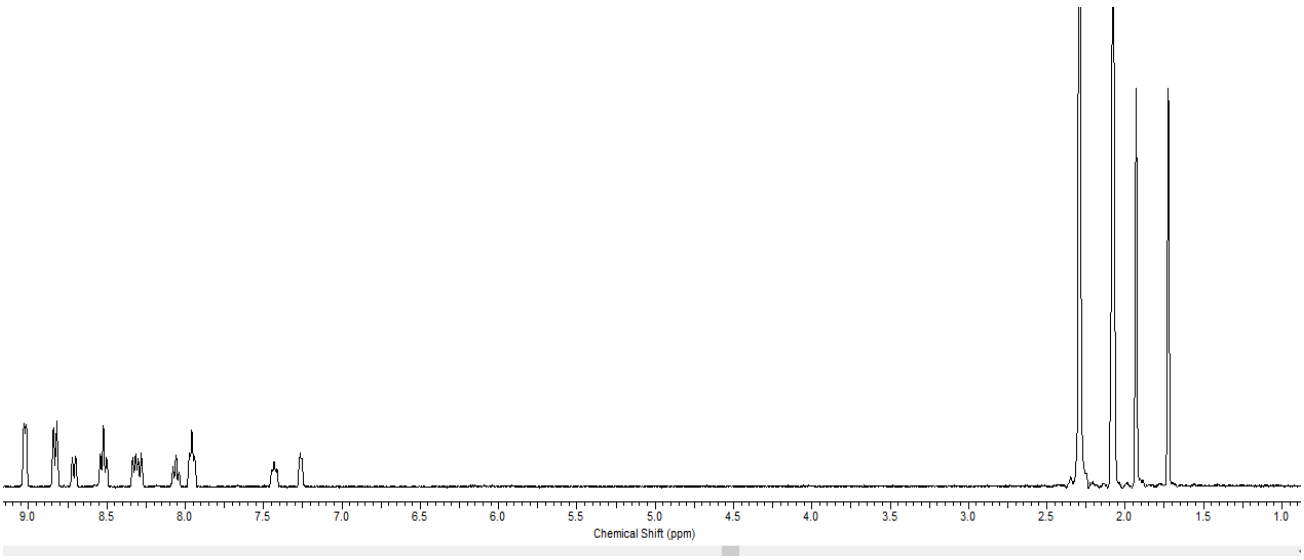
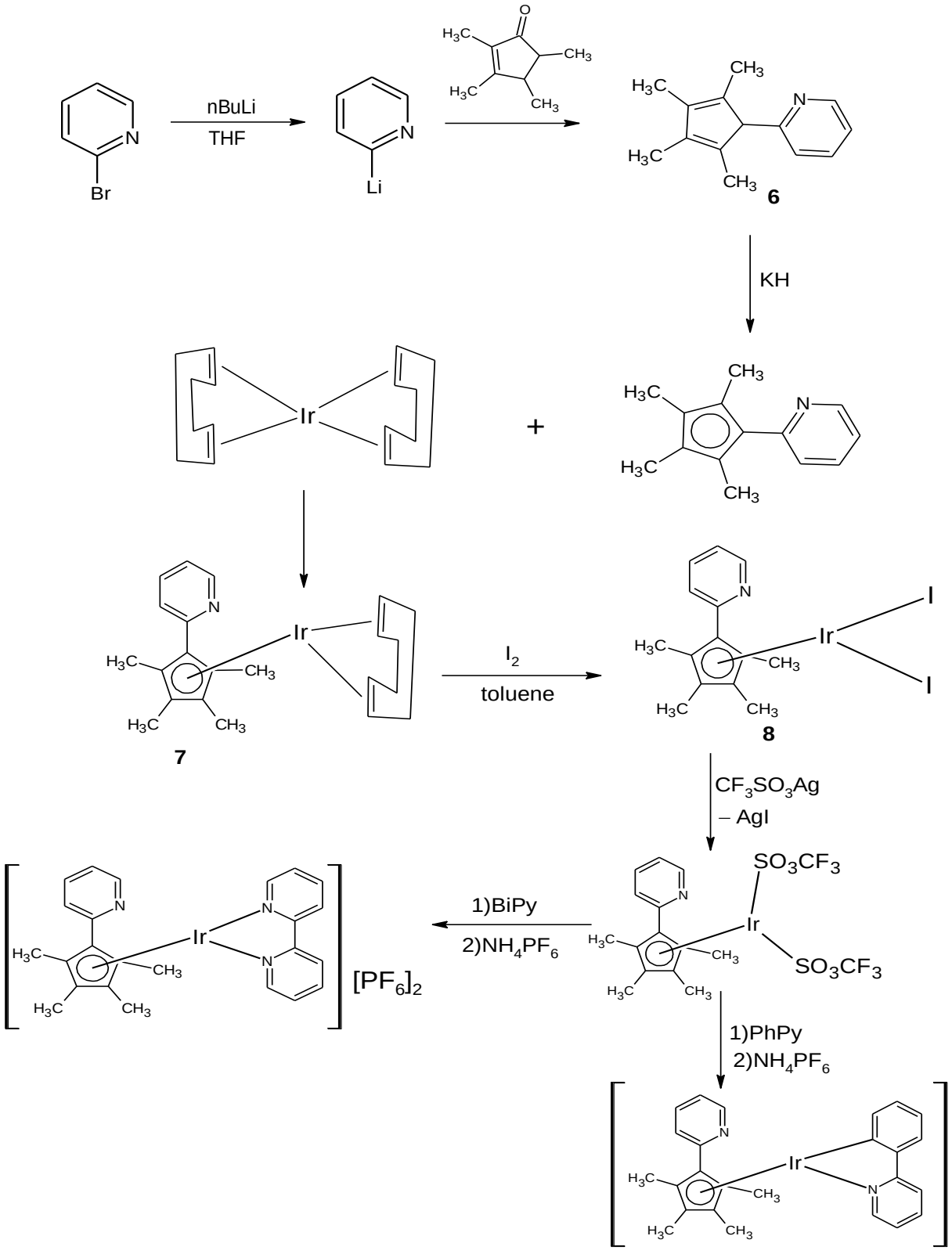


Figure 19. ^{1}H NMR of 4 (solvent: Acetone- d_6)

2.2 Synthesis of tetramethylcyclopentadienylpyridine compounds.



Scheme 2. Synthesis of tetramethylcyclopentadienepyridine iridium complexes

The same synthetic strategy, starting from 2,3,4,5-tetramethylcyclopenten-2-one, was used to synthesize **6**(Figure 19).

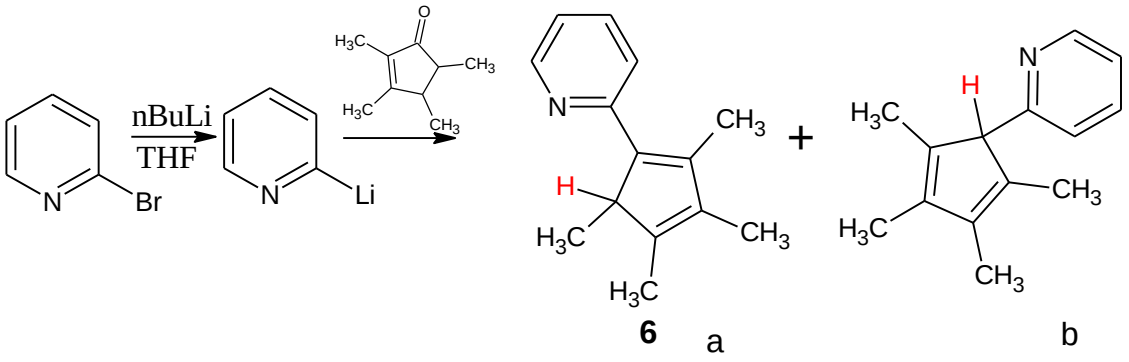


Figure 19. Synthesis of compound 6.

The fist synthetic step was the litiation of 2-bromopyridine, performed under rigorous nitrogen atmosphere. Then a solution of 2,3,4,5-tetrametylcyclopenten-2-one was added to the organolithium compound giving the corresponding lithium enolate. The mixture was subsequently hydrolysed with hydrochloric acid to convert the lithium enolate into the product **6**. The solution was neutralised and the organic layer was extracted with diethyl ether, then the crude product was distilled and characterized. Two different isomers could be unambiguously recognized. The major isomer **a** displays the cyclopentadiene double bonds in conjugation with the pyridine unit; it could be identified, by ^{1}H NMR spectroscopy, in view of the presence of a quartet signal at 3.53 ppm and a doublet at 0.97 ppm, assigned to the CH-CH_3 moiety.

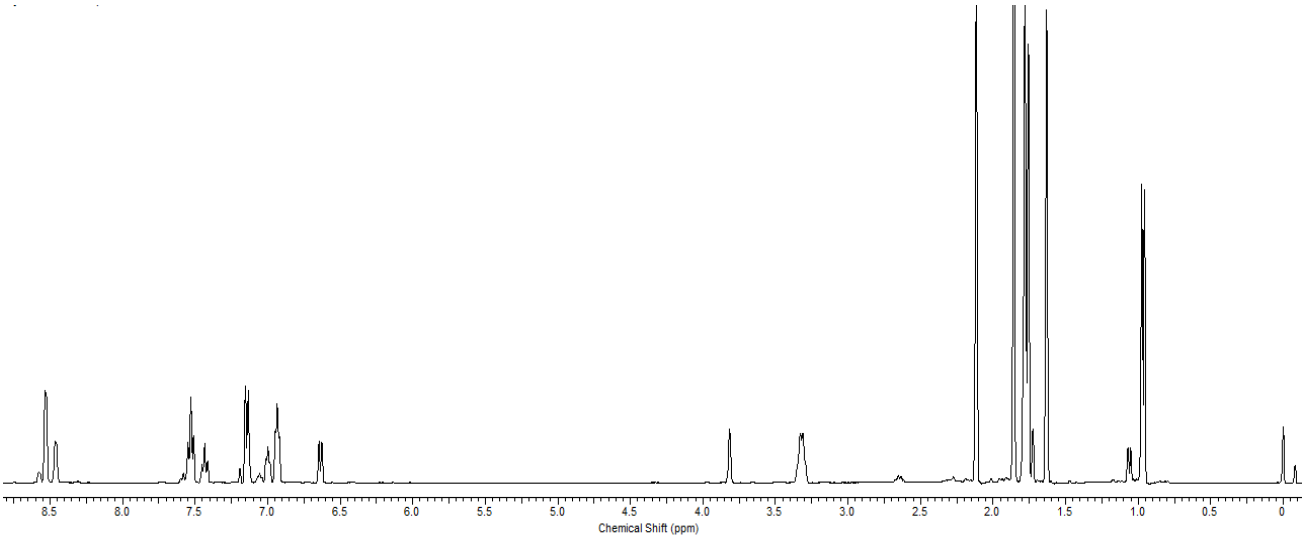


Figure 20. ^{1}H NMR spectrum of 6

The secondary isomer **b** was actually present as an impurity, as suggested by a resonance at 3.82 ppm. This unusual isomer distribution could be related to the additional stabilization imparted by the occurrence of an intramolecular hydrogen bond involving the nitrogen atom and the cyclopentadienyl CH, forming a five-membered ring (Figure 21).

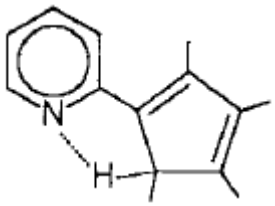


Figure 21. Hydrogen bound in compound 6

Previous results (Figure 22) suggested to test the direct reaction of the ligand **6** with $IrCl_3$-H_2O.[3]

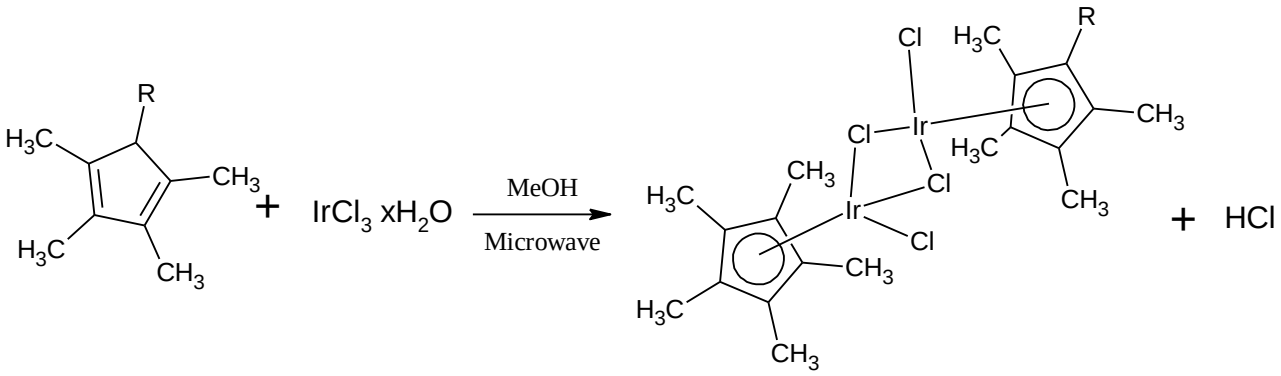


Figure 22. Synthesis of Ir complexes by Microwave-assisted reactions. (R=CH_3, Phenyle, Biphenyle).

The reaction depicted in Figure 22 was performed with the assistance of microwave irradiation. Microwave reactions represent a convenient strategy to accelerate organic[62] and organometallic syntheses; typically, microwave-assisted reactions are safer and may last only 5 minutes rather than 48h under high temperature conditions.[63] Both alcohols and water possess high dielectric loss tangents[64] and may be effective media in order to convert microwave energy into thermal energy.[65] It should be noted that the Iridium complexes

[62]R.J. Giguere, T.L. Bray, S.M. Duncan and G. Majetich, Tetrahedron Lett., 27 (1986) 4945. 2 R. Gedye, F. Smith, K. Westaway, H. Ali, L. Balderisa, L. Laberge and J. Rousell, Tetrahedron Lett., 21 (1986) 219

[63] M.A. Bennett and A.K. Smith, J. Chem. Sot. Dalton Trans., (1974) 233.

[64] Metaxas, A.C. Meredith, R.J.; IEE Power Engineering series 4, Industrial Microwave Heating. Peter Peregrinus, London, 1983.

[65] Bahgrust, D. R.; Mingos D.M.P.; Watson, M.J.; *Journal of Organometallic Chemistry*, **1989**,368, C43-C45

treated in the present thesis are soluble in methanol, therefore this can be used as solvent for the microwave-assisted reaction. Standard settings for this kind of syntheses are: 1) temperature = 140°C; 2) Power = 150 W; 3) time = 5m. The ligand and the $IrCl_3$-H_2O have to be charged in a suitable vessel in the microwave oven. After predetermined time a red solution is obtained, and the addition of pentane to the final step solution normally determines the precipitation of a red Iridium product. This method worked for Cp* and its phenyl and biphenyl derivatives as starting materials, as a quicker way to obtain $[Ir(\eta^5\text{-}C_5Me_4R)Cl_2]_2$ species.

Several attempts with compound **6** were done, but these were not successful (Figure 23).

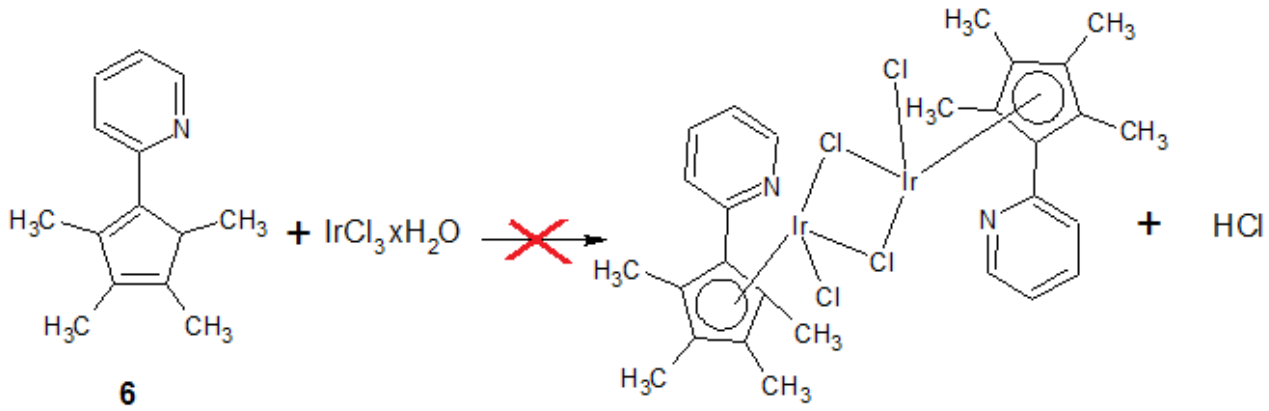


Figure 23. An example of microwave reaction with compound 6.

Since an important drawback to the occurrence of the reaction could be represented by the favourable interaction of the nitrogen atom with the metal centre in the high oxidation state +3, the reaction was tried in acidic conditions. The idea was to protonate the nitrogen atom thus preventing its interaction with Iridium. Another attempt was done by employing basic conditions, in order to help the deprotonation of cyclopentadiene; triethylammine was used to this purpose, but the results were not encouraging. Also the order of addition of the reagents and the time of reaction were varied, but without appreciable result. In all of the cases, the persistent presence of a doublet signal at 0.97 ppm (Figure 24), pointed that the deprotonation of the CpH moiety did not work.

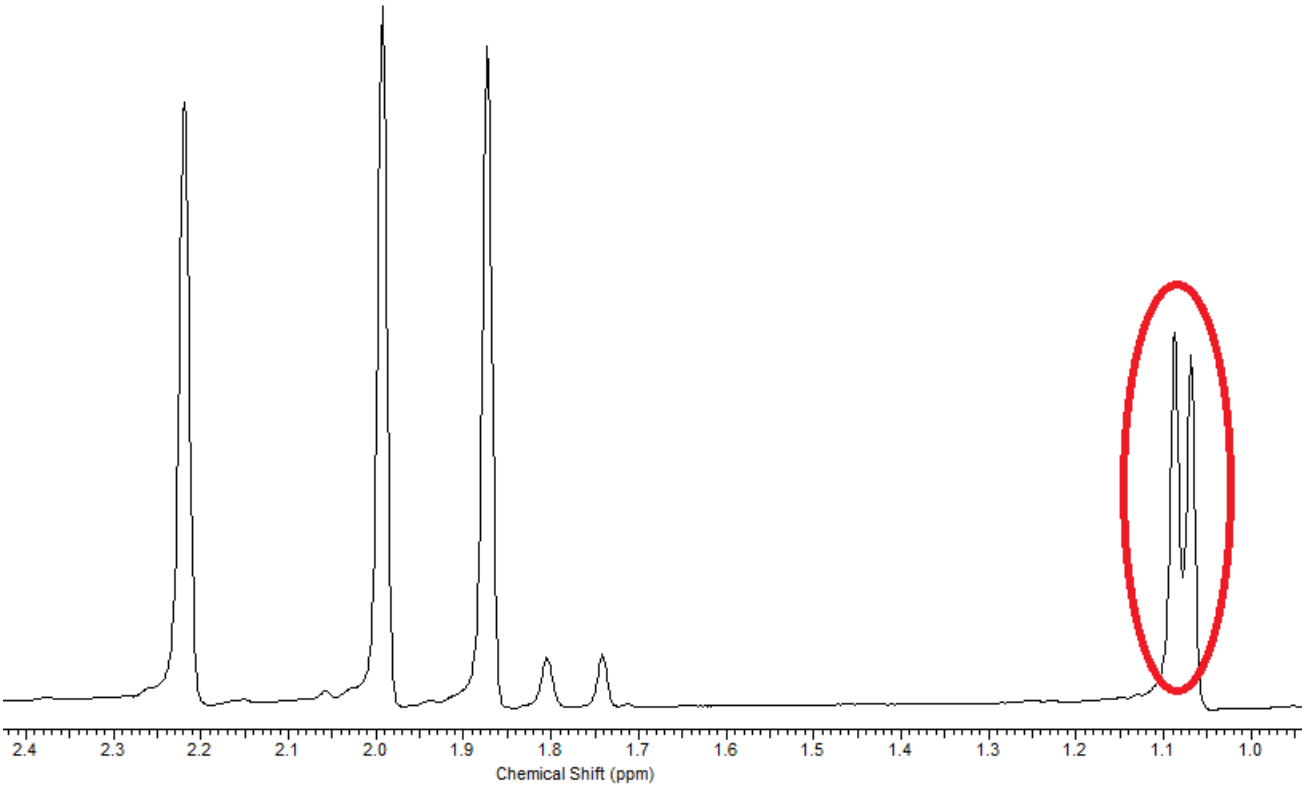


Figure 24. A particular of ^{1}H NMR($CDCl_3$) of 6 after Microwave reaction.

After the synthesis of the iridium complexes **4** and **5**, containing a quinoline-functionalized Cp ligand, the same approach was attempted with different ligands.

In principle, compound **6** should not be able to act as a hemi-labile ligand, on considering that the cyclopentadiene ring and the nitrogen donor atom are not separated by a sufficiently long spacer. As previously said, a C_2 or C_3 linker between the nitrogen atom and the Cp* ring atom is needed (Figure 25).

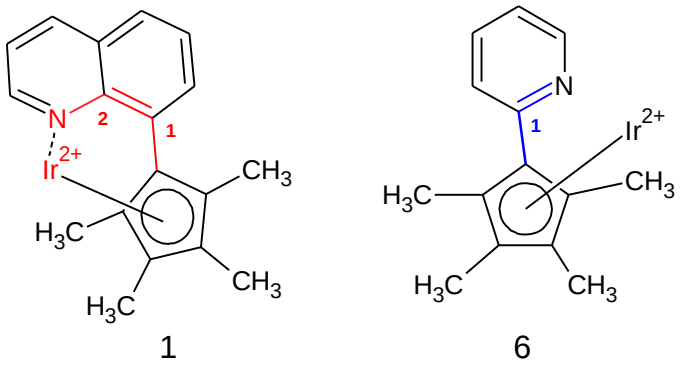


Figure 25. Chelating properties of compounds 1 and 6.

However, the nitrogen donor atom of an aromatic system such as pyridine could be able to bind tightly a metal centre in high oxidation state as is Iridium(III). The interaction between nitrogen and Ir(III) may be faster than the deprotonation of the cyclopentadiene fragment, and this latter could interfere with $IrCl_3$ action (Figure 23).

A solution of **6** was added to a suspension of potassium hydride at low temperature to form the anionic cyclopentadiene-derivative. After the addiction of $[Ir(COD)Cl]_2$, the Iridium (I) complex **7** was obtained (see Scheme 2). The ^1H NMR spectrum of the product, **7**, shows four different signals ascribable to the four Cp-bound methyl groups. In addition, the resonance at 4.08 ppm has been assigned to the alkene protons of the cyclooctadiene ligand. The identity of the product **7** was further corroborated by mass spectrum analysis.

After the addition of iodine and successive work-up (see Experimental for details), the ^1H NMR spectrum of **8** exhibited a resonance at 5.22 ppm, ascribable to free cyclooctadiene. This point suggests that cyclooctadiene was still present in solution, but presumably not binding to the metal.

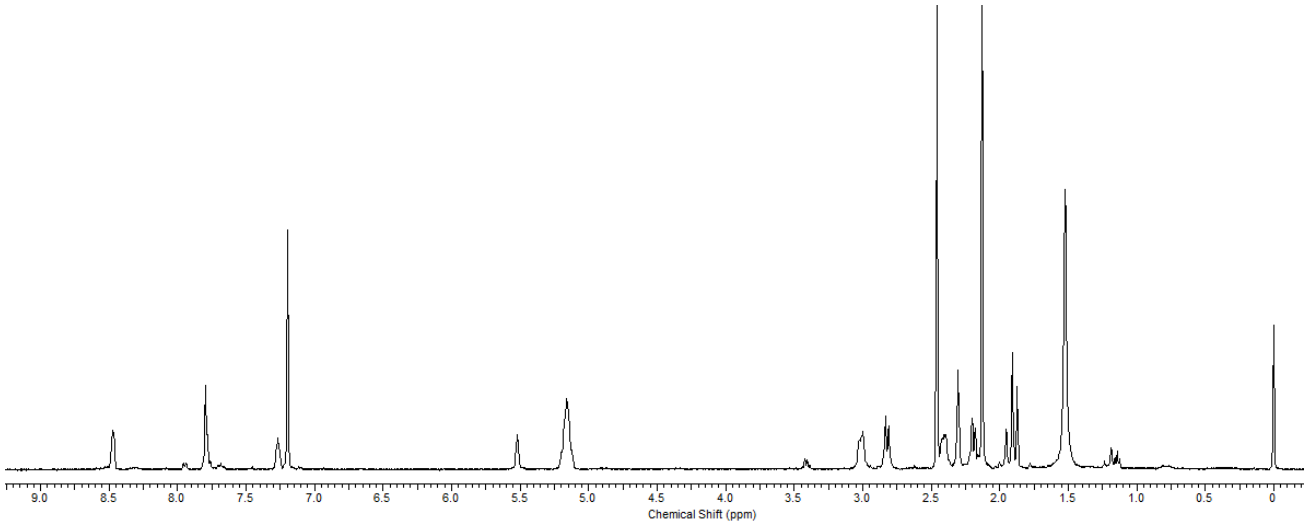
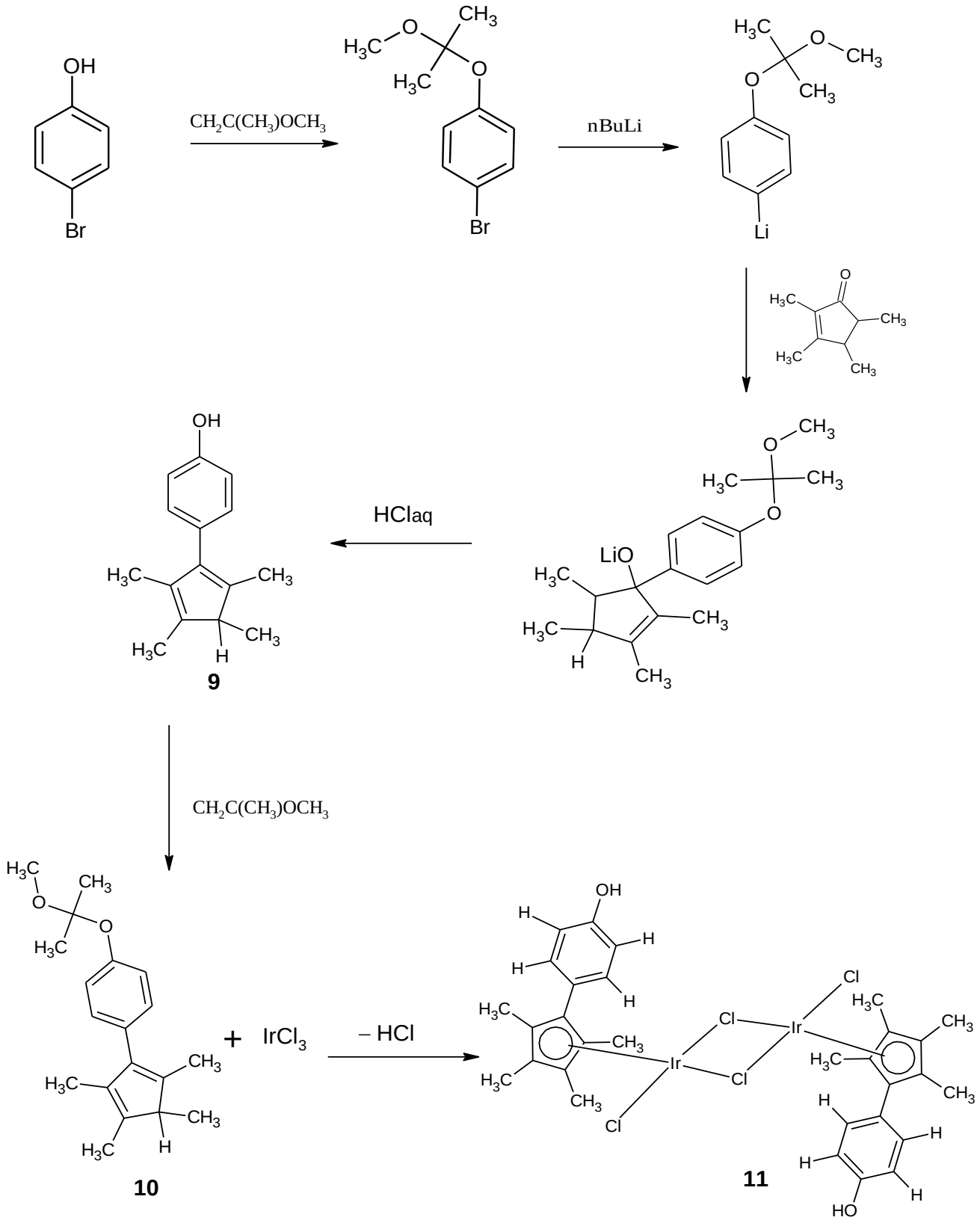


Figure 26. 1H NMR of 8.

According to the literature procedure, an attempt to synthesize complexes with 2-phenylpyridine and 2,2'-bipyridine was made, starting from **8**.[8] Silver triflate was used to precipitate silver iodide. The mixture was filtered and centrifuged, obtaining a colourless solution. A precipitate was obtained as hexafluorophosphate salts after the addiction of 2-phenylpyiridine and 2,2'-bipyridine.

Unfortunately, this synthetic protocol did not lead to a successful outcome; as a matter of fact, the mass spectra of the two isolated products both furnished the m/z value characteristic of **7**. It is likely that some problem occurred in the course of the oxidation reaction by iodine, but this could not be clearly ascertained.

2.3 Synthesis of tetramethylcyclopentadienylphenol compounds.



Scheme 3. Synthesis of tetramethylcyclopentadienilphenol Iridium complexes.

We prepared the cyclopentadiene compound **9**, functionalized with a phenol group. The introduction of the phenol group was designed for the following reasons:

1) the -OH group may confer enhance water solubility to the resulting complexes. Indeed water solubility of a metal complex may represent an important feature in view of its biological activity.

2) the -OH group may serve to the further functionalization of the ligand/complex. For instance, the esterification of alcoholic functions represent a well known strategy for the introduction of a bioactive fragment within a drug.[66] The bioactive fragment may tune the biological properties of the drug once in the physiological ambient, according to the cases.

One synthetic route to access **9** is described in the literature.[67] However, the reported procedure does not appear straightforward; moreover, no paper has followed reporting the use of **9** as ligand precursor by other authors. Therefore, a big effort was done in order to optimize the procedure.

The first synthetic step consists in the protection of the phenol function by etherification with 2-methoxypropene. This protection is necessary in order to avoid side reactions in the successive step, i.e. the lithiation of the C-Br site by using butyllithium. The resulting organolithium reagent, 1,4-$LiC_6H_4OCMe_2OMe$, was combined with commercial 2,3,4,5-tetramethylcyclopentenone. Then acidic hydrolysis was carried out to recover the final ligand **9** (Scheme 3). This product was characterized by NMR and IR spectroscopy. The ^1H NMR spectrum shows the three signals of $C\text{-}CH_3$ and a doublet signal at 0.93 ppm of methyl group of $CH\text{-}CH_3$ and a quartet at 3,16ppm of proton of $CH\text{-}CH_3$ (Figure 27).

In order to use **9** as a ligand precursor for the Ir metal centre, it was required to protect the alcohol group (with 2-methoxypropene) once again.

[66] Agonigi, A.; Riedel, T.; Zacchini, S.; Păunescu, E.; Pampaloni, G.; Bartalucci, N.; Dyson, P. J.; Marchetti, F. ; *Inorg. Chem*., **2015**, 54, 6504–6512

[67] Gibson, C.P.; Bem, D. S.; Falloon, S.B.; Hitchens, T.K.; Cortopassi, J.E.; *Organometallics*, **1992**, 11, 1742-1749.

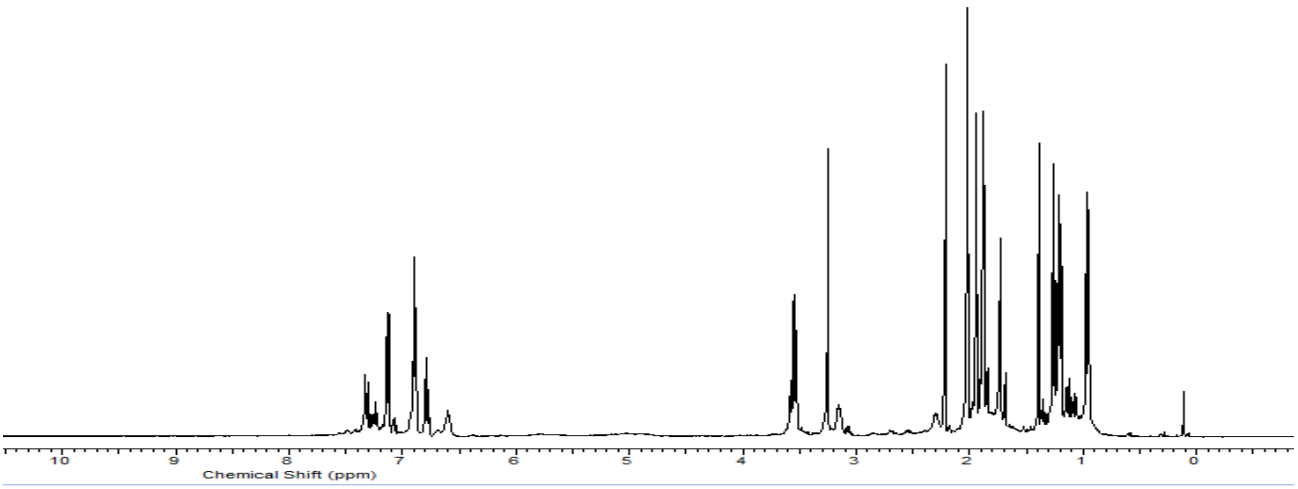


Figure 27. ^{1}H NMR in $CDCl_3$ of 10.

Then the protected pro-ligand **10** was allowed to react with iridium trichloride in methanol at reflux temperature for three days. It seems that hydrogen chloride released by the reaction between the cyclopentadiene reactant and Ir is responsible for the regeneration of the alcoholic function. The final metal complex was purified by silica chromatography and thus isolated as a pure product in ca. 60% yield. We tried different reaction conditions in order to try to increase the yield. The use of methanol at reflux temperature revealed to be the most convenient strategy while, for instance, performing the reaction in boiling isopropanol afforded **11** in a significantly lower yield. Complex **11** is well soluble in acetone and methanol and sparingly soluble in water. It was characterized by NMR and IR spectroscopy. The ^{1}H NMR spectrum displays two resonances accounting for two pairs of equivalent methyl groups. The ^{13}C NMR spectrum confirms the presence of a certain degree of symmetry within the complex. Only two resonances accounting for the four methyl groups are detectable, at 8.7 ppm and 9.8 ppm respectively, and consequently only two resonances for the four correspondent Cp-ring carbons (in $CDCl_3$: δ = 86.9, 89.9ppm). Even the aromatic part displays symmetry, and only four different signals for the six carbons have been detected (Figure 28).

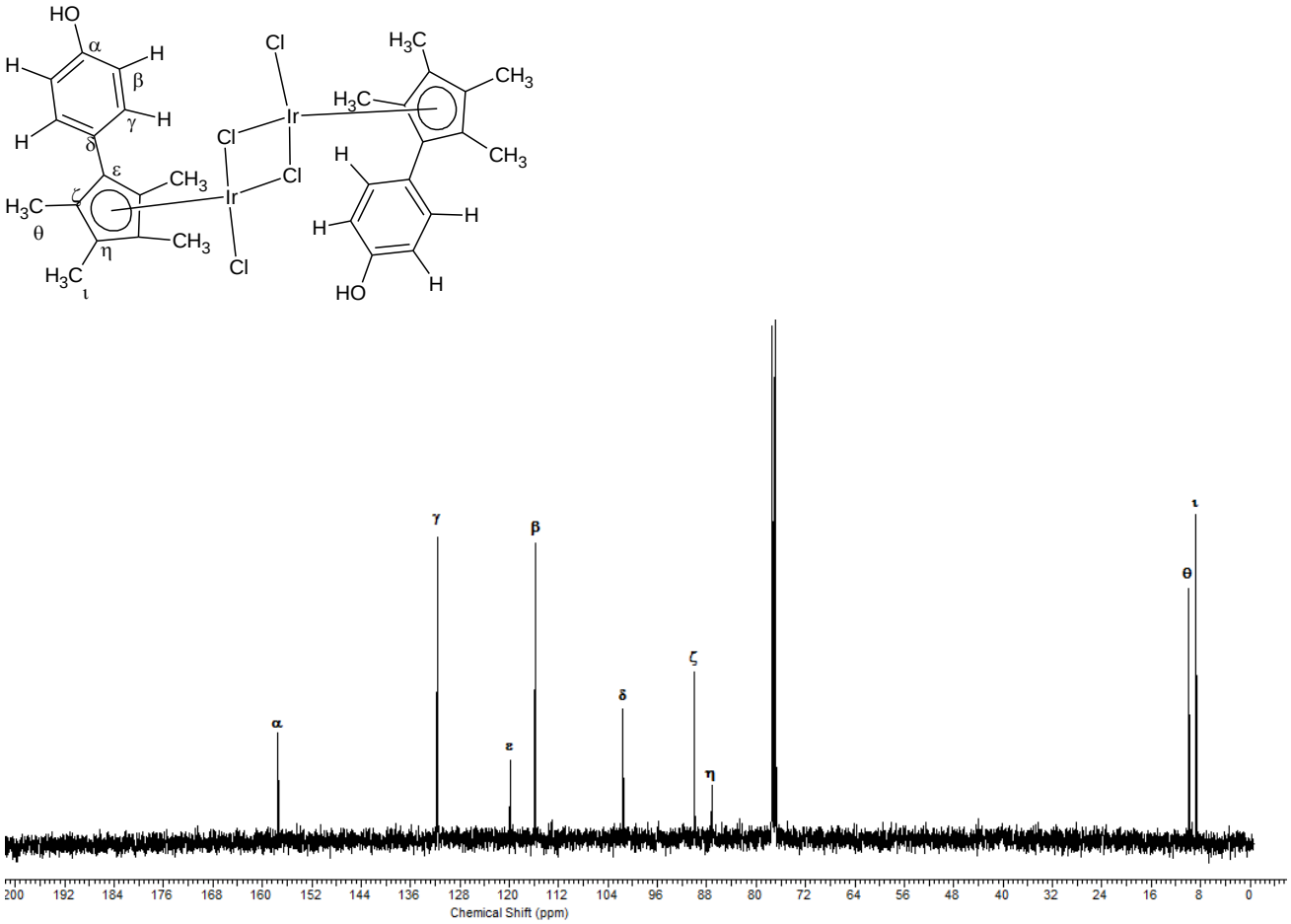


Figure 28. ^{13}C NMR in $CDCl_3$ of 11.

The IR spectrum of **11** is reported (Figure 29). The most salient feature is given by the broad band at 3146 cm^{-1}, that is characteristic of the stretching of the OH group.

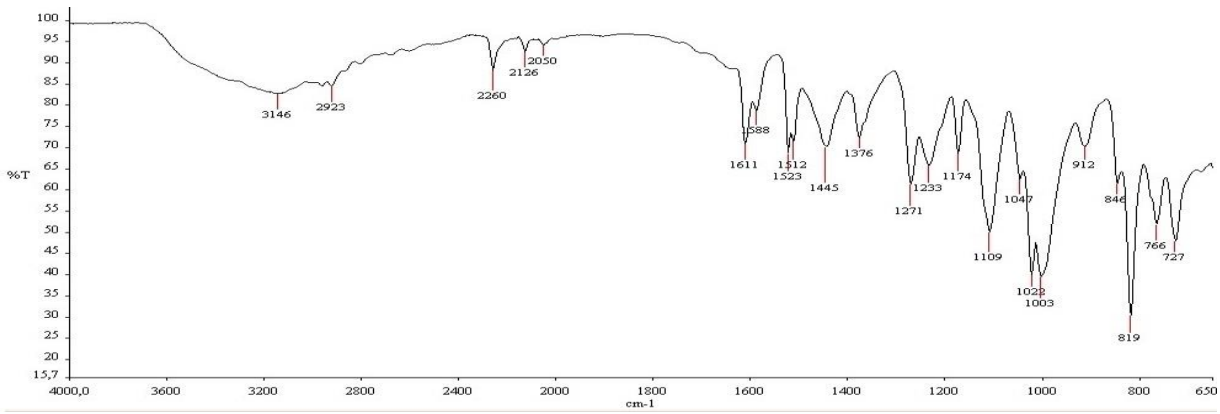


Figure 29. FTIR-ATR spectrum of 11.

CHAPTER 3

3. CONCLUSIONS

New cyclopentadiene pro-ligands have been designed and synthesized, then some related Ir(III) complexes has been obtained.

The new complexes possess a framework, which is typical of already reported Ir anticancer compounds, representing a new class of promising and powerful anticancer agents. The antiproliferative activity of the new complexes will be tested in vitro in a short time

In particular, the Cp-phenol Iridium complex **11** is interesting in view of the presence of a phenol group. This moiety confers slightly enhanced water solubility to the complex. Moreover, it may be exploited for the functionalization of the complex with bioactive compounds, in order to tune the anticancer activity.

CHAPTER 4

4. EXPERIMENTAL SECTION

Air- and/or moisture sensitive compounds were handled under nitrogen atmosphere by using standard Schlenk techniques. Solvents were purified by distillation from appropriate drying agents under an atmosphere of dry Ar. All the glassware was dried in a oven at 130°C and cooled under vacuum (10^{-2}mmHg).

4.1 Solvents

- Diethyl ether, $(C_2H_5)_2O$;
- Tetrahydrofuran (THF), C_4H_8O;
- Methanol, CH_3OH (only degassed);
- Dichloromethane, CH_2Cl_2 (drying agent: P_2O_5);
- Toluene, C_7H_8 (drying agent: Na);
- Deuterated chloroform, $CDCl_3$;
- Deuterated acetone, $(CD_3)_2CO$;
- Deuterated dimethylsulfoxide, $(CD_3)_2SO$;

4.2 Reagents

All the following materials were purchased from Sigma-Aldrich:

- n-Buthyllithium, C_4H_9Li (1.6M in hexanes or 2,5M in hexanes);
- Potassium hydride, KH in paraffine 50% w/w;
- 8-Bromoquinoline, C_3H_6BrN;
- 2-Bromopyridine, C_5H_4BrN;
- Iridium trichloride hydrate, $IrCl_3 \cdot 3H_2O$;
- 2,2-bypiridine, $C_{10}H_8N_2$;
- Bis(1,5-cyclooctadiene)diiridium(I) dichloride, $C_{16}H_{24}Cl_2Ir_2$;
- Iodine, I_2;
- 2-phenylpyridine, $C_{11}H_9N$;

- Ammonium hexafluorophosphate, NH_4PF_6;
- Sodium acetate, $NaCH_3COO$;
- Silver trifluorosulfonate (Silver Triflate), $AgCF_3SO_3$

2,3,4,5-tetramethylcyclopenten-2-one, $C_9H_{14}O$ (mixture cis and trans) was purchased from Apollo Scientific.

4.3 Analyses

IR spectra were recorded using a FT-IR Spectrum One Perkin Elmer with UATR. ^1H and ^{13}C NMR were acquired in 5mm NMR tubes at 298K on Bruker DPX400 (University of Warwick, UK) and Bruker Avance DRX 400 instrument equipped with a BBFO broadband probe.

Electrospray ionization mass spectra (ESI-MS) were obtained by preparing the sample in CH_3OH and infusing into the mass spectrometer (Bruker Esquire 2000). The mass spectra were recorded with a scan range of m/z 50-1000 for positive ions.

4.4 Synthesis

4.4.1 2,3,4,5-Tetramethyl-1-(8-quinolyl)Cyclopentadiene compounds. (Cp°quinoline)

2,3,4,5-Tetramethyl-1-(8-quinolyl)Cyclopentadiene[68] 1

A solution of 16.6 g ($8.00\cdot10^{-2}$mol) of 8-Bromoquinoline in 230 mL of THF was cooled to −78°C and 50mL of n-Butyllithium 1,6M in n-Hexane ($8.00\cdot10^{-2}$mol) were added dropwise with stirring. After stirring for 10 min, 12 mL ($8.00\cdot10^{-2}$mol) of 2,3,4,5-tetramhylcyclopent-2-enone was added dropwise. The green mixture was allowed to warm to room temperature and then was heated at reflux for 1h. After cooling down, 100g of ice and 16mL of HCl_{aq} (37%) were added and the orange mixture was stirred for 30m. It was subsequently alkalised with 8mL of aqueous ammonia (33%). The organic layer was extracted with Et_2O (3x100mL). $MgSO_4$ was added, then the organic layer was filtered and all the solvents were

[68] M.Enders, R. Rudolph, H. Pritzkow, *Chem. Ber.*, **1996**, 129, 459-463.

evaporated. An orange oil was obtained (20.109g).Purification of 4.00 g. Chromatography (silica gel): n-Hexane/Ethyl acetate, concentration's gradient 0-30%. Five fractions were separated, the 2nd was collected. Yield:1.756 g (43%).

NMR ^{1}H($CDCl_3$) δ: 8.81 (m, 2H, CH a-a'); 8.03 (m, 2H, CH c-c'); 7.54-7.72 (m, 3H, CH d-d'-e'); 7.18-7.46 (m, 3H, CH e-f-f'); 6.89 (m, 2H, CH b-b'); 5.41 (s, 1H, CH n); 3.86 (m, 1H, CH-CH3 m); 1.85 (s, 6H, CH_3 g-h); 1.81 (s, 6H, CH_3 h'-i'); 1.78 (s, 6H, CH_3 g'-l'); 1.58 (s, 3H, CH_3 i); 0.74 (d, 3H, CH_3 l) ppm.

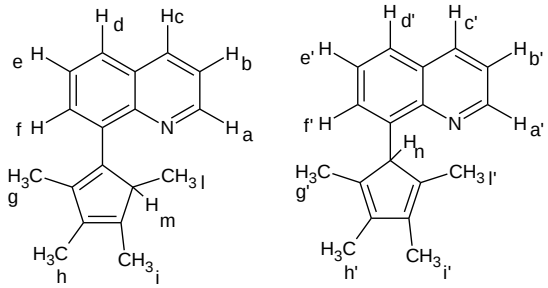


Chart 1. Naming of hydrogen atoms of 1 for ^{1}H assignments.

(1,5-cyclooctadiene)[η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl] Iridium(I)[69] 2

To a suspension of 0.074g of potassium hydride (KH in paraffin, 50% w/w) in 25 mL of THF, 0.233 g ($9.3\cdot10^{-3}$mol) of **1** was added. After stirring for 3h at room temperature, the violet suspension was added to a solution of 0.265g ($2.45\cdot10^{-2}$mol) of $[Ir(COD)Cl]_2$ in 25 mL of toluene, which was kept at −78°C. The resulting mixture was warmed to room temperature overnight. All solvents were evaporated. Chromatography.(Al_2O_3/5% H_2O): 100% toluene.. The solid was dried under vacuum. Yield: 0.226 g, (45%).

^{1}H NMR($CDCl_3$) δ: 8.81 (m, 2H, CH a-a'); 8.03 (m, 2H, CH c-c'); 7.54-7.72 (m, 3H, CH d-d'-e'); 7.18-7.46 (m, 3H, CH e-f-f'); 6.89 (m, 2H, CH b-b'); 4.08 (m, 4H, CH m-n); 2.83 (m, 8H, m-o); 1.88 (s, 3H, CH_3 h); 1.81 (s, 3H, CH_3 h); 1.78 (s, 3H, CH_3 i); 1.58 (s, 3H, CH_3 l)ppm.

[69] G.Kohl ,R.Rudolph, H.Pritkow, M.Enders, *Organometallics*, **2005**, 24, 4774-4781.

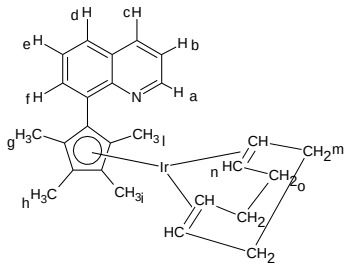


Chart 2. Naming of hydrogen atoms of 2 for ^{1}H assignments.

η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl]diiodoIridium(III)[70] 3

A solution of iodine (0.042g, $1.65\cdot10^{-3}$mol) in 5mL of toluene was added to a solution of **2** (0.094g, $1.71\cdot10^{-3}$mol) in 50 mL of toluene. The orange solution was stirred for 2h at room temperature. The product was precipitated by the addition of n-hexane (50 mL), separated by filtration, washed with n-hexane, and dried under vacuum. Yield: 0.104 g (93%).

MS: m/z 476 [Cp°quino Ir ($H_2O)_2$], 567.9 [Cp°quinoIr(III)I] ;

NMR ^{1}H($CDCl_3$) δ: 9.50 (d, 1H, CH a); 8.22 (d, 1H, CH c); 7.79 (t, 2H, CH d-e); 7.61(t, 1H, CH f); 7.36 (t, 1H, CH b); 2.07 (s, 6H, CH_3); 1.84 (s, 6H, CH_3).

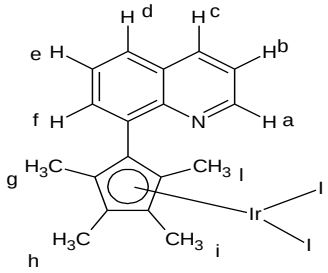


Chart 3. Naming of hydrogen atoms of 3 for ^{1}H assignments.

[η^5–2,3,4,5-tetramethyl-1-(8-quinolyl)cyclopentadienyl]bipyridyl Iridium(III)][PF_6]$_2$ 4

0.035g ($1.36\cdot10^{-4}$mol) of $AgCF_3SO_3$ was added to a solution of **4** (0.043g, $6.19\cdot10^{-5}$mol) in CH_2Cl_2 and stirred at room temperature overnight. The solution was filtered and centrifuged. 0.012g ($7.69\cdot10^{-5}$mol) of 2,2'-bipyridine was added to the yellow solution in CH_2Cl_2 and stirred for 3h. The solvent was evaporated and the solid was dissolved in methanol. 0.125g ($7.44\cdot10^{-4}$mol) of NH_4PF_6 was added. The yellow-green precipitate was filtered and stirred overnight in Et_2O, then filtered again and dried. 0.040g, yield = 87%.

MS: m/z 632 [Ir(Cp°quino)(BiPy)Cl]; 742 [Ir(Cp°quino)(BiPy)][PF_6]

[70] G.Kohl, H.Pritkow, M.Enders, *Eur.J. Inorg. Chem.*, **2008**, 4230-4235.

^{1}H NMR($(CD_3)_2CO$) δ: 9.02 (d, 2H, CH m-m'); 8.83 (d, 2H, CH a-c); 8.71 (d, 2H, CH o-o'); 8.50 (t, 2H, CH p-p'); 8.31(m, 1H, CH d); 8.06 (t, 1H, CH e); 7.96 (t, 2H, CH n-n'); 7.43(d,1H, CH f); 7.27 (t, 1H, CH b); 1.93 (s, 6H, CH_3 g-l),; 1.73 (s, 6H, CH_3h-i) ppm.

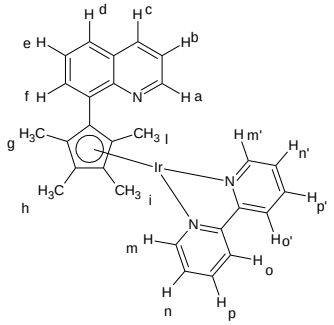


Chart 4. Naming of hydrogen atoms of 4 for ^{1}H assignments.

[η^5–2,3,4,5-tetramethyl-1-(8 quinolyl)cyclopentadienyl]phenylpyridyl Iridium(III)][PF_6] 5

0.037g (1,44·10^{-4}mol) of $AgCF_3SO_3$ was added to a solution of **3** (0.050g, 7.2·10^{-5}mol) in CH_2Cl_2 and stirred at room temperature overnight. The solution was filtered and centrifuged. 0.035g (4.27·10^{-4}mol) of CH_3COONa was added to the yellow solution and then 0.012g (7.79·10^{-4}mol) of 2-phenylpyridine was added and the solution was stirred for 2h at room temperature. The solution was filtered and dissolved in methanol. 0.118g (7.02·10^{-4}mol) of NH_4PF_6 was added. The green precipitate was filtered and stirred in Et_2O overnight, then filtered again and dried. Yield: 0.047g (88%).

MS: m/z 595 (Cp*quino phenylpyridyl Ir(III))

^{1}H NMR (CD_3OD) δ: 8.82 (d, 1H, CH a); 8.47 (d, 1H, CH m); 8.34(d, 1H, CH m'); 8.17(d, 1H, CH c); 8.02-8.11(m, 3H, p-p'-d); 7.87-7.94 (m, 2H, CH e-f); 7.34 (t, 1H, CH b); 7.29 (m, 2H, CH o-o'); 7.19 (m, 2H, CH n-n'); 2.21 (s, 3H, CH_3 g); 1.83 (s, 3H, CH_3 l); 1.71 (s, 3H, CH_3 i); 1.65 (s, 3H, CH_3 h) ppm.

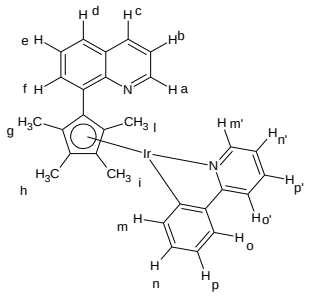


Chart 5. Naming of hydrogen atoms of 5 for ^{1}H assignments.

4.4.2 2-(2,3,4,5-tetramethylcyclopenta-1,3-dien-1-yl)pyridine compounds. (Cp°pyridine)

2-(2,3,4,5-tetramethylcyclopenta-1,3-dien-1-yl)pyridine 6

50 mL of a 1,6M solution of n-Butyllithium in hexane ($8.00 \cdot 10^{-2}$mol) was added dropwise with stirring to a solution of 7.8 mL ($8.18 \cdot 10^{-2}$mol) of 2-Bromopyridine in 140 mL of Et_2O cooled to –40°C. The brown solution was stirred for 1h at this temperature. A solution of 2,3,4,5-tetramethylcyclopent-2-enone ($7.90 \cdot 10^{-2}$mol) in 50 mL of Et_2O was added dropwise and the mixture was stirred for 1.30h at –40°C. The mixture turned to green after the addition. The mixture was allowed to warm to room temperature and it turned to brown. The mixture was hydrolysed with 80mL of water and 80mL of HCl_{aq} (37%) was added. The mixture was stirred at room temperature overnight. The yellow organic layer was separated. The aqueous phase was neutralised with potassium hydroxide and then extracted with Et_2O (3x50mL;1st fraction). The combined organic layers were dried with magnesium sulphate (Orange solution).The crude product was distilled (b.p. 80°C/0.01mbar).

Yield: 7.031g (45%).

^{1}H NMR($CDCl_3$) δ:8.54 (d, 1H, CH a) 7.55 (t,1H, CH c); 7.17 (d; 1H, CH d); 6.96 (t, 1H, CH b); 3.34 (q, 1H, CH-CH_3 e); 2.13 (s, 3H, CH_3 i) ; 1.87 (s, 3H, CH_3 h); 1.79 (s, 3H, CH_3 g); 0.97 (d, 3H, CH_3 f) ppm.

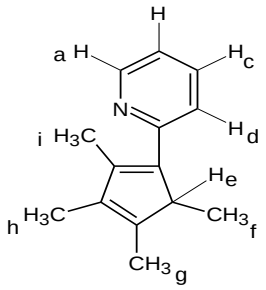


Chart 6. Naming of hydrogen atoms of 6 for ^{1}H assignments.

(1,5-cyclooctadiene)[η^5–(2,3,4,5-tetramethylcyclopentadienyl)-1-pyridine Ir(I) 7

To a suspension of 0.100g of potassium hydride (KH in paraffin, 50% w/w) in 25 mL of THF, 0.200 g ($1.01 \cdot 10^{-3}$mol) of **6** was added. After stirring for 3h at room temperature, the suspension was added to a solution of 0.341g ($3.16 \cdot 10^{-3}$mol) of $[Ir(COD)Cl]_2$ in 25 mL of

toluene, which was kept at −78°C. The resulting yellow-orange mixture was warmed to room temperature overnight. All solvents were evaporated.

MS: m/z 498.1 [Ir(Cp°Py) (COD)]

^{1}H NMR(($CDCl_3$) δ: 8.54 (d, 1H, CH a); 7.55 (t,1H, CH c); 7.17 (d; 1H, CH d); 6.96 (t, 1H, CH b); 4.08 (m, 4H, CH m-n) ; 2.84 (m, 8H, CH o-l) ;2.00 (s, 3H, CH_3 i) ; 1.89(s, 3H, CH_3 h) ; 1.66 (s, 3H, CH_3 g); 1.18 (s, 3H, CH_3 f) ppm.

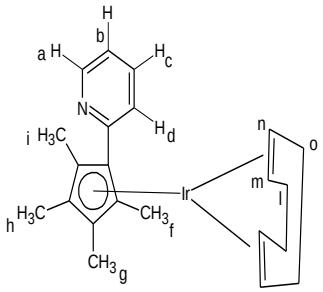


Chart 7. Naming of hydrogen atoms of 7 for ^{1}H assignments.

[η^5–2,3,4,5-tetramethyl-1-pyridilcyclopentadienyl)diiodoIridium(III)[71] 8

A solution of 0.140g ($5.51\cdot10^{-4}$mol) of iodine in 5mL of toluene was added to a solution of 0.290g ($5.82\cdot10^{-4}$mol) of **7** in 50mL of toluene. The orange solution was stirred for 1h at room temperature. The product was precipitated by the addition of n-hexane (50 mL), separated by filtration, washed with n-hexane, and dried under vacuum. 0.300g, yield = 80%.

MS: m/z 498.1 [Ir(Cp°Py)(COD)]

^{1}H NMR(($CDCl_3$) δ: 8.53 (d, 1H, CH a); 7.95 (t,1H, CH c); 7.84 (d; 1H, CH d); 7.33 (t, 1H, CH b); 5.22 (m, 4H, CH COD); 2.51 (s, 6H, CH_3 g-h); 2.18 (s, 6H, CH_3 f-i) ppm.

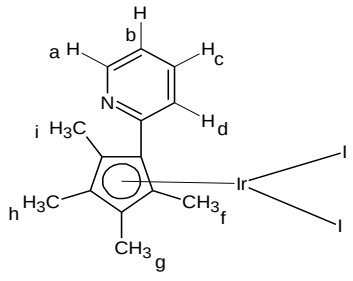


Chart 8. Naming of hydrogen atoms of 8 for ^{1}H assignments.

[71] G.Kohl, H.Pritkow, M.Enders, *Eur.J. Inorg. Chem*., **2008**, 4230-4235.

[η^5–2,3,4,5-tetramethylcyclopentadienyl]-1-pyridine bipyridyl Iridium(III)][PF_6]$_2$

0.075g ($2.92\cdot10^{-2}$mol) of $AgCF_3SO_3$ was added to a solution of **8** (0.100g, $1.55\cdot10^{-4}$mol) in CH_2Cl_2 and stirred at room temperature overnight. The solution was filtered and centrifuged. 0.024g ($1.53\cdot10^{-4}$mol) of 2,2-bipyridine was added to the yellow solution in CH_2Cl_2 and stirred for 3h. The solvent was evaporated and the solid was dissolved in methanol. 0.239g ($1.42\cdot10^{-3}$mol) of NH_4PF_6 was added. The pale yellow precipitate was filtered and stirred overnight in Et_2O, , then filtered again and dried (0.147g).

MS: m/z 498.1 [Ir(Cp°Py)(COD)]

[η^5–2,3,4,5-tetramethylcyclopentadienyl]-1-pyridine phenylpyridyl Iridium(III)][PF_6]

0.075g ($2.92\cdot10^{-2}$mol) of $AgCF_3SO_3$ was added to a solution of **8** (0.100g, $1.55\cdot10^{-4}$mol) in CH_2Cl_2 and stirred at room temperature overnight. The solution was filtered and centrifuged. 0.072g ($8.78\cdot10^{-4}$mol) of CH_3COONa was added to the pale yellow solution and then 0.023g ($1.49\cdot10^{-4}$mol) of 2-phenylpyridine was added and the solution was stirred for 2h at room temperature. The solution was filtrated and dissolved in methanol. 0.239g ($1.42\cdot10^{-2}$mol) of NH_4PF_6 was added. The white precipitate was filtered and stirred in Et_2O overnight, then filtered again and dried (0.234 g).

MS: m/z 498.1 [Ir(Cp°Py)(COD)]

4.4.3 4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)phenol compounds. (Cp°phenol)[72]

1-bromo-4-[(2-methoxypropan-2-yl)oxy]benzene

A 100mL flask was covered with aluminium foil in order to protect the contents from the light. The flask was then charged with 3mL ($3.13\cdot10^{-2}$mol) of 2-metoxypropene and 2.630g ($1.52\cdot10^{-2}$mol) of *p*-bromophenol. A drop of $POCl_3$ was added and the mixture was allowed to stir at room temperature for 1h. The reaction was quenched with five drops of NEt_3. The solvent was evaporated in vacuum and a colourless oil was obtained.

[72] G.Gibson, D.Bern, S.Falloon, T.Hitchens, J.Cortopassi, *organometallics,* **1992**, 11, 1742-1749.

4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)phenol 9

The colourless oil was dissolved in Et_2O. The mixture was cooled to −70°C with cooling bath of CH_3CH_2OH-$N_{2(l)}$. 6mL ($1.50 \cdot 10^{-2}$mol) of n-Butyllithium 2.5M was added dropwise. The solution was stirred for 1.30h and then was allowed to warm to room temperature. The solution was cooled down to −70°C and 2.30mL ($1.52 \cdot 10^{-2}$mol) of 2,3,4,5-tetramethylcyclopenten-2-one was added dropwise. The yellow solution was stirred overnight. The reaction was quenched with 10 mL of 10% HCl_{aq}. The yellow mixture was stirred for 1 h and then extracted with Et_2O. The ether extracts were first washed with $NaHCO_3$ and then water and finally were dried over $MgSO_4$. Removal of the ether in vacuum afforded a dark brown oil. Yield: 3.127g (96%).

^{1}H NMR ($CDCl_3$) δ: 7.32 (d, 1H, CH c); 7.14 (d, 1H, CH b); 6.92(d, 1H, CH d); 6.82 (d, 1H, CH a); 3.60; 3.45; 3.16 (q, 1H, CH f); 2.03 (s,3H, CH_3 i); 1,96 (s, 3H, CH_3 h); 1.89 (s, 3H, CH_3 e); 0.93 (d, 3H, CH_3 g) ppm.

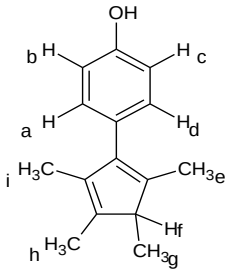


Chart 9. Naming of hydrogen atoms of 9 for ^{1}H assignments.

1-[(2-methoxypropan-2-yl)oxy]-4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)benzene 10

3mL ($3.13 \cdot 10^{-2}$mol) of 2-metoxypropene was added to **9**. After the addition of a drop of $POCl_3$, the solution was allowed to stir at room temperature for 1h. The reaction was then quenched with five drops of NEt_3. Evaporation of the solvent in vacuum afforded an orange-brown oil, which was purified. Chromatography (basic alumina): Et_2O. Yield: 3.637g (83%)

NMR ^{1}H($CDCl_3$) δ:7.31(d, 1H, CH c); 7.12 (d, 1H, CH b); 6.88(d, 1H, CH d); 6.79 (d, 1H, CH a); 3.52 (s, 3H, OCH_3 n); 3.15 (q, 1H, CH f); 2.01(s, 3H, CH_3),;1.94(s, 3H, CH_3),; 1.87(s, 3H, CH_3 ; 1.37 (s, 6H, CH_3 l-m); 0.96 (d, 3H, CH_3 g) ppm.

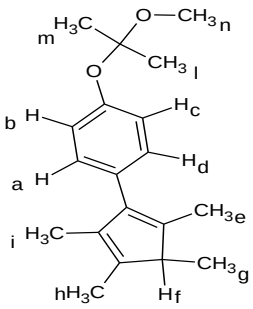


Chart 10. Naming of hydrogen atoms of 10 for ^{1}H assignments.

Bis(4-(2,3,4,5-tetramethylcyclopenta-1,4-dien-1-yl)phenol) diiridium (III)tetrachloride. 11

A solution of 0.400g ($1.40\cdot10^{-3}$mol) of **10** and 0.300g ($1.00\cdot10^{-3}$mol) $IrCl_3 \cdot 3H_2O$ in MeOH was heated under reflux for 72h. The reaction mixture was allowed to cool to room temperature. The solvent was evaporated and the brown oil was purified. Chromatography (silica gel). Four different fractions were collected. Elution with Et_2O:Acetone 1:1 afforded the product as an orange oil. Yield: 0.508g (64%).

IR (cm^{-1}): 819s, 912m, 1003s, 1109s, 1174s, 1233m, 1271m, 1376m, 1445m, 1512m, 1611m, 2050w, 2260w, 3146 br.

^{1}H NMR (DMSO-d^{6}) δ: 7.52 (d, 2H, CH b-c); 6.88 (d, 2H, CH a-d) ; 1.67 (s, 6H,CH_3 g-h); 1.60 (s, 6H, CH_3 e-i) ppm.

^{13}C NMR (DMSO-d^{6}): 157.2 (C α); 131.3 (C δ); 119.7 (C ε); 115.8 (Cβ); 101.5 (C γ); 89.9 (C ζ); 86.9 (C η); 9.8 (CH_3 g-h) ; 8.7 (CH_3 e-i).

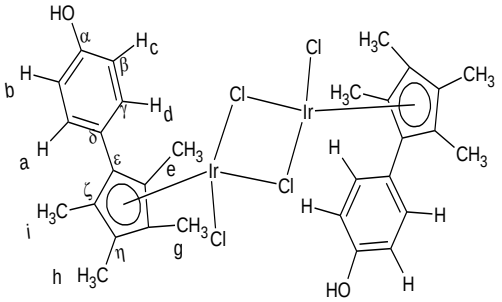


Chart 11 Naming of hydrogen and carbon atoms of 11 for ^{1}H and ^{13}C assignments.