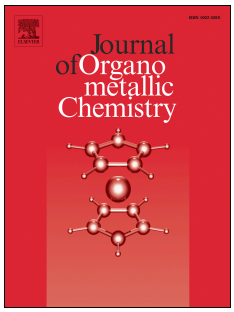
# Accepted Manuscript




Hydroxyl and amino functionalized cyclometalated Ir(III) complexes: synthesis, characterization and cytotoxicity studies

Zhaozhen Wu, Juanjuan Mu, Qiong Wang, Xing Chen, Lasse Jensen, Changqing Yi, Mei-Jin Li

PII: S0022-328X(15)30005-X

DOI: 10.1016/j.jorganchem.2015.05.035

Reference: JOM 19076

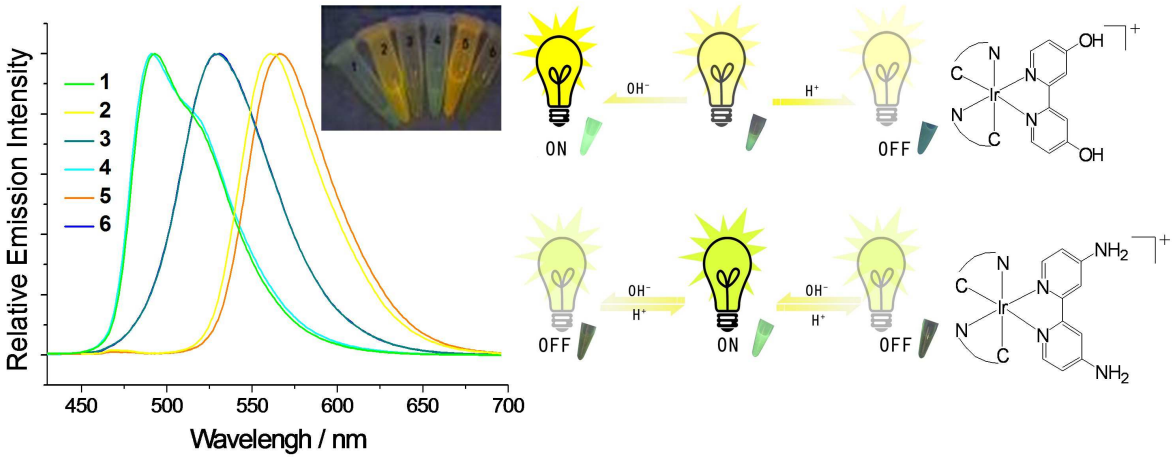
To appear in: *Journal of Organometallic Chemistry*

Received Date: 23 September 2014

Revised Date: 12 May 2015

Accepted Date: 23 May 2015

Please cite this article as: Z. Wu, J. Mu, Q. Wang, X. Chen, L. Jensen, C. Yi M.-J. Li Hydroxyl and amino functionalized cyclometalated Ir(III) complexes: synthesis, characterization and cytotoxicity studies, Journal of Organometallic Chemistry (2015), doi: 10.1016/j.jorganchem.2015.05.035.



# Hydroxyl and amino functionalized cyclometalated Ir(III) complexes: synthesis, characterization and cytotoxicity studies

**Zhaozhen Wu,[a] Juanjuan Mu,[a] Qiong Wang,[a] Xing Chen,[c] Lasse Jensen,[c] Changqing Yi,[b,*] Mei-Jin Li[a,*]**

[a] *Key Laboratory of Analysis and Detection Technology for Food Safety, Ministry of Education, Department of Chemistry, Fuzhou University, Fuzhou 350108, China*
[b] *Key Laboratory of Sensing Technology and Biomedical Instruments (Guangdong Province), School of Engineering, Sun Yat-Sen University, Guangzhou 510006, P.R.China*
[c] *Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, 16802, United States.*

**Abstract**

A series of Ir(III) complexes $(C\hat{}N)_2Ir(N\hat{}N)$ (N^N are 4,4'-dihydroxy-2,2'-bipyridine and 4,4'-diamino-2,2'-bipyridine, and C^N are phenylpyridine, benzo[h]quinolone, and 2-phenylquinoline) were synthesized and characterized. Two of the complexes were structurally characterized via X-ray crystallography. The photophysical and photochemical properties of these complexes were studied. Preliminary studies of their applications on pH sensing, and cell imaging were also performed.

**Keywords:** Iridium(III) complex / Synthesis / pH sensing / Cytotoxicity

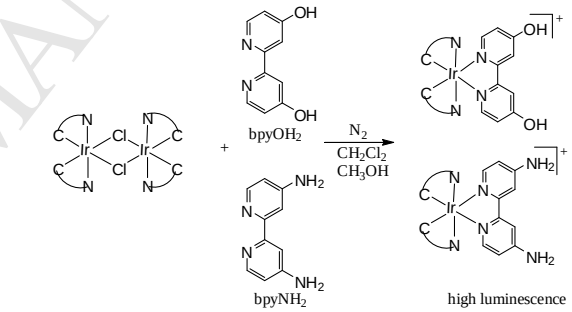
## 1. Introduction

Ir(III) complexes as luminescent materials have drawn considerable research attention because of their relatively long excited-state lifetime, high photoluminescence efficiency, and excellent color tuning.[1] In particular, tuning of the luminescence strength and wavelength of the complexes has been extensively studied.[2] The advantages of iridium(III) complexes make them potentially useful in applications such as photosensitizers, molecular sensing,[3] photocatalysis and organic light-emitting diodes (OLEDs).[4] Metal complexes with simple ligands show various electronic properties depending on the proton transfer reaction of the ligand.[5] Ligands such as 2,2'-bipyridinyl-4,4'-diol,[5a,6] 1,10-phenanthroline-4,7-diol,[7] and amino terpyridine ( tpy-φ-$NH_2$) [8] have been used in pH sensing studies due to their remarkable deprotonation ability. Based on the acid-base equilibrium of the phenolic hydroxyl and amino groups of the N^N ligands, the amino or hydroxyl functionalized Ir(III) complexes were expected to be potential pH sensors. In this paper, we report the synthesis, and characterization of a series of Ir(III) complexes containing 2,2'-bipyridinyl-4,4'-diol or 2,2'-bipyridinyl-4,4'-diamine. Preliminary studies of their applications on pH sensing, and cell imaging were also performed.

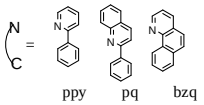
## 2.Results and discussion

### *2.1. Synthesis and characterization*

4,4'-Dihydroxy-2,2'-dipyridine ($bpy(OH)_2$) and 4,4'-diamine-2,2'-dipyridine ($bpy(NH_2)_2$) were synthesized according to the methods in the literature with slight modifications. Dichloro-bridged dimers $[Ir(C\hat{}N)_2\text{-}\mu\text{-}Cl]_2$ were conveniently prepared from a reaction of the appropriate ligand and $IrCl_3 \cdot xH_2O$.[9] The functionalized pyridine ligand was then reacted with $[Ir(C\hat{}N)_2\text{-}\mu\text{-}Cl]_2$ to provide the target complexes **1** to **6** (Scheme 1) as yellow or orange-yellow solids. The complexes were easily purified by crystallization to provide pure crystals. The identities of the complexes were confirmed by NMR spectroscopy, elemental analysis, and ESI mass spectrometry.




| complex | C^N | N^N |
|---|---|---|
| **1** | ppy | $bpy(OH)_2$ |
| **2** | pq | $bpy(OH)_2$ |
| **3** | bzq | $bpy(OH)_2$ |
| **4** | ppy | $bpy(NH_2)_2$ |
| **5** | pq | $bpy(NH_2)_2$ |
| **6** | bzq | $bpy(NH_2)_2$ |



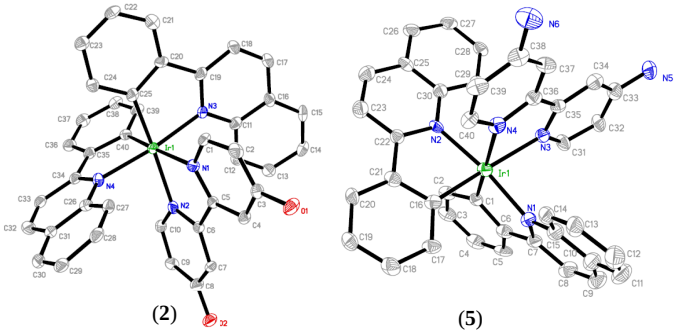

**Scheme 1**. Synthesis of the iridium(III) complexes.

### *2.2. Crystal structure determination*

The solid-state structures of **2** and **5** were determined by X-ray crystallography and their ORTEP illustrations are shown in Fig. 1. Selected bond distances and angles are listed in Table 1. The Ir(III) center adopts a distorted-octahedral geometry, as indicated by the crystal structures, with angles subtended by the N atoms of the N^N ligand at the Ir(III) center of 75.17° and 74.4° for complexes **2** and **5**, respectively. These angles are significantly smaller than the ideal angle of 90° adopted in octahedral geometry. Ir(III) is coordinated by two cyclometalated ligands pq and the dihydroxyl or amino functionalized bipyridine moiety. Selected bond lengths and angles for the molecules of complexes **2** and **5** are listed in Table 2. The bond distances of Ir-C(pq) were 1.993(6) and 2.003(5) Å for complex **2**, and 1.999(7) and 2.002(8) Å for complex **5**. The bond distances of Ir-N(pq) were 2.093(5) and 2.113(5) Å for complex **2**, and 2.060(6) and 2.089(6) Å for complex **5**, similar to those found in cyclometalated Ir(III) complexes.[10] All atoms of the modified bpy ligand, including the

* Corresponding authors: Fax: (+)86 591 22866135 (M. J. Li)
*E-mail addresses*: mjli@fzu.edu.cn (M. J. Li), yichq@mail.sysu.edu.cn (C. Q. Yi)

hydroxyl or amino moieties, are essentially coplanar, as indicated by the crystal structures.



(2) (5)

**Fig. 1.** ORTEP diagram of the complexes **2** and **5** with atom labeling scheme showing 30% thermal ellipsoids and the H atoms removed for clarity.

**Table 1**. Selected bond distances (Å) and angles (°) of complexes **2** and **5**.

| | | | |
|---|---|---|---|
| Ir(1)-C(25) | 1.993(6) | Ir(1)-C(16) | 1.999(7) |
| Ir(1)-C(40) | 2.003(5) | Ir(1)-C(1) | 2.002(8) |
| Ir(1)-N(3) | 2.093(5) | Ir(1)-N(1) | 2.060(6) |
| Ir(1)-N(4) | 2.113(5) | Ir(1)-N(2) | 2.089(6) |
| Ir(1)-N(1) | 2.143(4) | Ir(1)-N(4) | 2.162(6) |
| Ir(1)-N(2) | 2.160(5) | Ir(1)-N(3) | 2.167(6) |
| O(1)-C(3) | 1.344(7) | N(5)-C(33) | 1.365(9) |
| O(2)-C(8) | 1.335(7) | N6-C(38) | 1.364(9) |
| C(25)-Ir(1)-N(3) | 80.4(2) | C(1)-Ir(1)-N(1) | 80.6(3) |
| C(40)-Ir(1)-N(4) | 80.4(2) | C(16)-Ir(1)-N(2) | 80.1(3) |
| N(1)-Ir(1)-N(2) | 75.17(18) | N(4)-Ir(1)-N(3) | 74.4(2) |

*2.3. Absorption and photoluminescence properties of the Ir(III) complexes*

Electronic absorption data of the Ir(III) complexes were collected in DMSO (Table 3). The electronic absorption spectra of these complexes were mainly dominated by extremely high-energy absorption bands at ca. 270 to 370 nm with molar extinction coefficients in the order of 104 $dm^3mol^{-1}cm^{-1}$ and comparatively less intense low-energy absorption bands at ca. 420 nm to 448 nm. With reference to previous spectroscopic studies on the related cyclometalated Ir(III) diimine systems,[11] absorption bands with extinction coefficients in the order of $10^4$ $dm^3mol^{-1}cm^{-1}$ were ascribed to spin-allowed π-π* intraligand ($^1$IL) transitions of the cyclometalating ligands or substituted bpy ligands. The less-intense low-energy absorption bands at ca. 420 nm to 448 nm likely originated from dπ→π*(ligand) spin-allowed metal-to-ligand charge-transfer ($^1$MLCT) transitions, similar to those observed in previous studies of related Ir(III) diimine complexes.[12]

The complexes were found to emit strongly at room temperature upon excitation with both UV and visible light in DMSO solution with emission maxima ranging from 490 to 566 nm. Images of their luminescence under a UV lamp of 365 nm are shown in Fig. 2a, where the emission light is observably tuned from green to yellow. With reference to previous spectroscopic studies on other related cyclometalated Ir(III) diimine systems,[11a,11b,12a,12b,13] the strong emission was tentatively assigned to a spin-forbidden dπ(Ir)→π*(ligand) metal-to-ligand charge-transfer ($^3$MLCT) excited state, probably with some mixing of a spin-forbidden π→π* intraligand ($^3$IL) character. Photophysical data of the complexes in DMSO at room temperature are presented in Table 2. The emission lifetimes of complexes 1, 2, 3, 4, 5, and 6 are 180, 240, 470, 130, 240, and 270 ns, respectively, and their emission quantum yields in aerated DMSO are 15.0%, 29.0%, 3.0%, 16.0%, 23.0%, and 3.0%, respectively.

**Table 2**. Photophysical data of the new Ir(III) complexes.

| Complex | Solvent | $\lambda_{ab}$/nm (ε/$10^4$ $dm^3mol^{-1}cm^{-1}$) | $\lambda_{em}$/nm ($\tau_o$/μs) | $\varphi_{em}$ |
|---|---|---|---|---|
| 1 | DMSO | 271(2.39), 370(0.38) | 491 (0.18) | 0.15 |
| | $CH_3OH$ | | 489 | |
| | $CHCl_3$ | | 489 | |
| | glass | | 474 | |
| 2 | DMSO | 272(3.75),338(1.63), 445(0.32) | 560 (0.24) | 0.29 |
| | $CH_3OH$ | | 558 | |
| | $CHCl_3$ | | 561 | |
| | glass | | 547 | |
| 3 | DMSO | 273(4.00),331(1.47), 420(0.48) | 530 (0.47) | 0.03 |
| | CH3OH | | 523 | |
| | CHCl3 | | 521 | |
| | glass | | 500 | |
| 4 | DMSO | 270(5.77),350(0.98), 420(0.26) | 490 (0.13) | 0.16 |
| | $CH_3OH$ | | 489 | |
| | $CHCl_3$ | | 488 | |
| | glass | | 476 | |
| 5 | DMSO | 272(1.60),340(0.56), 448(0.21) | 566 (0.24) | 0.23 |
| | $CH_3OH$ | | 563 | |
| | $CHCl_3$ | | 561 | |
| | glass | | 552 | |
| 6 | DMSO | 275(8.25),344(2.92), 425(0.34) | 530 (0.27) | 0.03 |
| | $CH_3OH$ | | 523 | |
| | $CHCl_3$ | | 521 | |
| | glass | | 503 | |

The low-temperature photoluminescence spectra of all complexes in ethanol-methanol (4:1, v:v) glass were studied and shown in Fig. 2b. A blue-shift of emission maxima and vibronic structures in the emission bands from fluid solution (MeOH) at room temperature to rigid matrix at 77 K were observed. The blue shifts are about 350, 600 and 880 $cm^{-1}$ for pq (**2** and **5**), ppy (**1** and **3**) and bzq (**3** and **6**) complexes, respectively. Compared with the emission maxima at room temperature, a small blue-shift of the emission maxima of **2** and **5** at 77 K could be observed, which shows the excited-state properties of pq complexes are different from those of ppy and bzq complexes. The photoluminescence spectra of all complexes in different solvents were also investigated and the data are summarized in Table 2. The small dependence on the solvent polarity was observed, especially for pq complexes. This finding reflects that the emission of pq complexes originates from an excited state of predominantly $^3$IL (π→π*) (pq) character with some contribution of charge-transfer character.[14] While for ppy and bzq complexes, according to previous report,[14,15] the more sensitivity to the temperature of photoluminescence spectra shows the excited states for these complexes are mainly attributed to the $^3$MLCT states and the contribution from $^3$LC is relatively small. Gudel et al.[16] have reported the strong mixing between the MLCT and $^3$LC excited states in the similar cationic iridium complex $[Ir(ppy)_2bpy]^+$.

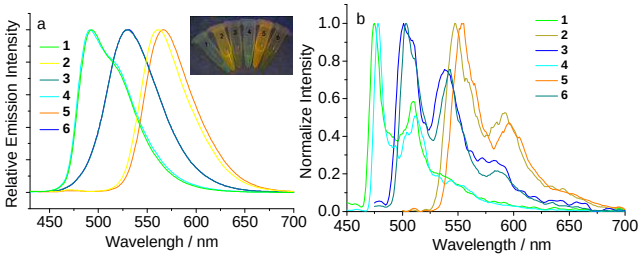



**Fig. 2.** Emission spectra and luminescent photograph of the Ir(III) complexes in DMSO (a); Emission spectra of the complexes in EtOH/MeOH (4:1, v/v) at 77 K (b).

*2.4. Photophysical properties of the complexes in acid/base solutions*

The complexes **1** and **4** were chosen for the preliminary studies of the effect of acid and base on the photophysical properties. No obvious changes were observed in the UV-vis spectra obtained upon addition of an aqueous solution of HCl to complexes **1** and **4** in DMSO/$H_2O$ (1:1, v/v). Upon addition of an aqueous solution of NaOH to the dihydroxyl complex **1**, the absorption intensity at ca. 271 nm increased obviously. No obvious changes were observed in the UV-vis spectra obtained upon addition of NaOH to complex **4**.

Fig. 3 shows luminescent changes of Ir(III) complex **1** upon addition of HCl and NaOH solutions. Upon addition of HCl solution to a DMSO/$H_2O$ (1:1, v/v) solution of Ir(III) complex **1**, the luminescence of the complex decreased in intensity at 492 nm with a reduction of ca. 99% in strongly acidic solution (pH<2), and a weak band appeared at around 550 nm. The lifetime is about 0.11μs in the acidic solution (pH=3.0). The luminescence intensity of complex **1** was quenched upon addition of HCl solution, which may be ascribed to protonation of the dihydroxy ligand $bpy(OH)_2$.[17] While the emission intensity of complex **1** was strongly enhanced over a range of pH from nearly neutral to highly alkaline with a small red shift of ca. 9 nm (Fig. 3) as a result of the strong influence of the two OH groups in the 4,4'-dihydroxyl-2,2'-bipyridine ligand of these complexes.[18] In the basic solution (pH=12), the lifetime is about 0.61 μs, which is longer than that in the acidic solution, probaly due to the changes of the excited state.

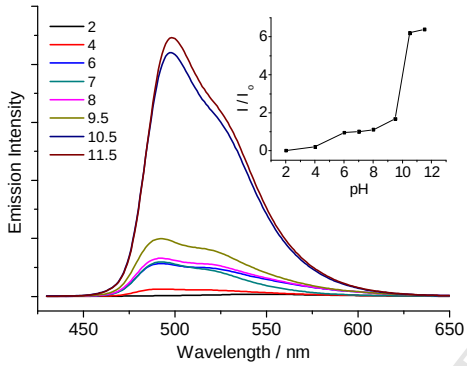



**Fig. 3.** Changes in the luminescence of Ir(III) complex **1** (6 μM) in a solution of DMSO and $H_2O$ (1:1, v/v) upon addition of various amounts of HCl or NaOH. Inset shows the changes in luminescence vs. pH.

Upon addition of HCl (0.1 M) to a solution of complex **4** (6 μM), the pH ranged from approximately 7 to 2 and the luminescence intensity was quenched with a reduction of about 95% (Fig. 4). The electron-donating $NH_2$ group becomes an electron-withdrawing ($NH_3^+$) group upon protonation, which quenches the emission intensity of the complexes because of the electron transfer effect.[19] Interestingly, upon addition of NaOH, the luminescence of complex **4** was also quenched with a reduction of approximately 96% (Fig. 4). These results differ from those observed in complex 1. The emission intensity of the amino functionalized Ir(III) complexes decreased in basic solution, probably due to the partial quinoid character resulted from delocalization of the negative charge on the aniline anion at high pH.[18,19] To differentiate the same emission intensity at different pHs, the emission lifetimes of complex 4 at pH=3 and 11 were investigated, and the lifetime is determined to be 0.60 and 0.35 μs, respectively**.** The results suggested that the energy gap might be affected by the pH, leading to the defferent lifetime in the acidic or basic solution.

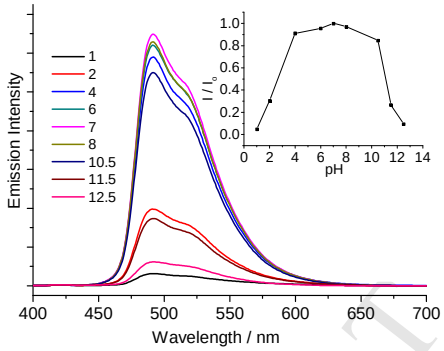



**Fig. 4.** Changes in the luminescence of Ir(III) complex **4** (6 μM) in a mixture solution of DMSO/$H_2O$ (1:1, v/v) upon addition of various amounts of NaOH or HCl. Inset shows the changes in luminescence vs. pH.

*2.5. The reversibility of the complex in acid/base solutions*

Fig. 5 illustrates the reversibility of complex **1** upon alternate addition of NaOH and HCl solution (pH 4.5 to pH 9.5) for six cycles. The luminescence intensity was enhanced after addition of NaOH, and the luminescence was turned off after addition of HCl, as shown in Fig. 5. The emission was then turned on again in basic solution. This on-off process was reversible, and the complex was extremely stable in acid/base solutions. Such results further demonstrate that the change in luminescence could be attributed to the electron-donating effect brought by the protonation and deprotonation of the dihydroxyl group. It is difficult to estimate the pKa of the ground state of the complexes, because of the tiny changes in the electronic absorption spectra in acidic/basic solutions. But multiple protonation-deprotonation equilibriums of complex **1** are clearly evident in Fig.3, the plots of the relative emission intensity vs. pH have two inflection points near pH 4.5 and 10.5, owing to the stepwise deprotonation of two hydroxyl groups.

The reversibility of complex **4** upon alternate addition of NaOH and HCl was also studied. The luminescence intensity of a solution of complex 4 was turned off upon addition of NaOH solution and recovered upon addition of HCl solution. The process was reversible and the complex remained stable after several cycles. These results indicate that amino functionalized Ir(III) complexes are fully and reversibly pH-controlled "off-on-off" luminescent signaling systems. From pH 4 to pH 11, the luminescence was almost constant. At around pH 0 to pH 4 and pH 11 to pH 14, the luminescence of the complexes was gradually switched off.

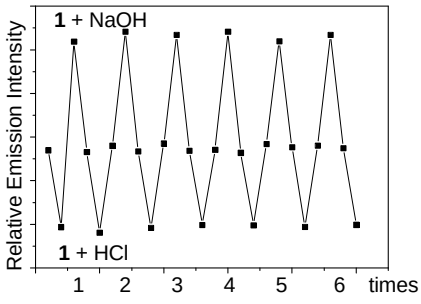



**Fig. 5**. Luminescent changes of Ir(III) complex **1** (6 μM) in a mixture solution of DMSO/$H_2O$ (1:1, v/v) upon alternate addition of NaOH and HCl for six cycles.

*2.6. Cytotoxicity and imaging studies*

Cell viability upon treatment with the new Ir(III) complexes was determined by testing mitochondrial enzyme functions according to the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Fig. 6 presents the viability of HepG2 cells after incubation with complexes **1** to **6** for 24 h in DMSO/PBS. Approximately >70% of the HepG2 cells remained alive after incubation for 24 h with complexes **2**, **4**, and **6** at concentrations lower than 25 μM, which reveals that the toxicities of these complexes are acceptable. By contrast, high toxicity to HepG2 cells was found with complexes **1** and **5** even at extremely low concentrations. In particular, complex **3** showed a significantly positive effect on cell proliferation without an obvious dose-dependent tendency. It is a usual phenomena for some complexes that show proliferative effects at low concentrations while showing cytotoxicity toward cells at high concentrations.[20] The exact molecular mechanism of cytotoxicity induced by Ir(III) complexes is still unclear. In general, the higher lipophilicity would result in the higher cytotoxicity of the complexes. It has been reported that the strong DNA binding,[21] the dissociation of the complexes,[22] or generation of intracellular ROS[23] should be responsible for the cytotoxicity induced by metal complexes.

The interaction of living cells with emissive iridiumIII) complexes **1** and **4** was also investigated by fluorescence microscopy. HepG2 cells were incubated with each complex in DMSO/PBS (pH 7.4, 99:1, v/v) for 1h at 37℃. Fig S2(Supporting information) shows the images of HepG2 cells incubated with the complex 1 at 50 μM and complex **4** at a concentration of 25 μM. Bright-field measurements after treatment with the Ir(III) complexes showed that the cells were visible throughout the imaging experiments. Interestingly, the complex **4** displayed intense intracellular luminescence, and the luminescence and bright-field images showed that the luminescence was evident in cytoplasm, not in the nucleus and membrane; whereas the complex **1** displayed weak or scattered luminescence in membrane and dead cell. These observations suggest that the complex **1** was hardly internalized into the living cells, and the complex **4** did better. The results were basically coincided with those of MTT cytotoxicity studies. It could be concluded that the hydrophilic and steric hindrance of the complexes collectively decided on the abilities of passing cell membrane.

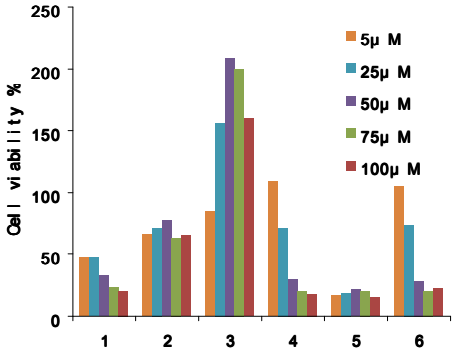



Fig. 6. Viability of HepG2 cells after incubation with complexes **1** to **6** in DMSO/PBS (pH 7.4, 99:1, v/v) for 24 h.

*2.7 Calculations*

Compared with the geometry parameters selected from the crystal structures, the equilibrium geometries optimized at B3LYP/6-31G*/LANL2DZ level provide the reliable descriptions in theory (Table S1). Therefore, we used the optimized $S_0$ geometry for the subsequent optical property investigations. As show in Table S2, the TDDFT calculations predict that two absorption bands are dominant in the high energy region with the wavelength from 400 to 200 nm, which well agree with the experimental results. The excitations are ascribed to metal-to-ligand charge transfer (MLCT) as shown in Fig. S3, which schematically depicts the molecular orbitals involved in the electron transitions.

As proposed by previous works, the emission energies of the iridium(III) complexes calculated by TDDFT from the $S_0$ geometries are sufficient to reproduce the experimental results.[24] We computed the $S_0$-$T_1$ vertical transition energies based on the optimized $S_0$ geometry excluding the solvent effect in consideration of the computational cost. The spin-orbit coupling leads to the spin-forbidden transition allowed, as the triplet manifold mixes with higher-energy singlets. The $S_0$-$T_1$ transition energies with non-neglected oscillator strength are shown in Table S3. The theoretically predicted emission energies deviates from the experimental values. It may arise from the instinct defect of B3LYP functional for the lack of long-range correction and the absence of solvent effects. The frontier molecular orbitals make the dominant contributions to the $S_0$-$T_1$ transition. As shown in Fig 7 (Fig. S4) and Table 4, the HOMOs are featured by the d orbital of iridium(III) in combination with orbital delocalized on the conjugated (C^N) ligands, and the LUMOs delocalized on the (N^N) ligands. It is evident that the singlet-triplet vertical transition has the mixed intraligand π–π* and MLCT character.

We further investigated the pH-dependent photophysical properties of complexes **1** and **4**. Two extreme cases were considered: the totally protonated species and alkalized species. The $T_1$ geometries of two protonated species and two alkalized species were optimized, and the energy difference between $S_0$ and $T_1$ is calculated based on the optimized $T_1$ geometry. For the protonated complexes **1** and **4**, the energy gap is only 0.05 and 0.14 eV, respectively. For the alkalized complex 1 whose energy gap is 2.10 eV matching the experimental value measured in high pH solution. The excitation is assigned to $\pi$–$\pi^*$ character (Table S4). While for the alkalized complex **4**, the $T_1$ is more stable than $S_0$. That means the phosphorescence signal is beyond the measured wavelength. Moreover, according to the Einstein theory of spontaneous emission, the radiative rate constant is proportional to the emission energy and transition dipole moment. As the nearly identical transition dipole moment for the Ir(III) complex in different pH solution, the tiny energy gap between $S_0$ and $T_1$ leads to the fast radiative rate. Accordingly, the nonradiative rate becomes enhanced and the nonradiative deactivation channel is facilitated.

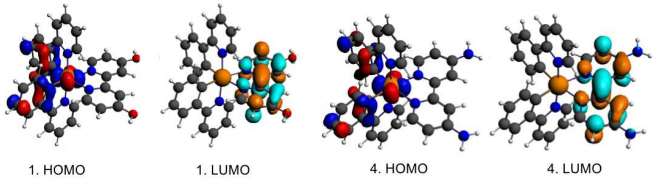



Fig .7. Calculated HOMOs and LUMOs involved in the singlet-triplet electron transitions of complexes **1** and **4**

## 3. Conclusions

A series of cyclometalated Ir(III) diimine complexes were successfully synthesized and characterized. The luminescence of the complexes containing hydroxyl groups could be switched off/on upon addition of acid and base, whereas the luminescence of the complexes functionalized with amino groups could be switched off/on/off upon addition of acid and base as a result of the protonation or deprotonation of amino and hydroxyl groups. The

results show that the complexes could be potentially used for the luminescence pH sensors. The cytotoxicity studies demonstrated that the toxicities of the complexes **2**, **4** and **6** were acceptable, and they have promising potential as specific cell-imaging agents.

## 4. Experimental Section

*4.1. Reagents*

All reagents were used as received from commercial sources. Solvents were purified to deoxygenization by dry nitrogen. Other solid or liquid reagents received from commercial sources were used without further purification. 4,4'-Diamine-2,2'-dipyridine was synthesized based on the method provided in the literature.[25] The ligand 4,4'-dihydroxy-2,2'-dipyridine ($bpy(OH)_2$) was synthesized according to the literature with slight modifications.[26] Cyclometalated Ir(III) chloride-bridged dimer $[(C\text{^}N)_2IrCl]_2$ was synthesized according to the literature.[1a,27]

*4.2. Physical Measurements and Instrumentation*

Physical measurements and instrumentation, cell culture and cell viability assay were the same to the previous report.[28]

*4.3. Synthesis*

A solution of $[(C\text{^}N)_2IrCl]_2$ (20 mg, 0.018 mmol) in oxygen-free $CH_2Cl_2/CH_3OH$ (10 mL; 1:1 v/v) was stirred under nitrogen, after which an excess of $bpy(OH)_2$ or $bpy(NH_2)_2$ (0.041 mmol) was added to it. The mixture was heated to reflux for 12 h to provide a fluorescent solution. Subsequently, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel.

*4.3.1. [Ir(ppy)$_2$[bpy(OH)$_2$]]Cl (**1**)*

The residue was purified by column chromatography on silica gel using a mixture of $CH_2Cl_2$ and methanol (20:1, v/v) as the eluent to afford the pure compound as a yellow solid with 75% yield. $^1$H NMR(400 MHz, $d_6$-DMSO), $\delta$ (ppm): 8.20 (d, 2H, $J$ = 8.2 Hz), 7.90 (t, 2H, $J$ = 8.2 Hz ), 7.84 ( d, 2H, $J$ = 7.8 Hz), 7.71 (d ,2H, $J$ = 5.8 Hz), 7.38 (s, 2H) , 7.20 (m, 4H), 6.94 (t, 2H, $J$ = 7.5 Hz ), 6.82 (t, 2H, $J$ = 7.4 Hz), 6.49 ( b, 2H), 6.18 (d, 2H, $J$ = 7.4 Hz). Positive Q-TOF HRMS: *m/z* found 689.1609 (calcd 689.1529) for $C_{32}H_{24}IrN_4O_2$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{32}H_{24}ClIrN_4O_2\cdot 2H_2O$: C 50.55, H 3.71, N 7.37. Found: C 50.65, H 3.62, N 7.60. $^{13}$CNMR($d_6$-DMSO), $\delta$ (ppm): 112.6, 116.2, 120.3, 122.3, 124.2, 125.4, 130.5, 131.6, 138.9, 144.4, 149.1, 151.0, 151.8, 157.4, 167.0, 167.6. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3423, 1616, 1553, 1471, 1268, 1024, 829, 764.

*4.3.2. [Ir(pq)$_2$[bpy(OH)$_2$]]Cl (**2**)*

The product was recrystallized from dichloromethane-hexane to provide red crystals with 99% yield. $^1$H NMR(400 MHz, $d_6$-DMSO) , $\delta$ (ppm): 12.0 (b, 2H) 8.53 (s, 4H), 8.24 (d, 2H, $J$ = 7.6 Hz ), 7.95 ( d, 2H, $J$ = 7.2 Hz), 7.77 (d ,2H, $J$ = 5.6 Hz), 7.56 (s, 2H) , 7.42 (m, 4H,), 7.13 (m, 4H), 7.00 (d, 2H, $J$ = 6.4 Hz), 6.77 ( t, 2H, $J$ = 7.0 Hz), 6.39 (d, 2H, $J$ =7.5 Hz). Positive Q-TOF HRMS: *m/z* found 789.1944 (calcd 789.1842) for $C_{40}H_{28}IrN_4O_2$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{40}H_{28}ClIrN_4O_2\cdot 4H_2O$: C53.59, H 4.05, N 6.25. Found: C 53.45, H 3.88, N 5.99. $^{13}$CNMR($d_6$-DMSO), $\delta$ (ppm): 110.6, 114.8, 117.3, 122.2, 125.0, 126.8, 127.3, 128.1, 128.8, 130.2, 130.3, 134.2, 139.6, 146.0, 147.2, 148.3, 151.8, 157.4, 166.9, 170.5. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3411, 2371, 1615, 1560, 1516, 1451, 1399, 1337, 1260, 1016, 826, 761.

*4.3.3. [Ir(bzq)$_2$[bpy(OH)$_2$]]Cl (**3**)*

The product was isolated as an orange solid with 90% yield. $^1$H NMR(400 MHz, $d_6$-DMSO) , $\delta$(ppm): 12.0 （b, 2H）, 8.56(d, 2H, $J$ = 7.1Hz), 8.12 (d, 2H, $J$ =5.4 Hz ), 8.02 ( d, 2H, $J$=2.5 Hz), 7.9 (m ,4H), 7.64 (m, 2H) , 7.49 (t, 4H, $J$ = 7.6 Hz), 7.13 (t, 2H, $J$ = 7.6 Hz), 6.94 (m, 2H), 6.19 ( d, 2H,$J$ = 7.2 Hz). Positive Q-TOF HRMS: *m/z* found 737.2121 (calcd 737.1529) for $C_{36}H_{24}IrN_4O_2$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{36}H_{24}ClIrN_4O_2\cdot H_2O$: C 52.33, H 3.66, N 6.78. Found：C 52.23, H 3.45, N 6.78. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3414, 2366, 1618, 1495, 1448, 1330, 1029, 835, 720, 620. The signal was very weak in $^{13}$CNMR spectrum due to the poor solubility of the complex.

*4.3.4. [Ir(ppy)$_2$[bpy(NH$_2$)$_2$]]Cl (**4**)*

The product was isolated as a yellow solid with 70% yield. $^1$H NMR(400 MHz, $d_6$-DMSO), $\delta$ (ppm): 8.22 (d, 2H, $J$ = 8.2 Hz), 7.92 (t, 2H, $J$ = 7.1 Hz ), 7.85 (d, 2H, $J$ = 7.1 Hz), 7.72 (d, 2H, $J$ = 5.2 Hz), 7.32 (s ,2H), 7.24 (t, 2H, $J$ = 6.6 Hz) , 7.17 (d, 2H, $J$ = 6.4 Hz), 7.06 (s, 4H), 6.96 (t, 2H, $J$ = 7.4 Hz ), 6.83 (t, 2H, $J$ = 7.4 Hz), 6.57 ( d, 2H, $J$ = 6.4Hz), 6.17(d, 2H, $J$ = 7.4 Hz). Positive Q-TOF HRMS: *m/z* found 687.1949 (calcd 687.1848) for $C_{32}H_{26}IrN_6$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{32}H_{26}ClIrN_6\cdot 2H_2O$: C 50.69, H 3.99, N 11.08. Found：C 50.99, H 4.04, N 10.78. $^{13}$CNMR($d_6$-DMSO), $\delta$ (ppm): $\delta$ 107.6, 111.9, 120.1, 121.9, 124.0, 125.3, 130.4, 131.7, 138.5, 144.5, 148.8, 149.9, 153.1, 156.0, 156.5, 167.8. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3414, 1622, 1503, 1474, 1265, 1029, 844, 759.

*4.3.5. [Ir(pq)$_2$[bpy(NH$_2$)$_2$]]Cl (**5**)*

The product was isolated as red crystals with 99% yield. $^1$H NMR (400 MHz, $CD_3OD$ ), $\delta$ (ppm): 8.36 (m, 4H), 8.10 (d, 2H, $J$ = 7.7 Hz ), 7.86 (d, 2H, $J$ = 7.2 Hz), 7.70 (d, 2H, $J$ = 9.0 Hz), 7.49 (d ,2H, $J$ = 6.4 Hz), 7.44 (t, 2H, $J$ = 7.4 Hz) , 7.12 (m, 4H), 6.99 (s, 2H), 6.75 (t, 2H, $J$ = 7.0 Hz ), 6.49 (m, 4H). 4.57 (b, 4H). Positive Q-TOF HRMS: *m/z* found 787.2262 (calcd 787.2161) for $C_{40}H_{30}IrN_6$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{40}H_{30}ClIrN_6\cdot 4H_2O$: C 53.71, H 4.28, N 9.50. Found：C 53.80, H 4.10, N 9.85. $^{13}$CNMR($d_6$-DMSO), $\delta$ (ppm): $\delta$ 106.5, 110.9, 117.1, 121.8, 125.5, 126.1, 126.6, 127.8, 128.6, 129.8, 130.1, 134.3, 139.3, 146.2, 146.3, 147.8, 153.2, 156.1, 156.4, 170.4. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3416, 2360, 1624, 1542, 1512, 1449, 1402, 1333, 1265, 1005, 826, 764.

*4.3.6 [Ir(bzq)$_2$[bpy(NH$_2$)$_2$]]Cl (**6**)*

The product was isolated as an orange solid with 90% yield. $^1$H NMR(400 MHz, $d_6$-DMSO) , $\delta$(ppm): 8.55 (d, 2H, $J$ = 7.1 Hz), 8.14 (d, 2H, $J$ = 5.4 Hz ), 7.89 (m, 4H), 7.68 (m, 2H), 7.47 (d ,2H, $J$ = 8.0 Hz), 7.35 (d, 2H, $J$ = 2.3 Hz) , 7.11 (m, 8H), 6.49 (m, 2H), 6.18 (d, 2H, $J$ = 7.1 Hz). Positive Q-TOF HRMS: *m/z* found 735.1951 (calcd 735.1848) for $C_{36}H_{26}IrN_6$ ({M-Cl}$^+$). Anal. Calcd (%) for $C_{36}H_{26}ClIrN_6\cdot 4H_2O$: C 51.33, H 4.07, N 9.98. Found：C 51.88, H 4.01, N 9.93. $^{13}$CNMR($d_6$-DMSO), $\delta$ (ppm): 107.7, 112.0, 120.0, 123.0, 124.5, 127.0, 128.9, 129.9, 130.0, 134.1, 137.4, 141.1, 148.5, 149.5, 149.8, 156.4, 156.5, 157.4. IR(KBr) $\nu_{max}$/cm$^{-1}$ 3417, 2363, 1624, 1503, 1444, 1330, 1095, 829, 724.

*4.4. Crystal Structure Determination*

Single crystals of complexes **2** and **5** were obtained via the liquid phase diffusion method by slow diffusion of n-hexane into dichloromethane/methanol (20/1, v/v) solutions of complexes **2** and **5**. Crystallographic data collections for complexes **2** and **5** were carried out on a beam line 3W1A at the Beijing Synchrotron Radiation Facility with a mounted MarCCD-165 detector using synchrotron radiation ($\lambda$ = 0.7501 Å). Data reduction and numerical absorption correction were applied using HKL2000 software.[29]

The structures were solved by direct methods, and all of the non-hydrogen atoms were refined anisotropically on $F^2$ by the full-matrix least squares technique using the SHELXH-97 crystallographic software package.[30] The asymmetric unit of complex **5** contains four Ir(III) complex molecules and four Cl$^-$ ions. The remainder of the unit is occupied by extreme electron density, which could be identified as free solvent molecules. Given that these guest solvent molecules in the crystal are too many and impossible to refine via conventional discrete-atom models, the SQUEEZE[31] subroutine of the PLATON software suite was

applied to remove the scattering from the highly disordered solvent molecules, and sets of solvent-free diffraction intensities were produced. SQUEEZE analysis showed that the void space was occupied by 49 electrons per formula, corresponding to 4 water molecules per formula unit. The final formula $(C_{40}H_{30}N_6Ir)Cl\cdot(H_2O)_4$ was calculated from the SQUEEZE results and elemental analysis. The crystal parameters are listed in Table 3.

**Table 3.** Crystallographic data for complexes **2** and **5**.

| Complex | **2** | **5** |
|---|---|---|
| Empirical formula | $C_{40}H_{28}ClIrN_4O_2\cdot H_2O\cdot CH_2Cl_2\cdot 0.5C_6H_{14}$ | $C_{40}H30ClIrN6$ |
| Formula weight | 970.34 | 822.35 |
| Space group | P21/n | P2(1)/c |
| a/Å | 14.482(3) | 19.246(2) |
| b/Å | 17.694(4) | 23.9560(10) |
| c/Å | 16.564(3) | 33.275(2) |
| α (°) | 90 | 90 |
| β(°) | 109.04(3) | 104.554(5) |
| γ(°) | 90 | 90 |
| V/Å | 4012.2(14) | 14849.4(19) |
| Z | 4 | 16 |
| ρcalcd/mg cm$^{-3}$ | 1.606 | 1.471 |
| μ/mm$^{-1}$ | 3.573 | 3.704 |
| Radiation, λ/Å | 0.75010 | 0.75010 |
| *T/K* | 203(2) | 194(2) |
| R1($F_o$)[a] | 0.0814 | 0.0494 |
| w$R_2$($F_o^2$)[b] | 0.2757 | 0.0866 |
| GOF | 1.144 | 1.372 |

[a] $R1=\sum| F_o-F_c|/\sum F_o$ [b] $wR2=\sum[w(F_o^2-F_c^2)^2]/\sum[w(F_o^2)^2]^{1/2}$.

*4.5 Cytotoxicity and imaging studies*

MTT assay was used to determine viability of HepG2 cells upon treatment with Ir(III) complexes, as described in detail elsewhere.[32] HepG2 cells were seeded in 96-well tissue culture plates at the density of $4 \times 10^6$ cells per well and incubated 3 days. After the treatment with Ir(III) complexes for 24h, the plates were washed twice with culture medium, and then MTT was added and incubated for another 4h. Cells without treatment of Ir(III) complexes were used as control. The relative cytotoxicity was expressed as percentage of $[OD_{sample} - OD_{blank}]/[OD_{control} - OD_{blank}] \times 100$. Data were collected from three separate experiments and expressed as means ± standard deviation (SD). The statistical differences were analyzed by a paired Student's t-test. P values less than 0.05 were considered to indicate statistical differences.

Cell imaging was performed with an inverted fluorescence microscope.HepG2 cells in growth medium ($1\times10^5$ cells mL$^{-1}$) were seeded in a 35-mm tissue culture dish and incubated at 37°C under a 5% $CO_2$ atmosphere for 48h. The culture medium was removed and replaced with medium/DMSO (99:1, v/v) containing the Ir(III) complexes at a concentration of 25 μM or 50 μM. After incubation for 1h, the medium was removed, and the cell layer was washed gently with PBS. The cell layer was then trypsinized and added up to a final volume of 3 mL with PBS.

*4.6 Computational details*

The singlet ground state (S0) and first triplet state (T1) were optimized by density functional theory. The exchange-correlation functional was chosen as B3LYP[33] with the LANL2DZ basis set[34] for iridium and 6-31G*[35] for the rest. The singlet-singlet vertical transition energies were calculated by the long-range corrected functional, LC-PBEh,[36] considering the excitations featured by the metal-to-ligand charge transfer. The solvent effects were taken into account by using the COSMO salvation model[37] in the excitation energy calculations. All these calculations were performed with NWChem 6.1.1.[38] For the S0-T1 vertical transition energies, SOC-TDDFT[39] and pSOC-TDDFT[40] calculations with the ZORA Hamiltonian[41] were carried out with ADF.[42] The B3LYP functional was applied with all electron Slater-type orbital basis sets,[43] TZP for iridium and DZ for the rest. 20 singlet and triplet excitations were investigated in SR-TDDFT calculations and the lowest 4 spin-mixed excitations were studied in the SOC-TDDFT calculations. HOMO and LUMO energy levels (eV) are showed in table 4.

Table 4. HOMO and LUMO energy levels (eV) and compositions (%) of iridium(III) complexes.

| Complex | $E_H$(eV) | $E_L$(eV) | HOMO | | | LUMO | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ir | C^N | N^N | Ir | C^N | N^N |
| 1 | -8.25 | -5.48 | 45.18 | 50.88 | 3.94 | 2.00 | 1.74 | 96.26 |
| 2 | -8.19 | -5.32 | 45.97 | 50.64 | 3.39 | 1.86 | 3.60 | 94.54 |
| 3 | -8.16 | -5.47 | 35.96 | 60.42 | 3.62 | 2.06 | 1.66 | 96.28 |
| 4 | -7.95 | -4.99 | 46.07 | 49.49 | 4.44 | 1.78 | 9.11 | 89.11 |
| 5 | -7.92 | -4.79 | 46.79 | 49.58 | 3.63 | 1.97 | 7.59 | 90.44 |
| 6 | -7.88 | -4.98 | 37.25 | 59.12 | 3.63 | 1.84 | 1.75 | 96.41 |

**Supporting Information**

The supporting information includes the synthetic procedure for the ligands, MS spectra of the Ir(III) complexes and X-ray crystallographic data in CIF format.

## Acknowledgments

This work was supported by the National Scientific Foundation of China (No. 21171038 and No. 31100723), the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1116), and the Key Laboratory Funding Scheme Guangzhou Municipal Government (2060204). The Beijing Institute of Research provided access to the equipment used for crystal structural determination.

[1] (a) M. J. Li, P. C. Jiao, W. W. He, C. Q. Yi, C. W. Li, X. Chen, G. N. Chen, M. S. Yang, Eur. J. Inorg. Chem. 2(2011), 197-200;
(b) Q. Zhao, T. Y. Cao, F. Y. Li, X. H. Li, H. Jing, T. Yi, C. H. Huang, Organometallics 26(2007), 2077-2081;
(c) D. Wang, H. Wang, Q. Wang, W. Yang, Y Ding, J. Organometillic Chem. 749(2014),41-46;
(d) T. H. Kwon, H. S. Cho, M. K. Kim, J. W. Kim, J. J. Kim, K. H. Lee, S. J. Park, I. S. Shin, H. Kim, D. M. Shin, Y. K. Chung, J. I. Hong, Organometallics 24(2005), 1578-1585;
(e) Y. Q. Wu, H. Jing, Z. S. Dong, Q. Zhao, H. Z. Wu, F. Y. Li, Inorg. Chem. 50(2011), 7412-7420;
(f) K. R. Schwartz, K. R. Mann, Inorg. Chem. 50(2011), 12477-12485;
(g) W. Y. Wong, C. L. Ho, Coord. Chem. Rev. 253 (2009), 1709–1758.

[2] (a) B. D. Muegge, M. M. Richter, Anal. Chem. 76(2004), 73-77;
(b) J. I. Kim, I. S. Shin, H. Kim, J. K. Lee, J. Am. Chem. Soc. 127(2005) 127, 1614-1615;
(c) Q. H. Shu, L. Birlenbach, M. Schmittel, Inorg. Chem. 51(2012), 13123-13127.

[3] (a)K. Y. Zhang, J. Zhang, Y. H. Liu , S. J. Liu , P. L. Zhang , Q. Zhao, Y. Tang, W. Huang. Chem. Sci. 6 (2015), 301-307;
(b)H. B. Sun , S. J. Liu, W. P. Lin, K. Y. Zhang , W. Lv, X. Huang , F. W. Huo , H. R. Yang , G. Jenkins , Q. Zhao , W. Huang. Nat. Commun. 5 (2014), 3601;
(c) W. J. Xu, X. Zhao, W. Lv, H. R. Yang, S. J. Liu, H. Liang, Z. Z. Tu, H. Xu, W. L. Qiao, Q. Zhao, W. Huang. Adv. Healthc. Mater. 3 (2014), 658-669;
(d) H. F. Shi, H. B. Sun, H. R. Yang, S. J. Liu, G. Jenkins, W. Feng, F. Y. Li, Q. Zhao, B. Liu, W. Huang. Adv. Funct. Mater. 23 (2013), 3268-3276 ;

(e) Y. Ma, S. J. Liu,H. R. Yang, Y. Q. Wu, H. B. Sun, J. X. Wang, Q. Zhao, F. Y. Li, W. Huang. J. Mater. Chem. B. 1 (2013), 319-329;
(f) Y. Tang, H. R. Yang, Sun, H. B. Sun , S. J. Liu, J. X. Wang, Q. Zhao, X. M. Liu, W. J. Xu, S. B. Li, W. Huang. Chem. Eur. J. 19(2013), 1311-1319;
(g) S. J. Liu, P. Wang, Q. Zhao, H. Y. Yang, J. Wong, H. B. Sun, X. C. Dong, W. P. Lin, W. Huang. Adv. Mater.24 (2012), 2901-2905;
(h) S. J. Liu, Z. H. Lin, Q. Zhao, Y. Ma, H. F. Shi, M. D. Yi, Q. D. Ling, Q. L. Fan, C. X. Zhu, E. T. Kang, et al . Adv. Funct. Mater. 21 (2011), 21, 979-985;
(i) W. J. Xu, S. J. Liu, H. B. Sun, X. Y. Zhao, Q. Zhao, S. Sun, S. Cheng, T. C. Ma, L. X. Zhou, W. Huang. J. Mater. Chem.21 (2011), 7572-7581;
(k) H. F. Shi, S. J. Liu, H. B. Sun, W. J. Xu, Z. F. An, J. Chen, S. Sun, X. M. Lu, Q. Zhao, W. Huang. Chem. Eur. J. 16 (2010), 12158-12167;
(l) W. J. Xu, Liu, S. J. Liu, X. Y. Zhao, S. Sun, S. Cheng, T. C. Ma, H. B. Sun, Q. Zhao, W. Huang. Chem. Eur. J. 16 (2010), 7125-7133.

[4] (a) P.-T. Chou, Y. Chi, Eur. J. Inorg. Chem. 2006, 3319-3332;
(b) R. C. Evans, P. Douglas, C. J. Winscom, Coord. Chem. Rev. 250(2006), 2093-2126;
(c) L. Flamigni, A. Barbieri, C. Sabatini, B. Ventura, F. Barigelletti, Top. Curr. Chem. 281(2007), 143-203;
(d) K. K.-W. Lo, M.-W. Louie, K. Y. Zhang, Coord. Chem. Rev. 254(2010), 2603-2622;
(e) Q. Zhao, F. Li, C. Huang, Chem. Soc. Rev.39( 2010), 3007-3030;
(f) C. L. Ho, H. Li, W. Y. Wong, J. Organomet. Chem. 751(2014), 261-285;
(g) G. Zhou, W. Y. Wong, S. Suo, J. Photochem. Photobio. C: Photochem. Rev. 11 (2010), 133-156;
(h) G. Zhou, W. Y. Wong, X. Yang, Chem. Asian J. 6 (2011), 1706-1727;
(i) L. Ying, C. L. Ho, H. Wu, Y. Cao, W. Y. Wong,. Adv. Mater. 26(2014), 2459-2473;
(j) X. Yang, G. Zhou, W. Y. Wong, J. Mater. Chem. C. 2 (2014), 1760-1778;
(k) C. L. Ho, W. Y. Wong, New J. Chem. 37(2013), 1665-1683.

[5] (a) Y. Himeda, N. Onozawa-Komatsuzaki, S. Miyazawa, H. Sugihara, T. Hirose, K. Kasuga, Chem. Eur. J. 14(2008), 11076-11081;
(b) N. Zhao, Y. H. Wu, H. M. Wen, X. Zhang, Z. N. Chen, Organometallics 28(2009), 5603-5611;
(c) H. J. Kim, Y. C. Jeong, J. R. Heo, K. J. Hwang, Bull. Korean Chem. Soc. 30(2009), 539-540;
(d) P. K. Lee, W. H. T. Law, H. W. Liu, K. K. W. Lo, Inorg. Chem. 50(2011), 8570-8579;
(e) E. Baranoff, B. F. E. Curchod, J. Frey, R. Scopelliti, F. Kessler, I. Tavernelli, U. Rothlisberger, M. Gratzel, M. K. Nazeeruddin, Inorg. Chem. 51(2012), 215-224.

[6] (a) S. Klein, W. G. Dougherty, W. S. Kassel, T. J. Dudley, J. J. Paul, Inorg. Chem. 50(2011), 2754-2763;
(b) S. Aoki, Y. Matsuo, S. Ogura, H. Ohwada, Y. Hisamatsu, S. Moromizato, M. Shiro, M. Kitamura, Inorg. Chem. 50(2011), 806-818.

[7] J. M. Price, W. Y. Xu, J. N. Demas, B. A. DeGraff, Anal. Chem. 70(1998), 265-270.

[8] D. C. Goldstein, Y. Y. Cheng, T. W. Schmidt, M. Bhadbhade and P. Thordarson, Dalton Trans., 40(2011), 2053-2061.

[9] S. Lamansky, P. Djarovich, D. Murphy, F. Abdel-Razzaq, H. E. Lee, C. Adachi, P. E. Burrows, S. R. Forrest, M. E. Thompson,J. Am. Chem. Soc. 123(2001), 4304-4312.

[10] Q. Shu, W. B. Jan, S. Michael, Inorg. Chem. 50(2011), 10531-10533.

[11] (a) K. K. W. Lo, J. S. W. Chan, C. K. Chung, V. W. H. Tsang, N. Y. Zhu, Inorg. Chem. Acta. 357(2004), 3109-3118;
(b) A. P. Wilde, R. J. Watts, J. Phys. Chem. 95(1991), 622-629;
(c) K. K. W. Lo, D. C. M. Ng, C. K. Chung, Organometallics 20(2001), 4999-5001;
(d) L. L. Wu, C. H. Yang, I. W. Sun, S. Y. Chu, P. C. Kao, H. H. Huang, Organometallics 26(2007), 2017-2023.

[12] a) S. Serroni, A. Juris. S. Campagna, M. Venturi, G. Denti, V. Balzani, J. Am. Chem. Soc. 116(1994), 9086-9091; b) G. D. Marco, M. Lanza, A. Mamo, I. Stefio, C. D. Pietro, G. Romeo, S. Campagna, Anal. Chem. 70(1998), 5019-5023.

[13] (a) G. Calogero, G. Giuffrida, S. Serroni, V. Ricevuto, S. Campagna, Inorg. Chem. 34(1995), 541-545; b) P. M. Griffiths, F. Loiseau, F. Puntoriero, S. Serroni, S. Campagna, Chem. Commun. 2000, 2297-2298; c) M. Maestri, V. Balzani, C. Deuschel-Cornioley, A. V. Zelewsky, Adv. Photochem. 17(1992), 1-68.

[14] (a) K. K.W. Lo, P. K. Lee, J. S. Y. Lau, Organometallics 27(2008), 2998-3006;
(b) J. S.Y. Lau, P. K. Lee, K. H. K. Tsang, C. H. C. Ng, Y. W. Lam, S. H. Cheng, K. K. W. Lo, Inorg. Chem. 48(2009), 708-718;
(c) A. Tsuboyama, H. Iwawaki, M. Furugori, T. Mukaide, J. Kamatani, S. Igawa, T. Moriyama, S. Miura, T. Takiguchi, S. Okada, M. Hoshino, K. Ueno, J. Am. Chem. Soc.125( 2003), 12971-12979.

[15] (a) F. Neve, M. L. Deda, A. Crispini, A. Bellusci, F. Puntoriero, S. Campagna, Organometallics 2004, 23, 5856-5863;
(b) Q. Zhao, S. Liu, M. Shi, C. Wang, M. Yu, L. Li, F. Li, T. Yi, C. Huang, Inorg. Chem. 45(2006), 45, 6152-6160.

[16] M. G. Colombo, A. Hauser, H. U. Gudel, Inorg. Chem. 32(1993), 3088-3092.

[17] (a) Y. Himeda, N. Onozawa–Komatsuzaki, H. Sugihara, K. Kasuga, Organometallics 26(2007), 702-712;
(b) Y. Himeda, N. Onozawa-Komatsuzaki, H. Sugihara, H. Arakawa, K. Kasuga, Organometallics 23(2004), 1480-1483.

[18] J. M. Price, W. Y. Xu, J. N. Demas, B. A. DeGraff, Anal. Chem. 70(1998), 265-270

[19] (a) S. Moromizato, Y. Hisamatsu, T. Suzuki, Y. Matsuo, R. Abe, S. Aoki, Inorg. Chem. 51(2012), 12697-12706;
(b) P. Diana, P. Barraja, A. Lauria, A. M. Almerico, G. Dattolo, G. Cirrincione, Tetrahedron. 2000, 56, 5177-5183;
(c) R. Stewart, M. G. Harris, J. Org. Chem. 43(1978), 3123-3126.

[20] M. J. Hoogduijn, M. J. Crop, S. S. Korevaar, A. M. A. Peeters, M. Eijken, et al. Transplantation. 86(2008), 1283-1291.

[21] M.-J. Li, T.-Y. Lan, X.-H. Cao, H-H. Yang, Y. Shi, C. Yi, G.-N. Chen, Dalton Trans., 43(2014),2789-2798.

[22] S. Tardito, I. Bassanetti, C. Bignardi, L. Elviri, M. Tegoni, C. Mucchino, O. Bussolati, R. Franchi-Gazzola, L. Marchio , J Am Chem Soc. 133 (2011), 6235.

[23] R. Scherz-Shouval, Z. Elazar, Trends Cell Biol. 17(2007) ,422–427.

[24] (a) B. Minaev, H. Ågren, F. De Angelis, Chem. Phys. 358(2009), 245-257;
(b) B. Minaev, V. Minaeva, H. Ågren, J. Phys. Chem. A 113(2009) 726-735;
(c) X. Li, Q. Zhang, Y. Tu, H. Ågren, H. Tian, Phys. Chem. Chem. Phys.12(2010), 13730-13736.

[25] (a) B. P. Sullivan, C. M. Bolinger, D. Conrad, W. J. Vining, T. J. Meyer, J. Chem. Soc., Chem. Commun. 18(1984), 1244-1245;
(b) G. Tapolsky, R. Duesing, T. J. Meyer, Inorg. Chem. 29(1990), 2285–2297;
(c) P. Kavanagh, D. Leech, Tetrahedron Letts. 45(2004), 121-123;
(d) D. Wenkert, R. B. Woodward, J. Org. Chem. 48(1983), 283-289;
(e) G. Maerker, F. H. Case, J. Am. Chem. Soc. 80(1958), 2745–2748.

[26] Y. R. Hong, C. B. Gorman, J. Org. Chem. 68(2003), 9019-9025.

[27] K. K.W. Lo, C. K. Chung, N. Y. Zhu, Chem. Eur. J. 9(2003), 475-483.

[28] M. J. Li, X. Liu, M. J. Nie, Z. Z. Wu, C. O. Yi, G. N. Chen, V. W. W. Yam, Organometallics, 31(2012), 4459-4466.

[29] Z. Otwinowski, W. Minor, Macromol. Crystallogr., Pt A 276(1997), 307-326.

[30] G. M. Sheldrick, SHELXH-97; Universita¨t Go¨ttingen: 1998.

[31] A. L. Spek, Acta Crystallogr., Sect. A 46(1990), C34.

[32] (a) Z. Zhang, Y Shi, Y. Pan, X. Cheng, L. Zhang, J. Chen,M.-J. Li, C. Yi, J. Mater. Chem. B 2(2014), 5020-5027;
(b) J. Carmichael , W. G. De Graff, A. F. Gazdar, et al.,Cancer. Res. 1987, 47, 936.

[33] A. D. Becke , J. Chem. Phys. 98(1993), 5648 –5652.

[34] (a) P. J. Hay and W. R. Wadt, J. Chem. Phys. 82(1985), 270–283;
(b)W. R. Wadt and P. J. Hay, J. Chem. Phys. 82(1985), 284–298;
(c)P. J. Hay and W. R. Wadt, J. Chem. Phys. 82(1985), 299-310.

[35] W. J. Hehre , R. Ditchfield , J. A. Pople , J. Chem. Phys. 56(1972) , 2257-2261.

[36] O. A. Vydrov, J. Heyd, A. Krukau, and G. E. Scuseria, J. Chem. Phys. 125 (2006), 074106.

[37] A. Klamt and G. Schüürmann, J. Chem. Soc. Perkin Trans. 2, (1993), 799-805;

[38] M. Valiev, E.J. Bylaska, N. Govind, K. Kowalski, T.P. Straatsma, H.J.J. van Dam, D. Wang, J. Nieplocha, E. Apra, T.L. Windus, W.A. de Jong, Comput. Phys. Commun. 181(2010), 1477-1489;

[39] F. Wang, T. Ziegler, E. van Lenthe, S. J. A. van Gisbergen and E. J. Baerends, J. Chem. Phys. 122(2005), 204103;

[40] F. Wang and T. Ziegler, J. Chem. Phys. 123(2005), 154102;

[41] (a) E. van Lenthe, E. J. Baerends and J. G. Snijders, J. Chem. Phys. 99(1993), 4597;
(b)E. van Lenthe, E. J. Baerends and J. G. Snijders, J. Chem. Phys. 101(1994), 9783.

[42] E. J. Baerends, T. Ziegler, J. Autschbach, D. Bashford, A. Bérces, F. M. Bickelhaupt, C. Bo, P. M. Boerrigter, L. Cavallo, D. P. Chong, L. Deng, R. M. Dickson, D. E. Ellis, M. van Faassen, L. Fan, T. H. Fischer, C. Fonseca Guerra, M. Franchini, A. Ghysels, A. Giammona, S. J. A. van Gisbergen, A. W. Götz, J. A. Groeneveld, O. V. Gritsenko, M. Grüning, S. Gusarov, F. E. Harris, P. van den Hoek, C. R. Jacob, H.

Jacobsen, L. Jensen, J. W. Kaminski, G. van Kessel, F. Kootstra, A. Kovalenko, M. V. Krykunov, E. van Lenthe, D. A. McCormack, A. Michalak, M. Mitoraj, S. M. Morton, J. Neugebauer, V. P. Nicu, L. Noodleman, V. P. Osinga, S. Patchkovskii, M. Pavanello, P. H. T. Philipsen, D. Post, C. C. Pye, W. Ravenek, J. I. Rodríguez, P. Ros, P. R. T. Schipper, G. Schreckenbach, J. S. Seldenthuis, M. Seth, J. G. Snijders, M. Solà, M. Swart, D. Swerhone, G. te Velde, P. Vernooijs, L. Versluis, L. Visscher, O. Visser, F. Wang, T. A. Wesolowski, E. M. van Wezenbeek, G. Wiesenekker, S. K. Wolff, T. K. Woo and A. L. Yakovlev, ADF2013 SCM, Theoretical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands, http://www.scm.com;

[43] E. van Lenthe and E. J. Baerends, J. Comput. Chem. 24(2003), 1142-1156.

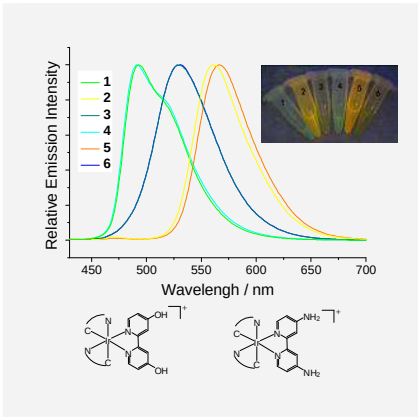
Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

**Entry for the Table of Contents** (Please choose one layout)

**Layout 1:**

**Key Topic**

A series of Ir(III) complexes with amino or hydroxyl groups were synthesized and characterized. Two of the complexes were structurally characterized via X-ray crystallography. The photophysical and photochemical properties of these complexes were studied. Preliminary studies of their applications on pH sensing, and cell imaging were also performed.




**Zhaozhen Wu, Juanjuan Mu, Qiong Wang, Xing Chen, Lasse Jensen, Changqing Yi,* Mei-Jin Li*** … Page No. – Page No.

Hydroxyl and Amino Functionalized Cyclometalated Ir(III) Complexes: Synthesis, Characterization and Cytotoxicity Studies

**Keywords:** Iridium(III) complex / Synthesis /pH sensing / Cytotoxicity

**Highlights**

- A series of Ir(III) complexes were successfully synthesized and characterized
- Two of the complexes were structurally characterized via X-ray crystallography
- The emission of the complexes could be switched off or on in acid or base solution
- The complexes could be potentially used for the luminescence pH sensors
- Some of the complexes have promising potential as specific cell-imaging agents

A series of Ir(III) complexes with amino or hydroxyl groups were synthesized and characterized. Two of the complexes were structurally characterized via X-ray crystallography. The photophysical and photochemical properties of these complexes were studied. Preliminary studies of their applications on pH sensing, and cell imaging were also performed.

# Supporting Information

# Hydroxyl and amino functionalized cyclometalated Ir(III) complexes: synthesis, characterization and cytotoxicity studies

**Zhaozhen Wu,[a] Juanjuan Mu,[a] Qiong Wang,[a] Xing Chen,[c] Lasse Jensen,[c] Changqing Yi, [b,*] Mei-Jin Li[a,*]**

[a] *Key Laboratory of Analysis and Detection Technology for Food Safety, Ministry of Education, Department of Chemistry, Fuzhou University, Fuzhou 350108, China*

[b] *Key Laboratory of Sensing Technology and Biomedical Instruments (Guangdong Province), School of Engineering, Sun Yat-Sen University, Guangzhou 510006, P.R.China*

[c]*Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, 16802, United States.*

## Content

# I. General Information

All reagents were used as received of commercial sources. Solvents were purified to deoxygenization by dry nitrogen. Other solid or liquid reagents received from commercial source were used without any further purification. 2-Phenylpyridine (**ppy**), 2-phenylquinoline (**pq**), benzo [*h*]quinoline (**bzq**) were all purchased from Alfa Aesar Reagent Co. Ltd. Iridium(III) chloride hydrate was obtained from Aladdin chemistry Co. Ltd. 4,4'-Dimethoxy-2,2'-bipyridine was obtained from Sigma-Aldrich Co. LLC. All of these reagents were used without any further purification.

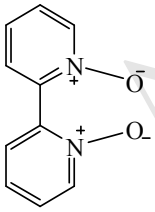
$^{1}$H NMR spectra were recorded on a Bruker Avance III (400 MHz) spectrometer using deuteriated solvents at room temperature with chemical shifts reported relative to tetramethylsilane(Me4Si). Positive Q-TOF mass spectra (MS) were recorded on an Agilent 6520 accurate mass spectrometer..

# II. The synthetic procedure for the ligands

## *Synthesis of 4,4'-dihydroxy-2,2'-dipyridine (bpy(NH$_2$)$_2$).*

4,4'-Diamine-2,2'-dipyridine was synthesized by the literature method.[1]

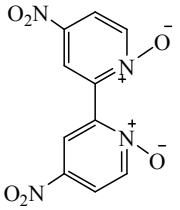
## *2,2'-Bipyridine-N,N-dioxide*



2,2'-Bipyridine-N,N-dioxide was prepared by heating 2,2'-bipyridine (1.0 g.), glacial acetic acid (7.5 ml.), and 30% hydrogen peroxide (1.3 ml.) together at 70-80℃ for 3 hr. An additional 0.9 ml. of 30% hydrogen peroxide was added and the temperature

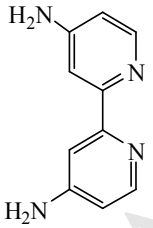
maintained at 70-80℃ for a further 19 hr. On addition of acetone, 2,2'-bipyridine N,N'-dioxide precipitated. The material was recrystallized from hot water by the addition of a large excess of acetone. Yield: 867mg of fine white needles (72%).

## *2,2'-Bipyridine N,N-dioxide*



3.6mL of concentrated sulfuric acid was added to 750mg of 2,2'-Bipyridine-N,N-dioxide. The mixture was first cooled in ice, before addition of 1.25mL of fuming nitric acid (CAUTION). The resulting solution was held at reflux (95–100 ℃) for 20 h before cooling to room temperature. The acidic mixture was then poured onto ice (-40 ℃), prepared by the addition of an excessive amount of liquid nitrogen onto 20mL of water with constant stirring. After continued stirring, a bright yellow precipitate formed, to which a further measure of liquid nitrogen was added. The solution was subsequently filtered and the yellow precipitate collected. The solid was washed successively with water and allowed to air dry. Yield: 920mg of yellow powder (83%).

## *5,6-diamino-1,10-phenanthrolin*



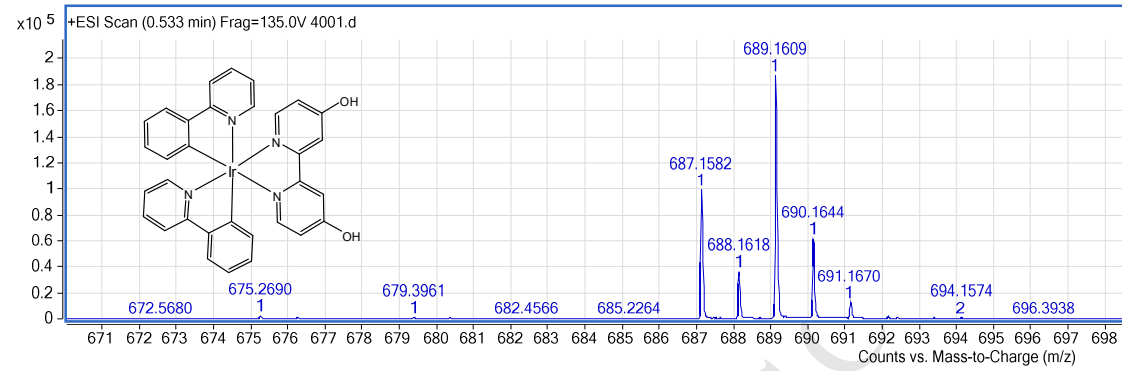
A mixture of 350mg of 2,2'-Bipyridine N,N-dioxide and 630 mg Pd/C (5%) in 38mL of ethanol was purged with $N_2$ gas. The suspension was then heated to reflux under nitrogen and, after the complex was completely dissolved, 2.7mL of hydrazine hydrate in 10mL ethanol was added dropwise over a period of 20min. The resulting solution was held at reflux for 15 h. When completed, the mixture was immediately filtered under reduced pressure, and washed with boiling ethanol. After removal of the solvent, the yellow

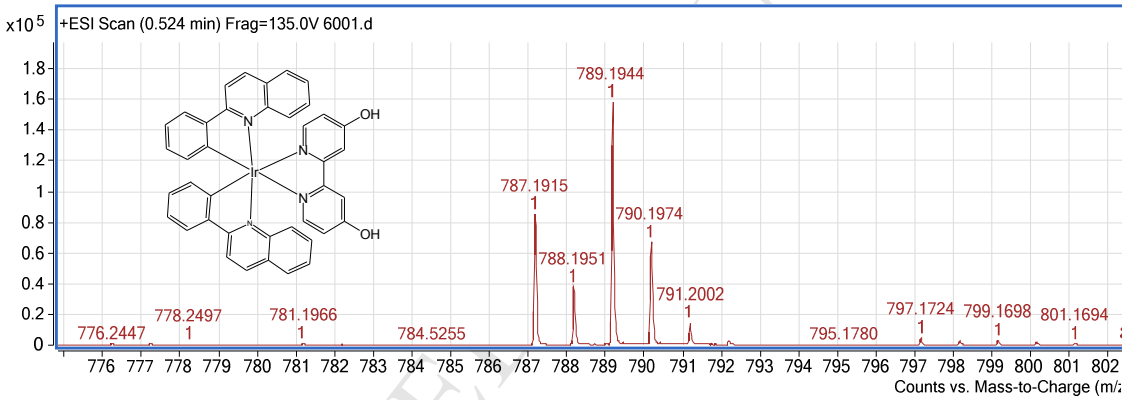
precipitate was ground in 20mL of water and left at 2℃ overnight. The white solid that separated was vacuum filtered, washed with cold water and dried at 50℃. Yield: 195 mg of white powder (83%).

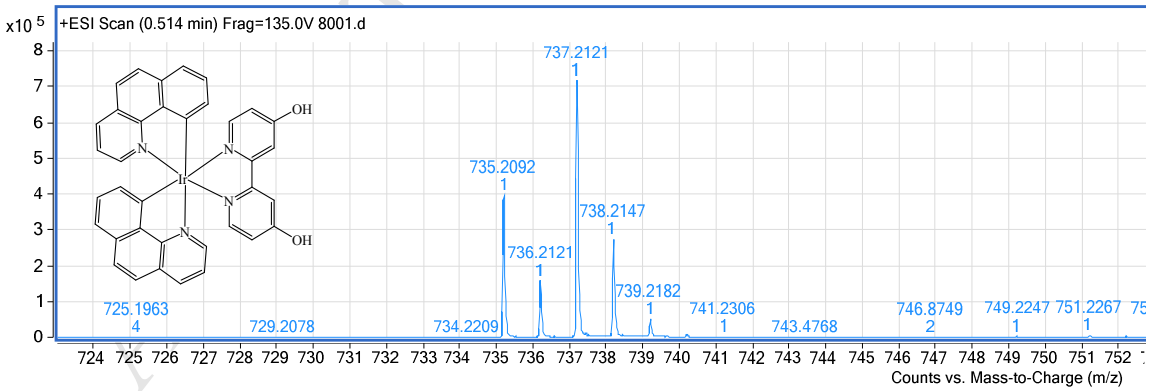
**Synthesis of 4,4'-dihydroxy-2,2'-dipyridine (bpy(OH)$_2$).** The ligand was synthesized according to the literature with slight modifications.[2] HBr solution (41 wt%, 0.554 mL, 2.77 mmol) was added to a mixture of glacial acetic acid (13.85 mL) and 4,4'-dimethoxy-2,2'-bipyridine (60 mg, 0.277 mmol); the resulting mixture was subsequently refluxed overnight. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in distilled water and neutralized to pH 6–7 by addition of aqueous ammonium hydroxide to provide a white precipitate with 64% (33 mg) yield.

**Synthesis of [(C^N)$_2$IrCl]$_2$.** Cyclometalated Ir(III) chloride-bridged dimer [(C^N)$_2$IrCl]$_2$ was synthesized according to the literature.[3] A mixture of $IrCl_3 \cdot 3H_2O$ and an excess of the desired C^N ligand, ppy, pq, or bzq, in oxygen-free 2-ethoxyethanol/$H_2O$ (3:1, v/v) was heated to reflux for 24 h in the dark under nitrogen to provide a crude chloride-bridged dimer. After cooling to room temperature, the precipitate was filtered off and washed with ethanol followed by an equal amount of acetone. The solid was dissolved and isolated to afford a colored powder.

# Ⅲ. MS spectra of the Ir(III) complexes



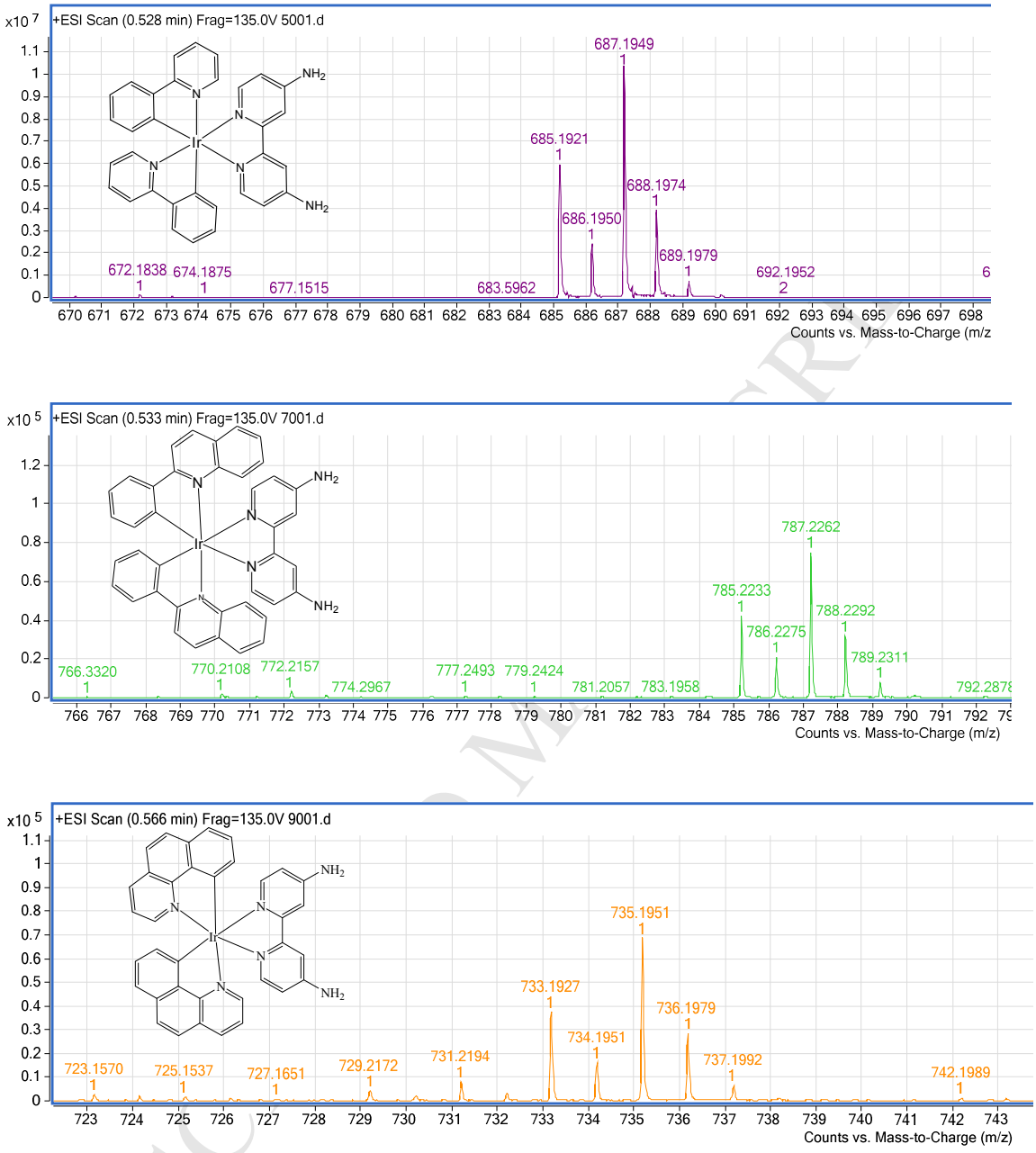



Fig.S1 MS spectra of the Ir(III) complexes

## IV. Imaging studies

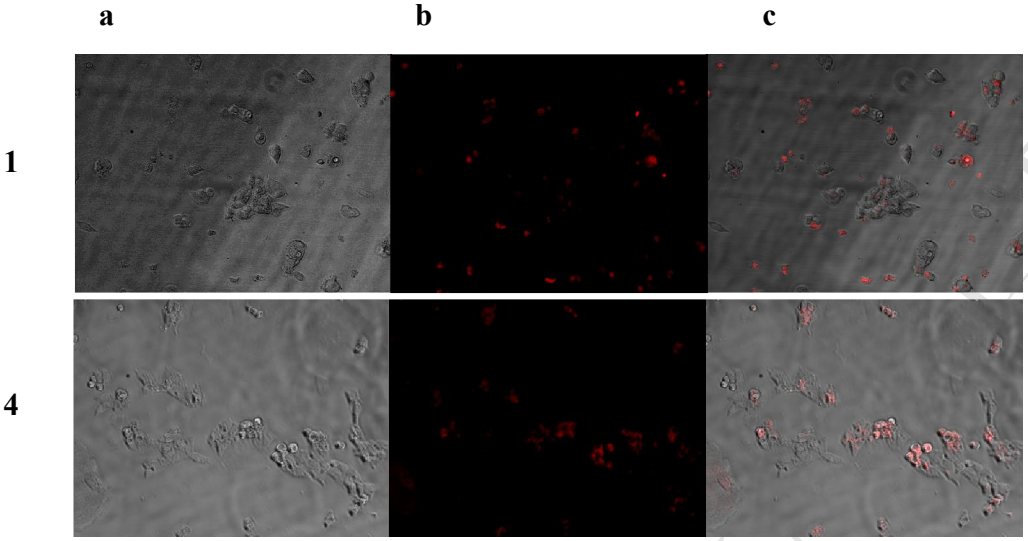



**Fig. S2.** Images of fluorescence microscopy of HpeG2 cells in the presence of Ir complexes **1 and 4**: (a) Brightfield images; (b) luminescence imaging of HepG2 cells incubated solely with 50 $\mu$M complex **1** (or 25$\mu$M **4**) in DMSO/PBS(pH 7.4, 99:1,v/v); (c) Merged image of (a) and (b).

## V. Calculations

Table S1. Selected geometry parameters from optimized geometries compared with the experimental data.

| Complex 2 | Geometry parameters | [1]Error (%) | Complex 5 | Geometry parameters | Error (%) |
|---|---|---|---|---|---|
| Ir(1)-C(25) | 2.010 | 0.85 | Ir(1)-C(16) | 2.010 | 0.65 |
| Ir(1)-C(40) | 2.007 | 0.20 | Ir(1)-C(1) | 2.008 | 0.30 |
| Ir(1)-N(3) | 2.138 | 2.11 | Ir(1)-N(1) | 2.137 | 3.60 |
| Ir(1)-N(4) | 2.141 | 1.31 | Ir(1)-N(2) | 2.141 | 2.43 |
| Ir(1)-N(1) | 2.272 | 5.68 | Ir(1)-N(4) | 2.265 | 4.55 |
| Ir(1)-N(2) | 2.258 | 4.34 | Ir(1)-N(3) | 2.253 | 3.82 |
| O(1)-C(3) | 1.346 | 0.15 | N(5)-C(33) | 1.361 | 0.29 |
| O(2)-C(8) | 1.346 | 0.82 | N(6)-C(38) | 1.361 | 0.22 |
| C(25)-Ir(1)-N(3) | 79.6 | 1.01 | C(1)-Ir(1)-N(1) | 79.7 | 1.13 |
| C(40)-Ir(1)-N(4) | 79.7 | 0.88 | C(16)-Ir(1)-N(2) | 79.7 | 0.50 |
| N(1)-Ir(1)-N(2) | 73.0 | 2.97 | N(4)-Ir(1)-N(3) | 73.0 | 1.92 |

[1]Error is defined as $|p_{cal}-p_{expt}/p_{expt}*100|$

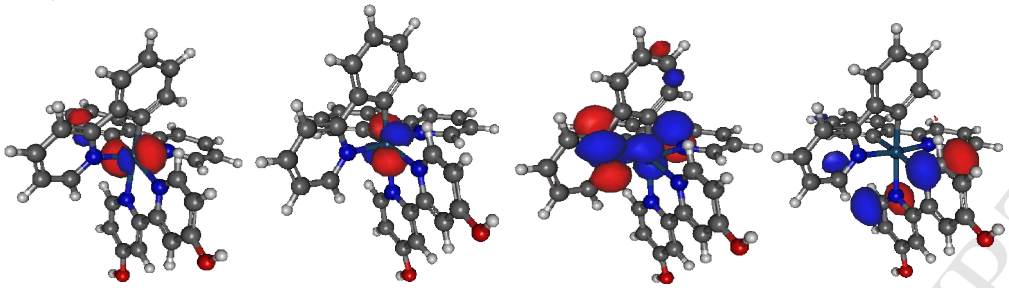
Table S2. Calculated singlet-singlet vertical transition energies (*E*) and wavelengths($\lambda$) with the large oscillator strengths (*f*) incorporated with transition assignment for the interested Ir(III) complexes in DMSO.

| Complex | *E*(eV), λ(nm) | *f* | Transition | Assignment |
|---|---|---|---|---|
| 1 | 3.70, 334.7 | 0.091 | 138-140 | MLCT |
| | 4.81, 257.9 | 0.232 | 134-140 | MLCT |
| | | | 138-142 | MLCT |
| 2 | 3.34, 371.6 | 0.108 | 164-165 | MLCT |
| | 4.28, 289.4 | 0.150 | 161-166 | MLCT |
| | | | 162-166 | MLCT |
| 3 | 3.66, 339.1 | 0.080 | 150-152 | MLCT |
| | 4.48, 276.9 | 0.268 | 147-153 | MLCT |
| | | | 148-152 | MLCT |
| 4 | 3.68, 337.7 | 0.090 | 138-140 | MLCT |
| | 4.79, 258.8 | 0.223 | 135-141 | MLCT |
| | | | 134-140 | MLCT |
| 5 | 3.30, 375.3 | 0.101 | 164-165 | MLCT |
| | 4.28, 289.8 | 0.147 | 162-166 | MLCT |
| | | | 160-166 | MLCT |
| 6 | 3.62, 342.4 | 0.078 | 150-151 | MLCT |
| | 4.41, 281.0 | 0.194 | 148-152 | MLCT |
| | | | 150-154 | MLCT |

Table S3. Calculated singlet-triplet vertical transition energies (*E*) and wavelengths($\lambda$) with the oscillator strengths (*f*).

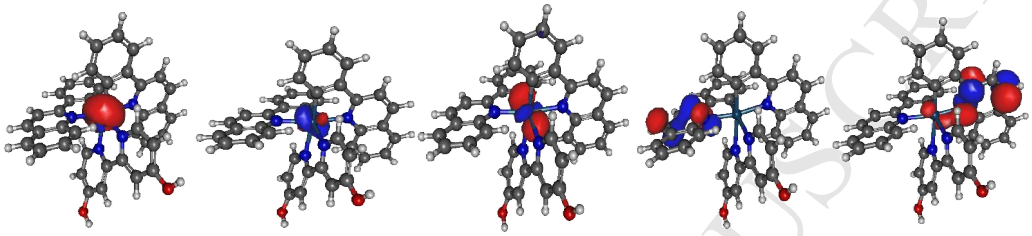
| Complex | *E*(eV), λ(nm) | *f* |
|---|---|---|
| 1 | 2.04, 606.5 | 0.0008 |
| 2 | 2.15,576.5 | 0.0005 |
| 3 | 2.02,612.3 | 0.0011 |
| 4 | 2.23,555.1 | 0.0012 |
| 5 | 2.34,529.3 | 0.0005 |
| 6 | 2.23,556.9 | 0.0019 |

complex 1



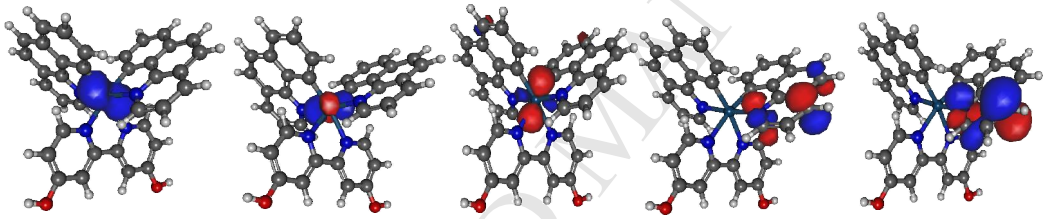
134 138 140 142

complex 2



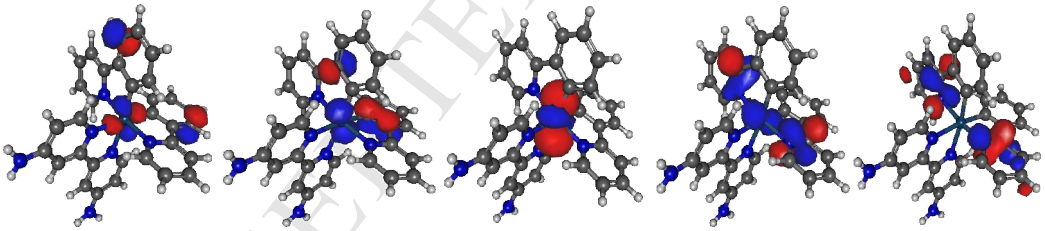
161 162 164 165 166

complex 3



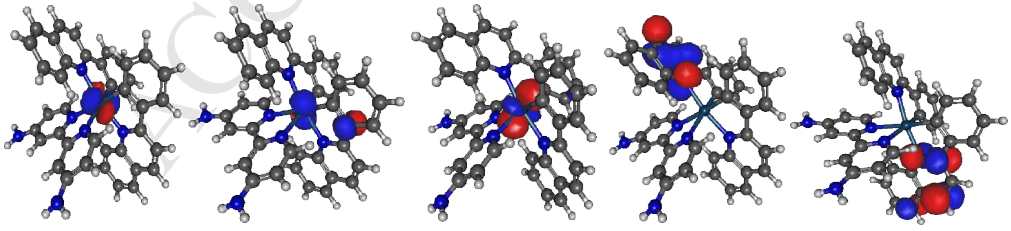
147 148 150 152 153

complex 4



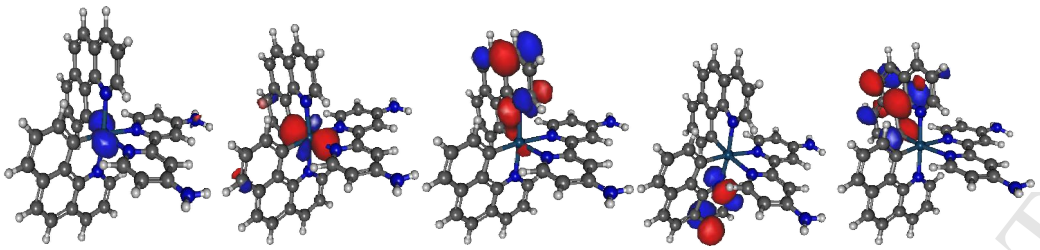
134 135 138 140 141

complex 5



160 162 164 165 166

complex 6



148 150 151 152 154

Fig. S3. Molecular orbitals involved in the singlet-singlet electron transitions with pronouced transition dipole moments.

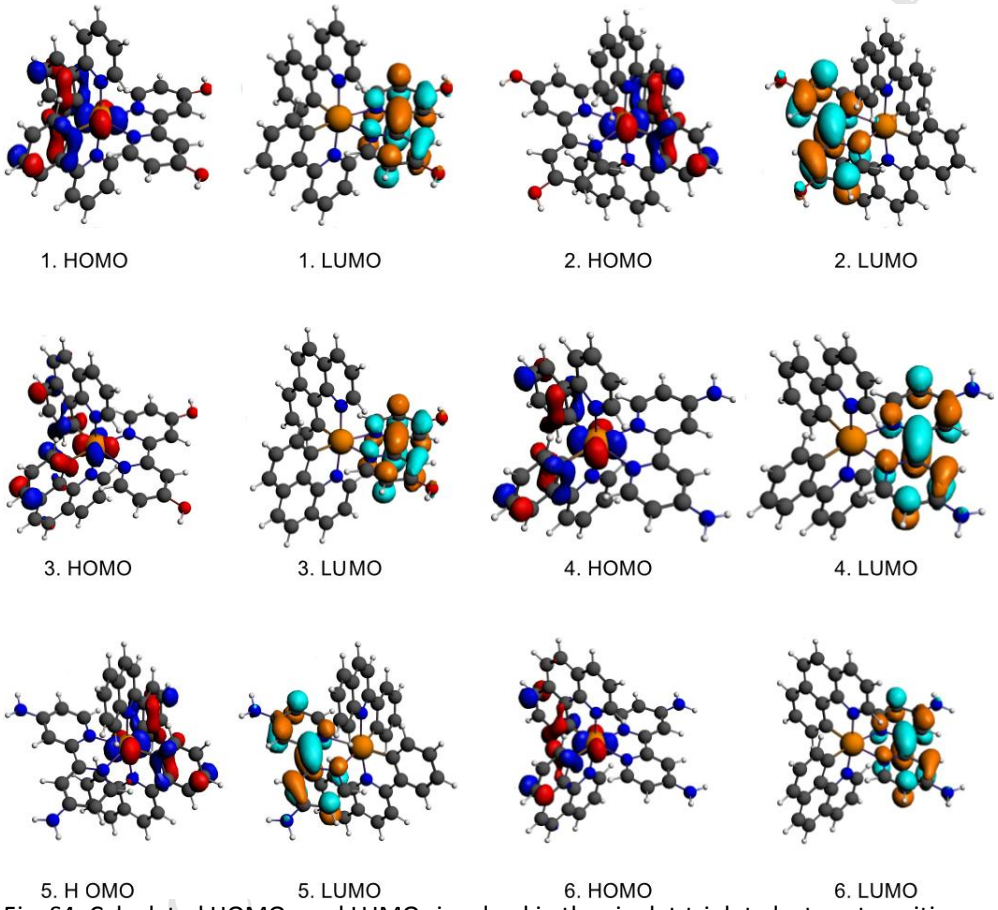
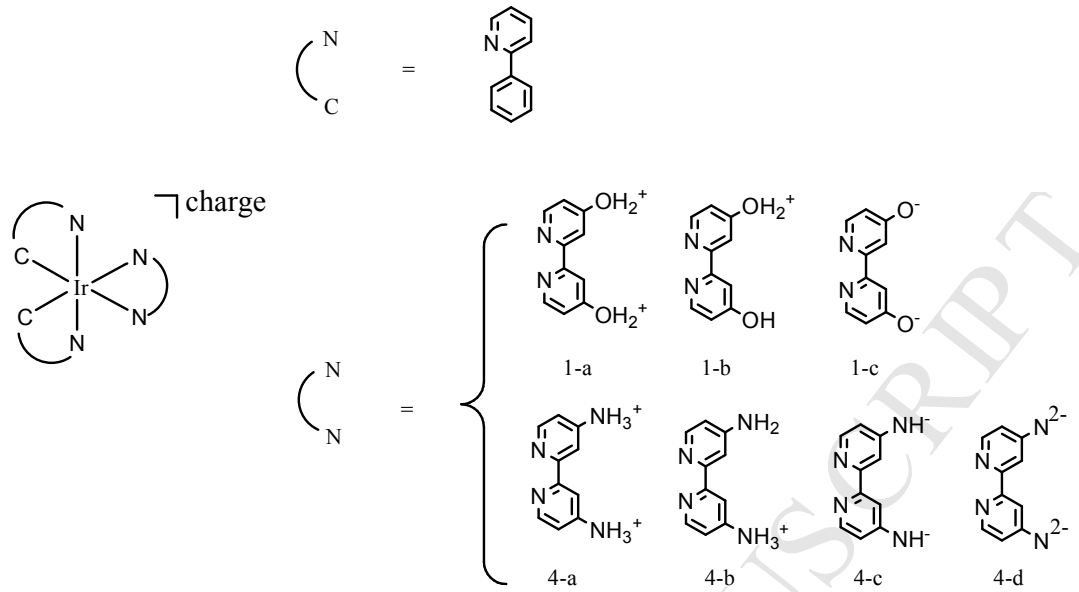



Fig. S4. Calculated HOMOs and LUMOs involved in the singlet-triplet electron transitions.




Scheme S1. Protonated and deprotonated forms of Iridium(III) complexes 1 and 4.

Table S4. Energy gap between the triplet and singlet states and compositions of iridium(III)complexes in HOMO and LUMO

| Complex | ΔE(eV) | HOMO | | | LUMO | | |
|---|---|---|---|---|---|---|---|
| | | Ir | C^N | N^N | Ir | C^N | N^N |
| 1-a | 0.04 | 33.19 | 61.46 | 5.35 | 0.31 | 0.09 | 99.60 |
| 1-b | -1.20 | 39.52 | 55.82 | 4.66 | 6.61 | 79.34 | 14.0 |
| 1-c | 2.10 | 6.05 | 4.00 | 89.95 | 1.94 | 9.08 | 88.98 |
| 4-a | 0.14 | 34.18 | 60.63 | 5.19 | 0.72 | 0.31 | 98.97 |
| 4-b | 1.00 | 40.12 | 55.24 | 4.64 | 5.94 | 74.79 | 19.27 |
| 4-c | 1.59 | 3.83 | 3.82 | 92.35 | 3.66 | 46.44 | 49.90 |
| 4-d | -1.90 | 6.04 | 4.00 | 89.96 | 1.94 | 9.08 | 88.98 |

# Reference

[1] a) P. G. Simpsox, A. Vinciguerra, J. V. Quagliano, *Inorg. Chem.* **1963**, 2, 282-286; b) P. Kavanagh, D. Leech, *Tetrahedron Let.* **2004**, 45, 121–123.
[2] Y. R. Hong, C. B. Gorman, *J. Org. Chem.* **2003**, 68, 9019-9025.
[3] K. K.W. Lo, C. K. Chung, N. Y. Zhu, *Chem. Eur. J.* **2003**, *9*, 475-483.