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Luminescent biscarbene iridium(III) complexes as living cell imaging reagents†

Cite this: DOI: 10.1039/c3cc40845k

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Received 29th November 2012,
Accepted 21st February 2013

DOI: 10.1039/c3cc40845k

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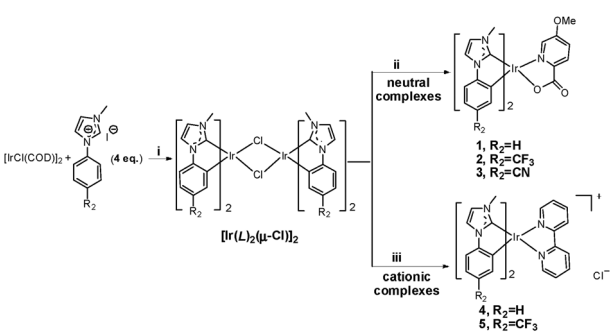
Five iridium(III) complexes with two N-heterocyclic carbene (NHC) ligands and an ancillary ligand have been designed and successfully synthesized. With multicolor photoluminescence and low toxicity, these carbene complexes were tested, for the first time, as living cell imaging reagents and showed promise for application beyond the OLED (organic light emitting diode) area.

Since the first stable imidazole-2-ylidene carbene was isolated by Arduengo *et al.* in 1991,[1] the chemistry of NHCs, derived from the replacement of the proton at C-2 in an imidazolium salt by a metal, has rapidly developed into an important area with an impact on catalysis,[2,3] materials science[4–6] and drug discovery.[7] Luminescence has been observed from a number of NHC metal complexes, such as ruthenium,[8] gold,[9] silver,[10] rhenium,[11] platinum,[12] and copper.[13] While the NHC complexes as catalysts, antimicrobial reagents, antitumor reagents and liquid crystalline materials have been extensively studied,[14,15] the application of the luminescence of NHC complexes has not been explored until Sajoto *et al.*[16] reported using NHC iridium(III) complexes as phosphorescent dopants of OLEDs.[17] The need for deep-blue phosphorescent emitters in OLED technology has added strong momentum to the growth of the luminescent NHC complexes. A number of tricarbene and biscarbene iridium(III) complexes have been synthesized and incorporated into OLED devices.[18–21]

In addition to the above-mentioned OLED application, general luminescent metal complexes found uses in biotechnology and life science. The demonstrated applications include bio-labeling,[22–26] bio-imaging,[27–29] protein staining,[30–32] *etc.* Several research groups[33,34] have extensively investigated biscyclometalated iridium(III) complexes, most of which are cationic, as cellular imaging reagents. These iridium(III) complexes exhibited large Stokes' shift and high photostability when used in the imaging process.[27,35] However, as a group of relatively new luminescent complexes with growing importance, the luminescence of the NHC complexes has not been exposed to the potentially vast opportunities beyond the OLED area. In this work, we demonstrate, for the first time, the utilization of a few novel luminescent biscarbene iridium(III) complexes as living cell imaging reagents.

The structures of the five biscarbene iridium(III) complexes and their general synthetic routes are shown in Scheme 1. The five compounds are grouped into two. **1–3** are electronically neutral and have a N^O ancillary ligand (picolate); **4–5** are positively charged and have a N^N ancillary ligand (2,2′-bipyridyl). The substitution groups $-CF_3$ and –CN were chosen because of their electron-withdrawing effects and potential effects on the lipophilicity. These carbene complexes were structurally determined using ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and TOF-MS (the crystal structure and crystallographic data of complex **3** are also shown in Fig. S1 and Table S1, ESI†). Their properties were further characterized spectroscopically and electrochemically in deaerated acetonitrile solutions and in PBS buffer solutions (Fig. 1; Fig. S2, S3 and Table S2, ESI†).



Scheme 1 The chemical structures of and the synthetic route to the biscarbene iridium(III) complexes used in this work. (i) NaOMe, 2-ethoxyethanol, 140 °C; (ii) K_2CO_3, 5-methoxypicolinic acid, DMF, 140 °C; and (iii) 2,2′-bipyridine, CH_3OH–CH_2Cl_2, reflux.

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† Electronic supplementary information (ESI) available: Details of experiments; crystal structure and crystallographical data of complex **3**; photophysical studies in aqueous solution; results of electrochemistry; summarized data of photophysical and electrochemical properties; theoretical calculations; MTT assay; flow cytometry studies and the stability study of complex **5** in HeLa cells. CCDC 909214. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc40845k

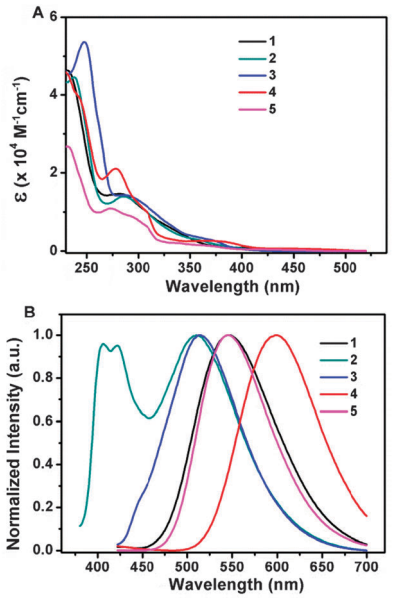

Fig. 1 Absorption (A) and normalized emission (B) spectra of complexes **1–5** in deaerated acetonitrile solutions (40 μM for absorption and emission spectra. λ_{ex} = 360 nm).

As illustrated in Fig. 1A and Table S2 (ESI†), both neutral and cationic biscarbene iridium(III) complexes show very strong intra-ligand absorption bands (π–π*) in the range of 250–300 nm (ε > 10 000 M^{-1} cm^{-1}) and weaker MLCT transition bands from 350 to 450 nm. Such a feature is similar to those of other heteroleptic iridium(III) complexes reported earlier.[16,19,20,36] While the electron-withdrawing effect of $-CF_3$ and –CN can hardly be linked to the features of the absorption spectra, it did have a consistent impact on the emission of these complexes. In both neutral (**1–3**) and cationic (**4–5**) series, the introduction of the substituents (–CN, $-CF_3$) into the main ligand shifts the emission maxima hypsochromically. However, no general trend was found in aqueous solution with respect to the substitution effect.

Table 1 Cytotoxicity (IC_{50}, 24 hours, towards HeLa and A549 cell lines) and lipophilicity ($\log P_{o/w}$) of **1–5**

	IC_{50} (μM)		
Complex	HeLa cell	A549 cell	$\log P_{o/w}$
1	117.03 ± 1.08	>200	1.33
2	73.61 ± 0.89	>200	1.28
3	>200	>200	1.57
4	109.83 ± 0.48	120.30 ± 1.26	0.89
5	49.80 ± 0.92	62.57 ± 0.93	0.56

As for the electrochemical properties (Fig. S3 and Table S2, ESI†), the neutral compounds **1–3** displayed a reversible one-electron oxidation wave at 0.43, 0.71 and 0.74 V, respectively. For the cationic complexes **4** and **5**, although the electrochemical oxidation occurred, the reversibility is poor at a potential scan rate of 100 mV s^{-1}. On the other hand, both **4** and **5** displayed a reduction wave *ca.* −1.8 V with a high reversibility.

Before exploring their use as imaging reagents, we evaluated the toxicity and lipophilicity of these NHC complexes. The cytotoxicity of **1–5** toward HeLa and A549 cell lines has been studied using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cell viability after exposure to the complexes for 24 hours *versus* the concentration of **1–5** is shown in Fig. S4 in ESI† and the half maximal (50%) inhibitory concentration (IC_{50} values) are listed in Table 1. All these complexes exhibit low cytotoxicity. **2** and **5** (both having $-CF_3$ groups on the main ligands) show the highest toxicity toward the HeLa cell line, but compound **2** did not show the same toxicity toward the A549 cell line. The lipophilicity ($\log P_{o/w}$ value) is an important factor for an imaging reagent to permeate through the cell membranes. It was measured by the relative solubility of a compound in oil and water (*n*-octanol–PBS, see Table 1). The $\log P_{o/w}$ values of **1–5** range from 0.56 to 1.57, similar to many other cyclometalated iridium(III) complexes with good cell permeability.[28,37–39] The neutral compounds (**1–3**) have larger values than the cationic compounds (**4–5**). It should be noted that although complex **5** has higher toxicity among these complexes, for imaging studies at a concentration of 20 μM, the cellular viabilities are still assessed to be greater than 75% according to Fig. S4 (ESI†). Based on the toxicity and lipophilicity, we consider these luminescent compounds to be suitable for living cell imaging.[28,37–39]

The imaging experiments with HeLa cells are reported here. HeLa cells were incubated with fresh Dulbecco's Modification Eagle's Medium (DMEM, with 1% pen/strep and 10% FBS) containing 20 μM iridium(III) complexes (in DMSO/culture medium, 1/99, v/v) for 2 hours at 37 °C, and then washed gently with PBS (1 mL × 3), the images were taken using a Nikon A1R confocal laser scanning microscope. According to the emission spectra shown in Fig. 1B, we selected two channels (500–550 nm of FITC for **2**, **3**, **5** and 570–620 nm of TRITC for **1**, **4**) to investigate the cell-imaging under 403 nm excitation. The luminescent images of HeLa cells co-incubated with 20 μM iridium(III) complexes for 2 hours at 37 °C are shown in Fig. 2, which shows that the intracellular distribution of these five novel iridium(III) complexes with NHC ligands is mainly in the cytoplasm, rather than in the nucleus and membrane. As expected, the luminescence intensity remained unchanged in the cell incubation experiments for at least 24 hours (Fig. S6, ESI†). Further temperature-dependence experiments using flow cytometry

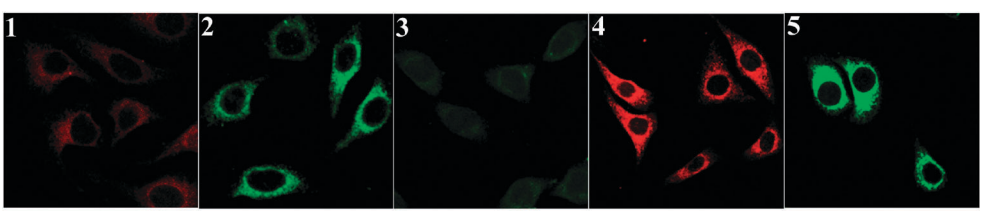

Fig. 2 Fluorescence images of HeLa cells incubated with complexes **1–5** for 2 hours at 37 °C.

Table 2 Mean emission intensities of HeLa cells incubated with blank medium and the iridium(III) complexes (20 μM) at 37 °C and 4 °C for 2 hours as determined using flow cytometry

	Mean emission intensity after incubation	
Complex	At 37 °C	At 4 °C
1	4352	61
2	4495	60
3	4267	60
4	5956	60
5	6491	56
Blank medium	60	55

(the results are shown in Table 2) demonstrate that living HeLa cells incubated with complexes **1–5** (20 μM) for 2 hours at 37 °C displayed intense intracellular luminescence. However, almost no intracellular luminescence was observed when the same incubation was carried out at 4 °C, suggesting that an energy-dependent process is the cellular uptake mechanism of these five complexes with NHC ligands.

It is worth noting that while the cyclometalated iridium(III) complexes are being extensively studied as imaging reagents, the research studies have been predominantly directed towards the cationic compounds with ancillary N^N bidentate ligands. The electronically neutral iridium(III) complexes were rarely reported.[40–42] The first one, bis(2-phenylbenzothiazole)(acetyl-acetonato)iridium(III), was reported by Wu *et al.*[40] Through the introduction of functional groups, Steunenberg *et al.*[41] have succeeded in increasing the cellular uptake of the neutral tris(2-phenylpyridine)iridium(III) complex and thus observed the luminescence of the amino acid-functionalized homoleptical neutral complexes from living cells. Our results demonstrated that without the need for ligand functionalization, the neutral carbene complexes, **1–3**, can penetrate the cell membranes and stain the cytoplasm exclusively.

In summary, a series of iridium(III) biscarbene complexes with an ancillary ligand were synthesized and characterized photophysically and electrochemically. Based on their emissive and biological properties, we demonstrated the applicability of these luminescent metal carbene complexes as multicolor living cell imaging reagents. The low cytotoxicity and good cell-permeability make biscarbene iridium(III) complexes, cationic and neutral, promising for further development.

The financial support for this work from National Science Foundation of China (NSFC) under Project No. 20902066, 20975074 and 21072218 is gratefully acknowledged. The authors thank Anxin Wang and Rui Cao for flow cytometry studies.

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