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Synthesis of water soluble PEG-functionalized iridium complex *via* click chemistry and application for cellular bioimaging

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ABSTRACT

A water soluble iridium (III) complex was prepared *via* click chemistry. It shows the bright red phosphorescence centered at 625 nm with a quantum yield of ~1.4% in the phosphate buffered saline (PBS) solution. Furthermore, it has low cytotoxicity, good membrane permeability and exclusive staining in cytoplasm, which can be an excellent promising candidate for the design of new generation luminescent bioprobes.

Fluorescence imaging is a powerfull technique which provides subcellular level information that cannot be resolved by ultrasound or magnetic resonance imaging [1–4]. Up to now, most of the fluorescent probes are organic fluorescent dyes, but there are some limitations because of their small stoke shift, easy photobleaching and interfering by autofluorescence [5]. Compared with organic fluorophores, phosphorescent complexes based on metal-to-ligand-charge-transfer mechanism (MLCT) have become the better candidate for bioimaging due to their large stoke shift, long life time and good stability [6–11].

Among the various phosphorescent complexes with MLCT, iridium complexes have been investigated in detail due to their versatile photophysical and photochemical properties [12,13]. Therefore, they have been applied in many fields such as organic light-emitting diodes, [14–16] solar energy conversion devices [17] and phosphorescent chemosensors [18–26]. For the application in luminescent imaging, Lo et al. reported the first example of luminescent cyclometalated iridium (III) complexes as labeling reagents for biological substrates [27]. Up to date, more detailed investigations in this field have been reported [28–32]. For example, Li et al. demonstrated two cationic iridium (III) complexes as phosphorescent dyes with good stability for imaging in living cells [33]. In order to improve the solubility of iridium (III) complexes in water, Bian et al. presented zwitterionic iridium complexes for cell imaging instead of cationic complexes [34].

Generally, luminescent probes should be water-dispersible, has low cytotoxicity and biocompatible. One of the most common methods is to combine a water soluble polymer with luminescent probes. Poly(ethylene glycol) (PEG) is a biocompatible polymer and has been widely regarded as a typical non-toxic, non-immunogenic, protein-resistant polymer [35]. Although π-conjugated polymer with iridium complexes has been widely applied in the field of organic light-emitting diodes, to the best of our knowledge, few reports of the synthesis and application of phosphorescent polymers containing iridium (III) complexes for cellular bioimaging have been published [36,37]. Click chemistry is a chemical philosophy introduced by K. Barry Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together [38]. In this paper, we present a strategy to prepare water soluble iridium (III) complexes containing PEG polymer as phosphorescent dyes for exclusive staining in the cytoplasm of living cells by click chemistry.

The synthetic route toward the water soluble iridium complex is outlined in Scheme 1. Click chemistry was used to attach the PEG chain to iridium (III) complexes. As shown in the IR spectrum of PEG-N_3 (Fig. 1), the strong absorption characteristic of the azide groups at 2102 cm^{-1} was observed, demonstrating that PEG-N_3 was obtained quite efficiently. After click reaction, the IR spectrum of complex 1 showed a strong and sharp C–O stretch ether linkage at 1107 cm^{-1}, which was due to the presence of the polyether backbone of PEG, while other important peaks were at 1587 cm^{-1} (weak, $\nu_{N=N}$), which confirmed the success of conjugation [39,40]. It is important that the strong absorption characteristic of the azide groups at

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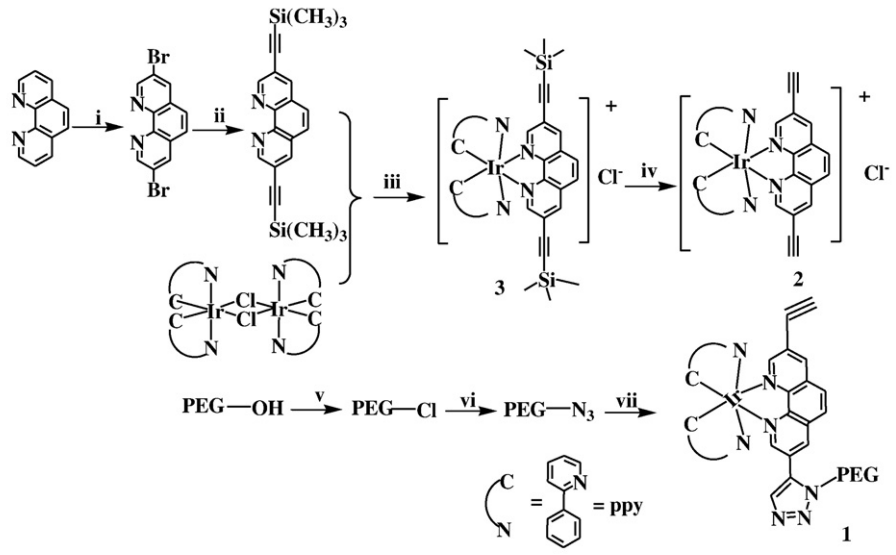

Reagents and conditions: (i) pyridine, S_2Cl_2, Br_2, 1-chlorobutane, 82°C, 46%; (ii) $Pd(pph_3)_4$,CuI, trimethylsilylacetylene, tetrahydrofuran-triethylamine (3:1 v/v), 85%; (iii) $[Ir_2(ppy)_4Cl_2]$, CH_2Cl_2/CH_3OH (2:1 v/v), reflux, 46%; (iv) $KF{\cdot}2H_2O$, CH_3OH, room temperature; (v) $SOCl_2$, 65°C, 73%; (vi) NaN_3, 100°C; (vii) complex 2, $CuSO_4$, sodium ascorbate, DMF/H_2O (2:1 v/v), 35°C, 85%;

Scheme 1. Synthesis of water soluble PEG-functionalized iridium complex 1.

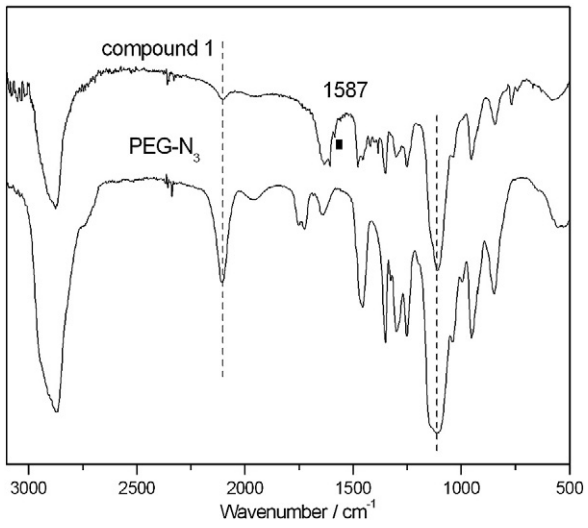

Fig. 1. FTIR spectra of PEG-N_3 and complex 1 at room temperature.

2102 cm^{-1} disappeared, and the typical weak absorption of 2110 cm^{-1} suggested the presence of –C≡CH group in complex 1. Combined IR and GPC data showed that one of the two alkynyl groups of complex 2 is conjugated by the click reaction.

The photophysical properties of 1 in $CHCl_3$, EtOH, THF and PBS were investigated. The data are summarized in Table 1. Fig. 2 shows the UV–vis absorption spectra recorded in different solvents. Complex 1 displays intense absorption bands below 370 nm in different solvents, which are assigned to spin-allowed intraligand LC ($\pi \rightarrow \pi^*$) transitions. The absorption bands in the lower energy region, starting around 370 nm and extending to around 600 nm, are observed. It is resulted from the weak and broad metal-to-ligand-charge-transfer (MLCT) transition of the iridium complexes. From the room temperature photoluminescence spectra in $CHCl_3$, EtOH and THF excited by 370 nm, the broad and structureless emission bands centered at 592 nm, while the emission peak shifted to 625 nm in the PBS solution. The phosphorescence quantum efficiencies in the PBS solution is 1.4% [41].

Generally, the cytotoxicity of complex 1 is critical to the biomedical application. The cytotoxicity of complex 1 was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay with the KB cell lines. The concentration-dependent effect of compound 1 on the cell viability at 12 and 24 h was determined as presented in Fig. 3. It is clear that the high cell viability can still be attained even at a concentration of 100 μM. This indicates that complex 1 can be considered to have low cytotoxicity and may be applied in the field of biology and medicine.

Due to the low energy MLCT absorption and emission properties in the visible region, it is convenient for bioimaging in living cells. After incubation of the KB cells with complex 1 at 37 °C under a 5% CO_2 atmosphere for 10 min, the efficient interiorization of 1 was observed by the laser-scanning confocal microscopy with an excitation wavelength at 488 nm as shown in Fig. 4a. The overlay of confocal luminescence and brightfield images confirmed that complex 1 was mainly located in the cytoplasm of living cells.

In conclusion, combined with click chemistry, a water soluble iridium (III) complex has been obtained with the incorporation of PEG polymer chain onto the neutral ligand. Its photophysical property in different solvent was investigated in detail. More importantly, it

Table 1
Photophysical data of compound 1 in $CHCl_3$, EtOH, THF and PBS at room temperature.

Solvent	Absorption (λ, nm)	Emission	
		(λ_{max}, nm)	φ^a (λ_{ex}, nm)
$CHCl_3$	272, 507	592	0.072 (365)
EtOH	271, 449	592	0.033 (295)
THF	270, 526	592	0.030 (325)
PBS	270, 410	625	0.014 (310)

[a]The quantum efficiency was measured with $[Ru(bpy)_3]Cl_2$ as an external standard ($\varphi = 2.8\%$).

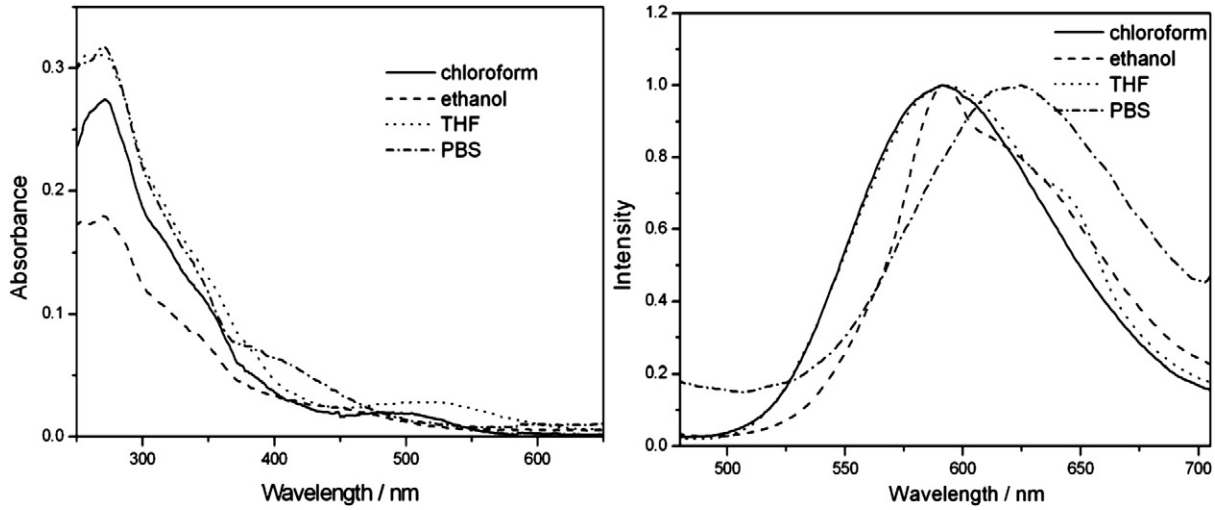

Fig. 2. UV–vis absorption spectra (Left) and normalized emission spectra (right) of 1 in different solvents at room temperature.

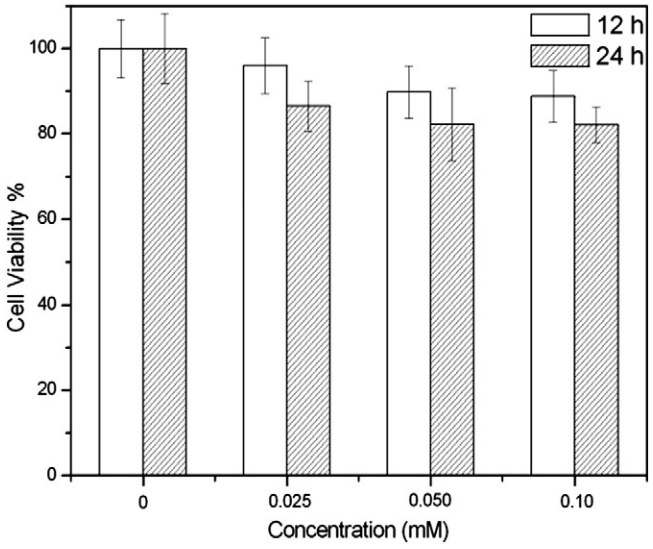

Fig. 3. In vitro cell viability of KB cells incubated with complex 1 with different concentrations for 12 and 24 h at 37 °C.

shows the bright red phosphorescence in the PBS buffer solution. Furthermore, the fast uptaking by live cells and low cytotoxicity make complex 1 a potentially useful tool in cytoplasm-related studies.

Acknowledgments

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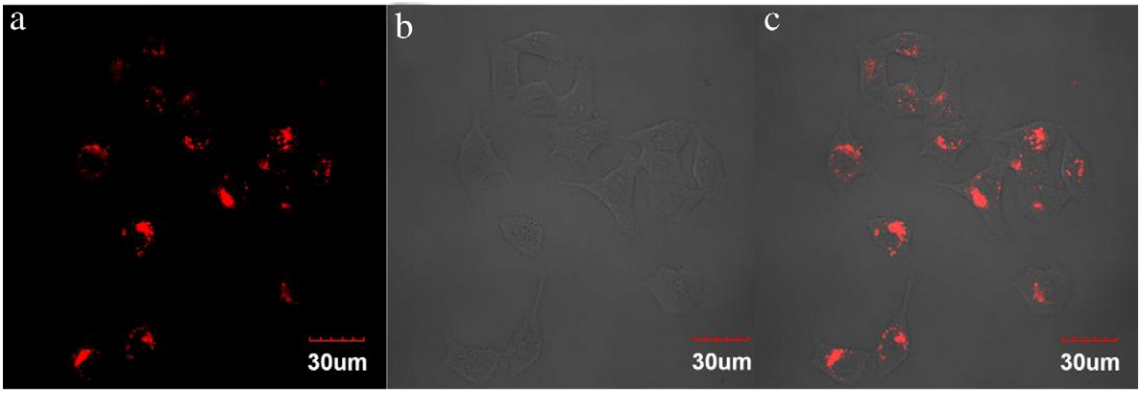

Fig. 4. Confocal luminescence (a) and brightfield image (b) of living KB cells incubated with 0.1 mM 1 in PBS for 10 min at 37 °C. Overlays of luminescence and brightfield image are shown in (c) (λex = 488 nm).

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