First Examples of Luminescent Cyclometalated Iridium(III) Complexes as Labeling Reagents for Biological Substrates

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*Summary: The syntheses, and photophysical and electrochemical properties of the three luminescent cyclometalated iridium(III) complexes [Ir(ppy)$_2$(phen-NCS)](PF$_6$) (**1**), [Ir(ppy)$_2$(phen-NHCOCH$_2$I)](PF$_6$) (**2**), and [Ir(ppy)$_2$(phen-NH$_2$)](PF$_6$) (**3**) (ppy$^-$ = 2-phenylpyridinate anion, phen-NCS = 5-isothiocyanato-1,10-phenanthroline, phen-NHCOCH$_2$I = 5-(iodoacetamido)-1,10-phenanthroline, phen-NH$_2$ = 5-amino-1,10-phenanthroline) are reported. Since the isothiocyanate and iodoacetamide groups can react with the primary amine and sulfhydryl groups of biomolecules, respectively, complexes **1** and **2** have been used to label amine- or sulfhydryl-modified oligonucleotides, and human serum albumin, to give luminescent bioconjugates.*

The photophysical properties of mononuclear[1] and binuclear/supramolecular[2] iridium(III) polypyridine complexes have been attracting much attention. The applications of these complexes as sensory materials for oxygen,[3a] protons,[3b] and chloride ions[3c] have also been reported recently. In view of the characteristic photoluminescence properties of iridium(III) polypyridine complexes,[4] we expect that these compounds can serve as promising candidates in various bioanalytical applications. On the other hand, despite labeling of biological substrates such as nucleic acids and proteins with organic dyes being a common procedure these days,[5] there are still disadvantages such as short fluorescence lifetimes, self-quenching effects, high photobleaching rate, and strong pH dependence. In this regard, transition-metal complexes such as those of ruthenium(II), osmium(II), and rhenium(I) have been covalently linked to different biomolecules in electron-transfer studies and other analytical applications.[6a−c] Related studies using the rhodium(III) system, especially on photoinduced electron transfers in biomolecules, have also been reported.[6d,e] However, to the best of our knowledge, the possibility of using luminescent iridium(III) complexes as labeling reagents for biomolecules or as probes for biomolecular structures/interactions has never been explored. We anticipate that this class of complexes can be developed as a new generation of biological labeling reagents because of their intense and long-lived emission, large Stokes shifts, and high stability. The intense emission allows higher detection sensitivity, while the long emission lifetimes of these complexes could be exploited in time-resolved detection techniques. Moreover, larger Stokes shifts are expected to minimize self-quenching effects that are commonly observed in biomolecules labeled with organic fluorophores. Most importantly, compared to their d^6 counterparts, luminescent iridium(III) complexes offer a wider range of emission energy, longer lifetimes, higher luminescence quantum yields, and higher structural variety,[4] which are all attractive advantages for the development of multicolor luminescent biological labels. Here we report the syntheses and photophysical and electrochemical properties of the three novel luminescent cyclometalated iridium(III) complexes [Ir(ppy)$_2$(phen-NCS)](PF$_6$) (**1**), [Ir(ppy)$_2$(phen-NHCOCH$_2$I)](PF$_6$) (**2**), and [Ir(ppy)$_2$(phen-NH$_2$)](PF$_6$) (**3**) (ppy$^-$ = 2-phenylpyridinate anion, phen-NCS = 5-isothiocyanato-1,10-phenanthroline, phen-NHCOCH$_2$I = 5-(iodoacetamido)-1,10-phenanthroline, phen-NH$_2$ = 5-amino-1,10-phenanthroline). The incorporation of the isothiocyanate and iodoacetamide groups into **1** and **2** allows them to react specifically with the primary amine and sulfhydryl groups of biological substrates, respectively.

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Table 1. Photophysical and Electrochemical Data for Complexes 1−3

	emission						electrochemistry[c]	
complex	medium (T/K)	λ_{em}/nm	τ_o/μs	Φ	k_r/s^{-1}	k_{nr}/s^{-1}	oxidn $E_{1/2}$ or E_a/V	redn $E_{1/2}$ or E_c/V
1	CH_2Cl_2 (298)	598	0.77	0.287	3.72×10^5	9.26×10^5	+1.27	−1.21,[d] −1.48
	CH_3CN (298)	608	0.41	0.0787	1.92×10^5	2.25×10^6		
	glass[a] (77)	512, 552, 594 sh[b]	93.52 (32%), 14.58 (68%)					
2	CH_2Cl_2 (298)	584	0.86	0.197	2.29×10^5	9.33×10^5	+1.25	−1.08,[d] −1.37,[d]
	CH_3CN (298)	590	0.59	0.228	3.87×10^5	1.31×10^6		−1.62
	glass[a] (77)	500, 536, 580 sh[b]	22.10 (23%), 4.99 (77%)					
3	CH_2Cl_2 (298)	564	9.52	0.428	4.50×10^4	6.01×10^4	+1.27[d]	−1.45[d]
	CH_3CN (298)	568	11.38	0.0792	6.96×10^3	8.09×10^4		
	glass[a] (77)	560, 602, 658 sh[b]	286.21					

[a] EtOH/MeOH (4:1 v/v). [b] Vibronically structured emission spectra. [c] In CH_3CN (0.1 mol dm^{-3} TBAP) at 298 K, glassy-carbon electrode, sweep rate 500 mV s^{-1}, all potentials vs SCE. [d] Irreversible wave.

Refluxing a mixture of $[Ir_2(ppy)_4Cl_2]$[2a] and phen-NH_2 in CH_2Cl_2/MeOH for 4 h, followed by metathesis with KPF_6 and recrystallization from acetone/diethyl ether, gave orange crystals of $[Ir(ppy)_2(phen\text{-}NH_2)](PF_6)$ (**3**) in 90% yield. Meanwhile, **1** and **2** were obtained, respectively, from the reaction of **3**, $SCCl_2$, and $CaCO_3$ in acetone at room temperature for 2 h and the reaction of **3** and $(ICH_2CO)_2O$ in CH_3CN at room temperature for 24 h. Both **1** and **2** were recrystallized from acetone/diethyl ether and isolated as air-stable orange-yellow crystals (yields 70−80%).[7]

The electronic absorption spectra of **1**−**3** display intense high-energy absorption bands (ϵ on the order of 10^4 dm^3 mol^{-1} cm^{-1}) at ca. 252−286 nm and weaker absorption shoulders at ca. 316−466 nm.[7] With reference to previous photophysical studies on related $[Ir(ppy)_2(diimine)]^+$ systems,[1a,c−e,2b,d,e,4] these absorption features are assigned to intraligand IL ($\pi \rightarrow \pi^*$) (diimine and ppy^-) and spin-allowed metal-to-ligand charge-transfer MLCT ($d\pi(Ir) \rightarrow \pi^*$(diimine and ppy^-)) transitions, respectively. In addition, the spectra also exhibit weaker absorption tails toward the lower energy region (ca. 466−550 nm), attributable to spin-forbidden MLCT ($d\pi(Ir) \rightarrow \pi^*$(diimine and ppy^-)) transitions.

Upon photoexcitation, the complexes exhibit intense and long-lived orange-yellow luminescence in fluid solutions at room temperature and in low-temperature glass (Table 1). The emission lifetimes at room temperature fall in the microsecond to sub-microsecond range and are significantly reduced in the presence of oxygen, indicating the phosphorescence nature of the emission. In view of the presence of low-lying π^* orbitals of the diimines, we assign the solution emission to originate from a charge-transfer CT triplet excited state involving π^*(diimine) as the acceptor orbitals.[1a,b,d,e,2b] Such an assignment is in agreement with the finding that the electron-withdrawing isothiocyanate and iodoacetamide groups render the emission of **1** and **2** to occur at lower energy than that of **3**, which contains an electron-donating amine group on the diimine ligand (Figure 1). This assignment is also in line with the electrochemical data (Table 1), which reveal that both **1** and **2** exhibit first reduction waves at less negative potentials than that of **3**. These waves are ascribed to reductions of the diimines[1a,c−e,2b,d,e] instead of the ppy^- ligands, since

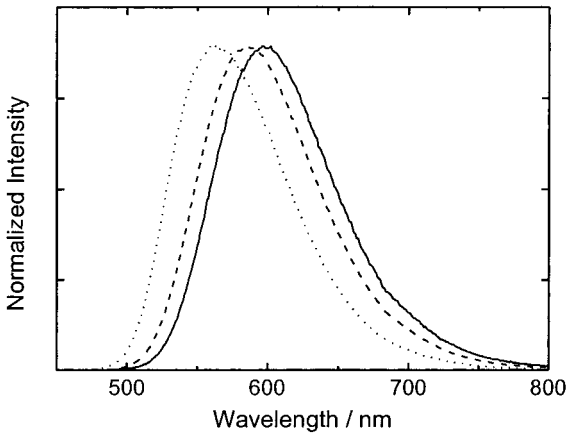

Figure 1. Emission spectra of **1** (—), **2** (- - -) and **3** (···) in degassed CH_2Cl_2 at 298 K.

reduction of the latter is well-known to occur at a more negative potential (ca. −2.0 V vs SCE).[1d,e,2b,e] The irreversible reduction wave for **2** at ca. −1.08 V vs SCE, which is absent for the other two complexes, is likely to be associated with the reduction of the iodoacetamide moiety. Nonetheless, further assignments of the reduction waves have not been attempted in view of their irreversible nature. Electrochemical studies also show that complexes **1** and **2** exhibit reversible oxidation couples at ca. +1.26 V vs SCE, typical of iridium(III)-based oxidations (Table 1).[1a,d,e,2b] An assignment of ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*$(diimine)) triplet emissive state is therefore proposed for these two complexes in consideration of their HOMOs being dominated by metal-based character.[1a,b,d,e,2b]

It is interesting to note that while both **1** and **2** display reversible iridium(IV/III) couples, the oxidation wave of **3** is irreversible with a sweep rate between 0.01 and 5 V s^{-1}. The irreversibility of this oxidation wave suggests the involvement of the coordinated phen-NH_2 ligand in the oxidation process.[8] On the other hand, irreversible oxidation waves have also been observed for $[Ir(ppy)_2(HAT)]^+$ (HAT = 1,4,5,8,9,12-hexaazatriphenylene) and related complexes,[1c,2e] for which a HOMO with substantial Ir−C σ-bond character has been identified and an emissive state of σ-bond-to-ligand charge-transfer (SBLCT) (σ(Ir−C) $\rightarrow \pi^*$ (diimine)) has been assigned.[1c,2e] Nevertheless, in view of the remarkably long emission

(7) Characterization and electronic absorption spectral data for complexes **1**−**3**, oligonucleotide- and HSA-labeling procedures, and the photophysical data for the duplexes (**1-M13-R**)·(**M13-R-comp**) and (**2-M13-R**)·(**M13-R-comp**) are given in the Supporting Information.

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lifetimes of **3** that result in much smaller radiative decay rate constants[9] compared to those of **1**, **2**, and other $[Ir(ppy)_2(diimine)]^+$ MLCT systems[1d,e] and the absence of a significant red shift in emission from low-temperature rigid glass to room-temperature fluid solutions, which is very commonly observed for cyclometalated iridium(III) diimine MLCT and SBLCT emitters,[1,2a,b,e] we believe that the excited state of **3** responsible for the room-temperature emission is mixed with ligand-centered (diimine) character.[1f,3b,c] For the low-temperature glass emission, both **1** and **2** exhibit dual luminescence decays. The possibility of the presence of impurities in the samples is ruled out on the basis of the characterization data of the complexes. The observation of the dual emission decays is likely to result from two close-lying nonequilibrated charge-transfer states, $^3[d\pi(Ir) \rightarrow \pi^*(diimine)]$ and $^3[d\pi(Ir) \rightarrow \pi^*(ppy^-)]$. Similar observations have also been reported in the photophysical studies of related luminescent cyclometalated iridium(III) diimine systems at low temperature.[1b] However, for the case of complex **3**, a single-exponential decay with a longer lifetime (286.21 μs) is observed. It is conceivable that similar charge-transfer states of **3** are shifted to higher energy at low temperature, and the lowest energy emissive state of this complex in a 77 K glass is dominated with ligand-centered (diimine) triplet character, resulting in long-lived emission with a single-exponential decay.

Complexes **1** and **2** have been used to label a universal M13 reverse sequencing primer, **M13-R** (5′-AACAGCTATGACCATG-3′), modified with an amine or a sulfhydryl group via a C_6 linker at the 5′ end.[7] Although the insolubility of free complexes **1** and **2** in water precludes the possibility of studying their photophysical properties in aqueous solutions, the labeled DNA species are soluble in aqueous buffer and exhibit intense and long-lived orange-yellow photoluminescence (in degassed 50 mM Tris-Cl pH 7.4, $\lambda_{ex} = 355$ nm; **1-M13-R** emits at 580 nm, biexponential decay $\tau_1 = 0.51$ μs (35%), $\tau_2 = 0.16$ μs (65%); **2-M13-R** emits at 572 nm, biexponential decay $\tau_1 = 0.44$ μs (15%), $\tau_2 = 0.06$ μs (85%)). The observation of double-exponential and multiexponential emission decays is not uncommon for luminescent inorganic complexes attached to biomolecules such as oligonucleotides and proteins.[10] The labeled oligonucleotides are hybridized with the unmodified complementary oligonucleotide **M13-R-comp** (5′-CATGGTCATAGCTGTT-3′). The melting temperatures of the duplexes (**1-M13-R**)·(**M13-R-comp**) and (**2-M13-R**)·(**M13-R-comp**) show a decrease of ca. 4 °C, indicative of little influence of the labels on the hybridization. The photophysical data of the duplexes are similar to those of the labeled probes,[7] and we tentatively assign the emission to arise from an ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*(diimine)$) excited state.

Human serum albumin (HSA) has also been labeled with **1** and **2**.[7] Under our reaction conditions, iridium-to-protein ratios of ca. 1.6 and 0.4 are obtained for the conjugates **1-HSA** and **2-HSA**, respectively. Irradiation of **1-HSA** and **2-HSA** at 355 nm in degassed 50 mM Tris-Cl pH 7.4 at 298 K results in intense orange-yellow emission (**1-HSA** $\lambda_{em} = 562$ nm, biexponential decay $\tau_1 = 0.90$ μs (33%), $\tau_2 = 0.15$ μs (67%); **2-HSA** $\lambda_{em} = 570$ nm, biexponential decay $\tau_1 = 0.66$ μs (19%), $\tau_2 = 0.07$ μs (81%)). The emission of the conjugates is suggested to originate from an MLCT ($d\pi(Ir) \rightarrow \pi^*(diimine)$) triplet excited state. It is interesting to note that while the emission wavelengths of **2-M13-R** (572 nm), (**2-M13-R**)·(**M13-R-comp**) (570 nm), and **2-HSA** (570 nm) in aqueous buffers are all very similar, there is a blue shift in emission energy from the oligonucleotides **1-M13-R** (580 nm) and (**1-M13-R**)·(**M13-R-comp**) (580 nm) to **1-HSA** (562 nm). Although the reasons for this observation are not completely known, in view of the solvatochromism (λ_{em} at higher energy in a more nonpolar solvent) exhibited by the free complexes **1** and **2** (Table 1) and the hydrophobicity associated with HSA, a possible explanation is that the HSA molecule provides a more hydrophobic environment for **1** than **2** and/or the luminescent labels in **1-HSA** are more sensitive to changes in the local surroundings than those in **2-HSA**. Finally, we notice that, in contrast to the free labels, the lifetimes of the serum conjugates **1-HSA** and **2-HSA** exhibit only a small decrease in the presence of oxygen (**1-HSA** $\tau_1 = 0.83$ μs (29%), $\tau_2 = 0.12$ μs (71%); **2-HSA** $\tau_1 = 0.60$ μs (14%), $\tau_2 = 0.06$ μs (86%)). It appears that the labels conjugated to HSA are well-shielded within the protein matrix and a low exposure to the solvent environment results in inefficient quenching by the oxygen molecules.[10b−e]

The present work describes the first utilization of luminescent iridium(III) polypyridine complexes as labeling reagents for biomolecules. Varying the diimine and cyclometalating ligands is expected to generate a series of multicolor labeling reagents. Applications of these luminescent labels in different bioanalytical applications will be investigated.

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Supporting Information Available: Text giving characterization and electronic absorption spectral data of complexes **1**−**3**, oligonucleotide- and HSA-labeling and purification procedures and the photophysical data of the duplexes (**1-M13-R**)·(**M13-R-comp**) and (**2-M13-R**)·(**M13-R-comp**). This material is available free of charge via the Internet at http://pubs.acs.org.

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