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A Novel Free Radical Probe Based on a Preluminescent Iridium Complex Bearing a Nitronyl Radical Moiety

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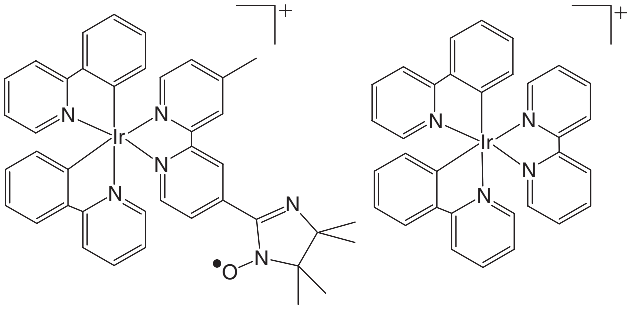
A novel iridium complex with an iminonitroxide bipyridine ligand, [Ir(ppy)$_2$(MebpyIN)]Cl (ppy = 2-phenylpyridine and MebpyIN = 4-methyl-4′-(1-oxyl-4,4,5,5-tetramethylimidazolin-2-yl)-2,2′-bypyridine), was prepared as a preluminescent probe to detect free radicals. Although a related material, [Ir(ppy)$_2$(bpy)]Cl, exhibits strong red luminescence, the emission of [Ir(ppy)$_2$(MebpyIN)]Cl was quenched nearly completely by the nitronyl radical moiety in the ligand. However, the carbon-centered free radicals, generated from azobisbutyronitrile by UV irradiation, brought about a dramatic recovery of the luminescence of [Ir(ppy)$_2$(MebpyIN)]Cl, with a linear relation between the luminescence intensity, and the free radical concentrations up to 120 mM. EPR measurements indicated that the luminescence recovery was caused by the free radical trapping at the nitronyl-radical moiety.

Recent attention has been paid to the detection of free radical species in various research fields such as biomedical science/technology. It is important to perform in situ analysis of NO and OH radicals in biological systems and to elucidate their biochemical functions in living cells.[1–4] Among the various techniques of free radical detection,[5] namely, EPR (electron spin resonance spectroscopy),[6] luminescence,[7–10] UV–vis spectroscopy,[11] electrochemical methods,[12,13] etc., luminescence measurements are the most analytically sensitive, as well as being cheap and convenient. Indeed, various photoluminescence-based sensors and probes have been developed to directly monitor free radicals.[14,15] For example, Scaiano et al. have prepared prefluorescent probes for radical detection in homogeneous and heterogeneous systems,[16–18] combining fluorophores such as quinoline derivatives[19] and CdSe nanoparticles,[20,21] and a nitronyl free radical that is covalently bound to the fluorophores. The nitronyl moiety quenches the fluorescence, and the interactions between this radical moiety and carbon-centered free radicals bring about recovery of the fluorescence.

Photoemissions of iridium complexes such as [Ir(ppy)$_2$(bpy)]$^+$ (**2**, ppy = 2-phenylpyridine, Figure 1), have been intensively studied because of their high quantum yields.[22,23] These materials have potential application to emitters in electroluminescent devices[24–28] and to sensors/probes for singlet oxygen[29–31] and biomolecules such as histidine.[32] In the present work, we synthesized a photofuctional iridium complex possessing a radical ligand, [Ir(ppy)$_2$(MebpyIN)]$^+$ (**1**, MebpyIN = 4-methyl-4′-(1-oxyl-4,4,5,5-tetramethylimidazolin-2-yl)-2,2′-bypyridine, Figure 1), and examined the performance of this iridium complex as a probe for carbon-centered free radicals.



1 **2**

Figure 1. Chemical structures of [Ir(ppy)$_2$(MebpyIN)]$^+$ (**1**) and [Ir(ppy)$_2$(bpy)]$^+$ (**2**).

Experimental

Materials. MebpyIN was prepared from 4-formyl-4′-methyl-2,2′-bipyridine and 2,3-bis(hydroxyamino)-2,3-dimethylbutane, using a conventional method in the literature.[33] The reaction of the chloride-bridged iridium dimer complex [Ir(ppy)$_2$Cl]$_2$ with MebpyIN ligand led to the synthesis of the mononuclear iridium complex **1**•Cl. For control experiments, we also prepared a well-known iridium complex [Ir(ppy)$_2$(bpy)]Cl (**2**•Cl).[34,35]

4-Methyl-4′-(1-oxyl-4,4,5,5-tetramethylimidazolin-2-yl)-2,2′-bypyridine (MebpyIN). A mixture of 2,3-bis(hydroxyamino)-2,3-dimethylbutane (0.35 g, 2.39 mmol) and 0.21 g of 4-formyl-4′-methyl-2,2′-bipyridine (1.06 mmol) in 25 mL of methanol was stirred at 100 °C for 3 days. The precipitate was filtered off to obtain a precursor MebpyNOH [4-methyl-4′-(1,3-dihydroxy-4,4,5,5-tetramethylimidazolin-2-yl)-2,2′-bipyridine]. To a suspension of MebpyNOH (110 mg, 0.33 mmol) in dichloromethane (12 mL) was added an aqueous solution (12 mL) of sodium periodate (65 mg, 0.30 mmol). The biphasic mixture was stirred for 1 h. The product was extracted with dichloromethane, and the organic layer was dried over magnesium sulfate. After chromatography (silica, $CH_3CO_2C_2H_5$),

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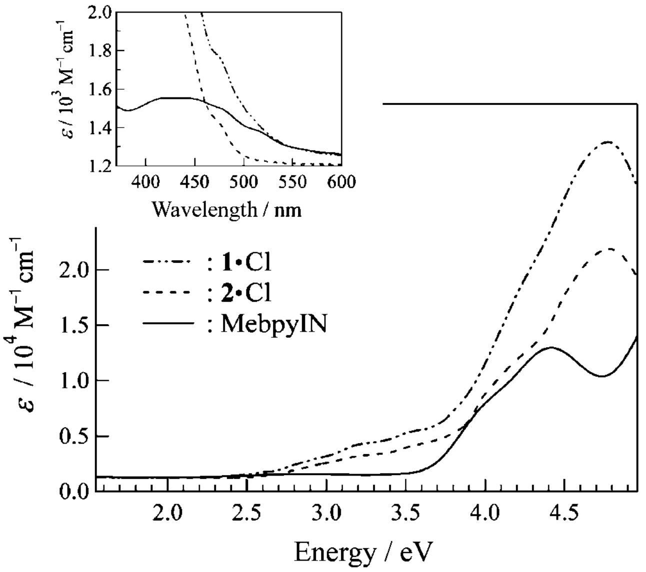

Figure 2. UV–vis absorption spectra (molar extinction coefficient vs. Energy) of [Ir(ppy)$_2$(MebpyIN)]Cl (**1**•Cl), [Ir(ppy)$_2$(bpy)]Cl (**2**•Cl), and MebpyIN at 0.025 mM in acetonitrile solution. The inset shows a magnification of the absorption spectra (molar extinction coefficient vs. wavelength) in the region between 380 and 600 nm.

the red fraction was evaporated and red powders of MebpyIN were obtained in 30% yield. ESR (room temperature, dichloromethane): $a_{N1} = 8.8$ G, $a_{N2} = 4.4$ G at $g = 2.0048$, FAB$^+$ m/z 310.39 [M + H$^+$].

[Ir(ppy)$_2$(MebpyIN)]Cl (1•Cl). [Ir(ppy)$_2$Cl]$_2$ (50.0 mg, 0.12 mmol) and MebpyIN (78.2 mg, 0.24 mmol) were dissolved in dichloromethane (5 mL), and the solution was stirred for 12 h under nitrogen at ambient temperature. The solvent was then removed on a rotary evaporator, and the remaining solids were washed with diethyl ether and hexane. The crude product was recrystallized from a mixture of dichloromethane and toluene to give 20.8 mg of **1**•Cl (20.3% yield). EPR (room temperature, dichloromethane): $a_{N1} = 8.8$ G, $a_{N2} = 4.4$ G at $g = 2.0048$, ESI-MS (in acetonitrile) m/z 810.27 [M + H$^+$], Calcd for [$C_{40}H_{37}IrN_6O$]$^+$: 809.84, Elemental analysis: Calcd for $C_{40}H_{37}ClIrN_6O_2 \cdot 1.6CH_2Cl_2 \cdot 0.1C_6H_5CH_3$: C, 50.5; H, 4.1; N, 8.3%. Found: C, 50.7; H, 3.9; N, 8.0%.

Physical Property Measurements. Photoluminescence and UV–vis absorption spectra were recorded on a Hitachi F-4500 and a JASCO V-570 spectrometer, respectively. EPR measurements were carried out using a JEOL X-band spectrometer JES-FA200. Electrospray ionization (ESI) and fast-atom bombardment (FAB) mass spectra were measured on a QSTAR (PE Biosystems) and a JMS-700 (JEOL) mass spectrometer, respectively.

Results and Discussion

The radical-ligand iridium complex **1**•Cl was prepared by the reaction of the chloride-bridged iridium dimer complex [Ir(ppy)$_2$Cl]$_2$ with MebpyIN. Though the crystal growth of **1**•Cl was unsuccessful, we indentified the compound by mass spectroscopy, UV–vis absorption spectra, and EPR. The results of the EPR and mass spectroscopy are shown in the experimental section. Figure 2 shows the UV–vis absorption spectra of the complexes, **1**•Cl and **2**•Cl, and the free radical, MebpyIN, at 0.05 mM in acetonitrile. The inset of this figure shows the spectra in the range of 380–600 nm on an enlarged scale. MebpyIN exhibits a broad absorption around 450 nm, which is ascribable to an n → π* transition. The absorption of **1**•Cl can be essentially understood as a superposition of the absorptions of MebpyIN and **2**•Cl. Figure 3 provides a comparison between the solution EPR spectra of **1**•Cl and MebpyIN in dichloromethane at room temperature. These spectra agree with each other, commonly showing a typical seven-line hyperfine structure of the iminonitronyl radical. These results suggest that there is no significant electronic interaction between the iridium core and the iminonitronyl moiety in the ground state.

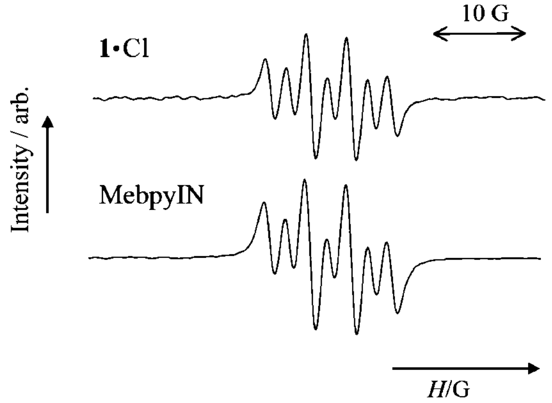

Figure 3. EPR spectra of [Ir(ppy)$_2$(MebpyIN)]Cl (**1**•Cl) and MebpyIN at 0.05 mM in dichloromethane solution.

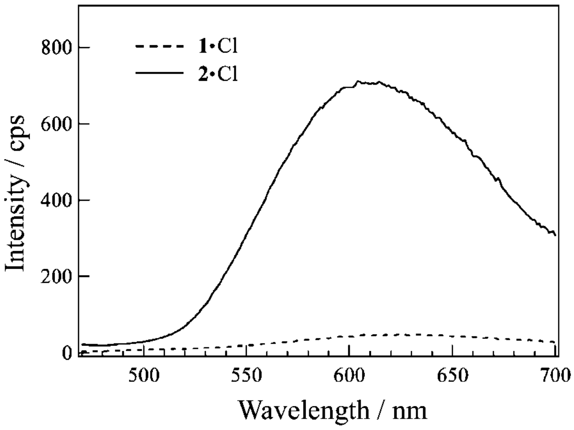

Figure 4. Luminescence spectra of [Ir(ppy)$_2$(MebpyIN)]Cl (**1**•Cl) and [Ir(ppy)$_2$(bpy)]Cl (**2**•Cl) at 0.003 mM in acetonitrile solution.

Figure 4 shows the luminescence spectra for **1**•Cl and **2**•Cl in acetonitrile solution at room temperature ($\lambda_{ex} = 362$ nm). The compound **2**•Cl exhibits an intense, structureless luminescence band at $\lambda_{max} = 611$ nm, which has been assigned to an admixture of ^{3}MLCT and π → π* transitions;[34,35] in contrast, the luminescence of **1**•Cl is suppressed almost completely. It is clear that the luminescence of the iridium complex is efficiently quenched by the iminonitronyl radical moiety in the MebpyIN ligand, while quenching of the triplet

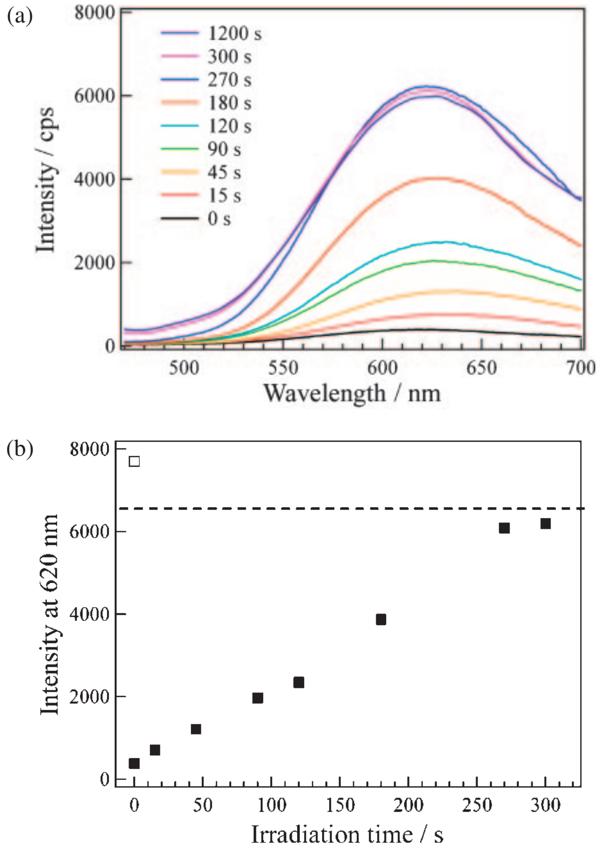

Figure 5. Radical-mediated luminescence recovery of the acetonitrile solutions of 0.3 mM [Ir(ppy)$_2$(MebpyIN)]Cl (**1**·Cl) and 30 mM AIBN with the UV irradiation time. (a) Luminescence spectra with each irradiation time. (b) Change in the luminescence intensities at 620 nm with irradiation time (solid square). The open square at 0 s shows the luminescence intensity of complex **2**·Cl, and the broken line indicates the saturation value obtained from Figure 5a.

state in the iridium complexes by nitronyl radicals has not yet been reported. In general, there are two possible mechanisms for luminescence quenching: intramolecular energy transfer and electron transfer. Regarding **1**·Cl, however, energy transfer is not a cause of the quenching because the broad n → π^* transition in MebpyIN around 450 nm has no overlap with the luminescence band of the iridium complex.

We elucidated **1**·Cl as a preluminescent probe for free radicals; we generated carbon-centered free radicals by UV irradiation in the solutions of **1**·Cl and examined the effects of the free radicals on its photoemissions. We adopted azobisbutyronitrile (AIBN) as a free radical source, which is well known to generate carbon-center radicals photochemically or thermally.[18] An acetonitrile solution including 0.3 mM **1**·Cl and 30 mM AIBN was irradiated with a xenon lamp ($\lambda_{\max} =$ 340 nm). These experiments were carried out in an ice bath in order to prevent thermal decomposition of the iridium complexes and the generated radicals. Figure 5a illustrates the recovery of the luminescence against the exposure time. The luminescence intensity shows a significant increase under irradiation, with little change in the line shape of the emission band. Figure 5b shows a plot of the luminescence intensities at 620 nm versus the UV irradiation time, together with the emission intensity of **2**·Cl (open square). The intensity shows a linear increase up to 270 s, at which point it is comparable to that of **2**·Cl; it then exhibits a saturation that is maintained even after 1200 s. These results are indicative of the stability of the luminescent compound, even with increases in the number of radical species under illumination.

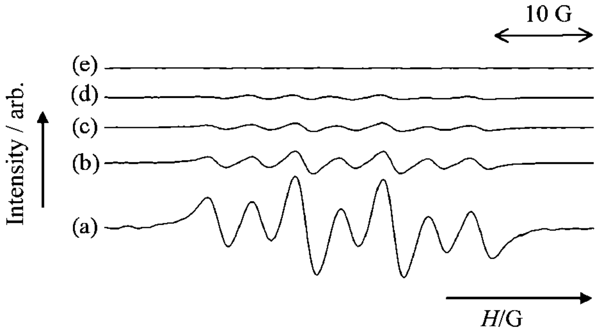

Figure 6. EPR spectra of the acetonitrile solutions of 0.3 mM [Ir(ppy)$_2$(MebpyIN)]Cl (**1**·Cl) and 30 mM AIBN after UV irradiation times of (a) 0, (b) 30, (c) 90, (d) 270, and (e) 900 s.

Figure 6 shows the effects of photoirradiation on the EPR spectra of an acetonitrile solution, including 0.3 mM **1**·Cl and 30 mM AIBN. The EPR shows a gradual decrease in intensity, without a change in line shape, *g*-factor, or hyperfine coupling constants. This suggests an annihilation of the iminonitronyl radicals by the carbon-center radicals generated by UV irradiation. All the peaks nearly disappear after 270 s, which is consistent with the peaks in the luminescence spectra.

We analyzed the compounds obtained after the reaction between **1**·Cl and the photo-generated radical species, by means of electron spray-ionization positive-ion mass spectrometry. The spectrum of an acetonitrile solution of **1**·Cl and AIBN after irradiation of 270 s includes a new peak at m/z 656.1, which is consistent with the spectrum of a compound (**3**, see Figure 7) formed by covalent bonding between isobutyronitrile and the imidazoline in **1**. In other words, the non-radical compound **3** should exhibit a strong luminescence due to no radical quenching.

As a control experiment, an acetonitrile solution of **1**·Cl was exposed to UV irradiation, but there was no enhancement of the luminescence. This confirms that the recovery of the luminescence of the chromophore is surely due to the annihilation of the iminonitroxide radicals in **1** caused by the photo-generated isobutyronitrile radicals.

In order to examine the performance of **1**·Cl as a free radical sensor, we investigated the dependence of the luminescence enhancement on the free radical concentration. The solutions of 0.3 mM **1**·Cl and various concentrations of AIBN ranging from 0 to 300 mM were irradiated by UV light for 180 s, and their luminescence spectra were then recorded. Figure 8 shows the plots of the luminescence intensity at 620 nm vs. the AIBN concentrations. The intensity shows a linear increase up to 60 mM of AIBN, suggesting a quantitative radical detection by **1**·Cl. Note that since two isobutyronitrile radicals are generated from one AIBN molecule (Figure 7), the critical radical

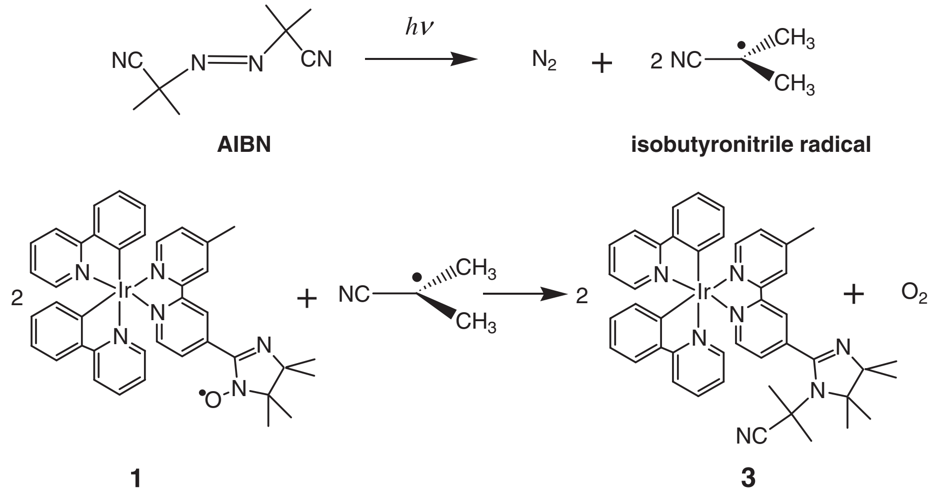

Figure 7. Reaction between **1** and the photo-generated radical species from AIBN.

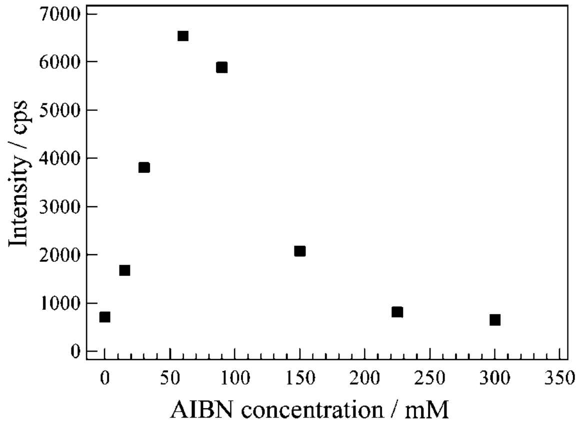

Figure 8. Luminescence recovery of the acetonitrile solutions of 0.3 mM [Ir(ppy)$_2$(MebpyIN)]Cl (**1**•Cl) and AIBN with various concentrations after UV irradiation for 180 s. Solid squares show emission intensities at 620 nm dependent on each AIBN concentration.

concentration should be 120 mM. Above 90 mM AIBN however, the intensity shows a decrease, which is probably caused by self-quenching of excess isobutyronitrile radicals.

While photo-generated free radicals in a solution of 130 mM AIBN were previously probed by CdSe quantum dots functionalized by 4-amino-2,2,6,6-tetramethylpiperidin-1-yl oxide radical,[20] the present study has demonstrated a quantitative detection of these free radicals using luminescent iridium complexes. Although it is hard to apply **1**•Cl to radical sensing at a micro molar concentration, which is required for biomedical applications,[1–4] it would be useful for the millimolar-range radical sensing without being damaged by an excess of UV light or an excess of free radicals.

Conclusion

In summary, a novel iridium complex **1**•Cl with an iminonitroxide bipyridine ligand was designed and synthesized as a preluminescent probe to detect free radicals. Although **1**•Cl showed no luminescence due to quenching by the nitronyl radical on the ligand molecule, the luminescence was dramatically enhanced by the photo-generated isobutyronitrile radicals. Our investigation of the radical concentration dependence of enhanced luminescence intensity indicated that compound **1**•Cl can quantitatively detect free radicals up to 120 mM. It can be concluded that the iridium complexes bearing the nitronyl radical moiety can act as a free radical probe through the luminescence recovery, which is stable over a long irradiation time.

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