Electronic Supporting Information

A G-triplex luminescent switch-on probe for the detection of Mung Bean nuclease activity

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MATERIAL AND METHODS

Materials. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia). Mung Bean nuclease was purchased from New England Biolabs Inc. (Beverly, MA, USA). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China).

General experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. Circular dichroism (CD) spectra were collected on a JASCO-815 spectrometer.

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d_6: ^{1}H δ 2.05, ^{13}C δ 29.8). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

Photophysical measurement. Emission spectra and lifetime measurements for **1** was performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter.

Error limits were estimated: λ (±1 nm); τ (±10%); φ (±10%). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw.

Luminescence quantum yields were determined using the method of Demas and Crosby (1) $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

Synthesis. The following complexes were prepared according to (modified) literature methods. All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

[Ir(phq)$_2$(2,9-dmphen)] (1) was prepared according to a reported literature method (2). A suspension of $[Ir_2(phq)_4Cl_2]$ (0.2 mmol) and the corresponding N^N ligand 2,9-dimethyl-1,10-phenanthroline (2,9-dmphen), (0.44 mmol) in a mixture of DCM:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was then allowed to cool to room temperature, and filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaporation until precipitation of the crude product occurred. The precipiate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound.

Complex **1**. Yield: 58%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.53 (d, J = 8.0 Hz, 2H), 8.47 (d, J = 8.0 Hz, 2H), 8.35 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.82-7.79 (m, 4H), 7.44 (d, J = 8.0 Hz, 2H), 7.37 (t, J = 4.0 Hz, 2H), 7.08 (t, J = 8.0 Hz, 2H), 6.99 (t, J = 8.0 Hz, 2H), 6.81 (t, J = 8.0 Hz, 2H), 6.49 (d, J = 8.0 Hz, 2H), 2.81 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 171.8, 165.4, 149.2, 148.9, 148.6, 147.1, 141.0, 139.5, 134.0, 131.5, 131.1, 130.1, 130.0, 128.6, 128.4, 128.0, 127.4, 127.3, 124.8, 123.5, 118.2, 25.2; HRMS: calcd. for $C_{44}H_{32}IrN_4$ $[M–PF_6]^+$: 809.2256, found: 809.2304. Elemental analysis ($C_{44}H_{32}N_4IrPF_6$+2H_2O) cal: C, 53.38; H, 3.67; N, 5.66, found C, 53.18; H, 3.49; N, 5.65.

Total cell extract preparation. The TRAMPC1 (ATCC® CRL2730™) cell line were purchased from American Type Culture Collection (Manassas, VA 20108 USA). Prostate cancer cells were trypsinized and resuspended in TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). After incubation on ice for 10 min, the lysate was centrifuged and the supernatant was collected.

Detection of MB nuclease activity. For assaying MB nuclease activity, 50 μL of 1×MB nuclease

reaction buffer (30 mM NaCl, 50 mM sodium acetate, 1 mM $ZnSO_4$, pH 5.0) with the indicated concentrations of MB nuclease were added to a solution containing the C-rich DNA oligonucleotide ON1 (20 μM). The mixture was heated to 37 °C for 60 min to allow the MB nuclease-catalyzed digestion of the single-stranded substrate to take place. The MB nuclease was deactivated by heating the mixture to 95 °C for 10 min. Then, 20 μM ON2 was added into the reaction solution, and the mixture was subsequently diluted using phosphate buffer (10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA, pH 7.0) to a final volume of 500 μL. Finally, 0.5 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 515−725 nm range using an excitation wavelength of 310 nm.

For assaying MB nuclease activity in cell extract, 50 μL of 1×MB nuclease reaction buffer (30 mM NaCl, 50 mM sodium acetate, 1 mM ZnSO4, pH 5.0) with the indicated concentrations of MB nuclease were added to a solution containing the C-rich DNA oligonucleotide ON1 (20 μM) and cell extract. The mixture was heated to 37 °C for 60 min to allow the MB nuclease-catalyzed digestion of the single-stranded substrate to take place. The MB nuclease was deactivated by heating the mixture to 95 °C for 10 min. Then, 20 μM ON2 was added into the reaction solution, and the mixture was subsequently diluted using phosphate buffer (10 mM potassium phosphate, 70 mM KCl, 0.2 mM EDTA, pH 7.0) to a final volume of 500 μL. Finally, 0.5 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 515−725 nm range using an excitation wavelength of 310 nm.

Table S1. DNA sequences used in this project:

	Sequences
ON1	5′-CCCGCCCTACCCA-3′
ON2	5′-TGGGTAGGGCGGG-3′
CCR5-DEL	5′-CTCAT$_4$C$_2$ATACAT$_2$A$_3$GATAGTCAT-3′
ds17	5′-C$_2$AGT$_2$CGTAGTA$_2$C$_3$-3′ 5′-G$_3$T$_2$ACTACGA$_2$CTG$_2$-3′
PS2.M	5′-TGGGTAGGGCGGGTTGGG-3′
ON2$_m$	5′-T*CT*GTA*CT*GC*CT*G-3′
ON1$_m$	5′-C*AG*GC*AG*TAC*AG*A-3′

Table S2 Photophysical properties of iridium(III) complex **1**.

Complex	Quantum yield	λ_{em}/ nm	Lifetime/ μs	UV/vis absorption λ_{abs}/nm (ε / dm^{3}mol^{-1}cm^{-1})
1	0.5298	575	0.472	228 (8.1× 10^4), 273 (8.5 × 10^4), 346 (1.7 × 10^4), 440 (5.1 × 10^3)

Fig. S1 UV/vis absorption and normalized emission spectra of complex **1** (2.5 μM) in acetonitrile solution at 298 K.

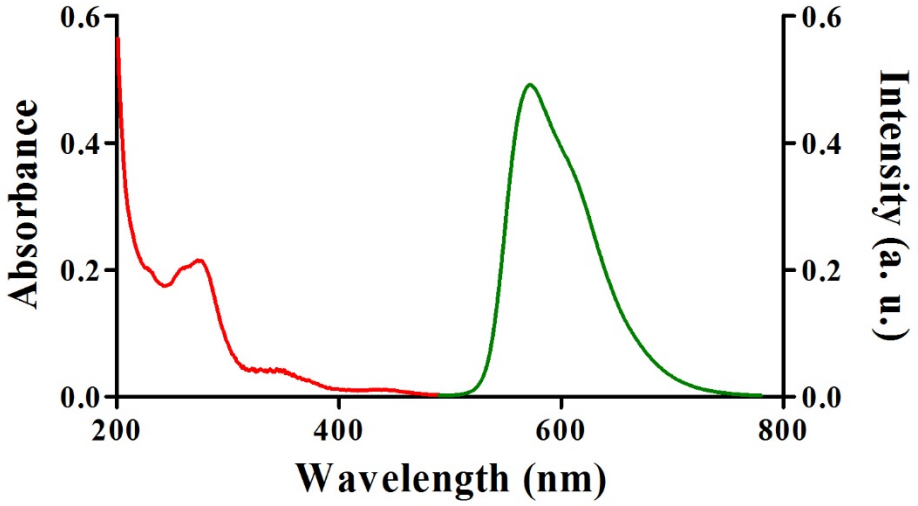


Fig. S2 Diagrammatic bar array representation of the luminescence enhancement of complex **1** (0.5 μM) for 5 μM of ssDNA (CCR5-DEL), dsDNA (ds17), G3 DNA and G4 DNA (PS2.M), respectively. Error bars represent the standard deviations of the results from three independent experiments. ("I" means the fluorescence intensity of the system in the presence of 5 μM of different kinds of DNA, "I_0" means the fluorescence intensity of the system in the absence of DNA.).

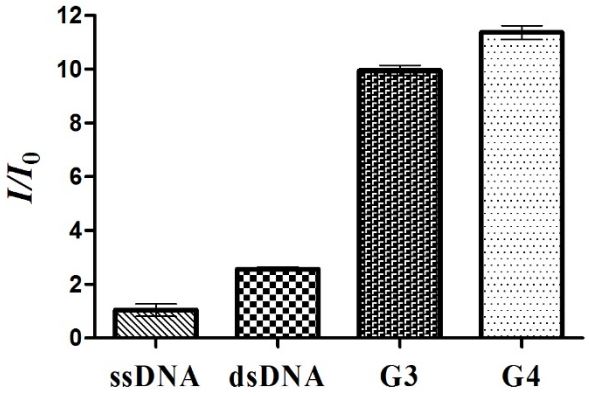


Fig. S3 a) Emission spectra of complex **1** (1 μM) in the presence of 0, 0.2, 0.5, 1, 2, 3, 4 or 5 μM of G-triplex DNA (ON2). b) Luminescence response of complex **1** at λ_{max} = 575 nm *vs.* ON2 concentration. Error bars represent the standard deviations of the results from three independent experiments.

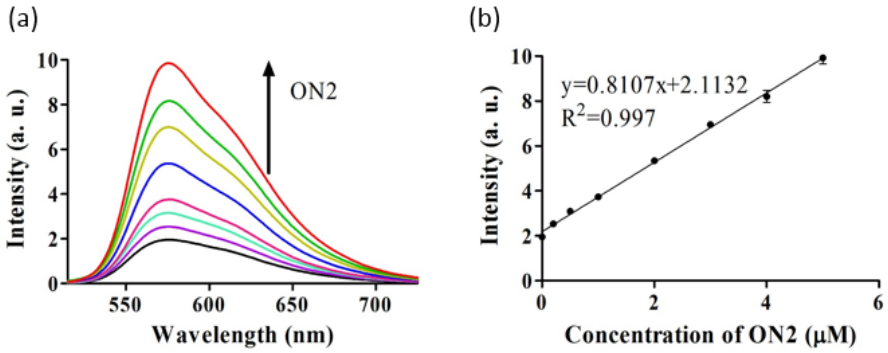


Fig. S4 Luminescence response of the system with the complex alone ([complex **1**] = 0.5 μM) in the absence and presence of MB nuclease (40 and 80 U/mL).

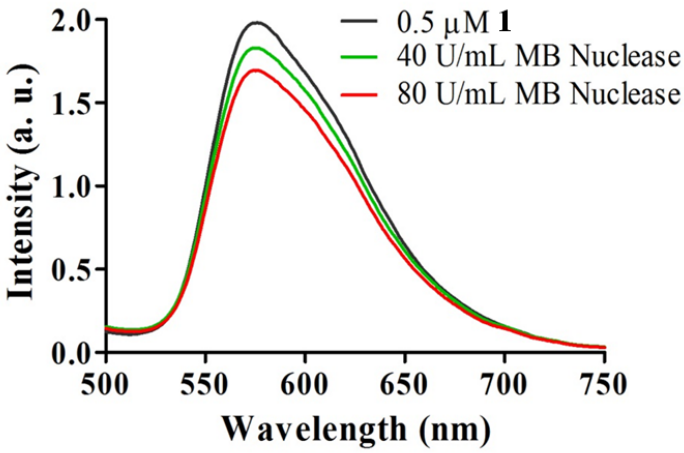


Fig. S5 Relative luminescence response of complex **1** (0.5 μM) in the presence of MB nuclease (2 U/mL) and ON1/ON2 or $ON1_m/ON2_m$ mutant (2 μM). Experimental conditions: 0.5 μM of complex **1** in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments. ("I" means the fluorescence intensity of the system in the presence of 2 U/mL MB nuclease, "I_0" means the fluorescence intensity of the system in the absence of MB nuclease).

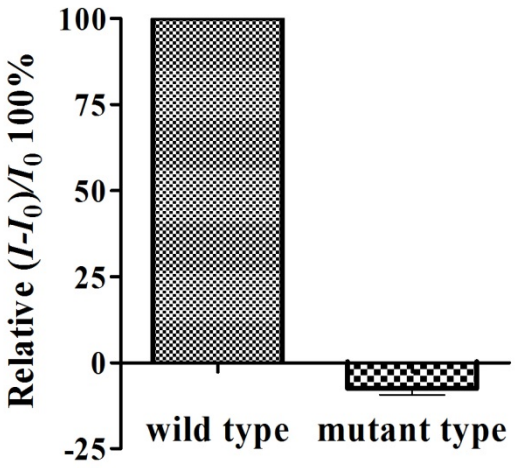


Fig. S6 Circular dichroism (CD) spectra of 4 μM ON1/ON2 in the absence of MB nuclease (green), 4 μM of ON2 added after the 1 hour incubation of 4 μM ON1 with 40 U/mL MB nuclease (red) and 4 μM of ON2 in the absence of MB nuclease (black), were recorded in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0). The CD spectrum of the duplex ON1/ON2 in the absence of MB nuclease exhibits an intense positive peak at around 280 nm and a strong negative peak at 240 nm, which is characteristic for duplex DNA. Since most of the ON1 has been digested by the incubation with 40 U/mL MB nuclease, the input of ON2 only got the G-triplex DNA structure. So the system containing ON1, MB nuclease and ON2 exhibited a similar CD spectrum as the system which only contain ON2, revealling a positive band at around 265 nm, and a weak negative peak at around 240 nm in CD spectrum.

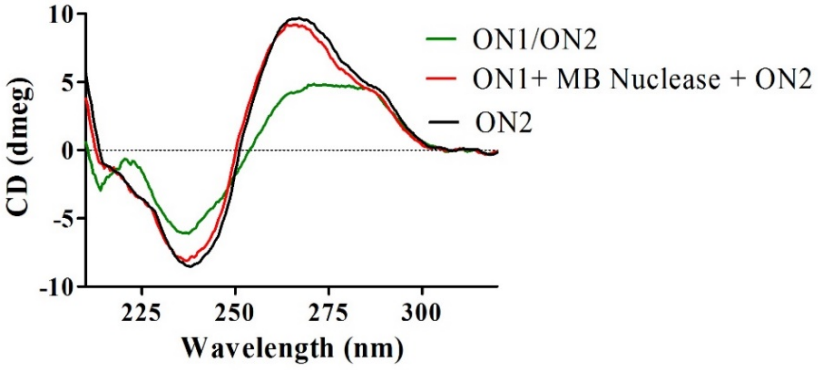


Fig. S7 Relative fold change of the system in the absence or presence of MB nuclease (2 U/mL) at various concentrations of complex **1** (0.25, 0.5, 0.75 and 1.0 μM). 0.5 μM of complex **1** offered the highest luminescence fold-change response compared to 0.25, 0.75 or 1.0 μM of complex **1**. Experimental conditions: ON1 (2 μM) and ON2 (2 μM) in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments. ("I" means the fluorescence intensity of the system in the presence of 2 U/mL MB nuclease, "I_0" means the fluorescence intensity of the system in the absence of MB nuclease).

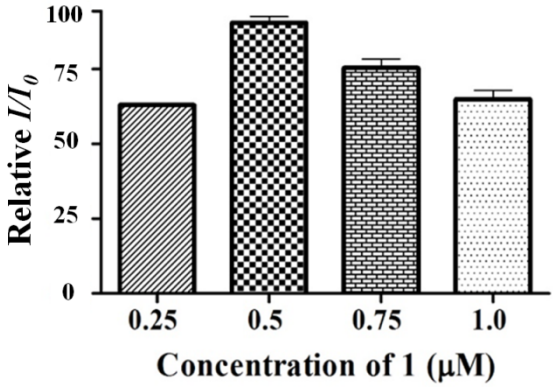


Fig. S8 Relative fold change of the system in the absence or presence of MB nuclease (2 U/mL) at various concentrations of ON1 and ON2 (0.5, 1.0, 2.0 and 4.0 μM, respectively). It was observed that the luminescence response of the system was highest at 2 μM of ON1 and ON2. Experimental conditions: complex **1** (0.5 μM) in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments. ("I" means the fluorescence intensity of the system in the presence of 2 U/mL MB nuclease, "I_0" means the fluorescence intensity of the system in the absence of MB nuclease).

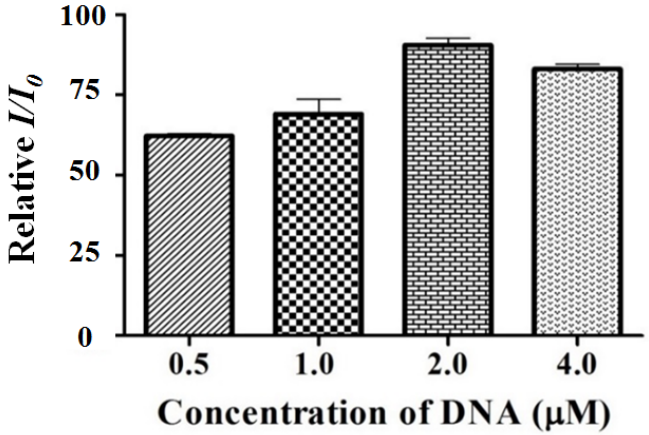


Fig. S9 Relative luminescence response of the system at different times of reaction with MB nuclease (2 U/mL and 4 U/mL). It was observed that the luminescence response of the system reaches a plateau at 40 min. Experimental conditions: 0.5 μM complex **1**, 2.0 μM ON1 and 2.0 μM ON2 in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0). Error bars represent the standard deviations of the results from three independent experiments. ("I" means the fluorescence intensity of the system at different reaction times, "I_0" means the fluorescence intensity of the system at the starting reaction time).

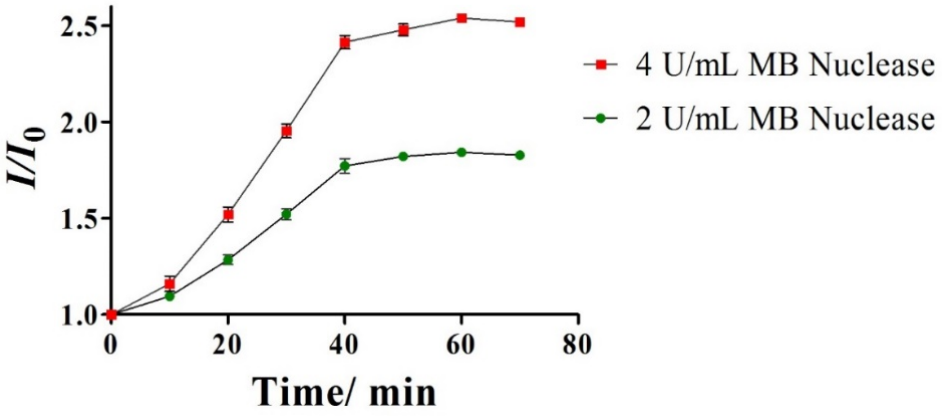


Fig. S10 Emission spectral traces of complex **1** (0.5 μM), 2 μM ON2, and 2 μM ON1 upon incubation with MB nuclease (0.5 U/mL) in 10 mM potassium phosphate buffer (70 mM KCl and 0.2 mM EDTA, pH 7.0), showing a signal-to-noise ratio greater than 3.

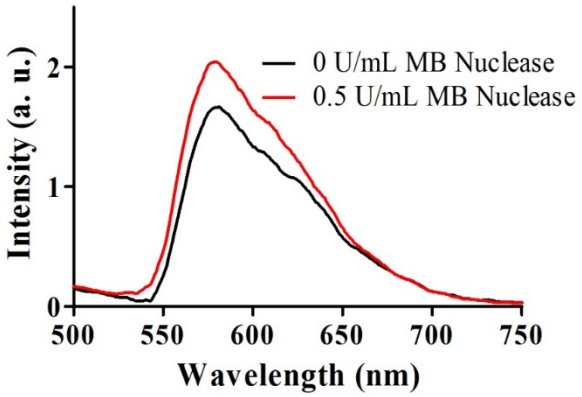
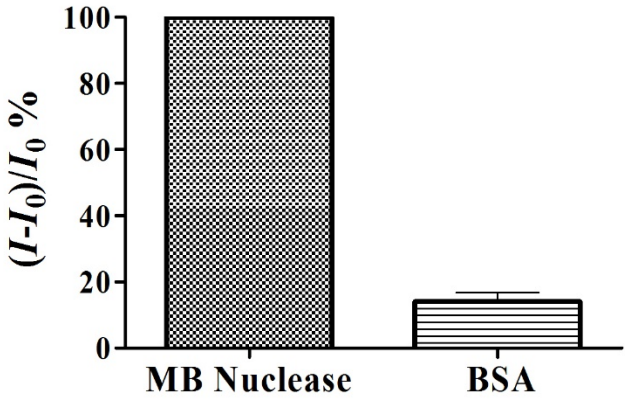


Fig. S11 Relative luminescence response of the system in the presence of 2 U/mL MB nuclease or 0.15 μM BSA.



References

1. Crosby, G.A. and Demas, J.N. (1971) Measurement of photoluminescence quantum yields. Review. *J. Phys. Chem.*, **75**, 991-1024.
2. Dragonetti, C., Falciola, L., Mussini, P., Righetto, S., Roberto, D., Ugo, R., Valore, A., De Angelis, F., Fantacci, S., Sgamellotti, A. *et al.* (2007) The Role of Substituents on Functionalized 1,10-Phenanthroline in Controlling the Emission Properties of Cationic Iridium(III) Complexes of Interest for Electroluminescent Devices. *Inorg. Chem.*, **46**, 8533-8547.