Electronic Supporting Information

A long-lifetime iridium(III) complex for lysosome tracking with high specificity and a large Stokes shift

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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. ^{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 2.09, ^{13}C δ 205.87, 30.60; DMSO-d_6: ^{1}H δ 2.50, ^{13}C δ 39.5). 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.

Photophysical measurement. Emission spectra and lifetime measurements for complexes were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm). 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.

Materials and cells lines. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate ($IrCl_3 \cdot xH_2O$) was purchased from Precious Metals Online (Australia). Lyso-Tracker was purchased from Beyotime (Nantong, China). Hoechst 33342 was purchased from Thermo Fisher Scientific Co. (Fair Lawn, NJ). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco BRL (Gaithersburg, MD). Cells were cultured in DMEM supplemented with 10% FBS and in 5% CO_2 in a 37 °C incubator.

Time-resolved emission spectra (TRES) measurement. 2 μL of 1 mM CM460 was added into 20 μM complex **Ir-Ly** in 0.5 mL PBS buffer (0.1 mM, pH 4.5). TRES was measured with a Horiba Fluorolog TCSPC spectrophotometer under an excitation wavelength of 355 nm.

pH effect study. 0.1 mM of complex **Ir-Ly** stock solution was prepared in dimethyl sulfoxide (DMSO). The complex was then added into PBS buffer with different pH values to a final concentration of 4 μM in a cuvette. Luminescence emission spectra were recorded on a PTI QM-4 spectrofluorometer (Photo Technology International, Birmingham, NJ) at 25 °C, with the slits for both excitation and emission set at 2.5 nm.

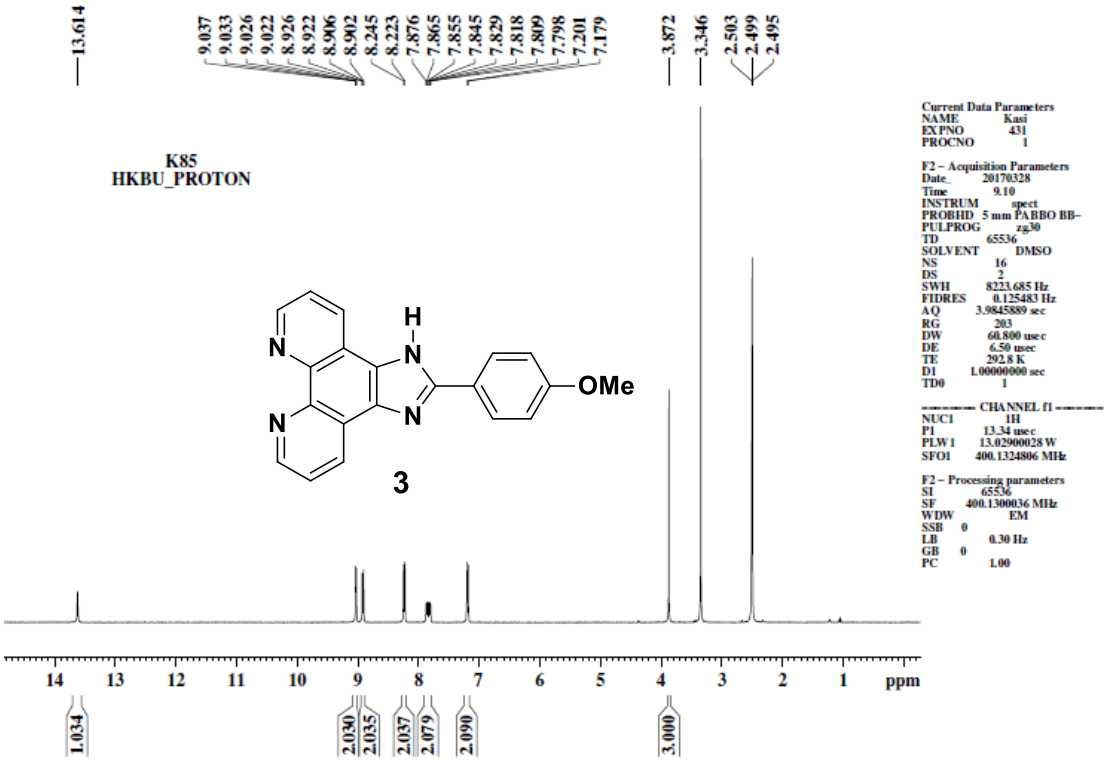
Specificity study. 0.1 mM of **Ir-Ly** stock solution was prepared in DMSO. Different concentrations of Na^+, various metal cations (0.1 mM), various anions (0.1 mM) or various amino acids (0.1 mM) were added to containing complex **Ir_Ly** (4 μM) in PBS buffer in a cuvette. Luminescence emission spectra were recorded on a PTI QM-4 spectrofluorometer (Photo Technology International, Birmingham, NJ) at 25 °C, with the slits for both excitation and emission set at 2.5 nm.

Cell viability assay. HeLa cells were seeded in 96-well plates at the density of 5,000 cells per well and incubated for 12 h. DMSO-dissolved **Ir-Ly** was added to 96-well plates at indicated concentrations for 6 h. 10 μL of MTT reagent (5 mg/mL) were then added to each well. After 4 h incubation in the dark, 100 μL of DMSO were added to each well, and the intensity of absorbance was determined by a SpectraMax M5 microplate reader at a wavelength of 570 nm.

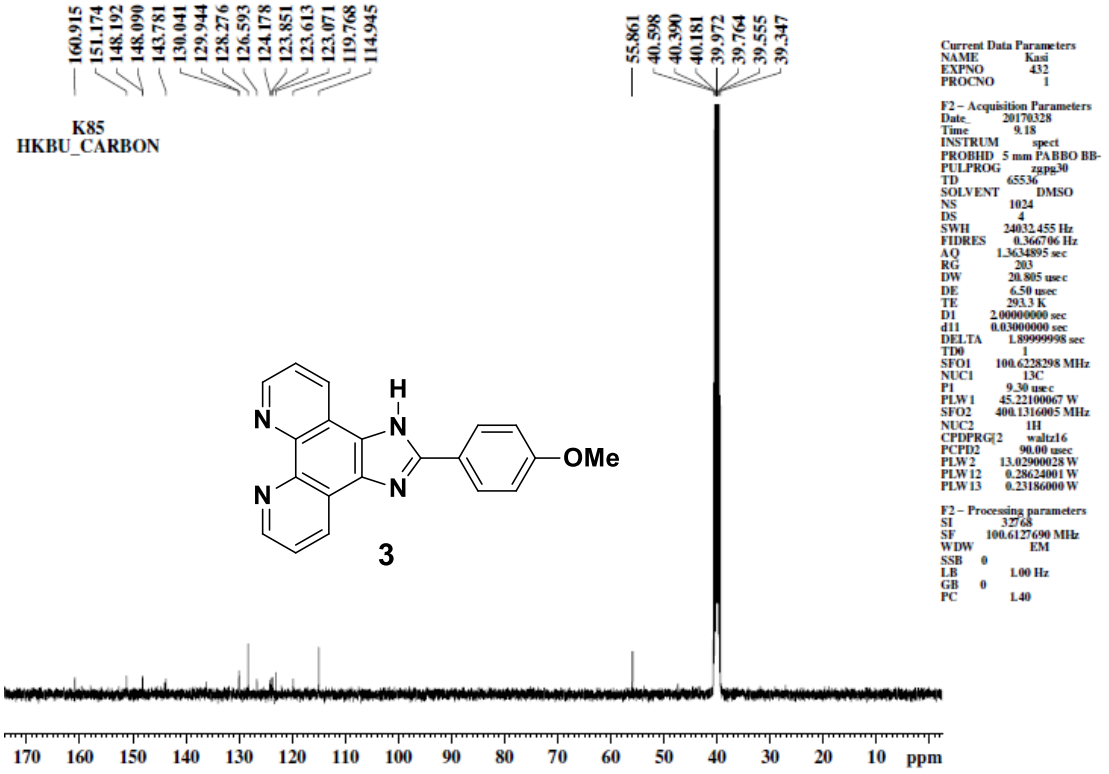
Confocal imaging. Cells were seeded into a glass-bottomed dish (35 mm dish with 20 mm well). After 12 h, cells were incubated with **Ir-Ly** at the indicated concentrations for 1 h. The cells were then stained with Lyso-Tracker (1:15,000) for 30 min and Hoechst 33342 (1:2,000) buffer for 10 min, followed by washing with phosphate buffer three times. The luminescence of **Ir-Ly** in different cell lines was imaged by a Leica TCS SP8 confocal laser scanning microscope system. The excitation wavelength was 405 nm.

Synthesis of compound 3.

(2-(4-methoxyphenyl)-1*H*-imidazo[4,5-*f*][1,10] phenanthroline): A mixture of 4-methoxybenzaldehyde (0.5 g, 3.67 mmol), 1,10 phenanthroline-5,6-dione (0.7 g, 3.67 mmol), ammonium acetate (5.6 g, 73 mmol) and glacial acetic acid (20 mL) was refluxed with stirring for 1 h. After completion of reaction, the cooled reaction mixture was filtered, diluted with ice cold water and neutralized with concentrated aqueous ammonia. The precipitate was collected and washed with methanol and diethyl ether gives the compound **3.** Yield: 85%. ^{1}H NMR (400 MHz, DMSO) δ 13.61 (s, 1H), 9.03 (d, *J* = 1.7 Hz, 1H), 9.02 (d, *J* = 1.7 Hz, 1H), 8.92 (d, *J* = 1.7 Hz, 1H), 8.90 (d, *J* = 1.7 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 2H), 7.86 (dd, *J* = 8.2, 4.3 Hz, 1H), 7.81 (dd, *J* = 8.1, 4.3 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 160.92, 151.18, 148.19, 144.04, 143.78, 130.04, 129.95, 128.28, 126.60, 124.18, 123.85, 123.62, 123.07, 121.99, 119.77, 114.95, 55.86.



^{1}H NMR spectrum of compound **3**.

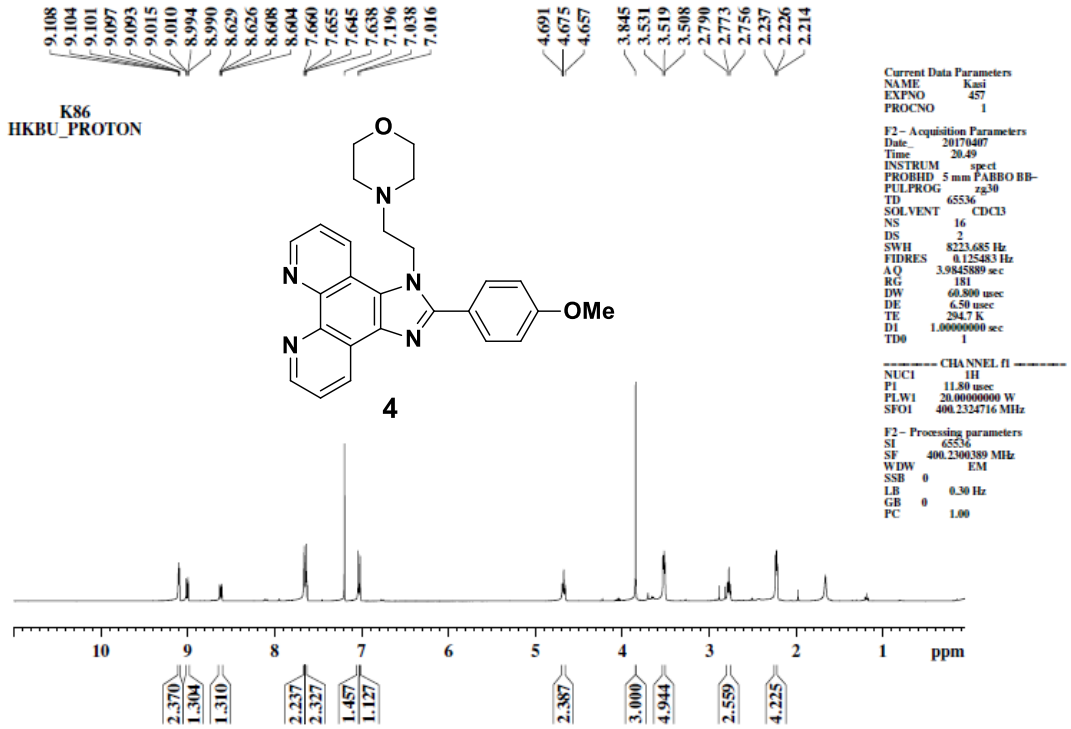


^{13}C NMR spectrum of compound **3**.

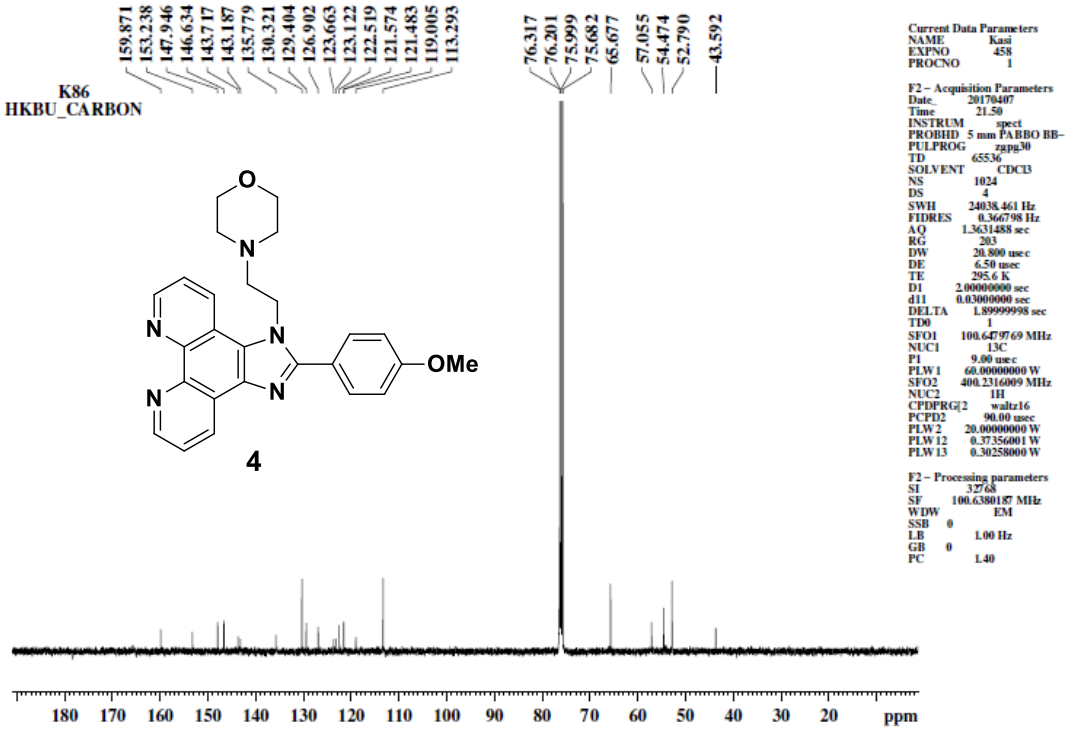
Synthesis of compound 4.

(4-(2-(2-(4-methoxyphenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthrolin-1-yl)ethyl)mor

pholine): The mixture of 4.9 mmol of sodium hydride (NaH) and 50 mL of anhydrous *N*,*N*-dimethylformamide (DMF) was stirred for 1 h, then 2.45 mmol of compound **3** and 3.65 mmol of 4-(2-chloroethyl)morpholine in 50 mL of DMF were added into reaction mixture. The reaction mixture was heated to reflux for 24 h. After cooling, the mixture was poured into cold water and extracted with dichloromethane (DCM). After the evaporation of solvent, the residue was further purified by column chromatography using DCM/MeOH (98/2) gives expected product **4** (0.6 g, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (m, 1H), 9.10 (d, *J* = 1.7 Hz, 1H), 9.00 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.62 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.66 (m, 2H), 7.64 (t, *J* = 2.1 Hz, 2H), 7.04 (m, 1H), 7.02 (m, 1H), 4.67 (t, *J* = 6.8 Hz, 2H), 3.85 (s, 3H), 3.52 (m, 4H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.23 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.87, 153.24, 147.95, 146.64, 143.72, 143.19, 135.78, 130.32, 129.41, 126.90, 123.66, 123.12, 122.52, 121.58, 121.48, 119.01, 113.30, 65.68, 57.06, 54.47, 52.79, 43.59.



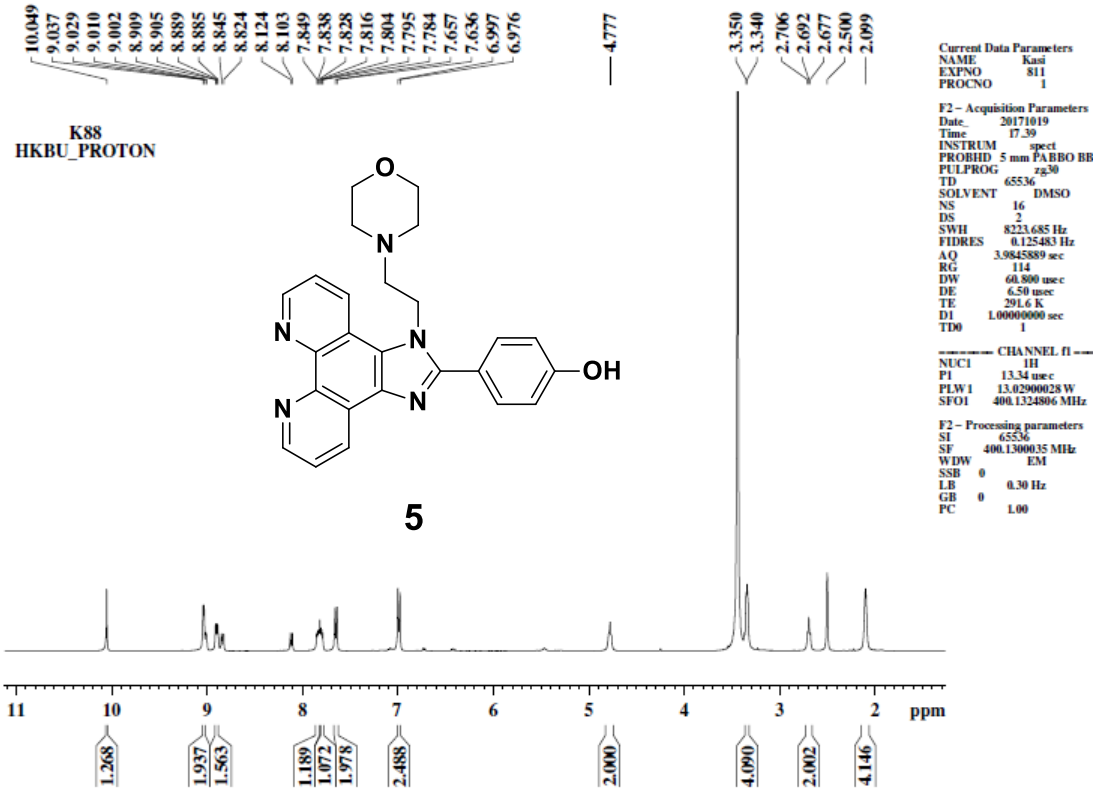
^{1}H NMR spectrum of compound **4**.



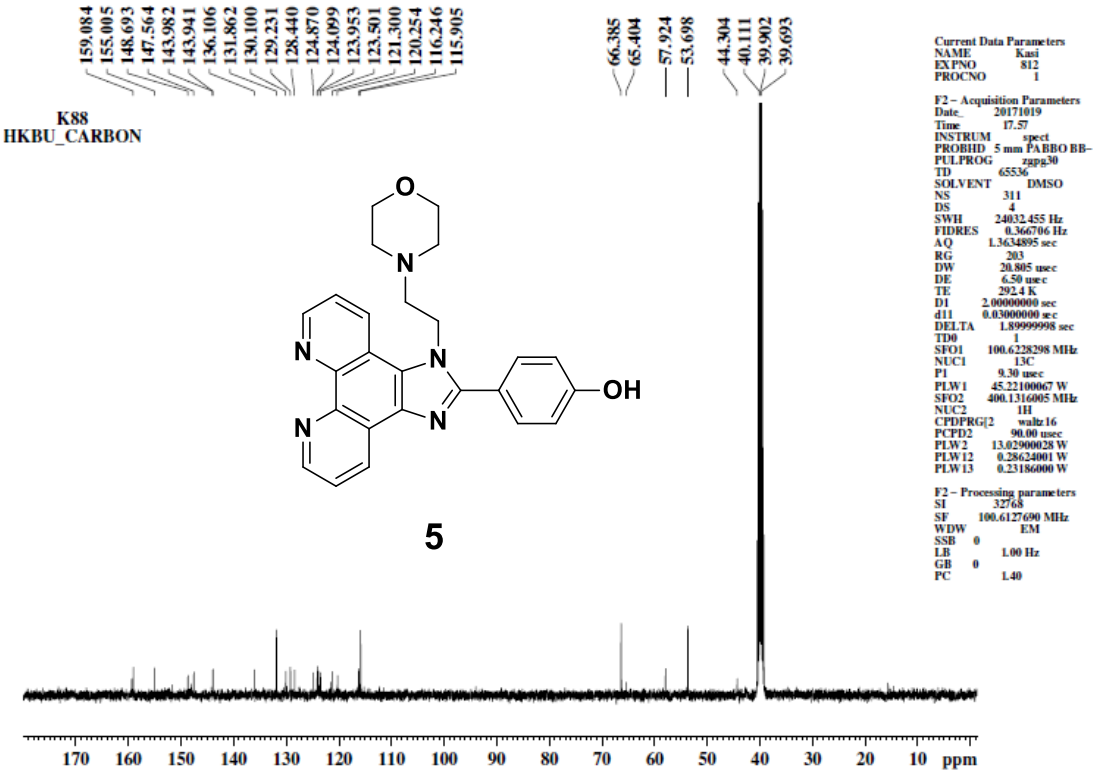
^{13}C NMR spectrum of compound **4**.

Synthesis of compound 5.

(4-(1-(2-morpholinoethyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthrolin-2-yl)phenol): A two neck round bottom flask was charged with compound **4** (0.2 g, 0.45 mmol), *N*-methyl-2-pyrrolidone (NMP) (3 mL), 2-aminobenzenethiol (0.047 mL, 0.45 mmol), and Cs_2CO_3 (8 mg, 5%), sealed with a septum, and purged with argon. The mixture was then heated to 185 °C for 30 min. Upon cooling, 5 mL of water was added to induce precipitation. The solid was collected by vacuum filtration and purified by flash chromatography eluting with DCM/MeOH (95/5). Gives the ligand **5** with 0.100 g, 50% yield.^{1}H NMR (400 MHz, DMSO) δ 10.05 (s, 1H), 9.03 (d, *J* = 3.1 Hz, 2H), 8.89 (m, 2H), 8.11 (d, *J* = 8.6 Hz, 1H), 7.82 (s, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 4.78 (m, 2H), 3.34 (m, 4H), 2.70 (m, 2H), 2.10 (brm, 4H). ^{13}C NMR (101 MHz, DMSO) δ 159.09, 155.01, 148.70, 147.57, 143.98, 136.11, 131.86, 130.10, 129.23, 128.44, 124.87, 124.10, 123.95, 123.50, 121.30, 120.26, 116.25, 115.91, 66.39, 57.92, 53.70, 44.31.



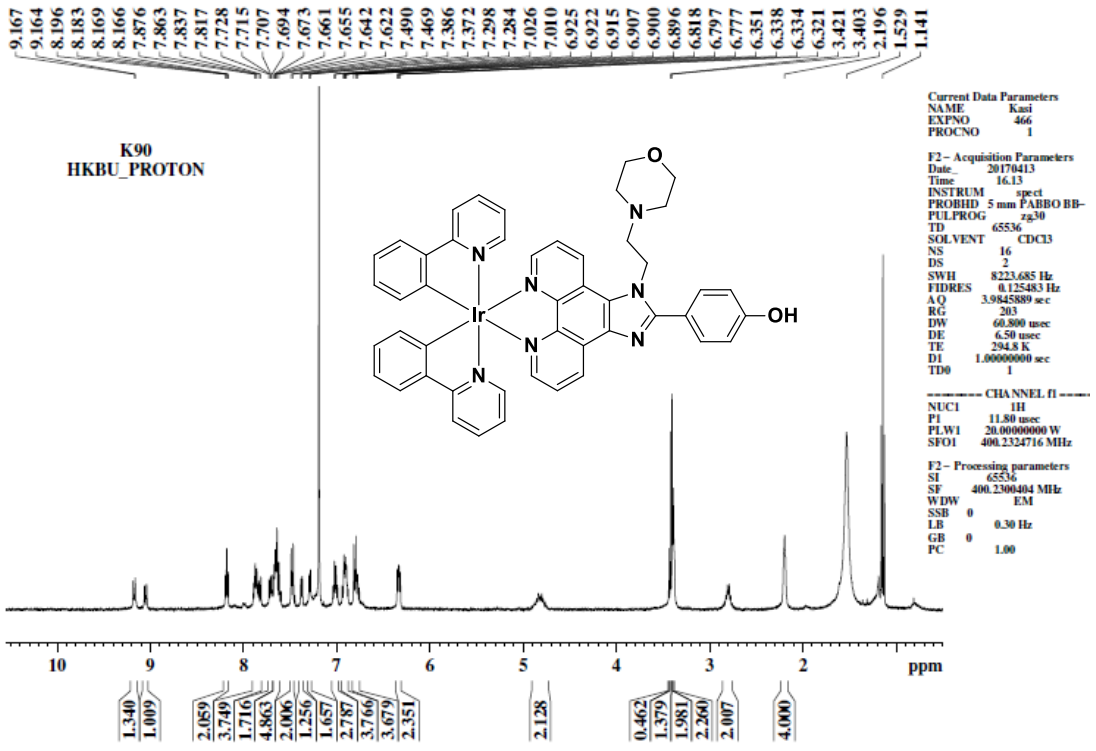
^{1}H NMR spectrum of compound **5**.



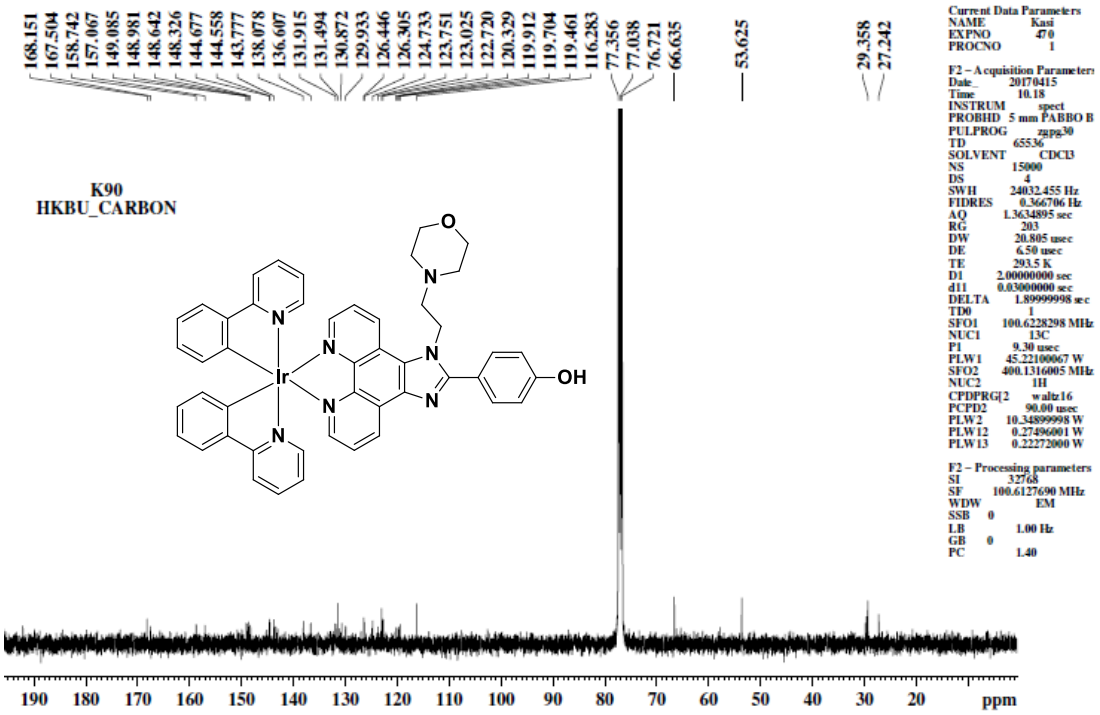
^{13}C NMR spectrum of compound **5**.

Synthesis of complex Ir-Ly.

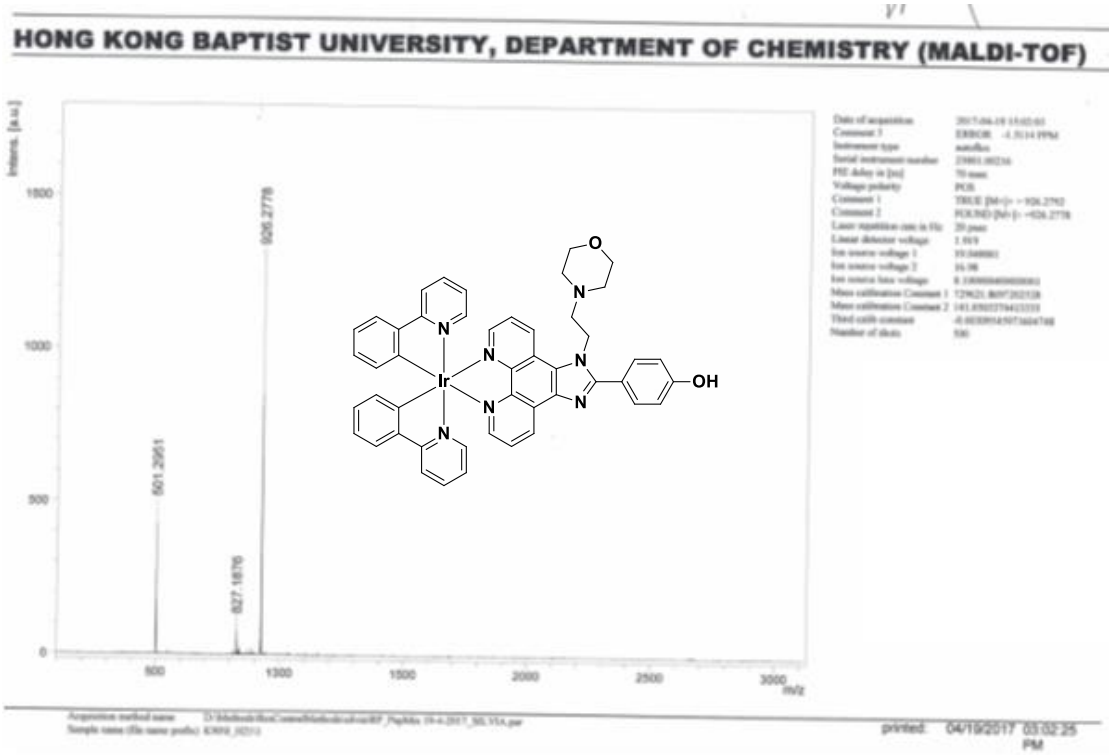
A mixture of $[Ir(ppy)_2Cl]_2$ (0.075 g, 0.069 mmol), compound **5** (0.060g, 0.14 mmol) and DCM/MeOH (1:1) were stirred for overnight. The reaction was monitored by TLC. When the reaction was completed, excess of NH_4PF_6 was added to the reaction mixture and allowed for 30 minutes. The reaction mixture was washed with small amounts of water and extracted with DCM and evaporated under reduced pressure. The final complex **Ir-Ly** was purified using column chromatography with MeOH/DCM (v/v, 98:2) as eluent. Yield: 0.098 g, 65%. ^{1}H NMR (400 MHz, DMSO) δ 9.26 (d, *J* = 8.2 Hz, 1H), 9.13 (d, *J* = 8.6 Hz, 1H), 8.25 (t, *J* = 5.1 Hz, 2H), 7.95 (m, 2H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.79 (dd, *J* = 8.3, 5.1 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 3H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.45 (d, *J* = 5.6 Hz, 1H), 7.36 (d, *J* = 5.4 Hz, 1H), 7.09 (dd, *J* = 12.3, 7.3 Hz, 2H), 6.98 (dd, *J* = 16.3, 8.1 Hz, 4H), 6.90 (d, *J* = 8.3 Hz, 2H), 6.84 (m, 1H), 6.40 (m, 2H), 4.89 (m, 2H), 3.46 (s, 4H), 2.86 (m, 2H), 2.25 (brm, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.15, 167.49, 158.72, 157.07, 149.07, 148.97, 148.84, 148.64, 148.30, 144.68, 144.56, 143.78, 143.42, 138.08, 136.69, 131.50, 131.50, 130.71, 129.93, 126.45, 126.30, 124.89, 124.73, 123.03, 123.03, 122.86, 122.72, 120.33, 119.87, 119.74, 119.46, 116.29, 116.29, 77.36, 77.24, 77.04, 76.72, 66.64, 53.63, 53.63, 29.36, 27.24. HRMS: Calcd. for $C_{47}H_{39}F_6IrN_7O_2P$ $[M–PF_6]^+$: 926.2792 Found: 926.2778.



^{1}H NMR spectrum of complex **Ir-Ly**.



^{13}C NMR spectrum of complex **Ir-Ly.**



HRMS (MALDI-TOF) spectrum of complex **Ir-Ly**.

Table S1. Photophysical properties of iridium(III) complex **Ir-Ly**.

Photo physical properties	Parameter
λ_{ex} (Max)	290 nm
λ_{em} (Max)	590 nm
Stocks shift	300 nm
Quantum yield	0.3481
Life time	4.196 μs

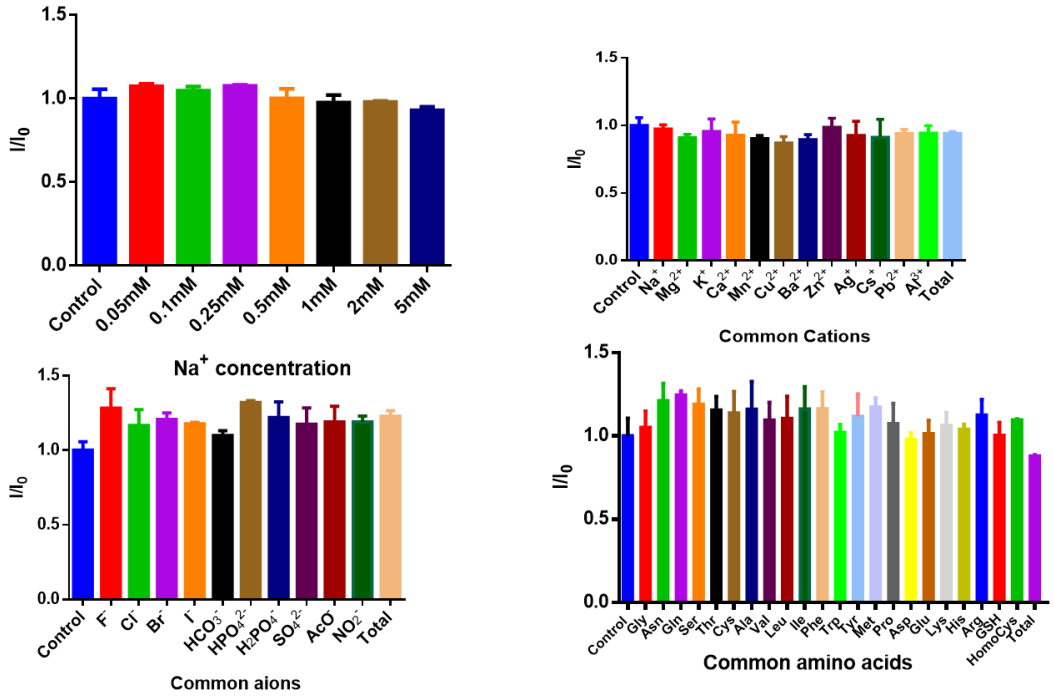


Fig. S1. Specificity study. Different concentrations of Na^+, various metal cations (0.1 mM), various anions (0.1 mM) or various amino acids (0.1 mM) were added to containing complex **Ir-Ly** (4 μM) in PBS buffer.

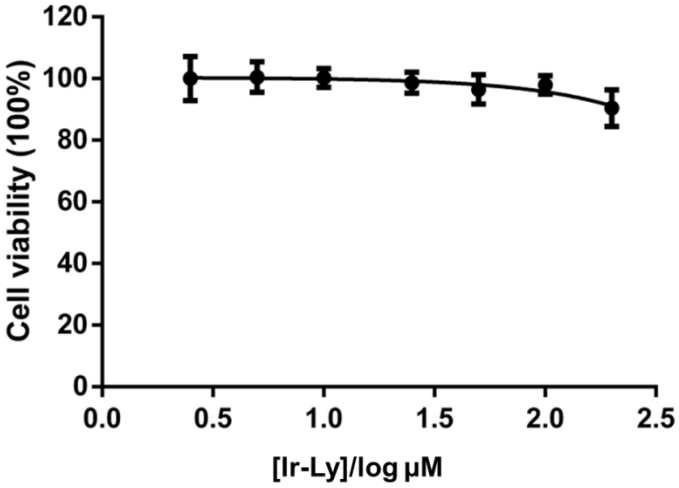


Fig. S2. Cytotoxicity of **Ir-Ly** towards HeLa cells, as determined by an MTT assay.

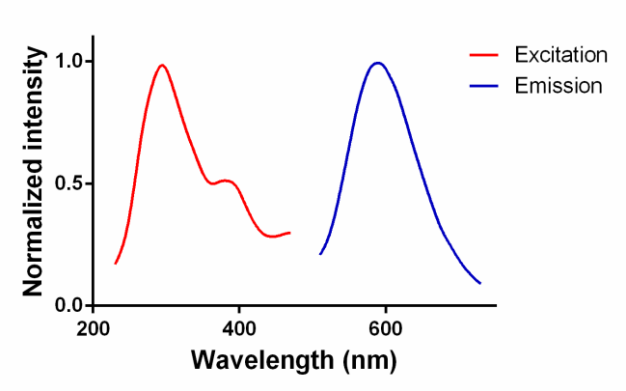


Fig. S3. Excitation and emission spectra of complex **Ir-Ly** in PBS buffer.