Supplementary Material (ESI) for Chemical Communications

Fluorescence investigation of a covalently linked phenanthridine-ruthenium(II) complex and its application as an RNA probe

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Experimental procedure

Steady-state and time-resolved luminescence measurements

Steady-state luminescence spectra were performed on a FL3-22 Fluorolog-3 spectrometer (J. Y. Horiba, Edison, NJ, USA). In a typical experiment, the spectra were obtained in solutions of 2.0 μM RuEth or 2.0 μM RuITC, with or without 0.25mg/ml Turolla Yeast RNA (Aldrich) in solutions of 10 mM Tris-HCl, 400 mM NaCl, 5mM $MgCl_2$ pH 7.5. An excitation wavelength of 450 nm was used. The quantum yield was determined using $Ru(phen)_3Cl_2$ (ϕ_F = 0.058) as the standard.

Time-resolved experiments were performed on either an OB920 single-photon counting spectrometer (Edinburgh Analytical Instruments) with a Picoquant 460 nm pulsed LED as excitation source or a streak camera system described in detail elsewhere.[1] Exponential fits were obtained with the programs included with the instruments.

Cell culture

MDA-MB-231 cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% bovine calf serum, and 1% antimycotic. Cells were maintained at 37 ºC in an atmosphere of 5% CO_2. For experiments, cells were seeded onto coverslips and left to adhere overnight prior to fluorescence assays.

Fluorescence imaging

Cells were plated onto coverslips in cell culture dishes and allowed to grow overnight. RuEth (2 mM dissolved in 1:1 DMF/water mixture) was added to culture media to a final concentration of 10 μM. After 24 h incubation, cells were then washed twice with 1X phosphate buffered saline (PBS) solution prior to fixation. Cells were fixed by incubation with 4% paraformaldehyde in PBS at room temperature for 20 min. Once fixed, cells were washed further in 1X PBS 4-5 times. Samples were mounted with Dako fluorescent mounting medium, put onto glass slides, air dried, and then visualized using an Olympus Fluoview FV1000 laser scanning confocal microscope. Images were acquired through a 60X 1.4 NA oil objective using 454 nm excitation and a variable bandpass emission filter set to 560-685 nm.

Synthesis

Materials and Instrumentation

All reactions were run under an atmosphere of dry nitrogen. Anhydrous solvents were transferred by oven-dried syringe needles purged with dry nitrogen. Flasks were flame dried *in vacuo*, or oven dried at 160 °C for greater than 2 hours. Dimethylformamide (DMF) was dried over activated 4Å sieves and placed under vacuum (0.01 – 0.02 torr) to remove low boiling amines and carbon monoxide. All solvents were purchased from the Sigma-Aldrich Company and used without further purification. NMR was performed on a Bruker 500 MHz instrument and the FT-IR on a Nicolet 8700 spectrometer.

3,8-diamino-5-(5-ammoniopentyl)-6-phenylphenanthridinium bromide (hydrobromide salt) (1). This was prepared according to the method described by Meunier.[2]

RuEth. To a solution of phenanthridinium **1** (50 mg, 0.094 mmol) in DMF (5 mL) was added Ruthenium isothiocyanate **RuITC** (75 mg, 0.094 mmol) and triethyl amine (100 mg, 0.94 mmol). The mixture was stirred overnight and precipitated by the addition of Et_2O. This precipitate was washed with water, ethanol and acetone to provide a red powder. This powder was purified by column chromatography on reverse phase C-18 silcia gel with an eluent of 20% methanol in chloroform. This provided 30 mg of a red powder (24% yield): δ_H (500 MHz, CD_3OD) 9.04 (d, *J*8.3, 1 H), 8.75 (m, 2 H), 8.72 (d, *J*8.1, 2 H), 8.62, (m, 1 H), 8.56 (m, 1 H), 8.49 (d, *J*8.4, 1 H), 8.23 (d, *J*5.2, 1 H),8.19 (m, 2 H), 8.15 (d, *J*5.3, 1 H), 8.09 (m, 2 H), 8.05 (d, *J*5.2, 1 H), 8.02 (d, *J*5.2, 1 H), 7.95 (m, 2 H), 7.88 (dd, *J*8.6 5.2, 1 H), 7.84-7.72 (m, 6 H), 7.66 (m, 3 H), 7.57 (m, 2 H), 7.49 (s, 1 H), 7.42 (s, 1 H), 7.36 (m, 2 H), 3.57 (m, 2 H), 2.08 (m, 2 H), 1.68 (m, 2 H), 1.55 (m, 2 H), 1.48 (m, 2 H); ν max(CaF)/cm^{-1} 3300, 3180, 3050, 2920, 2850, 1620, 1540, 1425; HRMS (FAB+) *m / z* calcd for $C_{57}H_{50}F_6N_{11}PRuS^+$(M+) 1167.27, found 1167.30.

References

1. Stevens, N. Ph.D. thesis, The City University of New York, New York, NY, **2006**.
2. Ross, S. A.; Pitie, M.; Meunier, B. *J. Chem. Soc. Perkins Trans. 1* **2000**, 571-574.

RuEth