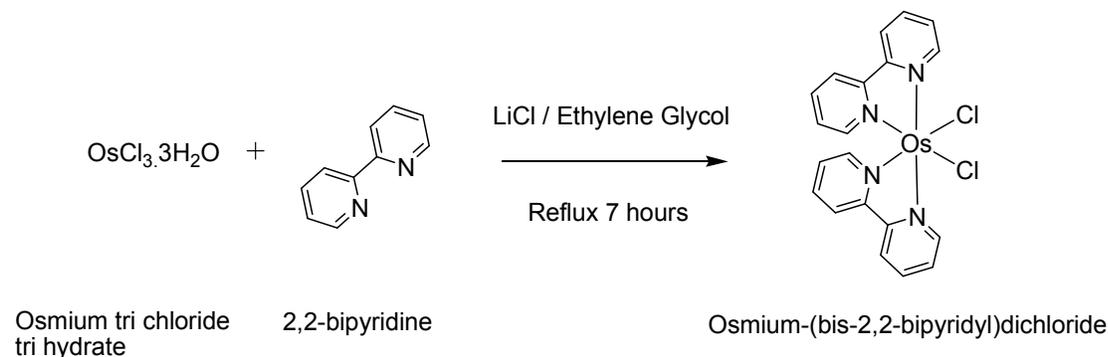


Supplementary Material

The preparation of osmium-(bis-2,2-bipyridyl)dichloride. [Os(bpy)₂Cl₂]

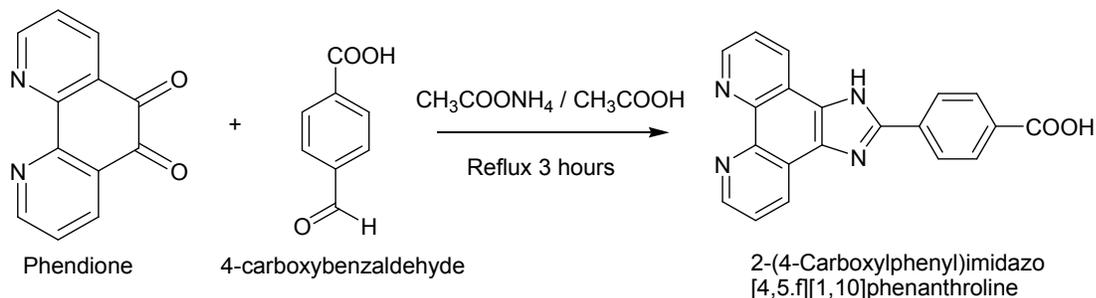


This compound was prepared according to a method modified from the literature.^[1] OsCl₃·3H₂O (409 mg, 1.38 mmol), 2,2'-bipyridine (431 mg, 2.76 mmol), lithium chloride (468 mg, 11.04 mmol) and degassed ethylene glycol (20 mL) were refluxed at 215 °C for 5.5 hours under a nitrogen environment. The reaction was cooled and triethylamine (800 μL) added and refluxed for a further 1.5 hours under a nitrogen environment. The reaction was cooled to room temperature, and an excess of acetone was added to the reaction mixture and was left in the freezer overnight to induce recrystallisation. The precipitate was collected and washed thoroughly with acetone. The product was then purified on a silica gel column using 10% MeOH/CH₂Cl₂ as mobile phase to yield purple-brown product (200 mg, 25%).

¹H NMR conformed to previous reported data;¹

¹H NMR (400 MHz, DMSO-d₆): δ (ppm) **8.77** (d, 4H), **7.94** (dd, 4H), **7.61** (dd, 4H), **7.41** (m, 4H).

The preparation of 2-(4-carboxylphenyl)imidazo[4,5-f][1,10]phenanthroline. [(picCOOH)]



The ligand was prepared according to a method modified from the literature.^[2] 1,10-Phenanthroline 5,6-dione (210 mg, 1 mmol), 4-carboxy-benzaldehyde (180 mg, 1.2 mmol), ammonium acetate (1540 mg, 20 mmol) and glacial acetic acid (25 ml) were added to a 100ml round bottomed flask and were left refluxing at 130 °C for 3 hours. The solution was then cooled to room temperature and excess water added to induce recrystallization. The precipitate was collected by vacuum filtration and washed with deionised water, methanol and diethyl ether to afford the ligand as a yellow solid (231 mg, 68%).

¹H NMR (400 MHz, DMSO-d₆): δ (ppm) **13.96** (s, 1H), **13.09** (s-broad, 1H), **9.05** (d, 2H), **8.95** (d, 2H, J = 2.4 Hz), **8.40** (d, 2H, J = 8.4 Hz), **8.17** (d, 2H, J = 8.4 Hz), **7.87** (m, 2H).

Supplementary Figure S1. Absorbance and emission spectra for $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{pic})]^{2+}$

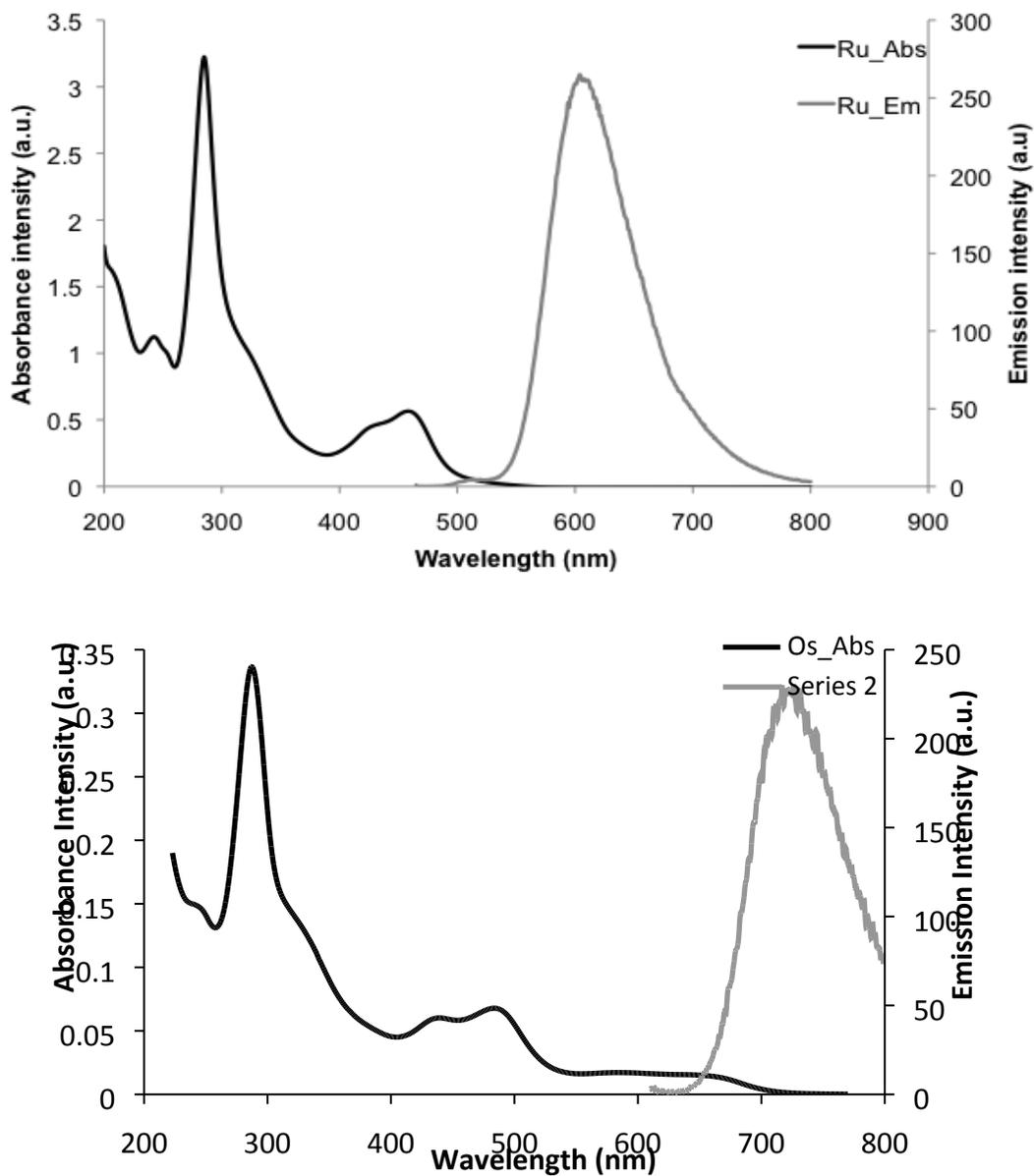
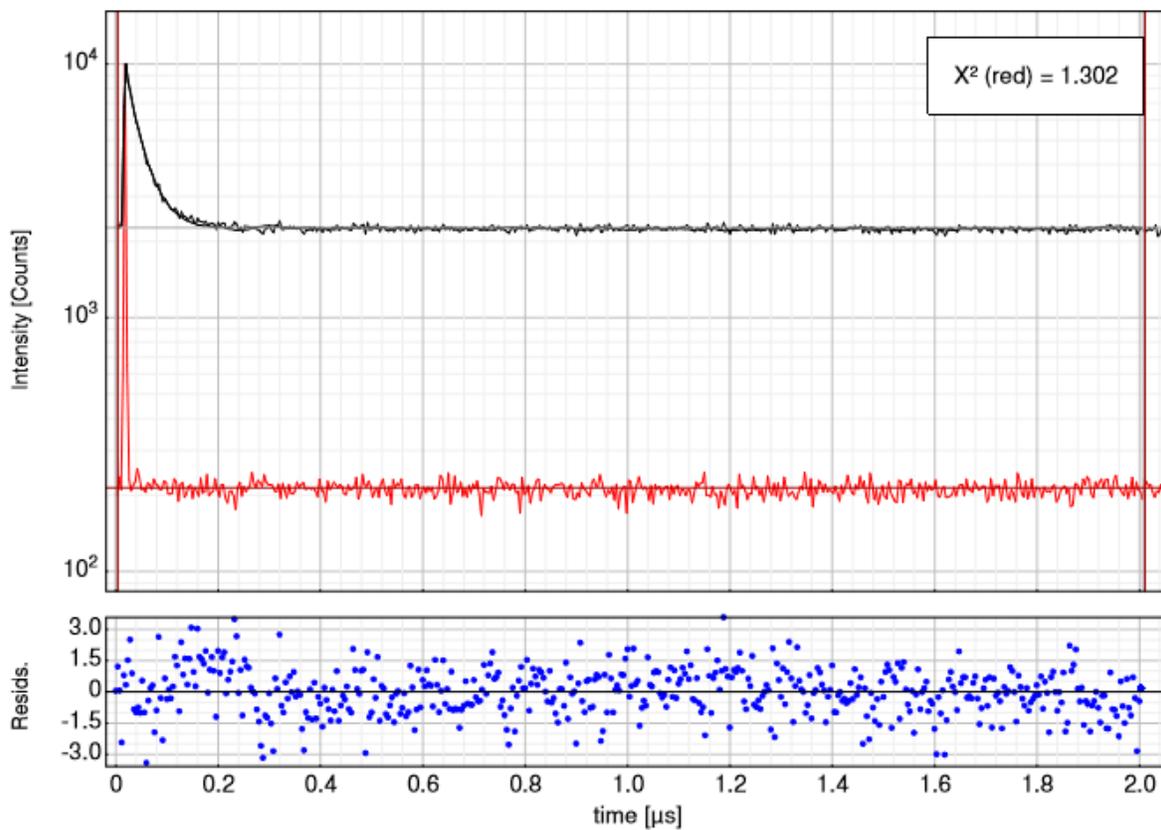


Figure S1. Absorbance and emission spectra of $[\text{Ru}(\text{bpy})_2(\text{pic})]^{2+}$ and $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ in PBS solution. Absorbance and emission spectra were measured using Jasco V-670 spectrophotometer and Varian Cary Fluorimeter.

Supplementary Figure S2. Luminescent lifetime decay of osmium parent and Os-Arg₈.



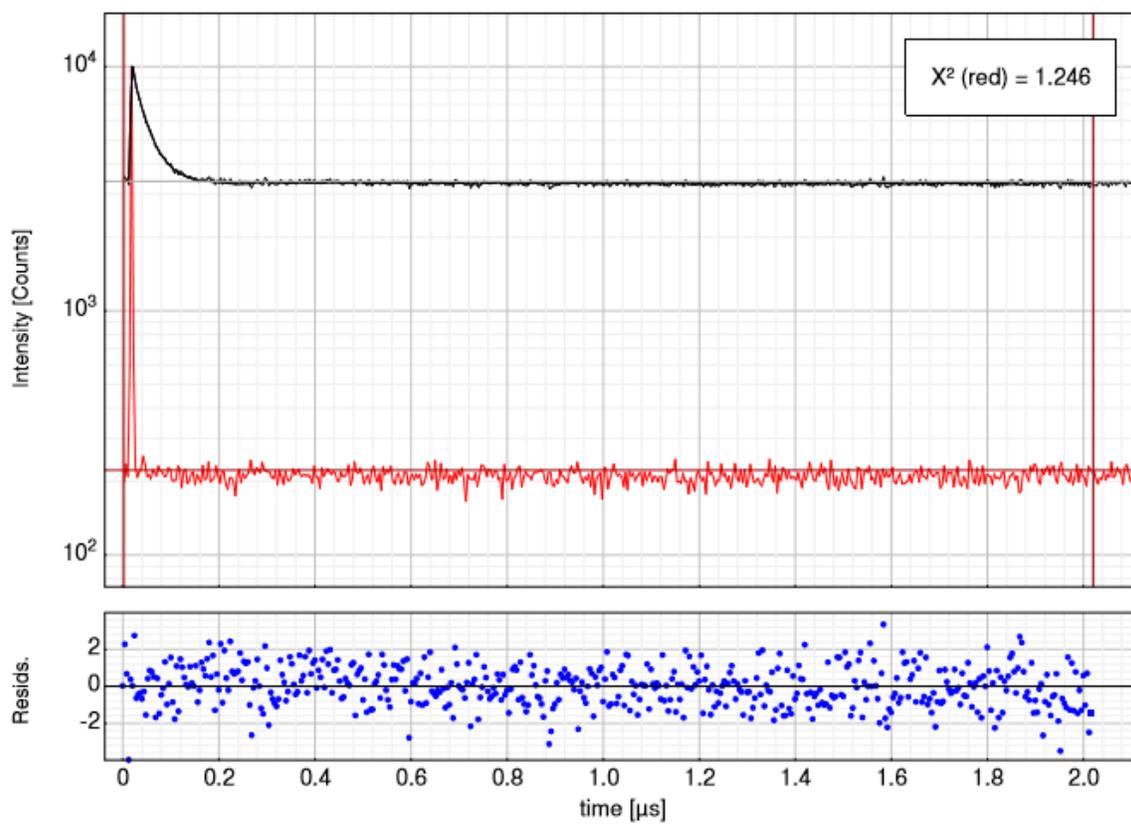


Figure S2. Time-correlated single photon counting (TCSPC) for osmium compounds. The luminescent lifetime decays for $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ (A) and $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ (B) measured in PBS buffer (1% DMSO for parent complex) using NanoHarp 2.1, FluoTime100 (PicoQuant) and fitted using PicoQuant Fluofit software.

Supplementary Figure S3. Emission of osmium complex is oxygen independent

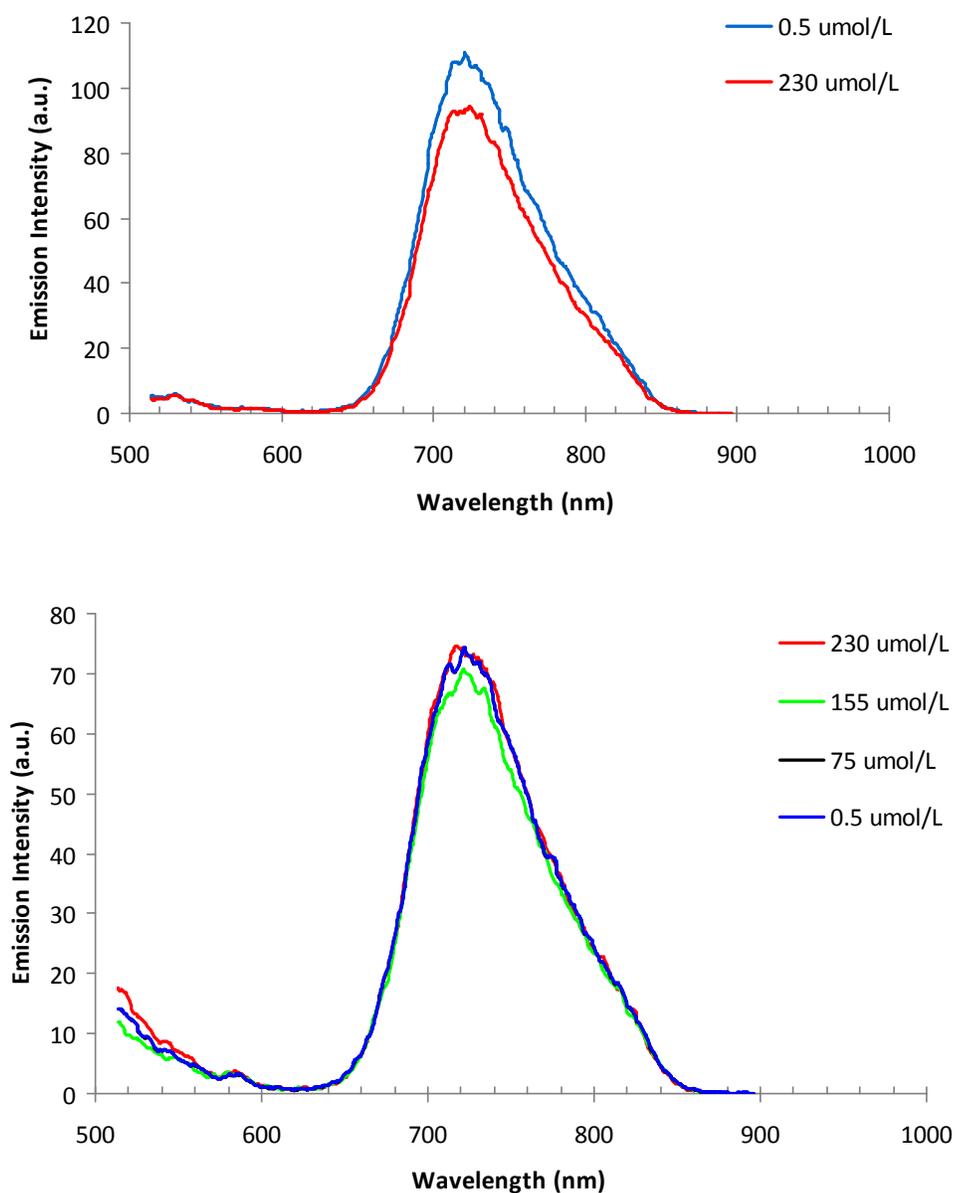


Figure S3. Emission intensity of the osmium complexes is not dependent on oxygen concentration. Solutions of $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ (A) and $[\text{Os}(\text{bpy})_2(\text{pic-arg8})]^{10+}$ (B) both in PBS (1% DMSO for parent complex) were deaerated with nitrogen gas and oxygen concentration were measured (using a micro needle fiber-optic probe, Presens) before and after emission intensity was read (using a Varian Fluorimeter).

Supplementary Figure S4

Videos of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ up take by CHO cells (A) 0-5min, (B) 5-7min, and (C) 7-12min

Supplementary Figure S5: Real Time uptake of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$

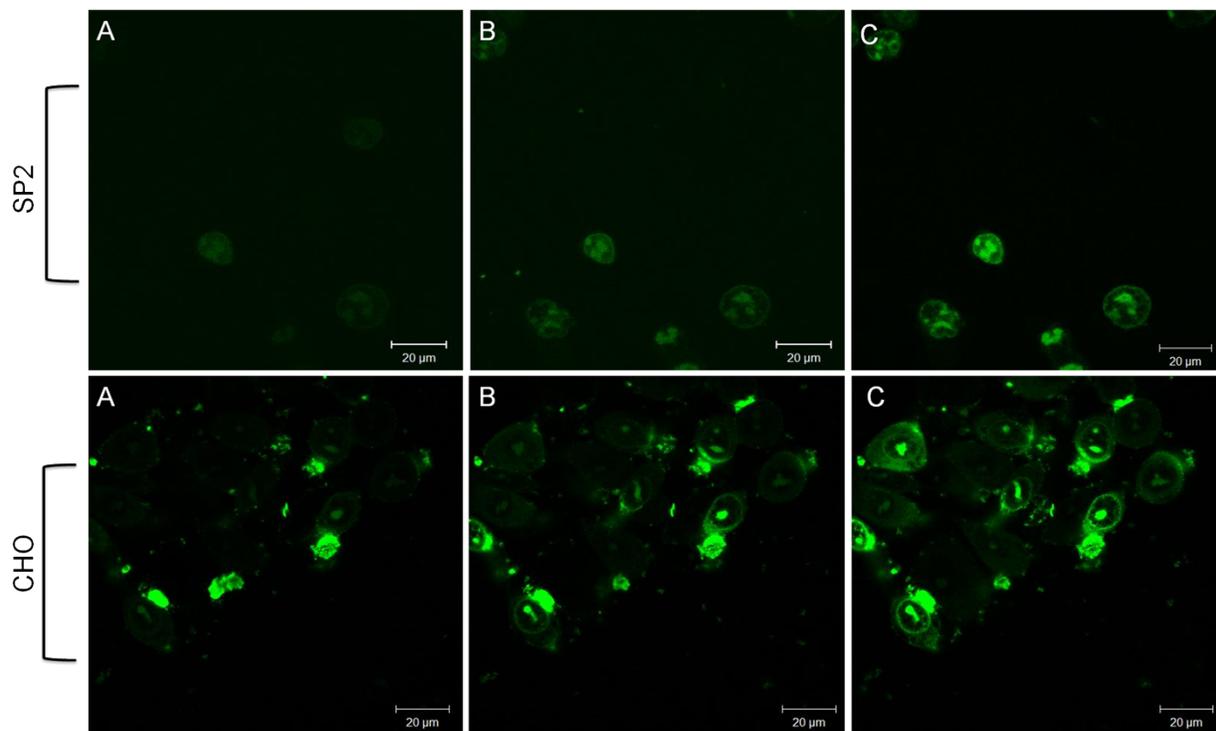


Figure S5 Confocal imaging of real-time uptake studies of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ in SP2 and CHO cells. 70 μM complex was added to cells and imaged continuously to monitor uptake. In both cell lines, initial uptake was seen after 3 minutes exposure (A), with further uptake seen after 10 minutes (B), and at 17 minutes (C) of continuous confocal scanning. Cells were excited with 488 nm argon laser and emission collected using long pass 650 nm filter set

Supplementary Figure S6. $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ in live CHO cells

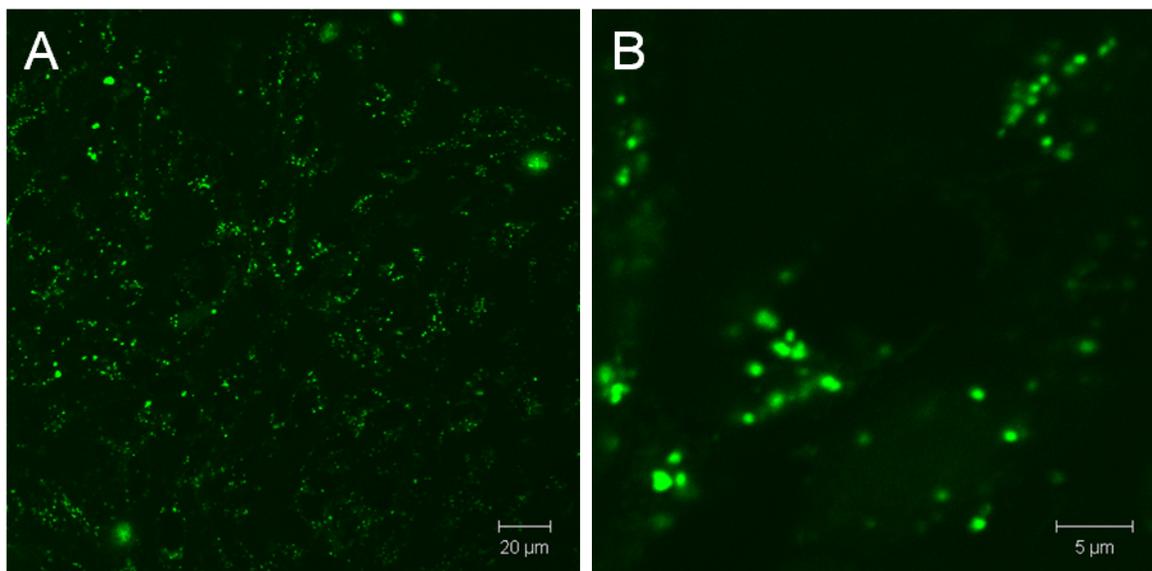


Figure S6. Confocal imaging of live CHO cells incubated with $70 \mu\text{M} [\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ for 2 h. Cells were washed x2 with PBS supplemented with 1.1 mM MgCl_2 and 0.9 mM CaCl_2 . Cells were excited with 488 nm Argon laser, and emission collected with long pass 650 nm filter set. (A) at zoom = 1, and (B) at zoom =4.

Supplementary Figure S7. Co-localisation studies of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$

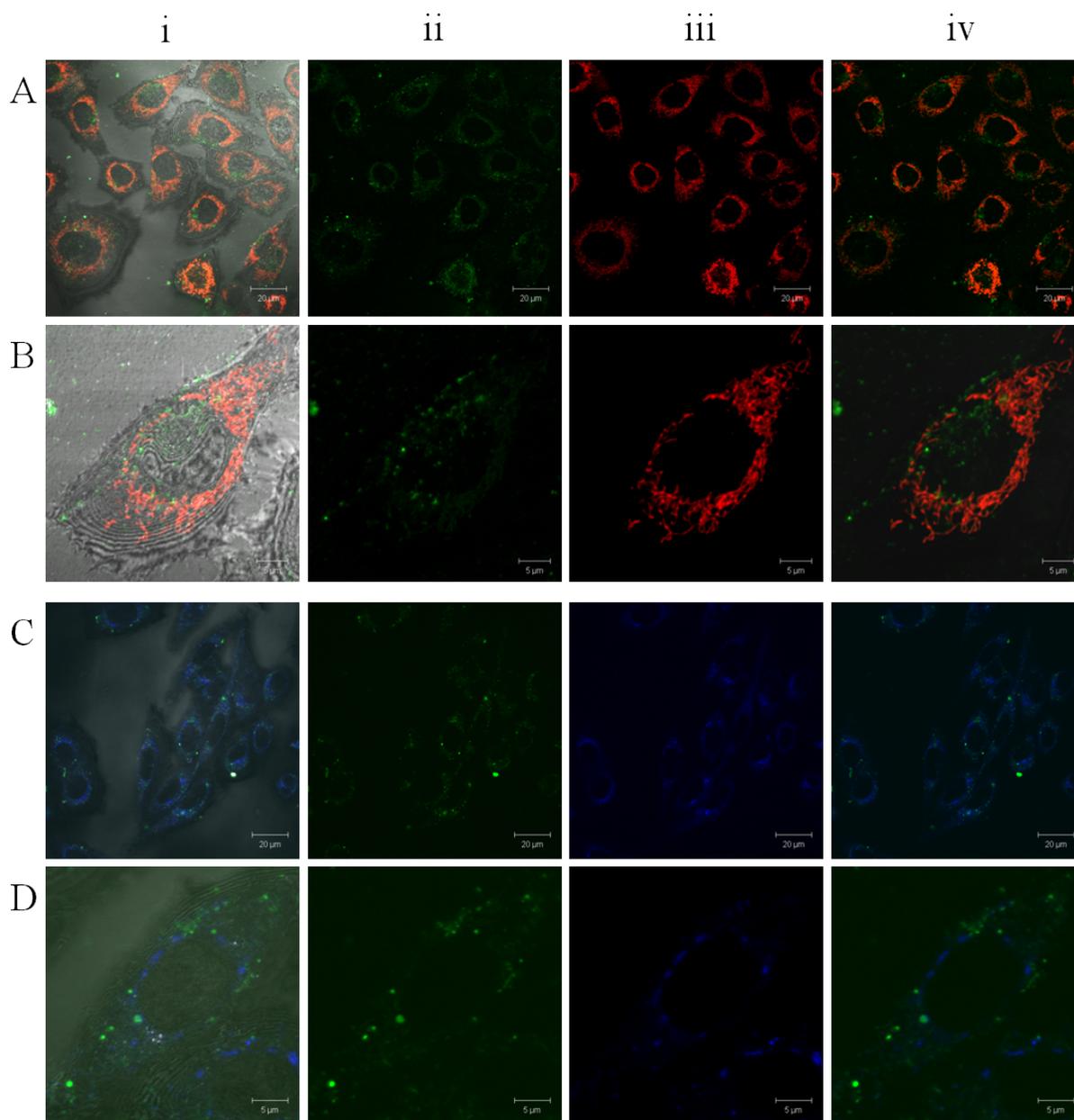


Figure S7 Co-localizing studies of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ carried out with MitoTracker Deep Red (500nM) (A and B) and LysoTracker Green (70nm) (C and D). Row (i) exhibits both the osmium channel, localizing dye channel and background channel; row (ii) osmium channel only; row (iii) localizing dye channel only; row (iv) overlap of Osmium with localising dye excluding background channel. CHO cells were incubated with 70 μM complex for 2h before co-localizing complexes were added.

Supplementary Figure S8. Fluorescent lifetime imaging of osmium in SP2 and CHO cells

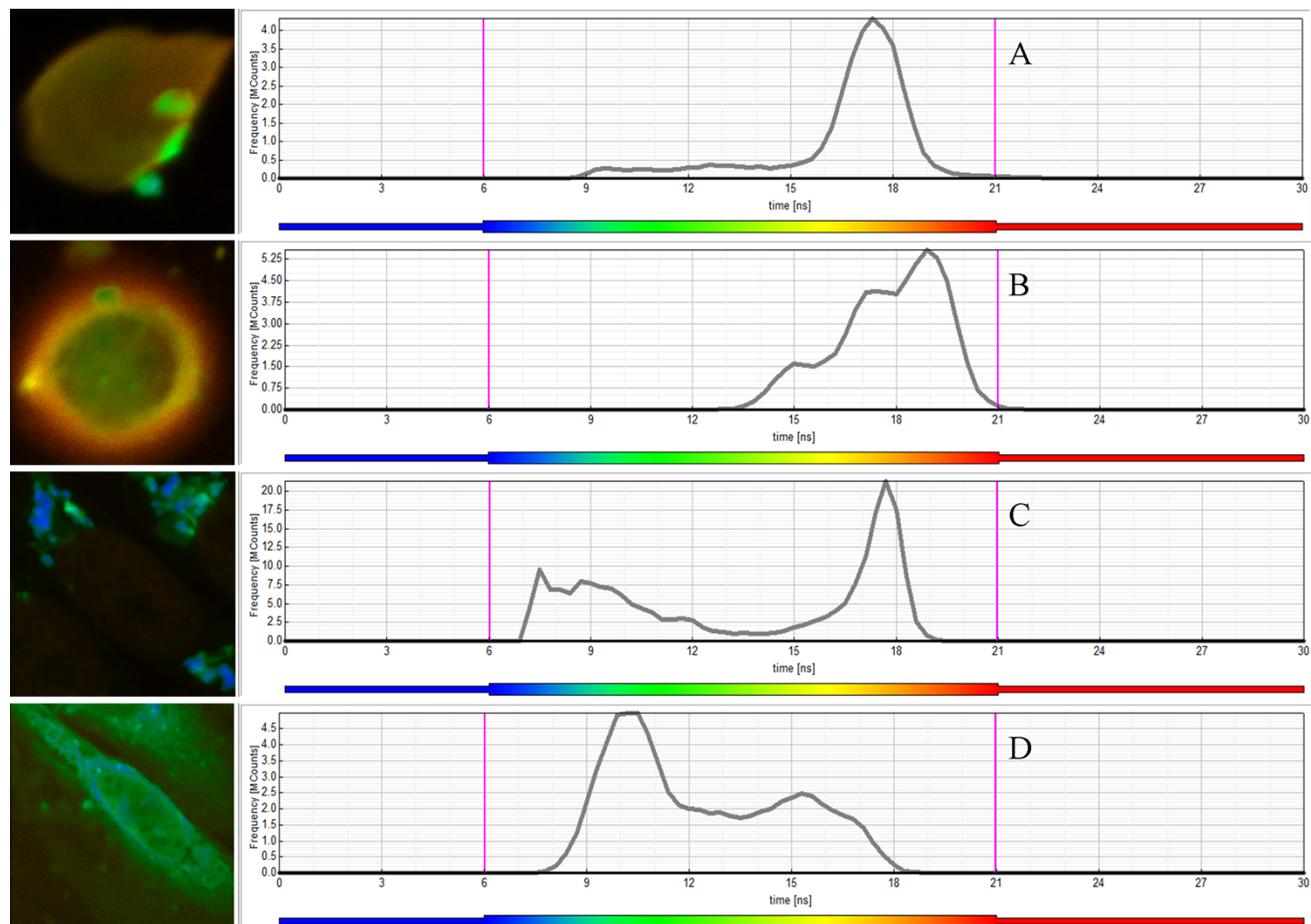


Figure S8. Fluorescent lifetime imaging of $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ and $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ in live SP2 (A and B, respectively) and live CHO cells (C and D, respectively). CHO cells were incubated with 70 μM complex for 24 h. Samples were excited using 405 nm laser and emission collected using long pass 530 nm filter for both complexes. Lifetimes were collected by scanning samples for 30 minutes.

Supplementary Figure S9 – Photostability of $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{pic})]^{2+}$

Comment [AF]: Charges are incorrect in figure

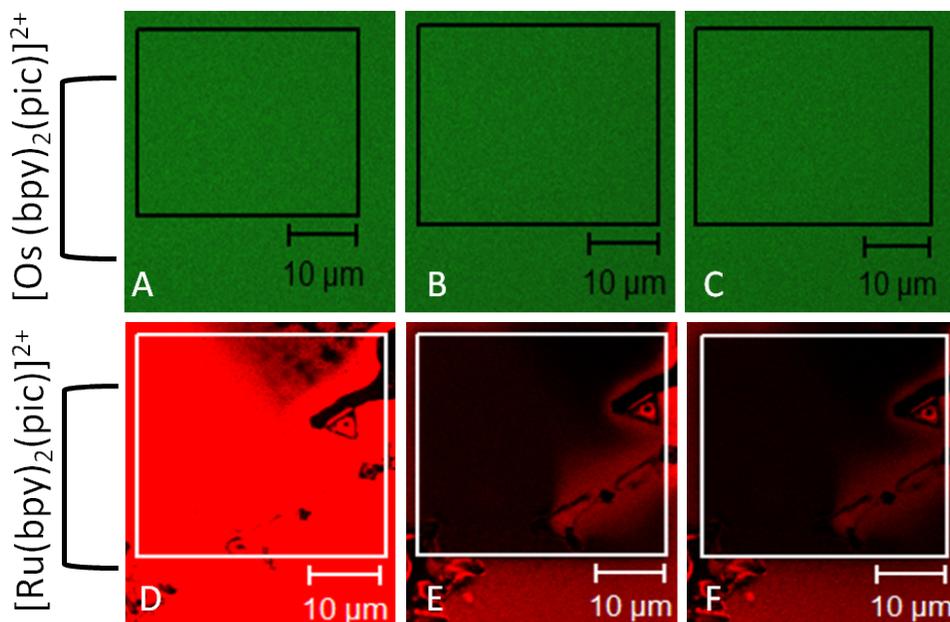


Figure S9 Photostability of $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{pic})]^{2+}$ in PBS solution was assessed using confocal microscopy laser scanning. Photobleaching was performed by exciting $[\text{Os}(\text{bpy})_2(\text{pic})]^{2+}$ with 488 nm laser and 458 nm laser for $[\text{Ru}(\text{bpy})_2(\text{pic})]^{2+}$ with the same incident energy. Both complexes were scanned using 30% laser power at 10 minute intervals. The complexes were imaged at the lowest laser power of 1% before scanning (A and D), after 10 minutes (B and E), and 20 minutes scanning (C and F). (scale bar 20 μM). As shown, extensive bleaching of the ruthenium complex occurred but the osmium was completely photostable under these conditions.

Supplementary Figure S10 A and B. ¹H NMR spectra of osmium parent (S10A) and osmium peptide conjugate (S10B).

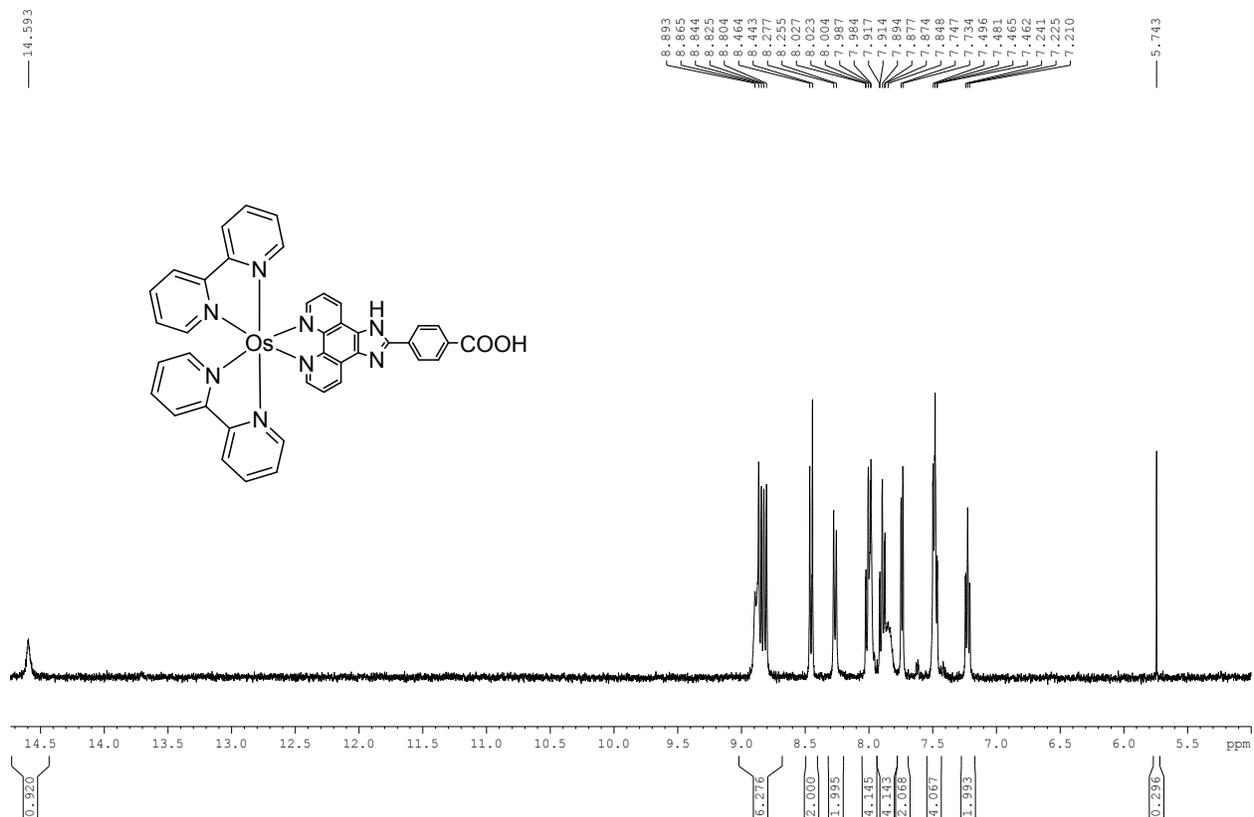


Figure S10A ¹H NMR of [Os(bpy)₂(pic)]²⁺·(ClO₄)₂ in DMSO-d₆.

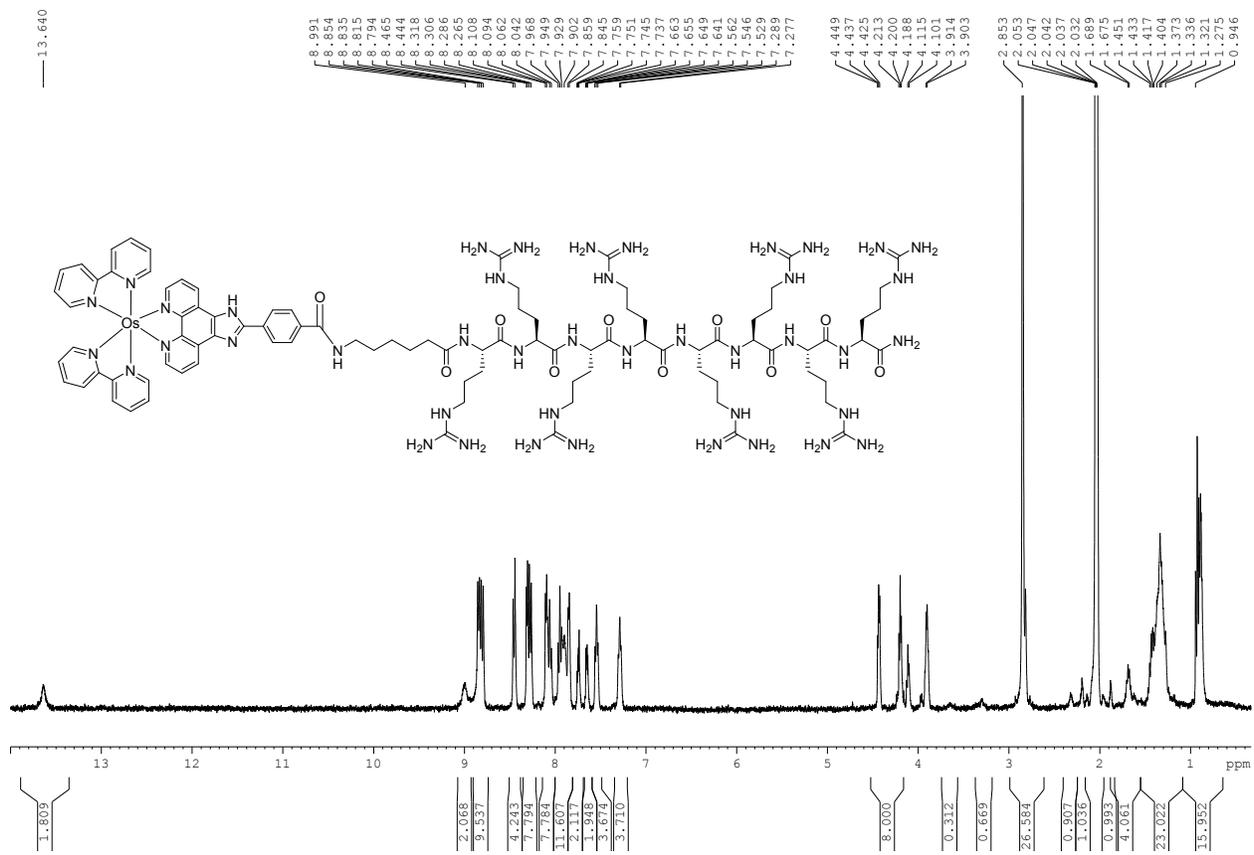


Figure S10B ^1H NMR of $[\text{Os}(\text{bpy})_2(\text{pic-arg}_8)]^{10+}$ in acetone- d_6 .

Supplementary Figure S11. HPLC chromatograms of osmium parent and osmium conjugate

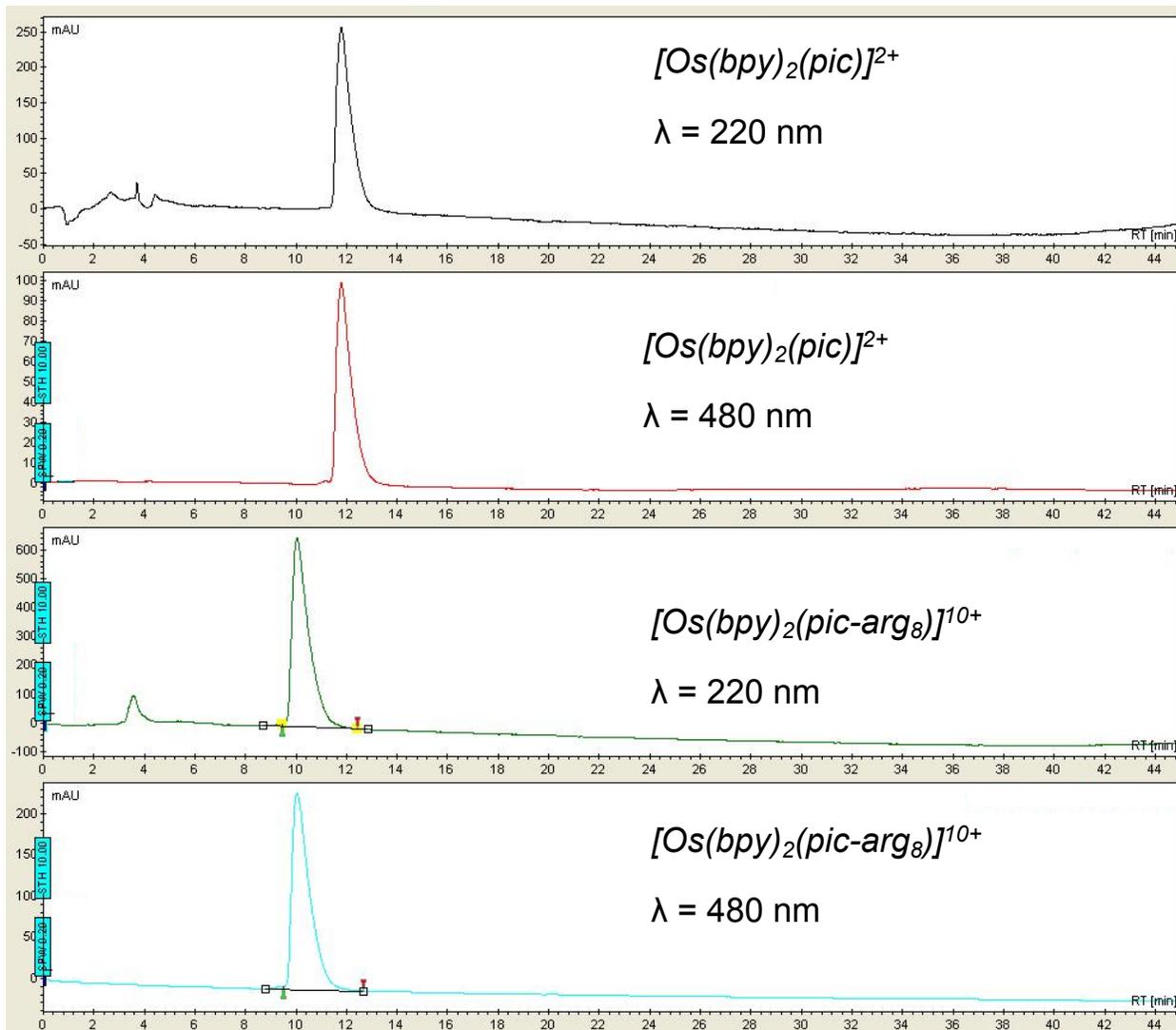


Figure S11 HPLC chromatograms (Hichrom C18 250, mobile phase A DI H₂O (with 0.1% v/v trifluoroacetic acid (TFA)) and mobile phase B acetonitrile (with 0.1% v/v TFA). The mobile phase gradient was initially set for 5%:95% (solvent A:solvent B) and ended up as a 50%:50% (solvent A:solvent B) mixture over the 45 min run time) of osmium parent complex and osmium-peptide conjugate observed at 220 nm and 480 nm. Osmium parent has a retention time of 11.79 min and osmium conjugate of 10.04 min, respectively.

References

[1] Geno, M. J. K.; Dawson, J. H. *Inorg. Chim. Acta* 1985, 97, 241.

[2] Pellegrin, Y.; Forster, R. J.; Keyes, T. E. *Inorg. Chim. Acta* **2009**, 362, 1715-1722.

[3] Pellegrin, Y.; Forster, R. J.; Keyes, T. E. *Inorg. Chim. Acta* **2008**, 361, 2683-2691.