Mitochondria localizing DNA binding biscyclometalated phenyltriazole iridium(III) dipyridophenazene complexes: syntheses and cellular imaging properties

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SUPPORTING INFORMATION

Cellular uptake of 1^+ and 2^+ in A2780 cells:

When subjected to tissue culture experiments with Ovarian cancer cell line (A2780) both the complexes showed instant cellular uptake at a concentration starting from 50 μM when subject to a 24 hour incubation period and the extent of intracellular uptake was determined. The Wide Field Fluorescence Microscopy images acquired shows the localization of the **1^+** and **2^+** mostly in the cytosol region of the A2780 cells.

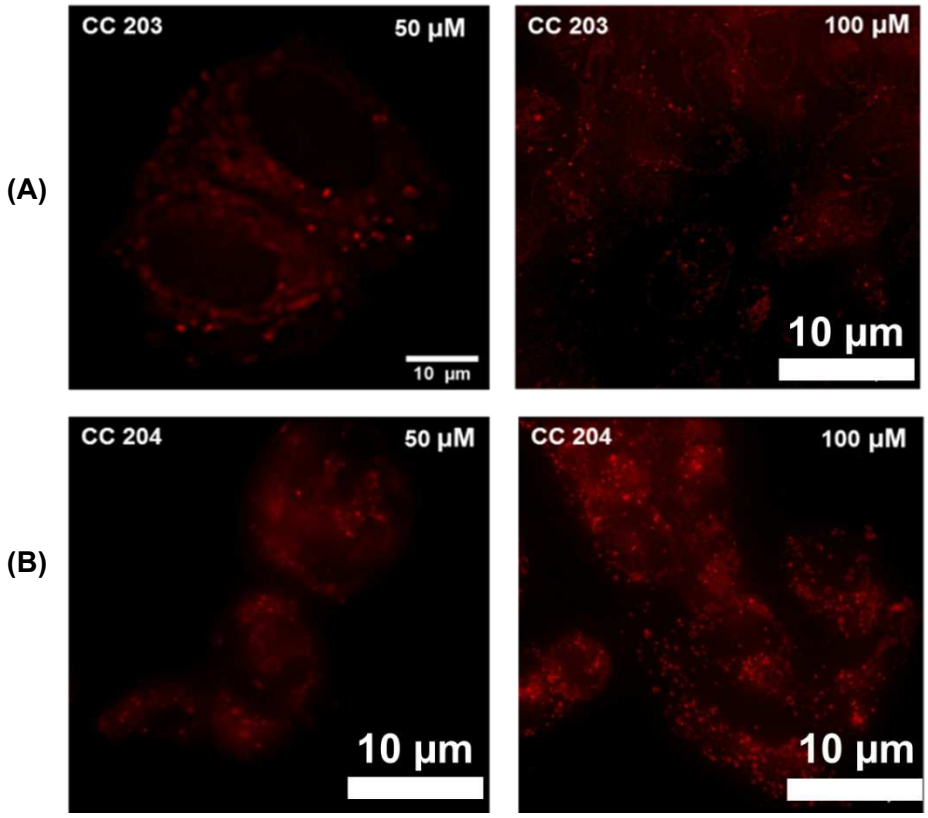


Fig. S1 Comparison images cellular uptake of **(A) 1^+** and **(B) 2^+** (50 μM and 100 μM) in A2780 cells (Pseudo colouring has been employed in all the images)

Intensity of emission signals coming from cytosol:

The Intensity v Distance plot generated offline by using Fiji software indicated that both $\mathbf{1}^{+}$ and $\mathbf{2}^{+}$ were not localizing in the nucleus but they might be localizing over other intracellular organelles present in the cytosol regions of both the cell lines. The Deconvolved Wide Field images obtained by using the Dual cam Nikon Wide Field Microscope indicated structures similar to that of Mitochondria

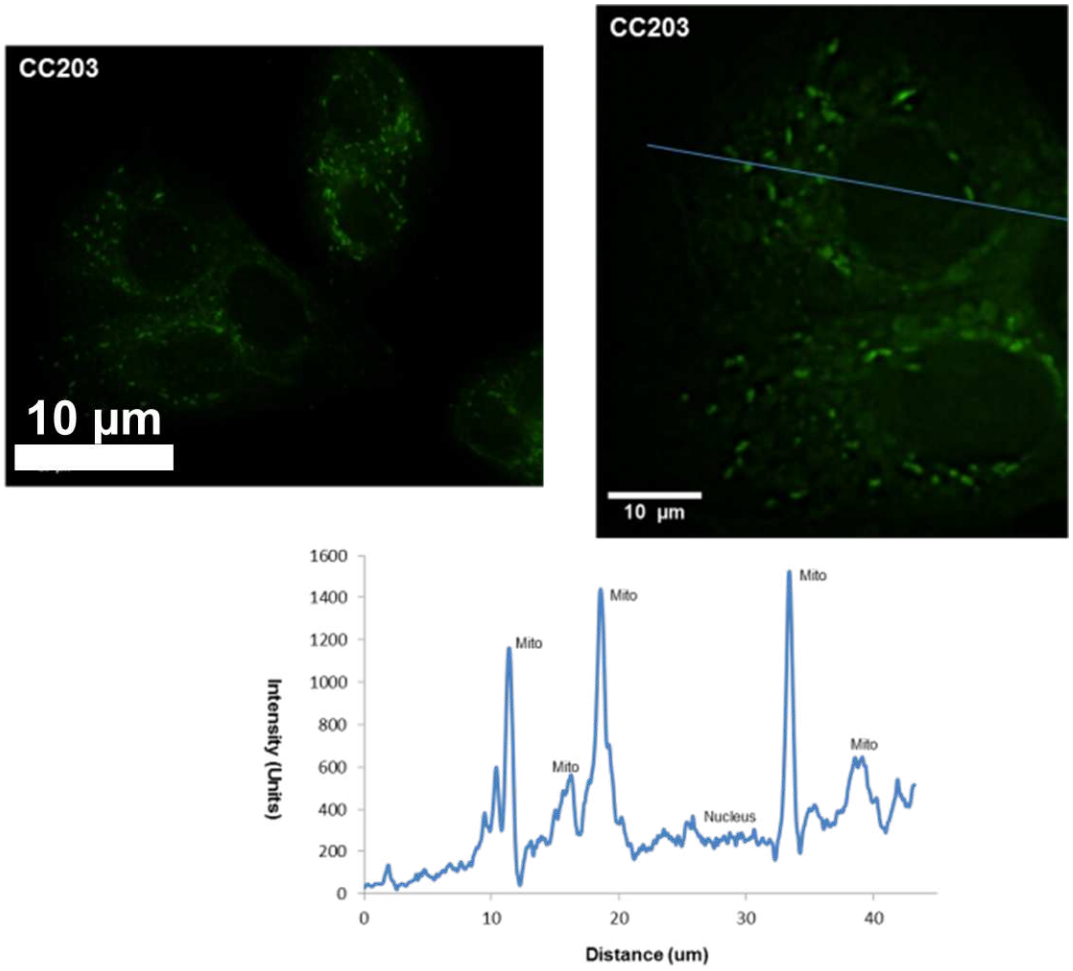
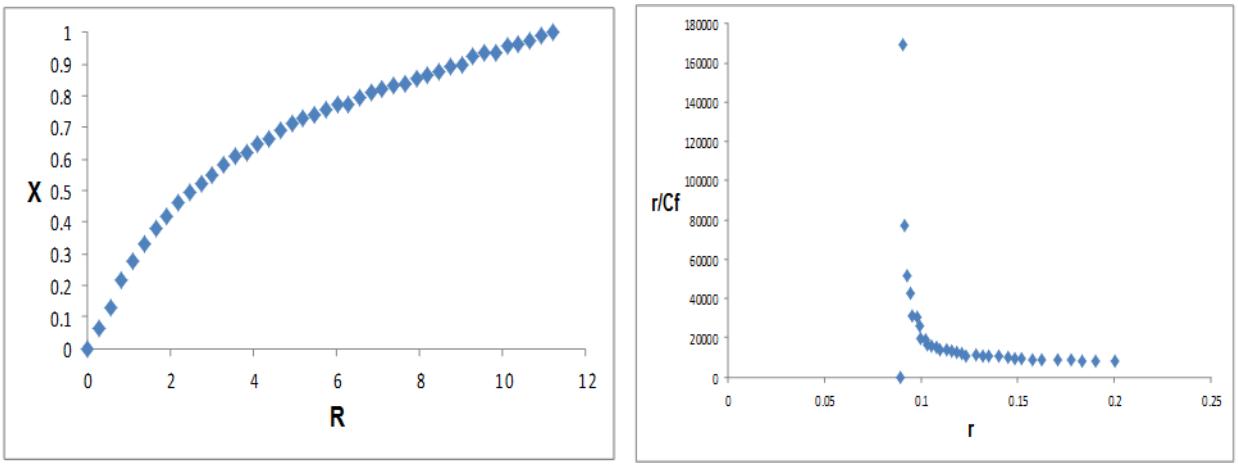


Fig. S2 Intensity v Distance plot of Intracellular uptake of $\mathbf{1}^{+}$ in A2780 cells (Pseudo colouring has been employed in all the images)

DNA binding curves and constants

A



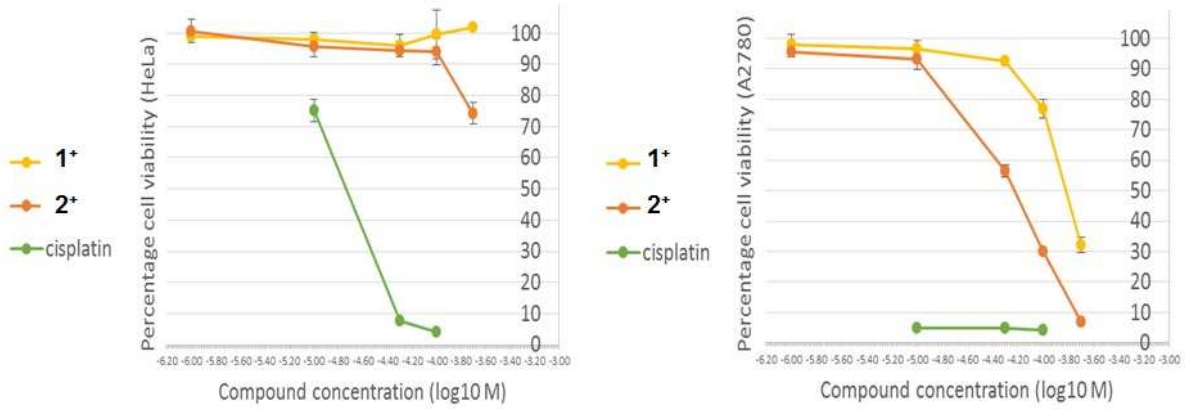
B

Compound	K (M^{-1})	n (bp)
CC 203	2.38 X 10^4	2.7
CC 204	5.37 X 10^4	9.5

Fig. S3 Left to Right: A Theoretical binding curve for CC 203 binding to CT-DNA (were, **X** = Fraction bound, **R** = Ratio of Concentration of DNA and Concentration of metal complex), Scatchard plot for the same (were, **r/C_f**, **r** = Ratio of Concentration of metal complex bound to DNA and Concentration of DNA, **C_f** = Concentration of free metal complex in solution). **(B)** Table showing Binding constants and the number of base pairs binding to CC 203 and CC 204 respectively

Cytotoxicity:

The extent of toxicity against HeLa cells and A2780 cells as illustrated below shows that both **1^+ and 2^+** shows very less cytotoxicity. Against the HeLa cell line, there was no significant cytotoxicity in the most concentrated treatment of 200 μM there were still 74±3% of cells (**1^+**) or 102±1% (**2^+**) still viable. Against the A2780 cell line, there was appreciable but, in comparison to cisplatin, low cytotoxicity – IC_{50} values of 59 μM (**1^+**) and 151 μM (**2^+**). This indicates that **1^+** and **2^+** are less toxic to cell lines and could be used as a safe cellular imaging agent.



Cell Line	1^+	2^+
HeLa	>200	>200
A2780	59	151

Fig. S4 Cytotoxicity studies of the complexes on **A2780** cells and **HeLa** cells

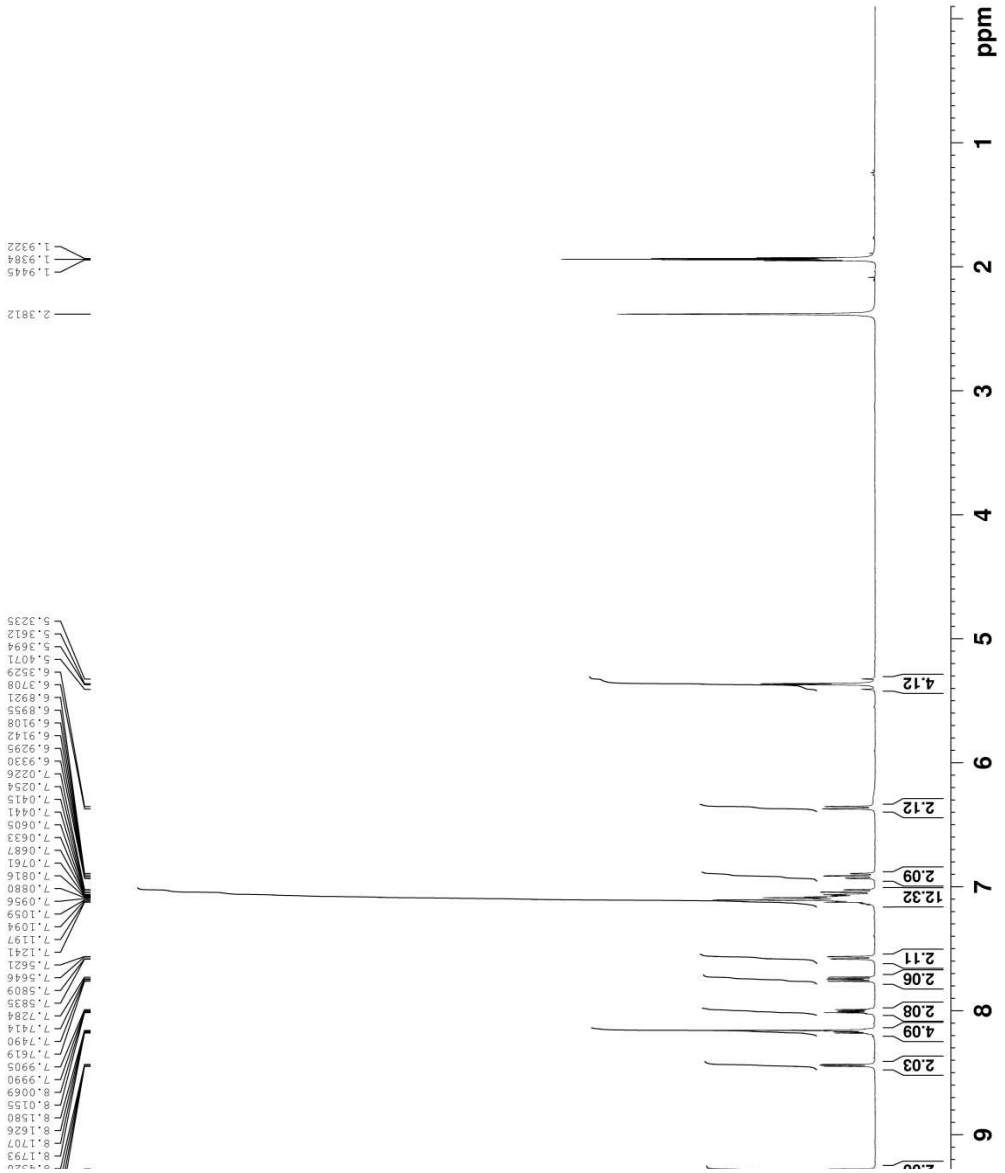


Fig. S5 ^{1}H NMR (400 MHz) spectrum of **1**.[PF_6] in d_3-MeCN.

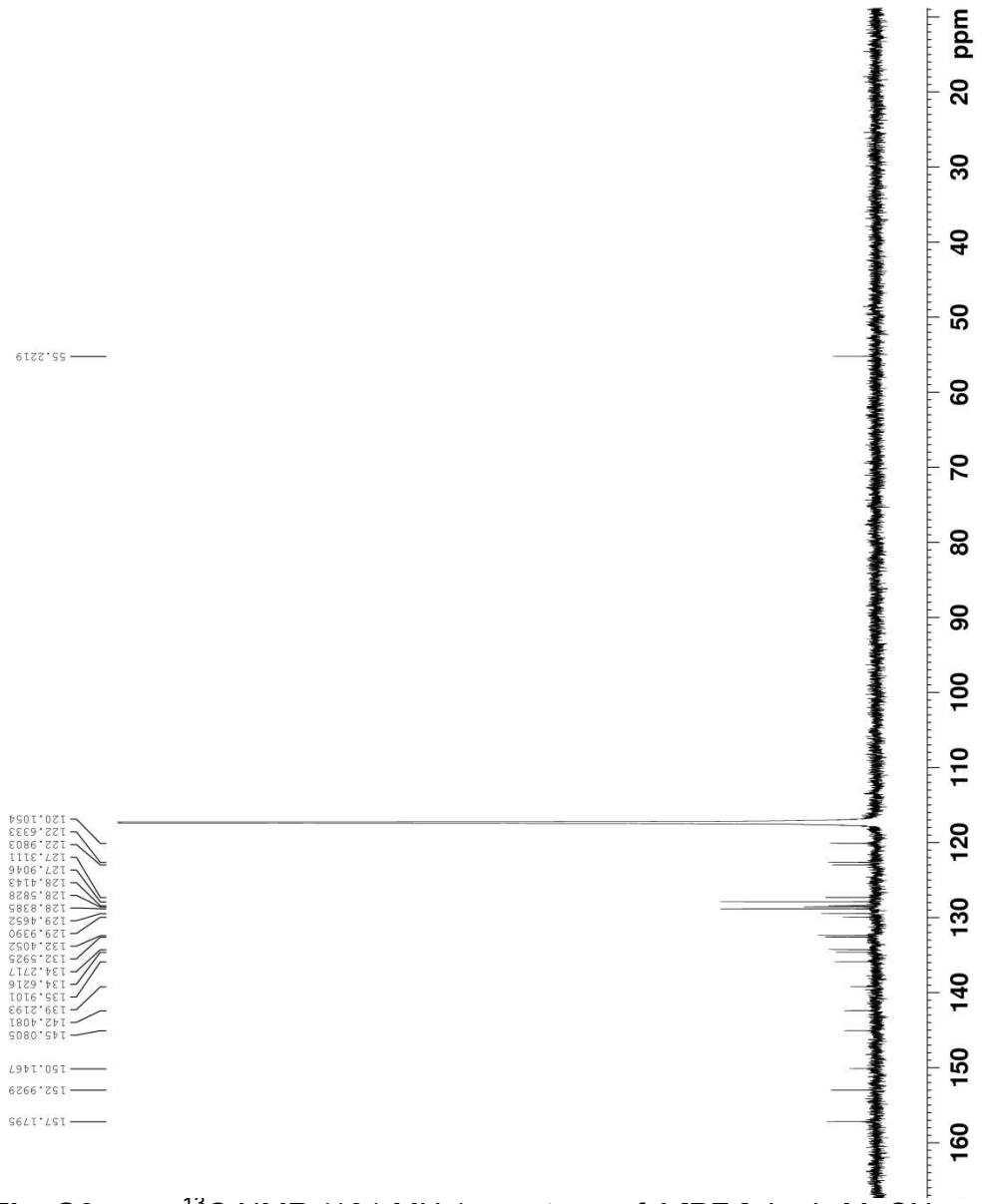


Fig. S6 ^{13}C NMR (101 MHz) spectrum of **1**.[PF_6] in d_3-MeCN.

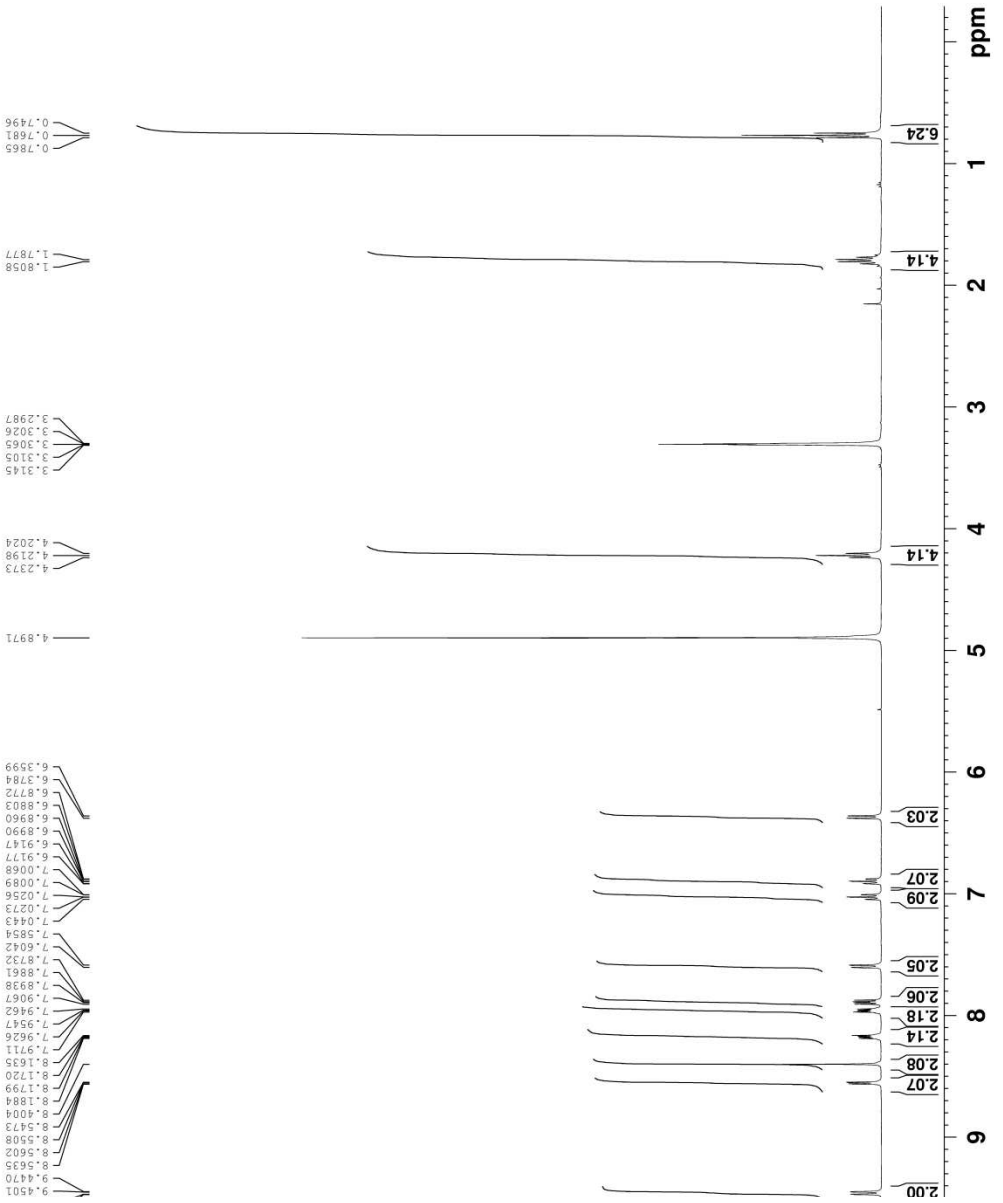


Fig. S7 ^{1}H NMR (400 MHz) spectrum of **2**.[PF_6] in d_4-MeOH.

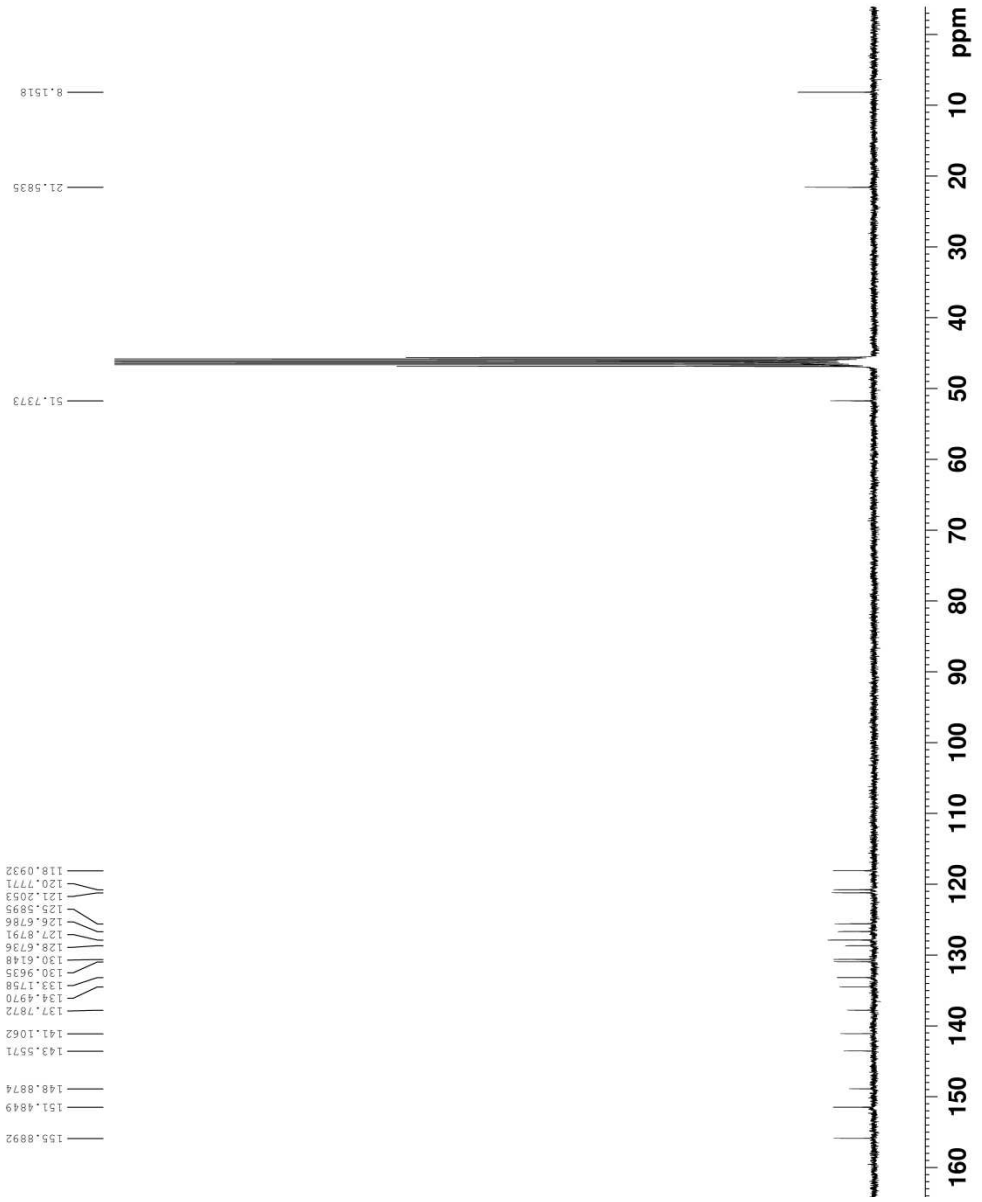


Fig. S8 ^{13}C NMR (101 MHz) spectrum of **2**.[PF_6] in d_4-MeOH.

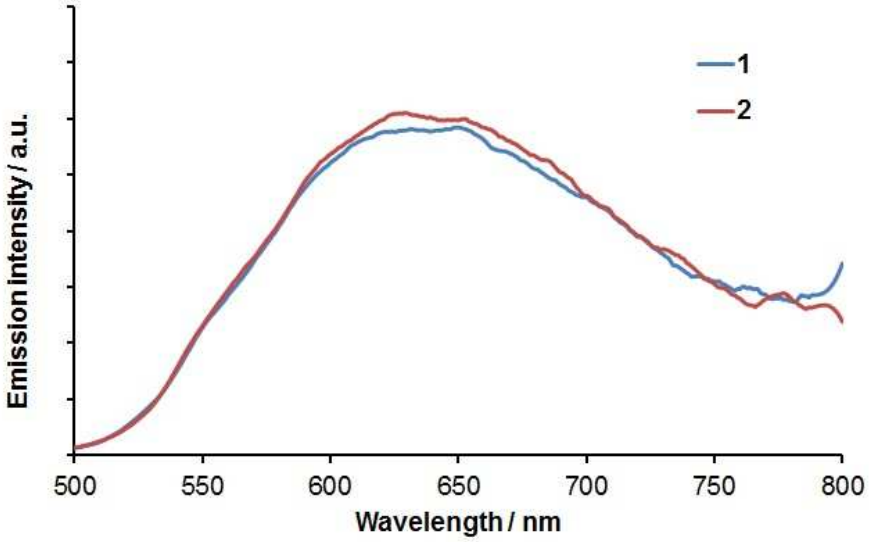


Fig S9. Emission spectra for $\mathbf{1}^{+}$ and $\mathbf{2}^{+}$ as their hexaflourophosphate slats in dichloromethane at room temperature.

Table S1. Summarised key crystallographic parameters for **1**$[NO_3].2.5CH_2Cl_2$.

Formula	$2(IrC_{48}H_{34}N_{10}).2(NO_3).5(CH_2Cl_2)$
a / Å	13.6282(8)
b / Å	16.6165(11)
c / Å	22.7142(14)
α / °	90
β / °	103.912(2)
γ / °	90
Cell volume / $Å^3$	4992.8(5)
Space group	P21/n
M_r	2434.75
Density / g cm^{-3}	1.620
Z	2
μ / mm^{-1}	2.997
Nref	12374
2θmax	56.562
S	1.050
R (reflections)	0.0584(7890)
wR2 (reflections)	0.1178(12374)

Single crystal X-Ray diffraction data were collected on a Bruker D8 Venture diffractometer equipped with a graphite monochromated Mo(K_α) 0.071073 nm radiation source and a cold stream of N_2 gas. Summarised crystal and refinement data are presented in Table S1. Preliminary scans were employed to assess the crystal quality, lattice symmetry, ideal exposure time *etc*. prior to collecting a full sphere of diffraction intensity data using SMART[1] operating software. Intensities

were integrated from several series of exposures, merged and corrected for Lorentz and polarisation effects using SAINT[2] software. Solutions were generated by conventional Patterson heavy atom or direct methods and refined by full-matrix non-linear least squares on F^2 using SHELXS-97 and SHELXL[3] software respectively. Empirical absorption corrections were applied based on multiple and symmetry-equivalent measurements using SADABS.[4] The structure was refined until convergence (max shift/esd <0.01) and in each case, the final Fourier difference map showed no chemically sensible features. The structure for **1** contained large anisotropic displacement parameters which could not be successfully modelled as disorder. As a result four atoms (C44 – C47) were constrained using *DELU*. *SIMU* and *ISOR* in the least squares refinement. One of the chloride atoms of a dichloromethane solvent atom lies on a special position and the resulting $–CH_2Cl$ unit was modelled as 10.5 occupancy.

References

1. SMART Diffracometer Control Software, Bruker Analytical X-ray Instruments Inc., Madison, WI, 1998.

2. SAINT Integration Software, Siemens Analytical X-ray Instruments Inc., Madison, WI, 1994.

3. SHELXTL Program System, Version 5.1, Bruker Analytical X-ray Instruments Inc., Madison, WI, 1998.

4. Sheldrick, G. M. SADABS: A Program for Absorption Correction with Siemens SMART System, University of Gottingen, Germany, 1996.