Supplementary Material

S1. Experimental

Chromatographic analysis and purification were performed on a BioCAD SPRINT Perfusion Chromatography Workstation (PerSeptive Biosystems) using Gemini Reverse-Phase columns (Phenomenex, 110Å, 5microns, C18, 4.6mmd/250mmL, 100mmd/250mmL, for the analytic and semi-preparative columns, respectively). Buffers used were mobile phase A: 0.1% TFA in water; mobile phase B: 0.1% TFA in acetonitrile with a gradient: 2 to 60% B in 18 column volumes (analytical) or 5 column volumes (semi-preparative) with a flow rate: 1 ml/mn (analysis) or 5 ml/mn (semi-preparative) and single wavelength detection at 214 nm.

The peptides were characterised by Matrix Assisted Laser Desorption Ionisation – Time Of Flight – Mass Spectrometry (a-cyano-4-hydroxy-cinnamic acid matrix).

UV/Vis spectra were recorded on a Shimadzu UV-Vis/NIR 3100 spectrophotometer.

Steady-state emission spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer, and luminescence lifetimes were obtained using a Picoquant Fluotime 100 TCSPC system exciting at 470 nm and detecting at 600 nm using a narrow band pass dielectric filter.

Confocal luminescence Images (Myeloma) were recorded with a confocal fluorescence microscope (LSM 50, Zeiss) using a 64x oil immersion objective. An argon ion laser provided 458 nm and 488 nm excitation wavelengths. The scanned images contain 512 x 512 pixels.

Resonance Raman spectra and images were collected with a high resolution micro-Raman setup (HR800, Jobin Yvon, Horiba). The 458 nm line of an argon ion laser (Coherent, Innova) was used to excite in the metal-to-ligand charge transfer band of the Ru complexes. The scattered light was collected in a 180º alignment through a 50x microscope objective using a 1800 groves/mm grating and a cooled CCD camera. Typical integration times were 5s and the spectral window was set to display the most intense Raman bands between 1115-1770 cm^{-1}. Typical Raman maps were recorded with a step size of 1 μm. Data analysis was performed using LabSpec software.

Materials

2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline (PIC), Dipyrido-[3,2-a:29,39-c]-phenazine (DPPZ) and $[Ru(dppz)_2]Cl_2$ were prepared according to literature procedures.[iii, iii]

HBTU: (O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate), HOBt: N-hydroxybenzotriazole, DIEA: N,N-diisopropyl ethylamine, NMP: N-methylpyrrolidone, PyBOP: benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, TFA: trifluoroacetic acid, TIA: triisopropylsilane, TA: thioanisole, Fmoc: (9-fluorenylmethyl) carbamate, Pbf: 2,2,4,6,7-pentamethyl-dihydrobenzofurane-6-sulfonyl.) were all purchased from Sigma Aldrich and used as received.

Synthesis

$[Ru(dppz)_2(PIC)]ClO_4$:

1.63 mmol of both $Ru(dpp)_2Cl_2$ (1.12g) and PIC ligand (0.55g) were reacted under reflux in ethylene glycol overnight, during which the solution turned dark orange. Aqueous lithium perchlorate was added to precipitate the complex. The suspension was vacuum-filtered and washed thoroughly with deionised water. The crude material yielded was dissolved with 50/50 dichloromethane/methanol and the filtrate was then evaporated and a suspension of the complex was formed in ether. The dark orange powder was washed with deionised water and dried with ether. Yield: 72.2% (1.30g) ESI-MS: M^{2+} 1005.6 m/z, $M^{2+}/2$ 503.5 m/z ^{1}HNMR (400 MHz, d6-DMSO): □ 9.70 (m, 4H), 9.16 (d, 2H), 8.58 (m, 4H), 8.53 (m, 2H), 8.41 (t, 2H), 8.25, m, 10H), 7.98 (m, 2H), 7.87 (m, 2H).)

$[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$

The peptides were synthesised by standard Solid Phase Peptide Synthesis according to the Fmoc-tBu strategy described previously with HBTU/HOBt/DIEA coupling chemistry in NMP solvent.[iv] The arginine oligomers with spacer were synthesised on Fmoc rink amide MBHA resin using an Applied Biosystems 433A Automated Peptide Synthesiser in single coupling cycles. 1 mmol (ten excess) of the Fmoc protected forms of the aminohexanoic acid spacer and the amino acid arginine (with Pbf as a side chain protecting group) were utilised for the synthesis. Fmoc

deprotection was achieved using piperidine and the carboxy terminus of the incoming amino acid was activated using HBTU/HOBt chemistry (0.1 M concentration of both) in conjunction with DIEA/NMP (70:130). The ruthenium label was coupled to the resin-bound arginine oligomer using HOBt/PyBOP chemistry (0.015 mM), DMF and DIEA with the ruthenium label at a concentration of 0.15 mM (ten excess) and allowed to react for four hours. Resin cleavage was performed using TFA as a cleaving agent and TIA, TA and deionised water as scavengers. The labelled peptide was precipitated using ether, separated via centrifugation and lyophilised. The labelled arginine oligomer was purified via HPLC. Resulting fractions were combined and lyophilised and analysed via MALDI-TOF mass spectrometry. (Purity was assessed and confirmed using HPLC, Yield: 1.7mg(4.8%) ESI-MS: M^+ 2284 m/z.

Preparation of large unlamellar vesicles

These were prepared from dipalmitoylphosphatidylglycerol (dppg) according to the literature procedure:

Dppg (1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)) phospholipids (Sigma Aldrich) were dissolved in 10 mM TRIS/ 50mM KCL and PBS buffer (pH 7.4). The solution was then sonicated at approximately 58^{o}C (as lipid phase transition temperature of dppg is 44^{o}C). The solution became clear and less viscous. The liposomes were then extruded at 58^{o}C through 0.1μm (Avanti Lipids) polycarbonate filters. The resulting liposomes were stored at 4^{oC} and analyzed for uniformity using a Malvern High Performance Particle Sizer.

The complexes and conjugate in buffer were added after the liposomes were prepared and characterised. After the addition of each luminophore, the final concentration of liposomes corresponded ~1 mg/ml and to a luminophore absorbance of 0.1 (5μM).

S2: Photophysical Properties of complexes

	$[Ru(dppz)_2PIC]^{2+}$	$[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$
UV/VIS Absorbance Maxima (nm)	*447*	*440*
	330	*330*
	258	*258*
Emission Maximum (nm)	*613*	*614*
Emission Lifetime (μs)	*0.68 ±0.017*	*0.78 ±0.022*

Table 1 Photophysical characterisation of $[Ru(dppz)_2PIC]^{2+}$ and $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$ in 9/1 acetonitrile/dmso following dearation with N_2.

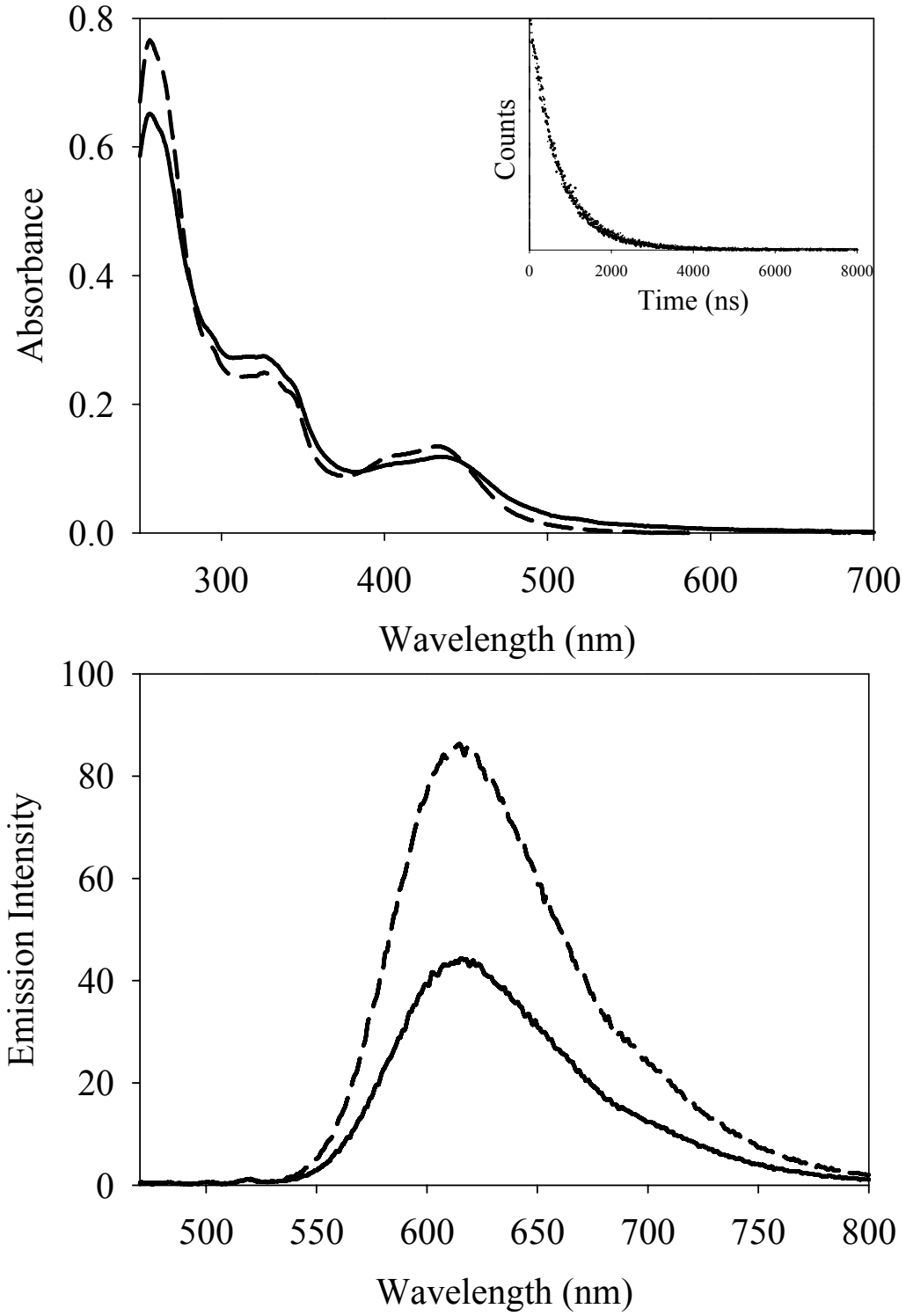

Figure 1 UV/VIS Spectra (top), emission spectra (bottom) and lifetime data (insert) of $[Ru(dppz)_2PIC]^{2+}$ (solid line, ——) and $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$, (dashed line, - - -) in 9/1 V/V acetonitrile/DMSO. (All solutions were absorbance matched and solutions for lifetime data were degassed with nitrogen).

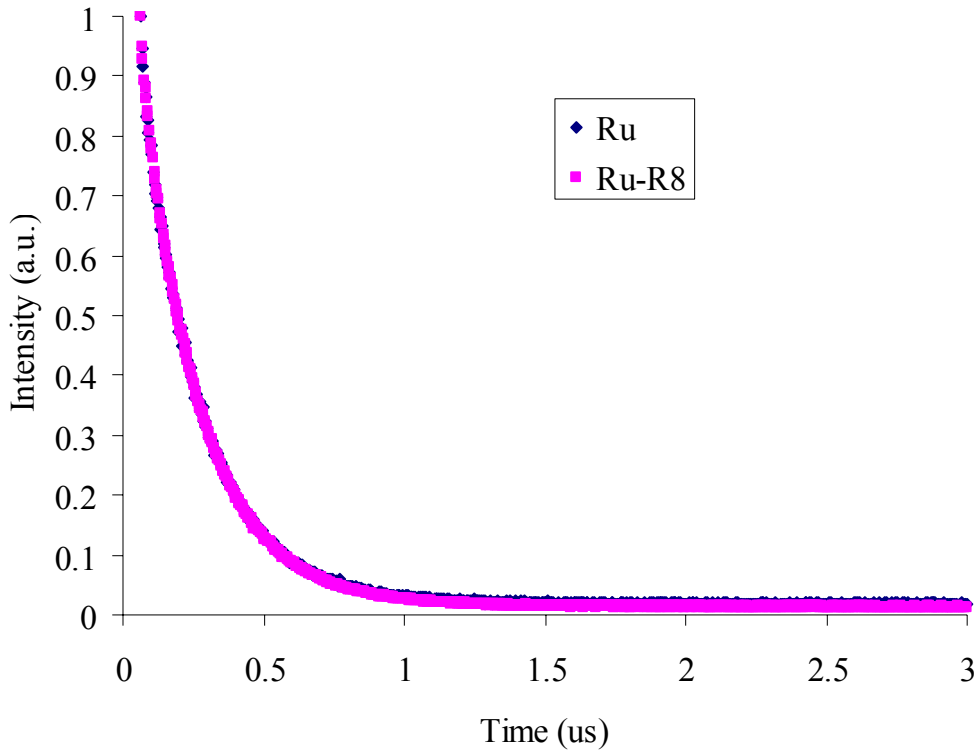

Figure 2 TCSPC lifetime data of [Ru(dppz)2PIC]2+ and $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$, measured in aerated 9:1 V/V acetonitrile:DMSO. All decays were fit using FluoFit software.

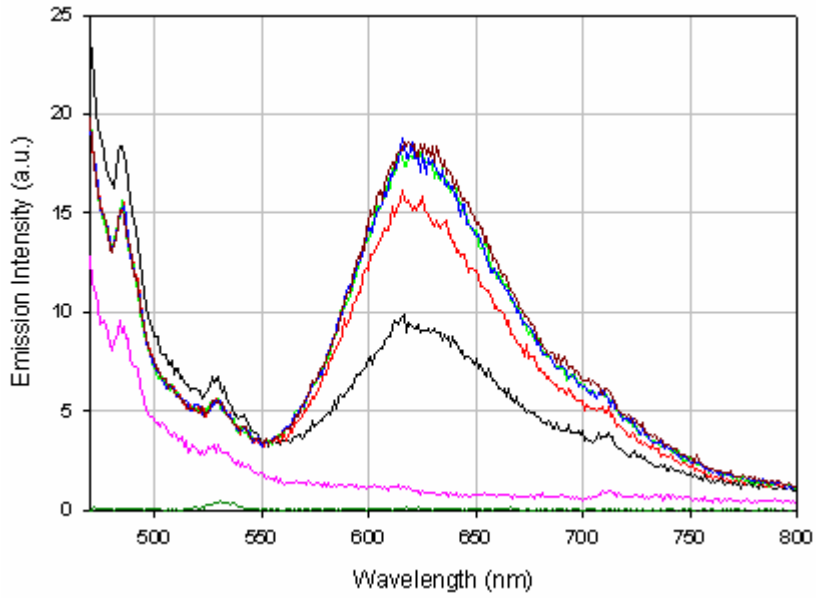

Figure 3 Luminescence Spectra 1 x 10^{-4} M $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$ in the presence of Large DPPG unilamellar vesicle in PBS buffer. Green line shows the emission from $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$ in buffer prior to addition of liposome, pink blank liposome solution, prior to addition of dye. Black luminescence spectrum 2 hours after addition of $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$ to liposome solution, subsequent spectra show emission 1 (red) to 5 days after addition of $[Ru(dppz)_2PIC\text{-}Arg_8]^{10+}$.

S3: NMR Characterisation of Precursors.

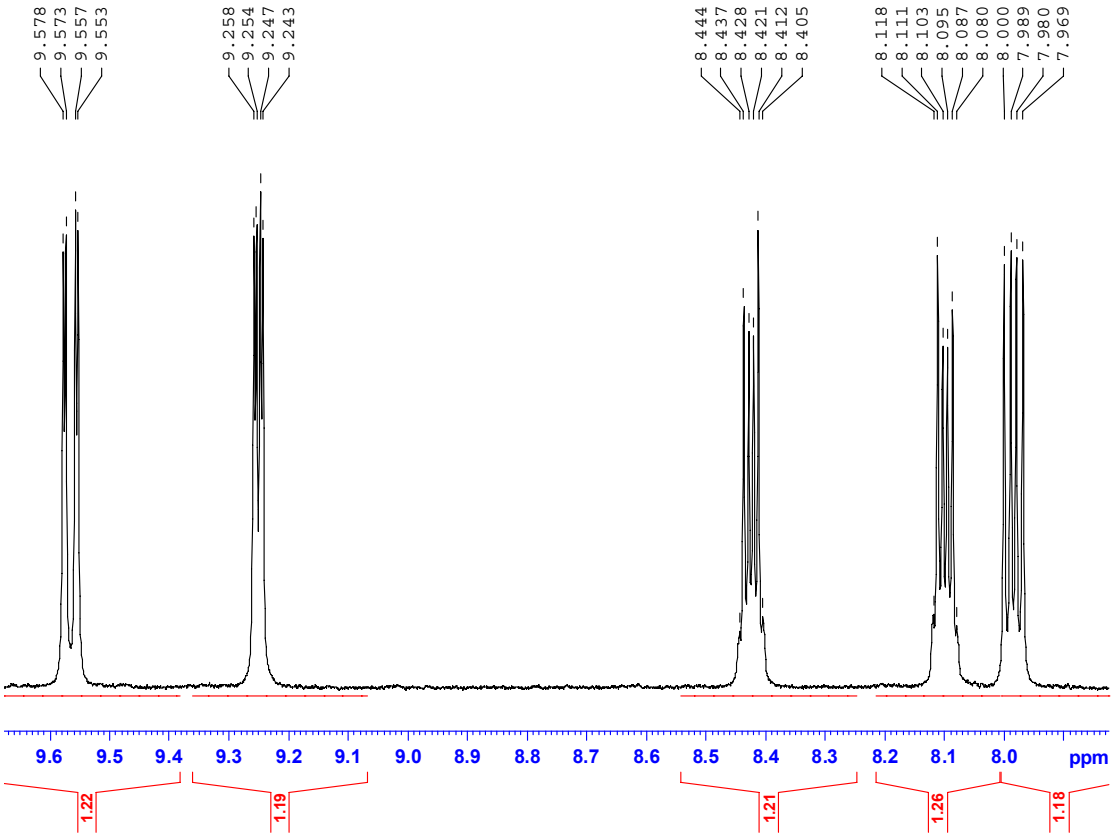

Figure 4 Proton NMR of ligand DPPZ (dipyrido-[3,2-a:29,39-c]-phenazine) in deuterated DMSO.

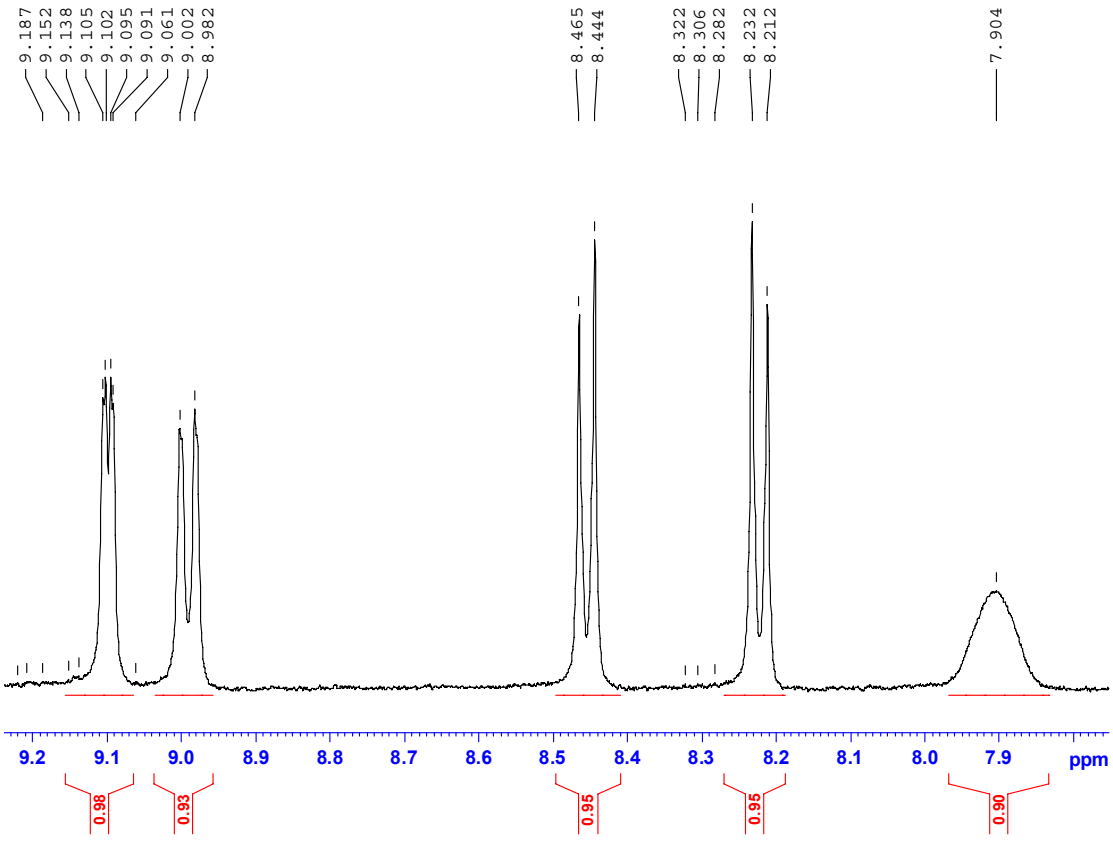

Figure 5 Proton NMR of ligand PIC in deuterated DMSO.

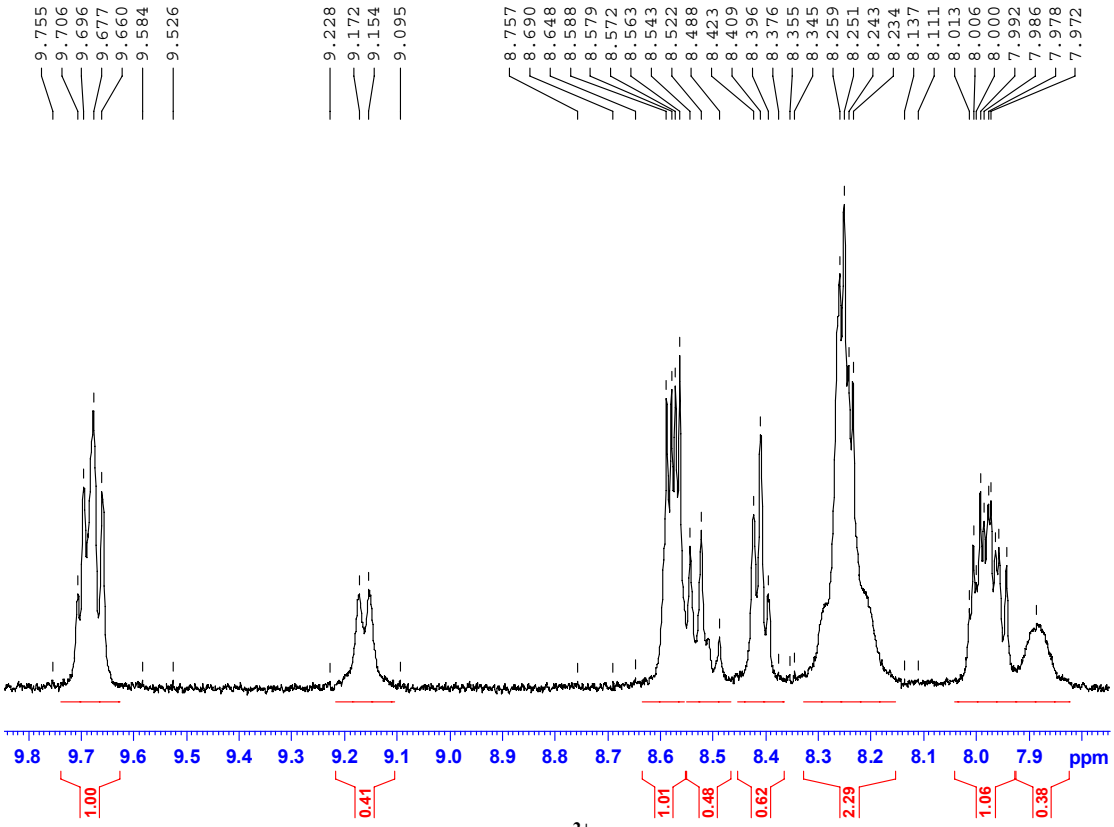

Figure 6 Proton NMR of complex $[Ru(dppz)_2PIC]^{2+}$ in deuterated DMSO.

References

[i] Y. Pellegrin, R.J. Forster, T. E Keyes. Inorg. Chim. Acta, 2009, 362, 1715.
[ii] J.E. Dickeson and L.A. Summers, Aust. J. Chem. 1970, 23, , 1023.
[iii] B.P. Sullivan, D.J. Salmon, T. J., and Meyer, Inorg. Chem, 1978, 17, 3334.
[iv] U. Neugebauer, Y. Pellegrin, M. Devocelle, R. J. Forster, W. Signac, N. Moran and T. E. Keyes, Chemical Communications, 2008, 5307-5309.)