Supplementary Information for:

Cyrene™ as a green alternative to N,N'-dimethylformamide (DMF) in the synthesis of MLCT-emissive ruthenium(II) polypyridyl complexes for biological applications

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Experimental

General Information

All chemical reagents and solvents were purchased from commercial sources (Sigma-Aldrich, ThermoScientific) and used as supplied. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker Advance III 500 MHz Nuclear Magnetic Resonance Spectrometer. HRMS (high resolution mass spectroscopy) samples were analysed at the EPSRC UK National Mass Spectrometry Facility at Swansea University using a ThermoScientific LTQ Orbitrap XL 1 Mass Spectrometer. Fourier Transform Infrared Spectra were run on a Perkin Elmer FT-IR Spectrometer Spectrum TWO. Elemental analysis was performed by the Elemental Analysis Service at London Metropolitan University. Microwave-assisted synthesis was performed on a CEM Discover 2.0 microwave synthesis reactor operating at a power maximum level of 150 W. The reactions were carried out in sealed glass tubes (15 mL) equipped with stirring bars.

Synthesis and characterisation

$Ru(N^N)_2Cl_2$ complexes:

$RuCl_3.3H_2O$ (100 mg, 1 molar equivalent), LiCl (113 mg, 7 mol. eq.), ascorbic acid (68 mg, 0.5 mol. eq.) and the required N^N ligand (2 mol. eq.) were dissolved into 5 mL Cyrene with stirring and gentle heating. In the case of N^N = bpy, no ascorbic acid was employed. The mixture was heated to the desired temperature for the stated reaction time. The reaction was cooled to room temperature, excess acetone added (20-50 mL) and stored overnight at 4 °C. The precipitated solid was collected by vacuum filtration and washed thoroughly with water and ether. Reactions using DMF were performed as described previously,[1] using a modification of the original method described by Sullivan et al.[2]

$Ru(bpy)_2Cl_2$

Cyrene™ conditions: Yield: 145 mg, 79%; dark purple solid; Elemental analysis (%) calcd (found) for $Ru(bpy)_2Cl_2.H_2O$: C 47.82 (47.53), H 3.61 (3.78), N 11.15 (10.11). HRMS: [M-Cl]+ calcd 449.01, found 449.0105.

DMF conditions: Yield: 125 mg, 68%; dark purple solid; Elemental analysis (%) calcd (found) for $Ru(bpy)_2Cl_2.H_2O$: C 47.82 (47.9), H 3.61 (3.31), N 11.15 (10.72). HRMS: [M-Cl]+ calcd 449.01, found 449.0100.

$Ru(phen)_2Cl_2$

Cyrene™ conditions: Yield: 134 mg, 67%; dark purple solid; Elemental analysis (%) calcd (found) for $Ru(phen)_2Cl_2.H_2O$: C 52.37 (52.12), H 3.30 (3.52), N 10.18 (8.57). HRMS: [M-Cl]+ calcd 497.01, found 497.0009.

DMF conditions: Yield: 160 mg, 80%; dark purple solid; Elemental analysis (%) calcd (found) for $Ru(phen)_2Cl_2.H_2O$: C 52.37 (51.28), H 3.30 (2.3), N 10.18 (10.39). HRMS: [M+Na]+ calcd 554.97, found 554.9678.

$Ru(4,4'\text{-}dmbpy)_2Cl_2$

Cyrene™ conditions: Yield: 101 mg, 49%; brown/black solid; Elemental analysis (%) calcd (found) for $Ru(4,'4\text{-}dmbpy)_2Cl_2$: C 53.3 (51.0), H 4.48 (3.53), N 10.4 (9.07). HRMS: [M-Cl]+ calcd 505.07, found 505.0736.

DMF conditions: 111 mg, Yield: 54%; brown/black solid; Elemental analysis (%) calcd (found) for $Ru(4,4'\text{-}dmbpy)_2Cl_2$: C 53.3 (46.57), H 4.48 (3.4), N 10.4 (9.62). HRMS: [M-Cl]+ calcd 505.07, found 505.0731.

$Ru(5,5'dmbpy)_2Cl_2$

Cyrene™ conditions: Yield: 138 mg, 67%; brown/black solid; Elemental analysis (%) calcd (found) for $Ru(5,5'\text{-}dmbpy)_2Cl_2$: C 53.3 (48.84), H 4.48 (4.09), N 10.4 (9.41). HRMS: [M-Cl]+ calcd 505.07, found 505.0729.

DMF conditions: Yield: 82 mg, 40%; brown/black solid; Elemental analysis (%) calcd (found) for Ru(5,5'-dmbpy)$_2$Cl$_2$: C 53.3 (48.86), H 4.48 (3.91), N 10.4 (9.41). HRMS: [M-Cl]+ calcd 505.07, found 505.0730.

Ru(tmphen)$_2$Cl$_2$

Cyrene™ conditions: Yield: 135 mg, 55%; dark purple solid; Elemental analysis (%) calcd (found) for Ru(tmphen)$_2$Cl$_2$.3H$_2$O: C 55.01 (53.44), H 5.48 (3.85), N 8.02 (7.5). HRMS: [M]+ calcd 644.10, found 644.1013.

DMF conditions: 199 mg, 81%; dark purple solid; Elemental analysis (%) calcd (found) for Ru(tmphen)$_2$Cl$_2$: C 59.6 (61.91), H 5.0 (4.31), N 8.7 (9.19). HRMS: [M]+ calcd 644.10, found 644.1042.

[Ru(N^N)$_3$]$^{2+}$ complexes:

[Ru(bpy)$_3$](PF$_6$)$_2$

cis-Ru(bpy)$_2$Cl$_2$ (100 mg, 0.206 mmol) was reacted with 2,2-bipyridine (32 mg, 0.205 mmol) in a minimum volume of ethylene glycol (~7 mL) for 25 minutes at 160 °C in the microwave reactor. The solution was poured over water (10 mL) and excess NH_4PF_6 was added. The precipitate was collected by vacuum filtration and recrystallised in 1:1 methanol:water. The solid was washed with water and diethyl ether, before drying in an oven. Yield: 83 mg, 47%; Orange solid; ^{1}H NMR (500 MHz, DMSO) δ 8.85 (d, *J* = 8.1 Hz, 1H), 8.18 (t, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 5.4 Hz, 1H), 7.54 (t, *J* = 6.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 157.01, 151.67, 138.39, 128.35, 124.94. HRMS: [M-PF6]+ calcd 715.07, found 715.0757. [M-2PF6]2+ calcd 285.06, found 285.0556. Elemental analysis (%) calcd for [Ru(bpy)$_3$](PF$_6$)$_2$ (found): C 41.92 (40.42), H 2.81 (2.42), N 9.78 (7.74). HPLC purity: 99.07%, retention time = 1.403 min.

[Ru(phen)$_3$](PF$_6$)$_2$

The same method as for [Ru(bpy)$_3$](PF$_6$)$_2$ was employed using *cis*-Ru(phen)$_2$Cl$_2$ (60 mg, 0.113 mmol) and 1,10 phenanthroline (20 mg, 0.113 mmol) in ethylene glycol (~5 mL). Yield: 47 mg, 42%; Orange solid; ^{1}H NMR (500 MHz, DMSO) δ 8.78 (dd, *J* = 8.3, 1.1 Hz, 1H), 8.39 (s, 1H), 8.09 (dd, *J* = 5.2, 1.1 Hz, 1H), 7.77 (dd, *J* = 8.2, 5.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 153.22, 147.71, 137.28, 130.91, 128.51, 126.77. HRMS: [M-PF6]+ calcd 787.07, found 787.0760. [M-2PF6]2+ calcd 321.06, found 321.0558. Elemental analysis (%) calcd for [Ru(phen)$_3$](PF$_6$)$_2$ (found): C 46.41 (46.16), H 2.6 (1.89), N 9.02 (8.30). HPLC purity: 99.8%, retention time = 1.535 min.

[Ru(4,4'-dmbpy)$_3$](PF$_6$)$_2$

The same method as for $[Ru(bpy)_3](PF_6)_2$ was employed using *cis*-$Ru(4,4'\text{-}dmbpy)_2Cl_2$ (50 mg, 0.092 mmol) and 4,4-dimethyl bipyridine (17 mg, 0.092 mmol) in ethylene glycol (~5 mL). Yield = 32 mgs, 37%; Orange solid. ^{1}H NMR (500 MHz, DMSO) δ 8.69 (s, 1H), 7.54 (d, *J* = 5.8 Hz, 1H), 7.34 (d, *J* = 5.6 Hz, 1H), 2.51 (s, 4H). ^{13}C NMR (126 MHz, DMSO) δ 156.65, 150.71, 149.76, 128.90, 125.41, 21.18. HR HRMS: [M-PF6]+ calcd 799.17, found 799.1707. [M-2PF6]2+ calcd 327.10, found 327.1029. Elemental analysis (%) calcd for $[Ru(4,4'\text{-}dmbpy)_3](PF_6)_2$ (found): C 45.8 (46.74), H 3.85 (2.79), N 8.91 (7.99). HPLC purity: 99.8%, retention time = 1.982 min.

$[Ru(tmphen)_3](PF_6)_2$

The same method as for $[Ru(bpy)_3](PF_6)_2$ was employed using *cis*-$Ru(tmphen)_2Cl_2$ (95 mg, 0.14 mmol) and 2,3,7,8 tetramethyl 1,10 phenanthroline (33 mg, 0.14 mmol) in ethylene glycol (~7 mL). Yield = 80 mg, 51%; Brown solid. ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 1H), 7.68 (s, 1H), 2.77 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 152.39, 146.48, 144.57, 134.91, 129.20, 124.74, 18.06, 15.01. HRMS: [M-PF6]+ calcd 955.26, found 955.2654. [M-2PF6]2+ calcd 405.15, found 405.1498. Elemental analysis (%) calcd for $[Ru(tmphen)_3](PF_6)_2$ + PF6 (found): C 46.31 (46.27), H 3.89 (3.61), N 6.75 (6.82). HPLC purity: 96.04%, retention time = 4.893 min.

$[Ru(N\text{^}N)_2(N\text{^}N)]^{2+}$ complexes:

$[Ru(tmphen)_2(DIP)](PF_6)_2$

The same method as for $[Ru(bpy)_3](PF_6)_2$ was employed using *cis*-$Ru(tmphen)_2Cl_2$ (124 mg, 0.19 mmol) and 4,7-Diphenyl-1,10-phenanthroline (64 mg, 0.19 mmol) in ethylene glycol (~7 mL). Yield: 182 mg, 80 %; Orange/brown solid; ^{1}H NMR (500 MHz, CD_3CN) δ 8.42 (s, 2H), 8.19 (s, 2H), 8.04 (d, *J* = 5.5 Hz, 1H), 7.84 (s, 1H), 7.79 (s, 1H), 7.70 (s, 1H), 7.68 – 7.57 (m, 8H), 7.55 (d, *J* = 5.5 Hz, 1H), 2.82 (s, 4H), 2.30 (s, 4H). ^{13}C NMR (126 MHz, CD_3CN) δ 153.44, 153.17, 152.55, 149.49, 148.96, 147.07, 145.17, 136.40, 135.40, 135.20, 130.40, 130.12, 129.66, 126.52, 126.27, 124.67, 17.36, 14.62. HRMS: [M-PF6]+ calcd 1051.26, found 1051.2633. [M-2PF6]2+ calcd 453.15, found 453.1492. Elemental analysis (%) calcd for $[Ru(tmphen)_2(DIP)](PF_6)_2$ + PF6 (found): C 50.16 (51.69), H 3.16 (3.05), N 6.27 (6.11). HPLC purity: 90.7%, retention time = 13.428 min.

$[Ru(bpy)_2(dppz)](PF_6)_2$

Dppz and $[Ru(bpy)_2(dppz)](PF_6)_2$ were synthesised as described previously.[3]

Dppz: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.70 (dd, *J* = 8.1, 1.6 Hz, 1H), 9.32 (d, *J* = 3.1 Hz, 1H), 8.40 (dd, *J* = 6.5, 3.4 Hz, 1H), 7.97 (dd, *J* = 6.5, 3.4 Hz, 1H), 7.85 (dd, *J* = 8.1, 4.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.44, 142.58, 141.08, 134.08, 130.81, 129.61, 127.74, 124.31.

$[Ru(bpy)_2(dppz)](PF_6)_2$: ^{1}H NMR (500 MHz, CD_3CN) δ 9.65 (d, *J* = 8.2 Hz, 1H), 8.58 (dd, *J* = 15.8, 8.1 Hz, 2H), 8.46 (dd, *J* = 6.5, 3.4 Hz, 1H), 8.31 – 8.09 (m, 3H), 8.10 – 7.86 (m, 3H), 7.79 (d, *J* = 5.5

Hz, 1H), 7.61 – 7.46 (m, 1H), 7.31 (m, *J* = 6.6 Hz, 1H). ^{13}C NMR (126 MHz, CD_3CN) δ 157.60, 154.31, 152.58, 151.05, 143.29, 140.58, 138.59, 134.06, 133.09, 131.42, 130.19, 128.23, 128.02, 124.88. HRMS: [M-PF6]+ calcd 841.10, found 841.0974. [M-2PF6]2+ calcd 348.07, found 348.0664. Elemental analysis (%) calcd for $[Ru(bpy)_2(dppz)](PF_6)_2$ (found): C 46.3 (46.65), H 2.66 (2.45), N 11.37 (11.16). HPLC purity: 96.2%, retention time = 2.537 min.

10,11-dmdppz

1,10-Phenanthroline-5,6-dione (0.136 g, 0.647 mmol) and 3,4-dimethyl-o-phenylenediamine (0.0993 g, 0.729 mmol) were dissolved in chloroform (7 mL). The mixture was refluxed under nitrogen for 3 hours before being cooled to room temperature. The light-yellow precipitate was filtered under vacuum and washed with ether. The solid was recrystallised with hot dichloromethane:ethanol (1:1), and then dried in an oven. Yield: 0.0952 g, 47%; Pale yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.68 (dd, *J* = 8.1, 1.6 Hz, 1H), 9.63 (dd, *J* = 8.1, 1.6 Hz, 1H), 9.32 – 9.24 (m, 2H), 8.10 (d, *J* = 8.7 Hz, 1H), 7.88 – 7.77 (m, 2H), 7.75 (d, *J* = 8.7 Hz, 1H), 2.98 (s, 3H), 2.65 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.21, 152.16, 148.21, 148.12, 141.58, 141.42, 139.68, 139.63, 138.60, 134.69, 134.20, 133.72, 133.60, 128.09, 127.75, 126.30, 124.07, 20.76, 13.15. HRMS: $[M+H]^+$ calcd 311.13, found 311.1289.

$[Ru(bpy)_2(10,11\text{-}dmdppz)](PF_6)_2$

$Ru(bpy)_2Cl_2$ (0.193 g, 0.399mmol) and 10,11-dmdppz (0.0832g, 0.268mmol) were added to a mixture of ethanol: water 1:1 (15 mL). The mixture was refluxed under nitrogen for 16 hours, then cooled to 4^{o}C for 24 hours. Ethanol was removed by rotary evaporation and a saturated aqueous solution of ammonium hexafluorophosphate (1 mL) was added. The brown/orange precipitate was collected by vacuum filtration, washed with DI water and recrystallised using hot acetonitrile. The solid was collected by vacuum filtration, washed with ether, and dried *in vacuo.* Yield: 0.1736 g, 63 %; Orange solid; ^{1}H NMR: (500 MHz, Acetone) δ 9.86 (d, J = 8.2 Hz, 1H), 9.74 (d, J = 8.2 Hz, 1H), 8.86 (dd, J = 17.3, 8.2 Hz, 4H), 8.54 (d, J = 5.0 Hz, 2H), 8.27 (dd, J = 17.2, 8.5 Hz, 3H), 8.18 (dd, J = 16.0, 6.9 Hz, 4H), 8.13 – 8.06 (m, 4H), 8.04 (d, J = 8.8 Hz, 1H), 7.66 (t, J = 6.5 Hz, 2H), 7.42 (t, J = 6.6 Hz, 2H), 3.03 (s, 3H), 2.71 (s, 3H). ^{13}C NMR: (126 MHz, Acetone) δ 157.52, 157.28, 153.63, 153.59, 152.29, 152.11, 141.74, 140.80, 138.71, 138.23, 138.12, 135.92, 134.94, 134.92, 133.81, 133.52, 131.28, 130.95, 127.93, 127.76, 127.59, 126.46, 124.51, 124.43, 19.91, 12.33. HRMS: $[M - 2PF_6]^{2+}$ calcd 362.08, found 362.0820. Elemental analysis (%) calcd for $[Ru(bpy)_2(dppz)](PF_6)_2$ (found): C 47.39 (52.38), H 2.98 (3.65), N 11.05 (11.05). HPLC purity: 97.36%, retention time = 2.551 min.

$[Ru(5,5'\text{-}dmbpy)_2(10,11\text{-}dmdppz)](PF_6)_2$

The same method as for $[Ru(bpy)_2(10,11\text{-}dmdppz)](PF_6)_2$ was employed using $[Ru(5,5\text{-}dmbpy)_2Cl_2]$ (0.148 g, 0.274 mmol) and 10,11-dmdppz (0.0641 g, 0.207mmol). Yield: 0.120 g, 54 %; Orange solid; ^{1}H NMR: (500 MHz, Acetone) δ 9.86 (d, J = 7.7 Hz, 1H), 9.74 (d, J = 8.0 Hz, 1H), 8.70 (dd, J = 20.8,

8.4 Hz, 6H), 8.52 (d, J = 5.1 Hz, 3H), 8.28 (d, J = 8.9 Hz, 2H), 8.12 – 8.04 (m, 7H), 7.99 (d, J = 14.0 Hz, 4H), 7.90 (d, J = 7.8 Hz, 3H), 3.06 (s, 3H), 2.74 (s, 3H), 2.29 (s, 3H). ^{13}C NMR: (126 MHz, Acetone) δ 154.77, 151.91, 151.70, 138.67, 138.58, 138.28, 123.42, 123.31, 17.62, 17.39, 12.32. HRMS: [M – PF_6]$^+$ calcd 925.19, found 925.1928. [M – 2PF_6]$^{2+}$ calcd 390.12, found 390.1138. Elemental analysis (%) calcd for [Ru(bpy)$_2$(dppz)](PF_6)$_2$ (found): C 49.40 (54.47), H 3.58 (4.26), N 10.47 (11.36). HPLC purity: 98.71%, retention time = 4.918 min.

HPLC

Reverse-phase HPLC was carried out on a Perkin-Elmer Altus HPLC system fitted with an Altus A-10 UV/Vis Detector and Waters XBridge C18 (130 Å, 3.5 μm, 4.6 x 150 mm) analytical column. Mobile phases: A = H_2O [HPLC grade] (+ 0.1% trifluoroacetic acid, TFA, v/v) and B = CH_3CN [HPLC grade] (+ 0.1% TFA, v/v). An isocratic HPLC method (50% A: 50% B) was used.

DNA preparation

Calf thymus DNA (Sigma) was dissolved in Tris buffer (5 mM Tris, 200 mM NaCl, pH 7.5) and sonicated for 2 x 15 mins. Absorbances at 260 nm and at 280 nm were measured using UV/VIS spectrometer and $A_{260\ nm}/A_{280\ nm} > 1.8$ indicated a protein free sample. The concentration of the CT DNA solution was determined by $A_{260\ nm}/\varepsilon_{260\ nm}$, where $\varepsilon_{260\ nm}$ = 6600 dm^3 mol^{-1} cm^{-1}. Luminescence titrations were performed as described in a recent publication.[4] 5' Cy5.5-labeled oligonucleotides (sequence: AGGGTTAGGGTTAGGGTTAGGG) were purchased from Merck (HPLC purification standard) and used as supplied. G-quadruplex DNA was prepared as described in Waller et al. employing a 100 mM KCl and 10 mM Tris.HCl buffer at pH 7.4.[5] For DNA studies, complexes were converted to their chloride salts and dissolved in Tris buffer.

Photophysical characterisation

Absorption spectra were recorded on a UV/VIS Lambda 365 Spectrometer (Perkin Elmer) and fluorescence spectra were run on an LS 55 Fluorescence Spectrometer (Perkin Elmer). Lifetime measurements were recorded on a FS5 Spectrofluorometer (Edinburgh Instruments) equipped with Multi-Channel Scaling and an EPL450 laser.

Relative luminescence quantum yields were calculated by Equation 1, where Φ_D is quantum yield, m is the gradient of luminescence versus absorbance, η is a solvent refractive index. Subscripts D and std refer to donor and standard, respectively. Commercial $[Ru(bpy)_3]^{2+}$ was employed as the standard (Φ_{std} = 0.018 ± 0.002 and 0.040 ± 0.002 at λ_{ex} = 450nm in acetonitrile and water, respectively[6]). DNA bound quantum yields for **1** and **2** were obtained in 5 × 10^{-5} M calf-thymus DNA solution in 5 mM Tris.HCl, 200 mM NaCl, pH 7.5.

$$\Phi_D = \Phi_{std}\left(\frac{m_{dD}}{m_{std}}\right)\left(\frac{\eta_D^2}{\eta_{std}^2}\right) \quad \textit{Equation 1}$$

Spectral overlap, J, was determined employing the normalized fluorescence emission spectrum of Cy5.5 and absorption spectrum of **1** or **2** scaled by peak molar absorption coefficient. Microsoft Excel was used to calculate the spectral overlap. Förster distance or radius, R_0, was determined by Equation 2 where J = spectral overlap, κ^2 = orientation factor, Φ_D = donor luminescence quantum yield, n = refractive index of the medium.

$$R_0^6 = 0.021 J \kappa^2 \Phi_D n^{-4} \quad \textit{Equation 2}$$

FRET binding assay

To determine binding, a concentration gradient of each compound (0.1 - 20 μM) was treated with 1 μM of Cyanine 5.5 labelled G-quadruplex DNA in 96 well plates (black, optical bottom, Thermo). 2x concentrations were prepared in advance and mixed 1:1 in each well to achieve the desired concentrations. After 30 mins, fluorescence spectra (λ_{ex} = 450 nm, λ_{em} = 550-850 nm) were recorded by Tecan Infinite M Nano plate reader. The intensity of the FRET emission peak (λ_{em} = 710 nm) for each compound concentration was measured, background subtracted and normalized to the maximum FRET intensity. Binding curves were generated, fit to a sigmoidal binding model (Origin) and K_d values extrapolated as the concentration required for 50 % binding, as described by Jarmoskaite et al.[7] All fits possessed R-squared values of 0.99 or greater.

Cell culture

All cell lines were obtained from West China Hospital of Sichuan University (ChengDu, China). Cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic. Cells were maintained at 37 °C under a humified atmosphere containing 5% CO_2 and passaged using Trypsin.

Antiproliferative assay

Cells were seeded at a density of 5×10^3 cells per well in 96-well plates. After 24 h incubation, complex was added to cells at the indicated concentrations. After 48 h, 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) reagent was added to each well. After 4 h incubation in the dark, 150 μL of DMSO was added to each well, and the intensity of absorbance was determined by a microplate reader microplate reader (BioTek Eon, America).

Confocal imaging

Cells were seeded into a cell culture confocal dish for 24 h, then pretreated with complex at 37 °C in a CO_2 incubator. After washing with PBS three times, the luminescence of complex in cells was imaged by a Leica TCS SP5 confocal laser scanning microscope system. For fixed cell imaging, the cells were treated with 4% PFA for 30 minutes before adding the compound for imaging. Excitation wavelength 485 nm, emission wavelength 550-750 nm.

STED imaging

STED nanoscopy experiments were performed under Leica DMi8 confocal microscopy equipped with Leica TCS SP5 STED-ONE unit and the compound was excited under STED laser, the emission signals were collected using HyD reflected light detectors (RLDs). Specimen cells were prepared using a similar method as normal confocal microscopy described previously, and donut laser was used in 775 nm STED laser (50% power), with 2048*2048 pixels and *100 scanning speed. Excitation wavelength 485 nm, emission wavelength 550-750 nm.

Supplementary Figures

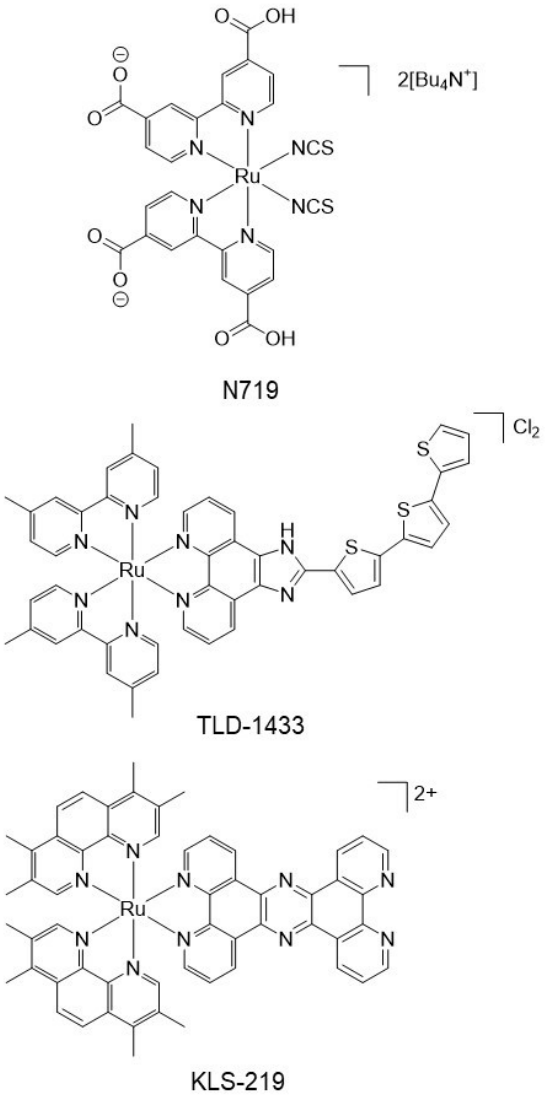

Fig. S1 Structures of N719, TLD1433 and KLS-219.

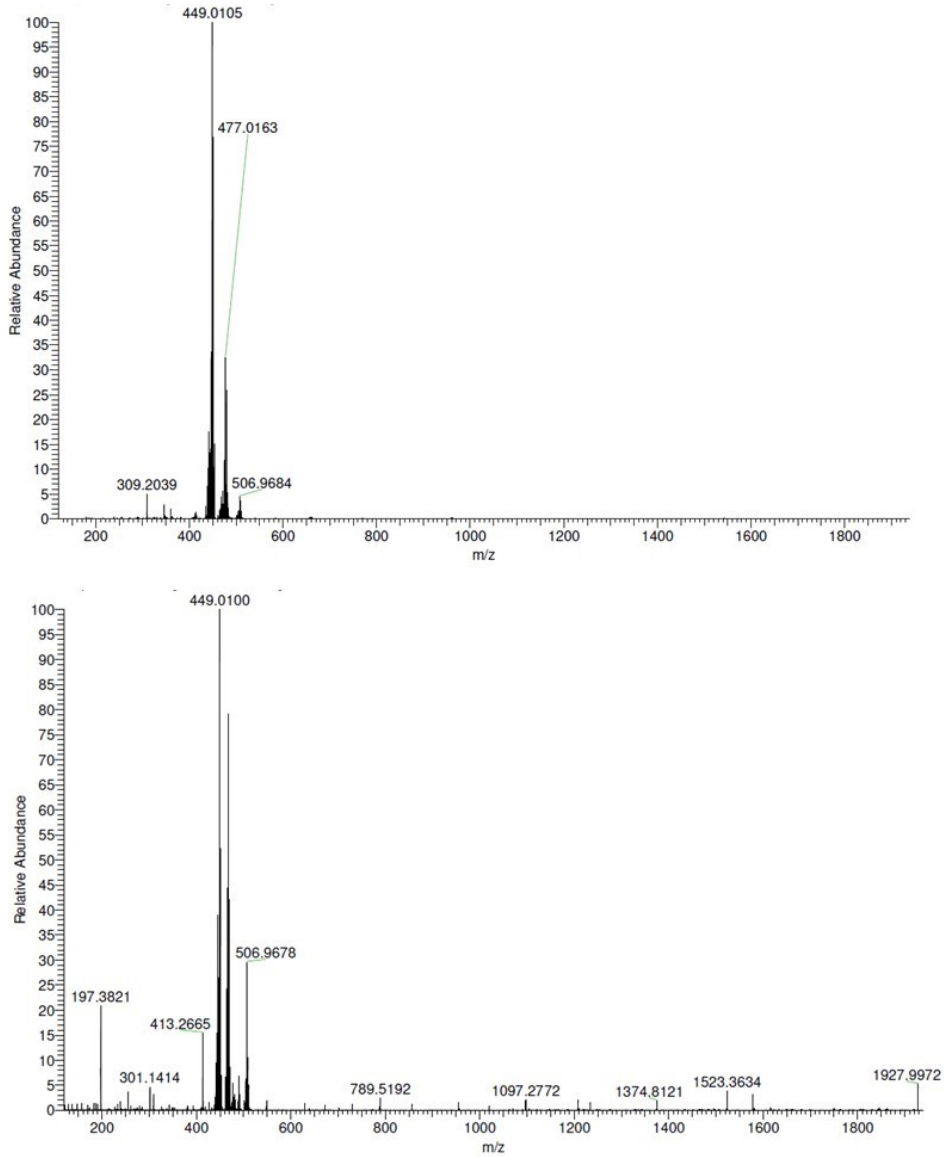

Fig. S2 HRMS spectrum of $Ru(bpy)_2Cl_2$ prepared via Cyrene™ (top) or DMF (bottom) pathways.

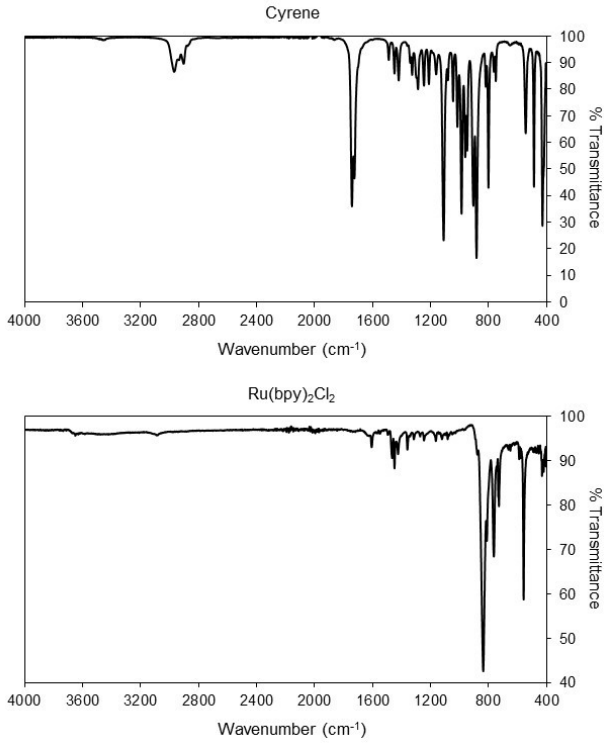

Fig. S3 FT-IR of Cyrene™ (top) and $Ru(bpy)_2Cl_2$ prepared via Cyrene™ pathway (bottom).

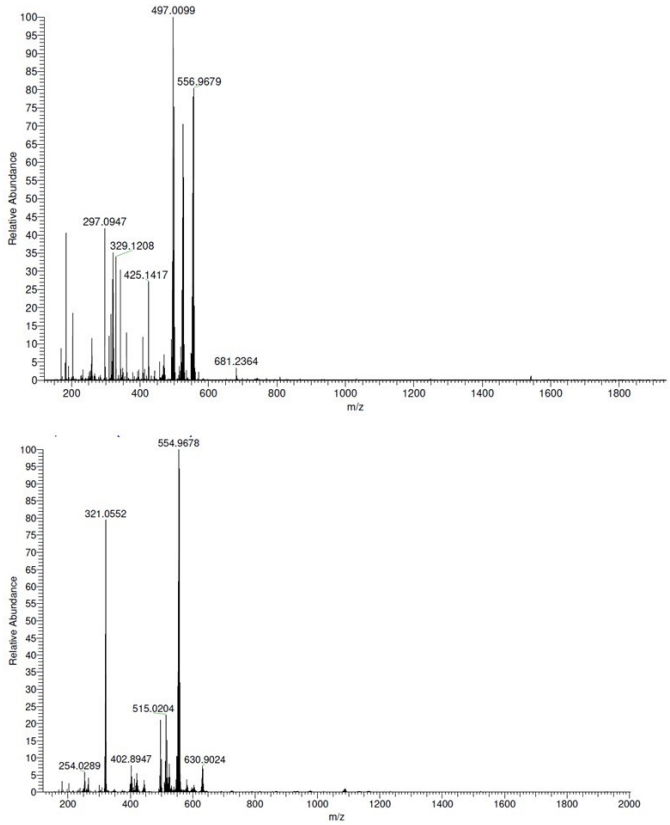

Fig. S4 HRMS spectrum of $Ru(phen)_2Cl_2$ prepared via Cyrene™ (top) or DMF (bottom) pathways.

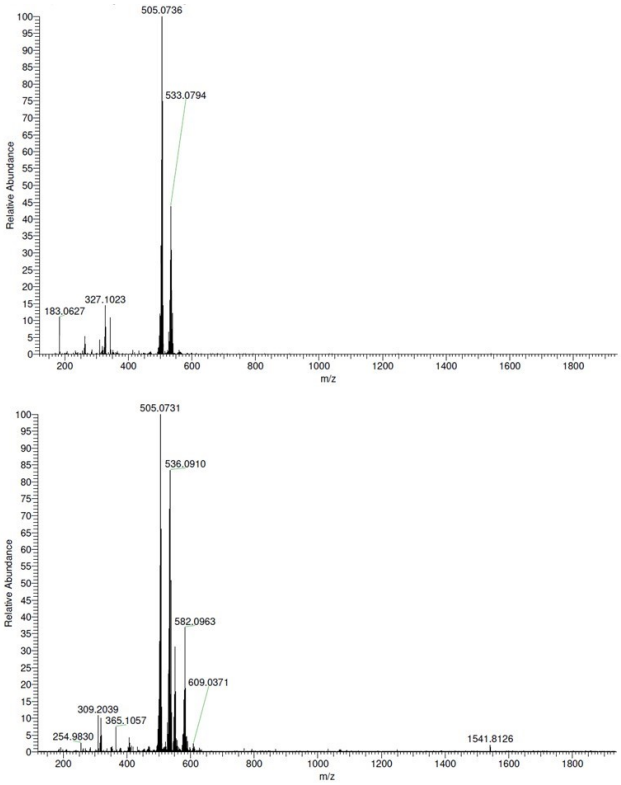

Fig. S5 HRMS spectrum of Ru(4,4'-dmbpy)$_2$Cl$_2$ prepared via Cyrene™ (top) or DMF (bottom) pathways.

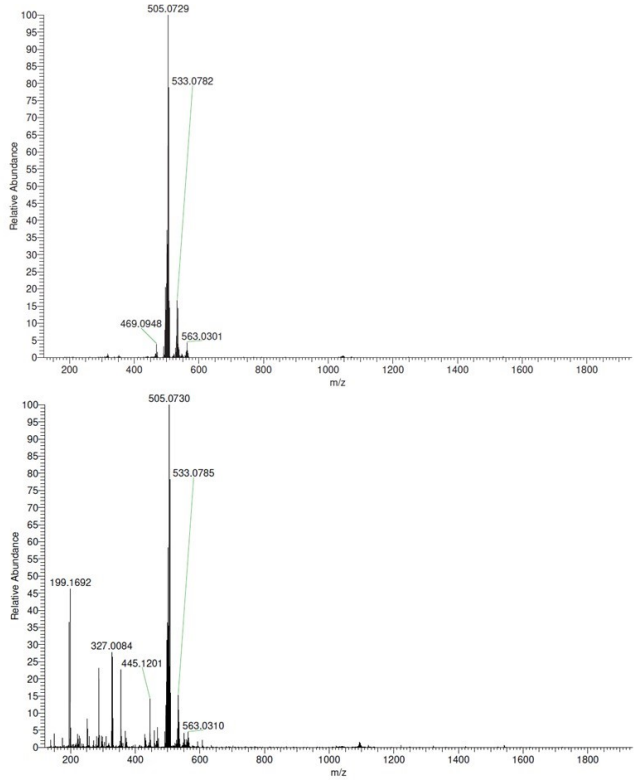

Fig. S6 HRMS spectrum of Ru(5,5'-dmbpy)$_2$Cl$_2$ prepared via Cyrene™ (top) or DMF (bottom) pathways.

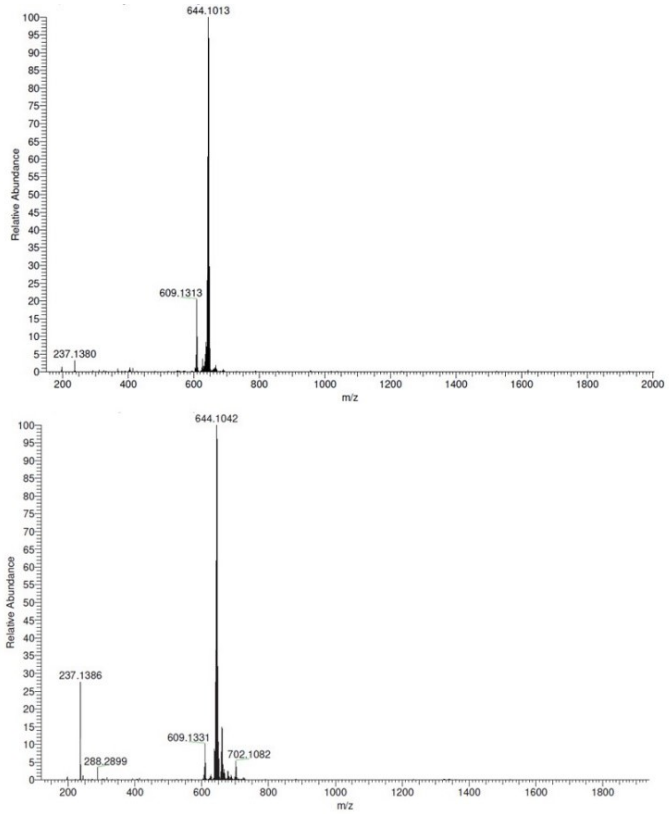

Fig. S7 HRMS spectrum of $Ru(tmp)_2Cl_2$ prepared via Cyrene™ (top) or DMF (bottom) pathways.

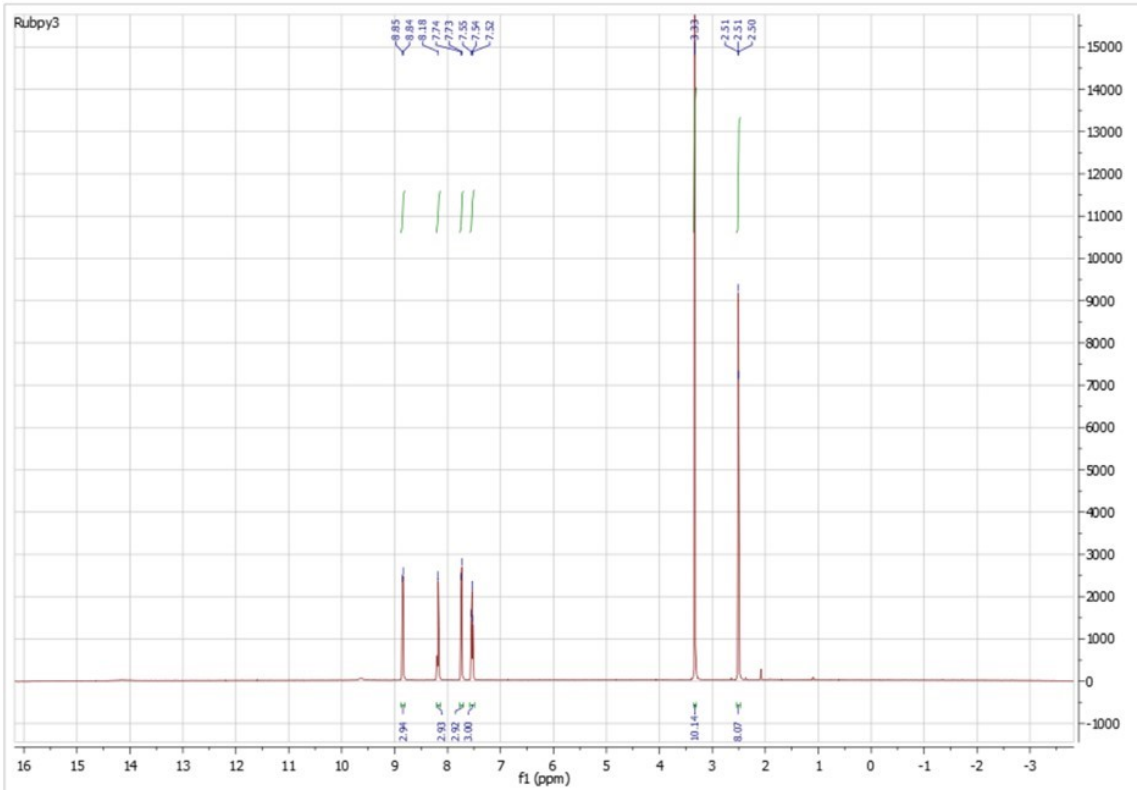

Fig. S8 ^1H NMR (500 MHz, DMSO) spectrum of $[Ru(bpy)_3](PF_6)_2$.

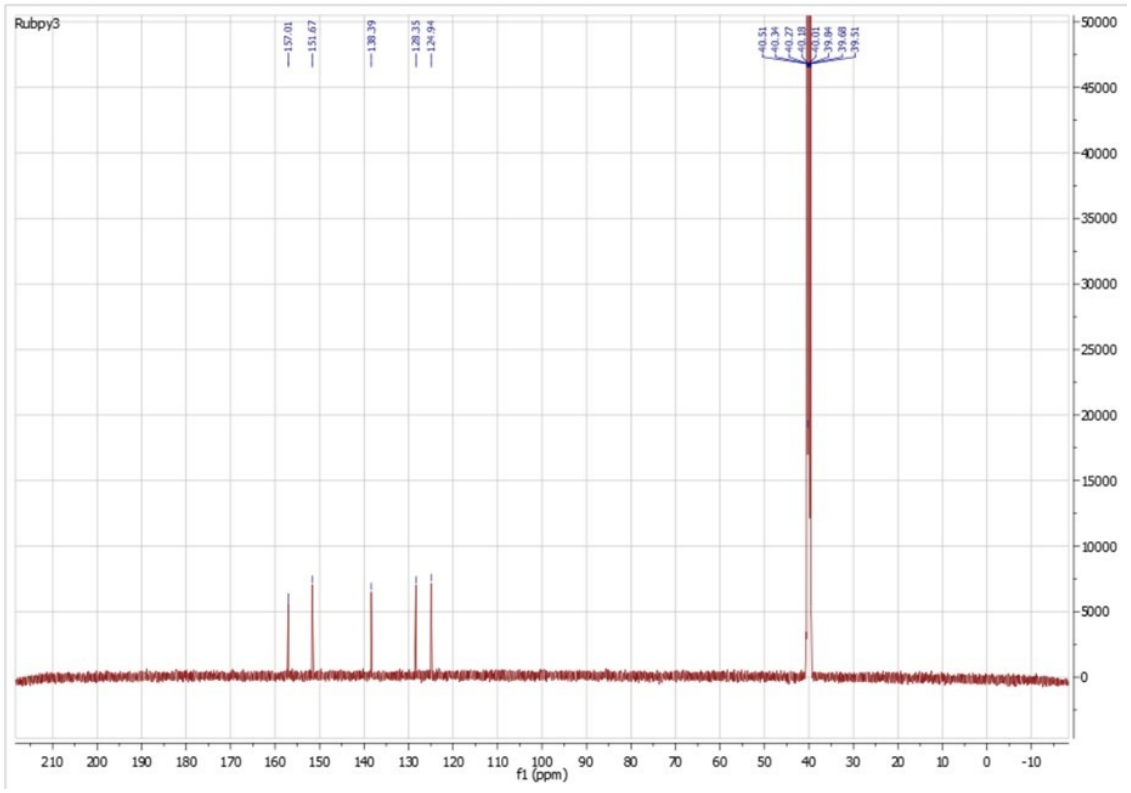

Fig. S9 ^{13}C NMR (126 MHz, DMSO) spectrum of $[Ru(bpy)_3](PF_6)_2$.

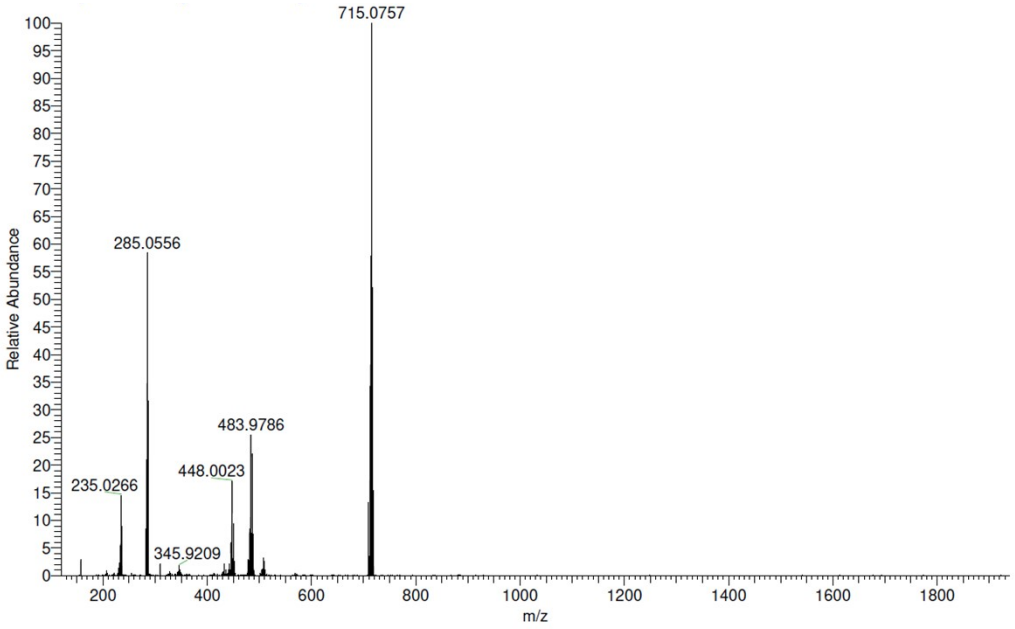

Fig. S10 HRMS spectrum of $[Ru(bpy)_3](PF_6)_2$.

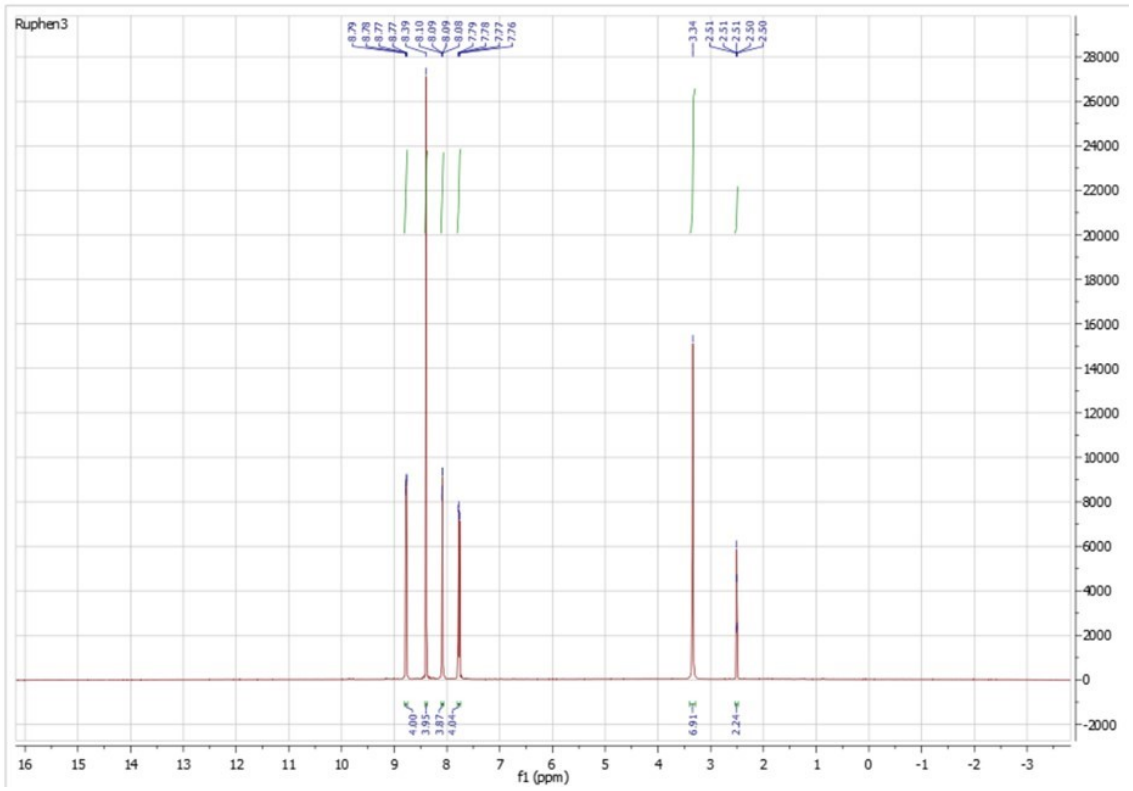

Fig. S11 ^{1}H NMR (500 MHz, DMSO) spectrum of $[Ru(phen)_3](PF_6)_2$.

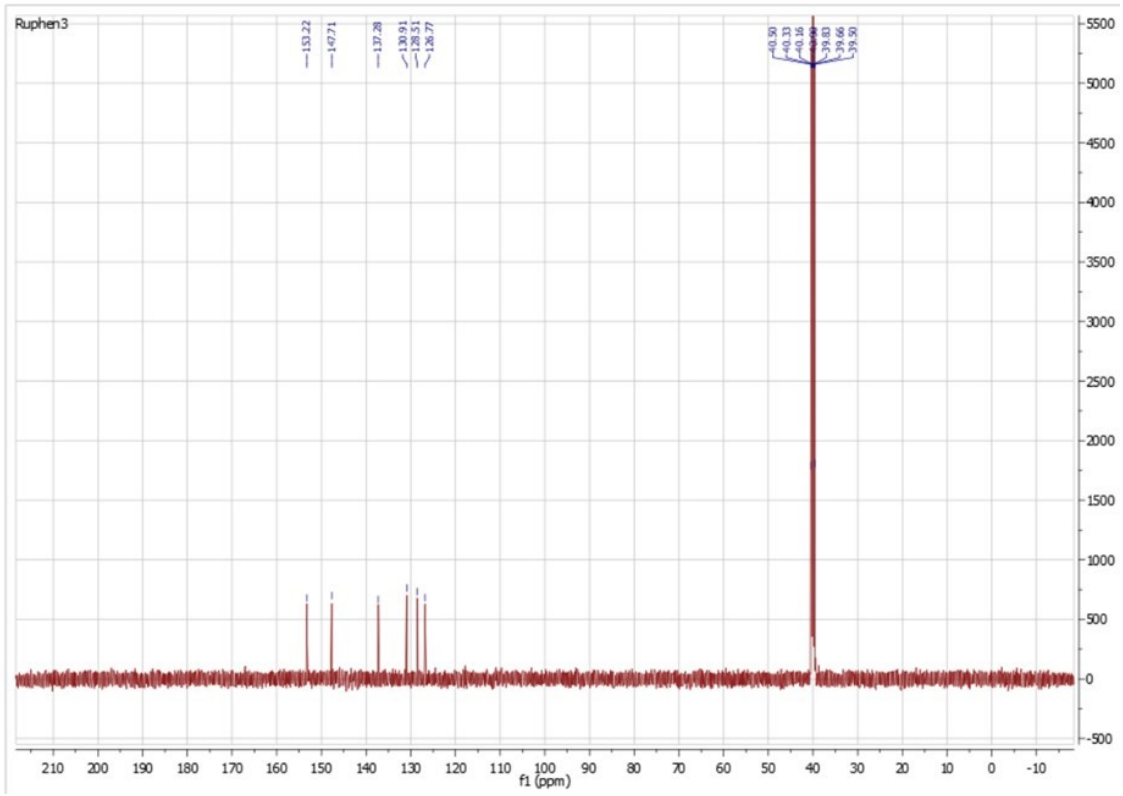

Fig. S12 ^{13}C NMR (126 MHz, DMSO) spectrum of $[Ru(phen)_3](PF_6)_2$.

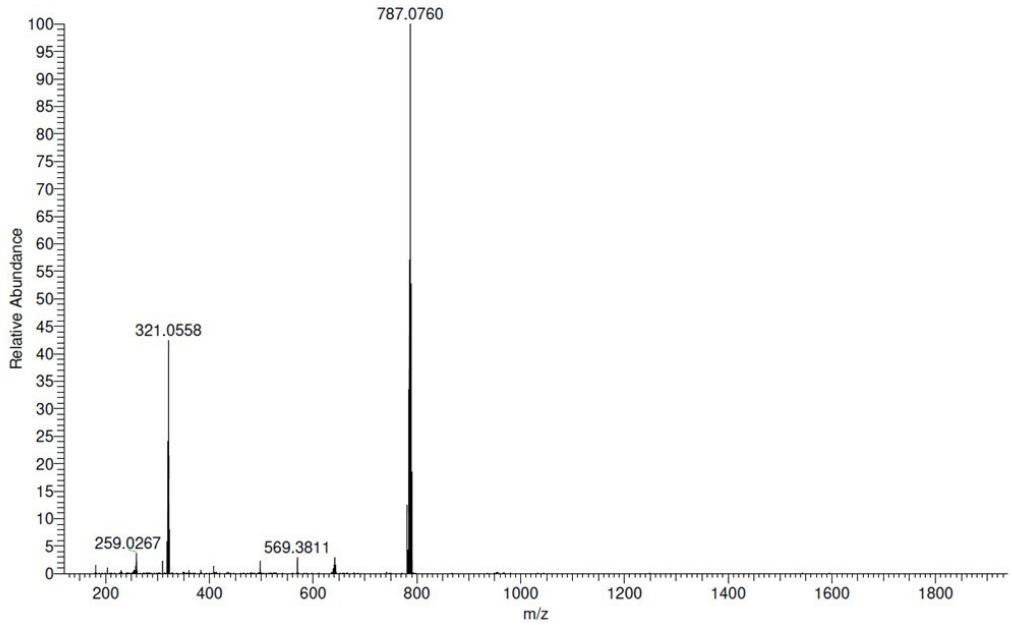

Fig. S13 HRMS spectrum of $[Ru(phen)_3](PF_6)_2$.

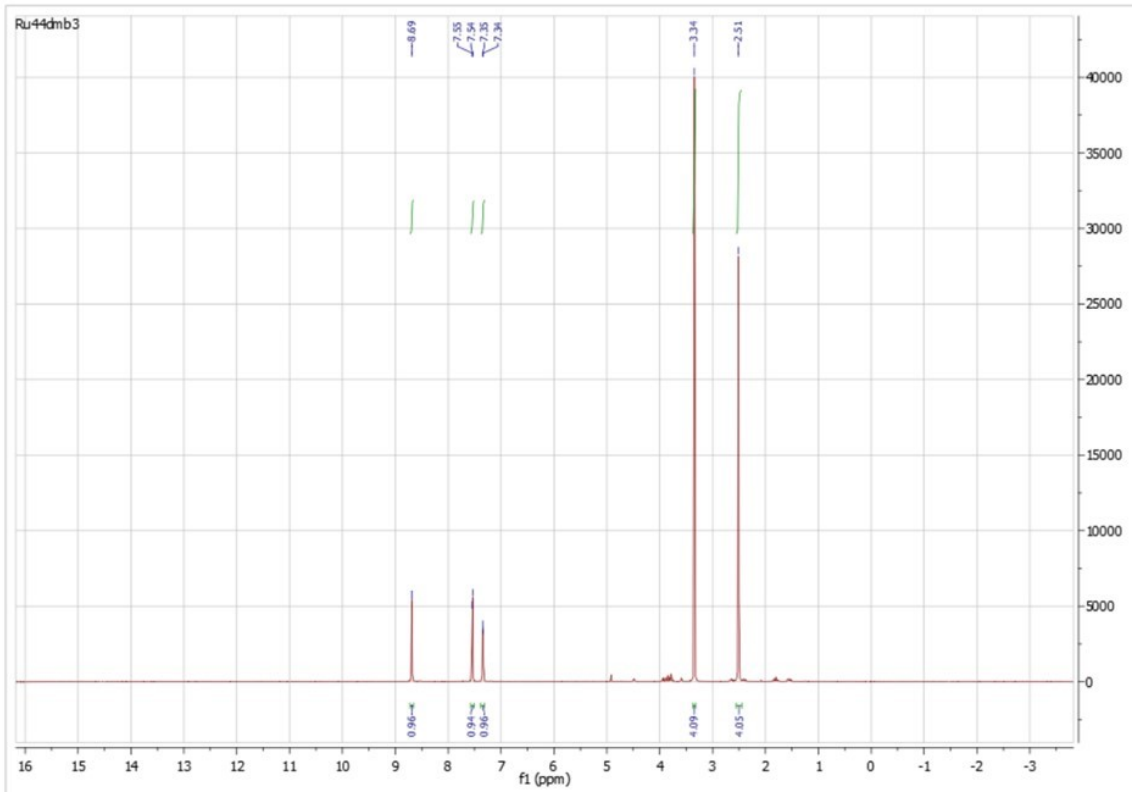

Fig. S14 ^{1}H NMR (500 MHz, DMSO) spectrum of $[Ru(4,4'\text{-dmbpy})_3](PF_6)_2$.

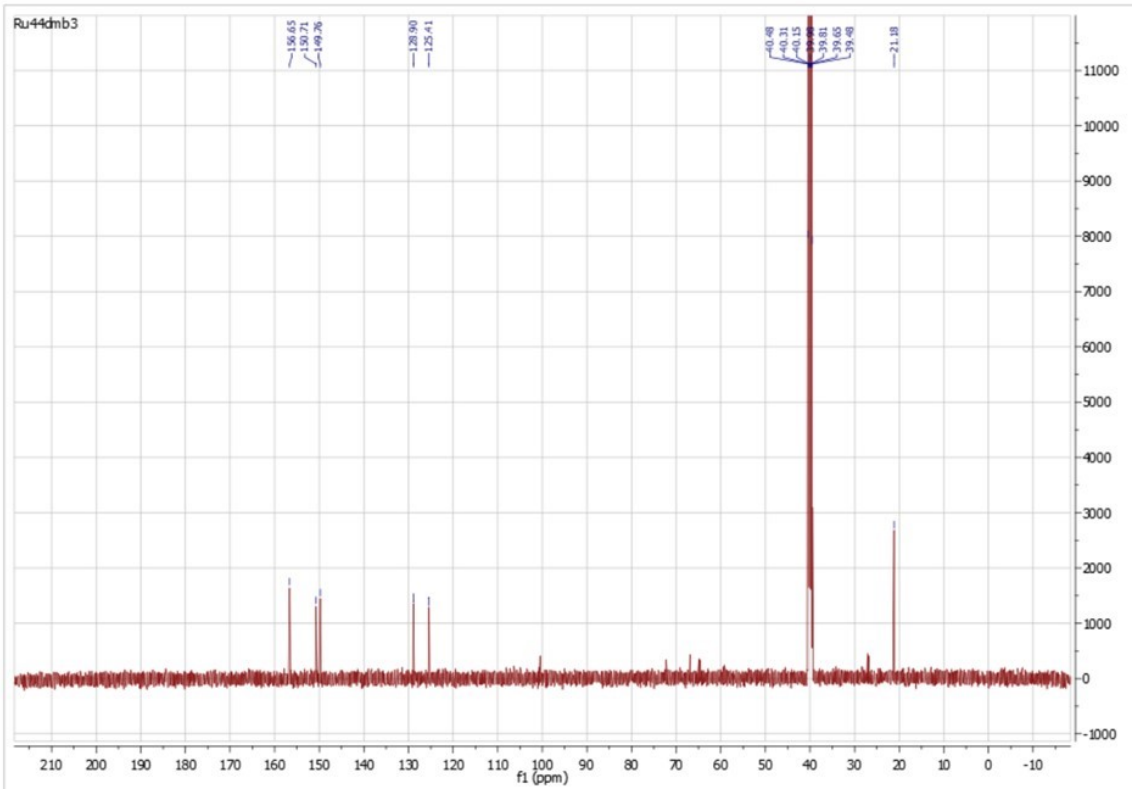

Fig. S15 ^{13}C NMR (126 MHz, DMSO) spectrum of $[Ru(4,4'\text{-dmbpy})_3](PF_6)_2$.

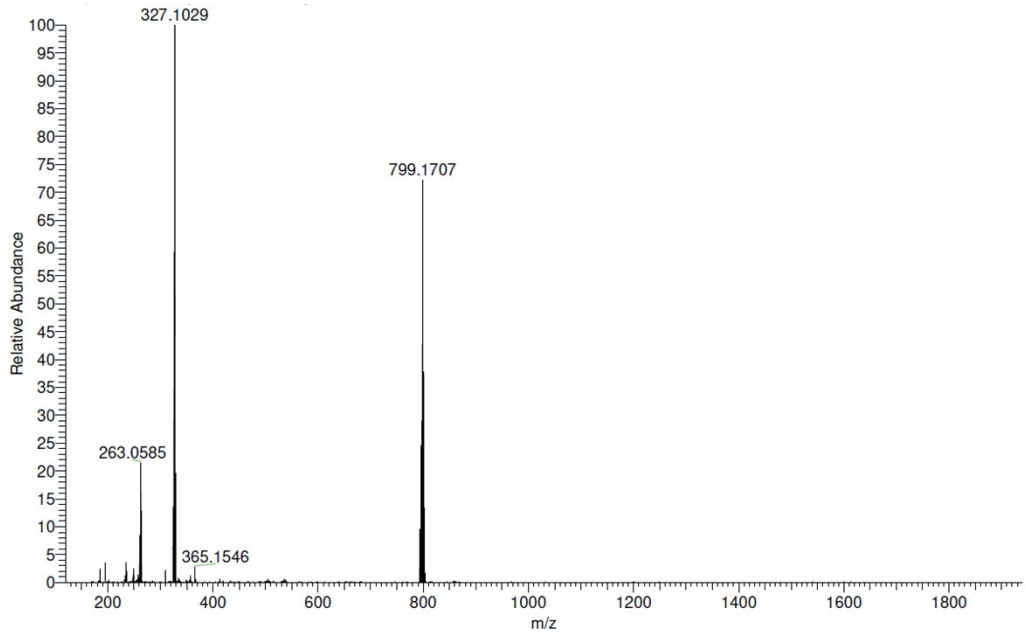

Fig. S16 HRMS spectrum of $[Ru(4,4'\text{-dmbpy})_3](PF_6)_2$.

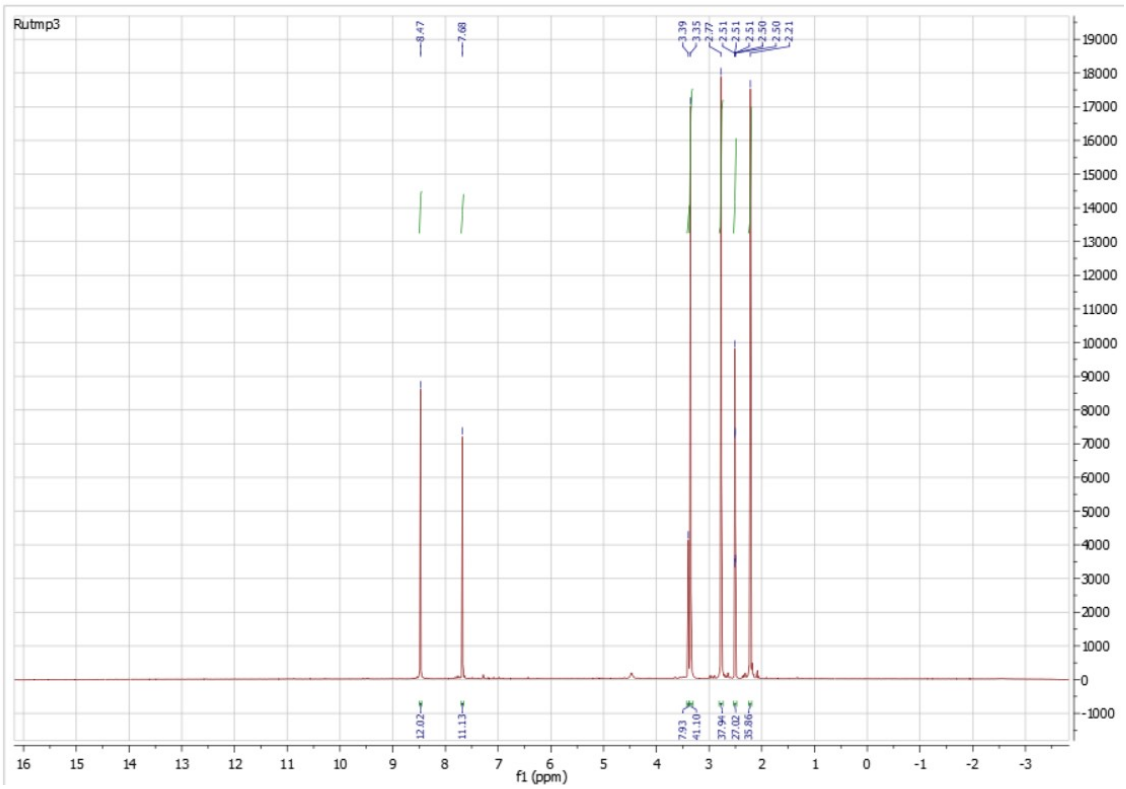

Fig. S17 ^{1}H NMR (500 MHz, DMSO) spectrum of $[Ru(tmphen)_3](PF_6)_2$.

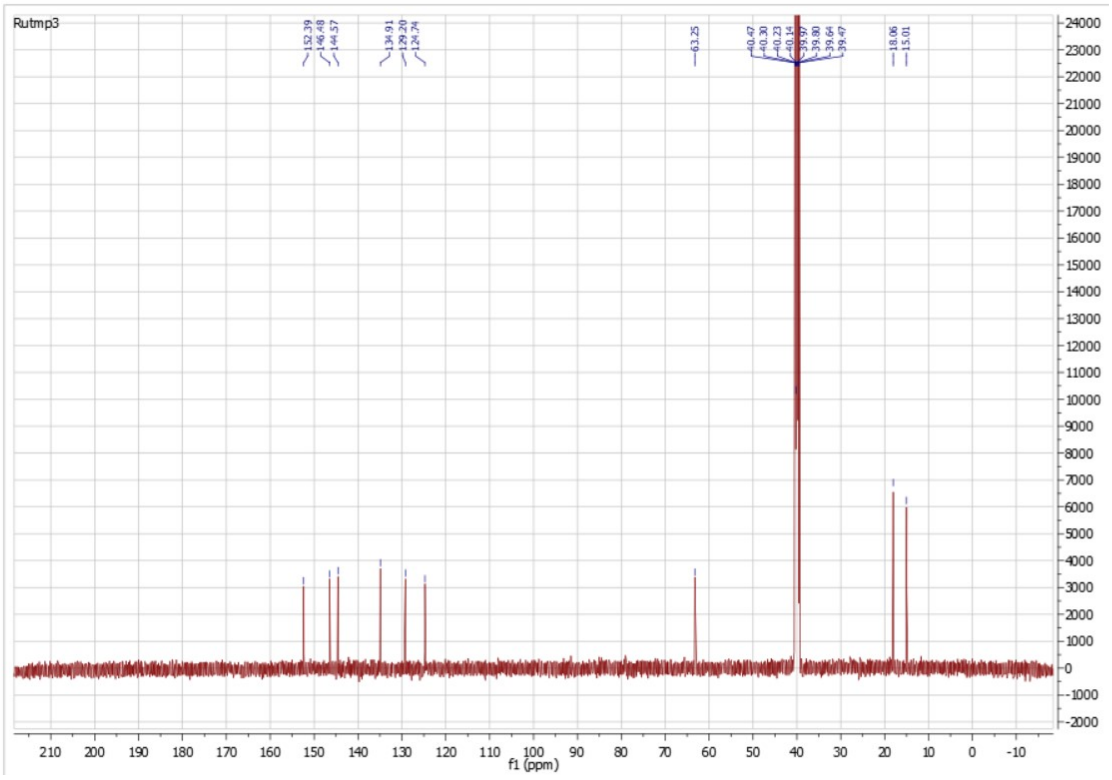

Fig. S18 ^{13}C NMR (126 MHz, DMSO) spectrum of $[Ru(tmphen)_3](PF_6)_2$.

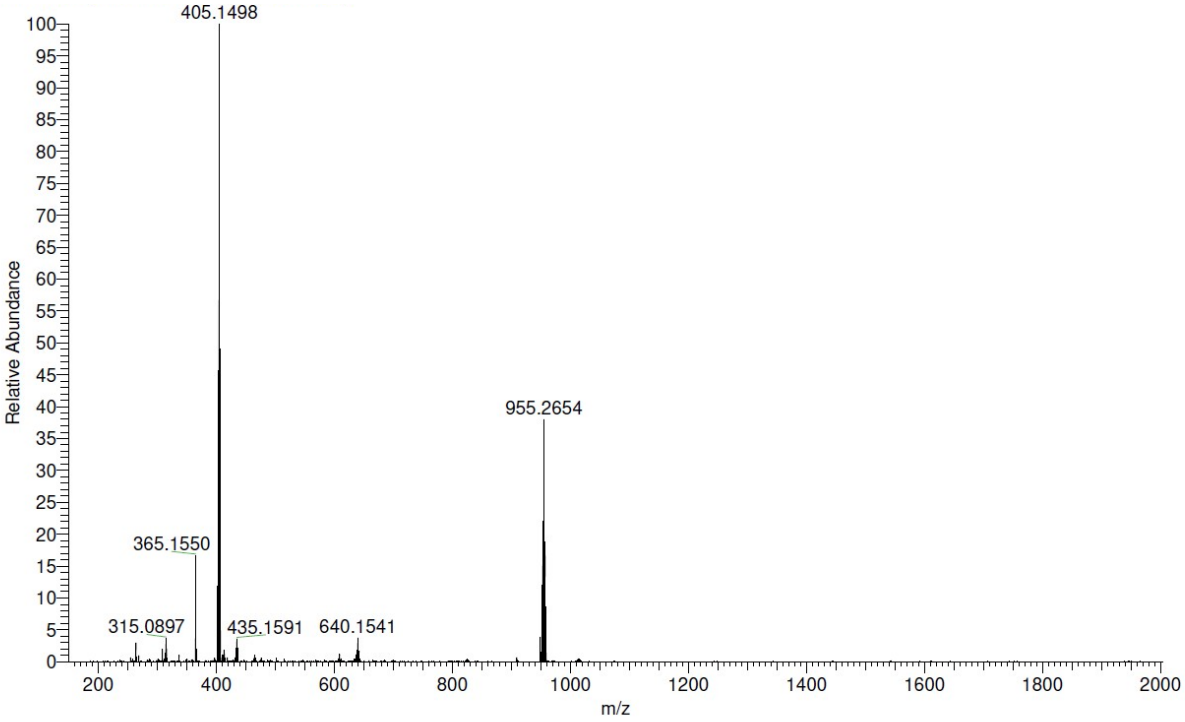

Fig. S19 HRMS spectrum of $[Ru(tmphen)_3](PF_6)_2$.

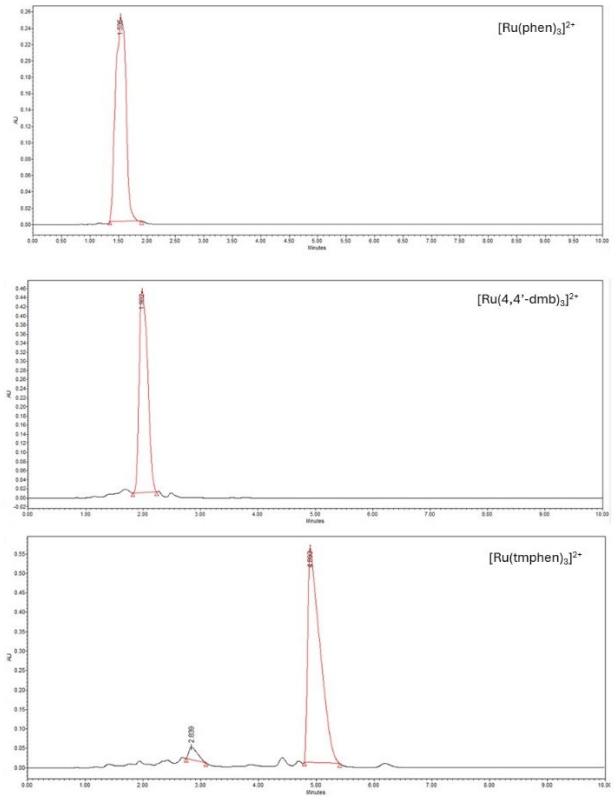

Fig. S20 HPLC of $[Ru(phen)_3](PF_6)_2$ (top), $[Ru(4,4'\text{-}dmbpy)_3](PF_6)_2$ (middle) and $[Ru(tmphen)_3](PF_6)_2$ (bottom).

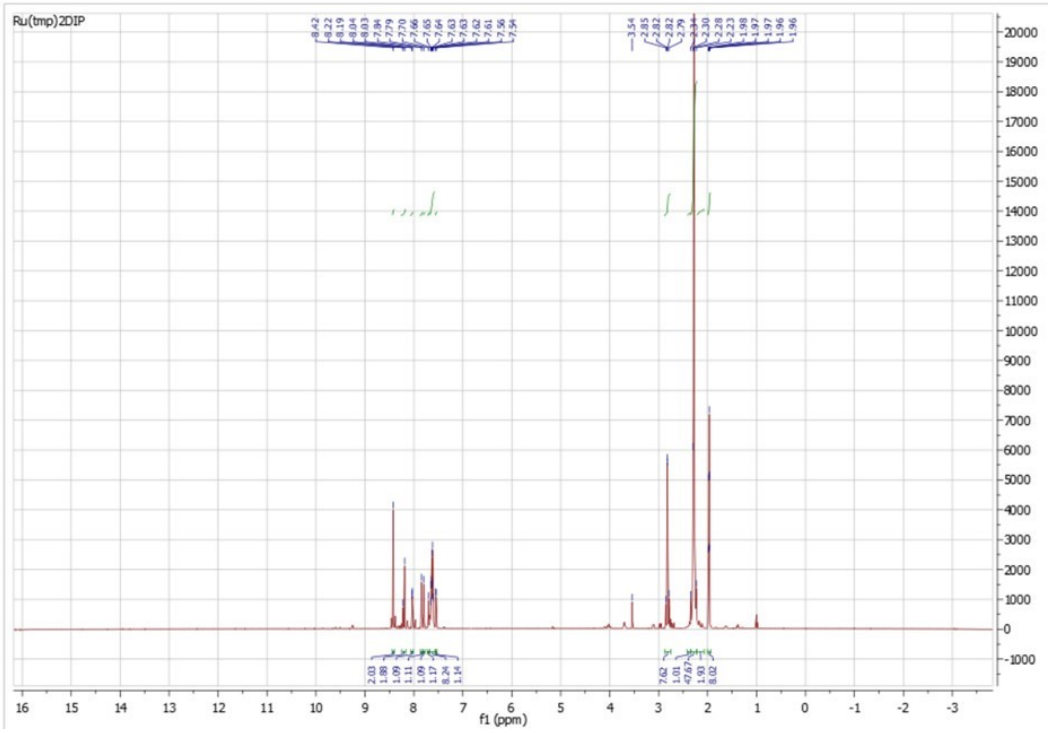

Fig. S21 ^{1}H NMR (500 MHz, CD_3CN) spectrum of $[Ru(tmphen)_2(DIP)](PF_6)_2$.

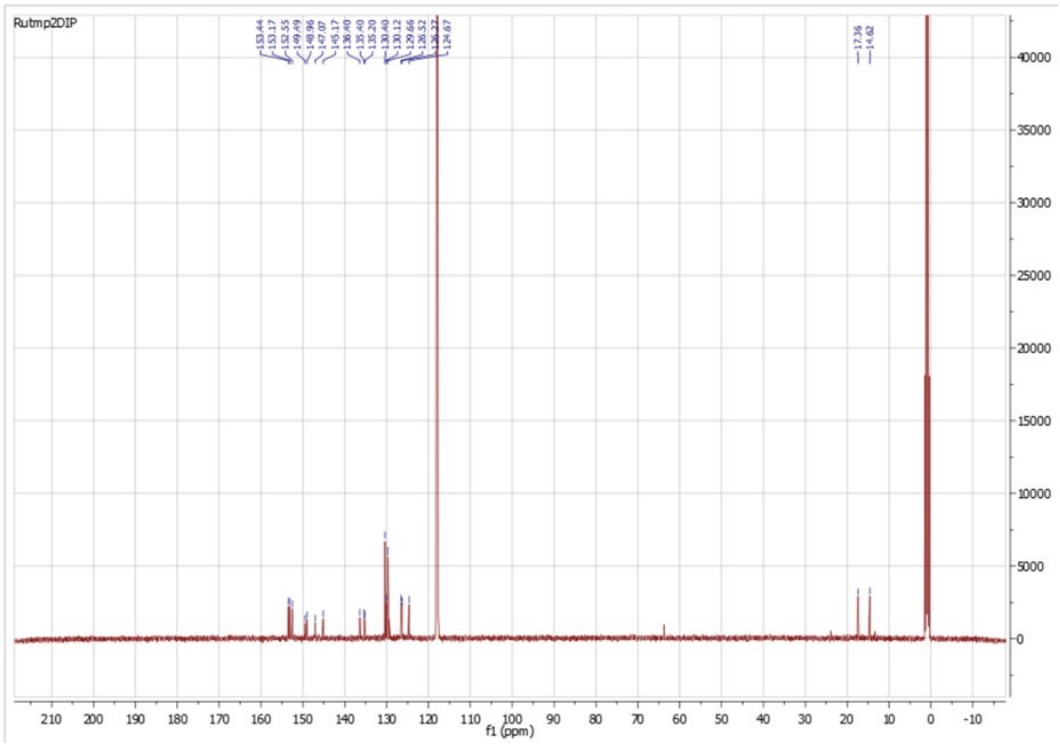

Fig. S22 ^{13}C NMR (126 MHz, CD_3CN) spectrum of $[Ru(tmphen)_2(DIP)](PF_6)_2$.

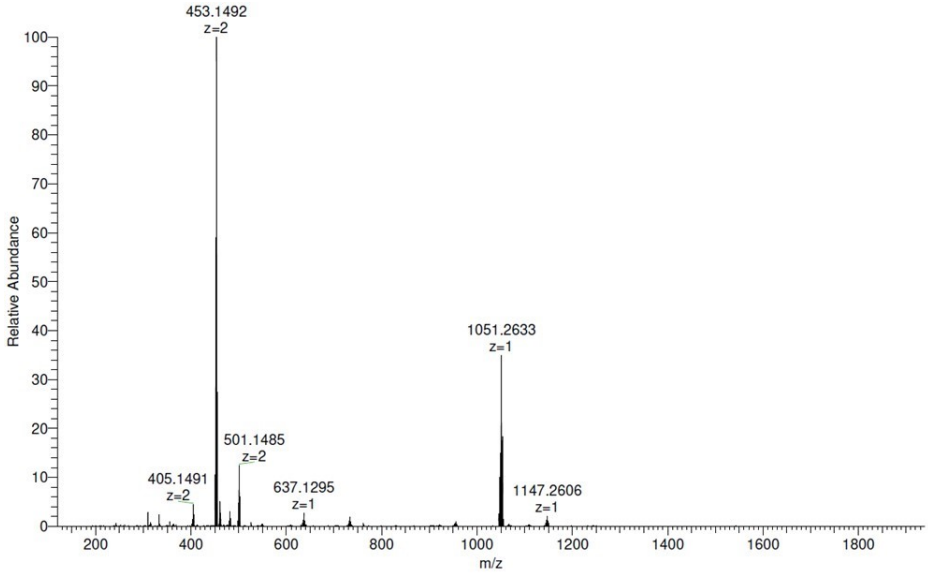

Fig. S23 HRMS spectrum of $[Ru(tmphen)_2(DIP)](PF_6)_2$.

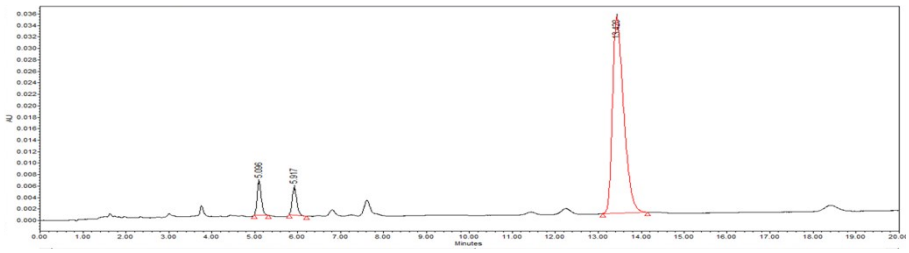

Fig. S24 HPLC of $[Ru(tmphen)_2(DIP)](PF_6)_2$.

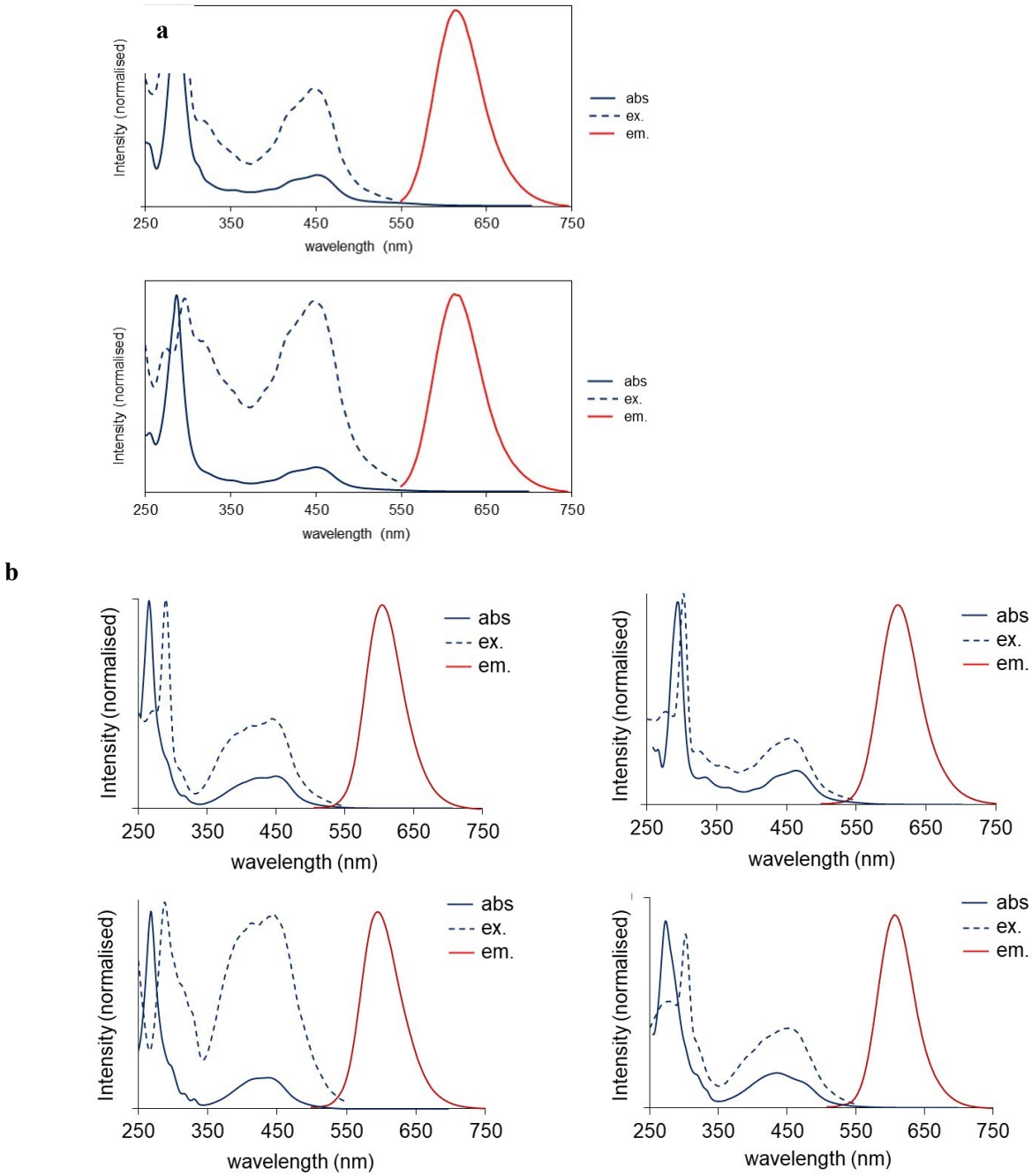

Fig. S25 (a) Overlayed absorption (blue, solid), excitation (blue, dashed, λ_{em} = 610 nm) and emission (red, solid, λ_{ex} = 450 nm) spectra of $[Ru(bpy)_3](PF_6)_2$ (top) and commercial reference (bottom). (b) Overlayed absorption (blue, solid), excitation (blue, dashed, λ_{em} = 610 nm) and emission (red, solid, λ_{ex} = 450 nm) spectra of $[Ru(phen)_3](PF_6)_2$ (top left), $[Ru(4,4'\text{-}dmbpy)_3](PF_6)_2$ (top right), $[Ru(tmphen)_3](PF_6)_2$ (bottom left) and $[Ru(tmphen)_2(DIP)](PF_6)_2$ (bottom right) in acetonitrile.

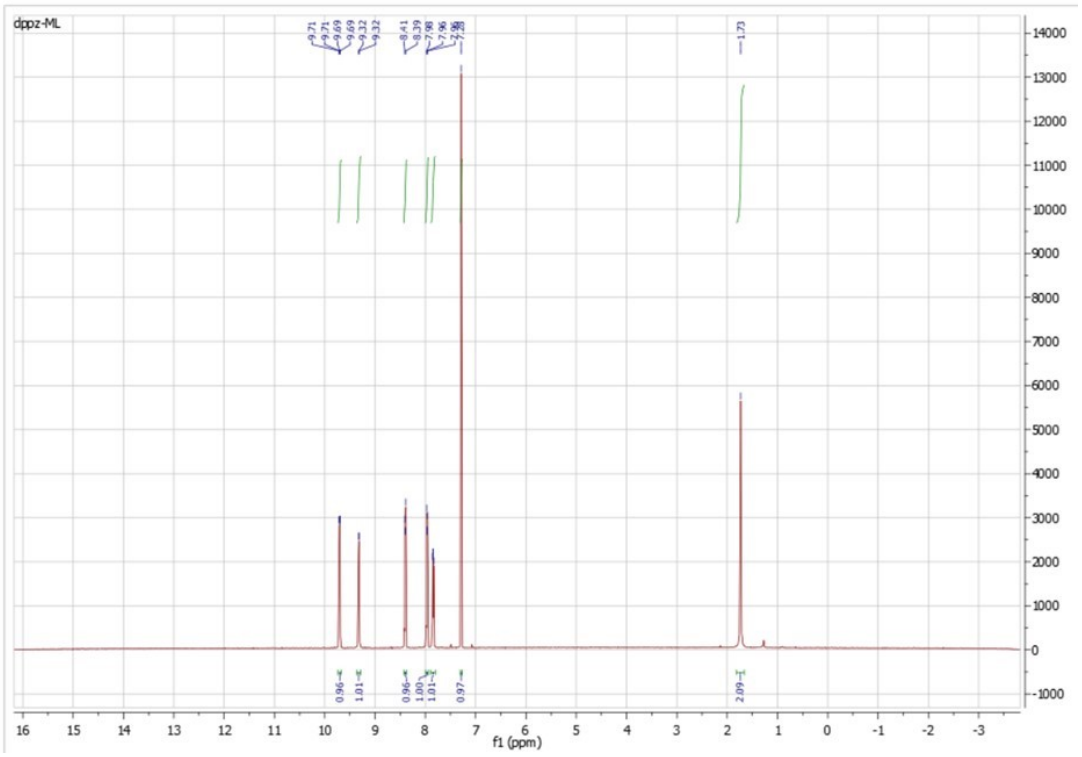

Fig. S26 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of dppz.

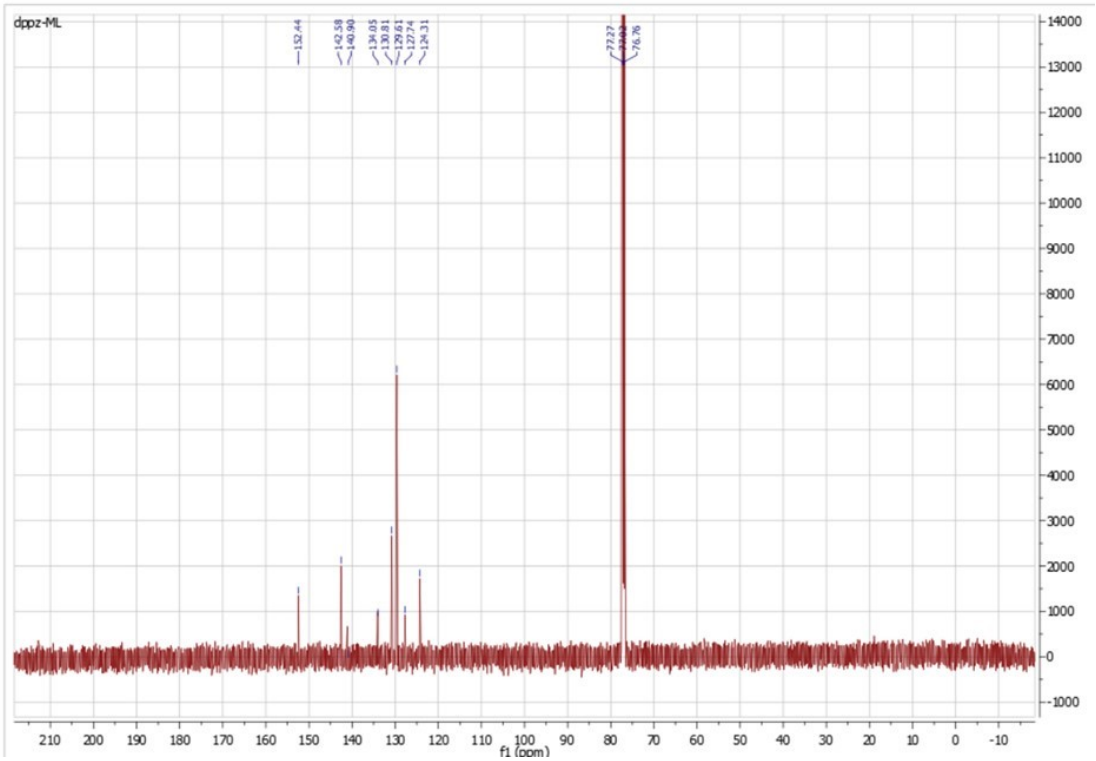

Fig. S27 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of dppz.

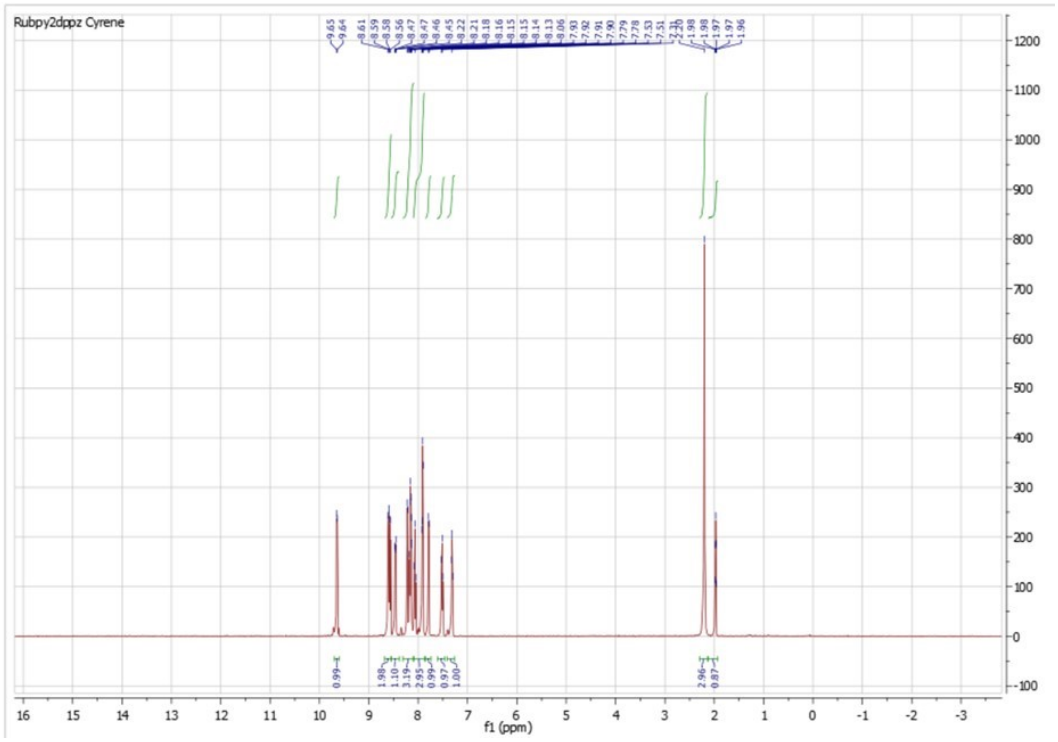

Fig. S28 ^{1}H NMR (500 MHz, CD_3CN) spectrum of $[Ru(bpy)_2(dppz)](PF_6)_2$.

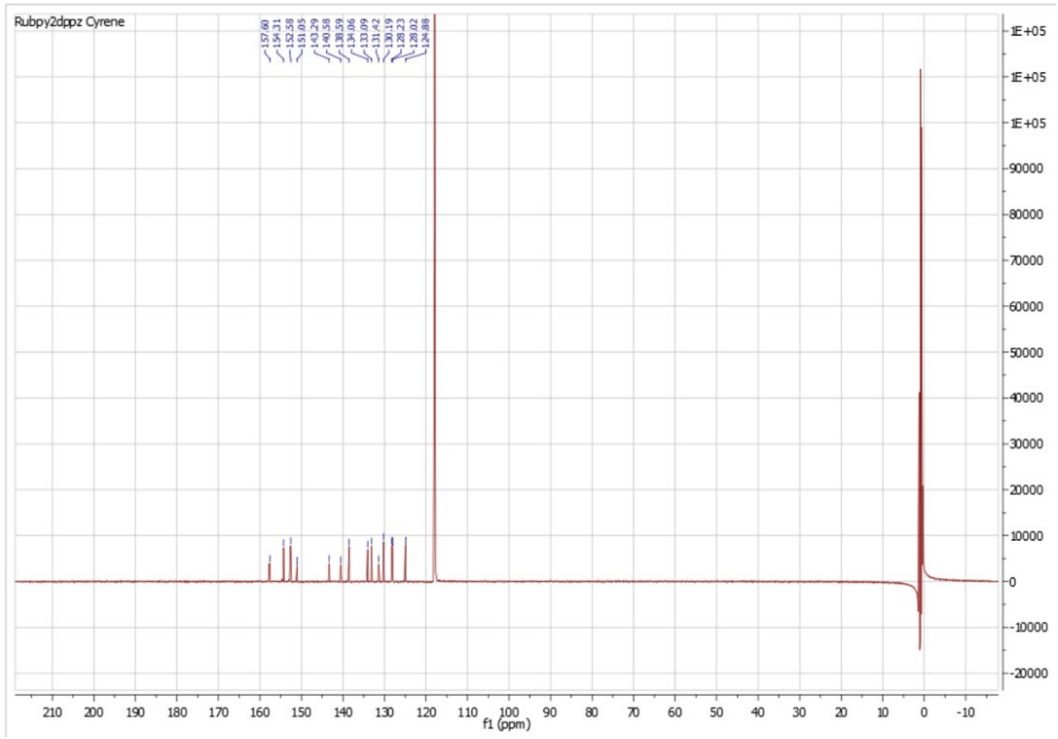

Fig. S29 ^{13}C NMR (126 MHz, CD_3CN) spectrum of $[Ru(bpy)_2(dppz)](PF_6)_2$.

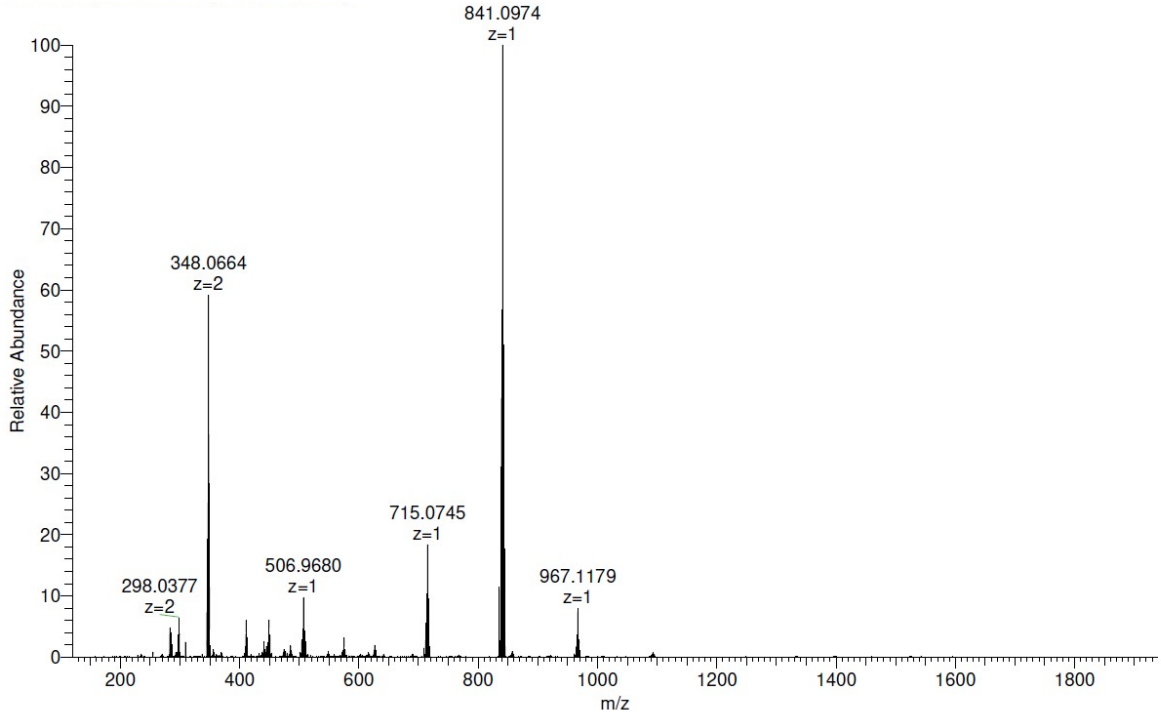

Fig. S30 HRMS spectrum of $[Ru(bpy)_2(dppz)](PF_6)_2$.

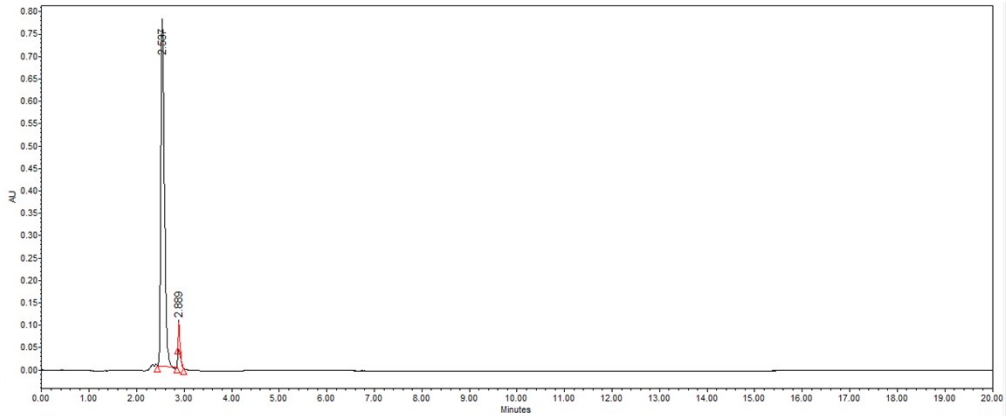

Fig. S31 HPLC of $[Ru(bpy)_2(dppz)](PF_6)_2$.

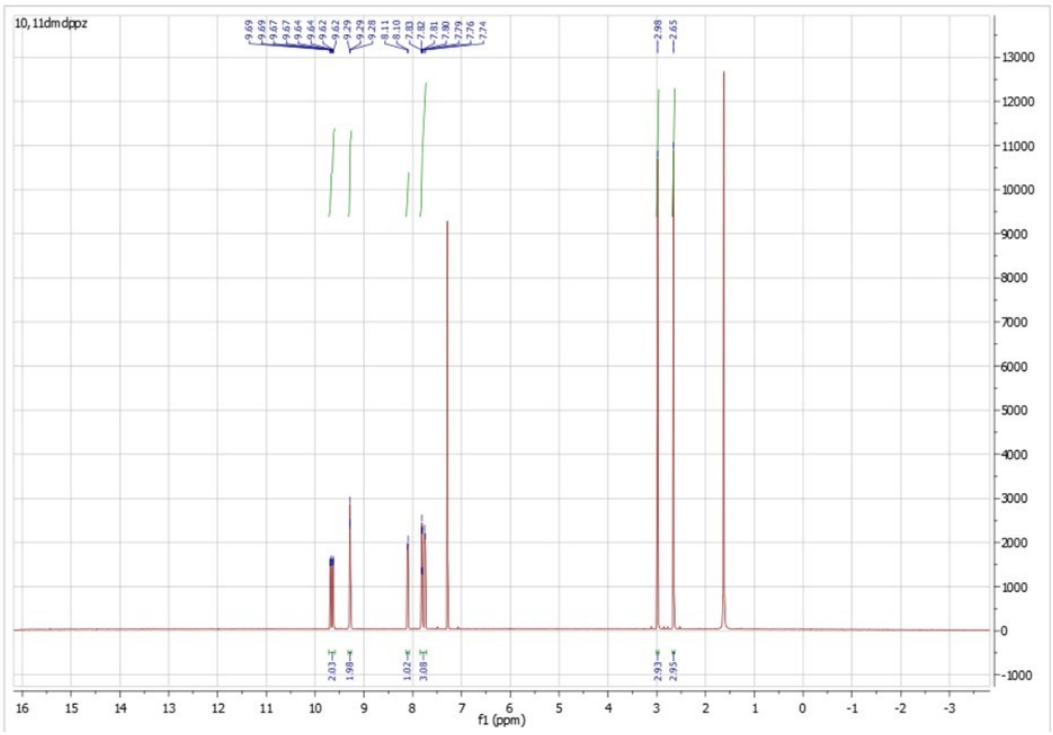

Fig. S32 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 10,11-dmdppz.

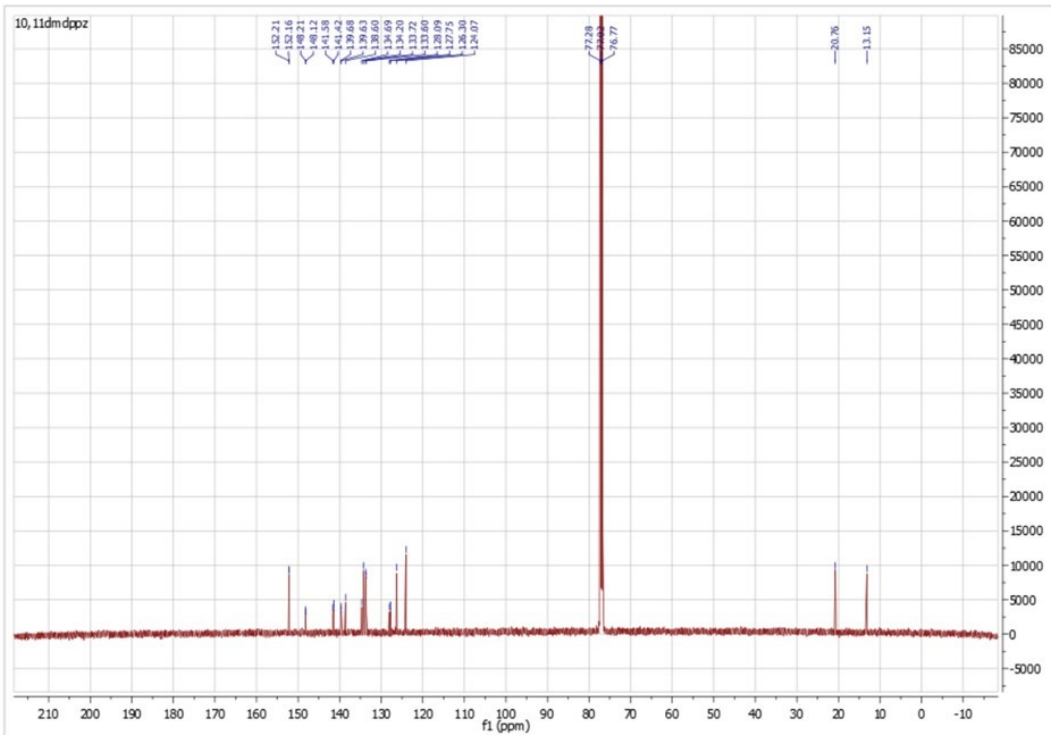

Fig. S33 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 10,11-dmdppz.

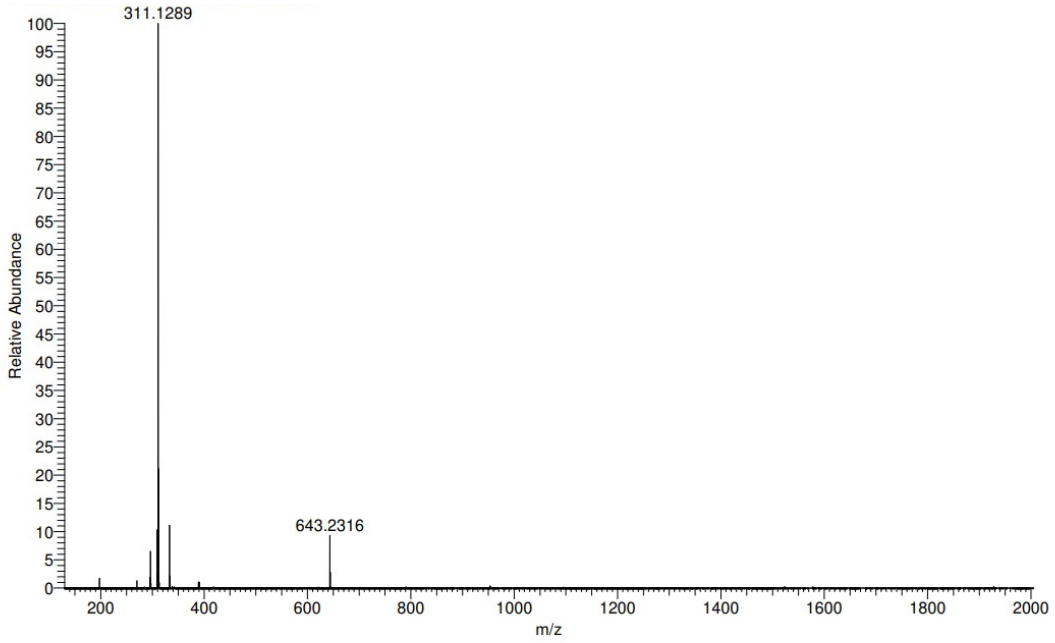

Fig. S34 HRMS spectrum of 10,11-dmdppz

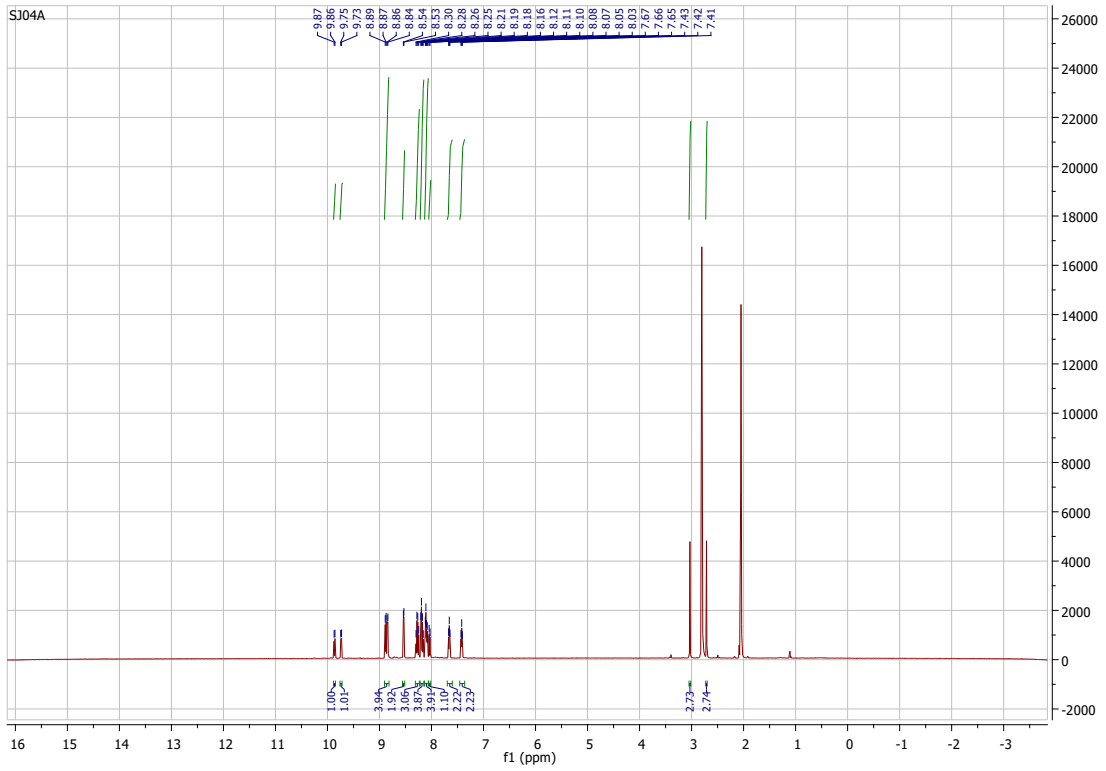

Fig. S35 ^{1}H NMR (500 MHz, Acetone) spectrum of $[Ru(bpy)_2(10,11\text{-dmdppz})](PF_6)_2$.

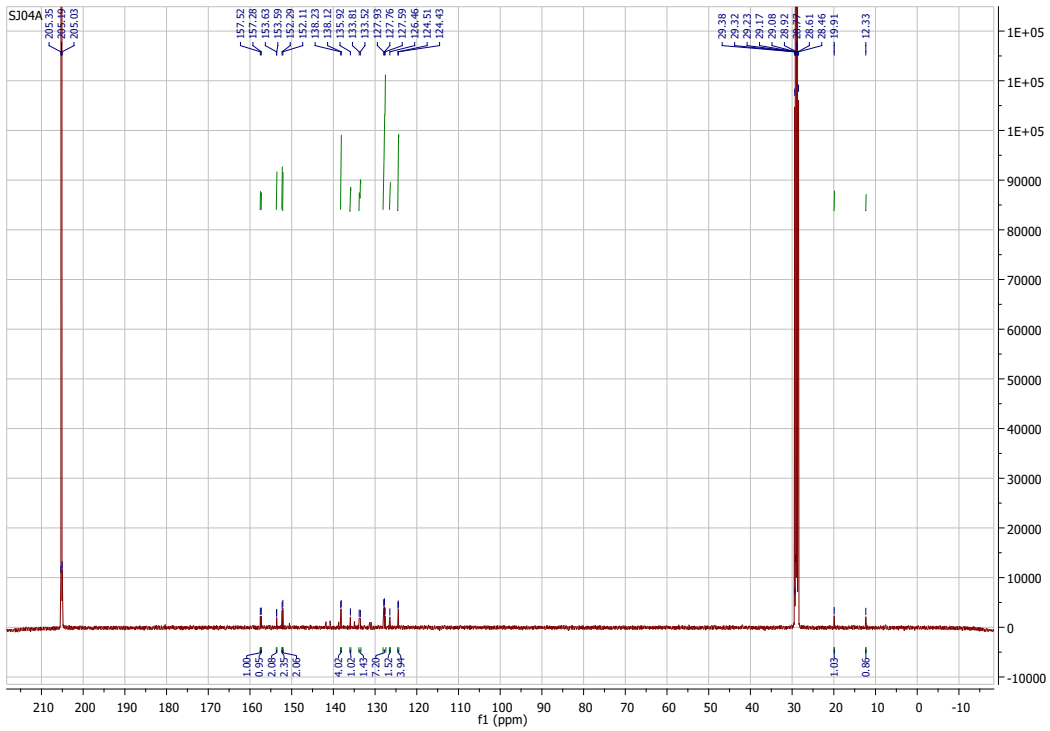

Fig. S36 ^{13}C NMR (126 MHz, acetone) spectrum of [Ru(bpy)$_2$(10,11-dmdppz)](PF$_6$)$_2$.

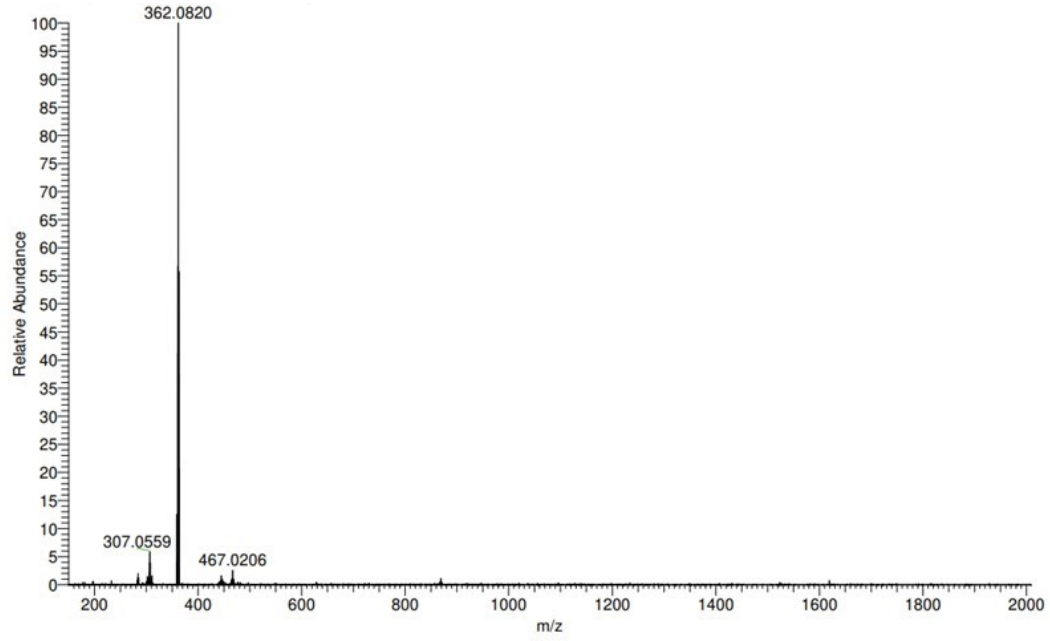

Fig. S37 HRMS spectrum of [Ru(bpy)$_2$(10,11-dmdppz)](PF$_6$)$_2$.

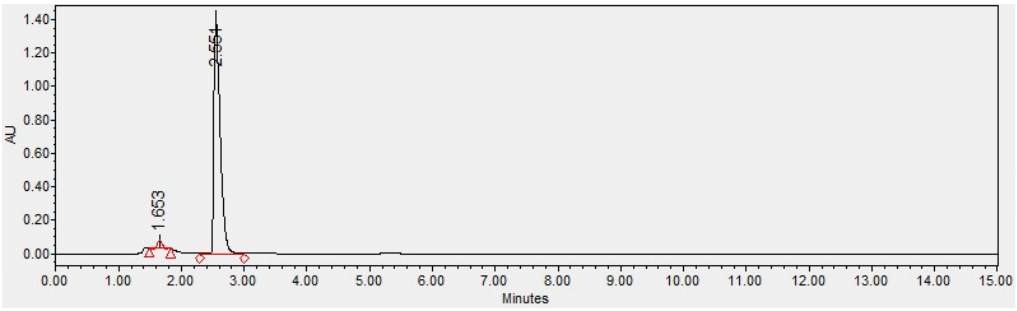

Fig. S38 HPLC of $[Ru(bpy)_2(10,11\text{-dmdppz})](PF_6)_2$.

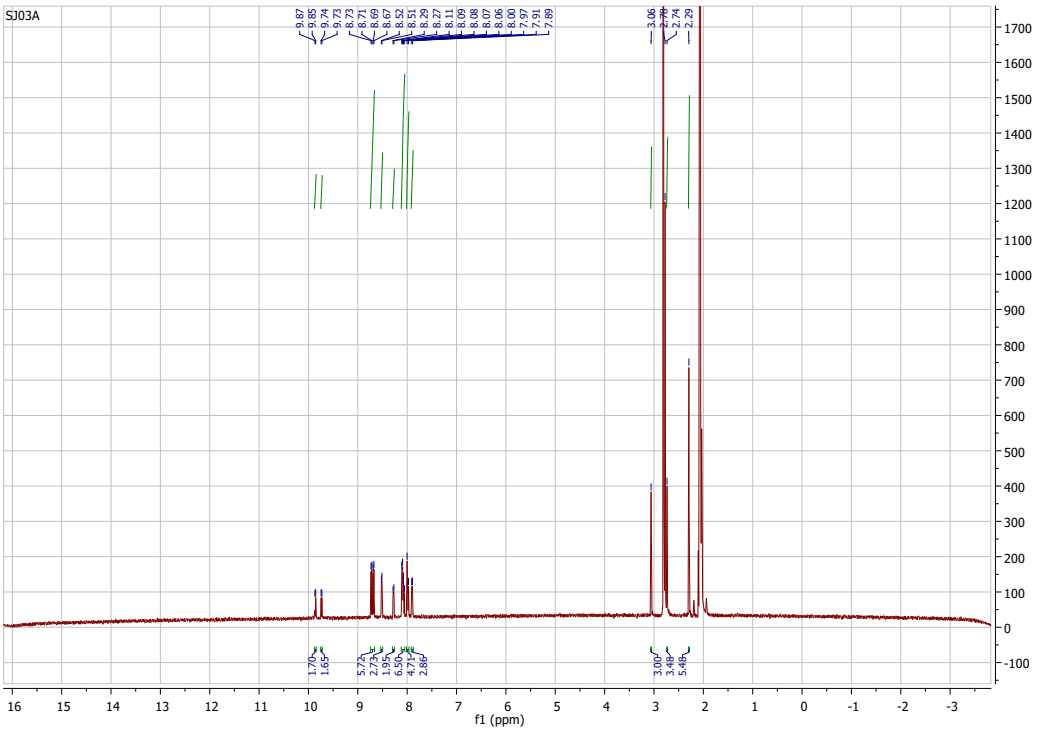

Fig. S39 ^{1}H NMR (500 MHz, Acetone) spectrum of $[Ru(5,5'\text{-dmbpy})_2(10,11\text{-dmdppz})](PF_6)_2$.

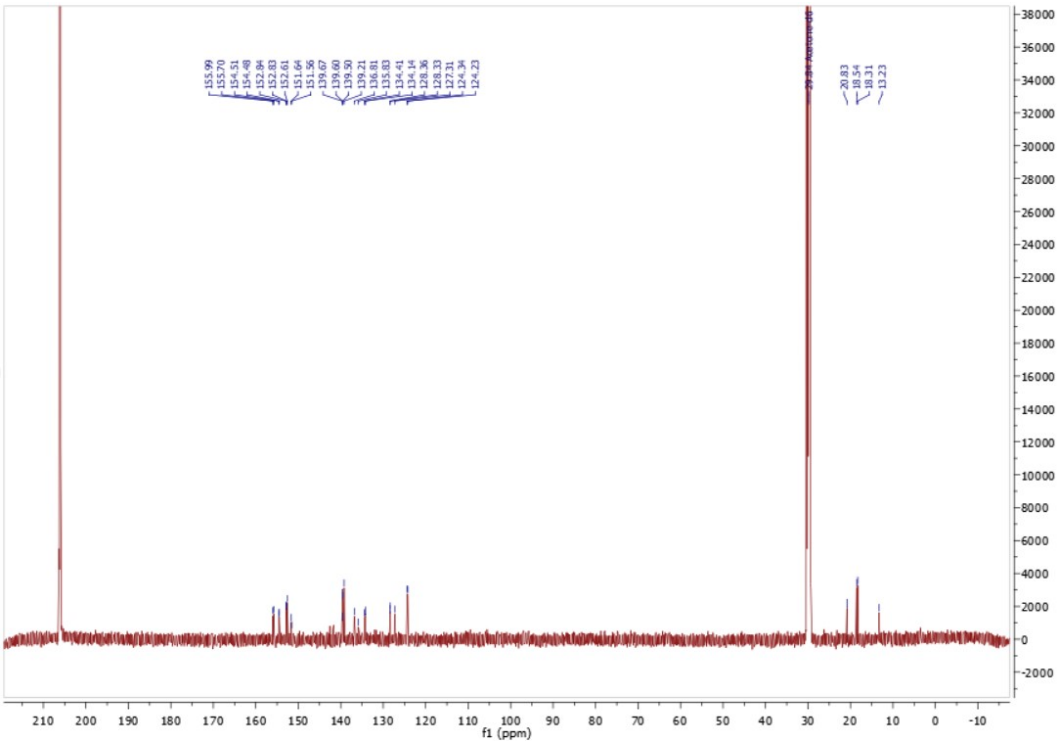

Fig. S40 ^{13}C NMR (126 MHz, acetone) spectrum of [Ru(5,5'-dmbpy)$_2$(10,11-dmdppz)](PF$_6$)$_2$.

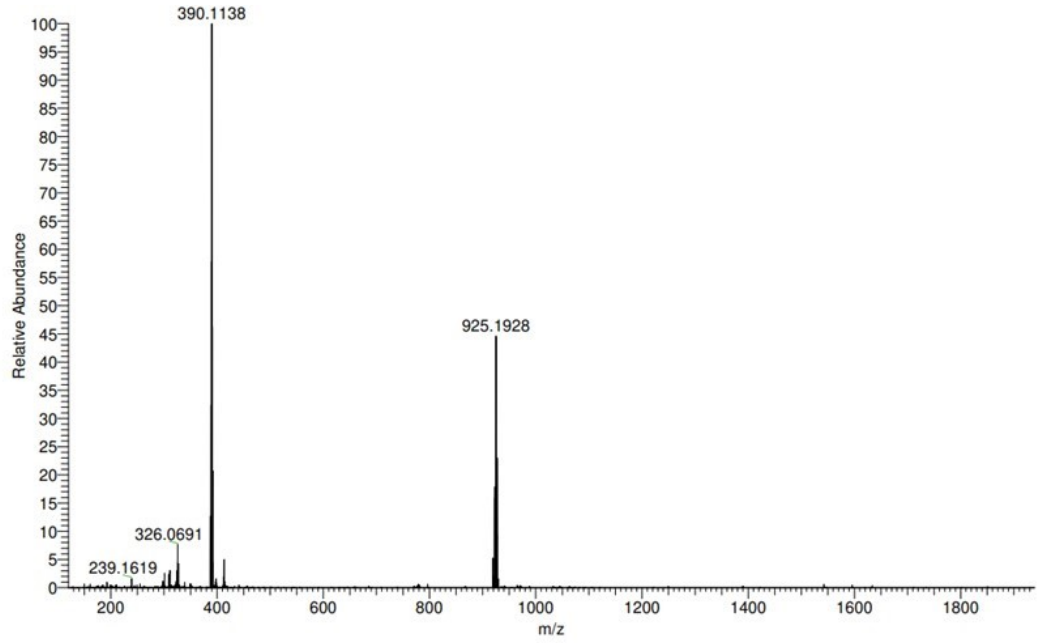

Fig. S41 HRMS spectrum of [Ru(5,5'-dmbpy)$_2$(10,11-dmdppz)](PF$_6$)$_2$.

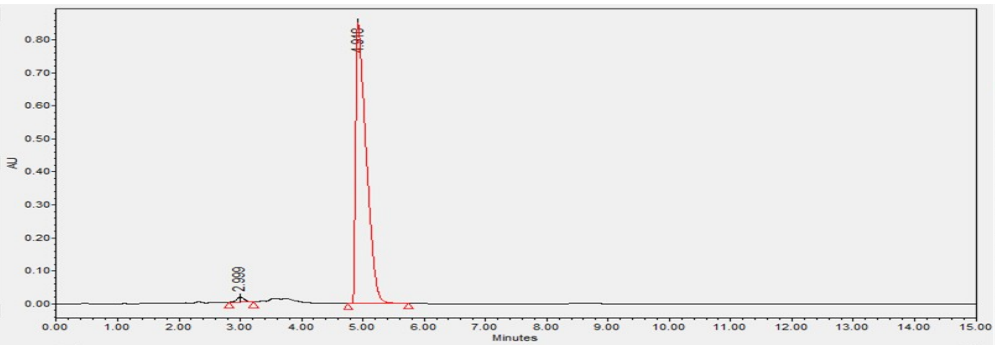

Fig. S42 HPLC of $[Ru(5,5'\text{-dmbpy})_2(10,11\text{-dmdppz})](PF_6)_2$.

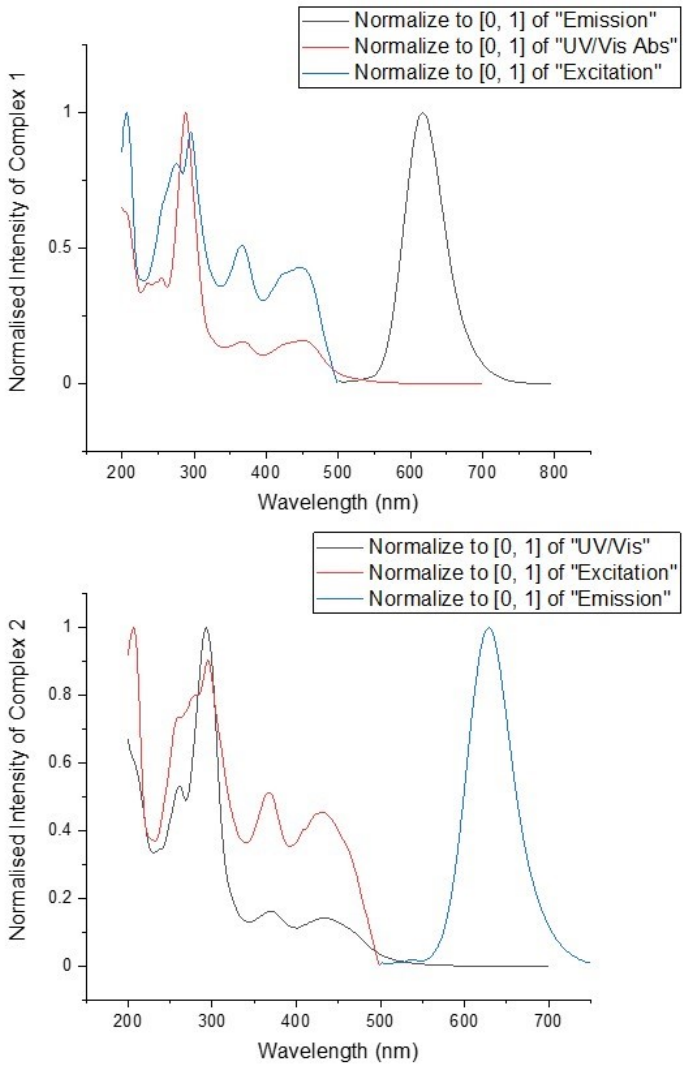

Fig. S43 Overlayed absorption, excitation (λ_{em} = 610 nm) and emission (λ_{ex} = 450 nm) spectra of **1** and **2** in acetonitrile.

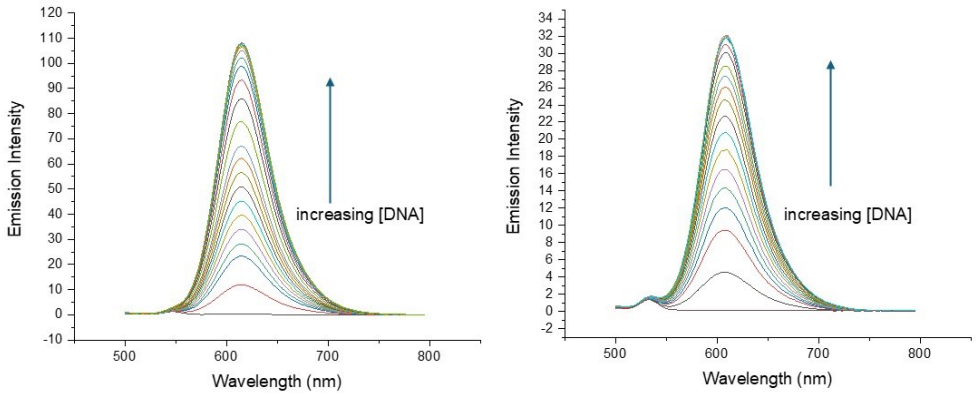

Fig. S44 Emission of **1** and **2** in Tris buffer with addition of DNA (λ_{ex} = 450 nm).

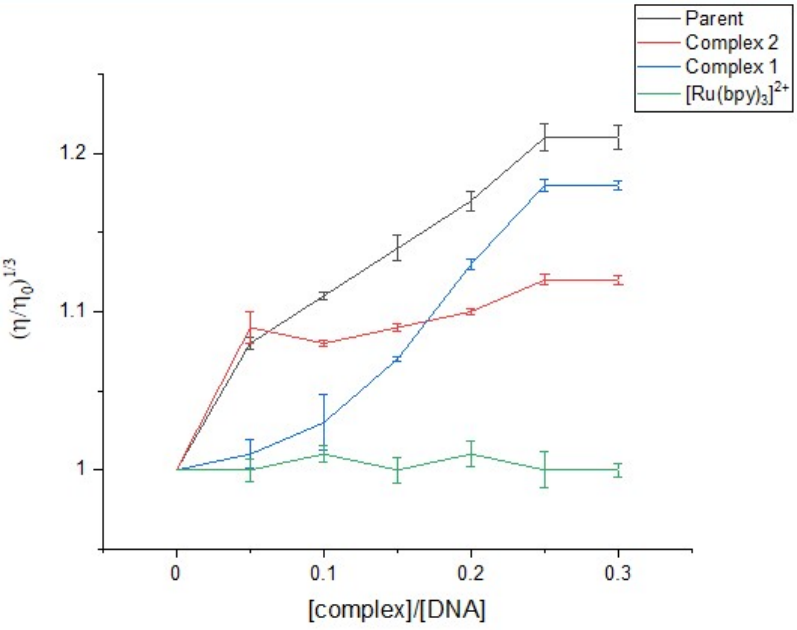

Fig. S45 Plot of relative viscosity $(\eta/\eta_0)^{1/3}$ of DNA versus [complex]/[DNA] upon addition of **1** or **2**. Intercalator $[Ru(bpy)_2(dppz)]^{2+}$, "parent", and non-intercalator $[Ru(bpy)_3]^{2+}$ employed for comparison.

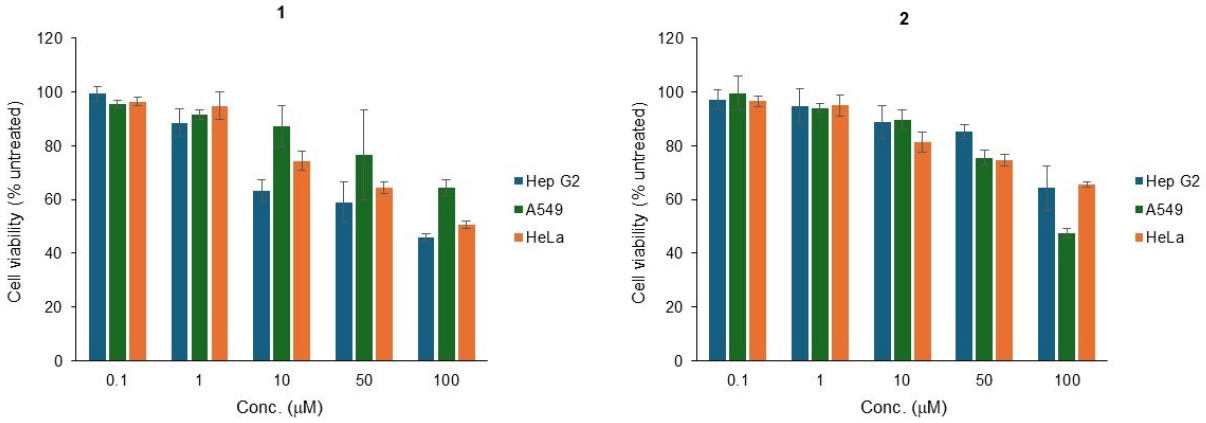

Fig. S46 Cytotoxicity of **1** and **2** towards Hep G2 human hepatocellular carcinoma, A549 human lung cancer or HeLa human cervical carcinoma cells. Cell viability determined by MTT assay (48 h incubation time).

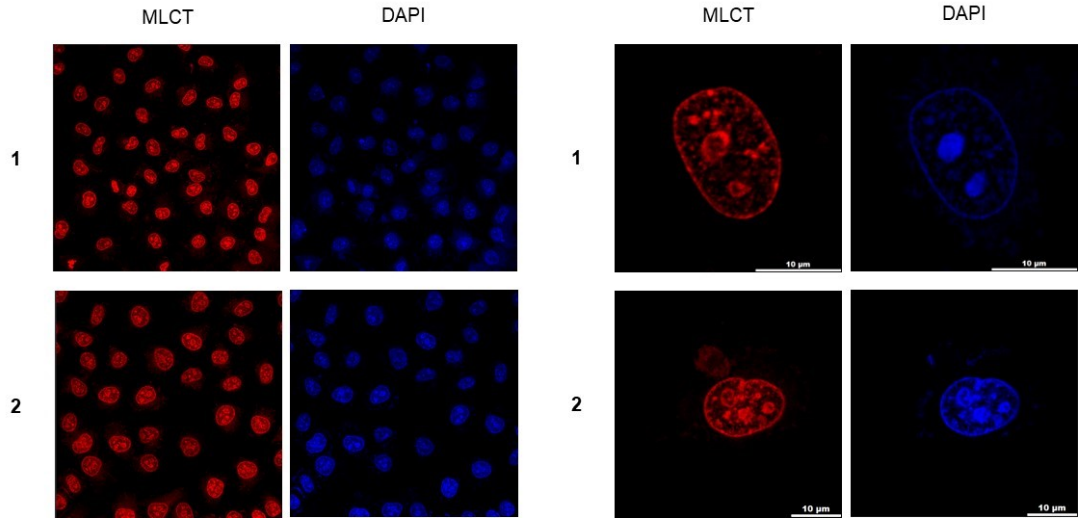

Fig. S47 Fixed HeLa cells co-stained with **1** or **2** and DAPI.

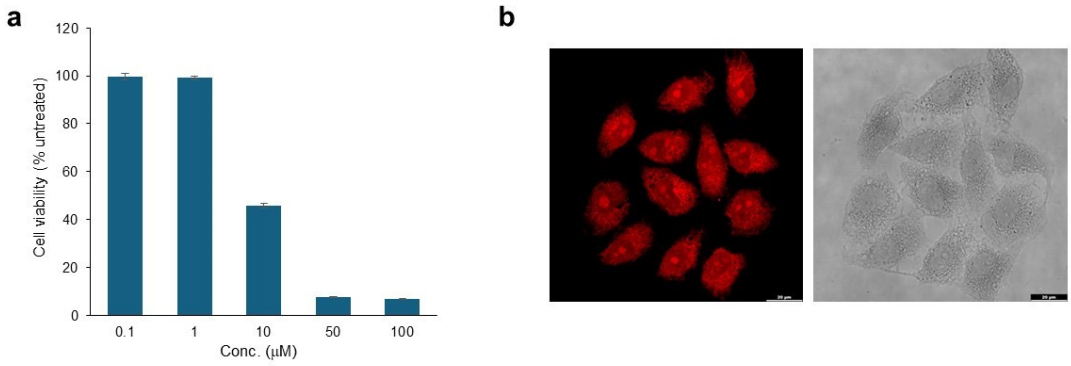

Fig. S48 a) Cytotoxicity of $[Ru(tmphen)_2(DIP)](PF_6)_2$ towards HeLa human cervical carcinoma cells. Cell viability determined by MTT assay (48 h incubation time). b) Fixed HeLa cells stained with $[Ru(tmphen)_2(DIP)](PF_6)_2$ (10 μM, 30 min).

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