Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Ratiometric ECL of heterodinuclear Os/Ru dual-emission labels

Shiguo Sun,[a]* Wei Sun,[a] Daozhou Mu[a], Na Jiang[a] and Xiaojun Peng[a]

[a] *State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Ling gong Road, Dalian 116024, P.R. China.*
E-mail: shiguo@dlut.edu.cn

Materials and reagents

4-Methyl-2,2′-bipyridine was made according to the literature procedure,[1] 1.6 M n-butyllithium in hexane was purchased from Aldrich, PF_6^- salt of $Ru(bpy)_3^{2+}$ and $Os(bpy)_3^{2+}$ were synthesized in our lab [2]. Other chemicals and solvents were all of reagent grade for synthesis and used directly as received.

Preparation of TPA samples

The 1.0 mol L^{-1} standard stock solution of TPA was prepared by accurately measuring the volume 4.77 mL of TPA, adding it into a 25 mL volumetric flask and dissolving it in 10 mL acetonitrile, followed by diluting with acetonitrile to the 25 mL scale. Then, the stock solutions of TPA were diluted with acetonitrile to obtain three standard solutions, *i.e.* 3.0×10^{-3}, 7.0×10^{-3}, and 1.1×10^{-2} mol L^{-1} respectively. All the solutions were maintained at room temperature.

Precision determination

The TPA samples were tested with **1b** and two reference molecules $Ru(bpy)_3^{2+}$ and $Os(bpy)_3^{2+}$ according to the method mentioned in experimental section respectively. The intra-day precision was determined by employing 5.0×10^{-5} mol L^{-1} **1b,** $Ru(bpy)_3^{2+}$ and $Os(bpy)_3^{2+}$ to analyze the three TPA samples three times on the same day respectively, while inter-day precision was determined by employing 5.0×10^{-5} mol L^{-1} **1b** to analyze the three TPA samples daily for 6 days over a period of one week. Average of three samples, each sample was measured repeatedly at least 7 times, and the averaged readings were used.

Apparatus

NMR spectra were obtained on a Varian INOVA 400 MHz spectrometer, using TMS as internal standard. The electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a HP1100 LC/MSD, and high resolution mass spectrometry (HRMS) was detected on a Q-Tof mass spectrometer (Micromass, Manchester, England). The absorption spectra were recorded on a HP 8453 spectrophotometer. Fluorescence spectra were recorded by a Perkin-Elmer LS 55 luminescence spectrometer. Cyclic voltammetry (CV) was measured on a CHI 660D instrument using a three-electrode system consisting of a Ag/ Ag^+ (0.01 M $AgNO_3$ in CH_3CN) as reference electrode, a platinum wire as counter electrode, and a freshly polished glassy carbon (GC, diameter 3 mm) as working electrode. ECL measurements were performed on a homemade jointing system of electrochemistry and fluorescence spectrometer. All experiments were carried out at room temperature.

ECL measurements were performed on a homemade jointing system of electrochemistry and fluorescence spectrometer. Cyclic potential sweep experiments were carried out in the potential region from 0 to 1.8 V, and then back to 0 V at a scan rate of 10 mV/s, the ECL signals vs time were collected between 550 and 850 nm, and the light intensity recorded every 0.015 V. Maximum intensities were obtained at +1.1 V vs Ag/$AgNO_3$ (±0.1V) and then start recording ECL spectrum, and these values used to generate intensity vs concentration profile. The data were measured repeatedly for at least 3 times, and the averaged readings were used for the creation of plots. [2]

Synthesis of Ruthenium(II) and Osmium(II) Tris-bipyridyl Complexes 1.

Complexes **1a** and **1b** were synthesized according to the literature procedure.[3] In short, 4-methyl-2,2′-bipyridine and the appropriate bihalogenoalkane were used as starting material for the synthesis of the corresponding ligands, and then they were employed toward the complexation of binuclear ruthenium and osmium using $Ru(bpy)_2Cl_2$ and $Os(bpy)_2Cl_2$ as precursor, leading to **1a** and **1b** with high purity. **1a,** yield: 73%. ^{1}H-NMR (400 MHz, δ ppm, Acetone-d_6): 1.23-1.42 (m, 12H, C_3H_2, C_4H_2), 1.60-1.77 (m, 4H, C_2H_2), 2.86-2.96 (m, 4H, bpy-CH_2), 7.32-7.64 (m, 12H, bpy-H, bpy'-H), 7.79 (d, J = 5.9 Hz, 1H, bpy'-H), 7.83-8.30 (m, 21H, bpy-H, bpy'-H), 8.65-8.89 (m, 12H, bpy-H, bpy'-H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 30.03, 30.24, 34.71, 34.90, 124.31, 124.40, 124.55, 124.59, 124.70, 127.73, 127.84, 127.93, 128.23, 128.27, 128.31, 137.23, 137.28, 137.95, 150.21, 150.78, 150.83, 150.88, 150.93, 151.04, 151.67, 151.73, 151.78, 154.41, 154.95, 156.75, 157.24, 157.26, 157.30, 157.38, 158.62, 159.18, 159.21, 159.25, 159.34. HRMS (ESI, *m/z*): $1/3[(M-3PF_6^-)]^{3+}$ calculated for $C_{70}H_{66}N_{12}F_6PRuOs$ 504.4611, found 504.4632. **1b,** yield: 73%. ^{1}H-NMR (400 MHz, δ ppm, Acetone-d_6): 1.22-1.44 (m, 20H, C_3H_2, C_4H_2), 1.62-1.78 (m, 4H, C_2H_2), 2.87-2.97 (m, 4H, bpy-CH_2), 7.32-7.65 (m, 12H, bpy-H, bpy'-H), 7.79 (d, J = 6.0 Hz, 1H, bpy'-H), 7.84-8.28 (m, 21H, bpy-H, bpy'-H), 8.60-8.91 (m, 12H, bpy-H, bpy'-H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 30.96, 31.17, 34.33, 35.62, 35.82, 125.21, 125.31, 125.44, 125.50, 125.61, 128.64, 128.73, 128.83, 129.14, 129.21, 138.15, 138.19, 138.86, 151.13, 151.70, 151.75, 151.80, 151.84, 151.96, 152.58, 152.64, 152.69, 155.32, 155.86, 157.67, 158.16, 158.18, 158.21, 158.30, 159.55, 160.13, 160.18, 160.25. HRMS (ESI, *m/z*): $1/3[(M-3PF_6^-)]^{3+}$ calculated for $C_{74}H_{74}N_{12}F_6PRuOs$ 523.1487, found 523.1474.

The mechanism of ratiometric ECL spectral change

In the case of $[Ru(bpy)_3]^{2+}$ and TPA, one of the ECL mechanism is:

$TPA - e \rightarrow TPA^{\bullet+}$ (1)

$TPA^{\bullet+} - H^+ \rightarrow TPA^{\bullet}$ (2)

$[Ru(bpy)_3]^{2+} - e \rightarrow [Ru(bpy)_3]^{3+}$ (3)

$[Ru(bpy)_3]^{3+} + TPA^{\bullet} \rightarrow [Ru(bpy)_3]^{2+*} + P$ (4)

$[Ru(bpy)_3]^{2+*} \rightarrow [Ru(bpy)_3]^{2+} + hv$ (5)

Similarly, the mechanism of $[Os(bpy)_3]^{2+}$ and TPA can be described as the following:

$TPA - e \rightarrow TPA^{\bullet+}$ (1)

$TPA^{\bullet+} - H^+ \rightarrow TPA^{\bullet}$ (2)

$[Os(bpy)_3]^{2+}$ - e → $[Os(bpy)_3]^{3+}$ (3)

$[Os(bpy)_3]^{3+}$ + $TPA^{\bullet}$ → $[Os(bpy)_3]^{2+*}$ + P (4)

$[Os(bpy)_3]^{2+*}$ → $[Os(bpy)_3]^{2+}$ + *hv* (5)

The mechanism outlined above is dominated when the co-reactant is in large excess. According to the literature, the mechanism of ratiometric ECL spectral change of **1** is proposed as follows:

TPA - e → $TPA^{\bullet +}$ (1)

$TPA^{\bullet +}$ - H^+ → $TPA^{\bullet}$ (2)

1 – e → $\mathbf{1}^+$ (3)

$\mathbf{1}^+$ + $TPA^{\bullet}$ → **1*** + P (4)

1* → **1** + *hv* (5)

In addition, at 1.1 V, **1** affords two excited states, namely *Ru-Cn-Os and Ru-Cn-*Os (Eq. a and b). The reductive quenching of *Ru-Cn-Os by an intramolecular electron transfer process (Eq. 1d) and the building of Ru-Cn-*Os by the intramolecular energy transfer process (Eq. 1e) are also taken into account**:**

Ru-Cn-Os → *Ru-Cn-Os, (a)

Ru-Cn-Os → Ru-Cn-*Os, (b)

*Ru-Cn-Os → Ru-Cn-Os + *hv*, (c)

*Ru-Cn-Os → Ru-Cn-Os, (d)

*Ru-Cn-Os → Ru-Cn-*Os, (e)

Ru-Cn-*Os → Ru-Cn-Os + *hv*. (f)

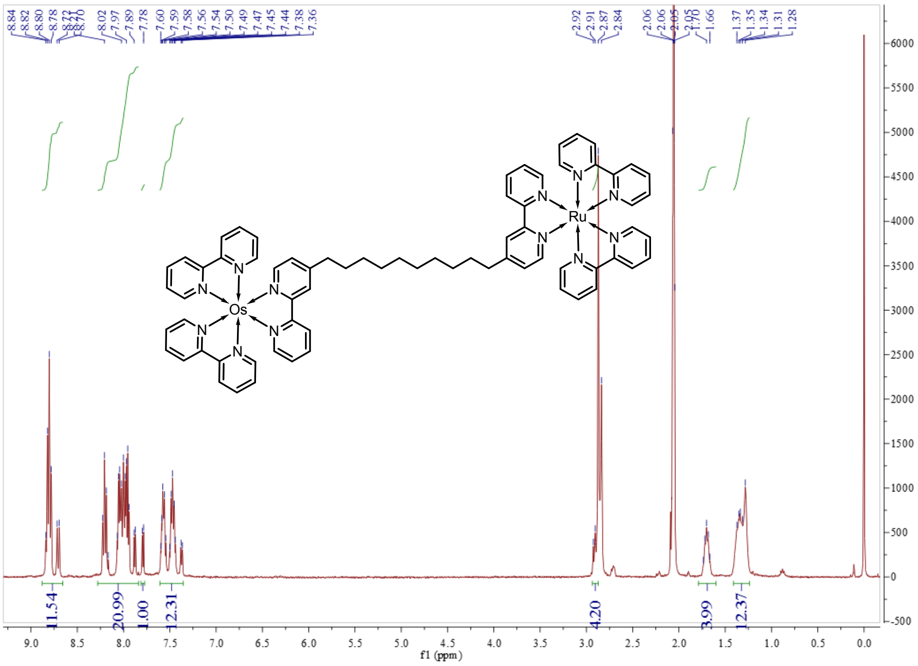


Figure S1. ^{1}H NMR spectrum of **1a** in CD$_3$OCD$_3$.

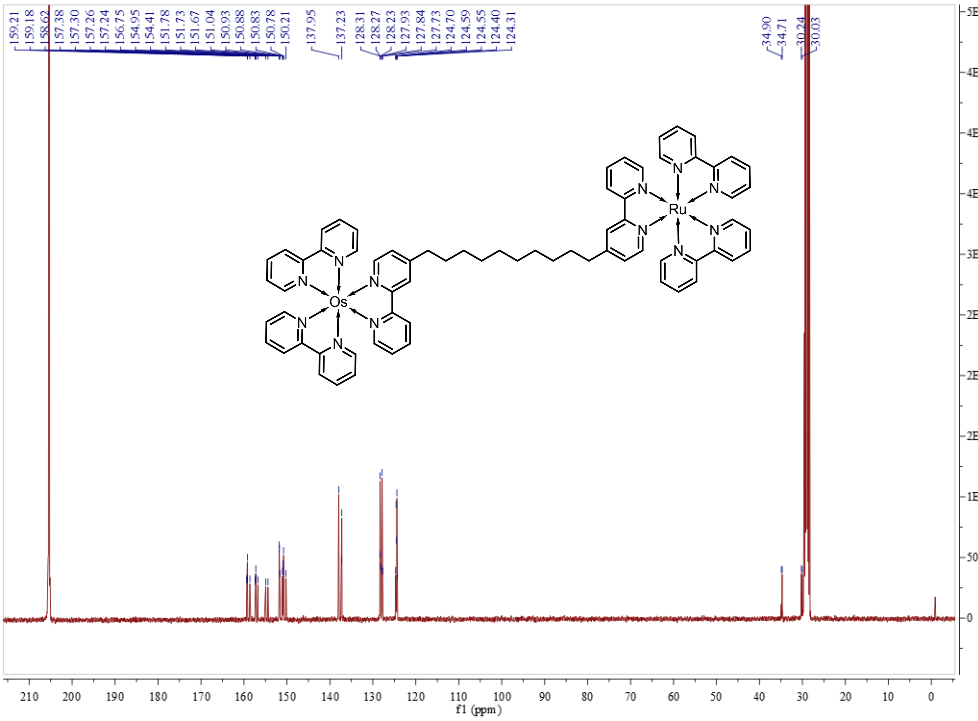


Figure S2. ^{13}C NMR spectrum of **1a** in CD$_3$OCD$_3$.

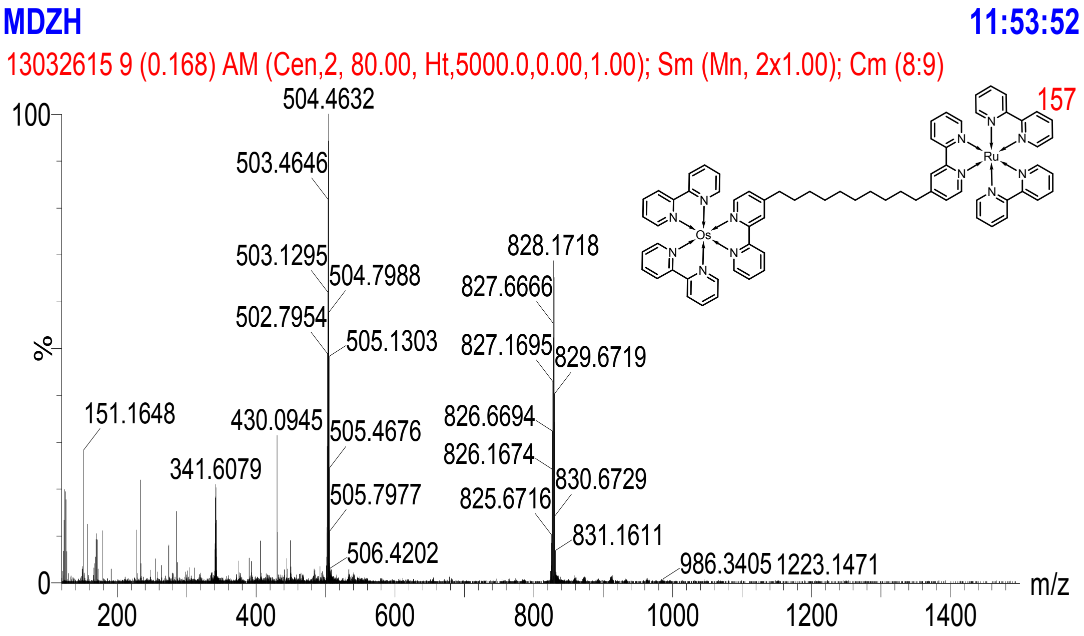


Figure S3. ESI-MS of **1a**

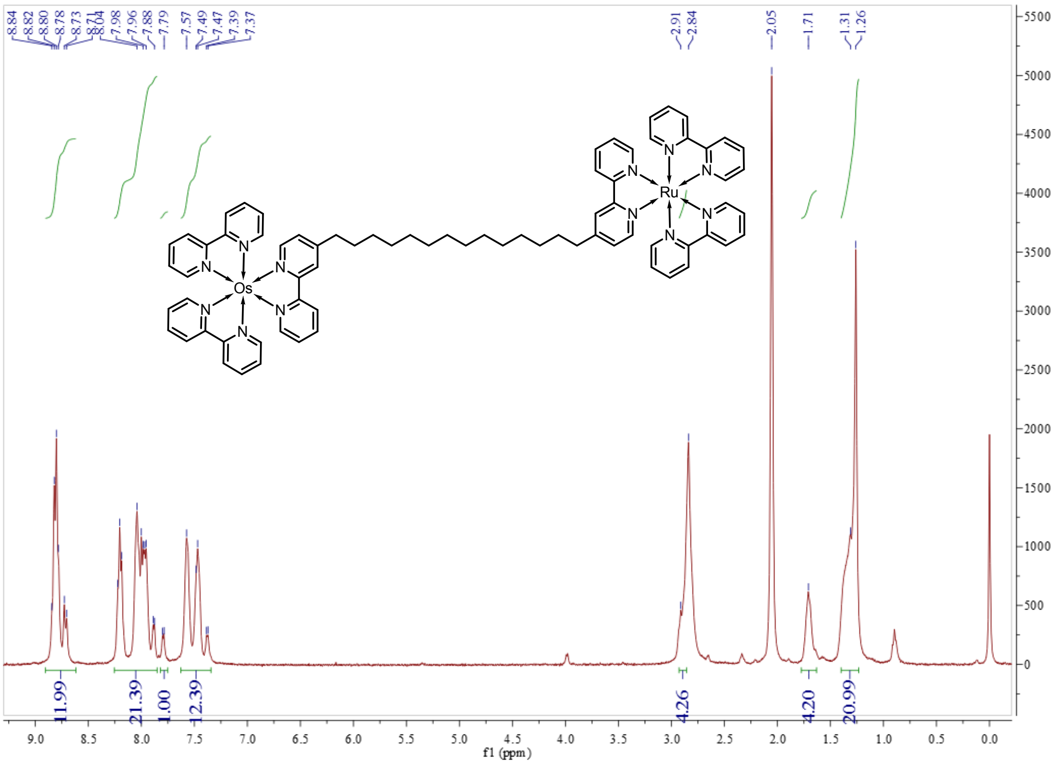


Figure S4. ^{1}H NMR spectrum of **1b** in CD_3OCD_3.

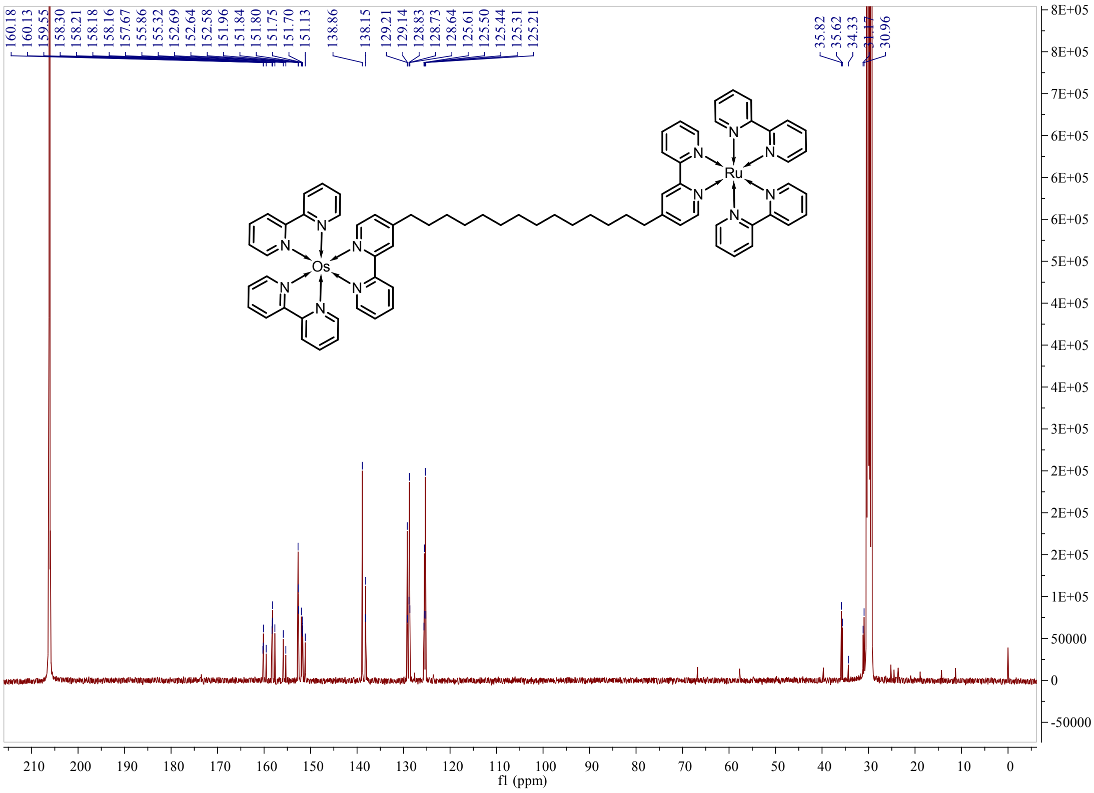


Figure S5. ^{13}C NMR spectrum of **1b** in CD_3OCD_3.

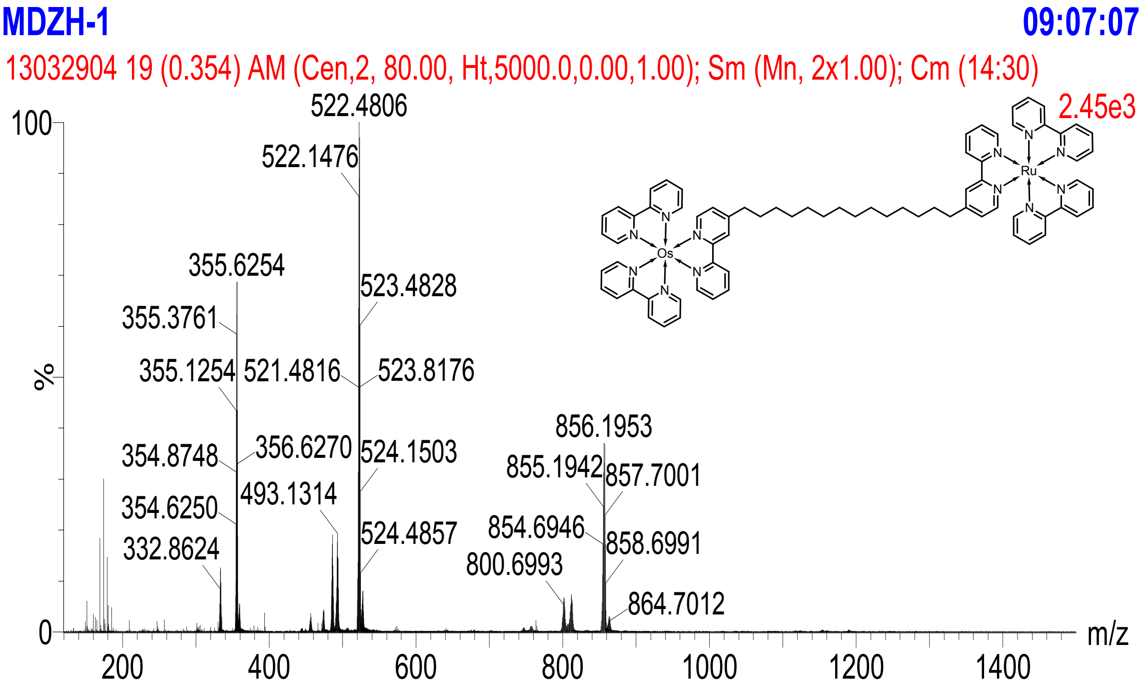


Figure S6. ESI-MS of **1b**

Table S1. Absorption maxima, emission maxima and molecular extinction coefficient of $Ru(bpy)_3^{2+}$, $Os(bpy)_3^{2+}$ and **1**

	Absorption λ_{max}(nm)	Excitation λ(nm)	Emission λ_{max}(nm)	ε ($\times 10^4$ L/mol)
	244	455	728	2.58
$Os(bpy)_3^{2+}$	290	-	-	7.74
	479	-	-	1.09
	244	455	610	2.45
$Ru(bpy)_3^{2+}$	287	-	-	7.45
	451	-	-	1.26
	244	455	611/730	4.25
1a	289	-	-	12.2
	450	-	-	1.99
	244	455	615/715	4.92
1b	289	-	-	13.9
	451	-	-	2.36

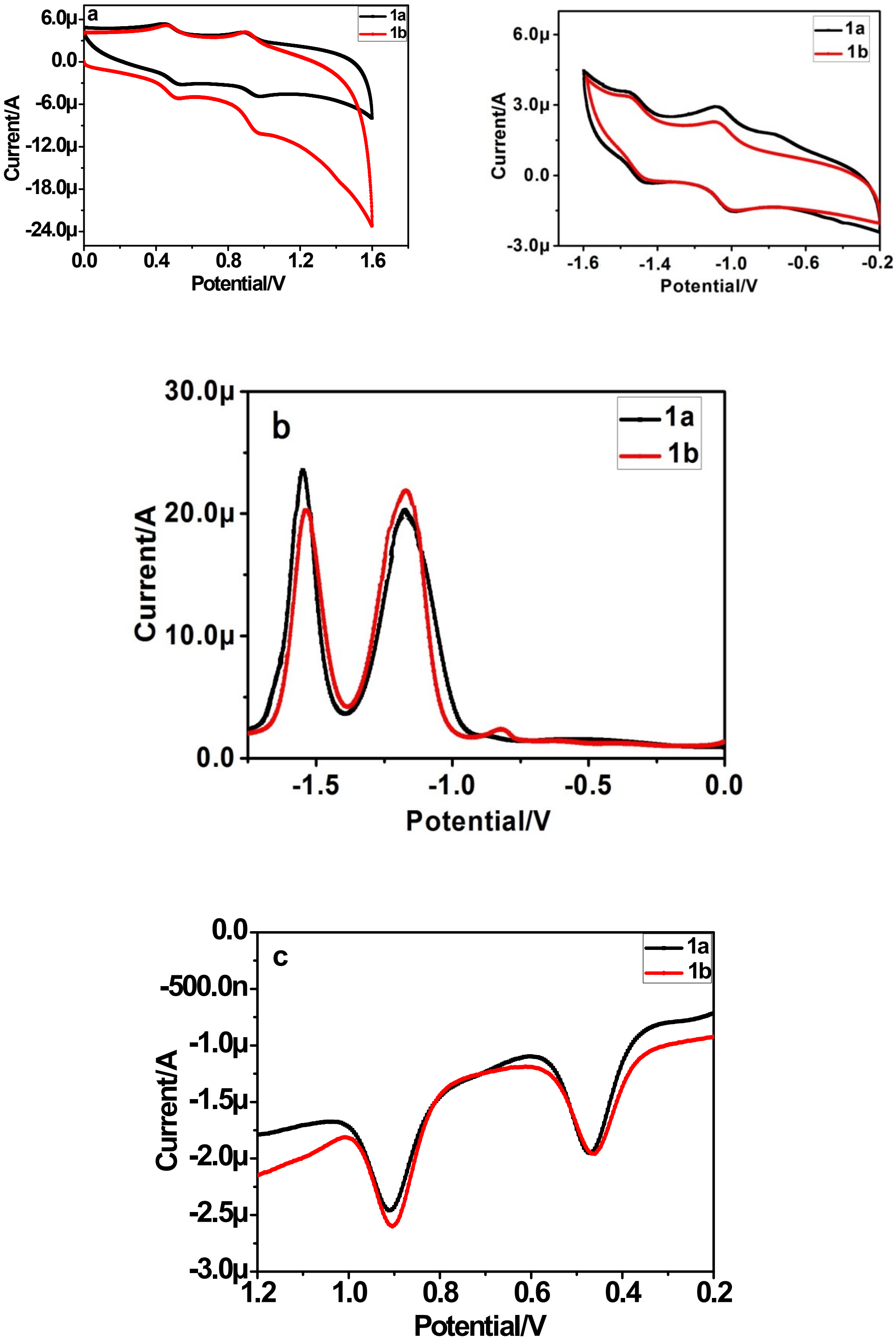


Figure S7. (a) The cyclic voltammetry (CV) curve and (b, c) the differential pulse voltammetry (DPV) curve of **1a** and **1b** in acetonitrile. The complex concentration was 5×10^{-5} M.

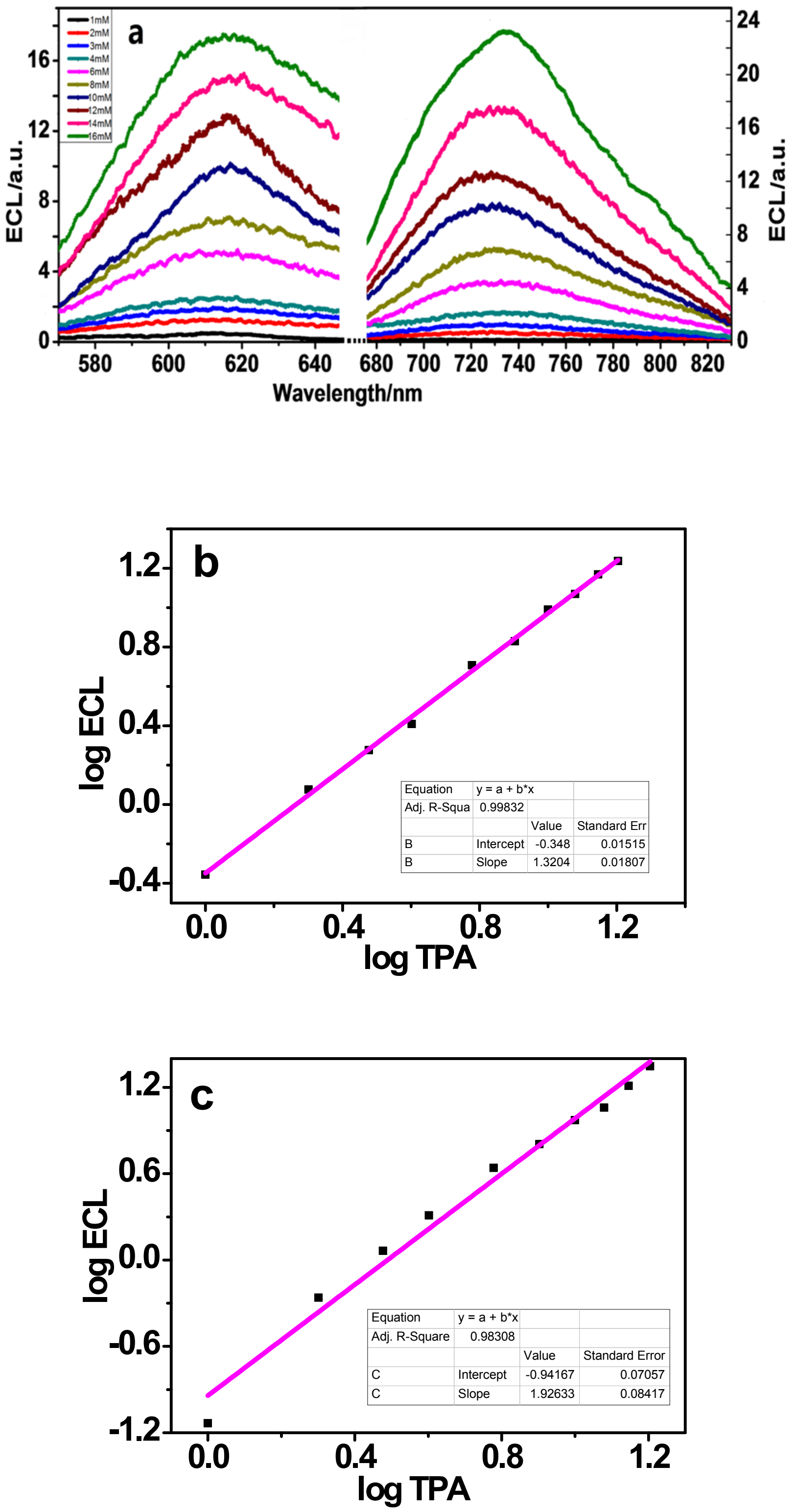


Figure S8. ECL spectra changes of Ru(bpy)$_3^{2+}$, Os(bpy)$_3^{2+}$ (5×10^{-5} M) along with the titration of TPA from 1 mM to 16 mM, 0.1 M $(n\text{-Bu})_4NPF_6$ in acetonitrile (a); Calibration curves of $[Ru(bpy)_3]^{2+}$ (b) and $[Os(bpy)_3]^{2+}$ (c) between the log ECL and log TPA added over a concentration range in acetonitrile, respectively.

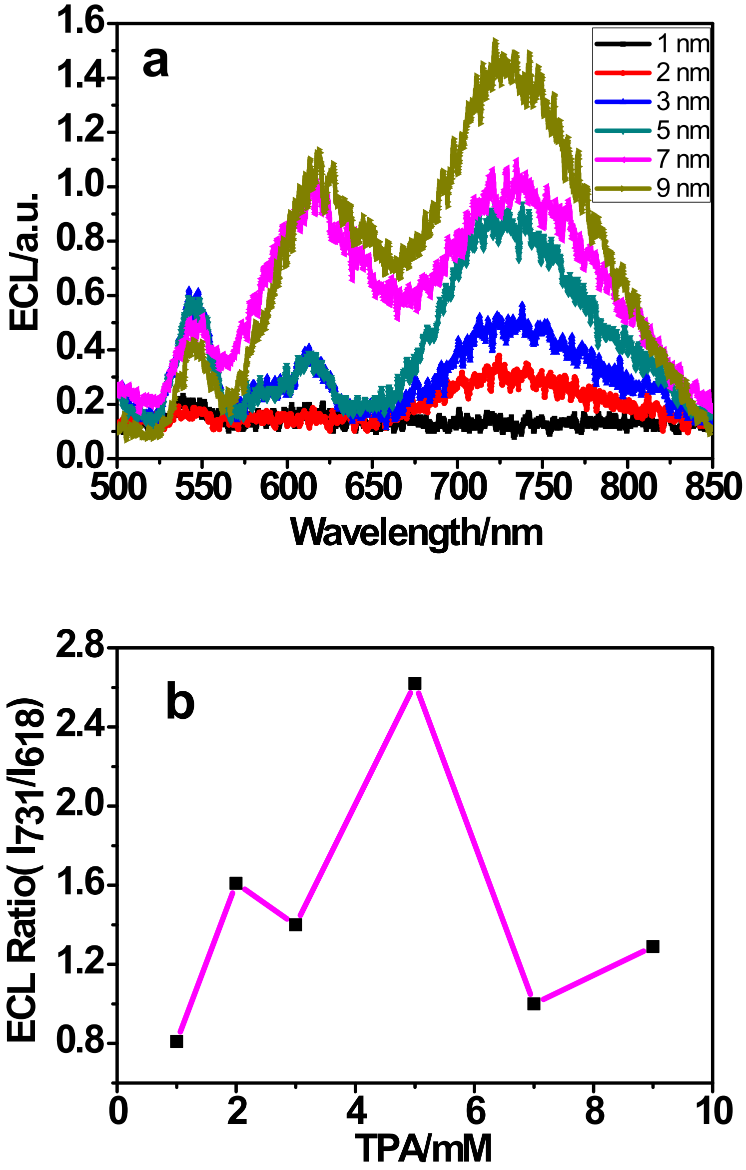


Figure S9. (a) ECL titration curve of Ru(bpy)$_3$$^{2+}$ and Os(bpy)$_3$$^{2+}$ in 1:1 with the addition of TPA and (**b**) A calibration curve between the ECL I_{731}/I_{618} for the TPA from 1 mM to 9 mM. rate, v = 0.01 V·s^{-1}, Scan range 0-1.4V. 0.1 M (n-Bu)$_4$NPF$_6$ in acetonitrile.

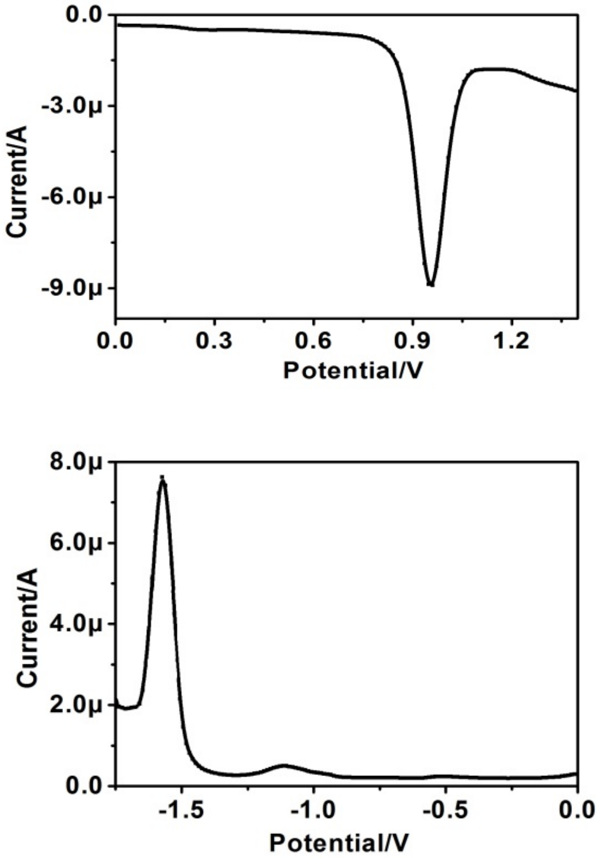


Figure S10 The differential pulse voltammetry (DPV) curve of reference complex $[(bpy)_2Ru(bpy)(CH_2)_{10}(bpy)]^{2+}$ in acetonitrile. The complex concentration was 5×10^{-5} M.

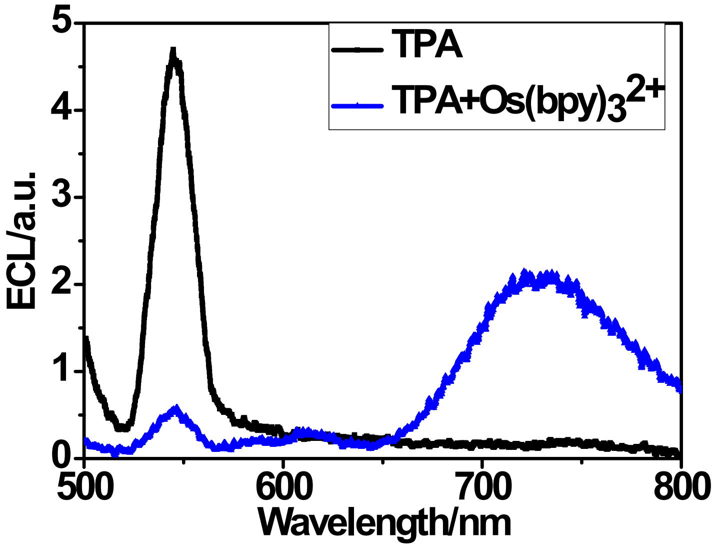
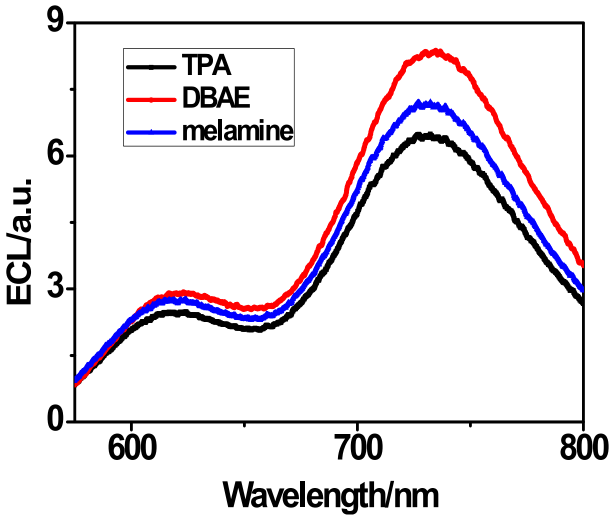


Figure S11 ECL spectra of 5 mM TPA (black line) in the absence or presence of 5×10^{-5} mM 5×10^{-5} mM $Os(bpy)_3^{2+}$.



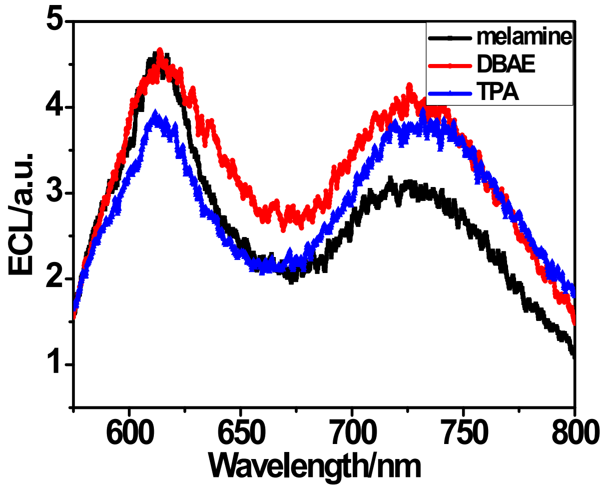


Figure S12 ECL spectra of **1a** and **1b** with the presence of 5 mM TPA, DBAE and melanmine in 0.1 M (n-Bu)$_4$NPF$_6$ acetonitrile, the concentration was 5×10^{-5} M.

Table S2. Analytical results of **1b** ECL in the actual TPA operation.

	inter-day			**alternate-day**		
TPA added (mM)	3	7	11	3	7	11
ECL Ratio (I_{731}/I_{618})	0.884	1.196	1.503	0.885	1.189	1.505
	0.886	1.196	1.503	0.885	1.190	1.505
	0.885	1.196	1.504	0.885	1.190	1.505
average	0.885	1.196	1.504	0.885	1.190	1.505
TPA detected (mM)	2.983	7.044	11.060	2.979	6.958	11.078
RSD (%)	0.567	0.629	0.545	0.700	0.600	0.7090

Table S3. Analytical results of Ru(bpy)$_3^{2+}$ and Os(bpy)$_3^{2+}$ ECL in the actual TPA operation.

	Ru(bpy)$_3^{2+}$			**Os(bpy)$_3^{2+}$**		
TPA added (mM)	3	7	11	3	7	11
	1.842	6.133	11.044	1.125	7.159	11.079
ECL	1.912	6.074	11.053	1.168	7.300	10.990
	1.867	6.161	11.101	1.133	7.102	11.052
average	1.873	6.123	11.066	1.142	7.187	11.041
Log ECL	0.273	0.787	1.044	0.058	0.857	1.043
TPA detected (mM)	2.953	7.237	11.330	3.303	7.187	10.722
RSD (%)	1.567	3.386	3.000	10.100	2.671	2.527

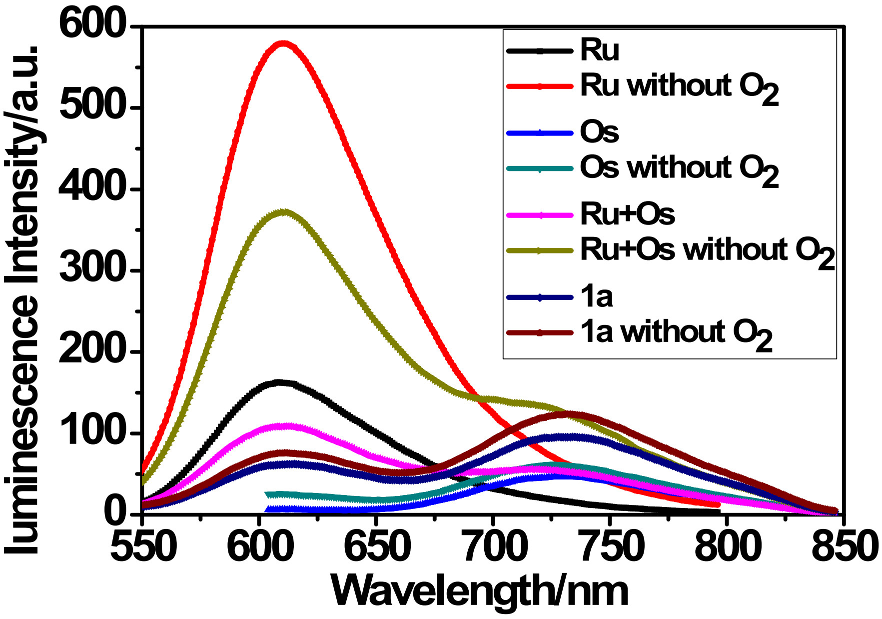


Figure. S13 The luminescence spectra of **1a**, Ru alone, Os alone and the mixture of one metallic Ru(bpy)$_3^{2+}$ and Os(bpy)$_3^{2+}$ in 1:1 ratio before and after ruling out the oxygen.

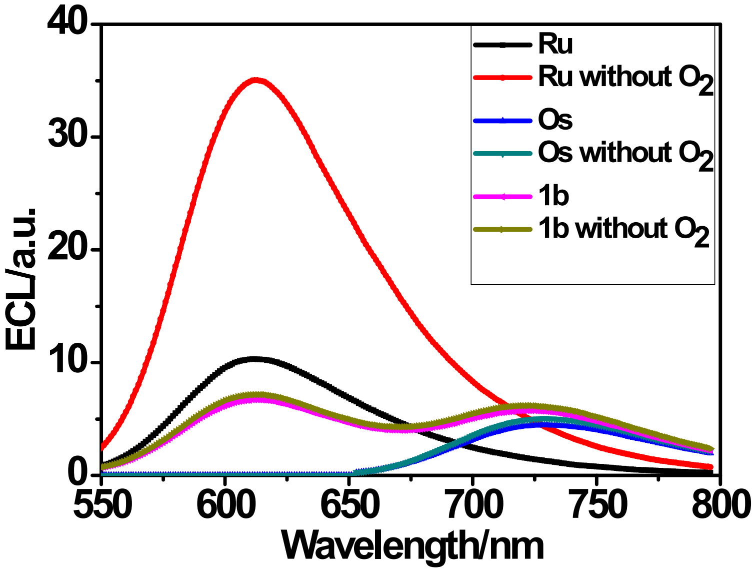


Figure. S14 The ECL of **1b**, Ru alone and Os alone before and after ruling out the oxygen.

References

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2. (a) Workman, S.; Richter, M. M. Anal. Chem. 2000, 72, 5556-5561. (b)McCall, J.; Alexander, C.; Richter, M. M. Anal. Chem. 1999, 71, 2523.
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