Asian Journal of Chemistry Vol. 11, No. 4 (1999), 1149–1154

Synthesis and Properties of Two Novel Ru(phen)$_3^{2+}$-based Probes for Electrochemiluminescent Immunoassay

PENG WANG and GUOYI ZHU*
Laboratory of Electroanalytical Chemistry
National Analytical Research Centre of Electrochemistry and Spectroscopy
Institute of Applied Chemistry, Chinese Academy of Sciences
Changchum 130 022, P.R. China

Two novel Ru(phen)$_3^{2+}$-based probes for electrochemiluminescent immunoassay, Ru(phen)$_2$(phen-NHCOCH$_2$CH$_2$COOH)(PF$_6$)$_2$ and Ru(phen)$_2$ (phen-NHCOCH$_2$CH$_2$CH$_2$COOH)(PF$_6$)$_2$, were designed, synthesized and characterized. Electrochemistry, Fluorescence, and electrochemiluminescence of the two complexed are also reported.

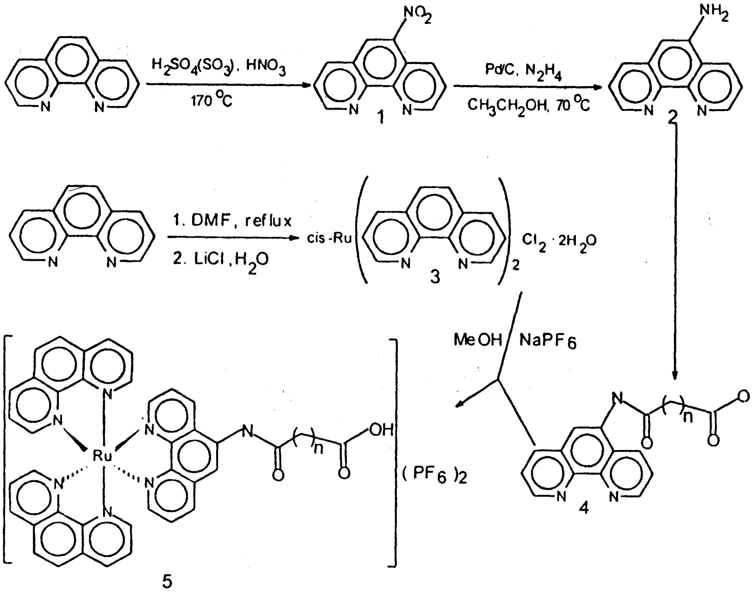
INTRODUCTION

Electrochemiluminescence (ECL) is a highly specific and sensitive detection protocol used in a diversity of analytical applications. These include bioassays in clinical diagnostics and high-throughout screening for drug discovery[1–4]. ECL of polypyridine transition-metal complexes such as Ru(bpy)$_3^{2+}$ has attracted interest from both theoretical and practical points of view.[5–7] Ru(bpy)$_2$(Me-bpy-CH$_2$ CH$_2$CH$_2$COOH)(PF$_6$)$_2$ has been widely used in ECL immunoassay.[1]

It is known[8] that ECL efficiency of Ru(phen)$_3^{2+}$ is higher than that of Ru(bpy)$_3^{2+}$. For analytical work, there is a continuous and expanding need for more highly sensitive and specific method of qualitative and quantitative detection. Thus, we designed and synthesized two novel Ru(Phen)$_3^{2-}$-based probes for ECL immunoassay. Synthesis of the two complexes is outlined in Scheme 1.

EXPERIMENTAL

IR spectra were recorded in KBr pellets on Bio-RAD FTS-7 infrared spectrometer. ^{1}H NMR spectra were carried at room temperature on a Varian 400 spectrometer, and samples were dissolved in DMSO-d$_6$ and chemical shifts were expressed in ppm using TMS as the internal standard. ESI-MS spectra were performed on a Finningan MCT LCQTM mass spectrometer using DMF as the matrix. Fluorescence spectra were taken on a Shimadzu RF-5000 fluorescence spectrometer at 20°C. Cyclic voltammetry experiments were measured on a CHI 660 electrochemical station. The working electrode was Au disk and all potentials were measured relative to a SCE electrode. The counter-electrode was a Pt wire and the scan rate was 100 mV/S. ECL was performed on home-made ECL analyzer.



a n=2; a n=3

Scheme 1. Synthesis of $Ru(phen)_3^{2+}$-based probes for electrochemiluminescent immunoassay

The key starting materials, **1**, **2** and **3**, were synthesized according to the reported methods[9–11].

Synthesis of 5-(3-carboxylic acid-propionamide)-1,10-phenanthroline (4a)

The solution of 0.7530 g (7.5 mmol) succinic anhydride in 50 mL acetonitrile (dried over calcium hydride and distilled before use) was slowly added to 50 mL of dry acetonitrile containing 0.9761 g (5 mmol) **2**. After the solution was stirred overnight at room temperature, the product was collected by filtration and washed with water, and dried in a desiccator under vacuum overnight. TLC (alumina, 95% ethanol) showed a new spot moving slightly faster than **2**. All the starting material had transformed in 72% yield. IR/cm^{-1}: 3447 (OH), 3239 (NH), 1729 and 1658 (C=O); ^1H NMR δ_H: 2.74 (t, 2H, CH_2), 2.91 (t, 2H, CH_2), 7.87 (q, 1H, 8-H), 7.93 (q, 1H, 3-H) 8.27 (d, 1H, 6-H) 8.56 (d, 1H, 7-H), 8.76 (d, 4-H), 9.15 (d, 1H, 9-H), 9.24 (d, 1H, 2-H), 10.34 (s, 1H, NH), 12.36 (s, 1H, COOH); ESI-MS: 318.3 ([M + Na^+]).

Synthesis of 5-(4-carboxylic acid-butyramide)-1,10-phenanthroline (4b)

The solution of 0.858 g (7.5 mmol) glutaric anhydride in 50 mL acetonitrile (dried over calcium hydride and distilled before use) was slowly added to 50 mL of dry acetonitrile containing 0.9761 g (5 mmol) **2**. After the solution was stirred overnight at room temperature, the product was collected by filtration and washed with water, and dried in a desiccator under vacuum overnight. TLC (alumina, 95% ethanol) showed a new spot moving slightly faster than **2**. All the starting material had transformed in 74% yield. IR/cm^{-1} : 3442 (OH), 3234 (NH), 1728 and 1660 (C═O); ^{1}H NMR δ_H: 2.23 (m, 2H, CH_2), 2.68 (t, 2H, CH_2), 2.84 (t, 2H, CH_2), 7.86 (q, 1H, 8-H) 7.94 (q, 1H, 3-H), 8.28 (d, 1H, 6-H), 8.55 (d, 1H, 7-H), 8.76 (d, 1H, 4-H), 9.14 (d, 1H, 9-H), 9.25 (d, 1H, 2-H), 10.35 (s, 1H, NH), 12.35 (s, 1H, COOH); ESI-MS: 332.2 ([M + Na]$^+$).

Synthesis of Ru(phen)$_2$(phen-NHCOCH$_2$CH$_2$COOH)(PF$_6$)$_2$ (5a)

0.5684 g (0.01 mol) **3** and 0.4773 g (0.01 mol) **4a** were stirred in refluxing methanol (20 mL) and water (5 mL) for 9 h. The resulting solution was separated by filtration and washed with 8 mL methanol. The combined filtrate and wash solution were treated with a solution of 5.0 g sodium hexaflurophosphate in 25 mL water. The reaction solution was cooled in an ice bath for 3 h. The resulting precipitate of orange microcrystal was collected by filtration, and dried under vacuum in presenc of P_2O_5. IR/cm^{-1} 1730 and 1699 (C═O); ^{1}H NMR δH: 2.75 (t, 2H, CH_2), 2.96 (t, 2-H, CH_2), 7.82 (q, 1-H, 8-H of **4a**), 7.90 (m, 5-H, 3-H and 8-H of phen and 3-H of **4a**), 8.10 (d, 1-H, 7-H), 8.20 (m, 5-H, 4-H and 7-H of phen and 4-H of **4a**), 8.51 (d, 4-H, 5-H and 6-H of phen), 8.70 (s, 1-H, 6-H of **4a**), 8.88 (m, 5-H, 4-H and 7-H of phen and 7-H of **4a**), 9.03 (d, 1-H, 4-H of **4a**), 10.65 (s, 1-H, NH), 12.33 (s, 1-H, COOH); ESI-MS 901.6 ([M-PF$_6$]$^+$), 378.4 ([M-2PF$_6$]$^{2+}$).

Synthesis of Ru(phen)$_2$(phen-NHCOCH$_2$CH$_2$CH$_2$COOH)(PF$_6$)$_2$ (5b)

0.5684 g (0.01 mol) **3** and 0.4773 g (0.01) mole **4b** were stirred in refluxing methanol (20 mL) and water (5 mL) for 9 h. The resulting solution was separated by filtration and washed with 8 mL methanol. The combined filtrate and wash solution were treated with a solution of 5.0 g sodium hexafluorophosphate in 25 mL water. The reaction solution was cooled in an ice bath for 3 h. The resulting precipitate of orange microcrystal was collected by filtration, and dried under vacuum in presence of P_2O_5. IR/cm^{-1} 1731 and 1698 (C═O); ^{1}H NMR $\delta_{H:}$ 2.27 (m, 2-H, CH_2), 2.74 (t, 2-H, CH_2), 2.97 (t, 2-H, CH_2), 7.80 (q, 1-H, 8-H of **4a**), 7.89 (m, 5H, 3-H and 8-H of phen and 3-H of **4a**), 8.11 (d, 1-H, 7-H), 8.20 (m, 5H, 4-H and 7-H of phen and 4-H of **4a**), 8.52 (d, 4-H, 5-H and 6-H of phen). 8.71 (s, 1-H, 6-H of **4a**), 8.87 (m, 5-H, 4-H and 7-H of phen and 7-H of **4a**), 9.02 (d, 1-H, 4-H of **4a**), 10.60 (s, 1-H, NH), 12.35 (s, 1-H, COOH); ESI-MS 915.6 ([M-PF$_6$]^2), 385.4 ([M-2PF$_6$]$^{2+}$).

RESULTS AND DISCUSSION

Infrared spectroscopy: The IR adsorption peaks (—CONH—) of **5** appear at *ca.* 1699 cm^{-1}, which are abnormally blue-shifted 40 cm^{-1} compared with those of **4**., we suggest that these may be an important contribution of *d*-*π* feedback effect from metal Ru to ligand **4**.

Electrospray mass spectroscopy: One of the main characterizational tools was electrospray mass spectrometry. In the last few years this has become a popular tool for the characterization of high-nuclearity and/or highly charged coordination complexes.[12, 13] The principal mass spectral peaks in (see Experi mental section) in every case confirm the formulations of the complexes. Generally, loss of both the hexafluorophosphate anions was observed giving a doubly-charged fragment which appeared therefore at the *m/z* value corresponding to half the value that would be expected for a + 1 fragment.

Proton nuclear magnetic spectroscopy: The ^{1}H NMR of **5** were attributed by the technique of ^{1}H-^{1}H COSY. ^{1}H-^{1}H COSY spectrum in high field of **5a** is shown in Figure 1, and is almost the same as that of **5b**. H_2 and H_9 of ligands occur at higher field than H_4 and H_7 due to a local shielding effect from the spacial adjacent ligands.

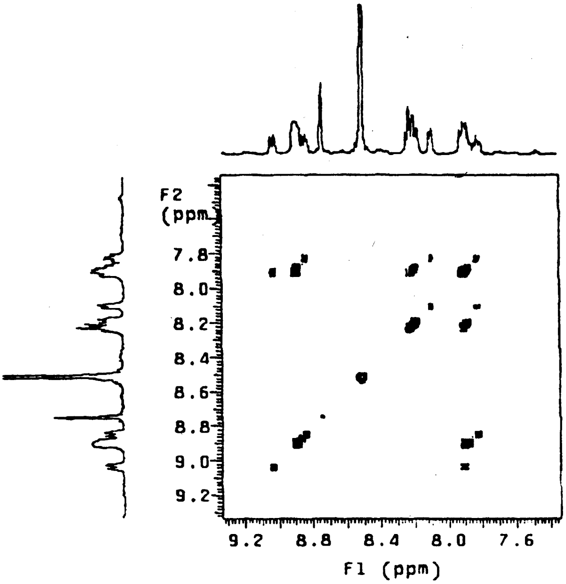
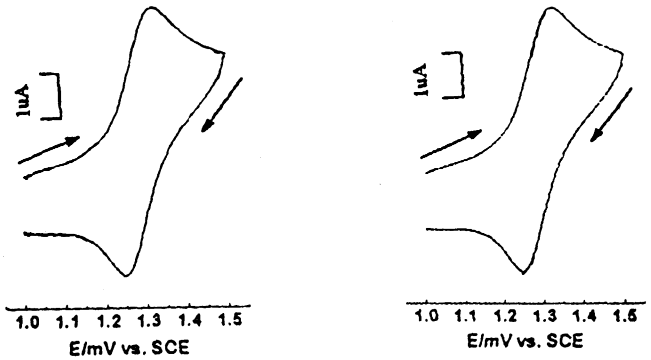


Fig. 1. $^{1\text{-}1}$H COSY spectrum of **5a**

Electrochemistry: Fig. 2 shows cyclic voltammograms of 1 mmol/L **5a** and **5b** in acetonitrile with 0.1 mol/L (TBA)PF_6 as supporting electrolyte. **5a** and **5b**

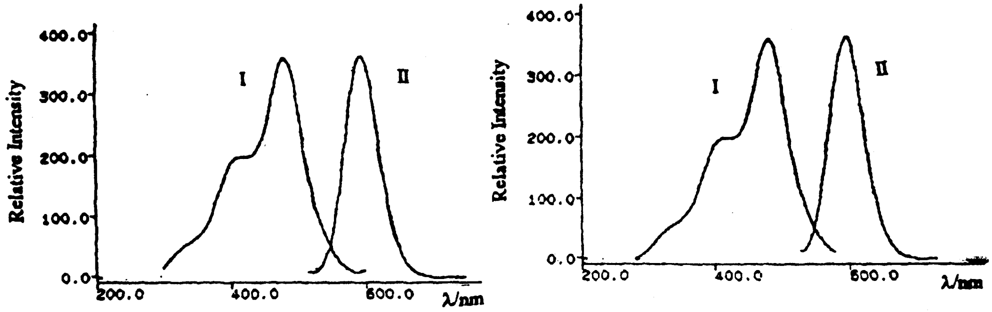


5a **5b**

Fig. 2. Cyclic Volbanmograms of **5a** and **5b**

both undergo a reversible single-electron oxidation when the potential scans from 1.0 to 1.5 V.

Fluorescence adsorption and emission: Fluorescence emission spectra of 1 mmol/L **5a** or **5b** in ethanol at room temperature are shown in Fig. 3. These intense adsorption bands are due to the MLCT transition where an electron is promoted from the metal centered t_{2g} orbital into a ligand centered π^* orbital. These intense emissions occur at lower energy than does the ligand centered $\pi^* \rightarrow \pi$ phosphorescence; here there is a significant contribution to the excited state from an interaction between the metal d orbitals and the ligand π system.[14] Many studies have indicated that the ECL spectrum of each Ru(II) complex is very similar to its fluorescence spectrum; so the fluorescence spectra of 5 hinted ECL spectra[15] of 5. We also noticed that the maximal emissio npeak of 5a or 5b is at the wavelength of 500 nm, which is different from that of Ru(bpy)$_3^{2+}$-based probe. This has the advantage that there would be very good signal separation in the event these two types of probes are employed together.



a

b

Fig. 3. Fluorescence spectra of **5a** and **5b**: I. adsorption spectrum; II. emission spectrum

Electrochemiluminescence: ECL intensity of **5a** is positively relative to its concentrations from 10^{-3} to 10^{-8} mol/L in 0.01 mol/L phosphate buffer aqueous solution containing (pH = 6.7) 0.5 mol/L *n*-tripropylamine when a 1.28 V voltage was applied to the Au working electrode, so is **5b**.

As expected from these results, $Ru(phen)_2(phen\text{-}NHCOCH_2CH_2COOH)(PF_6)_2$ and $Ru(phen)_2(phen\text{-}NHCOCH_2CH_2CH_2COOH)(PF_6)_2$ both behave as good probes for electrochemiluminescent immunoassay, and thus the further experiments are under way to detect some important biochemical substances using these probes.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Science and Technology of China for the financial support of this key project (GR/96-A23-03-01).

REFERENCES

1. D.R. Deaver, *Nature*, **377**, 758 (1995).
2. J.H. Kenten, J. Casadel and S.L. Lupold, *Clin. Chem.*, **37**, 1626 (1991).
3. L.J. Obenauer-Kutner, S.J. Jacobs and K. Kolz, *J. Immunol. Methods*, **206**, 25 (1997).
4. Y.T. Ma, T. Hsieh and M.E. Forbes, *J. Neurosci.*, **18**, 2097 (1998).
5. W.Y. Lee, *Microchim. Acta*, **127**, 19 (1997).
6. L.J. Kricka, *J. Bolumin. Chemilumin.*, **13**, 189 (1998).
7. G.C. Fiaccabrino, M. Koudelka-Hep and Y.T. Hsueh, *Anal. Chem.*, **70**, 4157 (1998).
8. H.J. Yang and S.R. Gudibande, *PCT WO* 96/35697, 20 (1996).
9. G.F. Smith and F.W.C. Jr., *J. Org. Chem.*, **12**, 781 (1947).
10. J.P. Lecomte and A.K.D. Mesmaeker, *J. Chem. Soc. Farady Trans.*, **89**, 3261 (1993).
11. B.P. Sullivan, D.J. Salmon and T.J. Meyer, *Inorg. Chem.*, **17**, 3344 (1978).
12. F. Bodar-Houillon, T. Humbert and A. Marsura, *Inorg. Chem.*, **34**, 5205 (1995).
13. M. Przybylski and M.O. Glocker, *Ang. Chem. Int. Ed. Engl.*, **35**, 807 (1996).
14. D.M. Roundhill, Photochemistry and Photophysics of Metal Complexes, Plenum Press, New York, p. 167 (1993).
15. J.G. Velasco, I. Rubinstein, R.J. Crutchley, A.B.P. Lever and A.J. Bard, *Inorg. Chem.*, **22**, 832 (1983).

(*Received:* 24 January 1999; *Accepted:* 2 July 1999) AJC-1767