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Comparative Study of Ruthenium(II) Tris(bipyridine) Derivatives for Electrochemiluminescence Application

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Several $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine and its derivatives) complexes were synthesized and compared electrochemically and spectroscopically in the search for better luminophores for electrochemiluminescence (ECL)-based analytical applications. ECL measurement in $[Ru(bpy)_3]^{2+}$/tripropylamine (TPA) aqueous buffer solutions has led to a conclusion that due to the complexity of the ECL generation process, the photoluminescence efficiency cannot be used to predict ECL intensity and there is no obvious relationship between the photoluminescence quantum yield and the ECL intensity. Under the present experimental condition, when compared with the pristine $[Ru(bpy)_3]^{2+}$, the ethoxycarbonyl-substituted derivative, $[Ru(bpy\text{-}COOEt)_3]^{2+}$, one of the most efficient luminophores under photoexcitation, did not generate reasonably intense ECL, whereas luminophores with lower photoluminescence quantum yields demonstrated higher ECL. These findings are useful for further efforts in the search for more efficient ECL luminophores.

Electrochemiluminescence or electrogenerated chemiluminescence (ECL)[1] of $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine and its derivatives[2]) complexes has applications in chemical and biochemical analysis.[3] For chemical analysis, the analytes are quantified based on either the emission intensity or the quenching of the emission in sample solutions that contain both the analytes and the ECL luminophores. For affinity-based bioanalytical assays, such as immunoassay and DNA probing in the well-developed commercial systems,[4−7] the analytes are captured by anti-analyte immobilized microbeads and the ECL of the Ru label is generated heterogeneously at the interface of the microbeads magnetically trapped on a working electrode. While the methodologies have been established based on the commercially available $Ru(bpy)_3Cl_2$ and Ru label for chemical analysis and bioanalytical assays, there is constant interest in searching for new ECL luminophores to reach higher performance of the ECL-based technologies. Since the syntheses of 2,2′-bipyridine derivatives have been studied extensively and $[Ru(bpy)_3]^{2+}$ represents a huge family,[8,9] there is ample room for searching for new luminophores and new labels with improved ECL emission properties, particularly the emission intensity. However, instead of exploring the rich chemistry of 2,2′-bipyridine derivation, significant efforts have focused on

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(1) (a) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons. Inc.: New York, 2000; pp 736−745. (b) Tokel, N. E.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 2862−2863.
(2) Throughout the paper, the formula $[Ru(bpy)_3]^{2+}$ unmistakably represents either the pristine ruthenium(II) tris(bipyridine) complex or, in general, any other analogues with 2,2′-bipyridine derivative(s) as ligand(s). The three ligands in $[Ru(bpy)_3]^{2+}$ i.e., bpy, can be the same or different when used in its general sense.
(3) For recent reviews of analytical applications, see, for example: (a) Fähnrich, K. A.; Pravda, M.; Guibault, G. G. *Talanta* **2001**, *54*, 531−559. (b) Kulmala, S.; Suomi, J. *Anal. Chim. Acta* **2003**, *500*, 21−69. (c) Richter, M. M. *Chem. Rev.* **2004**, 104, 3003−3036. (d) *Electrogenerated Chemiluminescence*; Bard, A. J., Ed.; Marcel Dekker: New York, 2004.
(4) (a) Blackburn, G. F.; Shah, H. P.; Kenten, J. H.; Leland, J.; Kamin, R. A.; Link, J.; Peterman, J.; Powell, M. J.; Shah, A.; Talley, D. B.; Tyagi, S. K.; Wilkins, E.; Wu, T.-G.; Massey, R. J. *Clin. Chem.* **1991**, *37*, 1534−1539. (b) Kenten, J. H.; Casadei, J.; Link, J.; Lupold, S.; Willey, J.; Powell, M.; Rees, A.; Massey, R. J. *Clin. Chem.* **1991**, *37*, 1626−1632.
(5) Deaver, D. R. *Nature* **1995**, *377*, 758−760.
(6) (a) Luppa, P. B.; Reutemann, S.; Huber, U.; Hoermann, R.; Poertl, S.; Kraiss, S.; von Bülow, S.; Neumeier, D. *Clin. Chem. Lab. Med.* **1998**, *36*, 789−796. (b) Erler, K. *Wien. Klin. Wochenschr*. **1998**, *110*, 5−10.
(7) Namba, Y.; Usami, M.; Suzuki, O. *Anal. Sci.* **1999**, *15*, 1087−1093.
(8) Meyer, T. J. *Pure Appl. Chem.* **1986**, *58*, 1193−1206.
(9) (a) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belser, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85−277. (b) Balzani, V.; Juris, A.; Venturi, M. *Chem. Rev.* **1996**, *96*, 759−833.

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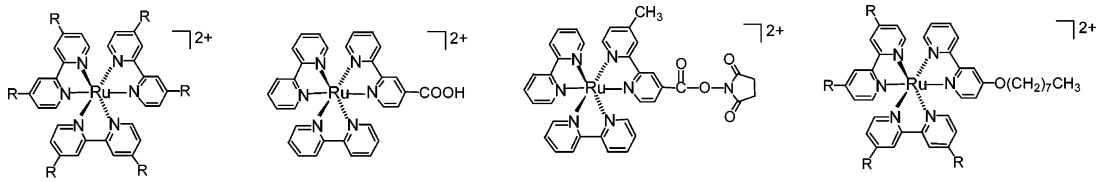
Scheme 1. Ruthenium Complexes Used in This Work



1 (R = H)
2 (R = COOEt)
3 (R = COOH)

4

5

6 (R = H)
7 (R = COOH)

Counter ion (Cl^- or PF_6^-) is indicated in the experiments when it is necessary.

other metal complexes, such as those of europium,[10a] rhenium,[10b] copper,[10c] osmium,[10d] aluminum,[11] terbium,[12] iridium,[13] platinum,[13b] etc., which have been found to be luminescent under electrochemical excitation. Despite the fact that a very large number of $[Ru(bpy)_3]^{2+}$ derivatives have been synthesized and investigated by electrochemical, photochemical, and photophysical methods, there is a relatively limited number of $[Ru(bpy)_3]^{2+}$ derivatives and other ruthenium *N*-*N* chelating complexes that have been tested for their ECL[14−19] or synthesized for labeling biomolecules.[4a,19−22] In his recent review, Richter[3c] has compiled a list of ECL luminophores and solution systems, in which the few $[Ru(bpy)_3]^{2+}$ luminophores studied for ECL contrasts considerably with the huge number of $[Ru(bpy)_3]^{2+}$ derivatives so far synthesized. Except for $(bpy)_2Ru(DC\text{-}bpy)^{2+}$ and $(bpy)_2Ru(DM\text{-}bpy)^{2+}$ (DC = 4,4′-dicarboxy-2,2′-bipyridine and DM = 4,4′-dimethyl-2,2′-bipyridine),[15] to the best of our knowledge, there has not been an intensive study on the ECL comparison of a larger number of $[Ru(bpy)_3]^{2+}$ luminophores in bioanalytically important buffer systems.

Three different approaches, i.e., annihilation,[1b] oxidative-reduction,[23] and reductive-oxidation,[24] were used to generate the luminescent excited state $[Ru(bpy)_3]^{2+*}$, i.e., the metal-to-ligand charge transfer (MLCT) state. A significant development for the practical applications is ECL with the participation of amines in the oxidative-reduction approach.[25] Particularly, the use of tripropylamine (TPA) as a co-reactant for the ECL generation of $[Ru(bpy)_3]^{2+}$ in buffers of physiological pH values made the ECL phenomenon a highly sensitive bioanalytical technology widely used in basic research laboratories, pharmaceutical industry, clinical settings, and homeland security.[3−7,26−28] Enhancement of the ECL signal level has continued to be a subject of interest in recent years. On one hand, efforts have been made in the syntheses and demonstration of labels bearing multiple $[Ru(bpy)_3]^{2+}$ units.[18,19,29] On the other hand, because the large family of $[Ru(bpy)_3]^{2+}$ was far from being exhausted, it is our objective to find $[Ru(bpy)_3]^{2+}$ luminophores with stronger ECL emission under the practical conditions. Here we report a comparative study of ECL from various $[Ru(bpy)_3]^{2+}$ complexes (shown in Scheme 1) with one or more *mono*-/*bi*-substituents at 4- and/or 4′-position(s) of bipyridine ring(s) in TPA-containing aqueous buffer solutions.

Experimental Section

Chemicals. For synthesis, reagent grade solvents and reactants were used as received unless otherwise specified. *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$ was prepared according to the procedure of Sullivan, et al.[30] 4,4′-Dimethyl-2,2′-bipyridine was prepared in the manner described in the literature.[31,32] 4-Carboxy-2,2′-bipyridine was received from EIC Laboratories, Inc. NH_4PF_6 (99.99%, Aldrich) was used to

(10) (a) Richter, M. M.; Bard, A. J. *Anal. Chem.* **1996**, *68*, 2641−2650. (b) Richter, M. M.; Debad, J. D.; Striplin, D. R.; Crosby, G. A.; Bard, A. J. *Anal. Chem.* **1996**, *68*, 4370−4376. (c) McCall, J.; Bruce, D.; Workman, S.; Cole, C.; Richter, M. M. *Anal. Chem.* **2001**, *73*, 4617−4620. (d) Bruce, D.; Richter, M. M.; Brewer, K. J. *Anal. Chem.* **2002**, *74*, 3157−3159.

(11) (a) Anderson, J. D.; McDonald, E. M.; Lee, P. A.; Anderson, M. L.; Ritchie, E. L.; Hall, H. K.; Hopkins, T.; Mash, E. A.; Wang, J.; Padias, A.; Thayumanavan, S.; Barlow, S.; Marder, S. R.; Jabbour, G. E.; Shaheen, S.; Kippelen, B.; Peyghambarian, N.; Wightman, R. W.; Armstrong, N. R. *J. Am. Chem. Soc.* **1998**, *120*, 9646−9655. (b) Gross, E. M.; Anderson, J. D.; Slaterbeck, A. F.; Thayumanavan, S.; Barlow, S.; Zhang, Y.; Marder, S. R.; Hall, H. K.; Nabor, M. F.; Wang, J.-F.; Mash, E. A.; Armstrong, N. R.; Wightman, R. W. *J. Am. Chem. Soc.* **2000**, *122*, 4972−4979.

(12) Kulmala, S.; Håkansson, M.; Spehar, A.-M.; Nyman, A.; Kankare, J.; Loikas, K.; Ala-Kleme, T.; Eskola, J. *Anal. Chim. Acta* **2002**, *458*, 271−280.

(13) (a) Bruce, D.; Richter, M. M. *Anal. Chem.* **2002**, *74*, 1340−1342. (b) Gross, E. M.; Armstrong, N. R.; Wightman, R. M. *J. Electrochem. Soc.* **2002**, *149*, E137−E142. (c) Kapturkiewicz, A.; Angulo, G. *Dalton Trans.* **2003**, 3907−3913. (d) Kim, J. I.; Shin, I.-S.; Kim, H.; Lee, J.-K. *J. Am. Chem. Soc.* **2005**, *127*, 1614−1615.

(14) (a) Tokel-Takvoryan, N. E.; Hemingway, R. E.; Bard, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 6582−6589. (b) Richter, M. M.; Bard, A. J.; Kim, W.; Schmehl, R. H. *Anal. Chem.* **1998**, *70*, 310−318. (c) Lai, R. Y.; Chiba, M.; Kitamura, N.; Bard, A. J. *Anal. Chem.* **2002**, *74*, 551−553.

(15) Bruce, D.; McCall, J.; Richter, M. M. *Analyst* **2002**, *127*, 125−128.

(16) Li, F.; Pang, Y.-Q.; Lin, X.-Q.; Cui, H. *Talanta* **2002**, *59*, 627−636.

(17) Wang, P.; Zhu, G. *Luminescence* **2000**, *15*, 261−265.

(18) Zhou, M.; Roovers, J. *Macromolecules* **2001**, *34*, 244−252.

(19) Zhou, M.; Roovers, J. Robertson, J.; P.; Grover, C. P. *Anal. Chem.* **2003**, *75*, 6708−6717.

(20) Terpetschnig, E.; Szmacinski, H.; Malak, H.; Lakowicz, J. R. *Biophys. J.* **1995**, *68*, 342−350.

(21) Peek, B. M.; Ross, G. T.; Edwards, S. W.; Meyer, G. J.; Meyer, T. J.; Erickson, B. W. *Int. J. Pept. Protein Res.* **1991**, *38*, 114−123.

(22) Kenten, J. H.; Gudibande, S,; Link, J.; Willey, J. J.; Curfman, B.; Major, E. O.; Massey, R. J. *Clin. Chem.* **1992**, *38*, 873−879.

(23) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 512−516.

(24) White, H. S.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 6891−6895.

(25) Leland, J. K.; Powell, M. J. *J. Electrochem. Soc.* **1990**, *137*, 3127−3131.

(26) Jameison, F.; Sanchez, R. I.; Dong, L.; Leland, J. K.; Yost, D.; Martin, M. T. *Anal. Chem.* **1996**, *68*, 1298−1302.

(27) Bruno, J. G.; Kiel, J. L. *Biosens. & Bioelectron.* **1999**, *14*, 457−464.

(28) Yu, H.; Raymonda, J. W.; McMahon, T. M.; Campagnari, A. A. *Biosens. & Bioelectron.* **2000**, *14*, 829−840.

(29) Staffilani, M.; Höss, E.; Giesen, U.; Schneider, E.; Hartl. F.; Josel, H.-P.; De Cola L. *Inorg. Chem.* **2003**, *42*, 7789−7798.

(30) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334−3341.

(31) Sprintschnik, G.; Sprintschnik, H. W.; Kirsch, P. P.; Whitten, D. *J. Am. Chem. Soc.* **1977**, *99*, 4947−4954.

(32) Ghosh, P.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 5543−5549.

convert the chlorides to hexafluorophosphates. In synthesis procedures where $RuCl_3 \cdot xH_2O$ ($x \leq 1$, Aldrich) was used, the calculations for reagent ratio and yields were based on $x = 1$. For spectral and electrochemical characterization, $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (Aldrich), KOH, acetonitrile (Aldrich), tetrabutylammonium hexafluorophosphate ($TBAPF_6$, Fluka, electrochemical grade), and phosphate buffer (ProCell from Roche Diagnostics, pH = 6.8, TPA 0.18 M, nonionic surfactant added) were used as received.

Synthesis of 4,4′-Dicarboxy-2,2′-bipyridine.[33] To stirred sulfuric acid (95∼98%, 125 mL), 4.9 g of 4,4′-dimethyl-2,2′-bipyridine was added. Then 24 g of potassium dichromate was added slowly in small portions. (**Caution.** The process is highly exothermic!) Occasional cooling with water was required to maintain the temperature of the mixture between 70 and 80 °C during the addition of dichromate. After all the potassium dichromate was added, the reaction mixture was continually stirred until the temperature fell to room temperature. The deep green mixture was poured into 900 mL of ice water and filtered. The resulting light yellow solid was then refluxed in 150 mL of 50% nitric acid for 4 h. The solution was poured into 400 mL of ice water and diluted to 600 mL. The white precipitate was filtered, washed with water (5×60 mL) and acetone (3×40 mL), and allowed to dry yielding 5.49 g (85%) white powder. The product is insoluble in all common solvents.

Synthesis of 4,4′-Bis(ethoxycarbonyl)-2,2′-bipyridine. This compound was prepared by a variation of a published procedure.[34] A solution of 1.35 g (5.53×10^{-3} mol) of 4,4′-dicarboxy-2,2′-bipyridine in a mixture of 15 mL of sulfuric acid (95∼98%) and 32 mL of absolute ethanol was refluxed for 6 h and was then cooled to room temperature and poured into 100 mL of ice water. The mixture was neutralized with 25% aqueous potassium hydroxide solution. The precipitate was collected after filtering, washing, and drying. The slightly gray powder was crystallized four times from absolute ethanol yielding 0.69 g of fine colorless needles. Concentrating the combined mother liquors and repeating the crystallization process afforded a further crop of 0.10 g. The combined yield is 48%. ^{1}H NMR (400 MHz, $CDCl_3$, 7.25 ppm) δ 8.95 (d, 2 H), 8.86 (d, 2 H), 7.91 (dd, 2 H), 4.45 (quartet, 4 H), 1.44 (t, 6 H).

Synthesis of 2-$(PF_6)_2$. We refluxed 0.11 g (4.88×10^{-4} mol) of $RuCl_3 \cdot xH_2O$ ($x \leq 1$) and 0.52 g (1.73×10^{-3} mol) of 4,4′-bis-(ethoxycarbonyl)-2,2′-bipyridine in 20 mL of absolute ethanol for 8 days. After the reaction flask cooled to room temperature, the unreacted bipyridine derivative was filtered out. The solvent was rotoevaporated and the solid was redissolved in 5 mL of deionized water. To the water solution was added NH_4PF_6 saturated aqueous solution. The precipitate was collected and washed with deionized water to afford 520 mg (82.5%) of orange powder. ^{1}H NMR (400 MHz, acetone-d_6, 2.05 ppm) δ 9.36 (d, 2 H), 8.37 (d, 2 H), 7.97 (dd, 2 H), 4.46 (quartet, 4 H), 1.38 (t, 6 H). Anal. Calcd for $C_{48}H_{48}O_{12}N_6RuP_2F_{12}$ (FW = 1291.93): C, 44.62; H, 3.74; N, 6.51. Found: C, 44.75; H, 3.65; N, 6.56.

Synthesis of 3-Cl_2. We refluxed 0.12 g (5.32×10^{-4} mol) of $RuCl_3 \cdot xH_2O$ ($x \leq 1$) and 0.45 g (1.84×10^{-3} mol) of 4,4′-dicarboxy-2,2′-bipyridine in 15 mL of anhydrous DMF for 24 h. After the reaction flask cooled to room temperature, the precipitate was filtered, washed with acetonitrile and dichloromethane, and vacuum-dried yielding 0.45 g (93%) of dark brown product. The product is soluble only in water. ^{1}H NMR (400 MHz, D_2O, 4.82 ppm, NaOH added) δ 8.89 (d, 2 H), 7.89 (d, 2 H), 7.68 (dd, 2 H).

Synthesis of 4. We refluxed 394 mg (7.57×10^{-4} mol) of *cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$ and 155 mg (7.74×10^{-4} mol) of 4-carboxy-2,2′-bipyridine in 15 mL of methanol/water (1:1) overnight. The reaction mixture was filtered and the filtrate was rotoevapaorated. The solid was redissolved in 6 mL of water and was acidified with 4 drops of concentrated hydrochloric acid. The solvent was rotoevaporated again and the solid was vacuum-dried to afford dark brown chloride **4**-Cl_2, which was then converted into hexafluorophosphate using NH_4PF_6 saturated aqueous solution. The vacuumed dried **4**-$(PF_6)_2$ has a yield of 582 mg (85%). ^{1}H NMR (400 MHz, CD_3CN, 1.94 ppm) δ 8.91 (d, 1 H), 8.65 (d, 1 H), 8.50 (d, 4 H), 8.07 (m, 5 H), 7.90 (d, 1 H), 7.79 (dd, 1 H), 7.68−7.76 (m, 5 H), 7.35−7.45 (m, 5 H). Anal. Calcd for $C_{31}H_{28}N_6O_4RuP_2F_{12}$ (FW = 939.59): C, 39.63; H, 3.00; N, 8.94. Found: C, 39.40; H, 3.17; N, 9.15.

Synthesis of Ru(bpy-COOH)$_2$Cl$_2$. We refluxed 140 mg (6.21×10^{-4} mol) of $RuCl_3 \cdot xH_2O$ ($x \leq 1$) and 300 mg (1.23×10^{-3} mol) of 4,4′-dicarboxy-2,2′-bipyridine were refluxed in 10 mL of anhydrous DMF for 6 h. After the reaction flask cooled to room temperature, the purple precipitate and DMF solution were poured into 100 mL of anhydrous acetone, filtered, washed with acetone, and vacuum-dried to afford 360 mg (89%) of dark purple powder.

Synthesis of 7-$(Cl)_2$. We refluxed 21.5 mg (7.56×10^{-5} mol) of 4-octoxy-2,2′-bipyridine and 50.5 mg (7.65×10^{-5} mol) of $Ru(bpy\text{-}COOH)_2Cl_2$ was refluxed in a mixture of 8 mL of methanol and 2 mL of water for 4 h. The solvent was evaporated and the solid was redissolved in 2 mL of methanol. The solution was dropped into dry ether through a 0.2 μm syringe filter. The precipitate was collected and dissolved in 5% KOH water solution. The solution was acidified with hydrochloric acid. The precipitate was filtered out and vacuum-dried to afford 45 mg (63%) of powder. ^{1}H NMR (400 MHz, D_2O, 4.82 ppm, NaOH added) δ 8.87 (d, 4 H), 8.46 (d, 1 H), 8.04 (t, 1 H), 7.86−7.96 (m, 5 H), 7.76 (d, 1 H), 7.64−7.77 (m, 4 H), 7.47 (d, 1 H), 7.38 (t, 1 H), 6.85 (dd, 1 H), 4.06 (t, 2 H), 1.47 (b, 2 H), 1.04 (b, 2 H), 0.7−0.9 (m, 8 H), 0.55 (t, 3 H). Anal. Calcd for $C_{42}H_{40}N_6O_9RuCl_2$ (FW = 944.79): C, 53.39; H, 4.27; N, 8.90. Found: C, 52.14; H, 4.42; N, 8.81.

Characterization. NMR spectra were recorded on a Varian Unity Inova spectrometer at a resonance frequency of 399.961 MHz for ^{1}H and 100.579 MHz for ^{13}C. 1D and 2D NMR spectra were obtained using a 5 mm indirect detection probe equipped with pulsed field gradients. A 5 mm broadband probe was used for acquiring the 1D ^{13}C NMR (^{1}H noise decoupled). For each analysis, the deuterated solvent signals were used as the reference except for the ^{13}C NMR spectrum where 2,2-dimethyl-2-silapentane-5-sulfonate-d_6 sodium salt (DSS) was used as the internal reference (0 ppm). Elemental analyses were conducted with LECO's CHNS-932 instrument.

UV−vis spectra were recorded on a Hewlett-Packard 8453 spectrophotometer. Emission spectra were obtained under 460 nm excitation with a FLUOROLOG spectrofluorometer. Both absorption and emission measurements were carried out in deaerated acetonitrile (30 min of argon bubbling) and buffer solutions at room temperature in a quartz cell with 1 cm optical path. The emission quantum yields, ϕ_R, were measured at 20 °C according to the equation (for buffer solutions, no refractive index correction was made)[35]

(33) (a) Kocian, O.; Mortimer, R. J.; Beer, P. D. *Tetrahedron Lett.* **1990**, *31*, 5069−5072. (b) Oki, A. R.; Morgan, R. J. *Synth. Commun.* **1995**, *25*, 4093−4097.

(34) (a) Maerker, G.; Case, F. H. *J. Am. Chem. Soc.* **1958**, *80*, 2745−2748. (b) Ciana, L. D.; Gressick, W. J.; von Zelewsky, A. *J. Heterocycl. Chem.* **1990**, *27*, 163−165.

(35) (a) Strouse, G. F.; Schoonover, J. R.; Duesing, R.; Boyde, S.; Jones, W. E.; Meyer, T. J. *Inorg. Chem.* **1995**, *34*, 473−487. (b) Casper, J. V.; Meyer, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 5583−5590.

$$\phi_R = \phi_{ref} (I_s A_{ref} / I_{ref} A_s)$$

where I is the emission intensity calculated from the area under the emission spectrum from 500 to 800 nm, A is the absorbance, and the subscripts s and ref stand for the samples and reference, respectively. An acetonitrile solution of $[Ru(bpy)_3](PF_6)_2$ was used as a standard with $\phi_{ref} = 0.062$.[35]

Cyclic voltammetry was performed in a three-electrode configuration using a Pt disk (diameter 1 mm, area 0.785 mm^2) sealed in a soft glass rod as the working electrode. It was polished with diamond polishing paste (0.25 μm), rinsed thoroughly with water and acetonitrile, and dried by a warm air flow. For measurement in buffer solutions, Pt wires and Ag/AgCl (in 3 M NaCl, from Applied Biosystem) were used as counter and reference electrodes, respectively, while for measurement in anhydrous acetonitrile, Pt and Ag wires were used as counter and quasi-reference electrodes, respectively. The electrochemical cell preparation was described previously.[18] Potentials versus the Ag quasi-reference electrode were then calibrated with the ferrocene/ferrocenium (Fc/Fc^+) redox couple by taking $E°_{Fc/Fc+} = 0.35$ V vs Ag/AgCl. A PARSTAT 2263-2 Advanced Electrochemical System with PowerSUITE software was used for electrochemical measurement and control.

For ECL measurement, a platinum foil as a working electrode with an effective area of 28.3 mm^2 was placed in the center of the bottom of a Teflon electrochemical cell. A coiled platinum wire (total surface 157 mm^2) and Ag/AgCl (in 3 M NaCl, from Applied Biosystem) were used as counter and reference electrodes, respectively. A Hamamatsu photomultiplier tube (PMT, H7468-20) module with a spectral response range of 300−900 nm served as a photodetector, which was vertically directed toward the ECL working electrode and interfaced with a computer via a homemade LabView-based data acquisition program. A potential step technique was employed to oxidize ruthenium complexes and TPA in order to generate ECL through the oxidative-reduction mechanism.[25] Before each measurement, the working electrode was cleaned by a procedure involving repeated potential steps at 2.0 V for 4 s, 0.0 V for 0.5 s and −1.2 V for 2 s in a KOH (1 wt %) water solution, followed by a conditioning procedure of twice-repeated potential steps at 1.0 V for 1 s, −1.2 V for 1 s, and 0.0 V for 1 s in the phosphate buffer solution.

Results and Discussion

Synthesis. Among the ruthenium complexes studied in this work, **1** and **5** are commercially available. The synthesis and photophysical characterization of **2**,[36] **3**,[37a] **4**,[37b] and **6**[18] have been reported before. Only **7** is a new compound, which was synthesized following well-established methods as described in the Experimental Section. ^1H NMR spectra confirmed the structures of all the compounds synthesized in this work. It should be mentioned that the chloride of **3** and **7** are insoluble in common solvents but slightly soluble in water and very soluble in alkaline water, so the NMR spectra were obtained in D_2O with added NaOH. Compound **4**, which was intended to be a potential luminescent label with the smallest molecular weight among the $[Ru(bpy)_3]^{2+}$ family, was further

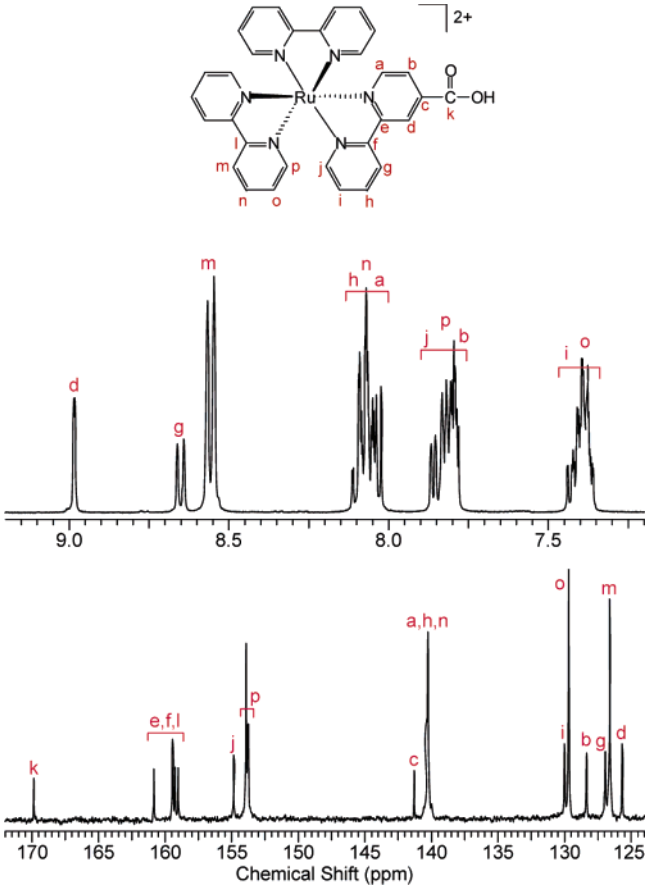


Figure 1. ^1H and ^{13}C NMR spectra of **4**-Cl_2 in alkaline D_2O (4.82 ppm). Chemical shift displacements of ^{13}C signals were referenced to the internal standard DSS.

structurally confirmed by the carboxy signal at 169.87 ppm in ^{13}C NMR spectrum as shown in Figure 1. The detailed assignment can be found in the Supporting Information.

Absorption and Emission. The electronic absorption spectra of **1**, **2**, **4**, **5**, and **6** in acetonitrile are compared in Figure 2. Due to the compound's insolubility in common organic solvents, the absorption spectra of **3** and **7** were obtained in aqueous buffer. For compounds **1** and **2**, the absorption spectra in both solvents were recorded and were found to be the same within the experimental error (see Table 1). The following observation regarding the absorption should be mentioned.

First, all compounds show a strong ligand centered $\pi \rightarrow \pi^*$ transition in the UV range and a MLCT $d \rightarrow \pi^*$ transition in the visible range. Second, for **1**, **4**, **5**, and **6**, the $\pi \rightarrow \pi^*$ transition is located at 286−288 nm, indicating the influence of the substitution with $-CH_3$, −COOH, or −OR at 4-position of a single bipyridine ring is very small. However, 4,4′-di-substitution with carboxy or carboxylate groups of more than one bipyridine rings shifts the ligand centered $\pi \rightarrow \pi^*$ transition to 303−308 nm, as can be seen from the absorbance of **2**, **3**, and **7**. For **7**, an interesting shoulder at ∼288 nm indicates the nature of the ligands with different $\pi \rightarrow \pi^*$ transition. Third, the substitution has a similar effect on the extinction coefficients of the MLCT transition, i.e., the monosubstitution at 4-position as in the cases of **4**, **5**, and **6** does not change the extinction coefficients of MLCT

(36) (a) Elliott, C. M.; Hershenhart, E. J. *J. Am. Chem. Soc.* **1982**, *104*, 7519−7526. (b) Wacholtz, W. F.; Auerbach, R. A.; Schmehl, R. H. *Inorg. Chem.* **1986**, *25*, 227−234.

(37) (a) Kalyanasundaram, K.; Nazeeruddin, Md. K.; Grätzel, M.; Viscardi, G.; Savarino, P.; Barni, E. *Inorg. Chim. Acta* **1992**, *198*−*200*, 831−839. (b) Patterson, B. T.; Keene, F. R. *Aust. J. Chem.* **1998**, *51*, 999−1002.

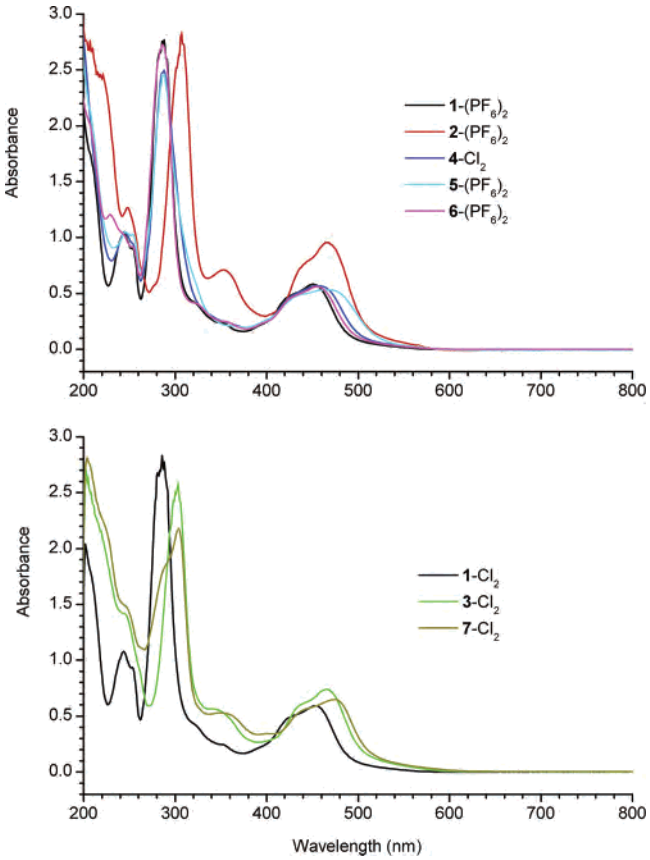


Figure 2. Absorption spectra of ruthenium complexes (40 μM) in acetonitrile (above) and aqueous buffer (pH 6.8) solutions (below).

maxima very much, in contrast to the cases of **2**, **3**, and **7**. Fourth, the MLCT absorption maxima of all the compounds with substituted bipyridine ligand(s) are red-shifted from 451 nm of the pristine $[Ru(bpy)_3]^{2+}$ to longer wavelengths.

While the absorption maxima of d→π* transition for the seven compounds ranges from 451 to 474 nm (Table 1), the emission spectra were obtained with a single excitation light at 460 nm. This choice is a compromise to enable the luminophores to be compared under identical conditions. Figure 3 demonstrates the emission spectra of **1**−**7** in both buffer and acetonitrile solutions. In comparison with the spectrum of **1**, the spectra of all luminophores studied here showed a red shift, which corresponds with the red shift observed in their absorption spectra.

Unlike the absorption spectra, the emission spectra of these compounds show bigger variation in both intensity and peak position. In Table 1, the emission maxima in acetonitrile and buffer solutions are presented, and the relative intensities compared. The emission intensity, *I*, was taken as the total recorded number of photons from 500 to 800 nm. In acetonitrile, all the compounds, except **2**, show weaker emission than **1**. However, in the buffer solutions, both **2** and **3** show a more-intense emission than **1**, whereas the emission of **5** is the same as **1**. Interestingly, contrary to the perception that the emission of $[Ru(bpy)_3]^{2+}$ in aqueous solutions was less efficient than in acetonitrile,[38] we found the fluorescent intensities of all the compounds, except **2**, were approximately doubled in the buffer solutions. For **2**, the emission intensity is even slightly reduced in the buffer solution in comparison with the emission in acetonitrile. It is possible that due to the anodically shifted potentials for all its redox states (see the following section),[36] the reductive quenching[39a] of $[Ru(bpy)_3]^{2+}$* to $[Ru(bpy)_3]^{+}$ by water is more likely to happen for **2** than for any other compounds. It should also be pointed out that between **2** and **3**, the fluorescent intensity, relative to each other, varies greatly in acetonitrile and buffer solutions. We believe the hydrolysis equilibrium of the ethoxycarbonyl groups of **2**, and its MLCT state, in the buffer solution is the possible reason. It should be noted that the buffer solutions used in this work has a pH value of 6.8. As demonstrated previously, the photophysical properties of carboxylic acid derivatives of $[Ru(bpy)_3]^{2+}$ are dependent on the pH value of solution.[39b]

Based on the extinction coefficients at 460 nm and the emission intensities, we have calculated the quantum yields using **1** as a standard (0.062, in acetonitrile). As can be seen from Table 1, the quantum yields vary greatly in acetonitrile (from 0.028 for **7** to 0.145 for **2**, whereas in the buffer solutions they change from 0.092 for **7** to the highest value of 0.15 for **3**.

Electrochemistry. Cyclic voltammetric measurements were first conducted in the buffer solutions to provide basic information on the subsequent ECL investigation. Because of the electrochemical oxidation of water, a relatively high concentration (5 mM) of $[Ru(bpy)_3]^{2+}$ was chosen to show the $[Ru(bpy)_3]^{2+/3+}$ wave imposed on the water oxidation wave. However, due to the very limited solubility of **2** in the buffer of pH = 6.8, we were unable to prepare a solution for the voltammetric study of **2**.

The voltammograms of compounds **1**, **3**, **4**, **6**, and **7** in the buffer solutions at room temperature are presented in Figure 4. As can be seen from the oxidation wave of $[Ru(bpy)_3]^{2+} \rightarrow [Ru(bpy)_3]^{3+}$, the E_{pa} of compounds **1**, **4**, **6**, and **7** are very close to each other ranging from 1.14 V for **6** to1.24 V for **4**. It is interesting to note that compound **3** does not show a perceptible oxidation wave up to the potential of 1.8 V. The cyclic voltammogram of **3** is very much the same as that of the buffer itself. Elliott et al.[36a] and Schmehl et al.[36b] have reported $E_{1/2}$ of compound **2** in acetonitrile; their consistent results indicated significant anodic shifts for all the redox states of **2** in comparison with **1**. For $\mathbf{2}^{2+/3+}$ redox process in acetonitrile, the $E_{1/2}$ was found to be 0.3 V higher than that of $\mathbf{1}^{2+/3+}$, i.e., 1.54 V vs SCE. It is possible that compound **3** undergoes an oxidation at an even higher potential. It is also understandable that the redox potentials for **3** in aqueous buffer solutions are dependent on the pH value because of the six carboxy groups. Furthermore, due to the hydrolysis equilibrium of the ethylcarboxy group of **2**, it is also likely that **2** and **3** have more similarities in terms of the redox potentials in aqueous buffer, as they do in terms of the emission properties.

(38) The perception may be based on the quantum yields measured in acetonitrile (0.062) and pure water (0.042) at 25 °C. See ref 35.

(39) (a) Creutz, C.; Sutin, N. *Inorg. Chem.* **1976**, *15*, 496−499. (b) Lay, P. A.; Sasse, W. H. F. *Inorg. Chem.* **1984**, *23*, 4123−4125.

Table 1. Spectroscopic Data in Acetonitrile and Aqueous Buffer (pH 6.8) Solutions at 293 K

	absorption, ϵ (10^4 M^{-1} cm^{-1})		emission (460 nm excitation)			
compd./solvent	($\pi\rightarrow\pi^*$), (d$\rightarrow\pi^*$) @ λ (nm)	@ 460 nm	λ_{max} (nm)	I_s/I_{ref} (MeCN)	I_s/I_{ref} (buffer)	ϕ_R
1-$(PF_6)_2$/MeCN	6.93 @ 288, 1.46 @ 451	1.34	606	1.0		0.062[c]
1-Cl_2/buffer	7.08 @ 288, 1.48 @ 453	1.41	607		1.0	0.125
2 -$(PF_6)_2$/MeCN	7.08 @ 307, 2.39 @ 467	2.30	626	4.02		0.145
2-$(PF_6)_2$/buffer[a]	7.14 @ 308, 2.47 @ 469	2.32	632		1.48	0.112
3-Cl_2/MeCN[a]			630	0.80		0.037[b]
3-Cl_2/buffer	6.45 @ 303, 1.85 @ 466	1.80	622		1.54	0.150
4-Cl_2/MeCN	6.24 @ 288, 1.42 @ 456	1.41	624	0.80		0.047
4-Cl_2/buffer			627		0.87	0.109[b]
5-$(PF_6)_2$/MeCN	6.18 @ 288, 1.33 @ 469	1.32	620	0.51		0.032
5-$(PF_6)_2$/buffer			624		1.01	0.135[b]
6-$(PF_6)_2$/MeCN	6.82 @ 286, 1.40 @ 455	1.37	617	0.77		0.047
6-Cl_2/buffer			619		0.85	0.109[b]
7-Cl_2/MeCN[a]			667	0.51		0.028[b]
7-Cl_2/Buffer	5.56 @ 303, 1.66 @ 474	1.53	637, 660		0.80	0.092

[a] **2**-$(PF_6)_2$ is only slightly soluble in the buffer. **3**-Cl_2 and **7**-Cl_2 are not soluble in MeCN. The MeCN solutions were prepared by diluting the concentrated buffer solutions with MeCN. Therefore, the MeCN solutions for **3**-Cl_2 and **7**-Cl_2 were not anhydrous. [b] The data were obtained by assuming the same absorbance at 460 nm in MeCN and in buffer. [c] See ref 35.

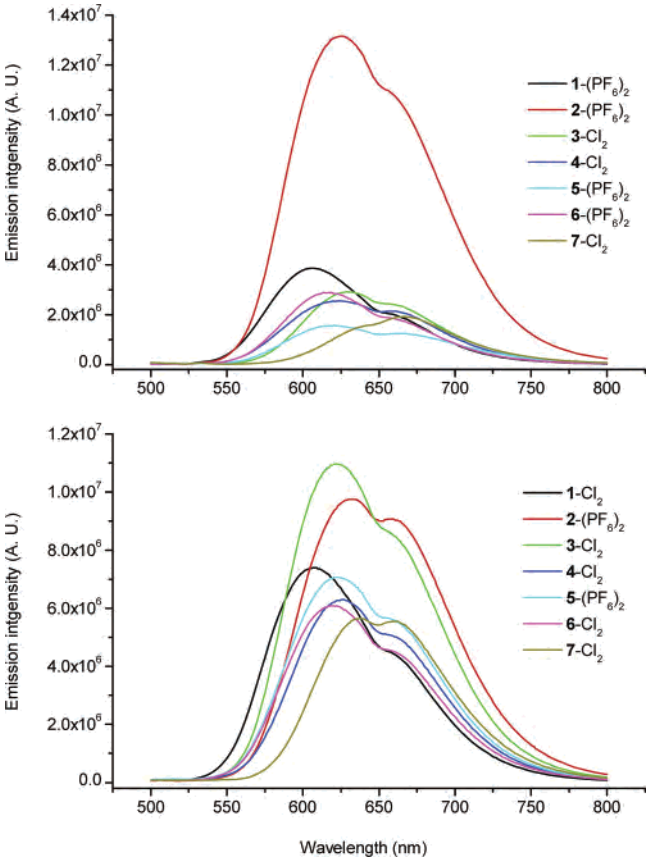


Figure 3. Emission spectra of ruthenium complexes (10 μM) in acetonitrile (above) and aqueous buffer (below, pH 6.8).

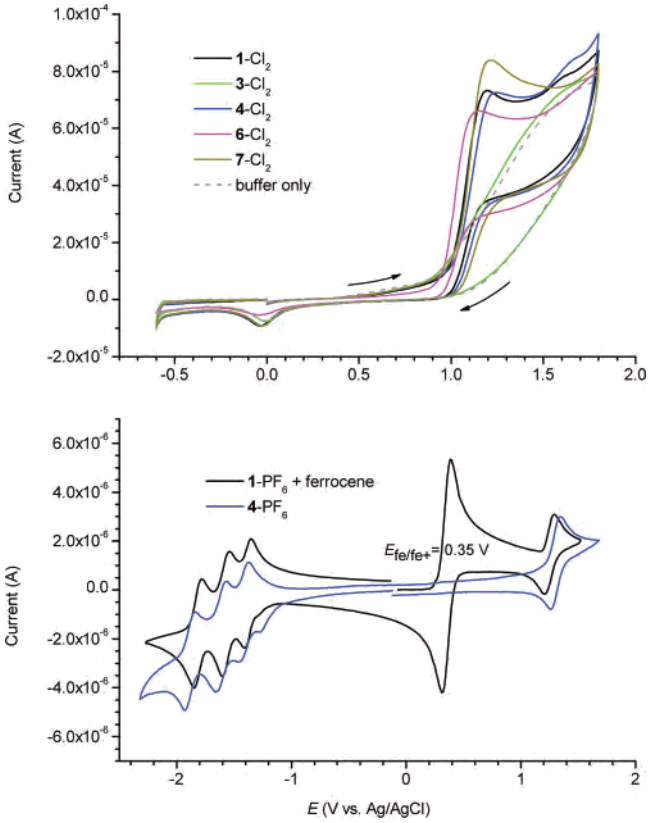


Figure 4. Cyclic voltammograms of ruthenium complexes in buffer (5 mM Ru complexes, pH = 6.8) (above) and in acetonitrile (1 mM Ru complexes, 0.1 M $TBAPF_6$) (below) solutions. Pt electrode 0.785 mm^2, scan rate 100 mV s^{-1}.

As for the compound **5**, due to the limited amount received, we were unable to carry out the voltammetric experiments using high concentration solutions. However, based on its similarity[40] to **4** and extensive literature information,[9] we can assume the $[Ru(bpy)_3]^{2+/3+}$ redox process for **5** occurs at a potential close to that demonstrated for **1**, **4**, **6**, and **7** in Figure 4. This allows us to apply the same potential when comparing the ECL of these compounds in the potential step experiments.

Regarding the voltammograms in the buffer solutions, one may reasonably raise questions as to where the oxidation wave of TPA is and whether the observed waves are from the oxidation of TPA, rather than $[Ru(bpy)_3]^{2+}$. It is our findings that the TPA oxidation wave could not be seen in the present buffer solution at pH 6.8. However, similar to those halide ions enhanced oxidation waves,[41] we did observe

(40) Due to hydrolysis, the *N*-hydroxysuccinimide ester has a half-life on the order of hours in the buffer of pH 6.8. So in buffer, **5** actually existed in carboxylic form after the solution was prepared and stored for more than 1 day.

(41) Zu, Y.; Bard, A. J. *Anal.Chem.* **2000**, *72*, 3223−3232.

a very strong irreversible TPA oxidation wave with a peak potential at 0.75−.80 V in PBS buffers (pH 7.4 or 9.0), which were used in our previous work and, upon adding $[Ru(bpy)_3]^{2+}$, a superimposed peak from $[Ru(bpy)_3]^{2+/3+}$ process. There is no doubt that the observed oxidation waves with peak potentials ranging from 1.14 V to1.24 V are from the oxidation of $[Ru(bpy)_3]^{2+}$, although the peak currents are not exactly the same, possibly due to the complication induced by the adsorption[42] of nonionic surfactant on the platinum electrode.

In addition to the above results obtained in the buffer solutions, we provide a cyclic voltammogram of **4** in comparison with **1** in acetonitrile. Unsurprisingly, compound **4** demonstrates redox behavior that is very similar to that of the pristine **1**, except for a small irreversible reduction potential at about −1.27 V. In view of the existence of the carboxy group, it is likely that the irreversible wave is linked to the reduction of the −COOH or H^+. It should be noted that, for **4**, the metal-centered redox potential is more positive than that of **1**, whereas the three ligand-centered redox potentials are more negative than those of **1**.[18,19] The $E_{1/2}$ values of these redox processes are 1.29 V (ΔE_p= 0.087 V), −1.42 V (ΔE_p= 0.080 V), −1.62 V (ΔE_p= 0.086 V), and −1.89 V (ΔE_p= 0.088 V), respectively.

Electrochemiluminescence. ECL can be generated by applying different electrochemical excitation in different systems. For the oxidative-reduction type involving TPA as coreactant in aqueous buffers, both potentiodynamic[15,41,43,44] and poteniostatic[6b,18,19,29] methods were used to characterize the ECL. To provide comparative data that is of particular interest to the practical bioanalytical use, we evaluated the ECL intensity using a procedure that is similar to what is described in a commercial immunoassay system.[6b] After the electrode underwent a cleaning and conditioning procedure, the potential steps were applied to generate ECL and the emission intensities were recorded as a function of time.

It is well recognized that a positive potential causes the formation of an oxide layer on Pt electrodes.[45] This stable and inert oxide can be cathodically reduced (see the small reduction wave at about 0.0 V in Figure 4) and a reproducible electrode surface can be prepared by applying alternatively positive and negative potential pulses.[46] In our comparative ECL study, it was critical to maintain a high reproducibility of the electrode and thus the luminescence measurement. We have established a protocol including the electrode cleaning and conditioning, which is similar to the procedure used in a commercial system.[6b] The electrode cleaning was exercised

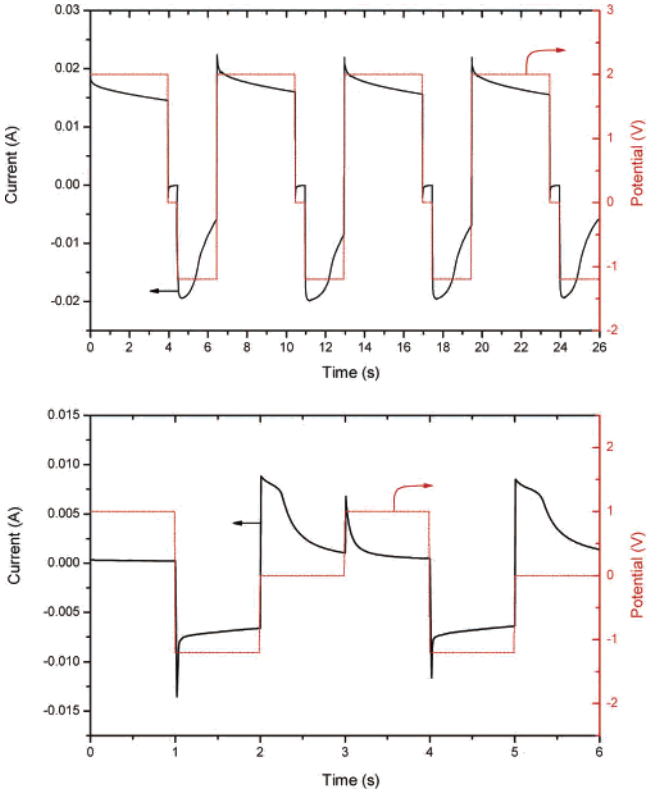


Figure 5. Current responses during the cleaning (above, in 1% KOH aqueous solution) and conditioning (below, in the buffer solution).

in alkaline solution each time after an ECL measurement. After 4 cycles of potential excursion (2.0 V/4 s → 0.0 V/0.5 s → −1.2 V/2 s) in 1% KOH water solution (see Figure 5), the electrode was then conditioned in a $[Ru(bpy)_3]^{2+}$-free buffer solution to undergo a further potential excursion between −1.2 to 1.0 V prior to each measurement, as illustrated in Figure 5. After such an electrochemical treatment in both alkaline and the buffer solution, the electrode can be regenerated with a highly reproducible surface property characterized by a ECL standard deviation of < 5%.

Depending on the concentration of $[Ru(bpy)_3]^{2+}$ and coreactants, the ECL intensity of $[Ru(bpy)_3]^{2+}$ has a wide linear dynamic range. It is, however, not the purpose of the present study to thoroughly investigate the ECL intensities as functions of concentrations of various $[Ru(bpy)_3]^{2+}$ compounds shown in Scheme 1. Instead, we compared the ECL with the solutions of a $[Ru(bpy)_3]^{2+}$ concentration at 1 μM. Figure 6 illustrates the ECL intensities, which were recorded as the PMT anode response in an arbitrary unit, when the working electrode potential was stepped to 1.4 V, a potential chosen for the comparative study. It is found that ECL intensities of these compounds varied widely under the experimental conditions, ranging from a level of lower than 100 (arbitrary unit) for compound **2** to about 4000 for compounds **4** and **5**. If we set the integrated ECL intensity for the period of the first 8 s of the pristine $[Ru(bpy)_3]^{2+}$ as 1, the compounds show the following sequence in terms of the relative ECL intensity (indicated in parentheses, integrated for the same period of time), i.e., **5** (1.24) > **4** (1.22) > **1** (1) > **7** (0.56) > **3** (0.091) > **6** (0.089) > **2** (0.029).

(42) (a) Li, F.; Zu Y. *Anal. Chem.* **2004**, *76*, 1768−1772. (b) Zu Y.; Bard, A. J. *Anal. Chem.* **2001**, *73*, 3960−3964. (c) Factor, B.; Muegge, B.; Workman, S.; Bolton, E.; Bos, J.; Richter, M. M. *Anal. Chem.* **2001**, *73*, 4621−4624. (d) Cole, C.; Muegge, B. D.; Richter, M. M. *Anal. Chem.* **2003**, *75*, 601−604.
(43) Miao, W.; Choi, J. P.; Bard, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 14478−14485.
(44) Yin, X.-B.; Qi, B.; Sun, X.; Yang X.; Wang, E. *Anal.Chem.* **2005**, *77*, 3525−3530.
(45) (a) Anson F. C.; Lingane, J. J. *J. Am. Chem. Soc.* **1957**, *79*, 4901−4904. (b) Tilak, B. V.; Conway, B. E.; Angerstein-Kozlowska, H.; *Electroanal. Chem. Inter. Electrochem.* **1973**, *48*, 1−23.
(46) Johnson, D. C.; LaCourse, W. R. *Anal. Chem.* **1990**, *62*, 589 A−597 A.

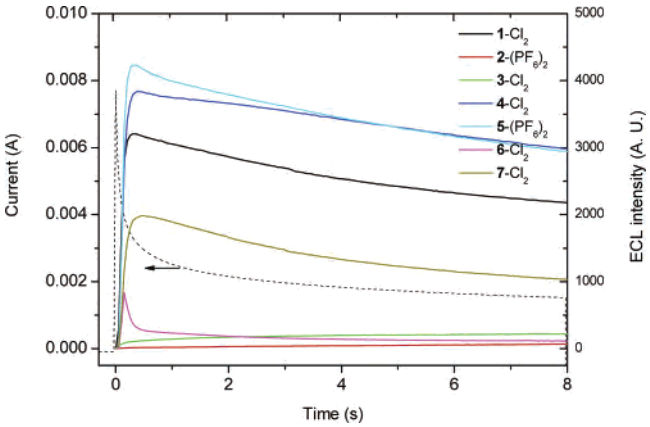


Figure 6. ECL intensities of 1 μM $[Ru(bpy)_3]^{2+}$ derivatives in buffer solutions, Potential step at 1.4 V. Dashed line represents the electrochemical current response.

Contrary to our expectation, the ECL intensity of **2** was the lowest among these compounds under the experimental conditions. Obviously, this is not the same order of the photoluminescence intensity demonstrated in Figure 3 and enumerated in Table 1. Furthermore, no obvious relationship between the photoluminescence efficiency and the ECL intensity can be straightforwardly established from these results.

In the ECL research, the ECL emission efficiency is defined as the number of photons generated from an electrochemical event. This definition is meaningful for the ECL generated from the annihilation process, e.g., between $[Ru(bpy)_3]^{3+}$ and $[Ru(bpy)_3]^{+}$. However, for the oxidative-reduction type ECL of $[Ru(bpy)_3]^{2+}$ involving the coreactant TPA in aqueous solutions, the ECL efficiency is ill-defined and hard to determine. As a matter of fact, the ECL is one of the results of a cascade of electrochemical and chemical reactions involving both $[Ru(bpy)_3]^{2+}$ and TPA derivatives. The situation is further complicated by three facts: first, the oxidation of $[Ru(bpy)_3]^{2+}$ occurs at potentials where the water is oxidized, so the contribution of $[Ru(bpy)_3]^{2+/3+}$ to the anodic current is hard to determine precisely; second, $[Ru(bpy)_3]^{3+}$, once formed, can react with water and the reaction is pH dependent; third, TPA derivatives can also react with water.[47] Therefore, in our comparative study of various $[Ru(bpy)_3]^{2+}$ compounds, we simply gave the PMT anode signal intensity as a measure in relation to the pristine $Ru(bpy)_3Cl_2$.

We have to point out that although the potential 1.4 V was equally applied to all the compounds, the effects on the generation of $[Ru(bpy)_3]^{3+}$ from the oxidation of $[Ru(bpy)_3]^{2+}$ are not the same for these compounds. It is possible that due to the very close E_{pa} of compounds **1**, **4**, **5**, **6**, and **7**, as discussed in the above section, the ECL generated at the same potential of 1.4 V for these compounds is comparable. However, for compounds **2** and **3**, this potential is not high enough to initiate a significant oxidation of $[Ru(bpy)_3]^{2+}$, which is the one of the first steps in the oxidative-reduction mechanism (eqs 1−5)[25,43,48] leading to ECL.

$$[Ru(bpy)_3]^{2+} - e^- \rightarrow [Ru(bpy)_3]^{3+} \quad (1)$$

$$N(C_3H_7)_3 - e^- \rightarrow N(C_3H_7)_3^{\bullet+} \quad (2)$$

$$N(C_3H_7)_3^{\bullet+} - H^+ \rightarrow CH_3CH_2C^{\bullet}HN(C_3H_7)_2 \quad (3)$$

$$CH_3CH_2C^{\bullet}HN(C_3H_7)_2 + [Ru(bpy)_3]^{3+} \rightarrow [Ru(bpy)_3]^{2+*} + ? \quad (4)$$

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

The photoluminescence efficiency of **1**, **4**, **5**, **6**, and **7** in the same buffer solutions is in the following order (ϕ_R in parentheses): **5** (0.135) > **1** (0.125) > **4** and **6** (0.109) > **7** (0.092). Not only is the sequence different from the ECL intensity sequence, but also the variation (from 0.092 to 0.135) of the photoluminescence efficiency is much smaller than that of the ECL intensity (from 0.089 to 1.24, arbitrary unit).

As the issue of much higher values of E_{pa} for **2** and **3** is raised when comparing the ECL at the same potential, we measured the ECL transient of **2** as a function of the applied potential. It is demonstrated in Figure 7 that both the ECL intensity and its transient behavior with time depend on the potential applied on the working electrode. It is very interesting to see an almost linear increase of ECL intensities with the time when the potentials are equal to or lower than 1.6 V. Such a linear transient were also found in other $[Ru(bpy)_3]^{2+}$/buffer systems if the applied potentials were low. With the potential stepped to 2.0 V, we observed an ECL decay pattern similar to, but not the same as, that of **1**, **4**, **5**, and **7** (Figure 6), with an intensity, integrated for the first 8 s, of 0.85 relative to 1.0 of compound **1** at 1.4 V. We have to point out that the potential step of 2.0 V did not cause the highest ECL emission for other compounds. Detailed research of the relationship between the ECL intensity and the applied potential is beyond the scope of the present paper. To compare these $[Ru(bpy)_3]^{2+}$ derivatives under the condition of the commercial systems,[6b] we chose only 1.4 V to create ECL.

The results obtained in this research clearly indicate that there is no direct relation between the ECL intensities and the photoluminescence efficiencies of the $[Ru(bpy)_3]^{2+}$ luminophores, due to the complexity of the ECL generation process. This is particularly true, if the photoluminescence efficiencies are obtained in solvents (such as the commonly used acetonitrile) that are different from the ECL solutions, which are often complex buffer systems. A plausible explanation is that in the TPA-containing buffer solution, the kinetic behaviors of the electrogenerated $[Ru(bpy)_3]^{3+}$ and the excited state $[Ru(bpy)_3]^{2+*}$ can be quite different and the ligand(s) with substitution group(s) also play a role in the chemical reactions.

It should be noted that, in order for us to have a straightforward comparative result toward an application to the existing analytical systems, we used a nonclassic phosphate buffer that contains a nonionic surfactant. The enhancement effect of surfactants on the ECL of $[Ru(bpy)_3]^{3+}$

(47) Smith, P. J.; Mann, C. K. *J. Org. Chem.* **1969**, *34*, 1821−1826.
(48) Wightman, R. M.; Forry, S. P.; Maus, R.; Badocco, D.; Pastore, P. *J. Phys. Chem. B* **2004**, *108*, 19119−19125.

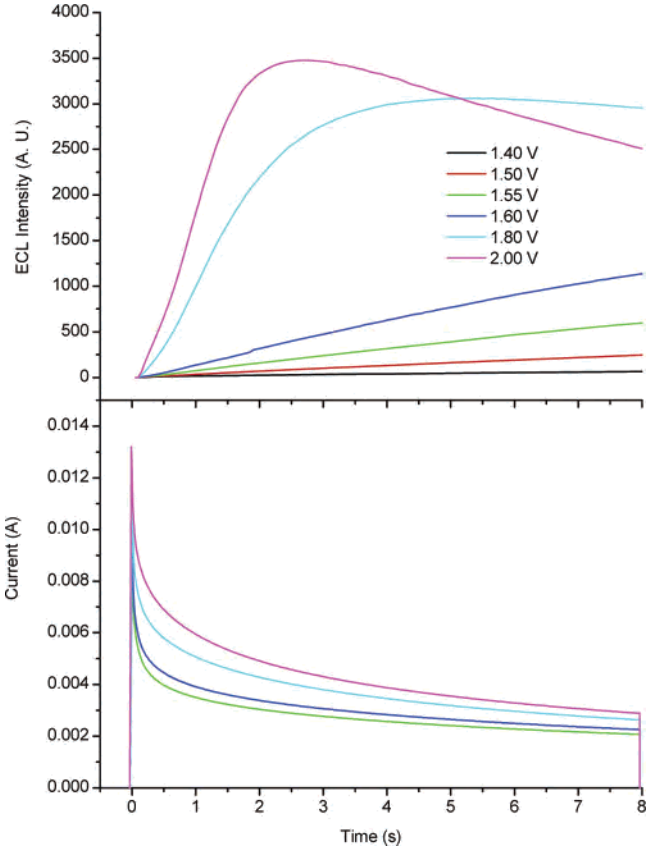


Figure 7. ECL intensity (above) of 1 μM of **2**-$(PF_6)_2$ in the buffer and the current responses (below) at different potentials.

and tris(2-phenylpyridine)iridium(III) have been thoroughly investigated.[42] It is possible that the observed sequence of ECL intensities may vary in other buffer systems but it is unlikely that the above conclusion, i.e., no obvious nonstatistical correlation between the photoluminescence quantum yield and the ECL intensity, can be changed in a particular solvent system. A recent ECL study[13d] of iridium(III) complexes led to the same conclusion, i.e., the photoluminescence efficiencies of iridium(III) complexes obtained in dichloromethane did not show a direct relationship with the ECL measured in TPA containing acetonitrile solutions.

Conclusions

The photophysical and electrochemical data were obtained for several $[Ru(bpy)_3]^{2+}$ derivatives. Contrary to the general perception, the photoluminescence quantum yields of the $[Ru(bpy)_3]^{2+}$ complexes, except the ethoxycarbonyl-substituted derivative $[Ru(bpy\text{-}COOEt)_3]^{2+}$, were found to be higher in the buffer solutions than in the deaerated acetonitrile solutions. The comparative ECL study led to a conclusion that because the ECL of $[Ru(bpy)_3]^{2+}$/TPA is a result of a cascade of electrochemical and chemical reactions, there is no nonstatistical correlation between the photoluminescence efficiency and ECL intensity within the $[Ru(bpy)_3]^{2+}$ family. Compounds **4**, **5**, and **1** showed the highest ECL under the conditions described in this work.

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Supporting Information Available: NMR assignment analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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