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Synthesis, Characterization, Spectroscopic, and Electrochemiluminescence Properties of a Solvatochromic Azacrown-Containing Cyanoruthenate(II): Potential Applications in Separation and Indirect Photometric Detection of Cations and Amino Acids in HPLC

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Abstract: A new anionic ruthenium(II) complex, $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ (tpyA18C6 = *N*-[4′-(2,2′:6′,2″-terpyridyl)]-1,4,7,10,13-pentaoxa-16-azacyclohexadodecane), has been synthesized and characterized. The complex was found to show pronounced solvatochromic behavior and, when dissolved in solution, changed its color from purple to yellow when the solvent system was varied from pure acetonitrile to pure water. Its absorption and emission energies in various solvents showed a linear dependence of the Gutman's acceptor number. The characteristic photoluminescence and electrochemiluminescence (ECL) of the complex were also found to be progressively quenched as the proportion of water in a water/acetonitrile mixture increased. Large changes in the chemical shifts of the ^1H NMR and ^{13}C NMR signals of $[Ru(tpyA18C6)(CN)_3]^-$ in different solvents were observed. The complex has also been demonstrated to serve as a mobile-phase additive in high-performance liquid chromatography for separation of metal cations and amino acids. Comparison studies with the crown-free analogue, $(Et_4N)[Ru(tpy)(CN)_3]$ (tpy = 2,2′:6′,2″-terpyridine), showed that other than the ion-pair effect, the allosteric host–guest interaction provided by the presence of the pendant crown was essential to the separation performance of the complex. Indirect detection of nonabsorbing analytes has been achieved by monitoring the absorbance changes of the eluent at the metal-to-ligand charge-transfer (MLCT) absorption band maximum of the complex at 445 nm. The effects of pH, ionic strength, and polarity of the mobile phase as well as the complex concentration on the selectivity and resolution have also been studied.

Keywords: electrochemiluminescence · high-performance liquid chromatography · photoluminescence · ruthenium · solvatochromism

Introduction

Supramolecular host–guest interactions and molecular recognition are subjects of considerable interest, and the recognition of cations is one such example, due to its implications in numerous fields, such as chemistry, biology, medicine, and environmental studies.[1] Such interactions may find applications when the host molecules are able to express the recognition signal by invoking a change in one or more properties of the system, such as absorption, emission, or redox potential characteristics. In recent years, much attention has been paid to the design of inorganic/organometallic sensors,[2–11] in particular those with metal-to-ligand charge-transfer (MLCT) chromophores in the visible region, as they could provide an easy spectrochemical and luminescence handle for the selective and specific monitoring of substrates. Electrochemiluminescence (ECL) properties of cationic ruthenium(II)–polypyridine complexes have been extensively

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. The Supporting Information contains [^1H-^{13}C] HETCOR and HMBC of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in D_2O and CD_3CN in Figures S1 and S2. The effect of MeOH content in the mobile phase on the analyte retention factor for the separation of selected amino acids using $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ as an additive is shown in Figure S3. The chromatograms of the separation of alkali and alkaline-earth metal ions on a PRP-1 column adsorbed with $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ are shown in Figure S4.

studied in both aqueous and nonaqueous solution,[12] but to our knowledge, no investigation has previously been reported on the ECL behavior of cyanoruthenate complexes. Besides, most of these studies were focused on the synthesis and spectroscopic changes that occur upon cation binding, with relatively few studies involving application of anionic complexes with a pendant crown as both inorganic host and ion-pairing reagent to separate cations by high-performance liquid chromatography (HPLC). As an extension of our previous work on the utilization of transition-metal complexes containing crown ethers for spectrochemical and luminescence chemosensing,[13–15] herein we report the preparation of a new anionic solvatochromic cyanoruthenate(II) complex bearing an azacrown pendant, $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ (Figure 1), which exhibits both chromophoric properties and

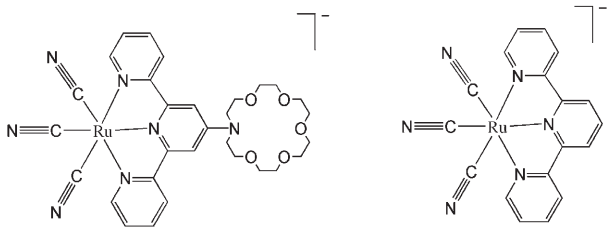


Figure 1. Structures of $[Ru(tpyA18C6)(CN)_3]^-$ and $[Ru(tpy)(CN)_3]^-$.

cation-binding ability. We believe that the incorporation of an additional cation-binding site, such as a crown ether, would give rise to allosteric effects that would provide the selectivity, specificity, and improved resolution for cation separation and detection in comparison to complexes with no binding site. The complex was also found to display interesting photoluminescence, electrochemiluminescence, and pronounced solvatochromic behavior. The applications of the complex as a mobile-phase additive for liquid chromatography separation and indirect photometric detection (IPD) for mixtures of inorganic and organic analyte cations are also reported.

Results and Discussion

Synthesis and characterization: The complex was prepared in good yield according to the modified literature methods.[16,17] The newly synthesized complex gave satisfactory elemental analyses and was characterized by UV/Vis spectroscopy, negative ESI-MS, IR and ^{1}H NMR spectroscopy. The IR spectrum of the newly synthesized complex shows three ν(C≡N) stretches in the region of 2030–2090 cm^{-1}, which are typical of the coordinated cyano groups and are similar to those found for $(Et_4N)[Ru(tpy)(CN)_3]$.[16]

Electronic spectroscopic properties and solvatochromic studies: The electronic absorption spectra of $[Ru(tpyA18C6)(CN)_3]^-$ in various solvents at 298 K show intense absorption bands at 280–300 nm and moderately intense bands at 320–570 nm. The typical electronic absorption spectra and color of $[Ru(tpyA18C6)(CN)_3]^-$ in various solvents are shown in Figure 2 (top), and the electronic absorption data in different solvents are listed in Table 1. With reference to previous spectroscopic work on $[Ru(CN)_3(tpy)]^-$,[16] the intense high-energy absorption bands with molar extinction coefficients in the order of 10^4 dm^3 mol^{-1} cm^{-1} at about 280–300 nm are assigned to the intraligand $\pi\rightarrow\pi^*$ transition of tpyA18C6, and the bands at 320–400 nm are ascribed to the admixture of the intraligand transition of tpyA18C6 and the $d\pi(Ru^{II})\rightarrow\pi^*$(tpyA18C6) metal-to-ligand charge-transfer (MLCT) transition. The absorption bands at 430–570 nm with molar extinction coefficients in the order of 10^3 dm^3 mol^{-1} cm^{-1} in the visible region are assigned to the $d\pi(Ru^{II})\rightarrow\pi^*$(tpyA18C6) MLCT transition. The strong solvatochromism of Ru^{II}–cyano-containing

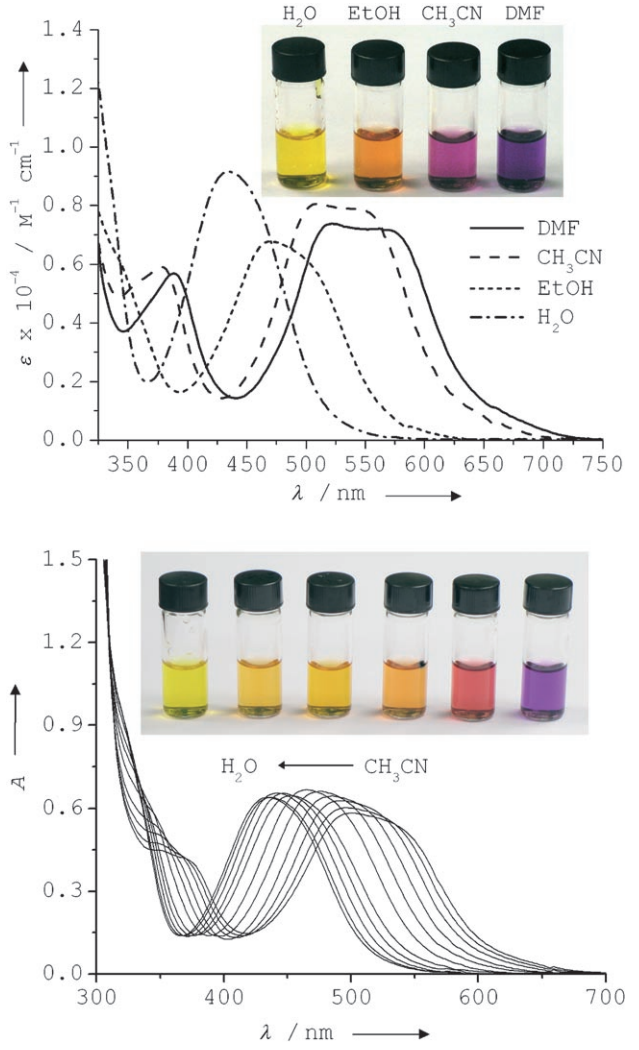


Figure 2. Top: Electronic absorption spectra and color of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in different solvents. Bottom: Solvent dependence of the MLCT absorption band and color of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ on changing from pure acetonitrile to pure water.

Table 1. Variations with solvent of λ_{abs} and λ_{em} for $(Et_4N)[Ru(tpyA18C6)(CN)_3]$.

Solvent	Acceptor number[25]	Absorption λ_{max} [nm] ($\varepsilon \times 10^{-3}$ [$M^{-1}cm^{-1}$])	Emission[a] λ_{max} [nm]	$\phi \times 10^4$
acetone	12.5	380 (4.43), 518 (5.56), 556 (5.62)	765	6.0
pyridine	14.2	306 (29.46), 389 (5.60), 523 (7.29), 565 (7.08)	760	5.8
DMF	16.0	298 (36.27), 388 (5.70), 522 (7.39), 562 (7.20)	755	6.0
CH_3CN	19.3	295 (38.40), 378 (6.02), 508 (8.15), 536 (8.04)	740	1.8
CH_2Cl_2	20.4	294 (29.96), 376 (4.39), 504 (6.11), 534 (5.71)	730	<1
propanol	33.5	284 (38.06), 345 (6.18), 478 (7.34)	685	<1
EtOH	37.1	284 (34.75), 344 (6.04), 470 (6.77)	675	<1
MeOH	41.3	282 (35.60), 344 (6.17), 460 (6.81)	645	<1
H_2O	54.8	284 (40.02), 324 (12.46), 434 (9.16)	_[b]	_[b]

[a] All are corrected values. The lifetime in different solvents is less than 0.1 μs. [b] Too weak to be measured.

polypyridyl complexes has been reported previously.[18] Similar to $[Ru(tpy)(CN)_3]^-$,[16] the MLCT absorption band of $[Ru(tpyA18C6)(CN)_3]^-$ exhibited a strong energy dependence on solvents, which arose from the interaction of the externally directed lone pairs on the cyano ligands with the solvent.[19–21] The visible color of the complex was also found to change dramatically from purple to yellow on varying the solvent composition from neat acetonitrile to neat water (Figure 2, bottom). The ^{1}MLCT absorption maximum (λ_{abs}) was found to shift from 508 to 434 nm, equivalent to an energy difference of approximately 3350 cm^{-1}. Apart from UV/Vis spectral changes in different solvents or solvent mixtures, the MLCT absorption bands of $[Ru(tpyA18C6)(CN)_3]^-$ were also found to shift in different pH buffer solutions. Figure 3

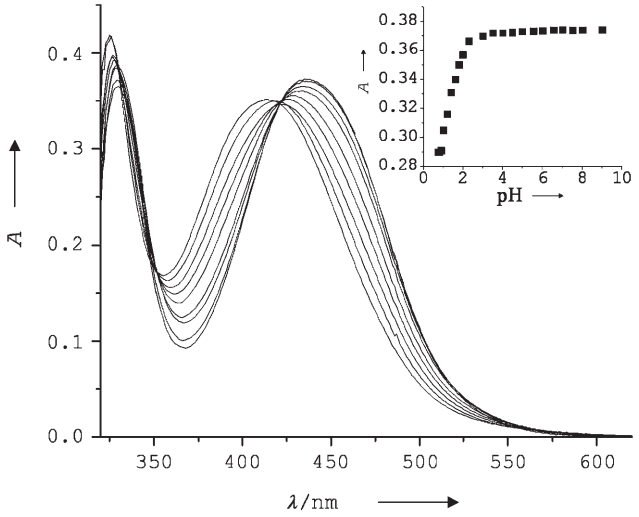


Figure 3. Spectral traces of the MLCT absorption band of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in aqueous buffer solutions as a function of pH. Inset: Change of absorbance at 435 nm as a function of pH.

shows the spectral traces of the MLCT absorption bands of $[Ru(tpyA18C6)(CN)_3]^-$ as a function of pH and the inset displays the effect of pH (0.8–10.0) on the absorbance of the MLCT absorption band at 435 nm. At the indicated measured pH, on going from basic to acidic conditions, the ^{1}MLCT band maximum shifted to higher energy ($pH < 4$) due to protonation at the CN^- sites, and a noticeable isosbestic point at 420 nm was observed. It was found that protonation of the cyano ligand occurred at an apparent pK_a of about 1.9, comparable to the literature pK_a value for $[Ru(bpy)(CN)_4]^{2-}$.[22]

Like other ruthenium(II) complexes, excitation of $[Ru(tpyA18C6)(CN)_3]^-$ in both the UV and visible region gave rise to ^{3}MLCT emission. Emission wavelengths and quantum yields of $[Ru(tpyA18C6)(CN)_3]^-$ in different solvents are summarized in Table 1. In general, the ^{3}MLCT emission bands are blue-shifted with respect to the crown-free complex of $[Ru(tpy)(CN)_3]^-$, and this is probably due to the presence of electron-donating aza–oxa crown moiety on the terpy ligand. Excitation of solid samples of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ at 500 nm at room temperature gave rise to a very weak ^{3}MLCT emission at 740 nm, while at 77 K, the complex was found to emit strongly in the red at 680 nm with fine vibronic structures. In an ethanol/methanol (4:1 v/v) glass at 77 K, vibronic structures with progressional spacings (ν_M) of about 1500 cm^{-1} were observed. These vibrational progressions are assigned to aromatic C=C vibrations and are commonly observed in ruthenium(II)–polypyridine complexes at low temperature.[23] The ^{3}MLCT emission band was also found to be sensitive to the solvent, similar to that of the ^{1}MLCT absorption. The emission band maxima (λ_{em}) was found to gradually shift from 715 to 630 nm on changing the solvent from acetonitrile to methanol, and the emission became greatly reduced in intensity (Table 1). Unlike the crown-free $[Ru(tpy)(CN)_3]^-$ complex, the emission intensity of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ was also found to be very sensitive to the presence of water and was greatly reduced with increasing water content in an acetonitrile solution of the complex (Figure 4). This might be ascribed to the efficient quenching of $[Ru(tpyA18C6)(CN)_3]^-$ by water through hydrogen-bonding interactions between the water molecule and the nitrogen and oxygen atoms on the tpyA18C6 ligand, as well as the increased electron density at the cyano nitrogen atoms as a result of the good electron-donating ability of the aza-crown moiety.[24]

When the transition energy (E_{abs}) of the absorption band and ^{3}MLCT emission energy (E_{em}) of the complex in different solvents were plotted against the Gutman's acceptor number (AN)[25] of a solvent, a linear relationship[16] was obtained (Figure 5). In general, the higher the AN of a solvent is, the greater is the blue shift of the MLCT absorption band relative to that in solvents of lower AN; this result is due to the greater interaction between lone pair electrons on the nitrogen atoms of the cyano ligands and the solvent molecules of high AN, resulting in a lowering of σ-donating ability as well as an increase in the π-acceptor ability of the

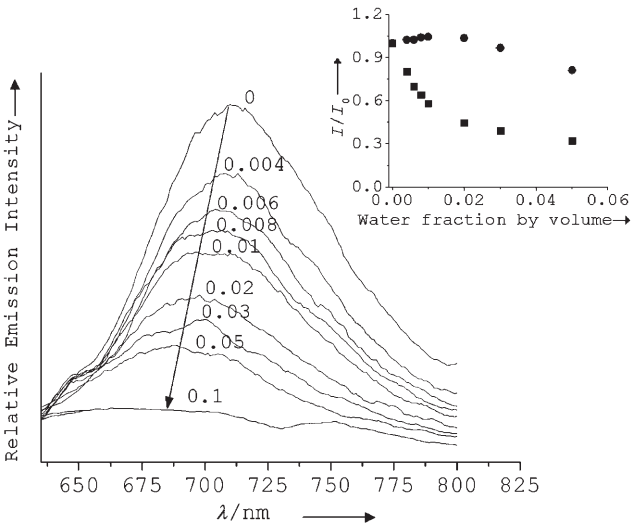


Figure 4. Emission spectra of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in acetonitrile with a trace amount of water (uncorrected spectra). Excitation at isosbestic wavelength of 540 nm. Inset: Plot of emission peak intensities of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ (■) and $(Et_4N)[Ru(tpy)(CN)_3]$ (●) against the water fraction by volume in acetonitrile.

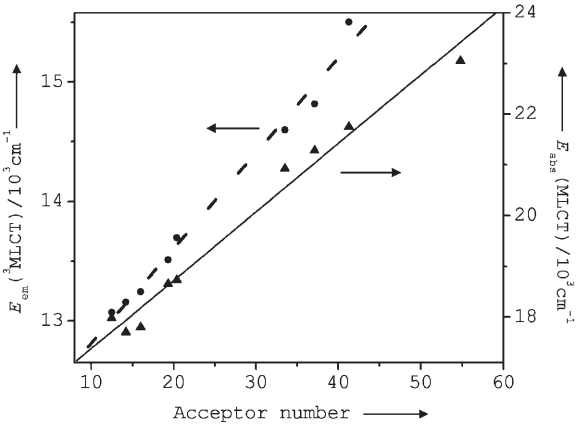


Figure 5. Shifts of E_{abs}(MLCT) (▲) and E_{em}(^{3}MLCT) (●) for $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ with acceptor numbers of different solvents.

cyano ligands and, hence, the stabilization of $d\pi[Ru^{II}]$ orbital. Specific donor–acceptor interactions could also be reflected by the absorption energy dependence of the $\pi\rightarrow\pi^*$ transition, $E_{abs}(\pi\rightarrow\pi^*)$, even though the underlying transition was apparently centrosymmetric and ligand-localized. Its sensitivity to acceptor number was only about 25% that of E_{abs}(MLCT), but still far greater than that of a typical $\pi\rightarrow\pi^*$ band in the free ligand and in $[Ru(bpy)_3]^{2+}$.[26] This $\pi\rightarrow\pi^*$ solvent dependence probably arises from a mixing with the MLCT transitions. Comparison of $d\pi\rightarrow\pi^*$ MLCT transition energies (ca. 20000 cm^{-1}) with $\pi\rightarrow\pi^*$ energies (ca. 30000 cm^{-1}) showed that the $d\pi(Ru^{II})$ and π(tpyA18C6) orbitals were closer in energy than the $d\pi(Ru^{II})$ and π^*(tpyA18C6) orbitals; these results point to $d\pi$–π mixing as the dominant effect. Higher acceptor number solvents would stabilize $d\pi(Ru^{II})$, which stabilizes both π(tpyA18C6) and π^*(tpyA18C6) by orbital mixing. The fact that the $\pi\rightarrow\pi^*$ transition energy was found to increase with acceptor number showed that the π orbital was stabilized more than the π^* orbital. The slope obtained from the linear correlation between the E_{abs}(MLCT) and acceptor number ($\Delta E_{abs}/\Delta AN$) was found to be $135\pm7\ cm^{-1}\,AN^{-1}$, which is much larger than that of the crown-free $[Ru(tpy)(CN)_3]^-$ complex ($\Delta E_{abs}/\Delta AN = 94\pm5\ cm^{-1}\,AN^{-1}$).[16] These results suggest that the complex with crown pendant shows a higher sensitivity to the nature of the solvent, rendering it a more sensitive probe for sensing subtle environmental changes. The slopes, $\Delta E_{em}/\Delta AN$, obtained from linear correlation between E_{em}(MLCT) and the acceptor number (Figure 5) was found to be $80\pm4\ cm^{-1}\,AN^{-1}$, which was also much larger than that found for the crown-free $[Ru(tpy)(CN)_3]^-$ complex ($\Delta E_{em}/\Delta AN = 58\pm4\ cm^{-1}\,AN^{-1}$).[16] It is interesting to note that the relative sensitivity of the shift in the energy of the emission band in $[Ru(tpyA18C6)(CN)_3]^-$ with respect to the crown-free complex to solvent polarity is very much to the same extent as that observed in the electronic absorption spectroscopy.

Eletrochemiluminescence (ECL) properties: Like the $[Ru(bpy)_3]^{2+}$/TPA system (TPA = tri-*n*-propylamine),[12] the $[Ru(tpyA18C6)(CN)_3]^-$/TPA system also generated ECL signals when the potential was swept to $>+1.0$ V; the cyclic voltammogram and ECL response of a 10 μM $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in acetonitrile containing 0.10 M Bu_4NPF_6/0.10 M TPA are shown in Figure 6 (top). Similar to $[Ru(bpy)_3]^{2+}$ in nonaqueous solvent systems,[27] the ECL generated from the present system is mainly produced by the reaction of the oxidized complex, $[Ru^{III}(tpyA18C6)(CN)_3]$, with the TPA radical, and the intensity was found to increase with increasing potential, finally forming a broad peak at $+1.2$ V with a half width of about 700 mV [Eqs. (1)–(4)]. On the reverse scan, a larger ECL signal at similar peak potential was observed.

$$[Ru(tpyA18C6)(CN)_3]^- - e^- \rightarrow [Ru^{III}(tpyA18C6)(CN)_3] \quad (1)$$

$$TPA - e^- \rightarrow [TPA^{\bullet+}] \rightarrow TPA^{\bullet} + H^+ \quad (2)$$

$$[Ru^{III}(tpyA18C6)(CN)_3] + TPA^{\bullet} \rightarrow [Ru(tpyA18C6)(CN)_3]^-{}^* + \text{products} \quad (3)$$

$$[Ru(tpyA18C6)(CN)_3]^-{}^* \rightarrow [Ru(tpyA18C6)(CN)_3]^- + h\nu \quad (4)$$

Richter et al. have previously studied the ECL properties of $[(bpy)_2Ru(bphp)]^{2+}$/TPA and $[\{(bpy)_2Ru\}_2(bphp)]^{4+}$/TPA systems in acetonitrile and an acetonitrile/H_2O (1:1 v/v) mixture[12b] and found that the ECL intensities were almost the same in both pure solutions. However, it is interesting to find that the ECL intensity of the present complex, $(Et_4N)[Ru(tpyA18C6)(CN)_3]$, was dependent on the water

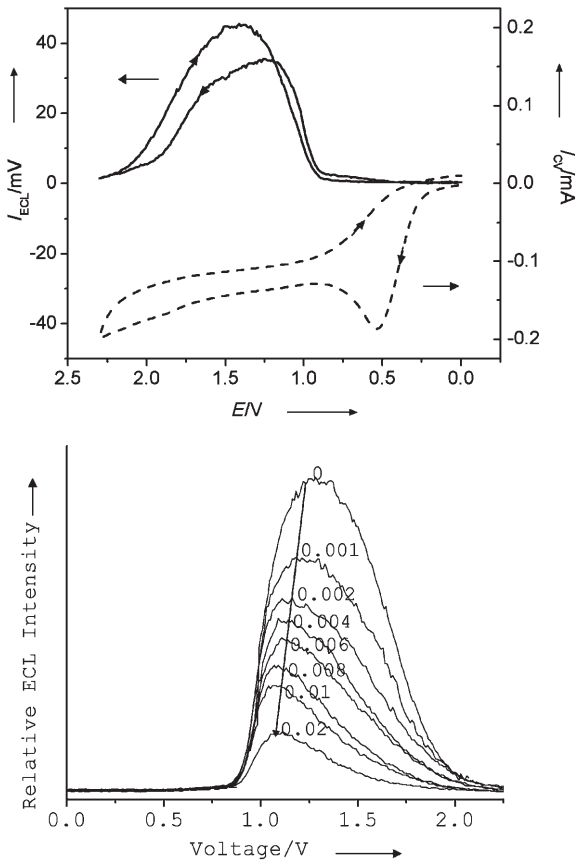


Figure 6. Top: Cyclic voltammogram (——) and the corresponding ECL response (-----) obtained from a solution of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in acetonitrile (10 μM) containing Bu_4NPF_6 (0.10 M) and TPA (0.10 M) on 2.2 mm diameter Pt electrode at a scan rate of 100 mVs^{-1}. Bottom: ECL responses of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ during cyclic voltammetry in acetonitrile with different water fraction by volume.

content in nonaqueous acetonitrile. The ECL responses from the first scanning segments of a 10 μM $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ with different water contents in acetonitrile containing 0.10 M Bu_4NPF_6/0.10 M TPA are shown in Figure 6 (bottom). It was observed that the ECL intensity of the complex was reduced with increasing water content. Such high sensitivity of ECL intensity towards water is probably because the excited state generated during such electrochemical reaction is similar to that formed during photoluminescence. To our knowledge, this is also the first report on the effect of water on ECL intensity in a nonaqueous system by such anionic cyanoruthenate(II) complexes.

NMR spectroscopy: The solvatochromic effects have also been probed by ^1H and ^{13}C NMR spectroscopy. Large changes in the chemical shifts of $[Ru(tpyA18C6)(CN)_3]^-$ were observed in the ^1H NMR spectra in different solvents. Typical examples of the solvent effect on the chemical shifts of the complex are shown in Figure 7a. It was found that the H_6 and $H_{3'}$ protons were the most strongly affected upon changing the solvent from D_2O to $[D_3]$acetonitrile, $[D_6]$methanol, and $[D_6]$acetone. It was established from previous investigations on cyanoruthenate and cyanoferrate complexes[28] that the cyano ligands played a major role in donor–acceptor interaction with the solvents and that the effects were transmitted to the terpyridine ligands through the metal center. Thus the H_6 protons are more susceptible to the magnetic anisotropy effects, owing to their close vicinity to the metal center and the cyano ligands. When the solvent was changed from D_2O to $[D_6]$acetone, a large change in chemical shift ($\Delta\delta$) of the $H_{3'}$ protons was observed (~0.4 ppm), which was much larger than that of the crown-free complex of $[Ru(tpy)(CN)_3]^-$ ($\Delta\delta = 0.05$ ppm).[17] The protons on the crown ether pendant were also found to be affected by the solvent. All these observations showed that the presence of crown ether pendant on the tpyA18C6 ligand and the large solvent effects of the nitrogen and oxygen atoms on the tpyA18C6 crown ether are essential to enhance the solvatochromic sensitivity of $[Ru(tpyA18C6)(CN)_3]^-$. The effect of solvent on the cyano group was also studied by ^{13}C NMR spectroscopy. Typical ^{13}C NMR spectra of $[Ru(tpyA18C6)(CN)_3]^-$ in D_2O and CD_3CN are provided in Figure 7b; the complete ^{13}C NMR spectral assignment obtained from [^1H-^{13}C] HETCOR and HMBC measurements are available in the Supporting Information (Figures S1 and S2). Prominent shifts of signals of cyano carbons, particularly at the C_{20} position, confirm their strong interaction with the solvent.

Liquid chromatography: Since the crown-containing complex $[Ru(tpyA18C6)(CN)_3]^-$ is anionic, water-soluble, stable, and absorbs in the visible region, it can be used as a mobile-phase additive for the separation of analyte cations. We believe that as the complex is anionic and possesses allosteric cation-binding sites, it would not only serve as the additive to separate cations by ion-pair and host–guest interactions, but that it would also act as a chromophore for the indirect photometric detection of the analytes. Preloading of the complex onto the stationary phase of the PRP-1 column was accomplished by monitoring the absorbance of the mobile-phase buffer containing $[Ru(tpyA18C6)(CN)_3]^-$ at 445 nm, until an equilibrium was established between the complex in the mobile phase and that adsorbed onto the stationary phase by hydrophobic interactions. It is proposed that a double-layer structure would be formed,[29,30] with the first layer containing the anionic $[Ru(tpyA18C6)(CN)_3]^-$ complex held by hydrophobic interactions onto the stationary phase of the column, and the second diffuse layer composed of counterions and electrolyte ions (C^+) by electrostatic attraction with the first layer. When an analyte ion (X^+) is introduced into the mobile phase, it would compete with C^+ in the secondary layer according to cation-exchange-like selectivity (Scheme 1A). Other than the nonspecific electrostatic interaction, a second host–guest binding interaction between the crown cavity and the analyte cations of the right size (M^+) would exist to form a binding associate complex (Scheme 1B). As the analyte cations pass through the column, each type of cation would form an ion-paired complex of different association constant with the anionic [Ru-

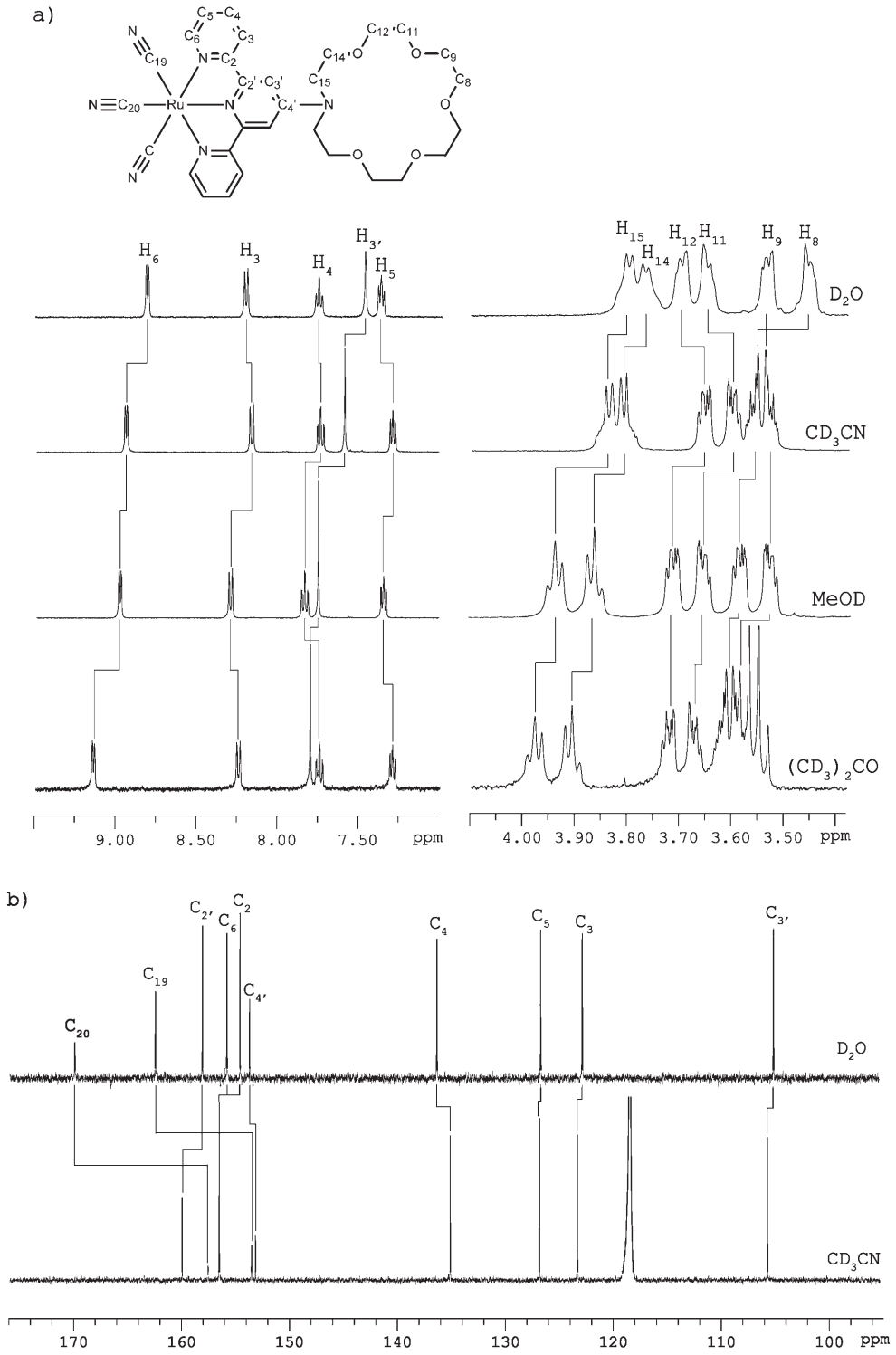
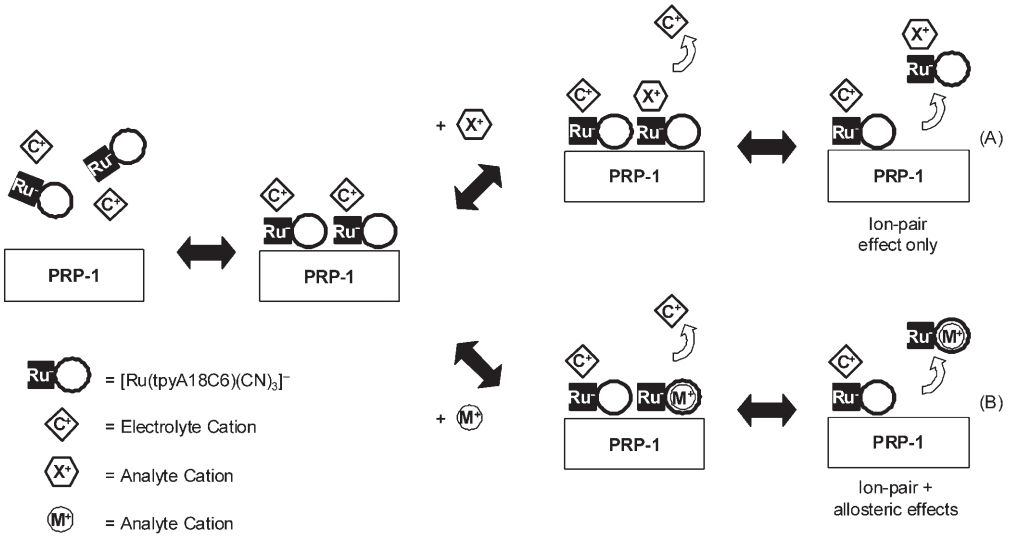


Figure 7. a) ^1H and b) ^{13}C NMR spectra of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in different solvents.

$(tpyA18C6)(CN)_3]^-$ complex, augmented by secondary allosteric effects with the azacrown unit, causing a difference in the retention time for each type of cation as well as a change in the amount of adsorbed complex, resulting in a change in the absorbance detected as the analyte ions are eluted.

Effects of complex concentration, ionic strength, and organic solvent in the mobile phase: As the concentration of $[Ru(tpyA18C6)(CN)_3]^-$ in the mobile phase was increased, the amount of the complex adsorbed onto the column material was found to increase over the concentrations studied from 0.01 mM to 0.2 mM. On increasing the complex concentra-



Scheme 1. Schematic representation illustrating the separation of analyte cations by ion-pair and allosteric effects of $[Ru(tpyA18C6)(CN)_3]^-$.

tion, the number of cation exchange sites was found to increase, resulting in a higher analyte-cation retention capacity by both electrostatic attraction and host–guest-binding associate complex formation. High complex concentration would not be recommended because of the high background absorbance and relatively small analyte peak heights and areas.[31] In general, a complex concentration of 0.05 mM was found to be optimal in terms of separation ability and selectivity, and was used throughout the study. An increase in ionic strength of the mobile phase by addition of Et_4NCl at a given concentration of $[Ru(tpyA18C6)(CN)_3]^-$ was also found to increase the analyte-cation retention factor; this result is due to the higher complex loading on the PRP-1 column surface, resulting in an increased apparent cation exchange capacity. However, a higher loading of the complex on the column did not afford great improvement on the column efficiency due to distinct peak broadening. Thus, a concentration of 2.5 mM of Et_4NCl was chosen for all the chromatograms to control the ionic strength for the different pH condition. The solvent effect on the retention factor was also investigated by using a mobile phase with different ratios of methanol/buffer (0:100 to 40:60 v/v), containing 0.05 mM $[Ru(tpyA18C6)(CN)_3]^-$ at pH 8.0. The analyte-cation retention factor was found to decrease with increasing methanol content. This could be explained by the removal of the $[Ru(tpyA18C6)(CN)_3]^-$ complex from the PRP-1 column, leading to a reduction of the apparent cation exchange capacity.

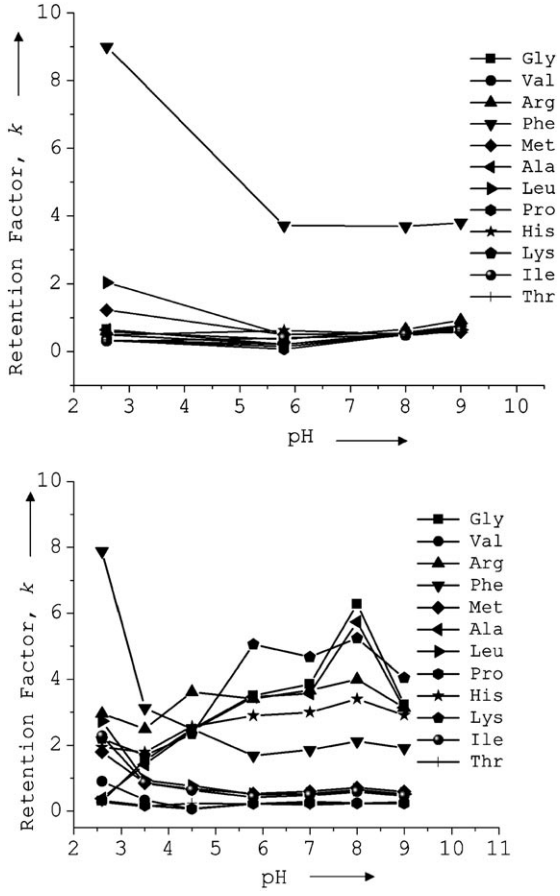


Figure 8. Effect of pH on the retention factor of amino acids using $(Et_4N)[Ru(tpy)(CN)_3]$ (0.05 mM; top) and $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ (0.05 mM; bottom) as an additive in a mobile phase containing Et_4NCl (2.5 mM).

Separation of amino acids: Separation of an amino acid mixture by using $[Ru(tpy)(CN)_3]^-$ as an additive in mobile phase is shown in Figure 8 (top). Almost all amino acids were eluted out at similar retention times and were poorly resolved, exhibiting virtually the same pattern over the whole measured pH range. The poor resolution was probably due to the nonspecific ion-pair effect, which did not exhibit any selectivity on the separation of amino acids. However, phenylalanine, which contains a hydrophobic phenyl ring, was well separated and its retention factor was found

to increase with increasing protonation of the amino group. This could be explained by the fact that the retention factor for amino acids was affected not only by the ionic adsorption on the cation exchange sites, but also by the hydrophobic adsorption onto the resin matrix.

A change in pH was shown to affect protonation of not only the amino acids, but also the azacrown moiety on the $[Ru(tpyA18C6)(CN)_3]^-$ complex. Although we were not able to determine the pK_a for protonation of the nitrogen atom at the azacrown moiety in our experiments, based on a knowledge of the pK_a of $C_6H_5N(CH_3)_2$ ($pK_a = 4.38$), one could assume that it would be lower than 5 due to their similar structures. Compared to an average value of pK_a for the α-ammonium group of amino acids of pH 9.47,[32] the nitrogen on the azacrown moiety would be much more difficult to protonate, because of conjugation between the lone pair electrons on nitrogen and the π electrons of the phenyl ring and large steric hindrance of the tertiary amine group, making the tpyA18C6 ligand a considerably weaker base. Figure 8 (bottom) displays the separation profiles for different amino acids by using the $[Ru(tpyA18C6)(CN)_3]^-$ complex as an additive in the mobile phase. At low pH (pH < 5), the complex became neutral on protonation of the azacrown moiety, leading to diminished ion-pair effect and domination of hydrophobic interactions in amino acid separation. When the pH (pH ~ 6) was close to the pK_a values of the amino acids, the amino acids mainly existed as zwitterions and the complex mostly as anions. Under such conditions, the unprotonated crown complex could interact with the amino acids both by host–guest and ion-pair interactions. These synergistic effects would lead to an increase in analyte retention, especially for glycine and lysine in which the ω-amino groups were less sterically hindered and more remote from the α-carboxyl groups, resulting in less electrostatic repulsion. At high pH (pH > 8), analyte retention factors for most amino acids (pK_a ~ 9–10) were found to decrease, probably due to electrostatic repulsion between the anionic complex and the deprotonated amino acids. The retention factor for proline was found to be independent of pH. Such phenomenon might be due to the steric bulk of proline, making it unfavorable to form a binding associate complex with the $[Ru(tpyA18C6)(CN)_3]^-$ complex. Under optimized conditions, five amino acids could be separated with good resolution and a column efficiency at pH 5.8; the retention times are given in Table 2. The ion-binding and ion-pair effects exerted a synergistic effect on the retention of the amino acids, resulting in complete separation within 15 min.

Table 2. Retention time of five selected amino acids on a PRP-1 column adsorbed with $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ in a mobile phase at pH 2.6, pH 5.8, and pH 8.0 containing 0.05 mM complex.

pH	Retention time [min]				
	Gly	Val	Met	Lys	Phe
2.6	3.3	4.8	7.0	8.0	22.2
5.8	9.6	2.6	3.3	12.9	5.7
9.0	9.4	2.8	3.5	11.1	6.4

Figure 9 shows the effect of pH on the separation of a mixture of three selected amino acids of histidine, arginine, and lysine. When the pH was 2.6, arginine, which has a long

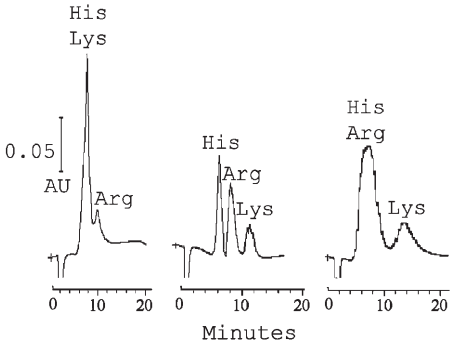


Figure 9. Separation of three amino acids at (left) pH 2.6 (chloroacetate buffer), (middle) pH 5.8 (succinate buffer), and (right) pH 9.0 (Tris-HCl buffer) using $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ (0.05 mM) as an additive in a mobile phase containing Et_4NCl (2.5 mM).

alkyl side-chain, was strongly retained due to its strong hydrophobic interaction with the column matrix, but the peaks of histidine and lysine were not resolved. The elution time was prolonged a little when the pH of the mobile phase was increased to 5.8 and all three amino acids were well resolved with elution sequence of histidine, arginine, and lysine. The larger increase in retention factor of lysine than those of arginine and histidine could be explained by the better formation constant of the associate complex, resulting from the aminobutyl group on the side chain of lysine and the poorer binding interaction of the imidazolyl and guanidinyl groups of histidine and arginine with the azacrown moiety of the complex. When the pH was 9, distinct peak broadening was observed in the chromatograms because the concentration of protonated amino acids decreased significantly, resulting in poor separation and overlapping of the peaks.

Metal-ion separation: Liquid chromatography of alkali and alkaline-earth metal ions was also performed using the modified PRP-1 column with water/methanol mixtures as the mobile phase. Typical retention times for the ion chromatography with $[Ru(tpyA18C6)(CN)_3]^-$ as additive are given in Table 3; these times show good separation of five alkali metal ions and three alkaline-earth metal ions. Use of pure aqueous buffer allowed separation of the alkali metal ions, with the retention time in the order of $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$. The retention behavior agrees well with the cation-complexing properties of the [18]crown-6 ring.[33] Increasing the methanol content in the mobile phase generally was found to decrease the retention of the metal ions, leading to the removal of the complex from the column and poor separation of the alkali metal ions. Separation of alkaline earth metal ions such as Mg^{2+}, Ca^{2+}, and Ba^{2+} was also performed. Since Mg^{2+}, Ca^{2+}, and Ba^{2+} are divalent ions, when they replace the Et_4N^+ due to the charge action, the equilibrium amount of retained $[Ru(tpyA18C6)(CN)_3]^-$ on

Table 3. Retention time of alkali and alkaline-earth metal ions on a PRP-1 column adsorbed with $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ or $(Et_4N)[Ru(tpy)(CN)_3]$ in a mobile phase of MeOH/Tris-HCl buffer (pH 8.0) (40:60, v/v) containing 0.05 mM complex at a flow rate of 1.0 mL min^{-1}.

Additive	Retention time [min]							
	Li^+	Na^+	K^+	Rb^+	Cs^+	Mg^{2+}	Ca^{2+}	Ba^{2+}
$[Ru(tpyA18C6)(CN)_3]^-$	2.5	4.6	18.7	12.5	6.8	3.8	9.8	21.2
$[Ru(tpy)(CN)_3]^-$	1.6	1.8	2.4	2.3	2.4	2.5	3.0	3.2

column increases; this results in a decrease of $[Ru(tpyA18C6)(CN)_3]^-$ concentration in the analyte band by an equivalent amount and negative absorbance peaks are obtained. To further confirm the cation-complexing property of the crown ligand, a control experiment was performed by using $[Ru(tpy)(CN)_3]^-$ as the mobile-phase additive and we were unable to achieve separation for both alkali and alkaline-earth metal ions by nonspecific ion-pair effects.

Conclusion

The spectroscopic and ECL properties of a new solvatochromic complex, $(Et_4N)[Ru(tpyA18C6)(CN)_3]$, have been studied and compared with its crown-free analogue, $(Et_4N)[Ru(tpy)(CN)_3]$. The presence of the azacrown moiety renders the $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ complex more sensitive to solvent change, which makes it a good candidate for sensing subtle humidity changes. We have also demonstrated that $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ serves as a mobile-phase additive for the separation of inorganic and organic cations on a Hamilton PRP-1 column by HPLC; the selectivity is provided by the ion-pair interactions between the anionic complex and analyte cations, and the host–guest interaction between the crown ether moiety and cations of right sizes. The use of this multifunctional $[Ru(tpyA18C6)(CN)_3]^-$ complex as a chromophoric reagent for IPD provides a new strategy for the separation and detection of non-chromophoric inorganic and organic analytes.

Experimental Section

Materials: The amino acids and tri-*n*-propylamine (TPA) were of analytical grade and were purchased from Aldrich Chemical Co. The alkali and alkaline-earth nitrate salts, tetraethylammonium chloride, and tris(hydroxymethyl)aminomethane (Tris) were of analytical grade and all solvents were of HPLC grade. Tetra-*n*-butylammonium hexafluorophosphate was used as supporting electrolyte for ECL measurements and was recrystallized three times from ethanol and dried in vacuum before use. Acetonitrile for all measurements was purified by refluxing over calcium hydride over 24 h under nitrogen. The aqueous buffers were prepared from oxalate (pH 0.7–2.0), citrate (pH 2.0–7.0), and phosphate (pH 6.0–10.0) buffer solutions. The ligand, tpyA18C6, was prepared by a literature method.[34] $[Ru(tpyA18C6)Cl_3]$ was prepared according to a literature procedure for the synthesis of $[Ru(tpy)Cl_3]$,[35] with tpyA18C6 used in place of the 2,2′:6′,2″-terpyridine ligand. $(Et_4N)[Ru(tpy)(CN)_3]$ was prepared according to a literature reported method[16,17] for control studies.

Synthesis of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$: The synthesis of $(Et_4N)[Ru(tpyA18C6)(CN)_3]$ was accomplished by following a previously described method[16,17] for the synthesis of $(Et_4N)[Ru(tpy)(CN)_3]$ with modification. $[Ru(tpyA18C6)Cl_3]$ (160 mg, 0.23 mmol) and $(Et_4N)CN$ (303 mg, 2 mmol) were heated to reflux in a mixture of ethanol (10 mL), water (10 mL), and DMF (1.5 mL) for 4 days. The solution was allowed to cool and the solvents were removed under vacuum. The residue was dissolved in ethanol and was subjected to chromatography on a basic alumina column with ethanol as eluent. The purplish-brown band was collected, and the solvent was removed under reduced pressure to afford the product. Recrystallization by slow diffusion of diethyl ether vapor into a solution of the complex in methanol gave the product as brown needles. ^{1}H NMR (300 MHz, [D$_4$]methanol, 298 K): $\delta = 1.29$ (t, $J = 7.2$ Hz, 12H; -CH_3), 3.30 (m, 8H; -CH_2-), 3.52 (m, 4H; -CH_2O-), 3.58 (m, 4H; -CH_2O-), 3.64 (m, 4H; -CH_2O-), 3.71 (m, 4H; -CH_2O-), 3.86 (t, $J = 10.2$ Hz, 4H; -CH_2O-), 3.94 (t, $J = 8.4$ Hz, 4H; -N(-CH_2-)$_2$), 7.33 (q, $J_1 = 8.4$ Hz, $J_2 = 5.5$ Hz, 2H; terpy), 7.76 (s, 2H; terpy), 7.84 (t, $J = 8.4$ Hz, 2H; terpy), 8.28 (d, $J = 8.4$ Hz, 2H; terpy), 8.98 ppm (d, $J = 5.5$ Hz, 2H; terpy); Negative ESI-MS (methanol): m/z: 674 $[M]^-$; elemental analysis calcd (%) for $(Et_4N)[Ru(tpyA18C6)(CN)_3]\cdot 3H_2O$: C 53.19, H 7.05, N 13.06; found: C 53.24, H 6.93, N 13.19; IR (KBr disk): $\tilde{\nu} = 2091$ (m), 2060 (vs), 2035 cm^{-1} (s; C≡N).

Physical measurements and instrumentation: The UV/VIS spectra were recorded on a Hewlett–Packard 8452 A diode array spectrophotometer, and steady-state excitation and emission spectra on a Spex Fluorolog 111 spectrofluorometer. Low-temperature (77 K) spectra were recorded by using an optical Dewar sample holder. Proton NMR spectra were recorded on a Bruker DPX-300 Fourier Transform NMR spectrometer with chemical shifts reported relative to tetramethylsilane. ^{13}C NMR spectra, ^{1}H-^{13}C HETCOR, and HMBC two-dimensional spectra were recorded on a Bruker DPX-600 Fourier Transform NMR spectrometer. Negative-ion EI mass spectra were recorded on a Finnigan MAT95 mass spectrometer. Elemental analysis of the new complex was performed on a Carlo Erba 1106 elemental analyzer at the Institute of Chemistry of the Chinese Academy of Sciences in Beijing. Cyclic voltammetry (CV) and electrochemiluminescence (ECL) experiments were performed with the model 600 A electrochemical workstation (CH Instruments, Austin, TX). A 2 mm-diameter platinum electrode was employed as the working electrode. The reference electrode was a $Ag/AgNO_3$ electrode, and a Pt wire was used as the counter electrode. Before each experiment, the Pt working electrode was subjected to repeated scanning in the potential range of −0.65 to 1.2 V (vs. SCE) in 0.1 M phosphate buffer until reproducible voltammograms were obtained, then rinsed with Milli-Q water and HPLC acetonitrile. TPA (0.1 M) was used as the ECL co-reactant. The ECL signals along with the CV were measured with a photomultiplier tube (PMT, Hamamatsu R928) installed under the electrochemical cell. A voltage of −800 V was supplied to the PMT with the Sciencetech PMH-02 (Sciencetech Inc., Hamilton, Ontario, Canada). All potentials reported were with reference to $Ag/AgNO_3$.

The high-performance liquid chromatograph (HPLC) system consists of a Waters 600E multisolvent delivery system with a Waters ILD in-line degasser, a 20 μL sample injector (Waters Associates U6K), and a Waters 2487 dual λ absorbance detector. The detection wavelength was 445 nm, which corresponded to the MLCT absorption maximum of the complex. A macroporous poly(styrenedivinylbenzene) 150 mm × 4.1 mm, 10 μm spherical, prepacked column (PRP-1) was obtained from Hamilton Co. Chromatograms were recorded and analyzed by Millenium[32] software from Waters Instrument Inc. The analyte concentrations were 0.1 mg mL^{-1}. The concentration of the complex in a mobile phase of 0.1 mM succinate buffer (pH 5.8) was 0.05 mM and chromatography was carried out at a flow rate of 0.5 mL min^{-1} at room temperature unless otherwise specified. The column was conditioned prior to use with the

desired mobile phase by passing the mobile phase until equilibrium was reached. The retained complex could be rapidly removed from the PRP-1 column with methanol after the experiment.

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