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Red-Emitting Ru(II) Metal–Ligand Complexes

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A series of $Ru(N–N)_2$(dcbp or mcmabp) complexes were synthesized where N-N represents a diimine ligand, dcbp is 4,4′-dicaboxylic 2,2-bipyridine, and mcmabp is 4,4′-2,2′-bipyridine monocarboxy amide. The N-N ligand was either 2,2′-bipyridine (bpy), 4,4′-dimethyl-2,2′-bipyridine (dmbp), or 4,4′,5,5′-tetramethyl-2,2′-bipyridine (tmbp). We observed a red shift in both the absorption and emission with the presence of dcbp in the mixed ligand complex when compared to $Ru(bpy)_3$. Conversion of one of the carboxylic groups in dcbp to amide resulted in further red shifts. A red shift in the absorption and the emission was also observed with increasing numbers of electrons donating methyl substitutions on bpy. The lifetimes of the complexes decreased as expected with the red shift emission. The tris heteroleptic Ru (tmphen)(tmbp)(dcbp or mcmabp) were the most red-shifted among the complexes. Because of the red shift in the absorption, the maxima in the fundamental anisotropies of the mixed ligand Ru complexes are now coincident with the maxima in the absorption. Overall we have been able to make an Ru complex which emits at about 700 nm in all aqueous environment and has a lifetime of about 220 ns.

KEY WORDS: Ru MLCT; heteroleptic; red; anisotropy; mixed ligand.

INTRODUCTION

There has been considerable progress in developing and adapting Ru- and Re-based metal–ligand complexes (MLCs) as suitable luminophores for biochemical and biophysical applications. These MLCs have lifetimes of a few 100 ns to several microseconds in solution, good photostability, large Stoke's shift in the emission, and intrinsic polarization (see reviews in references [1–4]). The long lifetimes allow suppression of background fluorescence, which typically decays in less than 10 ns. The longer lifetimes also facilitate measurements of slower events in macromolecules not seen using nanosecond-lifetime fluorophores. These include domain motions in proteins, diffusion in membranes, and hydrodynamics of large macromolecules, [5–9]. Most Ru(II) complexes that are made up with either 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen) emit around 600 nm. One characteristic of these MLCs is that the nature of the ligands on a Ru(II) complex affects the photophysical properties of the complex. Typically, the lifetimes follow the so-called energy gap law [10,11] which results in a decrease in the lifetime and the quantum yield as the emission shifts to longer wavelengths. Hence, we are attempting to develop Ru(II) complexes, that emit beyond 680 nm and have reasonably high quantum yields.

There are several advantages in using red-absorbing and red/near-infrared (NIR)-emitting fluorophores. The biological materials are significantly more transparent and there is much less background fluorescence at red and NIR wavelengths. There are red-emitting MLCs that are based on Os with diimine ligands with emission maxima approximately 750–800 nm. However, these Os com-

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ABBREVIATIONS: dcbp, 4,4′-dicaboxylic-2,2-bipyridine: mcmabp, 4,4′-2,2′-bipyrodine monocarboxy amide; dmbp, 4,4′-dimethyl-2,2′-bipyridine; tmbp, 4,4′,5,5′-tetramethyl-2-2′-bipyridine; bpy, 2,2′-bipyridine; ns, nanosecond; μs, microsecond; tmphen, 3,4,7-8-tetramethyl-1, 10-phenanthroline; MLCT, metal to ligand charge tranfer; DCC, N,N′-dicyclo-hexylcarbodimide; RQY, relative quantum yield.

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plexes are also short-lived, with lifetimes typically less than 50 ns in aqueous solution and quantum yields less than 0.005 (unpublished results). The goal to make Ru complexes that absorb and emit significantly to the red of $Ru(bpy)_3$ with marginal loss in quantum yield and lifetime is an open area of research [12,13].

The excited-state properties of these MLCs are dependent on the pattern of empty low-lying electronic levels that are ligand dependent, and in particular dependent on the ligand π^* energies and the $d\pi$ levels at the metal [1–4]. For mixed ligand Ru complexes, it is therefore possible to some extent fine tune the photophysical properties by the choice of ligands. For example, the use of a ligand with electron-withdrawing subsititution such as– $-COOH$ or $COOC_2H_5$ will lead to emission at higher wavelengths because of their lower π^* levels, and hence smaller energy gap between the $d\pi$ orbitals of the metal center and the π^* orbital of the ligand. The use of ligands that have electron-donating substitutions such as methyl groups will stabilize the hole at the metal center and increase the energy gap, which will increase the quantum yield and lifetimes. Several such examples have been reported [12–18]. There are Ru(II) complexes in the literature, as expected, which emit at longer wavelengths when compared to $Ru(bpy)_3$ or $Ru(phen)_3$. Almost all of the published reports of such red-emitting Ru complexes are in organic solvents and in the absence of oxygen. In many cases the ligands in these complexes are bulky and hydrophobic, which limits their use in biochemical systems. It is also important to consider the different possible photophysical properties of such complexes in polar and nonpolar solvents, which can be quite different.

To make brighter red-emitting Ru complexes, we synthesized a series of MLCs in which 4,4′-dicarboxylic acid-2,2′-bipyridine (dcbpy) was the lowest π^* ligand, whereas the remaining ligands were bpy, dimethyl bipyridine (dmbp), or tetramethyl bipyridine (tmbpy). Because our main interest is in biophysical applications, we also derivatized one of the carboxyl groups into an amide (4-carboxy-4′-amide bipyridine [mcmabp]) to represent the probe when conjugated to a macromolecule. To improve the quantum yields, we also made a tris heteroleptic Ru (tmphen)(tmbp)(dcbp) and its amide derivative Ru(tmbp)(tmphen)(mcmabp), where tmphen is tetramethyl 1,10-phenanthroline. The use of 1,10′-phenanthroline to replace a 2,2′-bipyridine was based on literature data [1,2], which reported higher quantum yields with phen ligands. In this communication, we report their luminescence properties including lifetimes in aqueous environment in both the presence and absence of oxygen. Some of the anisotropies at $-55°C$ in glycerol are also reported.

EXPERIMENTAL

All of the chemicals were obtained from Aldrich and used as such unless noted otherwise. The absorption spectra were taken on a HP8453 diode array spectrophotometer (Hewlett Pachard). The steady-state fluorescence measurements were carried out with a Cary Eclipse fluorescence spectrophotometer with the slits set at 5 nm. The absorption of the solutions was near 0.05 optical density (OD). The lifetimes were measured on a ISS K2 frequency domain instrument with a blue light-emitting diode (LED) light source in a setup similar to that used by Sipior *et al.* [19]. The excitation was selected through a 450-nm centered bandpass filter. Rhodamine B aqueous solution with a lifetime of 1.68 ns was used as a reference. To remove dissolved oxygen, nitrogen was flushed through a closed cuvette for 40 min for aqueous solutions. We did not attempt to eliminate the last traces of oxygen, although we expect solutions to be essentially free from dissolved oxygen. Mass spectrometry (MS/MS) data were obtained using a Finnegan/Thermoquest LCQ-MS ion trap instrument in the School of Pharmacy, University of Maryland, Baltimore.

Synthesis

tmbp

Ten grams of 3,4-lutidine (98%) was mixed with 0.8 g of Pd on carbon (5%). The mixture was allowed to reflux under nitrogen for 4 days. The mixture became highly viscous at the end of this period. It was cooled to room temperature and 50 mL of methylene chloride was added. Then the reaction sample was filtered to remove the catalyst and carbon. The filtrate was collected and the solvent and the unreacted lutidine were removed under reduced pressure. The product was recrystallized in ethyl acetate. ^1H NMR ($CDCI_3$, 300 MHz): δ 2.32 (s, 6H), 8.11 (s, 2H), 8.38 (s, 2H).

$Ru(bpy)_2Cl_2$ $Ru(dmbp)_2Cl_2$, and $Ru(tmbp)_2Cl_2$

These three compounds were prepared by the method of Togando *et al.* [20] for $Ru(bpy)_2Cl_2$ and were used without purification.

$Ru(N\text{-}N)_2(dcbp)(PF_6)_2$

Two hundred milligrams of $Ru(N\text{-}N)_2Cl_2$ (where N-N is either bpy or dmbp or tmbp) and 1.1 equivalent of dcbp were dissolved in 30 mL of ethanol/water (1:1

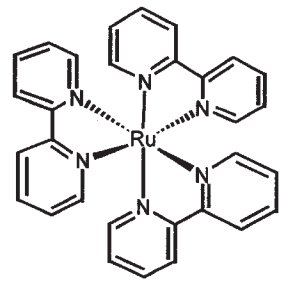
vol/vol) and heated at reflux for 10 hr to give a red solution. After filtration, the red solution was treated with an aqueous solution of ammonium hexafluorophosphate (150 mg in 30 mL of water). A bright orang-red precipitate was formed. This precipitate was filtered off, washed with water, and then washed with ether. The product was obtained after running a silica gel column with a mixture of methylene chloride and methanol (1:1 vol/vol) as the eluent. The Ru(II) complexes were identified by coupling two stages of mass analysis (MS/MS) conducted on a Finnegan/Thermoquest LCQ-MS ion trap mass spectrometer. Data obtained were follows: $[Ru(bpy)_2(dcbpy)](PF_6)_2$ Mol. ion (*m/z*) calculated: 946.57, found: 946.26; $[Ru(dm\text{-}bpy)_2(dcbpy)](PF_6)_2$ Mol. ion (*m/z*) calculated: 1003.68, found: 1003.44; $[Ru(tm\text{-}bpy)_2(dcbpy)](PF_6)_2$ Mol. ion (*m/z*) calculated: 1059.78, found: 1059.39.

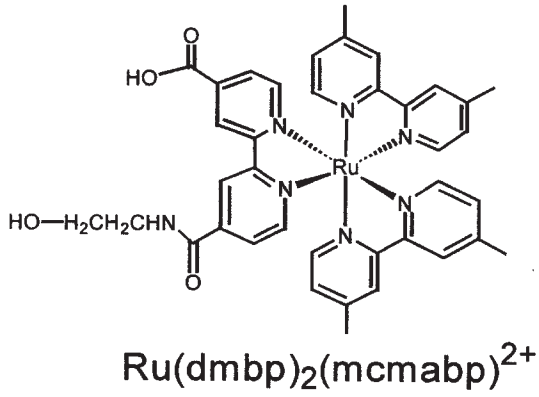
Ru(tmphen)(tmbp)(dcbp)(PF6)2

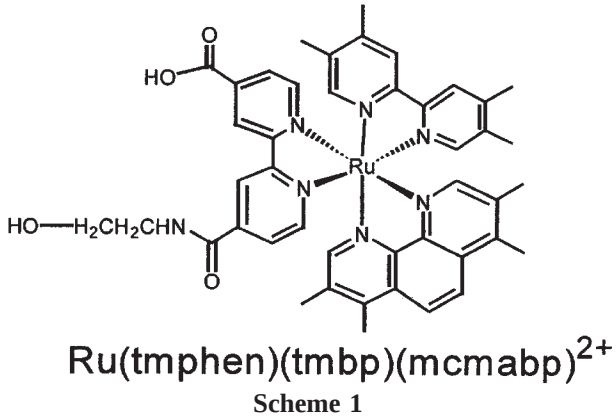
This compound was synthesized according to the method of Hesek *et al.* [21] and purified on a short silica gel column. Data obtained were as follows: $[Ru(tm\text{-}bpy)(tm\text{-}phen)(dcbpy)](PF_6)_2$ Mol. ion (*m/z*) calculated: 1083.80, found: 1083.48.

Amidation of the Carboxylic Group

The amidation of one of the carboxylic groups in the bpy ligand was done according to standard procedures. Typically, 5 mg of the Ru complex was reacted with 1.1 equivalent *N,N′*-dicyclohexyl carbodimide (DCC) and *N*-hydroxysuccinimide in a small volume of dry dimethyl formamide (DMF). After 24 hr, an equimolar amount of dry triethylamine and ethanolamine were added and the reaction was allowed to proceed for another 24 hr. The products were isolated on a semipreparative reversed-phase high performance liquid chromatography (RP-HPLC) using the C-18 column and only the central region of the peak. The gradient was made up of acetonitrile in water, both containing 0.1% trifluoroacetic acid. The starting Ru(II) complex was clearly separated by its much longer retention time.







Scheme 1

RESULTS AND DISCUSSION

The structures of a bis heteroleptic and a tris heteroleptic Ru complex along with that of $Ru(bpy)_3$ are given in Scheme 1. The absorption spectral properties are summarized in Table I and some representative examples are presented in Fig. 1. For clarity, we chose to show the absorption spectra of $Ru(bpy)_3$, $Ru(dmbp)_2(mcmabp)$, and Ru(tmphen)(tmbp)(mcmabp), which cover the entire range. The $Ru(bpy)_3$ is characterized by a ligand-centered absorption around 287 nm, a shallow valley in the 300- to 400-nm region, a metal-to-ligand charge transfer (MLCT) band (which has a shoulder around 425 nm), and a peak at 455 nm. There is little absorption beyond 520 nm. Upon replacing one bpy with a dcbp, we observed two changes. There is a significant increase in the

Table I. Room-Temperature Luminescence Properties of Mixed-Ligand Red Ru Complexes in 1.0 M tris HCI, pH 7.0

Sample	MLCT (nm)	λ_{em} (nm)	RQY[a]	τ_{air} (ns)	τ_{N2} (ns)
$Ru(bpy)_3$	455	610	1.00	370	583
$Ru(bpy)_2(dcbp)$	480	690	0.40	264	315
$Ru(dmbp)_2(dcbp)$	474	695	0.35	230[a]	280
$Ru(tmbp)_2(dcbp)$	480	694	0.25	177	197
$Ru(bpy)_2(mcmabp)$	482	690	0.37	256[b]	285[b]
$Ru(dmbp)_2(mcmabp)$	490	699	0.24	162[b]	180[b]
$Ru(tmbp)_2(mcmabp)$	503	715	0.13	113[b]	119[b]
Ru(tmphen)(tmbp)(dcbp)	485	672	0.35	294	N/A[c]
Ru(tmphen)(tmbp)(mcmabp)	505	700	0.31	219	N/A
$Os(diSO_3\text{-}dpphen)_3$		731	0.08	65	N/A

[a] Relative quantum yield (RQY) are accurate to ± 0.05.
[b] Heterogeneous $\langle\tau\rangle$.
[c] These compounds were strongly quenched by oxygen, so the oxygen-free lifetimes were difficult to determine.

absorption in the 300- to 400-nm region and the MLCT absorption maxima shifts to red. The values of the maxima of the lowest energy MLCT band are 455, 482, 474, and 480 nm, respectively, for the series $Ru(bpy)_3$, $Ru(bpy)_2(dcbp)$, $Ru(dmbp)_2(dcbp)$, and $Ru(tmbp)_2(dcbp)$. Upon converting one of the carboxyls to an amide, a further red shift is observed. The maxima for the series are now 482, 490, and 503 nm. In addition, the long wavelength end of the absorption spectrum in the most red-shifted derivatives goes past 560 nm. For the tris heteroleptic Ru complex, there is an additional increase in absorption in the 300- to 400-nm region. The shallow valley disappears and the maxima for dcbp and mcmabp derivatives are 485 and 502 nm, respectively.

The maxima of the emission in a buffer solution of 1.0 M tris HCl (pH 7.0) of these complexes are summarized in Table I and some representative emission spectra are given in Fig. 2. The emission maxima are 610, 690, 665, and 694 nm, respectively, for $Ru(bpy)_3$, $Ru(bpy)_2(dcbp)$, $Ru(dmbp)_2(dcbp)$, and $Ru(tmbp)_2(dcbp)$. Upon amidation of one of the carboxylic groups, the maxima become 690, 699, and 715 nm, respectively, for the complexes containing bis bpy, dmbp, and tmbp. For the tris heteroleptic complexes, the emission maxima are at 672 and 700 nm, respectively, for the dcbp and mcmabp ligand. As seen in Fig. 2, significantly more emission is observed above 700 nm, and in most of these complexes it extends up to 860 nm. For comparison, we have also included the emission spectrum of one of the brighter and water-soluble Os complexes, $Os(diSO_3\text{-}dpphen)_3$, which has a maximum at about 730 nm.

The red shift in the absorption and the emission with the use of dcbp in our Ru(II) complexes was expected. The ligand dcbp has significantly lower π^* levels when compared to bpy, which in turn will result in lowered energy gap. A similar red shift in the absorption and the

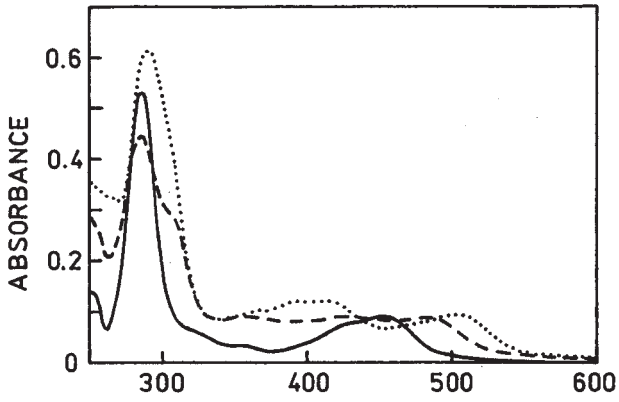


Fig. 1. The absorption spectra of complexes — Ru $(bpy)_3$, - -- $Ru(dmbp)_2(mcmabp)$, and ·····Ru(tmphem)(tmbp)(mcmabp) in 1.0 M tris HCI, pH 7.0. The concentrations were 1.4×10^{-5} M.

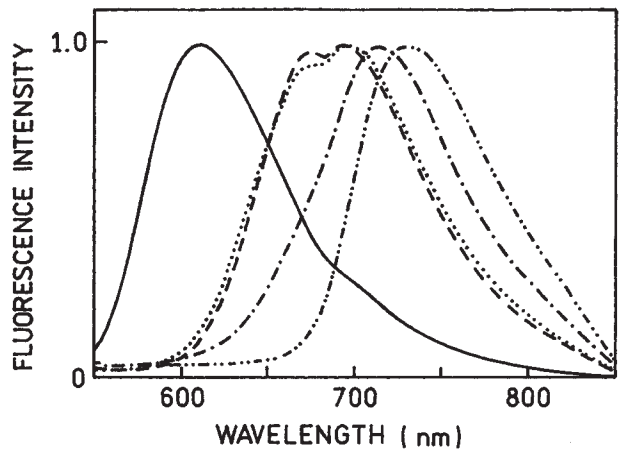


Fig. 2. The emission spectra of — Ru $(bpy)_3$, - -- $Ru(dmbp)_2(mcmabp)$, ·····$Ru(dmbp)_2(mcmabp)$, - · - · - Ru(tmphen)(tmbp)(mcmabp), and ·· - ·· $Os(diSO_3\text{-}dpphen)_3$ in 1.0 M tris HCI, pH 7.0.

emission has been seen in several mixed-ligand Ru complexes in which the emitting ligand has lower π^* energies than the ligand it replaced [12–18]. In our case, the other two ligands in our Ru complexes are the ones with electron-donating groups, such as dmbp, tmbp, and tmphen. The purpose of using electron-donating ligands is to increase the ground state (t_{2g}) energy level of the complex. Therefore, in our bis heteroleptic Ru complexes containing methylated diimine ligands and dcbp, the energy gap between the t_{2g} of ruthenium center and π^* of the dcbp is further reduced. Amidation of one of the two carboxylates in dcbp breaks the symmetry of the ruthenium polypyridyl complexes. The resulting degeneration of the excited states also seems to lead to red-shifted emission.

The relative quantum yield (RQY) in buffer in the presence of oxygen for all of these complexes is given in Table I. The complex $Ru(bpy)_3$ was taken as unity. In the dcbp series, the RQY values decreased to 0.40, 0.35, and 0.25, respectively, for the bis bpy, dmbp, and tmbp Ru(II) complexes. Upon amidation of one of the carboxylic groups, the values are 0.37, 0.24, and 0.13. There was little effect of amidation on $Ru(bpy)_2$(dcbp) complex. This is not surprising because there was also little change in the emission spectrum. However, in cases of complexes containing dmbp and tmbp, we observe a significant decrease in the RQY. Because there were also significant red shifts in the emission of these two complexes upon amidation, these decreased RQYs are probably a direct consequence of the energy gap law [10]. Overall, we achieved a red shift of approximately 80–90 nm with a concomitant decrease in RQY by a factor of 3. An additional red shift of about 15 nm in the case of $Ru(tmbp)_2$(mcmabp), however, also decreased the RQY by half, a rather steep decline. For the tris heteroleptic Ru complexes, the RQY values are 0.35 and 0.31, respectively, for dcbp and mcmabp complexes. Even though both the $Ru(tmbp)_2$(mcmabp) and Ru(tmphen)(tmbp)(mcmabp) emit around 700 nm, the tris heteroleptic complex has a higher RQY value. This may be due to a decrease of the nonradiative decay rate by using a more rigid ligand (tmphen) in the tris hereroleptic complex. There are examples in the lifetime that show that replacement of a bpy by an analgous phen can increase quantum yields in Ru complexes [12]. It seems that it still holds true in a mixed ligand Ru complex.

Figure 3 shows excitation anisotropy spectra for $Ru(dmbm)_2$(mcmabp) and Ru(tmphen)(tmbp)(mcmabp) at −55°C in glycerol. The anisotropy values decrease to close to zero in the initial part of the MLCT band from about 425–430 nm and gradually increase to about 0.20 around 490 nm. At higher wavelengths there is a

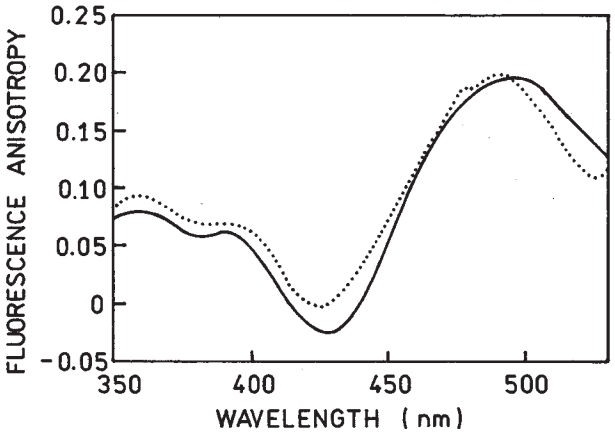


Fig. 3. The excitation anisotropy spectra at −60°C in glycerol of —— $Ru(dbbp)_2$(mcmabp) and ····Ru(tmphen)(tmbp)(mcmabp).

decline in the anisotropy. The shape and the absolute values of the fundamental anisotropies seen with these two Ru complexes are similar to those seen with conjugated derivatives of Ru complexes made up of either mono- or dicarboxylic acid bpy [7]. A noteworthy feature of these mcmabp derivatives is that both the absorption maxima and the wavelengths of anisotropy maxima are similar. It should therefore be possible to excite these complexes with a light source emitting around 490 nm, which is commonly used to excite fluorescein, and excite the maximum of both the absorption and the limiting anisotropy of the complex. Because the limiting anisotropy of the mixed complexes seems to depend on the ligand with the lowest π^* energies, it should be possible to obtain even higher anisotropy by replacing dcbp with a ligand of lower π^* energy.

The lifetime values for all of the complexes in a buffer solution are given in Table I. The decays were either monoexponential (the main component greater than 99% of the intensity) or somewhat heterogeneous, in some cases involving dmbp and tmbp ligand. However, even in these cases the main component was the long-lived one. In the presence of oxygen in the buffer, the lifetimes are 370, 264, 230, and 177 ns, respectively, for $Ru(bpy)_3$, $Ru(bpy)_2$(dcbp), $Ru(dmbp)_2$(dcbp), and $Ru(tmbp)_2$(dcbp). For the amidated derivative, the lifetime values in presence of oxygen are 256, 162, and 113 ns, respectively, for bpy, dmbp, and tmbp derivatives. The general trend is similar to that seen in quantum yields. Upon removal of the dissolved oxygen, the lifetime values increased to 583, 315, 280, and 197 ns, respectively, for $Ru(bpy)_3$, $Ru(bpy)_2$(dcbp), $Ru(dmbp)_2$(dcbp), and $Ru(tmbp)_2$(dcbp). In amidated series, the lifetime values are 285, 180, and 119 ns in the absence of oxygen. For tris heteroleptic Ru complexes, the lifetime values are 294 and 219 ns for

dcbp and mcmabp ligand, respectively, in the presence of oxygen. It should be noted that the increases upon oxygen removal are more pronounced for longer-lived complexes, as expected. It is difficult to compare our luminescence lifetimes in an aqueous environment with published values for mixed-ligand red-emitting Ru complexes made up with different low π^* ligands because all of the published values are in organic solvents. The main reason is probably that the MLC excited states are sensitive to the local environment.

CONCLUSIONS

We have been able to shift the emission of Ru complexes by 80–90 nm to the longer wavelength with less than three-fold decrease in the quantum yield. The accompanied decreases in lifetimes are less than two-fold. For example, Ru(tmphen)(tmbp)(mcmabp) has a lifetime of about 219 ns when compared to 370 ns for $Ru(bpy)_3$, whereas the emission is red shifted by 90 nm. The dcbp may not be the best ligand of choice. The commercial availability became the driving factor. We can visualize replacing it with 2,2′-bipyridine, 5,5′-C-C-benzoic acid. This ligand has even lower π^* energies and the excited electron in the Ru complex made up with this ligand will be highly delocalized. The electronic delocalization effect results in both an increase in the lifetime and red-emitting complex [12]. A tris heteroleptic complex provides a great deal of freedom in tailoring the properties of the final Ru complex. We believe it should be possible to make even longer lived, more red, and still brighter Ru(II) complexes that are able to conjugate with macromolecules.

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REFERENCES

1. K. Kalayanasundaram (1992) Photochemistry of Polypyridine and Porphyrin Complexes, Academic Press, New York.
2. A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Besler, and A. Von Zelevky (1998) Ru(II) polypyridine complexes: Photophysics, Photochemistry Electrochemistry and chemiluminescence, *Coord. Chem. Rev.* **84**, 85–227.
3. J. N. Demas and B. A. Degraff (1994) Design and applications of highly luminescent transition metal complexes in J. R. Lakowicz (Ed.), *Topics in Fluorescence Spectroscopy: Probe Design and Chemical Sensing*, Vol. 4, Plenum Press, New York, pp. 71–107.
4. E. Terpetschinig, H. Szmacinski, and J. R. Lakowicz (1997) Long lifetime metal-ligand complexes as probes in biophysics and clinical chemistry. *Methods Enzymol.* **278**, 294–321.
5. E. Terpetschinig, H. Szmacinski, H. Malak, and J. R. Lakowicz (1995) Metal-ligand complexes as a new class of long lived fluorophores for protein hydrodynamics. *Biophys. J.* **68**, 342–350.
6. H. Szmacinski, F. N. Castellano, E. Terpetschinig, J. D. Dattelbaum, J. R. Lakowicz, and G. J. Meyer (1998) Long-lifetime Ru(II) complexes for the measurement of high molecular weight protein hydrodynamics. *Biochim. Biophys. Acta* **1383**, 151–159.
7. H. Szmacinski, E. Terpetschinig, and J. R. Lakowicz (1996) Synthesis and evaluation of Ru-complexes as anisotropy probes for protein hydrodynamics and immunoassays of high molecular weight antigens. *Biophys. Chem.* **62**, 109–120.
8. L. Li, F. N. Castellano, I. Gryczynski, and J. R. Lakowicz (1999) Long lifetime lipid rhenium metal-ligand complex for probing membrane dynamics on the microsecond timescale. *Chem. Phys. Lipids* **99**, 1–9.
9. J. Kusba, L. Li, I. Gryczynski, G. Piszczek, and J. R. Lakowicz (2002) Lateral diffusion coefficients in membranes measured by resonance energy transfer and a new algorithm for diffusion in two dimensions. *Biophys. J.* **82**, 1358–1372.
10. J. V. Casper and T. J. Meyer (1983) Photochemistry of MLCT excited states. Effect of nonchromophoric ligand variations on photophysical properties in the series cic $Ru(bpy)_2L$. *Inorg. Chem.* **22**, 2444–2453.
11. E. M. Kober, J. V. Casper, R. S. Lumpokin, and T. J. Meyer (1986) Application of the energy gap law to excited state decay of osmium(II)-polypyridine complexes: calculation of relative nonradiative decay rates from emission spectral profiles. *J. Phys. Chem.* **90**, 3722–3734.
12. G. F. Strouse, J. R. Schoonover, R. Duesing, S. Boyde, W. E. Jones, Jr., and T. J. Meyer (1995) Influence of Electronic Delocalization in Metal to Ligand Charge Transfer Excited States. *Inorg. Chem.* **34**, 473–487.
13. P. A. Anderson, G. B. Deacon, K. H. Haarman, F. R. Keene, T. J. Meyer, D. A. Reitsma, B. W. Shelton, G. F. Strouse, N. C. Thomas, J. A. Treadway, and A. H. White (1995) Designed Synthesis of Mononuclear Tris(heteroleptic) Ruthenium Complexes Containing Bidentate Polypyridyl Ligands. *Inorg. Chem.* **34**, 6145–6157.
14. G. F. Strouse, P. A. Anderson, J. R. Schoonover, T. J. Meyer and F. R. Keene (1992) Synthesis of polypyridyl complexes of ruthenium(II) containing three different bidentate ligands. *Inorg. Chem.* **31**, 3004–3006.
15. W. F. Wacholtz, R. A. Aeurbach, and R. H. Schmehl (1986) Independent control charge transfer and metal centered excited states in mixed ligand polypyridine ruthenium(II) complexes via specific ligand design. *Inorg. Chem.* **25**, 227–234.
16. K. Kalyanasundaram, M. K. Nazeeruddin, M. Gratzel, G. Viscardi, P. Savarino, and E. Barni (1992) Synthesis and photophysical characterization of highly luminescent complexes of ruthenium(II) containing 4,4'-di (p-carboxyphenyl)-2,2'-bipyridine. *Inorg. Chem. Acta.* **198–200**, 831–839.
17. P. A. Anderson, G. F. Strouse, J. A. Treadway, F. R. Keene, and T. J. Meyer (1994) Black MLCT Absorbers. *Inorg. Chem.* **33**, 3863–3864.
18. A. E. Curtright and J. K. McCusher (1999) Static and Time Resolved Spectroscopic Studies of Low Symmetry Ru(II) Polypyridyl Complexes. *J. Phys. Chem. A* **103**, 7032–7041.
19. J. Sipior, G. M. Carter, J. R. Lakowicz, and G. Rao (1997) Blue light emitting diode demonstrated as an ultraviolet excitation source for nanosecond phase modulation flourescence lifetime measurements. *Rev. Sci. Instrum.* **68**, 2666–2670.
20. T. Togano, N. Nagao, M. Tsuchida, H. Kumakura, K. Hisamatsu, F. S. Howell, and M. Mukaida (1992) One pot and selective synthesis of a series of $[RuCl_{6-2n}L_n]$ (L bidentate ligand n = 0-3) types of complexes with polypyridyl ligands, another example of the synthetic utility of 'ruthenium-blue' solution. *Inorg. Chim. Acta* **195**, 221–225.
21. D. Hesek, Y. Inoue, S. R. L. Everitt, H. Ishida, M. Kunieda, and M. G. B. Drew (2000) Novel Synthetic Routes to Several New, Differentially Substituted Ruthenium Tris(4,4'-disubstituted-2,2'-bipyridine) Complexes *Inorg. Chem.* **39**, 308–316.