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Ruthenium(II) Bipyridyl Complexes as Photolabile Caging Groups for Amines

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The synthesis and characterization of a series of ruthenium bis(bipyridine) complexes where the inorganic moiety acts as a photolabile protecting group is described. Complexes of the type $[Ru(bpy)_2L_2]^+$ where bpy = 2,2′-bipyridine and L = butylamine, γ-aminobutyric acid, tyramine, tryptamine, and serotonin were studied by nuclear magnetic resonance, cyclic voltammetry, and electronic absorption spectroscopy. In all cases, ligands are coordinated by the amine group. The complexes are stable in water for several days and deliver one molecule of ligand upon irradiation with visible light (450 nm). These properties make them suitable for their use as biological caged compounds.

Introduction

Caged compounds are powerful experimental tools in physiology since they provide a means for rapid and localized delivery of bioactive substances.[1] In the last few years, a number of photolabile protecting groups have been developed for the caging of a variety of biomolecules.[2] Chemical bonds established between bioactive ligands and proper protecting groups should be strong enough to be water-stable and weak enough to be broken with low-energy, tissue-innocuous photons. Most available caged compounds are based on the use of nitrobenzyls or nitrophenyls as protecting groups.[2] These caged compounds show releasing wavelengths below 350 nm, which can damage living tissue. It is also desirable for protecting groups to be easily derivatizable in order to allow versatile interaction with inorganic or organic structures such as polymers or cell membranes.

In a recent work, we presented the use of metal complexes as photolabile protecting groups for biomolecules, giving birth to a new family of inorganic caged compounds.[3] The first member of that family was the potassium channel blocker 4-aminopyridine (4AP) caged compound $[Ru(bpy)_2(4AP)_2]^{2+}$. The coordination bond between the ruthenium and the aromatic nitrogen of 4AP is water-stable and can be broken with visible light around 470 nm. The bipyridines provide easy chemical derivatization. The general relevance of the advantages conveyed by inorganic caged compounds relies on the diversity of biomolecules that may be coordinated to the metal center.

On this occasion, we show that ruthenium polypyridines can also act as protecting groups for amines, extending the scope of action for this class of caged compounds. We report the synthesis and characterization of ruthenium bis(bipyridine) caged compounds of the neurotransmitters serotonin (5HT) and γ-aminobutyric acid (GABA), together with those of the analogues tryptamine, butylamine, and tyramine (see Chart 1).

Experimental Section

All reagents were commercially available and used as received. $Ru(bpy)_2Cl_2$ was synthesized according to the literature.[4] The UV−vis spectra were taken with a HP8452A diode-array spectrometer. NMR spectra were obtained using a 500 MHz Bruker AM-500. Fluorescence measurements were made with a PTI Quantamaster spectrofluorometer.

Voltagrams were obtained in CH_3CN/0.1M $TBAPF_6$ using a three-electrode potentiostat based on an operational amplifier TL071

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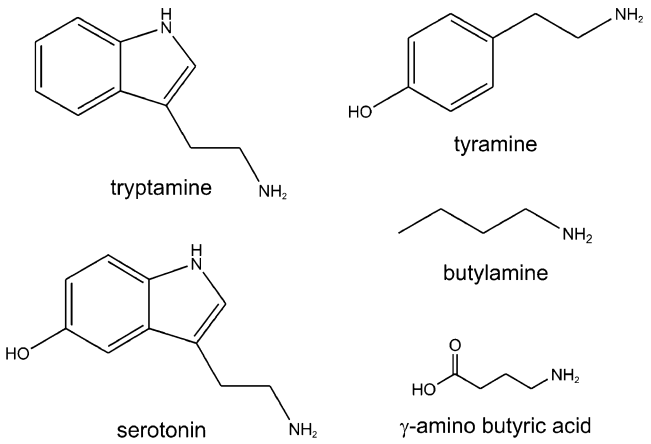
Chart 1. Ligands Used in This Work



in current-to-voltage configuration[5] and an acquisition software written in QB 4.5. A Pt wire with a diameter of 500 μm was used as the work electrode. All syntheses were done by degassing the solutions with N_2 prior to heating to prevent oxidation of the Ruthenium aquo complexes. Visible light irradiation of samples was performed using a Luxeon Star III Royal Blue high power light-emitting diode (LED).

Synthesis. 1. $[Ru(bpy)_2(L)_2](PF_6)_2$ for L = Butylamine, Tryptamine, Tyramine, and Serotonin (5HT). $Ru(bpy)_2Cl_2$ (100 mg) was suspended in 10 mL of distilled water; N_2 was bubbled for 15 min, and the suspension was heated at 80 °C until total dissolution. The formation of the $[Ru(bpy)_2(H_2O)_2]^{2+}$ complex was determined by its band at 480 nm.[6] After formation of the diaquo complex, 5−10 equivalents of the ligand dissolved in a small amount of EtOH was added, and the solution was heated until no further changes in the UV−vis spectrum at pH 12 were apparent. The solution was filtered to remove any insoluble particles and precipitated with NH_4PF_6 saturated solution after cooling. The precipitate was washed with several portions of cold water. NMR (acetone-d_6), L = butylamine: ^{1}H δ 0.65 (t, 6H), 1.04 (m, 2H), 1.37 (m, 2H), 1.96 (m, 2H), 2,05 (m, 4H), 2.13 (m, 2H), 3.86 (t, 2H), 4.06 (t, 2H), 7.32 (t, 2H), 7.78 (d, 2H), 7.94 (t, 2H), 7.97 (t, 2H), 8.33 (t, 2H), 8.60 (d, 2H), 8.79 (d, 2H), 9.46 (d, 2H).

2. $[Ru(bpy)_2(L)_2]Cl_2$ for L = Butylamine, Tryptamine, Tyramine, and Serotonin (5HT). The PF_6^- salt was dissolved into a minimum amount of acetone. Drops of tetrabutylammonium chloride saturated in acetone were added until total precipitation of the chloride salt. The precipitate was washed with several portions of acetone and dried. NMR (D_2O), L = tyramine: ^{1}H δ 1.92 (m, 2H), 2.28 (m, 2H), 2.45 (m, 4H), 3.07 (t, 2H), 3.19 (t, 2H), 6.68 (d,4H), 6.73 (d, 4H), 7.05 (t, 2H), 7.47 (d, 2H), 7.68 (t, 2H), 7.75 (t, 2H), 8.13 (t, 2H), 8.24 (d, 2H), 8.39 (d, 2H), 8.66 (d, 2H). NMR (D_2O), L = serotonin: ^{1}H δ 1.91 (m, 2H), 2.41 (m, 2H), 2.62 (m, 4H), 2.88 (t, 2H), 3.13 (t, 2H), 6.31 (s, 2H), 6.85 (dd, 2H), 6.90 (s, 2H), 6.94 (t, 2H), 7.29 (d, 2H), 7.33 (d, 2H), 7.46 (t, 2H), 7.61 (t, 2H), 7.85 (d, 2H), 7.87 (d, 2H), 7.91 (t, 2H), 8.54 (d, 2H). NMR (D_2O), L = tryptamine: ^{1}H δ 2.02 (m, 2H), 2.37 (m, 2H), 2.75 (m, 4H), 3.05 (t, 2H), 3.12 (t, 2H), 7.02 (s, 2H), 7.04 (t, 2H), 7.12 (m, 4H), 7.39 (m, 4H), 7.42 (t, 2H), 7.56 (d, 2H), 7.73 (t, 2H), 7.94 (t, 2H), 8.03 (d, 2H), 8.06 (d, 2H), 8.49 (d, 2H).

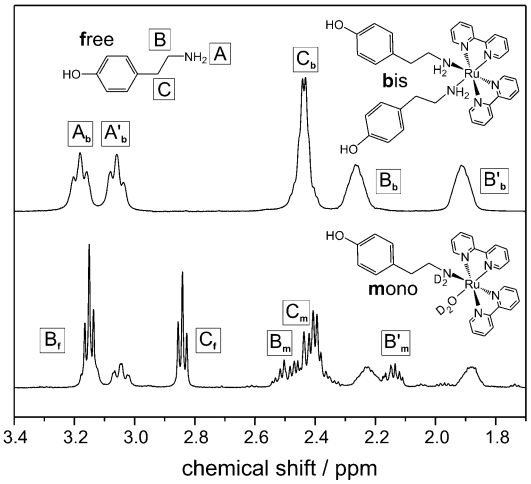


Figure 1. ^{1}H NMR spectra of $[Ru(bpy)_2(tyr)_2]^{2+}$ in D_2O showing tyramine signals. The upper trace shows the disubstituted complex in the dark (bis, b). The lower trace shows the partial photolysis of $[Ru(bpy)_2(tyr)_2]^{2+}$ with visible light around 450 nm, after proton exchange, yielding the monosubstituted complex $[Ru(bpy)_2(tyr)(H_2O)]^{2+}$ (mono, m) and free tyramine (free, f).

3. $[Ru(bpy)_2(GABA)_2](PF_6)_2$. The previous procedure was followed, but precipitation was achieved by using 60% HPF_6 in water and leaving the suspension on ice for 1 h. No further anion changes were performed. NMR (D_2O): ^{1}H δ 1.65 (m, 4H), 1.85 (m, 2H), 2.03 (m, 2H), 2,17 (t, 4H), 3.12 (t, <1H), 3.27 (t, <1H), 7.20 (t, 2H), 7.66 (d, 2H), 7.88 (t, 2H), 7.93 (t, 2H), 8.29 (t, 2H), 8.42 (d, 2H), 8.62 (d, 2H), 9.16 (d, 2H).

Results and Discussion

The identity of the complexes and their irradiation photoproducts were inferred by ^{1}H NMR. Both acetone-d_6 and D_2O solutions produced the free ligands under irradiation with 450 nm light. For acetone studies, the PF_6^- salts were used, while chloride salts were preferred in D_2O measurements. The only exception was the GABA complex, in which the PF_6^- salt can be solubilized in D_2O at neutral or basic pH. The photocleavage of the complexes was also studied by ^{1}H NMR, and the results confirmed that the unique photoproducts were the monoaquo complex and the free amine ligand, with no detectable side products. This can be seen in Figure 1. The fact that there are no side products is very important in terms of the application of these compounds to physiological studies.

In the aromatic region (see Supporting Information), the 8 signals that correspond to the bipyridyl protons split into 16 after irradiation, showing that the symmetric bis-substituted complex leads to an asymmetric monosubstituted product. Figure 1 (top) shows that the signals of the methylene B in the aliphatic chain of the bis-substituted complex appear at 1.92 and 2.28 ppm, displaying different chemical environments for each proton. The signals of the methylene C appear at 2.45 ppm. On the other hand, in the free tyramine, these two methylene signals appear at 3.15 and 2.84 ppm, respectively (Figure 1, bottom). The monosubstituted complex shows intermediate displacements for these signals.

Even in D_2O, the signals of the amine protons of butylamine, tyramine, tryptamine, and 5HT are visible and

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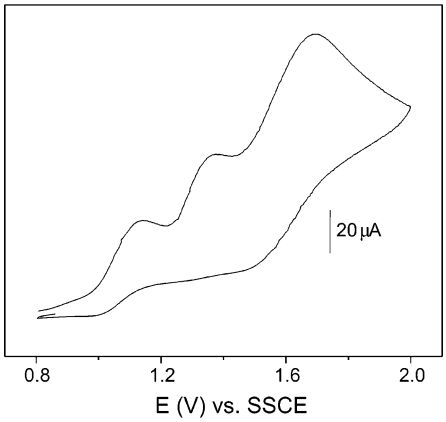

Figure 2. Cyclic voltammetry of native $[Ru(bpy)_2(tyr_2)_2]^{2+}$ at 100 mV/s on Pt wire electrode in CH_3CN containing 100 mM $TBAPF_6$

Table 1. Redox Potentials for All the Complexes in CH_3CN Containing 100 MM $TBAPF_6$ at 298 K[a]

	$E_{1/2}$ (V)		
complex	1st	2nd	3rd
$[Ru(bpy)_2(tyr)_2]^{2+}$	1.10	1.38p	1.69p
$[Ru(bpy)_2(buNH_2)_2]^{2+}$	1.04	1.25	1.48
$[Ru(bpy)_2(GABA)_2]^{2+}$	1.06p	1.25	1.55p
$[Ru(bpy)_2(tryp)_2]^{2+}$	1.11p	1.36p	1.67p
$[Ru(bpy)_2(5HT)_2]^{2+}$	1.03p	1.11p	1.64p

[a] Values are shown vs SSCE. $dE/dt = 100$ MV/s. In the cases where the reversibility is poor, peak potentials (p) are reported

integrate for four protons in the bis-substituted complex. This behavior indicates that the amine group is involved in the coordination to ruthenium, in a way that no further protonation is possible and no proton exchange can occur. If the synthesis is done in deuterated water, proton exchange on the ligand's free amine occurs prior to the coordination, so the proton signals do not appear in the NMR spectrum (see Supporting Information). That the coordination bond is established through the amine group is consistent with the fact that nonaminic carboxylic acids such as butyric, propionic, and benzoic do not coordinate to the metal in aqueous solutions.

After irradiation, the signals of the amine protons in the monosubstituted complex are apparent at 2.97 and 2.63 ppm, but they disappear after a few hours in the dark, suggesting that, in the monoaquo complexes, some equilibrium via labilization of the ligand is taking place (see Supporting Information). A similar result was seen in the GABA complex, but in this case, some proton exchange exists even in the bis-GABA compound. ^1H NMR spectra of all the studied compounds are given as Supporting Information.

Cyclic voltammetry (CV) was used to examine the electrochemical behavior of the complexes. The redox potential for the Ru^{2+}/Ru^{3+} couple and subsequent oxidation of the amines in the bis-tyramine complex can be seen in Figure 2.

The results of all the complexes studied in this work are shown in Table 1. We obtained the expected values for the Ru^{2+}/Ru^{3+} couples, which are in agreement with previous studies of similar compounds that assign the first oxidation wave to the bis-amine complex, the second one to the amine−nitrile complex, and the third one to the bis-nitrile complex.[7]

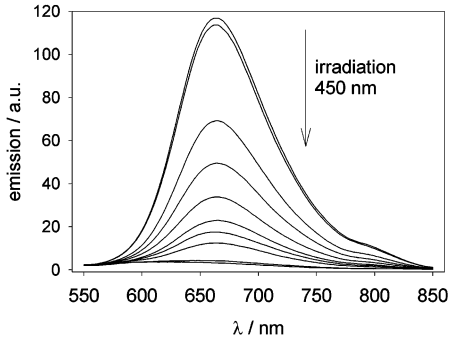

Figure 3. Fluorescence of $[Ru(bpy)_2(tyr_2)_2]^{2+}$ in water at pH 7 at different irradiation times; excitation wavelength = 450 nm.

Table 2. Extinction Coefficients at Maximum Absorption, Quantum Yield of Emission, and Wavelength of Maximum Emission in Water at 293 K

complex	$\epsilon_{max}/M^{-1}\,cm^{-1}$	ϕ	λ_{max}/nm
$[Ru(bpy)_2(tyr)_2]^{2+}$	8940	1.9×10^{-3}	664
$[Ru(bpy)_2(buNH_2)_2]^{2+}$	7910	9.7×10^{-4}	670
$[Ru(bpy)_2(GABA)_2]^{2+}$	8955	1.5×10^{-3}	674
$[Ru(bpy)_2(tryp)_2]^{2+}$	9720	1.8×10^{-3}	666
$[Ru(bpy)_2(5HT)_2]^{2+}$	9880	1.7×10^{-3}	662

The $[Ru(bpy)_2L_2]^{2+}$ complexes usually present fluorescence,[8] and the involved electronic states structure is related with their photoreleasing capabilities. We have measured fluorescence in all the bis-amine complexes, with the quantum efficiency being between 10^{-3} and 2×10^{-3}.

$[Ru(bpy)_3]Cl_2$ ($\phi = 0.042$) was used as an emission standard given its absorption overlap with the analyzed compounds. Figure 3 shows the emission spectra in aqueous solution for $[Ru(bpy)_2(tyr)_2]^{2+}$ at increasing times of irradiation. The monoaquo complex does not present fluorescence, suggesting the charge transfer (CT) excited state is decaying completely through nonradiative paths, possibly through solvent-coupled vibrations via the aquo ligand.

The other complexes investigated shared the same behavior. The corresponding data is presented in Table 2.

The absorption spectra of all synthesized complexes were similar, showing the metal-to-ligand charge transfer (MLCT) band in the visible region at 488 nm. In all the studied compounds, irradiation on the MLCT bands led to the heterolytic cleavage of one of the ligands with quantum efficiencies ~0.03, which is high for the release of a biomolecule at this mild wavelength. In comparison, the quantum efficiency for the release of pyridine in $[Ru(bpy)_2py_2]^{2+}$ is ~10 times greater[9] because of the lower basicity of the ligand, which shifts the MLCT band to higher energies, promoting an easier population of the dissociative d−d state. The monosubstituted complexes, in which a solvent molecule replaces the original ligand, do not present significant photodecomposition, as can be deduced from the isosbestic points appearing in all the irradiation spectra.

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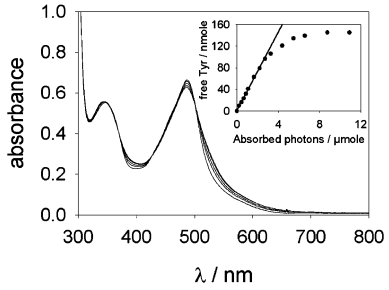

Figure 4. UV−vis spectra of an aqueous solution of $[Ru(bpy)_2(tyr)_2]^{2+}$ at neutral pH, under irradiation at 450 nm. The inset shows the degree of photoconversion.

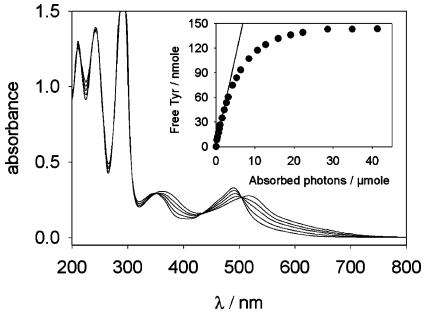

Figure 5. UV−vis spectra of an aqueous solution of $[Ru(bpy)_2(tyr)_2]^{2+}$ at pH12, under irradiation at 450 nm. The inset shows the degree of photoconversion.

To determine the quantum efficiency of the photouncaging, series of UV−visible spectra of the complexes in aqueous solution were taken after irradiation at 450 ± 20 nm, using a high-power blue LED. The solutions were made by dissolving the PF_6^- salts into a minimal drop of acetone and further addition of distilled water or by dissolving the chloride salts directly into water. Both procedures gave the same results. The spectra for $[Ru(bpy)_2(tyr)_2]Cl_2$ is shown in Figure 4. From the analysis of the complete spectra, it is possible to determine the degree of advance of the photoreaction. The inset of Figure 4 depicts the results of this calculation.

As can be noticed from Figure 4, the final spectra of the bis-substituted and monosubstituted complexes are almost identical in aqueous solution at neutral pH. The very small differences between the two species make the uncaging analysis quite difficult, forcing every source of noise to be avoided.

If the pH of the irradiated solution is increased, the acidic aqueous complex loses a proton, yielding the hydroxyl complex $[Ru(bpy)_2(L)(OH)]^+$. This species presents a characteristic red-shifted spectrum that is readily distinguishable from that of the disubstituted species. The uncaging results for $[Ru(bpy)_2(tyr)_2]^{2+}$ at pH 12 are shown in Figure 5.

The quantum efficiency of photodissociation, obtained from the initial slope of the photoconversion plots, seems to be slightly dependent on the pH in the studied range. The values corresponding to all the complexes synthesized are depicted in Table 3.

Although many biomolecules contain the amine group as an important active part, most photolabile protecting groups developed until now are limited to the caging of carboxylates. The use of ruthenium bipyridine fragments as caging groups for amines offers a general way for making phototriggers of these relevant biomolecules. The tryptamine and the 5HT

Table 3. Quantum Yield of Uncaging in Aqueous Solution at 293 K

complex	ϕ pH 7	ϕ pH 12
$[Ru(bpy)_2(tyr)_2]^{2+}$	0.028	0.016
$[Ru(bpy)_2(buNH_2)_2]^{2+}$	0.044	0.016
$[Ru(bpy)_2(GABA)_2]^{2+}$	0.036	0.032
$[Ru(bpy)_2(tryp)_2]^{2+}$	0.018	0.016
$[Ru(bpy)_2(5HT)_2]^{2+}$	0.023	

complexes were tested in an acute preparation of leech (*Hirudo medicinalis*) ganglia in concentrations up to 1 mM in a isoosmotic saline solution[3] The compounds were not toxic in these conditions according to the nontoxicity exhibited by the first complex of this series $[Ru(bpy)_2(4AP)_2]Cl_2$.

It has not escaped our notice that caged compounds of dopamine, histamine, or octopamine, molecules of high biological relevance, may be obtained this way. Such synthesis are currently being pursued.

Generally speaking, the syntheses here presented are very convenient, since they can be performed in aqueous solution in a one-step batch from the amine to be caged and the $Ru(bpy)_2Cl_2$ precursor. The fact that active caged compounds present fluorescence while their photoproducts do not allows the continuous monitoring of the bulk and local concentrations during the experiment.

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Supporting Information Available: ^1H NMR spectra of all the studied compounds can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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