Influence of the Steric Bulk and Solvent on the Photoreactivity of Ruthenium Polypyridyl Complexes Coordinated to L-Proline

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Supporting Information

**ABSTRACT:** Ruthenium polypyridyl complexes are good candidates for photoactivated chemotherapy (PACT) provided that they are stable in the dark but efficiently photosubstitute one of their ligands. Here the use of the natural amino acid l-proline as a protecting ligand for ruthenium-based PACT compounds is investigated in the series of complexes Λ-[Ru(bpy)(1-prol)]PF₆ ([1a]PF₆, bpy = 2,2'-bipyridine and 1-prol = l-proline), Λ-[Ru(bpy)(dmbpy)(1-prol)]PF₆ ([2a]PF₆ and [2b]PF₆, dmbpy = 6,6'-dimethyl-2,2'-bipyridine), and Λ-[Ru(dmbpy)(1-prol)]PF₆ ([3a]PF₆). The synthesis of the tris-heteroleptic complex bearing the disymmetric proline ligand yielded only two of the four possible regioisomers, called [2a]PF₆ and [2b]PF₆. Both isomers were isolated and characterized by a combination of spectroscopy and density functional theory calculations. The photoreactivity of all four complexes [1a]PF₆, [2a]PF₆, [2b]PF₆, and [3a]PF₆ was studied in water (H₂O) and acetonitrile (MeCN) using UV–vis spectroscopy, circular dichroism spectroscopy, mass spectrometry, and ¹H NMR spectroscopy. In H₂O, upon visible-light irradiation in the presence of oxygen, no photosubstitution took place, but the amine of complex [1a]PF₆ was photooxidized to an imine. Contrary to expectations, enhancing the steric strain by the addition of two ([2b]PF₆) or four ([3a]PF₆) methyl substituents did not lead, in phosphate-buffered saline (PBS), to ligand photosubstitution. However, it prevented photooxidation, probably as a consequence of the electron-donating effect of the methyl substituents. In addition, whereas [2b]PF₆ was photostable in PBS, [2a]PF₆ quantitatively isomerized to [2b]PF₆ upon light irradiation. In pure MeCN, [2a]PF₆ and [3a]PF₆ showed non-selective photosubstitution of both the l-proline and dmbpy ligands, whereas the non-strained complex [1a]PF₆ was photostable. Finally, in H₂O–MeCN mixtures, [3a]PF₆ showed selective photosubstitution of l-proline, thus demonstrating the active role played by the solvent on the photoreactivity of this series of complexes. The role of the solvent polarity and coordination properties on the photochemical properties of polypyridyl complexes is discussed.

**INTRODUCTION**

Because of their unique photophysical and photochemical properties, ruthenium polypyridyl complexes have found many applications in supramolecular chemistry, molecular imaging, chemical biology, and medicinal chemistry. Notably, several groups are studying the biological activity of ruthenium-based photoactivated chemotherapy (PACT) prodrugs. These compounds are non-toxic or poorly toxic in the dark, but they become highly cytotoxic, or more cytotoxic, upon visible-light irradiation. Unlike in photodynamic therapy, another phototherapeutic technique where phototoxicity comes from the light-induced generation of activated oxygen species such as singlet oxygen, in PACT light activation occurs via an oxygen-independent mechanism that often relies on ligand photosubstitution reactions. Ligand photosubstitution in polypyridyl complexes is typically attributed to the thermal promotion of photogenerated triplet metal-to-ligand charge-transfer (3MLCT) excited states into dissociative, low-lying triplet metal-centered (3MC) excited states. In many reported examples, ruthenium PACT compounds are based on complexes of the [Ru(bpy)₃]²⁺ family, where the photosubstituted ligand is a sterically hindered 2,2'-bipyridine (bpy) ligand such as 6,6'-dimethyl-2,2'-bipyridine (dmbpy). The increased cytotoxicity is generally attributed to the intracellular formation of the cis-bis(aqua) complex [Ru(bpy)₃(OH₂)₂]²⁺, which is believed to be the cytotoxic species. It should be noted, however, that the free dmbpy ligand is also generated upon light irradiation of [Ru(bpy)₃(dmbpy)]²⁺, the biological properties and cytotoxicity of which have not been evaluated yet.

In order to specifically address the question of the cytotoxicity of the metal-containing fragment, we embarked on investigating whether natural amino acids such as l-proline...
(l-prol), instead of hindered bipyridyl ligands, could be used to cage a cis-bis(aqua)ruthenium species. Amino acids are naturally present in a cell, so that the photochemical generation of 1 equiv of such ligands is not expected to have any impact on cell survival. For amino acid caged ruthenium polypyridyl complexes, any light-induced toxicity would be solely attributed to the metal fragment. In the literature, several examples of cis-ruthenium(II) diimine complexes coordinated to deprotonated L-amino acids were described that, upon light irradiation, interconvert between the $\Lambda$-L and $\Delta$-L isomers. However, to our knowledge, photosubstitution of an amino acid by solvent molecules has not been described yet. As reported for complexes with similar N,O-chelating ligands, the strong $\sigma$-donor properties of the carboxylate moiety usually increase the $e_g$ level of the metal complex and, therefore, the gap between the 3MLCT and 3MC states. Such an increased gap enhances the photostability of the complex by quenching photosubstitution reactions involving the 3MC states. In order to recover ligand photosubstitution properties, sterically hindered chelates such as 6,6'-dmbpy can be reintroduced but, if possible, as spectator ligands to see whether the 3MC states are low enough in energy to come in the vicinity of the photochemically generated 3MLCT states.

Of course, octahedral complexes bearing chiral and/or dissymmetric bidentate ligands such as amino acids can lead to the formation of many different isomers. Thus, the preparation of such complexes is a priori challenging, although diastereoselective coordination reactions making use of interligand repulsion, and chromatographic separation techniques, have been described in the past. Here, we report on the synthesis of a series of l-prol-bound ruthenium complexes comprising $\Lambda$-[Ru(bpy)$_2$(l-prol)]PF$_6$ ([1a]PF$_6$), $\Lambda$-[Ru(bpy)-(dmbpy)(l-prol)]PF$_6$ ([2a]PF$_6$ and [2b]PF$_6$), and $\Lambda$-[Ru-(dmbpy)$_2$(l-prol)]PF$_6$ ([3a]PF$_6$; Figure 1). In this series, the number of sterically hindering methyl groups increases from zero in [1a]PF$_6$ to two in [2a]PF$_6$ and [2b]PF$_6$ to four in [3a]PF$_6$. The influence of the solvent on the photoreactivity of these complexes was also investigated.

### RESULTS AND DISCUSSION

#### Synthesis and Characterization.

The four l-prol-coordinated ruthenium polypyridyl complexes were prepared as shown in Scheme 1. Complexes [1a]PF$_6$ and [3a]PF$_6$ were synthesized by reacting the C$_2$-symmetric precursor rac-[Ru(bpy)$_2$Cl$_2$] and rac-[Ru(dmbpy)$_2$Cl$_2$] (4), respectively, with l-prol. As reported by Meggers et al., coordination of the chiral ligand l-prol to these racemic mixtures is diastereoselective and leads to the $\Lambda$-L diastereomer as a major (+) or sole (+) product. However, to our knowledge, photosubstitution of an amino acid by solvent molecules has not been described yet. As reported for complexes with similar N,O-chelating ligands, the strong $\sigma$-donor properties of the carboxylate moiety usually increase the $e_g$ level of the metal complex and, therefore, the gap between the 3MLCT and 3MC states. Such an increased gap enhances the photostability of the complex by quenching photosubstitution reactions involving the 3MC states. In order to recover ligand photosubstitution properties, sterically hindered chelates such as 6,6'-dmbpy can be reintroduced but, if possible, as spectator ligands to see whether the 3MC states are low enough in energy to come in the vicinity of the photochemically generated 3MLCT states.29

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#### Scheme 1. Structures of the Complexes [1a]PF$_6$, [2a]PF$_6$, [2b]PF$_6$, and [3a]PF$_6$

Figure 1. Crystal structures of (a) [2b]PF$_6$ and (b) [2b-2H]PF$_6$. Hexafluorophosphate counteranions, lattice H$_2$O, and disorder have been omitted for clarity.
Limited ligand scrambling was observed, resulting in a sample containing also Δ-L and Λ-D isomers of \([\text{3a}]\text{PF}_6^+\), which means that they both have the Δ octahedron configuration. As a consequence, these isomers are necessarily complexes \([2a]\text{PF}_6^+\) and \([2b]\text{PF}_6^+\) in a ratio of 85:15 (Figures S2b,c, S3, and S4). Circular dichroism (CD) spectra of these two isomers in water (H\(_2\)O) showed a positive band at 300 nm for both isolated species (Figure S9), which means that they both have the Δ octahedral configuration. As a consequence, these isomers are necessarily complexes \([2a]\text{PF}_6^+\) and \([2b]\text{PF}_6^+\) (Figure S26).

In other words, the \(\alpha\) proton and methyl substituent on the dmbpy are closer in complex \([2b]\text{PF}_6^+\) than in complex \([2b]\text{PF}_6^+\). Finally, single crystals suitable for X-ray structure determination were obtained for \([2b]\text{PF}_6^+\) by slow crystallization in H\(_2\)O. The space group (P1) was chiral, and the X-ray structure contained a single configuration of the coordination octahedron (A). The molecular structure, shown in Figure 1a, showed a long N5—C26 single bond [1.510(5) Å] for the proline ligand, and the oxygen atom of \(\text{p}r\)ol was found trans to the dmbpy ligand (Table 1). Thus, the nature of the isomer \([2b]\text{PF}_6^+\) was unequivocally confirmed, and as a consequence, \([2a]\text{PF}_6^+\) was analyzed as the \(\Lambda\)-isomer having the oxygen atom trans to the \(\text{h}py\) ligand.

Density functional theory (DFT) calculations of both diastereoisomers \(\Lambda\)-\(l\) and \(\Delta\)-\(l\) of \([1]^+\) and \([3]^+\), and the four possible diastereoisomers of \([2]^+\), were performed in H\(_2\)O using the conductor-like screening model (COSMO) to simulate solvent effects (see the Supporting Information). The optimized structures, their energies in H\(_2\)O, and their
Table 1. Selected Bond Lengths (Å) and Angles (deg) for [2b]PF₆ and [2b-2H]PF₆

|          | [2b]PF₆     | [2b-2H]PF₆  |
|----------|-------------|-------------|
| Ru1−O1   | 2.100(3)    | 2.111(1)    |
| Ru1−N1   | 2.024(3)    | 2.047(1)    |
| Ru1−N2   | 2.067(4)    | 2.066(2)    |
| Ru1−N3   | 2.074(3)    | 2.074(2)    |
| Ru1−N4   | 2.098(4)    | 2.067(2)    |
| Ru1−N5   | 2.143(3)    | 2.046(1)    |
| N5−C26   | 1.510(5)    | 1.305(3)    |
| C25−C26−N5 | 115.5(2) | 106.0(3)     |
| C23−N5−C26−C27 | 122.1(4) | −174.4(2) |

The photoreactivity of [1a]PF₆ was studied first. The evolution of the UV–vis spectrum of a solution of [1a]PF₆ in phosphate-buffered saline (PBS) was studied upon irradiation at 493 nm under air. An hypsochromic shift in the 1MLCT band was observed, with a change in the absorption maximum from 495 to 467 nm and an isosbestic point at 486 nm (Figure 2a). Furthermore, the MS spectrum after irradiation showed a peak at m/z 526.1 (Figure 3a), which is two units smaller than the starting complex (calc m/z 528.1). These two units correspond to the loss of two hydrogen atoms. According to Keene et al., these hydrogen atoms may correspond to the α-hydrogen and amine hydrogen of proline, i.e., the imine complex [Ru(bpy)(1,1-prol-2H)]PF₆ ([7]PF₆) was formed. A quantum yield (φph) of 0.0010 was calculated for this photoreaction in PBS (Figure S14); and a dark control experiment at 37 °C did not show any change in the UV–vis spectrum over time (Figure S11), which excludes a thermal reaction under light irradiation. The oxidative nature of the photoreaction was confirmed by performing the same photoreaction under argon. No change in either the UV–vis (Figure 2b) or MS (Figure 3c) spectrum was observed in the absence of oxygen. When following the irradiation by NMR under argon, a new doublet appeared at 8.91 ppm, which corresponds to the Δ·l isomer [1b]⁺ (Figure S10). In addition, a decrease in the band at 300 nm in the CD spectra was observed upon irradiation under the same conditions (Figure S13). Finally, the addition of the antioxidant glutathione (GSH) before...
irradiation in air partially slowed down the photoreaction (Figures 2c and S12a). In such conditions, MS after 180 min of irradiation (Figure 3b) showed a mixture of [1a]+ (*m/z* 528.1) and [7]+ (*m/z* 526.1) because the relative intensity of the *m/z* 528.1 peak in the isotopic pattern of [7]PF₆ was slightly higher than expected, as shown in the calculated isotopic pattern for a given mixture of 7:3 [1]+/[7]+ in Figure S15. In order to confirm that irradiation led to photooxidation and compare our results under light irradiation to that obtained using electrochemical oxidation by Yamaguchi et al., a spectroelectrochemistry analysis of [1a]PF₆ was performed. Chromoamperometry of a solution of [1a]PF₆ in PBS with a constant potential of +0.645 V vs Ag/AgCl using carbon sponges as working and counter electrodes was followed by UV–vis spectroscopy. After 2 h, the current stabilized at 0.05 mA, and the oxidative reaction was considered to be finished. As shown in Figures S23b and S25, the UV–vis and MS spectra showed the same changes as those upon light irradiation, i.e., a hypsochromic shift from 495 to 466 nm in the MLCT band with an isosbestic point at 486 nm and a peak at a *m/z* 526.1. Thus, as shown in Scheme 3, upon light irradiation of [1a]+ under argon, partial photoisomerization from Λ-L to Δ-L takes place, as has been described extensively in the literature for cis-ruthenium(II) diimine complexes coordinated to a deprotonated amino acid. However, in the presence of dioxygen, the coordinated ligand L-prol is oxidized to its imino analogue [7]+, as described for the complex [Ru(bpy)₂(2-(1-aminoethyl)(pyridine))(PF₆)₂] by Keene et al. or for [Os(bpy)₂(2-aminoethanesulfonate)](PF₆) by Tamura et al. Although the exact mechanism of photooxidation is unclear, we suggest that the amine may be oxidized by the singlet oxygen (¹O₂) generated in the presence of light and molecular oxygen because it has been demonstrated that ¹O₂ is a much better oxidant than the ground state ³O₂. More in-depth studies would be needed to confirm this hypothesis.

In a second step, the reactivity of the more hindered complexes, [2a]PF₆, [2b]PF₆, and [3a]PF₆, was investigated. When a solution of [3a]PF₆ was irradiated in PBS at 493 nm under air, no change in the UV–vis or MS spectra was observed (Figures 4a and S12d). Like for [1a]+, partial isomerization from Λ-L to Δ-L occurred as shown by the decrease of the band at 300 nm in the CD spectrum (Figure S17). Thus, for complex [3a]PF₆, photooxidation did not occur in PBS, which represents a dramatic change compared to the photoreactivity of [1a]PF₆. Suprisingly, the much higher steric hindrance of the complex did not lead to photosubstitution reactions either. On the other hand, when a solution of [2a]PF₆ in PBS was irradiated with a 1000 xenon lamp equipped with a 450 nm blue-light filter and followed by ¹H NMR, a doublet at 9.1 ppm, characteristic of the 6′ proton of the bpy ligand in [2b]PF₆, arose upon 15 min irradiation. In such conditions, photoconversion of [2a]PF₆ to [2b]PF₆ was complete after 150 min.

**Scheme 3.** Scheme of the Photoisomerization and Photooxidation Observed upon Visible-Light Irradiation of [1a]PF₆ in PBS at 298 K with a 493 nm LED at 12.0 mW·cm⁻²

![Scheme 3](image)

**Figure 4.** (a) Evolution of the absorption at 500 nm of a solution of [1a]PF₆ (0.078 mM, red circles), [2a]PF₆ (0.032 mM, green squares), and [3a]PF₆ (0.077 mM, black triangles) in PBS upon irradiation under air with a 493 nm LED at 12.0, 8.7, and 11.0 mW·cm⁻², respectively. The conditions are detailed in Table S2. (b) Evolution of the ¹H NMR of a D₂O solution of (b) [2a]PF₆ (2.7 mg in 0.7 mL, circles) and (c) [2b]PF₆ (2.6 mg in 0.7 mL, triangles) upon light irradiation with the beam of a xenon lamp filtered with a 450 nm blue-light filter under air. The conditions are detailed in the Supporting Information.
of irradiation (Figure 4b). By contrast, no change in the \(^1\)H NMR spectrum was observed upon irradiation of \([2b]\)PF\(_6\) in the same conditions (Figure 4c). Thus, isomer \([2a]\)PF\(_6\), which is a kinetic product formed thermally by the coordination of l-prol to \([\text{Ru}(\text{bpy})(\text{dmbpy})(\text{MeCN})_2]^{2+}\), isomerizes photochemically into \([2b]\)PF\(_6\), which is the thermodynamically most stable isomer of \([2]\)\(^+\). According to the UV–vis spectral evolution in Figures 4a and S12b,c, isomerization of \([2a]\)\(^+\) to \([2b]\)\(^+\) is not the only process occurring upon irradiation, and photooxidation takes place as well. However, this process occurs at a much slower rate than it does for \([1a]\)\(^+\).

When a solution of \([2a]\)PF\(_6\) in H\(_2\)O slowly crystallized in the presence of dimmed daylight, single crystals were obtained that could be analyzed by crystallography. The crystal structure showed a short N5–C26 bond in the proline ligand.1.305(3) Å; Table 1) characteristic of a double N\(_\Lambda\) means that the NMR spectrum was observed upon irradiation of \([174.4(2)\]Å; Table 1\) characteristic of a double N\(_\Lambda\) means that the.

Furthermore, the torsion angle C23–N5–C25–C26 was 174.4(2)° in the new crystal (vs 122.1(4)° in the crystal structure of \([2b]\)PF\(_6\)), which confirmed the quasi-planar geometry of N5 and C26 in the new crystal and thus the oxidation of proline in an imine. In addition, like in \([2b]\)\(^+\), the carboxylato group was found to be trans to dmbpy, which confirmed the photochemical isomerization of \([2a]\)\(^+\) to \([2b]\)\(^+\) during crystallization. Thus, the obtained crystal structure corresponds to the imine complex \([2b-2H]^+\). It should be noted that, because this ruthenium complex crystallized in a space group that contained an inversion center (PT), it is a racemate. Because NMR experiments showed that irradiation of \([2b]\)\(^+\) did not lead to the Δ isomer \([2d]\)\(^+\), finding both enantiomers in the crystal structure of \([\text{Ru}(\text{dmbpy})(\text{bpy})(\text{l-prol}-2\text{H})](\text{PF}_6)\cdot\text{H}_2\text{O}\) means that the \(\Lambda\)-to-\(\Delta\) racemization occurred after photoisomerization of \([2a]\)\(^+\) to \([2b]\)\(^+\) and after photooxidation.

According to Gomez et al., the acidity of the amine of the coordinated l-prol ligand may have a crucial effect on the rate of dehydrogenation for amino acids coordinated to ruthenium polypyridyl complexes.53 The more acidic the amine is, the faster dehydrogenation takes place. In our case, more methyl substituents on the bpy ligands clearly lead to lower proline photooxidation rates. A plausible interpretation of this observation is that the methyl substituents are electron-donating. More methyl substituents will thus increase the electron density on ruthenium and hence decrease the acidity of the coordinated proline amine. At that stage, however, it remains impossible to say whether or not the steric effects of the methyl groups contribute as well to the dramatic switch in the photoreactivity observed in H\(_2\)O between \([2a]\)\(^+\), \([2b]\)\(^+\), and \([3a]\)\(^+\) and the non-hindered complex \([1a]\)\(^+\).

At that point, the absence of any photodesubstitution reaction upon irradiation of all four complexes in an aqueous medium may be surprising because the X-ray structure of \([2b]\)\(^+\) and the DFT-minimized geometries of the hindered molecules \([2a]\)\(^+\), \([2b]\)\(^+\), and \([3a]\)\(^+\) were distorted enough to suggest low-lying MC states. In order to investigate further this question, irradiation was performed in MeCN, which is a much less polar solvent than H\(_2\)O, as well as an excellent ligand for ruthenium(II). When an MeCN solution of \([1a]\)PF\(_6\) was irradiated at 493 nm under argon, no change in the maximum absorbance of the MLCT was observed (Figure 5a), which confirmed the photostability observed in H\(_2\)O. However, when the same experiment was performed using \([2a]\)PF\(_6\), \([2b]\)PF\(_6\), or \([3a]\)PF\(_6\), a clear photoreaction was observed by UV–vis spectroscopy, characterized by a hypsochromic shift of the MLCT band of all three complexes (Figure 5). For the heteroleptic complex \([2a]\)\(^+\), the maximum absorbance of the MLCT band shifted from 509 to 432 nm (Figure 5b), and the MS spectrum after irradiation showed peaks at m/z 185.4, 261.9, 452.2, and 669.2 (Figure S16a). These peaks correspond to the free ligand \(\{6,6′\text{-dmbpy}+\text{H}\}\)\(^+\) (calc. m/z 185.2),
[Ru(bpy)(dmppy)(MeCN)$_2$]$^{2+}$ (calcd $m/z$ 262.1), [Ru(bpy)-(l-prol-2H)(MeCN)$_2$]$^{3+}$ (calcd $m/z$ 452.1), and [{[Ru(bpy)-(dmppy)(MeCN)$_2$]PF$_6$}$]^+$ (calcd $m/z$ 669.1), respectively. Thus, in MeCN, both bidentate ligands l-prol and dmppy are photosubstituted by two solvent molecules. Similar results were found when a MeCN solution of [3a]PF$_6$ was irradiated at 493 nm. A shift in the absorbance maximum of the MLCT band occurred from S16 to 444 nm (Figure 5d), and the MS spectrum after irradiation showed peaks at $m/z$ 185.5, 276.3, 480.2, and 697.2, which corresponded to the free ligand [6,6-$\eta^1$]-dmppy+H$^+$ (calcd $m/z$ 185.2), [Ru(dmppy)(MeCN)$_2$]$^{2+}$ (calcd $m/z$ 276.1), [Ru(dmppy)-(l-prol-2H)(MeCN)$_2$]$^+$ (calcd $m/z$ 480.1), and [{[Ru(dmppy)(MeCN)$_2$]PF$_6$}$]^+$ (calcd $m/z$ 697.1), respectively (Figure S16b). Thus, also for [3a], irradiation in MeCN triggers the non-selective photosubstitution of both the l-prol and dmppy ligands. When the reaction was performed at a lower light intensity, the photosubstitution rate was lowered and a first isosbestic point at 493 nm could be observed during the first 10 min of the reaction (Figure S19a). A MS spectrum measured at that time point showed no peak corresponding to free dmppy (Figure S19b), suggesting that l-prol is substituted more rapidly than dmppy. Overall, in MeCN, the steric strain in the hindered complexes [2a]$^+$ and [3a]$^+$ indeed triggered the expected photosubstitution reactions that were not observed in PBS. However, these photoreactions are not selective and lead to the substitution of both proline and dmppy.

Considering the discrepancy between the photoreactivity observed in an aqueous buffer and that observed in MeCN, photosubstitution was also studied for [3a]$^+$ in H$_2$O mixtures containing large amounts (1–80 vol %) of MeCN, thus in pseudo-first-order conditions. As shown in Figure S20, in all cases photosubstitution occurred, as demonstrated by an isosbestic point at 388 nm, two sequential isosbestic points at 457 and at 479 nm showing a two-stage reaction, and the overall shift of the maximum absorbance of the 1MLCT band from 504 to 445 nm. Interestingly, MS spectra measured after the first stage of the reaction showed, next to the peaks at $m/z$ 275.8 and 697.5 corresponding to the final photoproduce [Ru(dmppy)$_2$(MeCN)$_2$]$_2$ (calcd $m/z$ 276.1) and [{[Ru(dmppy)$_2$(MeCN)$_2$]PF$_6$}$]^+$ (calcd $m/z$ 697.1), an additional peak at $m/z$ 313.3 characteristic for an intermediate where one of the bidentate ligands is bound in a monodentate fashion and one MeCN is coordinated, e.g., [{[Ru(dmppy)$_2$(MeCN)$_2$]PF$_6$}$]^+$ (calcd $m/z$ 313.1, Figure S21). MS spectra measured at the steady state did not show this intermediate $m/z$ 313.3 peak or any free dmppy ligand. Clearly, the two-step photochemical reaction observed by UV–vis corresponds to the initial substitution of one coordinating atom of l-prol by one MeCN ligand, followed by the selective substitution of the second coordinating atom of l-prol by a second MeCN ligand. The absorbance of the solution at 500 nm evolved linearly with the irradiation time during the first 5 min of all experiments, showing that in such conditions the reaction rate was constant (Figure S22a and Table S3). Surprisingly, the observed rate constants ($k_{obs}$) for formation of the final photoproduce [Ru(dmppy)$_2$(MeCN)$_2$]$_2$ (calcd $m/z$ 276.1) and [{[Ru(dmppy)$_2$(MeCN)$_2$]PF$_6$}$]^+$ (calcd $m/z$ 697.1) are the same for all concentrations in H$_2$O (Figure S22b), which discards a fully dissociative mechanism for such a two-step ligand photosubstitution. Because an associative mechanism is unlikely due to the crowdedness of the strained complex [3a]$^+$, we suggest that photosubstitution may take place via an interchange mechanism, although further kinetic studies should be performed to differentiate between a dissociative interchange and an associative interchange mechanism. Overall, an important observation is that the selectivity of the photosubstitution reaction in a 8:2 MeCN/H$_2$O mixture was different from that observed in pure MeCN: in the former case, photosubstitution was selective and only the proline ligand left the complex, whereas in the latter case, both dmppy and proline were photosubstituted.

The different photoreactivities of [2a]$^+$, [2b]$^+$, and [3a]$^+$ in PBS, MeCN, and H$_2$O/MeCN mixtures were puzzling, but they may be rationalized by different hypotheses. First, the coordinating properties of MeCN molecules toward ruthenium(II) are better than those of H$_2$O. Because photosubstitution of l-prol or dmppy seems to proceed via intermediates having $\eta^1$-coordinated bidentate ligands, more coordinating monodentate ligands may stabilize these intermediates, lower the overall activation barrier, and thus increase the photosubstitution rates in the presence of MeCN. Second, the carboxylate group of l-prol is highly polar, and it has excellent hydrogen-bond-accepting properties. Putative intermediates, where l-prol is coordinated in a $\eta^1$-$\kappa^N$ fashion, may hence be stabilized in the presence of H$_2$O, which would enhance the rate of l-prol photosubstitution versus that of dmppy. In contrast, in MeCN these [Ru(dmppy)$_2$(\eta$^1$-$\kappa^N$-proline)]$^+$ intermediates may be comparatively destabilized, while photosubstitution of the less polar dmppy ligands may occur via stabilization [Ru(\eta$^2$-dmppy)(\eta$^1$-proline)(\eta$^1$-dmppy)-(MeCN)]$^+$ intermediates. Finally, the different triplet excited states involved in the photosubstitution reactions are stabilized to a different extent in polar versus apolar solvents. The 3MLCT states are charge-transfer states that will be stabilized by the solvent with a higher polarity (H$_2$O), while the 3MC states are not charge-transfer excited states and will be less stabilized by high-polarity solvents. Thus, in H$_2$O, the 3MLCT–3MC energy gap should be enhanced compared to MeCN, and hence the rate of photosubstitution reactions will be lower. Low photosubstitution rates mean that slow photooxidation and photoisomerization reactions will be observed, whereas in pure MeCN, photosubstitution outcompetes these processes. Thorough—and challenging—theroretical studies, including triplet-state modeling with explicit solvent molecules, will be needed to evaluate the contribution of these three different effects on the solvent dependence of the photosubstitution reactions.

### CONCLUSION

In this work, we demonstrated that heteroleptic complexes bearing the N$_2$O-dissymmetric l-prol ligand can be prepared stereoselectively, isolated, and characterized. In complex [1a]$^+$, the absence of steric hindrance and the electron-rich oxygen ligand of proline quench any photosubstitution reaction, both in a chloride-containing aqueous solution and in MeCN. Instead, photooxidation occurs in the presence of air, leading to the formation of a double N=C bond. In parallel, partial isomerization of the ruthenium center from $\Lambda$ to $\Delta$ occurs, as reported for other amino acidato analogues. Increasing the steric strain, as in [2a]$^+$, [2b]$^+$, and [3a]$^+$, did not promote photosubstitution in an aqueous solution (PBS), unlike that demonstrated with other ruthenium complexes such as [Ru(bpy)$_2$(dmppy)]$^{2+}$ or [Ru(bpy)(dmppy)(L)]$^{2+}$, $^{23,26}$ In such conditions, increasing the number of methyl groups on the bipyridine ligands strongly slows photooxidation of the...
proline ligand probably because of the electron-donating effect of the methyl groups. It was necessary to add an excess of MeCN in H₂O to trigger the selective photosubstitution of l-prol in [3a]⁺. In pure MeCN, however, the increased strain in [2a]⁺, [2b]⁺, and [3a]⁺ did promote photosubstitution reactions, but two ligands were photosubstituted in a non-selective fashion, i.e., l-prol and dmbpy. The influence of the solvent opens interesting mechanistic questions for the photosubstitution reactions of ruthenium poly(pyridyl) complexes. It also increases the complexity of the speciation of photosubstitution reactions of ruthenium polypyridyl complexes. It also increases the complexity of the speciation of photosubstitution reactions of ruthenium polypyridyl complexes.

EXPERIMENTAL SECTION

Materials and Methods. The ligands 2,2′-bipyridine (bpy), 6,6′-dimethyl-2,2′-bipyridine (dmbpy), and l-proline (l-prol), as well as monopotassium phosphate (KH₂PO₄), sodium chloride (NaCl), and cis-bis(2,2′-bipyridine) dichlororuthenium(II) hydrate [cis-Ru(bpy)(H₂O)₂]Cl₂ were purchased from Sigma-Aldrich. Lithium chloride (LiCl) and potassium hexafluorophosphate (KPF₆) were purchased from Alfa-Aesar, and potassium carbonate (K₂CO₃) was obtained from Merck. RuCl₃·3H₂O was provided by Prof. Dr. E. Bouwman. All reagents and solvents were used without further purification. All reactions and solvents were used without further purification. The syntheses of cis-[Ru(bpy)(dmbpy)]Cl₂ ([4]) and [1]PF₆ were carried out according to literature procedures. Size-exclusion chromatography was performed using Sephadex LH-20.

Electrospray mass spectrometry (ES MS) spectra were recorded using a Thermo Finnigan EQA spectrometer and a MSQ Plus spectrometer, and CD spectra were recorded using a Bio-Logic MOS-500 spectrometer with a Bio-Logic ALX-300 lamp. For irradiation experiments of NMR tubes, the light of a LOT 1000 W xenon arc lamp mounted with an IR filter and either a 400 nm long-pass or a 450 nm 450FS10-50 filter from Andover Corp. was used. UV−vis experiments were performed on a Cary Varian spectrometer. When following photoreactions by UV−vis, MS, or CD, a light-emitting-diode (LED) light source (λem = 493 nm, with a full width at half-maximum of 14 nm) with a light intensity between 8.0 and 11.5 mW·cm⁻² was used. For spectrophotometry, UV−vis−light-source Avantes-DH-S-BAL and Avantes Avaspec-2048 spectrometers were used. An Autolab PGSTAT101 potentiostat was used to perform chronoamperometry.

All ¹H NMR spectra were recorded on a Bruker DPX-300 or DMX-400 spectrometer. Chemical shifts are indicated in parts per million relative to the residual solvent peak. For NMR experiments under argon, NMR tubes with polytetrafluoroethylene stoppers were used. For some NMR reactions, deuterated PBS was used as the solvent. A 10 mM PBS with 110 mM NaCl was prepared by dissolving KH₂PO₄ (6.5 mg, 0.047 mmol), K₂HPO₄ (36.8 mg, 0.211 mmol), and NaCl (160.8 mg, 2.752 mmol) in D₂O (25 mL) to reach a final pH of 7.54 at 22 °C. The pH was measured with a pH meter, taking into account that the measured pD = pH + 0.4. For the rest of the irradiations followed by UV−vis, MS, or CD, a 10 mM PBS with 110 mM NaCl was prepared by dissolving KH₂PO₄ (64.3 mg, 0.472 mmol), K₂HPO₄ (353.6 mg, 2.030 mmol), and NaCl (1.605 g, 27.64 mmol) in Milli-Q H₂O (250 mL) to reach a final pH of 7.35 at 22 °C.

Syntheses. [Ru(bpy)₃](l-prol-2H)PF₆ (1PF₆). The synthesis of complex [7]PF₆ was adapted from a literature procedure. [1a]PF₆ (3.0 mg, 0.005 mmol) was dissolved in 50 mL of PBS (pH 7.35) and transferred to one of the compartments of the cell. Oxidation at a constant potential of +0.645 V versus Ag/AgCl reference electrode was carried out under argon in a two-compartment cell with a Nafion membrane. Carbon sponge electrodes were used as working and counter electrodes. Electrolysis was continued until the current remained stable. Then, complex [7]PF₆ was extracted with DCM (3 × 20 mL) and dried over MgSO₄. After evaporation of the solvent by reduced pressure, an orange solid was obtained (2.6 mg, 93%). [1h NMR (300 MHz, methanol-d₄); δ 8.72 (d, J = 5.6 Hz, 1H), 8.66 (d, J = 8.1 Hz, 2H), 8.59−8.50 (m, 3H), 8.21 (ttd, J = 12.1, 7.9, and 1.5 Hz, 2H), 7.97−7.70 (m, 5H), 7.57 (d, J = 5.8 Hz, 1H), 7.33−7.20 (m, 2H), 3.88 (s, 1H), 3.20−3.02 (m, 1H), 2.97−2.79 (m, 1H), 2.30 (m, J = 3.4 Hz, 1H), 2.05 (m, 1H). ES MS (calcd): m/z 526.1 (526). 

rac-[Ru(bpy)(dmbpy)]PF₆ (5PF₆). A suspension was isolated as 85% pure containing traces of [3]PF₆ as shown by ¹H NMR (Figure S3) and 1H NMR (Figure S3) and.
The temperature of the data collection was controlled using a Cryosys system (manufactured by Oxford Instruments). The hydrogen atoms were placed at calculated positions (unless otherwise specified) using the instructions AFIX 23, AFIX 43, or AFIX 137 with isotropic displacement parameters having values of 1.2 or 1.5 Å.

Irradiation Experiments Followed by 1H NMR. Irradiation of (1a)[PF6]. A stock solution of [1a]PF6 in deuterated PBS (1.5 mg, 5.0 mL, 0.045 mM) was prepared and degassed under argon. Then, 650 μL were transferred, under argon, to a NMR tube. The tube was irradiated at 310 K with a LDT 1000 W xenon lamp equipped with IR short-pass and >400 nm long-pass filters. In addition, a control experiment without white-light irradiation was performed, in which no reaction was observed after 5 h. The reactions were monitored by 1H NMR at various time intervals.

Irradiation Experiments Followed by MS, UV–vis, and CD. UV–vis spectroscopy was performed using a UV–vis spectrometer equipped with the temperature control set to 298 K and a magnetic stirrer. The irradiation experiments were performed in a quartz cuvette containing 3 mL of a solution. A stock solution of the desired complex was prepared using either MeCN or PBS, which was then diluted in the cuvette to a working solution concentration. When the experiment was carried out under argon, the sample was degassed 15 min by gentle bubbling of argon and the atmosphere was kept inert during the experiment by a gentle flow of argon on top of the cuvette. A UV–vis spectrum was measured every 30 s for the first 10 min, every 1 min for the next 10 min, and eventually every 10 min until the end of the experiment. Data were analyzed with Microsoft Excel. The quantum yields for the photooxidation of [1a]PF6 in PBS were calculated by modeling the time evolution of the absorbance spectrum of the solution using the Glotaran software (Figure S14). The experimental conditions are detailed in Table S2.

Spectroelectrochemistry. A solution of [1a]PF6 in PBS (0.1 mM) was transferred into the working compartment of a two-compartment cell separated by a Nafion membrane, whereas the countercompartment contained only PBS. Carbon sponge with a resistance lower than 10 Ω were used as working and counter electrodes. A Ag/AgCl electrode was used as the reference electrode. Once the solution was degassed by bubbling argon for 15 min, the UV–vis probe was submerged in the working solution. Chronoamperometry was performed at a constant potential of +0.645 V vs Ag/AgCl reference electrode, taking points every second, while UV–vis

Inorganic Chemistry

Crystal Growth and X-ray Structure. Complex (2b)[PF6] Crystal growth: [2b]PF6 (2.0 mg, 0.003 mmol) was dissolved in H2O (0.7 mL) in a GC vial. After 2 weeks, single crystals suitable for X-ray diffraction were obtained. X-ray structure: All reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with an Atlas detector) with Cu Kα radiation (λ = 1.54178 Å) under the program CrysAlisPro (version 1.171.36.32, Agilent Technologies, 2013). The structure was solved by direct methods with SHELXS-2014/7 (Sheldrick, 2015) and was refined on F2 with SHELXL-2014/7 (Sheldrick, 2015). The hydrogen atoms were placed at calculated positions (unless otherwise specified) using the instructions AFIX 23, AFIX 43, or AFIX 137 with isotropic displacement parameters having values of 1.2 or 1.5 Å.

Additional notes: (i) The asymmetric unit contains two crystallographically independent ruthenium molecules, two PF6− counterions, and two lattice H2O solvent molecules. (ii) Both PF6− counterions are disordered over two orientations, and the occupancy factors of the major components of the disorder refine to 0.52(3) and 0.777(9). (iii) The structure refines in the space group P1. The absolute conformation is established by anomalous dispersion effects in diffraction measurements on the crystal. The Flack parameter refines to 0.013(2).

Oxidized Complex (2b–2H)PF6 Crystal growth: [2a]PF6 (2.0 mg, 0.003 mmol) was dissolved in H2O (0.7 mL) in a GC vial and left in dimmed daylight. After 6 weeks, single crystals suitable for X-ray diffraction were obtained.
spectra were recorded every 2 min. When the current of the chronoamperometry was constant, the experiment was terminated.

DFT Calculations. Electronic structure calculations were performed using DFT, as implemented in the ADF program (SCM). The structures of all possible isomers of [1], [2], and [3] were optimized first in vacuum and then in H2O using COSMO to simulate the effect of the solvent. The PBE0 functional and a triple-ζ potential basis set (TZP) were used for all calculations.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorg-chem.6b02794.

1H NMR, MS, and CD spectra of [1a]PF6, [2a]PF6, [2b]PF6, and [3a]PF6 crystal growth and X-ray structures, UV–vis, MS, CD, and 1H NMR spectra of the irradiation of [1a]PF6, [2a]PF6, [2b]PF6, [3a]PF6 spectroelectrochemistry of [1a]PF6, DFT calculations, and Cartesian coordinates (PDF)

Electronic structure calculations were performed using DFT, as implemented in the ADF program (SCM). The complexes as molecular machine prototypes.

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