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Synthesis of ruthenium tris(2,2′-bipyridine)-type complexes tethered to peptides at 5,5′-positions

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ABSTRACT

Utilization of 5′-amino-2,2′-bipyridine-5-carboxylic acid allows molecular design of ruthenium tris(bipyridine)-type complexes bearing two different functional groups. In this study, a novel ruthenium tris(bipyridine) derivative bearing viologen and tyrosine as an electron acceptor and donor, respectively, is synthesized. This synthesis exemplifies the effectiveness of the molecular design for functionalizing ruthenium bipyridine-type complexes. The photophysical properties are discussed in comparison with a reference ruthenium complex which has neither the electron acceptor nor donor.

Ruthenium polypyridyl complexes have widely been investigated and utilized in the fields such as artificial photosynthesis, solar energy conversion, photoredox catalyses, phosphorescence optical sensors, and light emitting diodes owing to the favorable photophysical and photochemical properties.[1–10] Although numerous works on the synthesis of ruthenium polypyridyl derivatives have been reported to tune the photophysical/electrochemical properties, the number of the reports for the ruthenium complexes of 5,5′-disubstituted-2,2′-bipyridyl derivatives is limited probably due to the synthetic difficulty.[11–18] In the 5,5′-disubstituted-2,2′-bipyridyl derivatives, 5′-amino-2,2′-bipyridine-5-carboxylic acid (H-**5Bpy**-OH) is attractive because two different functional groups can be attached to the amino and carboxylic groups (Chart 1). The metal complexes with the peptide, in which the unnatural amino acid **5Bpy** is incorporated, are also expected to retain high biocompatibility. However, there are only a few reports on the ruthenium complex with the **5Bpy** derivatives as the ligand, and even basic photophysical property remains unknown.[15,17] One of the reasons why such reports are very few is low reactivity of the amino group.[19] For example, even in the solid phase peptide synthesis using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), which is known as a strong coupling reagent, we could not couple any amino acids with the amino group of the unnatural amino acid, **5Bpy**. We noticed that Fmoc-AA-Cl (AA: amino acids) could be coupled with H-**5Bpy**-OH, however these reagents were not applicable to the common solid-phase peptide synthesis. We have overcome this issue by using a dipeptide, Fmoc-Leu-**5Bpy**-OH, which is separately synthesized in solution. We report herein the synthesis of a novel ruthenium complex (V^{2+}–Ru^{2+}–Y) with a hexapeptide having a viologen group and a tyrosyl residue as an electron acceptor and donor, respectively (Chart 1). We have also synthesized a reference complex (Ac–Ru^{2+}–F) without any electron accepting and donating groups. We describe the photophysical properties including the emission lifetimes, the quantum yields and the transition absorption spectra of the excited states of these ruthenium complexes.

The unnatural amino acid, **5Bpy**, was prepared from 5,5′-dimethyl-2,2′-bipyridine according to the literature[20] with a modified procedure: the solvent ratio of toluene/EtOH in the reaction of hydrazine with diethyl 2,2′-bipyridine-5,5′-dicarboxylate was changed from 3:1 to 2:1, improving the yield (80–95% yield).[15] Fmoc-Leu-**5Bpy**-OH was synthesized as shown in Scheme 1. Fmoc-Leu-Cl was reacted with H-**5Bpy**-OEt, which was prepared by an esterification of H-**5Bpy**-OH in the presence of sulfuric acid as an acid catalyst, giving Fmoc-Leu-**5Bpy**-OEt. The compound was then hydrolyzed with NaOH in aqueous EtOH to yield H-Leu-**5Bpy**-OH. This compound was again protected with Fmoc group, affording Fmoc-Leu-**5Bpy**-OH (58% yield). This seemingly circuitous synthetic route was an advantageous way for the synthesis of Fmoc-Leu-**5Bpy**-OH.

As the electron acceptor, the viologen group possessing carboxylic acid was synthesized. 1-Propyl-4,4′-bipyridinium bromide, which was prepared according to the literature,[21] was refluxed

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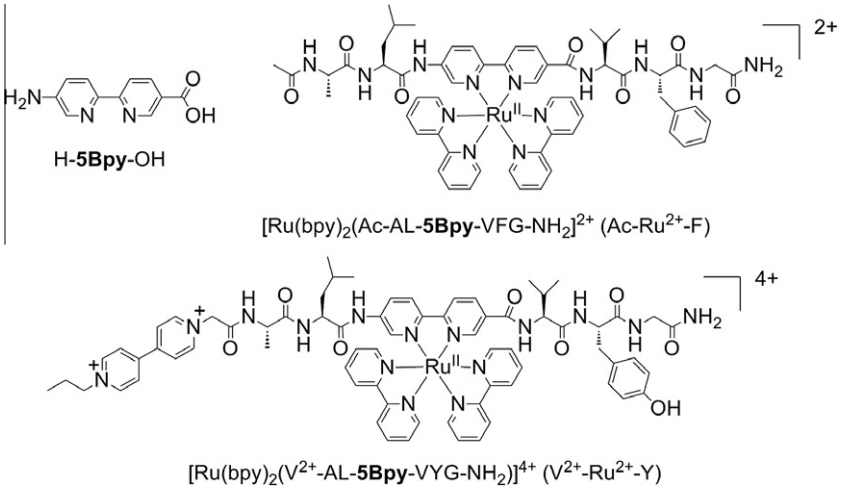
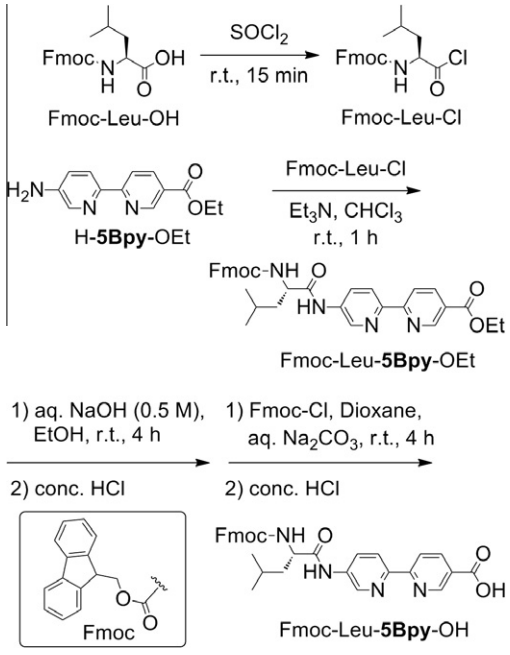


Chart 1.



Scheme 1. Synthesis of Fmoc-Leu-**5Bpy**-OH.

with ethyl 2-bromoacetate in acetonitrile. Then the compound was hydrolyzed with NaOH. After acidified with HCl, followed by treatment with $NaPF_6$, 1-carboxymethyl-1′-propyl-4,4′-bipyridinium dication (V^{2+}-COOH) was obtained as hexafluorophosphate salt (86% yield).

Peptides were synthesized by standard automated Fmoc solid-phase peptide synthesis from a Rink-amide solid support (Watanabe Chem. Ind. Ltd) on an Aapptec peptide synthesizer, Vantage. Fmoc group was deprotected with 20%-piperidine/DMF. Coupling of Fmoc-amino acid was carried out by using 2-(1*H*-benzotriazoyl-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIEA) in *N*-methylpyrrolidone (NMP)/DMF (3:2). Fmoc-Tyr(^{t}Bu)-OH was used as the side chain protected amino acid. V^{2+}-AL-**5Bpy**-VYG-NH_2 was synthesized by a reaction of V^{2+}-COOH with the resin-supported *N*-deprotected peptide in DMF containing *N*,*N*′-dicyclohexylcarbodiimide (DCC) and HOBt for 7 h. For the synthesis of Ac-AL-**5Bpy**-VFG-NH_2, the resin-supported peptide was treated with 50%-acetic anhydride/NMP. The peptides were cleaved and deprotected with trifluoroacetic acid (TFA)/*m*-cresol/thioanisole (25:1:2) for 1 h. The peptides were precipitated and washed with cold diethyl ether. Ac-AL-**5Bpy**-VFG-NH_2 was further washed with water for purification (67% yield). V^{2+}-AL-**5Bpy**-VYG-NH_2 was dissolved in methanol, followed by adding the concentrated aqueous $NaPF_6$, yielding the PF_6 salt (40% yield).

The ruthenium complexes, V^{2+}–Ru^{2+}–Y and Ac–Ru^{2+}–F, were obtained by refluxing ethanol solution of these peptide ligands with an equimolar $Ru(bpy)_2Cl_2$[22] (bpy = 2,2′-bipyridine) under Ar for 2 h. They were purified on a semipreparative reversed-phase HPLC column (Tosoh TSKgel ODS-80Ts (5 μm; 20 mm i.d. × 250 mm) equipped with a guard column TSKgel ODS-80Ts (20 mm i.d. × 50 mm)) using the water/acetonitrile mobile phase containing 0.1% (v/v) TFA. These eluted solutions were freeze-dried and dissolved again in water–methanol, followed by adding the concentrated aqueous $NaPF_6$, affording V^{2+}–Ru^{2+}–Y (21% yield) and Ac–Ru^{2+}–F (54% yield). These ruthenium complexes are in principle obtained as diastereomeric mixtures, which have chirality (Δ/Λ) around the metal center with the ligand chirality originated from L-amino acid. The HPLC technique could not recognize the diastereomers of V^{2+}–Ru^{2+}–Y at all, while the ones of Ac–Ru^{2+}–F were slightly recognized but not completely separated. Electrospray ionization-time-of-flight (ESI-TOF) mass spectra of these complexes showed the signals corresponding to $\{[V^{2+}–Ru^{2+}–Y](PF_6)_2\}^{2+}$ (m/z = 830.72) and $[Ac–Ru^{2+}–F]^{2+}$ (m/z = 578.70) with some peaks which were assignable to cationic species with PF_6 or $NaPF_6$ salts. The isotopic patterns being the characteristic of ruthenium were also observed (see Supplementary data).

The absorption and emission spectra of V^{2+}–Ru^{2+}–Y and Ac–Ru^{2+}–F in acetonitrile are depicted in Figure 1. Both the ruthenium complexes have intense bands around 290 nm with shoulders at 315 nm which are assigned as π–π* transition in the ligands. A difference between these complexes is observed in the absorption spectra around 250 nm, because the π–π* band based on the viologen in V^{2+}–Ru^{2+}–Y overlaps. They also exhibit metal-to-ligand charge transfer (MLCT) bands at 454 nm. When excited at the MLCT bands, the ruthenium complexes emit phosphorescence even at room temperature, which are observed as broad bands around 650 nm. The emission intensity for Ac–Ru^{2+}–F is high, while that for V^{2+}–Ru^{2+}–Y is low. The photophysical properties are summarized in Table 1. The time-resolved emission lifetime of V^{2+}–Ru^{2+}–Y shows a biexponential profile with time constants of 35 ns (80%) and 664 ns (20%). A representative viologen, 1,1′-dimethyl-4,4′-bipyridinium dication, of which the reduction potential is −0.46 V

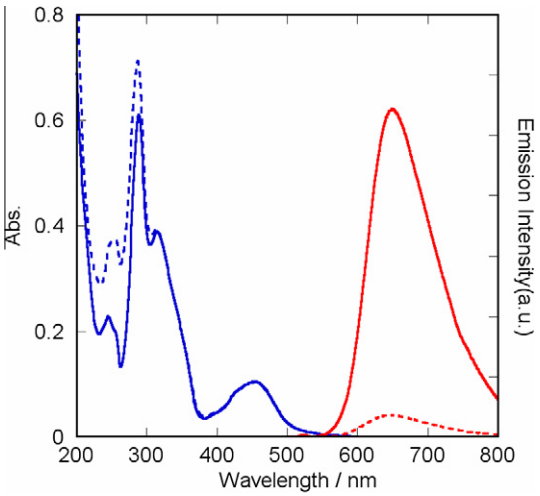


Figure 1. Absorption (blue) and emission (red) spectra of Ac–Ru^{2+}–F (solid line) and V^{2+}–Ru^{2+}–Y (dashed line) at 298 K in deaerated acetonitrile.

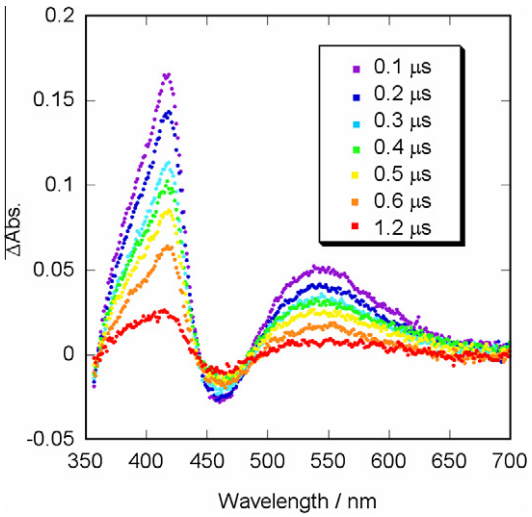


Figure 2. Transient absorption spectra of V^{2+}–Ru^{2+}–Y in deaerated acetonitrile; λ_{ex} = 355 nm.

Table 1
Photophysical properties of the ruthenium–peptide complexes in deaerated acetonitrile

Complex	λ_{abs}/nm	λ_{em}/nm	Φ	τ/ns
V^{2+}–Ru^{2+}–Y	454	649	0.007	35.1 (79.8%) 664 (20.2%)
Ac–Ru^{2+}–F	454	649	0.056	667

versus SCE in acetonitrile, is known to become a good quencher for oxidative quenching of $[Ru(bpy)_3]^{2+*}$.[1] On the other hand, the oxidation potential of tyrosine in water is 0.93 V versus NHE at pH 7,[23,24] which is more positive than the excited state oxidation potential ($E^*(Ru^{2+*}/Ru^+)$ = 0.84 V vs NHE) but more negative than the ground state oxidation potential ($E(Ru^{2+}/Ru^{3+})$ = 1.26 V vs NHE) of $[Ru(bpy)_3]^{2+}$.[1] This indicates that the reductive quenching of tyrosine is negligible and electron transfer occurs from tyrosine to the oxidized ruthenium complex, which generates by oxidative quenching with the viologen unit. Therefore, the major shorter-lived component as well as the smaller quantum yield than Ac–Ru^{2+}–F arises from the electron transfer from the excited states of the ruthenium complex to the viologen.[25] From the lifetime of the major short-lived component of V^{2+}–Ru^{2+}–Y, the intramolecular electron transfer rate (k_{ET}) in V^{2+}–Ru^{2+}–Y is estimated to be $\sim 2.70 \times 10^7$ s^{-1}.[26]

Covalently linked $[Ru(bpy)_3]^{2+}$–viologen dyads have been synthesized as physical models for the photosynthetic reaction center.[27–29] The lifetimes for the charge separation (CS) states have been observed in the range of 300 ps–3 ns. On the other hand, a $[Ru(bpy)_3]^{2+}$–tyrosine conjugate has been synthesized as a model for the photosystem II, where a tyrosine group (Tyr_Z) donates an electron to the primary electron donor ($P680^+$).[24] In the work, the electron transfer from the tyrosine group to $[Ru(bpy)_3]^{3+}$, which is generated with oxidation quenching by viologen, is researched. However, to the best of our knowledge, there are few studies for covalently linked viologen–$[Ru(bpy)_3]^{2+}$–tyrosine molecules. To estimate the lifetime for the CS state, nanosecond transition absorption spectra of V^{2+}–Ru^{2+}–Y in acetonitrile were measured (Fig. 2). The spectra showed a unique broad band at 500–600 nm, which had never been observed in the ones for ruthenium tris(bipyridyl) derivatives. However, it was also observed for Ac–Ru^{2+}–F (compare Fig. 2 with Fig. S5 in Supplementary data). This indicates that the broad band at 500–600 nm comes from the excited triplet states of the ruthenium complex and suggests that the lifetime for the CS state is within a couple of nanoseconds.

In conclusion, novel ruthenium tris(2,2′-bipyridine)-type complexes tethered to peptides containing the unnatural amino acid **5Bpy** were synthesized. This molecular design allows synthesis of the ruthenium tris(2,2′-bipyridine) derivatives bearing two different functional groups. In order to demonstrate this strategy, a novel ruthenium tris(bipyridine) derivatives having viologen and tyrosine were synthesized. Attaching the viologen to the ruthenium complex afforded the short lifetime of the excited states compared with the reference complex without both viologen and tyrosine, indicating that the electron transfer occurred from the excited states to the viologen. Preliminary photophysical results showed that the lifetime of the charge separation state is within a couple of nanoseconds. Further study on pico-second time-resolved spectroscopy will be planned for these ruthenium complexes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.117.

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25. The minor longer-lived component has the same lifetime as Ac–Ru^{2+}–F, indicating that the sample contains the species which do not occur the electron transfer. The HPLC for V^{2+}–Ru^{2+}–Y demonstrates that the sample does not contain major impurity (see Fig. S2 in the Supplementary data). This may arise from a conformer, but this is not actually understood.
26. The decay rate for the excited triplet states of Ac–Ru^{2+}–F is represented as $k(\text{Ac–Ru}^{2+}\text{–F}) = 1/\tau(\text{Ac–Ru}^{2+}\text{–F}) = k_r + k_{nr}$, where k_r and k_{nr} are radiative and non-radiative rate constants, respectively. For V^{2+}–Ru^{2+}–Y, the decay rate becomes $k(\text{V}^{2+}\text{–Ru}^{2+}\text{–Y}) = 1/\tau(\text{V}^{2+}\text{–Ru}^{2+}\text{–Y}) = k_r + k_{nr} + k_{ET}$, where k_{ET} is the electron transfer rate constant from the excited ruthenium complex to the viologen group. Accordingly, k_{ET} is written as $k_{ET} = 1/\tau(\text{V}^{2+}\text{–Ru}^{2+}\text{–Y}) - 1/\tau(\text{Ac–Ru}^{2+}\text{–F})$.
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