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Highly Effective DNA Photocleavage by Novel "Rigid" Ru(bpy)$_3$-4-nitro- and -4-amino-1,8-naphthalimide Conjugates

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The synthesis of the two novel 1,8-naphthalimideruthenium conjugates **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** and their photophysical evaluation upon interaction with DNA is reported. Significant changes were seen in both the absorption and emission spectra upon interaction of both conjugates with DNA, from which large binding constants were determined. Moreover, highly efficient DNA cleavage was observed upon irradiation for 5 min, during which supercoiled DNA was converted to nicked and linear DNA by **Ru-Nap-NH$_2$**.

The ability to selectively target and cleave DNA with high affinity and to report on the binding event by changes in luminescence is of great current interest.[1,2] To this end, ruthenium(II) polypyridyl metal complexes have received significant attention because of their excellent photophysical properties and defined structure,[1,2] with ligands such as dppz giving rise to strong binding to DNA, with large concomitant luminescent enhancements.[3] More recently, ruthenium(II) complexes with appended intercalating groups or tethered organic chromophores (bichromophore complexes) have also been used for targeting DNA.[4] Such modified designs can give rise to selective DNA targeting, enhanced photophysical properties, and DNA cleavage. With this in mind, we set out to develop novel Ru(bpy)$_3$ conjugates, based on the 4-nitro- or 4-amino-1,8-naphthalimide structure (**Nap**), which have well-understood photophysical properties[5] and are known to be effective binders and photoreactive reagents for DNA as well as showing promise as cancer therapeutic agents.[6,7] Herein, we demonstrate that our designs, **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$**, bind strongly to DNA with enhancement in the ruthenium(II) emission but also effectively cleave supercoiled DNA upon short irradiation. These are, to the best of our knowledge, the first examples of such naphthalimide conjugates, as DNA cleavers.

In the design of these conjugates, three principle factors were considered: (i) the nature of the linker/spacer between the complex and the chromophore, (ii) the structure of the polypyridyl complex, and (iii) the substitution pattern of the naphthalimide unit. In principle, of these, the linker can strongly influence the nature of the DNA binding as well as the photophysical properties of the resulting conjugate. In this study, the rigid linker that was used would allow for rotational flexibility while at the same time placing the naphthalimide in close proximity to the metal complex. This was achieved using the aniline-modified bipyridine ligand **1**. The substituents on the naphthalimide core profoundly affect the photophysical properties of such structures, where 4-amino (**Ru-Nap-NH$_2$**) substituents give rise to strong internal charge-transfer excited states, while the 4-nitro analogues (**Ru-Nap-NO$_2$**) are electron-deficient and, hence, alter the ability of the conjugate to bind to DNA as well as scissoring it upon photoradiation.

We have extensively explored the chemistry and photophysical properties of naphthalimide structures for fluorescent and colorimetric sensing of ions and molecues.[8] The synthesis of **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** is shown in Scheme 1. Ligand **1** was prepared according to the literature procedure.[9]

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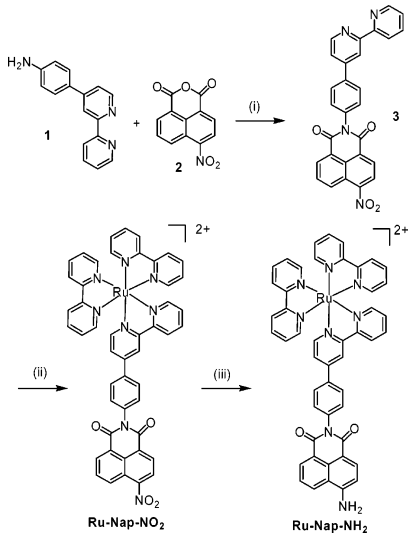
Scheme 1. Synthesis of **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** from **1**[a]



[a] (i) EtOH, Ar, Δ; (ii) Ru(bpy)$_2$Cl$_2$, DMF/H$_2$O, Ar, Δ; (iii) Pd/C, MeOH.

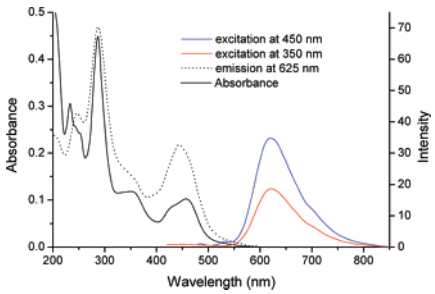


Figure 1. Absorption, excitation, and emission spectra of **Ru-Nap-NO$_2$** (6.7 μM) when recorded in a 10 mM phosphate buffer at pH 7.0.

The bipyridinenaphthalimide ligand **3** was prepared by the reaction of **1** with 4-nitro-1,8-naphthalic anhydride **2**, in anhydrous ethanol under argon. Ether precipitation followed by filtration afforded the product as a pale-yellow solid in 84% yield. The refluxing of **3** in the presence of ruthenium bisbipyridine chloride followed by precipitation from water using excess ammonium hexafluorophosphate yielded the crude complex **Ru-Nap-NO$_2$**, which was purified by column chromatography [flash silica; 40:4:1 $CH_3CN/H_2O/NaNO_3$-(sat)] to give the desired product in 69% yield. Hydrogenation of **Ru-Nap-NO$_2$** in methanol with 10% Pd/C afforded **Ru-Nap-NH$_2$** in 98% yield. Synthesized as their chloride salts, both complexes were found to be water-soluble. As such, the ground- and excited-state spectra of the complexes were explored in water and buffered solutions.

The characteristic absorbance spectra, together with the excitation and emission spectra of **Ru-Nap-NO$_2$**, are shown in Figure 1 (see the Supporting Information for **Ru-Nap-NH$_2$**). The absorption spectrum showed a band centered at 350 nm characteristic of 4-nitro-1,8-naphthalimide and a metal-to-ligand charge-transfer (MLCT) band at 460 nm for the metal complex. Exciting the complex at either of these wavelengths results in a MLCT emission centered at 635 nm. Typically, excitation of 4-nitro-1,8-naphthalimide results in a weak emission centered at 450 nm, which overlaps well with the MLCT absorption band. However, excitation at 350 nm in the case of **Ru-Nap-NO$_2$** results in no such naphthalimide centered emission. This suggests strong electronic communication between the two centers and is attributed to the mixing of low-lying isoenergetic triplet states, ^{3}MLCT and ^{3}Nap, as described by Castellano et al.[10] The quantum yield (air-saturated aqueous solution) for the metal-based emission was determined as $\phi_{MLCT} = 0.004$. This is significantly lower than that measured for the parent Ru(bpy)$_3^{2+}$ ($\phi_{MLCT} = 0.028$). This could be due to quenching of the MLCT excited state by electron transfer to the naphthalimide moiety.[11] The excitation spectrum ($\lambda_{em} = 625$ nm) yielded a spectrum almost identical with that of the absorption spectrum and reveals that both the naphthalimide and MLCT regions are the source of the emission. In the case of **Ru-Nap-NH$_2$**, the absorption spectrum possessed a single dominant band in the visible originating from both the aminonaphthalimide center and the MLCT band of the metal complex. Excitation at 450 nm again results in ruthenium-based emission at 635 nm. While excitation at 450 nm was expected to excite into both the naphthalimide core and the metal complex, no naphthalimide centered emission was detectable; again the excitation and absorption spectra are effectively superimposable. The quantum yield determined for the MLCT emission of **Ru-Nap-NH$_2$** was also higher than that for **Ru-Nap-NO$_2$**, $\phi_{MLCT} = 0.019$.

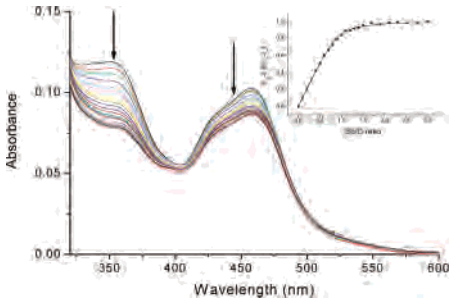


Figure 2. Changes in the UV/visible spectrum of **Ru-Nap-NO$_2$** (6.7 μM) with an increasing concentration of St-DNA (0−60.3 μM). Inset: changes in the naphthalimide absorption as a function of [DNA].

Both **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** were expected to interact with DNA through a combination of electrostatic and π-stacking interactions, with the possible anchoring of the naphthalimide unit through intercalation. Such interactions typically result in changes in the electronic spectra of such molecules, with these changes being greatest in the case of intercalation. To determine the binding affinity of these complexes for DNA, a series of DNA titrations was carried out. This was indeed found to be the case because the addition of salmon testes DNA to both **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** in 10 mM phosphate buffer at pH 7.0 resulted in significant changes to the absorption spectra of both complexes. In the case of **Ru-Nap-NO$_2$** (Figure 2), a 34% hypochroism was observed for the naphthalimide band, while the MLCT band was hypochromically shifted by 15%, which is significantly larger than that seen for Ru(bpy)$_3$, clearly demonstrating the importance of the naphthalimide moiety

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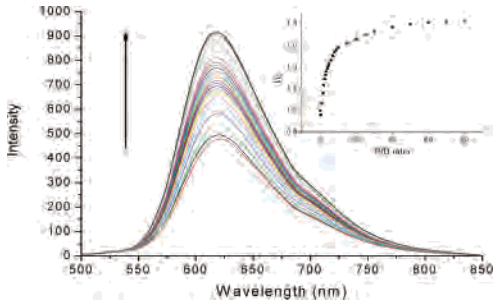

Figure 3. Changes in the emission spectrum (λ_{ex} = 450 nm) of **Ru-Nap-NH$_2$** (6.7 μM) with an increasing concentration of St-DNA (0−670 μM), in a 10 mM phosphate buffer, pH 7. Inset: changes in the MLCT emission as a function of P/D.

in the binding to DNA, which most likely interacts with the DNA through intercalation. The amino complex **Ru-Nap-NH$_2$** (see the Supporting Information) also exhibits significant changes, with ca. 30% hypochromic shift being observed upon titration with DNA. The intrinsic binding constant (K) and binding site size (n) were determined from the changes in the ground-state titration data using the model of Bard et al. (see the Supporting Information),[12] which showed that **Ru-Nap-NO$_2$** had a high affinity for DNA with $K = 4.5 \times 10^6$ ($\pm$0.7) and $n = 0.49$ ($\pm$0.01), while **Ru-Nap-NH$_2$** gave $K = 3.0 \times 10^6$ ($\pm$1.0) M^{-1} and $n = 0.44$ ($\pm$0.03). Moreover, significant changes were also seen in the emission spectra of both complexes. Interestingly, for **Ru-Nap-NO$_2$**, the MLCT emission was initially (P/D = 0−2) found to decrease by 30%, but this was followed by rapid ca. 40% luminescence enhancements (P/D = 3−40; see the Supporting Information). We attribute this to multiple binding interactions, as has been observed for cationic porphyrins.[15] In contrast to these results, the MLCT emission of **Ru-Nap-NH$_2$** exhibited monophasic interaction (Figure 3), where it was ca. 60% enhanced for P/D = 0−20. Furthermore, both complexes were found to effectively displace ethidium bromide from bound DNA, from which binding constants of 5.2×10^6 and 1.1×10^7 M^{-1} (see the Supporting Information) were calculated for **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$**, respectively, demonstrating their strong affinity for DNA.[13] Thermal denaturation experiments further supported the interaction of these complexes with DNA (see the Supporting Information). Here, **Ru-Nap-NO$_2$** was found to stabilize the DNA at both high and medium loading (P/D = 10 or 20). **Ru-Nap-NH$_2$** also stabilized the DNA at P/D = 20; T_m was shifted from 69 to >73 °C. However, the DNA melting transition was on both occasions achieved earlier than that for **Ru-Nap-NO$_2$**. These results suggest that these complexes interact with the DNA in a different manner, complementing the above results.

To shed light on these different binding modes, the ability of both complexes to photocleave DNA was examined using

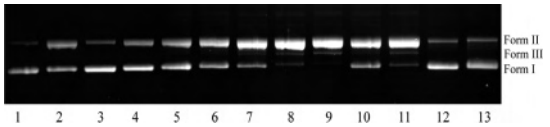

Figure 4. Agarose gel electrophoresis of pBR322 DNA (1 mg/mL) after 5 min of irradiation at G > 390 nm in a 10 mM phosphate buffer, pH 7. Lane 1: plasmid DNA control. Lane 2: Ru(bpy)$_3^{2+}$ (P/D = 50). Lane 3: 10 mM histidine. Lanes 4−6: **Ru-Nap-NO$_2$** (P/D = 50, 30, and 10, respectively). Lanes 7−9: **Ru-Nap-NH$_2$** (P/D = 50, 30, and 10, respectively). Lanes 10 and 11: histidine (10 mM) + **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** (P/D = 10), respectively. Lanes 12 and 13: **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$** (P/D = 10) in the dark.

agarose gel electrophoresis of pBR322 plasmid DNA. Both complexes cleaved DNA under aerobic conditions, after 5 min of irradiation (Figure 4). When DNA was incubated with these complexes in the dark, no cleavage was observed. Furthermore, significantly greater cleavage efficiency is observed for **Ru-Nap-NH$_2$** over that of **Ru-Nap-NO$_2$**. While this may reflect the relative quantum yields of the two complexes, where **Ru-Nap-NO$_2$** was found to be less emissive, it also reflects the difference in which the nature of the naphthalimide substituents has on the DNA binding, as demonstrated above. These results show that **Ru-Nap-NO$_2$** converts supercoiled DNA (form I) into nicked DNA (form II). However, in comparison, **Ru-Nap-NH$_2$** converts the supercoiled DNA into both nicked and linear (form III) DNA. Moreover, at P/D = 10, the supercoiled DNA has been completely converted, showing that **Ru-Nap-NH$_2$** is a significantly better DNA cleaver. These results also suggest the possible photo-adduct[14] formation, and this we have investigated by carrying out preliminary photolysis (see the Supporting Information) on **Ru-Nap-NH$_2$** in the presence and absence of *ct*-DNA. We are currently investigating this phenomenon in greater detail. The use of histidine, as a singlet oxygen scavenger, did not give rise to cleavage of the DNA. However, in the presence of either **Ru-Nap-NO$_2$** or **Ru-Nap-NH$_2$**, such cleavage was observed, suggesting that singlet oxygen was not the primary reason for the cleavage of the DNA (see the Supporting Information).

In conclusion, we have synthesized and studied two novel rigid bichromophore complexes, **Ru-Nap-NO$_2$** and **Ru-Nap-NH$_2$**, that bind strongly to DNA and cleave plasmid DNA with high affinity after 5 min of irradiation. These are, to the best of our knowledge, the first examples of such rigid Ru^{II}-Nap conjugates to interact with DNA. We are currently making other analogues of these complexes.

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Supporting Information Available: Synthesis and characterization of the complexes and Figures S1−S12. This material is available free of charge via the Internet at http://pubs.acs.org.

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