DNA Intercalators

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# A Multifunctional Light Switch: DNA Binding and Cleavage Properties of a Heterobimetallic Ruthenium–Rhenium Dipyridophenazine Complex**

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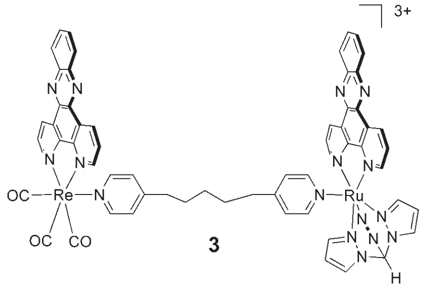
Polypyridyl-based transition-metal cations that interact with DNA are excellent probes for physical properties of DNA.[1] The "DNA light switch" $[Ru(phen)_2(dppz)]^{2+}$ (**1**; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine, phen = 1,10-phenanthroline) has attracted particular attention, as it displays a huge luminescence enhancement ($>10^4$) upon intercalation into DNA.[2] The excited states of such systems are insufficiently oxidizing to cleave DNA directly, and although they can generate singlet oxygen[3]—a species that can cause DNA damage and even some cleavage[4]—this process has low quantum yields, particularly for intercalated $[Ru^{II}(dppz)]$ systems,[5] and strand cleavage by secondary $^1O_2$ oxidation is usually only fully accomplished by subsequent treatment with alkali or piperidine.[6] Ground-state [Ru(dppz)] systems in which the metal is in a higher oxidation state—generated by electrochemistry or flash–quench procedures—have been shown to produce direct frank cleavage.[6,7] However these later systems are not light switches. Previous studies revealed that the related $Re^I$ complex $[Re(CO)_3(py)(dppz)]^+$ (**2**; py = pyridine) is capable of direct cleavage, but it possesses a greatly reduced binding affinity relative to that of **1**.[8,9] Furthermore, **2** is not a true light switch: its weak $\pi\rightarrow\pi^*$-based emission in water shows a much lower enhancement (×13) upon DNA binding[9] relative to **1** and its DNA-bound emission quantum yield is two orders of magnitude lower than that of **1**.[10]

Dinuclear [M(dppz)] systems are known. For example, Nordén and co-workers reported dinuclear $[Ru^{II}(dppz)]$ systems connected through dppz moieties which intercalate by a threading mechanism.[11] However, the construction of such enantiopure architectures from coordinatively saturated monomers is demanding and binding affinities are not greatly affected by chirality.

We are exploring more-facile methods for the synthesis of oligomeric metallointercalators.[12,13] For example, by using achiral, coordinatively unsaturated $[Ru(tpm)(dppz)(L)]^{2+}$ units (tpm = tris(1-pyrazolyl)methane, L = N-donor ligand) with binding parameters comparable to that of **1**,[14] we have developed a generalized method for the construction of bimetallic systems, the first example of which is $[\{Ru(tpm)(dppz)\}_2(\mu\text{-dpp[5]})]^{4+}$ (dpp[5] = 4,4′-dipyridylpentane), which binds to extended duplex sequences (> 6 base pairs).[12] This method has been extended to obtain the first hetero-dinuclear dppz complex.

The $Ru^{II}$–$Re^I$ system $[\{Ru(tpm)(dppz)\}(\mu\text{-dpp[5]})\{fac\text{-}(CO)_3Re(dppz)\}]^{3+}$ (**3**) was synthesized from the known mononuclear complexes[7–9,13] $[Ru(tpm)(dppz)(dpp[5])]^{2+}$ and $[ReCl(CO)_3(dppz)]$ through the two-step method used to obtain $[\{Ru(tpm)(dppz)\}_2(\mu\text{-dpp[5]})]^{4+}$.[12]



**3**

The UV/Vis absorption spectrum of **3**-$(PF_6)_3$ in $CH_3CN$ shows high-energy $\pi\rightarrow\pi^*$ transitions at 277 and 315 nm. Below 320 nm a superposition of metal(dπ)→dppz(π*) MLCT and dppz(π→π*) intraligand (IL) transitions is observed.[1,2,10,15] Excitation at 430 nm results in unstructured luminescence characteristic of the Ru(dπ)→dppz(π*) $^3$MLCT manifold (Figure 1).

However, emission from **3** is red-shifted by approximately 15 nm relative to that of a solution of $[Ru(tpm)(dppz)(dpp[5])]^{2+}$. This shift is consistent with the properties of other ligand-bridged, dinuclear $d^6$-metal complexes with interacting metal centers.[16] Excitation at 370 nm populates MLCT and IL excited states on both the $Re^I$ and $Ru^{II}$ metal centers,[1,2,10] thus resulting in enhanced emission intensity from **3**. Notably, there is no evidence of the weak, structured $^3$dppz-based $\pi\rightarrow\pi^*$ $^3$IL-based emission centered at 556 nm that is observed in **2**.[10,15] Furthermore, the emission decay fits a single exponential with a lifetime ($\tau_{em} = 77$ ns) identical to that of

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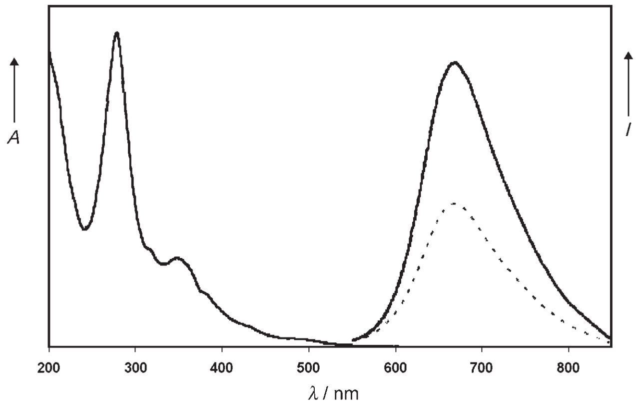

***Figure 1.*** UV/Vis absorption spectrum (left) and normalized emission spectra (right) of **3**-$(PF_6)_3$ in $CH_3CN$. Emissions shown for excitation at 370 nm (solid line) and 430 nm (dotted line).

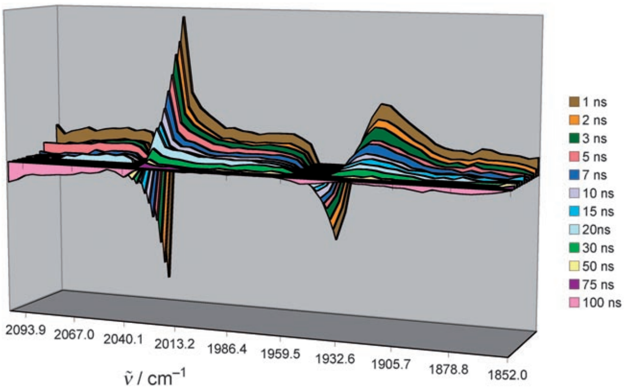

***Figure 2.*** ns-TRIR spectra obtained between 1 and 100 ns after excitation of an aqueous solution of **3**-$Cl_3$ ($\lambda_{ex} = 355$ nm).

the mononuclear complex $[Ru(tpm)(dppz)(dpp[5])]^{2+}$ in MeCN. The water-soluble salt **3**-$Cl_3$ was obtained by anion metathesis and, as would be expected from previous studies on other $[Ru^{II}(dppz)]$ systems, was found to be entirely nonemissive in aqueous solution.

Whereas luminescence spectroscopy is a convenient probe for the $Ru^{II}$ center of **3**, the carbonyl ligands of the $\{fac\text{-}(CO)_3Re(dppz)\}^+$ moiety facilitate IR spectroscopic studies.[15] Therefore, the development of Re-based excited states was probed by using nanosecond time-resolved infrared (ns-TRIR) spectroscopy.[17]

The ground-state FTIR spectrum of **3**-$(PF_6)_3$ in $CH_3CN$ displays two bands in the $\nu$(CO) stretching region at 2036 and 1932 $cm^{-1}$. Both bands occur at almost identical frequencies to those reported for **2**.[15] TRIR studies on **3** show the bleaching of these bands and the appearance of transient absorptions centered at 2028 and 1920 $cm^{-1}$. These features are consistent with analogous studies on **2** and indicate the formation of a dppz-centered $\pi \rightarrow \pi^*$ $^3$IL state. For the mononuclear complex, this state shows a slow biexponential decay ($\tau = 500$ ns and 3.5 μs).[15] In contrast, the lifetimes of **3** are significantly shorter ($\tau_{IL} = 108$ and 968 ns).

The decay of the $\pi \rightarrow \pi^*$ $^3$IL state is around two orders of magnitude more rapid in water than in MeCN (Figure 2; $\tau_{IL} =$ 4 and 23 ns). Schanze and co-workers suggested that the nonluminescence of $[fac\text{-}(CO)_3Re(dppz)(Mepy)]^+$ (MePy = 4-methylpyridine) in water is due to deactivation of the IL state via the close-lying and (in water) short-lived Re(dπ)→dppz(π*) MLCT manifold.[10] This latter state has been observed in TRIR studies on **2** in MeCN.[15]

In contrast, our TRIR studies offer no evidence for the formation of such a state; only $^3$IL decay is observed. Again, the facts that **3** is not luminescent in water and that no Re(dπ)→dppz(π*) $^3$MLCT manifold is observed are consistent with energy transfer. In both MeCN and water, the dppz-centered $\pi \rightarrow \pi^*$ $^3$IL state of the Re unit is being deactivated via the Ru(dπ)→dppz(π*) $^3$MLCT state, which is extremely short-lived in water.[1,2] We then investigated the interaction of **3** with calf-thymus DNA (CT-DNA) in aqueous buffer (25 mM NaCl, 5 mM tris(hydroxymethyl)aminomethane, pH 7.0).

Addition of aliquots of CT-DNA results in distinctive changes in the UV/Vis spectrum of **3** with several bands between 280 and 450 nm showing large hypochromicity and significant bathochromic shifts. Furthermore, progressive addition of **3** to CT-DNA solutions results in increases in relative viscosity—at a ligand/base pair molar ratio of 0.4, viscosity is increased by 25 % (see the Supporting Information). These two phenomena are characteristic of an interaction of a metallointercalator with DNA.[1,2,18]

As expected, **3** is nonluminescent in aqueous solution. On addition of DNA to **3**, the intense luminescence of **3** is restored (Figure 3). This observation is also consistent with the intercalation of the $\{Ru^{II}(dppz)\}$ unit, as is the biexponential decay of this excited state.[1,2] The lifetimes ($\tau_{em} = 36$ and 117 ns) are comparable to the values obtained for $[Ru(tpm)(dppz)(dpp[5])]^{2+}$ (32 and 102 ns).

By fitting the absorption and emission data to the McGhee–von Hippel model for binding to an isotropic lattice,[19] the DNA binding affinity $K_b$ of **3** was estimated as $6 \times 10^5\,M^{-1}$. Interestingly, despite the lower cationic charge of **3**, this value is similar to that of the corresponding dinuclear

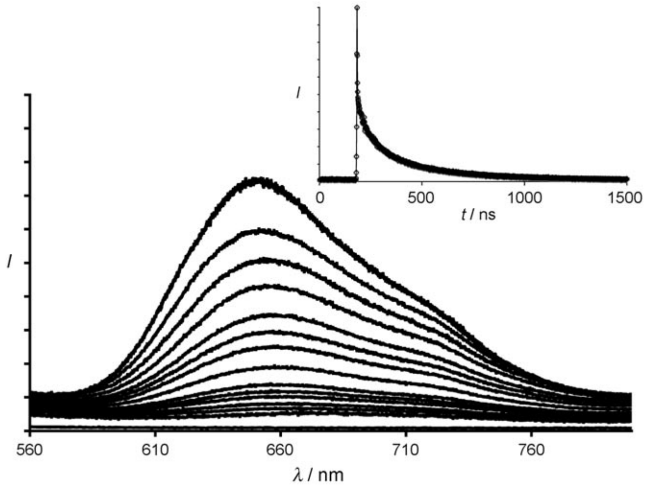

***Figure 3.*** Changes in luminescence of **3**-$Cl_3$ in aqueous buffer upon addition of CT-DNA. Inset: Luminescent decay of excited state on saturation binding.

tetracation $[\{Ru(tpm)(dppz)\}_2(dpp[5])]^{4+}$.[14] As this value represents more than an order of magnitude enhancement in binding affinity relative to **2**—which itself is known to cleave DNA directly—the possibility that **3** could also display cleavage properties was investigated.

Although no nicking was observed in control experiments with untreated plasmid (see the Supporting Information), it was found that addition of **3**-$Cl_3$ to aqueous buffer solutions of pBR322 plasmid DNA and subsequent photoirradiation leads to the generation of relaxed singly nicked plasmid (Figure 4). Indeed, through extensive double nicking, higher loading of the complex rapidly results in complete degradation of the DNA. As far as we are aware, this is the first example of a complex that can function as both a light switch and as direct cleavage "scissors" of DNA.

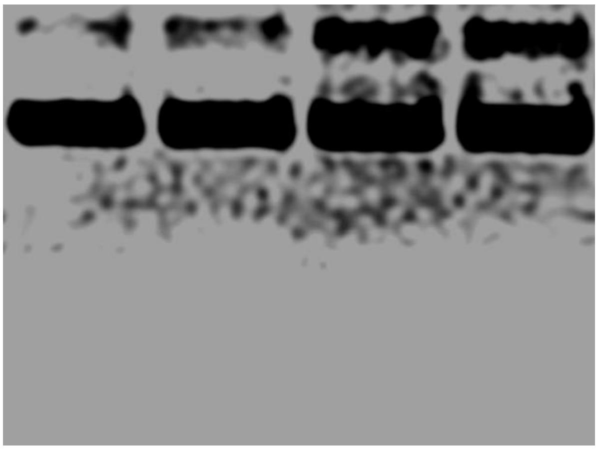

***Figure 4.*** Cleavage of pBR322 plasmid DNA by **3**-$Cl_3$. Conditions: Lane 1 is untreated plasmid. Lanes 2–4 show increasing cleavage over time (30, 60, 90 min) after addition of **3**-$Cl_3$ (0.1 μmol) and photoirradiation at 355 nm.

In summary, the first hetero-dinuclear dppz complex is reported. The excited states of both metal centers can be monitored independently by spectroscopy; these studies reveal energy transfer from the $Re^I$ to the $Ru^{II}$ center. Complex **3** binds to duplex DNA with good affinity and displays both DNA light switch and cleavage properties. These unique properties result from the synergy between two metal centers: the $\{Ru^{II}(dppz)\}$ unit supplies the light-switch function and enhances the binding affinity of the $\{Re^I(dppz)\}$ unit, while this latter moiety provides a hitherto unobserved functionality for light-switch systems, that of "scissors" for DNA. More detailed studies on **3** will investigate the nature of the DNA-cleavage mechanism. Work on related complexes and other specific DNA sequences, designed to investigate such issues as DNA-mediated energy-/electron-transfer processes, enhanced binding affinity, and any possible binding selectivity, will form the basis of future reports.

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