Efficient DNA photocleavage by $[Ru(bpy)_2(dppn)]^{2+}$ with visible light†

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The population and reactivity of two low-lying excited states in $[Ru(bpy)_2(dppn)]^{2+}$ (bpy = 2,2′-bipyridine, dppn = benzo[*i*]-dipyrido[3,2-*a*:2′,3′-*c*]phenazine), a weakly emissive ^{3}MLCT state and a long-lived ligand-centered $^3\pi\pi^*$ state, lead to efficient photoinduced DNA damage. Irradiation with visible light results in nearly complete DNA cleavage within 30 s ($\lambda_{irr} \geq 455$ nm), likely from the combined action of guanine oxidation and the production of reactive oxygen species derived from $^1O_2$.

Ruthenium complexes related to $[Ru(bpy)_3]^{2+}$ (**1**, bpy = 2,2′-bipyridine; Fig. 1) but possessing ligands with extended π-systems have been investigated extensively as probes of DNA and for numerous potential applications.[1–5] Changes in the ligation sphere around the ruthenium center can be used to attain diverse DNA binding modes and preference for particular sites or base sequences, with concomitant changes in the photophysical properties and excited state reactivity of the complex.[1–5] A well-known example is the "DNA light-switch" molecule, $[Ru(bpy)_2(dppz)]^{2+}$ (**2**, dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine; Fig. 1), with negligible emission in water and dramatic enhancement in the presence of DNA.[1*a*] A large number of complexes with dppz derivatives and related ligands have been investigated in order to elucidate the light-switch mechanism and discover new DNA probes.[1–5]

The reactive species produced upon irradiation of current agents for photodynamic therapy (PDT) is $^1O_2$, which results in the generation of other reactive oxygen species (ROS).[6] Although efficient sensitization of $^1O_2$ is a desirable property of a PDT agent, systems that exhibit dual reactivity are sought in order to improve drug efficacy.[7]

We recently reported new Ru(II) complexes possessing the tridentate ligand with extended π-system pydppn (3-(pyrid-2′-yl)-4,5,9,16-tetraaza-dibenzo[*a*,*c*]naphthacene), where the lowest-energy excited state was LC (ligand-centered) pydppn $^3\pi\pi^*$ instead of the typical ^{3}MLCT (metal-to-ligand charge transfer).[8] Because of the longer lifetime of the former, sensitized $^1O_2$ production with near 100% yield was observed, which results in efficient DNA photocleavage, DNA–protein, and protein–protein crosslinking within cells.[8] The present work focuses on $[Ru(bpy)_2(dppn)]^{2+}$ (**3**; dppn = benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]-phenazine; Fig. 1), where the dppn ligand possesses structural features similar to those of pydppn. As expected, a low-lying and long-lived LC $^3\pi\pi^*$ state was also recently reported for **3**, however, the photoinduced reactivity of the complex with DNA was not explored.[9]

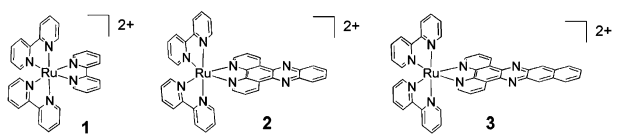

Fig. 1 Schematic representation of the molecular structures of **1–3**.

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Complexes **1–3** were synthesized and purified according to published methods (ESI†).[1*b*,9,10] The cyclic voltammograms of **1–3** exhibit one reversible metal-based oxidation in CH_3CN with similar values ($E_{1/2}([Ru]^{3+/2+})$ = 1.54–1.58 V *vs.* NHE, (Table 1).[9,11] However, **1–3** differ significantly in their ligand-based reduction potentials (Table 1), where dppn in **3** is the easiest to reduce followed by dppz in **2**, consistent with previous reports and with the extent of the π-delocalization of each ligand.[9,11]

The absorption spectrum of **2** shows the dppz-based $^1\pi\pi^*$ transitions with maxima at 359 nm (17 500 M^{-1} cm^{-1}) and 370 nm (17 200 M^{-1} cm^{-1}), whereas the dppn-centered peaks of **3** are observed at lower energies, 387 nm (9900 M^{-1} cm^{-1}) and 411 nm (13 400 M^{-1} cm^{-1}). These absorption features of **3** are similar to those of the free dppn ligand in $CHCl_3$ (Fig. S2, ESI†), with maxima at 390 nm (9400 M^{-1} cm^{-1}) and 414 nm (12 500 M^{-1} cm^{-1}). The typical ^{1}MLCT bands arising from Ru(dπ) → L(π*) transitions are prominent for **1–3** in the 444–450 nm range (Table 1 and Fig. S2, ESI†).[11] The ^{1}MLCT band of **3** clearly overlaps with the low energy $^1\pi\pi^*$ transitions of dppn. In contrast to the intense luminescence of **1** and **2** in CH_3CN, only weak ^{3}MLCT emission at 617 nm (τ = 803 ns) is observed from **3** (Table 1).[9] The well-defined vibronic structure of the luminescence spectrum of **3** at 77 K is consistent with the ^{3}MLCT character of the emission (Fig. S2, ESI†). The fluorescence spectra of dppn in $CHCl_3$ at 298 K and 77 K differ significantly from the respective emission spectra of **3** (Fig. S2, ESI†).

It should be noted that although shifts in the ^{1}H NMR spectrum of **3** in CD_3CN provide evidence of π-stacking at high concentrations (1 to 11 mM, Fig. S3, ESI†), similar to related systems,[12] these interactions are negligible at concentrations $\leq$0.5 mM in this solvent. The absorption and luminescence spectra of **3** were collected at concentrations well below this value (5–70 μM), such that the photophysical data are expected to correspond to monomeric **3** in solution.

The transient absorption spectra of **3** collected in the ns to μs timescale are similar to that previously reported for the complex and to that of the free dppn ligand, featuring a strong absorption band at 540 nm with τ = 33 ± 5 μs in CH_3CN (Fig. S4, ESI†).[9*b*] A lifetime of 18 ± 2 μs was measured for free dppn in $CHCl_3$, typical of $^3\pi\pi^*$ excited states of polycyclic aromatic heterocycles.[13] As previously discussed,[9*b*] the lowest-energy excited state of **3** is therefore assigned

Table 1 Photophysical and electrochemical data of **1–3**

Complex	λ_{abs}/nm ($\varepsilon \times 10^3$ M^{-1} cm^{-1})[a]	λ_{em}/nm (Φ_{em})[a]	Φ ($^1O_2$)[b]	$E_{1/2}$[c]/V
1	450 (13.0)	619 (0.062)	0.81[d]	+1.54, −1.07
2	445 (16.3)	629 (0.083)	0.16(2)	+1.57, −0.73
3	444 (13.5)	617 (0.003)[e]	0.88(5)	+1.58, −0.46
4[f]	474 (16.8)[g]	703 (3×10^{-5})[g]	0.92(2)	+1.59, −0.46

[a] In CH_3CN, at 298 K. [b] In CH_3OH. [c] In CH_3CN with 0.1 M Bu_4NPF_6, *vs.* NHE. [d] From ref. 19. [e] Error = ±0.001. [f] From ref. 8. [g] In H_2O.

as dppn-localized $^3\pi\pi^*$. In contrast, **2** is well known to exhibit ^{3}MLCT as the lowest excited state (τ = 750 ns) in CH_3CN.[14]

Ultrafast transient absorption spectra were collected to ascertain the presence of an emissive ^{3}MLCT state and non-emissive $^3\pi\pi^*$ in the same complex. Although not unprecedented,[8,9] such behavior is unusual. The signal at 370 nm is known to be associated with the ^{3}MLCT state, and its intensity remains relatively constant from 1 to 20 ps (Fig. 2a). The $^3\pi\pi^*$ absorption at 536 nm grows at early times with a risetime of 2.1(4) ps, then remains unchanged from 20 ps to 2 ns (Fig. 2). Some contribution from the broad ^{3}MLCT absorption in the 500–650 nm range is also expected, similar to that of **2** (Fig. S5, ESI†). Since the ^{3}MLCT signal does not decrease while that of the $^3\pi\pi^*$ state increases at early times (Fig. 2a), it may be concluded that the latter is not populated from the former. Therefore, it is believed that intersystem crossing (isc) results in population of both the ^{3}MLCT and $^3\pi\pi^*$, as previously described for related systems.[15,16] A slight decrease in the ^{3}MLCT signal is observed from 20 ps to 1 ns, which can be associated with the decay of that state and is also observed for **2** (Fig. S5, ESI†).

It has been shown that **2** and related complexes exhibit two or more low-lying ^{3}MLCT states possessing electron densities proximal (^{3}MLCT^{prox}) and distal (^{3}MLCT^{dis}) to the metal.[14] Fast equilibrium is expected between the ^{3}MLCT^{prox} and ^{3}MLCT^{dis} states of **3** at ambient temperature, with the former radiatively decaying to the ground state to generate the weak luminescence.[14,17] The triplet manifold is populated quickly

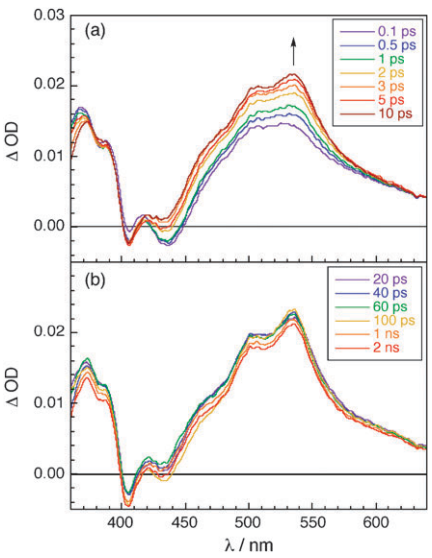

Fig. 2 Transient absorption spectra of 64 μM **[3]**$(PF_6)_2$ in CH_3CN collected at (a) 0–10 ps and (b) 20 ps–2 ns after the excitation pulse (λ_{exc} = 290 nm, fwhm = 300 fs).

(<1 ps) through intersystem crossing from the initially excited ^{1}MLCT and $^1\pi\pi^*$ states.[18] These states are shown in the Jablonski diagram for **3** in Fig. 3, with the addition of the lowest-lying $^3\pi\pi^*$ state. The energies of the ^{3}MLCT^{prox} and $^3\pi\pi^*$ states in Fig. 3 were calculated from the emission maximum of **3** and from reported theoretical calculations,[9b] respectively, whereas the energy of ^{3}MLCT^{dis} is uncertain. The energies of $^1\pi\pi^*$ and ^{1}MLCT in Fig. 3 were estimated from their corresponding absorption maxima.

The $^1O_2$ quantum yields of complexes **2–3** were measured using **1** as the standard (Φ = 0.81 in CH_3OH) and 1,3-diphenyl-isobenzofuran as a trapping agent.[19] As expected from its longer excited state lifetime, the $^1O_2$ quantum yield of **3** (0.88 ± 0.05) is significantly greater than that of **2** (0.16 ± 0.02), consistent with the reported value of **3** measured in CH_3CN[9b] and comparable to the related pydppn complexes, such as $[Ru(tpy)(pydppn)]^{2+}$ (**4**, tpy = [2,2′;6′,2″]-terpyridine), whose properties are also listed in Table 1.[8]

The DNA binding constant of Ru(II) complexes containing dppn ligands has been reported to be ~10^6 M^{-1},[9] several orders magnitude greater than that of **1** (700 M^{-1}).[20] The increase in the relative viscosity of DNA solutions upon addition of **3** unambiguously demonstrates that the complex is a DNA intercalator (Fig. S6, ESI†). The data also show the independence of the relative viscosity of DNA on the concentration of **1** (Fig. S6, ESI†), as expected for a non-intercalator.[20]

The photocleavage of 100 μM pUC18 by **1–3** is shown in Fig. 4a, where in the absence of complex (lane 1) the plasmid is in the supercoiled form (form I) with a small amount of nicked impurity (form II). No DNA cleavage was observed for 20 μM **1** and **2** upon irradiation (λ_{irr} ≥ 455 nm, 30 s) or in the dark (lanes 2–5). Complex **3** does not cleave the plasmid in the dark (lane 6), but results in complete cleavage of the supercoiled form within 30 s of irradiation (λ_{irr} ≥ 455 nm, lane 7). The wavelength dependence of the photocleavage by **3** reveals that significant DNA cleavage can still be obtained in 3 min with λ_{irr} ≥ 550 nm (Fig. S7, ESI†). Fig. 4b displays the DNA photocleavage of **3** in D_2O (lane 3), which shows only a slight enhancement compared to that in H_2O (lane 2). In a deaerated sample (lane 4), a small

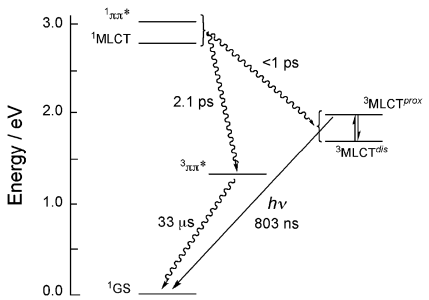

Fig. 3 Jablonski diagram of **3** in CH_3CN.

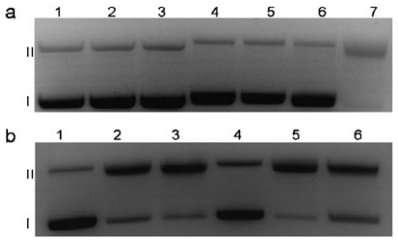

Fig. 4 Ethidium bromide stained agarose gels of the photocleavage of 100 μM pUC18 (5 mM Tris, 50 mM NaCl, pH = 7.5) and 20 μM of the chloride salt of each complex (a) in air: lane 1, plasmid only dark; lane 2, **1**, dark; lane 3, **1**, irr.; lane 4, **2**, dark; lane 5, **2**, irr.; lane 6, **3**, dark; lane 7, **3**, irr. ($\lambda_{irr} \geq 455$ nm, $t_{irr} = 30$ s) and (b) **3** ($\lambda_{irr} \geq 475$ nm, $t_{irr} = 50$ s), lane 1, dark; lane 2, air; lane 3, D_2O; lane 4, six freeze–pump–thaw cycles; lane 5, 2 mM NaN_3; lane 6, 2 units SOD.

amount of DNA photocleavage is observed, and the presence of NaN_3 (lane 5), a $^1O_2$ and $^{\bullet}OH$ scavenger, and superoxide dismutase (SOD, lane 6) has a small effect on the photocleavage of **3**. The data presented in Fig. 4b exclude the sole participation of $^1O_2$ and other ROS in the DNA cleavage mechanism. In addition, the quantum yields for $^1O_2$ generation of **1** and **3** are similar (Table 1), however, no photocleavage by **1** is observed in 30 s (Fig. 4a). This observation can be explained by the low DNA binding constant of **1**. For comparison, the pydppn complexes, with similar or greater production of $^1O_2$ that are also intercalators, do not cleave DNA as well as **3**.[8] These results point at a mechanism for the DNA reactivity by **3** that must encompass processes additional to ROS derived from $^1O_2$.

A possible explanation for the very efficient DNA photocleavage of **3** is the additional involvement of the ^{3}MLCT excited state. With $E_{00} \sim 2.1$ eV and $E_{1/2}([Ru]^{2+/+}) = -0.46$ V *vs.* NHE, an excited state reduction potential, E_{red}*, of $\sim$1.64 V *vs.* NHE can be estimated for **3**, making it a strong oxidizing agent. Using +1.29 V *vs.* NHE as the oxidation potential of guanine in water at pH = 7,[21] a favorable driving force of −0.35 V for the generation of G^+ from the ^{3}MLCT state of **3** is calculated. Guanine oxidation by the excited states of Ru(II) complexes has been previously shown to result in DNA cleavage and adduct formation.[3a,22] Although DNA photocleavage from guanine oxidation was reported for $[Ru(hat)_3]^{2+}$ (hat = 1,4,5,8,9,12-hexaazatriphenylene), a complex with similar excited state reduction potential as that of **3**, 80% DNA damage required 3 min irradiation at 436 nm.[22b] As shown in Fig. 4a, irradiation of **3** for only 30 s results in nearly quantitative DNA cleavage. Stern–Volmer plots of the changes in the emission lifetime (670 ns) of **3** as a function of GMP concentration in 50 mM deaerated Tris buffer (pH = 7.5) result in $k_q = 2.1 \times 10^8$ M^{-1} s^{-1} (Fig. S8, ESI†). As expected, no quenching of the lifetime of **1** by GMP is observed, with E_{red}* = 0.93 V *vs.* NHE (Fig. S8, ESI†).

Together these results point at an efficient DNA photocleavage mechanism that combines both the action of $^1O_2$ and guanine oxidation. A key feature to attain this dual reactivity is the presence of both a highly reactive ^{3}MLCT excited state for oxidation and the long-lived $^3\pi\pi$* state for sensitization of $^1O_2$. The investigation of the mechanistic details of this reactivity is currently underway, but it appears that the combined action of both excited states results in the high reactivity.

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