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$[Ru(bpy)_{3-n}(dpb)_n]^{2+}$: Unusual Photophysical Property and Efficient DNA Photocleavage Activity

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The sequential replacement of a bpy ligand (bpy = 2,2′-bipyridine) by a dpb ligand (dpb = 2,3-bis(2-pyridyl) benzoquinoxaline) in the series $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ ($n = 1-3$) leads to a remarkable increase of the excited state lifetime, the $^1O_2$ quantum yield, and the binding affinity toward dsDNA, rendering both $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ efficient DNA photocleavage activities upon red light irradiation ($\geq$600 nm).

Transition metal complexes that possess DNA photocleavage activities have drawn much attention by virtue of their utilities as DNA structure probes and as anticancer agents.[1] Among them, Ru(II) polypyridyl complexes were extensively studied owing to their tunable photophysical, photochemical, and redox properties.[2] Ru(II) complex-based DNA photocleavers generally show two distinct features, high singlet oxygen ($^1O_2$) quantum yield and strong binding ability to DNA. Both features favor their application in photodynamic therapy (PDT),[3] a tumor treatment strategy that uses the combination of a photosensitizer and visible or near-infrared (NIR) light to generate cytotoxic reactive oxygen species (ROS), mainly $^1O_2$.[4] Besides the high $^1O_2$ quantum yield, an ideal PDT photosensitizer should have strong absorptivity within the phototherapeutic window of 600−900 nm. However, most Ru(II) polypyridyl complexes suffer from short wavelength absorption, with the metal-to-ligand charge transfer (MLCT) absorption maximum shorter than 500 nm. Though the ligands having a delocalized π-system may shift the MLCT absorption to longer wavelengths,[5,6] shortened excited state lifetimes accompany them,[7] unfavorable for $^1O_2$ generation. For example, $[Ru(bpy)_2(dpb)]^{2+}$ (bpy = 2,2′-bipyridine, dpb = 2,3-bis(2-pyridyl) benzoquinoxaline, Scheme 1) exhibits a ^{1}MLCT maximum at 550 nm,[8] a 100 nm red shift compared to its parent complex $[Ru(bpy)_3]^{2+}$ (450 nm). However, both the ^{3}MLCT lifetime (66 ns) and the $^1O_2$ quantum yield (0.22) of $[Ru(bpy)_2(dpb)]^{2+}$[9] are much lower than those of $[Ru(bpy)_3]^{2+}$ (900 ns[10] and 0.57[11]). We recently synthesized a new Ru(II) polypyridyl complex, $[Ru(bpy)(dpb)(dppn)]^{2+}$ (dppn = 4,5,9,16-tetraaza-dibenzo[*a*,*c*]naphthacene), which exhibits not only a long wavelength ^{1}MLCT band centered at 548 nm but also a long ^{3}MLCT lifetime of 229 ns and a high $^1O_2$ quantum yield of 0.43.[9] The long ^{3}MLCT lifetime of $[Ru(bpy)(dpb)(dppn)]^{2+}$ originates from the long-lived (13 μs) triplet excited state of the dppn ligand, which is in close proximity to ^{3}MLCT(Ru→dpb) in energy, making an equilibrium established between the two states, i.e., the reservoir effect.[12]

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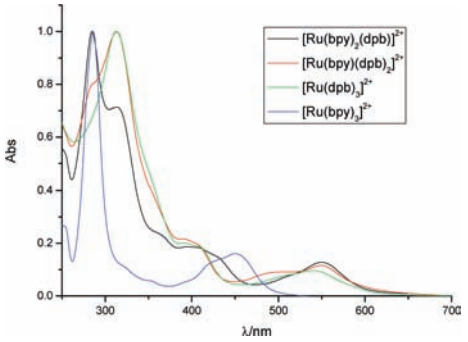
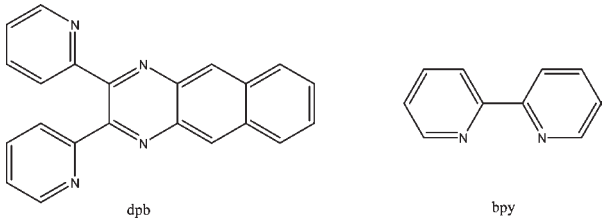


Figure 1. Normalized absorption spectra of the complexes in acetonitrile.

Scheme 1. Structures of dpb and bpy Ligands



To fully explore the capabilities of dpb-based Ru(II) complexes, we examined the photophysical and photochemical properties of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ ($n = 1$, 2, and 3). Both $[Ru(bpy)_2(dpb)]^{2+}$ and $[Ru(dpb)_3]^{2+}$ are known compounds;[8] however, their excited state lifetimes have never been reported (except our recent work on $[Ru(bpy)_2(dpb)]^{2+}$ [9]). To our surprise, with the increase of n from 1 to 3, the excited state lifetimes of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ extend from 66 ns to 330 ns to 530 ns, while the $^1O_2$ quantum yields increase from 0.22 to 0.45 to 0.52. Moreover, the binding affinities of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ toward double stranded DNA also increase with the increase of n. Consequently, both $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ exhibit efficient DNA cleavage abilities upon red light irradiation ($\geq$600 nm).

Figure 1 shows the normalized absorption spectra of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ ($n = 1-3$) and $[Ru(bpy)_3]^{2+}$. Comparison of these spectra can lead to the assignments of a $\pi \rightarrow \pi^*$ transition to the bpy ligand centered at 286 nm, a $\pi \rightarrow \pi^*$ transition to the dpb ligand centered at both 315 and 400 nm, and a ^{1}MLCT transition over the visible region, in good agreement with the previous results.[8] The dpb ligand renders the ^{1}MLCT of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ 100 nm red-shifted compared to that of $[Ru(bpy)_3]^{2+}$. In aqueous solutions, $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ undergoes a further bathochromic shift (Supporting Information), favorable for PDT application. The ^{3}MLCT emissions of $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ fall in the region of NIR (Table 1 and Supporting Information), recorded on a Confocal Laser Micro-Raman Spectroscope (532 nm excitation). On the same instrument, an emission centered at 619 nm was observed for $[Ru(bpy)_3]^{2+}$, in line with the result obtained on a conventional fluorescence spectrophotometer.

The electrochemical properties of these complexes were examined using cyclic voltammetry (Table 1 and Supporting Information). $[Ru(bpy)_2(dpb)]^{2+}$ displays a reversible Ru(III/II) based oxidation wave at +1.43 V versus SCE. The 0.14 V of anodic shift compared to that of $[Ru(bpy)_3]^{2+}$ (+1.29 V) may be attributed to the more electronegative character or stronger π-accepting feature of dpb than bpy. This is supported by the less negative reduction potential of -0.60 V for dpb compared to -1.33 V for bpy (Table 1). For $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$, the dpb ligand-based first reduction potentials appear at -0.50 V and -0.47 V, respectively, in accordance with the previous results.[8] The oxidation processes of $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ are no longer reversible with the peak potentials at 1.65 and 1.64 V, respectively. Carlson and Rorer Murphy ascribed the irreversible oxidation wave of $[Ru(dpb)_3]^{2+}$ to the oxidation of the dpb ligand.[8]

The DNA titration approach was used to examine the binding abilities of these complexes toward calf thymus DNA (CT-DNA). The absorption spectrum of $[Ru(bpy)_2(dpb)]^{2+}$ shows negligible changes upon the addition of DNA, indicative of a weak interaction. For $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$, the MLCT absorbance increased at first and then decreased continuously with the addition of CT-DNA. Such behavior was also observed for other Ru(II) complexes, probably due to the DNA-induced complex aggregation.[3g,5,13] Thus, an EB displacement assay was carried out to compare the DNA binding affinities of these complexes (Table 1 and Supporting Information). The binding constants of $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ are much higher than that of $[Ru(bpy)_2(dpb)]^{2+}$, presumably due to the more hydrophobic property of dpb than bpy (Supporting Information).[14]

The DNA photocleavage abilities of the dpb-based complexes were examined by agarose gel electrophoresis of the supercoiled pBR322 DNA upon red light irradiation ($\geq$600 nm; Figure 2). $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ show much higher DNA photocleavage activities than $[Ru(bpy)_2(dpb)]^{2+}$. Control experiments (Supporting Information) indicated that the DNA photocleavage was an $^1O_2$ mechanism. $^1O_2$ quantum yields of these complexes were determined to be 0.22 for $[Ru(bpy)_2(dpb)]^{2+}$, 0.45 for $[Ru(bpy)(dpb)_2]^{2+}$, and 0.52 for $[Ru(dpb)_3]^{2+}$ in CH_3CN, using $[Ru(bpy)_3]^{2+}$ as the standard (0.57 in CH_3CN)[11] and 1,3-diphenyl-isobenzofuran as the trapping agent of $^1O_2$. Obviously, the high $^1O_2$ quantum yield, the strong DNA binding affinity, and the long wavelength absorption of $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ are the main reasons for their efficient DNA photocleavage activities under red light irradiation. $[Ru(bpy)_2(dppz)]^{2+}$, a well-known DNA photocleaver that shows the activity within the visible region (< 550 nm), nearly did not photocleave DNA upon red light irradiation, due to its short wavelength absorption (Supporting Information).

To better understand the $^1O_2$ generation behaviors of these complexes, the time-resolved absorption spectra were measured (Supporting Information). $[Ru(bpy)_2(dpb)]^{2+}$ shows a ground-state bleaching band centered at 550 nm, and two positive absorption bands below 515 nm and over 580 nm, similar to the typical ^{3}MLCT T$-$T absorption spectra of Ru(II) polypyridyl complexes that do not emit or have a low quantum yield of emission.[3e,15] The bleaching band intensity

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Table 1. Photophysical and Electrochemical Properties, $^1O_2$ Quantum Yields, and Binding Constants Toward CT-DNA of the Examined Complexes[a]

complex	MLCT absorption maximum/nm	MLCT emission maximum/nm	$E_{1/2}$(ox)/V (vs SCE)	$E_{1/2}$(red)/V (vs SCE)[b]	τ_{TA}/ns[c]	$^1O_2$ quantum yield[d]	binding constant K_b/*10^6 M^{-1}[e]
$[Ru(bpy)_3]^{2+}$	449	619	1.29[b]	−1.33, −1.52, −1.76	900	0.57	
$[Ru(bpy)_2(dpb)]^{2+}$	551	863	1.43[b]	−0.60, −1.24, −1.58	66	0.22	1.7
$[Ru(bpy)(dpb)_2]^{2+}$	549	858	1.65[f]	−0.50, −0.78, −1.37	330	0.45	6.97
$[Ru(dpb)_3]^{2+}$	541	858	1.64[f]	−0.47, −0.64, −0.87	530	0.52	8.85

[a] In CH_3CN. [b] Half-wave potential for reversible process in CH_3CN. [c] Excited state lifetime obtained by transient absorption in CH_3CN. [d] Measured using $[Ru(bpy)_3]^{2+}$ as the standard and 1,3-diphenyl-isobenzofuran as the trapping agent of $^1O_2$ in CH_3CN. [e] Obtained by ethidium bromide displacement assay. [f] Peak potential for irreversible process in CH_3CN.

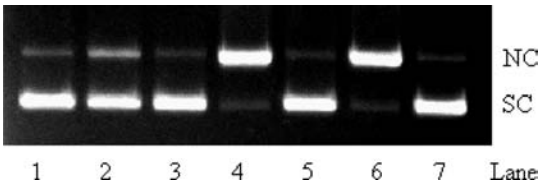

Figure 2. Agarose gel electrophoresis pattern of supercoiled pBR322 DNA (31 μM in base pair) upon visible light irradiation (≥600 nm) for 120 min (lanes 1, 2, 4, and 6) or dark control (lanes 3, 5, and 7) in an air-saturated Tris-CH_3COOH/EDTA buffer (pH = 7.4). Lane 1: DNA alone; lanes 2, 3: DNA + $[Ru(bpy)_2(dpb)]^{2+}$; lanes 4, 5: DNA + $[Ru(bpy)(dpb)_2]^{2+}$; lanes 6, 7: DNA + $[Ru(dpb)_3]^{2+}$. The concentration of the complex was 10 μM. SC and NC denote supercoiled circular and nicked circular forms, respectively.

of $[Ru(bpy)(dpb)_2]^{2+}$ is much lower than that of $[Ru(bpy)_2(dpb)]^{2+}$. In the case of $[Ru(dpb)_3]^{2+}$, no bleaching band was observed. The transient absorption spectra of these three complexes show single-exponential decay at all the wavelengths with the lifetimes of 66, 330, and 530 ns, respectively. The excited state lifetime of $[Ru(dpb)_3]^{2+}$ is among the longest for the Ru(II) complexes that have MLCT absorption maxima beyond 550 nm.[5,9] Considering the fact that the triplet excited state of the dpb ligand is long-lived (4 μs) and exhibits a strong positive band in the region of 300−570 nm, the transient absorption spectrum changes from $[Ru(bpy)_2(dpb)]^{2+}$ to $[Ru(dpb)_3]^{2+}$ seem the result of the augmented mixing of the triplet excited state of the dpb ligand with ^{3}MLCT. This unusual finding undoubtedly deserves further study, and theoretical calculations may shed light on the interesting behaviors. The long excited state lifetimes of $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ facilitate their photosensitized generation of $^1O_2$.

In summary, $[Ru(bpy)_{3-n}(dpb)_n]^{2+}$ ($n = 1-3$) not only possesses long wavelength MLCT absorption but also shows some unexpected properties; i.e., the sequential replacement of the bpy ligand by the dpb ligand leads to a remarkable extension of the excited state lifetime, a significant enhancement of the $^1O_2$ quantum yield, and a marked improvement of the binding affinity toward dsDNA. As a result, both $[Ru(bpy)(dpb)_2]^{2+}$ and $[Ru(dpb)_3]^{2+}$ exhibit efficient DNA photocleavage activities upon red light irradiation (≥600 nm), showing application potential in PDT.

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Supporting Information Available: Synthesis and characterization of the complexes, crystal structure of $[Ru(bpy)_2(dpb)]^{2+}$, luminescence spectra at room temperature, cyclic voltammograms, EB displacement assay, time-resolved absorption spectra, and partition coefficient. This material is available free of charge via the Internet at http://pubs.acs.org.