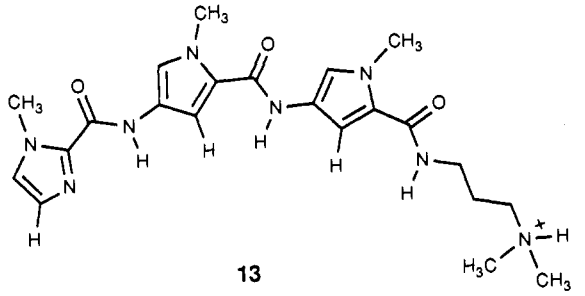
This is in contrast to previous studies of less specific imidazole-containing oligopeptides where the position of the GC base pairs within the ligand binding sites did not correlate with the prediction of hydrogen-bond formation between an imidazole N3 and guanine NH_2.

While this work was in progress, the distamycin A analog 2-ImN, (**13**), designed by Wade and Dervan,[19] was shown to bind specifically to TGACT·AGTCA.[20] This molecule binds with high



13

cooperativity to the minor groove of DNA (2:1 mode). The imidazole nitrogens of the two ligands each recognize a different guanine amino group. The results thus far contribute to our present understanding of sequence-specific recognition of DNA by minor groove binding ligands: (1) the 2:1 binding mode allows the ligands to optimize tight van der Waals contacts and hydrogen bonding with the minor groove surface independent of sequence-dependent variations in groove width; (2) the positive charge on the ligand is important for the initial attraction to DNA, but two positive charges at opposite ends of the ligand prevent the formation of the 2:1 complex; (3) the spacing of hydrogen donor and acceptor groups on the ligands must match those on the DNA allowing for stabilizing interactions similar to the "spine of hydration" in the free duplex.

Both 2-ImD and 2-ImN specifically recognize mixed GC- and AT-containing DNA sequences. The basic idea in the design of lexitropsins has therefore proven successful. We propose that binding to GC-containing sequences can be enhanced when a single hydrogen-bond acceptor per G amino group is strategically positioned for complexation. We emphasize the importance of the 2:1 binding mode for optimization of van der Waals contacts and of hydrogen bonding between ligands and DNA. On the basis of these findings, lexitropsin molecules can now be designed to specifically recognize many DNA sequences of four to five base pairs in length.

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Novel Dipyridophenazine Complexes of Ruthenium(II): Exploring Luminescent Reporters of DNA

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Abstract: A series of ruthenium(II) complexes have been prepared which contain two phenanthroline ligands and a third bidentate ligand which is one of a set of derivatives of the parent dipyrido[3,2-*a*:2′,3′-*c*]phenazine (DPPZ) ligand. The spectroscopic properties of these complexes in the presence and absence of DNA have also been characterized. The derivatives have been prepared by condensation of different diaminobenzenes or diaminopyridines with the synthetic intermediate bis(1,10-phenanthroline)(1,10-phenanthroline-5,6-dione)ruthenium(II). $[Ru(phen)_2DPPZ]^{2+}$, like $[Ru(bpy)_2DPPZ]^{2+}$, acts as a molecular "light switch" for the presence of DNA, displaying no detectable photoluminescence in aqueous solution but luminescing brightly on binding to DNA. None of the DPPZ derivatives prepared show comparable "light switch" enhancements, since some luminescence may be detected in aqueous solution in the absence of DNA. For some complexes, however, luminescence enhancements of a factor of 20–300 are observed on binding to DNA. For these and the parent DPPZ complexes, the large enhancements observed are attributed to a sensitivity of the ruthenium–DPPZ luminescent charge-transfer excited state to quenching by water; although these complexes show little or no luminescence in water, appreciable luminescence is found in acetonitrile. Other derivatives show little solvent sensitivity in luminescence, and these, like $Ru(phen)_3^{2+}$, display moderate enhancements (20–70%) on binding to DNA. $[Ru(phen)_2DPPZ]^{2+}$ and its derivatives all show at least biexponential decays in emission. Two binding modes have been proposed to account for these emission characteristics: a perpendicular mode where the DPPZ ligand intercalates from the major groove such that the metal–phenazine axis lies along the DNA dyad axis, and another, side-on mode where the metal–phenazine axis lies along the long axis of the base pairs.

There has been increasing attention given to the design of novel transition metal complexes which recognize and react with nucleic acids so as to develop new diagnostic and therapeutic agents.[1-7] Our laboratory has focused, in part, on the preparation of ruthenium complexes which bind to DNA by intercalation.[4-7] Ruthenium complexes provide very sensitive reporters of DNA in aqueous solution and may become particularly useful in de-

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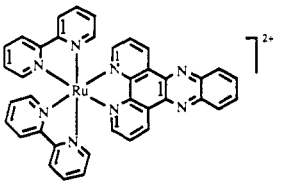
veloping new diagnostics based upon luminescent DNA probes.

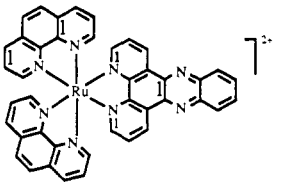
Polypyridyl complexes of ruthenium(II) are intensely colored owing to a well-characterized, localized metal-to-ligand charge-transfer (MLCT) transition.[8] Importantly this transition is perturbed on binding to DNA, which has led to the development of a family of spectroscopic probes of DNA structure. Extensive photophysical studies[5] have established two binding modes[9] for $Ru(phen)_3^{2+}$ with DNA: one corresponding to an intercalative interaction in which the excited-state lifetime is increased from 0.6 to 2 μs, and the second corresponding to a groove-bound interaction, which displays an excited-state lifetime comparable to that of the free form but which, when compared to free $Ru(phen)_3^{2+}$, is differentially quenched by anionic quenchers. The intercalative mode and groove bound modes are comparable energetically for $Ru(phen)_3^{2+}$, with the intercalative interaction favoring the Δ-isomer and the groove-bound form favoring the Λ-isomer.

More recently, the complex $[Ru(bpy)_2DPPZ]^{2+}$ (bpy = 2,2′-bipyridine, DPPZ = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) (**1**) was shown[6] to be a remarkable luminescent reporter of DNA structure. This probe, described as a molecular "light switch" for DNA, shows no photoluminescence in aqueous solution at ambient temperatures, but displays intense photoluminescence in the presence of double-helical DNA, to which the complex binds avidly. The complex also binds DNA through intercalation. Owing to the increased stacking area of the DPPZ ligand compared to phenanthroline, the affinity for DNA is enhanced by at least 3 orders of magnitude relative to $Ru(phen)_3^{2+}$ and groove binding becomes negligible.[10] The luminescence enhancement on binding to DNA for $[Ru(bpy)_2DPPZ]^{2+}$ is at least 10^4.[6] The complex therefore provides a unique spectroscopic probe for both B- and Z-DNA.[6,7]

Since the MLCT transition has been shown[11] to be localized onto the DPPZ ligand, and it is the intercalative interaction of the DPPZ ligand with DNA that is responsible for the luminescence enhancements, substitutions may be made in the ancillary ligands while still preserving the "light switch" effect.[12] Consequently, $[Ru(phen)_2DPPZ]^{2+}$ (**2**) similarly shows no detectable luminescence in aqueous solution but substantial luminescence on binding to DNA. Both complexes are illustrated below.



$[Ru(bpy)_2(DPPZ)]^{2+}$ (1)



$[Ru(phen)_2(DPPZ)]^{2+}$ (2)

Given the unique luminescent properties observed with ruthenium complexes of DPPZ, it becomes important to characterize further these luminescent characteristics and to explore the generality of the observation. Here we describe the synthesis of a series of derivatives of $[Ru(phen)_2DPPZ]^{2+}$ (**2**), where substitutions are made on the DPPZ ligand, as well as the characterization of their luminescent properties in the presence and absence of DNA. The results described here support the notion[6,11,13,14] that the sensitivity of the luminescent excited state to quenching by water is a key component of the "light switch" effect.

Experimental Section

Instrumentation and Materials. Ammonium hexafluorophosphate and the substituted *o*-diaminobenzene compounds were obtained from Aldrich. Calf thymus DNA was obtained from Pharmacia and dissolved in Tris/HCl buffer (pH 7.2), to a concentration of 1.25 mM in base pairs. 1,10-Phenanthroline-5,6-dione (phendione) was prepared according to the method of Amouyal et al.,[11] and $Ru(phen)_2Cl_2$ was prepared using the method[15] employed for the synthesis of $Ru(bpy)_2Cl_2$. $[Ru(phen)_2(O_3SCF_3)_2]$ was prepared from $Ru(phen)_2Cl_2$ by standard methods.[16] All other chemicals were of at least reagent grade and were used without further purification.

^1H NMR spectra were obtained on a JEOL GX-400 spectrometer. Chemical shifts were measured with reference to the residual solvent signals. Visible absorption spectra were measured on a Cary 2200 spectrophotometer in aqueous solution unless otherwise noted. Extinction coefficients were calculated using ruthenium concentrations that were determined as described below on a Varian AA-875 atomic absorption spectrophotometer equipped with a Varian GTA-95 graphite tube atomizer. Elemental analyses were performed by Galbraith Laboratories, Inc., and the FAB mass spectra were obtained at the Southern California Mass Spectrometry Facility at the University of California, Riverside.

Synthesis. $[Ru(phen)_2phendione](PF_6)_2·5H_2O$. $[Ru(phen)_2(O_3SCF_3)_2]$ (1.5 g) was dissolved in ethanol (75 mL), phendione (0.456 g) added, and the solution heated to reflux for 2 h. The yellow-brown solution was cooled to room temperature and filtered. The complex was precipitated by addition of NH_4PF_6. After cooling at −20 °C overnight, the solid material was isolated by filtration and washed with diethyl ether, yield 1.4 g. ^1H NMR (acetone-d_6): δ 8.88 (dd, 2 H), 8.77 (dd, 2 H), 8.67 (dd, 2 H), 8.60 (dd, 2 H), 8.42 (AB, 4 H), 8.32 (dd, 2 H), 8.28 (dd, 2 H), 7.98 (dd, 2 H), 7.76 (dd, 2 H), 7.68 (dd, 2 H). FABMS: $[MH^{2+} + PF_6^0]^+$ 819, MH^+ 673. Anal. Calcd for $C_{36}H_{32}N_6F_{12}O_7P_2Ru$: C, 41.11; H, 3.07; N, 7.99. Found: C, 41.06; H, 3.22; N, 7.90.

$[Ru(phen)_2DPPZ](PF_6)_2·2H_2O$ (2) and Derivatives. $[Ru(phen)_2phendione](PF_6)_2·5H_2O$ (150 mg) was suspended in ethanol (30 mL), the mixture heated to reflux, and the appropriately substituted *o*-diaminobenzene (1.1 equiv) added. Heating was continued for a further 15 min, during which time the brown solution became more red. The solution was cooled, NH_4PF_6 added, and the precipitated complex filtered and washed with diethyl ether. Crude yields varied from 100 to 160 mg. The complexes may be recrystallized by dissolution in a small volume of acetone and addition of an equal volume of aqueous NH_4PF_6, followed by slow evaporation of the acetone. Alternatively, in some cases the slow addition of concentrated aqueous LiCl precipitated the chloride salt. This material was taken up in methanol or ethanol and reprecipitated with NH_4PF_6. Reverse-phase HPLC (0.1 M triethylammonium acetate/CH_3CN eluent) was frequently employed to obtain high-purity samples for luminescence studies.

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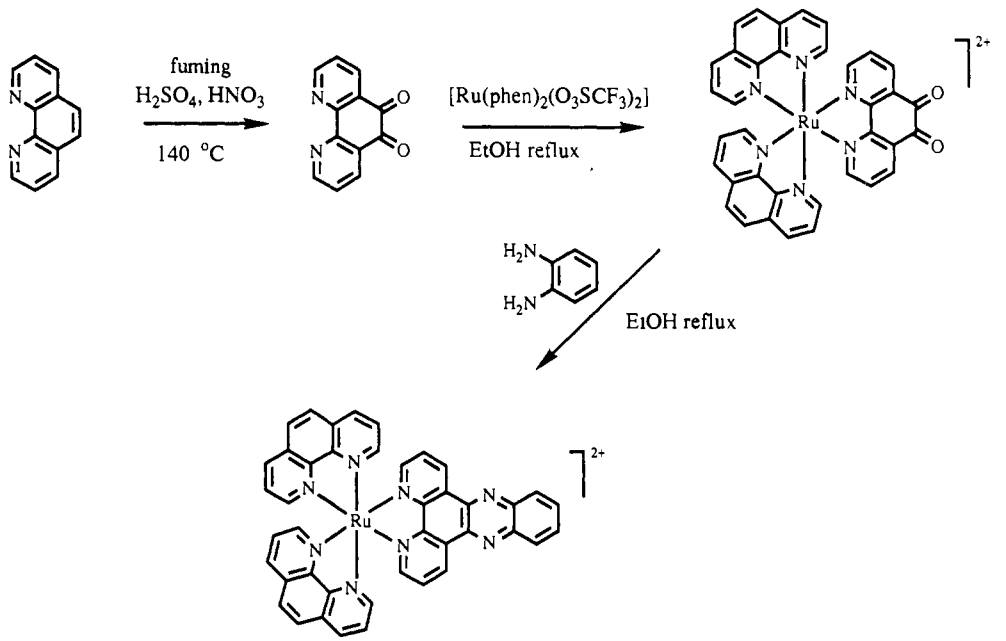

Figure 1. Synthetic route to $[Ru(phen)_2DPPZ]^{2+}$ and derivatives.

$[Ru(phen)_2DPPZ](PF_6)_2 \cdot 2H_2O$ (2): ^1H NMR (acetone-d_6) δ 9.73 (dd, 2 H), 8.82 (dd, 2 H), 8.79 (dd, 2 H), 8.60 (dd, 2 H), 8.50 (dd, 2 H), 8.48 (m, 2 H), 8.42 (s, 4 H), 8.39 (dd, 2 H), 8.18 (m, 2 H), 7.95 (dd, 2 H), 7.83 (dd, 2 H), 7.81 (dd, 2 H); FABMS, $[M^{2+} + PF_6^-]^+$ 889, M^+ 744. Anal. Calcd for $C_{42}H_{30}N_8F_{12}O_2P_2Ru$: C, 47.16; H, 2.83; N, 10.47. Found: C, 47.17; H, 2.88; N, 10.35.

$[Ru(phen)_2DPPX](PF_6)_2 \cdot 3H_2O \cdot {}^1/_4NH_4PF_6$: ^1H NMR (acetone-d_6) δ 9.68 (dd, 2 H), 8.81 (m, 4 H), 8.60 (dd, 2 H), 8.48 (dd, 2 H), 8.42 (s, 4 H), 8.40 (dd, 2 H), 8.23 (s, 2 H), 7.93 (dd, 2 H), 7.82 (dd, 2 H), 2.68 (s, 6 H); FABMS, $[M^{2+} + PF_6^-]^+$ 917, $[M - H]^+$ 771. Anal. Calcd for $C_{44}H_{37}N_{8.25}F_{13.5}O_3P_{2.25}Ru$: C, 45.69; H, 3.22; N, 9.99. Found: C, 45.90; H, 2.99; N, 9.62.

$[Ru(phen)_2DPPM2](PF_6)_2 \cdot 2H_2O \cdot {}^1/_3NH_4PF_6$: ^1H NMR (acetone-d_6) δ 9.81 (dd, 1 H), 9.72 (dd, 1 H), 8.81 (m, 4 H), 8.61 (m, 2 H), 8.51 (m, 2 H), 8.43 (s, 4 H), 8.41 (d, 2 H), 8.33 (d, 1 H), 8.05 (m, 2 H), 7.94 (dd, 2 H), 7.82 (m, 4 H); FABMS, $[M^{2+} + PF_6^-]^+$ 903, M^+ 758. Anal. Calcd for $C_{43}H_{33.3}N_{8.3}F_{14}O_2P_{2.3}Ru$: C, 45.38; H, 2.95; N, 10.26. Found: C, 45.45; H, 2.87; N, 10.22.

$[Ru(phen)_2DPPA](PF_6)_2$: ^1H NMR (acetone-d_6) δ 9.77 (m, 2 H), 9.07 (s, 1 H), 8.82 (m, 4 H), 8.62 (m, 2 H), 8.61 (AB, 2 H), 8.54 (m, 2 H), 8.43 (s, 4 H), 8.41 (m, 2 H), 7.98 (m, 2 H), 7.83 (m, 4 H); FABMS, $[M^{2+} + PF_6^-]^+$ 933, M^+ 788. Anal. Calcd for $C_{43}H_{26}N_8F_{12}O_2P_2Ru$: C, 47.92; H, 2.43; N, 10.40. Found: C, 48.02; H, 2.52; N, 10.63.

$[Ru(phen)_2DPPB](PF_6)_2 \cdot 2H_2O$: ^1H NMR (acetone-d_6) δ 9.76 (dd, 1 H), 9.72 (dd, 1 H), 8.81 (m, 4 H), 8.73 (d, 1 H), 8.64 (m, 1 H), 8.61 (m, 2 H), 8.53 (m, 2 H), 8.50 (m, 1 H), 8.43 (s, 4 H), 8.40 (d, 2 H), 7.97 (m, 4 H), 7.81 (m, 5 H), 7.67 (m, 2 H); FABMS, $[M^{2+} + PF_6^-]^+$ 993, M^+ 848. Anal. Calcd for $C_{49}H_{34}N_8F_{12}O_3P_2Ru$: C, 50.14; H, 2.92; N, 9.55. Found: C, 50.18; H, 2.84; N, 9.68.

$[Ru(phen)_2DPPP2](PF_6)_2 \cdot 3H_2O$: ^1H NMR (acetone-d_6) δ 9.72 (m, 2 H), 9.53 (dd, 1 H), 8.92 (dd, 1 H), 8.81 (m, 4 H), 8.62 (dd, 2 H), 8.54 (d, 2 H), 8.43 (s, 4 H), 8.40 (dd, 2 H), 8.18 (dd, 1 H), 7.98 (m, 2 H), 7.84 (dd, 2 H), 7.82 (dd, 2 H); FABMS, $[M^{2+} + PF_6^-]^+$ 890, M^+ 745. Anal. Calcd for $C_{41}H_{31}N_8F_{12}O_3P_2Ru$: C, 45.23; H, 2.87; N, 11.58. Found: C, 45.05; H, 2.79; N, 11.47.

$[Ru(phen)_2DPPP3](PF_6)_2 \cdot 5H_2O$: ^1H NMR (acetone-d_6) δ 9.89 (s, 1 H), 9.75 (m, 2 H), 9.09 (d, 1 H), 8.82 (m, 4 H), 8.61 (d, 2 H), 8.57 (m, 2 H), 8.43 (s, 4 H), 8.41 (d, 2 H), 8.35 (d, 1 H), 8.0 (m, 2 H), 7.82 (m, 4 H); FABMS, $[M^{2+} + PF_6^-]^+$ 890, M^+ 745. Anal. Calcd for $C_{41}H_{35}N_8F_{12}O_5P_2Ru$: C, 43.78; H, 3.14; N, 11.21. Found: C, 43.83; H, 2.86; N, 11.23.

$[Ru(phen)_2DPPN](PF_6)_2 \cdot H_2O$: ^1H NMR (acetone-d_6) δ 9.72 (dd, 2 H), 9.19 (s, 2 H), 8.82 (m, 4 H), 8.66 (dd, 2 H), 8.48 (dd, 2 H), 8.43 (s, 4 H), 8.41 (m, 4 H), 7.95 (dd, 2 H), 7.86 (dd, 2 H), 7.81 (m, 4 H); FABMS, $[M^{2+} + PF_6^-]^+$ 939, M^+ 794. Anal. Calcd for $C_{46}H_{30}N_8F_{12}OP_2Ru$: C, 50.15; H, 2.74; N, 10.17. Found: C, 50.03; H, 2.71; N, 10.10.

Determination of Extinction Coefficients. Small amounts of the complexes were dissolved in 3 drops of DMSO. The solutions were then diluted to 2 mL with H_2O and filtered through 0.2 μm syringe filters. The absorbances of these solutions were measured, and final solutions were prepared in which the optical density was approximately 0.2 at 440 nm and the concentration of nitric acid was approximately 40%. The solutions were allowed to stand for at least 3 days before the ruthenium concentration was measured by atomic absorption. Solutions of $[Ru(bpy)_3]Cl_2$ (ϵ_{428} = 14600 M^{-1} cm^{-1} [17]) and $[Ru(phen)_3]Cl_2$ (ϵ_{447} = 19600 M^{-1} cm^{-1} [5]) that had been similarly treated were employed as references. Measurements were made for six different volumes of complex solutions, made up to a constant volume with H_2O. Comparison of the slopes of plots of ruthenium absorbance versus volume of complex solution with those of the regularly determined standards allowed the extinction coefficients to be calculated for the various complexes.

Luminescence Studies. Steady-state luminescence intensities were measured on an SLM Instruments 8000C spectrofluorometer, with irradiation at 440 nm. Intensity measurements and emission maxima were calibrated using aqueous $[Ru(bpy)_3]Cl_2$ solutions (10 μM). Peak integrals were obtained using the SLM software package, and the value for the appropriate solvent blank was subtracted. DNA titrations were performed on 600-μL solutions that were 10 μM in complex. Volumes of 1.25 mM base pair calf thymus DNA solution were added, and the luminescence intensity was measured. No attempt was made to eliminate oxygen prior to the emission experiments.

Luminescence lifetime measurements were performed on an instrument constructed in the Laser Resource Center for the Beckman Institute, which has been described previously.[7] Various laser powers between 0.05 and 5 mJ were used, without significant differences in the results obtained. Emission decays were averages of at least 500 shots (more if the luminescence intensity was low). Decays were fit to single-exponential or multiexponential functions using a nonlinear least-squares minimization. Solution volumes and titration methods were identical to those employed in the steady-state measurements.

Results

Synthesis. $[Ru(bpy)_2DPPZ]^{2+}$ has been prepared previously.[11] The synthetic route involved the preparation and isolation of the free DPPZ ligand[18] and subsequent reaction with $Ru(bpy)_2Cl_2$. The ligand itself was prepared by condensation of phenanthroline-5,6-dione with *o*-phenylenediamine. By changing the order of these reactions, using $[Ru(phen)_2(O_3SCF_3)_2]$ instead of the bipyridyl complex, and by employing a variety of commercially available substituted *o*-diamino compounds, a wide variety of substituted $[Ru(phen)_2DPPZ]^{2+}$ complexes have now been prepared.

The new synthetic route is outlined in Figure 1. The key intermediate, $[Ru(phen)_2phendione]^{2+}$, is prepared from [Ru-

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Table I. Spectroscopic Properties of the Ruthenium Dipyridophenazine Complexes[a]

complex	absorbance max (nm)	extinction coeff ($\times 10^{-4}$ M^{-1} cm^{-1})[b]	emission max in H_2O (nm)[c]	rel int[c,d] (20 °C)	τ_{H_2O} (ns)[e]	emission max in CH_3CN (nm)[c]	rel int[c,d] (20 °C)
$[Ru(phen)_2phendione](PF_6)_2$	436	1.68	624	0.170	450	610	0.026
$[Ru(phen)_2DPPZ](PF_6)_2$	372, 439	2.48, 2.23	–	0	–	618	0.844
$[Ru(phen)_2DPPX](PF_6)_2$	382, 440	2.56, 2.10	620	0.065	50[f]	610	0.729
$[Ru(phen)_2DPPM2](PF_6)_2$	373, 441	2.27, 2.25	610	0.010	10[f]	615	0.807
$[Ru(phen)_2DPPA](PF_6)_2$	377, 438	2.78, 2.34	606	0.012	32[f]	612	0.878
$[Ru(phen)_2DPPB](PF_6)_2$	379, 439	2.49, 2.14	612	0.010	–	660	0.034
$[Ru(phen)_2DPPP2](PF_6)_2$	362, 439	2.82, 2.12	613	0.030	460	620	0.040
$[Ru(phen)_2DPPP3](PF_6)_2$	376, 439	1.72, 2.11	609	0.041	460	616	0.019
$[Ru(phen)_2DPPN](PF_6)_2$	391, 411, 443	2.42, 2.93, 2.56	609	0.160	500	606	0.037

[a] Measurements made on solutions 10 μM in complex. [b] Estimated error: ±0.05–0.1 × 10^4 M^{-1} cm^{-1}. [c] Relative to 10 μM $[Ru(bpy)_3]^{2+}$ in aqueous solution, excitation at 440 nm. [d] Estimated error: greater of ±10% or ±0.005. [e] Estimated error: ±10%. [f] 50% uncertainty is associated with these values, given the low overall luminescent intensity and the contribution at these low intensities of a second component (τ = 450 ns) which is likely contaminating $Ru(phen)_2(phendione)^{2+}$.

Table II. Luminescence Properties of the Ruthenium Dipyridophenazine Complexes in the Presence of DNA

complex	emission max with DNA (nm)[a]	rel int[a,b]	enhancement[a,c]	obsd lifetimes (ns)[a,d]	lifetime ratios[e]
$[Ru(phen)_2phendione](PF_6)_2$	605	0.21	1.2	450, 1140	50:50
$[Ru(phen)_2DPPZ](PF_6)_2$	618	0.56	>10^4	120, 750	80:20
$[Ru(phen)_2DPPX](PF_6)_2$	609	1.46	22.5	270, 1030	60:40
$[Ru(phen)_2DPPM2](PF_6)_2$	609	3.07	307	470, 1450, 4200	36:45:19
$[Ru(phen)_2DPPA](PF_6)_2$	625	0.12	10	60, 380	70:30
$[Ru(phen)_2DPPB](PF_6)_2$	611	0.02	2.0	370, 1250	60:40
$[Ru(phen)_2DPPP2](PF_6)_2$	603	0.05	1.7	400, 1400	45:55
$[Ru(phen)_2DPPP3](PF_6)_2$	603	0.06	1.5	350, 1300	50:50
$[Ru(phen)_2DPPN](PF_6)_2$	607	0.20	1.3	350, 1200	30:70

[a] 600 μL 10 μM complex solution with 1.25 mM base pair calf thymus DNA solution titrated in to give a final concentration of 8.3 μM complex, 0.21 mM base pair DNA, excitation at 440 nm, at 20 °C. [b] Relative to 10 μM $[Ru(bpy)_3]^{2+}$ in aqueous solution, excitation at 440 nm (estimated error: greater of ±10% or ±0.005). [c] Relative to 10 μM complex in aqueous solution (Table I, column 4, estimated error: ±10%). [d] Estimated error: ±10%. [e] Calculated from the magnitudes of the preexponential factors produced by the fitting program.

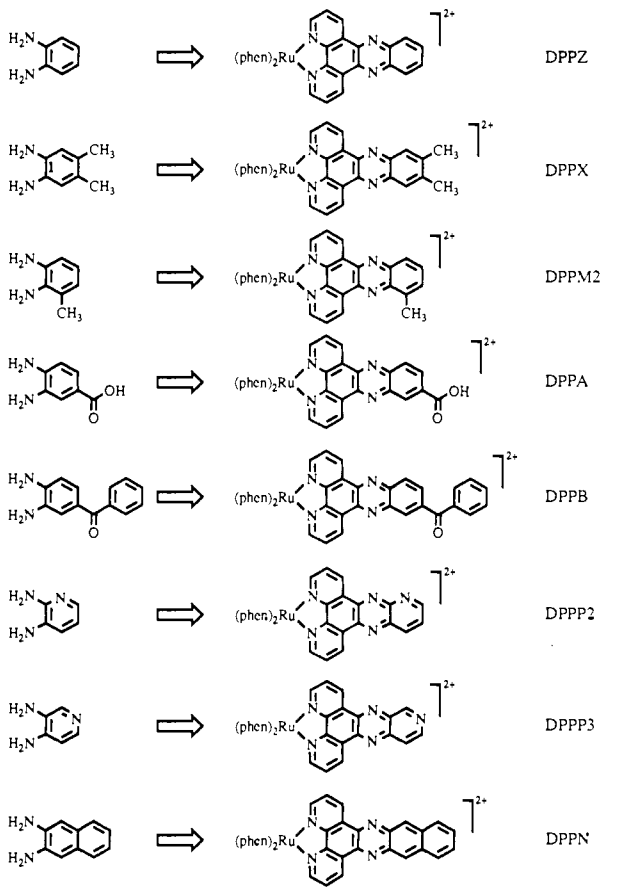

Figure 2. Shown (from left to right) are the ortho-diamines used in constructing DPPZ derivatives, the resulting complexes, and the ligand abbreviations.

$(phen)_2(O_3SCF_3)_2]$ and phenanthroline-5,6-dione and may be readily isolated by precipitation as the hexafluorophosphate salt. $[Ru(phen)_2(H_2O)_2]^{2+}$, obtained from aquation of $Ru(phen)_2Cl_2$, is also a satisfactory starting material. This intermediate complex can then be condensed with the appropriate *o*-diamino compound (see Figure 2) to give the desired substitution pattern on the DPPZ ligand in a straightforward reaction. The crude products were also isolated as the hexafluorophosphate salt and purified by recrystallization. The most common impurity is $[Ru(phen)_3]^{2+}$ (by ^1H NMR spectroscopy).

The ^1H NMR spectra of these complexes are quite similar, except that, as expected, the spectra of the unsymmetrically substituted complexes are somewhat more complex. Even in these cases, nonetheless, the similarity of the complexes is clear. The complexes have been characterized further by elemental analysis and FAB mass spectrometry (see Experimental Section).

Spectroscopic Studies. The results of the spectroscopic studies on this series of complexes are shown in Table I. The visible spectra typically display two maxima. An MLCT band, typical of tris(bipyridyl)ruthenium(II) complexes, is present at approximately 440 nm and does not vary substantially in position or intensity with substitution on the DPPZ ligand. The remaining visible band common to these complexes lies at higher energy (360–380 nm) and shows a much greater dependence on the nature of the substituents. This transition, characteristic of phenazines, is ligand localized. The visible spectra do not differ significantly with acetonitrile as solvent.

With the exception of the parent complex, $[Ru(phen)_2DPPZ]^{2+}$, all the complexes luminesce in aqueous solution when irradiated in the MLCT transition. The emission maxima vary, as does the intensity, but all are in the range of 600–625 nm. All of the complexes luminesce in acetonitrile; however, the position and intensity of the maxima vary even more widely and seem to show little correlation with those values obtained in aqueous solution. No attempt was made to eliminate oxygen from these emission experiments, since the focus of the study of these complexes was with a view to assessing their utility as probes for DNA.

The luminescent properties of the complexes are perturbed as duplex DNA is titrated into the solution. The emission maxima and intensities all change, and the decays in luminescence, measured in time-resolved experiments, are no longer single ex-

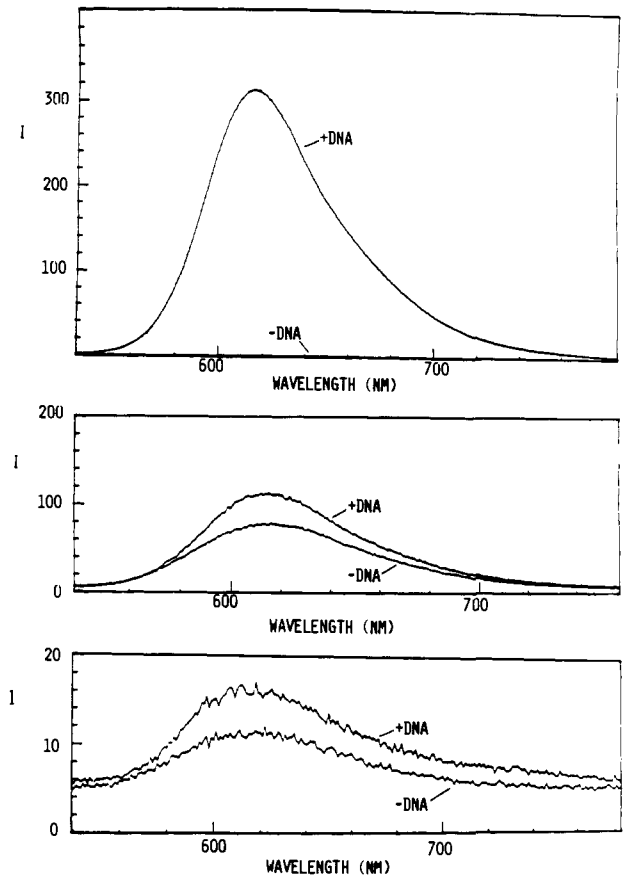

Figure 3. Luminescent spectra of (from top to bottom) $[Ru(phen)_2DPPM2]^{2+}$, $[Ru(phen)_2DPPN]^{2+}$, and $[Ru(phen)_2DPPB]^{2+}$ in the presence and absence of DNA in buffered aqueous solution. Ruthenium concentrations were 8 μM, and DNA concentrations were 210 μM base pairs. Excitation was at 440 nm.

ponential in nature. The emission data and excited-state lifetimes for the complexes in the presence of saturating concentrations of DNA (~25 base pairs/complex), where all the ruthenium may be considered in the bound form,[10] are shown in Table II.

There are also some differences in behavior among these complexes, which may be observed over the course of the DNA titrations, and this is illustrated in the spectra shown in Figure 3 and the spectral titrations plotted in Figure 4. Solutions of the complexes of DPPZ (**2**), DPPX, DPPM2, and DPPA exhibit a substantial increase in luminescence intensity when DNA is first added. The emission maxima and luminescence decay lifetimes rapidly approach their final values, with comparatively large enhancements in luminescence intensity over that seen in aqueous solution in the absence of DNA. For these complexes it is noteworthy that their luminescent intensity in acetonitrile is much greater than that in aqueous solution. In contrast, the luminescence of the complex of DPPB shows only a small change with increasing DNA. With complexes of DPPP2, DPPP3, DPPN, and phendione, an initial decrease in luminescence intensity is actually observed, reaching a minimum at DNA concentrations of ~3–4 base pairs/complex, before steadily increasing to a final somewhat enhanced value with increasing DNA. The enhancement in luminescence in the presence of excess DNA is much lower in these latter cases, being less than a factor of 3. The luminescent enhancement in a nonaqueous solvent in comparison to water is similarly small, if the luminescence is enhanced at all.

Most of the complexes exhibit biexponential luminescent decays in the presence of DNA. The exception to this is $[Ru(phen)_2DPPM2]^{2+}$. This less symmetrical complex required a triexponential model to obtain a satisfactory fit to the decay data. Data for two of the other unsymmetrically substituted complexes, $[Ru(phen)_2DPPP2]^{2+}$ and $[Ru(phen)_2DPPP3]^{2+}$, also do not fit satisfactorily to biexponential decay curves, but with these complexes the low intensity of the luminescence and consequent poor signal-to-noise ratio make such assignments difficult.

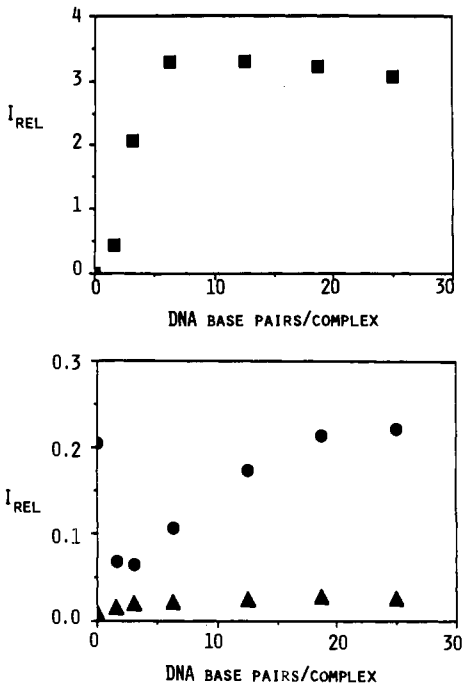

Figure 4. Plots of the relative luminescent intensities for $[Ru(phen)_2DPPM2]^{2+}$ (squares), $[Ru(phen)_2DPPN]^{2+}$ (circles), and $[Ru(phen)_2DPPB]^{2+}$ (triangles) in the presence of increasing concentrations of DNA. Ruthenium concentrations were 8 μM.

Discussion

The sensitivity of the luminescence properties of $[Ru(bpy)_2DPPZ]^{2+}$ (**1**) and $[Ru(phen)_2DPPZ]^{2+}$ (**2**) to environment has led to considerable interest in these complexes as photoluminescent probes for the presence of DNA. In order to obtain further information on these complexes and their interactions with DNA, a variety of derivatives of **2** have been prepared. These complexes have been synthesized by a different route from that first employed for the preparation of DPPZ complexes.[11] In this new scheme, the three parts that together make up the complex, the $(phen)_2Ru^{2+}$ moiety, phenanthroline-5,6-dione, and *o*-phenylenediamine, are assembled in a different order. The substitutions on the DPPZ ligand are thereby introduced in the final step, onto a common intermediate, by use of the appropriately substituted *o*-diamino compound (see Figure 2).

The goals of the study of these complexes were 3-fold. First, we were interested in determining whether substitution on the DPPZ ligand would give rise to new "light switch" complexes differing in their photophysical parameters. Changes in the ancillary ligands can be made with variation in excited-state lifetime but without affecting the remarkable luminescent enhancement seen with DNA. The second, related goal was to gain some insight into the factors contributing to the uniquely useful luminescence properties of the parent compound by studying those of the derivatives. Finally, the study of the interaction of these complexes with DNA as a function of substitution onto the complex should allow some conclusions to be drawn concerning the modes by which the DPPZ complexes bind to DNA.

None of the new derivatives described here may be considered to be "molecular light switches" for the presence of DNA; the complexes all luminesce to some degree in aqueous solution in the absence of DNA. A number of them do, however, display significant enhancements in luminescent intensity when DNA is added to the solution. This, together with the long luminescence lifetimes observed for some of these complexes, may lead to their application as diagnostic probes for DNA.

The electronic structure of $[Ru(bpy)_2DPPZ]^{2+}$ (**1**) has been described in terms of a coupling of a $[Ru(bpy)_3]^{2+}$ chromophore to a phenazine electron acceptor.[11] One of the pieces of evidence that led Amouyal and co-workers to this conclusion was the ab-

sence of a red shift in the MLCT of **1** relative to that of $[Ru(bpy)_3]^{2+}$, despite the presence of a better π-accepting ligand (DPPZ). Since little variation in the energy of the MLCT bands with substitution on the DPPZ ligand is observed, the same description may hold for the complexes described here. Irradiation in the MLCT band results in a directed electron transfer from the metal to the DPPZ ligand. Emission from the localized excited state then occurs.

It is the differential quenching of this excited state in aqueous solution versus nonaqueous media (including DNA intercalation) that appears responsible for the "light switch" characteristics. Excited-state quenching owing specifically to interactions of the phenazine nitrogen atoms with water have been implicated.[6,11,13,14] These studies include differential luminescence quenching[13] with D_2O versus H_2O as well as luminescence studies[14] in various alcohols. The studies described here provide support for this proposal. The differential quenching does not appear to be the result of a change in excited-state pK_a in the presence of DNA (or nonaqueous media). Inspection of Tables I and II shows that there does not appear to be any direct relationship between the intensity of luminescence in aqueous solution and the increase or decrease in pK_a that would be expected with substitution of an electron-donating or -withdrawing group.

The photophysical properties of the new complexes prepared differ somewhat from those of the parent DPPZ complexes. In particular the new complexes, in contrast to the parent forms, all luminesce to some degree in aqueous solution. The complexes fall into two basic categories: (i) those that luminesce in aqueous solution to some extent without DNA but show substantial enhancements in nonaqueous environments (nonaqueous solvents or bound to DNA), and (ii) those that luminesce only to a small extent both in aqueous and nonaqueous media. Molecules in the first category include complexes of DPPX, DPPM2, and DPPA. These complexes display steady-state luminescence intensities in acetonitrile that are similar to those of $[Ru(phen)_2DPPZ]^{2+}$, but are not similarly quenched completely in aqueous solution. For these complexes, steric bulk in the vicinity of the phenazine nitrogen atoms may limit the interactions with water that are necessary for efficient quenching (some steric bulk is also evident with DPPB complexes). In the second category are ruthenium complexes of DPPB, DPPP2, DPPP3, and DPPN. These complexes show much lower steady-state luminescence intensities in *nonaqueous* solution than $[Ru(phen)_2DPPZ]^{2+}$ (**2**). This effect may be due to alternative efficient quenching pathways, a much greater sensitivity to the presence of water, or a difference in the nature of the emitting state. The water sensitivity may be ruled out since that would result in the total quenching of luminescence in aqueous solution. A change in the emitting state is possible since the substituents in these complexes are all of a nature that could alter the π-system of the DPPZ ligand to some degree, but this explanation is unlikely since, except for DPPB, the maximum wavelengths for emission do not vary substantially. The insensitivity of the luminescent intensities of these complexes to environment therefore is likely derived from competition with an alternate relaxation pathway.

How do the variations in photophysical properties affect the luminescent characteristics of the complexes on DNA binding? We attribute the luminescent enhancement seen with $[Ru(bpy)_2DPPZ]^{2+}$ (**1**) and $[Ru(phen)_2DPPZ]^{2+}$ (**2**) to the protection of the phenazine nitrogen atoms from solvent water as the DPPZ ligand intercalates between the base pairs of DNA. The observed luminescence shows a biexponential decay, indicative of the presence of at least two modes of binding to DNA. $[Ru(phen)_3]^{2+}$ also shows a biexponential decay in luminescence in the presence of DNA but shows a relatively small enhancement (~60%) in steady-state luminescence.[5] Also $Ru(phen)_3^{2+}$ luminescence is relatively solvent-insensitive.[8] For $Ru(phen)_3^{2+}$ the biexponential decay has been attributed to the presence of both intercalated and surface-bound modes; these assignments were made in part through the observation that the surface-bound mode, but not the intercalated mode, was easily quenched by $[Fe(CN)_6]^{4-}$ ion and, unlike the longer lived intercalated mode, the surface-bound species showed no retention in polarization when excited with polarized light.[5] The surface-bound mode is quenched much more rapidly due to its greater exposure to the solvent, while the more protected intercalated species is unaffected. In the case of the parent DPPZ complexes, both luminescent species are unaffected by the addition of $[Fe(CN)_6]^{4-}$ ion and retain polarization in emission, implying that both may be due to intercalative binding.[7,19] The coefficients found in fitting the biexponential decay curves furthermore suggest that the two binding modes are comparably populated.[20] Complexes of DPPX, DPPM2, and DPPA resemble $[Ru(phen)_2DPPZ]^{2+}$ (**2**) in that there is a significant increase in the luminescence intensity on addition of DNA, and this enhancement also appears to be the result of the protection of the ligand from quenching by water upon intercalation. A high solvent dependence in luminescence is also apparent. For complexes of DPPB, DPPP2, DPPP3, and DPPN, the luminescent intensity is less sensitive to solvent and there is no correlation evident between hydrophobicity and luminescent intensity. Furthermore, no remarkable enhancements in luminescent intensity are observed on DNA binding. Instead it is likely that the small luminescent enhancements observed resemble those seen[5] with $Ru(phen)_3^{2+}$ and may similarly be attributed to a decrease in vibrational modes of relaxation owing to intercalation.

Our proposal for the assignments of DNA binding modes for DPPZ complexes is illustrated in Figure 5.[21] The large aromatic DPPZ ligand can achieve good overlap with the DNA bases, either through stacking between the base pairs from the major groove with the metal–phenazine axis perpendicular to the base pair long axis, or by adopting a "side-on" approach, with the metal–phenazine axis more in line with the base pair long axis. In the first case, both the phenazine nitrogen atoms are largely protected from the solvent, which would yield a longer excited-state lifetime if indeed water is responsible for the major quenching. The side-on model will result in one of the phenazine nitrogen atoms being protected and the other being partially exposed in the DNA major groove. This exposure to solvent would yield a shorter excited-state lifetime on the basis of water quenching. A side-on model for intercalation of somewhat analogous rhodium intercalators, $[Rh(phen)_2phi]^{3+}$ and $[Ru(phi)_2bpy]^{3+}$ (phi = 9,10-phenanthrenequinone diimine), which cleave DNA upon photoactivation, has also been proposed on the basis of cleavage product analyses of oligonucleotides.[22] The importance of the degree of exposure to the solvent in determining the intensity and excited-state lifetime of the luminescence of the ruthenium complexes may perhaps be gauged by comparing the values obtained for complexes of DPPX, DPPM2, and DPPA. The most hydrophobic complex in the region of the phenazine nitrogen atoms is $[Ru(phen)_2DPPM2]^{2+}$, and this complex displays the greatest intensity and longest excited-state lifetimes of these complexes in the presence of DNA. Next is $[Ru(phen)_2DPPX]^{2+}$, in which its two methyl groups are placed somewhat further from the phenazine nitrogen atoms, and finally, $[Ru(phen)_2DPPA]^{2+}$, which, with a relatively hydrophilic acid substituent, shows the shortest excited-state lifetimes and lowest intensity among these complexes. The parent complex, $[Ru(phen)_2DPPZ]^{2+}$ (**2**), shows parameters between those of the latter two, as would be expected on the basis of its relative hydrophobicity.

A key result in support of these assignments lies in the luminescence decay of $[Ru(phen)_2DPPM2]^{2+}$. Like **2**, the luminescence of this complex is enhanced in the presence of DNA, but in this case the decay fits most favorably a triexponential rather than biexponential curve. This more complex decay pattern would be expected on the basis of our model. Since the methyl group

(19) Given the relative binding constants of the tris(phenanthroline) and DPPZ complexes (log $K \sim 4$ and 7, respectively), contributions from a groove-bound interaction are likely to be negligible under the conditions tested.

(20) The biexponential decays cannot be attributed to differential binding by enantiomers, since under conditions where the ruthenium complexes are fully bound, nonidentical populations of the two modes are observed.

(21) Further support for these assignments is obtained in studies with different nucleic acid conformations.[13]

(22) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.

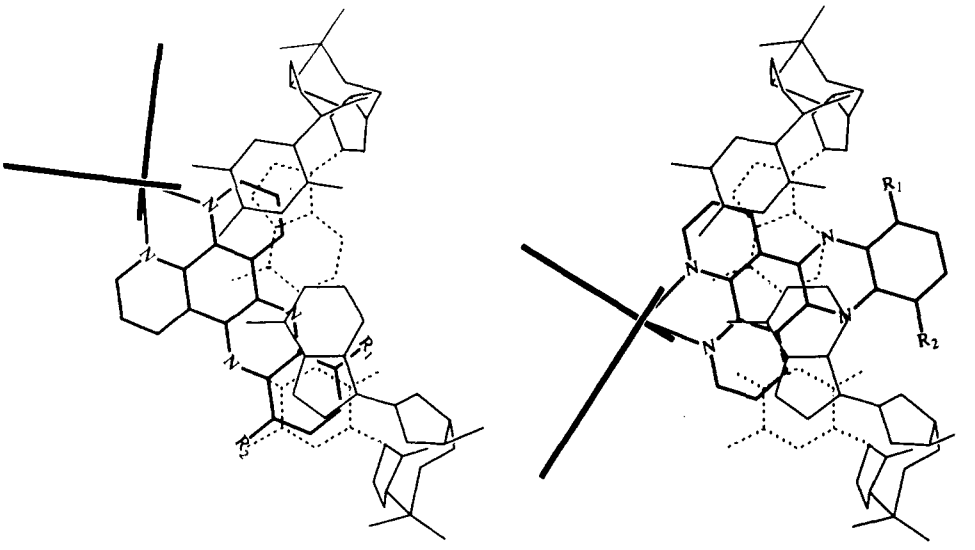

Figure 5. Illustrations of the side-on (left) and perpendicular (right) models proposed for intercalation of the complexes in DNA. The view is shown along the DNA axis (major groove is to the left) and illustrates the possible overlap of the DPPZ ligand with the base pairs above and below for the two proposed binding orientations. The DPPZ complex is shown schematically in bold lines. Note in particular the relative accessibility of the phenazine nitrogen atoms to solvent interactions for the two models. For the side-on model, with asymmetric substutions (R1 ≠ R2), solvent quenching through interactions with each of the phenazine nitrogen atoms would likely differ substantially.

is not symmetrically placed about the phenazine, there are now four possible binding orientations, as illustrated in Figure 5 (R_1 = H, R_2 = Me). Each of them would be expected to differ in excited-state lifetime owing to a differential accessibility of each form to water. If two of these lifetimes are close in value, then the numerical analysis would result in a triexponential fit, as is observed. It is not possible to assign unambiguously the binding modes to the three lifetimes, but two possibilities are as follows. In both assignments the short lifetime is attributed to the side-on binding mode in which the methyl group is pointing away from the major groove (R_1 = Me for side-on approach). This leaves the phenazine nitrogen atom exposed to solvent in the major groove, resulting in efficient quenching and a consequently short lifetime. In one model the two perpendicular binding modes would be assumed to be quite similar and would be assigned to the intermediate lifetime. The long lifetime would be assigned to the side-on mode in which the hydrophobic methyl group would be helping to protect the phenazine nitrogen atom from the solvent (R_2 = Me for perpendicular approach). Alternatively, one may postulate that a single localized water molecule is responsible for deactivation of the excited state in the side-on binding modes, and that the same is true for the perpendicular modes. Positioning the hydrophobic methyl group close to this water molecule may interfere with the deactivation, to the extent that the lifetime increases by a factor of 3 relative to that of the first side-on mode. Thus the alternate side-on binding mode, in which the methyl group is pointed into the major groove, would be assigned the lifetime of approximately 1400 ns ($\sim 3 \times 470$ ns). The perpendicular binding mode with the methyl group pointing away from the critical water molecule would also be assigned a lifetime of 1400 ns. The attenuation of the interaction with the water molecule by the methyl group in the other perpendicular modes would then similarly increase the lifetime by a factor of 3, to 4200 ns.

Conclusions

A series of derivatives of $[Ru(phen)_2DPPZ]^{2+}$ may be assembled with substitution on the DPPZ ligand using $[Ru(phen)_2$-phendione$]^{2+}$ as a key synthetic intermediate. In contrast to the parent $[Ru(phen)_2DPPZ]^{2+}$, none of the newly prepared complexes function as molecular "light switches" for DNA, although an enhancement on binding to DNA by as much as 300 times is observed. Protection from excited-state quenching by water through intercalation of the DPPZ ligand into duplex DNA is implicated as leading to the remarkable "light switch" characteristics of the parent complex. For the new derivatives, some show no similar sensitivity to luminescent quenching by water. Others display a high level of solvent sensitivity in quenching but yield a small but detectable level of luminescence in aqueous solution in the absence of DNA; these complexes give substantial increases in luminescence on DNA binding and may prove useful in the development of DNA-based diagnostics. On the basis of the luminescence data for all the DPPZ complexes, two intercalative binding modes are proposed, one perpendicular mode where the DPPZ ligand intercalates such that the metal–phenazine axis lies along the DNA dyad axis, and another, side-on mode where the metal–phenazine axis lies along the long axis of the base pairs.

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