Inorg. Chem. **2004**, *43*, 4570–4578

$[Ru(bpy)_2(L)]Cl_2$: Luminescent Metal Complexes That Bind DNA Base Mismatches

Eva RUba, Jonathan R. Hart, and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received January 16, 2004

Here we report the synthesis of luminescent ruthenium complexes that bind DNA base pair mismatches. $[Ru(bpy)_2(tpqp)]Cl_2$ (tpqp = 7,8,13,14-tetrahydro-6-phenylquino[8,7-*k*][1,8]phenanthroline), $[Ru(bpy)_2(pqp)]Cl_2$ (pqp = 6-phenylquino[8,7-*k*][1,8]phenanthroline), and $[Ru(bpy)_2(tactp)]Cl_2$ [tactp = 4,5,9,18-tetraazachryseno[9,10-*b*]-triphenylene] have been synthesized, and their spectroscopic properties in the absence and presence of DNA have been examined. While $[Ru(bpy)_2(pqp)]^{2+}$ shows no detectable luminescence, $[Ru(bpy)_2(tpqp)]^{2+}$ is luminescent in the absence and presence of DNA with an excited-state lifetime of 10 ns and a quantum yield of 0.002. Although no increase in emission intensity is associated with binding to mismatch-containing DNA, luminescence quenching experiments and measurements of steady-state fluorescence polarization provide evidence for preferential binding to oligonucleotides containing a CC mismatch. Furthermore, by marking the site of binding through singlet oxygen sensitized damage, the complex has been shown to target a CC mismatch site directly with a specific binding affinity, $K_b = 4 \times 10^6\ M^{-1}$. $[Ru(bpy)_2(tactp)]^{2+}$, an analogue of $[Ru(bpy)_2(dppz)]^{2+}$ containing a bulky intercalating ligand, is luminescent in aqueous solution at micromolar concentrations and exhibits a 12-fold enhancement in luminescence in the presence of DNA. The complex, however, tends to aggregate in aqueous solution; we find a dimerization constant of $9.8 \times 10^5\ M^{-1}$. Again, by singlet oxygen sensitization it is apparent that $[Ru(bpy)_2(tactp)]^{2+}$ binds preferentially to a CC mismatch; using a DNase I footprinting assay, a binding constant to a CC mismatch of $8 \times 10^5\ M^{-1}$ is found. Hence results with these novel luminescent complexes support the concept of using a structurally demanding ligand to obtain selectivity in targeting single base mismatches in DNA. The challenge is coupling the differential binding we can obtain to differential luminescence.

Introduction

Base mismatches occur naturally in the genome as a result of either polymerase errors or DNA damage by ultraviolet radiation, ionizing radiation, and numerous genotoxic chemicals.[1] The various known sources of spontaneous base damage are estimated to alter about 25000 bases per human genome per cell per day out of the 3×10^9 bases in the genome. In most cases the cell corrects these errors using a complex repair system. Failure of these repair mechanisms can lead to serious consequences, as in the human hereditary diseases xeroderma pigmentosum, hereditary nonpolyposis colon cancer, and some forms of breast cancer.[2]

The detection and targeting of single base mismatches in DNA therefore provides an avenue for the rational development of new diagnostics and chemotherapeutics. However, such development also represents a challenging problem. Recently, we reported the construction of a mismatch recognition agent $[Rh(bpy)_2(chrysi)]^{3+}$ that binds mismatch sites in DNA specifically and, upon photoactivation, cleaves the DNA backbone neighboring the site.[3] The source of preferential binding is the sterically bulky chrysi intercalating ligand, which is too wide to intercalate readily into B-form DNA, but binds the destabilized regions associated with base mismatches. Specific DNA cleavage is observed at over 80% of mismatch sites in all sequence contexts and the complex

* To whom correspondence should be addressed. E-mail: jkbarton@caltech.edu.

(1) (a) Goodman, M. F.; Creighton, S.; Bloom, L. B.; Petruska, *J. Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 83−126. (b) Leonard, G. A.; Booth, E. D.; Brown, T. *Nucleic Acids Res.* **1990**, *18*, 5617. (c) Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. *Biochemistry* **1995**, *34*, 16148. (d) Brown, T. *Aldrichimica Acta* **1995**, *28*, 15.

(2) Hoeijmakers, J. H. J. *Nature* **2001**, *411*, 366.

(3) (a) Jackson, B. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 12986. (b) Jackson, B. A.; Alekseyev, V. Y.; Barton, J. K. *Biochemistry* **1999**, *38*, 4655. (c) Jackson, B. A.; Barton, J. K. *Biochemistry* **2000**, *39*, 6176.

10.1021/ic0499291 CCC: $27.50
Published on Web 05/26/2004

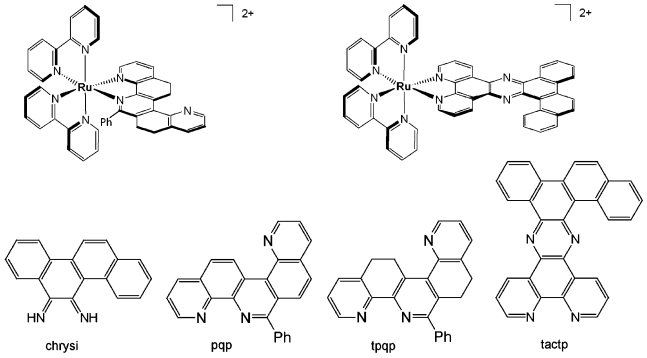

Figure 1. Structures of $[Ru(bpy)_2(tpqp)]^{2+}$ (**1**) and $[Ru(bpy)_2(tactp)]^{2+}$ **3** as well as the ligands.

was shown to target a single base mismatch in a 2725 base pair linearized plasmid heteroduplex.

A next step would be the development of fluorescent small molecules that preferentially target single-base mismatches, as a means of detecting single base mismatches within the cell. Octahedral polypyridyl complexes of ruthenium have attracted much attention because of their favorable photophysical and photochemical properties.[4] They have shown their potential utility as molecular light switches,[5] in chemotherapy and photodynamic therapy,[6] and as probes for charge transport through DNA.[7] Here, we describe efforts to develop a ruthenium complex containing a bulky intercalating ligand as a fluorescent probe for mismatches (Figure 1).

Experimental Section

Materials. Commercially obtained chemicals were used as received. $RuCl_3 \cdot nH_2O$ was obtained from Pressure Chemical. Bipyridine, $[K_4Fe(CN)_6]$, $[Ru(bpy)_3]Cl_2$, and rose bengal were purchased from Aldrich. Calf thymus DNA was purchased from Amersham and was dialyzed against a buffer of 5 mM Tris, 50 mM NaCl, pH 7.5. Phosphoramidites were from Glen Research and were used as received.

Metal Complex Synthesis. The ligands tpqp and pqp were prepared according to literature protocols,[8] as were chrysenequinone,[9] 5,6-diamino-1,10-phenanthroline,[10] $Ru(bpy)_2Cl_2 \cdot 2H_2O$,[11] and $[Ru(bpy)_2(tpqp)][PF_6]_2$.[12]

$[Ru(bpy)_2(pqp)][PF_6]_2$ was synthesized by heating $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (102 mg, 0.209 mmol) with pqp (75 mg, 0.209 mmol) in 15 mL of ethanol/water (3:1) for 12 h, whereupon the color changed from dark purple to orange. The solvent was reduced to 50% under vacuum, and an excess of NH_4PF_6 was added in 10 mL of water to precipitate the complex. The orange precipitate was filtrated, washed with water, methanol, and diethyl ether, and dried under vacuum. The complex was purified by column chromatography (neutral Al_2O_3, eluent acetonitrile/toluene, 3:1) (yield: 75%) and afterward converted to the soluble Cl^- salt by anion exchange chromatography on Sephadex QEA. The complexes were further purified via HPLC. ^1H NMR (CD_3CN, 300 MHz, δ, ppm): 11.53 (d, $J_{HH} = 9.2$ Hz, 1H, tpqp), 9.41 (m, 1H, tpqp), 9.11 (m, 4H, bpy), 8.67 (m, 3H, bpy), 8.52 (m, 2H, tpqp, bpy), 8.43−8.34 (m, 5H, tpqp, bpy), 8.1−7.89 (m, 6H, bpy, tpqp), 7.81−7.59 (m, 4H, bpy, tpqp), 7.49−7.37 (m, 2H, $tpqp^{ph}$), 6.92−6.80 (m, 2H, $tpqp^{ph}$), 6.17 (d, $J_{HH} = 7.9$ Hz, 1H, $tpqp^{ph}$). ESI-MS (cation): 916 ($M - PF_6$) observed, 916 calculated.

4,5,9,18-Tetraazachryseno[9,10-*b*]triphenylene (tactp) was synthesized by refluxing 5,6-diamino-1,10-phenanthroline (40 mg, 0.19 mmol) and chrysenequinone (49 mg, 0.190 mmol) in ethanol for 4 h. A yellow precipitate was filtered and washed with ethanol. Yield: 52%. ESI-MS (cation): 433 $(M + 1)^+$.

$[Ru(bpy)_2(tactp)]Cl_2$ was synthesized by heating $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (35 mg, 0.069 mmol) with tactp (30 mg, 0.069 mmol) in 15 mL of ethanol/water (3:2) for 24 h, whereupon the color changed from dark purple to orange. The solvent was reduced to 50% under vacuum, and an excess of NH_4PF_6 was added in 10 mL of water to precipitate the complex. The orange precipitate was filtrated, washed with water, a small amount of methanol, and diethyl ether, and dried under vacuum. The complex was purified by column chromatography (neutral Al_2O_3, eluent acetonitrile/toluene, 3:2) (yield: 63%), and afterward converted to the soluble Cl^- salt by anion exchange chromatography on Sephadex QEA. The complexes were further purified via HPLC. ^1H NMR (CD_3CN, 600 MHz, δ, ppm): 10.74 (d, $J_{HH} = 8.8$ Hz, 1H, tactp), 9.83 (d, $J_{HH} = 7.8$ Hz, 1H, tactp), 9.62 (d, $J_{HH} = 7.8$ Hz, 1H, tactp), 9.56 (d, $J_{HH} = 8.8$ Hz, 1H, tactp), 9.7 (m, 2H, tactp), 8.65−8.56 (m, 4H, bpy), 8.27 (m, 2H, tactp), 8.21−8.14 (m, 3H, bpy, tactp), 8.10−7.80 (m, 12H, tactp, bpy), 7.67 (m, 1H, tactp), 7.54 (m, 2H, bpy), 7.35 (m, 2H, bpy). ESI-MS (cation): 991.2 ($M - PF_6 + H_2O$) observed, 991 calculated.

Ten replicates of concentration and absorbance for each sample were used to calculate extinction coefficients at 450 nm as follows: $Ru(bpy)_2(tpqp)^{2+}$ (**1**) 20800 (600) M^{-1} cm^{-1}; $Ru(bpy)_2(pqp)^{2+}$ (**2**), 33000 (400) M^{-1} cm^{-1}; $Ru(bpy)_2(tactp)^{2+}$ (**3**), 15400 (400) M^{-1} cm^{-1}. Accurate measurements of ruthenium concentrations were made using a Perkin-Elmer/Sciex Elan 5000A ICP-MS and $[Ru(bpy)_3]Cl_2$ as calibrant, and absorbance measurements were collected using a Varian 300 Bio spectrophotometer.

Oligonucleotide Synthesis. Oligonucleotides were synthesized on an ABI 392 DNA/RNA synthesizer, using standard phosphoramidite chemistry. DNA was synthesized with a 5′-dimethoxy trityl (DMT) protecting group and was purified on Poly-Pak II cartridges and further purified by HPLC using a Dynamax 300 Å C18 reverse-phase column (Rainin) on a Hewlett-Packard 1100 HPLC (95% 50 mM NH_4OAc/5% acetonitrile to 70% 50 mM NH_4OAc/30% acetonitrile over 30 min). Quantification was done on a Beckman DU 7400 spectrophotometer using the ϵ_{260} values estimated for single stranded DNA.

UV−Visible Spectroscopy. Electronic spectra were recorded on a Beckman DU 7400 UV−visible spectrophotometer (Beckman Coulter). The pH dependent titrations were carried out as follows: 20 mL of a solution of the complex (10−20 μM) in pH 1.5 buffer (50 mM tris, 20 mM NaAc, 18 mM NaCl) was stirred while being

(4) Juris, A.; Balzani, V.; Barigeletti, V.; Campagna, S.; Belser, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85.
(5) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Hartshorn, R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919. (c) Gupta, N.; Grover, N.; Neyhart, G. A.; Liang, W.; Singh, P.; Thorp, H. H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1048. (d) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 10286. (e) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448. (f) Turro, C.; Bossmann, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1995**, *117*, 9026 and references therein.
(6) Clarke, M. J. *Coord. Chem. Rev.* **2003**, *236*, 209 and references therein.
(7) (a) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777. (b) Núñez, M. E.; Barton, J. K. *Curr. Opin. Chem. Biol.* **2000**, *4*, 199−206.
(8) Keuper, R.; Risch, N.; Flörke, U.; Haupt, H.-J. *Liebigs. Ann.* **1996**, 705.
(9) Greabe, V. C.; Hönigsberger, F. *Liebigs Ann.* **1900**, *311*, 257.
(10) Bodige, S.; McDonnell, F. *Tetrahedron Lett.* **1997**, *38*, 8159.
(11) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.
(12) Risch, N.; Keuper, R. *Z. Naturforsch.* **1995**, *50b*, 1115.

monitored with a pH meter. After addition of 30−200 μL aliquots of 1 N NaOH to the solution, the pH was recorded and 1 mL of the solution was withdrawn for recording the UV/vis spectrum. After scanning, the solution was returned, and this cycle was repeated in order to monitor spectral changes at pH intervals of 0.1 to 1 pH unit up to pH 11.5.

The absorption titrations with DNA were carried out as follows: A solution of metal complex (10−20 μM) in buffer (50 mM NaCl, 5 mM Tris, pH 7.5) was placed in the sample cell of the spectrometer while buffer alone was placed in the reference cell. The spectrum of the free metal complex was obtained. Then an aliquot of DNA solution (DNA concentration 0.8−45 μM) was added to the sample and the reference cell. The DNA solution added to the sample cell also contained metal complex of the same concentration as that in the cell. After addition of DNA, the solutions were agitated, and, after 5 min, the spectrum was obtained. This process was repeated until no further change was observed in the spectrum. From these spectra, the red shift and % hypochromicity upon binding to DNA were determined.

Luminescence. Luminescence data were obtained on an ISS-K2 spectrofluorometer. Emission intensities were determined by integration of the luminescence spectrum and standardized against $[Ru(bpy)_3]Cl_2$ as a calibration for the instrument. Excitation spectra were obtained by monitoring at the emission maximum while varying excitation wavelength from 250 to 600 nm. For luminescence polarization data, samples consisted of 20 μM racemic metal complex in 20 mM Tris, 50 mM NaCl, pH 7.5. When present, DNA concentration was 1 mM nucleotides, and glycerol samples contained 60% glycerol by volume. All luminescence polarization measurements were taken a minimum of 10 times, and the averages and standard deviations were noted. In no measurement did the standard deviation exceed 10% of the nominal value. Samples were irradiated at 450 nm, and emission was monitored at 610 nm using a 495 nm cutoff filter. Luminescence quenching experiments were carried out using 10 μM metal complex in 5 mM Tris, 50 mM NaCl, pH 7.5, DNA concentration of 1.7 mM nucleotides. $K_4Fe(CN)_6$ as a quencher was added to the solution, and the emission was monitored as a function of quencher concentration.

Luminescence titrations were performed to determine affinity constants for complex **3** to DNA (synthetic oligonucleotide 17mer, containing either a CC mismatch or a GC base pair). DNA ranging from 10^{-8} to 10^{-4} M^{-1} was titrated into solutions containing the metal complex, 10 mM tris, 50 mM NaCl, pH 7.5. Multiple experiments were conducted at a constant metal concentration from 0.25 to 7 μM. An excitation wavelength of 450 nm was used, and total luminescence intensity was recorded from 500 to 800 nm. The change of intensity was plotted against the concentration of DNA to analyze the data.

To determine excited-state lifetimes, time-resolved emission measurements were conducted using a pulsed YAG-OPO laser (λ_{ex} = 470 nm). Laser powers ranged from 3 to 4 mJ/pulse. To obtain luminescence lifetimes τ, time-resolved emission data were fit to a single-exponential decay according $y(t) = 100[C_1 \exp(-t/\tau_1) + (1 - C_1) \exp(-t/\tau_2)]$ ($C_1 = 1$) by a nonlinear least-squares method with convolution of the instrument response function using in-house software as described previously.[13] Errors in lifetimes and percent contributions are estimated to be ±10%.

Assays of $^1O_2$ Damage. Single strands were 5′-^{32}P end-labeled using standard protocols [14] and annealed to complementary strands in an aerated buffer of 5 mM Tris, 100 mM NaCl, pH 8.0. Oligonucleotide duplexes (5 μM) containing different concentrations of racemic metal complex (0.05−100 μM) were irradiated at 440 nm with a He/Cd laser for 10 min. After irradiation, samples were treated with 10% piperidine at 90 °C for 30 min, dried, and electrophoresed through 20% denaturing polyacrylamide gel. The extent of $^1O_2$ damage was quantitated by phosphorimagery (ImageQuant). Binding affinities were determined by plotting the amount of damage at the guanine nearest to the mismatch versus metal concentration and fitting the data to a sigmoidal curve using OriginPro software. The binding constant, K_b, was derived from the metal concentration at the inflection $[Ru_{50\%}]$ point of the curve and calculated according to $K_b = 1/([Ru_{50\%}] - 0.5[DNA_0])$. Variance from the fitted curve is used to calculate the standard deviation of the inflection point and hence the standard deviation of K_b; typically this standard deviation is 10−20%.

DNAse I Footprinting. All reactions were carried out in a total volume of 20 μL. Single strands were 5′-^{32}P end-labeled using standard protocols[14] and annealed to complementary strands in an aerated buffer of 5 mM Tris, 50 mM NaCl, 5 mM $MgCl_2$, and 5 mM $CaCl_2$ pH 8.0. Oligonucleotide duplexes (5 μM) containing different concentrations of racemic metal complex (0.1−100 μM) were incubated for 3 h; then 0.2 unit of DNAse I was added and incubated at 22 °C for 5 min. The reactions were stopped by adding 10 μL of a solution containing EDTA, NaOAc, and CT DNA and ethanol precipitated. The samples were electrophoresed through 20% denaturing polyacrylamide gel and exposed to a phosphor screen. The extent of damage was quantitated by phosphorimagery (ImageQuant). Data were analyzed as reported previously.[3,15] Given the apparent association of **3** in solution, the metal concentration was adjusted on the basis of the dimerization constant constant $K_D = 9.8 \times 10^5$ M^{-1}, for the formation of dimeric Ru in aqueous solutions.

Results

Synthesis and Spectroscopic Characterization. The metal complexes $[Ru(bpy)_2(tpqp)]Cl_2$ (tpqp = 7,8,13,14-tetrahydro-6-phenylquino[8,7-*k*][1,8]phenanthroline) (**1**), $[Ru(bpy)_2(pqp)][Cl]_2$ (pqp = 6-phenylquino[8,7-*k*][1,8]phenanthroline) (**2**), and $[Ru(bpy)_2(tactp)][Cl]_2$ (tactp = 4,5,9,18-tetraazachryseno[9,10-*b*]triphenylene) (**3**) were synthesized by refluxing $Ru(bpy)_2Cl_2 \cdot 2H_2O$ with 1 equiv of the corresponding ligand in ethanol/water mixtures for 12 to 24 h as described.[12] For **3** the amount of ethanol and the reaction time were increased due to the low solubility of the free ligand in these solvents. The orange complexes were isolated in high yields and purified as the PF_6^- salts by column chromatography on alumina. The ^{1}H NMR spectrum of **1** is in accordance with published data.[12] The spectrum of complex **2** exhibits similar features in the aromatic region but, as expected, lacks signals for protons in the aliphatic region. The signal at 11.5 ppm is characteristic and can be assigned to the proton in the coordinated phenanthroline unit opposite to the unbound nitrogen. The large downfield shift is due to a strong interaction of this proton with the nitrogen lone pair in the planar ligand.[16] For both complexes, the

(13) (a) Holmlin, R. E.; Barton, J. K. *Inorg. Chem.* **1995**, *34*, 7. (b) Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 5236.

(14) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Habor Laboratory: New York, 1989.

(15) Brenowitz, M.; Senear, D. F.; Shea, M. A.; Ackers, G. K. *Methods Enzymol.* **1986**, *130*, 132.

signals for the protons of the phenyl group in the 6 position, between 7.00 and 6.1 ppm, are shifted to high field due to π interactions with the ancillary bpy ligand; in the free ligand, the signals for these protons appear between 8 and 7.45 ppm. The ^{1}H NMR spectrum of **3** displays characteristic signals at 10.74 ppm, 9.83, 9.62, and 9.56 ppm that can be attributed to the protons H^m, H^n, H^c, and H^d, respectively. The signals corresponding to protons on the ligand tactp are highly dependent on concentration and temperature, a phenomenon that has been described for similar complexes.[17,18] They can be shifted downfield by as much as 0.4 ppm with increasing concentrations. This concentration effect can be attributed to an aggregation of the mononuclear species by stacking of the tactp ligands in solution. This effect is also observed when gradually increasing the amount of D_2O of a CD_3CN solution of the complex. The signals of the tactp protons are shifting downfield and broaden similar to the trend observed when increasing the concentration.

The electronic spectra of the complexes **1** and **2** both contain the characteristic metal to ligand charge transfer (MLCT) transition in the visible region at 453 nm (ϵ_{453} = 20800 M^{-1} cm^{-1}) for **1** and at 450 nm (ϵ_{453} = 33000 M^{-1} cm^{-1}) for **2**. Intense ligand-centered transitions in the UV region are observed at 245 and 292 nm, respectively. The spectrum of **3** retains most of the features of $Ru(bpy)_3^{2+}$ and tactp. It displays a strong MLCT band between 400 and 500 nm with a maximum at 440 nm (ϵ_{440} = 15400 M^{-1} cm^{-1}). A sharp band between 310 and 360 nm corresponds to tactp centered transitions.

Solutions containing $[Ru(bpy)_2(tpqp)]Cl_2$ (**1**) exhibit luminescence at ambient temperature upon excitation at 450 nm with a maximum of emission at 640 nm and a quantum yield of 0.002. The intensity of the emission is somewhat solvent dependent, being higher in aqueous solvents and lower in CH_2Cl_2 and acetonitrile. The location of the maximum is also dependent on the pH. When changing from pH 2.0 to pH 4 a shift of the maximum from 730 nm (protonated species) to 630 nm (deprotonated species) with an isobestic point at 670 nm is observed. The plot of emission maximum versus pH is in accordance with the data observed for the shift in the absorbance maximum. The lifetime of the excited state is 10 ns. $[Ru(bpy)_2(pqp)]Cl_2$ (**2**) shows no luminescence at ambient temperatures. The substituent in the 2 position of the phenanthroline ligand likely weakens the ligand field and so decreases the excited-state lifetimes and quantum yields for the MLCT transition. This effect has been studied recently for a series of ruthenium complexes containing phenanthroline ligands with aryl groups in the 2 position.[19]

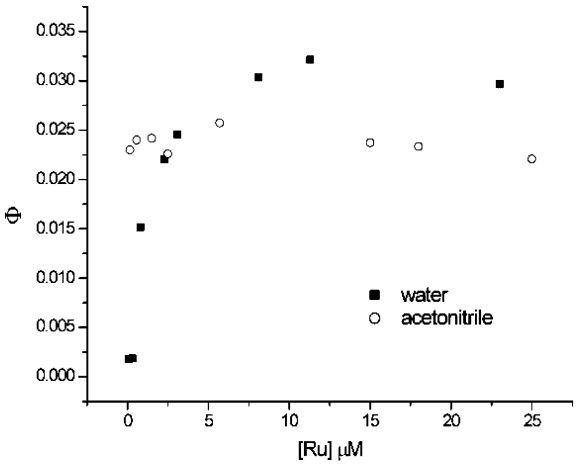

Figure 2. Concentration dependence of the quantum yield of **3** in water and acetonitrile.

$[Ru(bpy)_2(tactp)]Cl_2$ (**3**) was designed analogously to $[Ru(bpy)_2(dppz)]Cl_2$, which exhibits bright luminescence in nonaqueous solvents but is quenched by hydrogen bonding of the nitrogens of the phenazine moiety to water molecules.[5] **3** exhibits bright emission with a maximum at 610 nm upon excitation at 450 nm at ambient temperature. In this case the quantum yield is significantly higher and similar to $[Ru(bpy)_3]Cl_2$ with 0.024 in acetonitrile. The quantum yield of the complex in water is highly concentration dependent. At concentrations below 1 μM, the quantum yield is estimated to be 0.002; at higher concentrations it is similar to the values observed in acetonitrile. The excited state of the complex seems to be quenched by water, but at higher concentrations aggregates are formed, presumably by stacking interactions of the tactp ligands[17] so that the nitrogens of the ligands are protected. Figure 2 shows a plot of quantum yield versus concentration. From this plot, a dimerization constant of 9.8 $\times$ 10^5 M^{-1} for compound **3** in aqueous solution can be obtained. The emission decay kinetics of **3** are monoexponential in acetonitrile with a lifetime of 480 ns.

Absorption Titration with DNA. Binding of intercalators to DNA can be characterized in part through absorption titration. For metallointercalators, DNA binding is associated with hypochromism and a red shift in the MLCT and ligand bands.[20] Absorption titrations were carried out with complex **1** using two synthetic oligomers containing either a regular Watson−Crick base paired duplex DNA or a similar DNA containing a single CC mismatch. For both DNAs, a hypochromism of 8% and a red shift <3 nm are observed. In the case of **3** a hypochromism of 14% and again only a small red shift of <3 nm are found. In rhodium complexes containing 9,10-phenanthrenequinonediimine (phi), a ligand that is known to stack well between the base pairs of DNA, a red shift of <13 nm and a hypochromism of 25% are associated with binding to DNA.[20] For ruthenium complexes a hypochromism of 12% for $Ru(phen)_3Cl_2$,[21] 15% for Ru-

(16) Zhen, Q.-X.; Ye, B.-H.; Zhang, Q.-L.; Liu, J., G.; Li, H.; Ji, L.-N.; Wang, L. *J. Inorg. Biochem.* **1999**, *76*, 47.
(17) Bolger, J.; Gourdon, A.; Ishow, E.; Launay, J.-P. *Inorg. Chem.* **1996**, *35*, 2937.
(18) (a) Koch, K. R.; Sacht, C.; Lawrence,*C. J. Chem. Soc., Dalton Trans.* **1998**, 689. (b) Shetty, A. S.; Zhang, J.; Moore, J. S. *J. Am. Chem. Soc.* **1996**, *118*, 1019. (c) Arena, G.; Monsu Scolaro, L.; Pasternack, R. F.; Romeo, R. *Inorg. Che*m. **1995**, *34*, 2994. (d) Ishow, E.; Gourdon, A.; Launay, J.-P.; Chiorboli, C.; Scandola, F. *Inorg. Chem.* **1999**, *38*, 1504. (e) Gourdon, A.; Launay, J.-P. *Inorg. Chem.* **1998**, *37*, 5336. (f) Steullet, V.; Dixon, D. W. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1547.
(19) Wu, F.; Riesgo, E.; Pavalova, A.; Kipp, R. A.; Schmehl, R. H.; Thummel, R. P. *Inorg. Chem.* **1999**, *38*, 5620.
(20) Pyle, A. M.; Rehmannn, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.
(21) Barton, J. K.; Danishefsky, A. T.; Goldberg, J. M. *J. Am. Chem. Soc.* **1984**, *106*, 2172.

Table 1. Luminescence Polarization with DNA[a]

	buffer	DNA	60% glycerol	DNA/60% glycerol
$[Ru(bpy)_3]^{2+}$ [b]	0.0004	0.005	0.001	0.006
$[Ru(bpy)_2(dppz)]^{2+}$ [b]		0.029		0.070
$[Ru(bpy)_2(tpqp)]^{2+}$	0.0006	0.016 (GC)	0.033	0.048 (GC)
		0.025 (CC)		0.052 (CC)
$[Ru(bpy)_2(tactp)]^{2+}$	0.005	0.006 (GC)	0.01	0.010 (GC)
		0.005 (CC)		0.011 (CC)

[a] Samples consisted of 20 μM racemic metal complex in 5 mM tris, 50 mM NaCl, pH 7.5. When present, DNA concentration, containing a GC base pair or a CC mismatch as indicated, was 50 μM duplex, and glycerol samples contained 60% glycerol by volume. Samples were excited at 440 nm, and emission was monitored at 610 nm. Uncertainties are estimated to be 10%. [b] Literature values.[24b]

$(NH_3)_4(dppz)Cl_2$,[22] and 25% for $Ru(bpy)_2(taptp)Cl_2$[16] has been observed.

Luminescence in the Presence of DNA. Upon addition of either matched or mismatched DNA to a solution of **1**, a small decrease (<5%) in intensity is observed; the changes are too small to determine a binding constant with certainty. The luminescence lifetime of the complex also does not change significantly upon intercalation to DNA.

While only small changes in emission lifetime are evident on DNA binding, luminescence polarization experiments reveal significant variations associated with DNA binding. As shown in Table 1, in the absence of DNA, there is no significant polarization observed for complex **1**. In the presence of DNA, however, an increase in polarization is observed. Noteworthy is that the values for polarization are somewhat higher for samples containing DNA with a mismatch than for those without a mismatch. Thus the complex may be bound somewhat more tightly or rigidly within the mismatched site. It should also be noted that the polarization values are much smaller than expected; ethidium, which has a comparable excited-state lifetime (20 ns),[23] exhibits a polarization value of 0.2 on binding calf thymus DNA.

Differential luminescence quenching was also utilized in monitoring DNA binding. A highly negatively charged quencher is expected to be repelled by the negatively charged phosphate backbone, and therefore a DNA-bound cationic molecule should be protected from quenching; free complexes should be readily quenched.[24] The experiment was carried out using a 15 μM solution of **1** in buffer (5 mM Tris, 50 mM NaCl, pH 7.5) in the presence of DNA (50 μM duplex). The quencher, in this case $K_4Fe(CN)_6$, was added to the solution, and the steady-state emission was monitored. The resulting Stern−Volmer plots are shown in Figure 3.

The plots for complexes **1**and **3** in solution are linear; the emission is readily quenched by ferrocyanide ion. In the presence of well-matched DNA, the quenching of **1** is somewhat decreased. In the presence of mismatch-containing DNA, however, the plot for quenching is further decreased from that of the well-matched DNA and shows clear curvature. This observation is consistent with the model of closer binding of the complex to a CC mismatch compared to B-DNA.

For $[Ru(bpy)_2(tactp)]Cl_2$ (**3**) a different luminescence behavior upon binding to DNA is observed. When DNA is added to a 200 nM solution of the complex in buffer (5mM tris, 50 mM NaCl, pH 7.5), up to a 12-fold increase in the luminescence intensity is evident. Given the concentration dependent luminescence of **3** even in the absence of DNA, however, these results cannot be ascribed to preferential binding to a mismatch site. Indeed luminescence enhancements are evident also with fully matched DNA. In this system, with this oligonucleotide containing the CC mismatch, given a dimerization constant for **3** of $9.8 \times 10^5\ M^{-1}$, we estimate the binding affinity of **3** to be $8 \times 10^5\ M^{-1}$ for DNA containing the CC mismatch.

In time-resolved experiments we also observe a significant increase in the excited-state lifetime from 480 ns (free form) to 1230 ns (bound form). The emission decay becomes biexponential as has been observed for $Ru(phen)_3^{2+}$ and many other Ru(II) polypyridyl complexes.[4,24] Luminescence polarization experiments to distinguish between bound and unbound forms of the complex are not useful in this case, again owing to aggregation in solution; here there is some retention of polarization even in the absence of DNA, again likely reflecting multimeric aggregation. In the luminescence quenching experiment, however, we can easily distinguish between bound and unbound forms of **3** (Figure 2). The free complex is readily quenched by $K_4Fe(CN)_6$. Complex bound to DNA is fully protected, and virtually no quenching is observed. Similar results have been seen previously with tightly bound ruthenium intercalators.[24] Again, in this case, however, it is not possible to distinguish between duplexes containing a mismatch and those that are fully matched.

Singlet Oxygen Sensitization To Mark DNA Binding Sites. In an effort to establish whether the ruthenium complexes do indeed specifically target the mismatched site, gel electrophoresis experiments were conducted to monitor $^1O_2$-mediated damage of DNA. Upon photoexcitation, polypyridyl ruthenium complexes can sensitize the formation of singlet oxygen, whose subsequent reaction with DNA can be utilized to mark the site of ruthenium binding.[25−27] Two caveats are required, however. First, since $^1O_2$ is a diffusible species, damage can ensue several base pairs out from the site of sensitization. Second, $^1O_2$ reaction with DNA is revealed after piperidine treatment and is base sequence dependent; the rate of reaction varies in the series $G \gg T > A, C$.[28]

Here we utilize this methodology to determine the binding site of $Ru(bpy)_2tpqp^{2+}$ and to explore its ability to recognize single base pair mismatches. Samples of DNA were irradiated at 442 nm aerobically, followed by treatment with hot

(22) Nair, R. B.; Teng, E. S.; Kirkland, S. L.; Murphy, C. J. *Inorg. Chem.* **1998**, *37*, 139.
(23) LePecq, J.-B.; Paoletti, C. *J. Mol. Biol*. **1967**, *27*, 87.
(24) (a) Kumar, C. V., Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc*. **1985**, *107*, 5518. (b) Delaney, S.; Pascaly, M.; Bhattacharya, P. K.; Han, K.; Barton, J. K. *Inorg. Chem*. **2002**, *41*, 1966.
(25) Fleisher, M. B.; Mei, H. Y.; Barton, J. K. *Nucleic Acids Mol. Biol.* **1988**, *2*, 65.
(26) Mei, H. Y.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1339.
(27) Chow, C. S.; Barton, J. K. *Methods Enzymol.* **1992**, *212*, 219−242.
(28) Rodgers, M. A. J.; Snowden, P. T. *J. Am. Chem. Soc*. **1982**, *104*, 5541.

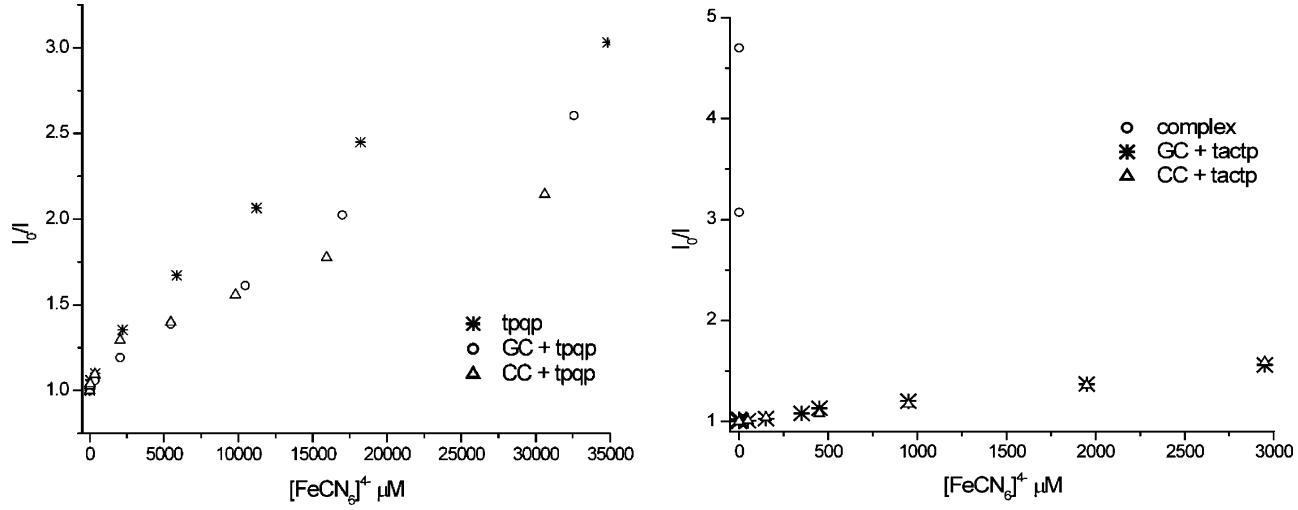

Figure 3. Luminescence quenching experiment for $[Ru(bpy)_2(tpqp)]^{2+}$ (**1**)(left) and $[Ru(bpy)_2(tactp)]^{2+}$ (**3**) (right) with $K_4Fe(CN)_6$. Samples consisted of 20 μM **1** or 5 μM **3** in 5 mM Tris, 50 mM NaCl, pH 7.5. When present, DNA (matched-GC mismatched-CC) concentration was 100 μM duplex. Samples were irradiated at 440 nm; emission was monitored at 610 nm.

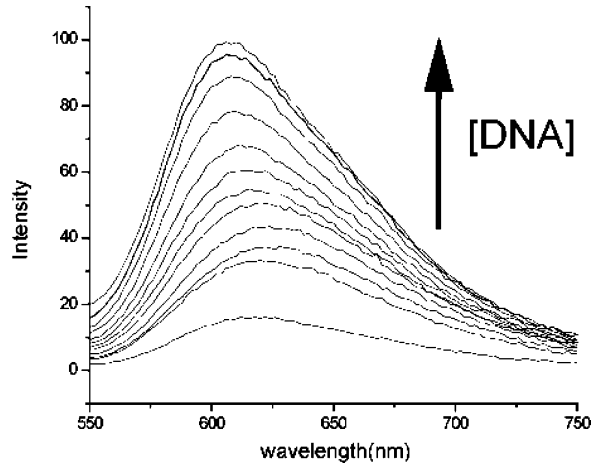

Figure 4. Luminescence titration for $[Ru(bpy)_2(tactp)]^{2+}$ (**3**) with an oligomer containing a CC mismatch. Samples consisted of 200 nM **3** in 5 mM Tris, 50 mM NaCl, pH 7.5. Increasing amounts of CC mismatch-containing DNA are added as indicated. Samples were excited at 440 nm; emission was monitored between 500 and 800 nm.

piperidine. Figure 5 shows an example for a typical electrophoresis experiment. For a duplex containing a CC mismatch, preferential damage at the guanine neighboring the CC mismatch is observed. The level of damage is 2.5-fold higher neighboring the mismatch site compared to other guanines. Furthermore, the extent of damage is doubled if the reaction is carried out in D_2O, consistent with the increase in $^1O_2$ lifetime in D_2O compared to H_2O.[28] In the oligomer lacking a mismatch, damage occurs mainly at the end of the duplex, where the bases may be more accessible to reaction with $^1O_2$.

That the differential damage reflects preferential binding of the metal complex is confirmed in parallel studies using rose bengal, an efficient $^1O_2$ sensitizer,[29] that does not bind to DNA. Upon photolysis of the mismatch-containing oligomer in the presence of rose bengal, no preferential reaction near the mismatch compared to other guanine sites is observed. Hence, the mismatch site is not inherently more reactive. Interestingly, some preferential reaction is obtained with $Ru(bpy)_3^{2+}$, which binds electrostatically in the groove of DNA (data not shown).

This assay was used also to determine the specific binding affinity for the complex to the CC mismatch. To determine the site-specific binding affinity, damage was quantitated at the guanine neighboring the mismatch. A binding constant of 4.2×10^6 M^{-1} is obtained for binding of **1** to a single CC mismatch site. No significant concentration-dependent damage and therefore binding are observed with DNA lacking a mismatch. It is noteworthy that the CC mismatch is the most thermodynamically destabilizing single base mismatch.[30]

Corresponding experiments were also carried out with **3**. In Figure 6, we see preferential damage at the guanine next to the CC mismatch compared to guanines in well-matched duplexes, again about 2.5-fold higher levels as compared to guanines in fully matched sequence contexts. Because this complex is a more efficient singlet oxygen sensitizer, the irradiation time was decreased to 4 min. Nevertheless at high metal concentrations a significant amount of damage of other guanines in the duplex is observed. This can be attributed either to damage by $^1O_2$ generated by unbound metal complex or to nonspecific binding of the complex to DNA at high metal to DNA ratios. Therefore this assay could not be used to determine an accurate binding constant to DNA.

Footprinting of Bound Ruthenium Complexes Using DNase I. Specific binding of the complexes to a CC mismatch was also revealed by a DNAse I footprinting assay.[15,31] Duplex DNA in a buffer of 5 mM tris, 50 mM NaCl, 5 mM $MgCl_2$, and 5 mM $CaCl_2$, pH 8 is incubated with different concentrations of **3** for 3 h and then treated

(29) (a) Wu, F. Y.-H.; Wu, C.-W. *Biochemistry* **1973**, *12*, 4343. (b) Schagen, F. H. E.; Moor, A. C. E.; Cheong, S. C.; Cramer, S. J.; van Ormondt, H.; van der Eb, A. J.; Dubbelman, T. M. A. R.; Hoeben, R. C. *Gene Ther.* **1999**, *6*, 873.

(30) Peyret, N.; Seneviratne, P. A.; Allawi, H. T.; SantaLucia, J., Jr. *Biochemistry* **1999**, *38*, 3468.

(31) (a) Parks, M. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6147. (b) Trauger, J. W.; Baird, E. E.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6160.

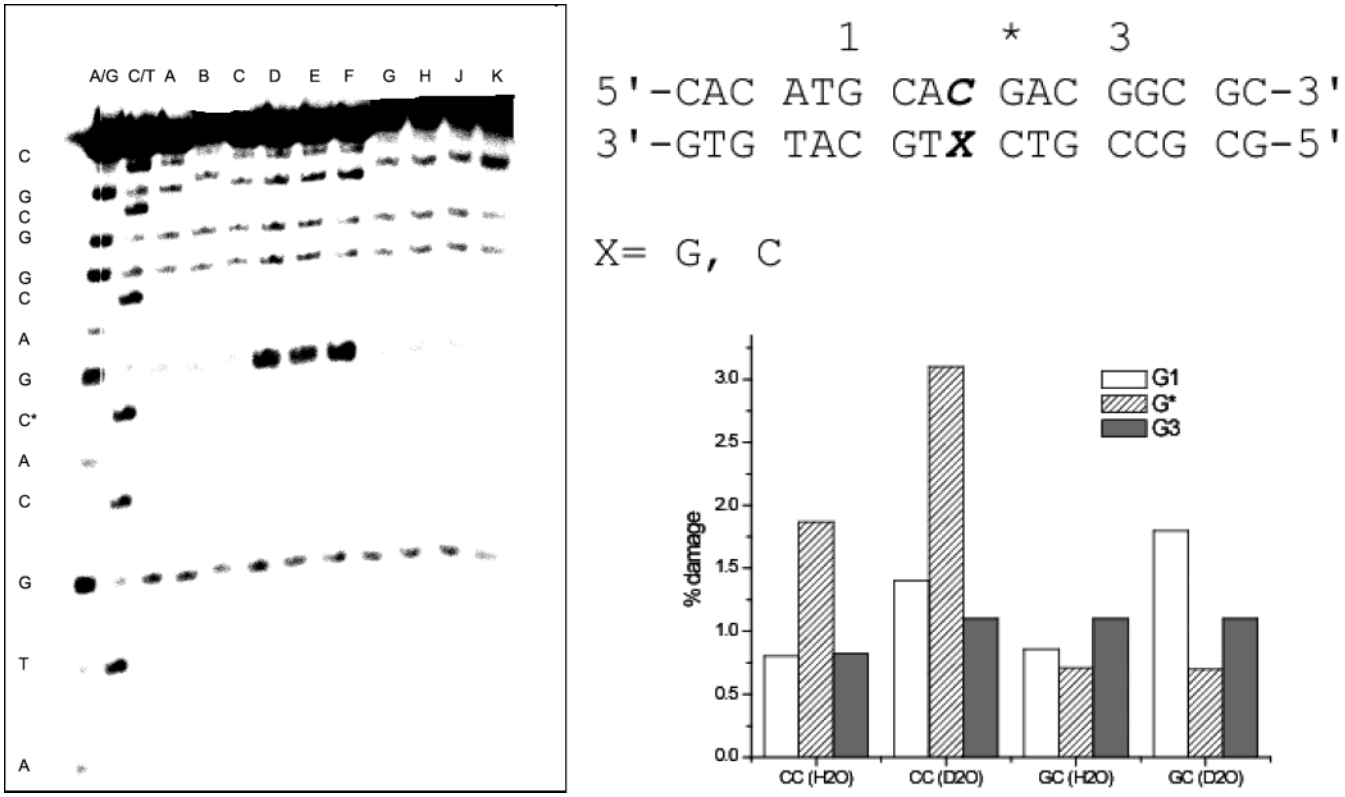

Figure 5. Singlet oxygen sensitization by $[Ru(bpy)_2(tpqp)]^{2+}$ (**1**). Shown at the top is the sequence of oligonucleotides used for the electrophoresis experiments. The DNA was ^{32}P labeled at the 5′-end of the upper strand. Shown at left is the autoradiogram. All samples were irradiated as in the Experimental Section. For the following lanes, Maxam−Gilbert sequencing A+G/C+T; A, CC-mismatched DNA (CC) in the absence of Ru; B, GC-matched DNA (GC) in the absence of Ru; CC, 1 μM Ru; D, CC, 25 μM Ru; E, CC, 50 μM Ru; F, CC, 25 μM Ru, D_2O; G, GC, 1 μM Ru; H, GC, 25 μM Ru; I, GC, 50 μM Ru; J, GC, 25 μM Ru, D_2O. The diagram shows a comparison of the amount of damage at different guanines in the duplex.

with DNase I. Sites that are protected due to the binding of the metal complex are not available for the cleavage reaction. The result is displayed on a denaturing polyacrylamide gel. This assay has successfully been used before to determine the binding constant of a small molecule to bulges[32] and mismatches.[33] In this type of experiment DNA is cleaved in the absence of metal complex at bases around the mismatch with different specificity. When increasing the concentration of complex **3**, we see a decrease in the amount of damage at bases close to the mismatch as displayed in Figure 7. Mainly three bases around the mismatch site are protected from reaction with the enzyme. Only a slight decrease in the amount of cleavage is observed at high concentrations of metal complex also in DNA not containing a mismatch. Using this assay a binding constant to a CC mismatch was determined, also taking into account the formation of dimers (K_a of 9.8×10^5 M^{-1}) measured by luminescence experiments. A binding constant to a CC mismatch under these conditions is 7.9×10^5 M^{-1}. When complex **1** was used in a similar study, no protection of DNA from digestion by DNAse I under these conditions was observed.

Discussion

Design of a Luminescent Metal Complex Targeted to Mismatches. Octahedral metal complexes containing an aromatic flat ligand, such as phenanthrenequinone diimine (phi) or dipyridophenazine (dppz), bind avidly to double-helical DNA by intercalation.[7a] Rhodium complexes containing the chrysene-5,6-quinone diimine (chrysi)[3] or benzo[*a*]-phenazine-5,6-quinone diimine (phzi)[34] were designed to target thermodynamic instabilities in the double helix caused by single base pair mismatches. The site specificity of these complexes is derived from the fact that they are more bulky intercalators with a width exceeding that of the well-matched base pair.

Here we have explored this strategy in an effort to design a luminescent octahedral metal complex that specifically binds to single base pair mismatches. Polypyridyl complexes containing ruthenium(II) in many cases exhibit favorable excited-state properties, leading to luminescence at ambient temperature with high quantum yields.[4] However ruthenium complexes containing phenanthrenequinone diimine (phi) are not luminescent at ambient temperature[35] nor are ruthenium complexes containing chrysi.[36] For this reason we chose ligands that coordinate to the metal center via a phenanthroline moiety. The ligands tpqp, pqp, and tactp contain dimensions similar to those of chrysi and phzi. They also contain an aromatic heterocycle, which should improve the binding affinity of the intercalator due to better stabilization

(32) Nakatani, K.; Sando, S.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 2172.
(33) (a) Nakatani, K.; Sando, S.; Kumasawa, H.; Kikuchi, J.; Saito, I. *J. Am. Chem. Soc.* **2001**, *123*, 12650. (b) Nakatani, K.; Sando, S.; Saito, I. *Nat. Biotechnol.* **2001**, *19*, 51.
(34) Junicke, H.; Hart, J. R.; Kisko, J.; Glebov, O.; Kirsch, I. R.; Barton, J. K. *PNAS* **2003**, *100*, 3737.
(35) (a) Pyle, A. M.; Barton, J. K. *Inorg. Chem.* **1987**, *26*, 3820. (b) Pyle, A. M.; Chiang, M. Y.; Barton, J. K. *Inorg. Chem.* **1990**, *29*, 4487.
(36) Rüba, E. Unpublished results.

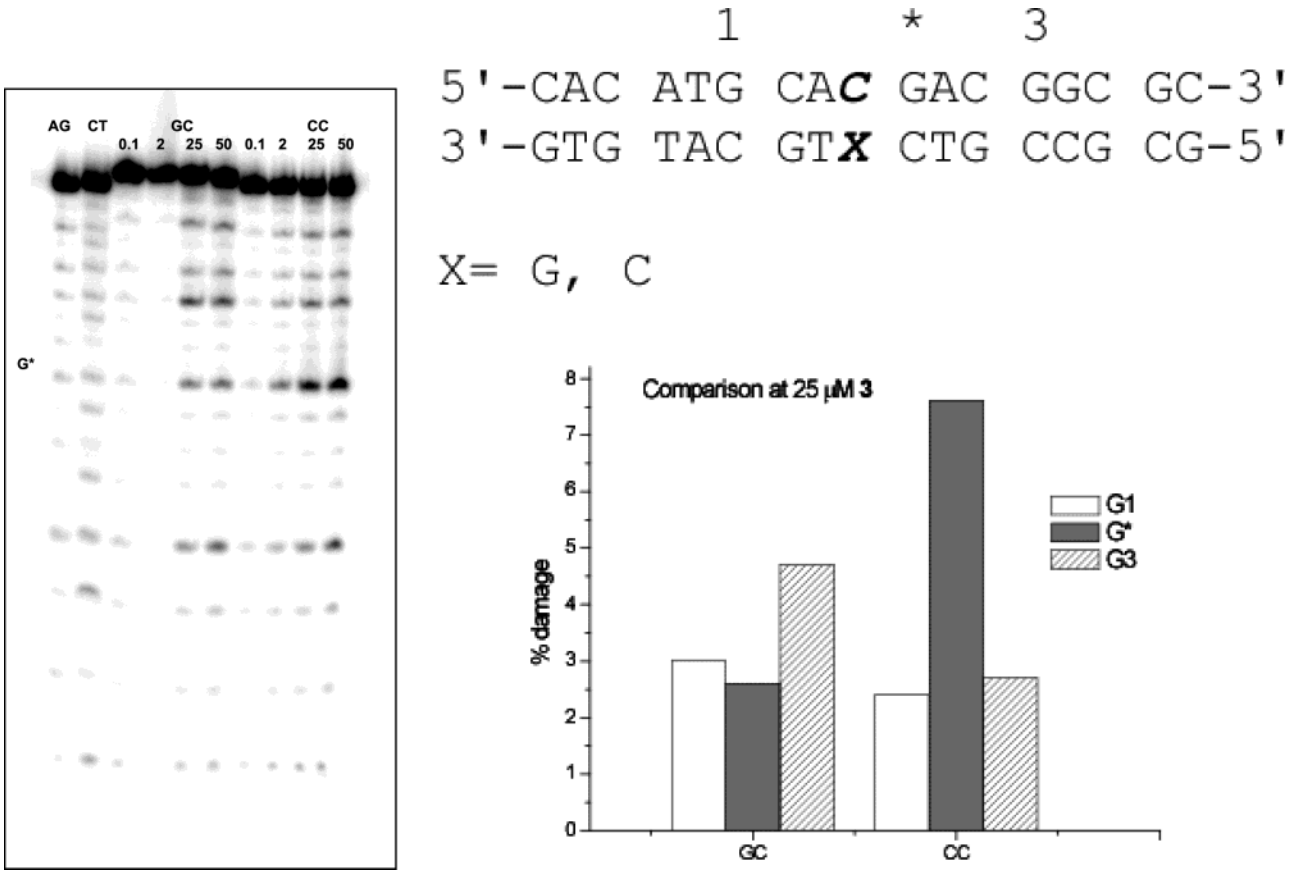

Figure 6. Singlet oxygen sensitization via $[Ru(bpy)_2(tactp)]^{2+}$ (**3**). Shown at the top is the sequence of oligonucleotides used for the electrophoresis experiments. The DNA was ^{32}P labeled at the 5′-end of the upper strand. Shown at left is the autoradiogram. All samples were irradiated as in the Experimental Section. Lanes 1 and 2 Maxam−Gilbert sequencing A+G/C+T; lanes 3−6 GC (well-matched DNA) with 0.1, 2, 25, 50 μM **3**, lanes 7−10, CC (mismatched DNA) with 0.1, 2, 25, 50 μM **3**. A comparison of the amount of damage at different guanines in the duplexes is shown in the graph.

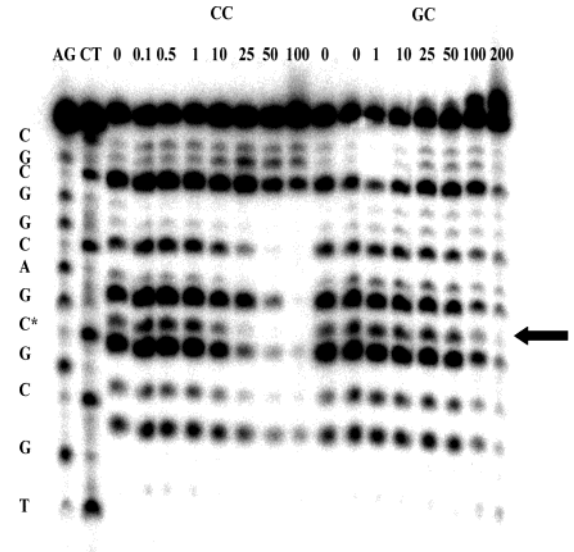

Figure 7. DNase I footprinting assay with $[Ru(bpy)_2(tactp)]^{2+}$ (**3**) and 5′-^{32}P-end labeled 17mer DNA duplex containing either a GC or a CC base pair. Shown is the autoradiogram with the following lanes. Lanes 1 and 2 Maxam−Gilbert sequencing A+G/C+T; lanes 3−8 CC with 0, 0.1, 0.5, 1, 10, 25, 50, 100 μM **3**, lanes 10−17, GC with 0, 1, 10, 25, 50, 100, 200 μM **3.** All samples were treated with DNase I as described in the Experimental Section. The arrow indicates the position used to determine the affinity of complex **3** to DNA.

upon stacking.[34] In addition, bpy ligands were employed in ancillary positions because their complexes are known to be luminescent, are relatively straightforward to coordinate, and also usually have favorable nonspecific binding affinities to DNA. In the case of $[Ru(bpy)_2(tactp)]^{2+}$ (**3**) we envisioned designing a molecular light switch that is specific for mismatches analogously to ruthenium complexes containing dppz as the intercalating ligand.[5]

Both **1** and **2** are found to exhibit similar electronic spectra in water, but only $[Ru(bpy)_2(tpqp)]^{2+}$ (**1**) exhibits weak luminescence at ambient temperature. Similar results have been reported in a study of the influence of aryl substituents in the 2 position of phenanthroline, where it is suggested that nonradiative relaxation in solution is dominated by rapid thermally activated internal conversion from the initially populated ^{3}MLCT state to a ligand field state that decays rapidly to the ground state.[19] The phenyl group stabilizes the complex compared to other substituents in this position by π interactions with the bpy ligands, orienting itself nearly perpendicular to the phenanthroline ring, as shown by ^{1}H NMR spectroscopy. Compound **3** emits brightly with a maximum of 620 nm in acetonitrile upon excitation at 450 nm. However, the complex forms aggregates especially in aqueous solutions at concentrations higher than 10^{-7} M. This is evident both by NMR and by luminescence. The luminescence quantum yield of this complex in water is highly concentration dependent, ranging from 0.002 at concentrations below 5×10^{-7} M to 0.029 at concentrations above 10^{-5} M; the latter value is comparable to the quantum yield in acetonitrile. We attribute this behavior to the formation

of aggregates by stacking of the tactp ligand which protects the nitrogens on the ligand from quenching with water.

Binding to DNA and Specificity for a Mismatch. $[Ru(bpy)_2(tpqp)]^{2+}$ binds to DNA preferentially at the mismatched base pair site. Hypochromism and a small red shift in the MLCT of the complex are associated with DNA binding. The small change in the absorbance compared to other ruthenium-based intercalators likely reflects weak binding by intercalation. The presumably intercalating bulky ligand has only a small aromatic surface area, with the nonplanar CH_2 groups that might interfere sterically with the bases so as to prevent deeper intercalation.

There is, however, no increase in emission of **1** upon addition of DNA. Different reasons for an increase of emission intensity when bound to DNA for ruthenium complexes have been reported. $[Ru(bpy)_2(dppz)]^{2+}$ is essentially nonluminescent in aqueous solvents, but upon intercalation, the phenazine nitrogens are protected from aqueous quenching and the complex emits.[5] This is not the case for complex **1**; the complex exhibits lower quantum yields in organic solvents. As has been described for $[Ru(phen)_3]^{2+}$,[37] intercalative binding can also reduce nonradiative deactivation of the excited state of the complex by reducing the vibrational motions and protecting the complex from solvent. Presumably in the case of **1**, intercalation is not very deep, the complex is not held with great rigidity, and hence extensive motion within the DNA binding site is still possible.

Given the absence of discrimination in luminescence, different studies were needed to examine any preference for binding near a mismatch. The luminescence polarization and the quenching experiments both indicate this preferential binding of **1** to well-matched DNA and a CC mismatch-containing DNA. The complex seems to be more closely bound to a CC mismatch, which results in better protection from the anionic quencher and slightly higher values of polarization.

For **3** we observe a significant increase in the quantum yield and the lifetime upon binding of the complex to DNA and the protection from the anionic quencher when bound to DNA. These results can all be attributed to tight intercalative binding of the complex to DNA. However **3** forms aggregates in aqueous solutions in the absence of DNA, consistent with the significant concentration-dependent luminescence of the complex in water. This complicates the interpretation of luminescence titrations with DNA and the determination of differences in the binding affinity of the complex to a mismatch or to regular matched DNA. Since some enhancements are evident also with DNA lacking a mismatch, given the significant expanse of the tactp ligand in two dimensions, partial intercalation at well-matched sites in DNA may still be feasible; alternatively, the DNA polymer may provide a template for cooperative aggregation.

The specificity for targeting a mismatch site was also examined with this complex **3** using singlet oxygen sensitization. By this experiment, preferential damage at a guanine next to a CC mismatch is indeed found with the bulky ruthenium intercalator. Furthermore no preferential damage to guanines at the same position but in a duplex lacking a mismatch is observed. Thus preferential targeting of the CC mismatch is observed by this assay. Presumably **3** is bound intercalatively more tightly and/or closely at the mismatched site.

Further evidence for the preferential binding of **3** to a single base mismatch is found by DNase I footprinting experiments, where selective protection of bases close to the mismatch is evident only in the presence of metal complex; at the same concentration no protection of duplexes containing a fully matched base pair is found. Analogous footprinting is not seen for complex **1**, and we attribute this effect to a weaker intercalative interaction of this complex with DNA which might be easily displaced by DNase I.[38]

Conclusions. In this study we have shown the development of luminescent metal complexes specifically targeted to mismatches in DNA. Two structurally very different ligands have been used as potential intercalative ligands that should provide the basis for discrimination between regular B-form DNA sites and sites that contain a single base mismatch. Both ligands are sterically demanding and therefore should have a higher affinity for sites in DNA that are thermodynamically and kinetically destabilized. This basis for discrimination has successfully been applied using rhodium complexes; we explored here coupling these characteristics to the well-known and frequently adopted luminescent properties of polypyridyl ruthenium complexes.

This attempt has in part been successful as has been shown by this work. The complexes do target CC mismatches in oligonucleotides. Both complexes have been shown to bind with some level of specificity to a CC mismatch. Hence the concept of using a structurally demanding ligand to obtain selectivity for a mismatch holds. The challenge however is in coupling the differential binding we can obtain to differential luminescence. In the case of $[Ru(bpy)_2(tpqp)]^{2+}$ (**1**), likely owing to poor intercalation, little enhancement in luminescence is coupled to binding. In the case of $[Ru(bpy)_2(tactp)]^{2+}$ (**3**), the opposite problem holds, in that luminescence enhancements are found to be associated not only with binding but also simply with dimerization of metal complexes in the aqueous environment.

Overall these studies again illustrate the utility of transition metal complexes in designing new probes for nucleic acids. Their rigid coordination framework, their stability in aqueous solution, and their rich photophysical properties provide useful characteristics to vary in fashioning novel nucleic acid probes. The many biological applications of these luminescent mismatch probes need now to be considered and optimized.

Acknowledgment. We gratefully acknowledge the NIH (GM33309) for financial support of this work. E.R. also thanks the FWF for a postdoctoral fellowship.

IC0499291

(37) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc*. **1986**, *108*, 2081.

(38) Suck, D.; Oefner, C. *Nature* **1986**, *321*, 620.