Photophysics of $Ru(phen)_2(PHEHAT)^{2+}$: A Novel "Light Switch" for DNA and Photo-oxidant for Mononucleotides

Cécile Moucheron, Andrée Kirsch-De Mesmaeker,*,† and Sylvia Choua‡

Physical Organic Chemistry, CP 160/08, Université Libre de Bruxelles, 50 Av. F. D. Roosevelt, B-1050 Brussels, Belgium

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The spectroelectrochemical properties of a novel light switch for DNA, $Ru(phen)_2(PHEHAT)^{2+}$ (phen = 1,10-phenanthroline; PHEHAT = 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene), are examined and compared to those of $Ru(phen)_2(DPPZ)^{2+}$ (DPPZ = dipyrido[3,2-a;2′,3′-*c*]phenazine) and $Ru(phen)_2(HAT)^{2+}$ (HAT = 1,4,5,8,9,12-hexaazatriphenylene). The excited $Ru(phen)_2(PHEHAT)^{2+}$ luminesces in organic solvents but not in water. It is shown that the orbitals involved in the absorption and luminescence spectroscopy are not the same as those in the electrochemistry. In aqueous solution, this complex luminesces upon intercalation of the PHEHAT ligand into the stacking of the DNA bases. Two modes of distribution of the complex on DNA can be evidenced from the titration curves of the complex with DNA. Laser flash photolysis experiments show that the excited state is able to abstract an electron from GMP (guanosine-5′-monophosphate) with a rather low efficiency, leading to the reduced complex and oxidized GMP. However, this process is not accompanied by the formation of photoproduct with GMP and cannot be detected with DNA on the time scale of the experiments.

Introduction

During the last decade, the interaction of polypyridyl ruthenium(II) complexes with DNA has been the focus of several research works.[1−11]

Generally, for many complexes, the emission intensities and the excited-state lifetimes increase by binding to DNA.[1a,2b,4a,5a,7,8] However, the background luminescence of the free complexes in aqueous solution and their relatively weak binding constants represent limiting factors for their application as nonradioactive nucleic acid probes. More recently, $Ru(bpy)_2(DPPZ)^{2+}$ and $Ru(phen)_2(DPPZ)^{2+}$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, and DPPZ = dipyrido[3,2-*a*;2′,3′-*c*]phenazine) have been described as molecular "light switches" for DNA: they exhibit a negligible background emission in water but luminesce in the presence of double-stranded DNA.[11a,c,12] On the basis of the unwinding results[13] and considering the structure of the DPPZ ligand, an intercalative binding mode with insertion of the DPPZ ligand between the base pairs of the DNA helix has been proposed. Intercalation of $Ru(phen)_2(DPPZ)^{2+}$ is further supported by linear dichroism[14] and ^{1}H NMR studies,[15]

* Author to whom correspondence should be addressed.
† Director of Research at the National Fund for Scientific Research (Belgium).
‡ Present address: Département de Chimie Physique, Quai Ernest Ansermet 30, 1211 Genève 4, Switzerland.
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(1) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Basile, L. A.; Barton, J. K. *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1989; pp 31−103. (c) Pyle, A. M.; Barton, J. K. *Progress in Inorganic Chemistry, Bioninorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1990; Vol. 38, pp 413−475. (d) Chow, S. C.; Barton, J. K. *Methods in Enzymology*; Lilley, D. M. J., Dahlberg, J. E., Eds.; Academic: San Diego, 1992; Vol. 212, pp 219−242. (e) Turro, N. J.; Barton, J. K.; Tomalia, D. A. *Acc. Chem. Res*. **1991**, *24*, 332.
(2) (a) Tossi, A. B.; Görner, H.; Schulte-Frohlinde, D. *Photochem. Photobiol*. **1989**, *50*, 585. (b) Görner, H.; Tossi, A. B.; Stradowski, C.; Schulte-Frohlinde, D. *J. Photochem. Photobiol., B: Biol.* **1988**, *2*, 67.
(3) Carter, M.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc*. **1989**, *111*, 8901.
(4) (a) Tossi, A. B.; Kelly, J. *Photochem. Photobiol*. **1989**, *49*, 545. (b) Kelly, J. M.; Tossi, A. B.; McConnell, D.; OhUigin, C.; Hélène, C.; Le Doan, T. *Free Radicals, Metal Ions and Biopolymers*; Beaumont, P. C., Deeble, D. J., Rice-Evans, C., Eds.; Richelieu Press: London, 1989, p 143−156.
(5) (a) Kirsch-De Mesmaeker, A.; Orellana, G.; Barton, J. K.; Turro, N. J. *Photochem. Photobiol*. **1990**, *52*, 461. (b) Orellana, G.; Kirsch-De Mesmaeker, A.; Barton, J. K.; Turro, N. J. *Photochem. Photobiol*. **1991**, *54*, 499.
(6) (a) Gupta, N.; Groverf, N.; Neyhart, G. A.; Singh, P.; Thorp, H. H. *Inorg. Chem*. **1993**, *32*, 310. (b) Gupta, N.; Groverf, N.; Neyhart, G. A.; Liang, W.; Singh, P.; Thorp, H. H. *Angew. Chem. Int. Ed. Engl*. **1992**, *31*, 1048.
(7) (a) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *31*, 2573. (b) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9320.
(8) (a) Kelly, J. M.; Feeney, M.; Tossi, A. B.; Lecomte, J.-P.; Kirsch-De Mesmaeker, A. *Anti-Cancer Drug Des.* **1990**, *5*, 69. (b) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Demeunynck, M.; Lhomme, J. *J. Chem. Soc., Faraday Trans*. **1993**, *89*, 3261. (c) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Orellana, G. *J. Phys. Chem*. **1994**, *98*, 5382. (d) Kirsch-De Mesmaeker, A.; Lecomte, J. P.; Kelly, J. M. *Top. Curr. Chem.* **1996**, *177*, 24.
(9) (a) Baker, A. D.; Morgan, R. J.; Strekas, T. C. *J. Chem. Soc., Chem. Commun*. **1992**,1099. (b) Morgan, R. J.; Chatterjee, S.; Baker, A. D.; Strekas, T. C. *J. Am. Chem. Soc*. **1991**, *30*, 2687.
(10) (a) Barton, J. K.; Danishefsky, A. T.; Goldberg, J. N. *J. Am. Chem. Soc*. **1984**, *106*, 2172. (b) Hiort, C.; Norden, B.; Rodger, A. *J. Am. Chem. Soc*. **1990**, *112*, 1971. (c) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc*. **1989**, *111*, 3051.
(11) (a) Friedman, A.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res*. **1991**, *19*, 2595. (b) De Buyl, F.; Kirsch-De Mesmaeker, A.; Tossi, A. B.; Kelly, J. M. *J. Photochem. Photobiol., A.: Chem*. **1991**, *60*, 27. (c) Hartshorn, R. H.; Barton, J. K. *J. Am. Chem. Soc*. **1992**, *114*, 5919. (d) Tysoe, S. A.; Morgan, R. J.; Baker, A. D.; Strekas, T. C. *J. Phys. Chem*. **1993**, *97*, 1707. (e) Naing, K.; Takahashi, M.; Taniguchi, M.; Yamagashi, A. *J. Chem. Soc., Chem. Commun*. **1993**, 402.
(12) (a) Chambron, J.-C.; Sauvage, J.-P.; Amouyal, E.; Koffi, P. *New. J. Chem*. **1985**, *9*, 527. (b) Amouyal, E.; Homsi, A.; Chambron, J.-C.; Sauvage, J.-P. *J. Chem. Soc., Dalton Trans.* **1990**, 1841. (c) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett*. **1991**, *182* (6), 603. (d) Turro, C.; Bossmann, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc*. **1995**, *117*, 9026.
(13) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc*. **1990**, *112*, 4960.
(14) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc*. **1993**, *115*, 3448.
(15) (a) Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc*. **1994**, *116*, 10286. (b) Eriksson, M.; Leijon, M.; Hiort, C.; Norden, B.; Graslund, A. *Biochemistry* **1994**, *33*, 5031.

S0020-1669(96)00931-7 CCC: $14.00

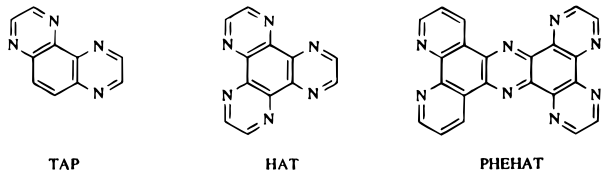


Figure 1. Structures of the different π-acceptor ligands.

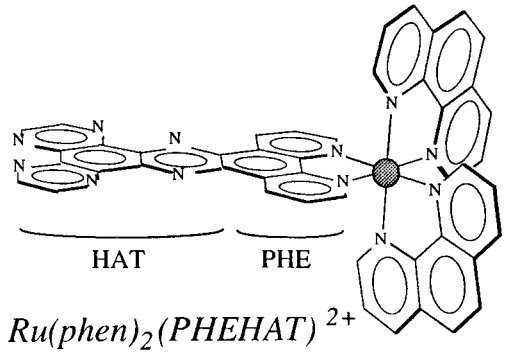


Figure 2. Bis(1,10-phenanthroline)(1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene)ruthenium(II) cation.

as well as by relative DNA viscosity measurements and fluorescence energy transfer experiments.[16]

Our laboratory has focused its interest more particularly on the photoreactions of tap (tap = 1,4,5,8-tetraazaphenanthrene) and hat (hat = 1,4,5,8,9,12-hexaazatriphenylene) ruthenium(II) complexes (Figure 1) with DNA.[17] Some of these complexes, whose metal-to-ligand charge transfer (^{3}MLCT) excited state is very oxidizing,[18] photocleave the DNA backbone [4a,19] and form photoadducts with nucleic acids.[8a,17,20] It has been demonstrated that the primary process which initiates DNA cleavages or adduct formation corresponds to a photoinduced electron transfer, generally from the guanine to the excited complex.[21] Although these properties make these ruthenium(II) complexes very attractive, their relatively weak binding constant to DNA limits their use as very efficient photoreagents of DNA bases. In order to cumulate the properties of a photoreagent with those of an excellent intercalator, we have designed, in this work, a novel Ru(II) compound containing a ligand which should not only induce intercalation but should also make the complex oxidizing in the excited state, so that it could photoreact with DNA. The targeted ligand is called PHEHAT (PHEHAT = 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene), where a phenanthroline is fused to an hexaazatriphenylene motif (Figure 1). Thus, in this report, we present the synthesis of $Ru(phen)_2PHEHAT^{2+}$ (Figure 2) and its photophysics in the absence and in the presence of DNA. The properties of the targeted complex are compared with those of the well-known $Ru(phen)_2(DPPZ)^{2+}$ and $Ru(phen)_2(HAT)^{2+}$ complexes in the same conditions.

Experimental Section

Instrumentation. ^{1}H NMR spectra were recorded on a Bruker Cryospec W 250 MHz and a Varian Unity 600 MHz instrument. The chemical shifts were measured versus $(CH_3)_4Si$ as an internal standard.

The electrospray mass spectrum (ESMS) was obtained with a VG-BIO-QUAD spectrometer at the University Louis Pasteur (Strasbourg, France).

Absorption spectra were recorded on a HP 8452A diode-array spectrometer and treated with a Macintosh computer. The molar absorption coefficients of the complex were determined by Ru titration with atomic emission from plasma atomization DCP (Spectrametric Spectrospan IV instrument); the Ru emissions (at 372.8 nm) of the samples were compared with those of $Ru(bpy)_3^{2+}$ ($\epsilon_{452\,nm}$ = 14 600 $dm^3\,mol^{-1}\,cm^{-1}$) as a standard.[22] Emission spectra were recorded with a Shimadzu RF-5001 PC spectrometer equipped with a Hamamatsu R-928 photomultiplier tube. Relative emission quantum yields were determined by integrating the corrected emission spectra over the frequency range.

The luminescence lifetimes were determined by using a modified Applied Photophysics laser kinetic spectrometer equipped with a Hamamatsu R-928 photomultiplier tube and a neodymium (Nd) YAG laser (Continuum NY 61-10 λ = 355 nm, 170 mJ per pulse) as the excitation source. Kinetic analyses of the luminescence decays were performed by a nonlinear least-squares regression using Marquardt's algorithm.[23]

Laser flash photolysis experiments were performed in a crossbeam configuration by using the pulsed Nd YAG laser described above and a xenon lamp as the monitoring source, with kinetic analysis of the decays performed as described above. This experimental setup does not allow for the detection of transient species shorter than a few tens of nanoseconds.

Cyclic voltammetry was carried out on a platinum disk electrode (approximate area = 20 mm^2) in dried acetonitrile solutions with dried ($^tBu_4N$)(PF_6) (0.5 M) as the supporting electrolyte; the counter electrode was a large area platinum grid. The potential of the working electrode, scanned at 200 mV s^{-1} between -2 and $+2$ V, was controlled by a homemade potentiostat *vs* a saturated calomel electrode (radiometer K701) separated from the solution by a Tacussel bridge.

Continuous illumination of the complex solution, in order to detect the formation of a photoproduct or photoadduct with GMP or DNA, was performed with a 2000 W quartz−halogen lamp (Philips) with a $NaNO_2$ UV cut off filter.

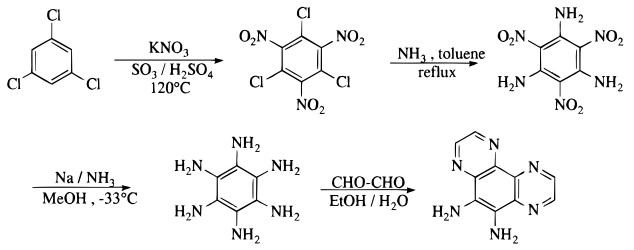
Chemicals. High-purity reagents and solvents (analytical grade) were used without further purification except for that used in the electrochemistry: acetonitrile (Aldrich, p.a.) was refluxed and distilled several times, first from P_2O_5 and afterward from CaH_2 before each electrochemical measurement.

Water was purified with a Millipore Milli-Q system. The Tris buffer (Tris(hydroxymethyl)aminomethane) was purchased from Aldrich. Calf thymus DNA (CT-DNA, Pharmacia LKB Biotechnology) was dialyzed extensively, first against phosphate buffer and afterward against water. [Poly(dA-dT)]$_2$ (Pharmacia LKB Biotechnology) was used as received. The polynucleotide phosphate concentration was determined spectrophotometrically (for CT-DNA: $\epsilon_{260\,nm}$ = 6600 $M^{-1}\,cm^{-1}$ and for [poly(dA-dT)]$_2$: $\epsilon_{262\,nm}$ = 6600 $M^{-1}\,cm^{-1}$).[24] The guanosine-5′-monophosphate (Aldrich) was used without further purification as the sodium salt.

Syntheses. A. Precursors. 9,10-Diamino-1,4,5,8-tetraazaphenanthrene. Scheme 1 shows the different steps for the preparation of the

(16) Haq, I.; Lincoln, P.; Suh, D.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788.
(17) Lecomte, J. P.; Kirsch-De Mesmaeker, A.; Feeney, M. M.; Kelly, J. M. *Inorg. Chem.* **1995**, *34*, 6481.
(18) (a) Kirsch-De Mesmaeker, A.; Nasielski-Hinkens, R.; Maetens, D.; Pauwels, P.; Nasielski, J. *Inorg. Chem.* **1984**, *23*, 377. (b) Masschelein, A.; Jacquet, L.; Kirsch-De Mesmaeker, A. *Inorg. Chem.* **1990**, *29*, 855. (c) Jacquet, L.; Kirsch-De Mesmaeker, A. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2471.
(19) Kelly, J.; McConnell, D.; OhUigin, C.; Tossi, A.; Kirsch-De Mesmaeker, A.; Masschelein, A.; Nasielski, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1821.
(20) (a) Feeney, M.; Kelly, J. M.; Tossi, A. B.; Kirsch-De Mesmaeker, A.; Lecomte, J.-P. *J. Photochem. Photobiol. B: Biol.* **1994**, *23*, 69. (b) Jacquet, L.; Kelly, J. M.; Kirsch-De Mesmaeker, A. *J. Chem. Soc., Chem. Commun.* **1995**, 913.
(21) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Tossi, A. B.; Kelly, J. M.; Görner, H. *Photochem. Photobiol.* **1992**, *55*, 681.
(22) Lin, C. T.; Boettcher, W.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6536.
(23) (a) Bevington, P. R. *Data Reduction and Errors Analysis for the Physical Sciences*; McGraw Hill: New York, 1969. (b) Demas, J. N. *Excited State Lifetime Measurement*; Academic Press: New York, 1983; pp 59−62.
(24) *(24) Pharmacia LKB Biotechnology Cataloge*; Pharmacia P-L Biochemicals, Inc.; Uppsala, Sweden, 1990.

Scheme 1



9,10-diamino-1,4,5,8-tetraazaphenanthrene precursor. Ammonolysis[25] of 1,3,5-trichloro-2,4,6-trinitrobenzene (obtained by nitration of 1,3,5-trichlorobenzene[26] with an 84% yield after sublimation) gave 1,3,5-triamino-2,4,6-trinitrobenzene (92%), which can be reduced with sodium in liquid ammonia[27] and produce hexaaminobenzene in excess of 90%.

A solution of 856 mg of 30% aqueous glyoxal (4.4 mmol) in 34 mL EtOH was added dropwise to 367 mg (2.2 mmol) hexaaminobenzene in H_2O (80 mL) and EtOH (40 mL) under nitrogen atmosphere. After the solution was stirred for 2 h at 50 °C, the cooled mixture was poured on water and extracted with chloroform. This was dried over $MgSO_4$ and filtered, and evaporation of the solvent gave 582 mg of a crude solid, which contained 9,10-diamino-1,4,5,8-tetraazaphenanthrene and 1,4,5,8,9,12-hexazatriphenylene. Pure 9,10-diamino-1,4,5,8-tetraazaphenanthrene (238 mg, 51%) was obtained after chromatography over neutral alumina (98:2 $CHCl_3$/EtOH).

1,10-Phenanthroline-5,6-dione[14] and bis(1,10-phenanthroline)dichlororuthenium(II)[28] were prepared as described previously.

Bis(1,10-phenanthroline)(1,10-phenanthroline-5,6-dione)ruthenium(II). The bis(hexafluorophosphate) salt was prepared from 1,10-phenanthroline-5,6-dione and bis(1,10-phenanthroline)dichlororuthenium(II), similar to the procedure already described[11c] but from the intermediate $Ru(phen)_2(H_2O)_2^{2+}$ instead of $Ru(phen)_2(O_3SCF_3)_2$. ^1H NMR (250 MHz, CD_3CN): 8.70 (2H, dd, H^P_4, $J_{3,4} = 8.3$), 8.59 (2H, dd, H^P_7, $J_{7,8} = 8.3$), 8.49 (2H, dd, H^D_γ, $J_{\beta,\gamma} = 8.0$), 8.33 (2H, dd, H^P_2, $J_{2,4} = 1.2$), 8.27 (4H, AB, $H^P_{5,6}$, $J_{5,6} = 8.9$), 7.91 (4H, dd, H^P_9 and H^D_α, $J_{7,9} = J_{\alpha,\gamma} = 1.2$), 7.82 (2H, dd, H^P_3, $J_{2,3} = 5.2$), 7.59 (2H, dd, H^P_8, $J_{8,9} = 5.4$), 7.49 (2H, dd, H^D_β, $J_{\alpha,\beta} = 5.6$). (H^P refers to protons on 1,10 phenanthroline and H^D refers to protons on 1,10-phenanthroline-5,6-dione. For the numbering of the different protons, see Scheme 2). The different protons were attributed from analysis of a $^1H-^1H$ COSY spectrum.

B. Polypyridyl Ruthenium(II) Complexes. The syntheses of $Ru(phen)_2(HAT)^{2+}$ [18c] and $Ru(phen)_2(DPPZ)^{2+}$ [11c] have already been described.

Bis(1,10-phenanthroline)(1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene)ruthenium Bis(hexafluorophosphate). The dione complex (103 mg, 0.11 mmol) was dissolved in 10 mL of acetonitrile and heated to reflux. 9,10-Diamino-1,4,5,8-tetraazaphenanthrene (25 mg, 0.12 mmol) in 4 mL of 1:1 acetonitrile/ethanol was added, and the medium was kept refluxing for 1 h and 15 min. After the mixture was cooled, the product was precipitated with a large excess of diethyl ether. The precipitate was filtered off, and the counterions were exchanged for chlorides before purification on Sephadex SP-C25, as already described.[8b,18b,c] The purity of the complex, obtained with 42% yield, was checked by high-performance liquid chromatography (HPLC) and ^1H NMR. MS (ESMS, CH_3CN, $M_w = 1137.8$): m/z = 992.6 (14, $M - PF_6^-$), 423.8 (100, $M - 2(PF_6^-)$). ^1H NMR (250 MHz, CD_3CN): 9.82 (2H, dd, H^{PH}_γ, $J_{\alpha,\gamma} = 1.0$), 9.35 (4H, s, $H^{PH}_{\delta,\epsilon}$), 8.62 (4H, dd, $H^P_{4,7}$, $J_{3,4} = J_{7,8} = 8.3$), 8.27 (4H, s, $H^P_{5,6}$), 8.22 (2H, dd, H^P_2, $J_{2,4} = 8.9$), 8.20 (2H, dd, H^{PH}_α, $J_{\alpha,\beta} = 1.2$), 8.04 (2H, dd, H^P_9, $J_{7,9} = 1.2$), 7.87 (2H, dd, H^{PH}_β, $J_{\beta,\gamma} = 5.2$), 7.65 (4H, 2dd, H^P_8 and H^P_3, $J_{2,3} = 5.6$, $J_{8,9} = 5.4$). (H^P refers to protons on 1,10 phenanthroline and H^{PH} refers to protons on 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene. For the numbering of the different protons, see Scheme 2). The different protons were attributed from a $^1H-^1H$ COSY spectrum.

Results

Synthesis. The synthesis of 1,10-phenanthroline-5,6-dione is described in the literature.[14] The other ligand precursor, 9,10-diamino-1,4,5,8-tetraazaphenanthrene, was prepared following a method that was more efficient than the one described previously.[29] Nitration of commercial 1,3,5-trichlorobenzene, substitution of the chlorine atoms for amino groups, and reduction of the nitro groups gave rise to the very reactive hexaaminobenzene, which easily reacts with 2 equiv of glyoxal to lead to the targeted molecule. Similar to the procedure described previously for $Ru(bpy)_2(DPPZ)^{2+}$,[12a,b] first the free ligand itself was synthesized by condensation of 1,10-phenanthroline-5,6-dione with 9,10-diamino-1,4,5,8-tetraazaphenanthrene. However, the poor solubility of this ligand prevented the subsequent reaction with $Ru(phen)_2Cl_2$. Therefore, we followed the synthetic route outlined in Scheme 2, described for the DPPZ complex.[11c] The desired complex was isolated as the hexafluorophosphate salt and purified by chromatography. It was characterized unambiguously by NMR and electrospray mass spectrometry.

Absorption and Emission. The absorption data for $Ru(phen)_2(PHEHAT)^{2+}$ and, for comparison purposes, for $Ru(phen)_2(DPPZ)^{2+}$ and $Ru(phen)_2(HAT)^{2+}$ are collected in Table 1. The UV−vis absorption spectrum of $Ru(phen)_2(PHEHAT)^{2+}$ in water (Figure 3) is characterized, as for the other tris(bipyridyl)ruthenium(II) complexes, by intense ligand-centered transitions in the UV and metal-to-ligand charge transfer (MLCT) transitions in the visible (λ_{max}: 440 nm, ϵ = 22 700 M^{-1} cm^{-1}). Moreover, intraligand (IL) transitions of the PHEHAT chromophore are also observed at rather long wavelengths (λ: 374 nm, ϵ = 28 600 M^{-1} cm^{-1} ; λ: 356 nm, ϵ = 24 400 M^{-1} cm^{-1}). As the free ligand is insoluble (no absorption data available), this attribution was made by comparison with the $Ru(phen)_2(DPPZ)^{2+}$.[12a,b] In the literature, comparison of the absorption spectrum of $Ru(bpy)_2(DPPZ)^{2+}$ [11c] with that of the parent complex $Ru(bpy)_3^{2+}$ shows that the MLCT transition band is unchanged by the annelation of a phenazine moiety to the bpy fragment. The same phenomenon is observed in this case, and the annelation of a HAT moiety to a phen fragment does not modify the MLCT band of the resultant $Ru(phen)_2(PHEHAT)^{2+}$ complex as compared to $Ru(phen)_3^{2+}$.

The luminescence has been examined in water, acetonitrile, and 2-propanol. In contrast to $Ru(phen)_2(HAT)^{2+}$ and most tris(bipyridyl)ruthenium(II) complexes, $Ru(phen)_2(PHEHAT)^{2+}$ does not luminesce in water, as $Ru(phen)_2(DPPZ)^{2+}$ does, but shows appreciable luminescence in acetonitrile and 2-propanol. The emission maxima, relative quantum yields, and lifetimes are compiled in Table 2, along with the data for the reference complexes; the values for the radiative (k_r) and non radiative (k_{nr}) rate constants have been calculated from ϕ/τ under argon. The emission energy decreases from $Ru(phen)_2(DPPZ)^{2+}$ to $Ru(phen)_2(PHEHAT)^{2+}$ to $Ru(phen)_2(HAT)^{2+}$. The relative luminescence quantum yields in both solvents follow the sequence $Ru(phen)_2(DPPZ)^{2+}$ > $Ru(phen)_2(HAT)^{2+}$ > $Ru(phen)_2(PHEHAT)^{2+}$.

Electrochemistry. The redox potentials for the ground and excited states (estimated from the lowest limit of the ΔE_{00} energy, *i.e.*, the emission maximum) of the three complexes

(25) Kohne, B.; Praefcke, K. *Liebigs Ann. Chem.* **1987**, 265.
(26) Hill, M. E.; Taylor, F. Jr. *J. Org. Chem.* **1960**, *25*, 1037.
(27) Rogers, D. Z. *J. Org. Chem.* **1986**, *51*, 3904.
(28) $Ru(phen)_2Cl_2$ was prepared using the method employed for the synthesis of $Ru(bpy)_2Cl_2$ in Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.
(29) Nasielski-Hinkens, R.; Benedek-Vamos, M.; Maetens, D.; Nasielski, J. *J. Organomet. Chem.* **1981**, *217*, 179.

Scheme 2

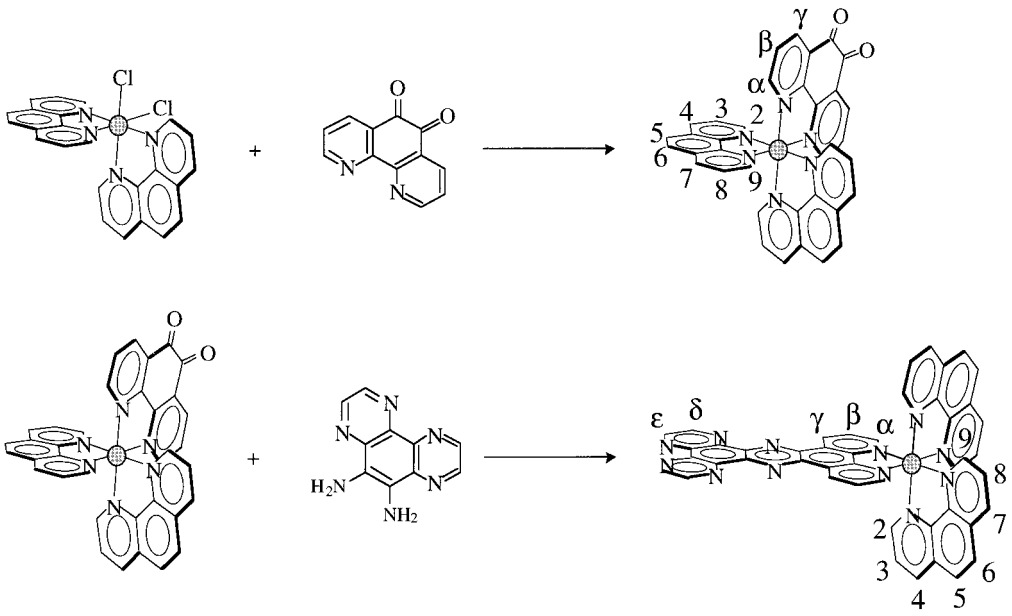


Table 1. Absorption Data for the Ruthenium(II) Complexes[a]

	λ_{max}/nm (ϵ/10^{-4} M^{-1} cm^{-1})		
complex	H_2O	MeCN	2-propanol
$Ru(phen)_3^{2+}$	421, 443(2.0)[b]	262, 446[c]	
$Ru(phen)_2(DPPZ)^{2+}$[d]	264, 278 sh, 318 sh, 358 sh, 372, 440 (2.34)	264, 276 sh, 316, 352, 360, 368, 440	266, 278 sh, 318 sh, 368, 440
$Ru(phen)_2(HAT)^{2+}$[e]	262, 430 (1.44), 494 sh	262, 420, 480 sh	264, 416, 476 sh
$Ru(phen)_2(PHEHAT)^{2+}$	264, 276 sh, 312 sh, 356, 374, 440 (2.27)	264, 278 sh, 312 sh, 354 sh, 370, 438	266, 276 sh, 312 sh, 350 sh, 372, 440

[a] Experimental error for the ϵ values is 10%; sh = shoulder. [b] From ref 10c and Lin, C-T.; Böttcher, W.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6536−6544. [c] From Staniewicz, R. J.; Sympson, R. F.; Hendricker, D. G. *Inorg. Chem.* **1977**, *16*, 2166. [d] Our data. See also ref 11c. [e] Our data. See also ref 18c.

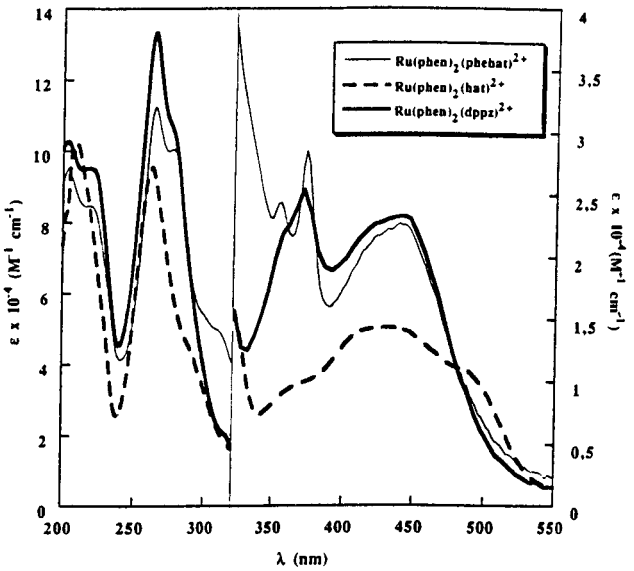


Figure 3. UV−vis absorption spectra of $Ru(phen)_2(PHEHAT)^{2+}$, $Ru(phen)_2(HAT)^{2+}$, and $Ru(phen)_2(DPPZ)^{2+}$ in aqueous solution at room temperature.

and of $Ru(phen)_3^{2+}$ are gathered in Table 3. The comparison between the Ru^{III}/Ru^{II} redox potentials of $Ru(phen)_3^{2+}$, $Ru(phen)_2(DPPZ)^{2+}$, and $Ru(phen)_2(PHEHAT)^{2+}$ suggests that the PHEHAT and DPPZ ligands behave as a phen ligand, as far as the oxidation of the metallic center is concerned. Reductively, the facility of the addition of the first electron on the complex ligand proceeds according to the π-acceptor ability of the ligand (*i.e.*, first HAT and PHEHAT, then DPPZ, and finally phen). In the excited state, $Ru(phen)_2(PHEHAT)^{2+}$ shows the strongest oxidation power among the three complexes.

Table 2. Emission Data for the Ruthenium(II) Complexes[a]

complex	λ_{max}/ nm[b]	τ_{air}/ ns	τ_{Ar}/ ns	ϕ_{air} × 10^3[c]	ϕ_{Ar} × 10^3[c]	k_r × 10^{-6} s^{-1}[f]	k_{nr} × 10^{-6} s^{-1}[g]
Acetonitrile							
$Ru(phen)_2(DPPZ)^{2+}$[d]	630	180	643	21	78	0.121	1.43
$Ru(phen)_2(HAT)^{2+}$[e]	696	371	776	17	43	0.055	1.23
$Ru(phen)_2(PHEHAT)^{2+}$	662	191	262	11	26	0.099	3.72
2-Propanol							
$Ru(phen)_2(DPPZ)^{2+}$[d]	610	200	391	20	48	0.123	2.43
$Ru(phen)_2(HAT)^{2+}$[e]	705	377	742	10	17	0.023	1.32
$Ru(phen)_2(PHEHAT)^{2+}$	658	164	215	8	10	0.046	4.61

[a] The luminescence decays correspond to strict single exponentials. Experimental errors for the lifetimes are ±5%. [b] Corrected λ_{max} of emission. [c] ϕ = emission quantum yields, measured relative to $Ru(bpy)_3^{2+}$ in an aerated acetonitrile solution ($\phi = 0.012$), from Calvert, J. M.; Casper, J. V.; Binstead, R. A.; Westmoreland, T. D.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 6620. Approximate experimental error is 20%. [d] Our data. see also ref 11c. [e] Our data. See also ref 18c. [f] Determined from ϕ/τ under Ar. [g] $k_{nr} = 1/\tau - k_r$ under Ar.

Absorption and Emission of $Ru(phen)_2(PHEHAT)^{2+}$ with Polynucleotides. Absorption Titration. Figure 4 shows the absorption spectrum of $Ru(phen)_2(PHEHAT)^{2+}$ (at constant concentration of complex) in the presence of increasing amounts of CT-DNA and $[poly(dA\text{-}dT)]_2$. By increasing the polynucleotide concentration, and thus the P/Ru ratio ([phosphate]/[Ru(II) complex]), two different behaviors are distinguished. The first phase (Figure 4A) is characterized by a hypochromic effect: the absorption at 440 nm decreases linearly when the polynucleotide concentration increases from zero up to P/Ru

Table 3. Electrochemical Data for the Ruthenium(II) Complexes in the Ground and in the Excited States[a]

complex	E_{red}/V[b]	E_{ox}/V	E_{ox}*/V[c]	E_{red}*/V[c]
$Ru(phen)_3^{2+}$ [d]	−1.35	+1.27		
$Ru(phen)2(DPPZ)^{2+}$ [e]	−1.00	+1.30	−0.67	+0.96
$Ru(phen)_2(HAT)^{2+}$ [f]	−0.86	+1.53	−0.25	+0.92
$Ru(phen)_2(PHEHAT)^{2+}$	−0.84	+1.35	−0.55	+1.10

[a] The redox potentials (V/SCE) were determined by cyclic voltammetry in acetonitrile, with 0.1 M $(^tBu_4N)^+PF_6^-$ as the supporting electrolyte and a Pt working electrode. [b] First reduction wave. [c] Oxidation and reduction potentials in the excited state have been estimated with the emission maxima. [d] From Barigelletti, F.; Juris, A.; Balzani, V.; Belser, P.; von Zelewsky, A. *Inorg. Chem.* **1987**, *26*, 4115−4119. [e] Determined with 0.1 M $(Et_4N)^+ClO_4^-$ in acetonitrile. [f] From ref 18c, in acetonitrile.

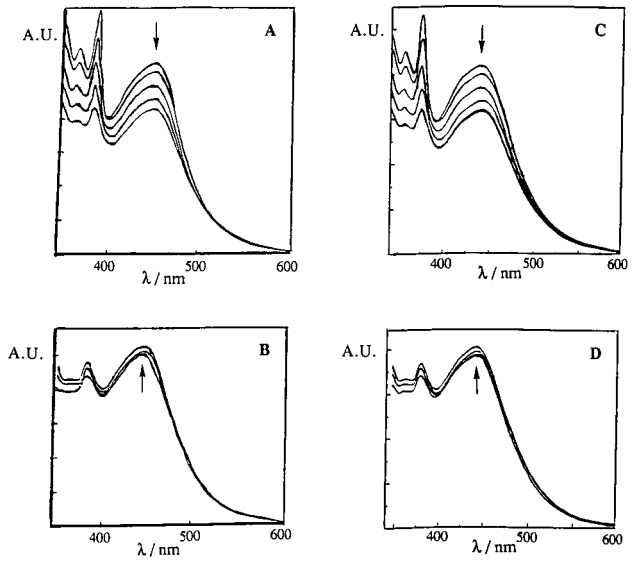


Figure 4. Absorption spectra (arbitrary units) of $[Ru(phen)_2(PHEHAT)]Cl_2$ (A and B) in the presence of calf thymus DNA ([complex] = 25 μM; [Tris buffer] = 3 mM) and (C and D) in the presence of $[poly(dA\text{-}dT)]_2$. The mixing ratios [DNA phosphate]/[complex] are the following: (A and C) from top to bottom 0 (no DNA present), 1, 1.5, 2, 3, and 5 and (B and D) from bottom to top: 10, 20, 25, and 50.

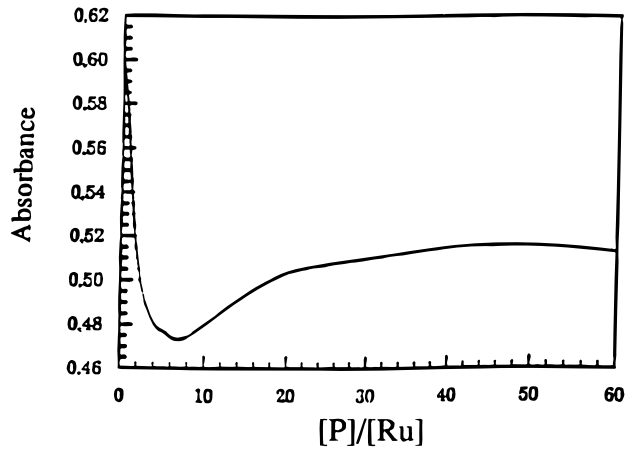


Figure 5. Absorbance of $Ru(phen)_2(PHEHAT)^{2+}$ at constant concentration (25 μM) in 3 mM Tris buffer, at 440 nm, versus increasing ratios of [DNA phosphate]/[complex].

≈ 7; in the second phase (Figure 4B), for higher P/Ru ratios, the absorption increases and reaches a plateau value at P/Ru ≈ 20 . These two phases of events are illustrated in Figure 5, which exhibits the change of absorption at 440 nm for increasing CT-DNA concentrations.

The absorption spectrum of the complex recorded with GMP (3×10^{-2} M) or with CT-DNA (P/Ru ratio = 4) does not change as a function of the illumination time of the complex. No photoproduct or photoadduct is thus formed.

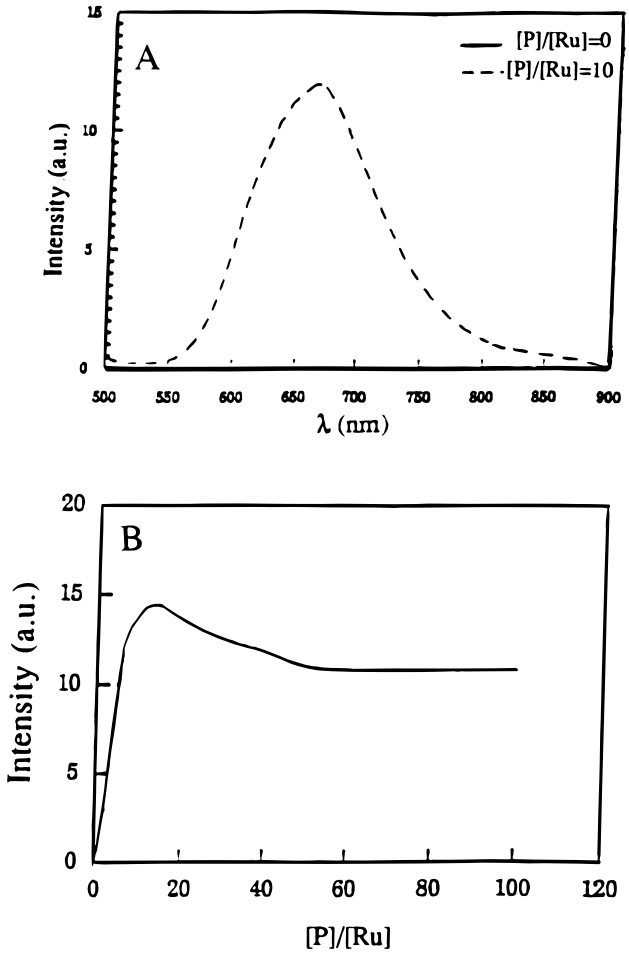


Figure 6. (A) Emission spectra of $[Ru(phen)_2(PHEHAT)]Cl_2$ in the absence and in the presence of calf thymus DNA. (B) Emission of $[Ru(phen)_2(PHEHAT)]Cl_2$ at constant concentration of 25 μM, in 3 mM Tris buffer, at 658 nm, versus increasing ratios of [DNA phosphate]/[complex].

Luminescence Titration. $Ru(phen)_2(PHEHAT)^{2+}$, carefully purified by chromatography, does not luminesce in Tris buffer, while it emits after addition of polynucleotides. Figure 6A exhibits the occurrence of luminescence from the complex by DNA addition for P/Ru = 10, and Figure 6B shows, for the same complex concentration, the occurrence of luminescence as a function of the addition of increasing amounts of DNA (P/Ru increasing). Again, for increasing CT-DNA concentration, two phases of events are observed. At low P/Ru ratios, the luminescence intensity shows an important enhancement for increasing polynucleotide concentrations: the emission at 658 nm increases linearly with increasing amount of polyelectrolyte up to a P/Ru ratio ≈ 13. A second phase, where the luminescence decreases by increasing the concentration of DNA, is observed for P/Ru ratios between 15 and 40; from P/Ru ≥ 50, no further changes are observed. These two phases of events also occur when the study is performed with synthetic $[poly(dA\text{-}dT)]_2$ instead of CT-DNA. In Figure 7, three luminescence titration curves with CT-DNA and with $[poly(dA\text{-}dT)]_2$ have been plotted for the three complexes $Ru(phen)_2(DPPZ)^{2+}$, $Ru(phen)_2(HAT)^{2+}$, and $Ru(phen)_2(PHEHAT)^{2+}$, for the same percentage of absorbed light at the same excitation wavelength.

The affinity of $Ru(phen)_2(PHEHAT)^{2+}$ for CT-DNA has been determined from a luminescence titration of DNA, present in concentration of 5 μM in base pairs[14] ([Tris buffer] = 1 mM, [NaCl] = 10 mM), using the Mc Ghee−Von Hippel relation.

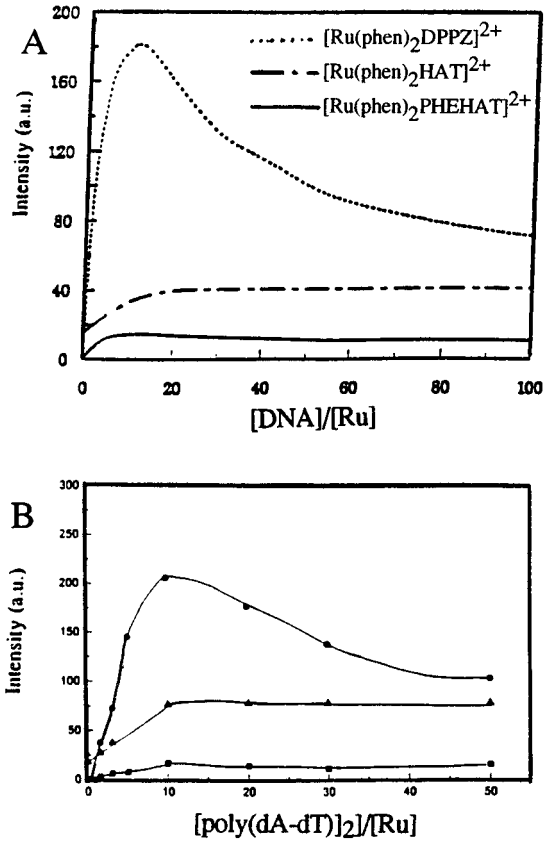


Figure 7. (A) Emission of $Ru(phen)_2(PHEHAT)^{2+}$ (658 nm, —), $Ru(phen)_2(HAT)^{2+}$ (700 nm, ···), and $Ru(phen)_2(DPPZ)^{2+}$ (613 nm, -·-) versus increasing ratios of [DNA phosphate]/[complex]. (B) Emission of $Ru(phen)_2(PHEHAT)^{2+}$ (658 nm, ■), $Ru(phen)_2(HAT)^{2+}$ (700 nm, ▲), and $Ru(phen)_2(DPPZ)^{2+}$ (613 nm, ●) versus increasing ratios of [[poly(dA-dT)]$_2$]/[complex]. In both cases, the complex concentration is constant and the experimental set up comparable.

The best fit was obtained with a site−size parameter equal to three base pairs, the value already obtained for $Ru(phen)_2(DPPZ)^{2+}$.[16] The binding constant calculated according to this model corresponds to 2.5×10^6 M^{-1}, which is comparable to the values obtained in the literature for $Ru(phen)_2(DPPZ)^{2+}$ (also summarized in ref 30).

Laser Flash Photolysis. In order to determine whether $Ru(phen)_2(PHEHAT)^{2+}$ could induce redox reactions in the ^{3}MLCT excited state with nucleotidic bases, laser flash photolysis experiments have been carried out in the absence and in the presence of mononucleotides and DNA. According to its redox potentials, $Ru(phen)_2(PHEHAT)^{2+}$ in the excited state could indeed oxidize the guanines of DNA (reduction potential of the excited complex: +1.1 V/SCE, Table 3).

First, laser flash photolyses have been performed with the complex alone in organic solvent to detect the excited state absorption. Comparisons have been made with $Ru(phen)_2(DPPZ)^{2+}$ in the same conditions. The differential transient absorptions obtained for the two complexes alone in acetonitrile under argon show positive signals at ∼350 nm and depletions in the 370−500 nm region (Figure 8). These transients decay according to monomolecular processes, with rate constants corresponding to the luminescence lifetimes in acetonitrile (*i.e.*, 260 ns for $Ru(phen)_2(PHEHAT)^{2+}$ and 640 ns for $Ru(phen)_2(DPPZ)^{2+}$); they are, thus, characteristic of the ^{3}MLCT excited states. No data can be obtained in aqueous solution (Tris buffer (0.5 M) at pH = 7) as both complexes have lifetimes that are too short in water (no emission). Moreover, because of the absence of emission, no quenching rate constants by guanosine-5′-monophosphate can be obtained in aqueous solution.

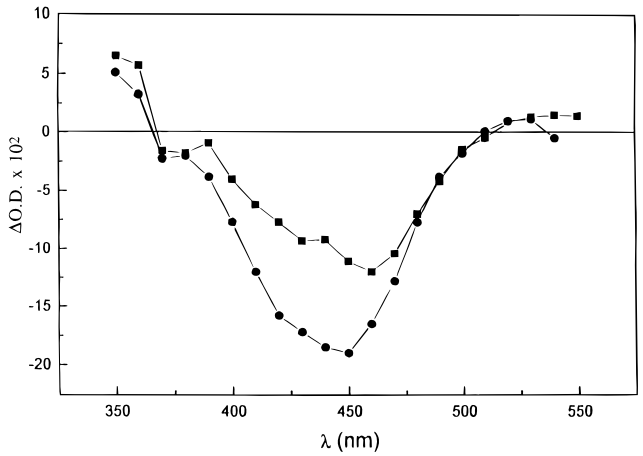


Figure 8. Transient differential absorption spectra for $Ru(phen)_2(PHEHAT)^{2+}$ (■) and $Ru(phen)_2(DPPZ)^{2+}$ (●) in acetonitrile just after the laser pulse, under argon.

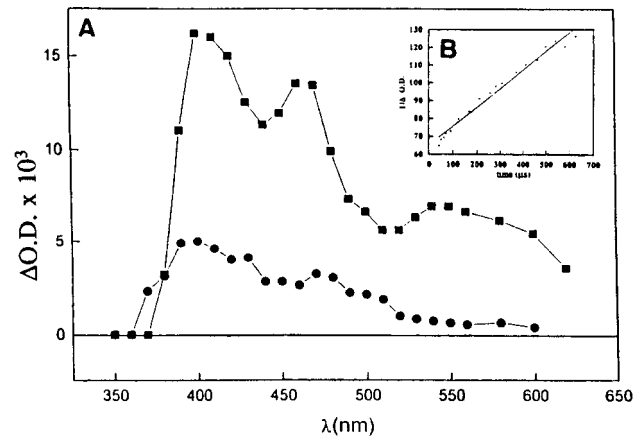


Figure 9. (A) Transient differential absorption spectra obtained with $Ru(phen)_2(PHEHAT)^{2+}$ (10^{-4} M) in the presence of 50 mM GMP (■) under argon 5 μs after the pulse and (●) under oxygen 80 μs after the pulse, in 0.5 M Tris buffer, pH = 7. (B) Decay at 400 nm under argon.

In contrast, laser flash photolysis of $Ru(phen)_2(PHEHAT)^{2+}$ in buffered solution in the presence of 50 mM GMP under argon produces a transient of a few hundreds of microseconds, absorbing in the 350−600 nm region (Figure 9A). No transient is detected for $Ru(phen)_2(DPPZ)^{2+}$ in the same conditions, in the presence of GMP. The transient with the PHEHAT complex decays according to a bimolecular process (Figure 9B). Moreover, when the flash photolysis experiments with GMP are performed with an oxygen-saturated solution at pH 7, two decays are observed. The first one decays during a few tens of microseconds, and the second one disappears over a few hundreds of microseconds. The differential transient absorption spectrum recorded after the first decay (Figure 9A) is in accordance with the transient absorption spectrum of the deprotonated GMP radical cation that was reported in the literature, from pulse radiolysis experiments.[21,31]

In order to confirm the attribution of the fast component in Figure 9A to monoreduced $Ru(phen)_2(PHEHAT)^{2+}$, experiments have been carried out in the presence of hydroquinone. This

(30) Norden, B.; Lincoln, P.; Akerman, B.; Tuite, E. *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 33, pp 177−252.

(31) (a) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *11*, 1094. (b) Jovanovic, S. V.; Simic, M. *Biochim. Biophys. Acta* **1989**, *1008*, 39.

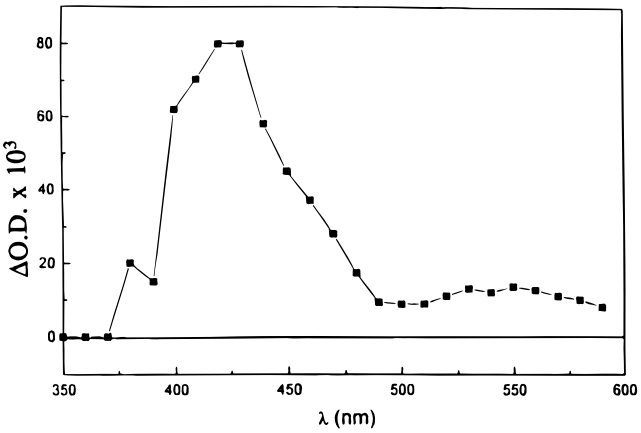


Figure 10. Transient differential absorption spectrum recorded for $Ru(phen)_2(PHEHAT)^{2+}$ (13 μM) in a deaerated solution (Tris buffer 0.01 M, pH = 7.0) in the presence of hydroquinone 0.05 M, 4 μs after the laser pulse.

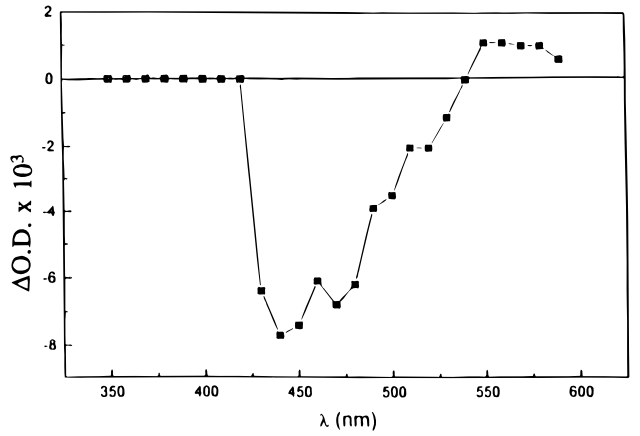


Figure 11. Transient differential absorption spectrum (3 μs after the pulse) for $Ru(phen)_2(PHEHAT)^{2+}$ (10^{-4} M) in the presence of calf thymus DNA (1 mM) in a Tris buffer solution (0.01 M, pH = 7.0) under argon.

neutral reductant is indeed able to reductively quench the ^{3}MLCT state of oxidizing Ru(II) complexes, such as $Ru(TAP)_3^{2+}$.[32] When hydroquinone (0.05 M) is added to the $Ru(phen)_2(PHEHAT)^{2+}$ solution, a positive transient absorption is detected in the 350−600 nm wavelength range 4 μs after the laser pulse (Figure 10). The absorption between 480 and 600 nm is characteristic of the monoreduced complex and is similar to the transient observed with GMP (Figure 9A); the absorption at ∼400−450 nm (in Figure 10) is attributed to semiquinone that is produced from hydroquinone oxidation.[32a]

In the presence of CT-DNA, $Ru(phen)_2(PHEHAT)^{2+}$ produces, after the laser pulse, a very weak transient absorption around 550 nm and a depletion in the 420−520 nm region (Figure 11).

Discussion

Properties of $Ru(phen)_2(PHEHAT)^{2+}$. Generally, a spectroelectrochemical correlation is observed for most polypyridyl ruthenium(II) complexes.[33] In such correlations, the optical excitation is regarded as the oxidation of the metal ion and reduction of the most π accepting ligand. Consequently, the plot for a series of complexes of the energy of the lowest MLCT transition in absorption or emission as a function of $E_{Ru^{2+/3+}} - E_{Ru^{2+/1+}}$ ($\Delta E_{1/2}$) gives a straight line. Figure 12 shows this correlation for the TAP and HAT complexes synthesized in our laboratory with the data for $Ru(phen)_2(PHEHAT)^{2+}$ and $Ru(phen)_2(DPPZ)^{2+}$. It is clear that these two complexes do not follow the correlation. This indicates that the orbitals involved in the spectroscopic and redox processes are not the same, as also concluded from the electrochemical and spectroscopic results outlined below.

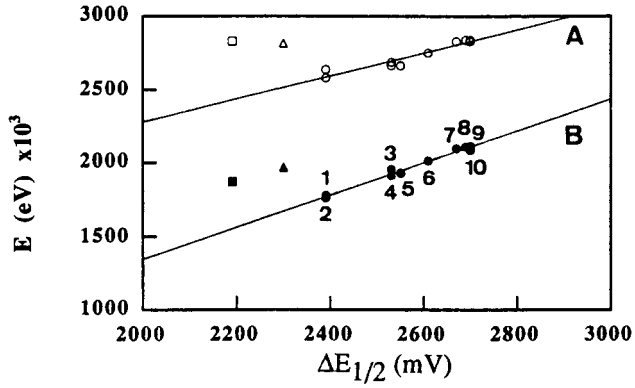


Figure 12. Energies of the lowest MLCT transition in the (A) absorption and (B) luminescence spectra for tap and HAT complexes as a function of $\Delta E_{1/2}$ (difference in oxidation and reduction potentials) for a series of complexes in acetonitrile at room temperature. (□ and ■) $Ru(phen)_2(PHEHAT)^{2+}$, (△ and ▲): $Ru(phen)_2(DPPZ)^{2+}$, (1) $Ru(bpy)_2(TAP)^{2+}$, (2) $Ru(bpy)_2(HAT)^{2+}$, (3) $Ru(bpy)(tap)(HAT)^{2+}$, (4) $Ru(tap)_2(bpy)^{2+}$, (5) $Ru(HAT)_2(bpy)^{2+}$, (6) $Ru(bpy)_3^{2+}$, (7) $Ru(HAT)_2(tap)^{2+}$, (8) $Ru(HAT)_3^{2+}$, (9) $Ru(tap)_3^{2+}$, (10) $Ru(tap)_2(HAT)^{2+}$.[18c]

In electrochemistry, the similarity between the oxidation potentials of $Ru(phen)_3^{2+}$ (or $Ru(phen)_2(DPPZ)^{2+}$) and $Ru(phen)_2(PHEHAT)^{2+}$ (Table 3) suggests that the PHEHAT has the same chelating characteristics as phen (or DPPZ). Thus, the HAT motif of PHEHAT (or the phenazine fragment of the DPPZ)[12a,b] does not affect the metal-centered dπ level. Reductively, the first wave for the PHEHAT complex is observed at the same potential as that for the HAT compound (∼ −0.85 V/SCE), *i.e.*, 0.5 V more positive than that for $Ru(phen)_3^{2+}$, in agreement with the important π acceptor character of the HAT fragment. This behavior shows that reductively, in contrast to oxidatively, the process is controlled by the HAT fragment of the PHEHAT ligand. These electrochemical considerations suggest that in order to observe a spectroelectrochemical correlation with the other TAP and HAT complexes, the absorption of a photon should lead to the transfer of the electron from the metal-centered dπ orbital, fixed by the phen motif, to a ligand π* orbital characteristic of the HAT motif. This is not the case as outlined below. Indeed, the λ_{max} of the most bathochromic MLCT absorption of $Ru(phen)_2(PHEHAT)^{2+}$ is similar to that of $Ru(phen)_3^{2+}$ or $Ru(phen)_2(DPPZ)^{2+}$. This suggests that the Frank−Condon transition involved in the absorption corresponds to a MLCT transition of a Ru−phen chromophore (the phen belonging to the PHEHAT). If the HAT-type π* orbitals were involved, the absorption maximum should be more bathochromic, as observed with $Ru(phen)_2(HAT)^{2+}$.

Similarly, in order to observe a spectroelectrochemical correlation with the TAP and HAT complexes, the emission from the excited state should correspond to an electron transfer

(32) (a) Tan-Sien-Hee, L.; Kirsch-De Mesmaeker, A. *J. Chem. Soc., Dalton Trans.* **1994**, 3651. (b) Masschelein, A.; Kirsch-De Mesmaeker, A. *New J. Chem.* **1987**, *11*, 329. (c) Kirsch-De Mesmaeker, A.; Maetens, D.; Nasielski-Hinkens, R. *J. Electroanal. Chem.* **1985**, *182*, 123.

(33) (a) Caspar, J. V.; Meyer, T. J. *Inorg. Chem.* **1983**, *22*, 2444. (b) Rillema, D. P.; Allen, G.; Meyer, T. J.; Conrad, D. *Inorg. Chem.* **1983**, *22*, 1617. (c) Juris, A.; Belser, P.; Barigelletti, G.; von Zelewsky, A. B.; Balzani, V. *Inorg.Chem.* **1986**, *25*, 256. (d) Wallace, L.; Rillema, D. P. *Inorg. Chem.* **1993**, *32*, 3836.

from a HAT-type π^* orbital (HAT belonging to the PHEHAT) to a dπ orbital characteristic of $Ru(phen)_3^{2+}$. This does not seem to be the case. Indeed, instead of having an emission energy for $Ru(phen)_2(PHEHAT)^{2+}$ close to or even lower than that of $Ru(phen)_2(HAT)^{2+}$, the luminescence energy is higher; actually, it lies between that of the DPPZ and that of the HAT complexes. In the excited state, the properties are, thus, intermediate between those of these two compounds. These considerations underline the particular photophysical properties of this new class of complexes.

Finally, the comparison of the emission data for the three complexes in organic solvents (Table 2) indicates that $Ru(phen)_2(PHEHAT)^{2+}$ is not a good luminophore in acetonitrile and in 2-propanol. This would originate from the higher nonradiative rate constants calculated for this complex, as compared to the two other compounds.

$Ru(phen)_2(PHEHAT)^{2+}$ and DNA. Absorption changes of the MLCT bands of $Ru(phen)_2(PHEHAT)^{2+}$ and the occurrence of luminescence with the addition of CT-DNA and $[poly(dA\text{-}dT)]_2$ obviously indicate the binding of $Ru(phen)_2(PHEHAT)^{2+}$ to the polynucleotide.

The changes in the absorption spectrum upon addition of CT-DNA or $[poly(dA\text{-}dT]_2$ are important in the IL band (31% hypochromicity with $[poly(dA\text{-}dT)]_2$ at 376 nm, at the plateau value) and appear to a lesser extent in the MLCT band (Figure 4). These important hypochromicities would indicate intercalation of the PHEHAT ligand between the stacking of bases, as expected with such an extended planar ligand.

At constant complex concentration, the emission, starting at base line, increases with increasing amounts of CT-DNA (or $[poly(dA\text{-}dT)]_2$), reaches a maximum (P/Ru $\approx$ 13), and decreases to a plateau value (P/Ru $\approx$ 50) for lower degrees of occupancy of the polynucleotide by $Ru(phen)_2(PHEHAT)^{2+}$. This suggests different distributions of the complex on the polynucleotide. The luminescence at the plateau value would correspond to that of the isolated complex bound to DNA. The extra enhancement of luminescence might originate from closely bound metal complexes, which would provide a further protection of the luminophore from water as compared to the isolated complex on the double helix. This second mode of interaction is also evidenced from the extra hypochromicity effect observed in the absorption as a function of the P/Ru ratio (Figure 5). A similar behavior in the absorption and emission has also been reported by Norden et al. for $Ru(phen)_2(DPPZ)^{2+}$ and was interpreted in the same terms.[14]

As compared to the literature data which demonstrate intercalation of $Ru(phen)_2(DPPZ)^{2+}$, the effects of CT-DNA and $[poly(dA\text{-}dT)]_2$ on the absorption and emission of $Ru(phen)_2(PHEHAT)^{2+}$ also suggest that intercalation occurs for this complex. Detailed complementary data from studies with other methods will furnish further evidence for this geometry.

The comparison of the emission intensities of the three complexes, $Ru(phen)_2(HAT)^{2+}$, $Ru(phen)_2(PHEHAT)^{2+}$, and $Ru(phen)_2(DPPZ)^{2+}$, in the presence of CT-DNA or $[poly(dA\text{-}dT)]_2$ recorded in the same conditions (Figure 7) shows that $Ru(phen)_2(PHEHAT)^{2+}$ is the weakest emitter. Whether this behavior originates from a poorer interaction of the complex with DNA (thus less protection from the aqueous phase) or from the fact that this complex is intrinsically a weaker emitter can be answered from inspection of Table 2. The comparison of ϕ_{em} in acetonitrile and in 2-propanol indicates that these quantum yields follow the same sequence as the luminescence intensities with CT-DNA, *i.e.*, $Ru(phen)_2(DPPZ)^{2+} > Ru(phen)_2(HAT)^{2+} > Ru(phen)_2(PHEHAT)^{2+}$. This strongly suggests that the weaker luminescence of $Ru(phen)_2(PHEHAT)^{2+}$ in DNA is intrinsic to the complex and not caused by a weaker interaction with the polynucleotide, as demonstrated from the determination of the affinity constant.

Existence of a Photoinduced Electron Transfer with GMP. The redox potentials of the excited states (Table 3) show that $Ru(phen)_2(PHEHAT)^{2+*}$ is slightly more oxidizing than the other complexes.[34] The reduction potential of excited $Ru(phen)_2(PHEHAT)^{2+}$ is actually the same as that of the Ru(II) complexes containing two π-deficient ligands, such as TAP and HAT. As the emission of these latter complexes is quenched by guanosine-5′-monophosphate via a photoinduced electron transfer, excited $Ru(phen)_2(PHEHAT)^{2+}$ could behave similarly.

Flash photolysis of aqueous solutions of $Ru(phen)_2(PHEHAT)^{2+}$ with guanosine-5′-monophosphate produces a differential transient absorption which is typical of the presence of the monoreduced complex[31] and oxidized GMP. Guanidine reduces, thus, excited $Ru(phen)_2(PHEHAT)^{2+}$ according to the process shown in reaction 1.

$$Ru(phen)_2(PHEHAT)^{2+*} + GMP \rightarrow Ru(phen)_2(PHEHAT^{\bullet-})^{1+} + GMP^{\bullet+} \quad (1)$$

As the absorption coefficient at 480 nm of the deprotonated radical cation $GMP^{\bullet+}$ that is produced is much weaker than that of the monoreduced complex,[30a] the resulting global transient absorption is similar to that of the reduced complex[21,31a] and also similar to the transient absorption obtained with hydroquinone from 490 to 600 nm; below this wavelength region, with H_2Q as the quencher, the semiquinone that is formed after the photoinduced electron transfer is responsible for the absorption (λ_{max}: 420 nm).[31a]

When the flash photolysis experiment is performed with the complex and GMP under argon, the reduced complex disappears in a few hundreds of microseconds, by reoxidation by $GMP^{\bullet+}$ according to a bimolecular process (reaction 2).

$$Ru(phen)_2(PHEHAT^{\bullet-})^{1+} + GMP^{\bullet+} \rightarrow Ru(phen)_2(PHEHAT)^{2+} + GMP \quad (2)$$

In the presence of O_2, the escaped monoreduced complex is reoxidized by oxygen within a few microseconds, producing the initial complex and, presumably, the radical anion $O_2^{\bullet-}$ (and $HOO^{\bullet}$) whose absorption spectrum is negligible above 300 nm (reaction 3).

$$Ru(phen)_2(PHEHAT^{\bullet-})^{1+} + O_2 \rightarrow Ru(phen)_2(PHEHAT)^{2+} + O_2^{\bullet-} \quad (3)$$

The transient absorption recorded after the disappearance of the monoreduced complex is similar to the spectrum of the radical cation of guanine,[21,31] in accordance with process 1. The occurrence of an electron transfer with GMP (50 mM), while the excited complex does not even luminesce in water, sounds strange a priori. This quenching could actually originate from a static quenching in the ion pair $[Ru^{2+*}\cdots GMP]$. Such an association has been evidenced between $Ru(TAP)_3^{2+}$ and GMP,[21] with an equilibrium constant probably lower than that for the present PHEHAT complex:

(34) As discussed above, these values should be regarded as approximate because the orbitals involved in the spectroscopy and electrochemistry are different.

$$Ru(phen)_2(PHEHAT)^{2+} + GMP \rightleftharpoons [Ru(phen)_2(PHEHAT)^{2+}\cdots GMP] \quad (4)$$

It should be noted that the flash photolysis trace is rather weak, approximately ten times weaker than for the $Ru(TAP)_3^{2+}$ with GMP system. In addition, as no photoadduct can be detected after continuous illumination of the PHEHAT complex with GMP, these two observations would indicate that the efficiency of the photoinduced electron transfer is rather poor. The absence of a photoadduct could, of course, also be attributed to a lack of reactivity of the radical ion pair for the PHEHAT complex $[Ru(phen)_2(PHEHAT^{\bullet -})^{1+}\cdots GMP^{\bullet +}]$ compared to that for the TAP or HAT compounds.

The luminescence titration curve of the complex with CT-DNA does not indicate whether quenching by the guanine bases occurs within the DNA (by photoinduced electron transfer). Moreover, the comparison of the titration curves with CT-DNA and $[poly(dA\text{-}dT)]_2$ (Figure 7) does not suggest the presence of an important quenching by the guanines of CT-DNA as the luminescence intensity (in the same experimental conditions) is more or less the same with both polynucleotides. Therefore, flash photolysis experiments were carried out with CT-DNA. In this case, only a weak transient absorption (Figure 11) similar to that obtained with the complex alone in acetonitrile would indicate a T−T absorption in CT-DNA but not the existence of a long-lived reduced complex. The absence of such a transient with CT-DNA on the microsecond time scale could be attributed to the fact that with the present laser equipment (see Experimental Section), the time scale for the detection of the radical ions is too long. Indeed, the electron transfer could actually be present but a fast and efficient back reaction would take place. However, we may not totally exclude the possibility that although the electron transfer takes place with GMP, the process is still less efficient with CT-DNA. This behavior has indeed already been observed before with $Ru(TAP)_3^{2+}$. $Ru(TAP)_3^{2+}$ gives efficient photoinduced electron transfer with GMP and CT-DNA; with AMP, there is still some luminescence quenching ($k_q = 3 \times 10^8$ M^{-1} s^{-1}), whereas no quenching can be observed with $[poly(dA\text{-}dT)]_2$.[21] The absence of photoadduct formation for the PHEHAT complex with CT-DNA would also be in favor of a poorly efficient electron transfer as concluded with GMP.

Conclusion

For the novel complex $Ru(phen)_2(PHEHAT)^{2+}$, prepared by condensation of the ligand precursor 9,10-diamino-1,4,5,8-tetraazaphenanthrene with $Ru(phen)_2(phendione)^{2+}$, the molecular orbitals involved in the spectroscopic and redox processes are not the same. The Frank−Condon transition in the absorption involves the π^* orbitals localized on the phen subunit, whereas the electrochemical reduction takes place on a HAT π^*-type orbital.

$Ru(phen)_2(PHEHAT)^{2+}$ turns out to be a highly sensitive spectroscopic probe for DNA. In aqueous solution, its luminescence is switched on by interaction with DNA.

The laser flash photolysis demonstrates clearly the presence of a photoinduced electron transfer from GMP to the excited complex with, however, a poor efficiency. This process cannot be evidenced with DNA, at least with the flash photolysis conditions used in this work. Efforts will be made to make the PHEHAT complex more oxidizing in the excited state by changing the nature of the ancillary ligands.

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