Ruthenium(II) Complexes with 1,4,5,8,9,12-Hexaazatriphenylene and 1,4,5,8-Tetraazaphenanthrene Ligands: Key Role Played by the Photoelectron Transfer in DNA Cleavage and Adduct Formation

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The interaction and photoreaction of a series of ruthenium(II) complexes containing 1,4,5,8,9,12-hexaazatriphenylene (hat) and 1,4,5,8-tetraazaphenanthrene (tap) ligands with nucleotides and DNA have been studied. The rate constant of quenching of the excited states of the complexes by guanosine-5′-monophosphate (GMP) is shown to depend on the reduction potentials of the metal complex excited state, suggesting that the quenching is due to electron transfer from the guanine. The more strongly oxidizing metal complex excited state species are also quenched by adenosine-5′-monophosphate (AMP). Electron transfer has been verified for $Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, and $Ru(tap)_2(hat)^{2+}$ by laser flash photolysis, which indicates the formation of Ru(I) species and oxidized nucleotide intermediates with cage escape yields in the range 20−35%. Application of the Marcus theory yields a value of 1.16 V (vs NHE) for $E_{G^{\bullet+}/G}$ in GMP. The luminescence from $Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, $Ru(tap)_2L^{2+}$, or $Ru(hat)_2L^{2+}$ (L = 2,2′-bipyridine or 1,10-phenanthroline) is also quenched when the complexes are bound to DNA, and these oxidizing complexes are shown to be more efficient photosensitisers for single strand breaks in plasmid DNA. Covalently bound adducts are formed between the metal complexes and calf thymus DNA for those complexes whose excited states can oxidize guanine.

Introduction

The interaction of polypyridyl Ru(II) complexes with DNA has been the subject of intensive studies in the past few years.[1−9] The effect of size, shape, hydrophobicity, and charge on the binding of the complex to DNA has been analyzed by changing either the type of heteroaromatic ligands[1−10] or the nature of the metal center.[1,4] On the basis of these results, it has been concluded not only that the octahedral complexes bind to DNA electrostatically in the ionic atmosphere of the negatively charged phosphate backbone, but also that they surface bind in the minor or major grooves of DNA and, in favorable cases, that they intercalate at least partially one of their ligands between the stacked bases of the nucleic acid double helix.[11] Full insertion of the ligand is hindered sterically by the ancillary

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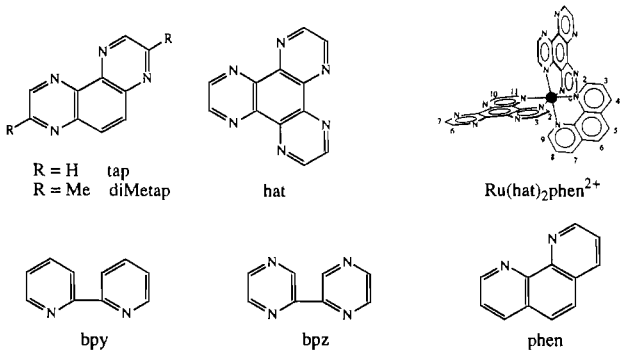

Figure 1. Structure of the different ligands used in the complexes.

ligands clashing against the phosphate backbone. The different modes of binding result from various types of forces or processes responsible for a decrease of the free energy of the system (electrostatic interactions, hydrogen bondings, London dispersion forces, and entropy increases for the hydrophobic interactions).

We have studied for several years the interactions and, more particularly, the photoreactions of tap (tap = 1,4,5,8-tetraazaphenanthrene) and hat (hat = 1,4,5,8,9,12-hexaazatriphenylene) (Figure 1) Ru(II) complexes with DNA.[5a-c,7a-c,11c] For a review on the photoreactions of Ru(II) complexes with the nucleic acids, see ref 7d.

Interestingly with $Ru(tap)_3^{2+}$, whose metal to ligand charge transfer (^{3}MLCT) excited state is particularly oxidizing,[12-14] it has been shown that this compound produces more efficient photocleavages of the DNA backbone than do most other Ru(II) polypyridyl complexes[15] and that it forms photoadducts with nucleic acids.[7a,16] These DNA photoreactions are thought to originate from a photoinduced electron transfer process from the guanine of DNA to the excited complex. The existence of such an electron transfer has been unambiguously demonstrated[7c] in the case of $Ru(tap)_3^{2+}$.

In this paper we present the photochemistry of a series of tap and hat Ru(II) complexes with modulated redox properties, in the presence of mononucleotides and DNA. Our aim is to demonstrate clearly the correlation between the occurrence of the photoelectron transfer process and the appearance of DNA cleavage and photoadduct formation. While it may be noted that photosensitized cleavages have been reported in the literature with other Ru(II) complexes containing phen (phen = 1,10 phenanthroline) or bpy (bpy = 2,2′-bipyridine) (Figure 1) ligands,[1f,3c] the efficiency is much lower than with some of the tap and hat complexes described here. Moreover for the bpy and phen complexes the base oxidation does not originate from a direct electron transfer to the excited complex, as proposed in this paper for the tap and hat compounds, but is attributed principally to singlet excited oxygen diffusing along the DNA strand and inducing DNA cleavage.[1f]

Although formation of adducts between metal complexes and DNA has been well-documented, especially for Pt(II) complexes, there have been very few reports of photoinduced reactions of this type. Recently, Morrisson and co-workers have reported the photoaddition of $Rh(phen)_2Cl_2^+$ to DNA by a photoanation process,[17] and we have reported adduct formation for $Ru(tap)_3^{2+}$,[7a,16] Irreversible photoinduced binding to DNA of the tap and hat Ru(II) complexes described in this work is not found for $Ru(phen)_3^{2+}$ or for most of the other Ru(II) polypyridyl complexes reported in the literature. These compounds may thus be regarded as potentially interesting photoreagents for nucleic acids.

Table 1. Luminescence Lifetimes and Quenching Rate Constants (k_Q) by GMP and AMP in Aqueous Solution (0.1 M Phosphate Buffer, pH 7, [Ru] $\approx 5 \times 10^{-5}$ (M)

	quencher = GMP			quencher = AMP	
complexes	τ buffer (Ar) (ns)	E^*_{red} (V vs SCE)	$k_Q \times 10^{-9}$ (M^{-1} s^{-1})	E^*_{red} (V vs SCE)	$k_Q \times 10^{-9}$ (M^{-1} s^{-1})
$Ru(hat)_3^{2+}$	176	1.46	2.16	1.46	0.87
$Ru(hat)_2(tap)^{2+}$	224	1.43	2.39	1.43	0.38
$Ru(tap)_2(hat)^{2+}$	327	1.36	2.16	1.36	0.13
$Ru(tap)_3^{2+}$	231	1.32	2.20	1.32	0.12
$Ru(bpz)_3^{2+}$	760	1.27[d]	1.98		
$Ru(Me_2tap)_3^{2+}$	98	1.24[e]	1.74		
$Ru(hat)_2(phen)^{2+}$	580	1.23	1.85[c]		
$Ru(hat)_2(bpy)^{2+}$	519	1.12	1.36		
$Ru(pzth)_2(hat)^{2+}$ [b]	343	1.18	0.84		
$Ru(tap)_2(bpy)^{2+}$	231	1.06	0.74		
$Ru(tap)_2(phen)^{2+}$	630	1.06	0.98[c]		
$Ru(phen)_2(hat)^{2+}$	108	0.87	0.024		
$Ru(bpy)_2(hat)^{2+}$	78	0.83	0.020		

[a] Values for the reduction potentials of the excited complexes (E^*_{red}) in acetonitrile ($E^*_{red} = E_{red} + \Delta E_{\lambda_{max}}$), estimated from the reduction potentials in the ground state (E_{red}) (measured by cyclic voltammetry) and the energy of the emission maximum ($\Delta E_{\lambda_{max}}$). [b] pzth, pyrazinothiazol: Orellana, G.; Quiroga, M.; Braun, A. *Helv. Chim. Acta* **1987**, *70*, 2073. [c] The ^{3}MLCT state is rather basic[13b] so that it starts being protonated at pH 7 (0.1 M phosphate buffer). This results in short luminescence lifetimes and makes the study of quenching by GMP rather difficult. Therefore the measurements are performed at pH 9 (0.1 M phosphate buffer). [d] Crutchley, R. J.; Lever, A. B. P. *J. Am. Chem. Soc.* **1980**, *102*, 7129. [e] Reference 20.

Experimental Section

Complexes and Reagents. The syntheses of the complexes (as Cl^- salts) $Ru(hat)_2tap^{2+}$,[14] $Ru(hat)_3^{2+}$,[14] $Ru(tap)_2hat^{2+}$,[14] $Ru(tap)_3^{2+}$,[12] $Ru(tap)_2bpy^{2+}$,[13] $Ru(bpy)_2tap^{2+}$,[13] $Ru(bpy)_3^{2+}$, $Ru(hat)_2bpy^{2+}$,[14] $Ru(bpy)_2hat^{2+}$,[18] $Ru(phen)_2hat^{2+}$,[14] $Ru(bpz)_3^{2+}$ (bpz = 2,2′-bipyrazine),[19] $Ru(Me_2tap)_3^{2+}$ (Me_2tap = 2,7-dimethyl-1,4,5,8-tetraazaphenanthrene),[20] $Ru(phen)_3^{2+}$,[21] and $Ru(tap)_2phen^{2+}$ [7b] have been described previously, as well as their absorption and emission properties. The preparation and characterization of $Ru(pzth)_2(hat)^{2+}$(pzth = pyrazinothiazol; Table 1) will be presented elsewhere. The synthesis of $Ru(hat)_2(phen)^{2+}$ was carried out from $Ru(phen)(DMSO)_2Cl_2$. This compound was prepared according to a classical method, from $Ru(DMSO)_4Cl_2$ and phen[21] and recrystallized in methanol; its ^{1}H NMR spectrum showed no presence of impuritiy. It was treated afterward during a few hours under reflux with an excess of hat in aqueous solution and then purified according to a procedure described previously.[14] The compound was characterized by its ^{1}H NMR spectrum (250 MHz in DMSO-d_6; ppm; for the numbering, see Figure 1): δ 8.26 (1H, d, $P_{2,9}$, $J_{2,3}$ = 5.2 Hz),

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7.78 (1H, dd, $P_{3,8}$, $J_{3,4}$ = 8.2 Hz), 8.84 (1H, d, $P_{4,7}$), 8.40 (1H, s, $P_{5,6}$), 8.27/8.57 (1H, d, $H_{2,11}$, $J_{2,3}$ = 3.0 Hz), 9.13/9.14 (1H, d, $H_{3,10}$), 9.46 (2H, s, $H_{6,7}$). Emission spectroscopy (corrected maxima of emission; nm): 657 (H_2O, 298 K), 650 (CH_3CN, 298 K), 652 (methanol/ethanol 1:4, 298 K), 583 nm (methanol/ethanol 1:4, 77 K). Luminescence lifetimes (ns): H_2O, 580 (air), 765 (argon); CH_3CN, 610 (air), 1710 (argon). Absorption spectrum in water [λ_{max}, nm (ϵ, M^{-1} cm^{-1})]: 210 (10.2 × 10^4), 272 (10.1 × 10^4), 408 (1.94 × 10^4), 470 (1.93 × 10^4). Redox potentials (CH_3CN, V vs SCE): oxidation, +1.86, reduction, −0.66, −0.87, −1.40, −1.54. The molar absorption coefficients were determined by Ru titration by atomic emission from plasma atomisation (Spectrometric Spectrospan IV instrument); the Ru emission intensities (at 372.8 nm) of the samples were compared to those of $Ru(bpy)_3(PF_6)_2$ (ϵ at 452 nm = 14 600 l mol^{-1} cm^{-1})[22] as standard. The samples were acidified before the measurements.

High molecular weight calf thymus DNA (CT-DNA) (Sigma) solutions were purified by standard procedures[23,24] to remove histone proteins (by phenol extraction) and small nucleic acids (by dialysis).[3c] The polynucleotide phosphate concentration was determined spectrophotometrically (for CT-DNA: ϵ_{260} = 6600 M^{-1} cm^{-1} [25]). Samples were deoxygenated by bubbling the solutions with argon for at least 20 min before the measurements.

Plasmid Photolysis. Photolysis solutions were prepared in sterile Eppendorf tubes by addition of appropriate volumes of stock solutions of complex and plasmid DNA in order to get a final volume of 10 μL and a plasmid DNA concentration (in phosphate equivalents) of 3.6 × 10^{-5} M. The samples were irradiated with 436 nm light (isolated from a PTI 200W Hg−Xe lamp source with $NaNO_2$ and $Cu(NH_3)_4^{2+}$ filters). For all the photolysis experiments, the irradiation beam was focused vertically down into the Eppendorf tube containing the photolysis solution. Samples were thermostated at 6 °C during the irradiation and were stored in the dark before and after photolysis. The samples were deposited onto electophoresis gels as soon as feasible after irradiation.

Agarose Gel Electrophoresis. Photoreacted plasmid DNA samples were separated using horizontal agarose gel electrophoresis in a TBE (90 mM Tris-borate, 2 mM EDTA, pH 8.0) buffer. A 0.8% (w/v) agarose solution was prepared by dissolving 0.2 g of agarose in 400 mL of TBE buffer. The agarose was melted by boiling, and the gel was poured while warm and left to cool. Electrophoresis was carried out at ca. 5 V/cm (40 mA, 90 V) to separate covalently closed circular (ccc), open circular (oc), and linear (lin) forms of the plasmid DNA. A loading dye solution composed of sucrose (40%), xylene−cyanol (0.25%), and bromophenol (0.25%) in TBE was added to the samples to help them sink in the wells of the gel. DNA migrated less rapidly than the xylene−cyanol dye (green), with bromophenol blue (violet) moving much more rapidly. Visualization of the DNA after electrophoresis was achieved by staining the gel for 90 min with an aqueous solution of ethidium bromide (0.5 μg/mL), which fluoresces strongly when bound to DNA. The dye within the gel was illuminated with a transilluminator (Bioblock 254 UV illuminator) and the gel photographed to provide a record of the distances migrated by the various DNA fragments. The ratio of ccc to oc forms, was estimated using a Gelman ACD-18 automatic computing densitometer.

Photolyses with CT-DNA were carried out in standard 1 cm square quartz cuvettes using visible light (λ > 400 nm) from a 250 W Hg lamp (MED/Thorn EMI) filtered with a $NaNO_2$ solution to remove the UV and IR portions of the lamp emission.[26] Absorption spectral changes were followed on Pye-Unicam SP8200 or 8800 UV/vis spectrophotometers. To monitor irreversible binding of the complex to CT-DNA, dialysis experiments were carried out by transferring the solution (typically 3 mL) to a dialysis bag (Spectra/por No. 132678; molecular weight cutoff 12 000, 14 000) and dialyzing with gentle stirring against 100 mL of buffer in the dark, with three changes of buffer over a 24 h period.

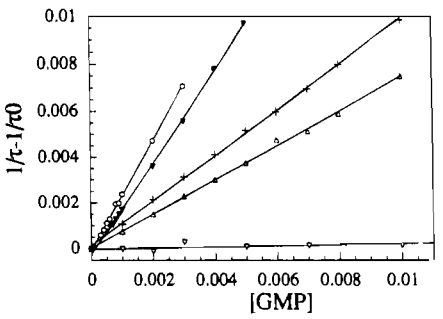

Figure 2. Stern−Volmer plots obtained from the luminescence lifetimes in the presence of GMP, in water, with a phosphate buffer 0.1 M, pH 7, and under air: ○, $Ru(hat)_2(tap)^{2+}$; ▼, $Ru(bpz)_3^{2+}$; +, $Ru(tap)_2(phen)^{2+}$; △, $Ru(tap)_2(bpy)^{2+}$; ▽, $Ru(hat)(phen)_2^{2+}$.

Emission lifetimes for the whole series of complexes were determined with a modified Applied Photophysics laser kinetic spectrometer equipped with a Hamamatsu R-928 photomultiplier tube and an excitation source composed of a frequency doubled neodymium-YAG laser (Continuum NY 61−10) coupled with a dye laser (Continuum ND60; dye, DCM, λ_{out} = 640 nm) with the mixing option (Continuum UVX), producing a 400 nm beam (10 ns pulse width, ca. maximum of 20 mJ per pulse). Fluorescence spectra and intensity measurements were recorded on a Perkin-Elmer MPF-44B fluorimeter.

Time-Resolved Absorption Spectra and Kinetic Studies. Laser flash photolysis experiments were carried out using the pulsed neodymium YAG laser mentioned above and the Applied Photophysics laser kinetic spectrometer described previously.[13] Kinetic analyses of the luminescence intensity decays were performed by nonlinear least-squares regression using Marquardt's algorithm.[27]

Pulse radiolysis was carried out with a 3 MeV van de Graaf accelarator, with a pulse width of 0.4 μs and an optical path length of 2 cm. G values of 2.8 were taken for the production of hydrated electrons and OH radicals.[7c]

Cyclic voltammetry was carried out on the apparatus described previously[14] in dried acetonitrile with $N(Bu^t)_4(PF_6)$ as supporting electrolyte.

Results

Luminescence Quenching with Mononucleotides. The complexes used in this study luminesce from their ^3MLCT[14] excited states with lifetimes of a few hundreds of nanoseconds in aqueous buffered solution (Table 1). The determination of their luminescence quenching rate constants by guanosine-5′-monophosphate (GMP) and adenosine-5′-monophosphate (AMP) was performed by the measurement of the excited state lifetimes for various quencher concentrations at room temperature in air saturated solutions. Figure 2 shows typical Stern−Volmer plots obtained for five complexes with GMP as quencher (aqueous solutions, 0.1 M phosphate buffer, pH 7, under air). In order to avoid variations of the ionic strength with increasing nucleotide concentrations, these experiments were performed in the presence of a high buffer concentration (0.1 M). The quenching rate constants for GMP and AMP are collected in Table 1, together with the respective reduction potentials of the excited complexes (E^*_{red}). As outlined in the Discussion, the trend of the k_q values with the variation of E^*_{red} indicates a quenching by electron transfer. With a E^*_{red} of ∼+1.30 V vs SCE, the quenching rate constant reaches a plateau value (k_Q = 2.2 × 10^9 M^{-1} s^{-1}) and becomes limited by diffusion. The

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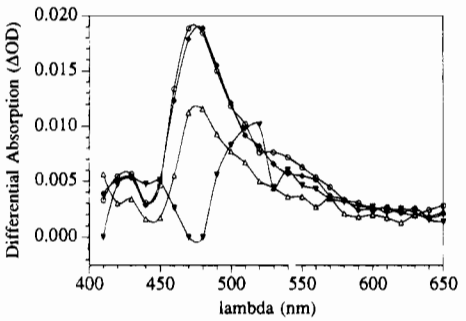

Figure 3. Differential transient absorption spectrum (pH 7, 0.1 M phosphate buffer) obtained after the laser pulse (1 μs) for the different complexes, in the presence of 10^{-2} M GMP: ♦, $Ru(tap)_2(hat)^{2+}$; ○, $Ru(tap)_3^{2+}$; △, $Ru(hat)_3^{2+}$; ▼, $Ru(hat)_2(bpy)^{2+}$.

diffusion rate depends on the ionic strength of the solution as the complex is positively charged and the mononucleotide negatively charged.[28] This is indeed clearly evidenced with one complex of the series $(Ru(tap)_2(hat)^{2+}$ with GMP) for which k_Q (in the plateau region) varies with the buffer concentration ([phosphate] = 0.01 M, $k_Q = 3.6 \times 10^9$ M^{-1} s^{-1}; [phosphate] = 0.1 M, $k_Q = 2.2 \times 10^9$ M^{-1} s^{-1}; [phosphate] = 1 M, $k_Q = 1.2 \times 10^9$ M^{-1} s^{-1}).

Transients Formed by Laser Flash Photolysis of the Complexes in the Presence of Mononucleotides. In order to show that the luminescence quenching originates indeed from a photoelectron transfer process, flash photolysis experiments with the complexes in the presence and in the absence of GMP or AMP were carried out. In the absence of nucleotide, differential transient absorptions of the ^{3}MLCT states of the complexes are observed. They exhibit absorptions at ~390 nm and depletions in the 400−450 nm region for $Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, and $Ru(tap)_2(hat)^{2+}$ and in the 408−472 nm region for $Ru(hat)_2(bpy)^{2+}$ (for the ground state absorption of these complexes see further, Figure 9). These transients decay according to monomolecular processes, with rate constants corresponding to the luminescence decays. In the presence of GMP or AMP (10^{-2} M, 0.1 M phosphate buffer, pH 7), with the most oxidizing excited complexes, the laser flash produces after ~1 μs (i.e., after complete decay of the excited state) a transient of a few hundreds of microseconds, absorbing in the 480−540 nm region. The corresponding differential transient absorption spectra recorded with $Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, $Ru(tap)_2(hat)^{2+}$, and $Ru(hat)_2(bpy)^{2+}$ in the presence of GMP are shown as examples in Figure 3, and the maxima of the differential absorption spectra for all complexes studied are collected in Table 2. No transients are observed in this long time domain for $Ru(bpy)_2(hat)^{2+}$ and $Ru(phen)_2(hat)^{2+}$ with GMP; only ^{3}MLCT states absorptions are detected on shorter time scales. This is consistent with the low extent of quenching by GMP. The dominant features characterizing the differential transient absorption spectra in the presence of GMP and AMP for the series of complexes $(Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, $Ru(tap)_2(hat)^{2+})$ are similar to those observed with $Ru(tap)_3^{2+}$ in the presence of hydroquinone; in this case the absorption at 480−500 nm induced by the laser pulse has been attributed to the presence of the reduced complex, $Ru(tap)_2(tap^{\bullet-})^{1+}$.[29] However, in the transient spectrum of $Ru(hat)_2(bpy)^{2+}$ with GMP, there is a minimum at 480−475 nm instead of a maximum. This is attributed to the ground state depletion of $Ru(hat)_2(bpy)^{2+}$ which exhibits a maximum of absorption at 472 nm (see further Figure 9b), red-shifted as compared to the maxima of absorption of the other complexes.

In order to determine the absorption spectrum and the molar extinction coefficients of some monoreduced complexes (Table 2), three complexes, $Ru(hat)_3^{2+}$, $Ru(tap)_2(hat)^{2+}$, and $Ru(tap)_3^{2+}$, were studied under pulsed radiolysis, under conditions where either solvated electrons, produced during the pulse, or the $(CH_3)_2{}^{\bullet}COH$ radicals act as the reducing agents.

It has previously been shown that $Ru(tap)_2(tap^{\bullet-})^+$ shows a strong absorption band at 485 nm which shifts to 480 nm at pH < 7, where it is protonated. Similar behavior is observed in the differential transient absorption spectra recorded 200 μs after the pulse for $Ru(hat)_3^{2+}$ and $Ru(tap)_2(hat)^{2+}$ in N_2O-saturated solution containing 0.1 M 2-propanol at pH 6 or 11. Both spectra show two maxima at ~360 and ~480 nm and a bleaching around 390 nm (Figure 4). Under these conditions, the reducing $(CH_3)_2{}^{\bullet}COH$ radical, initially formed via reactions 1 and 2, reacts with the complex according to reaction 3.

$$e_{aq}^- + N_2O + H_2O \rightarrow OH^{\bullet} + OH^- + N_2 \quad (1)$$

$$OH^{\bullet} + (CH_3)_2CHOH \rightarrow (CH_3)_2{}^{\bullet}COH + H_2O \quad (2)$$

$$(CH_3)_2{}^{\bullet}COH + [Ru(hat)_3]^{2+} \rightarrow [Ru(hat)_2(hat^{\bullet-})]^{1+} + (CH_3)_2C{=}O + H^+ \quad (3)$$

As the spectra for the reduced complexes in water obtained by pulse radiolysis are similar to the differential transient absorption spectra recorded after flash photolysis for the complexes in the presence of GMP or AMP, we may conclude that the monoreduced complexes are produced during the laser flash according to the process (for $Ru(hat)_3^{2+}$ for example):

$$[Ru(hat)_3]^{2+*} + GMP \rightarrow [Ru(hat)_2(hat^{\bullet-})]^+ + GMP^{\bullet+} \quad (4)$$

Differences, for a particular complex, between the transient absorption after the laser flash and pulsed radiolysis, may be ascribed to the absorption of oxidized GMP after the laser flash. Indeed deprotonated $GMP^{\bullet+}$ exhibits a maximum at 390 nm and a shoulder at 500 nm;[7c] this explains the positive absorption at ~400 nm in flash photolysis (Figure 3), whereas the absorption is negative in this wavelength region in pulsed radiolysis (Figure 4). Moreover, the much weaker absorption of deprotonated $GMP^{\bullet+}$ in the ~500 nm region does not distort much the spectrum around this wavelength in flash radiolysis as compared to the spectrum in pulsed radiolysis.

When the flash photolysis experiments are performed in the absence of O_2, the transient absorptions disappear in a few hundreds of microseconds according to a bimolecular equimolar process (reaction 5, for example for $Ru(hat)_2(hat^{\bullet-})^+$), as demonstrated by the linear relation between the inverse of the absorption and the time (not shown). As the absorption coefficients for the three photoreduced complexes and for the oxidized GMP (at pH 7 this will be in its deprotonated form) are known, the bimolecular rate constants k_2 (reaction 5) were determined (Table 2).

In contrast when the experiments are conducted with oxygen-saturated solutions, two decay components are observed: a fast decay during a few tens of microseconds and a slower one during a few hundreds of microseconds (not shown). The first decay originates from the reoxidation of the reduced complex by O_2 via reaction 6, and the second decay is attributed to the disappearance of the radical of the base.[7c] When a transient spectrum is recorded in this longer time scale (100 μs) under

(28) Chiorboli, C.; Indelli, M.; Rampi Scandola, M.; Scandola, F. *J. Phys. Chem.* **1988**, *92*, 156−163.

(29) (a) Masschelein, A.; Kirsch-De Mesmaeker, A. *New J. Chem.* **1987**, *11*, 329. (b) Tan-Sien-Hee, L.; Kirsch-De Mesmaeker, A. *J. Chem. Soc., Dalton Trans.* **1994**, 3651.

Table 2. Flash Photolysis and Pulse Radiolysis Data for the Different Complexes with GMP and AMP (See Text)

complexes	λ_{max}[a] (nm) GMP	AMP	$\lambda_{Ru^{1+}}$ (ϵ (M^{-1} cm^{-1}))[B1] (nm)	$\lambda_{Ru(H^+)^{1+}}$ (ϵ(M^{-1} cm^{-1}))[B2] (nm)	$k_2 \times 10^{-8}$ (GMP, pH 7) (M^{-1} s^{-1})
$Ru(hat)_3^{2+}$	480[a]	480[a]	480 (7.8×10^3)	490 (8.5×10^3)	9.3
$Ru(hat)_2(tap)^{2+}$	480[a]	470[a]			
$Ru(tap)_2(hat)^{2+}$	480[a]	470[a]	480 (7.8×10^3)	490 (8.5×10^3)	4.3
$Ru(tap)_3^{2+}$	475[a]	480[a]	480 (8.5×10^3)	485 (12×10^3)	12
$Ru(hat)_2(bpy)^{2+}$	520[a]				
$Ru(hat)(tap)(bpy)^{2+}$	510[b]				
$Ru(tap)_2(bpy)^{2+}$	505[b]				
$Ru(tap)_2(phen)^{2+}$	500[b]				

[a] Maxima of the transient absorption spectra obtained by laser flash photolysis in the presence of GMP or AMP: complexes 10^{-4} M, argon, GMP or AMP 0.01 M (*a*, phosphate buffer, 0.1 M, pH 7; b, phosphate buffer, pH 9−10). [b] Pulsed radiolysis data obtained at pH values where the reduced complexes are not protonated (*B1*, pH 11, Ru^{1+}) and protonated (*B2*, pH 6, $Ru(H^+)^{2+}$); k_2 is calculated at 475 nm using $\epsilon_{GMP^{\bullet+}} = 1500$ M^{-1} cm^{-1},[46] $\epsilon_{AMP^{\bullet+}} = 1000$ M^{-1} cm^{-1} (Vieira, A.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986).

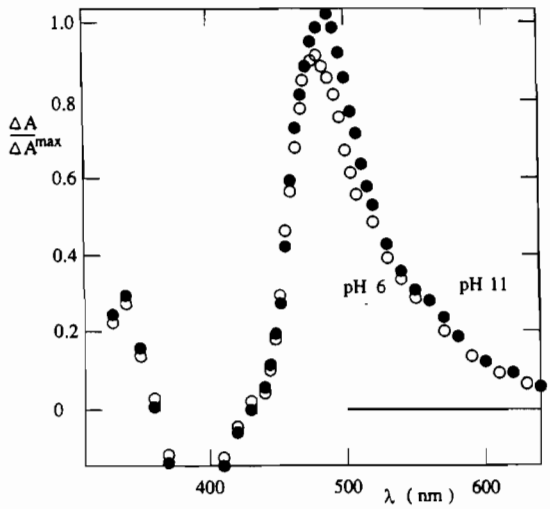

Figure 4. Differential transient absorption spectrum obtained 0.2 ms after the pulsed radiolysis for $Ru(hat)_3^{2+}$ (20 μM), (●) at pH 11 and (○) at pH 6, in N_2O-saturated aqueous solution in the presence of 2-propanol (0.1 M).

oxygen, it corresponds to the absorption of $GMP^{\bullet+}$.

$$[Ru(hat)_2(hat^{\bullet-})]^{1+} + \text{"}GMP^{\bullet+}\text{"} \rightarrow [Ru(hat)_3]^{2+} + GMP \quad (5)$$

$$[Ru(hat)_2(hat^{\bullet-})]^{1+} + O_2 \rightarrow [Ru(hat)_3]^{2+} + O_2^{\bullet-} \quad (6)$$

These experiments indicate that for each complex of this study, and when the corresponding excited state is sufficiently oxidizing (see Table 2), an electron transfer occurs from the nucleotide to the excited compound, leading to the formation of the corresponding reduced complex and radical cation of the base, the latter subsequently deprotonating at the pHs used.

With the absorption coefficient ϵ of the monoreduced form determined above, the quantum yield of formation of the reduced complex ($\phi_{Ru^{1+}}$) can be measured; $\phi_{Ru^{1+}}$ corresponds to the ratio between the number of reduced complex observed in solution after the laser pulse and the number of excited ^{3}MLCT states quenched by the mononucleotide. If it is assumed, as is the case for $Ru(bpy)_3^{2+}$, that the quantum yield of conversion of the ^{1}MLCT to the ^{3}MLCT is unity, $\phi_{Ru^{1+}}$ for 100% quenching can be calculated. This corresponds to the efficiency of cage escape from the ion pair. The ^{3}MLCT excited state of $Ru(bpy)_3^{2+}$ was chosen as the reference transient to determine the laser excitation intensity, as its quantum yield and its absorption coefficient are known.[30,31] If the absorbance of the

Table 3. Quantum Yield of Cage Escape of the Reduced Complex from the Ion Pair Formed by Electron Transfer from GMP or AMP to the Excited Complex (Argon Solution, pH 7, 0.1 M Phosphate Buffer)[a]

complex	$\phi_{Ru^{1+}}$ GMP	AMP
$Ru(tap)_3^{2+}$	0.30	0.28
$Ru(tap)_2(hat)^{2+}$	0.35	0.32
$Ru(hat)_3^{2+}$	0.23	0.20

[a] For the calculation of the concentration of reduced complex, we have used $[Ru^{1+}] = \Delta OD/\Delta\epsilon$ with $\Delta\epsilon = \epsilon_{Ru^{1+}} + \epsilon_{GMP^{\bullet+}} - \epsilon_{Ru^{2+}}$ (determined at 475 nm), $\epsilon^{475}_{GMP^{\bullet+}} = 1500$ M^{-1} cm^{-1},[43] $\epsilon^{475}_{AMP^{\bullet+}} = 1000$ M^{-1} cm^{-1} (Vieira, A.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986), $\epsilon_{Ru^{1+}}$ and $\epsilon_{Ru^{2+}}$ (see Table 2 and ref 14).

examined transient is ΔA_x and the absorbance of the reference transient ΔA_a, and if the same percentage of the exciting light is absorbed by the two systems, the $\phi_{Ru^{1+}}$ quantum yield[32] is given by

$$\phi_x = \epsilon_a(\Delta A_x \phi_a / \Delta A_a \epsilon_x)$$

where ΔA_x and ΔA_a are calculated for 100% quenching.

The values determined for the cage escape efficiency measured for three complexes for which we know the ϵ of the reduced species are reported in Table 3.

Absorption and Steady State Emission of the Complexes in the Presence of DNA. The absorption spectra of the series of racemic Ruthenium(II) complexes, the homoleptic and the heteroleptic compounds, were recorded in the presence of CT-DNA at a P/Ru ratio ([DNA phosphate]/[Ru]) of 50 in 10 mM phosphate buffer at pH 7. The effect of binding to DNA on the visible absorption spectra corresponds to a slight to moderate hypochromicity of the visible band accompanied by a widening of the band to longer wavelengths; examples are given in Figure 5. For $Ru(Me_2tap)_3^{2+}$, however, the absorption spectrum is essentially insensitive to CT-DNA (Figure 5D).

The emission spectra of the Ru complexes are not markedly affected in shape or position of the band maximum in the presence of CT-DNA (a small red shift of the maximum as well as a decrease in the band width at half-height can be observed in the presence of DNA). The amplitude of the emission on the other hand is considerably altered (either markedly enhanced or quenched) by increasing the DNA concentration (increase in the P/Ru ratio), as illustrated by the series $Ru(bpy)_m(tap)_{3-m}^{2+}$ (Figure 6a) and $Ru(bpy)_n(hat)_{3-n}^{2+}$ (Figure 6b) ($m = n = 0$, 1, 2, 3). Here two types of behavior are observed for both series:

(30) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belser, P.; von Zelewski, A. *Coord. Chem. Rev.* **1988**, *84*, 85.
(31) Yoshimura, A.; Hoffman, M. Z.; Sun, H. *J. Photochem. Photobiol., A: Chem.* **1993**, *70*, 29.
(32) Eaton, D. *Pure Appl. Chem.* **1991**, *63*, 290.

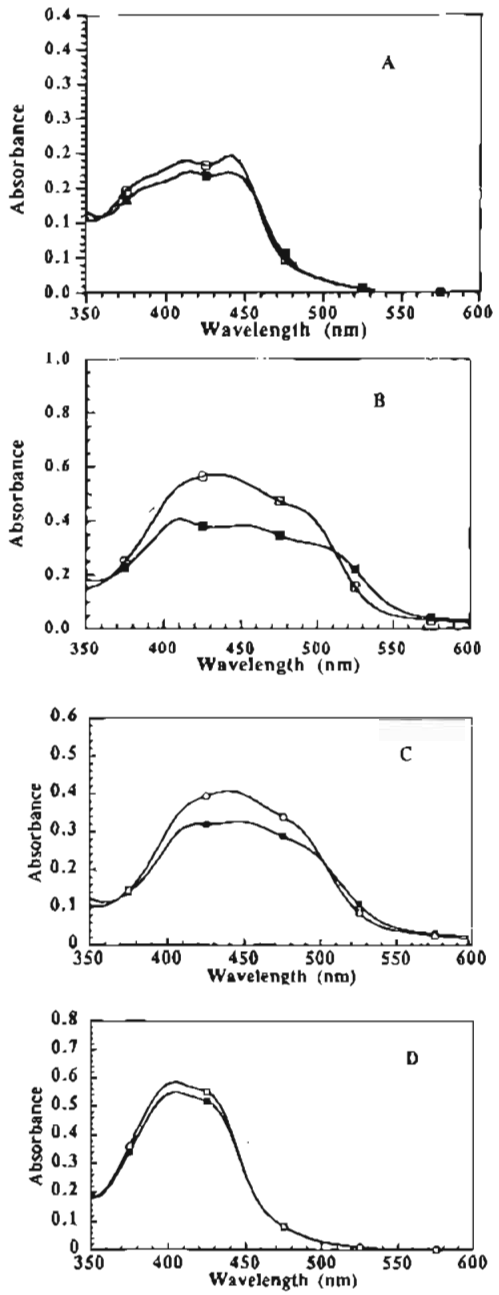

Figure 5. Changes in the visible absorption spectra of Ru(II) complexes with the addition of calf thymus DNA (□) without DNA and (■) with DNA [[P]/[Ru] = 50, 10 mM phosphate buffer, pH 7]: (a) $Ru(hat)_3^{2+}$, (b) $Ru(hat)(bpy)_2^{2+}$, (c) $Ru(tap)(bpy)_2^{2+}$, and (d) $Ru(Me_2tap)_3^{2+}$.

when $m = n = 2$, there is an emission enhancement, whereas when $m = n = 0$, 1 the emission is quenched. This can be rationalized by the occurrence of two different processes: (i) in the case of luminescence enhancement ($m = n = 2$), the complexes are protected by the double helix and the efficiency of the nonradiative deactivation processes of the excited states decreases;[5c] (ii) when $m = n = 0$, 1, the luminescent excited states are quenched by guanine-containing DNA,[5a,15] consistent with the photoelectron transfer process demonstrated above. In contrast to those cases, DNA has little effect on the luminescence intensity of $Ru(Me_2tap)_3^{2+}$ (Figure 6a).

Determination of Sensitized Photocleavage of Plasmid pBR 322. The series of complexes were screened for their effectiveness to photosensitize frank single strand breaks in DNA using 436 nm light by measuring the conversion of the ccc form of plasmid DNA to its oc form, as a function of time. Figure 7 illustrates the rate of appearance of the oc form for

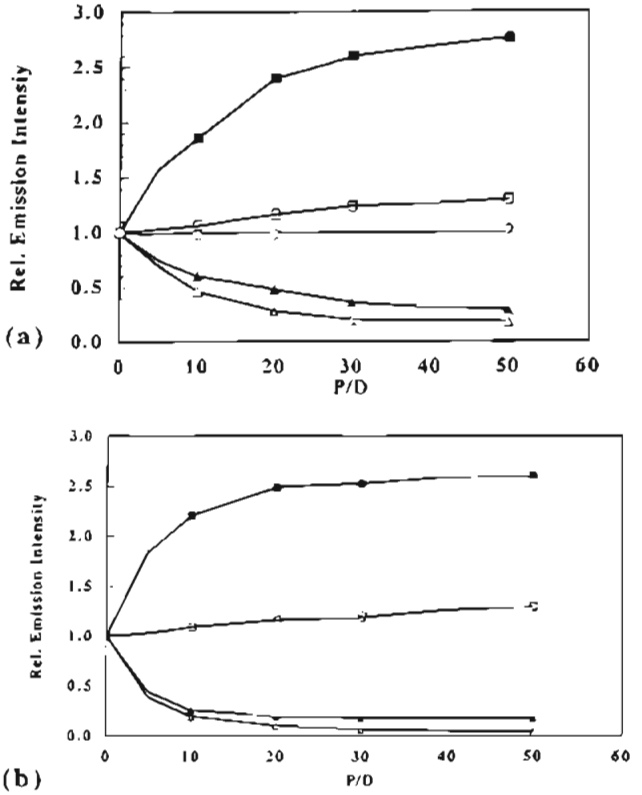

Figure 6. Variation of emission intensity with calf thymus DNA concentration, [Ru] = 1 × 10^{-6} M (P/D = phosphate/Ru): for (a) $Ru(bpy)_m(tap)_{3-m}^{2+}$ and for (b) $Ru(bpy)_n(hat)_{3-n}^{2+}$ in 3 mM phosphate buffer; for a and b, when m or n = 0 (△), 1 (▲), 2 (■), and 3 (□); for a (○), $Ru(Me_2tap)_3^{2+}$.

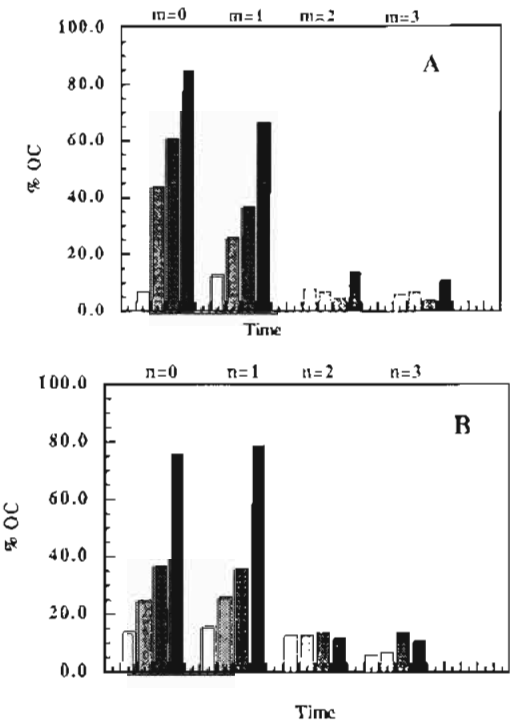

Figure 7. Estimation of % oc formed on visible irradiation of pBR322 in the presence of (A) $Ru(bpy)_m(tap)_{3-m}^{2+}$ and (B) $Ru(bpy)_n(hat)_{3-n}^{2+}$ (P/D = 10 in 10 mM phosphate buffer pH 7), where $m = n = 0$, 1, 2, and 3 and with (open bars) 0, (light hatched bars) 0.5, (dark hatched bars) 1, and (filled bar) 3 min of irradiation at 436 nm.

the various complexes, which is accompanied by the decrease of the ccc form. No linear DNA was detected under the conditions of short exposure time used. It may be observed

that, for both tap and hat complexes, $Ru(L)_3^{2+}$ and $Ru(L)_2(bpy)^{2+}$ are much more efficient photosensitisers than $Ru(bpy)_2L^{2+}$ (with L = tap or hat). It may thus be remarked that those complexes whose emission is quenched upon binding to DNA are the more efficient DNA cleaving agents. In agreement with this $Ru(tap)_2(hat)^{2+}$ and $Ru(tap)(hat)_2^{2+}$, whose luminescence is quenched by GMP (Table 1) and DNA, are also effective in converting the plasmid from its ccc form into its oc form. A more detailed examination of Figure 7, however, reveals that the relative effectiveness of these cleavers [$Ru(tap)_3^{2+}$ > $Ru(tap)_2(bpy)^{2+}$ > $Ru(hat)_2(bpy)^{2+}$ > $Ru(hat)_3^{2+}$] does not directly follow the percentage of quenching by GMP or DNA [$Ru(hat)_3^{2+}$ > $Ru(tap)_3^{2+}$ > $Ru(hat)_2(bpy)^{2+}$ > $Ru(tap)_2(bpy)^{2+}$].

Spectroscopic Monitoring of Photoproduct Formed with CT-DNA. As we have shown previously for $Ru(tap)_3^{2+}$,[16] UV/vis absorption measurements allow easy monitoring of the formation of photoproducts with CT-DNA under visible illumination of the complexes. Dialysis experiments also lead to conclusions concerning the type of photoproducts: if the products correspond to photoadducts covalently bound to the DNA, they will be retained in the dialysis bag, whereas otherwise they may pass through the membrane. Steady state illuminations were thus carried out with the complexes alone and in the presence of CT-DNA, and both samples subsequently dialyzed.

Complexes in the Absence of DNA. The tap and hat Ru(II) complexes undergo photosubstitution in aqueous and organic solvents by losing one of their chelating ligands.[13-14] It was shown previously that the quantum yield of this process increases with the increasing number of tap or hat in the complex.[13-14] In typical steady state illuminations in the absence of DNA the complexes containing two or three tap or hat ligands exhibit a bleaching of the ≈400 nm absorption band on irradiation and the occurrence of a new band at ~500 nm (Figure 8A,B as examples). Absorption in this wavelength region is expected for $Ru(L)_2XY^{n+}$, where X and Y are H_2O, Cl^-, or phosphate ions, and is typical for photodechelation in the absence of DNA.

Complexes in the Presence of CT-DNA. Changes of the visible absorption spectra after similar irradiation (λ > 400 nm) of the different complexes in the presence of CT-DNA (P/Ru = 50) are displayed in Figure 9 (The high P/Ru ratio was used in order to minimize the amount of unbound complex).

Figure 9 shows the absorption spectra of the complexes before illumination (○) and after irradiation (□). The spectra after illumination *and* dialysis (■) have also been plotted, together with the spectra without irradiation *and* after dialysis (●). With $Ru(tap)_3^{2+}$ for example (Figure 9A), it is observed that after illumination (□) the absorption has increased, especially around 400 nm, and remains approximately the same after dialysis of the irradiated sample (■). This indicates that the photoproduct is irreversibly attached to the CT-DNA. In contrast, for the sample which has not been illuminated (○, no photoproduct formed), but which has been dialyzed, the absorption almost disappears after the dialysis treatment (●).

From Figure 9, for all the complexes treated under these conditions, two different behaviors are again clearly distinguished for the series $Ru(bpy)_m(tap)_{3-m}^{2+}$ (Figure 9A–C). For $Ru(tap)_3^{2+}$ and $Ru(tap)_2(bpy)^{2+}$ a clear hyperchromic effect is evidenced upon irradiation (Figure 9A,B), with the maximum shifting slightly to shorter wavelengths. The new absorption band maintains the same shape even after longer illumination times. The lack of an increasing absorption at 500 nm indicates that the dechelation process is inhibited in the presence of CT-DNA. By contrast the absorption spectrum of $Ru(bpy)_2(tap)^{2+}$ is unaffected by photolysis in the presence of DNA (Figure 9C). For the series of hat complexes, similar effects of irradiation with DNA are observed with $Ru(hat)_3^{2+}$ and $Ru(hat)_2(bpy)^{2+}$ (Figure 9D,E), although $Ru(hat)_3^{2+}$ leads to a less important hyperchromic effect and some decomposition is detected by the occurrence of a weak absorption in the 500 nm region (Figure 9D). No photoreaction is found for $Ru(bpy)_2(hat)^{2+}$(Figure 9F).

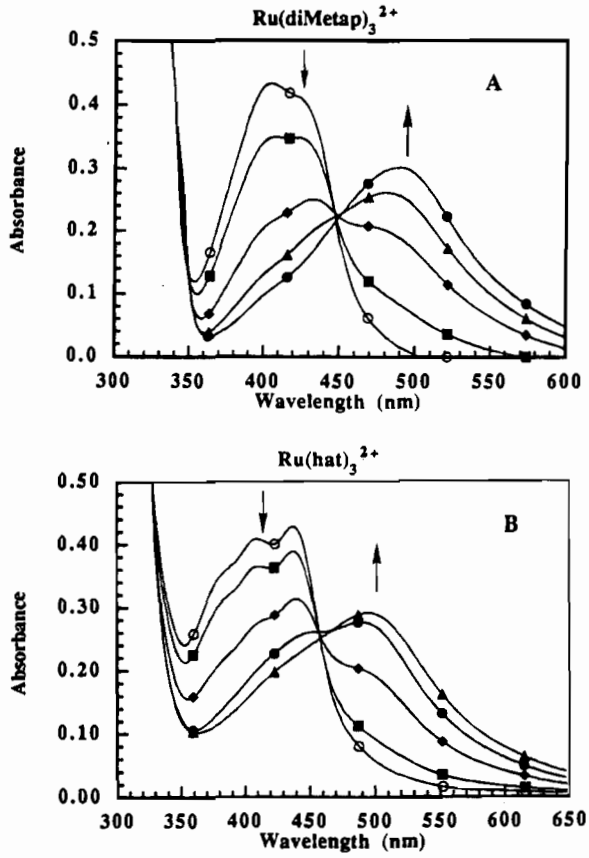

Figure 8. Absorption spectra of Ru(II) complexes following visible light irradiation in the absence of DNA: (A) for $Ru(Me_2tap)_3^{2+}$, (○) without irradiation, (■) after 10 min irradiation, (♦) after 30 min irradiation, (▲) after 60 min irradiation, and (●) after 120 min irradiation, 10 mM phosphate buffer, pH 7; (B) for $Ru(hat)_3^{2+}$, (○) without irradiation, (■) after 30 s irradiation, (♦) after 2 min irradiation, (●) after 5 min irradiation, and (▲) after 60 min irradiation.

Dialysis performed with unirradiated mixtures of the tap complexes and DNA caused the removal of the metal complex (Figure 9A–C). However the absorption is only reduced by 10–20% for $Ru(tap)_3^{2+}$ or $Ru(tap)_2(bpy)^{2+}$ after visible light irradiation in the presence of CT-DNA (Figure 9A,B), showing that most of the photoproducts are retained on the DNA in the dialysis bag, as would be expected for complexes covalently bound to the polynucleotide. By contrast, even after irradiation in the presence of DNA, $Ru(bpy)_2(tap)^{2+}$ is readily removed by dialysis, consistent with the inability of this complex to form photoadducts. Similar behavior is found for the hat series, although, as the hat complexes are more strongly noncovalently attached to DNA than their tap analogues, single pass dialysis is less effective at removing them from the DNA (e.g., compare Figure 9C,F). Complexes $Ru(bpy)(tap)(hat)^{2+}$ and $Ru(tap)_2(hat)^{2+}$ (data not shown) also exhibit the occurrence of a new absorbing species anchored to the DNA after irradiation.

Thus, these experiments show that only those tap and hat complexes capable of oxidizing guanine (with luminescence quenching) can form covalent adducts with DNA. $Ru(Me_2tap)_3^{2+}$ (Figure 9G) is a special case and behaves differently

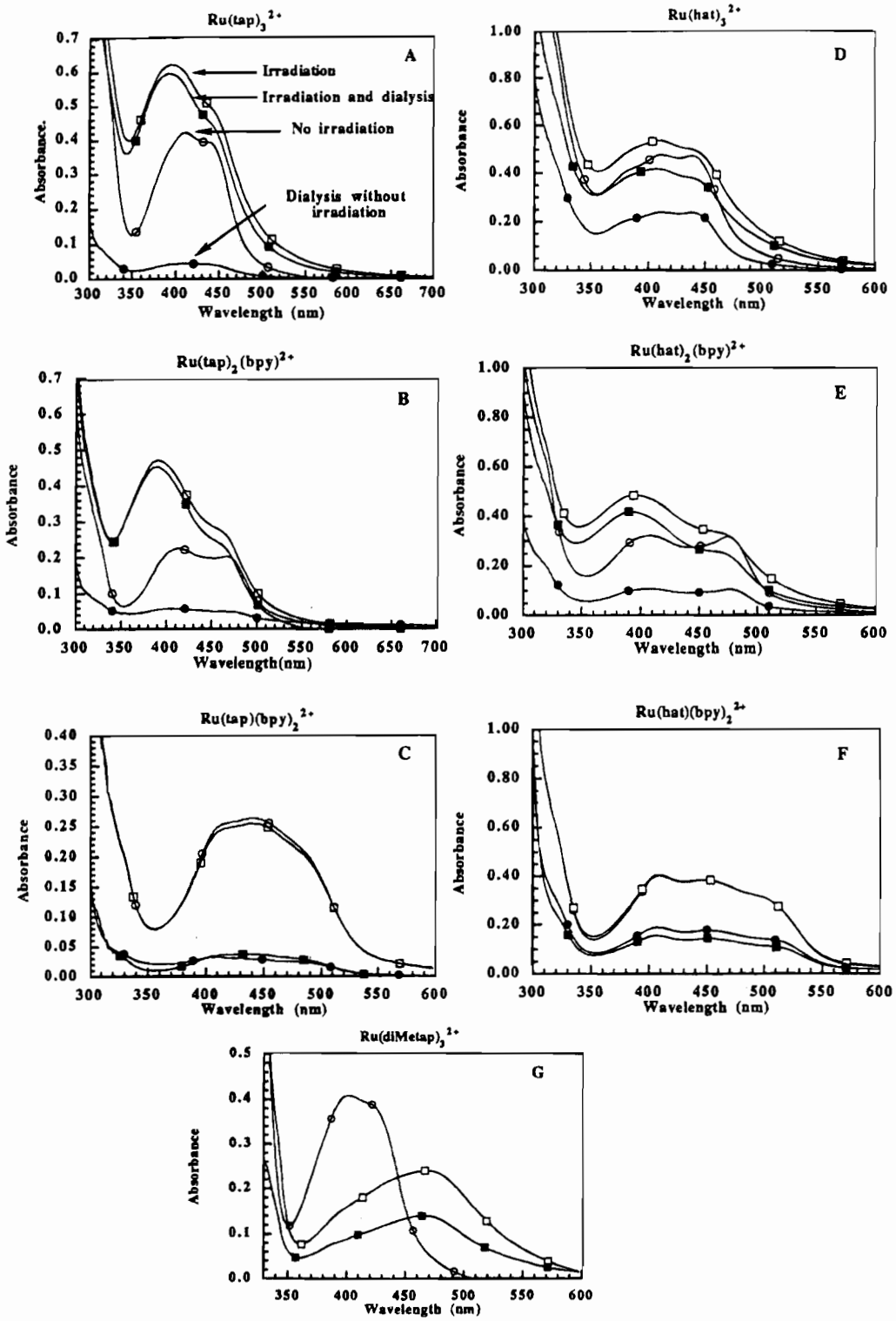

Figure 9. Changes in the absorption spectra with calf thymus DNA ([P]/[Ru] = 50 in 10 mM phosphate buffer, pH 7) of Ru(II) complexes (A) $Ru(tap)_3^{2+}$, (B) $Ru(tap)_2(bpy)^{2+}$, (C) $Ru(tap)(bpy)_2^{2+}$, (D) $Ru(hat)_3^{2+}$, (E) $Ru(hat)_2(bpy)^{2+}$, (F) $Ru(hat)(bpy)_2^{2+}$, and (G) $Ru(Me_2tap)_3^{2+}$ following 120 min visible light irradiation (□) and subsequent dialysis (■), compared with unirradiated samples dialyzed (●) and not dialysed (○).

than the above complexes: no photoadduct formation is observed, and photodechelation is rather important, although the luminescence of this complex is quenched by GMP (Table 1) but not by DNA (Figure 6). This case will be discussed further.

Discussion

Complexes in the Presence of Mononucleotides. (a) Luminescence Quenching. Ru(II) complexes containing heterocyclic π-deficient ligands such as tap and hat with low-lying unoccupied molecular orbitals (LUMOs) are strongly oxidizing in their ^{3}MLCT excited state,[14] with the excited state oxidizing potential increasing with the number of tap or hat ligands coordinated to the ruthenium (Table 1). The emission of many of these complexes is quenched by GMP, and as shown in Figure 10, the rate constant depends clearly on the E_{Ru^{2+*}/Ru^+} value for the particular complex, the rate constant reaching a plateau value which is determined by the diffusional rate

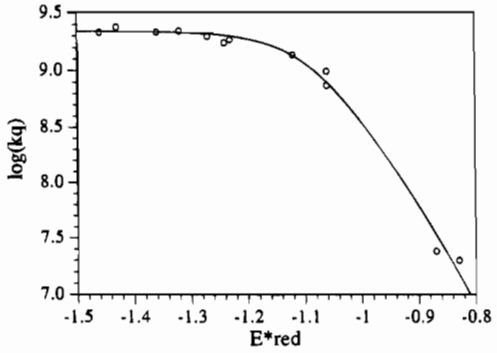

Figure 10. Plot of $\log(k_q)$, k_q = luminescence quenching rate constant, vs the calculated reduction potentials of the excited complexes of Table 1.

constant (k_d). This is strong evidence for electron transfer from the nucleotide to the metal complex excited state, and this proposal is validated by the laser flash photolysis experiments which indicate the formation of the monoreduced metal complex (see below).

Data such as that in Figure 10 can be analyzed using the Marcus eq 7,[33-36] where $\Delta G^{\ddagger}$ (the free energy of activation of the electron transfer step) is related to the free energy of the electron transfer process ΔG° and $\Delta G^{\ddagger}(0)$ (the intrinsic barrier)

$$\Delta G^{\ddagger} = \Delta G^{\ddagger}(0)[1 + (\Delta G^{0}/4\Delta G^{\ddagger}(0))]^{2} \qquad (7)$$

ΔG° (in kcal mol^{-1}) can be estimated by the Weller equation (ignoring the electrostatic work terms)[37,38]

$$\Delta G^{\circ} = 23.06(E^{\circ}_{G^{\bullet+}/G} - E^{\circ}_{Ru^{2+}/Ru^{+}} - \Delta E_{00}) \qquad (8)$$

where $E_{G^{\bullet+}/G}$ is for the oxidation of guanine in GMP and ΔE_{00} the energy of the 0−0 transition of the sensitizer (replaced in the present calculation by $\Delta E_{\lambda_{max}}$). This analysis can therefore be used to determine the oxidation potential of the nucleotide.

For the simplified kinetic scheme

$$[RuL_3^{2+*}...Q] \rightarrow RuL_3^{1+} + Q^{\bullet+} \quad (k_{el})$$

the quenching rate constant k_Q by electron transfer is given by

$$k_Q = k_d/(1 + k_d/k_{el}K_d) \qquad (9)$$

where $K_d = k_d/k_{-d}$ corresponds to the diffusional equilibrium and k_{el} is given by

$$k_{el} = \nu_n e^{-\Delta G^{\ddagger}RT} \qquad (10)$$

where ν_n is the frequency factor for a barrierless transfer and K, the transmission coefficient, is considered as equal to 1.

For the same family of electron acceptors (the excited complexes), in the presence of the same electron donor (GMP), k_d, k_{-d}, K_d, ν_n, and $\Delta G^{\ddagger}(0)$ may be considered as constants within the series, so that, under those conditions, k_{el} becomes simply a function of ΔG°. k_d has been chosen as equal to the plateau value of the quenching rate constant, i.e., 2.2×10^9 M^{-1} s^{-1}, and a value of 6×10^{12} s^{-1} [38,39] for ν_n has been used. The diffusion equilibrium constant K_d [40] has been assumed to be the same as the one in the ground state (20 M^{-1})

$$Q + RuL_3^{2+} \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} [RuL_3^{2+}...Q]$$

calculated from the fitting of the experimental curve $I_0/I = f$(GMP), obtained for $Ru(tap)_3^{2+}$, to the function $I_0/I = 1 + (K_{sv} + K_d)$[GMP] + $(K_{SV}K_d)$[GMP]^2.[41]

With those values for the different constants, the fitting of the experimental data to the Marcus equation has furnished a value for $\Delta G^{\ddagger}$ (0) of 7.5 kcal.mol^{-1} (≈31 kJ mol^{-1}) and a value of +0.92 V (vs SCE) [1.16 V (vs NHE)] for $E^{\circ}_{G^{\bullet+}/G}$(GMP). To the best of our knowledge, this is the first time this method has been used for determination of the oxidation potential of mononucleotides.

The oxidation potential of GMP so obtained may be compared to that obtained for guanine derivatives by other methods, including direct electrochemical measurements, mediated electrocatalysis, and pulse radiolysis. The values estimated by the earlier electrochemical methods are uncertain because of the irreversible nature of the guanine oxidation.[42,43] A more recent cyclic voltammetric study of the electrochemical oxidation of guanosine at a pyrolytic graphite electrode indicated that the oxidation led to 8-hydroxyguanosine and various di- and trinucleosides. The primary reaction appeared to involve a one-electron, one-proton reaction, leading to a deprotonated guanine radical.[44] The initial oxidation peak is found to occur at [1.16 V, at pH 0.05] (vs SCE)]—i.e., at 0.82 V vs SCE at pH 7. Another approach to the estimation of the one-electron oxidation potential of nucleotides is that based on a determination of the equilibrium between the nucleotides and oxidized species generated by pulse radiolysis methods.[45] Even so, the data is not simple to interpret because of the need to work at high pH so as to obtain sufficiently rapid electron transfer and the need therefore to take account of deprotonation of the nucleobases and of their radical cations to determine the values at pH 7.[46] A recent reestimation leads to a lower limit value of 1.15 V (vs NHE) for the one-electron, one-proton oxidation of guanosine to its deprotonated radical cation[47] and therefore to a value of $E^{\circ}_{G^{\bullet+}/G}$ of 1.33 V (vs NHE). Electrocatalytic studies using rhenium(V) complexes to oxidize guanine in DNA at neutral pH show that the oxidation potential for guanine (under these conditions) is between 0.90 and 1.00 V vs Ag/AgCl.[48] The value obtained at pH 7 by the electron transfer quenching method reported here (0.92 V vs SCE or 1.16 V vs NHE) is therefore well within the range of the values reported by these other methods.

Only the most oxidizing excited states are quenched by AMP, and our data therefore do not allow a full analysis such as that carried out for GMP. It is apparent, however, that the curves

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are shifted by about 400 mV higher than GMP. If the reorganization energy were approximately the same for both nucleotides, this would indicate that the oxidation potential of AMP would be more positive by about 400 mV vs GMP (i.e., ca. 1.32 V vs SCE; 1.56 V vs NHE).

The value of $\Delta G^{\ddagger}(0)$ can be used to determine the intrinsic barrier for the electron self-exchange in the couple GMP/GMP$^{\bullet+}$ as

$$\Delta G^{\ddagger}(0) = 0.5[\Delta G^{\ddagger}(0)_{GMP^{\bullet+}/GMP} + \Delta G^{\ddagger}(0)_{Ru^{2+*}/Ru^{+}}]$$

where $\Delta G^{\ddagger}(0)_{GMP^{\bullet+}/GMP}$ and $\Delta G^{\ddagger}(0)_{Ru^{2+*}/Ru^{+}}$ correspond to the intrinsic barriers for the electron self-exchange in the couples GMP$^{\bullet+}$/GMP and $RuL_3^{2+*}/[RuL_2L^{\bullet-}]^{+}$, respectively. It can be assumed[49] that the inner sphere term for the $RuL_3^{2+*}/[RuL_2L^{\bullet-}]^{+}$ couples is very small (because the distorsions are spread over many bonds) compared to the solvent reorganization energy. This latter is given in the literature for the couples RuL_3^{2+*}/RuL_3^{3+} [50] (4.2 kcal mol^{-1}) and has been employed for the couples $RuL_3^{2+*}/[RuL_2L^{\bullet-}]^{+}$.[35] Using this value and our determined value for $\Delta G^{\ddagger}(0)$ of 7.5 kcal mol^{-1} yields $\Delta G^{\ddagger}(0)_{GMP^{\bullet+}/GMP} = 10.8$ kcal mol^{-1}. This relatively high value of activation energy probably indicates that there are contributions from both the outer and inner sphere reorganization energies during the electron exchange in this couple.

(b) Flash Photolysis. The differential transient absorption spectra obtained by laser flash photolysis in the presence of the two nucleotides, GMP and AMP, are similar to the spectra obtained independently by reduction electrolysis of the complexes in their first reduction wave[29] or by reduction under pulsed radiolysis conditions and modified by the contribution of the transient absorption of the deprotonated cation radical of guanine GMP(H^{+})$^{\bullet}$ as mentioned above. As the absorption coefficient at 480 nm of this deprotonated radical cation is much weaker than that of the reduced complex,[46] this explains the global resemblance of the resulting total transient absorption in flash photolysis to the spectrum of the corresponding reduced complex. By analogy with the spectroscopic data of other reduced polypyridyl complexes based on the bpy, phen, bpz, or bipym (bipyrimidine) ligands,[51-56] the absorption bands of the reduced transients can be attributed to a mixture of MLCT transitions and $\pi-\pi^*$ transitions centered on the reduced ligand. More particularly for the reduced tap$^{\bullet-}$ and hat$^{\bullet-}$ [29b] the first $\pi-\pi^*$ absorption occurs in the 500 nm region, in accord with the flash photolysis results.

In the absence of oxygen, the reduced complex disappears by reoxidation by the oxidized guanine, according to a bimolecular process (Table 2, k_2, reaction 5). k_2 is close to the diffusion controlled value for the reduced form of $Ru(tap)_3^{2+}$, which should indeed be more easily reoxidized by the deprotonated GMP$^{\bullet+}$ than the reduced $Ru(tap)_2(hat)^{2+}$ or $Ru(hat)_3^{2+}$ (see k_2 values in Table 2).

In the presence of oxygen, the escaped reduced complex is reoxidized (reaction 6), producing the initial complex and presumably the radical anion $O_2^{\bullet-}$.

In conclusion, the rate constants of luminescence quenching by GMP and AMP and the laser flash photolysis results clearly demonstrate the presence of a photoinduced electron transfer from GMP and to a lesser extent from AMP to the excited state of the most oxidizing complexes in accord with the fact that the purine bases are more easily oxidized than the pyrimidine bases and guanine more easily than adenine.[57,58]

Interactions of the Complexes with DNA. Absorption changes in the visible MLCT bands and variations of the emission intensity of the complexes with the addition of CT-DNA indicate that most of the compounds bind to some extent to DNA. Whether they bind by surface binding or intercalation cannot be concluded solely from these spectroscopic data[1-3,5] so that we have to limit the discussion to general features which can be concluded only from the absorption and luminescence data.

The absorption spectra of the homoleptic complexes show only slight changes upon addition of DNA (Figure 5A), while the heteroleptic complexes of the type $Ru(bpy)(tap)(hat)^{2+}$ or $Ru(bpy/phen)_2(hat/tap)^{2+}$ show much more important changes (Figure 5B,C). $Ru(bpy)_2hat^{2+}$, for example, exhibits a 34% hypochromicity and a 17 nm red shift (Figure 5B).[11c] This might indicate intercalation of the hat ligand between the stacking of bases, as this could be expected for a ligand with such an extended aromaticity. Intercalation was tested in this case by enhancement of viscosity of CT-DNA in the presence of $Ru(bpy)_2(hat)^{2+}$.[59] However, the marked preference for binding to poly(d[A−T]),poly(d[A−T]) rather than to poly(d[G−C]), poly(d[G−C]) contrasts with the behavior of $Ru(phen/bpy)_2(dppz)^{2+}$(dppz = dipyridophenazine) for which Barton et al.[11a,b] and Norden et al.[60] suggested that intercalation is the main binding mode of this dppz ligand.

The luminescence data mentioned above can be divided into two categories: an enhancement of luminescence and an emission quenching in the presence of polynucleotide. The origin of luminescence increase by interaction with polynucleotides, with tap and hat complexes, has been examined in detail[5c] previously, in a study of luminescence lifetimes as a function of temperature in the absence and presence of polynucleotide and oxygen. $Ru(phen)_3^{2+}$ was used for comparison. It was demonstrated that the most important effect of DNA on the photophysics of $Ru(phen)_3^{2+}$ is the protection of its excited ^{3}MLCT state versus the oxygen quenching by the DNA double helix, which increases the excited state lifetime. In contrast, an oxygen effect was not found for tap and hat complexes; the increase of luminescence lifetime is due in that case to the DNA microenvironment on the deactivation rate constants controlling the photophysics. Thus, oxygen does not play a role versus the excited tap and hat complexes on DNA, at least at the level of the primary processes of the excited states.

$Ru(Me_2tap)_3^{2+}$ represents a special case as neither the absorption (Figure 5D) nor the emission (Figure 6a) is modified significantly by DNA. In this case, although this excited complex is quenched by GMP (Table 1), the absence of luminescence quenching by CT-DNA is attributed to a poor

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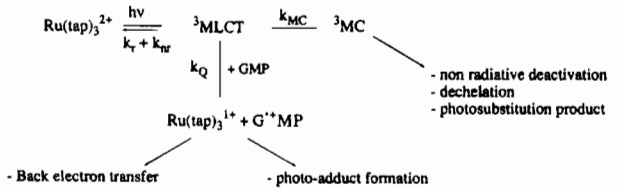

Figure 11. Schematic representation of the primary photochemical steps for $Ru(tap)_3^{2+}$.[13,14]

interaction with CT-DNA, due to the steric hindrances of the methyl groups. Presumably a direct access of the complex to the DNA bases is necessary for efficient quenching.

Photosensitized Reactions of DNA. The luminescence quenching by CT-DNA for the oxidizing complexes (E_{red}* > 1.1 V) originates from the oxidation of guanines. This quenching is mainly static as it leads to excited state lifetimes shorter than a few nanoseconds.[5a,b] The quenching process by the bulk oxygen cannot compete efficiently with the static quenching by the DNA bases, and therefore, the reactions of DNA photosensitized by such complexes are unlikely to originate from $^1O_2$* produced by energy transfer from the excited complexes. Rather we propose that they originate from the direct photoelectron transfer from the DNA bases to the excited metal complex.

The results show quite clearly that the occurrence of the luminescence quenching and electron transfer process is in all cases correlated with two different photosensitized DNA reactions: DNA strand cleavages and formation of photoadducts.

(a) Cleavage. The yield of single strand breaks is indeed strongly influenced by the number of tap or hat ligands in the complex (Figure 7) or, in other words, by the reduction potential of the excited complexes. Thus, when the oxidation power of the excited complex is not sufficient to induce a photoelectron transfer (as with $Ru(bpy/phen)_2(tap/hat)^{2+}$), the photocleavage efficiency is low. In contrast, those complexes which photooxidize guanines lead to much more efficient photocleavage. It is probable that the radical cation of guanine, $G^{\bullet+}$, produced by oxidation by the excited complex, undergoes a series of reactions similar to those reported for high-intensity irradiations of DNA,[61] leading to strand breaks as final steps. However, there is no simple correlation between the oxidizing power of the metal complex and its ability to cleave DNA. Thus, although $Ru(hat)_3^{2+}$ luminescence is very efficiently quenched by CT-DNA (Figure 6b), the photocleavage efficiency is lower than with $Ru(bpy)(hat)_2^{2+}$ and $Ru(bpy)(tap)_2^{2+}$ (Figure 7). The reasons for this are not clear but could be due to side photoreactions. It may be noted however that the plasmid assay is very sensitive and it is likely that the quantum yield for cleavage does not exceed 10^{-4}, and therefore other processes may intervene. Such a process is the formation of irreversible photoadducts or photodegradation.

(b) Photoadduct. Previous photophysical and photochemical studies with $Ru(tap)_3^{2+}$ [62] have shown (Figure 11) that the ^{3}MLCT state of this complex can easily reach, by thermal activation, the ^{3}MC state. This latter can give rise to the loss of a ligand which is substituted by two monodentate ligands such as water, Cl^-, or a nucleotide. This however cannot occur if the ^{3}MLCT is quenched by a photoelectron transfer process for example with GMP. As discussed below this process initiates formation of photoadduct which absorbs in the 400 nm region (Figure 9), whereas dechelation or photosubstitution product absorbs in the 500 nm region. Moreover, it was also demonstrated that, for the series of tap and hat complexes, the population of the ^{3}MC state increases with the number of tap or hat ligands in the complex. On the basis of these considerations, the data of Figures 8 and 9 can easily be interpreted. Without quencher, i.e., in the absence of GMP or CT-DNA, the complexes with high population of ^{3}MC state ($Ru(tap)_3^{2+}$, $Ru(Me_2tap)_3^{2+}$, and $Ru(hat)_3^{2+}$) produce photodechelation photosubstitution (Figure 8). This reaction is inhibited with GMP and CT-DNA (Figure 9) because of the photoelectron transfer process with the ^{3}MLCT state before the thermal activation.

The spectroscopic data (Figure 9) reveal the formation of adducts for oxidizing complexes such as $Ru(hat)_3^{2+}$, $Ru(tap)_3^{2+}$, $Ru(hat)_2(bpy)^{2+}$, and $Ru(tap)_2(bpy)^{2+}$. This again strongly suggests that the photoelectron transfer process is directly correlated with the formation of irreversible photoadducts. It is probable that this adduct is formed by reaction of the guanine radical with the radical anion of the ligand of the reduced complex (possibly also involving a proton transfer),[7c,16] a process that for GMP might occur by a bimolecular reaction or alternatively within the original ion pair. The structure of the photoadduct has been recently determined in the case of $Ru(tap)_3^{2+}$ illuminated in the presence of GMP[63] and shown to involve the binding of the 2-N of guanine to the 3-C of a tap ligand.

It is striking to observe that when the complex does not interact significantly with the bases of DNA, which is the case of $Ru(Me_2tap)_3^{2+}$, no photoadduct is formed and the photodechelation becomes very efficient, as detected by the occurrence of absorption at 500 nm (Figure 9G). This particular behavior of $Ru(Me_2tap)_3^{2+}$ is explained by the presence of the two methyl groups which, as outlined above, not only prevents a good interaction with DNA but also blocks the positions on the tap ligand where the guanines could be chemically attached; indeed the illumination of $Ru(Me_2tap)_3^{2+}$ in the presence of GMP does not produce formation of the photoadduct absorbing in the 400 nm region.[64]

In summary, the experiments reported here demonstrate that by variations of the coordinated ligands it is possible to increase the oxidizing power of ruthenium complexes so that they can photooxidize guanine (or in some cases adenine) in their nucleotides or in DNA. As well as enhancing the yield of photocleavage of the sugar−phosphate backbone of DNA, such oxidizing complexes also form covalent adducts. The formation of such complexes is expected to have a significant effect on the biological activity of nucleic acids.

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