Photoadducts of Metallic Compounds with Nucleic Acids − Role Played by the Photoelectron Transfer Process and by the TAP and HAT Ligands in the Ru^{II} Complexes

Romain Blasius,[a][‡] Cécile Moucheron,[a] and Andrée Kirsch-De Mesmaeker*[a]

Keywords: Photoadducts / Electron transfer / Ruthenium(II) complexes / GMP / DNA

The properties that are characteristic of TAP (1,4,5,8-tetraazaphenanthrene) and HAT (1,4,5,8,9,12-hexaazatriphenylene) Ru^{II} complexes under illumination are highlighted and compared with those of other metallic complexes. In particular, the photo-oxidizing power of their ^{3}MLCT states leads to a quenching of luminescence accompanied by a photoreaction when they are in presence of nucleic acids. The proton-coupled photoinduced electron transfer with the guanine nucleobases produces metallic adducts on DNA guanine units. The mechanism of formation, structure, and possible applications of these photoadducts are discussed and compared with other DNA metallic adducts currently in the literature.

Introduction

During the last 15 years, transition-metal complexes have been extensively studied in the presence of nucleic acids. The interest focused on these complexes stems mainly from their photophysical and photochemical properties, which can be easily tuned by the type of ligands and nature of the metallic centre. The platinum complexes such as *cis*-$[PtCl_2(NH_3)_2]$, known as cisplatin, and its derivatives are well-known examples of metallic compounds that have been widely applied in anticancer chemotherapy.[1−4] They have turned testicular cancer into a curable disease. The activity of these platinum complexes against a variety of cancers is

[a] Université Libre de Bruxelles, Chimie Organique et Photochimie, CP 160/08,
50 avenue F. D. Roosevelt, 1050 Brussels, Belgium
Fax: (internat.) + 32-2-650-3018
E-mail: akirsch@ulb.ac.be
[‡] Present address: Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg,
9 rue Edward Steichen, 2540 Luxembourg, Luxembourg

Romain Blasius was born in 1976 in Luxembourg. In 1995 he moved to the Université Libre de Bruxelles (Brussels, Belgium), where he received his BSc degree in chemistry in 1999. He then went on to complete his Ph.D. studies under the supervision of A. Kirsch and C. Moucheron in the area of photochemistry and photophysics of transition metal complexes with DNA and received his Ph.D. in 2003. He subsequently moved to the Laboratoire de Biologie Moléculaire et Cellulaire du Cancer in Luxembourg, where he is currently carrying out his research on the resistance of cancer cells towards chemotherapeutic drugs and the inhibition of this resistance through the use of natural compounds.

Cécile Moucheron was born in 1966 in Leuven. She received her doctoral degree in 1992 at the Université Libre de Bruxelles (ULB, Belgium) in the area of aromatic synthesis and coordination chemistry. She worked with Dr. J.-P. Sauvage on semicatenanes and copper(I) complexes at the Université Louis Pasteur in Strasbourg. She then joined the laboratory of photochemistry at the ULB. In 1994, she spent several months at the Université Joseph Fourier in Grenoble (UJF, France) where she contributed to research on new intercalating derivatives with Prof. J. Lhomme and Dr. M. Demeunynck. She continued her career as assistant professor at the ULB. From 2003, she was elected as Professor in the Department of Chemistry at the ULB. Her research includes the synthesis of polyazaaromatic complexes and their study with DNA or as antenna systems.

Andrée Kirsch-De Mesmaeker received her doctoral degree in 1972 at the Université Libre de Bruxelles (ULB, Belgium) in the area of photochemistry and electrochemistry. She subsequently moved to Germany at the Max Planck Institut für Strahlenchemie in Mülheim a.d. Ruhr to carry out post-doctoral research with Professor Schulte-Frohlinde and Dr von Sonntag on γ and pulsed radiolysis of sugar (1972−1974). She then moved to the University of North Carolina at Chapel Hill (USA) (1974−1975) where she contributed to research on dyes photochemistry with Dr. G. Wyman. Back at the ULB as FNRS researcher (Fonds National pour la Recherche Scientifique, Belgium), she performed research in photoelectrochemistry and has obtained an award from the Belgian Academy of Sciences. She continued her career as Director of Research at the FNRS and spent a sabbatical year at Columbia University in New York in 1989. From 1999 she was elected as full Professor in the Department of Chemistry at the ULB. Her current research includes photochemistry and photophysics of transition metal complexes with DNA.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

attributed to their reactivity with nucleic acids, mainly by substitution of their chloride ligands by water and ultimately by two adjacent guanines of DNA,[5,6] which results in covalent binding of the metallic species to the genetic material. However, treatment by these drugs leads to important toxic side effects. Therefore, there is still an urgent need for other metallic compounds that would be active mainly at the level of the genetic material and that could exhibit an equal or higher activity against other types of cancer but with a lower toxicity.

The use of a photoreactive metallic drug would be rather advantageous because the action of the drug could be triggered at a chosen time and at selected sites by using optical fibers. There is thus a motivation to develop metallic compounds that are photoreactive towards DNA. The illumination of metallic complexes that interact with DNA generally leads to cleavage (direct cleavage and a cleavage that is revealed by treatment with piperidine) or to the formation of photoadducts with a covalent bond between the metallic compound and a DNA base. These metallic photoadducts could play an important role in chemotherapy in the future, a role similar to that played by the Pt compounds. Some of them have indeed been shown to inhibit DNA replication or synthesis so that they may be regarded as potential antitumor drugs.[7]

In this review, we describe different mechanisms for the formation of metallic photoadducts with DNA and polynucleotides. The particular role played by the Ru^{II} complexes formed with the ligands TAP and HAT (TAP = 1,4,5,8-tetraazaphenanthrene, HAT = 1,4,5,8,9,12-hexaazatriphenylene, Figure 1) is highlighted. The Ru^{II} complexes can be used also as photoprobes of DNA structures or topologies, and for this particular aspect, the reader should refer to already published reviews.[8−14]

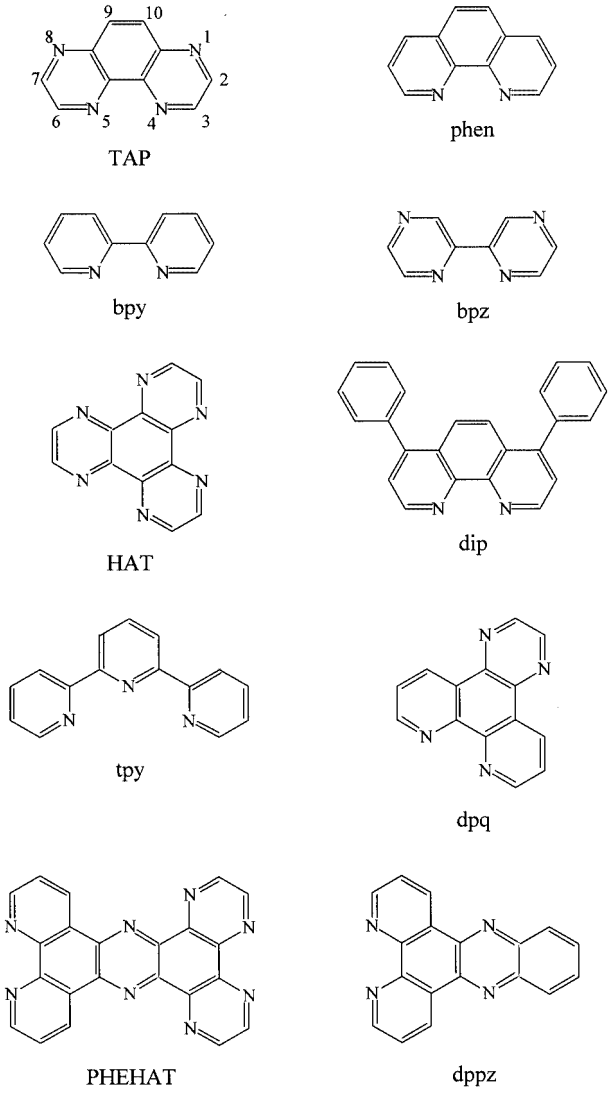

Figure 1. Structure of the different ligands

Different Types of Metallic Photoadducts with DNA

1. With Rhodium, Chromium, and Ruthenium Complexes. Photoadducts Formed by Ligand Substitution

Morrison's team has shown that irradiation of *cis*-dichlorobis(1,10-phenanthroline)rhodium(III) chloride (Figure 2) in the presence of 2′-deoxyguanosine (Figure 2) under aerobic conditions gives rise to the formation of a photoproduct in which the metal is bound to the N1 site of the deoxyguanosine (Figure 3, A); another photoproduct in which Rh^{III} is bound to the guanine either through the oxygen atom at C6 or the amino group at C2[15,16] is also formed. The illumination of the same starting complex, un-

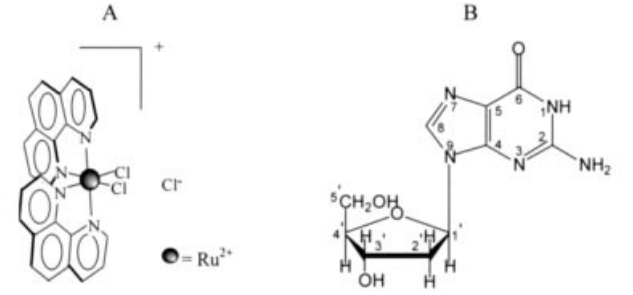

Figure 2. Structure of *cis*-dichlorobis(1,10-phenanthroline)-rhodium(III) (A) and 2′-deoxyguanosine (B)

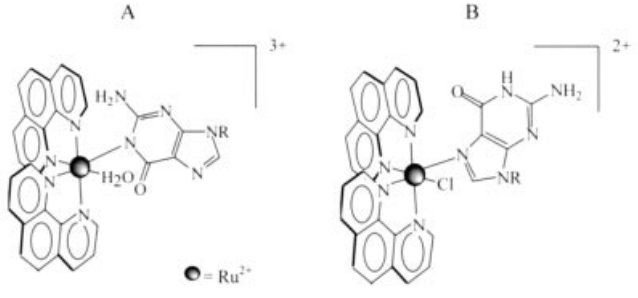

Figure 3. Proposed structure for the rhodium-guanine adducts; A: N1 bound adduct,[15] with 2′-deoxyguanosine, aerobic conditions; B: N7-bound adduct[17] with 2′-deoxyguanosine, anaerobic conditions; R represents the sugar group of dG

der aerobic conditions, in the presence of calf thymus DNA leads to the identical photoadduct (linked through the N1 position of the guanine) that was isolated after enzymatic digestion of the nucleic acid.[15]

Under anaerobic conditions, illumination of the Rh^{III} complex in the presence of 2′-deoxyguanosine also forms a photoadduct, but in this case, the metal centre is linked to the N7 atom of the guanine moiety (Figure 3B).[17] Furthermore, this photoreaction (in the absence of oxygen) proceeds with a quantum efficiency greater than 1, which has been explained by a chain reaction initiated by a photoinduced electron transfer.[17] However, the irradiation of *cis*-dichlorobis(1,10-phenanthroline)Rh^{III} chloride in the presence of calf thymus DNA under anaerobic conditions leads to the same photoadduct as that detected in the presence of oxygen.[18]

The key step of the mechanism for the formation of these photoadducts is an electron transfer from the guanine base to the excited-state metal complex (step 2).[15,16]

$$cis\text{-}[Rh^{III}(phen)_2Cl_2]^+ \xrightarrow{h\nu} cis\text{-}[Rh^{III}(phen)_2Cl_2]^{+*} \quad (1)$$

$$cis\text{-}[Rh^{III}(phen)_2Cl_2]^{+*} + dG \longrightarrow cis\text{-}[Rh^{II}(phen)_2Cl_2] + dG^{\bullet+} \quad (2)$$

A chain reaction begins with the loss of one Cl^- ligand from the Rh^{II} complex (step 3). In the absence of oxygen, a subsequent reaction with the most nucleophilic site of dG leads to an N7−guanine adduct on the Rh complex (step 4). Another electron transfer (in the dark) between the Rh^{II} adduct and the starting dichloro complex completes the first turnover of this chain reaction and leads to the formation of the final Rh^{III} photoadduct under anaerobic conditions (step 5). Finally, the chain reaction is terminated by a back electron-transfer from the Rh^{II} intermediate species to $dG^{\cdot+}$ (step 6).

Under aerobic conditions, however, oxygen can kill the chain propagation by oxidising the *cis*-$[Rh^{II}(phen)_2Cl]^+$ species formed during step 3, with the simultaneous addition of a water molecule and production of a chloroaquo complex and a superoxide anion (step 4a).

The formation of this chloroaquo compound and its reaction with $dG^{\cdot}$ (obtained by chemical oxidation−deprotonation of the guanosine) finally leads to the adduct.[16] Different sites have been proposed for the radical position on the guanine; the localisation on N1 explains the structure of the *cis*-$[Rh^{III}(phen)_2(N1-dG)(OH_2)]^{3+}$ adduct formed under aerobic conditions. Coupling with the radical at either the oxygen atom at C6 or the amino group at C2 has been postulated to explain the unidentified adduct.

In conclusion, the preferential binding of *cis*-$[Rh^{III}(phen)_2Cl_2]^+$ to guanine moieties rather than other nucleobases is due to the initiation step of the chain reaction, i.e. a primary electron transfer from a base to the excited complex. This process occurs, of course, preferentially with a guanine because it is the nucleobase with the lowest reduction potential for the cation radical.

Interestingly, thanks to this Rh photoreactivity, irradiation of *cis*-dichlorobis(1,10-phenanthroline)rhodium(III) chloride in the presence of packaging RNA (which forms a hexameric complex to gear the DNA translocation process) leads to some crosslinking of this RNA.[18,19]

It has also been reported that [*cis*-$Cr(phen)_2Cl_2$] upon excitation with visible light in the presence of DNA leads to the covalent attachment of the metal.[20] This Cr complex also undergoes aquation, which originates from both thermal and photochemical pathways. The photochemical pathway stems again from an electron transfer from dG to the excited state metal complex, with the exchange of one or two Cl^- ligands.[20]

The photoreactivity of complexes such as $[Ru(tpy)(X)Cl]^+$ {tpy = 2,2′:6′,2′′-terpyridine; X = bipyridine (bpy), 1,10-phenanthroline (phen) and dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz)},[21] has also been investigated in the frame of the search for novel photoreagents that are reactive towards DNA. When these Ru complexes are photolysed, the Cl^- ligand is easily substituted by a H_2O molecule, which leads to the aquaruthenium complex. This latter complex, like the Rh species, is reactive towards the DNA bases and forms a coordination bond with a heterocyclic nitrogen atom[22] of a nucleobase. The covalent binding of such aqua Ru complexes to DNA has been demonstrated by ultrafiltration, extensive dialysis and ethanol precipitation.[23,24] However, the reaction between $[Ru(tpy)(X)(H_2O)]^{2+}$ and double-stranded DNA proceeds with a rather poor yield ($[Ru]_{bound}$/[DNA-nucleotide] is approximately 0.02) because of the important steric hindrance due to the presence of the polypyridyl (X) ligands and the incoming reactive DNA base.[22]

2. The Ru^{II} Complexes Containing TAP and HAT Ligands

2a. Formation of Photoadduct by Substitution

In 1994 Lecomte et al. showed that $[Ru(TAP)_3]^{2+}$ can form photoadducts with AMP (adenosine 5′-monophosphate) by photosubstitution of one TAP ligand.[25] This

$$cis\text{-}[Rh^{II}(phen)_2Cl_2] \longrightarrow cis\text{-}[Rh^{II}(phen)_2Cl]^+ + Cl^- \quad (3)$$

$$cis\text{-}[Rh^{II}(phen)_2Cl]^+ + dG \longrightarrow cis\text{-}[Rh^{II}(phen)_2(N7\text{–}dG)Cl]^+ \quad (4)$$

$$cis\text{-}[Rh^{II}(phen)_2(N7\text{–}dG)Cl]^+ + cis[Rh^{III}(phen)_2Cl_2]^+ \longrightarrow cis[Rh^{III}(phen)_2(N7\text{–}dG)Cl]^{2+} + cis[Rh^{II}(phen)_2Cl_2] \quad (5)$$

$$cis\text{-}[Rh^{II}(phen)_2(N7\text{–}dG)Cl]^+ + dG^{\bullet+} \longrightarrow cis\text{-}[Rh^{III}(phen)_2(N7\text{–}dG)Cl]^{2+} + dG \quad (6)$$

$$cis\text{-}[Rh^{II}(phen)_2Cl]^+ + O_2 \xrightarrow{H_2O} cis\text{-}[Rh^{III}(phen)_2Cl(OH_2)]^{2+} + O_2^{\bullet-} \quad (4a)$$

photoreactivity is thus similar to that discussed above in which the initial chelation sphere around the Ru^{II} centre is transformed. The origin of this photoreactivity can easily be explained. It has been demonstrated (see later) that the ^{3}MLCT (metal to ligand charge-transfer) state of this complex is efficiently thermally activated to a ^{3}MC (metal-centred) state, which produces a rather high quantum yield of photodechelation (loss of a ligand under illumination).[26] Therefore, $[Ru(TAP)_3]^{2+}$ exhibits, under illumination, a higher probability of substitution of one of its TAP ligands by a DNA base than complexes with lower photodechelation quantum yields. The photoreactivity of this complex in the presence of AMP has been studied by UV/Vis absorbance and laser flash photolysis,[25] and it indicates the formation of $[Ru(TAP)_2(AMP)(X)]^{n+}$ (X = H_2O, Cl^-), which absorbs in the 500 nm region. It is important to note that such photosubstitution reactions are not observed with GMP as mononucleotide (see further for the explanation).

In conclusion, in the photoadducts described up to now in which a metal−nitrogen bond is formed, the chelation sphere around the metal centre is destroyed relative to that in the starting complex. This ligand substitution can be triggered either by a photoelectron transfer, as is the case for the Rh complexes, or by crossing from the ^{3}MLCT state to the ^{3}MC state at room temperature, as observed with $[Ru(TAP)_3]^{2+}$ in the presence of AMP.

2b. Formation of a New Type of Photoadduct

Quite different photoadducts were seen for the first time when analyses of the illuminated solution of $[Ru(TAP)_3]^{2+}$ in the presence of ^{32}P-labeled oligonucleotides were performed by denaturing polyacrylamide gel electrophoresis (PAGE). These PAGE experiments exhibit the unexpected appearance of bands of lower mobility than the starting 5′-^{32}P-labeled single-stranded oligomer,[27,28] whereas $[Ru(phen)_3]^{2+}$ or $[Ru(bpy)_3]^{2+}$ lead to photoinduced strand breaks by a mechanism involving singlet oxygen.[29,30] The slower bands were attributed to covalent photoadducts of $[Ru(TAP)_3]^{2+}$ with the oligonucleotide. Interestingly, such low mobility bands have also been observed with $[Ru(TAP)_2(bpy)]^{2+}$, but not with $[Ru(bpy)_2(TAP)]^{2+}$.[27] Further evidence for photoadduct formation was obtained by dialysis experiments coupled with detection by UV/visible spectroscopy.[28] The absorption spectrum indicates that the photoproduct cannot originate from dechelation/substitution, as discussed in Section 2a of this article, because no increasing absorption is detected around 500 nm, but a strong hypsochromic shift with a hyperchromic effect appears in the 350−400 nm region. The same spectral changes appear after illumination of $[Ru(TAP)_3]^{2+}$ in the presence of GMP, and are thus different from those described above in the presence of AMP.

Further evidence for the formation of a new type of photoadduct is furnished by electrospray mass spectrometry (ESMS).[31,32] Strong peaks corresponding to $[M - 2H - 2PF_6^-]^{2+}$ and $[M - 2H - PF_6^-]^+$, where M = [Ru(TAP)$_3$(PF$_6$)$_2$ + guanine or + guanosine monophosphate], are typical of the mass of the purified adduct as the PF_6^- salt, isolated after illumination of $[Ru(TAP)_3]^{2+}$ in the presence of GMP or $[Ru(TAP)_2(bpy)]^{2+}$ and DNA after hydrolysis of the DNA.[32] Thus, the results show that in the photoadduct, the chelation sphere has remained intact and a covalent bond between one of the ligands of the Ru complex and guanine is formed with the simultaneous loss of two hydrogen atoms.

In 1997, Vicendo et al. showed by ICP (inductively coupled plasma) and absorption spectroscopy that $[Ru(bpz)_3]^{2+}$ (bpz = 2,2′-bipyrazine) also forms photoadducts with calf thymus DNA[33] and dGMP. Spectroscopic changes similar to those described with the TAP complexes, appear after illumination of $[Ru(bpz)_3]^{2+}$ in the presence of DNA or dGMP. Subsequently, the same research team confirmed the occurrence of a photoadduct by gel electrophoresis experiments performed on illuminated $[Ru(bpz)_3]^{2+}$ with single- and double-stranded oligonucleotides.[34]

Quite recently, photoadduct formation has also been detected by visible illumination of $[Ru(HAT)_2(phen)]^{2+}$ in the presence of GMP, calf thymus DNA and synthetic oligonucleotides.[35] ESMS analyses of the isolated photoproduct with GMP confirms the addition of one GMP to the Ru complex, without loss of the polyazaaromatic ligands. Furthermore, photoadduct formation with $[Ru(HAT)_2(phen)]^{2+}$ appears only with guanine-containing synthetic oligonucleotides,[35] as shown by gel electrophoresis experiments.

In conclusion, TAP, HAT, and bpz Ru^{II} complexes containing at least two such ligands form photoadducts with GMP or G-containing polynucleotides that are quite different from those formed with Pt, Rh or other Ru complexes.

Mechanism for Formation of the New Photoadducts

In order to provide evidence for the primary processes responsible for the formation of the photoadducts, photophysical and redox properties of these particular complexes have to be considered. Figure 4 represents the classical photophysical scheme proposed for most Ru^{II} complexes with three bidentate ligands such as $[Ru(bpy)_3]^{2+}$.[36,37] Illumination of such polyazaaromatic complexes leads to the population of a ^{1}MLCT state, which deactivates rapidly

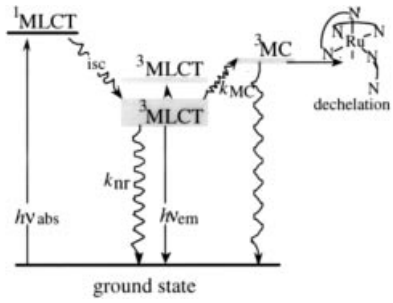

Figure 4. Photophysical scheme for $[Ru(bpy)_3]^{2+}$

(less than 1 ps) by intersystem crossing to a ^{3}MLCT state with a quantum yield of unity.[38] This ^{3}MLCT state can deactivate radiatively (k_r), giving rise to the emission of the complex, or nonradiatively (k_{nr}). A conversion into a higher ^{3}MC state can also take place by thermal activation from the ^{3}MLCT. The ^{3}MC state can either deactivate nonradiatively or lead to dechelation (the loss of one polyazaaromatic ligand). This process is important for $[Ru(TAP)_3]^{2+}$ and is responsible for the formation of the first type of photoadduct with AMP, as discussed in section 2a.

The emission and the excited state lifetime associated with the ^{3}MLCT state strongly depends on the environment. Generally, the interaction of such compounds with polynucleotides leads to an increase in the luminescence intensity and lifetime. This effect is attributed to the protection of the metallic complex by the DNA hydrophobic environment (protection from water and oxygen quenching). However, the behaviour of TAP or HAT complexes that contain at least two or three TAP (or HAT) ligands (thus with $[Ru(bpy)_n(TAP/HAT)_{3-n}]^{2+}$ when $n = 0$ or 1) is different. Instead of a luminescence enhancement, a luminescence quenching appears in the presence of CT-DNA and poly(dG-dC)$_2$[27,39,40] (see also Figure 5 for $[Ru(HAT)_2(phen)]^{2+}$). The emission is thus quenched by G-C containing polynucleotides. As described below this quenching is due to a photoelectron transfer. In Table 1 we have presented the results on the effect of increasing polynucleotide concentrations on the luminescence of different Ru^{II} complexes.

An emission quenching for the same complexes also appears in the presence of mononucleotides. Large quenching rate constants of luminescence (of the order of 10^9 M^{-1}·s^{-1}) by GMP are characteristic of complexes containing two or three TAP (or HAT) ligands, whereas the other complexes exhibit extremely weak or no quenching at all by GMP.

Actually, the ruthenium(II) complexes containing at least two π-deficient ligands (TAP or HAT) with low-lying unoccupied molecular orbitals centred on the ligand are strongly oxidising in their ^{3}MLCT state.[41] Therefore, this excited

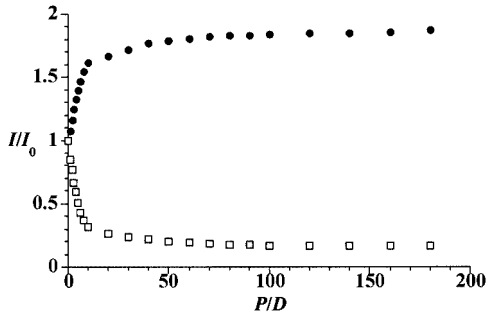

Figure 5. Effect of the polynucleotide/dye (P/D, D = ruthenium complex) ratio on the emission intensity of $[Ru(HAT)_2(phen)]^{2+}$; [Ru] = 5.2×10^{-5} M in 10 mm TRIS buffer; the excitation has been performed at 410 nm; I = emission intensity in the presence of the polynucleotide; I_0 = emission intensity in the absence of the polynucleotide; solid circles: effect of [poly(dA-dT)]$_2$. open squares: effect of DNA

species can abstract one electron from a guanine unit of DNA or GMP. The oxidising power can be estimated from the reduction potentials of the ground state and the energy of the emission maximum ($\Delta E_{\lambda_{max}}$) (Table 2):

$$E_{red}^* \approx E_{red} + \Delta E_{\lambda_{max}}$$

Taking into account the oxidation potential of GMP (E_{ox} = +0.92 V vs. SCE[11] or +1.35 V vs. SCE[42]) it is concluded that depending on the value, the excited complexes may or may not be able to oxidise GMP.

The plot of the logarithms of the quenching rate constants (k_q in water) as a function of the reduction potential of the excited state of different TAP and HAT complexes in MeCN (Figure 6) gives a curve typical of quenching by electron transfer; one where a plateau value is reached, and which corresponds to the diffusion rate constant. Actually, it has been demonstrated for some complexes that the electron transfer is coupled to a proton transfer in water,[43] as evidenced from a decrease in k_q from H_2O to D_2O. This

Table 1. Effect of increasing concentrations of various polynucleotides on the emission intensity of different ruthenium(II) complexes; ↑: increase of the luminescence; ↓: quenching of luminescence

Complex	CT-DNA	[poly(dG-dC)]$_2$	[poly(dA-dT)]$_2$	Reference
$[Ru(HAT)_3]^{2+}$	↓	–	↓	[39][40]
$[Ru(TAP)_3]^{2+}$	↓	↓	↑	[27][44]
$[Ru(HAT)_2(TAP)]^{2+}$	↓	–	↓	[39]
$[Ru(TAP)_2(HAT)]^{2+}$	↓	↓	↑	[39]
$[Ru(HAT)_2(phen)]^{2+}$	↓	–	↑	[35]
$[Ru(TAP)_2(phen)]^{2+}$	↓	↓	↑	[55]
$[Ru(HAT)(TAP)(bpy)]^{2+}$	↓	–	↑	[39]
$[Ru(HAT)_2(bpy)]^{2+}$	↓	–	↑	[40][56]
$[Ru(TAP)_2(bpy)]^{2+}$	↓	–	↑	[27]
$[Ru(bpy)_2(HAT)]^{2+}$	↑	–	↑	[40][56]
$[Ru(bpy)_2(TAP)]^{2+}$	↑	–	↑	[27][39]
$[Ru(phen)_2(PHEHAT)]^{2+}$	↑	–	↑	[57]
$[Ru(TAP)_2(dppz)]^{2+}$	↓	–	↑	[43]
$[Ru(phen)_2(dppz)]^{2+}$	↑	–	↑	[57]

Table 2. Oxidation (E_{ox}) and reduction (E_{red}) potentials of a series of complexes and the corresponding oxidation (E_{ox}*) and reduction (E_{red}*) potentials in the excited state (the data are expressed in V vs. SCE)

Complex	E_{ox}	E_{red}	E_{ox}*	E_{red}*	Reference
$[Ru(HAT)_3]^{2+}$	+ 2.07	− 0.62	− 0.01	+ 1.46	[41]
$[Ru(HAT)_2(TAP)]^{2+}$	+ 2.03	− 0.64	− 0.04	+ 1.43	[41]
$[Ru(TAP)_2(HAT)]^{2+}$	+ 2.02	− 0.68	− 0.02	+ 1.36	[41]
$[Ru(TAP)_3]^{2+}$	+ 1.94	− 0.75	− 0.12	+ 1.32	[26]
$[Ru(HAT)_2(phen)]^{2+}$	+ 1.86	− 0.66	− 0.03	+ 1.23	[40]
$[Ru(HAT)_2(bpy)]^{2+}$	+ 1.79	− 0.76	− 0.08	+ 1.12	[41]
$[Ru(HAT)(TAP)(bpy)]^{2+}$	+ 1.78	− 0.75	− 0.08	+ 1.11	[41]
$[Ru(TAP)_2(dppz)]^{2+}$	+ 1.77	− 0.80	− 0.18	+ 1.15	[43]
$[Ru(TAP)_2(phen)]^{2+}$	+ 1.73	− 0.83	− 0.16	+ 1.06	[40]
$[Ru(TAP)_2(bpy)]^{2+}$	+ 1.70	− 0.83	− 0.19	+ 1.06	[40]
$[Ru(bpy)_2(HAT)]^{2+}$	+ 1.56	− 0.84	− 0.11	+ 0.83	[41]
$[Ru(bpy)_2(TAP)]^{2+}$	+ 1.51	− 0.88	− 0.23	+ 0.86	[26]
$[Ru(phen)_2(PHEHAT)]^{2+}$	+ 1.35	− 0.84	− 0.52	+ 1.03	[57]
$[Ru(phen)_2(dppz)]^{2+}$	+ 1.30	− 1.00	− 0.76	+ 0.97	[57]
$[Ru(bpy)_3]^{2+}$	+ 1.28	− 1.35	− 0.73	+ 0.66	[26]

proton coupling could explain the appearance of quenching by electron transfer, even with an oxidation potential value for GMP as positive as +1.35 V/SCE.

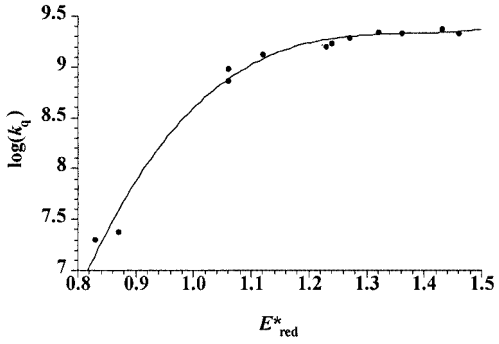

Figure 6. Plot of $\log(k_q)$ in water as a function of the estimated reduction potentials of the excited complexes (E^*_{red}) in MeCN; k_q = luminescence quenching rate constant

The electron transfer generates an oxidised $GMP^{\cdot+}$ species and a reduced complex $[Ru(L_2)(L^{\cdot-})]^+$ (the percentage of protonated reduced species depends on the pH). If instead of GMP, AMP is present, the excited ^{3}MLCT state cannot be quenched reductively, therefore its thermal crossing to the ^{3}MC state is possible and is accompanied by the loss of a TAP ligand and by a possible substitution by AMP (see section 2a).

The presence of an electron-transfer process with guanine units was demonstrated by laser flash photolysis experiments. The monoreduced complex and monooxidized GMP could be detected after the laser pulse.[40,44]

$$[Ru(L)_3]^{2+*} + GMP \rightarrow [Ru(L)_2(L^{\cdot-})]^+ + GMP^{\cdot+}$$
$$[Ru(L)_2(L^{\cdot-})]^+ + GMP^{\cdot+} \rightarrow [Ru(L)_3]^{2+} + GMP$$

The differential transient absorption measured at about 500−550 nm, characteristic of the absorption of the monoreduced complex, disappears according to a bimolecular, equimolecular process in the absence of oxygen, which indicates the presence of a back electron-transfer process in a few hundred μs. In the presence of oxygen, the decay of the transient at the same wavelength becomes pseudo-monomolecular and takes place in a few ten μs, due to the reoxidation of the reduced complex by oxygen. The remaining absorption still present after this decay corresponds to the absorption of the deprotonated radical cation of $GMP^{\cdot+}$. By flash photolysis experiments with DNA, a transient absorption at the same wavelength could also be observed; however, the amplitude of the decay is too small to perform a kinetic analysis of the signal, which indicates that the back electron-transfer is faster with DNA than with GMP. Moreover, the signal does not decay to zero but to a permanent absorption corresponding to that of the photoadduct.

In conclusion, the results with the mononucleotides and polynucleotides highlight a correlation between the reduction potential of the excited complex (thus the oxidation power of the excited state), the proton-coupled photoinduced electron transfer from guanine units, and the formation of photoadducts. Thus, the latter could result from the reaction of the intermediate radicals produced by the electron transfer. Determination of the structure of the photoadduct is a key element, which allows the confirmation of this conclusion.

Structure of the New Photoadducts

The determination of the structure of the photoadducts formed between the TAP and HAT complexes and GMP was performed mainly by NMR spectroscopy, first with the system $[Ru(TAP)_3]^{2+}$ and GMP.[31] From the integration of the different peaks in the NMR spectra, two protons are missing relative to the spectrum of one equivalent of $[Ru(TAP)_3]^{2+}$ and one equivalent of GMP. This indicates that the photoadduct, as concluded from the ESMS data, contains the starting complex plus one GMP, minus two H. One of the missing hydrogen atoms corresponds to that on position 2 of the TAP ligand (see Figure 1 for the numbering), which indicates the formation of a covalent bond between the position 2 of TAP and a guanine moiety. This is in agreement with the chemical shift of proton 3 of TAP, which is induced by this addition.[31] The other hydrogen atom missing belongs to the exocyclic amine (position 2 on GMP); the signal for the remaining amine proton is shifted downfield by δ = 5 ppm relative to that for the exocyclic amine protons of the free guanine. Thus, the covalent bond with the TAP ligand is formed with the exocyclic N atom of the guanine.[31] The structure of this photoadduct, as deduced from the NMR-COSY spectrum and the above arguments, is depicted in Figure 7.

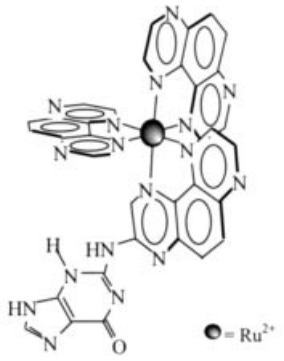

Figure 7. Structure of the photoadduct formed after illumination of $[Ru(TAP)_3]^{2+}$ in the presence of GMP; the structure shown corresponds to the photoadduct after acid hydrolysis to remove the sugar-phosphate moiety

The structure of the photoadduct formed between $[Ru(TAP)_2(bpy)]^{2+}$ and CT-DNA has also been studied. In that case, CT-DNA was hydrolysed or digested by enzymes after illumination.[32] The NMR spectrum obtained corresponds to a mixture of two products, i.e. two geometrical isomers (Figure 8), the covalent bond forms with the exocyclic-N of the guanine residue.[32] This was confirmed by mass spectrometry data that showed a fragmentation product corresponding to $[Ru(TAP\text{-}NH_2)(TAP)(bpy)]^{2+}$.

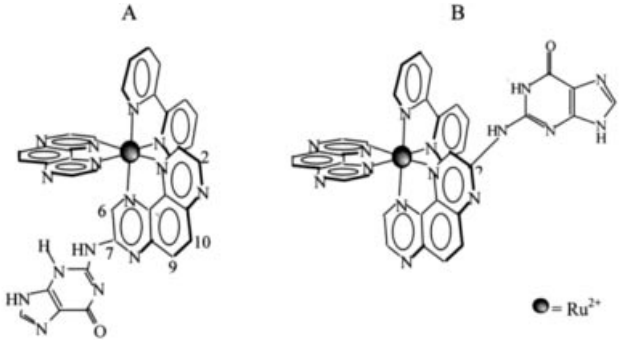

Figure 8. Structure of the two geometrical isomers for the photoadducts formed after illumination of $[Ru(TAP)_2(bpy)]^{2+}$ in the presence of CT-DNA; the structures have been obtained after enzymatic digestion and acidic treatment of CT-DNA

More recently, the structure of the photoadducts between a HAT-containing complex, i.e. $[Ru(HAT)_2(phen)]^{2+}$ and GMP, was examined.[35] In this case, two geometrical isomers were also detected from the NMR spectroscopic data, as with $[Ru(TAP)_2(bpy)]^{2+}$. However, a difference appears at the level of the protons of the guanine moiety, which suggests that the covalent bond with the HAT ligand is formed, in this case, with the O6 atom of the guanine unit. The difference in the structures of the photoadducts with the HAT and TAP complexes (i.e. addition at the O6 or at the exocyclic-N of the guanine unit) cannot be explained. With $[Ru(HAT)_2(phen)]^{2+}$ illuminated in the presence of GMP, in addition to the two geometrical isomers mentioned above, two supplementary photoadducts were detected by ESMS analyses :(i) a photoproduct containing a supplementary oxygen atom, which could correspond, for example, to the addition of an 8-oxoguanosine monophosphate to the complex; and (ii) a photoproduct not involving a 1:1 stoichiometry, i.e. a biadduct corresponding to the addition of two guanosine monophosphate moieties to one ruthenium(II) complex.[35] The structure of these species has not yet been determined.

On the other hand, the origin of the structures of the photoadducts with one GMP can be explained on the basis of the proton-coupled photoinduced electron transfer,[40,44] i.e.:

$$[Ru(TAP/HAT)_2phen]^{2+}* + GMP \rightarrow \{[Ru(TAP/HAT)(phen)(TAPH^{\cdot}/HATH^{\cdot}]^{+},(GMP-H)^{\cdot}\}$$

The proton transferred to the $TAP^{\cdot -}$ or $HAT^{\cdot -}$ moiety can stem from the GMP radical cation. Indeed the radical cation of guanine has a pK_a of 3.9, $GMP^{\cdot +}$ [45–47] thus deprotonates at pH 7 to give a guanine radical. Monoreduced $[Ru(TAP)_3]^{2+}$ has a pK_a of 7.6, which means that it should be protonated after electron transfer.[44] Similar pK_a values can be expected for complexes like $[Ru(TAP)_2(bpy)]^{2+}$ and $[Ru(HAT)_2(phen)]^{2+}$. In conclusion, the coupling of the two radical species resulting from the proton-coupled electron transfer, followed by rearomatisation, could explain the formation of the isolated photoadducts (reactions 7 and 8).

The formation of photoadducts with nucleic acids should obviously be preceded by the interaction of the two partners. The question which may be raised is whether the geometry of interaction of the complexes with the polynucleotides would play an important role in photoadduct formation.

Actually, it has been shown that $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2(bpy)]^{2+}$ [27,48] bind to the surface DNA grooves and interact with a rather low affinity (10^3-10^4 M^{-1}). In contrast, $[Ru(HAT)_2(phen)]^{2+}$, as probed by monitoring of the spectroscopic changes of the complex while interacting with DNA and by DNA viscosity measurements, intercalates one of its HAT ligands between the stacking of bases, and its affinity for the polynucleotides reaches $3.8 \cdot 10^5$ M^{-1}.[35] We may thus conclude that both geometries of interaction, adsorption or intercalation, produce photoadducts since they are detected in both cases.

Applications

One major disadvantage of metallic complexes that react in the dark with DNA, like cisplatin, or that photoreact

$$\{[Ru(TAP)(phen)(TAPH^{\bullet})]^{2+},GMP(-H)^{\bullet}\} \longrightarrow [Ru(TAP)(phen)(TAPH\text{-}GMP(-H))]^{2+} \quad (7)$$

$$[Ru(TAP)(phen)(TAPH\text{-}GMP(-H))]^{2+} \xrightarrow{-2H} [Ru(TAP)(phen)(TAP(-H)\text{-}GMP(-H))]^{2+} \quad (8)$$

with DNA, like the TAP and HAT complexes, is their non-specificity of (photo)reaction. Indeed, they do not interact with a specific targeted gene, thus a specific base sequence. This represents a drawback if the expression of this sequence has to be inhibited by using the Ru complex as DNA photoreagent. The antigene or antisense strategies are well-known to provide means to inhibit such genes. Unfortunately, after hybridization with the synthetic oligonucleotide, the system formed (double helix for the antisense and triple helix for the antigene strategy) is recognised by the enzymes and destroyed. Many efforts have been focused on different possible ways of increasing the stability of the pairing of the synthetic oligonucleotide with the targeted sequence. In this context, the use of synthetic oligonucleotides derivatised by photoreactive Ru^{II} complexes could be promising as one can take advantage of the formation of the photoadduct on the nucleobases. This strategy has been developed with complexes that show the two types of photoadducts (without and with destruction of the chelation sphere), thus (i) with the TAP complexes and (ii) with the precursors of aqua complexes. (i) A photoreactive TAP complex is chemically anchored to an oligonucleotide with a sequence complementary to the sequence of the targeted gene. After hybridization of the Ru^{II}-labeled sequence to the target sequence, and after illumination of the attached complex, the formation of the photoadduct can be "directed" towards a specific guanine unit of the target strand. Such a photoreaction should result in the photocrosslinking of the two strands and could inhibit the expression of the specific gene. The existence of such photocrosslinkings was demonstrated by gel electrophoresis with $[Ru(TAP)_2(dip)]^{2+}$ (dip = 4,7-diphenylphenanthroline) tethered on different probe sequences (Figure 9).[49−53] Moreover, it was shown that enzymes such as DNA polymerase and exonuclease III are blocked at the level of the photocrosslinking.[54] Thus, a 17-*mer* Ru^{II}-labeled oligodesoxyribonucleotide was hybridised to its complementary sequence located on the 5′-side of a 40-*mer* matrix. After illumination, the elongation of a 13-*mer* DNA primer hybridised to the 3′-end of the same matrix stopped at a position corresponding to the formation of the photoadduct with an efficiency of 100%.

(ii) On the other hand, oligonucleotide conjugates bearing a Ru^{II} complex such as $[Ru(tpy)(dppz)(CH_3CN)]^{2+}$ (tpy = 2,2′:6′,2″-terpyridine)[22] have also been tested. This complex, chemically attached to a probe sequence, can lead to successful crosslinkings with the complementary sequence. Actually, the attached $[Ru(tpy)(dppz)(CH_3CN)]^{2+}$ is simply transformed under illumination into the corresponding aqua complex $[Ru(tpy)(dppz)(H_2O)]^{2+}$ which reacts (dark reaction) with a base unit of the complementary strand.

It should be stressed at this stage that the use of the above complexes in the frame of the antisense strategy would be limited to treatments of specific cancer cells in dermatology or hematopoietic cells that can be illuminated outside the patient. Indeed the visible light (in contrast to IR wavelengths) does not penetrate deeply in tissues, which would prevent an efficient treatment. However, it should be noted

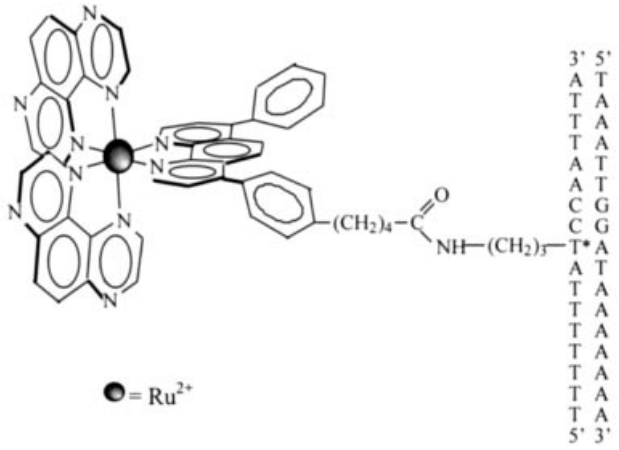

Figure 9. $[Ru(TAP)_2(dip)]^{2+}$-labeled oligonucleotide in the presence of its complementary sequence

that the recognition of a target nucleotidic sequence by an oligonucleotide probe derivatized with a photoreactive complex, followed by photocrosslinking between the two strands as described above, could also be applied in biotechnology without problems with the visible illumination in this case.

Conclusions

In this review, we tried to highlight the properties brought about by π-deficient TAP or HAT ligands in the resulting Ru^{II} complexes. Thus the complexes with at least 2 of these ligands induce a photoinduced electron-transfer with the guanine bases of DNA, a process that generates an adduct between the two partners. In this photoadduct, the complex and the guanine species are attached by a covalent bond without destruction of the chelation sphere.

One could speculate on the important role which could be played in the future by these complexes. For example, as outlined above, the Ru derivatised oligonucleotides could be applied in biotechnology or for treatments of specific cancers in the frame of the antigene or antisense strategy, whose effect could be triggered by light. The important problem which remains to be solved is the penetration and transport of the photoreactive agent into the cell. Research, with this aim, is presently being carried out.

Acknowledgments

R. B. thanks the Luxembourg Ministry of Education and Vocational Training for a Ph.D. research grant. The COST D14 and Action Lions Vaincre le Cancer are also acknowledged. The authors are grateful to the ARC program (02/07−286) and the LEA (Laboratoire Européen Associé, Belgian FNRS-French CNRS) for financial support.

[1] K. T. Douglas, *Chem. & Ind.* **1984**, 738−742.
[2] S. E. Sherman, S. J. Lippard, *Chem. Rev.* **1987**, *87*, 1153−1181.
[3] W. I. Sundquist, S. J. Lippard, *Coord. Chem. Rev.* **1990**, *100*, 293−322.

[4] Y. Mikata, Q. He, S. J. Lippard, *Biochemistry* **2001**, *40*, 7533−7541.
[5] J. Reedijk, *Chem. Commun.* **1996**, 801−806.
[6] T. W. Hambley, *J. Chem. Soc., Dalton Trans.* **2001**, *19*, 2711−2718.
[7] M. Pauly, I. Kayser, M. Schmitz, M. Dicato, A. Del Guerzo, I. Kolber, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Commun.* **2002**, 1086−1087.
[8] C. Moucheron, A. Kirsch-De Mesmaeker, J. M. Kelly, in *Structure and Bonding*, vol. 92 Springer Verlag, Berlin, **1998**.
[9] A. Kirsch-De Mesmaeker, C. Moucheron, N. Boutonnet, *J. Phys. Org. Chem.* **1998**, *11*, 566−576.
[10] A. Kirsch-De Mesmaeker, J.-P. Lecomte, J. M. Kelly, *Topics in Current Chemistry* **1996**, *177*, 25−76.
[11] C. Moucheron, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Photochem. Photobiol. B: Biol.* **1997**, *40*, 91−106.
[12] I. Ortmans, C. Moucheron, A. Kirsch-De Mesmaeker, *Coord. Chem. Rev.* **1998**, *168*, 233−271.
[13] K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.* **1999**, *99*, 2777−2795.
[14] B. Nordén, P. Lincoln, B. Akerman, E. Tuite, in *Metal Ions in Biological Systems*, vol. 33 (*Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules*) (Eds.: A. Sigel, H. Sigel), Marcel Dekker, Inc., New York, Basel, Hong Kong, **1996**.
[15] R. E. Mahnken, M. A. Billadeau, E. P. Nikonowicz, H. Morrison, *J. Am. Chem. Soc.* **1992**, *114*, 9253−9265.
[16] M. A. Billadeau, K. V. Wood, H. Morrison, *Inorg. Chem.* **1994**, *33*, 5780−5784.
[17] H. L. Harmon, H. Morrison, *Inorg. Chem.* **1995**, *34*, 4937−4938.
[18] H. Morrison, H. Harmon, *Photochem. Photobiol.* **2000**, *72*, 731−738.
[19] T. Mohammad, C. Chen, P. Guo, H. Morrison, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1703−1708.
[20] M. A. Billadeau, H. Morrison, *J. Inorg. Biochem.* **1995**, *57*, 249−270.
[21] N. C. Pramanik, K. Pramanik, P. Ghosh, S. Bhattacharya, *Polyhedron* **1998**, *17*, 1525−1534.
[22] D. Ossipov, S. Gohil, J. Chattopadhyaya, *J. Am. Chem. Soc.* **2002**, *124*, 13416−13433.
[23] N. Grover, N. Gupta, H. H. Thorp, *J. Am. Chem. Soc.* **1992**, *114*, 3390−3393.
[24] N. Grover, T. W. Welch, T. A. Fairley, M. Cory, H. H. Thorp, *Inorg. Chem.* **1994**, *33*, 3544−3548.
[25] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, *Bull. Soc. Chim. Belg.* **1994**, *5−6*, 193−200.
[26] A. Masschelein, L. Jacquet, A. Kirsch-De Mesmaeker, J. Nasielski, *Inorg. Chem.* **1990**, *29*, 855−860.
[27] J. M. Kelly, M. M. Feeney, A. B. Tossi, J.-P. Lecomte, A. Kirsch-De Mesmaeker, *Anti-Cancer Drug Des.* **1990**, *5*, 69−75.
[28] M. M. Feeney, J. M. Kelly, A. B. Tossi, A. Kirsch-De Mesmaeker, J.-P. Lecomte, *J. Photochem. Photobiol. B: Biol.* **1994**, *23*, 69−78.
[29] J. M. Kelly, A. B. Tossi, D. J. McConnell, C. OhUigin, *Nucleic Acids Res.* **1885**, *13*, 6017.
[30] M. B. Fleisher, K. C. Waterman, N. J. Turro, J. K. Barton, *Inorg. Chem.* **1986**, *25*, 3549−3551.
[31] L. Jacquet, J. M. Kelly, A. Kirsch-De Mesmaeker, *J. Chem. Soc., Chem. Commun.* **1995**, 913−914.
[32] L. Jacquet, R. J. H. Davies, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Am. Chem. Soc.* **1997**, *119*, 11763−11768.
[33] P. Vicendo, S. Mouysset, N. Paillous, *Photochem. Photobiol.* **1997**, *65*, 647−655.
[34] E. Gicquel, N. Paillous, P. Vicendo, *Photochem. Photobiol.* **2000**, *72*, 583−589.
[35] R. Blasius, H. Nierengarten, J.-F. Constant, E. Defrancq, P. Dumy, M. Luhmer, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Eur. J.,* submitted.
[36] T. J. Meyer, *Pure Appl. Chem.* **1986**, *58*, 1193−1206.
[37] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, A. von Zelewsky, *Coord. Chem. Rev.* **1988**, *84*, 85−277.
[38] J. N. Demas, D. G. Taylor, *Inorg. Chem.* **1979**, *18*, 3177−3179.
[39] A. Kirsch-De Mesmaeker, G. Orellana, J. K. Barton, N. J. Turro, *Photochem. Photobiol.* **1990**, *52*, 461−472.
[40] J.-P. Lecomte, A. Kirsch-De Mesmaeker, M. M. Feeney, J. M. Kelly, *Inorg. Chem.* **1995**, *34*, 6481−6491.
[41] L. Jacquet, A. Kirsch-De Mesmaeker, *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2471−2480.
[42] S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617−618.
[43] I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron, A. Kirsch-De Mesmaeker, *Dalton Trans.* **2004**, 668−676.
[44] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. B. Tossi, H. Görner, *Photochem. Photobiol.* **1992**, *55*, 681−689.
[45] J. Reynisson, S. Steenken, *Phys. Chem. Chem. Phys.* **2002**, *4*, 527−532.
[46] J. Reynisson, S. Steenken, *Phys. Chem. Chem. Phys.* **2002**, *4*, 5346−5352.
[47] S. C. Weatherly, I. V. Yang, H. H. Thorp, *J. Am. Chem. Soc.* **2001**, *123*, 1236−1237.
[48] J.-P. Lecomte, A. Kirsch-De Mesmaeker, M. Demeunynck, J. Lhomme, *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 3261−3269.
[49] O. Lentzen, J.-F. Constant, E. Defrancq, M. Prévost, S. Schumm, C. Moucheron, P. Dumy, A. Kirsch-De Mesmaeker, *ChemBioChem* **2003**, *4*, 195−202.
[50] S. Schumm, M. Prévost, D. Garcia-Fresnadillo, O. Lentzen, C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Chem. B* **2002**, *106*, 2763−2768.
[51] D. Garcia-Fresnadillo, N. Boutonnet, S. Schumm, C. Moucheron, A. Kirsch-De Mesmaeker, E. Defrancq, J.-F. Constant, J. Lhomme, *Biophysical Journal* **2002**, *82*, 978−987.
[52] I. Ortmans, S. Content, N. Boutonnet, A. Kirsch-De Mesmaeker, W. Bannwarth, J.-F. Constant, E. Defrancq, J. Lhomme, *Chem. Eur. J.* **1999**, *5*, 2712−2721.
[53] J.-F. Constant, E. Defrancq, J. Lhomme, N. Boutonnet, S. Content, I. Ortmans, A. Kirsch-De Mesmaeker, *Nucleosides & Nucleotides* **1999**, *18*, 1319−1320.
[54] O. Lentzen, E. Defrancq, J.-F. Constant, S. Schumm, D. Garcia-Fresnadillo, C. Moucheron, P. Dumy, A. Kirsch-De Mesmaeker, *J. Biol. Inorg. Chem.* **2003**, *9*, 100−108.
[55] A. Del Guerzo, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **2002**, *41*, 938−945.
[56] F. de Buyl, A. Kirsch-De Mesmaeker, A. B. Tossi, J. M. Kelly, *J. Photochem. Photobiol. A: Chem.* **1991**, *60*, 27−45.
[57] C. Moucheron, A. Kirsch-De Mesmaeker, S. Choua, *Inorg. Chem.* **1997**, *36*, 584−592.

Received June 9, 2004

