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Ru(II) complexes and light: molecular tools for biomolecules

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Ruthenium(II) (Ru(II)) complexes are the focus of numerous research works with various applications mainly due to their attractive photophysical and photochemical properties. In biological research areas, in particular, they are developed as efficient photoprobes or photosensors of biological molecules. More interestingly specific Ru(II) complexes bearing π-deficient ligands such as TAP = 1,4,5,8-tetraazaphenanthrene, bpz = 2,2′-bipyrazyl or HAT = 1,4,5,8,9,12-hexaazatriphenylene exhibit interesting photoreactivity with biomolecules (DNA, polypeptides). The photoreactions are initiated by a photo-electron transfer from the biomolecule to the excited metal complex. A back electron transfer (BET) succeeds this primary process. However in competition with this BET, a DNA photocleavage and/or formation of adducts of the complex with guanine units of DNA or with tryptophan (Trp) residues of polypeptides can take place. In this review we highlight the studies of these photo-adducts carried out by our laboratories, and connected to some applications. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: polyazaaromatic ruthenium complexes; photophysics; electron transfer; DNA; photochemical processes; photo-adduct; photocrosslinking; derivatized oligonucleotides

INTRODUCTION

The interesting photophysical and photochemical properties of Ruthenium(II) (Ru(II)) complexes have transformed them into attractive candidates for challenging applications.[1] Thus these metallic compounds have been tested as photosensitizers in solar cells[2–4] or in supramolecular assemblies mimicking antenna's systems for collecting light.[5–9] Moreover they have also been interestingly applied for the development of low-voltage devices such as light-emitting electrochemical cells (LECs)[10] or organic light-emitting diodes (OLEDs).[11]

Surprisingly, the Ru(II) complexes have played quite a significant role in biological research areas.[12–17] The sensitivity of their electronic absorption and emission to the solvents or more particularly to the different microenvironments of biomolecules such as DNA has motivated the researchers to develop novel applications in which the Ru compounds are used as photoprobes or photosensors.[18–23] Thus numerous experimental works have been devoted to studies of the interaction between Ru(II) complexes and the double-stranded DNA helix by using the changes in their luminescence properties as a method for probing different nucleic acid structures or topologies. Moreover a few theoretical approaches have been devoted to studies of the Ru complexes excited states behaviours[24–26] and provided suitable models[27–31] to describe their possible geometries of interaction with the genetic material. The so-called light-switch effect observed with Ru(II) complexes containing extended planar ligands is an interesting example which emerged from numerous studies.[32–38] Thus the absence of luminescence of the excited states of these complexes in water, and their restoration when they intercalate their planar ligand into the DNA double helix, illustrate the role of these Ru(II) compounds as DNA photoprobes. Other Ru(II) polypyridine complexes have also been shown to be excellent photoprobes for proteins.[39–42]

Among these numerous and different uses of Ru(II) complexes, only a few studies have been focused on their photoreactivity with biomolecules,[43–47] which is the main focus of this report. Thus it is shown how particular photoreactive Ru(II) complexes could be converted into useful biomolecular tools. The study of their photophysical and photoredox properties allowed to further exploit them for the photocrosslinking of two biomolecules.[48,49] Therefore, we will first consider the photophysical and the electrochemical properties of these metallic species. The results and conclusions will lead us to the understanding and applications of their photoreactivity with DNA, oligonucleotides and polypeptides, which will be discussed later.

The Ru(II) complexes that will be considered here are those formed with oxidizing π-deficient ligands such as the TAP = 1,4,5,8-tetraazaphenanthrene, bpz = 2,2′-bipyrazyl or HAT = 1,4,5,8,9,12-hexaazatriphenylene, in contrast to the well-known bpy = 2,2′-bipyridine or phen = 1,10-phenanthroline ligands, which are used as ancillary ligands. Dinuclear complexes with the bridging ligand TPAC = tetrapyrido[3,2-a:2′, 3′-c:3″,2″-h:2‴, 3‴-j]acridine will also be included in the discussion (Fig. 1).

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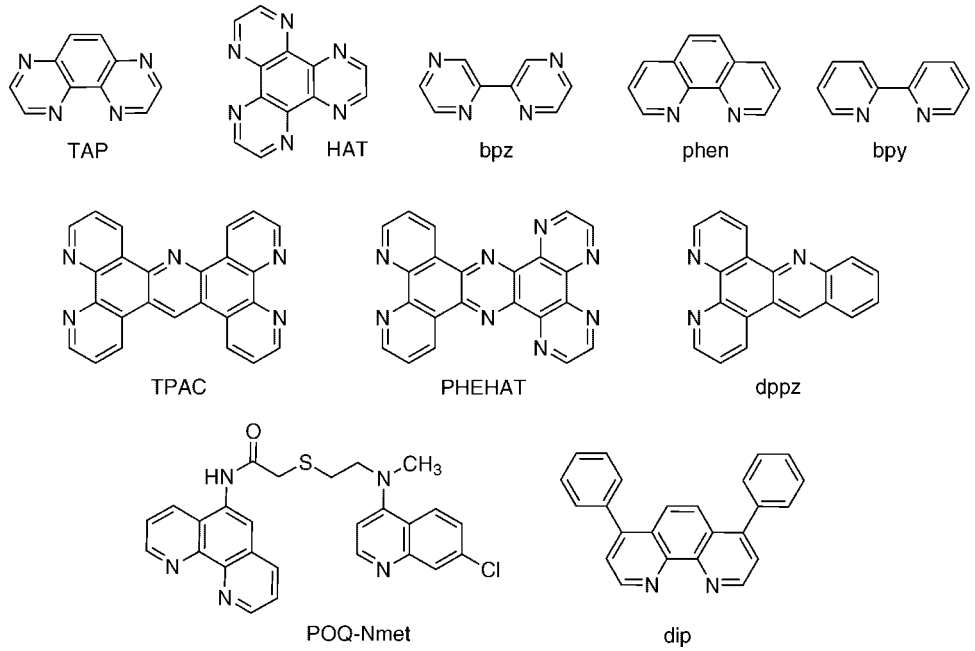

Figure 1. Structures of the ligands

GENERAL PHOTOPHYSICS OF RU(II) COMPLEXES WITH POLYAZAAROMATIC LIGANDS

The complexes are formed with three bidendate polyazaaromatic ligands; thus they have six coordinative bonds. They exhibit a D_3 symmetry at least for a homoleptic complex, where the central metal ion coordinates to the three identical ligands. Usually, electronic transitions between the different molecular orbitals of the complex are classified according to the characteristics of the involved orbitals, whether they have metallic or ligand characteristics. Thus the following transitions can be distinguished: centred on the ligands (ligand centred (LC)), centred on the Ru(II) ion (metal centred (MC)) or corresponding to a charge transfer from the metal to one of the ligands, that is where π^* antibonding orbital is the most stabilized (metal to ligand charge transfer (MLCT)).[50] The lowest transition in absorption and emission has an MLCT character, therefore we will mainly discuss the properties of the corresponding excited states because they control the photophysics and the photochemistry of the complexes.

The MLCT singlet excited state reached by absorption of a photon deactivates very rapidly, in approximatively 100 fs,[51] with a unitary quantum efficiency,[52] to three ^{3}MLCT states very close in energy (a few cm^{-1}) (Fig. 2).[53] Whereas these states can be distinguished at low temperature (below 77 K), they appear as one unique state at room temperature, which we denote as the lowest ^{3}MLCT state. Experiments also show the existence of a fourth ^{3}MLCT state at 400–1000 cm^{-1} above the three other states,[54,55] which will be also included into the lower energy ^{3}MLCT state for the rest of our discussion.

For complexes containing π-deficient ligands, such as HAT or TAP in $[Ru(TAP)_2phen]^{2+}$ or $[Ru(HAT)_2(phen)]^{2+}$, the excited electron in the lowest ^{3}MLCT state is localized on one of these ligands because their corresponding π^* orbital is more stabilized

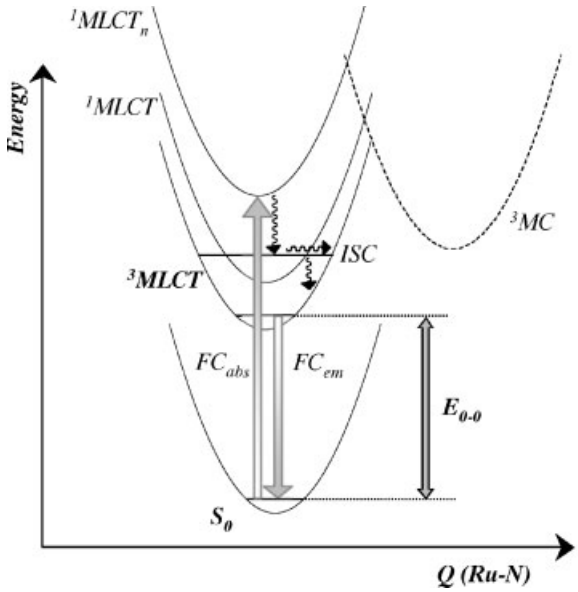

Figure 2. Simplified photophysical scheme of Ru polyazaaromatic complexes, where S_0 stands for the ground singlet state, $^1MLCT_n$ for high energetic singlet states, ^{1}MLCT and ^{3}MLCT for the lower singlet and triplet states, ^{3}MC for the metal centred state, $FC_{abs/em}$ for the Franck–Condon transition in absorption/emission, ISC for intersystem crossing and where $E_{0\text{-}0}$ is the energy of the $v'' = 0 \rightarrow v' = 0$ transition, usually approximated by the FC_{em} value

than that of a phen or bpy ligand. Dechelation of one ligand (loss of a ligand) is possible for $[Ru(TAP)_3]^{2+}$[56] by thermal activation from the ^{3}MLCT to the ^{3}MC state, which presents a distorted geometry. The so-produced photo degraded complexes, such as $[Ru(TAP)_2Cl_2]$ or $[Ru(TAP)_2(H_2O)_2]^{2+}$, have a maximum of absorption around 500 nm. This photodecomposition does not exist if a TAP ligand is replaced by a phen ligand, such as in

$[Ru(TAP)_2phen]^{2+}$. In this case, although the lowest unoccupied molecular orbital (LUMO) is still centred on the TAP ligand, the energy level of the Ru centred highest occupied molecular orbital (HOMO) is a bit destabilized due to the presence of the phen ligand. Therefore the MLCT transition is shifted bathochromically and the energy gap between the ^{3}MLCT and ^{3}MC states becomes greater than for $[Ru(TAP)_3]^{2+}$, so that the ^{3}MC state is no longer accessible. The thermally activated pathway from the ^{3}MLCT to the ^{3}MC state can actually be completely inhibited by quenching the ^{3}MLCT state by addition of increasing amounts of a reductive agent, as we will describe later.

The lifetime of the ^{3}MLCT state is typically of the order of a few hundreds of nanoseconds to 1 μs. This depends (i) on the nature of the ligands that directly influence the energy gap between the ^{3}MLCT and ^{3}MC states as illustrated above with $[Ru(TAP)_2phen]^{2+}$ and $[Ru(TAP)_3]^{2+}$ and (ii) on the solvent or microenvironment, which the complex probes.

Some complexes containing planar extended ligands such as dppz = dipyrido[3,2-a:2′,3′-c]phenazine or PHEHAT = 1, 10-phenathrolino[5,6-b]1,4,5,8,9,12-hexaazatriphenylene, like $[Ru(phen)_2dppz]^{2+}$, $[Ru(bpy)_2dppz]^{2+}$ or $[Ru(phen)_2PHEHAT]^{2+}$ exhibit a light-switch effect. This means that they do not emit in water but the luminescence is turned on by intercalating into the stack of DNA bases. For these complexes, a much more elaborated scheme was required than that described in Fig. 2: several luminescent excited states and even a dark state participate in the relaxation to the ground state. This is not the case of the complexes constructed with the planar TPAC ligand,[57,58] which in addition allows the bridging of two $Ru(TAP)_2^{2+}$ moieties, to yield a dinuclear complex [$(TAP)_2$ Ru TPAC $Ru(TAP)_2]^{4+}$.

We have to note that not only this latter complex but also the other complexes bearing TAP or HAT ligands behave as bases in their excited state. Indeed, the TPAC and TAP ligands contain hetero-aromatic nitrogens, potentially protonable when they are not chelated to the metal ion. As these complexes are used for their interaction with biomolecules, the knowledge of their pK_a values in the ground and excited states is important for their applications with the biomolecules. The pK_a values of the TPAC and TAP complexes have thus been studied.[57,59] For the excited state the pK_a* values can be determined (i) by the Förster's Cycle, in which the pK_a of the corresponding ground state has to be known,[60,61] or (ii) from the measurements of the emission as a function of pH.[59] For the TAP-based complexes described above, the Forster's pK_a* extends from 5.6 to 7.2. This means that the ^{3}MLCT states could be easily protonated, especially when they interact with DNA in which the microenvironment is slightly more acidic than the aqueous media (pH 4.5–5).[62,63] This process should further complicate the behaviours of the polyazaaromatic ligand based metal complexes under pulsed illumination, which are described below.

PHOTO-ELECTRON TRANSFER IN THE PRESENCE OF ELECTRON DONORS

In the presence of biomolecules containing guanine bases

From the photophysical properties of polyazaaromatic complexes in the presence of DNA or oligonucleotides, two types of behaviours are generally observed: on the one hand, enhancement of both the luminescence intensity and the lifetime of the ^{3}MLCT state, and on the other hand, the quenching of the emission intensity and thus the shortening of the ^{3}MLCT lifetime.

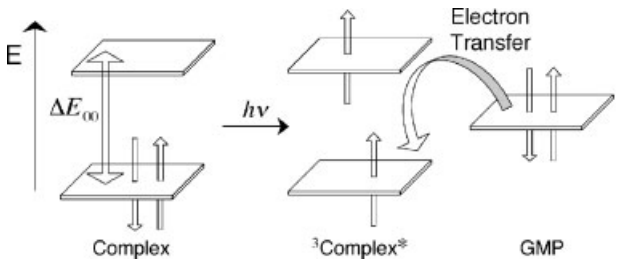

Figure 3. Schematic representation of the photo-electron transfer process between the HOMO orbital of a G base and the excited state of the Ru species under illumination of the complex

In the first case, for example with a system composed of $[Ru(phen)_3]^{2+}$ or $[Ru(TAP)_3]^{2+}$ mixed with $[poly(dA\text{-}dT)]_2$, an increase in the ^{3}MLCT lifetime along with the enhancement of the emission intensity was observed with increasing amounts of polynucleotide.[64,65] These effects are due to the protection of the excited state by the DNA microenvironment (i) from a quenching by molecular oxygen and (ii) from non-radiative deactivation essentially due to OH vibrators of the aqueous solvent. In contrast, with a system composed of $[Ru(TAP)_3]^{2+}$ and $[poly[(dG\text{-}dC)]_2$ for example,[65] the steady-state emission of the complex is efficiently quenched, and the lifetime is shortened. It has been experimentally evidenced that this quenching is due to a photo-electron transfer between G residues and the ^{3}MLCT state of the complex as represented in Fig. 3. Such a process occurs mainly with G moieties of polynucleotides (the most reducing base) and the complexes containing at least 2 π-deficient ligands, such as 2 TAP, 2 HAT or 2 bpz ligands (shown further in Table 1).

The existence of such a charge transfer process followed by a back electron transfer (BET) has been demonstrated by transient absorption spectroscopy in different time scales when the guanine electron donor can freely diffuse (case of GMP) or is simply a base moiety of DNA.[47,66–68] Thus a laser pulse of ≈9 ns on a solution of $[Ru(TAP)_3]^{2+}$ and GMP produces monoreduced complex and oxidized mononucleotide during the laser pulse because the process is diffusion controlled. The transient absorption spectrum recorded 5 μs after the pulse at around 500 nm is shown in Fig. 4 and is attributed to the reduced complex and the oxidized GMP.

The following kinetic scheme has been proposed, in which Eqn 1 represents the production of the ^{1}MLCT state, Eqn 2 the intersystem crossing (ISC), Eqn 3 the intermolecular electron transfer (ET) process with GMP and Eqn 4 the corresponding BET:

$$[Ru(II) - L_3]^{2+} \xrightarrow{h\nu} {}^1[Ru(III) - L_2L^{\cdot -}]^{2+*} \quad (1)$$

$${}^1[Ru(III) - L_2L^{\cdot -}]^{2+*} \xrightarrow{h\nu} {}^3[Ru(III) - L_2L^{\cdot -}]^{2+*} \quad (2)$$

$${}^3[Ru(III) - L_2L^{\cdot -}]^{2+*} + GMP \rightarrow [Ru(II) - L_2L^{\cdot -}]^{+} + GMP^{\cdot +} \quad (3)$$

$$[Ru(II) - L_2L^{\cdot -}]^{+} + GMP^{\cdot +} \rightarrow [Ru(II) - L_3]^{2+} + GMP \quad (4)$$

This ET with the G residues is observed only with the complexes which sufficiently oxidize in their ^{3}MLCT state as

Table 1. Reduction potentials (E_{red}), reduction potential in the excited state (E^*_{red}) for the discussed complexes and exergonicity of the ET with a guanine using the equation $\Delta G_{ET} \approx \left[E_{ox}(G/G^{\cdot+}) - E_{red}\left(RuL_3^{2+*}/RuL_3^{+}\right)\right]$ with the oxidation potential for guanine determined by cyclic voltammetry ($\Delta G^0_{ET}(1)$) or by pulsed radiolysis ($\Delta G^0_{ET}(2)$), as shown below for references and discussion on these values

Complex	E_{red} (V) *versus* SCE	E^*_{red} (V) *versus* SCE	$\Delta G^0_{ET}(1)$ (eV)	$\Delta G^0_{ET}(2)$ (eV)
1. $[Ru(HAT)_3]^{2+}$[69]	−0.62	+1.49	−0.24	−0.42
2. $[Ru(TAP)_3]^{2+}$[56]	−0.75	+1.32	−0.07	−0.25
3. $[Ru(bpz)_3]^{2+}$[70]	−0.80	+1.30	−0.05	−0.23
4. $[(TAP)_2$ Ru TPAC Ru$(TAP)_2]^{4+}$[58]	−0.76	+1.24	+0.01	−0.17
5. $[Ru(TAP)_2dppz]^{2+}$[71]	−0.80	+1.20	+0.05	−0.13
6. $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$[72,73]	−0.83	+1.16	+0.09	−0.09
7. $[Ru(TAP)_2dip]^{2+}$[74]	−0.82	+1.14	+0.11	−0.07
8. $[Ru(TAP)_2phen]^{2+}$[72]	−0.86	+1.13	+0.12	−0.06
9. $[Ru(bpy)_2phen]^{2+}$[75]	−1.35	+0.71	+0.54	+0.36
10. $[Ru(phen)_3]^{2+}$[76]	−1.35	+0.70	+0.55	+0.37
11. $[Ru(bpy)_3]^{2+}$[56]	−1.35	+0.65	+0.60	+0.42

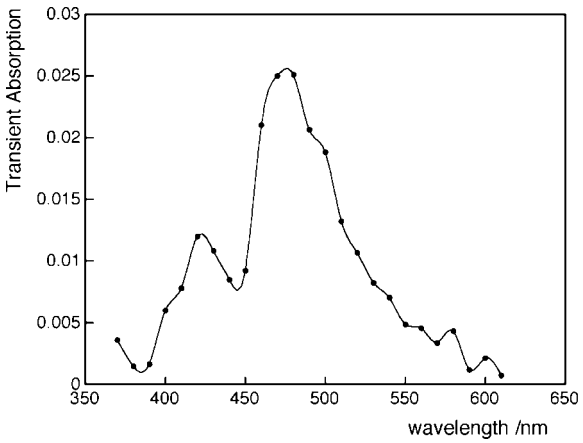

Figure 4. Transient absorption spectra of $[Ru(TAP)_3]^{2+}$ 1×10^{-4} M + GMP 1×10^{-2} M, 0.1 M Tris buffer, pH 7, under argon, recorded 5 μs after the laser pulse (≈8 mJ)

illustrated in Table 1 (entries 1–8) by the very positive values of the excited state reduction potentials or oxidizing powers. These latter have been estimated by using

$$E_{red}\left(RuL_3^{2+*}/\ RuL_3^{+}\right) = E^*_{red} = E_{red} + \Delta E_{00} \approx E_{red} + \Delta E_{\lambda_{max}} \quad (5)$$

in which E_{red} is the reduction potential of the ground state, $\Delta E_{0\text{-}0}$ the energy corresponding to the most bathochromic transition, which could be approximated by the energy of the emission maximum $\Delta E_{\lambda_{max}}$.

The rate constant of the direct ET (Eqn 3) is not experimentally accessible (at least with GMP) because it is diffusion controlled for the most photo-oxidizing complexes.[77] The reduced complex and oxidized GMP that are produced first diffuse away from each other and afterwards give rise to the BET (Eqn 4) in the hundreds of microseconds timescale (in the experimental conditions described in the legend of Fig. 4). Process 4 thus corresponds to a bimolecular equimolecular process.

If we consider the values of the reduction potentials of the excited state of the metal complexes and the oxidation potential of the guanine base, in order to verify the exergonicity of the charge transfer process (Eqn 3), we might conclude that the process is not exergonic. Actually the oxidation potential of the guanine species varies not only with the pH but depends also on the experimental methods (cyclic voltammetry or pulse radiolysis).[78–83] If we take the value determined for the guanosine by cyclic voltammetry, that is 1.25 V *versus* SCE,[80] then a calculation of the exergonicity of the ET process shows that it is unfavourable ($\Delta G^0 \sim 0$ eV), although the process was diffusion controlled with GMP. A possible explanation would be that the ET is actually coupled to a proton transfer (PCET = proton-coupled electron transfer), which favours the reaction. Indeed if we take the value determined by pulsed radiolysis (1.07 V *versus* SCE),[81] which corresponds to the reduction potential of the neutral guanosine radical, thus to the redox couple (G(–H)$^{\cdot}$/G) (i.e. G which has lost one electron and one proton, and this same species which has gained one electron and one proton), an exergonic process is found. This PCET has actually been observed experimentally with the system $[Ru(TAP)_2dppz]^{2+}$ and $[poly(dG\text{-}dC)]_2$ by time resolved studies in the picosecond time domain.[66] Indeed in that case an isotopic effect (H_2O/D_2O) on the ET rate constants has been detected. Picosecond transient absorption spectroscopy by monitoring the metal complex intermediate by electronic absorption and the oxidized guanine intermediate by IR absorption leads to a value for the direct PCET rate of $2.0 \times 10^9\ s^{-1}$ (in H_2O, with a corresponding lifetime of 506 ps) and for the back PCET to a value of $1.1 \times 10^8\ s^{-1}$ (in H_2O, with a corresponding lifetime of 9 ns). Thus a first-order rate constant is obtained because the complex is intercalated between the stack of DNA bases so that the transient species (the reduced protonated complex and the oxidized deprotonated guanine) cannot diffuse away from each other. This study thus highlights the following conclusion: instead of Eqns 3 and 4,

Eqns 3′ and 4′ have to be taken into consideration:

$$^{3}[Ru(III) - L_2L^{\cdot -}]^{2+*} + G \rightarrow [Ru(II) - L_2(L + H)^{\cdot}]^{2+} + G(-H)^{\cdot} \quad (3')$$

$$[Ru(II) - L_2(L + H)^{\cdot}]^{2+} + G(-H)^{\cdot} \rightarrow [Ru(II) - L_3]^{2+} + G \quad (4')$$

in which G base belongs to a mono- or polynucleotide and the processes correspond to a PCET.

In the presence of amino acids and proteins

The TAP complexes, with at least two or three TAP ligands, have also been shown to produce a photo-electron transfer process with some amino acids (tryptophan (Trp) and tyrosine).[84]

Thus the luminescence of $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2phen]^{2+}$ is quenched in the presence of Trp with a quenching rate constant of $4.7 \times 10^9\,M^{-1}\,s^{-1}$ as determined from the Stern–Volmer plots. By flash photolysis experiments, the characteristic spectral features of the monoreduced complex in the transient absorption spectra have afforded a direct evidence for the photo-induced charge transfer process. The thermodynamic parameters of the system that is $E^{+}/E_{Trp} = +0.78$ *versus* SCE at pH 7[85] and $E^{2+*}/E^{+}{}_{Ru} = +1.32\,V$ *versus* SCE for $[Ru(TAP)_3]^{2+}$ indicate that in this case, the process is thermodynamically favourable without proton transfer. In agreement with this, no deuterium effect on the rate constants has been observed.

CONSEQUENCES OF THE PHOTO-INDUCED ELECTRON TRANSFER

As mentioned above, the ET processes occurring between the excited complexes and the biomolecules (with a guanine or Trp moiety) are followed by a BET. However two other processes may compete with this back charge transfer. As observed with plasmid DNA, single-strand breaks followed by double-strand cleavages can occur. Moreover the formation of covalent adducts between the complexes and guanine bases or Trp residues are also detected.

Photocleavage of plasmid DNA

Several Ru(II) complexes can photo-induce with various efficiencies a single-strand break (nick) of DNA plasmids, which transforms the covalently closed circular (CCC) form into the open circular (OC) form of the plasmid.[77,86–90] This strand break could originate, at least for the complexes discussed in this report, from a leak reaction to process of Eqn 4, in which the guanine radical or radical cation escapes from the BET process and reacts with a sugar moiety for example. Many complexes have been investigated in the presence of DNA plasmids by agarose gel electrophoresis experiments because the CCC and OC forms migrate differently. In a more recent work,[91] topological modifications of plasmid DNA, induced by illumination of $[Ru(TAP)_3]^{2+}$, have been studied by atomic force spectroscopy (AFM) on mica. Kinetic studies of the photocleavages have been performed using both AFM and gel electrophoresis. Interestingly, the kinetic results obtained by counting the OC and CCC forms by AFM and electrophoresis as a function of the illumination time are the same. This shows that interesting investigations of plasmids topological changes could be carried out by the AFM technique.

Photo-adduct formation

With guanine species

Another reaction in competition with the BET (Eqn 4) has been detected. It corresponds to the formation of a 'new type' of photo-adduct identified between Ru(II) complexes bearing at least two TAP,[92] two HAT[93] or three bpz ligands[45] and GMP or polynucleotides containing a G-unit.[94] This new type of photo-adduct originates from the formation of a covalent link between one chelated TAP ligand and the exocyclic nitrogen of the G-unit (Fig. 5).[92]

It is proposed that this adduct is formed from the recombination of the two radicals resulting from the PCET (Eqn 3′) and rearomatization of the resulting adduct molecule[95]:

$$\left[Ru(II) - L_2(L + H)^{\cdot}\right]^{2+} + G(-H)^{\cdot} \xrightarrow{\text{rearomatization}} \text{photo} - \text{adduct} \quad (6)$$

The presence of this photo-adduct between the TAP complex and a G-unit (Fig. 5) has been demonstrated by several techniques including polyacrylamide gel electrophoresis experiments,[68,95] electrospray mass spectrometry (ESI/MS),[92,96] dialysis experiments monitored by UV–Vis spectroscopy[95] and steady-state illuminations followed by UV–Vis absorption spectroscopy.[77,95] The absorption spectra resulting from such illuminations show characteristic spectral features of adducts on TAP ligands, that is changes in the 350–390 nm region, with the appearance of hypsochromic and hyperchromic effects (as shown in Fig. 6, for example for $[(TAP)_2$ Ru TPAC $Ru(TAP)_2]^{4+}$ + GMP).

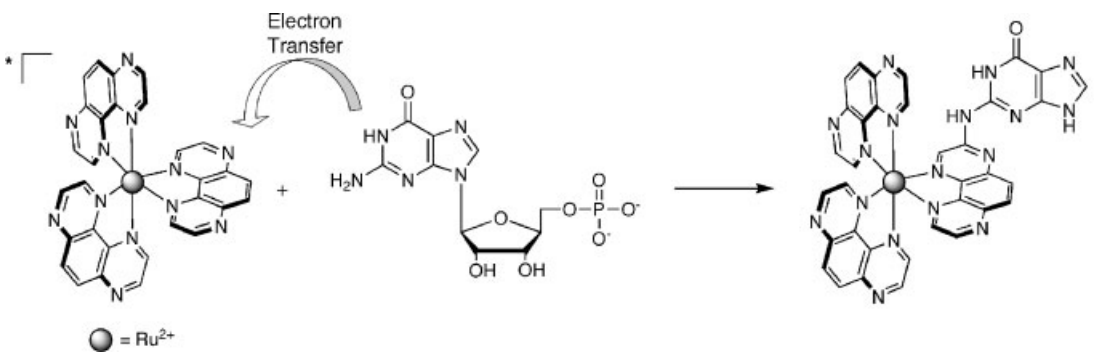

Figure 5. Structure of the photo-adduct formed upon illumination of $[Ru(TAP)_3]^{2+}$ in the presence of GMP, after hydrolysis to remove the sugar–phosphate unit

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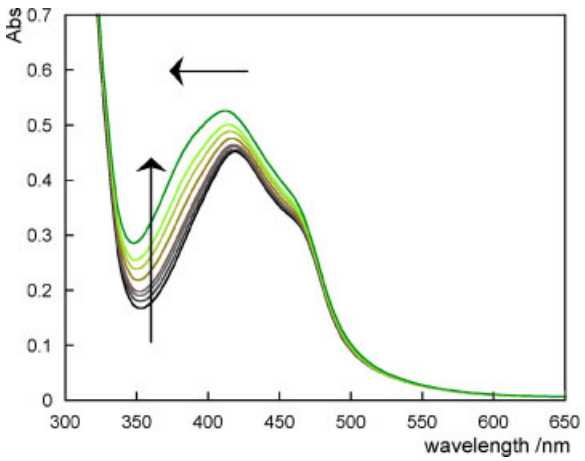

Figure 6. Spectral changes of the absorption of [(TAP)$_2$ Ru TPAC Ru(TAP)$_2$]$^{2+}$ in the presence of GMP 1×10^{-2} M at pH 5 in phosphate buffer solution under argon during a steady-state irradiation (from 0 to 90 min). This figure is available in colour online at www.interscience.wiley.com/journal/poc

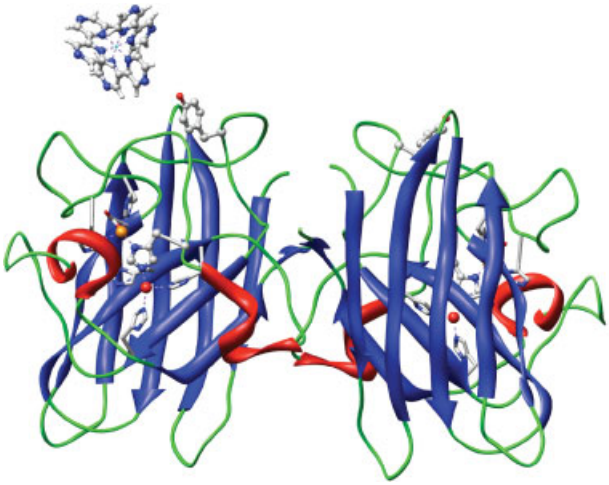

Figure 7. [Ru(bpz)$_3$]$^{2+}$ in the presence of the Cu/Zn superoxide dismutase protein (Cu/Zn SOD), (X-ray structure, PDB 1e9p),[113] with highlighted tyrosine residue at the surface

The structure of the photo-adduct for [Ru(TAP)$_3$]$^{2+}$ or [Ru(TAP)$_2$phen]$^{2+}$ and a G-unit (as shown in Fig. 5) has been determined by 1D and 2D ^{1}H NMR spectroscopy, and is in agreement with ESI/MS spectra.[92,96] In this adduct, the coordination sphere around the metal ion has remained intact. The carbon in '2' position of a TAP ligand (next to the α-position of the non-chelated nitrogen) is covalently linked to the exocyclic amino group of the guanine.

With tryptophan as amino acid

Interestingly, the ET between the excited complexes [Ru(TAP)$_3$]$^{2+*}$ or [Ru(TAP)$_2$phen]$^{2+*}$ and Trp leads to the formation of a photo-adduct.[95] Its presence has been confirmed by absorption spectroscopy after steady-state illumination. But in this case the $^1O_2$ produced by photosensitization by the excited complex plays a non-negligible role. Indeed, in air or oxygenated solutions, the large hyperchromic effect observed around 350–400 nm is mainly attributed to the formation of secondary products, *N*-FK (*N*-formylkynurenine) and HPI (3a-hydroxy-pyrolidinoindole), which result from the reaction of singlet oxygen with Trp.

The structure of the photoproduct, isolated in the absence of oxygen, has not yet been determined by NMR, but a mass corresponding in the case of [Ru(TAP)$_2$phen]$^{2+}$ to the resultant entity [Ru(TAP)$_2$phen+Trp-2H] has been detected by ESI/MS. Moreover, MS/MS analyses show the loss of CO_2 and $NH_2CHCOOH$ moieties, which demonstrates that the Trp is bound to a TAP ligand through its indole part.

In connection with 'the consequences of the photo-electron transfer', the results of a study of a photo-oxidizing complex in the presence of a protein are worthwhile to be mentioned.[43,97,98] The behaviour of the complex [Ru(bpz)$_3$]$^{2+}$ has been investigated in the presence of the Cu/Zn superoxide dismutase protein (Cu/Zn SOD) (Fig. 7).

In this case, several ET processes have been evidenced by flash photolysis and Electron Paramagnetic Resonance (EPR) spectroscopy.[43] A first quenching of the excited complex is observed in the presence of the Cu/Zn SOD, with the production of the monoreduced complex and oxidized enzyme. It is proposed that this photo-oxidation of the enzyme is due to oxidation of amino acids located on the surface of the protein. Indeed (i) the SOD contains at least one of the most reducing amino acid residues (i.e. Trp/tyrosine) on its surface and (ii) the Cu(II) is not accessible to a Ru complex for such a charge transfer process. This photoreduction of [Ru(bpz)$_3$]$^{2+}$ would modify the protein in such a way that the Cu(II) in the active site becomes, after this first ET, accessible to the complex which is indeed able to photoreduce Cu(II) into Cu(I), with generation of a [Ru(bpz)$_3$]$^{3+}$ species.

APPLICATIONS OF THE PHOTO-ADDUCT FORMATION

Some transition metal complexes are known as highly efficient chemotherapeutic agents targeting DNA. For example, the well-known *cis*-platin [Pt(NH$_3$)$_2$Cl$_2$] molecule is currently employed for the treatment of certain human cancer.[99,100] The biological activity of this compound is directly related to the formation of adducts with adenine and guanine bases yielding inter- or intra-strand crosslinks.[101] One of the main interest of the formation of a photo-adduct between a TAP ligand of a Ru complex and a guanine residue is that this type of adduct would be triggered exclusively under illumination. The nature of this photo-adduct as described above is quite different from the adducts formed with *cis*-platin as the photoreaction between Ru(II) complexes and GMP does not disrupt the coordination sphere around the metal ion. Such interesting photochemical properties of Ru(II) complexes make them interesting candidates for photodynamic therapy. For this purpose, the Ru(II) complexes have thus been studied either as a free complex or as conjugates of oligonucleotides, peptides and polymers. Such conjugations of Ru(II) complexes provide an interesting approach to modulate their photoreactions with selective biological agents.

Photo-adduct formation using the free complex

Reaction of compounds with DNA can produce dramatic effects on DNA functions and can disturb the gene expression. This is

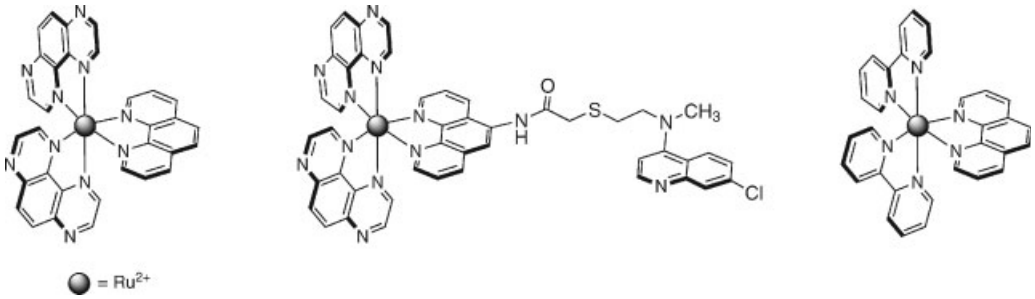

Figure 8. Structure of polyazaaromatic Ru(II) complexes used for *in vitro* assays: $[Ru(TAP)_2phen]^{2+}$, $[Ru(TAP)_2POQ\text{-}NMet]^{2+}$ and $[Ru(bpy)_2phen]^{2+}$

mainly due to the disruption of the binding of enzymes, which are involved in the gene transcription and replication processes. This is indeed of great interest for the inhibition of gene expression in cancerous cells. The photo-active Ru(II) complexes were thus studied as potential anti-cancer drug candidates. The inhibition of transcription was examined for three Ru(II) complexes using *in vitro* assays.[102] It was demonstrated that under illumination $[Ru(TAP)_2phen]^{2+}$ and $[Ru(TAP)_2POQ\text{-}NMet]^{2+}$ (POQ-NMet = 5-[4-[*N*-methyl-*N*-(7-chloroquinolein-4-yl)amino]-2-thiabutanecarboxamido]-1,10-phenanthroline) containing the highly π-deficient polyazaaromatic TAP ligands (Fig. 8) dramatically reduce the transcription rate of a RNA polymerase activity to around 50%, whereas $[Ru(bpy)_2phen]^{2+}$ (Fig. 8) reduced it only to 20%. As a control, no inhibition occurred in the dark. The better activity of the compounds $[Ru(TAP)_2phen]^{2+}$ and $[Ru(TAP)_2POQ\text{-}NMet]^{2+}$ is directly related to the photo-adduct formation whereas the complex $[Ru(bpy)_2phen]^{2+}$ is unable to form a photo-adduct. The effect of such photo-adducts formations on the cellular function could thus open a new way to develop novel potential drugs.

More recently, the dinuclear complex based on the bridging TPAC ligand and containing four TAP ligands, $[(TAP)_2Ru\ TPAC\ Ru(TAP)_2]^{4+}$, was tested under illumination in the presence of different types of nucleic acids, and turned out to be a very efficient photodamaging agent.[103]

Photo-adduct formation using the ODN conjugates

Transforming these photo-active metal complexes to sequence-specific DNA photo-reagents that could inhibit the function of a particular gene in the cell is of great interest. In the aim to achieve this challenging task, the photo-active Ru(II) complexes were conjugated to a particular oligodeoxyribonucleotide (ODN) sequence, which upon illumination could efficiently form the photo-adduct with the guanine base present in the complementary strand leading to the formation of a photocrosslinking between the two DNA strands (Fig. 9).

Furthermore, using these Ru-labelled ODNs, we should expect higher yields of photo-adduct formation due to the presence of the metal complex near to the guanine site of the double-stranded DNA as compared to the free complex in solution. Obviously, these systems are interesting in the area of antisense or antigene strategy for gene expression inhibition.[104]

The photo-adduct formation (resulting thus into a photo-crosslinking between the two DNA strands) is a two-steps phenomenon. The first step is clearly the ET, which depends directly on the ionization potential (IP) (or oxidation potential, as explained above) of stacked guanine residues that in turn are correlated with the percentage of luminescence quenching. The second step involves the recombination of the radicals formed in the ligand and the guanine moiety. Formation of such covalent bond is very much dependent on the relative position (distance) and orientation (overall geometry) between the guanine bases and the attached metal complex. These geometrical factors are mainly controlled by the length and the flexibility of the linker that define the degree of freedom of the complex along the duplex DNA.

Preparation of the Ru(II) metal complex–ODN conjugates

Various duplex DNA sequences containing guanine residues located at different positions of the complementary strand were

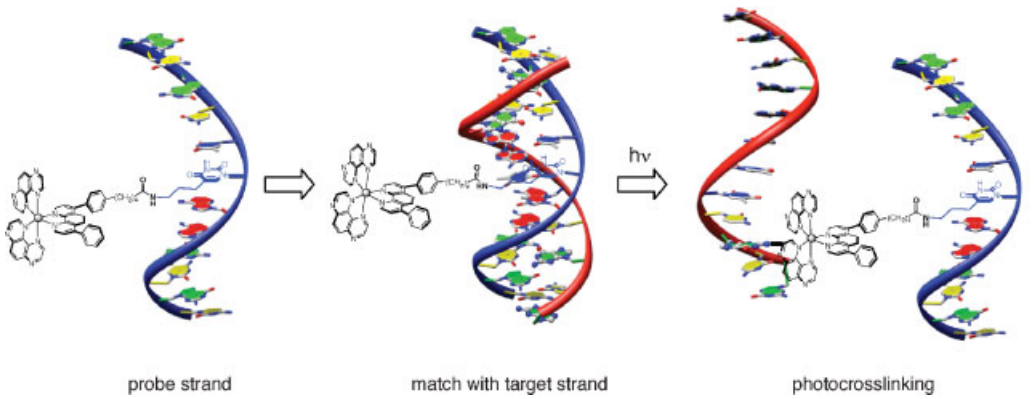

Figure 9. Schematic representation of the photocrosslinking for $[Ru(TAP)_2dip]^{2+}$ anchored to a modified thymidine in the middle of an oligonucleotidic sequence, after matching of the probe strand to the target strand

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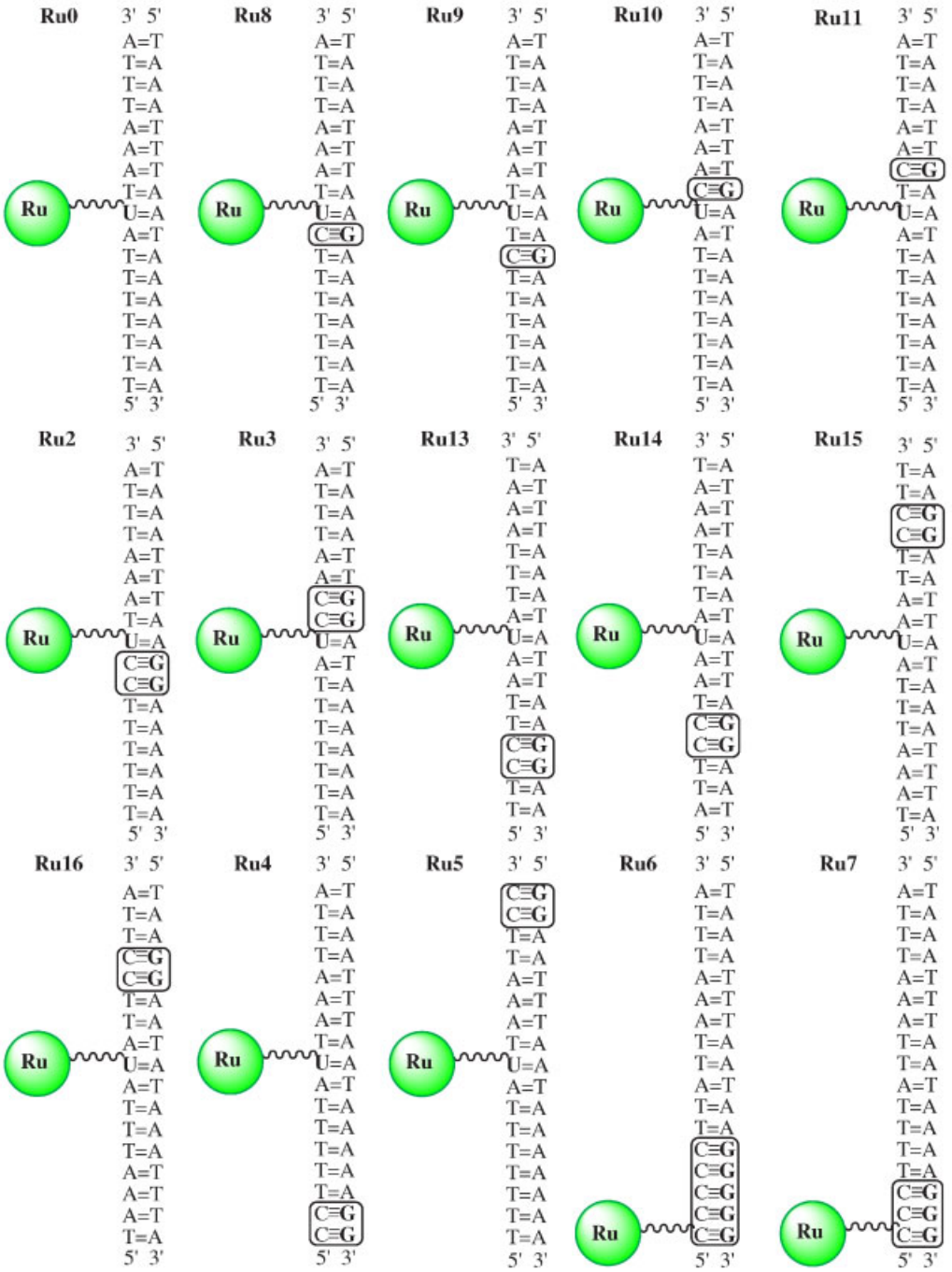

Figure 10. Duplexes conjugates containing the derivatized $[Ru(TAP)_2dip]^{2+}$ complex ('Ru') in the middle or at the 5′-extremity of the sequence. This figure is available in colour online at www.interscience.wiley.com/journal/poc

thus prepared to examine the dependence of the yield of photocrosslinking formation with the IP of the guanine residues as well as with the relative distance and orientation of the guanine bases with respect to the tethered metal complex (Fig. 10).

The metal complex $[Ru(TAP)_2dip]^{2+}$ (dip = 4,7-diphenyl-1,10-phenanthroline) was attached either to a modified thymidine at the central position of the ODN sequence or to the phosphate backbone at the 5′-end of the ODN stretch (Fig. 11).[48]

This would in fact change the micro-environment of the metal complexes along duplex DNA. The metal complex at the middle of the derivatized duplex would get the advantage in targeting the guanine moieties in both directions towards 3′- and 5′-ends. The complex would also be pre-oriented in the hydrophobic environment of the major groove. On the other hand, the metal complex at the 5′-extremity could approach guanine residues present only in the 3′-direction of the complementary ODN stretch. However, less chemical steps are needed for the preparation of 5′-conjugates as compared to the internal conjugation. The synthetic strategies for the preparation of the conjugates involved the coupling reaction of an amino-containing oligonucleotide with the Ru(II) complex bearing an

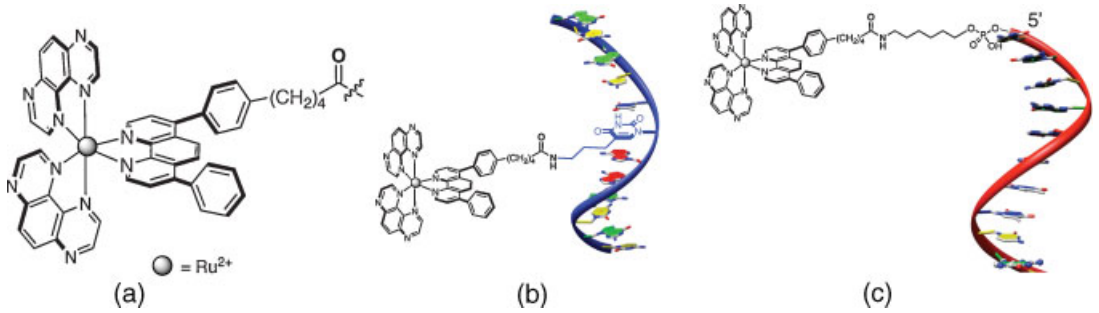

Figure 11. Structure of (a) derivatized $[Ru(TAP)_2dip]^{2+}$ and sites for modified ODNs, (b) inside the sequence and (c) at the 5′-extremity

activated ester on one of the ligands. In general, the amino-modified oligonucleotides were prepared by using the commercially available aminohexyl phosphoramidite at the 5′-end or the phosphoramidite of 5-aminopropyl-2′deoxyuridine for introduction in the middle of the sequence.[105] Such deprotected amino-modified oligonucleotides were subsequently coupled with the active ester functionalized Ru-complexes in H_2O/DMF mixture to form amide-coupled covalent Ru–ODN conjugates. The Ru–ODN conjugates were obtained in about 50% yield after reverse-phase HPLC purification.

However, this amide-coupling strategy involving the reaction between amine derivatized ODN with the activated ester containing metal complex is not very efficient. In fact, a large excess of metal complex is required for the completion of the reaction leading to tedious purification steps. A new and improved methodology has thus been developed to enhance the efficiency of conjugating a variety of biomolecules (i.e. oligonucleotides or peptides) with Ru(II) metal complexes. This involves the formation of oxime bond by reacting with either aldehyde containing ODN or peptide moiety with aminooxy containing metal complexes. Such a coupling strategy does not require any activation and stabilization step but produces a high efficiency of coupling via maintaining regiospecificity of both reactive functionalities.[106] This strategy has been successfully used to couple aminooxy containing $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2phen]^{2+}$ metal complexes at either 5′ or 3′ extremity of the oligonucleotides or *N*-terminus of peptides.[107]

Photochemical studies of the Ru(II) metal complex–ODN conjugates

The different duplex DNA sequences were studied by UV–Vis absorption spectroscopy, steady-state luminescence quenching, lifetime measurements of the excited metal complex (^{3}MLCT state) and denaturing polyacrylamide gel electrophoresis methods in order to characterize the photo-adduct formation by Ru–ODN conjugates with their complementary strands under steady-state illumination (Table 2). The occurrence of a retarded band that migrates like a duplex (i.e. a 34-mer) in the denaturing gel experiments indicates the formation of inter-strand photo-crosslinking upon illumination. Furthermore, the photo-adduct was purified and analyzed by ESI/MS to confirm the formation of such photocrosslinked duplexes.[108]

The photophysical studies of the duplexes revealed that the luminescence quenching can be correlated in first approximation to the IP of the involved guanine residues (Table 2).[109,110] For example, the guanine doublet in sequence **Ru2** has a calculated

Table 2. IP of guanine bases and percentage of luminescence quenching and ODN adduct formation for the $[Ru(TAP)_2phen]^{2+}$-labelled duplex ODNs

Duplex sequence	Calcd. IP (eV)	Quenching (%)	ODN adduct formation (%)	Relative position of G	Distance
Ru0		0	0	—	—
Ru2	6.32	59 ± 2	54 ± 5	3′	0
Ru3	6.42	49 ± 2	17 ± 4	5′	0
Ru4	—	0	0	3′	6
Ru5	—	0	0	5′	6
Ru6	6.17	87 ± 2	56 ± 5	5′	0
Ru7	6.26	81 ± 2	50 ± 4	5′	0
Ru8	6.55	38 ± 2	44 ± 4	3′	0
Ru9	6.55	30 ± 3	41 ± 4	3′	1
Ru10	6.60	31 ± 2	16 ± 4	5′	0
Ru11	6.65	23 ± 3	20 ± 4	5′	1
Ru13	—	4 ± 2	0	3′	4
Ru14	—	4 ± 2	13 ± 3	3′	3
Ru15	—	7 ± 2	4 ± 2	5′	4
Ru16	—	9 ± 2	9 ± 3	5′	3

Distance = number of base pairs between the G base and metal complex-anchored site.

IP value of 6.32 eV whereas for the sequence **Ru3** the calculated IP is 6.42 eV. These IP values are in agreement with the percentage of quenching where **Ru2** gives a quenching of 59 % whereas **Ru3** gives a quenching of 49 %. In addition to the IP values, the percentage of quenching does depend on the distance between the G-base (quencher) and the ODN tethered metal complex. This is evidenced by comparing the sequences **Ru8** and **Ru9** where the G-bases have similar IP values (6.55 eV) but the percentage of quenching is higher when the guanine residue is closer to the site of the anchored metal complex. Another extreme example is the case of the sequences **Ru4** and **Ru5** that contain the GG doublet at six base pairs away from the anchoring site. In both cases, no luminescence quenching is observed. In this case, the length and the flexibility of the linker do not allow the complex to reach the GG doublet located at the end of the duplex. This indicates that a direct contact between a guanine base and the complex is necessary for the photo-induced ET to take place.

The second step of the photo-adduct formation is the recombination of the radicals formed in the ligand and the guanine moiety that is mainly dependent on the geometrical factors. On the basis of geometric constraints of the linker, the metal complex reaches more easily the guanine residues directed towards the 3′-side of the complementary sequence than towards the 5′-side. Indeed the formation of photo-adduct with the guanine residue present at the 3′-side of the complementary sequence is favoured as compared to the guanine base present at the 5′-side. This geometric influence is exemplified by sequences **Ru2**, **Ru8** and **Ru9**, which give a larger amount of ODN adduct than sequences **Ru3**, **Ru10** and **Ru11**, respectively. The influence of the distance between the tethered metal complex and the guanine bases was observed by examining ODN sequences **Ru13**–**16**. Moving the guanine moieties away from the anchoring site of the metal complex induces a decrease in the photo-adduct formation as well as a less extent of luminescence quenching (values in Table 2). All the results clearly demonstrate that for ODN derivatized on a thymidine in the middle of the sequence, the complex could form a photo-adduct until the fourth base towards the 3′-direction and the fifth base towards the 5′-direction.

The 5′-derivatized duplexes **Ru6** and **Ru7**, as compared to a derivatization in the middle of the sequence, showed nearly the same behaviour. However, the yields of photo-adduct are lower in comparison to those expected based on the IP values. This observation stresses again the influence of the linker in directing the metal complex more towards the 3′-side of the complementary strand relative to the 5′-side.

In conclusion, the combination of all three factors such as an efficient ET, a close distance between electron donor and acceptor and a proper geometrical position of the linker could allow to achieve favourable photocrosslinkings between two DNA strands.

Biological effects of Ru-tethered ODN conjugates

One of the challenges to generate DNA damages by anti-tumour drugs is to make these damages stable in biological medium and able to block the activity of the enzymes involved in the DNA repair. The stability of the photocrosslinking product was verified by treating the gel-purified photocrosslinked duplexes **Ru2**, **Ru8** and **Ru10** with piperidine. This method is commonly used to detect oxidative DNA modifications and leads to the formation of an abasic site, which is a key intermediate of DNA repair, and occurs via the cleavage of the *N*-glycosidic bond of the damaged nucleobase. It was found that the *N*-glycosidic bond of the photo-adduct is resistant to piperidine treatment. Thus, we might reasonably extrapolate that the DNA damages caused by photocrosslinking should be stable in living cells. The photo-crosslinked duplexes **Ru2**, **Ru8** and **Ru10** were also incubated in the presence of exonuclease III (a typical exonuclease enzyme) to examine their resistance to 3′-exonucleolytic activity. The results obtained from gel electrophoresis clearly showed that the photo-adduct blocks the exonuclease enzyme activity with 100% efficiency. Both phenomena are quite interesting in view of applying these Ru–ODN conjugates as sequence-specific DNA photoreagents.

Photo-adduct formation using Ru(II)complex–polymer conjugates

Some of the free Ru(II) complexes are promising as anti-cancer drugs, but they exhibit major drawbacks. Generally, they are unable to penetrate through the cell membranes to reach the nucleus. We thus investigated the attachment of polymers to Ru(II) complexes for increasing their cellular uptake efficiency. Poly-[*N*-(2-hydroxyethyl)-L-glutamine] (PHEG) was selected as it is known to be a non-toxic, non-immunogenic polymer, able to accumulate in the tumour regions and penetrate into the cells by endocytosis.[111] Thus, attachment of these photoreactive Ru(II) complexes to this polymer type should enhance the cell penetrating ability and the efficiency for targeting tumour cells. The photo-oxidizing $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2phen]^{2+}$ complexes were anchored to the water soluble 6 or 80 kDa PHEG polymer, respectively by using the oxime bond ligation (Fig. 12).

It was demonstrated that both Ru(II) complexes keep their photoreactive properties towards the guanine moieties despite their attachment to the polymers (Fig. 13).

Further, the formation of the photo-adducts with guanine-containing ODN was clearly demonstrated by gel electrophoresis analyses.[112] As the conjugation of photo-active Ru(II) complexes to polymeric carriers such as PHEG does not disturb the photochemical properties, the investigation of the cell uptake efficiency of these Ru–PHEG conjugates should be carried out in the future to generate an efficient cell penetrating photoreactive drug.

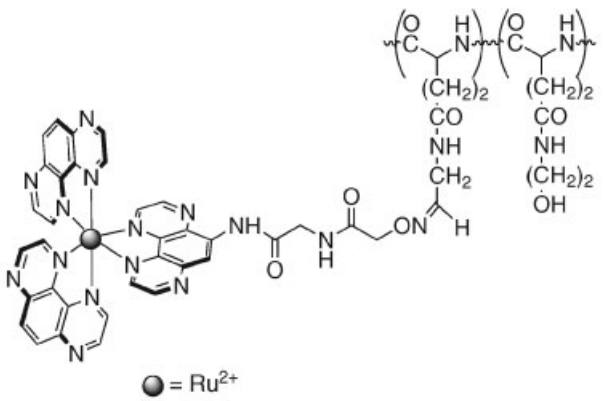

Figure 12. Structure of the derivatized $[Ru(TAP)_3]^{2+}$ anchored to the PHEG polymer through an oxime bond

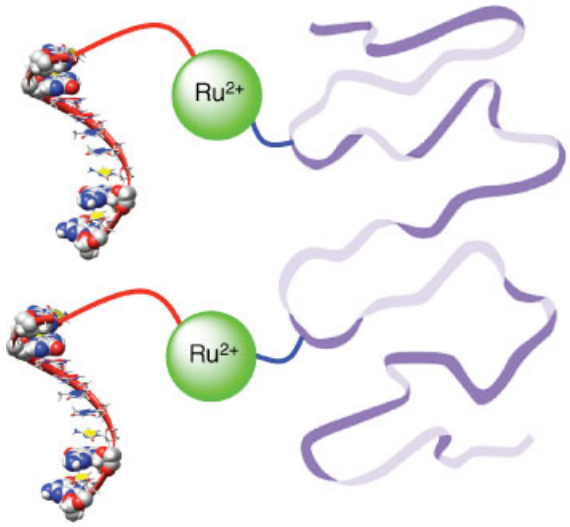

Figure 13. Schematic representation of the product of illumination of photo-oxidizing complexes anchored to the PHEG polymer in the presence of oligonucleotides (represented with space-filled guanines)

CONCLUSIONS AND PERSPECTIVES

The investigation of the different effects consequent to the ET process either from the G bases of DNA or from the Trp residues of peptides, induced by oxidative Ru(II) complexes under irradiation, opens the way to useful applications. Until now as described in this report, we have mainly exploited the formation of photo-adducts with G units, resulting from the primary charge transfer process. As explained, this study has been performed by preparing different Ru–ODN conjugates. In the future, the different pathways that generate such photo-adducts could be developed so that they would efficiently act on the cellular function. This research would allow the investigation of the potentialities of these metallic compounds as novel drugs activated by light. In this context, photoreactive Ru(II) complex–oligonucleotide conjugates are currently examined as gene-silencing agents.

On the other hand, the present results on the photo-adducts formation with Trp residues also offer many new possibilities of applications. In other words, with the Ru–ODN conjugate, one can, not only photocrosslink the two ODN stretches of a duplex moiety, but also a Ru–ODN species with a Trp containing polypeptide. Vice versa, a Ru–peptide conjugate (without Trp) could also be photocrosslinked with a G containing ODN. Thus, the $[Ru(TAP)_2phen]^{2+}$ complex has recently been anchored on a peptidic fragment of the TAT protein, which does not contain any Trp and which is a well-known vectorizing peptide, able to penetrate through the cellular membranes. This investigation should allow to test the photoreaction with different biological components.

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REFERENCES

[1] J. G. Vos, J. M. Kelly, *Dalton Trans.* **2006**, *41*, 4869–4883.
[2] M. K. Nazeeruddin, M. Grätzel, Transition metal complexes for photovoltaic and light emitting applications. *Struct. Bond.* **2007**, *123*, 113–175.
[3] J. N. Clifford, E. Palomares, M. K. Nazeeruddin, M. Grätzel, J. R. Durrant, *J. Phys. Chem. C* **2007**, *111*, 6561–6567.
[4] M. Grätzel, *Inorg. Chem.* **2005**, *44*, 6841–6851.
[5] F. Puntoriero, F. Nastasi, M. Cavazzini, S. Quici, S. Campagna, *Coord. Chem. Rev.* **2007**, *251*, 536–545.
[6] M. Falkenstrom, O. Johansson, L. Hammarstrom, *Inorg. Chim. Acta* **2007**, *360*, 741–750.
[7] S. Rau, D. Walther, J. G. Vos, *Dalton Trans.* **2007**, *19*, 915–919.
[8] S. Campagna, S. Serroni, S. Bodige, F. M. MacDonnell, *Inorg. Chem.* **1999**, *38*, 692–701.
[9] V. Balzani, S. Campagna, G. Denti, A. Juris, S. Serroni, M. Venturi, *Sol. Energy Mater. Sol. Cell.* **1995**, *38*, 159–173.
[10] G. Kalyuzhny, M. Buda, J. McNeill, P. Barbara, A. J. Bard, *J. Am. Chem. Soc.* **2003**, *125*, 6272–6283.
[11] K. C. Gordon, P. J. Walsh, E. M. McGale, *Curr. Appl. Phys.* **2004**, *4*, 331–334.
[12] L. Ronconi, P. J. Sadler, *Coord. Chem. Rev.* **2007**, *251*, 1633–1648.
[13] C. B. Spillane, N. C. Fletcher, S. M. Rountree, H. van den Berg, S. Chanduloy, J. L. Morgan, F. R. Keene, *J. Biol. Inorg. Chem.* **2007**, *12*, 797–807.
[14] A. R. Timerbaev, L. S. Foteeva, A. V. Rudnev, J. K. Abramski, K. Polec-Pawlak, C. G. Hartinger, M. Jarosz, B. K. Keppler, *Electrophoresis* **2007**, *28*, 2235–2240.
[15] R. F. Donnelly, N. C. Fletcher, P. J. McCague, J. Donnelly, P. A. McCarron, M. M. Tunney, *Lett. Drug Des. Discov.* **2007**, *4*, 175–179.
[16] G. L. Fiore, J. M. Edwards, S. J. Payne, J. L. Klinkenberg, D. G. Gioeli, J. N. Demas, C. L. Fraser, *Biomacromolecules* **2007**, *8*, 2829–2835.
[17] M. Brindell, G. Stochel, V. Bertolasi, R. Boaretto, S. Sostero, *Eur. J. Inorg. Chem.* **2007**, *16*, 2353–2359.
[18] K. K. W. Lo, *Struct. Bond.* **2007**, *123*, 205–245.
[19] F. Pierard, A. Kirsch-De Mesmaeker, *Inorg. Chem. Commun.* **2006**, *9*, 111–126.
[20] C. Metcalfe, J. A. Thomas, *Chem. Soc. Rev.* **2003**, *32*, 215–224.
[21] B. T. Patterson, J. G. Collins, F. M. Foley, F. R. Keene, *J. Chem. Soc. Dalton Trans.* **2002**, 4343–4350.
[22] K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.* **1999**, *99*, 2777–2795.
[23] C. S. Chow, J. K. Barton, *Meth. Enzymol.* **1992**, *212*, 219–242.
[24] F. Alary, J. L. Heully, L. Bijeire, P. Vicendo, *Inorg. Chem.* **2007**, *46*, 3154–3165.
[25] M. Atsumi, L. Gonzalez, C. Daniel, *J. Photochem. Photobiol. A: Chem.* **2007**, *190*, 310–320.
[26] G. Pourtois, D. Beljonne, C. Moucheron, S. Schumm, A. Kirsch-De Mesmaeker, R. Lazzaroni, J. L. Bredas, *J. Am. Chem. Soc.* **2004**, *126*, 683–692.
[27] N. Besker, C. Coletti, A. Marrone, N. Re, *J. Phys. Chem. B* **2007**, *111*, 9955–9964.
[28] S. Fantacci, F. De Angelis, A. Sgamellotti, A. Marrone, N. Re, *J. Am. Chem. Soc.* **2005**, *127*, 14144–14145.
[29] D. X. Han, H. Y. Wang, N. L. Ren, *Theochem-J. Mol. Struct.* **2004**, *711*, 185–192.
[30] Z. H. Xiong, P. Yang, *Theochem-J. Mol. Struct.* **2002**, *582*, 107–117.
[31] A. Broo, P. Lincoln, *Inorg. Chem.* **1997**, *36*, 2544–2553.
[32] J. Olofsson, B. Onfelt, P. Lincoln, *J. Phys. Chem. A* **2004**, *108*, 4391–4398.
[33] B. Onfelt, J. Olofsson, P. Lincoln, B. Norden, *J. Phys. Chem. A* **2003**, *107*, 1000–1009.
[34] M. K. Brennaman, J. H. Alstrum-Acevedo, C. N. Fleming, P. Jang, T. J. Meyer, J. M. Papanikolas, *J. Am. Chem. Soc.* **2002**, *124*, 15094–15098.
[35] C. G. Coates, J. J. McGarvey, P. L. Callaghan, M. Coletti, J. G. Hamilton, *J. Phys. Chem. B* **2001**, *105*, 730–735.
[36] E. J. C. Olson, D. Hu, A. Hormann, A. M. Jonkman, M. R. Arkin, E. D. A. Stemp, J. K. Barton, P. F. Barbara, *J. Am. Chem. Soc.* **1997**, *119*, 11458–11467.
[37] C. Moucheron, A. Kirsch-De Mesmaeker, S. Choua, *Inorg. Chem.* **1997**, *36*, 584–592.
[38] A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro, J. K. Barton, *J. Am. Chem. Soc.* **1990**, *112*, 4960–4962.

[39] M. Slim, N. Durisic, P. Grutter, H. F. Sleiman, *Chembiochem* **2007**, *8*, 804–812.
[40] K. K. W. Lo, K. H. K. Tsang, K. S. Sze, C. K. Chung, T. K. M. Lee, K. Y. Zhang, W. K. Hui, C. K. Li, J. S. Y. Lau, D. C. M. Ng, N. Zhu, *Coord. Chem. Rev.* **2007**, *251*, 2292–2310.
[41] M. Slim, H. F. Sleiman, *Bioconjug. Chem.* **2004**, *15*, 949–953.
[42] I. J. Dmochowski, B. R. Crane, J. J. Wilker, J. R. Winkler, H. B. Gray, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12987–12990.
[43] L. Bijeire, B. Elias, J. P. Souchard, E. Gicquel, C. Moucheron, A. Kirsch-De Mesmaeker, P. Vicendo, *Biochemistry* **2006**, *45*, 6160–6169.
[44] C. Moucheron, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Photochem. Photobiol. B: Biol.* **1997**, *40*, 91–106.
[45] P. Vicendo, S. Mouysset, N. Paillous, *Photochem. Photobiol.* **1997**, *65*, 647–655.
[46] A. Kirsch-De Mesmaeker, J.-P. Lecomte, J. M. Kelly, Photoreactions of metal complexes with DNA, especially those involving primary photo-electron transfer, in *Topics in Current Chemistry*, Springer Verlag, Berlin, **1996**, pp. 25–76.
[47] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. B. Tossi, H. Görner, *Photochem. Photobiol.* **1992**, *55*, 681–689.
[48] O. Lentzen, E. Defrancq, J. F. Constant, S. Schumm, D. Garcia-Fresnadillo, C. Moucheron, P. Dumy, A. Kirsch-De Mesmaeker, *J. Biol. Inorg. Chem.* **2004**, *9*, 100–108.
[49] D. Ossipov, S. Gohil, J. Chattopadhyaya, *J. Am. Chem. Soc.* **2002**, *124*, 13416–13433.
[50] T. J. Meyer, *Pure Appl. Chem.* **1986**, *58*, 1193–1206.
[51] S. Yoon, P. Kukura, C. M. Stuart, R. A. Mathies, *Mol. Phys.* **2006**, *104*, 1275–1282.
[52] C.-T. Lin, W. Böttcher, M. Chou, S. Creutz, N. Sutin, *J. Am. Chem. Soc.* **1976**, *98*, 6536–6544.
[53] H. Yersin, E. Gallhuber, *J. Am. Chem. Soc.* **1984**, *106*, 6582–6586.
[54] A. Harriman, G. Izzet, *Phys. Chem. Chem. Phys.* **2007**, *9*, 944–948.
[55] R. S. Lumpkin, E. M. Kober, L. A. Worl, Z. Murtaza, T. J. Meyer, *J. Phys. Chem.* **1990**, *94*, 239–243.
[56] A. Masschelein, L. Jacquet, A. Kirsch-De Mesmaeker, J. Nasielski, *Inorg. Chem.* **1990**, *29*, 855–860.
[57] L. Herman, B. Elias, F. Pierard, C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Chem. A* **2007**, *111*, 9756–9763.
[58] B. Elias, L. Herman, C. Moucheron, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **2007**, *46*, 4979–4988.
[59] A. Kirsch-De Mesmaeker, L. Jacquet, J. Nasielski, *Inorg. Chem.* **1988**, *27*, 4451–4458.
[60] E. Vander Donckt, *Progress in Reaction Kinetics*, Pergamon Press, Oxford, **1970**.
[61] T. Förster, *Z. Elektrochem.* **1950**, *54*, 42–46.
[62] G. R. Pack, L. Wong, *Chem. Phys.* **1996**, *204*, 279–288.
[63] G. Lamm, G. R. Pack, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9033–9036.
[64] J. M. Kelly, A. B. Tossi, D. J. McConnell, C. Oh Uigin, *Nucleic Acids Res.* **1985**, *13*, 6017–6034.
[65] J. M. Kelly, D. J. McConnell, C. Oh Uigin, A. B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein, J. Nasielski, *J. Chem. Soc. Chem. Commun.* **1987**, 1821–1823.
[66] B. Elias, C. Creely, G. W. Doorley, M. M. Feeney, C. Moucheron, A. Kirsch-De Mesmaeker, J. Dyer, D. C. Grills, M. W. George, P. Matousek, A. W. Parker, M. Towrie, J. M. Kelly, *Chem. Eur. J.* **2008**, *14*, 369–375.
[67] C. G. Coates, P. Callaghan, J. J. McGarvey, J. M. Kelly, L. Jacquet, A. Kirsch-De Mesmaeker, *J. Mol. Struct.* **2001**, *598*, 15–25.
[68] J. M. Kelly, M. M. Feeney, A. B. Tossi, J.-P. Lecomte, A. Kirsch-De Mesmaeker, *Anticancer Drug Des.* **1990**, *5*, 69–75.
[69] L. Jacquet, A. Kirsch-De Mesmaeker, *J. Chem. Soc. Faraday Trans.* **1992**, *88*, 2471–2480.
[70] M. A. Haga, E. S. Dodsworth, G. Eryavec, P. Seymour, A. B. P. Lever, *Inorg. Chem.* **1985**, *24*, 1901–1906.
[71] I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron, A. Kirsch-De Mesmaeker, *Dalton Trans.* **2004**, 668–676.
[72] J.-P. Lecomte, A. Kirsch-De Mesmaeker, M. Demeunynck, J. Lhomme, *J. Chem. Soc. Faraday Trans.* **1993**, *89*, 3261–3269.
[73] A. Del Guerzo, A. Kirsch-De Mesmaeker, M. Demeunynck, J. Lhomme, *J. Phys. Chem. B* **1997**, *101*, 7012–7021.
[74] I. Ortmans, *Ph.D.Thesis*, Université libre de Bruxelles, **1996**.
[75] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, A. von Zelewsky, *Coord. Chem. Rev.* **1988**, *84*, 85–277.
[76] J. Leveque, B. Elias, C. Moucheron, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **2005**, *44*, 393–400.
[77] J.-P. Lecomte, A. Kirsch-De Mesmaeker, M. M. Feeney, J. M. Kelly, *Inorg. Chem.* **1995**, *34*, 6481–6491.
[78] C. E. Crespo-Hernandez, D. M. Close, L. Gorb, J. Leszczynski, *J. Phys. Chem. B* **2007**, *111*, 5386–5395.
[79] S. Fukuzumi, H. Miyao, K. Ohkubo, T. Suenobu, *J. Phys. Chem. A* **2005**, *109*, 3285–3294.
[80] C. A. M. Seidel, A. Schulz, M. H. M. Sauer, *J. Phys. Chem.* **1996**, *100*, 5541–5553.
[81] S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617–618.
[82] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109–1151.
[83] S. V. Jovanovic, M. G. Simic, *J. Phys. Chem.* **1986**, *90*, 974–978.
[84] E. Gicquel, A. Boisdenghien, E. Defrancq, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Commun.* **2004**, 2764–2765.
[85] A. Harriman, *J. Phys. Chem.* **1987**, *91*, 6102–6104.
[86] L. F. Tan, H. Chao, *Inorg. Chim. Acta* **2007**, *360*, 2016–2022.
[87] L. F. Tan, H. Chao, Y. F. Zhou, L. N. Ji, *Polyhedron* **2007**, *26*, 3029–3036.
[88] H. Xizo, *Acta Chimi. Sin.* **2007**, *65*, 1464–1468.
[89] M. S. Deshpande, A. A. Kumbhar, A. S. Kumbhar, *Inorg. Chem.* **2007**, *46*, 5450–5452.
[90] B. Armitage, *Chem. Rev.* **1998**, *98*, 1171–1200.
[91] H. Uji-i, P. Foubert, F. C. De Schryver, S. De Feyter, E. Gicquel, A. Etoc, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Eur. J.* **2006**, *12*, 758–762.
[92] L. Jacquet, J. M. Kelly, A. Kirsch-De Mesmaeker, *J. Chem. Soc. Chem. Commun.* **1995**, 913–914.
[93] R. Blasius, H. Nierengarten, M. Luhmer, J.-F. Constant, E. Defrancq, P. Dumy, A. van Dorsselaer, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Eur. J.* **2005**, *11*, 1507–1517.
[94] R. Blasius, C. Moucheron, A. Kirsch-De Mesmaeker, *Eur. J. Inorg. Chem.* **2004**, 3971–3979.
[95] 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.
[96] L. Jacquet, R. J. H. Davies, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Am. Chem. Soc.* **1997**, *119*, 11763–11768.
[97] E. Gicquel, N. Paillous, P. Vicendo, *Chem. Commun.* **1998**, 997–998.
[98] E. Gicquel, N. Paillous, P. Vicendo, *Photochem. Photobiol.* **2000**, *72*, 583–589.
[99] B. K. Keppler, *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim and New York, **1993**.
[100] T. W. Hambley, *J. Chem. Soc. Dalton Trans.* **2001**, 2711–2718.
[101] P. Takahara, A. Rosenzweig, C. Frederick, S. Lippard, *Nature* **1995**, *377*, 649–652.
[102] M. E. Pauly, I. Kayser, M. Schmitz, M. Dicato, A. Del Guerzo, I. Kolber, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Commun.* **2002**, *21*, 1086–1087.
[103] L. Ghizdavu, S. Rickling, S. Le Gac, F. Pierard, M. Surin, C. Moucheron, A. Kirsch-De Mesmaeker, in press **2008**.
[104] D. D. F. Ma, T. Rede, N. A. Naqvi, P. D. Cook, *Biotechnol. Annu. Rev.* **2000**, *5*, 155–196.
[105] 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.
[106] Y. Singh, N. Spinelli, E. Defrancq, *Curr. Org. Chem.* **2008**, *16*, 263–290.
[107] M. Villien, S. Deroo, E. Gicquel, E. Defrancq, C. Moucheron, A. Kirsch-De Mesmaeker, P. Dumy, *Tetrahedron* **2007**, *63*, 11299–11306.
[108] O. Lentzen, J. F. Constant, E. Defrancq, M. Prevost, S. Schumm, C. Moucheron, P. Dumy, A. Kirsch-De Mesmaeker, *Chembiochem* **2003**, *4*, 195–202.
[109] S. Schumm, M. Prevost, D. Garcia-Fresnadillo, O. Lentzen, C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Chem. B* **2002**, *106*, 2763–2768.
[110] D. Garcia-Fresnadillo, N. Boutonnet, S. Schumm, C. Moucheron, A. Kirsch-De Mesmaeker, E. Defrancq, J.-F. Constant, J. Lhomme, *Biophys. J.* **2002**, *82*, 978–987.
[111] H. Maeda, L. Seymour, Y. Miyamoto, *Bioconjug. Chem.* **1992**, *3*, 351–362.
[112] S. Deroo, V. Toncheva, E. Defrancq, C. Moucheron, E. Schacht, A. Kirsch-De Mesmaeker, *Biomacromolecules* **2007**, *8*, 3503–3510.
[113] M. A. Hough, R. W. Strange, S. S. Hasnain, *J. Mol. Biol.* **2000**, *304*, 231–241.