Ru-Labeled Oligonucleotides for Photoinduced Reactions on Targeted DNA Guanines

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Abstract: As a strategy to synthesize new sequence-specific DNA photoreagents, oligodeoxyribonucleotides bearing a photoreactive $[Ru^{II}(tap)_2(dip)]^{2+}$ complex (tap = 1,4,5,8-tetraazaphenanthrene; dip = 4,7-diphenylphenanthroline) tethered to a central nucleotide base have been prepared and characterized. The resulting Ru-labeled oligonucleotides exhibit absorption and emission properties of the tethered metal complex and bind to complementary single-stranded DNA sequences. The thermal denaturation curves are not significantly affected by the chemical attachment of the complex. Steady-state and time-resolved emission data reveal a significant luminescence quenching upon hybridization of the Ru-labeled oligonucleotide with the complementary target strand, if the strand contains guanines. Based on the behavior of the free complex, the quenching process is attributed to a photoinduced electron transfer from the guanines of the target strand. This primary process is related to the formation of photoproduct(s) on the duplex that generate an irreversible photocrosslinking of the two strands. This work constitutes an initial step in the design of sequence-specific photocrosslinking agents.

Keywords: DNA oxidation · electron transfer · oligonucleotides · photooxidations · ruthenium

Introduction

There is considerable interest in the development of new chemical reagents which target nucleic acids in a sequence-specific fashion. One of the most attractive ways of achieving this goal involves tethering of the active compound to a DNA probe sequence.[1–4] In the resulting conjugate, the ability of DNA oligomers to bind specific complementary nucleic acid sequences is in principle retained, whereas ancillary reactive properties are introduced. This strategy has been successfully applied in several fields and particularly with transition metal species. Thus site-specifically platinated oligodeoxyribonucleotides were prepared by automated solid-phase synthesis.[5] Various Ru^{II} compounds such as $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine),[6] $[Ru(dip)_3]^{2+}$ (dip = 4,7-diphenylphenanthroline),[7–10] and enantiomerically pure $[Ru(phen)_2(dppz)]^{2+}$ (phen = 1,10-phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]-phenazine)[11] were anchored to synthetic DNA strands in order to prepare luminescent oligonucleotide probes. The role of DNA in the mediation of the electron transfer process was investigated with oligonucleotides derivatized by metal complexes.[12–15] Oxidative DNA damage and thymine dimer repair by intercalated Rh^{III}-phi and Ru^{III} complexes, attached to the 5′- or 3′- terminal phosphates of an oligonucleotide duplex, were also investigated.[16–18] Sequence-specific artificial nucleases were designed by tethering to single-stranded probe sequences, redox-active metal compounds such as Fe^{II}-EDTA,[19, 20] Cu^{II}-(*o*-phenanthroline)$_2$,[21, 22] Fe^{II}-bleomycin,[23] and several metalloporphyrins.[24–27]

Our research topic has been focused on the use of polypyridyl ruthenium(II) complexes based on tap and hat ligands as depicted in Figure 1 as efficient light-activated reagents of nucleic acids. These complexes were found to produce light-induced strand breaks in plasmid DNA.[28–30] Interestingly, these same tap and hat compounds such as $[Ru(tap)_3]^{2+}$ and $[Ru(tap/hat)_2L]^{2+}$ (L = bpy or phen) are also able to form photoadducts with guanosine-5′-monophosphate

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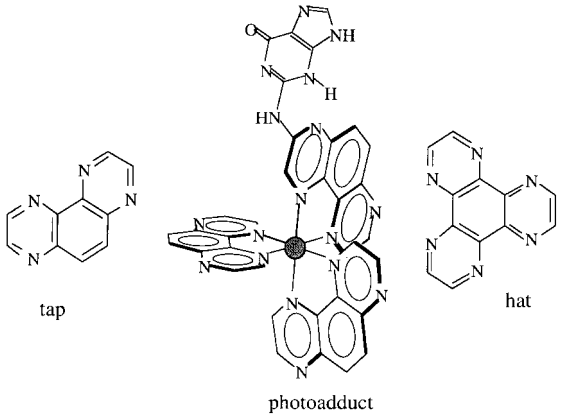


Figure 1. (tap) and (hat) ligands and photoadduct (tap = 1,4,5,8-tetraazaphenanthrene; hat = 1,4,5,8,9,12-hexaazatriphenylene).

(GMP) and the guanines in calf thymus DNA (CT-DNA) and synthetic polynucleotides.[28–31] Both types of DNA damages (photocleavage and photoadduct formation) are initiated by a direct photoinduced electron transfer process from the guanines to the excited complex.[32, 33] The structure of the photoadduct formed with the tap and hat compounds and guanosine-5′-monophosphate or CT-DNA was determined[34–36] (see for example photoadduct in Figure 1 obtained from $[Ru(tap)_3]^{2+}$ and isolated after acid hydrolysis). This photoreaction thus leads to a new mode of covalent binding of metallic species to DNA quite different from that of Pt compounds where the spheres of ligands around the metal change.[5] In order to target this photoreaction on a specific sequence, we have prepared photoreactive Ru-derivatized oligonucleotides.

The tethered metal complex corresponds to a derivative of $[Ru(tap)_2(dip)]^{2+}$ (Figure 2) which, based on previous results

Abstract in French: *En vue de synthétiser des nouveaux agents photoréactifs de séquences spécifiques d'ADN, des oligodéoxyribonucléotides portant un complexe du ruthénium photoactivable $[Ru^{II}(tap)_2(dip)]^{2+}$ (tap = 1,4,5,8-tétraazaphenanthrène; dip = 4,7-diphénylphénanthroline) attaché sur une base nucléotidique centrale ont été préparés et caractérisés. Ces oligonucléotides résultant, marqués au Ru, présentent les propriétés d'absorption et d'émission du composé métallique attaché et s'apparient avec les oligonucléotides simples brins de séquence complémentaire. Les courbes de dénaturation thermique ne sont pas significativement affectées par l'attachement chimique du complexe. Les données d'émission à l'état stationnaire et résolues dans le temps montrent une inhibition significative de la luminescence du composé métallique par hybridation de l'oligonucléotide marqué au Ru avec son brin complémentaire si ce dernier contient des guanines. En se basant sur le comportement du complexe libre, ce processus d'inhibition est attribué à un transfert d'électron photoinduit des guanines du brin cible. Ce processus primaire est lié à la formation de photoadduit(s) sur le duplexe ce qui produit un photo-ancrage irréversible des deux brins l'un à l'autre. Ce travail constitue une étape initiale vers la conception d'agents de photo-ancrage sur des séquences spécifiques.*

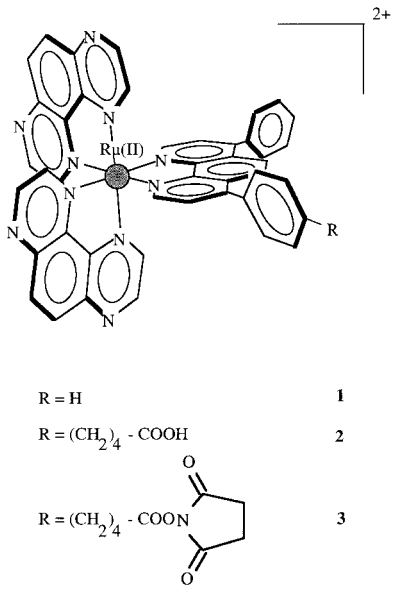


Figure 2. $[Ru(tap)_2(dip)]^{2+}$ and derivatives.

on free Ru^{II} complexes,[29–33, 36, 37] should be able to abstract electrons from guanines and generate guanine photoadducts. In contrast to most studies where compounds were tethered to a 3′- or 5′-terminal phosphate,[1, 2, 12, 13, 16–18] the photoreactive complex is attached to an internal modified base. This anchoring in the middle of a sequence, as also performed recently by other authors,[38] offers several advantages. It allows the targeting of guanines in both directions towards the 3′- and 5′-ends. Moreover, the microenvironment of the nucleobases in the middle of the derivatized duplex should be closer to that of normal double-stranded structures, as a minimum perturbation is introduced by the derivatization at position 5 of a uracil residue.

In this preliminary study, the target sequence includes several guanine residues (six G in total) both on the 3′- and 5′-sides (three G on each side), so as to increase the probability of observing photoreactions.

Results and Discussion

Synthesis of the Ru-labeled oligodeoxyribonucleotides: Two ruthenium-labeled 17-mer sequences were synthesized (Figure 3). The metalated sequence **S1** was designed in order to assess the photoreactivity of the single-stranded tethered metal complex towards guanines contained in the complementary target strand. The second sequence **S2** was prepared as a reference Ru-derivatized oligonucleotide in order to check the absence of photoreactivity of the metal complex in the absence of guanines in the target strand.

The Ru-labeled oligodeoxyribonucleotides were prepared from amino-modified oligomers containing a propylamine linker arm at the position 5 of a central uracil residue. This amino oligonucleotide was synthesized by standard automated solid-phase procedures using phosphoramidites as building blocks. The complex was introduced in a final stage on the fully deprotected oligonucleotides. Preparation of the amino-oligonucleotides required the preliminary synthesis of a

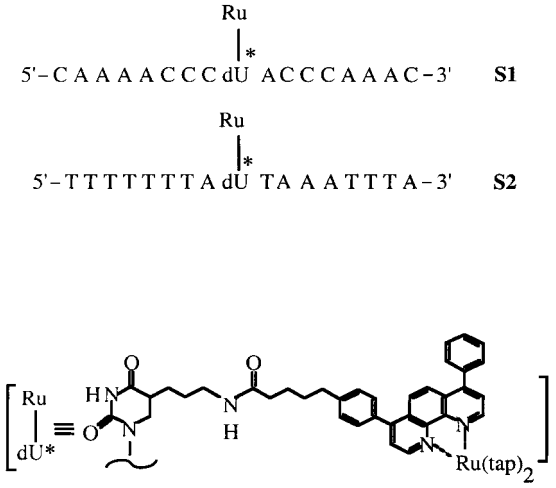
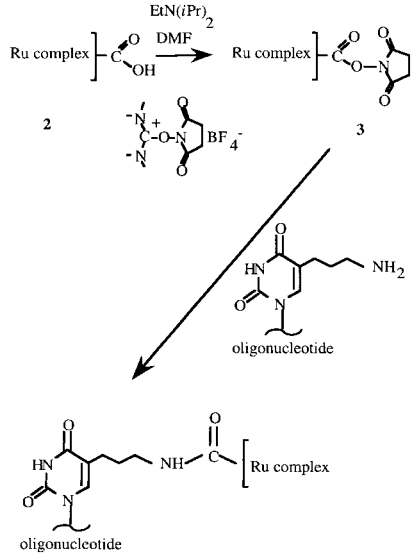


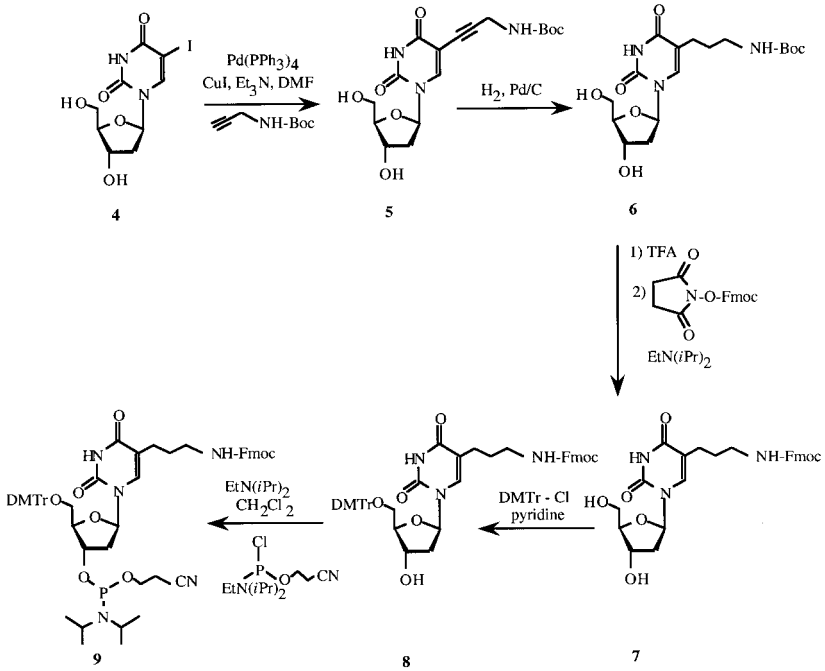
Figure 3. Base sequences of Ru-labeled oligomers **S1** and **S2**.

modified phosphoramidite precursor **9** (Scheme 1). The protected nucleoside **7** was prepared from 5-iodo-2′-deoxyuridine with the palladium(0) coupling procedure described by Hobbs.[39] The propynyl residue was reduced by catalytic hydrogenation. As the acidic conditions necessary for the Boc deprotection could lead to depurination, we decided to protect the amino group with an Fmoc group. The protection and phosphitylation were achieved by conventional means. The Ru compound was first activated before it was coupled with the deprotected amino-modified oligonucleotide. Thus, reaction of the carboxylic acid functionalized ruthenium(II) complex **2** with TSU (TSU = *N*,*N*,*N*′,*N*′-tetramethyl(succinimido)uronium tetrafluoroborate) yielded the corresponding activated ester **3** (Scheme 2), which was added directly to a solution of the amino-oligonucleotide. The coupling of the complex with the oligonucleotide was thus performed in solution. The activated ester reacted preferentially with the primary amine of the linker arm to form a stable amide bond. The resulting Ru-labeled oligonucleotides were purified by polyacrylamide gel electrophoresis and HPLC, and were characterized by electrospray (ES) mass spectrometry.



Scheme 2. Synthesis of the activated Ru^{II} complex and Ru-derivatized oligonucleotide.

Spectroscopic characteristics of the Ru-labeled single-strands: The spectroscopic data recorded for the Ru-labeled single strands are summarized in Table 1. The absorption and emission characteristics of the free $[Ru(tap)_2(dip)]^{2+}$ complex are also included for comparison. All measurements were performed at room temperature in a buffered aqueous solution (50 mM NaCl, 10 mM Tris, pH 7). Typical absorption spectra are displayed in Figure 4. The metalated oligonucleotides show the characteristic metal-to-ligand charge transfer (MLCT) bands of the tethered $[Ru(tap)_2(dip)]^{2+}$ complex at 420 and 460 nm. The UV domain of the spectrum is characterized by an intense band centered around 266–268 nm resulting from ligand-centered (LC) transitions of the metal compound mixed with $\pi-\pi^*$ transitions of the nucleotide bases. The Ru-DNA conjugates show a structureless emission band at room temperature ($\lambda_{max} = 652$ and 654 nm for **S1** and **S2**, respectively). This emission is typical of Ru^{II} polypyridyl complexes and can be attributed to a ^{3}MLCT excited state involving a tap li-



Scheme 1. Synthesis of the modified phosphoramidite precursor for the preparation of amino-oligonucleotides. Boc = *tert*-butoxycarbonyl; Fmoc = 9-fluorenylmethoxycarbonyl; DMTr = 4,4′-dimethoxytrityl.

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Table 1. Spectroscopic data for metalated single strands.[a]

Sample	Absorption λ_{max} [nm]		Emission[b] λ_{max} [nm][c]	Φ_{rel}[d]	Excited-state lifetimes[e] τ_1 [ns]	A_1 [%]	τ_2 [ns]	A_2 [%]
5′CAAAACCCdU*-RuACCCAAAC 3′ (**S1**)	266	420	652	1.5	315	16	707	84
5′TTTTTTTAdU*-RuTAAATTTA 3′ (**S2**)	268	420	654	2.1	721	58	1268	42
$[Ru(tap)_2(dip)]^{2+}$	278	418	652	1.0	580	100		

[a] All measurements were performed at room temperature in air-saturated buffer solutions (50 mM NaCl, 10 mM Tris, pH 7). [b] Excitation at 420 nm. [c] Emission maxima were corrected for the photomultiplier response. [d] Emission quantum yields were determined relative to the emission of free $[Ru(tap)_2(dip)]^{2+}$, which was taken as unity. [e] Luminescence decays monitored at 652 nm ($\lambda_{exc} = 337$ nm) were analyzed according to a biexponential function: $I_{em}(t) = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2)$; normalized preexponential factors ($A_i = \alpha_i/\Sigma\alpha_i$) reflect the contributions of the different decay components to initial emission. Error estimation: 3% for single-exponential and 10% for double-exponential decays.

Table 2. Melting temperatures characterizing metalated duplexes and reference double strands.[a]

Metalated duplex sample	T_m [°C]	Reference duplex sample	T_m [°C]
5′-CAAAACCC dU*-Ru ACCCAAAC-3′ (**S1**) 3′-GTTTTGGG A TGGGTTTG-5′	62	5′-CAAAACCCTACCCAAAC-3′ 3′-GTTTTGGGATGGGTTTG-5′	60
5′-TTTTTTTA dU*-Ru TAAATTTA-3′ (**S2**) 3′-AAAAAAAT A ATTTAAAT-5′	40	5′-TTTTTTTATTAAATTTA-3′ 3′-AAAAAAATAATTTAAAT-5′	40

[a] All duplexes were prepared in buffered aqueous solution (50 mM NaCl, 10 mM Tris, pH 7).

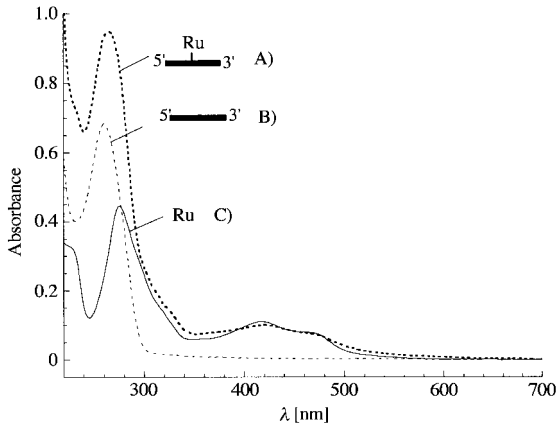

Figure 4. UV/Vis absorption spectra of Ru-labeled oligomer **S1** A), the unmodified single-stranded DNA corresponding to the same base sequence B), and the free $[Ru(tap)_2(dip)]^{2+}$ C). Spectra were recorded in aqueous buffer (50 mM NaCl, 10 mM Tris, pH 7).

gand.[30] Time-resolved luminescence studies indicate that the emission under pulsed illumination is characterized by a biexponential decay, which contrasts the monoexponential behavior characterizing the free excited $[Ru(tap)_2(dip)]^{2+}$ complex. This result reflects the existence of several conformations (at least two) of the Ru-labeled single-strand oligonucleotide. The longer lifetime indicates some protection of the excited state by the single-stranded oligonucleotide versus the aqueous phase. For **S2**, the shorter lifetime is close to the value for the free complex in solution ($\tau_0 = 580$ ns); this indicates a conformation where the complex is in the aqueous phase. The shorter lifetime in **S1** is shorter than τ_0. This could be attributed to the presence of a rapid equilibrium between two conformations of **S1**, in competition with the emission; τ_1 and τ_2 are then combinations of the different rate constants (those related to the excited state and those related to the equilibrium). Similar behavior, with protection or quenching of the excited state, was observed for single-stranded oligonucleotides derivatized at the 5′-end by Rhodamine 6G.[40, 41]

Stability and spectroscopic characterization of the Ru-labeled duplexes: Duplexes of ruthenium-labeled oligonucleotides **S1** and **S2** were formed upon addition of their complementary single-stranded DNA sequence. The addition of the complementary strand to the Ru-derivatized oligonucleotide (4 μM) induced a hypochromic effect of ~4% (absorption λ_{max} around 420 nm). This is in agreement with the behavior of the free[29–33] complex in the presence of double-stranded DNA for which we had concluded that binding takes place in the DNA grooves. The effect of ruthenium labeling on duplex formation and stability was examined on the basis of thermal denaturation profiles recorded by UV/Vis absorption spectroscopy, with unmetalated parent duplexes as reference samples. The melting temperature values (T_m) derived from these denaturation experiments are collected in Table 2. Typical UV absorbance versus temperature profiles recorded for the metalated duplexes and for the unmodified reference double strands are illustrated in Figure 5. The thermal denaturation profiles characterizing the duplexes of meta-

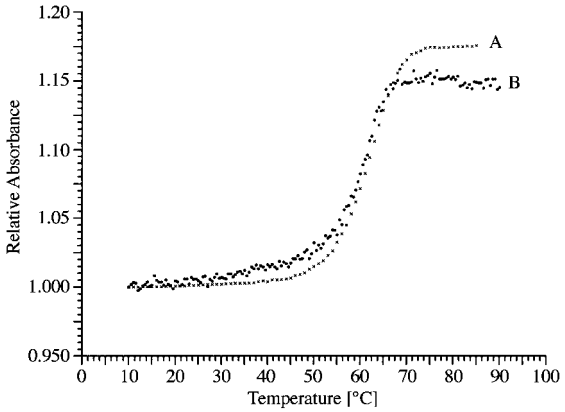

Figure 5. UV absorbance versus temperature profiles recorded for the metalated duplex derived from oligomer **S1** A) and for the corresponding unmetalated parent duplex B). The absorbance was monitored at 260 nm; the temperature was increased by 0.5 °C min^{-1}. Duplex solutions (4 μM) were prepared in aqueous buffer (50 mM NaCl, 10 mM Tris, pH 7).

Table 3. Spectroscopic data for metalated duplexes.[a]

Duplex sample	Emission[b] λ_{max} [nm][c]	Φ_{rel}[d]	Φ_{rel}[e]	Excited-state lifetimes[f] τ_1 [ns]	A_1 [%]	τ_2 [ns]	A_2 [%]	τ_3 [ns]	A_3 [%]
5′-CAAAACCC dU*-Ru ACCCAAAC-3′ (**S1**) 3′-GTTTTGGG A TGGGTTTG-5′	652	0.26	0.22	46	71	229	21	659	8
5′-TTTTTTTA dU*-Ru TAAATTTA-3′ (**S2**) 3′-AAAAAAAT A ATTTAAAT-5′	645	1.2	1.0	632	28	1176	72	–	–

[a] All measurements were performed at room temperature in air-saturated buffer solutions (NaCl 50 mM, Tris 10 mM, pH 7). [b] Excitation at 420 nm. [c] Emission spectra were corrected for the photomultiplier response. [d] Relative emission quantum yields were determined as a ratio of integrated emission spectra relative to the emission measured in the absence of target sequence. [e] Relative emission quantum yields were determined as a ratio of the integrated emission spectra relative to the emission measured with the Ru duplex formed with **S2**, taken as unity. [f] Luminescence decays monitored at 652 nm ($\lambda_{exc} = 337$ nm) were analyzed according to a multiexponential function: $I_{em}(t) = \alpha_i \exp(-t/\tau_i)$; normalized preexponential factors ($A_i = \alpha_i/\Sigma\alpha_i$) reflect the contributions of the different decay components to initial emission. Error estimation: 10% for double exponentials and 20–30% for triexponentials.

lated oligomers **S1** and **S2** are rather similar in shape, and their melting temperatures are similar to those recorded for the reference double strands. This similarity between the denaturation temperatures, in the absence and in the presence of the tethered complex, suggests that the $[Ru(tap)_2(dip)]^{2+}$ label associated to the duplex does not have a great influence on the thermodynamic behavior of the double strand under denaturation conditions, which is in agreement with the nonintercalating characteristics of the complex.[42, 43]

The luminescence data for the metalated duplex solutions recorded at room temperature are collected in Table 3. A slight steady-state emission increase was observed upon addition of the complementary strand of Ru-labeled oligonucleotide **S2** (Table 3). In contrast, annealing of Ru-labeled oligomer **S1** to its complementary strand induced an important decrease in steady-state emission intensity of **S1**. This is illustrated in Figure 6 (top) where the emission spectra of the metalated single strand **S1** are displayed in the presence and in the absence of the target complementary sequence. The corresponding emission quantum yields indicate a 74% inhibition upon duplex formation. In other words, a relative quantum yield of emission of the duplex can be determined either in comparison to the emission of **S1** measured in the absence of the target sequence (Table 3, $\Phi_{rel}^{d} = 0.26$), or in comparison to the emission of the duplex derived from **S2** where no quenching takes place (Table 3, $\Phi_{rel}^{e} = 0.22$). The intramolecular or intraduplex nature of the luminescence quenching process was confirmed by recording the emission intensity of the metalated duplexes as a function of temperature. Figure 6 (bottom) shows the resulting profile for the duplex of Ru-labeled oligonucleotide **S1**. This emission intensity profile exhibits a thermal transition corresponding to luminescence restitution induced by duplex denaturation in the 50–70 °C temperature range. The intensity decrease observed on each side of the denaturation temperature range may be attributed to the photophysics of $[Ru(tap)_2(dip)]^{2+}$ as a function of temperature. Indeed, a thermally activated crossing from the ^{3}MLCT state to the ^{3}MC (metal-centered) state[44, 45] is responsible for the decrease in emission with enhanced temperature.[46] Moreover, the fact that the denaturation temperature derived from absorption measurements at 260 nm (Figure 5) is the same as that measured from luminescence data (Figure 6, bottom) indicates clearly that the luminescence quenching may be attributed to the hybridization process with the complementary sequence containing guanines and does not result from any other intermolecular quenching process. As there is no luminescence quenching upon hybridization of **S2** with its complementary sequence, a curve as in Figure 6 (bottom) (thus with increased emission at the denaturation temperature) is not obtained. In this case the emission intensity decreases continuously with temperature (from 10 °C to 45 °C the drop in intensity corresponds to ~31% of the initial value, and is thus comparable to duplex

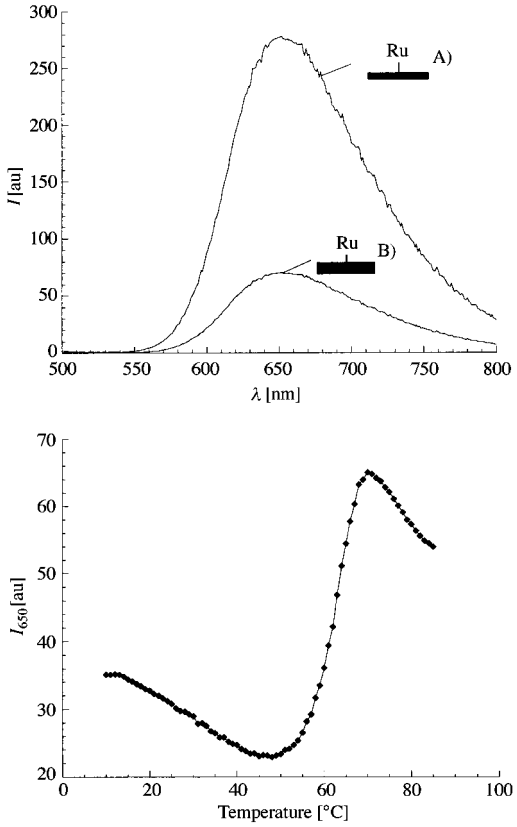

Figure 6. Top) Steady-state emission spectra of Ru-labeled oligomer **S1** in the absence A) and in the presence of the complementary target strand B). Bottom) Emission intensity versus temperature profile recorded for the metalated duplex derived from oligomer **S1.** Emission was monitored at 650 nm ($\lambda_{exc} = 420$ nm) and the temperature was increased by 0.5 °C min^{-1}. All measurements were performed in aqueous buffer (50 mM NaCl, 10 mM Tris, pH 7, and 4 μM oligonucleotide).

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S1 for the same temperature domain where there is no denaturation).

Time-resolved luminescence data recorded for the metalated duplexes are consistent with the steady-state emission results. A biexponential decay is observed for the duplex of **S2** where no luminescence quenching takes place (Table 3). In this case, the shorter lifetime approaches that of the free excited complex (Table 1). This indicates that a part of the tethered complex is not protected by the double helix and thus does not interact significantly inside the duplex. The longer component of the decay would correspond to the fraction of the excited states protected from water by the duplex. In contrast, the multiexponential decay obtained for the duplex of oligonucleotide **S1** is dominated by a short-lived component whose lifetime is significantly shorter than the values that characterize the free complex (compare results in Table 3 with those in Table 1). This indicates the existence of quenching which is in agreement with the steady-state luminescence data.

With regard to the excited-state lifetimes, the data for hybridized **S1** correspond to a triexponential decay (Table 3); thus the analyses according to biexponential decays did not furnish satisfactory fittings of the calculated curve with the experimental ones, whereas a treatment according to a triexponential decay was satisfactory. This does not exclude the fact that more than three excited species are responsible for the decay, rather it remains that most of the contribution originates from quenched species. On the other hand, one could be tempted to compare quantitatively the steady-state emission data with the time-resolved luminescence results. This is, however, not possible because the steady-state relative emission quantum yields and the excited-state lifetimes have not been measured at the same excitation wavelength, and different excitation wavelengths could produce variable contributions of the different excited species.

In conclusion, the emission results establish clearly the occurrence of luminescence quenching processes involving the guanine bases opposite to the ruthenium-labeled site in the double strand derived from **S1**. The quenching may be attributed to a photoinduced electron transfer from the targeted guanines to the excited state of the tethered metal compound. This interpretation is supported by earlier studies.[29, 30, 32, 33, 36, 37, 47]

Steady-state illuminations of the Ru-labeled duplexes

Photoproduct formation: Previous studies with $[Ru(tap/hat)_2L]^{2+}$ complexes in the presence of guanine-containing polynucleotides showed[29, 30, 36] that the luminescence quenching was directly correlated with the formation of a photoadduct of the tap and hat complexes with the guanine bases.[29, 30] The structure of one of these photoadducts with $[Ru(tap)_3]^{2+}$,[34, 35] which was determined by NMR spectroscopy (Figure 1) has been studied along with other $[Ru(tap/hat)_2L]^{2+}$ complexes.[35] Interestingly, the formation of these photoadducts can be verified very easily by the appearance under continuous illumination of a new band in the Ru^{II} complex absorption spectrum, which is hypsochromic as compared to the absorption of the starting material.[34, 35] This characteristic blue shift indicates that the photoproduct still contains the three bidentate ligands; otherwise the loss of a tap or a dip ligand with substitution by H_2O or Cl^- would have produced a bathochromic shift.[34, 35] Moreover, this hypsochromicity indicates that the π^* level involved in the MLCT absorption has been raised by the mesomeric donor effect of a substituent on the azaaromatic ligand; thus, in agreement with the presence of an amino function, and with the structure of the photoadduct shown in Figure 1.

In order to test whether these characteristic absorption changes could also be detected for the duplex formed with **S1**, steady-state illumination experiments of this Ru-labeled duplex have been carried out. The spectral changes observed upon illumination of the tethered Ru^{II} complex in duplex **S1** (Figure 7, top) are comparable to those obtained by illumination of free $[Ru(tap)_2(dip)]^{2+}$ in the presence of CT-DNA (Figure 7, bottom) and are characteristic of a photoadduct.[36] In contrast, no absorption changes were observed under continuous illumination of the **S2**-containing duplex, or under continuous illumination of the **S1** single-strand oligonucleotide. Quite clearly, a photoproduct is formed in the **S1** duplex and is related to luminescence quenching.

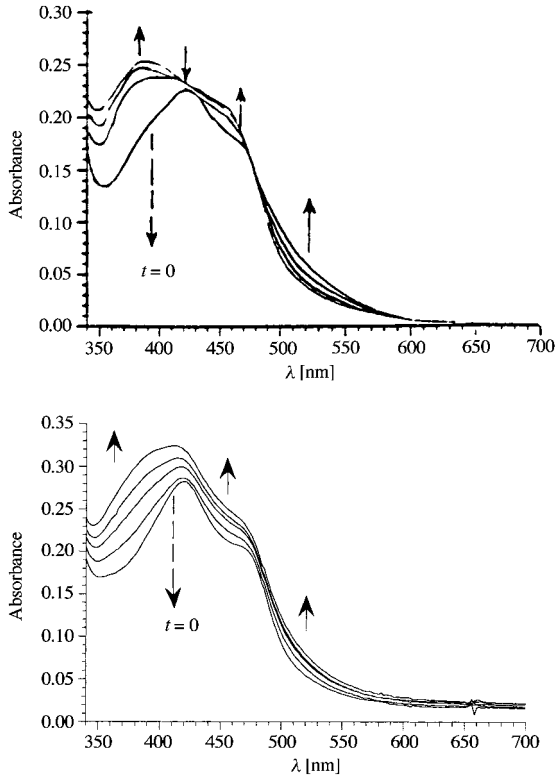

Figure 7. Top) Visible absorption spectra of the metalated duplex derived from oligomer **S1** as a function of steady-state illumination time (50 mM NaCl, 10 mM Tris, pH 7), $t = 0$, 60, 90, 120 min. Bottom) Visible absorption spectra of $[Ru(tap)_2(dip)]^{2+}$ in the presence of CT-DNA, as a function of steady-state illumination time (1.5 mM complex, 5 mM phosphate buffer, pH 7, DNA/complex = 100), $t = 0$, 40, 80, 120 min.

These experiments show the same correlation as previously observed with the free complex, in other words the photoelectron transfer quenching of the oligonucleotide-attached complex by the guanines of the complementary target strand, also induces the formation of photoproduct(s).

Photocrosslinking of the two strands: The similarity of the spectral characteristics of the photoproduct(s) formed on the **S1** duplex with those of the photoadduct formed between the same complex and DNA (Figure 7, bottom) suggests that the duplex photoproduct could be a photoadduct. Such a photoreaction would result in photocrosslinking between the two strands of the **S1** duplex since the ruthenium complex would be attached to one strand by the linker arm and to the target strand through the covalent bonding to a guanine. Figure 8

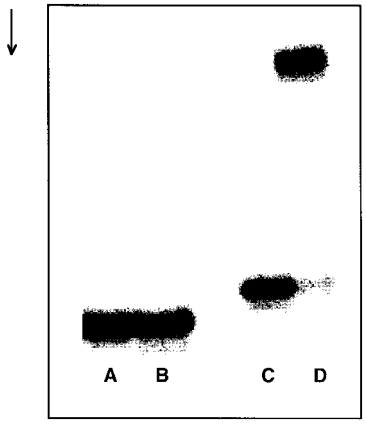

Figure 8. Autoradiogram of a 20% denaturing polyacrylamide gel showing the ^{32}P-end labeled 17-mer **S1** and **S2** duplexes where only the non-Ru-derivatized single-strand oligonucleotide (target strand) was labeled with ^{32}P. The reaction mixture contained 5 pmol 5′-^{32}P-labeled oligonucleotide duplex, 10 mM Tris-HCl (pH 7), 50 mM NaCl in a total volume of 10 μL. Lane A: duplex **S2**. Lane B: duplex **S2** illuminated for 1 hour, lane C: duplex **S1**, lane D: duplex **S1** illuminated for one hour. Arrow: direction of migration.

shows the autoradiogram from the illumination experiments of the **S1** and **S2** duplexes analyzed by using polyacrylamide gel electrophoresis (PAGE) in denaturing conditions. In such conditions, only the migration of the 5′-end labeled single-strand complementary to **S1** and **S2** (see Experimental Section) is visualized on the gel. Lanes A and B correspond to the **S2** duplex before and after continuous illumination, respectively. The electrophoretic mobility is not affected by the illumination treatment since this sequence is devoid of a guanine and does not lead to the formation of a photoproduct. Lane C corresponds to nonilluminated **S1** duplex. Lane D shows the effect of continuous illumination on the **S1** duplex which leads to formation of a retarded band that migrates like a duplex and corresponds to covalent crosslinking between the two strands.

Conclusion and Perspectives

The results presented in this paper establish the successful design of $[Ru(tap)_2(dip)]^{2+}$-DNA conjugates which retain the absorption and emission characteristics of the tethered metal complex and the hybridization properties of the DNA probe. Moreover, on the basis of the luminescence data, we can now answer the questions raised at the beginning of this work. The photooxidative behavior of the tethered metal complex towards target strands containing guanines is retained. The formation of photoproduct(s) under continuous illumination is confirmed by the occurrence of a hypsochromic absorption that increases with the illumination time and by the formation of interstrand linkage(s) evidenced by the retarded band observed in the PAGE experiment. We can thus conclude from the study of this model, in which six guanines residues are present in the vicinity of the chemically attached complex of the probe sequence, that these synthetic Ru-derivatized oligonucleotides exhibit the same characteristics and interesting properties as the free tap complexes. This oligonucleotide system thus allows the introduction of an irreversible modification on a targeted sequence. Moreover, it should also be suitable for examining the conditions for both the photoelectron transfer and the formation of photoadduct(s) with other sequences. It is clear that these conditions cannot be the same for both processes.

Experimental Section

Synthesis: ^{1}H NMR spectra were recorded on a Bruker 360 MHz or, for the Ru complexes, on a Varian Unity 600 MHz spectrometer. Chemicals and solvents were purchased from Sigma or Aldrich chemical companies, and Sephadex SP-C25 cation-exchange resin from Pharmacia. The activation agent *N*,*N*,*N*′,*N*′-tetramethyl(succinimido)uronium tetrafluoroborate (TSU) was prepared according to a previously described procedure.[7, 48] Anhydrous DMF, pyridine, and CH_2Cl_2 were stored over molecular sieves prior to use.

Ruthenium complex 2: 5-[*p*-(7-Phenyl-1,10-phenanthroline-4-yl)phenyl] pentanoic acid (86 mg, 0.2 mmol), corresponding to the carboxylic acid functionalized dip ligand, was added to a suspension of $[Ru(tap)_2Cl_2]$ (80 mg, 0.15 mmol) in refluxing EtOH/H_2O 1:1 (20 mL). The derivatized dip ligand was prepared according to a previously described procedure.[7] The $[Ru(tap)_2Cl_2]$ precursor was synthesized from $RuCl_3$ and tap analogously to other complexes.[49] After refluxing for 36 hours under argon, the reaction mixture was filtered and concentrated. Purification on a cation-exchange Sephadex SP-C25 column with aqueous NaCl as eluent provided complex **2** (42 mg, 29% yield). ^{1}H NMR ([D$_6$]DMSO); T = tap, D = dip: δ = 1.62 (m, 4H; CH_2-(CH_2)$_2$-CH_2), 2.37 (t, 2H; CH_2-COO), 2.72 (t, 2H; C_6H_4-CH_2), 7.48 (d, 2H of C_6H_4), 7.56 (d, 2H of C_6H_4), 7.64 (m, 5H; C_6H_5), 7.73 (2d, 2H; D_3,D_8), 8.25 (2d, 2H; D_5,D_6), 8.35 (2d, 2H; D_2,D_9), 8.40 (d, 2H; T_3,$T_{3'}$ or T_6,$T_{6'}$), 8.52 (d, 2H; T_3,$T_{3'}$ or T_6,$T_{6'}$), 8.68 (s, 4H; T_9,$T_{9'}$,T_{10},$T_{10'}$), 9.08 (d, 2H; T_2,$T_{2'}$ or T_7,$T_{7'}$), 9.12 (d, 2H; T_2,$T_{2'}$ or T_7,$T_{7'}$).

Activated ruthenium complex 3: TSU (7 mg, 0.024 mmol) and EtN(*i*Pr)$_2$ (5 μL, 0.03 mmol) were added to a solution of **2** (20 mg, 0.02 mmol) in dry DMF (1 mL) under exclusion of moisture. The mixture was stirred in the dark at room temperature for two hours under argon. The DMF was then removed under vacuum and the residue was suspended in Et_2O. After filtration, the residue was washed several times with Et_2O and dried to give **3** (18 mg, 85% yield), which can be used directly for the coupling with amino-modified oligonucleotides. ^{1}H NMR ($CDCl_3$); T = tap, D = dip: δ = 1.56 (m, 4H; CH_2-(CH_2)$_2$-CH_2), 2.72 (t, 2H; CH_2-COO), 2.80 (t, 2H; C_6H_4-CH_2), 3.00 (s, 4H; OC-(CH_2)$_2$-CO), 7.37 (d, 2H of C_6H_4), 7.48 (d, 2H of C_6H_4), 7.55 (m, 5H; C_6H_5), 7.76 (2d, 2H; D_3,D_8), 8.20–8.28 (m, 6H; T_3,$T_{3'}$ or T_6,$T_{6'}$, D_2,D_9,D_5,D_6), 8.50 (d, 2H; T_3,$T_{3'}$ or T_6,$T_{6'}$), 8.60 (s, 4H; T_9,$T_{9'}$,T_{10},$T_{10'}$), 9.14 (d, 2H; T_2,$T_{2'}$ or T_7,$T_{7'}$), 9.17 (d, 2H; T_2,$T_{2'}$ or T_7,$T_{7'}$); FAB MS: calcd 994; found 995 $[M^{2+}+e^-+H]^+$.

5-(3-*N*-(*tert*-Butoxycarbonyl)amino-1-propynyl)-2′-deoxyuridine (5): Products **5** and **6** were prepared analogously to the procedure in reference [50]. Et_3N (0.788 mL, 5.6 mmol), *N*-*tert*-butoxycarbonyl-1-amino-3-propyne (1.316 g, 8.4 mmol), and tetrakis(triphenylphosphane)palladium(0) (325 mg, 0.28 mmol) were added to a suspension of 5-iodo-2′-deoxyuridine (**4**) (1 g, 2.8 mmol) and copper(I) iodide (107 mg, 0.56 mmol) in dry DMF (15 mL). (*N*-*tert*-Butoxycarbonyl-1-amino-3-propyne was prepared from *N*-(*tert*-butoxycarbonyloxy)succinimide and propargylamine.) The mixture was stirred at room temperature under argon in the dark for 12 hours and then concentrated under vacuum. The oily residue

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was purified by flash chromatography (SiO_2, MeOH (9–11%)/CH_2Cl_2) to give **5** (833 mg, 78% yield); ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.39$ (s, 9H; C-(CH_3)$_3$), 2.11 (m, 2H; H$_{2'}$,H$_{2''}$), 3.58 (m, 2H; H$_{5'}$,H$_{5''}$), 3.79 (m, 1H; H$_{4'}$), 3.93 (d, 2H; $J = 5.7$ Hz, C-CH_2-NH), 4.22 (m, 1H; H$_{3'}$), 5.05 (t, 1H; $J =$ 5.0 Hz, C$^{5'}$-O*H*), 5.21 (d, 1H; $J = 4.3$ Hz, C$^{3'}$-O*H*), 6.11 (t, 1H; $J = 6.7$ Hz, H$_{1'}$), 7.27 (t, 1H; CH$_2$-N*H*-CO), 8.12 (s, 1H; H$_6$), 11.57 (br s, 1H; N^3-*H*).

5-(3-*N*-(*tert*-Butoxycarbonyl)aminopropyl)-2′-deoxyuridine (6): A mixture of **5** (1.45 g, 3.8 mmol) and 10% Pd/C (195 mg) in MeOH (125 mL) was stirred under H_2 pressure (40 psi) at room temperature. After five hours, another batch of Pd/C was added and the suspension was stirred for five hours. The mixture was filtered through celite and concentrated. Flash chromatography (SiO_2, MeOH (10%)/EtOAc) provided **6** (1.274 g, 87% yield). ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.38$ (s, 9H; C-(CH_3)$_3$), 1.52 (m, 2H; H$_{\beta}$,H$_{\beta'}$), 2.10 (t, 2H; H$_{\alpha}$,H$_{\alpha'}$), 2.16 (m, 2H; H$_{2'}$,H$_{2''}$), 2.90 (m, 2H; H$_{\gamma}$,H$_{\gamma'}$), 3.57 (m, 2H; H$_{5'}$,H$_{5''}$), 3.76 (m, 1H; H$_{4'}$), 4.24 (m, 1H; H$_{3'}$), 4.96 (t, 1H; $J =$ 5.1 Hz, C$^{5'}$-O*H*), 5.20 (d, 1H; $J = 4.2$ Hz, C$^{3'}$-O*H*), 6.17 (t, 1H; $J = 6.9$ Hz, H$_{1'}$), 6.74 (t, 1H; CH$_2$-N*H*-CO), 7.66 (s, 1H; H$_6$), 11.23 (s, 1H; N^3-*H*).

5-(3-*N*-(9-Fluorenylmethoxycarbonyl)aminopropyl)-2′-deoxyuridine (7): The synthesis of **7** was adapted from a previously published procedure.[51] Trifluoroacetic acid (1.8 mL) was added to a suspension of **6** (1.1 g, 2.9 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for one hour and additional trifluoroacetic acid (0.8 mL) was added. After stirring for one hour, the mixture was evaporated with MeCN (4 × 40 mL). The residue was suspended in water (15 mL) and a solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1.1 g, 3.3 mmol) in THF (15 mL) was added, followed by addition of EtN(*i*Pr)$_2$ (2.25 mL, 13.2 mmol). After stirring for 90 min at room temperature, the mixture was poured into water (10 mL) and extracted three times with CH_2Cl_2. A white solid corresponding to **7** was formed during the extraction. The combined organic layers were filtered, dried over $MgSO_4$, concentrated and purified by flash chromatography (SiO_2, MeOH (8–10%)/CH_2Cl_2) to provide an additional batch of the desired product. Filtration and flash chromatography gave **7** (1.266 g, 86% yield). ^{1}H NMR ([D$_6$]DMSO): $\delta =$ 1.56 (m, 2H; H$_{\beta}$,H$_{\beta'}$), 2.09 (t, 2H; H$_{\alpha}$,H$_{\alpha'}$), 2.19 (m, 2H; H$_{2'}$,H$_{2''}$), 2.98 (m, 2H; H$_{\gamma}$,H$_{\gamma'}$), 3.57 (m, 2H; H$_{5'}$,H$_{5''}$), 3.77 (m, 1H; H$_{4'}$), 4.21–4.23 (m, 4H; H$_{3'}$, O-CH_2-Ar, H$_9$ (Fmoc)), 4.97 (t, 1H; $J = 5.1$ Hz, C$^{5'}$-O*H*), 5.20 (d, 1H; $J = 4.2$ Hz, C$^{3'}$-O*H*), 6.17 (t, 1H; $J = 6.8$ Hz, H$_{1'}$), 7.25 (t, 1H; CH$_2$-N*H*-CO), 7.32 (t, 2H; $J = 7.2$ Hz, H$_3$,H$_6$ or H$_2$,H$_7$ (Fmoc)), 7.41 (t, 2H; $J = 7.2$ Hz, H$_3$,H$_6$ or H$_2$,H$_7$ (Fmoc)), 7.66 (s, 1H; H$_6$ (thymine)), 7.68 (d, 2H; $J = 7.2$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 7.88 (d, 2H; $J = 7.2$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 11.24 (s, 1H; N^3-*H*).

5-(3-*N*-(9-Fluorenylmethoxycarbonyl)aminopropyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (8): 4,4′-Dimethoxytrityl chloride (725 mg, 1.5 mmol) was added in small portions over one hour to a solution of **7** (520 mg, 1.5 mmol) in dry pyridine (10 mL) at room temperature under exclusion of moisture. After two hours stirring under argon at room temperature, the reaction mixture was evaporated with toluene (3 × 25 mL). Prior to the last evaporation step, MeOH (3 mL) was added. The residue was dissolved in CH_2Cl_2 (100 mL), poured into TEAA buffer (100 mL, 0.1M Et$_3$N, 0.1M AcOH, pH 7), and extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (SiO_2, MeOH (1–5%)/EtN(*i*Pr)$_2$ (0.5%)/CH_2Cl_2) to give **8** (853 mg, 81% yield). ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.40$ (m, 2H; H$_{\beta}$,H$_{\beta'}$), 1.92 (t, 2H; H$_{\alpha}$,H$_{\alpha'}$), 2.15–2.24 (m, 2H; H$_{2'}$,H$_{2''}$), 2.80 (m, 2H; H$_{\gamma}$,H$_{\gamma'}$), 3.07 (m, 2H; H$_{5'}$,H$_{5''}$), 3.71 (s, 6H; Ar-O-CH_3 (DMTr)), 3.87 (m, 1H; H$_{4'}$), 4.19–4.28 (m, 4H; H$_{3'}$, O-CH_2-Ar, H$_9$ (Fmoc)), 5.29 (d, 1H; $J = 4.6$ Hz, C$^{3'}$-O*H*), 6.19 (t, 1H; $J = 6.8$ Hz, H$_{1'}$), 6.86 (d, 4H; $J = 8.6$ Hz, H$_3$,H$_{3'}$,H$_5$,H$_{5'}$ (DMTr)), 7.16 (t, 1H; CH$_2$-N*H*-CO), 7.27 (d, 4H; $J = 8.6$ Hz, H$_2$,H$_{2'}$,H$_6$,H$_{6'}$ (DMTr)), 7.28–7.43 (m, 10H; H$_2$,H$_3$,H$_6$,H$_7$ (Fmoc), H$_{2''}$,H$_{3''}$,H$_{4''}$,H$_{5''}$,H$_{6''}$ (DMTr), H$_6$ (thymine)), 7.66 (d, 2H; $J = 7.3$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 7.88 (d, 2H; $J = 7.3$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 11.31 (s, 1H; N^3-*H*).

5-(3-*N*-(9-Fluorenylmethoxycarbonyl)aminopropyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (9): EtN(*i*Pr)$_2$ (0.26 mL, 1.5 mmol) and 2-cyanoethoxy-*N*,*N*-diisopropylamino-chlorophosphane (0.14 mL, 0.67 mmol) were added under the exclusion of moisture to a solution of **8** (400 mg, 0.5 mmol) in dry CH_2Cl_2. After the mixture was stirred at room temperature under argon for one hour, CH_2Cl_2 (15 mL) was added and the resulting mixture was poured into 5% $NaHCO_3$ aqueous solution (75 mL) and extracted three times with CH_2Cl_2. The combined organic layers were evaporated and the residue was purified by flash chromatography (SiO_2, EtOAc (70%)/EtN(*i*Pr)$_2$ (0.5%)/hexane) to provide phosphoramidite **9** as a mixture of two diastereoisomers (399 mg, 79% yield). ^{1}H NMR ([D$_6$]DMSO) selected signals of diastereoisomers: $\delta = 1.42$ (m, 4H; H$_{\beta}$,H$_{\beta'}$), 2.61 (t, 2H; $J = 5.9$ Hz, CN-CH_2-CH$_2$ (cyanoethyl)), 2.73 (t, 2H; $J = 5.9$ Hz, CN-CH_2-CH$_2$ (cyanoethyl)), 2.81 (m, 4H; H$_{\gamma}$,H$_{\gamma'}$), 3.24 (m, 4H; H$_{5'}$,H$_{5''}$), 3.45 (m, 1H; C*H*-(CH$_3$)$_2$ (*i*Pr$_2$)), 3.51 (m, 1H; C*H*-(CH$_3$)$_2$ (*i*Pr$_2$)), 3.58 (s, 6H; Ar-O-CH_3 (DMTr)), 3.70 (s, 6H; Ar-O-CH_3 (DMTr)), 3.97 (m, 1H; H$_{4'}$), 4.03 (m, 1H; H$_{4'}$), 4.18 (m, 2H; H$_9$ (Fmoc)), 4.25 (m, 4H; O-CH_2-Ar (Fmoc)), 4.53 (m, 2H; H$_{3'}$), 6.19 (m, 2H; H$_{1'}$), 6.85 (m, 8H; H$_3$,H$_{3'}$,H$_5$,H$_{5'}$ (DMTr)), 7.45 (s, 2H; H$_6$ (thymine)), 7.65 (d, 4H; $J = 7.7$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 7.88 (d, 4H; $J = 7.7$ Hz, H$_1$,H$_8$ or H$_4$,H$_5$ (Fmoc)), 11.32 (s, 2H; N^3-*H*); ^{31}P NMR ($CDCl_3$): $\delta = 148.61$ and 148.94.

Unmodified oligodeoxyribonucleotides: All oligodeoxyribonucleotides were synthesized on a Controlled Pore Glass solid support by using the phosphoramidite approach with an Applied Biosystems 394 DNA/RNA Synthesizer (1 μmol scale). Cleavage from the solid support and deprotection was performed by treatment with concentrated NH_4OH (30%) for two hours at room temperature and 30 min at 70 °C. The solid support and protecting groups were removed by standard procedures, and the resulting samples purified by gel electrophoresis with a 20% polyacrylamide, urea (7M) denaturating gel with a Tris-borate (89mM), and EDTA (2mM) buffer at pH 8.6.

Amino-modified oligodeoxyribonucleotides: The phosphoramidite **9** of 5-aminopropyl-2′-deoxyuridine was introduced as a building block to produce amino-modified oligonucleotides with the automated DNA synthesizer. Attachment of **9** proceeded with the same high yield as with unmodified standard phosphoramidites (as indicated by release of the trityl cation). After deprotection and replacement of the NH_4^+ ions by K^+ ions, the crude mixture could be used directly for the coupling in solution with the activated ruthenium complex **3**. The ion exchange was introduced in order to avoid any interference of the NH_4^+ ions during the coupling reaction.

Coupling procedure: In a 1.5 mL Eppendorf tube, 20 OD (~0.1 μmol) of crude amino-modified oligonucleotide was solubilized in DMF/dioxane/H_2O 3:2:1 (600 μL). After the addition of the activated complex **3** (16 mg, 15 μmol) and EtN(*i*Pr)$_2$ (6 μL, 0.035 mmol), the coupling was performed for 24 hours in the dark at room temperature with slow shaking. The reaction mixture was then evaporated to dryness under vacuum. The pellet was suspended in water (500 μL) and extracted four times with CH_2Cl_2 to remove excess unconverted Ru complex. The aqueous layer was concentrated and purified by gel electrophoresis under the same conditions as used for the natural oligonucleotides. The solution coupling of the ruthenium complex to the DNA fragments led to only one yellow-orange spot in polyacrylamide gel electrophoresis with a significant decrease in mobility compared with the NH_2-modified oligonucleotide. The Ru-modified oligonucleotide could thus be separated and isolated from the non-Ru-derivatized oligonucleotide. The yield of this coupling reaction in solution is in the order of 20%.

Mass spectrometry of oligodeoxyribonucleotides: Electrospray mass spectrometry analyses were run on a VG Platform II (Micromass) in the negative-ion mode. The eluent was 50% aqueous acetonitrile and the flow rate was 5 μL min^{-1}. Samples were prepared by dissolving the oligonucleotides in H_2O/CH_3CN 1:1 (10 μL, 50–100 μg mL^{-1}). Et$_3$N (1%) was added in order to reduce the amount of complexed alkali metal ions.[52] The MS data for the Ru-labeled oligonucleotide **S1** (5′-CAAAACCC(dU*-Ru)ACC-CAAAC-3′): calcd 5984.4 Da; found 5982.7 Da ± 1.00; for the Ru-derivatized oligomer **S2** (5′-TTTTTTTA(dU*-Ru)TAAATTTA-3′): calcd 6077.5 Da; found 6076.2 Da ± 0.81.

Spectroscopy: Absorption spectra were monitored with a UV/Vis Varian Cary 219 spectrophotometer. Emission spectra were recorded with a Shimadzu RF-5001PC spectrofluorimeter equipped with a Hamamatsu R928 red-sensitive photomultiplier tube. Emission lifetimes were determined by single-photon counting (SPC) with an Edinburgh Instruments FL900 spectrometer (Edinburgh, UK), equipped with a nitrogen-filled discharge lamp and a Hamamatsu R928 photomultiplier tube. The emission decays were analyzed by using the original Edinburgh Instruments software.

Steady-state illuminations: Ru-labeled duplex photolysis was carried out in a 600 μL quartz cuvette with visible light ($\lambda > 400$ nm) from two lamps perpendicular to each other. The first excitation source was a 2000 W

halogen lamp (Osram 64788) and the second, a super-pressure mercury lamp (Osram HBO 200 W). The two beams were focused on the sample with lenses. Water was allowed to circulate through a filter to cut off the IR radiation and a 0.2 M $NaNO_2$ solution was used to remove the UV part of the source. Absorption spectral changes were monitored on a UV/Vis Varian Cary 219 spectrophotometer. The illumination conditions for $[Ru(tap)_2(dip)]^{2+}$ in the presence of CT-DNA were different. In this case, only the 2000 W halogen lamp was used for the excitation with an illuminated volume of 1 mL. The illumination times may thus not be comparable.

Melting experiments: Duplex solutions were prepared from equimolar single-strand solutions in aqueous buffer (50 mM NaCl, 10 mM Tris, pH 7). Determination of the concentrations of metalated oligomers required a correction for the contribution to the absorbance at 260 nm of the tethered Ru^{II} complex itself. This was done by measuring the ratio of the absorbance of free $[Ru(tap)_2(dip)]^{2+}$ at 260 nm to that at 418 nm (λ_{max}). The absorption of the metalated oligonucleotide at 260 nm was corrected on the basis of the above ratio of $[Ru(tap)_2(dip)]^{2+}$ absorbances and the absorbance of the labeled oligomer at 420 nm (λ_{max}). UV absorbance versus temperature profiles were recorded with a Hewlett-Packard HP 8452A diode-array UV/Vis spectrophotometer. An Oxford instruments DN 1704 nitrogen cryostat was used to control temperature during the measurements. The temperature of the analyzed solution was increased from 10 to 85 °C at a heating rate of 0.5 °C min^{-1}. Experiments were performed at least twice and melting temperatures were averaged.

Photocrosslinking experiments

Enzymes and chemicals: Bacteriophage T4 polynucleotide kinase was purchased from Pharmacia (9500 U mL^{-1}) and [γ^{32}P]-ATP (specific activity 3000 Ci $mmol^{-1}$) from Isotopchim.

Oligodeoxyribonucleotides: Ru-derivatized oligonucleotides were labeled at the 5′-end by treatment with [γ^{32}P]-ATP and polynucleotide kinase at 37 °C for 30 min. These ^{32}P-labeled oligonucleotides were purified by precipitation in 70 % ethanol and annealed to the Ru-containing complementary strand by incubating at 90 °C for 5 min and then at room temperature for 1 h.

Assay for photoreactivity: The reaction mixture contained 10 πmol of 5′-^{32}P-labeled oligonucleotide duplex, 10 mM Tris-HCl buffer (pH 7), 50 mM NaCl, in a total volume of 10 mL. The illumination was performed at 4 °C for 1 hour with a mercury/xenon lamp (Oriel, 200 W) by using a KNO_3 2 M filter (optical path = 6 cm, $\lambda > 320-330$ nm). After evaporation to dryness, these materials were dissolved in urea (10 mL, 7 M) containing an aqueous dye (0.1 % xylene cyanol, 0.1 % bromophenol blue). The reaction products were analyzed by electrophoresis through a 20 % denaturing (7 M urea) polyacrylamide gel (19:1 ratio of acrylamide to bisacrylamide) using TBE (90 mM Tris-borate, pH 8, 2 mM EDTA). DNA fragments were visualized by autoradiography using Kodak X-OMAT AR film.

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[46] The intensity decrease in Figure 6 (bottom) is slightly less pronounced at lower temperatures (below 40 °C); this observation is in agreement with previous studies indicating reduced crossing to the ^{3}MC state upon complex interaction with double-stranded polynucleotides.[45] As at lower temperatures the complex is part of a duplex, the temperature dependence of the Ru^{II} complex excited state exhibits less pronounced crossing to the ^{3}MC state than at higher temperatures, where the two strands are separated.

[47] Excited free complexes containing at least two tap or two hat ligands, $[Ru(tap/hat)_2L]^{2+}$, are quenched reductively by GMP. Thus luminescence of $[Ru(tap)_2(dip)]^{2+}$ (reduction potential of the excited state = $+1.08\ V\,SCE^{-1}$ in MeCN) is quenched in water by GMP with a rate constant (k_q) of $0.7 \times 10^9\ M^{-1}s^{-1}$ (ΔG° is estimated to -0.26 eV or $+0.01$ eV, depending on the value for the oxidation potential of GMP).[32, 33, 36, 37] On the other hand, laser flash photolysis[32, 36, 37] of free $[Ru(tap)_2(dip)]^{2+}$ with GMP has shown that the monoreduced complex with a maximum of absorption at 500 nm was formed after the laser pulse.

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