Photoaddition of $Ru(tap)_2(bpy)^{2+}$ to DNA: A New Mode of Covalent Attachment of Metal Complexes to Duplex DNA

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Abstract: Near-UV or visible light irradiation of $Ru(tap)_2(bpy)^{2+}$ (**1**) (tap = 1,4,5,8-tetraazaphenanthrene; bpy = 2,2′-bipyridyl) in the presence of duplex DNA induces the formation of covalent adducts with guanine. The adduct has been isolated from the photomodified DNA as both its nucleotide and nucleobase derivatives by using a combination of enzymatic and acid hydrolytic procedures in conjunction with HPLC. Characterization by electrospray mass spectrometry and NMR spectroscopy shows that two isomeric covalent adducts are formed in which the exocyclic amino group of a guanine nucleobase is linked to the C2 or C7 position of one of the tap ligands. It is proposed that the products are generated from the reduced ruthenium complex and the guanine radical cation resulting from photoinduced electron transfer between **1** and guanine.

Introduction

There is an expanding interest in the interaction of transition metal complexes with nucleic acids, a major reason being the effectiveness of such complexes as chemotherapeutic agents.[1,2] Of principal importance in this context are square planar Pt(II) derivatives, especially cis-platin $Pt(NH_3)_2Cl_2$, which are employed for the treatment of testicular, ovarian, and certain other human cancers.[3,4] The action of these complexes has been shown to derive from their coordinative binding to the DNA bases (especially guanine and adenine).[5−7]

By contrast, studies of octahedral complexes, most notably those of ruthenium and rhodium, have focused on their potential as photophysical and photochemical probes for DNA.[8−11] Thus by exploiting the different shapes and electronic properties of the complexes it has been possible to obtain molecules that bind selectively to particular DNA sequences and different DNA conformers, including Z-DNA, hairpin structures, and cruciforms. The planarity of the heteroaromatic ligands in many of the complexes suggests that they might intercalate between the base pairs of DNA, and this has been confirmed for $Ru(phen)_2(dppz)^{2+}$ (phen = 1,10-phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine).[12−14] With complexes such as $Ru(phen)_3^{2+}$, which have less extended aromatic ligands, full intercalation is unlikely although there is still significant interaction of the ligand with the DNA base pairs.[15−17] The distinctive luminescent properties of many ruthenium complexes make them useful molecular probes as exemplified by $Ru(phen)_2(dppz)^{2+}$, which is nonluminescent in aqueous solution but emits strongly when bound to DNA. This property has been exploited in the study of the physical properties of DNA, including electron transfer between its base pairs.[18,19]

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Chemical DNA damage photosensitized by ruthenium complexes has also been studied. Most attention has focused on the induction of DNA strand breaks, which are readily monitored in plasmid DNA.[16,20−22] The quantum yield of frank strand breaks is low for molecules such as $Ru(phen)_3^{2+}$, although photochemically-induced base oxidation is much more efficient and subsequent treatment of the exposed DNA with piperidine causes DNA strand scission primarily at guanine bases.[23] By contrast, there have been few reports of photoinduced formation of adducts between the sensitizing metal complex and DNA. We have previously demonstrated by a combination of electrophoresis, dialysis, and optical spectroscopy that 1,4,5,8-tetraazaphenanthrene (tap) or 1,4,5,8,9,12-hexaazatriphenylene (hat) complexes such as $Ru(tap)_3^{2+}$ or $Ru(hat)_2(bpy)^{2+}$, which undergo visible light-induced electron transfer from guanine, yield photoadducts.[24−26] Photoadducts produced by UV irradiation of $Rh(phen)_2Cl_2^+$ and mononucleosides, mononucleotides, or DNA have also been isolated and characterized, and it has been shown that they are formed by loss of Cl^- from the complex and subsequent coordination of the metal directly to guanine.[27]

In this paper we present results of our investigation of the chemical nature of the species produced by the reaction of photooxidizing ruthenium(II) complexes with double-helical DNA. The products formed upon photolysis of $Ru(tap)_2(bpy)^{2+}$ in the presence of native (calf thymus) DNA have been enzymatically excised and the structure of the adduct has been compared to that determined in model studies where it was shown that photochemical reaction of $Ru(tap)_3^{2+}$ with guanosine 5′-monophosphate (GMP) causes formation of a covalent linkage between one of the tap ligands and the 2-NH_2 group of guanine.[26]

Experimental Section

Materials. Calf thymus DNA (Type I), DNase I, snake venom phosphodiesterase (*Crotalus atrox)*, 5′-dGMP[33] (free acid and disodium salt), and Sephadex ion exchangers were obtained from Sigma-Aldrich; nuclease S1 was from Boehringer-Mannheim. $[Ru(tap)_2(bpy)]Cl_2$ was prepared as described previously.[28]

Instrumentation. HPLC was carried out with a Waters multisolvent delivery system incorporating a Model 600E automated gradient controller, a U6K injector and Model 994 programmable photodiode array detector; elution profiles were routinely monitored at 288 nm. Electrospray mass spectra (ESMS) were recorded with a Fisons VG Quattro instrument operated at 3.89 kV with the cone voltage varying from 30 to 90 V; samples were dissolved in CH_3CN/H_2O (1:1). ^1H, $^1H-^1H$ COSY (double quantum filtered, pulse field gradient), HMQC, HMBC, and ^{13}C NMR spectra were acquired with a Varian 600-MHz spectrometer.

Preparation of Photomodified DNA. A continuously stirred solution (40 mL) containing 1 mM $[Ru(tap)_2(bpy)]Cl_2$ and 5 mM calf thymus DNA (as nucleotide phosphate) dissolved in 10 mM K_2HPO_4/KH_2PO_4 buffer, pH 6.0, was irradiated with a 125-W medium-pressure Hg lamp in a Pyrex glass photochemical reactor (Hanovia). A water-cooled jacket surrounding the lamp maintained the solution at room temperature (∼20 °C); no special precautions were taken to exclude air. The course of the photoreaction was monitored spectrophotometrically. After 7 days, the DNA was recovered and separated from unreacted $[Ru(tap)_2(bpy)]Cl_2$ by mixing the irradiated solution with ethanol (120 mL). The DNA which precipitated at −15 °C was collected and then subjected to several cycles of redissolution in 80 mM $MgCl_2$, 80 mM phosphate buffer at pH 6, followed by precipitation with 3 volumes of ethanol at −15 °C, until the supernatant was colorless.

Enzymatic and Acid Digestion. Each batch of photomodified DNA, prepared as above, was dissolved in 50 mL of 50 mM sodium acetate buffer, pH 4.5 (also containing 200 mM NaCl, 1 mM $ZnSO_4$, 4 mM $MgCl_2$, and 0.5% glycerol), and incubated at 37 °C, first with DNase I (10000 units) for 12 h and then with S1 nuclease (4000 units) for 24 h. The digestion products, comprising short oligonucleotides, were isolated by precipitation with 3 volumes of cold ethanol and then fractionated by semipreparative HPLC on a Hamilton PRP-1 reversed phase column (305 × 7 mm), using a linear gradient of 0−50% acetonitrile in 0.05% aqueous trifluoroacetic acid over 30 min at a flow rate of 2 mL min^{-1}. Eluate fractions containing ruthenium were identified from their absorbance at 400 nm by diode array detection, and they were then pooled and lyophilized. This material was finally subjected to hydrolysis either by mineral acid or by snake venom phosphodiesterase.

Degradation with snake venom phosphodiesterase was carried out by incubating the nuclease digestion products with the *Crotalus atrox* enzyme (0.5 units) at 37 °C, for 12 h, in proprietary pH 9 buffer. The mononucleotide adduct thus liberated was separated from incompletely digested material by its retention on a cation exchange chromatography column (10 × 1 cm) of Sephadex-SP C-25 which was eluted with 0.02 M $(NH_4)_2CO_3$, pH 7.0. The photoadduct isolated by lyophilization from late-eluting fractions was precipitated as its monohexafluorophosphate salt (by addition of KH_2PO_4 and KPF_6) and subsequently characterized by ESMS and ^1H NMR. For the latter purpose, it was converted to the more soluble chloride salt by anion exchange chromatography on a column of Sephadex-DEAE A-25.

Acid hydrolysis was performed by dissolving the nuclease digestion products in 2 M HCl (20 mL) and refluxing the solution for 2 h. After evaporation, the hydrolysate was analyzed by HPLC, using the same conditions as above. The Ru-containing fractions were combined and lyophilized to yield the guanine photoadduct as its trifluoroacetate salt. Its spectroscopic properties are detailed below.

Results and Discussion

Formation and Isolation of the DNA Photoadduct. The aim of the present study was to determine the nature of the photoadducts formed between native DNA and ruthenium complexes incorporating tap ligands. In model studies the photoaddition product of $Ru(tap)_3^{2+}$ with the monoribonucleotide 5′-GMP has been isolated as its guanine derivative, the linkage of the nucleobase and metal complex being shown to involve a covalent bond between the C2 of a tap ligand and the N2 of guanine.[26] We were therefore interested in establishing whether the structurally analogous adduct would be formed

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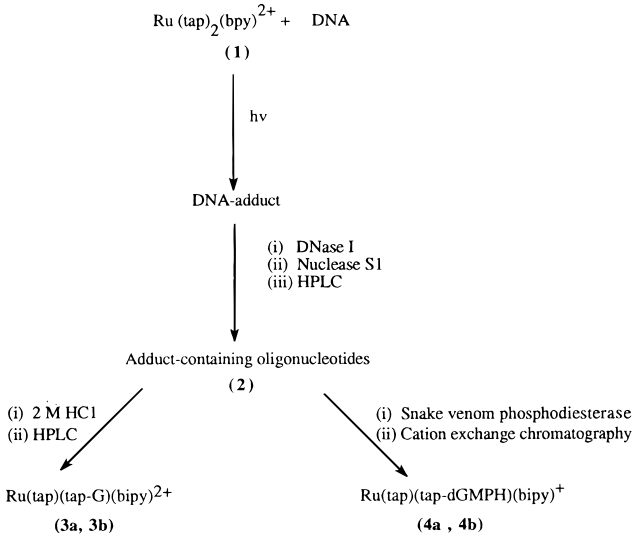
Scheme 1



with double-stranded DNA, since in this case the structure of the adduct might be influenced by the precise mode of non-covalent association of $Ru(tap)_2(bpy)^{2+}$ to DNA (which is probably similar to that of $Ru(phen)_3^{2+}$).[17] It might, for example, be quite different if the complex were bound in the minor rather than the major groove of the double helix. To carry out preparative experiments it is necessary to work with relatively high concentrations of the ruthenium complex. Equally, it is desirable not to work with excessive concentrations of DNA. We have chosen to work with 1 mM ruthenium complex and 5 mM DNA nucleotide and, given that calf thymus has a 42% G-C content, the ratio of Ru:G is 1.05. Preliminary experiments photolyzing $Ru(tap)_3^{2+}$ under these conditions were discontinued when it was observed that substantial amounts of dechelated materials were formed which inhibited subsequent endonuclease digestion. Such problems were not encountered with $Ru(tap)_2(bpy)^{2+}$ because it is more photostable.[24d]

The occurrence of the light-induced reaction between $[Ru(tap)_2(bpy)]Cl_2$ and native calf thymus DNA was followed by spectrophotometry. The observed changes closely paralleled those previously reported for this system on an analytical scale,[24d] with the maximum of the visible absorption band at 412 nm undergoing a marked hyperchromic effect (~30%) as it shifted progressively to ~390 nm. Irradiation was discontinued at this point and the photomodified DNA was recovered and freed from unbound ruthenium complexes by repeated precipitation from ethanol. From the magnitude of the absorbance changes occurring at 412 and 390 nm during irradiation and from the visible absorption of the precipitated DNA, it was estimated that >65% of the original $Ru(tap)_2(bpy)^{2+}$ had bound irreversibly to the DNA.

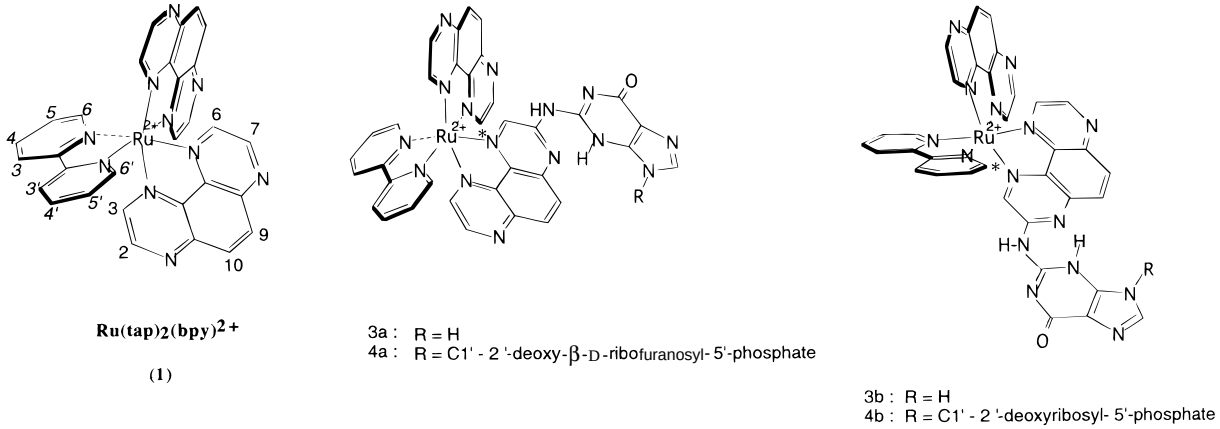
Our initial attempt to isolate the covalent adducts between the ruthenium complex and the nucleobases of DNA employed an acid hydrolysis procedure similar to that which had been successful for the adduct of $Ru(tap)_3^{2+}$ and 5′-GMP.[26] However it did not prove possible to separate the product directly from the acid hydrolysates of the photomodified DNA. Instead a stepwise procedure for removing the excess unaltered nucleotide residues was devised (Scheme 1). The DNA was first digested with the non-specific endonuclease DNase I, which normally yields 5′-phosphorylated oligonucleotides about 4 bases in length. The progress of this reaction could be traced by a gradual increase in the absorbance of the solution at 260 nm. It was followed by treatment with the endonuclease S1, which is specific for single stranded or partially denatured DNA and degrades it to 5′-mononucleotides. Owing to the presence of modified nucleotide residues which inhibit the activity of these enzymes, the limit nuclease digest contained a mixture of mononucleotides, dinucleotides, and longer oligomers (as evidenced by a series of peaks at high mass values in ESMS spectra). The digest was then fractionated by reversed phase HPLC and, although a complicated multicomponent elution profile was observed, diode array detection allowed the fractions (**2**) containing ruthenium to be identified (by their absorption at 400 nm) and collected selectively. The combined fractions from this purification step provided a nuclease digest that was highly enriched in photomodified nucleotide residues, thus facilitating their isolation and characterization.

Isolation of the dGMP Adduct (4). Treatment with snake venom phosphodiesterase (an exonuclease which cleaves DNA sequentially from a 3′-hydroxyl terminus generating 5′-mononucleotides) further degraded the mixture of ruthenium-containing oligonucleotides (**2**). This was shown by the changing pattern of retention times and A_{260}:A_{400} ratios of the peaks observed in the diode array HPLC chromatogram. However, when the chromatogram stabilized, it was clear from the profile that digestion to mononucleotides had not proceeded to completion; ESMS also confirmed the presence of oligonucleotide material. Cation exchange chromatography on Sephadex-SP C-25 was therefore employed to isolate mononucleotide components attached to the ruthenium complex. Being partially positively charged at neutral pH, these entities were bound by the column whereas longer oligonucleotide species possessing a net negative charge were not retained. Subsequent elution with volatile ammonium carbonate buffer yielded small quantities of a modified nucleotide which was identified as a photoadduct (**4**) of 5′-dGMP with $Ru(tap)_2(bpy)^{2+}$. When analyzed by reversed phase HPLC, it was found to consist of two components having identical absorption spectra which eluted as closely overlapping peaks with retention times of 24.03 and 24.58 min.

Isolation of the G Adduct (3). As an alternative to enzymatic degradation, the enriched nuclease digest (**2**) was also hydrolyzed with 2 M HCl at 100 °C to liberate modified nucleobase residues from the DNA backbone; the $Ru(tap)_2(bpy)^{2+}$ complex is chemically stable under these conditions. The hydrolysate was analyzed by reversed phase HPLC and the fractions comprising the Ru-containing photoadduct peak were combined and evaporated to yield the photoadduct (**3**) as its trifluoroacetate salt. The near UV/vis absorption profile of this material in water at pH 5.5 (λ_{max} 389 nm) coincided with the spectrum observed for the photomodified DNA (after removal of the unreacted ruthenium complex and before enzyme treatment) and with that of the dGMP adduct (**4**). They all show a distinctive hyperchromic and hypsochromic shift of the maximum relative to that of $[Ru(tap)_2(bpy)]Cl_2$ (λ_{max} 412 nm).

Detailed structural characterization of the photoadduct was accomplished by positive ion electrospray mass spectrometry in conjunction with one and two-dimensional NMR methods. As now discussed, the spectroscopic data show that the isolated photoadduct is a mixture of two geometrical isomers produced by substitution of the C2 or C7 position of one of the tap ligands of the complex by the exocyclic amino group of a guanine nucleobase in DNA. Studies were made on both the guanine adduct (**3**) obtained by acid hydrolysis and the dGMP adduct (**4**) obtained by digestion with snake venom phosphodiesterase. In the latter case, ^{13}C NMR measurements were precluded by the limited amount of material available, as only a small proportion of the total adduct present in the photomodified DNA

Chart 1



was ultimately purified to homogeneity. The isolation of **4** was complicated by lability of the deoxyriboside glycosidic bond, which was exacerbated by the weakly acidic conditions employed for HPLC. For this reason, the photoadduct was more readily characterized as the modified nucleobase derivative **3**.

Mass Spectrometry. The attribution of Ru-containing ions in the ES mass spectra was facilitated by their distinctive multiisotope profile; calculated masses for the most intense component are based on the most abundant Ru isotope with relative atomic mass 101.9. For the doubly charged guanine photoadduct (**3**), having a relative molecular mass (*M*) of 771.1, the spectrum observed with an extraction potential of 30 V comprised just the parent ion (*m/z* 385.8 (100%), calcd 385.6) and the triply charged $(M + H)^{3+}$ species (*m/z* 257.3 (40%), calcd 257.4); the relative abundance of other peaks did not exceed 5%. When the extraction potential was increased to 67 V, these ions were still prominent, but a more intense peak now corresponded to the singly charged $(M - H)^+$ ion (*m/z* 770.3 (90%), calcd 770.1). For all three ions, their observed isotopic profiles agreed very closely with the theoretical patterns. A number of fragment ions appearing in the latter mass spectrum were structurally informative. The presence of intact tap and bpy ligands in the photoadduct was indicated by dechelation giving rise to the species $(M - H - bpy)^+$ at *m/z* 614.4 (calcd 614.0) and $(M - H - tap)^+$ at *m/z* 588.3 (calcd 588.1); although weak, their relative abundances increased dramatically at an extraction potential of 90 V. However, the most significant ion observed with an extraction potential of 67 V was the base peak (100%) at *m/z* 318.7 (calcd 318.6), which arises by fragmentation of the guanine moiety to leave an amino group substituent on the modified tap ligand, $Ru(tap\text{-}NH_2)(tap)(bpy)^{2+}$. This provides convincing evidence for attachment of guanine to a tap ligand through its exocyclic amino group and corresponds to a documented major decomposition pathway of the guanine nucleus induced by electron impact ionization.[29] The identity of *m/z* 318.7 was confirmed by ions at *m/z* 240.0 (25%) and 227.4 (50%) formed by loss of an intact bpy or tap ligand to give respectively $Ru(tap\text{-}NH_2)(tap)^+$ (calcd 240.5) or $Ru(tap\text{-}NH_2)(bpy)^{2+}$ (calcd 227.5). Additional support for the point of attachment of guanine to the tap ligand was afforded by an intense ion at *m/z* 331.2 (60%) whose origin parallels the favored expulsion of cyanamide from an ionized guanine nucleus[29] to give $Ru(tap\text{-}NHCN)(tap)(bpy)^{2+}$ (calcd 331.0).

The mass spectrum recorded for the dGMP adduct (**4**) at an extraction potential of 35 V showed a prominent molecular ion at *m/z* 966.2 (50%) corresponding to the phosphate monoanion

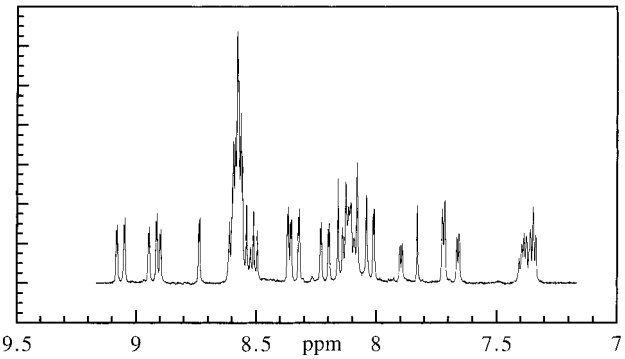


Figure 1. 600-MHz 1H NMR spectrum of photoadducts **3a** and **3b** in CD_3CN:D_2O (80:20).

$Ru(tap\text{-}dGMPH)(tap)(bpy)^+$ (calcd 966.1); a peak at *m/z* 502.8 (25%) was attributed to $Ru(tap\text{-}dGMPKH)(tap)(bpy)^{2+}$ (calcd 502.6). The main fragment ion at *m/z* 385.8 (100%) was derived, as expected, by glycosidic bond fission to give the molecular ion of **3** described above. It was replaced by the singly charged species at *m/z* 770.2 when the extraction potential was increased to 90 V; the cognate dechelated ions at *m/z* 588.3 and 614.2 (both 20%) were also evident.

NMR Analysis. The ^1H NMR spectrum of the guanine photoadduct (**3**), recorded in CD_3CN/D_2O, is shown in Figure 1. The molecular asymmetry created by connection of the guanine moiety to one of the tap ligands in the complex allows spectral resolution of the proton signals associated with all three ligands. Because the C2 and C7 atoms of the tap ligands in **1** are chemically nonequivalent, the adduct produced by reaction with guanine is a mixture of two geometrical isomers **3a** and **3b**. The self-consistent set of peak assignments for both isomers, given in Table 1, has been deduced from $^1H-^1H$ (COSY) and $^1H-^{13}C$ (HMQC and HMBC) correlation spectra (see Supporting Information), as well as by comparison with existing data for **1** and for the photoadduct of $Ru(tap)_3^{2+}$ with 5′-GMP.[26] Corroborating ^{13}C spectra were recorded in D_2O to avoid interference from the CD_3CN solvent signal at 118 ppm.

Integration of the ^1H NMR spectrum of the guanine photoadduct indicates a slight excess (55:45) of isomer **3a** over **3b**. With reference to the numbering scheme shown for **1**, the position of attachment to the guanine base is established as C7 for **3a** and C2 for **3b** by the absence of respective doublet signals for H7 and H2 in the spectrum. Furthermore, the absence of signals outside the aromatic region confirms that rearomatization of the substituted tap pyrazinic ring has occurred. Introduction of the guanine substituent has the effect of splitting the 9,10 proton signals into an AB system and causes the ^{13}C signal of the adjacent C3,6 atom to be shifted by ∼−9 ppm.

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Table 1. 600-MHz ^{1}H-NMR Data for Ru(tap)$_2$(bpy)$^{2+}$ (**1**) and Adducts **3a** and **3b** in CD_3CN/D_2O (80:20)

	Ru(tap)$_2$(bpy)$^{2+}$			adduct **3a**			adduct **3b**	
		δ (ppm)	J (Hz)	δ (ppm)	J (Hz)[d]		δ (ppm)	J (Hz)[d]
tap					tap			
2	2H(d)	9.11	2.9	9.05	2.9	1H	9.08	2.9
3	2H(d)	8.30	2.9	8.32	2.9	1H	8.35	2.9
6	2H(d)	8.11	2.9	8.36[b]	2.9	1H	8.20	2.9
7	2H(d)	8.90	2.9	8.91	2.9	1H	8.90	2.9
9	2H(d)	8.59		8.59	ab	1H	8.50	ab
10	2H	8.60		8.60	ab	1H	8.56	ab
tap					tap-G			
2	2H(d)	9.11	2.9	8.74	2.9			
3	2H(d)	8.30	2.9	8.01	2.9	1H	8.14[a]	
6	2H(d)	8.11	2.9	8.12[a]		1H	8.23	2.9
7	2H(d)	8.90	2.9			1H	8.95	2.9
9	2H(d)	8.59		8.56	ab	1H	8.53	ab
10	2H	8.60		8.60	ab	1H	8.60	ab
bpy								
6,6′	2H	7.74	5.7	7.66	6	1H	7.90[b]	6
				7.72	6	1H	7.72	6
5,5′	2H	7.38	5.7	7.35	(6)	2H	7.38	(6)
4,4′	2H	8.14	7.7	8.11	m	2H	8.12	m
3,3′	2H	8.6	7.7	8.56	m	2H	8.57	m
guanine								
H8	1H		7.58[c]	8.08		0.5H	8.16	
				8.04		0.5H	7.83	

[a] Tap position α to the connection to the exocyclic amino group of the guanine residue. [b] See text. [c] In DMSO-d_6. [d] ab: the 4 overlapping ab systems of the tap H9,10. m: bpy overlapping multiplet. Value of J ±10%; values in parentheses are ±30%.

The chemical shifts of the protons on the unmodified tap and bpy ligands are within 0.1 ppm of their values in **1** except for one of the tap H3,6 protons in **3a** and one of the bpy H6,6′ protons in **3b,** whose signals are shifted downfield by 0.25 and 0.16 ppm, respectively. These protons (asterisked in the formulae and denoted *b* Table 1) point toward the modified tap ligand and are therefore sensitive to changes in its ring current caused by substitution. As observed previously for the Ru(tap)$_3^{2+}$ adduct with 5′-GMP, the guanine H8 signals of both isomers are split into two components of equal intensity with slightly different chemical shifts; similar behavior has also been reported for aminobenzo[*a*]pyrene adducts with deoxyguanosine.[30] This is attributed to a tautomerization process (involving a prototropic shift between N1 and N3 of the guanine nucleus) that leads to slow rotation around the bond linking guanine to the tap ligand because the individual tautomers are stabilized by hydrogen bonding to N1,8 of the ligand. The HMQC and HMBC spectra permitted unequivocal assignment of the two H8 signals for each isomer.

To obtain a well resolved ^{1}H NMR spectrum of the 5′-dGMP photoadduct (**4**) isolated from DNA, it was converted to the chloride salt by anion exchange chromatography and dissolved in DMSO-d_6/D_2O (80:20). Measurements on the hexafluorophosphate salt were hampered by its very limited solubility in the normal NMR solvents. The aromatic region of the spectrum very closely resembled the spectrum of **3a,b** and showed the same distinctive features. Additional resonances due to the deoxyribose protons, including characteristic multiplets for anomeric protons at 6.13 and 6.19 ppm, were evident at higher field. The spectral assignments, supported by ^{1}H−^{1}H COSY measurements, were entirely consistent with the dGMP adduct comprising a mixture of the two isomers **4a** and **4b** in a 60:40 ratio.

Mechanism for Formation of Adducts. Our previous studies[24] have shown that the excited states of complexes such as Ru(tap)$_3^{2+}$ or Ru(tap)$_2$(bipy)$^{2+}$ are sufficiently oxidizing to undergo electron transfer with guanine (eq 1), and we have proposed that the adduct is formed by subsequent combination of the protonated reduced ruthenium complex and the deprotonated radical cation of guanine (eq 3) and rearomatization (eq 4).

$$\{RuL(tap)_2^{2+}*, G\} \rightarrow \{[RuL(tap)(tap^{\bullet -})]^+, G^{\bullet +}\} \quad (1)$$

$$\{[RuL(tap)(tap^{\bullet -})]^+, G^{\bullet +}\} \rightarrow \{[RuL(tap)(tapH^{\bullet})]^{2+}, G(-H)^{\bullet}\} \quad (2)$$

$$\{[RuL(tap)(tapH^{\bullet})]^{2+}, G(-H)^{\bullet}\} \rightarrow RuL(tap)(tap\text{-}GH_2)^{2+} \quad (3)$$

$$RuL(tap)(tap\text{-}GH_2)^{2+} \rightarrow RuL(tap)(tap\text{-}G)^{2+} \quad (4)$$

The experiments reported here with double-helical DNA provided no evidence for the formation of photoadducts with nucleobases other than guanine. The lack of such other products is consistent with the proposed photoredox mechanism, because electron transfer to the excited state of Ru(tap)$_2$(bpy)$^{2+}$ from adenine or the pyrimidine bases is very inefficient. It has previously been demonstrated by spectroscopic means that adducts can be formed between Ru(tap)$_3^{2+}$ and double stranded poly(dA-dT).[24c] The production of these species appears to be initiated by the loss of a tap ligand and subsequent coordination of the Ru(tap)$_2^{2+}$ fragment to adenine. Analogous products do not appear to be formed in significant quantities in the present study, due in part to the enhanced photostability of Ru(tap)$_2$(bpy)$^{2+}$ toward ligand loss.

The structure of the photoadducts **3** and **4** involves the covalent linking of guanine via its exocyclic amino group to the C atom, which is β to the coordinating N of the tap ligand. Similar structures have not been observed in the photoaddition reactions of other polypyridyl metal complexes with DNA and nucleosides, for example those involving [Rh(phen)$_2$Cl$_2$]$^+$.[27] In these latter cases the final result is a photosubstitution of the

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chloride ligand by the purine bases, even though it has recently been shown that the reaction involves photoinduced electron transfer. The formation of adducts **3** and **4** is consistent with the hypothesis of radical combination (eq 3). It is interesting to speculate on why the radical recombination does not lead to the product where the tap ligand is bonded to the O6 of guanine, as it is generally accepted that the tautomer having the radical center on the O6 is the most stable form of the deprotonated radical cation of guanine.[30] Were $Ru(tap)_2(bpy)^{2+}$ to be bound in the minor groove of DNA, then reaction with the N2 of guanine would be favored as the exocyclic amino group of the nucleobase is situated in the minor groove of B-DNA. However, the fact that this C2(tap)−N2(guanine) bond is also present in the adduct formed from $Ru(tap)_3^{2+}$ and 5′-GMP indicates that such preferred noncovalent association of the metal complex with DNA is unlikely to be the sole determining factor. Structurally analogous covalent adducts are also formed by dGMP with photooxidizing complexes containing hat (1,4,5,8,9,12-hexaazatriphenylene) or 2,2′-bipyrazine ligands.[32]

Conclusion

Definitive evidence has been obtained for a new type of site-specific covalent modification of double-helical DNA initiated by a photoexcited ruthenium complex. The reaction is targeted to the guanine bases in DNA which become linked, via their exocyclic amino functions, to the pyrazinic ring moieties of one of the tap ligands in the complex. The resulting photoadducts have been isolated from DNA and characterized as nucleobase and mononucleotide derivatives, by procedures that should be generally applicable to other photooxidizing ruthenium complexes. Their formation is inferred to occur by a mechanism where electron transfer to generate the guanine radical cation is a key step.

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Supporting Information Available: ESMS data of **3** and **4**; 600-MHz $^1H-^1H$ COSY, HMQC, HMBC, and ^{13}C NMR spectra of **3** (6 pages). See any current masthead for ordering and Internet access instructions.

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(33) Where the protonation of 5′-dGMP is specified we use $dGMPH_2$ to represent the free acid, and $dGMPH^-$ to represent the monoanion.