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Photolysis and Thermolysis of Platinum(IV) 2,2′-Bipyridine Complexes Lead to Identical Platinum(II)–DNA Adducts

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Abstract: Two Pt^{IV} and two Pt^{II} complexes containing a 2,2′-bipyridine ligand were treated with a short DNA oligonucleotide under light irradiation at 37 °C or in the dark at 37 and 50 °C. Photolysis and thermolysis of the Pt^{IV} complexes led to spontaneous reduction of the Pt^{IV} to the corresponding Pt^{II} complexes and to binding of Pt^{II} 2,2′-bipyridine complexes to *N7* of guanine. When the reduction product was $[Pt(bpy)Cl_2]$, formation of bis-oligonucleotide adducts was observed, whereas $[Pt(bpy)(MeNH_2)Cl]^+$ gave monoadducts, with chloride ligands substituted in both cases. Neither in the dark nor under light irradiation was the reductive elimination process of these Pt^{IV} complexes accompanied by oxidative DNA damage. This work raises the question of the stability of photoactivatable Pt^{IV} complexes toward moderate heating conditions.

Keywords: oligonucleotides · photolysis · platinum · reduction · thermolysis

Introduction

cis-Dichloridodiammineplatinum(II), *cis*-$[PtCl_2(NH_3)_2]$, and *cis*-tetrachloridodiammineplatinum(IV), *cis*-$[PtCl_4(NH_3)_2]$, were identified as the Pt compounds responsible for the inhibition of cell division by platinum complexes discovered by Rosenberg et al.[1] This led to *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) as one of the most widely utilized antitumor drugs. The anticancer activity of cisplatin is based on its ability to form intrastrand covalent adducts with DNA by binding of Pt to the *N7* atoms of two adjacent guanine (G) bases.[2,3] A serious drawback of cisplatin treatment is the intrinsic resistance of some tumors or the development of resistance of several tumors in the course of the treatment.[4] Although no Pt^{IV} compound has been approved yet as an anticancer drug, these six-coordinate complexes still draw attention as alternative platinum-based anticancer drugs.[5–9] They show anticancer activity and are toxic to tumors that are resistant to cisplatin. Pt^{IV} complexes are considered prodrugs of their corresponding Pt^{II} congeners. They react too slowly with targets to show any biological effect, because such reactions cannot compete with the faster reduction of Pt^{IV} to Pt^{II} with biological reducing agents.[10–13] However, as precursors of Pt^{II} complexes, they offer a number of additional properties. Variation of the axial ligand of the Pt^{IV} complexes allows the lipophilicity of the complex to be modulated and influences its capacity to penetrate cells.[14–16] The major advantages of Pt^{IV} drugs are their potential for oral administration, whereas cisplatin, carboplatin, and oxaliplatin are intravenously administered. Furthermore, one can take advantage of the release of two axial groups upon reduction within cells. The two released ligands can have their own mechanism of action. Two recent examples following this strategy have been reported.[17,18] On the other hand, axial positions may carry substituents that allow targeting and internalization of the platinum complex inside the appropriate cell.[19,20]

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In the above examples the release of the two axial groups is due to chemical reduction by the intracellular reductant pool, and the axial ligands are released as anions. However, reduction of Pt^{IV} complexes may be independent of the presence of reductants, and can also occur "spontaneously" upon light irradiation (photolysis).[21–32] In this case, the two electrons required for reduction of Pt^{IV} to Pt^{II} are provided by intramolecular electron transfer from two ligands to the platinum ion.[25,26,30] As with chemical reduction, a square-planar Pt^{II} complex results, but in this case, it is associated with two oxidized ligands. While some Pt^{IV} complexes can be reduced either by reductants or through photoactivation,[21,27,30] some others, more interestingly, are only reduced upon illumination.[22–24,28] It was reported that light-promoted photolysis, in addition to intracellular reduction, can potentiate the anticancer activity of a Pt^{IV} complex.[27] Except for some examples,[26,27,29] the nature and fate of the two oxidized ligands was not studied in detail. We reported that photolysis of Pt^{IV} complexes with a 2,2′-bipyridine ligand triggered the release of HOCl.[25] The formation of hypochlorous acid conceivably takes place through direct reductive elimination of HOCl or reductive elimination of Cl_2 and subsequent disproportionation of Cl_2 in H_2O. These diffusible oxidative species may be capable of damaging biomolecules in addition to the action of the Pt^{II} drug generated.

Additionally, in the absence of reductant, in vitro substitution of Pt^{IV} complex $[PtCl_4(dach)]$ (dach = 1,2-diaminocyclohexane) with an oxidizable biological ligand such as a guanine nucleobase (G) was reported to lead to inner-sphere two-electron transfer from guanine to the platinum ion.[33–36] Considering the redox potential of G (1.29 V vs. NHE), an outer-sphere electron-transfer mechanism is not possible between G and the Pt^{IV} complex.[37] An inner-sphere mechanism induced by coordination of Pt^{IV} to G must be considered. In this example, up to now, only guanine residues at the 5′-end of a DNA strand were shown to be reactive.[34] Besides, substitution reactions of Pt^{IV} complexes with G may be catalyzed by traces of Pt^{II}.[36,38] Although such a reaction may not be biologically relevant due to competition with intracellular reduction of the Pt^{IV} complex, it illustrates the potential oxidative behavior of Pt^{IV} complexes toward DNA.

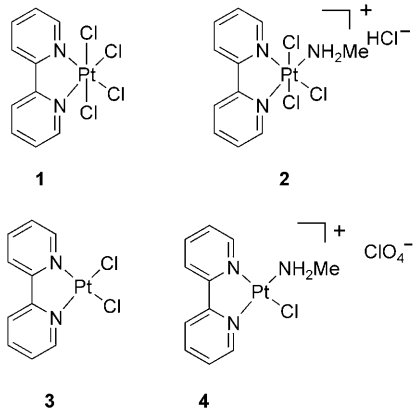
We have studied, in this work, the reactivity of two photoactivatable (Pt^{IV} complexes with a 2,2′-bipyridine ligand, $[PtCl_4(2,2'\text{-bpy})]$ (**1**), and $[PtCl_3(2,2'\text{-bpy})(MeNH_2)]Cl$ (**2**). Photolysis of **2** is known to generate the corresponding Pt^{II} complex $[PtCl(2,2'\text{-bpy})(MeNH_2)]^+$ with concomitant release of diffusible oxidative species (HOCl or Cl_2).[25]

Photolysis of Pt^{IV} complexes **1** and **2** in the presence of oligonucleotide DNA models induced reduction of the Pt^{IV} to the corresponding Pt^{II} complexes and subsequent binding of the Pt^{II} species to *N7* of G. Mono-oligonucleotide–Pt^{II} adducts and bis-adducts stemmed from **2** and **1**, respectively. Interestingly, we found that a heating step in the dark (50 °C) has the same effect as light irradiation and likewise causes reduction of Pt^{IV} complexes to the corresponding Pt^{II} complexes. Under the reaction conditions applied, reductive elimination of Pt^{IV} complexes was not associated with concomitant oxidative DNA damage.

Our data also support the view that the tested Pt^{IV} complexes do not undergo ligand substitution with G located either inside or at the 3′-end of a DNA strand, and are thus unable to induce guanine oxidation by inner-sphere electron transfer at these G residues. Spontaneous reduction upon photolysis and thermolysis is the major reaction route.

Results

The Pt^{IV} complexes with a 2,2′-bipyridine ligand, $[PtCl_4(2,2'\text{-bpy})]$ (**1**) and $[PtCl_3(2,2'\text{-bpy})(MeNH_2)]Cl$ (**2**), tested in the present work (Scheme 1) were shown previously to be light-



Scheme 1. Structures of the complexes applied in this study.

sensitive.[25] Light irradiation of **2** promotes its photoisomerization and photolysis.[25] Photolysis ($\lambda > 300$ nm) generates the corresponding Pt^{II} complex $[PtCl(2,2'\text{-bpy})(MeNH_2)]^+$ (**4**) with release of HOCl. Similarly, light irradiation of **1** is expected to lead to $[PtCl_2(2,2'\text{-bpy})]$ (**3**) and HOCl. HOCl may originate from direct reductive elimination of HOCl or from reductive elimination of Cl_2 and subsequent reaction with H_2O. The reactivity of Pt^{IV} complexes **1** and **2** in the presence of DNA models was studied under photolysis in order to detect DNA platinum adducts due to the released Pt^{II} complexes and oxidative DNA damage due to HOCl. Complexes **1** and **2** were also treated with DNA models under dark conditions in order to evidence any substitution reactions of the Pt^{IV} complexes with guanine nucleobase and, eventually, guanine oxidation by inner-sphere electron transfer within adducts of Pt^{IV} complexes with guanine.

Thus, adducts of DNA with Pt^{IV} and/or Pt^{II} and oxidative DNA damage were considered. The Pt^{IV} complexes (**1**, **2**) and their Pt^{II} analogues (**3**, **4**) were treated with 5′-CAGCTG oligonucleotide (ODN) in the dark and under light irradiation. The time course of the reactions was monitored by liquid chromatography coupled to UV/Vis detection or coupled to electrospray mass ionization (LC/ESI-

MS). This method is highly sensitive and has previously allowed us to detect about 1% of oxidative DNA damage.[39,40] Under these experimental conditions the short ODN was single-stranded.

Complex **2** is soluble in H_2O. Complexes **1**, **3** and **4** are soluble in DMSO. Therefore, initial dilution of these complexes in DMSO was followed by addition of water, and the reaction medium contained 1% DMSO. The complexes were never stored in solution to avoid ligand exchange. Buffers were not used to avoid complications arising from buffer coordination to platinum. The UV/Vis spectra of the tested complexes are shown in Figure S1 in the Supporting Information.

Cyclic voltammetry of Pt^{IV} complexes 1 and 2: The cyclic voltammogram of Pt^{IV} complex **1** in DMSO is shown in Figure S2 in the Supporting Information. This complex displays a first irreversible two-electron cathodic peak ($E_p = 0.12$ V/NHE) for the Pt^{IV}/Pt^{II} reduction process followed by a reduction pattern identical to that exhibited by [$PtCl_2$(2,2′-bpy)].[41] The Pt^{IV} complex, upon addition of two electrons, loses the two axial ligands, affording the corresponding platinum(II) species. The second reduction process is a one-electron reversible process ($E_{1/2} = -0.86$ V/NHE), which suggests that it is mainly localized on the 2,2′-bipyridine ligand. The cyclic voltammogram of Pt^{IV} complex **2** in aqueous media (see Figure S2 in the Supporting Information) exhibits a first irreversible two-electron cathodic peak ($E_p = 0.40$ V/NHE) for the Pt^{IV}/Pt^{II} reduction process. The features are the same as reported for other platinum complexes.[42] This reduction is irreversible due to loss of the axial ligands. Complex **1** was not soluble in H_2O at the typical concentration used for cyclic voltammetry. However, it is possible to predict the Pt^{IV}/Pt^{II} peak potential of **1** in aqueous solution[43] from the known electrochemical parameters of the separate ligands.[44] The predicted E_p of **1** in H_2O is consequently $E_p^{pred} = 0.11$ V/NHE.

According to the Pt^{IV}/Pt^{II} redox potentials, complex **1** ($E_p^{pred} = 0.11$ V/NHE) and **2** ($E_p = 0.12$ V/NHE) should be capable of G oxidation by inner-sphere electron transfer within a guanine–Pt^{IV} adduct provided they can coordinate at *N7* of G.[45]

Reaction of Pt^{IV} complexes 1 and 2 with 5′-CAGCTG: light-promoted formation of Pt–DNA adducts: Platinum(IV) complexes **1** and **2** were treated with 5′-CAGCTG (10 μM) in H_2O at 37 °C under light irradiation. The platinum complex was present either in stoichiometric amount (10 μM) with respect to the oligonucleotide or was added in tenfold excess (100 μM). The irradiated oligonucleotide showed no DNA damage.

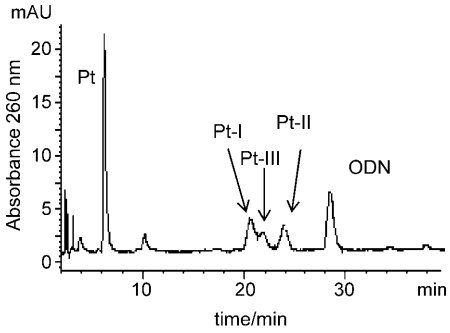


Figure 1. HPLC trace (260 nm) of the products formed during reaction of complex **1** (100 μM) with 5′-CAGCTG (10 μM) after 24 h under light irradiation (37 °C). Pt-I, Pt-II, and Pt-III eluting at 20–25 min are platinum–oligonucleotide adducts. Intact ODN 5′-CAGCTG was eluted at 28.5 min. Pt refers to excess platinum complex.

When Pt^{IV} complex **1** (100 μM) was treated with 5′-CAGCTG, the initial ODN (28.6 min) transformed into three different products eluted at shorter retention times (20-24 min, Figure 1). The ratio of the peaks corresponding to the modified ODNs did not change during the course of the reaction. Pt-I, Pt-III, and Pt-II accounted for approximately 45, 20 and 35%, respectively. Their increase was associated with a proportional decrease of the intact ODN. After 24 h, the area of these peaks (HPLC trace at 260 nm) accounted for about 60% of the starting ODN material (Table 1, entry 2). The area of the remaining ODN corresponded to about 40% of the initial area. The new products exhibited a dual absorbance at 260 and 320 nm compatible with covalent binding of a platinum complex to an oligonucleotide strand, as confirmed by LC-ESI/MS analysis (see below). For the three compounds, the on-line UV/Vis spectra were identical; one example is shown in Figure S3A in the Supporting Information. Assuming that the ε values of the starting ODN and the platinum modified ODN at 260 nm are on the same order, the area of the HPLC peaks of the adducts (Pt-I + Pt-II + Pt-III) gives the yield of the re-

Table 1. Yield of adducts (%, with respect to ODN) at different reaction time for the reaction of complex **1** and **2**, at 37 °C, in the dark (−) or under light irradiation (+). The concentration of the oligonucleotide (ODN) was constant and the concentration of the platinum complex was 10 or 100 μM. Data are the means (±standard deviation, SD) of three independent experiments.

Entry	Light	Complex (μM) /ODN (μM)	Complex	1 h	3 h	6 h	24 h
1	+	10/10	**1**				29 (±11)
2	+	100/10	**1**	10[a]	23 (±6)	41 (±6)	57 (±6)
3	+	10/10	**2**	10[a]	20 (±3)	29 (±6)	46 (±9)
4	+	100/10	**2**	60 (±10)	74 (±9)		
5	–	10/10	**1**	0	0	0	0
6	–	100/10	**1**		10 (±5)	19 (±6)	22 (±7)
7	–	10/10	**2**	5 (±1)	9.8 (±1)	14 (±2)	26 (±2)
8	–	100/10	**2**	15[a]	29	34 (±9)	54 (±6)

[a] One measurement.

action. The yield of reaction depended on the irradiation time and the concentration of the platinum complex. Platinum adducts Pt-I, Pt-II, and Pt-III reached about 30% after 24 h under light irradiation for a stoichiometric amount of **1** with respect to ODN (Table 1, entry 1).

The LC-ESI/MS analysis of the reaction mixture of the photolysis of **1** in the presence of 5′-CAGCTG shows the starting ODN 5′-CAGCTG with an m/z signal at 894.8 amu with $z=2$ (not shown). The three different platinum modified oligonucleotides exhibited the same m/z signals at 1069.2 amu ($z=2$; see Figure S4 in the Supporting Information). It was assigned to a $[Pt(2,2'\text{-bpy})ODN]^{2-}$ species compatible with a +2 oxidation state for platinum. As shown thereafter by NMR spectroscopy, for each of the three adducts, the platinum center is coordinated to two bases on the ODN (bis-adduct). However, the oxidation state of platinum is not certain, since reduction of metal complexes during ESI-MS analysis may be possible.[46]

As for complex **1**, the reaction of complex **2** with the short ODN 5′-CAGCTG under light irradiation led to formation of platinum–DNA adducts. The reaction was also dependent on light and on the concentration of the complex. However, the nature of the products, the kinetics, and yields were different. First, in the case of complex **2**, the platinum–DNA adducts were eluted after the starting ODN on the HPLC chromatograms, that is, they were structurally different from the previous ones. They appeared in the form of two peaks of almost equal intensity Pt-A and Pt-B (Figure 2). The UV/Vis specta of the two reaction products

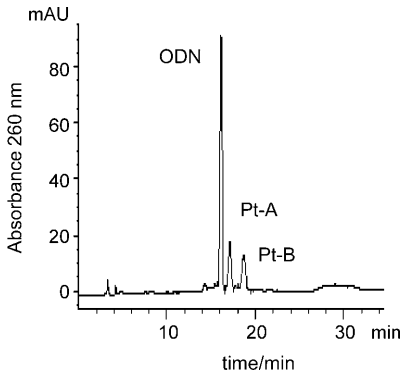


Figure 2. HPLC trace (260 nm) of the products formed during reaction of complex **2** (10 μM) with 5′-CAGCTG (10 μM) after 24 h of light irradiation (37 °C). Pt-A and Pt-B are platinum–oligonucleotide adducts.

were similar to that of the products from the reaction of complex **1** (Figure S3, Supporting Information). They were attributed also to platinum–DNA adducts. As in the case of complex **1**, the peaks corresponding to the platinated ODNs increased simultaneously during the reaction, and their formation was quantitative with the decrease of the initial ODN. Complex **2** proved more reactive than complex **1** (Table 1, entries 3 and 4).

The LC/ESI-MS analyses of platinum-modified oligonucleotides Pt-A and Pt-B were identical. Each exhibited two signals. One of them gave the same m/z signal, observed before for complex **1** adducts, at 1069.2 amu for a doubly charged ion (Figure S5, Supporting Information). As for the adducts of complex **1**, this ion was attributed to a $[Pt(2,2'\text{-bpy})ODN]^{2-}$ species. The second doubly charged ionic species observed at 1085.5 amu was attributed to a $[Pt(2,2'\text{-bpy})(MeNH_2)ODN]^{2-}$ ion, corresponding to a platinum adduct carrying one 2-2′-bipyrine and one methylamine ligand. Fragmentation (loss of methylamine ligand) in the mass spectrometer is likely the origin of the 1069.2 ion. Complex **2** formed two well-separated monoadducts (Pt-A, Pt-B) with identical mass spectra, each of which still carries one methylamine ligand. As was confirmed by enzymatic hydrolysis, they correspond to Pt^{II} adducts of the 5′-CAGCTG sequence at the two individual G sites.

In summary, complexes **1** and **2** are capable of platinating 5′-CAGCTG under light irradiation. The mass data are in favor of the platinum ion for all these DNA adducts being Pt^{II}, yet reduction of a Pt^{IV} species in the mass source may be possible. Consequently, the oxidation state of platinum–oligonucleotide adducts could not be determined with certainty. The formation of Pt^{II} adducts under light irradiation can be explained by photolysis of Pt^{IV} complexes **1** and **2** to the corresponding Pt^{II} species.[25] Oxidative damage of DNA associated with light-induced decomposition of Pt^{IV} complexes was never evidenced, even at high concentration of Pt^{IV} complex (100 μM). The nature and/or concentration of the diffusible oxidative species produced by the photolysis of Pt^{IV} complexes was probably not compatible with oxidative DNA damage. Indeed, DNA oxidation mediated by oxidative species such as H_2O_2 is observed at millimolar concentration of reagent, whereas in the present work HOCl (or Cl_2) is produced at micromolar concentration.

To determine the mechanism of the platination reaction mediated by the Pt^{IV} complexes under light and to unambiguously identify the Pt^{II}–oligonucleotide adducts, the corresponding Pt^{II} complexes **3** and **4** were treated with 5′-CAGCTG.

Reaction of Pt^{II} complexes 3, 4, and cisplatin with 5′-CAGCTG: The concentrations of ODN and Pt^{II} complexes **3** and **4** were 10 μM. Chromatograms of the reaction mixtures of complexes **1** and **3** and of complexes **2** and **4** showed the same adducts. Complex **3** led to the formation of Pt-I, Pt-II, and Pt-III (Figure S6A, Supporting Information). Complex **4** led to the formation of Pt-A and Pt-B (Figure S6B, Supporting Information). On-line UV/Vis spectra were also similar (Figure S7, Supporting Information). LC/ESI-MS analysis confirmed that the same adducts formed with the Pt^{IV} and the Pt^{II} complexes in each series (not shown). As expected, the reaction rate was much higher with Pt^{II} compared to the corresponding Pt^{IV} complexes. Under stoichiometric conditions (10 μM of ODN and 10 μM of Pt^{II} complex) adduct production reached more than 80% within 1 h for both complexes **3** and **4** (Figure S6,

Supporting Information). Thus, reduction of the Pt^{IV} complexes **1** and **2** to Pt^{II} complexes **3** and **4**, respectively, is likely to account for the formation of the observed platinum adducts upon incubation of the Pt^{IV} complexes with ODN. Furthermore, since the reactivity of the Pt^{II} complexes is high, the formation of DNA adducts is probably not the rate-determining step in the reaction of Pt^{IV} complexes with DNA under light irradiation.

Complexes **3** and **4** were compared with cisplatin. The yield of the cisplatin–ODN adduct was only 20% after 2 h of incubation of 5′-CAGCTG (10 μM) with cisplatin (10 μM) at 37°C, as opposed to almost total reaction with **3** or **4** (Figure S8, Supporting Information). However, when cisplatin was allowed to exchange the chloride ligands with water molecules before incubation in the presence of ODN, the yield reached 80% within 1 h, which is comparable to those of **3** and **4**. Under these two reaction conditions cisplatin led to only one peak of a Pt^{II}–DNA adduct on the HPLC trace, which was eluted before intact ODN (Figure S8, Supporting Information).

As previously reported, the 2,2′-bipyridine ligand decreases the electron density and increases the electrophilicity of the Pt^{II} center, which results in increased reaction rates for nucleophilic substitution reactions.[47]

In summary, the platinum-oligonucleotide adducts formed during photolysis of Pt^{IV} complexes **1** and **2** are adducts of the Pt^{II} species **3** and **4**.

Reaction of Pt^{IV} complexes 1 and 2 with 5′-CAGCTG in the dark: heat-promoted formation of Pt-DNA adducts: Control reactions in the absence of light were performed. Platinum(IV) complexes **1** and **2** were treated with 5′-CAGCTG (10 μM) in H_2O, at 37°C in the dark. As under light irradiation, the platinum complex was present either in stoichiometric amount (10 μM) with respect to the oligonucleotide or was added in a tenfold excess (100 μM). Complex **1** (100 μM) led to the formation of the three adducts, Pt-I, Pt-II, and Pt-III, that were previously observed under light irradiation and with Pt^{II} complex **3** (Figure S9A, Supporting Information). They formed in the same ratio (45/20/35) but with lower yield (Table 1, entries 5 and 6 and Figure S10A, Supporting Information). LC/ESI-MS of the dark reaction exhibited the usual 1069.2 amu ($z=2$) signal for Pt-I, Pt-II, and Pt-III (not shown). An excess of complex **1** with respect to the ODN was necessary to observe some reaction in the dark, and no reaction occurred between **1** (10 μM) and 5′-CAGCTG after 24 h of incubation in the dark.

The dark reactions of **2** with 5′-CAGCTG gave rise to Pt-A and Pt-B adducts (Figure S9B, Supporting Information). LC/ESI-MS analysis of the reaction showed the two signals at 1085.5 and 1069.2 amu ($z=2$) for Pt-A and Pt-B (not shown). As for complex **1**, the yields of the reactions were lower in the dark (Table 1, entries 7 and 8 and Figure S10B, Supporting Information). A minor product (ca. 2% of the initial ODN after 24 h) eluted before the ODN was evidenced in the HPLC trace of the reaction of **2** (100 μM) in the dark. It was attributed to an oligonucleotide strand carrying one abasic site due to loss of one guanine nucleobase on the basis of its on-line mass spectrum showing a signal at 828.4 amu.

The yields of reactions in the dark can be calculated in the same way as those under light irradiation because the formation of the platinum–DNA adducts was quantitative on the basis of the decrease of the initial ODN. The formation of Pt^{II} adducts under light irradiation can be explained by photolysis of Pt^{IV} complexes **1** and **2** to the corresponding Pt^{II} species.[25] The origin of Pt^{II} adducts in the dark reactions of the Pt^{IV} complexes is puzzling. One hypothesis is traces of Pt^{II} in the Pt^{IV} complex. However, this is not completely consistent with the data. Indeed, 2% of **3** may justify the observed 2.2 μM concentration of adducts when **1** (100 μM) reacts with ODN in the dark for 24 h (Table 1 entry 6 and Figure S10A in the Supporting Information). Similarly, 5% of **4** may be responsible for the formation of adducts (5.4 μM) when **2** (100 μM) was incubated with ODN in the dark (Table 1 entry 8 and Figure S10B in the Supporting Information). However, the reaction of **2** (10 μM) in the dark for 24 h led to 2.6 μM concentration of adducts (Table 1 entry 7 and Figure S10B in the Supporting Information), which is too high to be accounted for by a concentration of 0.5 μM (5%) of **4**. Additionally, the kinetics of the control reactions involving Pt^{II} complex **3** (2 μM) or **4** (5 μM) were more rapid than the corresponding reactions of 100 μM of Pt^{IV} complex **1** or **2**, respectively, in the dark (Figure S11, Supporting Information).

Micromolar concentration of reductant in the reaction medium might account for the generation of Pt^{II} species in a light-independent process. Indeed, ascorbate was able to reduce Pt^{IV} complexes **1** and **2** to the corresponding Pt^{II} complexes. However, the source of electrons in the dark reactions of the Pt^{IV} complexes is not obvious. Although DNA might be considered as a possible source of electrons through guanine oxidation, oxidation of 5′-CAGCTG was never observed. The abasic site seen with **2** in the dark (Figure S9B, Supporting Information) is not an oxidation product of DNA. Besides, the yield of its formation cannot compare with that of adducts Pt-A and Pt-B.

Alternatively, decomposition of Pt^{IV} to Pt^{II} complexes in the dark and at 37°C may be heat-promoted instead of light-promoted (thermolysis instead of photolysis). The Pt^{IV} complexes **1** and **2** were treated with 5′-CAGCTG in the dark at 50°C. Figure 3 and Table S1 in the Supporting Information show unambiguously that heating the reaction medium at 50°C in the dark leads to the same kinetics of platination as light irradiation whatever the concentration of Pt^{IV} complex.

In conclusion, moderate heating (37°C, 50°C) as well as light promote decomposition of Pt^{IV} complexes **1** and **2** to Pt^{II} complexes **3** and **4**, respectively.

Isolation and stability of the oligonucleotide–platinum adducts of 1 and 2 formed under light irradiation: Oligonucleotide–platinum adducts formed under light irradiation were isolated by semipreparative HPLC. In the case of ad-

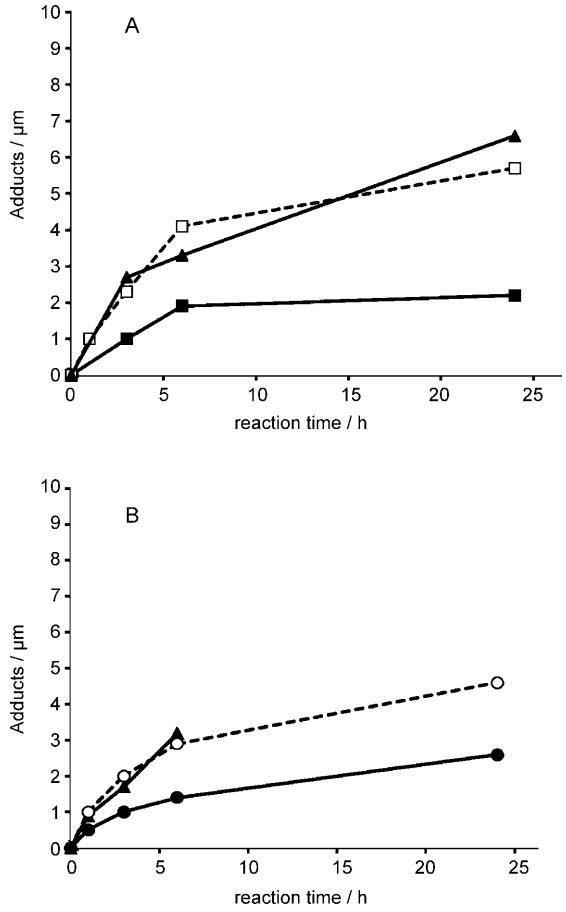


Figure 3. Kinetics of the formation of platinum adducts upon photolysis (empty symbols) compared to thermolysis (filled symbols) for Pt^{IV} complexes **1** (A) and **2** (B). Complex **1** (100 μM) and **2** (10 μM) were incubated with 5′-CAGCTG (10 μM) under light irradiation at 37 °C (□, ○) or in the dark at 37 °C (■, ●) and 50 °C (▲).

ducts from photolysis of Pt^{IV} complex **1**, although the HPLC peaks corresponding to the two major adducts Pt-I and Pt-II were well separated, neither product was recovered in pure form after lyophilization. Each product was contaminated by the other. These ODN–platinum adducts interconvert slowly. Adducts formed with Pt^{II} complex **3** behaved in the same way. This conversion was avoided by keeping the collected fractions at low temperature (4 °C).

The ODN–platinum adducts from photolysis of Pt^{IV} complex **2**, Pt-A and Pt-B, proved to be labile during the lyophilization step. Lyophilization of the chromatography solvent, which contained 10 mM triethylammonium acetate (TEAA) buffer, induced decoordination of platinum and led to 25–50 % of intact 5′-CAGCTG in the dried fractions. This problem was solved by introducing a desalting step (C18 Sep-Pak cartridge) before lyophilization. Finally, all adducts could be isolated in pure form and in suitable amount for characterization. Three isolated fractions of platinum–oligonucleotide adducts from photolysis of complex **1** were separated: one corresponded to the peak referred to as Pt-I, another to Pt-II, and third to a mixture of the peak referred to as Pt-III with some material from Pt-I. From the photolysis reaction of complex **2**, Pt-A and Pt-B were isolated separately.

The stability of the ODN–platinum adducts was studied further on the isolated products. Adducts Pt-I and Pt-II were incubated separately in 10 mM TEAA buffer pH 6.5 or 10 mM phosphate buffer pH 7.6 at 37 °C. After 4 h, each isolated adduct was found to be a mixture of the previous three forms of adducts of the platination reaction and with the same ratio (45, 20, and 35 % for adducts Pt-I, Pt-III and Pt-II, respectively). These three forms of adducts are clearly in equilibrium. The same stability test was performed in H_2O containing 100 mM NaCl at 37 °C. Again, isomerization took place following the same kinetics, with one pure adduct at the beginning of the incubation time giving rise to three products. Equilibrium is reached after 4 h and the mixture was then stable for 24 h. The three adducts were found to be conformers of platinum bisadducts on purine groups of 5′-CAGCTG (see NMR data below). Rotation about the Pt–purine bond is hindered by the 2,2′-bipyridine ligand, and this leads to slowly interconvertible rotamers. The same phenomenon was reported before for Pt^{II} bisadducts of complexes bearing bulky ligands designed to reduce the dynamic motion by destabilizing the transition state for Pt–G rotation.[48]

Similarly, adducts Pt-A and Pt-B were incubated separately in either 10 mM TEAA buffer pH 6.5 or 10 mM phosphate buffer pH 7.6 at 37 °C. They were relatively stable; only 10 % of intact 5′-CAGCTG was observed after 24 h of incubation. The two adducts did not interconvert with each other. No other product, except the unmodified oligonucleotide, was observed. It was identified by its on-line UV/Vis spectrum and by co-injection with a standard. Contrary to Pt-I, Pt-II, and Pt-III, adducts Pt-A and Pt-B were sensitive to chloride ions, with their lability increasing by a factor of 2–3 upon incubation in 100 mM NaCl at 37 °C. After 24 h 30 % 5′-CAGCTG formed. Long-term storage at −20 °C also led to 20 % labilization of the dried isolated adducts Pt-A and Pt-B.

In summary, as confirmed below by NMR data, bisadducts Pt-I, Pt-II, and Pt-III (from **3** or from photolysis or thermolysis of **1**) are interconvertible rotamers in slow exchange. Monoadducts Pt-A and Pt-B (from **4** or from photolysis or thermolysis of **2**) are relatively labile.

NMR characterization of the platinum adducts formed by photolysis of complex 1: The self-complementary 5′-CAGCTG oligonucleotide is single-stranded at 310 K in D_2O, as deduced from the temperature dependence of the chemical shifts. Assignments of the ^1H NMR resonances of the 5′-CAGCTG oligonucleotide were made on the basis of the established procedure developed for right-handed DNA duplexes[49] and the chemical shifts are listed in Table S2 in the Supporting Information. The ^1H NMR spectrum of 5′-CAGCTG is shown in Figure 4A.

The ^1H NMR spectrum shown in Figure 4B corresponds to the mixture of oligonucleotide–platinum adducts obtained

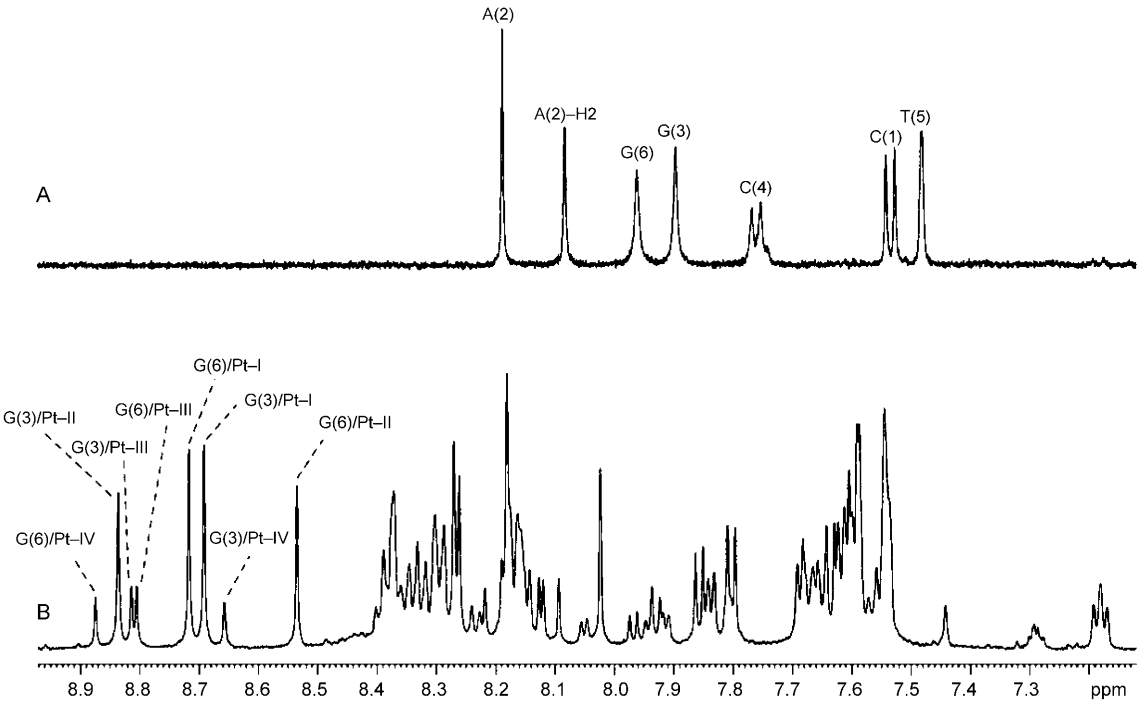


Figure 4. ^{1}H NMR spectra of the aromatic region A) of the 5′-CAGCTG oligonucleotide and B) of the mixture of adducts of **1** and 5′-CAGCTG at 310 K in D_2O. Assignments of H8/H6 base protons of the free 5′-CAGCTG oligonucleotide in (A) and of H8 protons of the platinated guanines of the four adducts in (B) are indicated.

from isolated Pt-II adduct that was equilibrated in D_2O at room temperature for several days. The binding of the metal complex to the 5′-CAGCTG oligonucleotide results in large chemical shift changes of selected oligonucleotide resonances. Although three different peaks of adducts were seen in the HPLC trace, four different adducts are evidenced in the NMR spectrum of the equilibrium mixture (Figure 4B) with a distribution of 44 (Pt-I), 34 (Pt-II), 12 (Pt-III), and 10% (referred to as Pt-IV), respectively. The Pt–oligonucleotide adducts are deduced to be single-stranded at 310 K from the temperature dependence of the chemical shifts and the broadening of resonances when lowering the temperature, due to equilibrium between single-stranded and double-stranded oligonucleotide.

Owing to the single-stranded nature of adducts and the elevated temperature, the oligonucleotides have a random structure and most of the internucleotide NOEs could not be observed. Furthermore, only very few NOEs between protons of the oligonucleotide and of the Pt 2,2′-bipyridine moiety were detected. Chemical-shift changes were only observed for protons in close proximity to the metal complex. Important chemical shift changes (>0.2 ppm) were only detected for H8 base proton and H1′, H2′, and H2″ deoxyribose protons of the nucleotides bound to the Pt complex and for H1′, H2′, and H2″ deoxyribose protons of their 5′ neighbor residues (Tables S3–S6, Supporting Information). Base protons of the residues not linked to the metal complex and H3′, H4′, H5′, and H5″ sugar protons, close to the phosphodiester backbone, were only slightly shifted by binding of the Pt complex.

Each set of deoxyribose proton resonances could be assigned with 2D TOCSY and 2D ROESY spectra and related to the corresponding base H8 or H6 protons by means of intranucleotide NOEs. Assignment of cytosine resonances was made possible by the characteristic correlation between H6 and H5 base protons observed in 2D TOCSY spectra. Distinction between C(1) and C(4) resonances was based on the fact that sugar protons of C(1) resonate at lower frequency than those of C(4) due to their position at the 5′-edge of the nucleotide and on the absence of ^{31}P couplings for H5′ and H5″ C(1) protons. Assignments of thymine T(5) were confirmed by means of correlations between H6 and methyl base protons detected in 2D TOCSY and 2D ROESY spectra. Purine resonances were assigned primarily by comparison with the free 5′-CAGCTG oligonucleotide. Guanine G(6) resonances could be identified with the aid of H3′ and H4′ sugar protons that resonate at lower frequency for guanine G(6) than for guanine G(3) and adenine A(2) due to their positions at the 3′-edge of the nucleotide. Resonances of adenine A(2) and guanine G(3) were distinguished on the basis of the H8 proton chemical shift of the purine base that is not linked to the metal complex ($\delta=8.27$, 8.26, 8.12, and 8.21 ppm for adducts Pt-I, Pt-II, Pt-III, and Pt-IV, respectively). These chemical shifts are close to the adenine A(2) H8 resonance ($\delta=8.20$ ppm) but seem too shifted compared to the guanine G(3) H8 resonance ($\delta=7.91$ ppm)

of free 5′-CAGCTG. The assignments of guanine G(3) and G(6) and adenine A(2) were confirmed in adduct Pt-I by a weak NOE between H6-C(4) and H2″-G(3) protons, in adduct Pt-II by NOEs between H8-G(3) and H1′-A(2) (Figure S12, Supporting Information) and between H8-G(3) and H2″-A(2) protons, and in adduct Pt-IV by NOEs between H8-G(3) and H1′-A(2) and between H8-G(6) and H1′-T(5) protons (Figure S12, Supporting Information). Distinction between resonances of the four different adducts was based on integration and confirmed by the few internucleotide NOEs detected. Furthermore, Pt-I and Pt-II and a mixture of Pt-I and Pt-III adducts were analyzed separately at 279 K (Figure 5). Assignment of Pt-III versus Pt-IV adducts, which

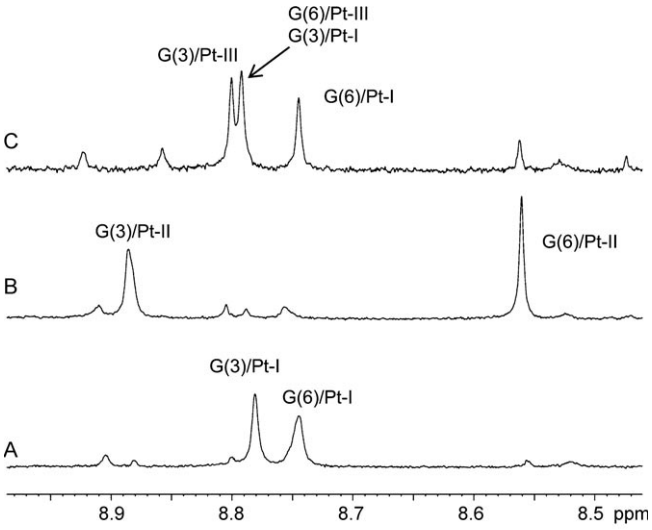


Figure 5. ^{1}H NMR spectra (guanine-H8 resonances only) of: HPLC fraction (A) of Pt-I adduct, (B) of Pt-II adduct, and (C) of a mixture of Pt-I and Pt-III adducts at 279 K in D_2O.

are in close ratio at 310 K, could be validated (Tables S8–S10, Supporting Information). Increasing the temperature from 279 to 310 K restored the distribution of the four Pt-5′-CAGCTG adducts after several minutes (Figure 6), supporting the hypothesis that adducts interconvert slowly to reach equilibrium.

In summary, we were able to detect and assign four different oligonucleotide platinum adducts isolated from the photolysis of complex **1** with 5′-CAGCTG that interconvert slowly at 310 K in D_2O. The four adducts are bis-adducts and show platination of G(3) and G(6) nucleotides of 5′-CAGCTG. No binding to the A(2) residue was detected, notably confirmed by the fact that only very slight shifts of the C(1) proton resonances were detected for the four Pt–ODN adducts (Tables S3–S6, Supporting Information). Pt binding through the *N7* position of guanine, as expected, is validated by 1) the high-frequency shifts of H8 resonances of G(3) and G(6) residues (between 0.5 and 1.0 ppm) and 2) by

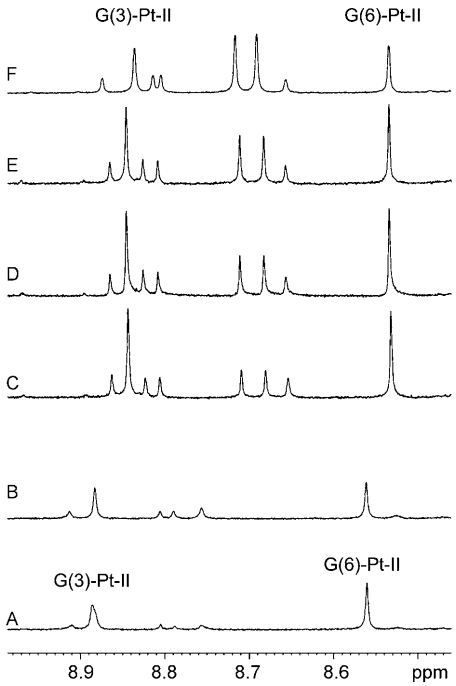


Figure 6. ^{1}H NMR spectra (guanine-H8 resonances only) of the HPLC fraction of adduct Pt-II A) at 279 K, B) after 15 h at 279 K, C) 15 min, D) 30 min, E) 45 min after increasing the temperature to 310 K, and F) after several days at room temperature (spectrum acquired at 310 K).

NOEs between H8 protons of G(3) and G(6) residues and H6 and H6′ protons of the 2,2′-bipyridine moiety (Figures S12–S14 and Table S7, Supporting Information).

Although determination of the fine structure of the four different adducts is beyond the scope of the present study, their presence could be explained by the possibility of obtain multiple Pt–oligonucleotide conformers (head-to-head and head-to-tail), as has been reported for other Pt–DNA adducts.[50,51] NOEs between H8-G(3) and H8-G(6) for Pt-IV indicate that Pt-IV is a head-to-head conformer, while the absence of NOEs between H8-G(3) and H8-G(6) for Pt-I and Pt-II may imply a head-to-tail conformation for the two major forms of adducts (Figure S15, Supporting Information). The conformation of Pt-III could not be assigned by NOE measurements because the two H8 signals are too close. No satellite signal for platinum was detected. The absence of coupling between ^{1}H and ^{195}Pt suggests a +2 oxidation state for platinum adducts, although an unambiguous assignment is not possible.

Enzymatic digestion of the platinum adducts from photolysis of complex 2: Under light irradiation, the reaction of complex **2** with 5′-CAGCTG gives rise to two platinum adducts, Pt-A and Pt-B. The two adducts were isolated as pure samples and subjected separately to enzymatic digestion by phosphodiesterase I (followed by alkaline phosphate). Phosphodiesterase I from snake venom removes nucleotide residues from the 3′-OH terminus of oligonucleotides in the form of nucleotides. Alkaline phosphatase removes the 5′-

phosphate of nucleotides. Pt-A proved completely resistant to digestion. The presence of intact Pt-A adduct at the end of the digestion time was checked by negative LC/ESI-MS analysis. The expected signals at *m/z* 1085.5 for an $[Pt(2,2'\text{-bpy})(MeNH_2)ODN]^{2-}$ ion associated with a signal at *m/z* 1069.5 for $[Pt(2,2'\text{-bpy})ODN]^{2-}$ ion were observed. These data allowed us to propose that Pt-A consists of an oligonucleotide with a platinum ion covalently bound at *N7* of guanine G(6) at the 3′-end of 5′-CAGCTG.

Figure 7 displays the HPLC trace of the products of enzymatic digestion of Pt-B (Figure 7B, C) compared to those of 5′-CAGCTG (Figure 7A). Digestion of Pt-B shows three

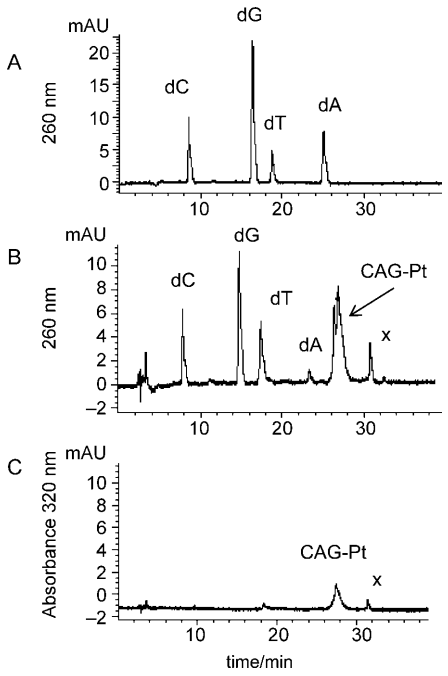


Figure 7. HPLC trace of the enzymatic digestion of enzymatic digestion of 5′-CAGCTG (A) and adduct Pt-B (B,C).

nucleosides dC, dG, and dT associated with a peak at 28 min whose mass spectrum in positive LC/ESI-MS analysis is consistent with a platinated trinucleotide 5′-CAG. Two main signals at *m/z* 1219.3 and 1250.1 account for $[Pt(2,2'\text{-bpy})(CAG)]^+$ and $[Pt(2,2'\text{-bpy})(MeNH_2)(CAG)]^+$ singly charged ions, respectively (Figure S16, Supporting Information). The unmodified trinucleotide 5′-CAG was also detected in the on-line mass spectrum of the trinucleotide adduct at *m/z* 870.5 due to adduct lability during mass analysis. Adduct Pt-B is thus a mono-adduct at G(3) in the middle of 5-CAGCTG with the platinum ion coordinated to a 2,2′-bipyridine ligand, a methylamine ligand, and N7 of guanine.

Discussion

It was previously reported that Pt^{IV} complexes **1** and **2** are reduced to the corresponding Pt^{II} species upon photolysis. This process is accompanied by loss of the axial ligands and release of HOCl due to reductive elimination of HOCl or reductive elimination of Cl_2 and further reaction of Cl_2 with H_2O.[25] The release of two axial ligands from Pt^{IV} complexes is likely to be quantitative with respect to the generated Pt^{II} species upon light irradiation. In this work, photolysis of Pt^{IV} complexes **1** and **2** was carried out in the presence of the short oligonucleotide 5′-CAGCTG. Upon photolysis, Pt^{IV} complexes gave rise to Pt^{II}–ODN adducts identical to those identified with their respective Pt^{II} complexes. Complex **1** was at the origin of complex **3**, which produced a mixture of four conformers of bis-adducts with *N7* of guanine bases G(3) and G(6) of 5′-CAGCTG. Complex **2** transformed into complex **4**, which formed two mono-adducts at G(3) or G(6) of 5′-CAGCTG. For all these adducts, the substitution reactions involved the chlorido ligands. The 2,2′-bipyridine ligand was still present in the bis-adducts Pt^{II}-ODN adducts of **3**. In the case of **4**, the Pt^{II}–ODN adducts carried one methylamine ligand and one 2,2′-bipyridine ligand. In the HPLC traces the folded bis-adducts were eluted first, and the linear mono-adducts after intact ODN 5′-CAGCTG.

Photoisomerization of complex **2** involving migration of the methylamine ligand to an axial position was evidenced in previous work.[25] In the present work we did not observe the formation of complex **3** upon light irradiation of **2**. Photolysis of **2** in the presence of 5′-CAGCTG never produced Pt-I, Pt-II, and Pt-III DNA bis-adducts. It may be concluded that Pt^{IV} complex with an axial methylamine ligand do not undergo photolysis.

We observed that the Pt^{IV} complexes were not stable at 37 °C and transformed spontaneously into their corresponding Pt^{II} complexes in the dark. The dark decomposition of Pt^{IV} complexes increased with increasing temperature. Thus, reduction of the Pt^{IV} bipyridine complexes can be promoted either by light irradiation or heat. Heating at 50 °C had the same efficiency as light irradiation under the tested experimental conditions. Complex **2** was more susceptible to spontaneous reduction than complex **1**.

We found that the reaction of the Pt^{II} complexes **3** and **4** (reduction products of **1** and **2**, respectively) with 5′-CAGCTG was faster than that of cisplatin. The rapid and quantitative reaction of Pt^{II} complexes with DNA allowed us to estimate the yield of spontaneous reduction (photolysis and/or thermolysis) of the Pt^{IV} complex. Indeed, the quantity of Pt^{II} complex generated upon photolysis of the Pt^{IV} complexes at 37 °C can be measured by means of the quantity of Pt^{II}–ODN adducts. Light irradiation of complexes **1** and **2** (10 μM) for 24 h yielded 29 and 46 % of Pt^{II} complex with respect to the starting Pt^{IV} complex. At higher concentration of Pt^{IV}, the oligonucleotide became a limiting factor under the present conditions.

Oxidative DNA damage was never evidenced when Pt^{IV} complexes **1** and **2** reacted with 5′-CAGCTG. The retention times and the mass data of oxidized ODN were known from previous work on 5′-CAGCTG.[39,40] Formation of an oxidized oligonucleotide strand (even 1 % yield, 0.1 μM) would have been detected by the method of analysis. The yield of

spontaneous reduction determines the subsequent concentration of diffusible oxidative species from the released axial ligands (HOCl or Cl_2). When the photolysis reaction was performed at 10 μM of Pt^{IV} complexes, about 3 and 5 μM of Pt^{II} complex formed with **1** and **2**, respectively. Consequently, no more than 3–5 μM of diffusible oxidative species is expected. Increasing the concentration of Pt^{IV} complex did not lead to detectable oxidative DNA damage either. The Pt^{IV} complexes were incubated at a concentration of 100 μM with 5′-CAGCTG (10 μM). The concentrations of Pt^{II}–oligonucleotide adducts were 5.7 μM for **1** after 24 h and 7.4 μM for **2** after 3 h, that is, at least 6–8 μM of Pt^{II} complex and associated potential oxidant were produced during photolysis. In no case was the concentration of diffusible oxidative species (HOCl or Cl2) high enough to allow us to detect oxidative DNA damage.

On the other hand, the Pt^{IV} 2,2′-bipyridine complexes were inert toward substitution reactions with 5′-CAGCTG. Thus, despite the fact that the 2,2′-bipyridine ligand confers to the Pt^{IV} complexes a high redox potential, no guanine oxidation by inner-sphere electron transfer was possible, since neither the intrastrand G nor 3′-G of 5′-CAGCTG coordinated to the Pt^{IV} center. Rather, reductive elimination to Pt^{II} species was the preferred pathway.

Conclusion

Photolysis ($\lambda > 300$ nm) and thermolysis in the dark (37–50 °C) of Pt^{IV} 2,2′-bipyridine complexes **1** and **2** led to reductive formation of the corresponding Pt^{II} complexes. In the presence of oligonucleotide 5′-CAGCTG, Pt^{II} complexes readily formed typical DNA–platinum(II) adducts. All adducts carried the 2,2′-bipyridine ligand and involved guanine *N7* as binding site. When the Pt^{II} species had two chlorido ligands, bisadducts at G(3) and G(6) of 5-CAGCTG were observed. When the Pt^{II} species had only a single chlorido ligand, in addition to a methylamine and a 2,2′-bipyridine ligand, only monoadducts formed.

The extent of spontaneous reduction under light irradiation at 37 °C or in the dark at 50 °C after 24 h was identical and reached about 30 and 50% with respect to Pt^{IV} complexes **1** and **2**, respectively. The Pt^{IV} 2,2′-bipyridine complexes **1** and **2** proved inert toward substitution. The question of the stability of other photoactivatable Pt^{IV} complexes under conditions of moderate warming deserves further studies.

Experimental Section

Chemicals: H_2O was of milliQ grade (Millipore). Oligodeoxyribonucleotide (ODN) 5′-CAGCTG was from Eurogentec SA (Belgium) and purified by HPLC if necessary. Concentration was determined by UV at 260 nm, $\varepsilon = 55\ 000\ M^{-1}cm^{-1}$.[52] Cisplatin was purchased from Merck. Phosphodiesterase I from *Crotalus adamanteus* venom was purchased from USB. Phosphatase, alkaline from *Escherichia coli* was obtainedfrom Sigma-Aldrich.

Platinum complexes: $[PtCl_4(2,2'\text{-bpy})]$ (**1**), $[PtCl_3(2,2'\text{-bpy})(MeNH_2)]Cl$ (**2**), $[PtCl_2(2,2'\text{-bpy})]$ (**3**), and $[PtCl(2,2'\text{-bpy})(MeNH_2)]C1O_4$ (**4**) were prepared according to published procedures.[25] They were characterized by NMR spectroscopy before use. They were never stored in solution to avoid ligand exchange. From an initial solution of complex in acetonitrile (**3**, **4**) or in H_2O (**2**), aliquots containing 100 nmol or 200 nmol were prepared in Eppendorf tubes and immediately dried under vacuum. Dry aliquots were stored in the absence of light at −20 °C. New solutions of complex **1**, dissolved in DMSO, were prepared before each experiment. UV/Vis [λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$)] of **1** in acetonitrile: 307 (10500), 319 (9500); **2** in H_2O: 308 (11000), 320 (11400); **3** in acetonitrile: 276 (18000), 312 (7000), 323 (8600); **4** in acetonitrile: 250 (15500), 308 (9700), 321 (13200).

Electrochemical experiments: Voltammetric measurements were carried out with a potentiostat Autolab PGSTAT100. Experiments were performed at room temperature in a homemade airtight three-electrode cell connected to a vacuum/argon line. The reference electrode consisted of a saturated calomel electrode (SCE) separated from the solution by a bridge compartment. The counterelectrode was a platinum wire of ca 1 cm^2 apparent surface area. The working electrode was a glassy carbon microdisk (0.5 mm diameter). In aqueous media, the platinum complex was dissolved to a final concentration of 1 mM in 0.1 M KCl, and the pH was adjusted to 7.0. In aprotic media, the supporting electrolyte tetrabutylammonium tetrafluoroborate (Fluka, 99% puriss electrochemical grade) and the solvent (DMSO) were used as received. Acetonitrile was freshly distilled over Na/benzophenone prior to use. The solutions used during the electrochemical studies were typically 1 mM in complex and 0.1 M in supporting electrolyte. Before each measurement, the solutions were degassed by bubbling Ar, and the working electrode was polished with a polishing machine (Presi P230). Experimental peak potentials were measured versus SCE and converted to the NHE scale by adding 0.242 V.

HPLC and HPLC coupled to electrospray mass analysis (LC/ESI-MS): The HPLC apparatus was a Hewlett Packard 1050 equipped with a diode-array detector. The oligonucleotide reactions were analyzed on a reverse-phase Nucleosil column (C18, 10 μm, 250×4.6 mm from Interchim, France) eluted with two different gradients: gradient 1: linear gradient from 5 to 12% of acetonitrile in 10 mM triethylammonium acetate buffer (TEAA), pH 6.5 for 20 min, followed by a linear gradient from 12 to 30% acetonitrile for 5 min; gradient 2: linear gradient from 5 to 10% of acetonitrile in 10 mM TEAA, pH 6.5 for 50 min, followed by a linear gradient from 10 to 30% acetonitrile for 5 min. Gradient 1 was used for analysis of the products of the reactions of complexes **2** and **4**, and gradient 2 for the products formed by complex **1**, complex **3**, and cisplatin. The enzymatic digestion media were analyzed on a reverse-phase Nucleosil column (C18, 5 μm, 250×4.6 mm from Interchim, France) with gradient 1. The flow rate was 1 mL min^{-1} and detection was at $\lambda = 260$ and 320 nm. LC/ESI-MS analysis was carried out with the same column, which was coupled to a Perkin-Elmer SCIEX-API 365 spectrometer equipped with a turbo ion spray source (negative mode for oligonucleotides, positive mode for nucleosides or trinucleotides). The temperature of the gas (N_2) stream was set at 450 °C. The chromatography conditions of LC/ESI-MS remained the same as above, but the flow was reduced to 0.5 mL min^{-1}. All the samples were directly injected into the HPLC system. However, in the case of enzymatic digestions a pre-column (reverse-phase) was added ahead of the column.

Reaction of the Pt complexes with oligonucleotide 5′-CAGCTG: The oligonucleotide (ODN) was stored as a 2 mM solution in H_2O at −20 °C. For the platinum complexes **1**, **3**, and **4**, a 1 or 10 mM fresh solution of the platinum complex was prepared before each reaction by dissolving 100 nmol of the complex in 100 or 10 μL of DMSO, respectively. In the case of complex **2** the same dilutions were done in H_2O before each reaction. The reaction medium was prepared by mixing 50 μL of a 100 μM ODN solution, 5 μL of the 1 mM (or 10 mM) platinum complex solution, and 445 μL of H_2O. The final concentrations of the ODN and the platinum complexes were 10 and 10 or 100 μM for 1:1 or 1:10 reactions, respectively. Except in the case of complex **2**, the reaction contained 1% DMSO. The reactions samples (in glass tubes) were incubated at 37 °C in

a glass, thermostated water bath in the dark or under light irradiation. A halogen lamp (40 W, Philips krypton) was mounted 10 cm to the side of samples. Light illumination through glass dishes removes light of wavelengths below about 300 nm.

Isolation of individual platinum adducts: The different adducts were collected when they were eluted from the chromatography column and immediately cooled in ice. The salts of the HPLC buffer were removed by a desalting step (C18 Sep-Pak cartridge from Waters). The desalting procedure consisted of dilution of the fraction with 10 mL H_2O, loading of the sample on the cartridge, washing with 15 mL H_2O, and elution of oligonucleotide with 5 mL H_2O/CH_3CN (95:5). After lyophilization, the dried material was dissolved in H_2O and quantified by UV/Vis spectroscopy, and the adducts were stored dry, as 1 nmol aliquots after a second lyophilization step. For NMR characterization, several reactions were carried out on a 10-fold scale, adducts were collected from HPLC, and fractions were pooled together until about 100 nmol of each adduct was isolated.

NMR spectra: NMR spectra were recorded on a Bruker Avance 600 spectrometer equipped with a 5 mm triple resonance inverse Z-gradient cryoprobe. All chemical shifts for ^1H are relative to the residual HOD resonance (relative to TMS). ^1H NMR spectra were collected at 310 K in D_2O. Presaturation was used to suppress the residual water signal. The 2D T-ROESY spectra were acquired with a mixing time of 250 ms and TOCSY spectra were recorded with a spin-lock time of 60 ms and 90 ms. Typically, 2048 t_2 data points were collected for 512 t_1 increments. Spectra processing was performed using Bruker Topspin software.

Enzymatic digestion: Each isolated, pure, platinum adduct Pt-A, Pt-B, and 5′-CAGCTG (1 nmol) was incubated with phosphodiesterase I (0.03 unit) in 0.18 mL (final volume) 100 mM Tris-HCl buffer pH 8.6, 100 mM NaCl, 14 mM $MgCl_2$ for 1 h at 37 °C. The reaction was quenched at 65 °C for 5 min, then 0.7 units of phosphatase alkaline was added and the sample was kept at 37 °C for 1 h. The analysis of the digestion products was performed by HPLC.

Acknowledgements

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