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Mass spectrometry analysis reveals the distinct reaction pathways of d(Cp^{ox}G) with a photoactivatable Pt(IV) anticancer prodrug

Jishuai Zhang,†[a,b] Ziqi Ma,†[a] Jiafan Lin,[a,b] Wenbing Li,[a] Xiaoqin Wu,[a] Yao Zhao, *[b,c] Fuyi Wang *[b,c,d] and Kui Wu *[a]

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The interactions between d(Cp^{ox}G) (^{ox}G = 8-oxo-guanine), a major form of the oxidatively damaged CpG island motif, and a photoactivatable anticancer Pt(IV) prodrug, *trans*,*trans*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(pyridine)$_2$] (**1**), were investigated using electrospray ionization mass spectrometry (ESI-MS). Surprisingly, the primary MS analysis showed that the major photooxidative products were the platinum-free dinucleotides d(CpGh) (**2a**)/d(CpIa) (**2b**) (possibly a mixture of the two isomers) and d(CpDGh) (**3**), in which the guanine was oxidized to 5-guanidino-hydantoin (Gh) or iminoallantoin (Ia) and 5-guanidino-dehydrohydantoin (DGh), respectively. Moreover, two mono-platinated adducts, {[CpGh] + **1′**}$^+$ (**4**) and {[CpDGh] + **1′**}$^+$ (**5**) (**1′** = [Pt^{II}(N$_3$)(py)$_2$]$^+$), and three Pt-crosslinked dinucleotide adducts, {[CpGh]$_2$ + **1″**}$^{2+}$ (**6**), {[CpGh] + [CpDGh] + **1″**}$^{2+}$ (**7**) and {[CpDGh]$_2$ + **1″**}$^{2+}$ (**8**) (**1″** = [Pt^{II}(py)$_2$]$^{2+}$), were observed as the main platinated adducts. Tandem mass spectrometry with collision induced dissociation (CID) demonstrated that **1′** bound at Gh or DGh in **4** and **5**, while the inter-dinucleotide crosslinks by **1″** between Ghs, Gh and DGh, or DGhs in **6**, **7** and **8** were implicated. Unexpectedly, the proposed platinated d(Cp^{ox}G) adducts were not observed, indicating that ^{ox}G preferentially undergoes further oxidation by the reactive oxygen species released during the photodecomposition of complex **1** rather than coordination with the reduced Pt(II). These results revealed the greater complexity of the photo-interaction of complex **1** with d(Cp^{ox}G) than with d(CpG), with the implication that ^{ox}G-containing DNA, in particular, the oxidative CpG island, might play a vital role in the mechanism of action of photoactivatable Pt(IV) prodrugs, which merits further exploration.

Introduction

The photoactivatable platinum(IV) anticancer prodrug *trans*,*trans*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(py)$_2$] (py = pyridine; **1**) has shown great clinical potential as a photoactivated chemotherapy (PACT) agent.[1,2] Complex **1** is inert in the dark even when incubated with glutathione under physiological conditions for several days, but upon irradiation with blue or green light, it is photoactivated to present promising anticancer activities even to cisplatin-resistant cancer cells.[3,4] This excellent light-controlled cytotoxicity has been attributed to its unique dual-action mode: coordination to biomolecules *via* the reduced Pt(II) species and induced oxidation of biomolecules by producing reactive oxygen species (ROS) including hydroxyl radicals (HO$^•$), singlet oxygen (^{1}O$_2$) and nitrene intermediates to initialize oxidative stress.[4–8] This brings high complexity to the photo-interactions of complex **1** with biomolecules, which might mask its molecular mechanism of action.

Given the virtues of high sensitivity, low sample consumption and chemical specificity, electrospray ionization mass spectrometry (ESI-MS) has been widely applied to elucidate the interactions of metal complexes with potential target DNA.[9–16] We previously performed a series of studies using ESI-MS to investigate the interactions of complex **1** with different DNA structures.[17–23] We showed that complex **1** could bind to and induce oxidation of all five nucleobases with the reactivity following the order G > A, C, U > T.[17] Complex **1** has also been observed to have similar activity in inducing base oxidation in its photoreactions with dideoxynucleotides,[18] 6-mer human telomeric unit (5′-d(TTAGGG)-3′)[20] and a 15-mer single-stranded oligodeoxynucleotide (ODN: 5′-d(CTCTCTTGTCTTCTC)-3′).[23]

[a]*Key Laboratory of Hubei Province for Coal Conversion and New Carbon Materials; School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, P. R. China. E-mail: wukui@wust.edu.cn*
[b]*Beijing National Laboratory for Molecular Sciences; CAS Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China. E-mail: fuyi.wang@iccas.ac.cn, yaozhao@iccas.ac.cn*
[c]*University of Chinese Academy of Sciences, Beijing 100049, P. R. China*
[d]*National Center for Mass Spectrometry in Beijing, Beijing 100049, P. R. China*
†These authors contributed equally to this work.

Interestingly, by investigating the interactions of the epigenetic dideoxynucleotide-pair d(CpG), the CpG island motif, and 5-cytosine methylated d(CpG) (referred to as d(^{5m}CpG)) with complex **1** using ESI-MS,[21] we demonstrated that cytosine methylation had no obvious effect on the platination mode of d(CpG) and d(^{5m}CpG), while the **1**-induced guanine oxidation pathways in d(CpG) and d(^{5m}CpG) differed from each other.[21] These implicated a differential response of the epigenetic DNA pairs to oxidative stress, which may in turn change the mechanism of action of Pt(IV) prodrugs.

8-Oxo-guanine/8-hydroxyl-guanine (^{ox}G) is the major oxidation adduct of guanine in cells, and has been suggested to be a vital biomarker of oxidative stress.[24–26] It is estimated that each cell undergoes an average of 10^5 ^{ox}G damage events in DNA per day,[27] and that the background level of ^{ox}G is about 1 per 10^6 guanines.[28] In particular, oxidative damage in CpG islands was demonstrated to be responsible for the hypermethylation of the CpG island in the promoter region and the global hypomethylation in cancer cells.[29] Due to its much lower redox potential (0.74 V *versus* NHE),[30] ^{ox}G will be preferentially oxidized further even in the presence of a large excess of guanines,[31,32] and is also much more sensitive to oxidative stress than dG, dA, dC, dT, or other modified bases.[33] These facts inspired us to examine the interactions of ^{ox}G-containing CpG dideoxynucleotide, 5′-d(Cp^{ox}G), with complex **1** under light irradiation. This will skip the oxidation process of G to ^{ox}G *via* photoreaction with complex **1**, providing novel insights for a better understanding of the potential roles of ^{ox}G and Cp^{ox}G in the mechanism of action of Pt(IV) prodrugs.

Materials and methods

Chemicals

Pt(IV) complex (*trans*,*trans*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(py)$_2$], **1**, Chart 1) was synthesized as previously reported.[2] HPLC-purified dideoxynucleotide 5′-d(Cp^{ox}G) (Chart 1) was purchased from Sangon Biotech (Shanghai, China). Acetonitrile (HPLC grade) and formic acid (HPLC grade) were purchased from Merck (Darmstadt, Germany). Irradiation was carried out with an LED array of 12 blue 5630 SMD LEDs (3 W; λ_{max} = 459 nm; 3000 lx). Aqueous solutions were prepared using MilliQ water (MilliQ Reagent Water System).

Sample preparations

Complex **1** and 5′-d(Cp^{ox}G) were dissolved in deionized water to give stock solutions of 5 mM and 1 mM, respectively. The typical reaction between 5′-d(Cp^{ox}G) and complex **1** (0.1 mM) was performed by mixing complex **1** and 5′-d(Cp^{ox}G) in a molar ratio of Pt/d(Cp^{ox}G) = 1 : 1 in water followed by irradiation under blue LED light for 1 hour. Each reaction mixture was then immediately diluted with an equal volume of acetonitrile containing 1% formic acid before infusion into the mass spectrometer for MS or MS/MS analysis under positive-ion mode.

Electrospray ionization mass spectrometry (ESI-MS)

Positive-ion electrospray ionization mass spectra were obtained using a Xevo G2 Q-TOF mass spectrometer (Waters, Manchester, UK). Typical source conditions were as follows: capillary voltage, 3.10 kV; sample cone, 40 V; extraction cone, 3.0 V; source temperature, 373 K; desolvation temperature, 623 K; desolvation gas (N_2) flow rate, 800 L h^{-1}. Leucine-enkephalin at a concentration of 2 ng μL^{-1} and a flow rate of 10 μL min^{-1} was used as the lockmass calibration. Postcalibrations using the respective native oligonucleotide for primary MS and the parent ion for MS/MS analysis were also applied, respectively. MassLynx (ver. 4.1) software was used for all the analysis and post-processing.

Results and discussion

First, the reaction mixture of complex **1** with 5′-d(Cp^{ox}G) in a 1 : 1 molar ratio in deionized water under dark conditions was injected directly for primary mass spectrometric analysis. As shown in Fig. 1A, only free d(Cp^{ox}G), its dimer, and dissociated fragments of d(Cp^{ox}G), such as [d(Cp^{ox}G)-C]$^+$ and [^{ox}G]$^+$, were identified, along with the dimer of free complex **1**; no platinated d(Cp^{ox}G) or other platinated products were observed. This means that no interaction occurred between d(Cp^{ox}G) and complex **1** in the absence of light irradiation, indicating the

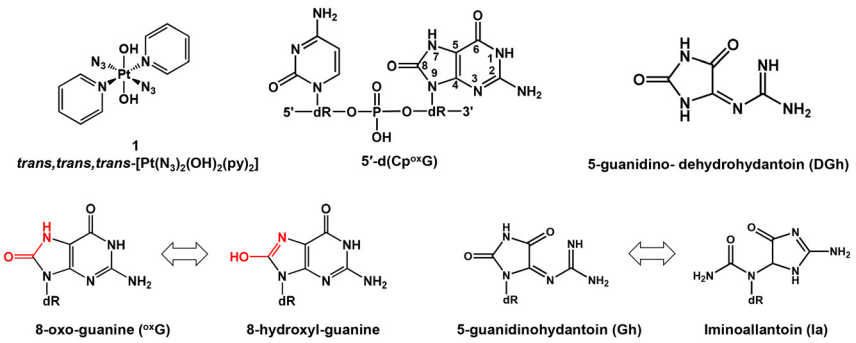


Chart 1 Chemical structures of photoactivatable diazido Pt(IV) prodrug (**1**), 5′-d(Cp^{ox}G) with the ^{ox}G ring atoms numbered, and the proposed oxidation adducts.

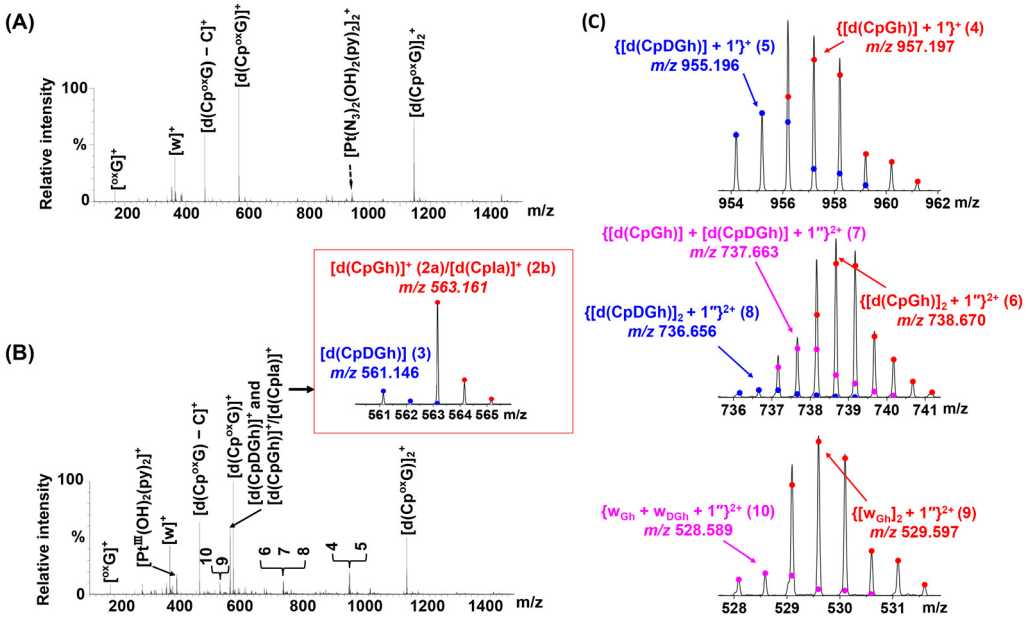


Fig. 1 MS spectra with the ion peaks labeled for the reaction mixtures of 5′-d(Cp^{ox}G) with complex **1** in the dark (A) or after blue light irradiation for 1 h (B). The inset with a red frame in (B) presents the isotopic models (dots) and mass spectra (lines) of d(CpGh) (**2a**)/d(CpIa) (**2b**) and d(CpDGh) (**3**). (C) The isotopic models (dots) and mass spectra (lines) of adducts **4**–**8** and the fragments **9**–**10** shown in (B). **1′** = *trans*-[Pt(N$_3$)(py)$_2$]$^+$ and **1″** = *trans*-[Pt(py)$_2$]$^{2+}$.

inertness and dark stability of complex **1**. These results are in line with previous reports.[21]

In contrast to the aforementioned inertness of complex **1** in the dark, after irradiation under blue light at 459 nm for 1 hour, in addition to the peaks observed under dark conditions (Fig. 1A), several new peaks corresponding to both platinum-free and platinated adducts formed by the photoreaction of 5′-d(Cp^{ox}G) with complex **1** were observed in the primary mass spectrum (Fig. 1B and Table S1 in the SI). Notably, the major product with high intensity was a platinum-free oxidative dideoxynucleotide at *m*/*z* 563.161 (insert in Fig. 1B), whose mass is 10 Da less than that of d(Cp^{ox}G) (calc. *m*/*z* 573.145). By referring to the potential oxidation forms of ^{ox}G reported previously,[33–35] this product was assigned as [d(CpGh)]$^+$ (**2a**) (Gh = 5-guanidino-hydantoin) or [d(CpIa)]$^+$ (**2b**) (Ia = iminoallantoin) (calc. *m*/*z* 563.161), or a mixture of **2a** and **2b**. Gh and Ia are tautomers; Gh retains the N7 atom, which is the preferential site for Pt(II) coordination, while Ia loses it. The pH condition is the dominant factor controlling the ratio of Gh to Ia during the oxidation of ^{ox}G.[36] At 22 °C, ^{ox}G consisted of 50% Gh and 40% Ia at pH = 4, while the ratio of Gh to Ia in the oxidated single-stranded oligomers was 1 : 1 at pH = 7.[36] Unfortunately, herein, we cannot distinguish these tautomers *via* MS analysis as they have the same molecular weight. Considering the reaction medium (pure water) of the reaction of complex **1** and d(Cp^{ox}G), and the weak acidity of dinucleotides, the ratio of Gh and Ia might be approximately 1.1. It is worth pointing out that a minor Pt-free product at *m*/*z* 561.146 was detected (insert in Fig. 3B) and is assignable to [d(CpDGh)]$^+$ (**3**, calc. *m*/*z* 561.145) (DGh = 5-guanidino-dehydrohydantoin), in which ^{ox}G was further oxidized to DGh; its abundance relative to adduct **2** is only 0.13.

The oxidation of guanine in DNA was driven by the ROS released from photodecomposition of complex **1**.[8,17] Taking the transient lifetimes of the ROS into account, close proximity of the bases to **1** was necessary for the oxidation of guanine, which implies that platination on a G base might take place prior to the oxidation of the G base.[4] However, herein, we found that the main products of the reaction of Cp^{ox}G with complex **1** under irradiation were Pt-free oxidative species. This indicates that the oxidated G base (referred to as ^{ox}G herein) is more susceptible to oxidation than to Pt(II) coordination, in line with previous reports.[31,32]

In addition to the Pt-free products **2** (**2a**/**b**) and **3**, we identified two groups of platinated adducts with lower abundance than the Pt-free adducts in the reaction mixture of Cp^{ox}G with complex **1** upon irradiation. The first group, which was associated with the singly-charged ion peaks at *m*/*z* 955.196 and 957.197 (representing the highest isotopic peak of each adduct; this convention will be used hereinafter), respectively, comprises two mono-platinated adducts. Based on our previous knowledge of the photoreaction of complex **1** with d(CpG) and d(^{5m}CpG),[21] the platinated adduct at *m*/*z* 957.197 was assumed to be the mono-platinated dideoxynucleotide with the bound Pt moiety bound as *trans*-[Pt(N$_3$)(py)$_2$]$^+$ (**1′**), *i.e.*, {[d(CpGh)] + **1′**}$^+$ (**4**, calc. *m*/*z* 957.212), in which ^{ox}G was oxidized to Gh. The adduct at *m*/*z* 955.196 was another mono-platinated adduct {[d(CpDGh)] + **1′**}$^+$ (**5**, calc. *m*/*z* 955.196) (Fig. 1C), in which the ^{ox}G base was further oxidized to DGh according to the isotopic pattern shown in Fig. 1C. Based on

the peak intensities of the two adducts, their abundance ratio is **4 : 5** = 1.7 : 1, indicating that ^{ox}G is more readily oxidized to Gh than DGh whether Pt(II) binds to it or not (referring to the result shown in the inset in Fig. 1B).

Interestingly, the products in the second group, for which doubly-charged ion peaks were observed in the range of *m*/*z* 735–741 (Fig. 1C), are assignable to three dideoxynucleotides crosslinked by *trans*-$[Pt(py)_2]^{2+}$ (**1″**), *i.e.*, $\{[d(CpGh)]_2 + \mathbf{1''}\}^{2+}$ (**6**, obs. *m*/*z* 738.670; calc. *m*/*z* 738.678); $\{[d(CpGh)] + [d(CpDGh)] + \mathbf{1''}\}^{2+}$ (**7**, obs. *m*/*z* 737.663; calc. *m*/*z* 737.670), and $\{[d(CpDGh)]_2 + \mathbf{1''}\}^{2+}$ (**8**, obs. *m*/*z* 736.656; calc. *m*/*z* 736.662). The relative abundance of the three Pt-crosslinked adducts decreases in the order 18.4 (**6**) > 6.7 (**7**) > 1 (**8**). These results are consistent with previous reports that *trans*-$[Pt(py)_2]^{2+}$, which is formed through two one-electron donations from the two N_3 ligands during the photodecomposition of complex **1**,[5] and its analogues[37] are bifunctional agents and can crosslink electron donors—such as guanines and their analogues[2,20,38]—to form 1,3-intrastrand crosslinks in the same way as *trans*-Pt(II) complexes.[39]

Moreover, two fragment ions of the aforementioned Pt-crosslinked adducts were observed in low abundance at *m*/*z* 529.579 and 528.589 (Fig. 1C). The former ion is assignable to $\{[w_{Gh}]_2 + \mathbf{1''}\}^{2+}$ (**9**), and the latter to $\{w_{Gh} + w_{DGh} + \mathbf{1''}\}^{2+}$ (**10**), where w_{Gh} and w_{DGh} are fragments possibly derived from the crosslinked adducts *via* the O-glycosidic bond breakage of cytosine in Cp^{ox}G (Fig. 2A, *vide infra*). This implies that the glycosidic bond in cytosine is more easily broken in CID than the phosphodiester bond bridging C and ^{ox}G when Pt(II) crosslinks the two oxidized guanine bases DGh and Gh.

It was worth pointing out that herein, the reaction between 5′-d(Cp^{ox}G) and complex **1** was performed at a molar ratio of Pt/d(Cp^{ox}G) = 1 : 1. In our previously-reported results of the photo-interaction between complex **1** and four natural nucleosides (guanosine, adenosine, cytidine and thymidine) under similar conditions, increasing the Pt/nucleoside molar ratio from 1.0 to 2.0 showed no effects on the mono-platinated nucleosides as the major products, but produced more di-platinated guanosine, cytidine adducts, and platinated oxidized adenosine and cytidine adducts, as well as more mono-platinated cytidine dimer adducts, although all these adducts were minor products.[17] According to these results, varying the Pt : DNA molar ratio might also affect the abundance of platinated products, including oxidized platinated adducts and Pt-crosslinking adducts derived from the photoreaction of 5′-d(Cp^{ox}G) and complex **1**.

To confirm the proposed structures of the mono-platinated adducts **4–5** and identify the platination sites, tandem mass spectrometry (MS/MS) using collision induced dissociation (CID) was applied by introducing these platinated adducts as parent ions (Fig. 2 and Table S2 in SI). Based on the fragmentation of native DNA[40] and platinated d(CpG) reported previously by us,[21] a representative CID fragmentation pathway of d(Cp^{ox}G) is proposed in Fig. 2A.

In the fragmentation spectrum of complex **4** (Fig. 2B), in addition to the parent ion, major fragment ions assignable to

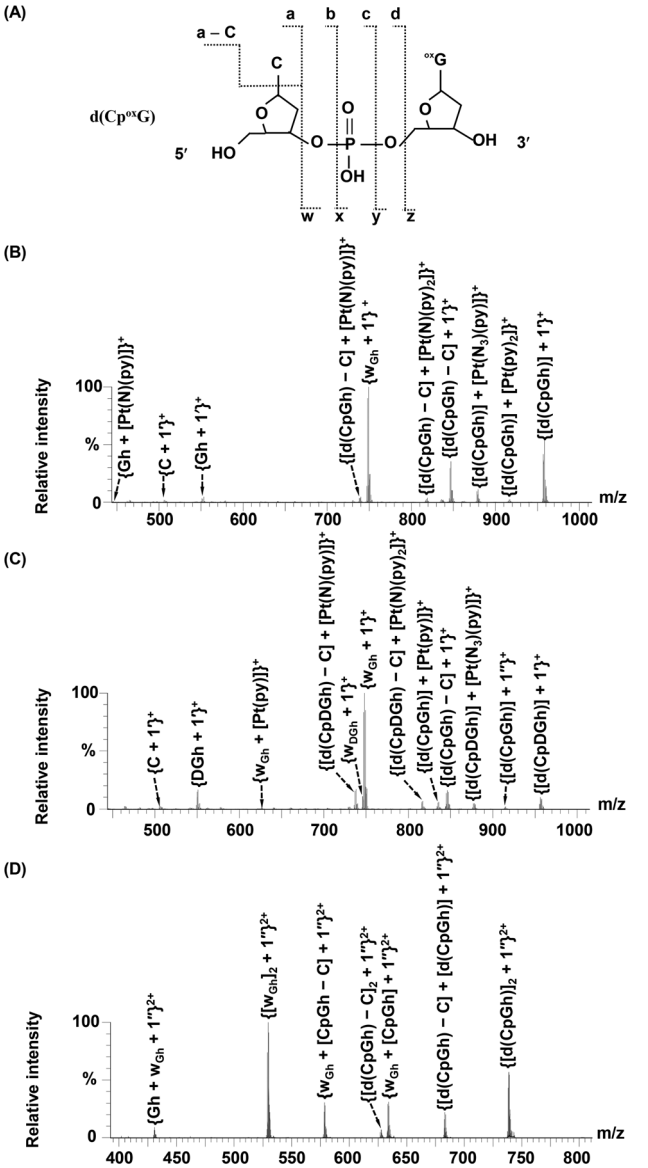


Fig. 2 (A) Representative fragmentation pathway of dideoxynucleotides during the CID fragmentation using 5′-d(Cp^{ox}G) as a model. (B, C and D) Tandem mass spectra of complexes **4** (B), **5** (C) and **6** (D) with the major fragment ions labeled.

$\{w_{Gh} + \mathbf{1'}\}^+$ and $\{[d(CpGh)\text{-}C] + \mathbf{1'}\}^+$ were observed; these indicate Gh to be the platination site. Other minor fragment ions assignable to $\{[d(CpGh)\text{-}C] + [Pt(N)(py)_2]\}^+$, $\{[d(CpGh)\text{-}C] + [Pt(N)(py)]\}^+$, $\{w_{Gh} + [Pt(N)(py)]\}^+$ and $\{w_{Gh} + [Pt(N)]\}^+$ also provide evidence that the oxidated guanine base was the platination site. This result was further verified by the direct detection of the platinated Gh fragments $\{Gh + \mathbf{1'}\}^+$ and $\{Gh + [Pt(N)(py)]\}^+$. Notably, a detectable amount of the $\{C + \mathbf{1'}\}^+$ fragment ion was also observed in the MS/MS spectra of adducts **4** (Fig. 2B and Table S2). This verifies the platination of cytosine by complex **1** upon light irradiation,[21] albeit much less favorably. However, we cannot exclude the possibility that the $\{C + \mathbf{1'}\}^+$ fragment ion is recombined during the collision induced dissociation in

the MS/MS chamber. CID was similarly applied to fragmentate adduct **5** (Fig. 2C), and the platination at the DGh site is directly evidenced by the $\{DGh + \mathbf{1}'\}^+$ fragment ion and indirectly supported by fragment ions such as $\{[d(CpDGh)\text{-}C] + [Pt(N)(py)_2]\}^+$, $\{[d(CpDGh]\text{–}C] + [Pt(N)(py)]\}^+$, $\{w_{DGh} + \mathbf{1}'\}^+$, $\{w_{DGh} + [Pt(py)_2]\}^+$, $\{w_{DGh} + [Pt(py)]\}^+$ and $\{DGh + [Pt(N)(py)]\}^+$. Due to the partial overlap of the isotope peaks between adducts **4** and **5** and the relatively lower intensity of adduct **5** (Fig. 3C), a few fragment ions derived from **4** were observed in the MS/MS spectrum of **5**, but these did not affect the identification of the platination site in **5**.

Similarly, tandem mass spectrometry was applied to identify the platination site in Pt(II)-crosslinked adduct **6** $\{[d(CpGh)]_2 + \mathbf{1}''\}^{2+}$ (Fig. 2D and Table S2). The major fragments were identified as $\{[w_{Gh}]_2 + \mathbf{1}''\}^{2+}$, $\{w_{Gh} + [d(CpGh)\text{-}C] + \mathbf{1}''\}^{2+}$, $\{w_{Gh} + [d(CpGh)] + \mathbf{1}''\}^{2+}$, $\{[d(CpGh)\text{-}C] + [d(CpGh)] + \mathbf{1}''\}^{2+}$, $\{Gh + w_{Gh} + \mathbf{1}''\}^{2+}$ and $\{[d(CpGh)\text{-}C]_2 + \mathbf{1}''\}^{2+}$, accompanied by other similar 1 + charged platinated fragments and free cytosine ion. While all fragment ions unambiguously indicate the platination of Gh by $[Pt(py)_2]^{2+}$, no information supporting the platination of cytosine in **6** was obtained. Although the classical H-bonding G : C pair can be maintained to form Pt-free dideoxynucleotide dimers, as shown in the primary mass spectrum (Fig. 1A), this seemed to be impossible in the present case based on a comparison of the strength of the H-bond and covalent bond in collision dissociation. Hence, a crosslinking mode between the two Ghs by $[Pt(py)_2]^{2+}$ in adduct **6** was more favored. Due to their similar structure, the same crosslinking mode between Gh and DGh *via* [Pt

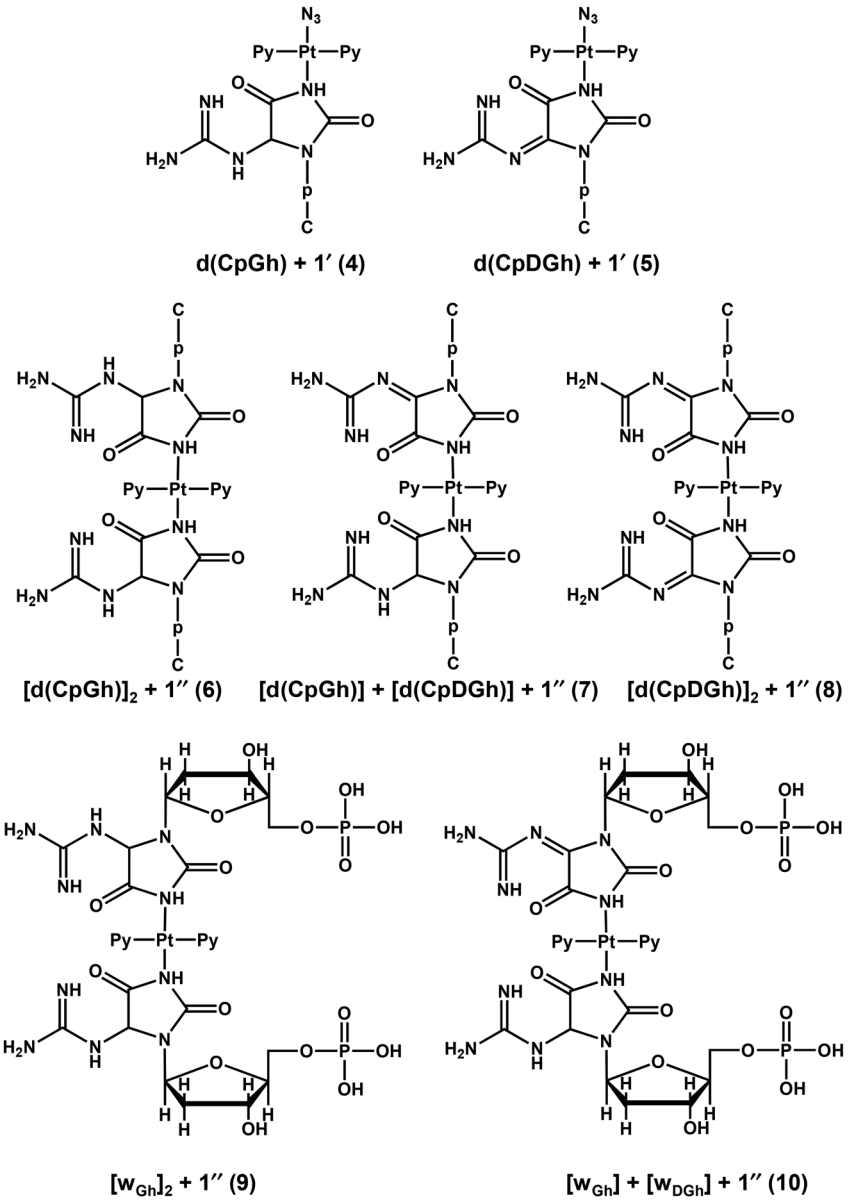


Fig. 3 Proposed structures of platinated adducts **4**–**8** and fragments **9**–**10**.

$(py)_2]^{2+}$ in **7** ({[d(CpGh)] + [d(CpDGh)] + **1″**}$^{2+}$), and between two DGhs *via* $[Pt(py)_2]^{2+}$ in **8** ({[d(CpDGh)]$_2$ + **1″**}$^{2+}$) is also implicated.

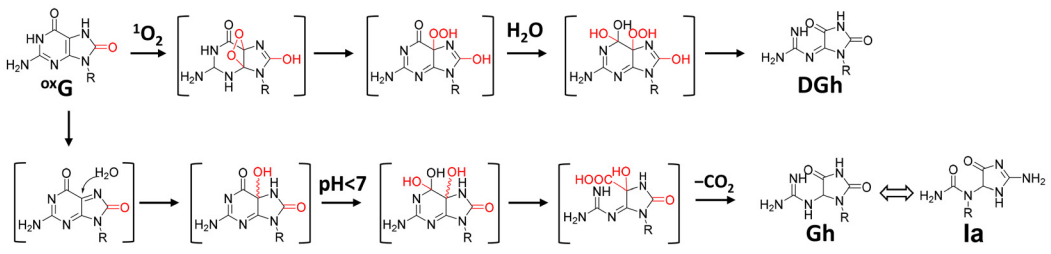
Cisplatin forms G–Pt–G and A–Pt–G intra-strand or G–Pt–G interstrand crosslinks in the *cis*-configuration, which are the pivotal cisplatin-DNA adducts causing cell death.[41] Notably, complex **1** and its analogues are in the *trans*-configuration, and their photo-reduced Pt(II) species ($[Pt(N_3)(py)_2]^+$ (**1′**) and $[Pt(py)_2]^{2+}$ (**1″**)) have been shown to crosslink guanines to form *trans*-$[Pt(py)_2(5'\text{-GMP})_2]^{2+}$ when interacting with 5′-GMP upon light irradiation.[2,4] One of our recent studies showed that 1,3-intrastrand crosslinking by *trans*-$[Pt(py)_2]^{2+}$ *via* G4 and G6 might be formed in the photo-interactions of complex **1** with the 6-mer human telomeric motif, 5′-$T_1T_2A_3G_4G_5G_6$,[20] and minor amounts of adenine–adenine, adenine–cytosine and cytosine–cytosine intrastrand crosslinks by *trans*-$[Pt(py)_2]^{2+}$ were also confirmed.[22] However, this is the first time that crosslinks between guanine oxidation adducts (Gh and/or DGh) by complex **1** (**1′** or **1″**) upon light irradiation have been observed. Notably, based on the MS/MS results, it was speculated that there may be two pathways for crosslink formation (Gh–Pt–Gh for example): (1) The formed mono-platinated adduct {[Cp(Gh)] + **1′**}$^+$ could be further attacked by free Cp (Gh) to form {[Cp(Gh)]$_2$ + **1″**}$^{2+}$ by replacing the azidyl ligand in **1′**; (2) two Cp^{ox}G could attack **1** at the same time upon irradiation accompanied with the release of both azidyl or hydroxyl ligands, and then oxidize to {[Cp(Gh)]$_2$ + **1″**}$^{2+}$.

Our studies described above unambiguously showed that complex **1** could oxidize ^{ox}G in d(Cp^{ox}G) to Gh/Ia and DGh (Scheme 1) to form the oxidative species d(CpGh)/d(CpIa) and d(CpDGh) under the examined conditions. Additionally, the reduced Pt(II) coordinated to Gh and DGh to produce platinated photochemical products upon light irradiation. This was in line with previous reports that ^{ox}G could be easily oxidized to produce DGh and Gh or Ia *via* electron transfer or singlet oxygen.[42–45] Additionally, the results showed that the oxidation process of ^{ox}G by complex **1** had no effects on the binding of **1′** to the further oxidized G bases DGh and Gh (Scheme 1 and Fig. 3). The tandem mass spectrometric results also indirectly provided evidence for the formation of these two minor guanine oxidation adducts in the photoreactions of complex **1** with d(CpG) and the corresponding proposed oxidation mechanism.[21]

The photoactivatable diazido Pt(IV) complex can bind to CpG and its analogues, including CpG and ^{5m}CpG,[21] and the Cp^{ox}G herein, inducing different degrees of oxidative damage. For instance, methylated CpG (^{5m}CpG) increased oxidation and maintained the same binding capability of oxidized guanine base to reduced Pt(II) species. The reactivity of the reduced Pt(II) species, $[Pt(N_3)(py)_2]^+$, in binding to the bases in CpG and ^{5m}CpG decreases in the order G-N7 > C-N3/^{5m}C-N3 > G-N1/C-N3/^{5m}C-N3, but cytosine methylation switched the oxidation products of guanine from Gh and DGh in CpG to DIz and Iz in ^{5m}CpG addition to the classic ^{ox}G and RedSp oxidation products. However, the already oxidized CpG (Cp^{ox}G) significantly promoted further oxidation, and unprecedentedly induced crosslinking between Gh and/or DGh. Both the $[Pt(N_3)(py)_2]^+$-bound mono-functional CpGh and CpDGh and the $[Pt(py)_2]^{2+}$-bound crosslinking adducts were clearly observed in this work. This broad spectrum of photoreaction products, including platination, crosslinking, and oxidation products, might significantly strengthen the anticancer capability of the Pt(IV) prodrugs.

Platinum coordination to DNA has been widely confirmed to exert anticancer effects. Additionally, the oxidation of DNA has also been shown to enhance anticancer effects. Oxidative damage to the epigenetic d(CpG) sequence could inhibit recognition in essential physiological processes due to the chemical structure alteration of the d(CpG) islands, especially in the promoter region.[46,47] For example, the oxidation in methylated d(CpG) could block the demethylase recognition and keep the gene silent.[48,49] Furthermore, the oxidation damage in d(Cp^{ox}G) generally presents a block to the DNA synthesis of Polymerase I, especially in DNA templates containing spiroiminodihydantoin (Sp) and Gh.[36] Compared to mildly mutagenic ^{ox}G, Gh possessed strong mutagenicity when encoding G → T and G → C transversion mutations *in vitro* and *in vivo*,[50] and it could also prevent polymerases from replicating DNA.[27,36]

Previous research measured the DNA-bound platinum to be 700 ± 84 fmol Pt μg^{-1} DNA for complex **1** upon irradiation in A2780 cancer cells, which was approximately 16-fold higher compared to the amount formed by cisplatin in the dark (43 ± 8 fmol Pt μg^{-1} DNA).[51] Using the background level of ^{ox}G (1/10^6 G),[28] the ratio of Pt to G was calculated to be about 1 : 1082, and that of Pt to ^{ox}G to be about 924 : 1 in cells treated with complex **1**. However, the much lower redox potential of ^{ox}G will cause it to be preferentially oxidized over other intact bases even in the presence of a large excess of guanines, *e.g.*,



Scheme 1 Proposed mechanism for the production of the oxidized species of ^{ox}G, Gh/Ia and DGh, in 5′-Cp^{ox}G by $^1O_2$ during the photodecomposition of complex **1** upon blue light irradiation for 1 h (the bound Pt units are omitted for clarity).

in the model duplex sequence 5′-TCATGGGTC^{ox}GTCGGTATA-3′*3′-AGTACCCAGCAGCCATAT-5′. Hence, the preferential further oxidation of ^{ox}G in Cp^{ox}G could occur in cancer cells exposed to complex **1** upon irradiation, perhaps playing a role in the action of complex **1** as well as its analogues.

Conclusion

In summary, the interactions between the photoactivatable anticancer Pt(IV) prodrug *trans*,*trans*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(py)$_2$] (**1**) and d(Cp^{ox}G) upon light irradiation were investigated using high resolution ESI-MS. The results showed that unlike the methylation of the motif in the CpG island, the oxidation of the G base in CpG (to Cp^{ox}G) changes the reaction pathway of the photoreaction of CpG with complex **1**. For instance, the oxidation of G makes the oxidized G, *i.e.*, ^{ox}G, more sensitive to the oxidative stress response to the ROS released from the photodecomposition of complex **1** than to Pt(II) coordination, although the oxidation does not change the coordination mode of Pt(II)–N7(–G). Moreover, the oxidation of G to ^{ox}G and even to Gh and DGh does not change the crosslinking feature of the oxidative G bases by Pt(II). The coordination of reduced Pt(II) species to Gh and DGh produced inter-dideoxynucleotide crosslinking between DGh and DGh, DGh and Gh, Gh and Gh by Pt(II). Given the key roles of oxidative damage on CpG islands in maintaining genome stability and in the development of cancers, our studies herein implicate that the epigenetic effects, in particular, the oxidative damage of CpG islands, deserves further exploration in order to better understand the unique molecular mechanism of anticancer Pt(IV) prodrugs. Additionally, our studies verify that the high sensitivity and high resolution of MS play a vital role in deciphering the interactions between small molecular drugs and biological molecules.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

All the data used are provided in the article and the SI. Supplementary information containing all the primary MS data and the MS/MS data is available. See DOI: **https://doi.org/10.1039/d5an00728c**.

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