[^{1}H, ^{15}N] NMR Studies of the Platination of Phosphorothioate Nucleotides – Monofunctional Sulfur Adducts versus Macrochelation

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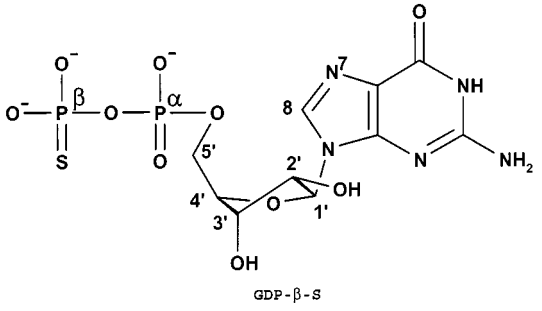
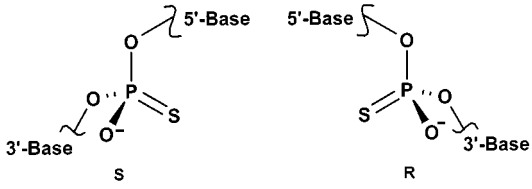
cis-[$PtI_2(^{15}NH_3)_2$] reacts rapidly with guanosine-5′-*O*-(2-thiodiphosphate) (GDP-β-S) to form a macrochelate in which Pt bridges the phosphorothioate S and guanine N7 atoms. Phosphorothioate(α-S)-containing self-complementary decamer oligonucleotides give rise to relatively long lived S-bound monofunctional adducts, the fate of which is sequence dependent.

Platinum complexes are now widely used in cancer chemotherapy.[1,2] In order to reduce toxic side effects it would be advantageous to target Pt to specific DNA base sequences on specific genes. Such a strategy is currently being explored by Lippert, Leng et. al.[3] using oligonucleotides labelled with *trans*-Pt^{II} diam(m)ine complexes, which subsequently cross-link guanine bases on the two strands. Since Pt^{II} drugs are known to have a high affinity for sulfur ligands[4] an alternative strategy might involve antisense or antigene recognition of the target gene using phosphorothioate-labelled oligonucleotides.[5–8] Phosphorothioate nucleotides are already of much clinical interest in view of their activity against HIV, for example.[9] One of our own interests is in targeting photoactivatable prodrugs to specific sites on DNA for potential photodynamic therapy.[10,11]

In the present work, we have studied the pathways and products from reactions of ^{15}N-labelled *cis*-[$PtI_2(^{15}NH_3)_2$] with the phosphorothioate guanosine-5′-*O*-(2-thiodiphosphate) [GDP-β-S, Chart 1] and three decamer oligonucleotides by NMR spectroscopy.

GDP-β-S was chosen for the study because of its ready availability. The oligonucleotides possess the self-complementary sequence 5′-TATGGCCATA-3′ (**I**) containing one phosphorothioate group either between the GG sequence 5′-TAT**GsG**CCATA-3′, **Is**, or between the CC sequence 5′-TATGG**CsC**ATA-3′, **IIs**. For comparison we also investigated the reaction of the diiodo Pt^{II} complex with the unmodified decamer **I**. Iodoplatinum complexes are also of interest because of their potential photoreactivity.[10,11]





Reactions of *cis*-[$PtI_2(^{15}NH_3)_2$] (0.8 mM) with guanosine-5′-*O*-(2-thiodiphosphate) [GDP-β-S] in a 1:1 molar ratio were investigated by ^{1}H NMR spectroscopy at pH 6.94 and 310 K for a period of 24 h. Within minutes, the ^{1}H NMR signals for *cis*-[$PtI_2(^{15}NH_3)_2$] and H8 of GDP-β-S decreased in intensity. After 5 h the reaction was finished and one major product was formed, giving rise to a singlet at δ = 8.99, which was assigned to H8 of Pt-bound GDP-β-S. A high-frequency shift for the H8 resonance of 5′-GMP is typical of metal coordination to the N7 of 5′-GMP (free 5′-GMP: δ = 8.17; Pt-bound 5′-GMP: δ = 8.52). The large high-frequency shift of 0.82 ppm observed for the H8 resonance is notable. The doublet for H1′ shifted to higher frequency from δ = 5.93 to 6.06 and the ^3J(H1′-H2′) coupling constant was dramatically reduced from 6.05 to 2.75 Hz. This suggested a major change in the sugar conformation. The signal for H2′, however, shifted to low fre-

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quency from δ = 4.84 to 4.52. The assignments of the sugar proton resonances were confirmed by homonuclear ^{1}H-^{1}H-{^{15}N}-DQF-COSY NMR spectroscopy (Table S1 in the Supporting Information). The 2D [^{1}H, ^{15}N] HSQC NMR spectrum of the final product showed the presence of two cross-peaks with ^{15}N/^{1}H shifts of δ = −67.85/4.18 and −46.35/4.00, compatible with NH_3 *trans* to N and S, respectively, in square-planar Pt^{II} complexes.[12] The 1D ^{1}H-{^{15}N} NOE NMR difference spectrum of this product showed that irradiation of H8 increased the signal intensity of H3′ by ca. 5% and H2′ by ca. 4%, which suggested that the flexible sugar ring of free GDP-β-S changed to a more rigid structure with a C3′-endo sugar geometry in the Pt-bound GDP-β-S product. A model that is consistent with all of these observations is shown in Figure 1.

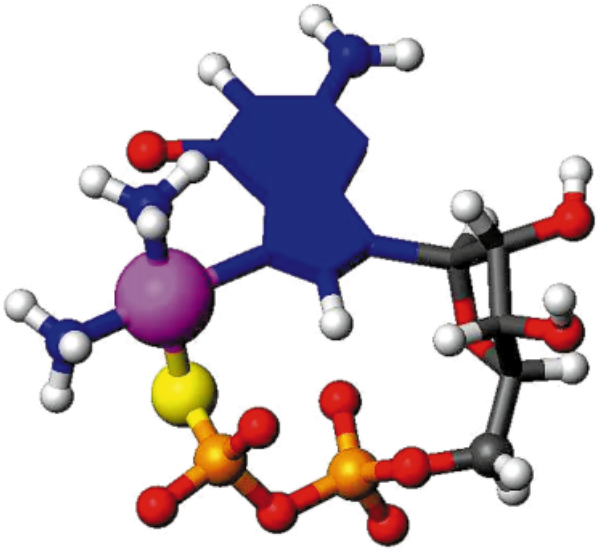

Figure 1. Model of the macrochelate complex *cis*-[$Pt(NH_3)_2$(GDP-β-S)-*N7,S*], the major adduct from the reaction of *cis*-[$PtI_2(NH_3)_2$] with the phosphorothioate GDP-β-S as deduced from NMR spectroscopic data; the macrochelate is formed by Pt binding to the S and N7 atoms of GDP-β-S

The model contains a square-planar *cis*-{$Pt(NH_3)_2$}$^{2+}$ centre bound to *N*7 and the S of GDP-β-S, thereby forming a 13-membered macrochelate ring. The composition was confirmed by ESI-MS analysis of the product of the reaction between *cis*-[$PtI_2(^{15}NH_3)_2$] and GDP-β-S (1:1 molar ratio, 100 μM) after 4 h incubation at 295 K. The negative ion electrospray mass spectrum of the product showed a cluster of ions related to the isotopic distribution of Pt, P and N at *m*/*z* = 687.1 associated with the molecular ion [$Pt(^{15}NH_3)_2C_{10}H_{12}N_5O_{10}P_2S]^-$ (calcd. 687.0) (Figure S1 in the Supporting Information), which corresponds to the macrochelate [$Pt(^{15}NH_3)_2$(GDP-β-S)*S,N*7].

Studies were also carried out to investigate the course of the reactions of *cis*-[$PtI_2(^{15}NH_3)_2$] with oligonucleotide duplexes containing a phosphorothioate group. The self-complementary oligonucleotides **I**, **Is** and **IIs** were shown to exist as duplexes in solution by the presence of imino-proton ^{1}H NMR resonances between δ = 12.00 and δ = 14.00. Reactions of *cis*-[$PtI_2(^{15}NH_3)_2$] (0.5 mM) with **Is** or **IIs** duplexes in a 1:1 molar ratio were studied by 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy in 10 mM KH_2PO_4, 100 mM $KClO_4$, at pH 6.1 and 310 K for a period of 24 h. In this case the sulfur is located on the α-phosphate in contrast to the β-phosphate in GDP-β-S.

For the reactions with **Is**, the ^{15}N/^{1}H cross-peak at δ = −52.12/3.99 of *cis*-[$PtI_2(^{15}NH_3)_2$] in the 2D [^{1}H, ^{15}N] HSQC NMR spectrum had already disappeared after 4 h and four new major cross-peaks were observed (Figure 2). These peaks had ^{15}N/^{1}H chemical shifts of δ = −54.92/4.07 (**1**) and δ = −54.33/4.04 (**2**) consistent with NH_3 *trans* to I in square-planar Pt^{II} complexes, and δ = −45.97/4.24 (**3**) and δ = −46.05/4.22 (**4**), which are characteristic of NH_3 *trans* to S.[12] The intensities of cross-peaks **1**/**3** and **2**/**4** increased simultaneously and were comparable at all times, suggesting that they were due to the formation of two diastereomeric mono-iodo, mono-sulfur Pt^{II} oligonucleotide adducts, {$Pt(^{15}NH_3)_2$}-**Is**A and {$Pt(^{15}NH_3)_2$}-**Is**B, respectively. Interestingly, the cross-peaks for {$Pt(^{15}NH_3)_2$}-**Is**A, **1**/**3**, were about twice as intense as the cross-peaks **2**/**4** for {$Pt(^{15}NH_3)_2$}-**Is**B, indicating that {$Pt(^{15}NH_3)_2$}-**Is**A is the favoured product.

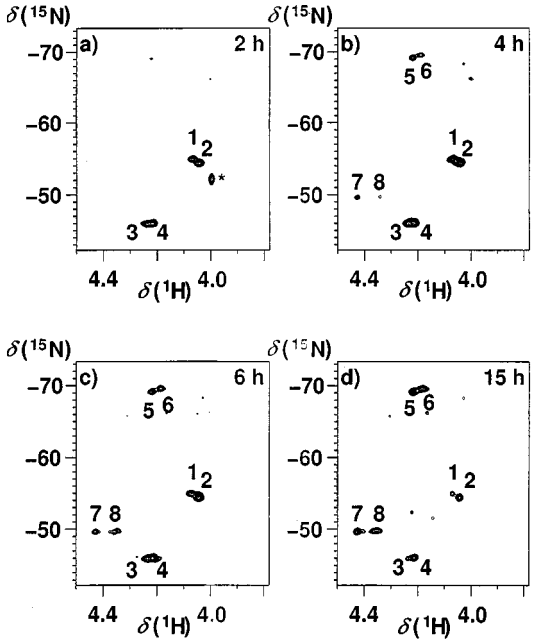

Figure 2. 2D [^{1}H, ^{15}N] HSQC NMR spectra showing the time course of the reaction between *cis*-[$PtI_2(^{15}NH_3)_2$] and **Is** at 310 K, pH 6.1: a) after 2 h; b) after 4 h; c) after 6 h; d) after 15 h; the cross-peak corresponding to the starting material is highlighted by an asterisk in a)

After 6 h, the intensities of peaks **1**/**3** and **2**/**4** decreased by ca. 27% and four new cross-peaks appeared, with intensities equivalent to the loss for signals **1**/**3** and **2**/**4**. The ^{15}N/^{1}H chemical shifts of δ = −68.98/4.22 (**5**) and δ = −69.45/4.19 (**6**) are consistent with NH_3 *trans* to N in Pt^{II} complexes, and those of the other two cross-peaks of δ = −49.69/4.43 (**7**) and δ = −49.80/4.35 (**8**) are compatible with NH_3 *trans* to S (Table S2 in the Supporting Information). These observations suggest that the mono-Pt adduct

with Pt^{II} bound to S is converted into a bifunctional-Pt adduct in which *cis*-$\{Pt(^{15}NH_3)_2\}^{2+}$ is bound to the phosphorothioate S and to N7 of a guanine base. Interestingly, the mono-iodo, mono-S Pt adducts (cross-peaks **1/3** and **2/4**) were still observable after several days, and are therefore long-lived.

We also studied the above reaction of *cis*-$[PtI_2(^{15}NH_3)_2]$ with **Is** by ^{31}P NMR spectroscopy. Oligonucleotide **Is** gave rise to two close ^{31}P NMR peaks at $\delta = 54.948$ and 54.446 due to the presence of two diastereomers (chiral P). These resonances decreased in intensity after the addition of the diiodo Pt^{II} complex, and a broad new multiplet appeared at $\delta = 41.06$. The large, low-frequency shift of the phosphorothioate ^{31}P resonance ($\delta = -13.8$) can be attributed to the direct coordination of P=S to Pt, as observed previously.[13,14] Any ^{195}Pt satellites appeared to be broadened beyond detection (by chemical shift anisotropy). The ^{31}P NMR chemical shifts of the resonances for the phosphate ^{31}P atoms of **Is** were little affected by the binding of Pt to the oligonucleotide.

The 2D [^{1}H, ^{15}N] HSQC NMR cross-peak for *cis*-$[PtI_2(^{15}NH_3)_2]$ had almost disappeared within 2 h of reaction with the oligonucleotide **IIs**, which contains the phosphorothioate group between the two cytosine residues (Figure S2 in the Supporting Information). Four new cross-peaks, **9/10** and **11/12** were then observed (^{15}N/^{1}H chemical shifts listed in Table S3 in the Supporting Information). The ^{15}N/^{1}H shifts of cross-peaks **9/10** and **11/12**) were similar to the intermediates giving rise to cross-peaks **1/3** and **2/4** in the reaction of *cis*-$[PtI_2(^{15}NH_3)_2]$ with **Is**. This suggested that two diastereomeric mono-iodo, mono-sulfur Pt^{II} adducts are also formed from **IIs** in a 2:1 (**9/10:11/12)** ratio. Surprisingly, the intermediates giving **9/10** and **11/12** for **IIs** were not as long-lived as they were during the reaction with **Is**. In particular the intermediate with cross-peaks **11/12** was short-lived compared to that with cross-peaks **9/10**. The rate of further reactions of these intermediates therefore appears to depend on the chirality at P. Cross-peaks **11** and **12** had disappeared completely after 12 h. After 8 h, five cross-peaks with ^{15}N/^{1}H shifts consistent with NH_3 *trans* to S (peaks **13**−**17**) and five cross-peaks with ^{15}N/^{1}H shifts consistent with NH_3 *trans* to N (peaks **18**−**22**; Table S3 in the Supporting Information) appeared simultaneously. This suggests that **9/10** and **11/12** undergo further reactions to form bifunctional Pt adducts in which *cis*-$\{Pt(^{15}NH_3)_2\}^{2+}$ is bound to S of the phosphorothioate and N from a base. After 12 h, two further species (cross-peaks **23**−**26**) were observed. These cross-peaks had similar ^{15}N chemical shifts to those for the products from the reaction of **Is**, indicating that *cis*-$\{Pt(^{15}NH_3)_2\}^{2+}$ is also bound to N and S. In all, about seven products appeared to form in this reaction, all of which were stable for 24 h (Table S3 in the Supporting Information) and contain Pt coordinated to S/N of the oligonucleotide. Over a longer period of time, it might be expected that some loss of coordinated NH_3 *trans* to S could occur.

For comparison, we followed the reaction of *cis*-$[PtI_2(^{15}NH_3)_2]$ with the unmodified oligonucleotide duplex **I** by 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy (Figure S3 in the Supporting Information). After 10 h, three cross-peaks with similar ^{15}N/^{1}H shifts of $\delta = -68.19/4.50$, $-67.93/4.57$ and $-68.24/4.52$ were observed, consistent with NH_3 *trans* to N. This suggested that a bifunctional adduct was formed in which *cis*-$\{Pt(^{15}NH_3)_2\}^{2+}$ was bound to the N7 atoms of the two adjacent guanine bases. This is consistent with reported observations for *cis*-$\{Pt(^{15}NH_3)_2\}^{2+}$ binding to similar oligonucleotides.[15,16]

cis-$[PtI_2(^{15}NH_3)_2]$ reacts relatively rapidly with nucleotide phosphorothioates, initially at sulfur. In the case of the mononucleotide GDP-β-S, further reaction to form the S,N7 macrochelate was relatively rapid, whereas for the oligonucleotide **Is**, the *S*-bound monofunctional adducts were very long lived. Such macrochelation by Pt in phosphorothioate oligonucleotide duplexes appears to be at least partially stereoselective. Molecular modelling studies (Figure S4 in the Supporting Information) support the proposal of chelate formation by the duplex. Studies of the platination of phosphorothioate nucleotides have been reported previously,[8,13,14] but our work appears to provide the first evidence for Pt-S,N7 macrochelation. NMR evidence for O,N7 macrochelation has been reported for unmodified mono- and oligo-nucleotides.[17−19] The reaction pathways for phosphorothioate oligonucleotides appear to be highly dependent on the position of the sulfur atom. Our finding that monofunctional adducts of phosphorothioate oligonucleotides have considerable stability suggests that this might be a useful approach for the introduction of photo-active Pt−I centres into DNA and for antisense strategies.

Experimental Section

Materials: Guanosine-5′-*O*-(2-thiodiphosphate), GDP-β-S, was purchased from Aldrich and used without further purification. The oligonucleotides 5′-d(TATGGCCATA)-3′, **I**, 5′-d(TAT**Gs**G**C**CATA)-3′, **Is** and 5′-d(TATGG**Cs**CATA)-3′, **IIs**, were supplied by Oswel. *cis*-$[PtI_2(^{15}NH_3)_2]$ was synthesised according to previously described procedures[10] and was fully characterised by elemental analysis, NMR spectroscopy and FAB-MS.

ESI-MS: Atmospheric pressure ionization (API) negative ion electrospray mass spectrometry (ESI-MS) was performed using a Platform II mass spectrometer (Micromass, Manchester, UK). The mass spectra were obtained by injecting samples (concentration 100 μM in water) using an infusion pump with a flow rate of 0.48 mL/h. A source temperature of 65 °C and a drying gas flow rate of 450 $L \cdot h^{-1}$ were found to be suitable parameters for analysis. A potential of −3.17 kV was applied to the probe capillary and a cone voltage of −20 V over 100−1000 Da was used. The quadrupole was scanned at 100 $amu \cdot s^{-1}$. The acquisition and deconvolution of data were performed on a Mass Lynx (version 2.3) for Windows NT data system using the maximum entropy (MaxEnt) software algorithm. The mass accuracy of all measurements was within 0.1 *m/z* units.

NMR Spectroscopy: NMR spectroscopic data were acquired using a 500 MHz Bruker DMX NMR spectrometer according to previously described methods.[15] Typically 2D [^{1}H, ^{15}N] HSQC NMR spectra were acquired over a ^{1}H frequency width of 2 kHz and a

^{15}N frequency width of 3.55 kHz centred at δ = −55 relative to external $^{15}NH_4Cl$. 16 transients were acquired into 1024 data points for each of 256 t_1 increments. The total experiment time was typically 2 h for the acquisition of each 2D [^{1}H, ^{15}N] HSQC NMR spectroscopic data set. This was extended to 4 h (32 transients per t_1 increment) at later reaction times. $^{31}P\{^1H\}$ NMR spectra were acquired with 4096 transients over a frequency width of 50 kHz into 41662 data points (acquisition time 0.413 s). All NMR spectroscopic data were processed using Xwin-NMR version 2.0 (Bruker Ltd.).

pH Measurements: pH Measurements were made using a Corning 145 pH meter equipped with an Aldrich micro-combination electrode calibrated with Aldrich buffer solutions of pH 4, 7 and 10. Values of pH were adjusted with 1 M $HClO_4$ or NaOH as appropriate.

Acknowledgments

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