The Dinuclear Complex [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}]Cl$_2$ forms a Unique Macrochelate Intrastrand Crosslink with d(GpG)

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Reaction of the dinuclear antitumour compound [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}]Cl$_2$ with d(GpG) results in the formation of the intrastrand macrochelate [{*trans*-Pt(NH$_3$)$_2$}$_2$(μ-H$_2$N(CH$_2$)$_6$NH$_2$)](d(GpG–N7,N7).

The mechanism of action of the clinically important anticancer drug *cis*-[PtCl$_2$(NH$_3$)$_2$] (cisplatin, cDDP) is thought to originate from its interaction with DNA.[1] The final adducts which result are crosslinks of three types: intrastrand, interstrand and DNA–protein, the most common adduct being the intrastrand crosslink bridging adjacent guanines at the N7 position.[2] Two significant drawbacks of cisplatin are that it has limited activity against many common human cancers and that it is susceptible to acquired drug resistance.[3]

The recently reported bis(platinum) complexes containing two platinum–amine units are of special interest, because they show high activity *in vitro* and *in vivo* against tumour cell lines resistant to cisplatin.[4] Early DNA-binding studies have shown that an important interaction, inaccessible to cisplatin, is the formation of interstrand cross-links through binding of the two platinum units to opposite DNA strands and this binding mode might well be responsible for overcoming the resistance.[5]

In this communication, the results of the reaction of the dinucleotide d(GpG) (known as the major target for cisplatin), with the bis(platinum complex [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}]Cl$_2$, (μ-hexanediamine-*N,N'*)-bis[*trans*-diamminechloroplatinum(II)] **1**, is reported. Fig. 1 shows the structure of the starting materials.

The 1 : 1 reaction product† shows two H8 resonances in the ^{1}H NMR spectrum, shifted downfield compared to d(GpG) (see Fig. 2).‡ A more complex pattern than the usual doublet known to occur for cisplatin chelates[6] is observed for the coupling pattern of the H1′ protons. Proton signals for the –CH$_2$– groups are found at −1.88, −1.58 and −0.77 ppm *vs.* TMA (1.30, 1.60 and 2.41 ppm *vs.* Me$_3$SiCD$_2$CD$_2$CO$_2$Na, TSP).

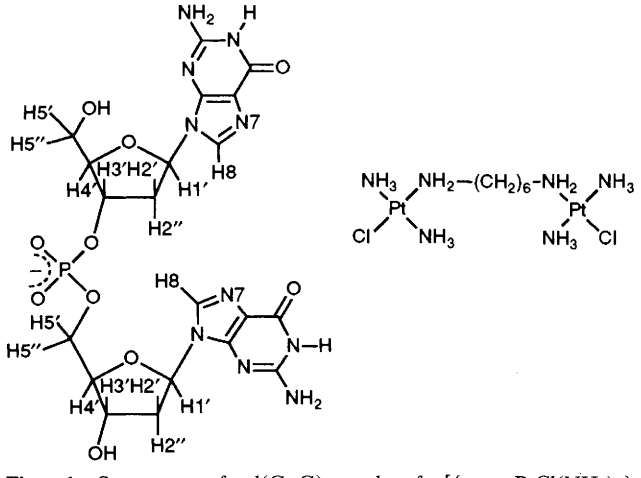

Fig. 1 Structure of d(GpG) and of [{*trans*-PtCl(NH$_3$)$_2$}$_2$-{μ-H$_2$N(CH$_2$)$_6$NH$_2$}]$^{2+}$

The pH dependence of the base proton chemical shift provides direct evidence for coordination of Pt at N7; no N7 protonation effect is observed around pH 2–3, whereas a clear N1 deprotonation occurs around pH 8.5.[7] The ^{31}P NMR spectrum§ shows one signal at δ −3.42, which is 0.68 ppm downfield compared to d(GpG), a common feature for such platinated oligonucleotides.[8] All data confirm the formation of a unique N7,N7 macrochelate. No interstrand crosslinks are found for this bis(platinum) complex in reaction with d(GpG). Considering the H8 chemical shifts of known platinated guanine residues in oligonucleotides, two 'groups' of compounds can be recognized.[9] One type of compound exhibits its H8 chemical shifts at δ 5.3(1), which is characteristic for compounds lacking steric strain. The deoxyribose ring adopts an S-type conformation, thus allowing coupling of the H1′ with both H2′and H2″. Compounds of the second type are the cDDP derivatives of oligonucleotides with an (–NpGpG–) sequence. In this group steric strain is highly likely to occur and as a result the two H8 resonances of the G-bases are not so near chemical equivalency, as they are in the first group. The 5′G deoxyribose ring adopts an N-type conformation, which is accompanied by the disappearance of one H1′–H2′ coupling. The macrochelate structure described here clearly corresponds to the group of compounds lacking steric strain. Both H8 resonances are *ca.* δ 5.3 and the H′ resonances show coupling to both H2′ and H2″. Using the FAB mass spectrometry techniques¶ it was possible to determine the (MH − 2NH$_3$)$^+$ peak at *m/z* 1138. The fragmentation pattern does not show the presence of other high molecular weight species. At lower mass the loss of the deoxyribose sugars as well as the phosphodiester linkage is observed, as has been found in other mass spectral studies of platinated (oligo) nucleotides.[10]

In reaction with 5′GMP a stepwise displacement of the chlorides was found and it has been proposed that the initial approach of a bis(platinum) complex to DNA must be monodentate binding to GN7.[11] The next step may involve a base on the opposite strand (Pt,Pt interstrand) or on the same

† d(GpG) was synthesized *via* an improved phosphotriester method and used as its sodium salt (G. A. van der Marel, C. A. A. van Boeckel, G. Wille and J. H. van Boom, *Tetrahedron Lett.*, 1981, 3887). [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}]Cl$_2$ was prepared according to literature procedures.[5a] [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}]Cl$_2$ was allowed to react with 1 equiv. of d(GpG) in doubly distilled water (pH 6.5) at 37 °C in 5 days in the dark. After removal of the solvent, the reaction product was dissolved in 0.5 ml of D$_2$O (99.8%, Merck) and the sample was lyophilized twice.

‡ NMR spectra were run on a Bruker Wm 300 spectrometer at 297 K (10 mmol dm^{-3} in D$_2$O, 99.95%, Merck). A trace amount of TMA (tetramethylammonium nitrate, 3.18 ppm downfield from tetramethylsilane) was added to the solvent and used as an internal reference. The pH dependence of the H8 chemical shift was monitored by adding trace amounts of DCl and NaOD (0.1 and 1.0 mol dm^{-3}). The pH was not corrected for deuterium isotope effects.

§ The ^{31}P NMR spectra (10 mmol dm^{-3} in D$_2$O at pH 5.6) were referenced to TMP (trimethyl phosphate).

¶ Positive ion FAB mass spectrometry was done with a VG7070 instrument, using a static FAB probe, with Ar gas (8 kV; 1 mA), and glycerol–triethanolamine (1 : 1) as a matrix.

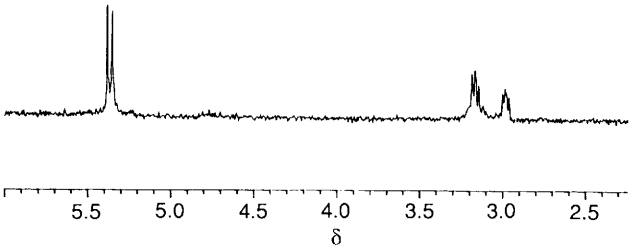

Fig. 2 300 MHz ^{1}H NMR spectrum of *trans*-{[{Pt(NH$_3$)$_2$}$_2${μ-H$_2$N(CH$_2$)$_6$NH$_2$}] {d(GpG)-N7(1),N7(2)}] showing the H8 and H1′ region

strand (Pt,Pt, instrastrand). Previous studies have identified the Pt,Pt interstrand crosslink both *in vivo* and *in vitro*.[4b,5] The present study clearly shows that **1** forms a chelate which is a model for the (Pt,Pt) intrastrand crosslink. Whether or not this is due to the particular chain length of the diamine linker, or its flexibility, is presently under investigation with other diamine linkers.

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