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# Isomer formation in the binding of [$PtCl_2$(*cis*-cyclohexane-1,3-diamine)] to oligonucleotides and the X-ray crystal structure of [$PtCl_2$(*cis*-cyclohexane-1,3-diamine)]·dimethylformamide †

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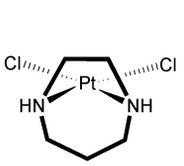
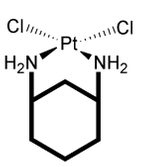
The crystal structure of [$PtCl_2$(*cis*-1,3-chxn)] (*cis*-1,3-chxn = (*cis*-cyclohexane-1,3-diamine)) as the dimethyformamide solvate is reported. When [$PtCl_2$(*cis*-1,3-chxn)] binds to d(GpG), two isomers are formed that are readily separated by HPLC. Both the HPLC and GFAAS studies of the products show that the isomers form in a 1 : 1 ratio. Competition experiments involving d(GpG) and the aquated and nonaquated forms of [$PtCl_2$(*cis*-1,3-chxn)] and [$PtCl_2(NH_3)_2$] showed that the slower binding of the former complex was due to slower aquation and not steric bulk. 1D and 2D NMR studies of the [Ptd(GpG)(*cis*-1,3-chxn)] isomers showed that both the dinucleotide and the diamine were highly fluxional, even at low temperatures, and this prevented formation of strong cross peaks in the NOESY and ROESY spectra and hence identification of the isomers. [$PtCl_2$(*cis*-1,3-chxn)] was reacted with a 52-mer oligonucleotide having six GpG binding sites and the products were enzymatically digested and separated by HPLC. The two [Ptd(GpG)(*cis*-1,3-chxn)] stereoisomers were the only significant platinated products, again forming in a 1 : 1 ratio although it had been anticipated that stereoselectivity would be observed in the reaction with the 52-mer because of the potential for steric interactions with the *cis*-1,3-chxn ligand. Molecular modelling revealed that the observed lack of stereoselectivity was due to the ability of the *cis*-1,3-chxn ligand to adopt a continuum of conformations that allow it to avoid severe steric clashes with the DNA.

## Introduction

It is generally accepted that Pt-based anticancer drugs such as cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) effect their action by binding to DNA.[1–7] The principal binding sites are the guanine–guanine (5′-GpG-3′) and adenine–guanine sequences (5′-ApG-3′).[8–11] Pt binding to DNA is kinetically controlled and it has recently been confirmed experimentally that the adduct distribution is controlled by the rate of monofunctional adduct formation.[12,13] The factors that control monofunctional adduct formation are not yet known, nor are the factors that control conversion of monofunctional adducts to bifunctional adducts. Formation of the bifunctional adducts is particularly important since they are believed to be the most effective at inducing cell death. We have been interested in the role that the steric bulk of the amine ligands can have on the conversion of monofunctional to bifunctional adducts.[14–18] For instance, we have recently reported that when the complex [$PtCl_2$(hpip)] (**1**) (hpip = 1,4-diazacycloheptane) binds to GpG sequences of DNA or duplex oligonucleotides, it forms two isomers in unequal amounts.[18] Such stereoselectivity has been reported previously for complexes with two chemically different amine groups,[19,20] but this is the first example where the stereoselectivity can be ascribed solely to steric effects. Using molecular modelling, we were able to show that steric clashes between the ligand and the DNA were probably responsible for the observed stereoselectivity,[18] and thereby establish a link between steric bulk and inhibition of bifunctional adduct formation.




**1**




**2**

The complex [$PtCl_2$(*cis*-1,3-chxn)] (**2**) (*cis*-1,3-chxn = *cis*-cyclohexane-1,3-diamine) has key similarities to [$PtCl_2$(hpip)]: both have bulky ligands and both have two chiral centres with opposite configurations. Any stereoselectivity in the DNA binding of these complexes must be due to steric factors alone. The difference between the two is that in hpip the amine groups are chiral and the steric bulk lies close to the Pt whereas in *cis*-1,3-chxn the carbon atoms to which the amine groups are bound are chiral, and the steric bulk is more removed from the Pt. We were interested in establishing whether [$PtCl_2$(*cis*-1,3-chxn)] exhibited stereoselectivity in its interactions with DNA similar to that seen for [$PtCl_2$(hpip)]. There is brief reference to [$PtCl_2$-(*cis*-1,3-chxn)] exhibiting such stereoselectivity in a paper published some years ago,[21] but to our knowledge there has been no full report on this work. It is also notable that the complexes of *cis*-1,3-chxn are somewhat less active than analogous complexes of *trans*-1,2-chxn such as the highly successful oxaliplatin (*trans*-cyclohexane-1,3-diamineoxalatoplatinum(II)).[22,23] Thus, the role of steric bulk in influencing this activity is also of interest.

Herein we describe the crystal structure of the dichloroplatinum(II) complex of *cis*-1,3-chxn, and digestion, HPLC, NMR and molecular modelling studies of the products from the reaction of this complex with both d(GpG) and the GpG sites of a 52-mer duplex oligonucleotide.

† Electronic supplementary information (ESI) available: figures showing HPLC chromatograms and NMR spectra. See http://www.rsc.org/suppdata/dt/b1/b104502b/

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## Experimental

### Materials

$K_2[PtCl_4]$ was purchased from Aithaca Chemical Corp. and all other starting materials and solvents were purchased from Sigma-Aldrich. The 2-deoxyguanylyl-(3′-5′)-2-deoxyguanosine (d(GpG)) was purchased from Sigma-Aldrich and the double stranded oligonucleotide (52-mer) containing 6-GpG sites was synthesised by Sigma Genosys. Cisplatin was synthesised using previously reported methods.[24]

### Instrumentation

$^1$H NMR spectra were recorded at 298 K on either a Bruker AC 200 MHz, a Bruker AMX 400 MHz spectrometer or a Bruker DRX 500 MHz spectrometer. $^{13}$C NMR spectra were recorded at 298 K on a Bruker AC 200 MHz spectrometer at 50.3 MHz. 2D NMR spectra were collected on a Bruker Avance DMX 600 MHz spectrometer. All spectra were recorded using commercially available solvents (Aldrich or Merck) of 99.6% isotopic purity or better and referenced to TMS, TPS or residual solvent isotopic impurities. Diffuse reflectance infrared Fourier transform spectra (DRIFTS) were recorded on a BIO-RAD FTS-40 spectrophotometer equipped with Win-IR Windows-based software. Potassium bromide was used as both the background and matrix over the range of 400–4000 cm$^{-1}$. HPLC was performed on a Bio-Rad Series 800 HRLC gradient system using v2.30.1a software. The system was fitted with a Bio-Rad model 2800 solvent delivery system, a Bio-Rad UV-1806 UV/vis detector and a Bio-Rad model 2110 fraction collector. The samples were chromatographed on either a Waters Spherisorb® S5ODS2 C18 reverse phase analytical column (4.6 × 250 mm) or a Waters RCM (100 × 250 mm) semi-preparative column. Platinum concentrations were determined using a Varian SpectrAA-20 absorption spectrometer graphite furnace (GFAAS), equipped with a GTA-96 graphite tube atomiser and a PC-56 autosampling system. GFAAS readings were measured between 0–302.7 ppb in an HCl matrix.

### Synthesis of *cis*-cyclohexane-1,3-diaminedichloroplatinum(II)

The synthetic precursor, *cis*-dichlorobis(dimethylsulfoxide)-platinum(II) ($[PtCl_2(DMSO)_2]$), was synthesised by the method of Price *et al.*[25] The synthesis of *cis*-1,3-chxn as the hydrochloride salt is described elsewhere.[26] The platinum(II) complex of *cis*-1,3-chxn was synthesised using a procedure modified by Fenton.[27] A solution of sodium hydrogencarbonate (0.27 g, 3.2 mmol) in a minimum volume of water was slowly added to the dihydrochloride salt (0.3 g, 1.6 mmol) of *cis*-1,3-chxn suspended in ethanol (100 mL). The mixture was stirred for 0.5 hour to ensure completion of reaction. The solvent was removed by rotary evaporation leaving a mixture of sodium chloride and the "free" ligand, to which ethanol was added to azeotropically remove residual water. The excess solvent was removed by rotary evaporation and methanol (10 mL) was added to the dried mixture. The filtered solution was added to a solution of *cis*-$[PtCl_2(DMSO)_2]$ (0.67 g, 1.6 mmol) suspended in methanol (64 mL) and the mixture stirred for several hours. Methanol was removed on a rotary evaporator, the resultant yellow residue was dissolved in water, and excess lithium chloride (0.5 g) was added. The solution was gently warmed on a steam bath until the volume had reduced to about 10 mL and the mixture was then cooled in an ice bath. The product, which formed as pale yellow crystals, was collected by suction filtration, washed with a small amount of ice-cold water, ethanol and then diethyl ether. It was then recrystallised from DMF. The crystals were air dried and then further dried by storing in a desiccator over silica gel. IR: (KBr; cm$^{-1}$) 3257 s, 3200 s, 3134 s, 2927 s, 2900 s, 2863 m, 1589 s, 1456 ms, 1363 ms, 1272 m, 1209 ms, 1174 ms, 1141 m, 1118 m, 1045 m, 995 m, 964 m, 931 m, 750 m.

**Table 1** Selected bond lengths (Å) and angles (°) for $[PtCl_2$(*cis*-1,3-chxn)]

| | | | |
|---|---|---|---|
| Pt(1)–Cl(1) | 2.323(1) | Pt(1)–Cl(2) | 2.315(1) |
| Pt(1)–N(1) | 2.041(5) | Pt(1)–N(2) | 2.037(5) |
| Cl(1)–Pt(1)–Cl(2) | 92.65(5) | Cl(1)–Pt(1)–N(1) | 87.4(1) |
| Cl(1)–Pt(1)–N(2) | 178.6(2) | Cl(2)–Pt(1)–N(1) | 177.4(2) |
| Cl(2)–Pt(1)–N(2) | 87.6(1) | N(1)–Pt(1)–N(2) | 92.2(2) |
| Pt(1)–N(1)–C(1) | 120.6(4) | Pt(1)–N(2)–C(3) | 119.7(4) |

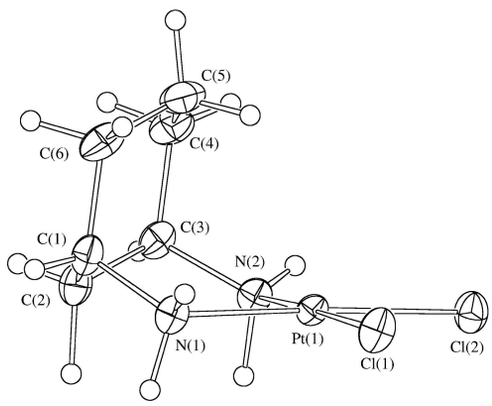


**Fig. 1** ORTEP plot of $[PtCl_2$(*cis*-1,3-chxn)] giving the crystallographic atom numbering. 30% probability ellipsoids are shown.

Satisfactory microanalytical data could not be obtained because of variable loss of the DMF solvate. $^1$H and $^{13}$C spectra are provided in the ESI. †

### Structure determination

**$[PtCl_2$(*cis*-1,3-chxn)]·DMF.** $C_9H_{21}Cl_2N_3OPt$, $M$ 453.28, triclinic, space group $P\bar{1}$ (no. 2), $a$ 7.690(1), $b$ 8.358(2), $c$ 12.149(4) Å, $\alpha$ 70.00(2), $\beta$ 80.87(2), $\gamma$ 83.58(2)°, $V$ 723.1(3) Å$^3$, $D_c$ 2.082 g cm$^{-3}$, $Z$ 2, crystal size 0.27 × 0.17 × 0.10 mm, yellow plate, $\lambda$(MoKα) 0.7107 Å, $\mu$(MoKα) 84.31 cm$^{-3}$, $T$(analytical)$_{min,max}$ 0.346, 0.448, $2\theta_{max}$ 50.0°, $hkl$ range −9 9, −9 9, 0 14, $N$ 2663, $N_{ind}$ 2531 ($R_{merge}$ 0.009), $N_{obs}$ 2411 ($I > 2.5\sigma(I)$), $N_{var}$ 202, residuals $R(F)$ 0.023, $R_w(F)$ 0.023, GoF(all) 2.76, $\Delta\rho_{min,max}$ −1.13, 1.88 e Å$^{-3}$.

**Data collection, structure solution and refinement.** A crystal of $[PtCl_2$(*cis*-1,3-chxn)] was selected and mounted onto a glass fibre. Data were collected at 294 K, cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections, measured and refined on an Enraf-Nonius CAD4-F diffractometer with graphite monochromated MoKα ($\lambda$ = 0.7107 Å) radiation. Data reduction and application of Lorentz, polarisation and analytical absorption corrections were carried out using teXsan.[28] The structure was solved by direct methods using SHELXS-86[29] and refined using full-matrix least-squares methods with teXsan.[28] Hydrogen atoms were refined with isotropic thermal parameters. Non-hydrogen atoms were refined anisotropically. Scattering factors and anomalous dispersion terms for Pt were taken from International Tables.[30] Anomalous dispersion effects were included in $F_c$;[31] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[32] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[33] All other calculations were performed using the teXsan[28] crystallographic software package of the Molecular Structure Corporation. Selected bond lengths and angles are listed in Table 1. The atomic nomenclature is defined on the ORTEP[34] plot in Fig. 1.

CCDC reference number 164932.

See http://www.rsc.org/suppdata/dt/b1/b104502b/ for crystallographic data in CIF or other electronic format.

### Reaction of the platinum complexes with d(GpG)

[$PtCl_2$(cis-1,3-chxn)] and cisplatin were each converted to the diaqua forms by reacting them with two equivalents of $AgNO_3$ in DMF at 37 °C for 16 hours as described by Berners-Price *et al*.[35] The AgCl that precipitated was removed by centrifugation. Stock solutions of d(GpG) were made up in autoclaved Millipore-filtered water ($2.0 \times 10^{-7}$ moles per 150 μL). Millipore-filtered water solutions of the dichloro- or diaquaplatinum complex (1.1 molar equivalents) were added to the dinucleotide (150 μL, $2.0 \times 10^{-7}$ moles) and the reactions were incubated for 7 days at 37 °C. For the competition experiments, 1.1 equivalents of each of the platinum complexes were added to a solution of the dinucleotide.

### Larger scale preparation of [Ptd(GpG)(*cis*-1,3-chxn)] for NMR analysis

For the larger scale sample preparation, the appropriate amount of the platinum complex (1.1 equivalents) was added to 4 mg of d(GpG). The reactions were incubated for 7 days at 37 °C, and the products separated using HPLC. All of the eluted samples for each of the peaks were combined and a small sample rechromatographed to confirm their purity. The ammonium acetate buffer was exchanged for triethylamine acetate buffer (10 mM, pH 5.5) by reloading the sample onto the HPLC and eluting with triethylamine acetate. The samples were then freeze dried twice to remove the buffer.

### Reaction of [$PtCl_2$(*cis*-1,3-chxn)] with the 52-mer duplex oligonucleotide

A 52-mer self-complementary oligonucleotide with the sequence $^{5'}$TAATTGGTATATTGGTATATACCAATATTGGTATATACCAATATACCAATTA$^{3'}$ was prepared by a denaturing and reannealing process. The process involved denaturing the DNA strands by heating to 95 °C for 5 minutes, followed by stepwise heating and cooling (cooling to 23 °C, heating to 65 °C for 10 minutes, cooling to 37 °C for 30 minutes, heating to 65 °C for 10 minutes, cooling to 37 °C and incubating for 4 h, before cooling to 23 °C). The prepared DNA was then frozen until required for use.

The 52-mer oligonucleotide (100 μg) and the platinum complex ($R_t$ = 0.05, in $NaClO_4$, 0.02 M pH 5.5) were incubated at 37 °C for 7 days. The sample was then treated with P1 nuclease (19.6 μL of 1 mg $mL^{-1}$ stock solution, 3100 U/mg solid in 50% 20 mM sodium acetate (U is units of activity), 50% glycerol buffer, Sigma) for 17 h at 37 °C. Tris buffer (tris(hydroxymethyl)methylamine 1 M, pH 9, 40 μL) and alkaline phosphatase (57.8 μL, 5 units, 2.7 mg protein $mL^{-1}$, 64 U/mg protein, suspended in $(NH_4)_2SO_4$, 2.5 M, Sigma) were added to the samples and incubated at 37 °C for 4 h. The DNA digests were freeze dried, resuspended in a minimal amount of $NaClO_4$ (0.02 M, pH 5.5) and separated by HPLC.

### NMR spectroscopy of the isomers of [Ptd(GpG)(*cis*-1,3-chxn)]

NMR experiments were run with approximately 1–2 mM solutions in both $D_2O$ and a 50 : 50 mixture of $D_2O$ and $CD_3OD$. Spectra were collected over a spectral width of 6000 Hz; quadrature detection was employed throughout for all. $^1$H 1D spectra were acquired using water pre-saturation. Two dimensional spectra were acquired in phase-sensitive mode with time-proportional phase incrementation. Double-quantum filtered (DQF) COSY spectra, NOESY spectra, and ROESY spectra, all with water suppression by pre-saturation, were acquired using standard Bruker pulse sequences. Spectra were acquired and processed using Bruker XWinNMR software, version 2.6 (Silberstreifer, Germany, 1999).

Spectra of isomers 1 and 2 were collected at 298, 303 and 305 K in $D_2O$. For the COSY spectra, 512 $t_1$ increments were acquired, with each free induction decay composed of 2048 data points, using a recycle delay of 1.8 s. For each isomer, NOESY spectra were acquired with mixing times ($\tau_m$) between 600 and 1000 ms. ROESY spectra were acquired for each isomer with mixing times between 250 and 600 ms. No crosspeaks were observed that provided any structural information. For both ROESY and NOESY experiments, data sets were acquired with 512 $t_1$ increments, with a recycle delay of 1.7 s and each free induction decay composed of 2048 data points. COSY, ROESY and NOESY spectra were also collected at higher temperature, using the above parameters. Data was subjected to shifted sine-bell weighting functions in $F1$ and $F2$ of $\pi/2$ and baseline corrected using Bruker XWinNMR software. For each isomer, resonances were assigned using the COSY spectra.

### Molecular mechanics calculations

The force field used has been fully reported previously,[36,37] and the starting models for the platinum/d(GpG) and platinum/8-mer complexes were taken from previously reported models.[15,18] The DNA fragment used in the modelling was an 8-mer duplex with the sequence 5′-GGGGGGGG-3′ : 3′-CCCCCCCC-5′ with the platinum complexes bound *via* the platinum to the N7 atoms of the central two guanines. Energy minimisation was carried out using MOMECSG[38] using the Newton–Raphson method for energy minimisation. Constraints used to generate the bifunctional adducts were applied using the method of Lagrangian multipliers.[39] Final models were viewed and analysed using HyperChem version 4.5.[40]

## Results

### Synthesis of *cis*-1,3-cyclohexanediaminedichloroplatinum(II)

Synthesis of the [$PtCl_2$(*cis*-1,3-chxn)] complex *via* the synthetic precursor, *cis*-[$PtCl_2(DMSO)_2$],[25] was readily accomplished. To ensure complete conversion from the synthetic precursor to the dichloroplatinum(II) complex, the sample was characterised by infrared (IR) spectroscopy in order to check for the absence of bands attributed to the DMSO group. The distinctive absorption bands at 431 $cm^{-1}$ ($\nu$ Pt–S) and 1156 $cm^{-1}$ ($\nu$ S=O)[41] are absent in the IR spectra of the complexes. The pale yellow complex was recrystallised from hot DMF, resulting in small translucent crystals within 2 hours. Broad peaks are observed in the $^1$H spectrum and are most likely to be the result of a dynamic process, such as conformational changes in the cyclohexane ring and/or the six membered chelate ring. Preparation of this complex from $K_2[PtCl_4]$ has been reported[42] and the spectroscopic data reported are consistent with those obtained here.

### Crystal structure

The asymmetric unit is made up of one complex and a molecule of DMF with an H bond between the formamide oxygen and one of the amine groups (O(1) ··· N(2) 2.893(7), O(1) ··· H(3) 2.15(6) Å). The complexes pack as hydrogen-bonded dimers (N ··· Cl 3.38–3.44 Å) with an intercomplex Pt ··· Pt distance of 3.4378(4) Å, similar to distances reported in a variety of Pt(II) and Pd(II) complexes.[37,43] The complex has an approximate mirror plane that passes through the Pt, C(2) and C(5) atoms. The cyclohexane ring adopts a chair conformation as does the chelate ring. As a consequence the cyclohexane ring is folded back over the metal resulting in short Pt ··· H and Pt ··· C separations of 2.71 and 3.328 Å respectively. In the crystal structure of bis(*cis*-1,3-chxn)palladium(II), the chelate ring is flattened and the Pd ··· H and Pd ··· C separations are 2.81 and 3.495 Å respectively.[44] This flattening is exemplified in the angle between the coordination plane and the "seat" of the chair shaped chelate ring which is 11.1° in the Pd structure and 27.2° in the Pt structure. Thus, it appears that this chelate ring is conformationally flexible and this aspect is discussed further

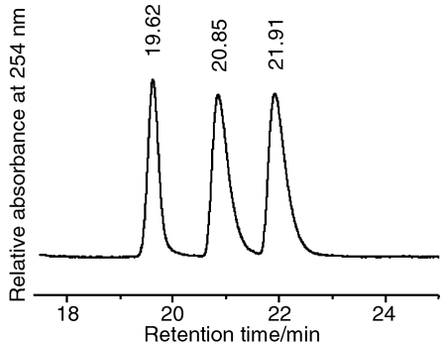


**Fig. 2** HPLC chromatogram for the products of the reaction between [$PtCl_2$(*cis*-1,3-chxn)] and d(GpG).

below. The Pt–Cl bond lengths (2.323(1) and 2.315(1) Å) and Pt–N bond lengths (2.041(5) and 2.037(5) Å) are normal and are not indicative of high strain.

### Reaction of the {Pt(*cis*-1,3-chxn)}-complexes with d(GpG)

Fig. 2 shows the HPLC chromatogram for the products of the reaction of [$PtCl_2$(*cis*-1,3-chxn)] and d(GpG). Spiking with d(GpG) showed that the first peak (19.6 minutes) was due to unreacted d(GpG). The other two peaks are assumed to be due to the two isomers of [Ptd(GpG)(*cis*-1,3-chxn)] and formed in a 1 : 1 ratio. These putative isomers have the chiral centre with the *R* configuration either *cis* or *trans* to the 5′ side of the d(GpG). The HPLC chromatogram for the products of the reaction between the diaqua complex, $[Pt(H_2O)_2$(*cis*-1,3-chxn)$]^{2+}$ and d(GpG) reveals only the latter two peaks, again in a 1 : 1 ratio. In the HPLC chromatogram for the reaction products from the competition experiment involving [$PtCl_2$(*cis*-1,3-chxn)], cisplatin and d(GpG) in a ratio of 1 : 1 : 1, three peaks eluted at 18.6, 20.7 and 21.7 minutes. Spiking with d(GpG) established that there was none left unreacted. Spiking with [Ptd(GpG)-(*cis*-1,3-chxn)] and *cis*-[Ptd(GpG)$(NH_3)_2$] in turn, showed that the peak that eluted at 18.6 minutes was due to *cis*-[Ptd(GpG)$(NH_3)_2$] and that the other two major peaks were due to the two isomers of [Ptd(GpG)(*cis*-1,3-chxn)]. The areas of the peaks for *cis*-[Ptd(GpG)$(NH_3)_2$] and [Ptd(GpG)(*cis*-1,3-chxn)] were found to be in a 3 : 2 ratio with the two isomers of [Ptd(GpG)(*cis*-1,3-chxn)] again forming in a 1 : 1 ratio. The HPLC chromatogram for the reaction products of the competition experiment involving the diaqua forms of the complexes, $[Pt(H_2O)_2$(*cis*-1,3-chxn)$]^{2+}$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, revealed the same three peaks with [Ptd(GpG)(*cis*-1,3-chxn)] and *cis*-[Ptd(GpG)$(NH_3)_2$] forming in a 1 : 1 ratio.

### Reaction of [$PtCl_2$(*cis*-1,3-chxn)] with a duplex 52-mer

Fig. 3(a) shows the HPLC chromatogram of the digested products of the reaction between [$PtCl_2$(*cis*-1,3-chxn)] and the 52-mer. Spiking experiments showed that the isomers of [Ptd(GpG)(*cis*-1,3-chxn)] eluted at 16.9 and 17.9 minutes. Samples collected every 20 s were analysed using GFAAS to determine the platinum content and the results are shown in Fig. 3(b). These show that the stereoisomers of [Ptd(GpG)-(*cis*-1,3-chxn)] are in a 1 : 1 ratio. There is also some platinum present in the peaks that eluted at 10.8 and 15.5 minutes, possibly due to species such as [PtCl(dG)(*cis*-1,3-chxn)] that would arise from any monofunctional adducts.

### NMR spectroscopy

NMR spectroscopy was carried out with the primary goal of assigning the two isomers of [Ptd(GpG)(*cis*-1,3-chxn)] on the basis of crosspeaks between the resonances due to the *cis*-1,3-chxn ligand and the d(GpG) in the 2D spectra. Rapid motion in both the dinucleotide and the diamine ligands resulted in broad

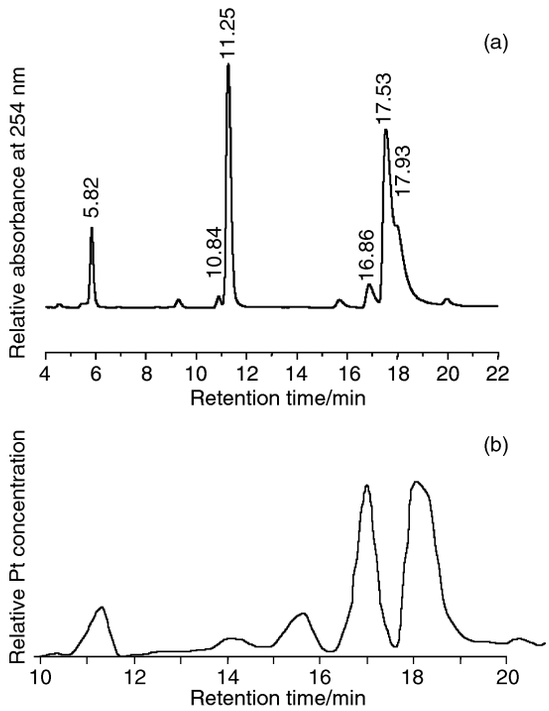


**Fig. 3** (a) HPLC chromatogram of the digested products of the reaction between [$PtCl_2$(*cis*-1,3-chxn)] and the 52-mer. (b) GFAAS analysis of the HPLC samples from the digested products of the reaction between [$PtCl_2$(*cis*-1,3-chxn)] and the 52-mer.

peaks and, more significantly, weak or non-existent interligand crosspeaks in the NOESY or ROESY spectra. A variety of solvents and a range of temperatures were investigated but in no case were significant crosspeaks observed.

Tentative assignments of the diamine and dinucleotide ligands were made using COSY spectra. The lack of even intramolecular NOESY or ROESY crosspeaks made determination of the directionality of the dinucleotide impossible. The characterisation of these isomers, though not a definitive identification, has been reported by Inagaki and Sawaki[45] and therefore, we have not reported details of our assignments here but have included them in the ESI. †

### Molecular modelling

Fig. 4 shows the molecular models of the isomers and conformers of [Ptd(GpG)(*cis*-1,3-chxn)] (only head-to-head conformers are shown). The main features that might be used to distinguish between the two isomers in the 2D NMR spectra are the distances between the H8 atoms of the closest guanine and the $CH_2$ protons of the *cis*-1,3-chxn ring. The closest distance between these two hydrogen atoms for the isomer with the *R*-carbon on the 5′ side are 5.2 (H(C2)–H8(5′-G)) and 3.2 Å (H(C2)–H8(5′-G)) for the folded over and the folded back conformations respectively. The closest distance between these two hydrogen atoms for the isomer with the *R*-carbon on the 3′ side are 2.7 (H(C5)–H8(5′G)) and 5.3 Å (H(C5)–H8(5′-G) for the folded over and the folded back conformations respectively. Thus, for both isomers these H ⋯ H contacts, that were expected to give crosspeaks in the NOESY spectra, vary by 2.0 Å or more as the conformation of the diamine ligand changes. Given that the dinucleotide is also in rapid motion it is not surprising that no such crosspeaks were observed.

Fig. 5 shows the molecular models of the two isomers of [Pt(8-mer)(*cis*-1,3-chxn)] with the *cis*-1,3-chxn ring sitting in the major groove. The conformations of the *cis*-1,3-chxn ring shown in these models are those with the $CH_2$ group (C5) sitting directly above the platinum atom (folded over conformation) and are similar to those conformations depicted in Figs. 4(a) and (c). There is potential hydrogen bonding present

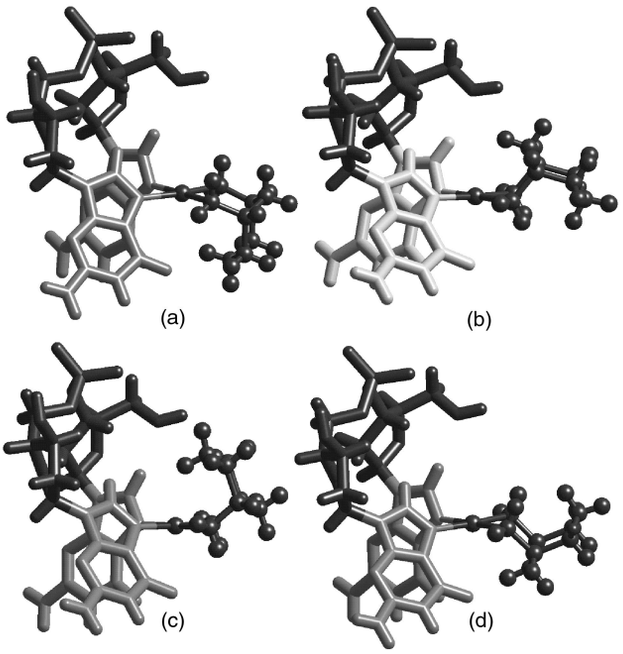


**Fig. 4** Molecular models of the isomers and conformers of [Ptd(GpG)(*cis*-1,3-chxn)] with (a) the folded over and (b) folded back conformations and the *R*-carbon on the 5′ side and (c) the folded over and (d) folded back conformations with the *R*-carbon on the 3′ side.

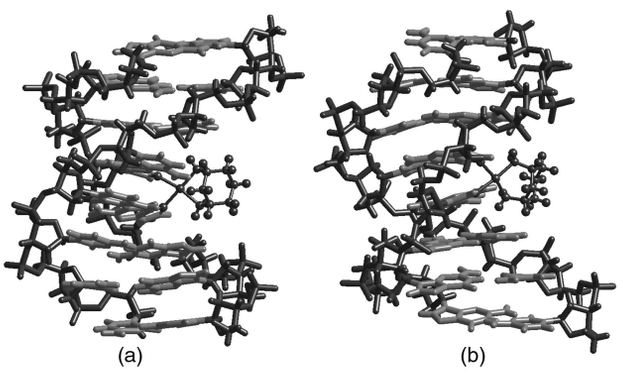


**Fig. 5** Molecular models of [Pt(8-mer)(*cis*-1,3-chxn)] with (a) the *R*-carbon on the 5′ side and (b) [Pt(8-mer)(*cis*-1,3-chxn)] with the *R*-carbon on the 3′ side.

between the amine group of the {Pt(*cis*-1,3-chxn)} and the O6 of the 3′-G in each of these isomers. There are also short contacts between protons on the nearby bases and protons on the *cis*-1,3-chxn ring. In each of these isomers, the 1,3-chxn ring can easily flip to its alternate folded back conformations (similar to those conformations depicted in Figs. 4(b) and (d)) or intermediate conformations. This reduces or removes clashes with the DNA, but hydrogen bonds are still possible.

## Discussion

To assess the rate of binding of [$PtCl_2$(*cis*-1,3-chxn)] relative to that of cisplatin and the role of aquation in this competition, these complexes in their dichloro and diaqua forms were reacted together with d(GpG). In the case of the dichloro complexes the product formed by cisplatin predominated by a ratio of 3 : 2 whereas in the case of the diaqua complexes the two products formed in equal amounts. Thus, it can be concluded that cisplatin reacts with the DNA at a faster rate than [$PtCl_2$(*cis*-1,3-chxn)], but once the rate determining aquation step is no longer a factor, the complexes react with d(GpG) at equal rates. Thus, slower aquation and not steric bulk is responsible for the slower reaction of [$PtCl_2$(*cis*-1,3-chxn)]. The difference in the rates of binding is not great and would not be likely to account for the observed difference in activity.

Both [$PtCl_2$(*cis*-1,3-chxn)] and [$Pt(H_2O)_2$(*cis*-1,3-chxn)]$^{2+}$ reacted with d(GpG) producing two isomers in a 1 : 1 ratio. This 1 : 1 ratio has also been noted when reacting d(GpG) with other platinum complexes such as (3-aminohexahydroazepine)-dichloroplatinum(II), [$PtCl_2$(ahaz)],[46] (1,4-diazacycloheptane)-dichloroplatinum(II), [$PtCl_2$(hpip)],[15] and (5,5,7-trimethyl-1,4-diazacycloheptane)dichloroplatinum(II), [$PtCl_2$(tmdz)][47] and indeed stereoselectivity is rarely observed in such reactions. This is probably due to the short, single-stranded dinucleotides not being able to exert any conformational constraints or unfavourable interactions that can be imposed by the geometry and greater rigidity of longer pieces of double-stranded DNA.

When [$PtCl_2$(ahaz)],[46] [$PtCl_2$(hpip)],[18] or [$PtCl_2$(tmdz)][48] complexes were reacted with a longer strand of duplex DNA, stereoselectivity was exhibited and the formation of the two isomers deviated from a 1 : 1 ratio. However, when [$PtCl_2$(*cis*-1,3-chxn)] was reacted with a 52-mer (Fig. 3) the two isomers still formed in a 1 : 1 ratio. This indicated that there was no stereoselectivity imposed on the binding of the complex by interactions between the ligand and the DNA. The lack of selectivity for this bulky compound is probably due to the conformational flexibility of the *cis*-1,3-chxn. This finding was also supported by the findings in the molecular modelling studies of {Pt(*cis*-1,3-chxn)} with an 8-mer (Fig. 5) where any short contacts between the cyclohexane ring of {Pt(*cis*-1,3-chxn)} and the DNA were alleviated when either isomer was in its folded back conformation.

The presence of equal amounts of the two isomers when [$PtCl_2$(*cis*-1,3-chxn)] is reacted with either d(GpG) or a longer 52-mer, together with the results from molecular modelling studies, indicates that there was no kinetic or steric preference for one isomer over the other. These findings for the two [Ptd(GpG)(*cis*-1,3-chxn)] isomers contradict a previous report[21] where the ratio of the two isomers formed on DNA was found to be 1 : 0.26. In this report, it was postulated that an "axially standing cyclohexane ring" causes steric hindrance that may impede its approach toward DNA and this may be the reason for its weaker antitumour activity and toxicity. The models described above show that the orientation of the cyclohexane ring is not constrained and so does not provide a clear rationale for any stereoselectivity.

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