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Synthesis, Characterisation and Biological Activity of Chiral Platinum(II) Complexes

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Four platinum(II) complexes of 1,10-phenanthroline (phen) and 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-Me_4phen), with the chiral ancillary ligands (1*R*,3*S*) and (1*S*,3*R*)-1,3-diamino-1,2,2-trimethylcyclopentane (*R*,*S*-tmcp and *S*,*R*-tmcp, respectively) have been synthesised and their biological activity quantified using an in vitro cytotoxicity assay against the L1210 murine leukaemia cell line. [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4-phen)]Cl_2 showed an increase in biological activity over their non-methylated complexes, [Pt(*R*,*S*-tmcp)(phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(phen)]Cl_2. Some chiral discrimination was observed in the in vitro cytotoxicity experiments with the complexes having (*S*,*R*) configuration showing higher biological activity in L1210 cells. Titrations of the metal complexes into ct-DNA and observation of the changes induced in the CD spectra were used to determine the binding constants. The binding of these metal complexes to the hexamer d(GTCGAC)$_2$ was studied using two-dimensional ^{1}H NMR spectroscopy. The addition of metal complexes to the hexamer produced upfield shifts of the metal complex of selected resonances, characteristic of intercalation for [Pt(tmcp)-(phen)]Cl_2, whereas the [Pt(tmcp)(3,4,7,8-Me_4phen)]Cl_2 complexes only partially intercalate and in a "side-on" fashion. Through the observation of NOE cross-peaks, two-dimensional NMR experiments provided some insight into the site and groove preferences of these complexes when binding to DNA. Here, we report the biological activity of platinum(II) complexes containing an intercalator and a chiral diamine, which influences the degree to which the complexes can interact with DNA.

Introduction

The interaction of positively charged metallointercalators with the base-pair stack of DNA has been an area of interest for some time.[1–7] Square-planar platinum(II) complexes derived from heterocyclic ligands have produced greater in vitro cytotoxicity against human ovarian cancer cell lines, than some current therapeutic agents.[8] These intercalating complexes introduce reversible binding, a mode that is different from that of cisplatin. Compounds like [Pt(en)(phen)]Cl_2, which bind reversibly and non-covalently, have also been widely studied using calorimetric studies and thermodynamic calculations,[9] circular dichroism (CD) polarimetry,[10] electronic absorption studies using nucleotides[11–12] and two-dimensional ^{1}H nuclear magnetic resonance (NMR) spectroscopy[13] to further understand their interaction with DNA.

We recently published[14] our investigation of a series of platinum(II) complexes, of the type [Pt(en)(phen)]Cl_2 containing methylated derivatives of the ligand 1,10-phenanthroline [where en = 1,2-diaminoethane, phen = 1,10-phenanthroline and phen is substituted with 4-methyl-1,10-phenanthroline (4-Mephen), 5-methyl-1,10-phenanthroline (5-Mephen), 4,7-dimethyl-1,10-phenanthroline (4,7-Me_2phen), 5,6-dimethyl-1,10-phenanthroline (5,6-Me_2phen) or 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-Me_4phen)] and determined that the position and number of methyl groups on the 1,10-phenanthroline structure influences biological activity. Viscometry, CD and NMR studies indicated that all the platinum(II) complexes intercalate into the DNA base pair stack.[14]

A dependence of the mutagenic and anticancer activity in covalent platinum(II) complexes upon the configuration of the ancillary ligand has been previously reported.[14,15] Cytotoxic activity of a series of chiral *cis*-diaminodichloroplatinum(II) complexes, in various cell lines, has shown an enantioselective trend.[16]

[Pt(en)(phen)]Cl_2 proved to be the most efficient intercalator of the platinum(II) complexes investigated, whereas [Pt(en)(3,4,7,8-Me_4phen)]Cl_2 was one of the least efficient.

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The wide range of biological activity seen for that series of platinum(II) complexes of 1,10-phenathrolines was encouraging and raised the question of whether other non-covalent binding platinum(II) complexes, with a wider range of substituting group-type, number and position/s on 1,10-phenanthroline and ancillary ligands have the potential to yield more active complexes.

Accordingly, we have prepared chiral platinum(II) complexes based on [Pt(en)(phen)]Cl_2 and [Pt(en)(3,4,7,8-Me_4phen)]Cl_2, where en is replaced by the 1*R*,3*S*- or the 1*S*,3*R*-form of 1,3-diamino-1,2,2-trimethylcyclopentane (*R*,*S*-tmcp and *S*,*R*-tmcp, respectively) (Figure 1). Each complex was examined for its growth inhibition against L1210 murine leukaemia cells, their effect on the viscometry of linear DNA, their induced circular dichroism upon addition to ct-DNA and ^{1}H NMR binding with the hexanucleotide d(GTCGAC)$_2$. The results are compared with those for the parent complexes [Pt(en)(phen)]Cl_2 and [Pt(en)(3,4,7,8-Me_4phen)]Cl_2.

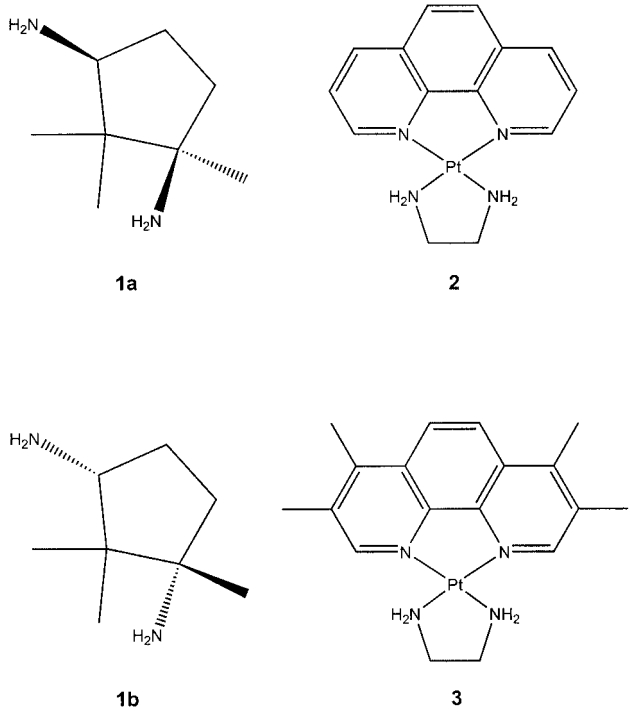
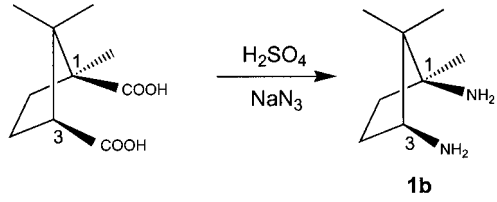


Figure 1. The structure of (1*R*,3*S*) (**1a**) and (1*S*,3*R*)-1,3-diamino-1,2,2-trimethylcyclopentane (**1b**) along with the metal complexes: [Pt(en)(phen)]Cl_2 (**2**) and [Pt(en)(3,4,7,8-Me_4phen)]Cl_2 (**3**).

Results and Discussion

Metal Complex Syntheses

The ligands **1a** and **1b** were prepared by an adapted Schmidt reaction from (*R*,*S*) and (*S*,*R*)-camphoric acid, respectively (Scheme 1). The yields and ^{1}H/^{13}C NMR of both compounds are consistent with published data.[14] The platinum(II) complexes were prepared by refluxing aqueous solutions of [Pt(phen)Cl_2], or [Pt(3,4,7,8-Me_4phen)Cl_2], with the appropriate chiral ligand, until the complex had completely dissolved. Despite their structural similarities, the methylated complexes [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2·4H_2O (**3a**) and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2·4H_2O (**3b**) took longer to react (24 hours) in comparison to [Pt(*R*,*S*-tmcp)(phen)]Cl_2·2H_2O (**2a**) and [Pt(*S*,*R*-tmcp)(phen)]Cl_2·2.5H_2O (**2b**) (16 hours). The variation in reaction time can be ascribed to the difference in pK_a, where the reactivity observed in each complex increases with a decrease in base character {[Pt(phen)Cl_2] pK_a = 4.96; [Pt(3,4,7,8-Me_4phen)Cl_2] pK_a = 6.31}.[17] All complexes were isolated as chloride salts to facilitate aqueous solubility for biological testing.



Scheme 1.

The complexes were characterised by NMR and CD spectroscopy. All ^{1}H NMR spectra were recorded in [D_6]-DMSO in the range of 0 to 10 ppm. The C2 methyl protons of [Pt(*R*,*S*-tmcp)(phen)]Cl_2·2H_2O (**2a**) and [Pt(*S*,*R*-tmcp)(phen)]Cl_2·2.5H_2O (**2b**), appeared at δ = 0.80 and 1.26 ppm. The C1 methyl proton occurred downfield at δ = 1.45 ppm as a consequence of shielding due to its proximity to the nitrogen and platinum(II) atoms. The C3 methine proton occurred as a multiplet at δ = 2.91 ppm, with the C5 protons further upfield at δ = 1.78 and 1.92 ppm. Assignment of the amine protons was based on 2D DQFCOSY experiments, with the two AB spin systems occurring at δ = 6.51 ppm for C(1)NH_2, and 6.78 ppm for C(3)NH_2. The C4 protons were observed at δ = 2.15 ppm through scalar coupling to the methine proton on C3. The coordination of the diamine species rendered the aromatic ligands asymmetric, producing further splitting of the characteristic proton signals. The signals of the phenanthroline ring resonated as two doublet of doublets at δ = 8.31 and 8.29 ppm for H7/14, and a singlet at δ = 8.35 ppm for H10/11. H6/15 and H8/13 appeared as a multiplet at δ = 9.15 ppm.

The aromatic protons of [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2·4H_2O (**3a**) and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2·4H_2O (**3b**), H6′/15′ appeared as a singlet at δ = 8.38 ppm, and for H10′/11′ two singlets in the ^{1}H NMR and were observed at δ = 9.06 and 8.99 ppm. The aromatic methyl groups were upfield at δ = 2.64 and 2.80 ppm. The signals for the chiral ancillary ligand occurred at similar chemical shifts to the non-methylated complex. ^{13}C and DEPT NMR spectra accounted for all carbon atoms present for the four complexes.

Table 1. Summary of the biological activity of the platinum(II) complexes.

Compound	IC_{50}[a] (μM, L1210)	$K_b \times 10^5$ at 230–232 nm[c]	n at 230–232 nm[c]	$K_b \times 10^5$ at 273–275 nm[c]	n at 273–275 nm[c]	Relative viscosity[g] $(\eta/\eta^0)^{1/3}$ $r = 0.125$	Relative viscosity[g] $(\eta/\eta^0)^{1/3}$ $r = 0.250$
Cisplatin	0.5					0.85	0.89
[Pt(en)(phen)]Cl_2	9.7 ± 0.4[b]	22.2 ± 4.6[d][e]	2.0 ± 0.3[d,e]			1.13	1.23
[Pt(*R*,*S*-tmcp)(phen)]Cl_2 (**2a**)	11.3 ± 1.8[b]	16.1 ± 9.9[d]	2.4 ± 0.1[d]	11.0 ± 0.1[f]	2.0 ± 0.1[f]	0.90	0.97
		6.6 ± 0.1[f]	2.2 ± 0.1[f]				
[Pt(*S*,*R*-tmcp)(phen)]Cl_2 (**2b**)	11.2 ± 2.1[b]	5.3 ± 2.2[d]	2.4 ± 0.1[d]	8.6 ± 0.2[f]	2.0 ± 0.1[f]	1.06	1.15
		5.7 ± 0.1[f]	2.0 ± 0.1[f]				
[Pt(en)(3,4,7,8-Me_4phen)]Cl_2	> 50[c]	7.4 ± 3.0[d,e]	6.0 ± 0.4[d,e]			1.08	1.12
[Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (**3a**)	11.5 ± 0.7[b]	3.1 ± 1.3[d]	2.5 ± 0.1[d]	2.6 ± 0.3[f]	1.8 ± 0.1[f]	0.86	0.90
		0.7 ± 0.1[f]	2.9 ± 0.3[f]				
[Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (**3b**)	8.5 ± 0.7[b]	3.5 ± 1.2[d]	3.1 ± 1.2[d]	3.3 ± 0.1[f]	1.9 ± 0.1[f]	0.81	0.87
		1.6 ± 0.1[f]	2.0 ± 0.1[f]				
DNA						1.00	1.00
Ethidium						1.11	1.13
Hoechst 33258						1.01	1.03

[a] IC_{50} values were determined for murine leukaemia L1210 cells and are reported for two experiments at 48 h continuous exposure to the metal complexes. [b] Replication = 2. [c] Replication = 4. [d] The intrinsic approach[18] was used to determine the values of α whereas the Scatchard model[18] was used to determine K_b and n for the CD ct-DNA titration data. [e] Data from ref.[14]. [f] Direct least-squares curve fitting, using a Levenberg–Marquardt method, to the same experimental points.[17] [g] Relative viscosity measurements were made for [metal]/[DNA] molar ratios equal to 0.125 or 0.250.

Cytotoxicity

[Pt(*R*,*S*-tmcp)(phen)]Cl_2 (**2a**) and [Pt(*S*,*R*-tmcp)(phen)]-Cl_2 (**2b**) produced an average IC_{50} values of 11.3 and 11.2 μM, respectively, a decrease in potency when compared to [Pt(en)(phen)]Cl_2 (IC_{50} = 9.7 μM) (Table 1). [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (**3a**) and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2, (**3b**) produced average IC_{50} values of 11.5 and 8.5 μM, respectively. This is a marked increase in the potency when compared to the achiral parent complex, [Pt(en)(3,4,7,8-Me_4phen)]Cl_2, with an IC_{50} value greater than 50 μM (Table 1).

Viscosity

Platinum(II) complexes are able to bind DNA by reversible non-covalent intercalation, partial intercalation, electrostatic association or groove binding. To investigate the mode of binding the viscosity of a linearized DNA (≈ 200 bp) solution was measured in the presence of complexes (such as, ethidium bromide, cisplatin and Hoechst 33258, which each have different, known DNA binding modes) as well as the platinum(II) complexes. This provided a common reference and verified the sensitivity of the measurements towards the different binding modes.

Ethidium bromide, a classical organic DNA intercalator, increased the relative viscosity (1.13 at a binding ratio of 0.250) due to an increase in the axial length and rigidity of DNA.[19,20] Cisplatin, in contrast, produced a decrease in the relative viscosity (0.89 at a binding ratio of 0.250) as a consequence of kinking and bending that results from geometry of the Pt-DNA covalent adduct.[21] Partial intercalators also kink the DNA reducing its axial length, which is observed as a reduction in relative viscosity.[22] Hoechst 33258, a groove binder, did not alter the relative viscosity (1.00 at a binding ratio of 0.250) as it does not change the axial length of DNA.[20,23] The above results confirm the sensitivity of viscosity as a method to differentiate the modes of binding.

The viscosity of the ct-DNA solution (200 bp), decreased for [Pt(*R*,*S*-tmcp)(phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(phen)]Cl_2 when compared to [Pt(en)(phen)]Cl_2 at binding ratios of 0.250 to 0.97, 1.15 and 1.23, respectively, as shown in Table 1. These results suggest that regardless of chirality, the bulk of the ancillary ligand impedes intercalation for both [Pt(*R*,*S*-tmcp)(phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(phen)]Cl_2. The *S*,*R*-form of the diamine in [Pt(*S*,*R*-tmcp)(phen)]Cl_2 may reduce the extent as to how far the aromatic rings can insert into the DNA helix and because [Pt(*R*,*S*-tmcp)(phen)]Cl_2 accentuates this trend, this would indicate that the orientation of the *R*,*S*-isomer further obstructs the phen from full intercalation.[14,22] The viscosity results therefore indicate that [Pt(*R*,*S*-tmcp)(phen)]$^{2+}$ undergoes partial intercalation.

[Pt(en)(3,4,7,8-Me_4phen)]$^{2+}$ intercalates with a relative viscosity of 1.08, but its ability to increase the axial length of linearized DNA is less than [Pt(en)(phen)]Cl_2. It is likely that the methyl groups on the 3,4,7,8-Me_4phen protrude into the binding site of the DNA duplex, obstructing intercalation. This interaction is further affected with the introduction of the bulky chiral ancillary ligands. The complexes [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 showed substantial decreases in viscosity 0.90 and 0.87, respectively (Table 1). Although the viscosity is similar to that of cisplatin it can be explained by kinking of the DNA[14] as the 3,4,7,8-Me_4phen

can only partially intercalate into the DNA helix. Here the change in structure, where the DNA bends (or kinks) to accommodate the ligand, is accompanied by an increase in the biological activity of the complex.

Circular and Induced Circular Dichroism (ICD)

CD spectra were recorded for each of the platinum(II) complexes upon titration into DNA, and the changes in ct-DNA structural conformation were observed upon binding. The titration curves for each of the complexes are presented in Figure 2 and Figure 3 as CD and ICD spectra. The initial CD is consistent with B-type DNA, with a positive peak at 275 nm, a negative peak at 245 nm, zero between the peaks at ca. 258 nm and a negative peak at 210 nm. Changes in the CD spectra for the complexes were observed, but these are more obvious when they are represented as ICD spectra (as shown in Figure 2 and Figure 3). The ICD titration spectra of each complex were used to determine the average affinity and stoichiometry of binding.

The CD spectrum of DNA from 200–300 nm, is because of the skewed orientation of the bases.[18] Changes to the secondary structure, such as unwinding of the helix, or inclination of the bases, due to interaction with a metal complex, led to an alteration in the shape of the spectrum. If the spectrum intensity changes, but the shape of the peak remains uniform, a single binding mode by the ligand can be expected.[24] If the spectrum, however, changes both in shape and intensity, a modification to the DNA-drug interaction may be taking place. Such effect may be attributed to a number of factors which include: 1) occupancy of more than one binding site as the drug concentration increases; 2) ligand–ligand interactions, 3) changes in the DNA conformation.

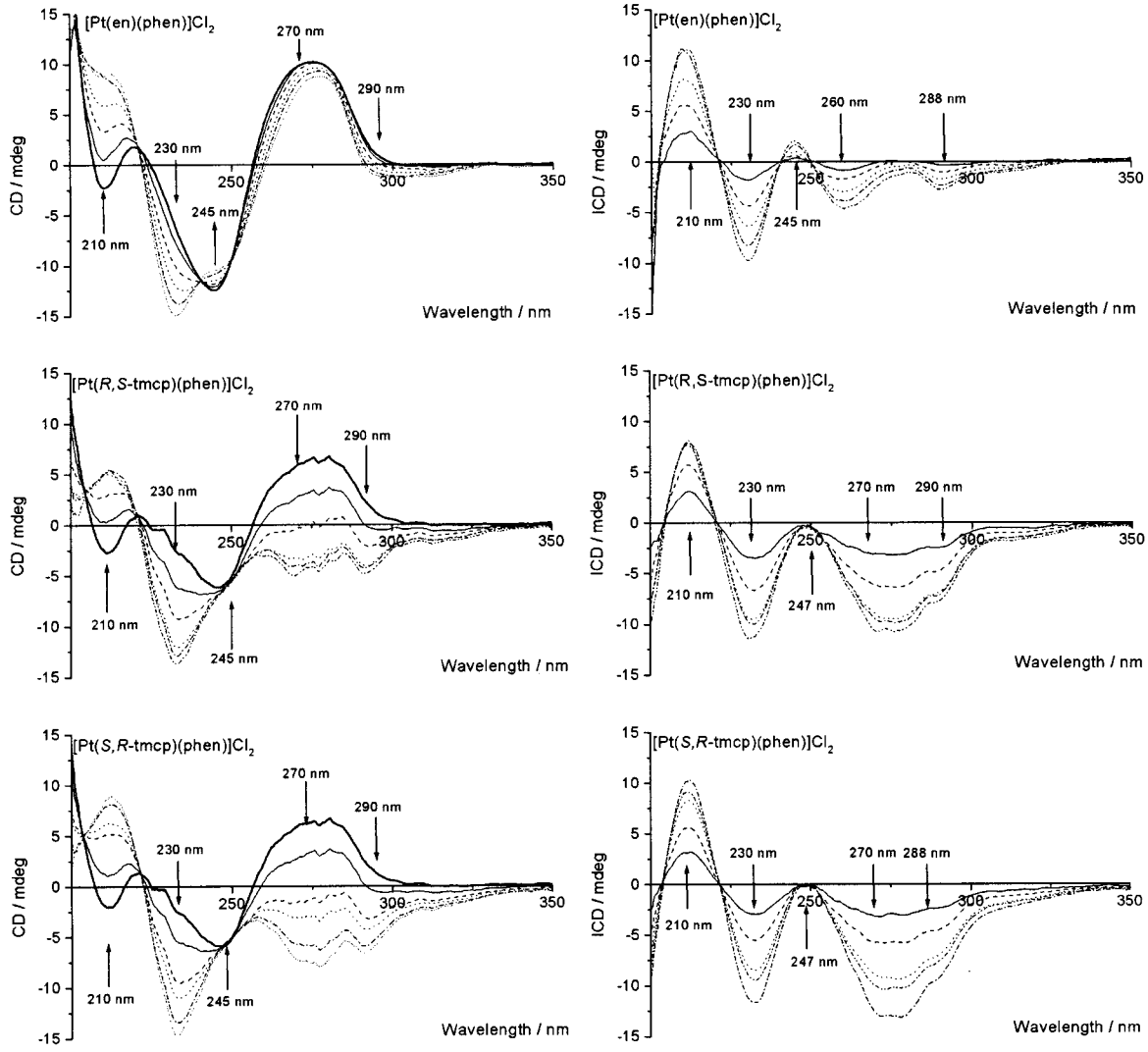


Figure 2. CD (left) and ICD (right) spectra of varying concentrations of the platinum(II) complexes, [Pt(en)(phen)]Cl_2 (**2**), [Pt(*R*,*S*-tmcp)(phen)]Cl_2 (**2a**) and [Pt(phen)(*S*,*R*-tmcp)]Cl_2 (**2b**) upon titration with ct-DNA solution in buffer (5 mM, Trizma, 50 mM NaCl, pH 7.5). The ratios of [Pt]/[DNA] are 0, 0.1, 0.2, 0.4. 0.5 and 1.0 and are indicated on the graph as –, –, ---, ···, ·-·, -··-, respectively.

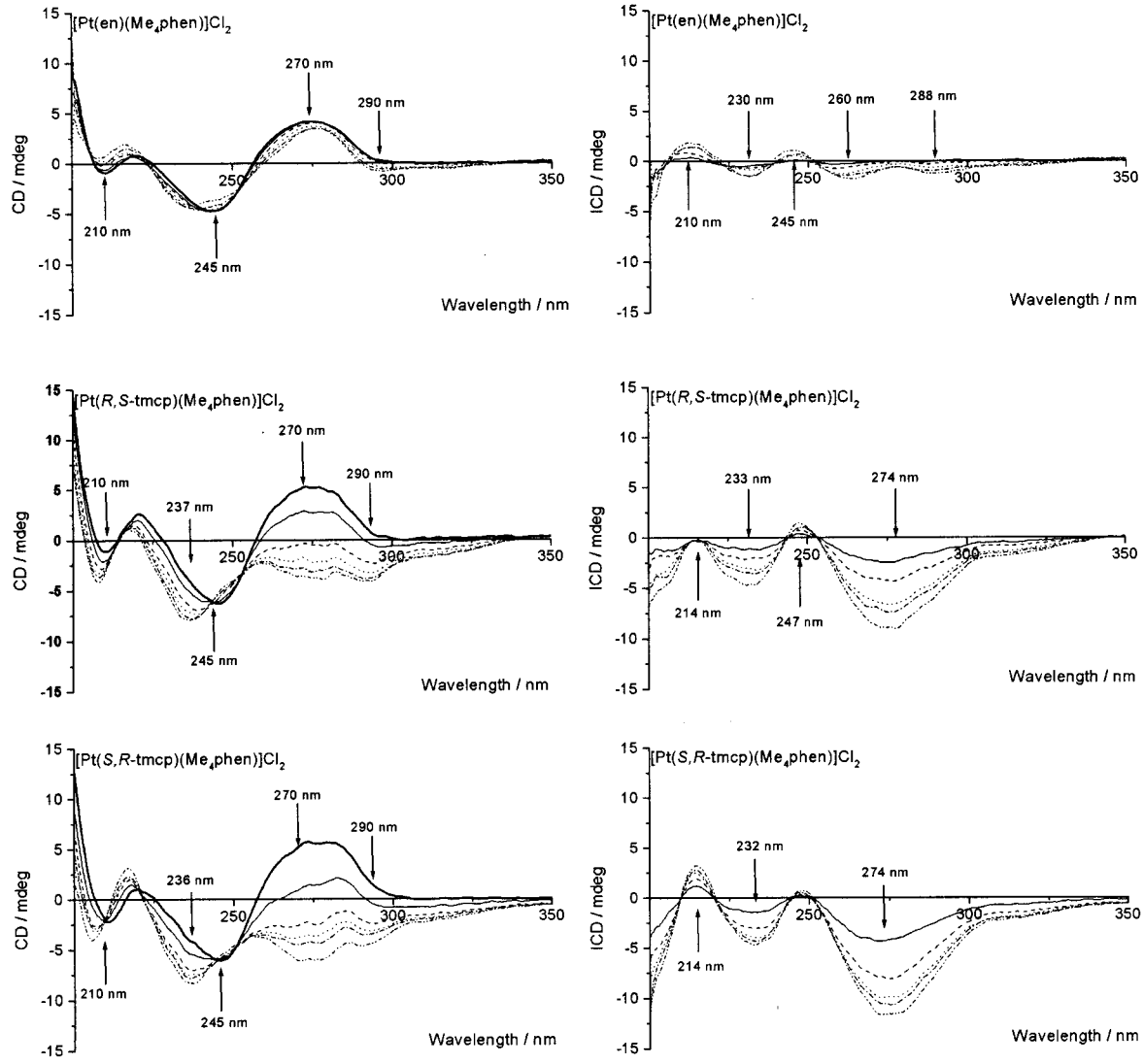


Figure 3. CD (left) and ICD (right) spectra of varying concentrations of the complexes, [Pt(en)(3,4,7,8-Me_4phen)]Cl_2 (**3**), [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (**3a**) and [Pt(*S*,*R*-tmcp) (3,4,7,8-Me_4phen)]Cl_2 (**3b**) upon titration with ct-DNA solution in buffer (5 mM, Trizma, 50 mM NaCl, pH 7.5). The ratios of [Pt]/[DNA] are 0, 0.1, 0.2, 0.4. 0.5 and 1.0 and are indicated on the graph as –, – –, ---, ···, ·-·, -··-, respectively.

The ICD of [Pt(en)(phen)]Cl_2 (**2**), [Pt(*R*,*S*-tmcp)(phen)]-Cl_2 (**2a**) and [Pt(*S*,*R*-tmcp)(phen)]Cl_2 (**2b**) change in comparable ways below 245 nm suggesting that the interaction in DNA is of a similar nature. Above 245 nm the two discrete peaks observed for [Pt(en)(phen)]Cl_2, develop into one broad peak for both [Pt(*R*,*S*-tmcp)(phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(phen)]Cl_2 (Figure 2). This peak is also observed for [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 and [Pt(*S*,*R*-tmcp)-(3,4,7,8-Me_4phen)]Cl_2 (Figure 3). The intensity of this broad peak is consistent with a deformation of the DNA helix by the complex as shown in viscosity, perhaps by partial intercalation, where $S,R > R,S$.

[Pt(en)(3,4,7,8-Me_4phen)]Cl_2 makes little impact on the structure of DNA as the ICD spectra show only very small peaks upon the addition of metal complex. Both [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 have a profound effect on the CD, which is made more obvious in the ICD. The spectra below 245 nm are significantly different from that of the phen analogues.

Binding constants were determined using the data obtained from the titrations. Previously we have used the method of Roger and Nordén[24] to determine the binding constants; however, here a different data analysis technique, which utilises direct least-squares curve fitting and a Levenberg-Marquardt method, was also employed.[18] Some inconsistencies were observed, but on the whole, the new method proved to be effective, reducing the time required to process the data from days to one hour. The inconsistencies were observed when attempts to apply a Scatchard plot to all of the data were unachievable. The calculated values for K_b and n using both methods are included in Table 1. This new method allowed us to review the results and investigate alternative wavelengths when calculations for K_b or n where not reasonable.

Binding of the Pt(tmcp) Complexes to the Hexamer d(GTCGAC)$_2$

Each platinum(II) complex was titrated into a solution containing the hexamer d(GTCGAC)$_2$, with one-dimensional ^{1}H NMR spectra recorded at a metal complex to hexamer ratio (R) of 0.2, 0.4, 0.6, 0.8 and 1.0 and two-dimensional spectra, DQFCOSY and NOESY, recorded at $R = 1$.

The addition of each platinum(II) complex to d(GTCGAC)$_2$ induced significant broadening of the hexamer and metal-complex resonances (for example see Figure 4), indicating that each of the [Pt(tmcp)(Me$_n$phen)]Cl$_2$ complexes bind with intermediate exchange kinetics (on the NMR timescale). The observed intermediate exchange kinetics is consistent with an intercalative binding mode and that observed in the previous study with the [Pt(en)(3,4,7,8-Me$_4$phen)]Cl$_2$ complexes.[14] For each platinum(II) complex, significant upfield shifts of all 3,4,7,8-Me$_4$phen resonances were observed upon hexamer binding (see Table 2), again consistent with intercalation. The upfield shifts of the resonances for the [Pt(*R*,*S*-tmcp)(phen)]Cl$_2$ and [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$ complexes were similar, as were the shifts for the [Pt(*R*,*S*-tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$ and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$ complexes; however, the upfield shifts for the phen ligand resonances were considerably larger for the phen complexes than the 3,4,7,8-Me$_4$phen complexes. This may indicate that the phen complexes intercalate more fully into the hexamer base-stack than do the 3,4,7,8-Me$_4$phen complexes. The binding of the *S*,*R* complexes to d(GTCGAC)$_2$ induced larger shifts for the hexamer resonances than the corresponding *R*,*S* complexes (see Table 3), with the phen complexes generally inducing larger shifts than the 3,4,7,8-Me$_4$phen complexes (see Table 4). As most nucleotide residues exhibited significant shifts, it was not possible to deduce the metal complex binding site from the changes in chemical shift.

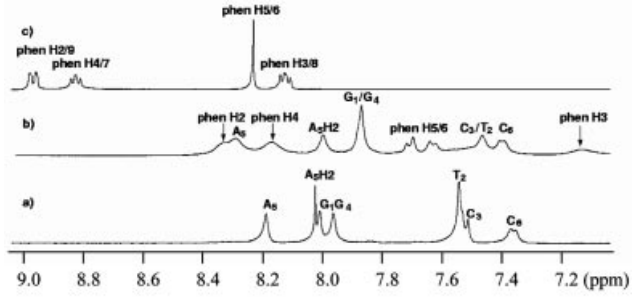


Figure 4. An expansion of the 1D NMR spectra of a) the free hexamer, b) [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$ bound to d(GTCGAC)$_2$ at R = 1 and c) [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$ in 10 mM phosphate buffer containing 1 mM EDTA and 20 mM NaCl at pH 7.0 and 25 °C.

NOESY spectra were recorded, at a range of temperatures and mixing times, in order to gain further information on the hexamer binding by the [Pt(tmcp)(3,4,7,8-Me$_4$phen)]-Cl$_2$ series of complexes. Figure 5 shows an expansion of the NOESY spectrum of the [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$ complex bound to d(GTCGAC)$_2$ at $R = 1$. In addition to the expected intra-duplex NOEs, a range of NOE cross-peaks are also observed between the metal complex and hexamer resonances, indicating that the platinum(II) complex fully intercalates from the minor groove. A range of relatively strong NOEs are observed from the tmcp methyl protons to the hexamer minor groove protons, ie. to the A$_5$H2 and the H1′ and H4′/H5′/H5′′ protons of all nucleotide residues. In addition, NOEs are clearly observed from the phen H3 and H4 resonances to the both major and minor groove protons, whereas NOEs are only observed from the phen H5/H6 protons to the T$_2$Me protons that are located in the major groove. As all the observed intermolecular NOEs from the phen protons are to T$_2$, C$_3$, G$_4$ and A$_5$ protons, it is concluded that [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$ mostly intercalates between the T$_2$·A$_5$ and C$_3$·G$_4$ base-pairs. The NOE data from the [Pt(*R*,*S*-tmcp)(phen)]Cl$_2$ complex bound to d(GTCGAC)$_2$ is similar, although the individual cross-peaks are weaker because of the greater degree of broadening of the metal complex resonances.

Due to the extreme broadening of all the resonances, it was difficult to resolve many of the intermolecular NOEs observed in NOESY spectra of the hexamer with either stereoisomer of [Pt(tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$; however, strong NOEs were observed from the tmcp-methyl protons to the hexamer A$_5$H2, H1′ and H4′/H5′/H5′′ protons. At temperatures between 40 and 50 °C a considerable number of metal complex-hexamer NOEs were clearly resolved. For

Table 2. Chemical shift [ppm] changes for the 1,10-phenanthroline ligand protons of the platinum(II) complexes [Pt(en)(phen)]Cl$_2$, [Pt(en)(3,4,7,8-Me$_4$phen)]Cl$_2$, [Pt(*R*,*S*-tmcp)(phen)]Cl$_2$, [Pt(*S*,*R*-tmcp)(phen)]Cl$_2$, [Pt(*R*,*S*-tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$ and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$, upon addition to the hexamer d(GTCGAC)$_2$ in 10 mM phosphate buffer containing 20 mM NaCl and 1 mM EDTA at 25 °C.

Metal complex Phen Proton	en-phen[a]	en-Me$_4$phen[b]	*R*,*S*-tmcp-phen	*S*,*R*-tmcp-phen	*R*,*S*-tmcp-Me$_4$phen	*S*,*R*-tmcp-Me$_4$phen
2,9	–0.66	–0.66	–0.59	–0.55	–0.44	–0.45
3,8	–0.90		–1.07	–1.03		
4,7	–0.64		–0.68	–0.59		
5	–0.51	–0.42	–0.60	–0.61	–0.31	–0.37
6	–0.51	–0.42	–0.55	–0.53	–0.40	–0.40
3/8-Me		–0.56			–0.46	–0.52
4/7-Me		–0.49			–0.40	–0.46

[a] Data taken from refs.[13,14]. [b] Data taken from ref.[14].

Table 3. Chemical shift [ppm] and changes in chemical shift (numbers in parentheses) of the non-exchangeable protons of $d(GTCGAC)_2$ in 10 mM phosphate buffer containing 20 mM NaCl and 1 mM EDTA at 25 °C upon binding by $[Pt(R,S\text{-tmcp})(phen)]Cl_2$ and $[Pt(S,R\text{-tmcp})(phen)]Cl_2$.

1:1 $d(GTCGAC)_2$/$[Pt(R,S\text{-tmcp})(phen)]^{2+}$						
Base	H8/H6	AH2	H1′	H2′	H2′′	H3′
G_1	7.93 (–0.08)		5.98 (–0.09)	2.63 (–0.10)	2.75 (–0.06)	4.82 (0.01)
T_2	7.48 (–0.05)		6.05 (–0.16)	2.25 (0.00)	2.45 (–0.14)	4.92 (–0.01)
C_3	7.51 (0.01)		5.59 (–0.13)	2.08 (–0.07)	2.33 (–0.09)	4.88 (0.01)
G_4	7.92 (–0.04)		5.52 (–0.12)	2.67 (–0.07)	2.71 (–0.08)	5.03 (0.00)
A_5	8.26 (0.07)	8.03 (0.01)	6.28 (–0.01)	2.74 (0.09)	2.90 (0.01)	5.04 (0.02)
C_6	7.36 (0.01)		6.10 (0.03)	2.13 (0.04)	2.13 (0.04)	4.48 (0.00)
1:1 $d(GTCGAC)_2$/$[Pt(S,R\text{-tmcp})(phen)]^{2+}$						
Base	H8/H6	AH2	H1′	H2′	H2′′	H3′
G_1	7.86 (–0.15)		5.92 (–0.15)	2.54 (–0.19)	2.68 (–0.13)	4.83 (–0.02)
T_2	7.45 (–0.08)		5.93 (–0.28)	2.27 (0.02)	2.36 (–0.23)	4.90 (–0.03)
C_3	7.50 (0.00)		5.51 (–0.21)	2.11 (0.06)	2.28 (–0.14)	4.91 (0.04)
G_4	7.87 (–0.09)		5.43 (–0.21)	2.55 (–0.19)	2.61 (–0.18)	5.02 (–0.01)
A_5	8.30 (0.11)	7.99 (–0.03)	6.20 (–0.09)	2.63 (–0.03)	2.88 (0.01)	5.07 (0.05)
C_6	7.38 (0.03)		6.10 (0.03)	2.18 (0.09)	2.18 (0.09)	4.51 (0.03)

Table 4. Chemical shift [ppm] and changes in chemical shift (numbers in parentheses) of the non-exchangeable protons of $d(GTCGAC)_2$ in 10 mM phosphate buffer containing 20 mM NaCl and 1 mM EDTA at 25 °C upon binding by $[Pt(R,S\text{-tmcp})(3,4,7,8\text{-}Me_4phen)]Cl_2$ and $[Pt(S,R\text{-tmcp})(3,4,7,8\text{-}Me_4phen)]Cl_2$.

1:1 $d(GTCGAC)_2$/$[Pt(R,S\text{-tmcp})(Me_4phen)]^{2+}$						
Base	H8/H6	AH2	H1′	H2′	H2′′	H3′
G_1	7.91 (–0.10)		5.98 (–0.09)	2.60 (–0.13)	2.73 (–0.08)	4.82 (–0.03)
T_2	7.41 (–0.11)		6.06 (–0.15)	2.19 (–0.06)	2.48 (–0.11)	4.88 (–0.05)
C_3	7.41 (–0.10)		5.60 (–0.12)	2.03 (–0.02)	2.35 (–0.07)	4.88 (0.01)
G_4	7.90 (–0.06)		5.60 (–0.04)	2.59 (–0.15)	2.68 (–0.11)	4.98 (0.05)
A_5	8.20 (0.01)	7.91 (–0.12)	6.24 (–0.05)	2.66 (0.01)	2.86 (–0.03)	5.02 (0.00)
C_6	7.36 (0.01)		6.06 (–0.01)	2.12 (0.03)	2.12 (0.03)	4.50 (0.02)
1:1 $d(GTCGAC)_2$/$[Pt(S,R\text{-tmcp})(Me_4phen)]^{2+}$						
Base	H8/H6	AH2	H1′	H2′	H2′′	H3′
G_1	7.80 (–0.21)		5.90 (–0.17)	2.54 (–0.19)	2.66 (–0.15)	4.84 (–0.01)
T_2	7.33 (–0.19)		5.95 (–0.26)	2.12 (–0.18)	2.40 (–0.19)	4.84 (–0.09)
C_3	7.40 (–0.11)		5.51 (–0.21)	2.09 (0.04)	2.40 (–0.02)	4.87 (0.00)
G_4	7.83 (–0.13)		5.52 (–0.12)	2.46 (–0.28)	2.67 (–0.12)	4.96 (–0.07)
A_5	8.20 (0.01)	7.86 (–0.17)	6.21 (–0.08)	2.69 (0.04)	2.82 (–0.07)	5.00 (–0.02)
C_6	7.40 (0.05)		6.04 (–0.03)	2.14 (0.03)	2.14 (0.03)	4.52 (0.04)

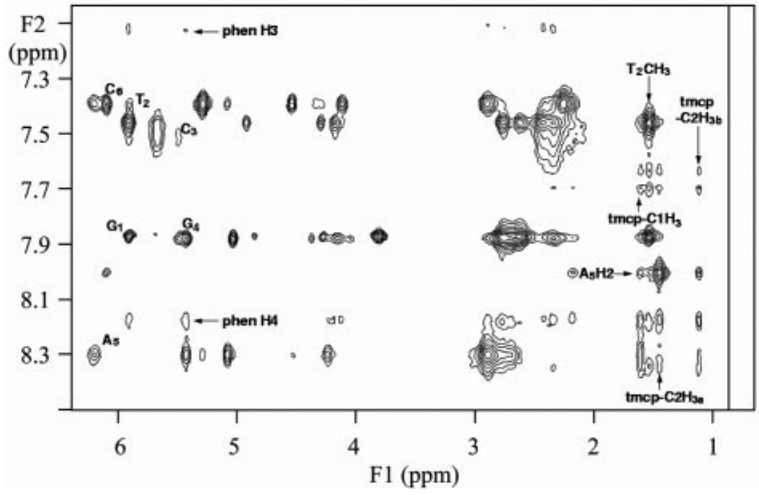


Figure 5. An expansion of the NOESY spectrum of the $[Pt(S,R\text{-tmcp})(phen)]Cl_2$ complex bound to $d(GTCGAC)_2$ at $R = 1$, in 10 mM phosphate buffer (pH, 7) containing 1 mM EDTA and 20 mM NaCl at 25 °C. The expansion shows the intermolecular connectivities between the metal complex phen and tmcp-methyl protons and the hexamer H1′ and A_5H2 protons, respectively.

the [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 complex, NOEs were observed from the H2/H9 protons to the G_1, T_2, C_3, G_4 and C_6 H1′ protons, with the strongest NOEs to the C_3 and G_4. In addition, NOEs were detected between the hexamer A_5H8 and the metal complex C3/C8 phenanthroline methyl protons. This data indicates that the [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 complex intercalates from the minor groove at all possible intercalation sites; however, based on the relative strengths of the intermolecular NOEs, with some preference for intercalation between the T_2·A_5 and C_3·G_4 base-pairs. For hexamer binding by [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2, a similar pattern of intermolecular NOEs, but of lower intensity, to the corresponding *S*,*R* complex was observed.

As the NOESY spectra only represent exchange-averaged NOEs for the hexamer binding by the [Pt(tmcp)(3,4,7,8-Me_4phen)]Cl_2 complexes, it is not possible to draw unambiguous conclusions on the intercalation geometry; however, the NMR spectroscopic data, coupled with the viscosity data, suggest that the [Pt(tmcp)(3,4,7,8-Me_4phen)]Cl_2 complexes only partially intercalate and in a "side-on" fashion. Simple molecular modelling indicates that if the metal complex fully intercalated in a "head-on" fashion, NOEs would not be observed from the tmcp-methyl protons to the A_5H2 and the H1′ and H4′/H5′/H5′′ protons. Alternatively, for partial and side-on intercalation (as illustrated in Figure 6), the observed NOEs from the A_5H2 to the C_2-methyls and the strong NOEs between the tmcp-methyl protons and the H1′ and H4′/H5′/H5′′ protons would be expected, as these distances are less than 5 Å.

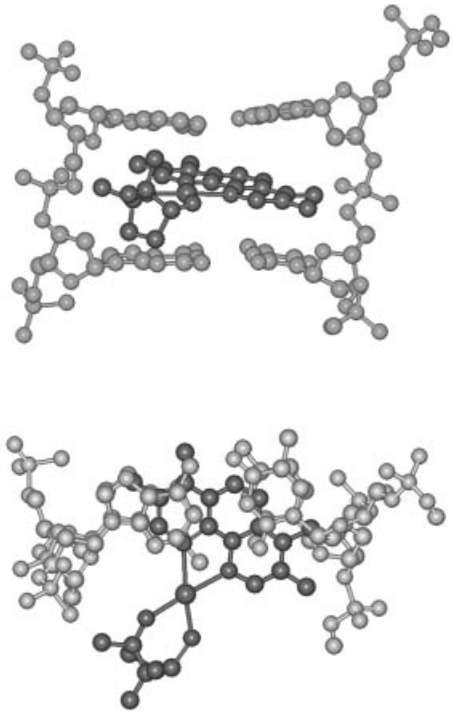


Figure 6. A HyperChem® molecular model of [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (dark grey) intercalated from the minor groove of d(GTCGAC)$_2$ (light grey). For simplicity, only the T_2 and C_3 base pairs have been shown.

Conclusions

An investigation into the biological activity of four platinum(II) complexes of 1,10-phenanthroline (phen) and 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-Me_4phen), with the ancillary ligands (1*R*,3*S*) and (1*S*,3*R*)-1,3-diamino-1,2,2-trimethylcyclopentane (*R*,*S*-tmcp and *S*,*R*-tmcp, respectively) has determined that the ancillary ligand can significantly influence biological activity.

In vitro cytotoxicity assays against the L1210 cell line, showed that [Pt(*R*,*S*-tmcp)(phen)]Cl_2 (**2a**) and [Pt(*S*,*R*-tmcp)(phen)]Cl_2 (**2b**) produced an average IC_{50} value of 11.3 and 11.2 μM, respectively, a decrease in potency when compared to [Pt(en)(phen)]Cl_2 with an average IC_{50} value of 9.7 μM; whereas [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 (**3a**) and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2, (**3b**) produced an average IC_{50} value of 11.5 and 8.5 μM, respectively, an increase in potency when compared to [Pt(en)(3,4,7,8-Me_4phen)]Cl_2 with an average IC_{50} value of >50 μM. This result suggests that the interaction between an intercalator, containing 3,4,7,8-Me_4phen, and DNA can be modulated in such a way as to increase activity.

Viscosity studies verified that the extent of intercalation had in fact been modulated, where the relative viscosities were reduced when compared to the parent complexes. Such that the viscosity of [Pt(*S*,*R*-tmcp)(phen)]Cl_2 and [Pt(*R*,*S*-tmcp)(phen)]Cl_2 were reduced to 1.15 and 0.97 respectively when compared to [Pt(en)(phen)]Cl_2 at 1.23 and [Pt(*R*,*S*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 and [Pt(*S*,*R*-tmcp)(3,4,7,8-Me_4phen)]Cl_2 were reduced to 0.90 and 0.87 respectively when compared to [Pt(en)(3,4,7,8-Me_4phen)]-Cl_2 at 1.12. The increase in activity of the 3,4,7,8-Me_4phen analogues may come about because the bending of DNA is of an appropriate proportion to halt DNA replication.

Titrations of the metal complexes into ct-DNA and the observation of the changes in ICD spectra was used to determine the binding constants. The observed changes in the ICD are consistent with an intimate interaction and indicate that some small, but different, structural/conformational changes result when these complexes interact with DNA.

For each of the metal complexes studied with d(GTCGAC)$_2$, intermolecular NOE data and observed changes in chemical shift suggests that the [Pt(tcmp)(3,4,7,8-Me_4phen)]Cl_2 complexes bind the hexamer by intercalation from the minor groove, and predominantly, between the T_2·A_5 and C_3·G_4 base-pairs.

Significant differences in biological activity that are achieved by four platinum(II) complexes of 1,10-phenathrolines and 3,4,7,8-tetramethyl-1,10-phenathrolines, suggest that the use of chiral ancillary ligands can affect biological activity. Of the two stereoisomers, the *S*,*R* ligand is more active than the *R*,*S* ligand and may be related to the degree that each isomer can prevent intercalation. From both the viscosity and NMR experiments it was shown that the *R*,*S* isomer prevented intercalation in the DNA to a greater extent than the *S*,*R* isomer. As both ligands are located in the DNA minor groove when the metal complex is intercalated,

it is possible the *R*,*S* ligand projects one of its methyl groups more deeply into the groove, thereby creating a steric clash. The results indicate that more active complexes might be developed by changing the chiral diamine ancillary ligands. Chiral ancillary ligands that have more steric bulk may result in a reduction in cytotoxicity, or the addition of stereospecific functional groups capable of forming hydrogen bonds with the DNA backbone may result in an increase in cytotoxicity.

Experimental Section

Instrumentation: The melting points were determined (uncorrected) with a Gallenkamp Melting Point apparatus. UV spectra were recorded with a Varian Cary 1E Spectrophotometer, and CD spectra were obtained with a Jasco J-810 Spectropolarimeter, both at room temperature. Viscosity measurements were carried out using a Cannon-Manning semi-micro viscometer maintained at a constant temperature of 25 °C in a water bath. ct-DNA was prepared and purified according to the method of Chaires et al.,[25] and DNA concentration was determined with a molar extinction coefficient (ε_{260}) of 12,824 $M^{-1}cm^{-1}$ per base pair.[25] 1D and 2D NMR spectra were recorded with a 300 or 400 MHz Varian Mercury NMR spectrometer at 25 °C, using commercially available solvents referenced to TMS or internal standards. DNA-NMR ^{1}H NMR spectra were recorded with a 400-MHz Varian Unityplus-400. Two-dimensional phase-sensitive NOESY spectra were acquired by the method of States et al.,[26] using 2048 data points in t_2 and 256–400 t_1 values with a pulse-repetition delay of 1.7 s. DQFCOSY experiments were accumulated using 2048 data points in t_2 for 256 t_1 values using a pulse-repetition delay of 1.7 s. Two-dimensional NMR spectroscopic data sets were zero-filled to 1024 points in the t_1 dimension and apodized with either a Gaussian or a shifted sinebell function. Microchemical analyses were performed at Australian National University, Canberra.

Materials: (*R*,*S*)- and (*S*,*R*)-camphoric acid, 1,10-phenanthroline (phen), 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-Me_4phen), calf thymus DNA (ct-DNA), IRA-400 (Cl) Amberlite ion exchange resin, Trizma [tris(hydroxymethyl)aminomethane hydrochloride] and all deuterated solvents were purchased from Aldrich Chemical Company. Potassium tetrachloroplatinate(II) was bought from Precious Metals Online Pty Ltd. CM-25 Sephadex and Waters C-18 Sep-Pak columns were obtained from Pharmacia. Oligonucleotides were purchased from Geneworks Inc. All other reagents were of analytical reagent or high purity grade.

Syntheses

(1*R*,3*S*)-1,3-Diamino-1,2,2-trimethylcyclopentane (*R*,*S*-tmcp) (1a): The compound was prepared by a procedure based on the method of Gillard et al.[27] To a vigorously stirred mixture of (1*R*,3*S*)-camphoric acid (20.00 g, 0.10 mol), in concentrated H_2SO_4 (50 mL) and ethanol-free chloroform (300 mL), at 50 °C, was added sodium azide (19.50 g, 0.30 mol) in small amounts over a period of 2 h. The mixture was then stirred for a further 18 h at 50 °C. The mixture was cooled, poured into H_2O (500 mL), and the aqueous phase made strongly basic with 12 M NaOH. The amine was extracted into $CHCl_3$ (2 × 500 mL), the organic extracts dried with anhydrous Na_2SO_4 and the chloroform removed in vacuo to give a clear oil. The oil was dissolved in diethyl ether (100 mL), the solution filtered, and the solvent removed in vacuo to give the product as a white solid (8.09 g, 57%). M.p. 128.1 °C. CD spectrum λ_{max} ($\Delta\varepsilon$/$mol^{-1}dm^3cm^{-1}$, water) = 217 nm (–0.12). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.98 (dd, ^3J = 6.9, 8.5 Hz, 1 H), 2.01 (m, 2 H), 1.62 (m, 4 H), 1.29 (m, 2 H), 1.01 (s, 3 H), 0.80 (d, ^3J = 3.6 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 61.0 (C), 60.8 (CH), 46.2 (C), 38.3 (CH_2), 30.2 (CH_2), 25.8 (CH_3), 22.2 (CH_3), 16.3 (CH_3) ppm.

(1*S*,3*R*)-1,3-Diamino-1,2,2-trimethylcyclopentane (*S*,*R*-tmcp) (1b): The ligand was prepared as described for **1a**, using (1*S*,3*R*)-camphoric acid, to yield the product as a white solid (7.21 g, 51%). M.p. 132.4 °C. CD spectrum λ_{max} nm ($\Delta\varepsilon$/$mol^{-1}dm^3cm^{-1}$, water) = 218 (+0.13). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.00 (dd, ^3J = 7.0, 8.7 Hz, 1 H), 2.02 (m, 2 H), 1.60 (m, 4 H), 1.31 (m, 2 H), 1.03 (s, 3 H), 0.81 (d, 6 H, ^3J = 3.5 Hz) ppm.

(1*R*,3*S*)-1,3-Diamino-1,2,2-trimethylcyclopentane(1,10-phenanthroline)platinum(II) Dichloride Dihydrate, [Pt(*R*,*S*-tmcp)(phen)]Cl_2·2H_2O (2a): The complex was prepared by a similar method as described previously.[14,28] [Pt(phen)Cl_2] (0.09 g, 0.20 mmol) and **1a** (0.23 g, 1.60 mmol) were refluxed in H_2O (20 mL) until all of the complex had dissolved to yield a pale yellow solution. This was concentrated to a small volume (5 mL) and chilled (4 °C) 1 M HCl (6 mL) was added, which caused the product to precipitate. The reaction mixture was cooled and left overnight at 4 °C before the yellow product was collected, washed with cold 1 M HCl and acetone, then dried in vacuo at room temperature (0.11 g, 88%). $C_{20}H_{26}Cl_2N_4Pt$·2H_2O (624.54): calcd. C 38.46, H 4.84, N 8.97; found C 38.39, H 5.02, N 8.83. Electronic spectrum: λ_{max} (ε/$mol^{-1}dm^3cm^{-1}$, water) = 227 (35769), 277 nm (30857). CD spectrum: λ_{max} = ($\Delta\varepsilon$/$mol^{-1}dm^3cm^{-1}$, water) = 212 (–1.42), 280 nm (+1.64). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.15 (m, 4 H), 8.35 (s, 2 H), 8.31 and 8.29 (2, dd, ^3J = 5.4, 8.3 Hz, 2 H), 6.78 (dd, ^3J = 11, 13.1 Hz, 2 H), 6.51 (dd, ^3J = 10.3, 10.6 Hz, 2 H), 2.91 (m, 1 H), 2.15 (m, 2 H), 1.92 (m, 1 H), 1.78 (m, 1 H), 1.45 (s, 3 H), 1.26 (s, 3 H), 0.80 (s, 3 H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 150.6 (CH), 150.3 (CH), 146.7 (2C), 141.3 (2CH), 130.7 (C), 130.6 (C), 128.1 (2 CH), 125.5 (CH), 125.4 (CH), 63.1 (C), 60.6 (CH), 47.6 (C), 34.5 (CH_2), 27.1 (CH_2), 25.1 (CH_3), 20.1 (CH_3), 16.3 (CH_3) ppm.

(1*S*,3*R*)-1,3-Diamino-1,2,2-trimethylcyclopentane(1,10-phenanthroline)platinum(II) Dichloride Dihydrate, [Pt(*S*,*R*-tmcp)(phen)]Cl_2·2.5H_2O (2b): The complex was prepared as described for **2a**, using **1b**, to yield the product as a yellow solid (0.10 g, 80%). $C_{20}H_{26}Cl_2N_4Pt$·2.5H_2O (633.55): calcd. C 37.92, H 4.93, N 8.84; found C 37.59, H 4.74, N 8.42. CD spectrum: λ_{max} ($\Delta\varepsilon$/$mol^{-1}dm^3cm^{-1}$, water) = 213 (+1.42), 281 nm (–1.83). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.11 (m, 4 H), 8.35 (s, 2 H), 8.31 and 8.28 (2, dd, ^3J = 5.5, 8.3 Hz, 2 H), 6.75 (dd, ^3J = 11.2, 13.2 Hz, 2 H), 6.50 (dd, ^3J = 10.4, 10.7 Hz, 2 H), 2.91 (m, 1 H), 2.12 (m, 2 H), 1.93 (m, 1 H), 1.75 (m, 1 H), 1.44 (s, 3 H), 1.26 (s, 3 H), 0.81 (s, 3 H) ppm.

(1*R*,3*S*)-1,3-Diamino-1,2,2-trimethylcyclopentane(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) Dichloride Tetrahydrate, [Pt(*R*,*S*-tmcp)(3,4,7,8-Me4phen)]Cl_2·4H_2O (3a): This complex was prepared as described for **2a**, using [Pt(3,4,7,8-Me_4phen)Cl_2][14,20] and **1a**, to yield the product as a green solid (0.12 g, 86%). $C_{24}H_{33}N_4PtCl_2$·4H_2O (715.69): calcd. C 40.27, H 5.78, N 7.83; found C 40.16, H 5.43, N 7.58. Electronic spectrum: λ_{max} nm (ε/$mol^{-1}dm^3cm^{-1}$, water) = 233 (29510), 283 nm (27070). CD spectrum: λ_{max} ($\Delta\varepsilon$/$mol^{-1}dm^3cm^{-1}$, water) = 218 (–1.53), 286 nm (+1.45). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 9.06 (s, 1 H), 8.99 (s, 1 H), 8.38 (s, 2 H), 6.62 (dd, ^3J = 10.2, 11.3 Hz, 2 H), 6.55 (dd, ^3J = 12.2, 11.3 Hz, 2 H), 2.91 (m, 1 H), 2.80 (d, ^3J = 2.9 Hz, 6 H), 2.64 (d, ^3J = 4.4 Hz, 6 H), 2.16 (m, 2 H), 1.93 (m, 1 H), 1.78 (m, 1 H), 1.48 (s, 3 H), 1.25 (s, 3 H), 0.80 (s, 3 H) ppm. ^{13}C NMR [D_6]-

DMSO: δ = 150.9 (CH), 150.6 (CH), 149.1 (C), 149.1 (H), 144.8 (C), 133.7 (C), 133.1 (C), 129.0 (C), 128.9 (C), 124.5 (CH), 63.2 (C), 60.5 (2 CH), 51.8 (C), 47.5 (C), 34.6 (CH_2), 27.3 (CH_2), 25.1 ($2CH_3$), 20.22 (CH_3), 17.6 (CH_3), 17.5 (CH_3), 16.2 (CH_3), 15.2 (CH_3) ppm.

(1*S*,3*R*)-1,3-Diamino-1,2,2-trimethylcyclopentane(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) Dichloride Tetrahydrate, [Pt(*S*,*R*-tmcp)(3,4,7,8-Me$_4$phen)]Cl$_2$·4H$_2$O (3b): This was prepared as described for **2a**, using [Pt(3,4,7,8-Me$_4$phen)Cl$_2$][14,20] and **1b**, to yield the product as a green solid (0.11 g, 83%). $C_{24}H_{38}Cl_2N_4O_2Pt$ (715.69): calcd. C 40.27, H 5.78, N 7.83; found C 40.26, H 5.50; N 7.69. CD spectrum: λ_{max} ($\Delta\varepsilon$/mol^{-1}dm^{3}cm^{-1}, water) = 223 (+1.47), 285 nm (–1.25). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.04 (s, 1 H), 8.98 (s, 1 H), 8.41 (s, 2 H), 6.60 (dd, ^3J = 10.3, 11.4 Hz, 2 H), 6.54 (dd, ^3J = 12.1, 11.2 Hz, 2 H), 2.91 (m, 1 H), 2.82 (d, ^3J = 2.9 Hz, 6 H), 2.62 (d, ^3J = 4.39 Hz, 6 H), 2.12 (m, 2 H), 1.95 (m, 1 H), 1.75 (m, 1 H), 1.45 (s, 3 H), 1.24 (s, 3 H), 0.82 (s, 3 H) ppm.

Cytotoxicity: In vitro cytotoxicity studies were carried out at the Peter MacCullum Cancer Institute, Melbourne by a standard method.[29] The chiral platinum(II) complexes were dissolved in warm distilled water and then diluted to the required concentrations. Results of the growth inhibition for murine leukaemia L1210 cells are reported for two experiments at 48 h continuous exposure to the metal complex. Cells were counted by Coulter Counting. The results of the growth inhibition assays of the platinum(II) complexes against the L1210 mouse leukaemia cell line are presented in Table 1. Values represent IC_{50} in μM. IC_{50} is the concentration of platinum(II) complex required to induce 50% inhibition of cell growth. All complexes were administered in water. Cytotoxicity of the new platinum(II) complexes are compared with cisplatin, and their individual parent compounds [Pt(en)(phen)]Cl$_2$ and [Pt(en)(3,4,7,8-Me$_4$phen)]Cl$_2$,[14] to assess the contribution of the chiral ancillary ligands.

Viscosity Studies: Samples were made to a volume of 300 μL and the flow time for the BPES buffer (6 mM, Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.0) used was 479 (±1) s. The ct-DNA solution (100 μM per base pair, molar absorptivity of 12824 M^{-1}cm^{-1}bp^{-1} at 258 nm)[25] gave a flow time of 518 (±1) and samples were prepared to give total platinum(II) complex/base pair ratios of 0.125 and 0.25. Flow times were measured after a thermal equilibration period of 20 min and ranged from 500 (±1) to 552 (±1) depending on the concentration and the complex added. Each sample flow time was measured three times and an average calculated. Relative viscosity is presented as $(\eta/\eta_o)^{1/3}$ in accordance with the theory of Cohen and Eisenberg.[30] The value η is the viscosity of the solution in the presence of a complex, and η_o is the viscosity of the ct-DNA solution alone. This viscosity η is calculated from the time for the sample (t) to flow through the viscometer subtracted by the time measured for the buffer (t_o) only.

DNA Binding and Induced Circular Dichroism: Titrations were performed to determine the affinity constants for the chiral platinum(II) complexes with ct-DNA (Figure 2 and Figure 3) using published methods.[24] The CD was recorded over the range 200 to 350 nm, for the ct-DNA solution (3000 μL, 28 or 35 μM) in buffer (5 mM, Trizma, 50 mM NaCl, pH 7.5), and after each titration with a small volume of metal-complex solution (10 or 5 mM). The small increase in the final volume was no more than 3%. Titrations were continued until no further change was observed in the CD spectrum, indicating that saturation of the DNA had been reached.

Using the information gained from the spectra and utilising the methods of Roger and Nordén,[24] binding constants for each of the complexes were determined as described previously.[14] CD data and the methodology for calculating the binding constant are included in the supporting information (for details see the footnote on the first page of this article). The calculated values for K_b and n [where K_b is the equilibrium binding constant and n is the number of binding sites (in base pairs)] are reported in Table 1. We have also developed a different data analysis technique, which utilises direct least-squares curve fitting, using a Levenberg–Marquardt method,[18] to our experimental points. Essentially we fit the equation below to the data, where the independent variable L_T is the ligand concentration, B_T the total DNA concentration, and the dependent variable ε the CD response. The calculated values for K_b and n (the number of bases per binding site) using this method are also included in Table 1.

$$\frac{\varepsilon}{\alpha} = 0.5\left[\frac{1}{K_b} + L_T + \frac{B_T}{n_s} - \sqrt{\left(\left\{\frac{1}{K_b} + L_T + \frac{B_T}{n_s}\right\}^2 - \frac{4B_TL_T}{n_s}\right)}\right]$$

Titration of d(GTCGAC)$_2$ by the Platinum(II) Complexes: The hexamer was converted into the sodium salt using a CM-25 Sephadex column after residual impurities were removed by elution through a C-18 Sep-Pak as described previously.[14] The hexamer was dissolved in 700 μL of phosphate buffer (10 mM, pH 7) containing 20 mM NaCl and 0.1 mM EDTA. For experiments carried out in D_2O the sample was repeatedly freeze-dried from D_2O and finally made up in 99.96% D_2O. The hexamer concentration was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1}cm^{-1} per nucleotide.[13,14]

Supporting Information (see footnote on the first page of this article): Supporting information is available in electronic format only giving a description of how the DNA binding constants were determined, including mathematical forumlae; DNA binding data; and figures of the DNA binding curves.

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[1] K. W. Jennette, S. J. Lippard, G. A. Vassiliades, W. R. Bauer, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3839–3843.
[2] J. K. Barton, S. J. Lippard, *Nucleic Acid-Metal Ion Interactions*, (Ed.: T. G. Spiro), Wiley-Interscience, New York, **1980**, p. 31–113.
[3] A. H. Krotz, L. Y. Kuo, T. P. Shields, J. K. Barton, *J. Am. Chem. Soc.* **1993**, *115*, 3877–3882.
[4] A. M. Pyle, J. K. Barton, *Prog. Inorg. Chem., Bioinorg. Chem.* **1990**, *38*, 413–475.
[5] S. J. Lippard, *Acc. Chem. Res.* **1978**, *11*, 211–217.
[6] B. P. Hudson, C. M. Dupureur, J. K. Barton, *J. Am. Chem. Soc.* **1995**, *117*, 9379–9380.
[7] M. Cusumano, M. L. Di Pietro, A. Giannetto, *Inorg. Chem.* **1999**, *38*, 1754–1758.
[8] G. Lowe, A. S. Droz, T. Vilaivan, G. W. Weaver, J. J. Park, J. M. Pratt, L. Tweedale, L. R. Kelland, *J. Med. Chem.* **1999**, *42*, 3167–3174.
[9] A. Odani, H. Masuda, O. Yamauchi, *Inorg. Chem.* **1991**, *30*, 4484–4486.
[10] M. Cusumano, M. L. Di Pietro, A. Giannetto, F. Nicolo, E. Rotondo, *Inorg. Chem.* **1998**, *37*, 563–568.
[11] A. Odani, R. Shimata, H. Masuda, O. Yamauchi, *Inorg. Chem.* **1991**, *30*, 2133–2138.

[12] O. Yamanchi, A. Odani, R. Shimata, Y. Kosaka, *Inorg. Chem.* **1986**, *25*, 3337–3339.
[13] J. G. Collins, R. M. Rixon, J. R. Aldrich-Wright, *Inorg. Chem.* **2000**, *39*, 4377–4379.
[14] C. Brodie, J. G. Collins, J. R. Aldrich-Wright, *Dalton Trans.* **2004**, 1145–1152.
[15] W. R. Leopold, R. P. Batzinger, E. C. Miller, J. A. Miller, R. H. Earhart, *Cancer Res.* **1981**, *41*, 4368–4377.
[16] E. M. Rezler, R. R. Fenton, W. J. Esdale, M. J. McKeage, P. J. Russell, T. W. Hambley, *J. Med. Chem.* **1997**, *40*, 3508–3515.
[17] R. C. Conrad, J. V. Rund, *Inorg. Chem.* **1972**, *11*, 129–134.
[18] F. H. Stootman, D. M. Fisher, J. R. Aldrich-Wright, manuscript in preparation.
[19] B. H. Long, J. K. Barton, *Acc. Chem. Res.* **1990**, *23*, 271–273.
[20] D. Suh, J. B. Chaires, *Bioorg. Med. Chem.* **1995**, *3*, 723–728.
[21] S. E. Sherman, D. Gibson, A. H.-J. Wang, S. J. Lippard, *Science* **1985**, *230*, 412–417.
[22] L. Kapicak, E. J. Gabbay, *J. Am. Chem. Soc.* **1975**, *97*, 403–408.
[23] L. P. G. Wakelin, M. J. Waring, *DNA intercalating agents*, in: *Comprehensive Medicinal Chemistry* (Ed.: P. G. Sammes), Pergamon Press, Oxford, UK, **1990**, *2*, 703.
[24] A. Rodger, B. Nordén, *Circular Dichroism and Linear Dichroism*, Oxford University Press, Oxford, **1997**.
[25] J. B. Chaires, N. Dattagupta, D. M. Crothers, *Biochemistry* **1982**, *21*, 3933–3940.
[26] R. A. States, R. A. Haberkorn, D. J. Ruben, *J. Magn. Reson.* **1982**, *48*, 286–292.
[27] R. D. Gillard, P. D. Newman, R. S. Vagg, P. A. Williams, *Inorg. Chim. Acta* **1995**, *233*, 79–84.
[28] W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos, V. A. Leopold, *J. Med. Chem.* **1985**, *28*, 1113–1116.
[29] G. B. Deacon, E. T. Lawrenz, T. W. Hambley, S. Rainone, L. K. Webster, *J. Organomet. Chem.* **1995**, *493*, 205–213.
[30] G. L. Cohen, H. Eisenberg, *Biopolymers* **1969**, *8*, 45–55.

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