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COORDINATION CHEMISTRY REVIEWS
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Review

Non-covalent interactions in adducts of platinum drugs with nucleobases in nucleotides and DNA as revealed by using chiral substrates

Giovanni Natile [a,*], Luigi G. Marzilli [b]

[a] *Department Farmaco-Chimico, University of Bari, Via E. Orabona 4, I-70125 Bari, Italy*
[b] *Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*

Received 26 July 2005; accepted 7 December 2005
Available online 24 January 2006

Contents

Abstract

Selective binding of coordinating agents (cisplatin) to target molecules (DNA) and the contribution of H-bonding, electrostatic interactions, and steric effects in such binding are fundamentally important subjects. To address some of these questions, simple models of the platinum–DNA

Abbreviations: 1-Me-5′-GMP, N1-methylguanosine-5′-monophosphate; A_2, two amines or a diamine ligand; bip, 2,2′-bipiperidine; C_2, two-fold rotation axis; CL, cross-link; CCC, chirality-controlling chelate ligands; dab, 2,3-diaminobutane; dach, 1,2-diaminocyclohexane; d(GpG), guanine bases N9-bridged by a deoxyribo-phosphodiester backbone; DS, double-stranded; en, 1,2-diaminoethane; FFC, first-to-first sphere communication; FSC, first-to-second sphere communication; G, guanine base, nucleoside, or nucleotide; GpG, guanine bases N9-bridged by a ribo-phosphodiester backbone; HH, head-to-head; HMG, high-mobility group; HMG1 or HMGB1, Protein containing HMG domain; HT, head-to-tail; Me_2dab, *N*,*N*′-dimethyl-2,3-diaminobutane; Me_2dap, *N*,*N*′-dimethyl-2,4-diaminopentane; Me_2en, *N*,*N*′-dimethyl-1,2-diaminoethane (Me_2dae); Me_2pip, *N*,*N*′-dimethyl-1,4-piperazine; NER, nucleotide excision repair; tn, trimethylenediamine (1, ,3-diaminopropane); SS, single-stranded; SSC, second-to-second sphere communication

* Corresponding author. Tel.: +39 080 5442774; fax: +39 080 5442230.
E-mail address: natile@farmchim.uniba.it (G. Natile).

0010-8545/$ – see front matter
doi:10.1016/j.ccr.2005.12.004

cross-links, such as *cis*-$(NH_3)_2PtG_2$ (G = detached or tethered guanine bases) complexes, have been investigated extensively. The fast interconversion between possible conformers on the NMR time scale led us to construct analogs of cisplatin with bulky ligands designed to reduce the dynamic motion by destabilizing the transition state for Pt-G rotation. The term "retro modeling" was introduced to emphasize that the models are more complicated than the relevant molecule, cisplatin. Retro models have been proved to be particularly useful in unraveling the existence of different conformations, and in the most favorable cases have also allowed the full characterization of the stereochemistry of single conformers. Moreover, several interligand interactions that escaped detection for a long time have been identified and grouped into three categories: First-to-First, First-to-Second, and Second-to-Second sphere Communication. A hierarchy ranking these interactions has been established. The new analysis has allowed us to rationalize computational, solution-state, and solid-state results on cross-linked oligonucleotides directly relevant to the *cis*-$(NH_3)_2Pt$ adducts themselves.

Keywords: Non-covalent interactions; Platinum–DNA; Antitumor drugs; Circular dichroism; Chiral diamines

1. Introduction

Rosenberg's discovery of the antitumor activity of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) [1–3] represented a breakthrough in tumor chemotherapy. Cisplatin, which is highly effective in the treatment of testicular and ovarian cancers, is used in association with other antitumor drugs in the treatment of oropharyngeal, bronchogenic, and cervical carcinomas, lymphoma, osteosarcoma, melanoma, bladder carcinoma, and neuroblastoma [4]. Since the introduction of cisplatin, a large number of new platinum compounds have been prepared and tested for antitumor activity. However, only a few of them have reached clinical trials [5], and only one, diammine(1,1-cyclobutanedicarboxylate-*O*,*O*′)platinum(II) (carboplatin), has achieved world-wide approval for routine clinical use. This new drug has a lower toxicity than cisplatin, but is unable to overcome cisplatin resistance in the same range of tumors. More recently, two other platinum compounds, namely diammine(glycolate-*O*,*O*′)platinum(II) (nedaplatin or 254-S) and (*R*,*R*)-1,2-diaminocyclohexane(oxalate-*O*,*O*′)platinum(II) (oxaliplatin or L-OHP), have received approval for use in some countries, the latter being used only for the secondary treatment of metastatic colorectal cancer [6].

1.1. Binding to target molecules

Cisplatin mainly targets DNA by binding to N7 of adjacent purines [5–10]. The resultant intrastrand adducts are thought to be the lesions that are responsible for cell death, but the mechanism of action is not entirely understood. Failure to fully understand the mechanism of antitumor activity could be responsible for the low success rate in the development of new platinum drugs able to overcome cisplatin resistance.

X-ray structures of double-stranded (DS) oligonucleotides containing a *cis*-A_2Pt moiety (A_2 = two amines or a diamine) cross-linking two G residues of the same strand [11–14] or of opposite strands [15] have been reported. In the intrastrand cross-links (Intra-CL) the *cis*-A_2Pt moiety is located in the major groove, and the six-membered rings of the two guanines are on the same side of the platinum coordination plane (Head-to-Head, HH, conformation, Figs. 1 and 2). In contrast, when platinum cross-links G residues of opposite strands (Inter-CL), the *cis*-A_2Pt moiety is located in the minor groove, and the six-membered rings of the two guanines are on opposite sides of the platinum coordination plane (Head-to-Tail, HT, conformation, Figs. 1 and 2). Solution NMR data have been interpreted by analogy with the results of the X-ray investigations [10,16–19]. A detailed description of these adducts appears in some excellent reviews [6–8]. However, even in the case of DS oligonucleotides, which are, per se, somewhat rigidly structured, some dispute exists as to the fine structure of more dynamic regions, and the contributions of the sugar-phosphate backbone and of interligand interactions in determining the overall stereochemistry remain rather uncertain. These interligand interactions within the coordination sphere of platinum can be investigated more

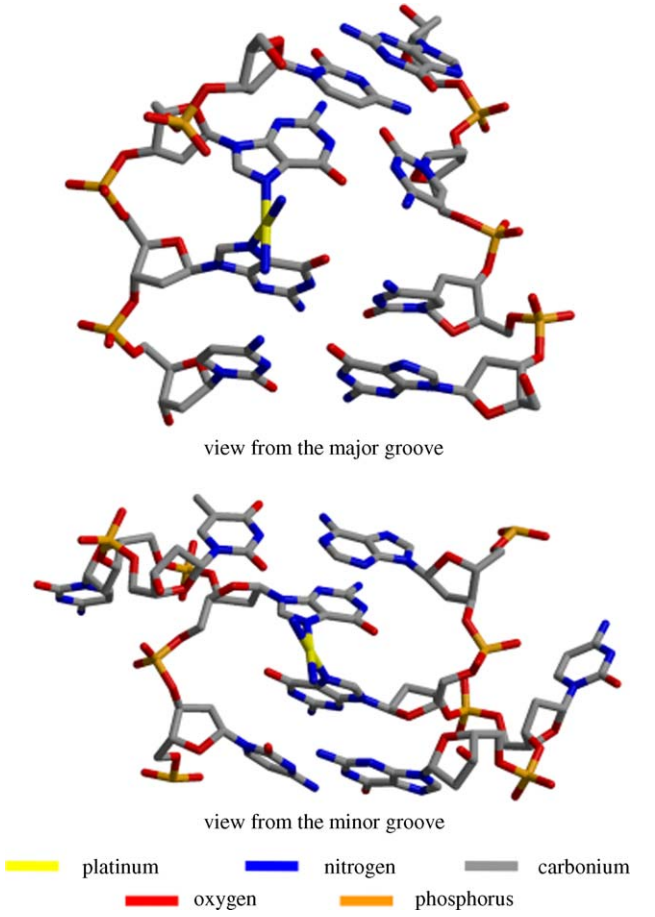

Fig. 1. Central portion (four base-pairs) of oligonucleotides containing G/G intrastrand (top) and interstrand (bottom) cross-links (based on data from Refs. [10,19]).

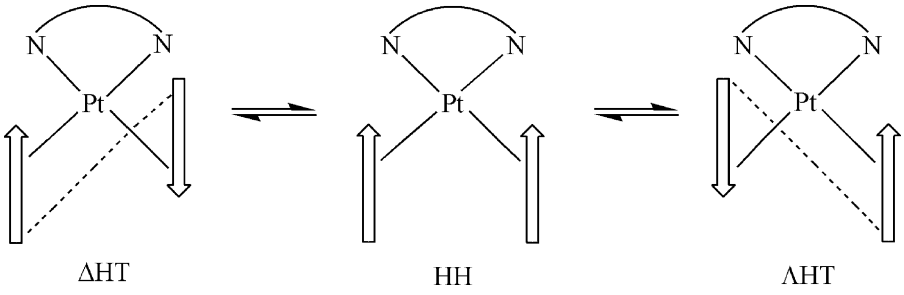

Fig. 2. HH, ΛHT, and ΔHT conformers. The chiralities of the two HT conformers are defined according to the handedness of two straight lines: one perpendicular to the coordination plane and passing through the platinum atom and the second connecting the O6 atoms of the two G's.

effectively in *cis*-A_2PtG_2 model systems in which A_2 are two monodentate amines or a diamine and G_2 are two detached or tethered guanine bases.

1.2. Model compounds

In the solid state, the large majority of *cis*-A_2PtG_2 complexes adopt the conformation with the six-membered rings of the two guanines on opposite sides of the platinum coordination plane [20–25]. In only a few cases (all complexes containing 9-ethylguanine except one containing 9-[(2-hydroxyethoxy)methyl]guanine), was the HH conformation found (Fig. 2) [21,26,27].

In solution, *cis*-A_2PtG_2 complexes usually exhibit free rotation about the Pt–N7 bond [28–31]. In a pioneering study, Cramer demonstrated that guanosine rotation can be slowed sufficiently to detect atropisomers on the NMR time scale, if A_2 is a sufficiently bulky chelate (e.g. *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Me_4en) [28]. Two G H8 signals were detected. In an insightful analysis, it was shown that, when the *cis*-A_2Pt moiety has local C_2 symmetry, the asymmetric sugar renders magnetically non-equivalent the two possible HT atropisomers, each with one H8 signal. Two H8 signals are also expected for the non-equivalent G's of the HH atropisomer. The observation of only two of four possible signals could be best explained by the presence of only the two HT atropisomers, consistent with most crystallographic results [20–25,32–35]. The bulky A_2 ligand(s) often lacked NH groups. Although *N*,*N*′-dimethylethylenediamine (Me_2en) complexes also formed HT atropisomers, the broad guanosine H8 signals revealed a rotation rate that is fast on the NMR time scale [30].

In conclusion, there is an intrinsic difficulty in investigating dynamic nucleos(t)ide complexes. The structure in the solid state may be very different from that in solution because of crystal-packing interactions. In solution, because of fast interconversion between possible conformers, only one set of signals, the average of those of individual conformers, is observed.

1.3. Retro modeling

The "dynamic motion problem" led us to construct analogs of cisplatin with bulky ligands designed to reduce the dynamic motion by destabilizing the transition state for Pt–N7 rotation (Fig. 3). An important feature of the design was to minimize steric effects in the ground state equilibrium species to allow conformers likely to be present in dynamic *cis*-A_2PtG_2 adducts to exist in the new adducts also. We have introduced the term "retro modeling" [36] to emphasize that the models we employ [36–47] are more complicated than the relevant molecule, cisplatin. By reducing rotation rates by a billion fold [36,38], retro models have enabled us to understand the adducts of the highly fluxional cisplatin drug with DNA constituents [45]. Retro-model results [36–40,43,44,46–48] have called into question the following two concepts that for many years were widely accepted from studies on dynamic platinum complexes: (i) untethered G's adopt an HT conformation in preference to the HH conformation [9], and (ii) single-stranded d(GpG) cross-links favor the HH form, which undergoes slow rotation about the Pt–N7 bond [49–53].

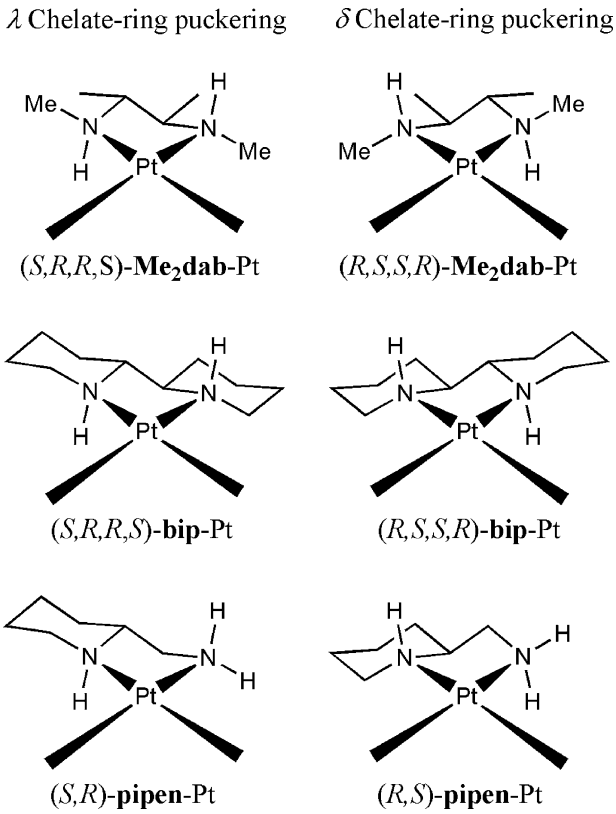

Fig. 3. Me_2dab, bip, and pipen ligands with in-plane bulk used to slow down rotation about the Pt–N7 bonds in *cis*-A_2PtG_2 compounds.

N,*N*′-Dimethyl-2,3-diaminobutane (Me_2dab) was the first carrier ligand used in such a detailed NMR retro-model study [40,54,55]. Me_2dab differs from Me_2en in having an additional methyl substituent on each carbon atom of the ethylene chain bridging the two nitrogens. These two methyl substituents not only increase the overall steric rigidity of the ligand, but also, by asymmetric induction, influence the configuration of the adjacent amine groups. Each N and each C in the ring is an asymmetric center. The Me_2dab retro models provided the opportunity to define the solution structure of conformers by NMR methods, allowing us to identify the HH form of a *cis*-A_2PtG_2 adduct for the first time [45] and also allowing us to use the known Me_2dab configuration to define the absolute conformation of the HT forms in solution for the first time [40].

The 2,2′-bipiperidine ligand (bip) is analogous to Me_2dab, but is much more sterically rigid. This rigidity has two components: first, the chelate ring is part of a three-ring system, decreasing its fluxional character with respect to the Me_2dab ligand. Second, the CH groups projecting toward the G coordination sites are unable to rotate away from the G bases during G base rotation around the Pt–N7 bond. In contrast, these CH's in Me_2dab are in N-methyl groups, which are able to rotate freely, in essence acting as a turnstile that permits the G O6 to pass by easily during G rotation. The bip ligand was able to decrease the dynamic motion roughly one billion times relative to $(NH_3)_2$ and ca. one hundred times relative to Me_2dab in *cis*-A_2PtG_2 complexes [36,38–40,47,48,56].

Most of our work involved the C_2-symmetrical isomers of Me_2dab and bip, in which the chiral N,C,C, and N centers in the chelate ring were *S*,*R*,*R*,*S* and *R*,*S*,*S*,*R*, respectively.

2. Retro models with untethered guanine bases

The use of Me_2dab and bip ligands allowed the simultaneous observation by ^{1}H NMR spectroscopy of all possible conformers (two HT and one HH conformer, Fig. 4) that can be formed in *cis*-A_2PtG_2 complexes with a C_2-symmetrical carrier ligand.

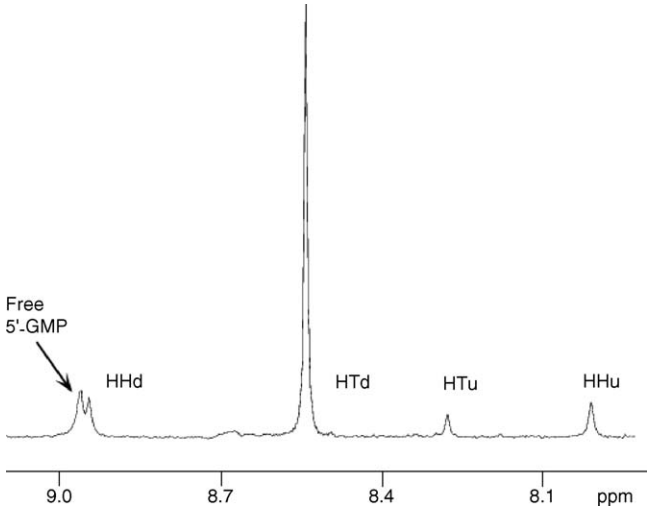

Fig. 4. H8 region of the ^{1}H NMR spectrum for (*R*,*S*,*S*,*R*)-Me_2dabPt(5′-GMP)$_2$ (Ref. [54] for related work).

Moreover, the conformers were present at equilibrium in different amounts depending upon the chiralities of the ligands; thus these ligands were named Chirality-Controlling Chelates (CCC). The relative amounts were the same for bip and Me_2dab compounds with a given chirality and they differ only in dynamics.

The H8 signal of the major HT form appeared downfield from the H8 signal of the minor HT form [36,38,40,47,54]. The H8 chemical shift is influenced by the positioning of the H8 atom with respect to the shielding cone of the *cis* G. The canting of the two equivalent bases in the direction that moves each H8 away from the *cis* G will lead to less H8 shielding and hence to a downfield H8 signal relative to the average H8 signal (this relationship, called "six-in" because the six-membered ring of each G moves closer to the *cis* G, is found in the major HT conformer). In contrast, a rotation of the two equivalent bases in the direction moving each H8 toward the *cis* G will lead to greater H8 shielding [51], and hence to a more upfield H8 signal (this relationship, called "six-out" because the six-membered ring of each G moves farther from the *cis* G, is found in the minor HT conformer). The finding that the conformer with the larger six-membered rings of the two *cis* G's closer together would be more stable was quite unexpected [23].

The major HT conformer found for Me_2dab and bip-PtG_2 adducts has the carrier-ligand NH located on the opposite side of the coordination plane from G O6 [40,47]. The favored form could not participate in a G O6/NH *cis* amine hydrogen bond, revealing that carrier-ligand H-bonding might not be so important [10,37,47].

The equivalent bases in each HT form had H8 chemical shifts intermediate between those of the non-equivalent bases in the HH form. The G base of the HH form with O6 on the same side of the coordination plane as the NH of the *cis* amine had an upfield H8 signal (HH_u), indicative of an H8 pointing toward the *cis* G ("six-out" canting). The base with O6 on the same side of the coordination plane as the N-CH_3 or N-CH_2-group at the *cis* amine had a downfield H8 signal (HH_d) [36,38,40,47,54], indicative of an H8 pointing away from the *cis* G ("six-in" canting).

2.1. Dipole–dipole interaction between cis guanines, FFC_a

The fact that the major HT conformer has the six-membered ring of each guanine on the same side as the N-alkyl substituent of the *cis* amine, while the minor HT conformer has the six-membered ring of each guanine on the same side as the NH of the *cis* amine, indicates that base/*cis* amine steric clashes are not large enough to destabilize the major HT conformer, just as any possible guanine O6/NH *cis* amine H-bonding is not sufficiently favorable to stabilize the minor HT form. The most plausible explanation for this behavior is that stabilization of the HT conformers stems from dipole(base)–dipole(base) interaction between *cis* bases, and this interaction is stronger for canted G bases with the six-membered ring of each guanine leaning toward the *cis* G ("six-in" canting). The "six-in" canting, however, will bring the H8 of each guanine close to the *cis* amine, and if an N-Me substituent is on the same side of the

platinum coordination plane as the H8, an unfavorable interaction between the two groups will limit the degree of "six-in" canting and the stability of this conformer. This lower degree of canting explains the lower stability of the minor HT conformer. Although in the minor HT conformer an H-bonding interaction between guanine O6 and NH of the *cis* amine is possible, such an interaction most probably does not play an important role because dipole–dipole interaction tends to bring the six-membered ring of each guanine closer to the *cis* guanine and farther from the *cis* amine. It is noteworthy that, in X-ray structures of HT conformers of *cis*-A_2PtG_2 complexes, much greater "six-in" canting can be found when the H8 of each guanine is on the same side of the platinum coordination plane as an NH of the *cis* amine [57,58] than when it is on the side of an N-Me of the *cis* amine [28,59]. A "six-in" canting of ca. 20° will bring the electron-rich O6 atom of each guanine closer to the electron-deficient H8 atom of the *cis* base. Moreover, greater canting also reduces the dihedral angle between the planes of the two guanines (for a canting angle of 80°, 70°, 60°, and 50° the dihedral angle is found to be 82°, 74°, 66°, and 56°, respectively).

Because the applicable interactions just discussed involve parts of ligands close to the metal, we call this class of interligand interactions "First-to-First sphere Communication" (FFC_a, Fig. 5).

2.2. Repulsion between O6 groups of cis guanines, FFC_{HH}

With the exception of very simple species such as *cis*-$(NH_3)_2Pt(d(GpG))$, an unusual but characteristic feature of larger oligonucleotides cross-linked at N7 by platinum(II) is a large difference (∼1 ppm) in the H8 signals with the 3′-G H8 signal downfield in single-strand adducts. The guanine orientation dictated by the sugar-phosphate backbone is responsible for the shift difference [49]. The same remarkable difference in H8 chemical shifts of the HH conformer was found in Me_2dab- and bipPtG_2 complexes with untethered guanines, suggesting that interligand interactions in the coordination sphere may dictate guanine orientations comparable to those induced by the sugar-phosphate backbone of the GpG moiety within larger oligonucleotides.

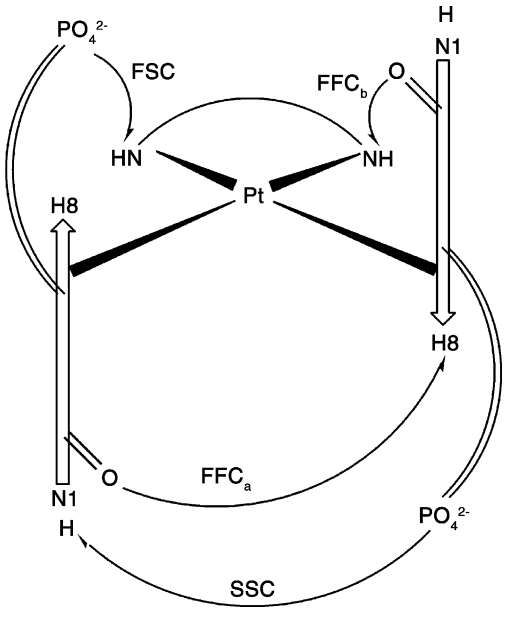

Fig. 5. Schematic drawing of possible internucleotide and nucleotide *cis* amine interactions. Because of the C_2 symmetry of the HT conformers, each interaction is doubled; however, for clarity, only one has been represented. The scheme does not intend to take into account the chirality of the HT conformer (Δ or Λ) or the position of the phosphate (3′ or 5′). The repulsion between O6 groups of *cis* guanines in the HH conformer, FFC_{HH}, is not represented (Ref. [86] for related work).

In Me_2dab- and bipPtG_2 models with untethered G's, the large dispersion of H8 chemical shifts for the HH conformer stems from a combination of electronic and steric effects. Electrostatic repulsion between the electron-rich O6 atoms of *cis* G's will tend to place the six-membered rings farther apart [60]. The stereochemistry of the Me_2dab and bip ligands (for *R*,*S*,*S*,*R* or *S*,*R*,*R*,*S* configurations at the N,C,C, and N asymmetric centers) places one NH and one N-CH_2X (X = H or R for Me_2dab and bip ligands, respectively) on each side of the platinum coordination plane. The guanine *cis* to NH can be canted "six-out" because there is no clash with an amine substituent and O6 can act as an H-bond acceptor from the NH. In contrast, the guanine *cis* to the N-CH_2X will be much less canted and probably canted away from the *cis* amine ("six-in" canting). The former guanine will have a strongly shielded H8 signal, whereas the second guanine will have an unshielded H8 signal.

It is noteworthy that the shift separation between the H8 signals of the two HT conformers or between the two H8 signals of the HH conformer is greatly reduced if in place of Me_2dab or bip we use more symmetrical bidentate ligands such as *N*,*N*′-dimethyl-1,4-piperazine (Me_2pip) [37,43,44], phenanthroline (phen), and 2,9-dimethylphenanthroline (Me_2phen) [61], or *N*,*N*,*N*′,*N*′-tetramethyl-substituted aliphatic diamines such as Me_4en [28], Me_4dab [62], and Me_4-1,2-diaminocyclohexane (Me_4dach) [63]. Under these circumstances, the two HT conformers have comparable ability to be canted "six-in" and the two guanines of the HH conformer will have comparable ability to be canted "six-out". The abundance of the HH conformer further decreases when there is a steric impediment to a "six-out" canting of at least one guanine, such as in the case of the Me_4en complex first investigated by Cramer et al. [28], and in the analogous complexes with Me_4dab and Me_4dach ligands [62,63]. On the other hand, removal of the electron-rich O6 atom of guanine, such as in the case of adducts with benzimidazole (B), not only increases the yield of the HH conformer with respect to the HT conformers (the dipole–dipole interactions stabilizing the latter conformers are greatly reduced if not absent, Fig. 6) but also greatly reduces the dispersion of the B H2 chemical shifts even in the case of Me_2dab or bip ligands (B H2 occupies the same position as H8 in G) [60]. Although the Me_2dab and bip ligands have one NH and one NCH_2X on each side of the coordination plane, this unsymmetrical distribution does not induce a great difference in the canting of the B bases of the HH conformer because there is no great repulsion between the six-membered B rings. Because the applicable interaction just discussed involves parts of ligands close to the metal, this interligand interaction is also called First-to-First sphere Communication. However,

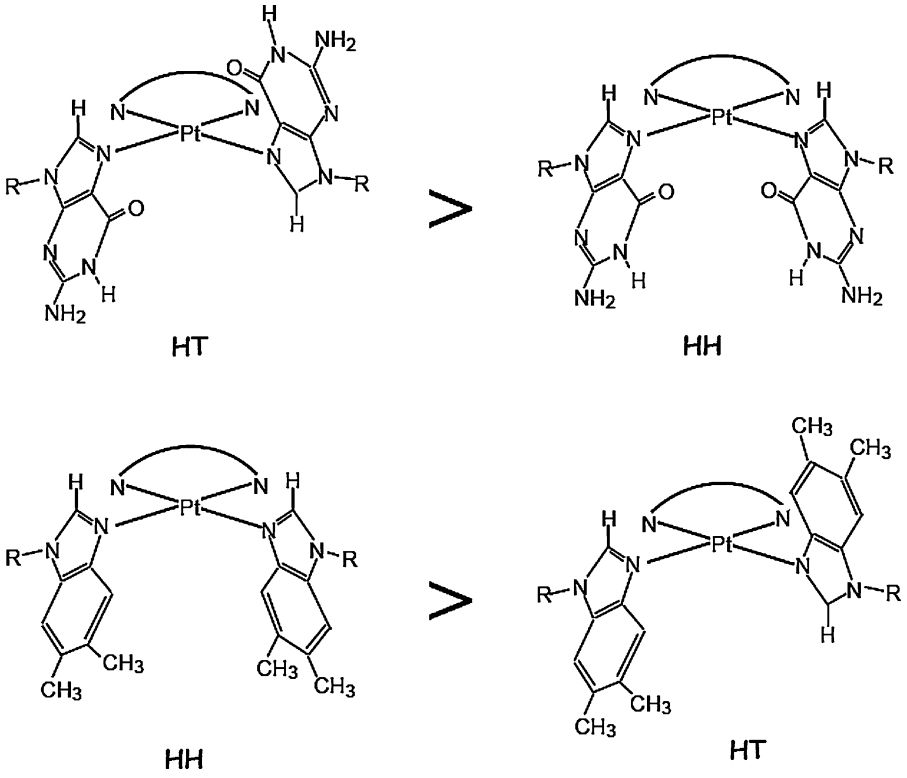

Fig. 6. Different ratio of HH and HT conformers in adducts with G (top) and 5,6-dimethylbenzimidazole (bottom). The scheme does not intend to take into account the chirality of the HT conformer (Δ or Λ) (Ref. [60] for related work).

because this interaction applies only to HH conformers while all other interactions discussed in this section concern mainly HT conformers, we have added the suffix HH (FFC_{HH}) and not included this interaction in Fig. 5.

2.3. Phosphate/N1H cis G H-bond interaction, SSC

Two relevant types of ionizable groups are present in *cis*-$A_2Pt(GMP)_2$ adducts. First, the monoanionic phosphates of the GMP ligands $[ROP(OH)O_2]^-$ become fully deprotonated by pH ∼7.5 [20,64–67]. The lower phosphate group pK_a of 5.6–6.2 versus 6.3 for free 5′-GMP [36,42,66,68–70] has been attributed either to H-bonding between the phosphate group and a *cis* amine hydrogen [53,71,72] or to the electrostatic effect of the positively charged platinum [73]. Second, the G N1H groups deprotonate with pK_a values of 8.7 and ∼9.4 for *cis*-$A_2Pt(5'-GMP)_2$ versus 9.5 for free 5′-GMP [66,67,70]. The lower N1H pK_a values of PtG_2 adducts are due to the inductive effects of the platinum ion bound to N7 [50,66,70,74].

For both the *R*,*S*,*S*,*R* and *S*,*R*,*R*,*S* chiralities of the Me_2dab- and bipPt(3′-GMP)$_2$ complexes, the percentage of the ΔHT conformer increased near pH 7 as compared to that at lower pH. For the 5′-GMP complexes, the percentage of the ΛHT conformer was found to increase near pH 7 [36,41–45].

We have unravelled the reasons for these changes in conformer percentage [47]. The phosphate group position is different in 5′-GMP versus 3′-GMP. For 5′-GMP adducts, the 5′-phosphate group projects toward the *cis* 5′-nucleotide in the ΛHT form, and away from the *cis* 5′-nucleotide in the ΔHT form (assuming that the nucleotide maintains the preferred *anti* conformation, Fig. 7). In contrast, for 3′-GMP adducts the 3′-phosphate is close to the *cis* 3′-nucleotide in the ΔHT form and away from it in the ΛHT form (this can be seen in a gedanken experiment in which the phosphates of Fig. 7 are detached from the 5′ position and attached to the 3′ position). Thus, the relative positions of phosphate groups to the *cis* G in a HT form with a given chirality are opposite for 3′-GMP versus 5′-GMP adducts. On this ground, the key factors stabilizing the ΔHT conformer for 3′-GMP derivatives and the ΛHT conformer for 5′-GMP adducts near pH 7 appear to be the phosphate/N1H *cis* G interactions. We call such interligand interactions "Second-to-Second sphere Communication" (SSC) because the interacting groups are at the periphery of the *cis* nucleotides [44,45,47]. These SSC interactions are optimal at pH 6–7, conditions in which the phosphate group is deprotonated and the N1 is protonated.

Full support of the conclusion that in *cis*-$A_2Pt(5'-GMP)_2$ complexes the ΛHT conformer is favored by G phosphate/N1H *cis* G hydrogen bonding (SSC) came also from our investigation of 1-Me-5′-GMP adducts [47]. When the pH of solutions of (CCC)Pt(1-Me-5′-GMP)$_2$ adducts is raised from 3 to 7, the ΛHT conformer decreases instead of increasing. This result is attributed to the impossibility of forming phosphate/N1H *cis* G H-bonds, accompanied by the possible repulsion between dianionic phosphates, which are closer in the ΛHT than in the ΔHT conformer when G = 5′-GMP. The ΔHT conformer concentration remains practically constant, while the HH conformer concentration undergoes an increase corresponding to the decrease

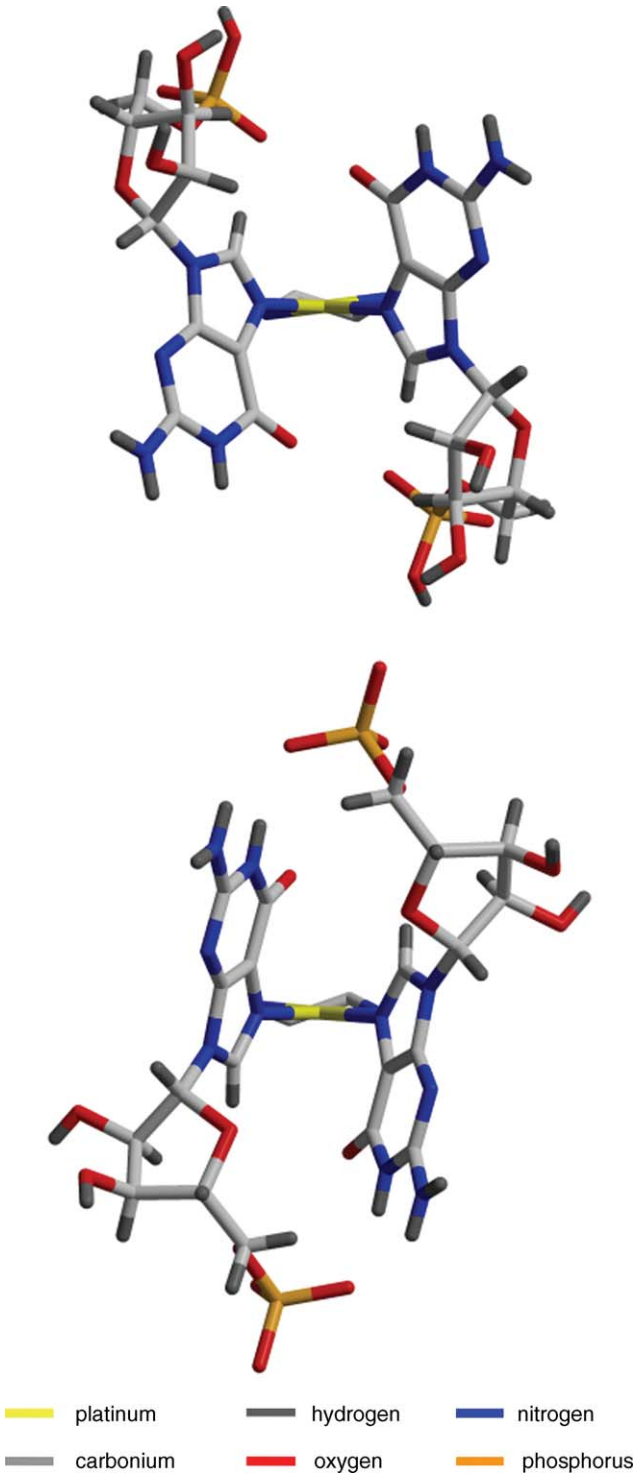

Fig. 7. X-ray structures of two *cis*-$A_2Pt(5'\text{-GMP})_2$ adducts showing how in the ΔHT conformer (top, based on data from Ref. [59]) the 5′-phosphate is directed towards the *cis* amine, while in the ΛHT conformer (bottom, based on unpublished data from Natile laboratory) the 5′-phosphate is directed towards the *cis* guanine. For simplicity only the chelate-ring atoms of the carrier ligands (Me_4dach in the former case and Me_4en in the latter case) are shown.

in the ΛHT conformer. This increase leads to an unusually abundant HH conformer for (CCC)Pt(1-Me-5′-GMP)$_2$ complexes at pH ∼7 (40–44% for the *S*,*R*,*R*,*S* and 27–29% for the *R*,*S*,*S*,*R* CCC ligand configurations). These findings clearly indicate that SSC stabilization of the ΛHT conformer is a key element influencing conformer distribution in 5′-GMP PtG_2 adducts.

2.4. Guanine O6/NH cis amine H-bond interaction, FFC_b

At basic pH (∼10), the N1H deprotonation eliminates the SSC phosphate/N1H *cis* G interactions that stabilize the ΛHT conformer for 5′-GMP adducts and the ΔHT conformer for 3′-GMP adducts. On the other hand, N1H deprotonation greatly increases the capacity of the G O6 to act as a hydrogen bond acceptor. It was observed that, upon N1H deprotonation at basic pH, the less abundant HT conformer of the moderately dynamic Me_2dabPt(3′-GMP)$_2$ model compounds becomes more favored (Table 1) [56]. The most abundant conformer at acidic and neutral pH is stabilized mainly by FFC_a and has the G O6 of each guanine on the same side of the coordination plane as the N-Me of the *cis* amine (when the major HT has Δ chirality, its concentration is further increased by SSC). The minor HT at acidic and neutral pH has the G O6 of each guanine on the same side of the platinum coordination plane as the NH of the *cis* amine; therefore, its increase in concentration at basic pH is likely to stem from G O6/NH Me_2dab hydrogen bonding, now favored as a result of an increased capacity of the G O6 to act as a hydrogen bond acceptor.

Because the latter interaction involves parts of ligands close to the metal (Fig. 5), we have called this interligand interaction "First-to-First sphere Communication" (FFC_b) and also have added a suffix b in order to distinguish this interaction from the dipole–dipole interaction, which we have called FFC_a, and the repulsion between the O6 groups of *cis* guanines, which we have called FFC_{HH}.

2.5. Phosphate/NH cis amine H-bond interaction, FSC

When G has a 5′-phosphate group, conformer distribution could also be influenced by phosphate/NH *cis* amine hydrogen-bonding interaction. Such an interaction should favor the ΔHT conformer because for this conformer the 5′-phosphate protrudes toward the *cis* amine (assuming the favored *anti* conformation for the nucleotides). For the moderately dynamic Me_2dabPtG_2 model compounds at acidic and neutral pH's, the conformer distribution of the Me_2dabPt(5′-GMP)$_2$ adducts could be understood by using much of the same reasoning as discussed for the corresponding Me_2dabPt(3′-GMP)$_2$ adducts in the previous section [56]. For both the 3′-GMP and 5′-GMP adducts, the conformer preference appeared to be dominated by FFC_a (and to some extent by SSC) at acidic and neutral pH's, as expected. However, at basic pH the ΔHT conformer was unexpectedly always preferred (Table 1) for both the (*S*,*R*,*R*,*S*)- and (*R*,*S*,*S*,*R*)-Me_2dabPt(5′-GMP)$_2$ adducts. As mentioned, the results for Me_2dabPt(3′-GMP)$_2$ adducts at basic pH can be attributed to FFC_b. However, FFC_b requires that the G O6 and the NH of the *cis* amine are on the same side of the platinum coordination plane; therefore, at basic pH FFC_b can stabilize the ΔHT conformer only in the case of (*S*,*R*,*R*,*S*)-Me_2dab, while for (*R*,*S*,*S*,*R*)-Me_2dab FFC_b would stabilize the ΛHT conformer, as observed for 3′-GMP adducts (Section 2.4). In contrast,

Table 1
Conformer percentages of Me_2dabPtG_2 complexes at different pH values, as measured from the H8 signal intensities

G	*S*,*R*,*R*,*S*				*R*,*S*,*S*,*R*			
	pH	ΛHT (%)	ΔHT (%)	HH (%)	pH	ΛHT (%)	ΔHT (%)	HH (%)
3′-GMP	3.4[a]	80	13	7	3.3	16	84	–[b]
	7.2[a]	60	35	5	6.9	4	96	–[b]
	9.6[a]	37	60	3	9.3	86	14	–[b]
5′-GMP	3.2	92	2	6	3.1	7	71	22
	7.0	98	1	1	7.3	12	70	18
	10.4	20	72	8	10.0	10	69	21

[a] At 5 °C; all others were at room temperature.
[b] Not determined.

the stabilization at basic pH of the ΔHT conformer also in the case of (*R*,*S*,*S*,*R*)-Me_2dabPt(5′-GMP)$_2$ is clear evidence for the occurrence of another type of interaction, most probably a phosphate/NH *cis* amine interaction, which favors the ΔHT conformation, as just mentioned. Indeed, for the ΔHT conformer of (*R*,*S*,*S*,*R*)-Me_2dabPt(5′-GMP)$_2$ the 5′-phosphate and the NH of the *cis* amine are on the same side of the platinum coordination plane, the situation required for the ΔHT conformation to be favored. In Me_2dabPt(3′-GMP)$_2$ adducts, conformers cannot be affected by phosphate/NH *cis* amine interaction for stereochemical reasons because the 3′-phosphate is too remote from the *cis* amine NH.

Because the 5′-phosphate/NH *cis* amine hydrogen bonding involves one part of the ligand close to the metal (the NH) and one part far from the metal (the phosphate), we have called this interaction "First-to-Second sphere Communication" (FSC).

Evidence in favor of FSC (phosphate to carrier-ligand NH hydrogen bonding) has been obtained in highly dynamic *cis*-A_2Pt(1-Me-5′-GMP)$_2$ complexes with symmetrical A_2 ligands such as $(NH_3)_2$, en(1,2-diaminoethane), and tn(1,3-diaminopropane) [45]. In these complexes, FFC interactions do not discriminate between ΛHT and ΔHT conformers, and the Me substituent on N1 also rules out SSC interactions. The only remaining interaction able to discriminate between ΛHT and ΔHT is FSC. Our experiments employing CCC ligands confirmed a role for this interaction that is, however, weak compared to FFC and SSC. In the solid state, numerous examples of ΔHT complexes of 5′-GMP and several related 5′-phosphate derivatives have been found not only for Pt with various carrier ligands, but also for other metals [21–23,26–28,32,34,51,57,58,75–80]. The nucleotides in the structures have very similar relationships, with the purine bases having the same relative positions and the phosphate group always hydrogen-bonded to the *cis* ligand in a similar manner. This contrast between the prevalence of FSC in the solid and its relative unimportance in solution supports the premise of our retro-model studies that the structures in the solid state of dynamic nucleotide complexes may be very different from the solution structure.

The investigation of highly dynamic systems required the use of CD spectroscopy (NMR is not applicable to highly dynamic systems). The following section will be devoted to the use of retro models for unraveling the CD signature of different conformers.

3. CD signature of different conformers

The presence of enhanced CD signals for some *cis*-A_2PtG_2 complexes was first reported in 1980 [81], and attempts were later made to interpret the results on a structural basis [82,83]. However, a convincing interpretation of the results has depended on subsequent studies with less dynamic complexes [9,38,40].

The CD spectra of (*S*,*R*,*R*,*S*)-Me_2dabPtG_2 complexes all had the same shape, i.e., positive peaks at ∼285 and 230 nm and negative peaks at 250 and 210 nm. Because all the 2D NMR studies showed that the dominant atropisomer was ΛHT, this type of CD signal was designated as Λ. The negative peaks at 285 and 230 nm and positive peaks at 250 and 210 nm in the CD spectra of (*R*,*S*,*S*,*R*)-Me_2dabPtG_2 complexes had signs opposite to those of the corresponding peaks in the (*S*,*R*,*R*,*S*)-Me_2dabPtG_2 CD spectra. Because the 2D NMR studies showed that the dominant atropisomer for (*R*,*S*,*S*,*R*)-Me_2dabPtG_2 complexes had the ΔHT conformation, this type of CD signal was designated as Δ. Furthermore, the CD signal intensity roughly correlated with the percentage of the major HT form [40].

Studies with the related bip complexes indicated that a mixture of 25% ΔHT, 50% HH, and 25% ΛHT had essentially no CD intensity [38]. The CD intensity increased as the favored HT form became dominant with time. In a 'pH jump' experiment on (*S*,*R*)-pipenPt(5′-GMP)$_2$ (Fig. 3), we monitored the change of atropisomer distribution by using both NMR and CD spectroscopy [42]. Changes in H8 NMR signal intensities corresponded to changes in CD intensity. On the basis of the above experiments, we interpret the sign of the CD signal as a reflection of the Δ or Λ conformation of the major HT form. In particular, the pattern of a positive Cotton effect around 285 nm and a negative Cotton effect around 250 nm is characteristic of a ΛHT conformer; the opposite pattern is characteristic of a ΔHT conformer [36].

3.1. Conformers in cisplatin adducts with 3′-GMP

NMR methods do not allow detection of individual conformers in fluxional *cis*-A_2PtG_2 complexes with two ammines or a primary diamine ligand. Because of this dynamic motion problem, we relied heavily on CD spectroscopy to assess

the chirality of the major HT conformer. Although CD spectroscopy is an empirical method at the current state of theoretical understanding of CD spectral transitions in such complexes, reasonable results were obtained which agree with those from better-defined, less dynamic *cis*-A_2PtG_2 complexes with chiral bulky diamine ligands described in previous paragraphs.

The CD signals of cisplatin adducts of G derivatives with no phosphate groups are weak. No conformer is favored because the two ammine ligands have no chiral property that can exert a stereochemical control favoring a particular conformation. This analysis applies not only to cisplatin but also to analogous A_2PtG_2 complexes with achiral primary diamines because these also have weak CD signals.

In contrast, at neutral pH, *cis*-$(NH_3)_2$Pt(3′-GMP)$_2$, tnPt(3′-GMP)$_2$, and enPt(3′-GMP)$_2$ all showed the Δ type CD signal [45], indicating that the major atropisomer is ΔHT (Fig. 8). The carrier ligand has a modulating effect on the CD intensity: weakest for enPt and strongest for *cis*-$(NH_3)_2$Pt. The 3′-phosphate group from one 3′-GMP can form a hydrogen bond with the N1H of the *cis* 3′-GMP in the ΔHT conformation (assuming the normally preferred *anti* conformation for the nucleotide), whereas such hydrogen bonding is less favorable in the ΛHT conformation. The preference for the ΔHT atropisomer for these three 3′-GMP complexes appears to be due to such hydrogen bonding (SSC).

Raising the pH from 3 to 7 increased the Δ type CD signal intensity, indicating that the ΔHT form is favored upon 3′-phosphate group deprotonation, most likely due to formation of stronger phosphate/N1H *cis* G hydrogen bonds at neutral pH.

Above pH 7, the Δ type CD signal starts to decrease, and the intensity is nearly zero at pH 9. At pH ∼9, where N1H deprotonation occurs [73], the N1 atom has lost its proton and no phosphate/N1H hydrogen bond can exist. As the pH is raised above 9.5, the CD signals of tnPt(3′-GMP)$_2$ and enPt(3′-GMP)$_2$ complexes inverted, suggesting that the major atropisomer is ΛHT [45]. Our NMR/CD studies with (CCC)Pt(3′-GMP)$_2$ [36] and pipenPt(3′-GMP)$_2$ [41] complexes support this interpretation. From examination of models, the distance between the 3′-phosphate group and the N1 atom of the *cis* 3′-GMP is shorter in the ΔHT than in the ΛHT atropisomer. Also the two 3′-phosphate groups are closer to each other in the ΔHT than in the ΛHT conformation. Repulsion between negatively charged groups could be greater in the ΔHT than in the ΛHT conformation, such that the latter conformer gains stability. These repulsions have been collectively called *cis* G repulsions [41]. However, we do not believe that the CD evidence by itself is a reliable indicator of the conformation for high pH forms, because the signals are weak and because the electronic transitions of the G bases are altered by deprotonation.

3.2. Conformers in cisplatin adducts with 5′-GMP

At neutral pH, the CD signals of *cis*-$(NH_3)_2$Pt(5′-GMP)$_2$, tnPt(5′-GMP)$_2$, and enPt(5′-GMP)$_2$ are of the Λ type, indicating

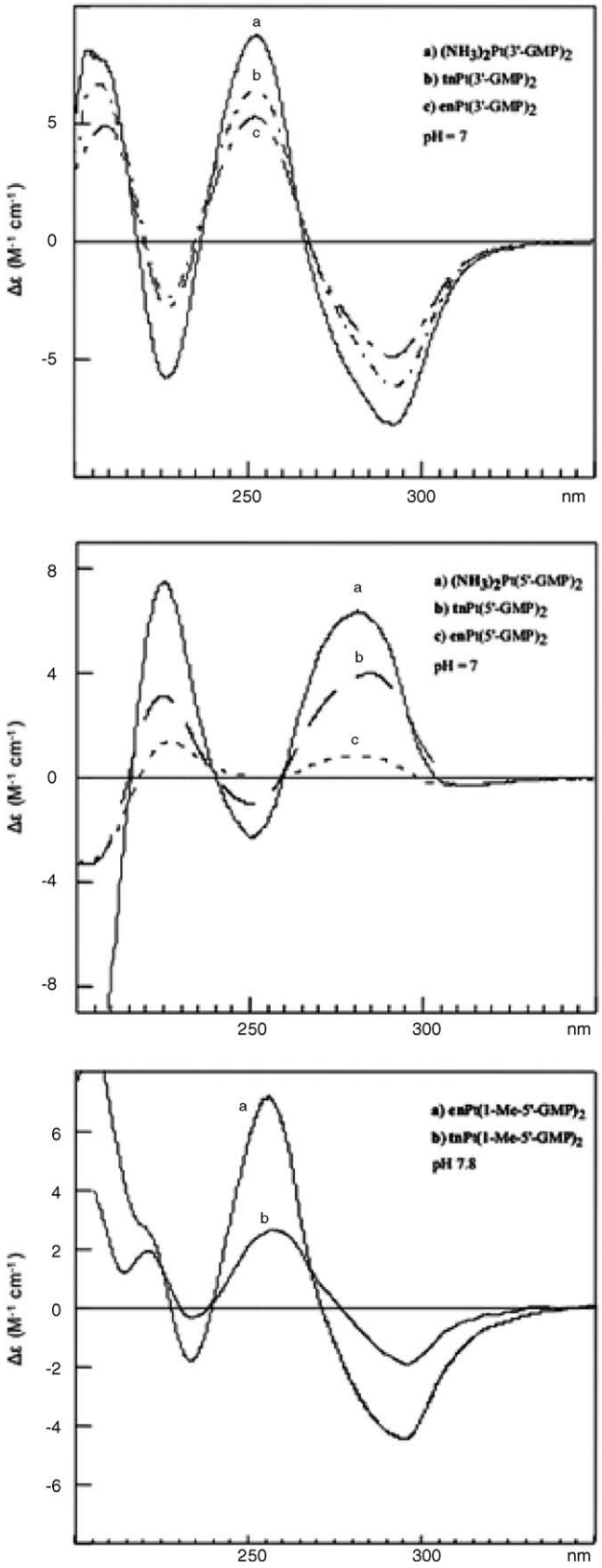

Fig. 8. CD spectra of *cis*-A_2PtG_2 compounds when $A_2 = (NH_3)_2$, tn, or en and G = 3′-GMP, 5′-GMP, or 1-Me-5′-GMP (Ref. [45] for related work).

that the major conformation is ΛHT (Fig. 8) [45]. Intramolecular hydrogen bonding between the phosphate group of one 5′-GMP and the N1H of the *cis* 5′-GMP is more favorable in the ΛHT than in the ΔHT conformer.

The CD data point to substantial differences in signal intensity among 5′-GMP adducts of different carrier ligands [*cis*-$(NH_3)_2$, en, tn]. One obvious potential factor is the bite angle of the carrier ligand(s). The very low CD intensity of the enPt(5′-GMP)$_2$ complex suggests that the low bite angle of the en ligand reduces the strength of the *cis* G interactions.

Raising the pH from ∼3 to 7 increased the Λ type CD signal intensity, indicating that the ΛHT form is more favored upon 5′-phosphate group deprotonation, possibly because of stronger phosphate/N1H hydrogen bonds at neutral pH. As the pH was raised further, the CD signals decreased in intensity and partially inverted at pH 10, suggesting a bias toward the ΔHT form after N1H deprotonation [45]. The explanation for this bias could be similar to that given for the 3′-GMP adducts at high pH, except that for 5′-GMP derivatives the *cis* G repulsions favor the ΔHT conformer. However, the mobility of a 5′-phosphate (attached to an exocyclic carbon) is greater than that of a 3′-phosphate (attached to an endocyclic carbon); therefore, *cis* G repulsions are expected to be less important for 5′-GMP derivatives. On the other hand, in a ΔHT conformation the 5′-phosphate is directed toward and can directly reach the *cis* amine and form an H-bond (this hydrogen-bonding interaction is found in relevant X-ray structures). It is therefore most likely that the presence of 5′-phosphate/NH *cis* amine hydrogen bonding is responsible for the stabilization of the ΔHT form at high pH (such hydrogen bonding is masked at acidic and neutral pH's by the dominating SSC interactions).

3.3. Conformers in cisplatin adducts with 1-Me-5′-GMP

The CD signals of tnPt(1-Me-5′-GMP)$_2$ and enPt(1-Me-5′-GMP)$_2$ had signs opposite to those of 5′-GMP adducts (Fig. 8) [45]. The signs indicate that the major atropisomer of the 1-Me-5′-GMP adducts is ΔHT.

Because no phosphate/N1H hydrogen bond is possible for 1-Me-5′-GMP derivatives, the only important interaction influencing the HT atropisomer population is phosphate/NH *cis* amine H-bonding. The ΔHT conformer has the 5′-phosphate protruding toward the *cis* amine and able to form a phosphate/NH *cis* amine H-bond.

The ΔHT conformer could also be favored by a smaller phosphate/phosphate repulsion between *cis* G's; however, such an internucleotide interaction would be expected to increase by increasing the bite angle of the A_2 carrier ligand but the CD signal was smaller in the tn than in the en derivative. Therefore, the latter interaction, if present, is less important than the former. This conclusion is also supported by the observation that in the absence of a *cis* G (which rules out any SSC interaction) such as in the complex Me_3dienPt(5′-GMP) (Me_3dien = *N*,*N*′,*N*″-trimethyldiethylenetriamine, with all three methyl groups on the same side of the platinum coordination plane), H-bond formation between phosphate and NH of the *cis* amine becomes the dominant interaction [84,85].

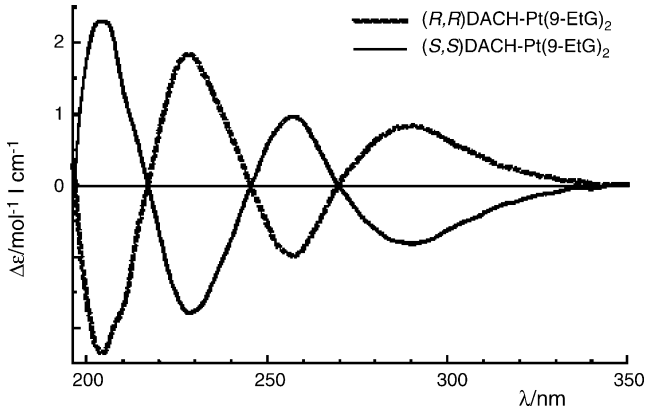

Fig. 9. CD spectra of (*R*,*R*)-dachPt(9-EtG)$_2$ (broken line) and (*S*,*S*)-dachPt(9-EtG)$_2$ (solid line) at pH 3, room temperature (Ref. [86] for related work).

3.4. Conformers in oxaliplatin adducts with guanines lacking a phosphate group

In an early investigation of the reaction of enantiomeric dach platinum complexes with guanine derivatives [82], Pasini and co-workers observed that the CD spectrum of (*R*,*R*)-dachPtG$_2$ (G = 9-methylguanine) was characterized by a positive Cotton effect centered at 280 and 230 nm and a negative Cotton effect centered at 260 nm; the corresponding Cotton effects for the (*S*,*S*)-dachPtG$_2$ enantiomer had the opposite sign (Fig. 9). The Cotton effect was assigned to coupling between π–π^* electronic transitions centered on the guanine bases and was taken as a clear indication of transmission of chirality from the dach ligand to the coordinated *cis* guanines mediated by the amine protons [82]. The details of the mode of transmission of chirality given at that time were not convincing and have remained obscure until very recently [86].

Cis-A_2PtG$_2$ complexes with G = a guanine derivative lacking a phosphate substituent can have only FFC interactions, as revealed by previous investigations on retro models. However, whereas each N in retro-model carrier ligands had substituents of very different bulk on the two sides of the platinum coordination plane, the dach ligand has an NH on each side of the plane. However, a slight difference in the stereochemistry of the two protons (one NH has "quasi axial" and the other "quasi equatorial" character) is sufficient to induce a significant change in the relative stabilities of the dachPtG$_2$ ΔHT and ΛHT conformers. At acidic and neutral pH the stability of HT conformers is governed by G to G dipole–dipole interaction, which is greater for the six-membered ring of each guanine leaning toward the *cis* G. Such a "six-in" canting of the two guanines can be hampered by the steric interaction between the H8 of each guanine and the substituent on the *cis* amine that is on the same side of the platinum coordination plane. Such a repulsion is greater for a "quasi equatorial" NH than for a "quasi axial" NH. Therefore, the ΔHT conformer is stabilized in the (*S*,*S*)-dachPtG$_2$ complex, while the ΛHT conformer is stabilized in the (*R*,*R*)-dachPtG$_2$ species. Indeed the CD spectrum is typical of a ΔHT conformer in the former case and of a ΛHT conformer in the latter case [86].

At basic pH, deprotonation of the guanine N1H renders the O6 a much better H-bond acceptor; therefore, the stability of the HT conformers is governed by the guanine O6/NH of the *cis* amine H-bond interaction. Such a guanine O6/NH *cis*-amine interaction is stronger for a "quasi axial" than for a "quasi equatorial" NH. As a consequence, at basic pH the CD spectrum of (*S*,*S*)-dachPtG$_2$ has the signature of a ΛHT conformer, while the CD spectrum of (*R*,*R*)-dachPtG$_2$ has the signature of a ΔHT conformer [86].

4. Retro models with tethered guanine bases

Cis-(NH$_3$)$_2$Pt(GpG) and *cis*-(NH$_3$)$_2$Pt(d(GpG)) complexes, the simplest G-linked models of the major cisplatin–DNA adduct, have been characterized by a number of techniques, including CD spectroscopy [50,87], ^{1}H NMR spectroscopy [49,87], and molecular modeling [51]. On the basis of the observation that both nucleotides of each complex were *anti* [87], the cross-links were initially assigned structures in the HH1 conformer class. Studies of retro models have shown that there are three other classes, namely, HH2, ΔHT1, and ΛHT2 (Fig. 10). Members of adjacent classes are separated by rotation of one G base about its Pt–N7 bond. Moreover, the G nucleotides can adopt *syn* or *anti* conformations (for a total of four possible combinations: *syn*/*syn*, *syn*/*anti*, *anti*/*syn*, and *anti*/*anti*), and the bases can have right-handed (R) or left-handed (L) canting. The 32 conceivable forms (4 classes × 4 conformations × 2 cantings), which may not all be in a local minimum and which are therefore referred to as variants, are designated by the 5′-G, then by the 3′-G conformation (e.g. *anti*/*anti* HH1 R).

Cisplatin is a very simple molecule, but the possibility of dynamic motion in cisplatin–DNA adducts complicates spectroscopic analysis. Thus, analogs of cisplatin have been constructed with bulky carrier ligands designed to reduce the dynamic motion. Variants in non-HH1 conformer classes have been found for bipPt(d(GpG)) and bipPt(GpG) adducts [39,46,48]. The stereochemistry of the bip carrier ligand influences the conformer distribution and controls cross-link handedness. (*S,R,R,S*)-bipPt(d(GpG)) and (*S*,*R*,*R*,*S*)-bipPt(GpG) both favored two variants, *anti*/*anti* HH1 L (with N and S puckers for the 5′- and 3′-G residues, respectively) and *anti*/*syn* ΔHT1 L (both sugars having mainly N puckers, Fig. 10) [46,48]. When these complexes were kept at pH 10 for several days, the ΔHT1 L variant became favored over the HH1 L variant, demonstrating that ΔHT1 L is more favorable when N1 is deprotonated [5,46]. The two major conformers for (*R*,*S*,*S*,*R*)-bipPt(d(GpG)) at physiological pH were *anti*/*anti* HH1 R and *anti*/*anti* HH2 R (for both conformers the sugar pucker was N and S for the 5′- and 3′-G, respectively, Fig. 10) [39]. When the sample was kept for 1 day at pH 10, where N1 is not protonated, the ΛHT2 R conformer became ∼30% abundant [46]. In contrast to the derivative with d(GpG), no HH2 R variant was observed for (*R*,*S*,*S*,*R*)-bipPt(GpG) at any pH, and only a small population of ΛHT2 R was observed at pH 10; [46] these results suggested that fewer abundant conformers are present in *cis*-A$_2$Pt(GpG) complexes than in *cis*-A$_2$Pt(d(GpG)) complexes.

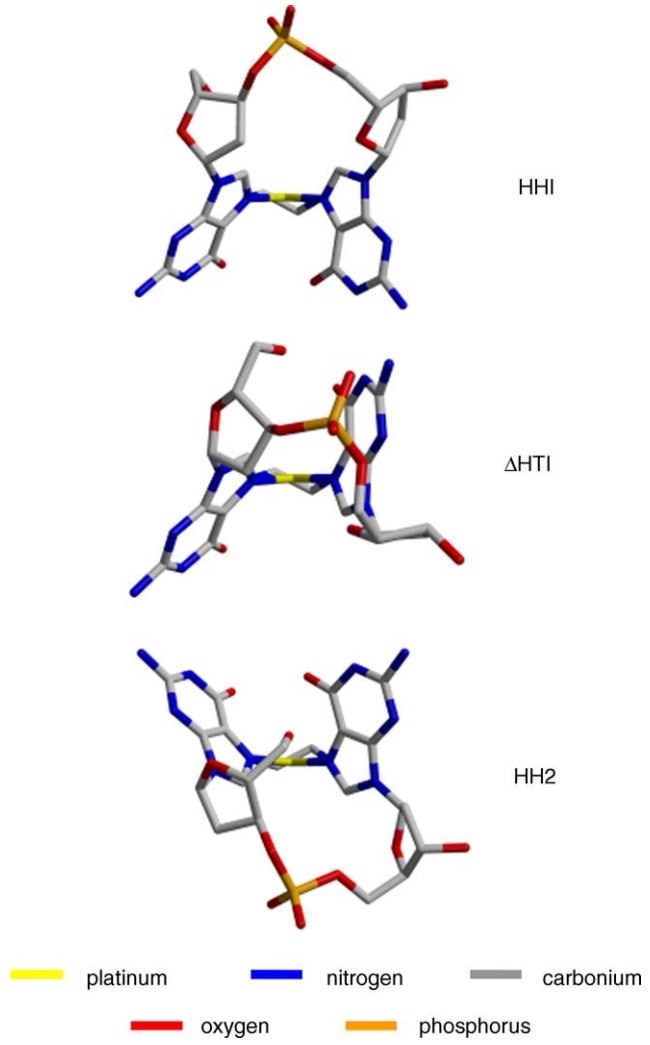

Fig. 10. Minimum energy models (from NMR-restrained MMD calculations) of *anti*/*anti* HH1 (top), *anti*/*syn* ΔHT1 (center), and *anti*/*anti* HH2 (bottom) forms of bipPt(dGpG)) adducts (the conformation of the 5′-G is given first, then that of 3′-G; only the chelate-ring atoms of the bip ligand are shown). The ΛHT2 conformer, having guanine orientation opposite to that of the ΔHT1 conformer, is not formed in significant quantity at acidic and neutral pH's and is not shown (based on data from Refs. [39,48]).

4.1. Comparison between d(GpG) and GpG adducts

(*S*,*R*,*R*,*S*)-bipPt(d(GpG)) and (*S*,*R*,*R*,*S*)-bipPt(GpG) compounds are a mixture of *anti*/*anti* HH1 L and *anti*/*syn* ΔHT1 L variants. The equilibrium ratio of these variants is nearly exactly the same in the two compounds, a result supporting the absence of an H-bonding effect for the 2′-OH group in the L variant [46].

The main difference found between the (*R*,*S*,*S*,*R*)-bipPt(d(GpG)) and the (*R*,*S*,*S*,*R*)-bipPt(GpG) adducts is the absence in the latter case of any variant of the unusual HH2 form. For the GpG derivative the hydrogen bond between the 5′-G 2′-OH and the phosphate group, which is possible for the *anti*/*anti* HH1 R variant but not for the *anti*/*anti* HH2 R variant, appears to stabilize the former conformer with respect to the latter one [46].

The three dominant bipPt(GpG) variants that were found at low pH have very similar spectral features, including the CD signals, as the three corresponding bipPt(d(GpG)) variants. Thus, the observed GpG and d(GpG) variants are structurally very similar.

4.2. Interligand interactions stabilizing different conformers in adducts with tethered nucleotides

Investigations of adducts of retro models with d(GpG) and GpG dinucleotides, not only revealed the dynamic behavior of these intrastrand cross-link models with the possibility for each guanine to undergo ca. 180° rotation about the Pt–N7 bond, but also highlighted the role of non-covalent interactions not unlike those discovered in the investigations with untethered *cis* guanines. In particular, in the HH conformer a key interaction appears to be the repulsion between the electron-rich O6 atoms of the two guanines. Because of such a repulsion, the six-membered ring of each guanine is forced to move farther from the *cis* guanine and closer to the *cis* amine (six-out relationship). If the *cis* amine has an NH on the same side of the platinum coordination plane as the O6 of the guanine, an H-bond could possibly be formed in the HH conformers [39]. The ΔHT1 conformer is a major component in the case of adducts with the (*S*,*R*,*R*,*S*)-bip ligand, which in theory could provide two NH's for H-bond formation with the O6 of the two guanines [48]. The ΛHT2 conformer can also form two guanine O6/NH *cis* amine H-bonds in adducts of (*R*,*S*,*S*,*R*)-bip; however, at acidic pH the ΛHT2 conformer is a minor form compared to the HH1 R and HH2 R (only for the d(GpG) adduct) conformers. The ΛHT2 conformer concentration increases at high pH, and this could be attributed to O6 becoming a better hydrogen bond acceptor when N1 is deprotonated [46]. Although invoking such H-bonding appeared to be reasonable for tethered PtG_2 adducts on the basis of the earliest results with retro models, later examination of adducts with other carrier ligands identified significant amounts of the ΔHT1 conformer, although no H-bonding was possible. Our evidence for G O6 H-bonding in tethered PtG_2 adducts is most clear for the canted G of HH forms.

4.3. Evidence for steric interligand clashes

Steric clashes between amine substituents and O6 of guanine can also influence the stability of a conformer, as found for the HH1 conformer of (*S*,*S*,*S*,*S*)- and (*S*,*S*,*S*,*R*)-Me_2dapPt(GpG) (Me_2dap = *N*,*N*′-dimethyl-2,4-diaminopentane). In general, the N-Me groups of a coordinated Me_2dap ligand prefer axial positions over equatorial positions [68,88]. Furthermore, many of the favored conformations of the Me_2dap ligands have one C-Me group in an axial position, thus the Me_2dap complexes typically possess carrier-ligand bulk that is significantly out of the coordination plane [88]. Such bulk can affect the GpG conformation in Me_2dapPt(GpG) adducts by disfavoring certain variants due to steric interligand clashes [89]. Moreover, in Me_2dapPtG_2 complexes, axial N-Me groups were calculated to affect the barrier to G rotation only slightly [68].

The *anti*/*anti* HH1 R variant was the almost exclusive form for (*R*,*R*,*R*,*R*)- and (*S*,*R*,*R*,*R*)-Me_2dapPt(GpG). In the latter case there are two equally populated linkage isomers having the 5′-G *cis* to the *S* and to the *R* nitrogens, respectively. The linkage isomer with 5′-G *cis* to the *S* nitrogen has the O6 of each guanine on the same side of the platinum coordination plane as the NH of the *cis* amine and sharper signals (an indication that interligand interactions are more favorable). In contrast, the linkage isomer with 5′-G *cis* to the *R* nitrogen has the O6 of each guanine on the same side as the N-Me of the *cis* amine and broader signals, indicating a more dynamic species [89].

For the (*S*,*S*,*S*,*S*)- and (*S*,*S*,*S*,*R*)-Me_2dapPt(GpG) complexes, steric clashes between the Me_2dap C-Me groups and the G O6 atoms appeared to disfavor the HH1 conformer (which is no longer the exclusive form) leading to a significant population of the *anti*/*syn* ΔHT1 conformer, as indicated by broad ^{1}H NMR signals and by ^{31}P NMR and CD data [89].

5. Cisplatin adducts with tethered guanine bases

The *cis*-$(NH_3)_2$Pt(GpG) complex is probably very dynamic, undergoing rapid rotation about the Pt–N7 bonds. The H8 signals of *cis*-$(NH_3)_2$Pt(GpG) are relatively sharp (∼10 and 4 Hz for 5′-G H8 and 3′-G H8, respectively) at 21 °C but broader (∼20 and 5 Hz, respectively) at 5 °C [46]. Therefore, exchange between conformer classes or between variants within a conformer class must be moderately fast at 21 °C and slower at 5 °C. Comparison to spectral data for bipPt(GpG) indicates that *cis*-$(NH_3)_2$Pt(GpG) exists as a mixture of variants from both the ΔHT1 and HH1 conformer classes [46]; thus, the most likely explanation for the broadening is exchange between conformer classes.

Analysis of the NMR shifts and CD signal shapes indicates that at low pH *cis*-$(NH_3)_2$Pt(GpG) exists mostly as the HH1 R conformer, as suggested previously in the literature [51]; however, other conformers account for perhaps one-third of the complex. At high pH, the conformer distribution changes. Although it appears to be reasonable that the HH1 L variant is now a more abundant form, it is not the exclusive form. Other forms likely to be present include the ΔHT1 form [46].

As found for the GpG adduct, *cis*-$(NH_3)_2$Pt(d(GpG)) also exists as a dynamic mixture of various forms of comparable populations, in contrast to the adducts with longer oligonucleotides, which exist mainly as the HH1 L variant [48]. In the solid state, *cis*-$(NH_3)_2$Pt(d(pGpG)) crystallizes with an equal population of HH1 R and HH1 L variants [90]. We believe that a mixture of these two variants also exists in solution. In addition, the computed *anti*/*syn* ΔHT1 model of *cis*-$(NH_3)_2$Pt(d(GpG)) is also structurally very similar to that computed for the analogous bip adduct. Furthermore, we computed that this conformer has one of the lowest energies of the *cis*-$(NH_3)_2$Pt(d(GpG)) models and we have found this form in many adducts containing a wide variety of carrier ligands; therefore, it is most likely that the ΔHT1 form is also part of the dynamic mixture of *cis*-$(NH_3)_2$Pt(d(GpG)) conformers.

5.1. Cisplatin adducts with extended oligonucleotides

On the basis of the unusual ^{31}P NMR signals observed in the LPt–d(TCTCGGTCTC) adducts (L = *cis*-$(NH_3)_2$ or en), it was concluded that this G/G cross-linked single-strand is a distorted coil with the 5′ residues "wrapped around" the Pt binding site [91]. The 5′-region distortion is probably caused both by electrostatic interactions between the negatively charged nucleotide sequence flanking the cross-link and the cationic $[cis\text{-}(NH_3)_2Pt]^{2+}$ or $[enPt]^{2+}$ moieties and by hydrogen bond formation between the phosphate and the Pt–NH groups; the resulting local distortion at phosphodiester groups explains the rather unusual ^{31}P NMR shifts [92]. Such "wrapping" of the cationic $[cis\text{-}(NH_3)_2Pt]^{2+}$ moiety by flanking residues results in more stable coils and in local "melted" regions of longer DNA, thereby decreasing the T_m of DNA [92,93].

Treatment of the self-complementary dodecamer $d(A_1T_2G_3G_4G_5T_6A_7C_8C_9C_{10}A_{11}T_{12})$ (G3) under conditions in which G3 is in the duplex (DS) form with cisplatin or $enPtCl_2$ gives single-stranded (SS) products: G_3/G_4 adducts in a coil form and G_4/G_5 adducts in a hairpin form stabilized by a favorable A_7–G_4 stacking interaction at the loop–stem junction (Fig. 11) [94–97].

The "wrapped" SS coil form becomes less stable in bipPt–G3 and Me_2dabPt–G3 adducts because the hydrophobic and bulky bip and Me_2dab carrier ligands prevent the flanking regions of the negative DNA strand from approaching the cationic Pt center as closely as these regions can approach the Pt in adducts with less hindered bisammine or primary diamine ligands. These unfavorable effects involving the bip and Me_2dab ligands do not occur in the duplex form; therefore, the bipPt–G3 and Me_2dabPt–G3 adducts with the cross-link in an HH1 conformation favor the duplex form (Fig. 11) [98].

In the case of (*S*,*R*,*R*,*S*)-Me_2dabPt–G3 both the G_3/G_4 and G_4/G_5 duplexes appear to exist in part in a single-strand (most likely coil) form. The facts that the *S*,*R*,*R*,*S* chirality favors L canting and that this canting is characteristic of coils suggest a reasonable explanation for this result. In the case of the more bulky (*S*,*R*,*R*,*S*)-bip ligand, the balance between canting effect favoring the coil form, and bulk effect favoring the duplex form is still in favor of the duplex form [98]. This investigation demonstrates that the conformation and annealing propensity of SS oligonucleotides with G/G intrastrand cross-links can be modulated by the stereochemistry of the platinum carrier ligands.

6. Dynamic behavior of cisplatin adducts with double-stranded oligonucleotides

Single-stranded N7–Pt–N7 cross-links generally have spectroscopic characteristics of an HH1 L variant [50,51,66,99,100]. In duplexes with *cis*-$(NH_3)_2$Pt-generated cross-links, the HH1 R variant appears to dominate [51]; however, NMR spectra contain features consistent with a high fluxional character, which could be due to rapid equilibria involving other variants [9]. It is of some interest that no two NMR studies on duplexes have led to the same proposed structure [9,16,17,101,102] and that the diverse proposed solution structures generally lacked the features recently found in the X-ray structure of a 16-mer bound to rat HMG1 [103]. These variations in structure may be caused by spectral complications arising from dynamic motion centered at the cross-link. For instance, the dihedral angle between cross-

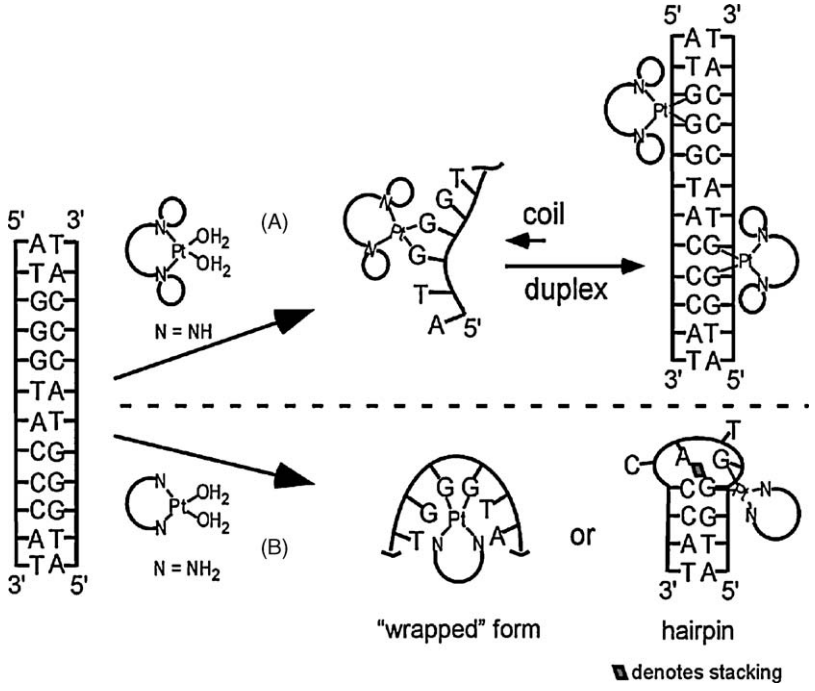

Fig. 11. Schematic representation of the reaction of G3: (A) platinum complexes with secondary nitrogen donors having steric bulk (small loops close to N). (B) Platinum compounds without steric bulk on nitrogen atoms (Ref. [98] for related work).

linked guanines has been found to span all values between 25° and 75°. However, in a relatively recent study of a 9-mer duplex, an NMR-based structural model with many of the elements of the X-ray structure of the 16 mer bound to rat HMG1 was reported [10], and this 9-mer has many spectral features common to duplexes with a pyrimidine–G–G–pyrimidine sequence.

If, as appears to be the case, the structure of cross-linked double-stranded oligonucleotides is far from being rigid, it is likely that the conformation is deeply influenced by non-covalent interactions which are difficult to detect, but which can result in a different biological activity. This appears to be the case for oxaliplatin, [Pt(oxalato)(dach)] (dach = 1,2-diaminocyclohexane), for which only the enantiomer with (*R*,*R*)-dach has been approved for clinical use [5,6]. We also investigated the mutagenic activity of several platinum compounds with chiral diamines and found that the *S*,*S* enantiomer was a far greater mutagenic agent than the *R*,*R* form, particularly in the case of dach and dab adducts (dab = 2,3-diaminobutane) [104].

6.1. Different processing of DNA cross-links with enantiomeric platinum drugs

Various damaged-DNA binding proteins, such as those containing the High-Mobility Group (HMG) domain, are attracted to DNA containing platinum adducts that induce stable directional bending and unwinding [103,105–107]. The binding of these proteins has been postulated to mediate the antitumor properties of the platinum drugs [103,107,108]. In addition, several reports have demonstrated that intrastrand CL's of cisplatin and its direct analogs are removed from DNA during Nucleotide Excision Repair (NER) reactions and that NER is also an important mechanism contributing to cisplatin resistance [109–111].

We investigated how HMG box proteins and the NER differentiate between major DNA adducts of cisplatin analogs having enantiomeric non-leaving ligands in in vitro reactions [112,113]. Electrophoretic mobility shift assays show that domains A and B of HMGB1 protein bind to (*R*,*R*)-dabPt-generated cross-links with a higher affinity than to those generated by (*S*,*S*)-dabPt. The cross-links of both enantiomers are removed by NER with a similar efficiency; however, HMGB1 protein significantly inhibits removal of the (*R*,*R*)-dabPt adduct, but not that of the *S*,*S* enantiomer. Thus, HMG domain proteins discriminate among different conformations of the G/G intrastrand cross-links of the two enantiomeric analogs of cisplatin, which results in different NER of these cross-links (Fig. 12) [113]. An error-prone repair of damaged DNA could account for the far greater mutagenic activity of the (*S*,*S*)-dab-Pt complex, while a greater inhibition of repair of damaged DNA could explain the better antitumor activity of the *R*,*R* enantiomer.

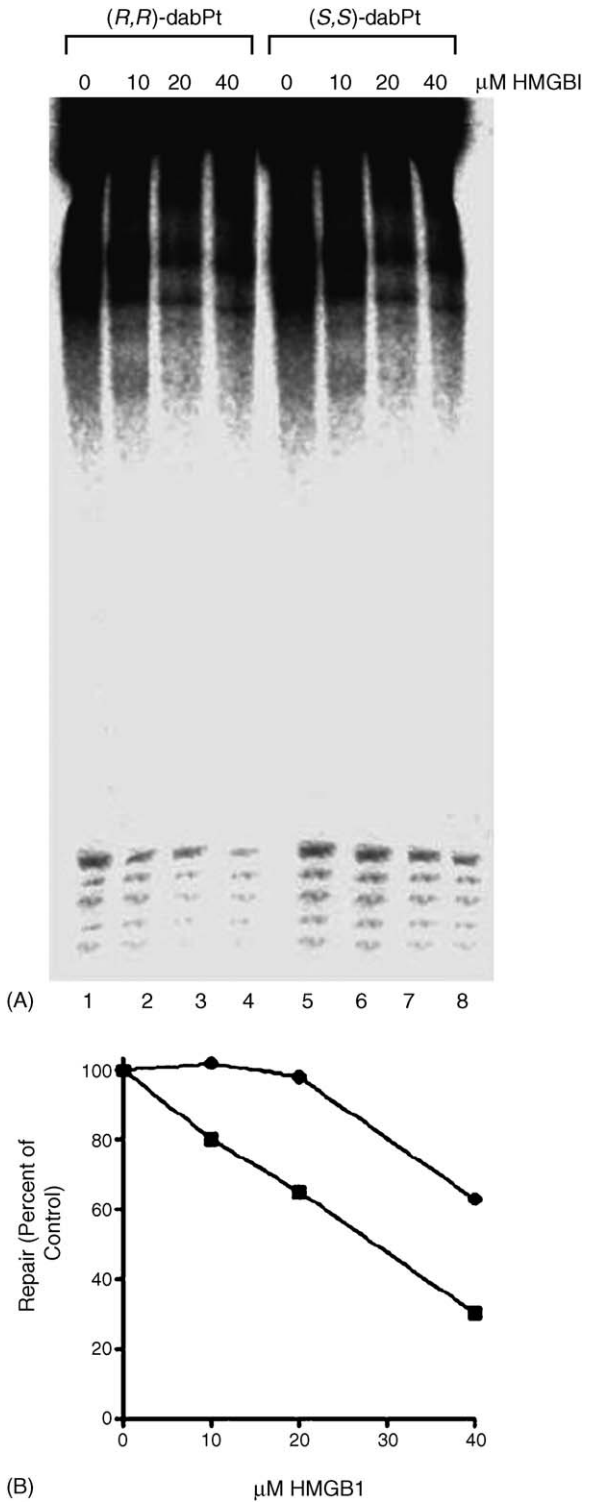

Fig. 12. (A and B) Effect of HMGB1 on NER of the G/G intrastrand CL of (*R*,*R*)-dabPt (■) or (*S*,*S*)-dabPt (●) adducts by rodent excinuclease (Ref. [113] for related work).

6.2. Conformational differences in DNA cross-links of enantiomeric platinum compounds

The details of the non-covalent interactions leading to a different conformation of the cross-link in DNA adducts of enantiomeric dach-Pt compounds are not completely understood. The crystal structure of a DNA dodeca-nucleotide duplex with a G/G intrastrand CL formed by oxaliplatin has been reported to have an overall geometry similar to that of the cisplatin analog; this structure of the oxaliplatin adduct shows hydrogen bonding between a pseudoequatorial NH group in the (*R*,*R*)-dach ligand and the O6 atom of the 3′-G of the CL [13,14].

Such an H-bond has not been confirmed in a subsequent NMR investigation which did not reveal the downfield shift and the sharpening of the NH proton signal expected for such an interaction in a rigid stereochemistry [114].

A theoretical investigation has disclosed the possibility that the different conformation of double helical DNA containing cisplatin analogs with enantiomeric (*R*,*R*)- and (*S*,*S*)-dab might stem from steric clashes between a C-Me of the dab ligand and a thymine adjacent to the cross-linked 5′-guanine [115]. The latter proposal does not envisage differences between the two enantiomers in different sequence contexts [116].

We addressed the question of conformational differences in DNA adducts of enantiomeric dab-platinum compounds, with several biophysical techniques comprising the use of chemical probes [112]. The results obtained with the dabPtCl_2 complex (the first we have investigated) have been rather exciting. It was found that the G/G intrastrand CL's of (*R*,*R*)- and (*S*,*S*)-dabPt not only destabilize DNA differently but also bend and unwind DNA to a different extent. In particular, chemical probes indicated that the distortion is more symmetrical and centered on the cross-link in the case of (*R*,*R*)-dab, while it is less symmetric and shifted on the 5′-side of the platinated strand in the case of (*S*,*S*)-dab.

7. Conclusions and perspectives

In general, adducts of cisplatin (and cisplatin analogs) with nucleotides and SS and DS oligonucleotides are highly dynamic systems that are difficult to investigate with conventional techniques. In most cases intermolecular interactions will affect the solid-state conformation; solution data are generally interpreted on the basis of a single model even when the system is likely to be a mixture of rapidly interconverting forms. Retro models have proven to be particularly useful in unraveling the existence of different conformations, and in the most favorable cases have also allowed the full characterization of their stereochemistry. Moreover, several interligand interactions, some of which had remained unrecognized for a long time, have been revealed and a hierarchy among them has been established.

Our analysis, based on solution results with adducts containing specially designed carrier ligands, has allowed us to rationalize computational, solution-state, and solid-state results on *cis*-$(NH_3)_2$Pt cross-link adducts in a more consistent manner than do previous interpretations based on the *cis*-$(NH_3)_2$Pt adducts themselves. This success verifies the value of the retro modeling approach. In addition, by using retro models we found dramatic differences in the relative stability of different forms of oligonucleotides (coil, hairpin, and duplex) dependent on the carrier ligand.

We believe that in the future the use of retro models for the stabilization of single conformers and the characterization of their stereochemistry will continue to play an important role in the interpretation of experimental data pertaining to dynamic systems and in the validation of results coming from theoretical investigations.

Acknowledgments

The authors are grateful for long and enduring support from NIH (GM 29222), the Italian Ministry for Education and Research (MIUR) and the University of Bari. The work described herein was conducted as part of an extensive series of studies with many of our co-workers and several collaborators over a number of years. Without the participation of these many talented individuals, the progress we have reported would not have been possible and we are most grateful to all of them. We are most thankful to Dr. Patricia Marzilli who has long played a role in this area and to Dr. Francesco Cannito, who provided valuable assistance in the preparation of the manuscript.

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