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Rotamer Stability in *cis*-[Pt(diA)G_2] Complexes (diA = Diamine Derivative and G = Guanine Derivative) Mediated by Carrier-Ligand Amine Stereochemistry as Revealed by Circular Dichroism Spectroscopy

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Abstract: Extensive investigations of *cis*-[Pt(diA)G_2] complexes (in which G = a guanine ligand; diA = a single diamine ligand) revealed the types of interactions between the two G ligands and between the G and the *cis*-amine substituents when diA is a diamine ligand with substituents on each nitrogen atom being a small hydrogen atom and a bulky group able to slow the rotation about the Pt–G bond. All these interactions are shown to apply also when diA = dach (1,2-diaminocyclohexane), even though this chiral primary diamine has only small N–H atoms on each side of the coordination plane. However, a slight difference in the stereochemistry of the two protons (one N–H has "quasi axial" and the other "quasi equatorial" character) is sufficient to induce a significant change in the relative stabilities of the [Pt(dach)G_2] ΔHT and ΛHT rotamers (HT = head-to-tail). The new results show that at acidic and neutral pH the induction of asymmetry from the dach ligand to the HT rotamers is governed by the G-to-G dipole–dipole interaction, which is greater for the six-membered ring of each guanine leaning towards the *cis*-G. Such a "six-in" canting of the two guanine ligands 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 coordination plane. Such a repulsion is greater for a "quasi equatorial" N–H than for a "quasi axial" N–H. Under basic pH conditions, deprotonation of the guanine N1–H renders the O6 atom a much better hydrogen-bond acceptor; therefore, the stability of the HT rotamers is governed by the hydrogen-bond interaction of guanine O6 and the *cis*-amine N–H group. Such a guanine O6/N–H *cis*-amine interaction is stronger for a "quasi axial" than for a "quasi equatorial" N–H group. In the head-to-head (HH) rotamer, in which the electrostatic repulsion between electron-rich O6 atoms, both on the same side of the platinum coordination plane, tends to place the six-membered rings of each guanine further from the *cis*-guanine and closer to the *cis*-amine, we can expect better N–H···O6 hydrogen bonding for the "quasi equatorial" N–H groups.

Keywords: antitumor agents · atropisomerism · circular dichroism · hydrogen bonds · platinum

Introduction

Rosenberg's serendipitous discovery of the antitumor activity of cisplatin (*cis*-diamminedichloroplatinum(II))[1–3] was a breakthrough in the chemotherapy of tumors. This highly effective drug for the treatment of testicular and ovarian cancers is also beneficial in association with other antitumor drugs in the treatment of many other types of tumors.[4]

Thousands of platinum compounds have been synthesized and tested for antitumor activity in the attempt to circumvent the acquired or intrinsic resistance to cisplatin in several tumors. Dozens of new platinum drugs have been used in human clinical trials,[5] but only carboplatin [*cis*-diammine(1,1-cyclobutanedicarboxylato-*O*,*O*′)platinum(II)], which is active in the same range of tumors as cisplatin, has achieved world-wide routine clinical use because of its lower

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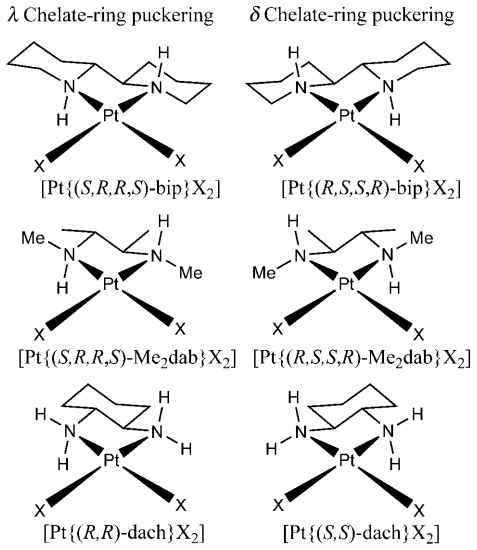
toxicity.[5] More recently two other platinum compounds have received approval for use in several countries: nedaplatin [*cis*-diammine(glycolato-*O*,*O*′)platinum(II), or 254-S][5] and oxaliplatin [(*R*,*R*)-1,2-diaminocyclohexane(oxalato-*O*,*O*′)platinum(II), or L-OHP].[5,6] The latter has been used in the secondary treatment of metastatic colorectal cancer.[5,6]

DNA remains the ultimate target for cisplatin, which forms adducts mainly with the N7 of adjacent purines.[6–10] The fact that the activity of *cis*-$[PtA_2X_2]$ compounds (in which A = NH_3, RNH_2, R_2NH, and R_3N; and X = anionic ligand)[11] appears to correlate with the number of amine hydrogen atoms led to speculation about hydrogen-bond formation between the amine N–H groups and a nucleotide phosphate or a guanine O6 atom of the cross-link. Structural investigations of platinated oligomers containing cisplatin[12] or oxaliplatin[13] suggest that hydrogen bonds involving the cisplatin NH_3 groups are weak and perhaps not important, and that the low steric hindrance of the carrier ligand, rather than its ability to form hydrogen bonds, could be the main factor influencing the antitumor activity of *cis*-$[PtA_2X_2]$ compounds.[10]

Although the role of N–H groups is unclear, small changes in the carrier ligand(s) can lead to significant differences in biological activity. For example, the *trans*-1,2-diaminocyclohexane (dach) ligand in oxaliplatin, which was recently approved for the second-line treatment of colorectal tumors in combination with 5-fluorouracil,[5] has two enantiomers. Different *trans*-lesion synthesis and nucleotide-excision repair of DNA as well as a very large difference (ten times or more) in mutagenic activity have been found for the platinum complexes with the two enantiomers.[14,15]

In an early investigation of the reaction of enantiomeric dach–platinum complexes with guanine derivatives,[16] Pasini and co-workers observed that the circular dichroism (CD) spectrum of [Pt{(*R*,*R*)-dach}G_2] (in which G = 9-methylguanine) was characterized by positive Cotton effect bands centered at 230 and 280 nm and a negative Cotton effect band centered at 260 nm; the corresponding Cotton effect bands for the [Pt{(*S*,*S*)-dach}G_2] enantiomer have the opposite sign. The Cotton effect, assigned to coupling between π–π* electronic transitions centered on the guanine bases, was taken as a clear indication of transmission of chirality from the dach ligand to the coordinated *cis*-guanine groups mediated by the amine protons.[16] The details of the chirality transmission mode given at that time have remained unclear up to now. Meanwhile, very detailed investigations have been carried out on less dynamic *cis*-[Pt(diA)G_2] compounds [diA = chiral diamines of C_2 symmetry with *N*-substituents of different bulk on the two sides of the coordination plane; e.g., *N*,*N*′-dimethyl-2,3-diaminobutane (Me_2dab)[17,18] and 2,2′-bipiperidine (bip);[19,20] these ligands were named chirality controlling chelates, CCC, Scheme 1]. These studies allowed us to establish a correlation between the Cotton effects at long wavelengths (≥245 nm) and the chirality of the dominant head-to-tail (HT) rotamer as revealed by the NMR spectra. In particular, the spectral pattern of a positive Cotton effect around 255 nm and a negative Cotton effect around 290 nm is characteristic of a *Δ*HT rotamer; the opposite pattern is characteristic of a *Λ*HT rotamer.[19]



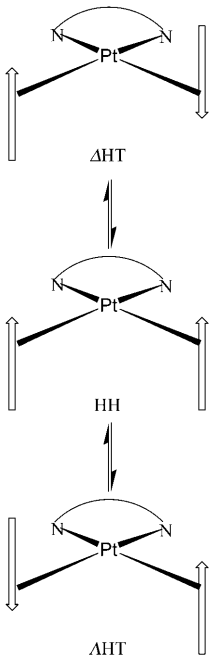
Scheme 1. Schematic representation of bip, Me_2dab, and dach ligands coordinated to a platinum(II) moiety.

Moreover, these investigations revealed different types of interligand interactions that influence the stability of different rotamers. These interactions have been grouped into three classes: FFC, SSC and FSC.[21–23] FFC (first-to-first sphere communication) involves internucleotide and nucleotide–amine interactions close to the metal center (e.g., the dipole–dipole interaction between *cis*-guanines, and hydrogen-bond formation between the guanine O6 atom and N–H of the *cis*-amine). SSC (second-to-second sphere communication) involves interactions far from the metal center and between *cis*-nucleotides (i.e., the hydrogen-bond interaction between the phosphate of a nucleotide and the N1–H group of the *cis*-guanine). Finally, FSC (first-to-second sphere communication) involves interactions between residues, one far from (nucleotide) and the other close to (*cis*-amine) the metal center (i.e., the hydrogen-bond interaction between the 5′-phosphate of a nucleotide and the N–H group of the *cis*-amine).[23]

We have focused on highly dynamic [Pt(dach)G_2] complexes. NMR spectroscopy is not useful for elucidating the interligand interactions due to fast interconversion between rotamers.[24] However, CD spectroscopy allowed us to determine the conformation of the dominant rotamer at different pH values. From this information we were able to assess the relative strength of different interligand interactions (FFC, SSC, and FSC) and, in turn, to elucidate how amine protons can mediate the induction of chirality from the dach backbone to the coordinated *cis*-nucleotides.

Results

Platinum adducts with two *cis*-guanine ligands and a bidentate carrier ligand of C_2 or higher symmetry, can form three different rotamers: *Δ*HT, *Λ*HT and HH (Scheme 2; HT = head-to-tail, HH = head-to-head). If the interconversion between rotamers is fast on the NMR timescale, only one set of signals, which is the time average of the signals of the three rotamers, is observed. Under these circumstances, CD spectroscopy can be used to study the equilibria between rotamers.[21,22,24] At longer wavelengths (≥245 nm) the major contribution to the CD is given by coupling between π–π* electron transitions of the nucleobases, while the contributions of the carrier ligand and the sugar substituents (in the case of nucleosides and nucleotides) are negligible.[25] Of the three possible rotamers, only the two HT rotamers exhibit strong Cotton effects, because the two HT rotamers are intrinsically chiral, whereas the HH rotamer is intrinsically achiral due to a symmetry plane between the two *cis*-coordinated purines. In the last case, a source of chirality can be introduced by canting (either left-handed, L, or right-handed, R) of the two guanines, and inversion of canting will result in inversion of the CD signal. However, the canting effect is second order, and CD spectra of HH rotamers are generally much less intense than those of HT rotamers.[26–28]



Scheme 2. Schematic representation of HH, *Δ*HT, and *Λ*HT atropisomers for *cis*-$[PtA_2X_2]$ complexes.

$[Pt(dach)(9\text{-EtG})_2]^{2+}$: We used ^{1}H NMR spectroscopy to monitor the formation of the bisadduct starting from $[Pt\{(R,R)\text{-dach}\}(D_2O)_2]^{2+}$ and 9-EtG (D_2O, molar ratio 1:2, pH 3.0, at 22 °C). Soon after mixing, two new H8 spectral peaks were observed: one signal at $\delta = 8.25$ ppm is assigned to the monoadduct, $[Pt\{(R,R)\text{-dach}\}(D_2O)(9\text{-EtG})]^{2+}$, while the other signal at $\delta = 8.03$ ppm is assigned to the bisadduct, $[Pt\{(R,R)\text{-dach}\}(9\text{-EtG})_2]^{2+}$. During the reaction there was a steady decrease of the H8 spectral peak of free 9-EtG and an increase of the H8 peak of the bisadduct. The intensity of the H8 peak assigned to the monoadduct first increased, and then decreased to zero.

The CD spectrum of the final solution shows positive Cotton effects at 230 and 287 nm and negative Cotton effects at 208 and 255 nm; the shape of the CD bands at 255 and 287 nm is a clear indication that the *Λ*HT conformer is dominant at pH 3.0. As the pH increases, the intensities of the CD bands at 255 and 287 nm decrease, reach zero, and above pH 7.5 invert sign. These results indicate that as pH increases, the *Λ*HT rotamer, initially more abundant, decreases in concentration and becomes less abundant at basic pH than the *Δ*HT rotamer (Figure 1a).

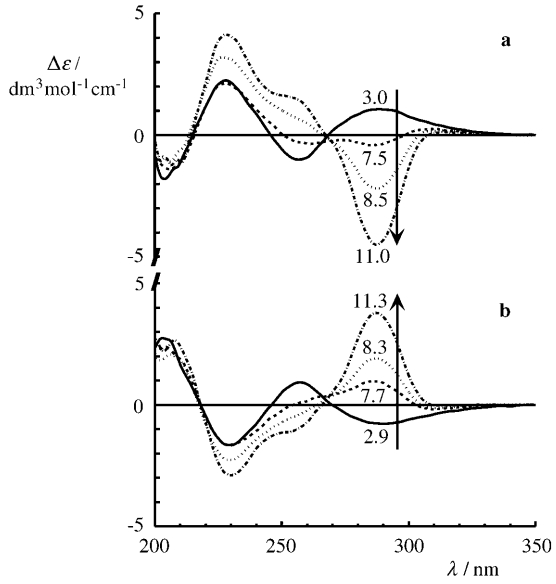


Figure 1. CD spectra conducted in H_2O. a) $[Pt\{(R,R)\text{-dach}\}(9\text{-EtG})_2]^{2+}$ and b) $[Pt\{(S,S)\text{-dach}\}(9\text{-EtG})_2]^{2+}$, at different pH values.

The $[Pt\{(S,S)\text{-dach}\}(9\text{-EtG})_2]^{2+}$ formation reaction, followed by NMR spectroscopy, shows exactly the same time course as that observed for the formation of the enantiomeric complex, $[Pt\{(R,R)\text{-dach}\}(9\text{-EtG})_2]^{2+}$. As expected, the CD spectra of $[Pt\{(S,S)\text{-dach}\}(9\text{-EtG})_2]^{2+}$ acquired at different pH values show the opposite trend to that observed for the $[Pt\{(R,R)\text{-dach}\}(9\text{-EtG})_2]^{2+}$ complex (Figure 1b), in accord with an opposite trend in the stability of the HT rotamers (*Δ*HT favored at acidic pH and *Λ*HT favored at basic pH).

$[Pt(dach)(3'\text{-GMP})_2]$: The formation of the bisadduct starting from $[Pt\{(R,R)\text{-dach}\}(D_2O)_2]^{2+}$ and 3′-GMP (D_2O, molar ratio 1:2, pH 3.0, at 22 °C) was also monitored by NMR spectroscopy. As in the case of 9-EtG, two new H8 peaks

became evident soon after mixing. The signal at $\delta =$ 8.60 ppm is assigned to the monoadduct, [Pt{(*R*,*R*)-dach}-(D_2O)(3′-GMP)]$^+$, and another signal at $\delta = 8.38$ ppm is assigned to the bisadduct, [Pt{(*R*,*R*)-dach}(3′-GMP)$_2$]. During the course of the reaction there is a steady decrease of the H8 peak of free 3′-GMP and a steady increase of the H8 peak of the bisadduct. The intensity of the H8 peak of the monoadduct first increases and then decreases to zero.

The CD spectrum of the final solution shows negative Cotton effects at 230 and 295 nm and positive Cotton effects at 210 and 255 nm. The shape of the CD bands at 255 and 295 nm indicates that the *Δ*HT conformer is more abundant at pH 3.0. Increasing the pH to 7.5 resulted in a steady increase of the Cotton effect band intensities. Above pH 7.5 the intensities of the CD bands decreased and approached zero at pH 11.5. Thus, the concentration of the *Δ*HT conformer, which is greater than that of the *Λ*HT rotamer at pH 3, further increases on raising the pH to 7.5. At higher pH the *Δ*HT concentration starts to decrease, and a pseudoracemic situation is reached at pH 11.5 (Figure 2).

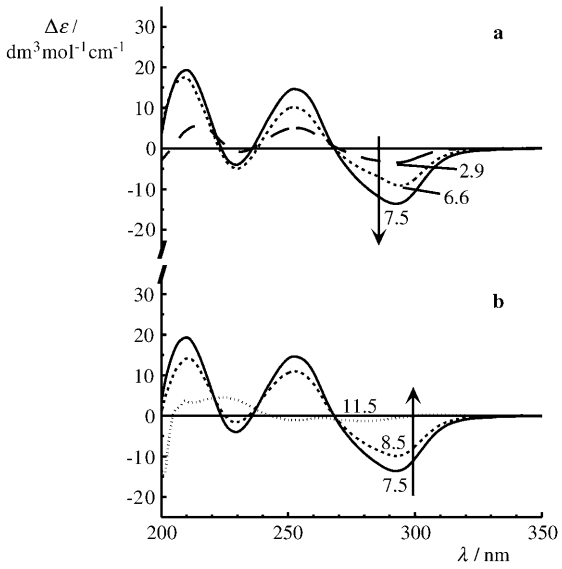


Figure 2. CD spectra conducted in H_2O of [Pt{(*R*,*R*)-dach}(3′-GMP)$_2$] at pH values in the range a) 2.9–7.5 and b) 7.5–11.5.

In the case of [Pt{(*S*,*S*)-dach}(3′-GMP)$_2$] the formation reaction, followed by NMR spectroscopy, evolves in a similar manner to that observed for the diastereoisomeric complex [Pt{(*R*,*R*)-dach}(3′-GMP)$_2$] (the H8 signal at $\delta = 8.53$ ppm for [Pt{(*S*,*S*)-dach}(D_2O)(3′-GMP)]$^+$ and at $\delta = 8.38$ ppm for [Pt{(*S*,*S*)-dach}(3′-GMP)$_2$]).

The CD spectrum of the final solution of the bisadduct shows negative Cotton effects at 230 and 290 nm and positive Cotton effects at 208 and 255 nm. The positive band at 255 nm and the negative band at 290 nm are typical of a *Δ*HT rotamer. As we increased the pH from 3.0 to 7.3, the intensities of the CD bands also increased. However at higher pH the intensities start to decrease, and at pH 9.5 we observed an inversion of the sign of the bands at 255 and 290 nm. It can be concluded that the *Δ*HT conformer, dominant at acidic pH, increases in concentration on raising the pH from 3.0 to 7.3. At higher pH the *Λ*HT rotamer gains stability and becomes dominant at pH > 10.0 (Figure 3).

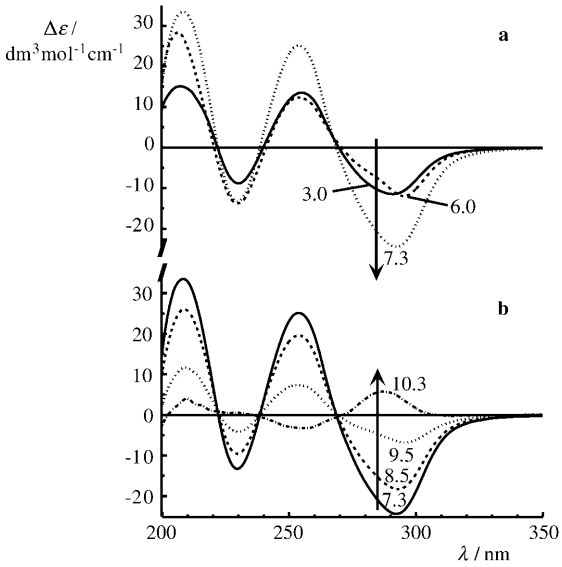


Figure 3. CD spectra in H_2O of [Pt{(*S*,*S*)-dach}(3′-GMP)$_2$] at pH values in the range a) 3.0–7.3 and b) 7.3–10.3.

[Pt(dach)(5′-GMP)$_2$]: The bisadduct [Pt{(*R*,*R*)-dach}(5′-GMP)$_2$] was obtained by reaction of [Pt{(*R*,*R*)-dach}-(D_2O)$_2$]$^{2+}$ and 5′-GMP (D_2O, molar ratio 1:2, pH 3.0, at 22 °C). Two new H8 peaks are evident after mixing; we assigned the signal at $\delta = 8.60$ ppm to the monoadduct [Pt-{(*R*,*R*)-dach}(D_2O)(5′-GMP)]$^+$ and the other signal at $\delta =$ 8.46 ppm to the bisadduct [Pt{(*R*,*R*)-dach}(5′-GMP)$_2$]. As the reaction proceeds, the H8 peak of free 5′-GMP decreases while the H8 peak of the bisadduct increases. The intensity of the H8 peak of the monoadduct first grows and then decreases to zero.

The CD spectrum of the final solution shows positive Cotton effects at 230 and 290 nm and negative Cotton effects at 208 and 255 nm. The negative band at 255 nm and the positive band at 290 nm are characteristic of a *Λ*HT rotamer. As the pH is increased, the absolute intensities of the CD bands first increase, reaching a maximum at pH 7.5, then start to decrease, and at pH > 10.0 the intensity of the signal at 290 nm is practically zero (Figure 4). The *Λ*HT conformer of the bisadduct can be concluded to be slightly more abundant than the *Δ*HT rotamer at pH 3.0. The concentration of the *Λ*HT rotamer further increases as the pH is increased and reaches a maximum value at neutral pH. At basic pH the *Δ*HT rotamer gains stability, and a pseudoracemic situation is reached at pH > 10.0.

The formation reaction of [Pt{(*S*,*S*)-dach}(5′-GMP)$_2$], monitored by NMR spectroscopy, is similar to that observed for the diastereoisomeric complex, [Pt{(*R*,*R*)-dach}(5′-GMP)$_2$] (as evidenced by the H8 signal at $\delta = 8.63$ ppm for

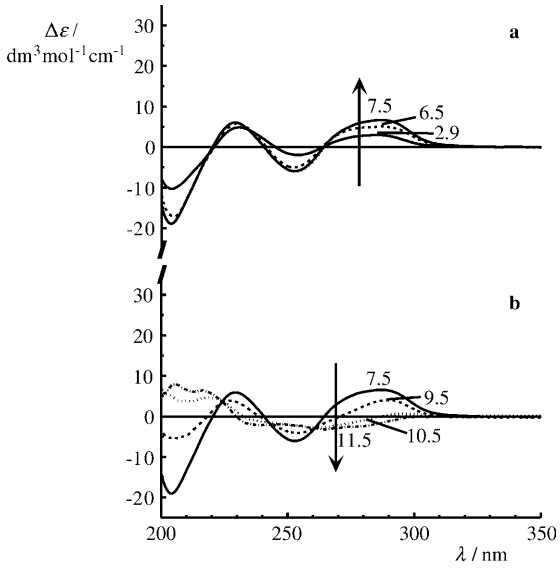


Figure 4. CD spectra conducted in H_2O of [Pt{(*R*,*R*)-dach}(5′-GMP)$_2$] at pH values in the range a) 2.9–7.5 and b) 7.5–11.5.

[Pt{(*S*,*S*)-dach}(D_2O)(5′-GMP)]$^+$, and at $\delta = 8.52$ ppm for [Pt{(*S*,*S*)-dach}(5′-GMP)$_2$]).

The CD spectrum of the reaction solution shows a positive Cotton effect at 260 nm and a negative Cotton effect at 295 nm, indicative of a dominant *Δ*HT rotamer. On increasing the pH the Cotton band intensities decreased, and at pH 5.0 the intensity of the lowest energy band at 290 nm tended towards zero (Figure 5). Further increase in pH did not cause significant changes in the spectral region above 280 nm. The *Δ*HT conformer of the bisadduct appears to be slightly more abundant than the *Λ*HT conformer at pH 3.0. A pseudoracemic situation between HT rotamers is attained above pH 5.0.

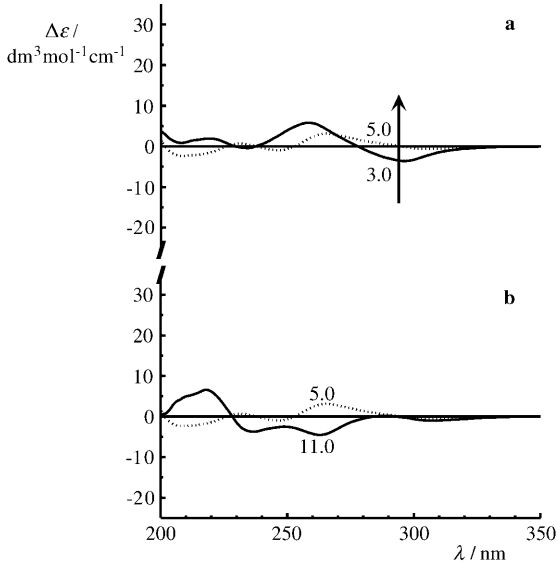


Figure 5. CD spectra conducted in H_2O of [Pt{(*S*,*S*)-dach}(5′-GMP)$_2$] at pH values in the range a) 3.0–5.0 and b) 5.0–11.0.

Discussion

9-EtG complexes: For the [Pt{(*R*,*R*)-dach}(9-EtG)$_2$]$^{2+}$ complex, the *Λ*HT rotamer dominates at acidic pH, while the *Δ*HT rotamer dominates at basic pH. The opposite trend is observed for the [Pt{(*S*,*S*)-dach}(9-EtG)$_2$]$^{2+}$ complex. The same relationships were observed for the [Pt(CCC)(9-EtG)$_2$]$^{2+}$ complexes with *R*,*R* and *S*,*S* configurations at the asymmetric carbon centers, respectively (in which CCC = bip or Me_2dab).[20] However the intensities of the Cotton effect bands revealed that the abundance of the dominant rotamer was much greater (approximately ten times) for CCC complexes than for the dach complexes considered here.

Because the 9-EtG ligand lacks a phosphate group, only two types of interligand interactions can take place. One is interbase dipole–dipole interaction (the positively charged H8 of each guanine is close to the negatively charged O6 of the *cis*-guanine). This interaction favors a canted HT conformation in which the six-membered ring of each guanine is leaning towards the *cis*-guanine ("six-in" canting).[23,25] The other possible interaction is hydrogen-bond formation between the O6 atom of a guanine and an N–H group of the *cis*-amine located on the same side of the platinum coordination plane. The latter interaction favors a "six-out" canting of the guanine (the six-membered ring of the guanine leaning towards the *cis*-amine). Both interactions were classified as FFC because in both cases the two interacting groups are close to the platinum center. To distinguish between the two types of interactions, we will use the abbreviations FFC_a and FFC_b, respectively (Figure 6).

The extensive investigations of compounds with CCC ligands led to the conclusion that FFC_a dominates at acidic and neutral pH. Moreover, the strength of FFC_a depends upon the degree of "six-in" base canting,[29] which can be reduced by steric interaction between the H8 terminus of each G and substituents on the *cis*-amine.[23] Thus, for an *S*,*R*,*R*,*S*

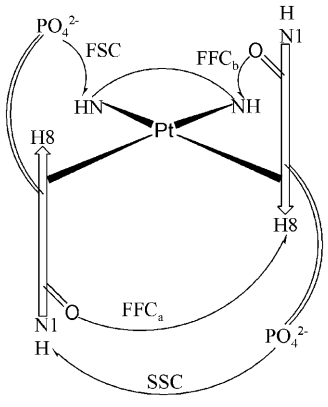


Figure 6. Schematic drawing of possible internucleotide and nucleotide *cis*-amine interactions (FFC_a, FFC_b, SSC, and FSC). Because of the C_2 symmetry of the HT rotamers, each interaction occurs twice; however, for clarity, each is represented only once. In this figure we do not take into account the chirality of the HT rotamer (*Λ* or *Δ*), the position of the phosphate (3′ or 5′), or the number of protons on the diamine N-donors.

configuration of bip or Me_2dab (λ-puckering of the diamine chelate ring) the ΛHT rotamer, having an H8 on the side of the N−H of the *cis*-amine, is favored over the ΔHT rotamer, having H8 on the side of the alkyl substituent of the *cis*-amine. Conversely, for an *R*,*S*,*S*,*R* configuration of the CCC ligand, FFC_a stabilizes the ΔHT over the ΛHT rotamer.

FFC_b was found to dominate at basic pH as a consequence of deprotonation of the N1−H of the guanine base.[23] Such a deprotonation greatly increases the nucleophilicity of the guanine O6 atom, which becomes a good acceptor of a hydrogen bond from an N−H of the *cis*-amine ligand. Consequently, at basic pH the rotamer in which the O6 atom of each guanine is on the same side of the N−H of the *cis*-amine ligand with respect to the platinum coordination plane becomes more stable. Therefore, FFC_b stabilizes the ΔHT conformer for the *S*,*R*,*R*,*S* configuration of CCC ligands, and the ΛHT conformer for the *R*,*S*,*S*,*R* configuration of CCC ligands.

Because FFC_a (dominating at acidic and neutral pH) favors the rotamer with H8 on the same side of the coordination plane as the N−H of the *cis*-amine, while FFC_b (dominating at basic pH) favors the rotamer with the O6 atom of each guanine on the same side of the coordination plane as the N−H of the *cis*-amine, a change in the conformation of the dominating HT rotamer (Δ or Λ) is observed on going from acidic and neutral pH to basic pH for the $[Pt(CCC)(9\text{-}EtG)_2]^{2+}$ complexes.

In the $[Pt(dach)(9\text{-}EtG)_2]^{2+}$ complexes the diamine chelate ligand has N−H protons on both sides of the coordination plane albeit one "quasi axial" and the other "quasi equatorial" (bottom of Scheme 1); therefore, we did not expect a behavior similar to that of CCC ligands with N donors with an N−H group on one side and an *N*-alkyl group on the other side of the platinum coordination plane. However, the behavior at acidic pH indicates that the HT conformation with the H8 of each guanine on the side of the "quasi axial" N−H of the *cis*-amine (ΛHT and ΔHT for *R*,*R* and *S*,*S* configuration of dach, respectively) is favored over the HT conformer with the H8 of each guanine on the side of the "quasi equatorial" N−H of the *cis*-amine. In contrast, the behavior at basic pH indicates that the rotamer with the O6 atom of each guanine on the side of a "quasi axial" N−H moiety (ΔHT for (*R*,*R*)-dach and ΛHT for (*S*,*S*)-dach) is favored over the rotamer with an O6 on the same side of a "quasi equatorial" N−H moiety. We can conclude that the rotamer in which the H8 of each guanine is on the side of a "quasi axial" N−H moiety of the *cis*-amine can attain a greater "six-in" canting of the two guanines and therefore is favored at acidic pH, at which FFC_a dominates. On the other hand, the rotamer in which the O6 atom of each guanine is on the side of the "quasi axial" N−H moiety is favored at basic pH, at which FFC_b dominates. These results clearly indicate that in an HT rotamer the hydrogen-bonding interaction between the O6 atom of each guanine and the N−H group of the *cis*-amine is greater for a "quasi axial" than for a "quasi equatorial" N−H group. Theoretical calculations appear to support this conclusion.[29] Therefore, FFC_a and FFC_b can fully explain the behavior of $[Pt(dach)(9\text{-}EtG)_2]^{2+}$ complexes, with the "quasi equatorial" N−H groups of dach playing the role of the *N*-alkyl substituents in the corresponding compounds with CCC ligands.

3′-GMP complexes: 3′-GMP displays another type of interaction; the possibility for the 3′-phosphate of one nucleotide to form a hydrogen bond with the N1−H group of the *cis*-nucleotide. Because nucleotides have a preference for an *anti* conformation, the 3′-phosphate group is directed towards the *cis*-nucleotide only in the ΔHT conformer. This type of phosphate/N1−H interaction between *cis*-G ligands has been already discovered in $[Pt(CCC)G_2]$ complexes, and was classified as second-to-second sphere communication (SSC, Figure 6), because it involves groups that are both far from the platinum center.[20–23]

For the $[Pt(dach)(3'\text{-}GMP)_2]$ adduct the ΔHT rotamer was preferred at acidic and neutral pH, regardless of the configuration of the diamine (*R*,*R* or *S*,*S*); therefore SSC appears to dominate over FFC_a. The greatest abundance of the ΔHT rotamer is reached at $pH \approx 7$, at which the phosphate is completely deprotonated and can give the strongest interaction with the N1−H of the *cis*-G. A greater stabilization of the ΔHT rotamer is observed in the *S*,*S* isomer with respect to the *R*,*R* enantiomer, due to the additional contribution of FFC_a, which favors the ΔHT rotamer in (*S*,*S*)-dach, and the ΛHT rotamer in (*R*,*R*)-dach.

Above pH 8, the (*S*,*S*)-dach complex exhibits a slight prevalence of the ΛHT rotamer, while the (*R*,*R*)-dach complex attains a pseudoracemic situation. N1−H deprotonation occurring at basic pH precludes phosphate/N1−H interactions between *cis*-guanines, while favoring guanine O6/N−H of the *cis*-amine interactions (FFC_b). FFC_a and FFC_b are the only interactions occurring at basic pH. In the (*S*,*S*)-dach complex, FFC_a favors the ΔHT rotamer and FFC_b favors the ΛHT rotamer, and the net result is a slight preference for the ΛHT rotamer (an indication that FFC_b is slightly stronger than FFC_a). In contrast, in the (*R*,*R*)-dach complex FFC_a favors ΛHT and FFC_b favors ΔHT resulting in a comparable amount of the two rotamers (a pseudoracemic mixture). In the latter case an FFC_b stronger than FFC_a would have led to a slight preference for the ΔHT rotamer (corresponding to the slight preference for of the ΛHT rotamer observed in the (*S*,*S*)-dach complex); however, this result was not observed, probably because at high pH the ΔHT rotamer is destabilized by an electrostatic repulsion between the negatively charged 3′-phosphate of one nucleotide and the deprotonated N1 of the *cis*-nucleotide.[22,23,30]

5′-GMP complexes: In complexes with 5′-GMP, the equilibrium between rotamers is further complicated by the high mobility and the extended conformational "reach" of the 5′-phosphate, properties which allow the 5′-phosphate to form a hydrogen bond not only with the N1−H group of the *cis*-G, but also with the N−H of the *cis*-amine. The interaction between the 5′-phosphate of a nucleotide and the N−H group of the *cis*-amine involves a group close to the plati-

num center (the N–H group of the amine) and a group far from the platinum center (the 5′-phosphate) and has been classified as first-to-second sphere communication (FSC). FSC always stabilizes the *Δ*HT rotamer in which the 5′-phosphate protrudes toward the *cis*-amine (assuming that the nucleotide adopts the preferred *anti* conformation). On the other hand, SSC, which in the case of 3′-GMP stabilizes the *Δ*HT rotamer, stabilizes the *Λ*HT rotamer in the case of 5′-GMP, because this rotamer has the 5′-phosphate protruding toward the *cis*-nucleotide.[22,23,30] Therefore, the number of possible interligand interactions increases continuously on going from 9-EtG (FFC_a and FFC_b) to 3′-GMP (FFC_a, FFC_b, and SSC) and to 5′-GMP derivatives (FFC_a, FFC_b, SSC, and FSC).

For [Pt{(*R*,*R*)-dach}(5′-GMP)$_2$] at acidic and neutral pH, FFC_a and SSC favor the *Λ*HT rotamer; FSC favors *Δ*HT (FFC_b is only active at basic pH). We observed a preference for the *Λ*HT rotamer, which reaches the highest concentration at pH of approximately 7.5. At more basic pH, N1–H deprotonation of the guanines precludes SSC (favoring *Λ*HT) and increases FFC_b (favoring *Δ*HT). As a consequence, the *Δ*HT rotamer gains stability over the *Λ*HT rotamer and becomes slightly preferred at very basic pH.

For the [Pt{(*S*,*S*)-dach}(5′-GMP)$_2$] species at acidic and neutral pH, SSC favors *Λ*HT, whereas FFC_a and FSC favor *Δ*HT. The *Δ*HT rotamer dominates at acidic pH, while a pseudoracemic mixture is attained at neutral pH because of an increased contribution of the SSC interaction favoring *Λ*HT. At basic pH, N1–H deprotonation precludes SSC (favoring *Λ*HT), but allows for FFC_b (also favoring *Λ*HT); as a consequence, no significant change in HT rotamer distribution was observed.

Comparison with corresponding cis-[Pt(NH$_3$)$_2$G$_2$] adducts and with {Pt(dach)}–DNA cross-links: In the adducts of G ligands with cisplatin, the ammine carrier ligands have no chiral centers and hence no stereocontrolling effect upon the FFC interactions. As a consequence, the CD spectra of *cis*-[Pt(NH$_3$)$_2$G$_2$] adducts reflect the sole dominance of SSC and FSC interactions on rotamer distribution. SSC interactions stabilize the *Δ*- and *Λ*HT rotamers for 3′- and 5′-GMP adducts, respectively, and are precluded at basic pH (simultaneously with guanine N1–H deprotonation) or by guanine N1 methylation. FSC interactions are restricted to 5′-GMP derivatives and favor *Δ*HT.[24] In this respect the interpretation of CD spectra for *cis*-[Pt(NH$_3$)$_2$G$_2$] species is significantly less complex than for analogous compounds with chiral carrier ligands.

The new finding that at basic pH (guanines deprotonated at N1) the guanine O6 preferentially forms a hydrogen bond with a "quasi-axial" amine proton may appear to contradict previous observations reporting hydrogen-bond formation between guanine O6 and a "quasi-equatorial" N–H of the *cis*-amine. For instance, the structure (elucidated by X-ray diffraction) of a Pt{(*R*,*R*)-dach} cross-linked dodecamer duplex[13] showed the formation of a hydrogen bond between the more canted "six-out" 3′-guanine and a "quasi-equatorial" *cis*-N–H of the dach ligand. Such an interaction had been proposed previously to explain the greater destabilization on the 3′-side of duplexes cross-linked by Pt{(*R*,*R*)-dab} as compared to those cross-linked by Pt{(*S*,*S*)-dab} (dab = 2,3-diaminobutane).[31] Although more recently an NMR solution study on a Pt{(*R*,*R*)-dach} intrastrand cross-linked dodecamer duplex[32] has confirmed the greater "six-out" canting of the cross-linked guanine on the 3′-side (thereby placing the guanine O6 near the "quasi-equatorial" N–H of dach), the dach N–H signals did not show the downfield shift and narrow line-width expected to result from hydrogen bonding. In our opinion, the stereochemistry of guanine O6/N–H *cis*-amine hydrogen-bonding interaction is also dependent upon the type of rotamer (HT or HH). In HT rotamers the *cis*-G dipole–dipole interaction favors the conformation with the six-membered ring of each guanine leaning toward the *cis*-G ("six-in" canting). In contrast, in the case of HH rotamers the electrostatic repulsion between the electron-rich O6 atoms of the *cis*-guanines, both on the same side of the platinum coordination plane, tends to place the six-membered ring of the guanines further from one another ("six-out" canting). Such a difference in canting could well result in stronger guanine O6/NH *cis*-amine interactions for the "quasi-axial" N–H in the case of HT rotamers and for the "quasi-equatorial" N–H in the case of HH rotamers.

It should also be noted that in the present study the guanine O6/N–H *cis*-amine hydrogen bond involves an N1-deprotonated guanine, whereas in Pt{(*R*,*R*)-dach} cross-linked duplexes the guanines are protonated at N1. Moreover, adducts with untethered nucleotides are much more flexible than those with conformationally more constrained DNA duplexes.

Conclusion

In this work we have demonstrated that all the interactions between *cis*-coordinated nucleobases and between these bases and *cis*-amine ligands discovered in the extensive investigations carried out on [Pt(CCC)G$_2$] complexes (FFC_a, FFC_b, SSC, and FSC) apply also to chiral primary diamines such as dach.

In [Pt(CCC)G$_2$] compounds the transmission of chirality from the diamine to the *cis*-nucleobases was mediated by the markedly unsymmetric distribution of the N-donor substituents with respect to the coordination plane (a proton on one side and an alkyl substituent on the other side of this plane) and resulted in a remarkable difference in the percentages of the two HT rotamers, with the dominating HT rotamer determining the shape of the CD spectra.

In [Pt(dach)G$_2$] complexes the transmission of chirality from the diamine to the nucleobases is mediated by the two N-donors with a proton on both sides of the platinum coordination plane; however, one N–H has "quasi-axial" and the other "quasi-equatorial" character. The overall effect of the dach carrier ligand chirality on the abundances of different rotamers is still significant, but smaller than in the case

of [Pt(CCC)G_2] complexes. FFC_a is favored by "six-in" canting of the nucleobases, and the degree of canting can be reduced by repulsion between the H8 proton of each guanine and the substituent on the *cis*-amine, which is on the same side of the platinum coordination plane as the H8 proton. The repulsion between the H8 proton and a "quasi-equatorial" N–H group has been found to be greater than that between H8 proton and a "quasi-axial" N–H group. Also, FFC_b (guanine O6/N–H *cis*-amine hydrogen-bond interaction) becomes relevant only after N1–H deprotonation of the guanine at high pH. We note that the O6 atom of the guanine has been found to interact more strongly with a "quasi-axial" than with a "quasi-equatorial" N–H group of the *cis*-amine. SSC (hydrogen-bond formation between the phosphate of one base and the N1–H group of the *cis*-base; such an interaction favors Δ- and Λ-HT rotamers for 3′- and 5′-GMP derivatives, respectively) is dominant at acidic and neutral pH. This interaction loses strength at basic pH simultaneously with deprotonation of N1–H. For 3′-GMP at basic pH, an electrostatic repulsion between the phosphate of one nucleotide and the deprotonated N1 atom of the *cis*-nucleotide appears to slightly destabilize the ΔHT rotamer. Finally, FSC (nucleotide phosphate/N–H *cis*-amine interaction favoring the ΔHT rotamer) is restricted to the case of 5′-phosphates and appears to be slightly weaker than SSC at both acidic and neutral pH.

The present investigation also explains the CD data initially reported by Pasini and co-workers on [Pt(dach)(9-MeG)$_2$]$^{2+}$ complexes in a different way.[16] The shape of the CD spectra is dependent upon the dominance of an HT rotamer (Λ or Δ) and not upon the canting of the nucleobases (right- or left-handed). Moreover, the key factor influencing the stability of the dominant HT rotamer at acidic or neutral pH is the interaction between the H8 proton of each guanine and the N–H group of the *cis*-amine rather than the hydrogen-bond formation between the O6 atom of each guanine and the N–H group of the *cis*-amine.

This work also confirms the usefulness of CD spectroscopy in the study of chiral platinum cross-link models that are too dynamic to be fully assessed in NMR investigations. Without the CD technique, factors intervening in the transmission of chirality from the dach ligand to the *cis*-G bases could not have been elucidated. Such a transmission of chirality from the carrier ligand to the coordinated nucleobases might play a role in influencing the activity and selectivity of antitumor agents, for example, the different activities of the two oxaliplatin enantiomers.

Experimental Section

Starting materials: 5′-GMP, 3′-GMP and 9-EtG (Sigma) were used as received.

Preparation of compounds: [Pt{(*R*,*R*)-dach}Cl_2] and [Pt{(*S*,*S*)-dach}Cl_2] were prepared by a standard procedure.[33]

[Pt{(*R*,*R*)-dach}$(H_2O)_2$]SO_4: [Pt{(*R*,*R*)-dach}Cl_2] (362.5 mg, 0.954 mmol) was suspended in water (50 cm^3) and treated with Ag_2SO_4 (300.0 mg, 0.954 mmol). The mixture was stirred overnight in the dark and the solution was filtered to remove AgCl. The pale yellow residue obtained by evaporation of the solvent was the desired [Pt{(*R*,*R*)-dach}$(H_2O)_2$]SO_4 product. Yield 400 mg, 95%; elemental analysis calcd (%) for $C_6H_{18}N_2O_6PtS$: C 16.3, H 4.1, N 6.3; found: C 16.2, H 4.1, N 6.2; ^{1}H NMR (D_2O): δ_H = 1.14, 1.31, 1.57, 2.06, (m, each signal integrates for 2H, methylene protons), 2.41 ppm (m, 2H, C*H*N).

[Pt{(*S*,*S*)-dach}$(H_2O)_2$]SO_4: The synthesis of this compound was carried out as already described for the *R*,*R* enantiomer. Yield 94%; elemental analysis calcd (%): C 16.2, H 4.1, N 6.2; found: C 16.3, H 4.0, N 6.2. ^{1}H NMR data were the same as those of the enantiomeric species reported above.

Solution experiments: Stock solutions of G and [Pt(dach)$(H_2O)_2$]SO_4 (10–25 mM in D_2O) were prepared and adjusted to acidic pH with diluted D_2SO_4 in D_2O. The selected pH was always approximately 2.5–3.0, except in the case of 9-EtG, for which a more acidic pH of around 1.6 was required in order to ensure complete dissolution of the base in water. Aliquots of these stock solutions were transferred into an NMR tube in order to have a final G:Pt ratio slightly higher than two. The formation of [Pt(dach)G_2] complexes was monitored by ^{1}H NMR spectroscopy. The concentration of the platinum complex in the NMR tube was 4–10 mM.

Aliquots of the NMR solutions were brought to a concentration of 3–5 × 10^{-5} M by addition of water. Na_2SO_4 was added in order to maintain a constant ionic strength (~50 mM). The pH was varied from 3 to 11, and the changes in rotamer composition were monitored by CD spectroscopy.

Spectroscopy: ^{1}H NMR spectra were recorded on a Bruker Avance DPX 300 instrument. The number of scans per spectrum was at least 128 in order to have a high signal-to-noise ratio. CD and UV-visible spectra were registered on a Jasco J-810 spectropolarimeter in the range 200–350 nm. Each spectrum was the average of 4–16 different scans in order to increase the signal-to-noise ratio; the path length of the cell was 0.5 cm.

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