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Structural consequences of a 3' → 3' DNA interstrand cross-link by a trinuclear platinum complex: unique formation of two such cross-links in a 10-mer duplex

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Abstract

Combined multidimensional nuclear magnetic resonance spectroscopy and electrospray mass spectrometry was used to analyze the platinated DNA adduct of the phase II anticancer drug [*trans*-PtCl(NH₃)₂]₂-μ-*trans*-Pt(NH₃)₂(NH₂(CH₂)₆NH₂)₂](NO₃)₄ (BBR3464) with [5'-d(ACG*TATACG*T)-3']₂. Two 1,2-interstrand cross-links were formed by concomitant binding of two trinuclear moieties to the oligonucleotide. The four DNA-bound platinum atoms coordinated in the major groove at N7 positions of guanines in the 3' → 3' direction and the central platinum unit is expected to lie in the DNA minor groove. This is the first report of such a DNA lesion. The melting temperature of the adduct is 76 °C and is 42 °C higher than that of the unplatinated DNA. The sugar residues of the platinated bases are in the N-type conformation and the G9 nucleoside is in the *syn* orientation, while the G3 nucleoside appears to retain the *anti* configuration. The secondary structure of DNA was significantly changed upon cross-linking of the two BBR3464 molecules. Base destacking occurs between A1/C2 and C2/G3 and weakened stacking is seen for the C8/G9 and G9/T10 bases. The lack of Watson–Crick base pairing is also seen for A1–T10 and C2–G9 base pairs, whereas Watson–Crick base pairs in the central sequence of the DNA (T4 → A7) are well maintained. While DNA repair proteins may “see” different platinated adducts as bulky “lesions”, the subtle differences involved in base pairing and stacking, as summarized here, may extend to their role as a substrate for repair enzymes. Thus, differences in protein recognition and repair efficiency among the various interstrand cross-links are likely and a subject worthy of detailed exploration.

Keywords

NMR spectroscopy; Interstrand cross-link; Platination; DNA; BBR3464

Introduction

The anticancer drug cisplatin (CDDP; *cis*-[PtCl₂(NH₃)₂]; Fig. 1) forms several structurally distinct DNA adducts, including 1,2-GG intrastrand cross-links and 1,2-GC interstrand cross-links (IXLs), which are considered ultimately responsible for its biological activity [1–3]. Protein recognition of the ensuing conformational distortions is an attractive pathway for differential repair of cellular CDDP-DNA adducts [4–6]. The trinuclear BBR3464 (TPC; Fig.

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1) belongs to the polynuclear class of Pt-based anticancer agents, and has undergone phase II clinical trials [7–9]. DNA adducts of polynuclear Pt complexes differ significantly in structure and type from those of mononuclear Pt [10–13]. Especially, long-range (Pt,Pt) IXLs are formed in both the (normal) 5' → 5' and the (unusual) 3' → 3' direction. Maxam–Gilbert footprinting experiments confirmed the formation of directional isomers with cross-links formed in both directions, and the directionality is dependent on the nature of the cross-link. The 1,2-IXL forms in only the 3' → 3' direction and the 1,4-IXL is formed in both directions in approximately equal proportions, while the 1,6-IXL forms in only the 5' → 5' direction [14]. To our knowledge, this is the only example of directional isomers for cross-linking reagents. These results are significantly different from those of the CDDP 1,2-IXL [15]. They also contrast with the properties found for adducts of the simpler dinuclear compounds (DPC4, DPC6; Fig. 1), which form only 5' → 5' cross-links [14]. Long-range IXLs persist over time, suggesting a lack of DNA repair, which may contribute significantly to the cytotoxicity of the polynuclear species [16,17]. It is therefore important to compare the structures of the various Pt-induced IXL formed in different DNA sequences to assess possible contributions at the molecular level to downstream cellular processes such as protein recognition and repair. Herein we report on the structural consequences of a 1,2-IXL in [5'-d(ACGTATACGT)-3']₂ formed by TPC and formally in the 3' → 3' direction, the first report of such a DNA lesion.

Materials and methods

Sample preparation

TPC was prepared according to literature procedures [10]. The self-complementary decamer 5'-d(ACGTATACGT)-3' was purchased from the Midland Certified Reagent (Midland, TX, USA). The oligonucleotide was annealed by suspending it in a 1.5-ml microfuge tube which was placed in a standard heatblock at 90 °C for 2 min; the tube was then removed from the heatblock and allowed to cool to room temperature on the workbench. The slow cooling to room temperature took 50 min. The sample was stored on ice until it was ready to use. The cross-linked 10-bp oligonucleotide was prepared by reacting the 5'-d(ACGTATACGT)-3' duplex (0.5 mM) with TPC (0.5 mM) in 100 mM NaClO₄ solution at room temperature. The reaction was followed by high-performance liquid chromatography using a µBondapak C18 reverse-phase column for 4 days (there were no changes after 3 days). The major adduct was collected on a preparation column and extensively dialyzed for 24 h against cold water (4 °C) to remove any salts. The sample was lyophilized to yield 3.0 mg cross-linked DNA (13% based on starting DNA). Solutions of the unplatinated and the platinated oligonucleotide in D₂O containing 10 mM NaClO₄ were prepared by lyophilizing them twice from 99.999% D₂O and redissolving the samples in 0.65 ml 99.999% D₂O (0.59 mM). Where spectra in H₂O were required, the D₂O solutions were lyophilized and the samples were redissolved in 92% Milli-Q H₂O/8% D₂O.

Mass spectrometry

Electrospray ionization (ESI) mass spectrometry was performed at The University of Arizona (Tucson, TX, USA) and Virginia Commonwealth University. The sample (approximately 0.10 µg) was dissolved in 1 ml H₂O/MeOH (1:5) with 5% NH₄OH at room temperature and electrosprayed from a 50-µm fused-silica capillary pulled to a fine tip and remotely coupled to a potential of approximately –2,100 V with a flow rate of 300 nl/min. The electrospray tip was positioned directly in front of the ESI source, modified to accept a heated metal capillary that was held at 200 °C. The mass spectrometer was an IonSpec (Irvine, CA, USA) 4.7 T Fourier transform ion cyclotron resonance mass spectrometer.

Melting point

The unplatinated oligonucleotide and the Pt–oligonucleotide adduct (4×10^{-6} M in duplex) were dissolved in 0.1 M NaClO₄ solution. The data were recorded with a JASCO V-550 UV–vis spectrometer by measuring the absorbance at 260 nm and collected at 1 or 2 °C intervals with 3-min equilibration. The value of the melting temperature was determined as the temperature corresponding to a maximum on the first-derivative profile of the melting curves.

Circular dichroism

Circular dichroism (CD) spectra were taken using a JASCO 600 CD spectrophotometer and a 10-mm quartz sub-micro-cuvette. The unplatinated oligonucleotide and the Pt–oligonucleotide adduct (1×10^{-5} M in duplex) were dissolved in 0.1 M NaClO₄ solution. Each sample was scanned in the 200–350-nm range thrice and the spectra were averaged. The background was subtracted electronically.

NMR analysis

NMR spectra were run obtained with a Varian Unity 500 MHz spectrometer at 5 and 25 °C. The following parameters were used for data acquisition of nonexchangeable protons: double quantum filtered correlation spectroscopy (DQF-COSY), 900 complex increments in t_1 , each with 2,048 complex points in t_2 , sweep width 7,486.4 Hz, 64 transients; total correlation spectroscopy (TOCSY), mlev-17 pulse sequence, 300 complex t_1 increments, 2,048 complex t_2 points, sweep width 7,486.4 Hz, 64 transients, relaxation delay 1 s, mixing time 0.8 s; nuclear Overhauser enhancement spectroscopy (NOESY), 350 complex t_1 increments, 2,048 complex t_2 points, sweep width 7,486.4 Hz, 64 transients, relaxation delay 1 s, mixing time 100, 200, 300, and 400 ms, respectively.

The exchangeable proton 2D-NOESY experiment was carried out in H₂O/D₂O (92:8) solution with a spectral width of 7,486.4 Hz. A total of 2,048 points were acquired in t_2 for each of 350 complex t_1 increments with a recycle delay of 2 s and a mixing time of 200 and 400 ms, respectively, each data point being the average of 64 transients.

The data were processed with Felix 2004 NMR software on a Fuel SGI workstation. The assignments were made using through-bond DQF-COSY and TOCSY, and through-space NOESY peaks.

Molecular modeling

Energy minimizations were performed on the adduct of the 1,2-interstrand cross-linked DNA, [d(ACGTATACGT)₂–{(*trans*-Pt(NH₃)₂)₂–μ-*trans*-Pt(NH₃)₂(NH₂(CH₂)₆NH₂)₂}]₂, hereafter referred to as (TPC)₂–DNA, using DISCOVER_3 (Accelrys, San Diego, CA, USA) with the Amber force field and a nonbonding cutoff of 9.0 Å. The calculation was continued until the gradient norm requirement dropped to below 0.01 kcal/mol.

Results and discussion

Physical measurements

Characterization—The molecular mass of the Pt–DNA adduct was determined by negative-ion ESI mass spectrometry to be 7,880 (±2) amu, corresponding to two TPC units (less the four Cl[−] displaced upon Pt binding) per duplex of DNA, [(TPC)₂–DNA] (Fig. 2a). The 4[−], 5[−], and 6[−] states are clearly seen (represented by diamonds in Fig. 2a). The large multiple isotopic distributions further confirmed the presence in the molecule of six Pt atoms (see Fig. 2b). Figure 2a also shows peaks at $m/z = 1,742/4^-$ and $1,393/5^-$ (represented by circles), corresponding to a molecular mass of 6,967 (±2) amu, corresponding to one TPC unit per duplex of DNA.

However, the peaks were relatively small and an additional species was not observed in the NMR experiments. The melting temperature of the adduct is 42 °C higher than that of the unplatinated DNA, 76 and 34 °C, respectively, indicating the formation of DNA IXLs. The DNA IXLs increase the DNA melting point, in contrast to the behavior of intrastrand cross-links [18]. The increase in melting temperature is similar to that observed previously (38–44 °C) for long-range Pt,Pt-DNA 1,4-IXLs [12,19]. The CD spectrum of the adduct appears to change from the B form, the unplatinated DNA (Fig. 3, blue line), to an unknown form (Fig. 3, magenta line) [20,21], where the intensity of the negative band at 240 nm decreases and the positive band at 269 nm shifted to 279 nm with decreasing intensity, and also a new positive band appears at 257 nm (Fig. 3, magenta line). In comparison with 5' → 5' 1,4-IXL, Fig. S1 shows CD spectra of the IXL adduct of TPC with [5'-d(ATGTACAT)-3']₂ (Fig. S1, purple line) and unplatinated 8-mer DNA (Fig. S1, green line). The maximum positive peak broadened slightly and shifted from around 268 nm to approximately 271 nm (Fig. S1, purple line). The CD spectra implicated extensive conformational changes in 3' → 3' 1,2-IXL compared with 5' → 5' 1,4-IXL.

Structural analysis of the Pt-oligonucleotide adduct

NMR spectroscopy—2D-NOESY, DQF-COSY, and TOCSY spectra in D₂O were used to assign the shifts of all nonexchangeable protons. For the sequential assignment, we used the 2D-NOESY spectrum beginning with the cross-peak region of aromatic protons to H1', then extending to aromatic protons to H2'/H2'' and all regions of the spectrum. Once the intranucleotide and sequential nuclear Overhauser enhancements (NOEs) have been assigned, there should be no unassigned cross-peaks left in the NOESY spectra of the DNA duplex in D₂O, with the possible exception of some interstrand NOEs between H2–H2 or H2–H1' of adenines. The assignment of base-pair protons was aided by the 2D-NOESY cross-peaks. Analysis of these data sets resulted in assignments for most of the exchangeable and nonexchangeable ¹H peaks for [(TPC)₂–DNA]. The chemical shifts of the assigned peaks are listed in Table 1.

NMR spectroscopy gave a picture of the local distortions and revealed a number of interesting observations. Figure 4 shows the 2D-TOCSY spectrum containing the hexanediamine region. The hexanediamine linkers are not equivalent in the (TPC)₂–DNA adduct (Fig. 4c), unlike in free TPC (Fig. 4a). There were also more multiplicities than observed in the previously characterized 1,4-IXL (5' → 5') formed between TPC and the 8-mer oligonucleotide d(ATGTACAT)₂ (Fig. 4b) [12]. However, the differences were very small between L1 and L3, also L2 and L4, but were large between L1 and L2, and similarly L3 and L4. The complication of multiple TOCSY peaks is caused by the presence of the two TPC units cross-linking to DNA and may also be caused by the fact that there are two distinct guanines, G3 and G9, involved in bonding. In contrast, there was only one sequential assignment pathway in the NOESY spectrum for the duplex DNA in the adduct (Fig. 5, arrows), except for H2 protons of A5 and A7, where chemical shifts were different on the two strands (Fig. 5, Table 1). The downfield chemical shifts of the guanine H8 protons (8.53 and 8.41 ppm, G3 and G9) in comparison with those of the unplatinated duplex (Δδ 0.66 and 0.53 ppm for G3 and G9, respectively; Fig. 5, Table 1) indicated platination of the guanine bases at their N7 positions.

The sugar residues of the platinated bases were in the N-type conformation and the G9 nucleoside was in the *syn* orientation, as indicated by strong G9 H8/H1' and weak G9 H1'/T10H6 NOE cross-peaks. In contrast the G3 nucleoside appears to retain the *anti* configuration (Fig. 5). NOE cross-peaks between A1H2/C2 H1', A5H2/A5 H1', A5H2/T6H1', and A5'H2/A5 H1' were also observed. An unusual cross-peak from G3H1' to A7H2 was also observed (marked as an asterisk in Fig. 5), which is attributed to an interstrand connectivity. The H6 and H1' protons of the neighboring C2, T4, C8, and T10 bases undergo relatively large chemical

shifts, indicating altered environments upon guanine platination (Fig. 6, Table 1). It is noteworthy that all chemical shift changes are downfield from the free oligonucleotide, except for T10H1', shifted upfield by 0.54 ppm (Fig. 6). NOEs between aromatic protons provide further confirmation of the assignments of the aromatic protons and the presence or absence of base stacking. Figure 7a represents a normal B-form DNA with base stacking starting from A1 and ending at T10 without interruption, as shown by arrows representing interresidue NOE cross-peaks between the adjacent bases. In contrast, Fig. 7b shows clearly that base destacking occurred in the adduct between A1/C2 and C2/G3 and weakened stacking was seen for the C8/G9 and G9/T10 bases, as evidenced by the lack of NOE cross-peaks for A1/C2 and C2/G3 and weak NOE cross-peaks for C8/G9 and G9/T10. This result is consistent with the CD spectra (Fig. 3).

Figures 8 and S2 show aspects of the 2D-NOESY spectrum in water. The assignment of the exchangeable-proton NMR spectrum was aided by the connectivities. Watson–Crick base pairs in the central sequence of the DNA (T4 → A7) are well maintained as evidenced by the relatively strong NOE connectivities from the imino protons of T6 and T4 to the amino protons and H2 of A5 and A7 (Fig. 8). The interstrand connectivities were also shown by NOE cross-peaks of H2 of A7'/A5 and A5'/A7 (Fig. 7b), but not in unplatinated DNA (Fig. 7a). Usually, the protons in position 2 of adenine are assigned from NOE connectivities with exchangeable protons. However, for DNA segments containing only adenine and thymine, a continuous assignment pathway for AH2 can also be obtained from experiments in D₂O. This relies on the short sequential distance of H2 of A_i–A_{i+1} and on the short interstrand H2 distance of A_{strand1}–A_{strand2} [22]. This case is also observed in a stack of several A/T base pairs [23]. In the present case, the stronger NOE cross-peak of A5H2/A7'H2 than that of A5'H2/A7H2 (Fig. 7b) implies that the interstrand distance of the H2 pair of A5–A7' was shorter than that of A5'–A7 in the (TPC)₂–DNA adduct and that both distances were shorter than those in unplatinated DNA. Note that these interstrand connectivities were only seen in the (TPC)₂–DNA adduct (Fig. 7). Also, there was a NOE connectivity between A1H2 and C2H6 in unplatinated DNA (Fig. 7a) but not in the cross-linked adduct (Fig. 7b). Figure 7, in summary, indicates that the secondary structure of DNA was significantly changed upon cross-linking by two TPC units. The absences of NOE connectivities in imino or amino protons of A1, C2, G9, and T10 indicated missing Watson–Crick base pairs in A1–T10 and C2–G9. The loss of A1–T10 hydrogen bonding could be due to “end fraying” because of the exposed terminal base pair and rapid exchange of the imino and amino protons with water. However this would not necessarily be the case for C2–G9, where the effect may be expected to be caused by Pt bonding [24, 25]. Furthermore, an unusual NOE cross-peak from the imino proton of G3 to H2 of A1 (even though very weak; Fig. 8) indicated that the strand around G3 is distorted. The central Pt unit is suggested to lie in the DNA minor groove as many NOE cross-peaks were observed from the “central” Pt–NH₃ and hexanediamine protons of the linkers to the sugar H1– protons (Fig. S2a) and H2 protons of adenines (Fig. S2b). It was not surprising to see many NOE connectivities around G3 and G9 to TPC since they were the bonding sites (Fig. S2). It was not possible to unambiguously assign all of the NOE cross-peaks between TPC and DNA, owing to overlap at the field strength used.

All of the 2D NMR data indicated that the two strands of DNA in the platinated adduct were essentially the same as expected from a symmetric duplex, except that (as stated) the H2 of A5 and A7 were not the same as those of A5' and A7'. The difference was smaller for the pair of A5 protons ($\Delta\delta$ 0.09, A5'H2–A5H2) than for the A7 pair ($\Delta\delta$ 0.18, A7'H2–A7H2). Likewise, there were also smaller chemical shifts compared with those for unplatinated DNA: $\Delta\delta$ –0.04, 0.05, 0.29, and 0.47 for H2 of A5, A5', A7, and A7', respectively (Table 1). To visualize the situation, the 3D graph of base pairs was generated by 3DNA [26] from molecular model of (TPC)₂–DNA and is displayed in Fig. 9. The results showed that the H2 protons of A5/A7', and A5'/A7 pointed in slightly different directions (Fig. 9a). The distances were 3.79 Å from

H2 of A5 to A7' and 3.46 Å from H2 of A5' to A7 and thus the differences were consistent with the results from NMR spectroscopy as summarized above.

How do the conformational changes as delineated compare with those from similar lesions? The 1,2-IXL formed by CDDP results in loss of base stacking and pairing, producing a localized Z-like conformation (Table 2), where the complementary cytosine bases of the platinated guanines are “flipped” out of the helix [25]. In that sequence, d(CATAG₅C₆TATG)₂, with G₅C₆ representing the platinated GC base pairs, large upfield chemical shifts of approximately 3 ppm for 5GH1' are diagnostic of the platinated but unpaired guanine base. Comparable shifts in the present work were Δδ 0.39 and 0.06 ppm for G9 and G3, respectively. Thus, by analogy, these significantly smaller shifts suggest that the conformational distortions suffered by the cytosine bases in the present structure are not sufficient to cause a complete “flipping out” of the helix (Fig. 9b) as seen for the CDDP case [25].

Table 2 also compares the structural effects of 1,2- and 1,4-IXLs formed by polynuclear Pt complexes. The 5' → 5' 1,2-IXL from DPC4 and [5'-d(CATG*CATG)]₂ adopts a dumbbell shape in which two hairpin-like structures are stacked end-over-end and the platinated guanines are in the *syn* conformation [24]. In contrast, the major feature of the long-range 5' → 5' 1,4 cross-links formed by both DPC6 and TPC in [5'-d(ATGTACAT)-3']₂ is a delocalized structure with a *syn* conformation of both the intervening and adjacent adenine nucleotides not directly involved in the cross-link [12,13,19]. The G–C base pairing of the platinated guanine is maintained in the 1,4-IXL. The differences in DNA conformations between 3' → 3' 1,2 cross-links and 5' → 5' 1,4 cross-links formed by TPC are clearly shown in the CD spectra (Figs. 3, S1).

By monitoring the annealing of a “bottom” strand to a “top” strand containing a discrete monofunctional platinated site, molecular biology studies showed that the kinetics of 1,2- and 1,4-IXL formation were similar and faster than the formation of the analogous 1,6-IXL [11, 14]. The shorter 1,2-IXL is formed with an exclusive preference for the 3' → 3' direction, whereas both 3' → 3' and 5' → 5' IXLs were formed for the longer-range adducts [11]. The combined results suggest that the formation of two simultaneous 1,2-IXLs of TPC with [5'-d(ACGTATACGT)-3']₂ may be kinetically favored over the theoretically possible 1,6-IXL (G3 → G8) in the present structure. Note that the reaction was performed at 1:1 molar ratio [5'-d(ACGTATACGT)-3' duplex/TPC] and unplatinated 10-mer was left after 3 days, confirmed by collection by high-performance liquid chromatography and comparison of NMR and mass spectra. It is possible that the 1,6-IXL Pt–DNA adduct is the source of the small peaks of the 1:1 (TPC/DNA) product observed by ESI mass spectrometry. This cross-link could be formed by transformation under the mass spectrometry conditions or during any stages of preparation of the Pt–DNA complex. However, it was not observed in solution by NMR spectroscopy and not definitively confirmed.

This, to our knowledge, is the first DNA structural characterization containing more than one covalent adduct of a DNA-binding ligand as well as being the first to structurally characterize the molecular details of an IXL in the 3' → 3' direction. In summary, unique features of this altered DNA structure include the differences in platinated guanines, where G9 adopts a *syn* conformation and yet the “complementary” G3 remains in the *anti* conformation; the loss of Watson–Crick base pairing in G9–C2 but not G3–C8, and the intrastrand base destacking in C2/G3 as well as weakened stacking for C8/G9.

The physical constraints induced on DNA by interstrand cross-linking as well as the necessity that repair must occur on both strands of DNA makes interstrand cross-linking agents especially damaging to cells [27]. Clinical studies have strongly implicated the involvement of DNA repair capacity as a factor in inherent antitumor drug sensitivity to Pt agents, as well as acquired

drug resistance [2]. Nucleotide excision repair is a major mechanism of platinated adduct removal from template DNA [28,29]. The use of chemical probes and comparison of cleavage patterns by DNase I footprinting have shown subtle differences in the conformational distortions suffered by the 5'–5' and 3'–3' 1,4-IXLs [14,30]. Neither cross-link is an efficient substrate for repair by cell extracts [14]. While DNA repair proteins may “see” different platinated adducts as bulky “lesions,” the subtle differences involved in base pairing and stacking, as summarized here, may extend to their role as substrates for repair enzymes [31]. Thus, differences in repair efficiency among the various IXLs are likely and a subject worthy of detailed exploration, although the biological relevance of the 3' → 3' cross-link compared with the 5' → 5' adduct is still unknown.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Structures of Pt compounds and sites of interstrand cross-link adduct formation on the 10-mer duplex of DNA, 5'-d(ACGTATACGT)₂

Fig. 2.

a Mass spectrum of adducts of platinumated DNA. The peaks labeled with *diamonds* and *circles* were assigned, respectively, as formed from binding of two TPC units and one TPC unit (less the Cl^- displaced upon Pt binding) per duplex of DNA, $[\text{d}(\text{ACGTATACGT})_2\{(\text{trans-Pt}(\text{NH}_3)_2)_2\text{-}\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{NH}_2(\text{CH}_2)_6\text{NH}_2)_2\}_2]$ and $[\text{d}(\text{ACGTATACGT})_2\{(\text{trans-Pt}(\text{NH}_3)_2)_2\text{-}\mu\text{-trans-Pt}(\text{NH}_3)_2(\text{NH}_2(\text{CH}_2)_6\text{NH}_2)_2\}]$, molecular weight 7,880 and 6,967 (± 2). **b** The isotopic distribution at 1,970 m/z of the major adduct, confirming the pattern expected for the presence of six Pt atoms

Fig. 3. Circular dichroism spectra of the 10-mer duplex of DNA, 5'-d(ACGTATACGT)₂ (*blue line*) and the adduct of platinated DNA (*magenta line*)

Fig. 4. 2D total correlation spectroscopy spectrum of the hexanediamine resonances region of **a** TPC, **b** (TPC)—d(ATGTACAT)₂ [12], and **c** [d(ACGTATACGT)₂—(TPC)₂]. The spectra show the non-equivalence of the four hexanediamine linker proton environments designated as *L1*, *L2*, *L3*, and *L4* in **c**

Fig. 5. 2D nuclear Overhauser enhancement spectroscopy (NOESY) spectrum of the [d (ACGTATACGT)₂-(TPC)₂] adduct at a mixing time of 200 ms, showing the sequential assignment pathway and symmetric DNA. The strong G9H1'–G9H8 nuclear Overhauser enhancement (NOE) cross-peak and very weak G9H1'–T10H6 NOE cross-peak suggests a *syn* glycosyl conformation for G9

Fig. 6.

The drug-induced perturbations to ^1H chemical shift values of $\text{H1}'$ and H6/H8 . Negative shift differences correspond to resonances that move upfield on ligand binding. The significant chemical shifts of G3H8 and G9H8 indicate that Pt binds to GN7 as expected. The large chemical shifts of $\text{C2H1}'$, $\text{G3H1}'$, $\text{C8H1}'$, and $\text{G9H1}'$ show the major distortion at G/C areas

Fig. 7. 2D-NOESY spectrum containing the H8/H6 cross-peak region of **a** 10-mer duplex of DNA, 5'-d(ACGTATACGT)₂ and **b** [d(AC GTATACGT)₂-(TPC)₂] adduct. The spectra show the base stacking where present. Secondary structure DNA distortion in the DNA-Pt is shown by the destacking (C8/G9, G9/T10) and the absence of stacking (A1/C2 and C2/G3). A cross-peak was also observed between A5'H2/A7H2 and A7'H2/A5H2

Fig. 8.

The exchangeable-proton 2D-NOESY spectrum containing the imino–amino cross-peak region of $[d(ACGTATACGT)_2-(TPC)_2]$ showing the relatively strong NOE connectivities from the imino protons of T6 and T4 to the amino protons and H2 of A5 and A7 and an unusual NOE cross-peak from the imino proton of G3 to H2 of A1. No cross-peak is observed for the A1–T10 and C2–G9 base pairs

Fig. 9.

3D graphs showing **a** differences in chemical environment of the H2 proton pairs of A5/A7' and A7/A5' and **b** the 3' → 3' 1,2-interstrand cross-link between the adjacent guanines [half molecule showing one of the two TPC moieties per 10-mer duplex of DNA, 5'-d (ACGTATACGT)₂] and the orientation of the cytosine opposite the platinated guanine

Table 1

^1H chemical shifts of 5'-d(A1C2G3T4A5T6A7C8G9T10)₂ in the cross-linked adduct

	H6/H8	H5/H2/CH ₃	H1'	H2'/H2''	GHI/TH3
A1	8.17 (0.01)	8.00 (0.03)	6.29 (0.11)	2.74/2.73 (-0.03/0.12)	
C2	7.69 (0.33)	5.78 (0.45)	6.27 (0.86)	2.63/2.36 (0.30/0.22)	
G3	8.53 (0.66)		6.02 (0.06)	2.75/2.61 (0.01/0.00)	12.97 (0.24)
T4	7.65 (0.44)	1.38 (-0.05)	5.92 (0.19)	2.66/2.31 (0.15/0.16)	13.66 (0.23)
A5	8.27 (0.00)	7.21 (-0.04)	6.17 (0.02)	2.89/2.62 (0.01/0.03)	
		7.30 (0.05)			
T6	7.25 (0.12)	1.43 (0.07)	5.62 (0.01)	2.43/2.13 (0.02/0.12)	13.46 (0.20)
A7	8.20 (0.02)	7.89 (0.29)	6.19 (0.08)	2.87/2.57 (0.08/0.00)	
		8.07 (0.47)			
C8	7.36 (0.19)	5.24 (0.09)	6.15 (0.61)	2.28/2.08 (0.07/0.28)	
G9	8.41 (0.53)		6.37 (0.39)	2.48/2.48 (-0.15/-0.15)	12.72 (-0.08)
T10	7.49 (0.17)	1.44 (-0.08)	5.63 (-0.54)	1.89/1.87 (-0.29/-0.31)	

^1H referenced to tetramethylsilane. The stereospecific assignment has been made for H2'/H2'' protons. Numbers in *parentheses* indicate the difference between the chemical shift of cross-linking adduct and free oligonucleotide resonances

Table 2

Base and sugar conformation of various interstrand cross-linked adducts of Pt complexes with DNA

Complex ^a	DNA	Interstrand cross-link	<i>syn</i> orientation base	N-type sugar	G*-C pair	Base destacking	Weakened base stacking	Minor groove interaction	Melting point change	References
CDDP	CATAG*CTATG	1,2	G ₅	G ₅	No	G ₅ CT	NA	Yes	NA	[25]
DPC4	CATG*CATG	1,2	G ₄	G ₄	No	TG ₄ C	C ₅ A	Yes	NA	[24]
DPC6	ATG*TACAT	1,4	A ₁ , G ₃ , A ₅ , A ₇	G ₃ , A ₇	Yes	TG ₃	G ₃ T	Yes	+38 °C	[12,13,19]
BBR3464	ATG*TACAT	1,4	A ₁ , G ₃ , A ₇	G ₃ , A ₇	Yes	ATG ₃	G ₃ T	Yes	+44 °C	[12,13,19]
BBR3464	ACG*TATACG*T	1,2	G ₉	G ₃ , G ₉	Yes, no	CG ₃	A ₁ C, CG ₉ T	Yes	+42 °C	This work

NA not available

^aSee Fig. 1