Supporting Information for:

DNA Binding by Antitumor *trans*-[$PtCl_2(NH_3)$(thiazole)]. Protein Recognition and Nucleotide Excision Repair of Monofunctional Adducts

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Bending and Unwinding. Important structural motifs that attract HMG-domain and NER proteins to 1,2-intrastrand CLs of cisplatin are the bending and unwinding of the helix axis (*1-5*). For DNA adducts of cisplatin, the structural details responsible for bending and unwinding and subsequent protein recognition have recently been elucidated (*6, 7*). Given the recent advances in our understanding of the structural basis for the bending of DNA caused by cisplatin CLs, it is of considerable interest to examine how frequent monofunctional adducts of *trans*-PtTz affect conformational properties of DNA such as bending and unwinding. In this work we further performed studies on the bending and unwinding induced by single, site-specific monofunctional adduct of *trans*-PtTz using electrophoretic retardation as a quantitative measure of the extent of planar curvature. Molecular modelling had previously indicated that such bending might be possible for the *trans*-planar compounds (*8*).

The oligodeoxyribonucleotide duplexes TGT(16, 20-23) (16, 20 – 23 bp long shown in Figure 1E) were used for the bending and unwinding studies of the present work. All

sequences were designed to leave a one nucleotide overhang at their 5'-ends in double-stranded form. These overhangs facilitate polymerization of the monomeric oligonucleotide duplexes by T4 DNA ligase in only one orientation, and maintain a constant interadduct distance throughout the resulting multimer. Other experimental details of these studies are given in our recent reports (*9-11*). Autoradiograms of electrophoresis gels revealing resolution of the ligation products of nonplatinated 20 – 23 bp duplexes or containing a unique monofunctional adducts of *trans*-PtTz are shown in Figure S1A. A significant retardation was observed for the multimers of all platinated duplexes. The *K* factor is defined as the ratio of calculated to actual length. The variation of the *K* factor *versus* sequence length obtained for multimers of the duplexes 20 – 23 bp long and containing the unique monofunctional adduct of *trans*-PtTz is shown in Figure S1B. Maximum retardation was observed for the 21-bp adducted duplex. Interestingly, the 21-bp curves had only a slightly greater slope than 22-bp curves, whereas the 20-bp curve differed more pronouncedly. This asymmetry is consistent with a significant DNA unwinding due to the formation of the monofunctional adduct of *trans*-PtTz. This observation suggests that the natural 10.5-bp repeat of B-DNA and that of DNA perturbed by the monofunctional adducts of *trans*-PtTz are different as a consequence of DNA unwinding (*12*). Importantly, the plot of the *K* factor *versus* sequence length obtained for multimers of the duplex 16 bp long and containing the unique monofunctional adduct of *trans*-PtTz rose only slightly and then leveled off (not shown), indicating an imperfectly phased bend unit. This behavior was observed apparently because the platinum adducts in these multimers were almost perfectly dephased so that any directed bends added destructively, preventing any appreciable anomalous mobility shifts (*12*).

The exact helical repeat of the duplexes containing the monofunctional adduct of *trans*-PtTz and from it the unwinding angle were calculated by interpolation with the use of the *K* *versus* interadduct distance curve (Figure S1C) as described in the previous papers for the 1,2-

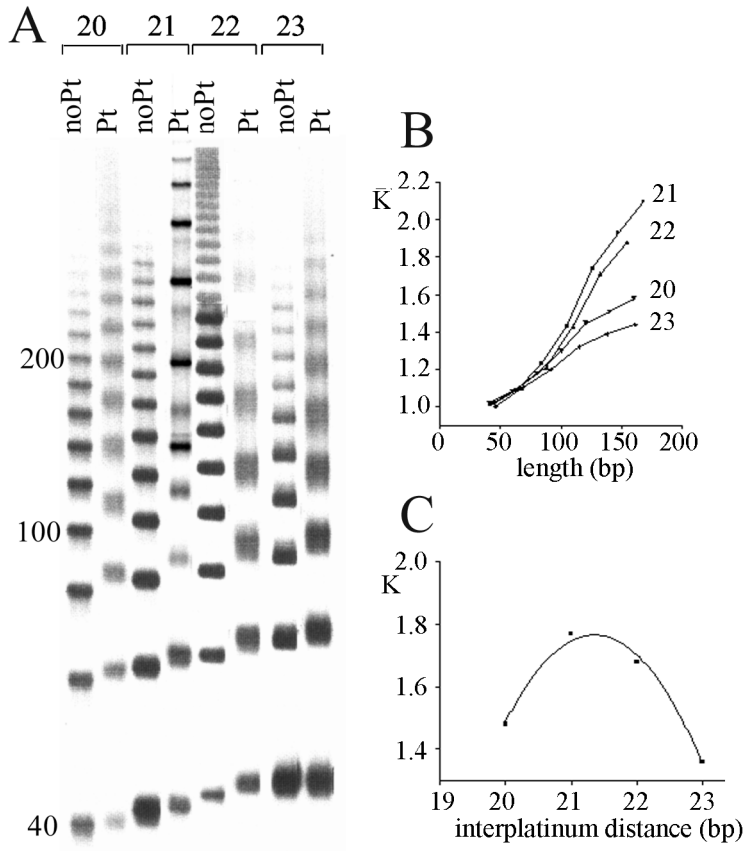

FIGURE S1. The mobility of the ligation products of duplexes TGT(20-23)] containing single, site-specific monofunctional adduct of *trans*-PtTz in an 8% PAA gel. A. Phosphorimage of the ligation products. The duplexes contained a unique monofunctional adduct of *trans*-PtTz at the central G in the top strand. Lanes: noPt, unplatinated duplexes; Pt, platinated duplexes. B. Plots showing the relative mobility *K versus* sequence length curves for the oligomers 20-23 bp long denoted respectively as 20, 21, 22 and 23. C. A plot showing the relative mobility *K versus* interadduct distance in bp for the oligomers 20-23 bp long with a total length of 126 bp. The experimental points represent the average of three independent electrophoresis experiments. The curves represent the best fit of these experimental points to the equation $K = ad^2 + bd + c$ (*12*).

GG intrastrand CL of cisplatin in the duplexes containing the central GG sequence (*12, 13*). The maximum of these curves constructed for the duplexes containing the monofunctional adduct of *trans*-PtTz with a total length of 120 bp were determined to be 21.34 ± 0.01. Total sequence lengths other than 120 bp (110 and 130 bp) were examined and gave identical

results. To convert the interadduct distance in bps corresponding to the curve maximum into a duplex unwinding angle in degrees, the value is compared with that of the helical repeat of B-DNA, which is 10.5 ± 0.05 bp (*14, 15*). The difference between the helical repeat of B-DNA and the DNA containing the monofunctional adduct of *trans*-PtTz, therefore, is [(21.34 ± 0.01) – 2(10.5 ± 0.05)] = 0.34 ± 0.06 bp. There are 360 deg/10.5 bp, so the DNA unwinding due to one intrastrand CL of cisplatin is 12 ± 2°. This unwinding angle is very similar to that afforded by the 1,2-GG interstrand CL of monofunctional adduct of [PtCl(dien)]Cl which only unwinds DNA negligibly (*16, 17*).

The evaluation of the relationship between interadduct distance and phasing for self-ligated multimers composed of the identical number of monomeric duplexes (bend units) resulted in a bell-shaped pattern (Figure 5B) characteristic for bending (*9, 18-23*). The quantitation of the bend angle of the monofunctional adduct of *trans*-PtTz was performed in the way described previously (*9, 18-23*) utilizing the empirical equation

$$K - 1 = (9.6 \times 10^{-5} L^2 - 0.47)(\mathrm{RC})^2 \quad (1)$$

where L represents the length of a particular oligomer with relative mobility K and RC the curvature relative to a DNA bending induced at the tract of A residues (A tract) (*18, 24*). Application of Eq. 1 to the 126-bp multimers of the 21-bp oligomers of TGT duplexes containing the single, monofunctional adduct of *trans*-PtTz leads to a mean curvature of 0.85, relative to the A tract. The average bend angle per two helix turns can be calculated by multiplying the relative curvature by the absolute value of the A tract bend [20° (*18, 19, 24, 25*)]. The results indicate that the bend induced by the monofunctional adduct of *trans*-PtTz is about 34°. Application of Eq. 1 to the 110-bp and 130-bp multimers of the 21-bp oligomers yielded the same result. We assigned the bend direction by reference to an A tract, which is bent by ~20° toward the minor groove (*24*) using the same procedure as in the previously published paper (*22*). The duplex [TGT+$(A/T)_5$](32) (Figure 1D) was used which also

contained, besides the single monofunctional adduct of *trans*-PtTz, the A tract located "in phase" from the adduct (the platinated site and the center of the A tract were separated by 11 bp). In these platinated multimers, the adducts or the A tracts were separated by 33 bp, corresponding to about three helical turns after the incorporation of the estimated 12° of unwinding at the lesion (*vide supra*). The platinated multimers of duplexes [TGT+$(A/T)_5$](32) were in all cases less retarded than their unplatinated counterparts (not shown). Hence, the effective bend of the helix axis at the site of the monofunctional adduct of *trans*-PtTz is in the opposite direction as that at the center of the A tract, i.e. the monofunctional adduct of *trans*-PtTz bends DNA toward the major groove. Other details of the calculations of the unwinding and bending angles are given in the previously published papers (*9, 18-23*).

Also produced in ligations of monomers 20-23 bp long investigated in this work were separate bands arising from small DNA circles that migrate close to the top of the gel (see the bands marked by asterisk in Figure S1A, lane Pt for the 21-mer as example). The occurrence of small DNA circles was even better evident if platinum complex was removed from the products of the ligation reaction by NaCN (not shown). The highest tendency to yield DNA circles was observed for the 21-bp intrastrand crosslinked multimers confirming a close match between the 21-bp sequence repeat and the helix screw (*18, 26*). Interestingly, the ligation products of the intrastrand crosslinked 21-bp duplexes contained several types of DNA circles.

The bending and local unwinding induced by the monofunctional adduct of *trans*-PtTz is very similar to those afforded by the 1,2-GG interstrand CL of cisplatin using the same experimental procedure (32-34° and 13°, respectively (*12, 13*), but in a distinct contrast to the monofunctional adduct of [PtCl(dien)]Cl which does not bend DNA and unwinds it only negligibly (*16, 17*).

Chemical Probes of DNA Conformation. Our studies have revealed (*vide supra*) that the monofunctional adduct of *trans*-PtTz distorts DNA conformation. Therefore, further studies of the present work were also focused on analysis of this distortion by chemical probes of DNA conformation using the duplex containing the single, site-specific monofunctional adduct of *trans*-PtTz. The platinated duplex TGT (Figure 1B) was treated with several chemical agents that are used as tools for monitoring the existence of conformations other than canonical B-DNA. These agents include $KMnO_4$, DEPC and bromine. They react preferentially with single-stranded DNA and distorted double-stranded DNA (*21, 27-30*). As we used for this analysis exactly the same methodology described in detail in our recent papers aimed at DNA adducts of various antitumor platinum drugs (*11, 31*), the results are only commented briefly.

$KMnO_4$ is hyperreactive with thymine (T) residues in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (*27, 29, 32, 33*). The platinated duplex showed strong reactivity of the 5‘ T residue adjacent to the adduct (Figure S2C, lane trans-PtTz).

DEPC carbetoxylates purines at the N(7) position. It is hyperreactive with unpaired and distorted adenine (A) residues in DNA and with left-handed Z-DNA (*27, 29, 34, 35*). Within the duplex containing the monofunctional adduct of *trans*-PtTz the A residue complementary to the strongly reactive T residue of the top strand was also strongly reactive (Figure S2B, lane trans-PtTz).

Bromination of C residues and formation of piperidine-labile sites are observed when two simple salts, KBr and $KHSO_5$ are allowed to react with single-stranded or distorted double-stranded oligonucleotides (*28*). Within the duplex containing the monofunctional adduct of *trans*-PtTz the C residue in the bottom strand complementary to the platinated G residues was strongly reactive (Figure S2A, lane trans-PtTz).

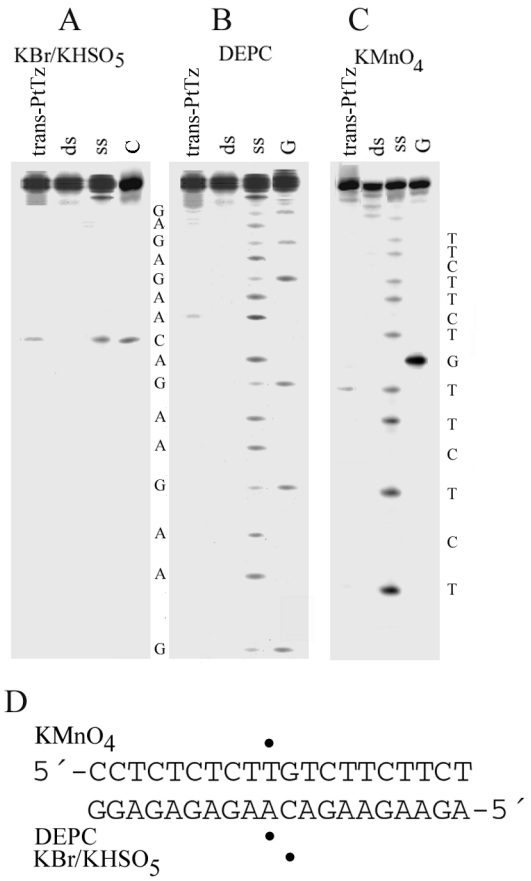

FIGURE S2. Chemical probes of DNA conformation. Piperidine-induced specific strand cleavage at $KBr/KHSO_5$-modified (A) and DEPC-modified (B) and $KMnO_4$-modified (C) bases in the 20-bp duplex TGT (Figure 1B) unplatinated or containing single, monofunctional adduct of *trans*-PtTz. The oligomers were 5'-end labeled at their top (C) or bottom (A,B) strands. Lanes: ss, the unplatinated strand; ds, the unplatinated duplex; trans-PtTz, the duplex containing a unique monofunctional adduct of *trans*-PtTz at the central G in the top strand; C and G, a Maxam-Gilbert specific reaction for the unplatinated duplex. D. Summary of the reactivity of chemical probes.

The results of the analysis of the duplex TGT containing the monofunctional adduct of *trans*-PtTz by chemical probes are summarized in Figure S2D. These results indicate that this adduct induces in DNA the distortion that extends over at least 2 bp and is localized mainly at the platinated base pair and that on its 5' side.

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