DNA-protein cross-linking by *trans*-[$PtCl_2$(*E*-iminoether)$_2$]. A concept for activation of the *trans* geometry in platinum antitumor complexes

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Supplementary material

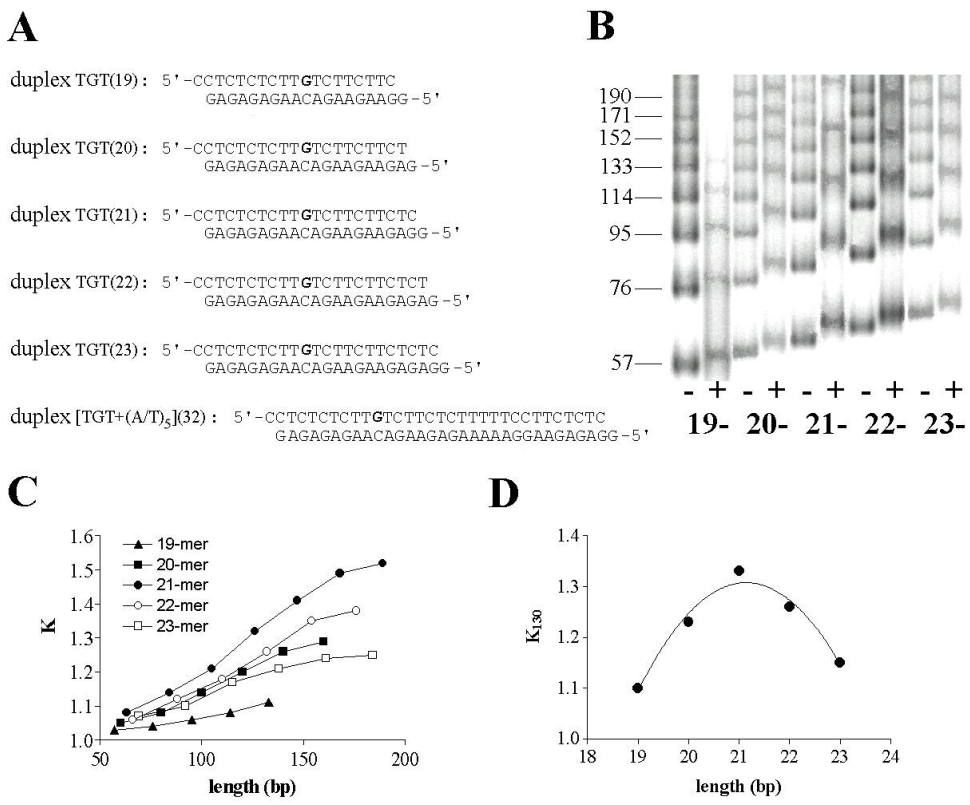

Figure S1. Analysis of the mobility of the ligation products of the 19-23 bp duplexes TGT unplatinated or containing a single, monofunctional adduct of *trans-EE* in an 8 % PAA gel. A. sequences of the synthetic oligodeoxyribonucleotides with their abbreviations. The top and bottom strands of each pair are designated top and bottom, respectively, in the text. The bold letter (G) in the top strands of all duplexes indicate the location of the monofunctional adduct after modification of the oligonucleotides by *trans-EE* in the way described in the experimental section. B. Autoradiogram of the ligation products of the TGT duplexes. Lanes: minus, unplatinated duplexes; plus, the duplexes containing the adduct. C. Plots showing the relative mobility *K versus* sequence length curves for the oligomers containing the adduct. D. Plots showing the relative mobility *K versus* interadduct distance in bp for the oligomers TGT(19-23) modified by *trans-EE* with a total length of 130 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 $K = ad^2 + bd + c$ (Bellon, S.F., Coleman, J.H. and Lippard, S.J. (1991) DNA unwinding produced by site-specific intrastrand cross- links of the antitumor drug cis-diamminedichloroplatinum(II). *Biochemistry*, **30,** 8026-8035).

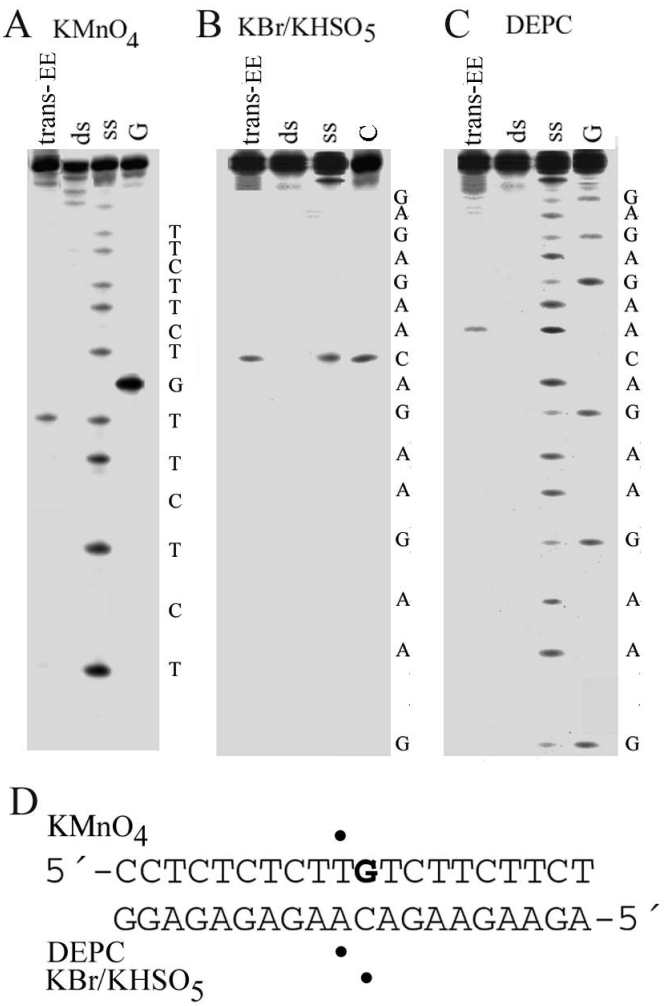

Figure S2. Chemical probes of DNA conformation. Piperidine-induced specific strand cleavage at $KMnO_4$-modified (A), $KBr/KHSO_5$-modified (B) and DEPC-modified (C) bases in the 20-bp duplex TGT (Figure S2D) unplatinated or containing single, monofunctional adduct of *trans-EE*. The oligomers were 5'-end labeled at their top (A) or bottom (B,C) strands. Lanes: ss, the unplatinated strand; ds, the unplatinated duplex; trans-EE, the duplex containing a unique, monofunctional adduct of *trans-EE* 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.

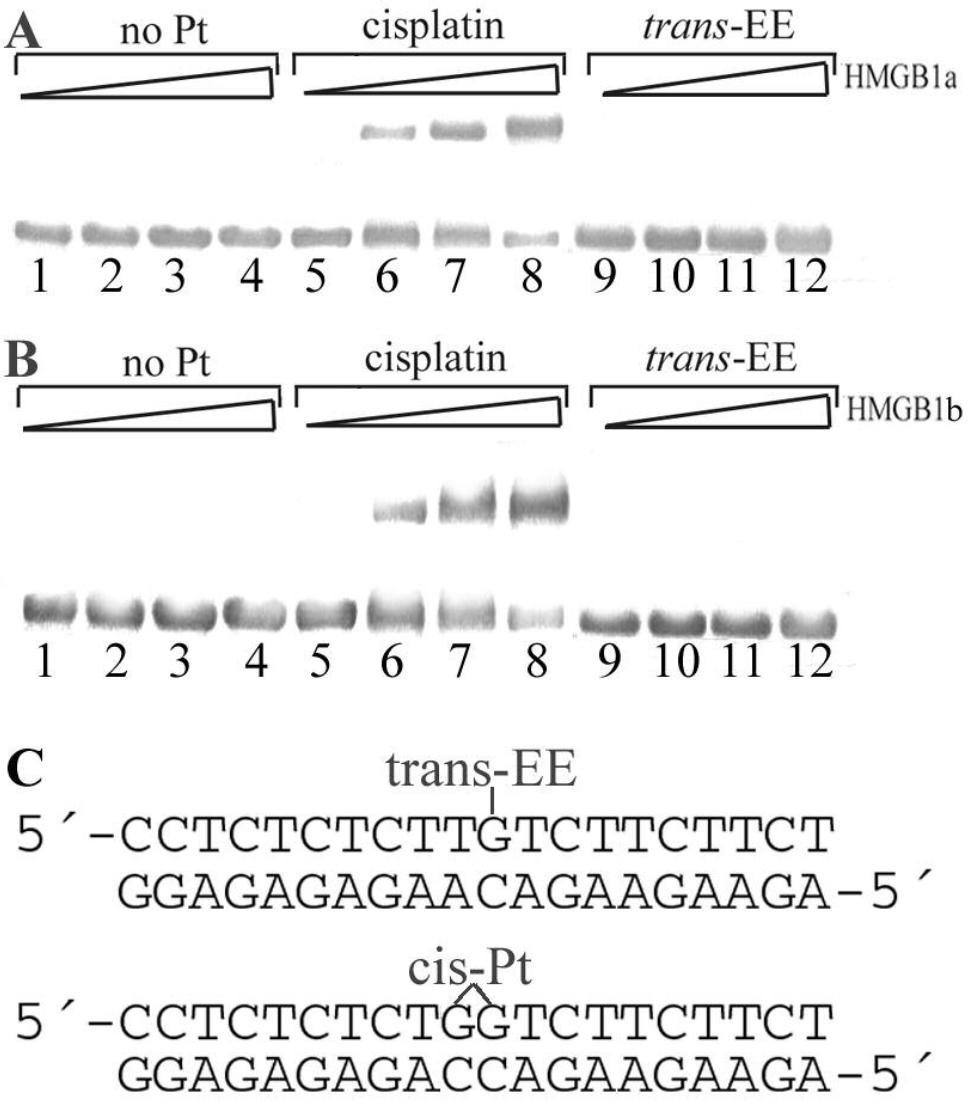

Figure S3. Analysis of the binding affinity of platinated 20-bp DNA probes containing monofunctional adduct of *trans-EE* or 1,2-GG intrastrand cross-link of cisplatin to HMGB1 domain proteins in a 6% PAA gel. A. HMGB1a. B. HMGB1b. The oligonucleotide duplexes (see Figure S3C for their sequences) (10 nM) were either nonplatinated (lanes1-4) or contained 1,2-GG intrastrand cross-link of cisplatin (lanes 5-8) or monofunctional adduct of *trans-EE* (lanes 9-12). Lanes in the panel A. 1, 5, 9: no protein; 2, 6, 11: 9.6 nM HMGB1a; 3, 7, 11: 28 nM HMGB1a; 4, 8, 12: 96 nM HMGB1a. Lanes in the panel B. 1, 5, 9: no protein; 2, 6, 10: 0.1 μM HMGB1b; 3, 7, 11: 0.5 μM HMGB1b; 4, 8, 12: 1.0 μM HMGB1b.