Supporting Information for:

Solution Structure of a DNA Duplex Containing the Potent Anti-Poxvirus Agent Cidofovir

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Primer Extension Analysis. The CDV-containing and control dodecamers were first separately ligated to an 18-mer template extension oligonucleotide [5′-phosphate-

d(AGAAGAGAGAGAAGAGAG)-3′] in the presence of an IRDye700®-labelled primer [5′-IRDye700®-d(CTCTCTTCTCTCTCTTCTGCGTA)-3′] to create primer-template substrates (Figure S1A). Briefly, 21 nmol of template extension oligonucleotide, 21 nmol of CDV-containing or control oligonucleotide and 7 nmol of labelled primer were mixed, incubated at 55°C for 5 min and cooled to room temperature. One unit of T4 DNA ligase (Fermentas) was added and the reactions incubated overnight at 16°C. Negative control reactions without ligase were also prepared in parallel. Each reaction mixture was purified by phenol extraction, the aqueous layers desalted using G-50 MicroSpin columns (GE Healthcare), and the DNA collected by ethanol precipitation. After resuspension in 49 µl ddH_2O, primer extension reaction mixtures were prepared by the addition of polymerase buffer [30 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 70 mM NaCl, 1.8 mM dithiothreitol, 80 µg/ml bovine serum albumin], 50 µM of each dNTP and 25 ng/ml purified vaccinia virus DNA polymerase.[1] Reaction mixtures were incubated at 37°C and at 0, 1, 2, 5, 10 and 15 min, 10 µl aliquots were removed and added to 5 µl gel loading buffer [80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml bromophenol blue]. Reaction products were separated on a 15% denaturing polyacrylamide gel run at 50 W for 1 hr in Tris-borate-EDTA. The gel was transferred to cellophane and scanned using an Odyssey scanner (Li-Cor) at 169 µm resolution, medium quality and an intensity of 5.0.

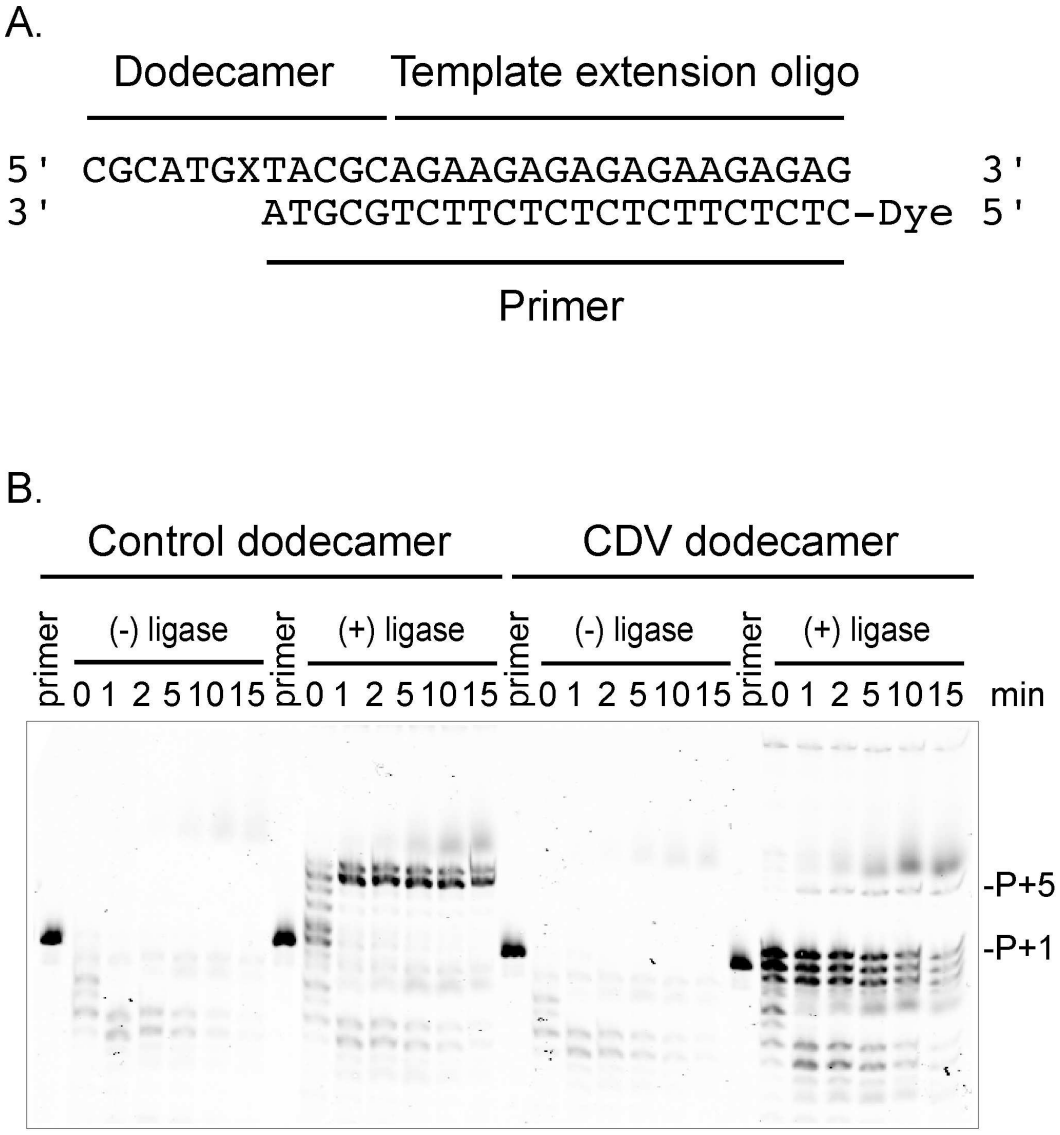

Figure S1. The presence of CDV in the template strand inhibits DNA synthesis across the drug lesion. (A) Primer-template substrates. "X" in the dodecamer indicates CDV; this residue is replaced by dCMP in the control DNA dodecamer. The dodecamers and template extension oligonucleotide are not ligated in the negative control substrates. (B) Primer extension assay. The primer-template substrates shown in (A) were incubated with vaccinia virus DNA polymerase and 200 μM dNTPs at 37°C. Samples were taken at 0, 1, 2, 5, 10 and 15 min, stopped with gel loading buffer and the reaction products resolved by denaturing PAGE.

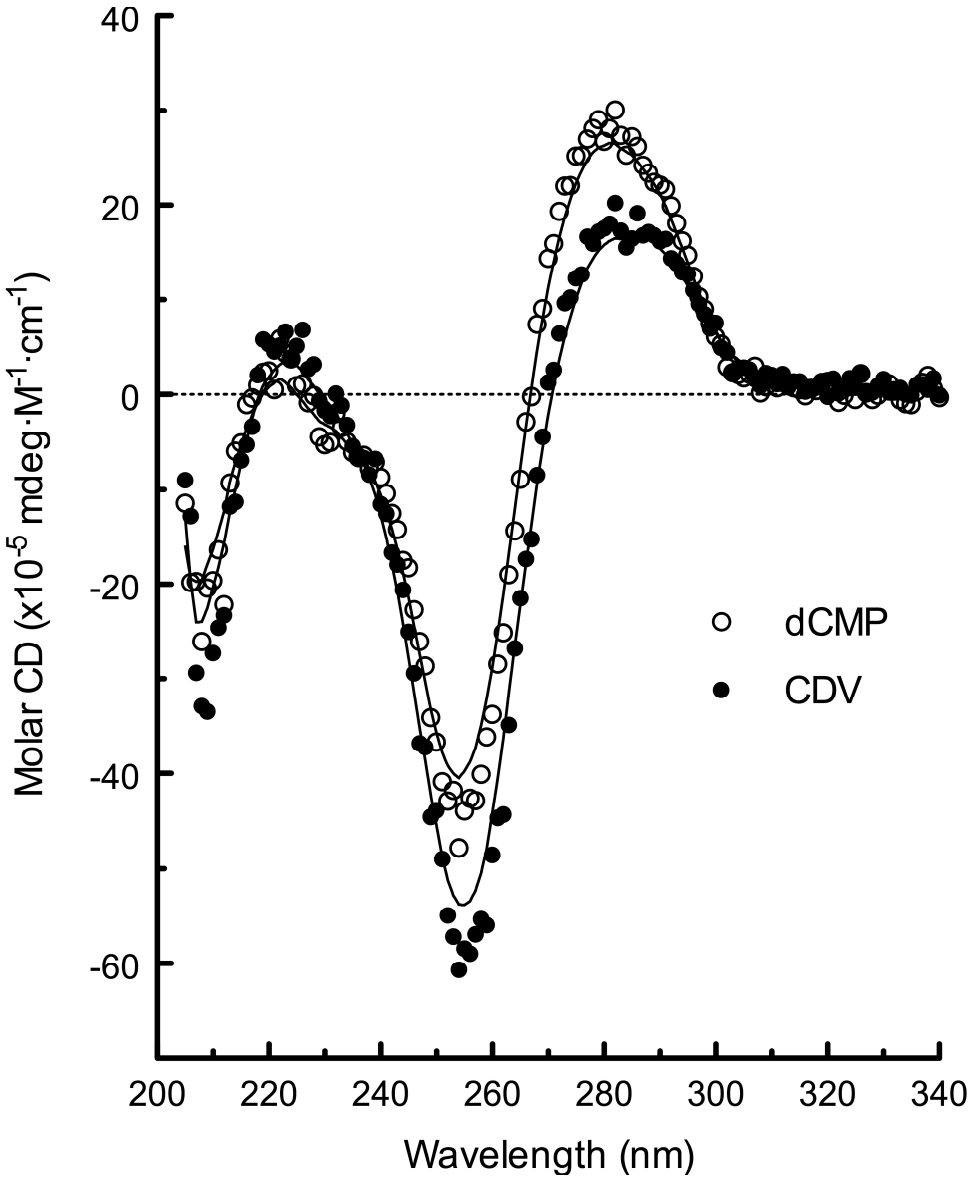

Figure S2. CD spectra of CDV- and dCMP-containing DNA duplexes at 20°C. Each sample was dissolved in 10 mM sodium phosphate (pH 7.3), 185 mM sodium chloride and scanned from 205 to 360 nm in 1-nm increments. A buffer baseline spectrum was also recorded and subtracted from the data presented here. The CDV-containing DNA exhibits spectral properties characteristic of a well-ordered B-DNA duplex, although some perturbation of the structure is illustrated by alterations in the peak intensities at 255 and 282 nm.

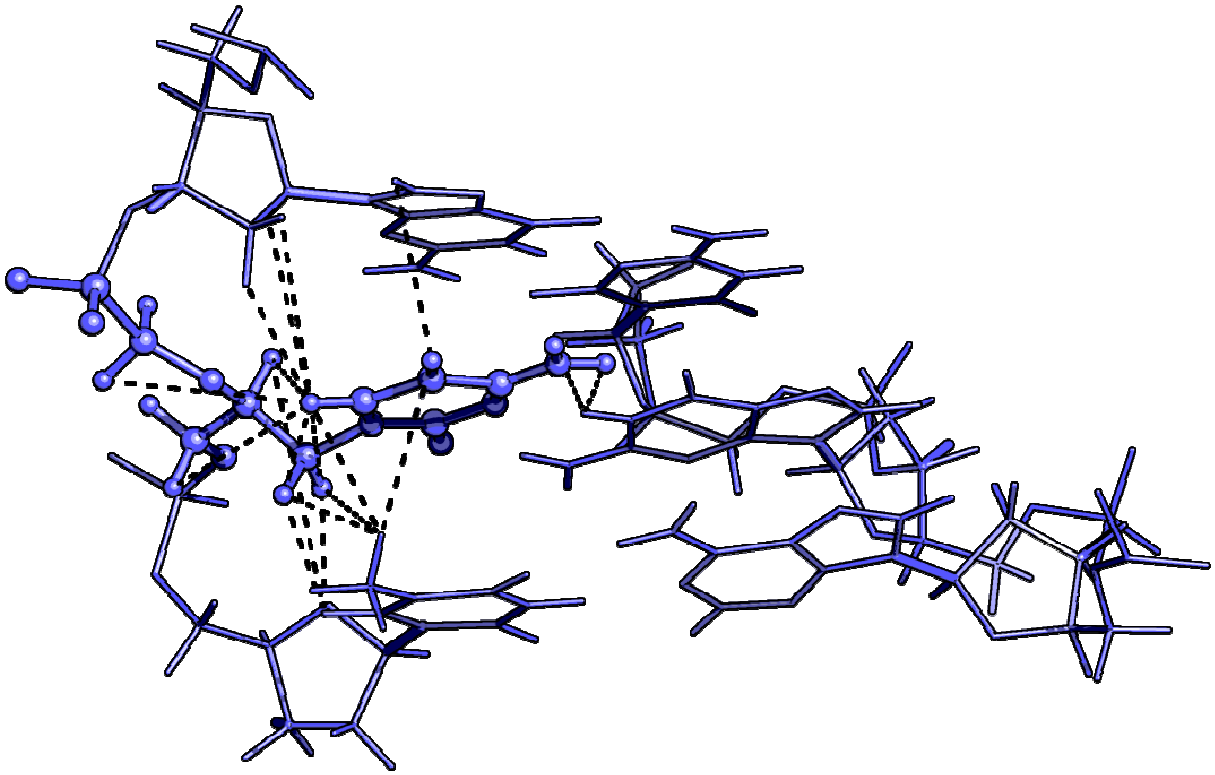

Figure S3. The position of X7 in the DNA duplex is defined by 18 NOE distances represented by black dashed lines: (7.H5, 6.H8), (7.H6, 6.H1′), (7.H6, 6.H2′), (7.H6, 6.H2″), (7.H6, 7.H4′), (7.H6, 7.H2′), (7.H6, 7.H3″), (7.H6, 7.H1′), (7.H6, 7.H1″), (8.H6, 7.H2′), (8.H6, 7.H1′), (8.H6, 7.H1″), (8.M7, 7.H1′), (8.M7, 7.H1″), (8.M7, 7.H5), (8.M7, 7.H6), (18.H1, 7.H42), (18.H1, 7.H41).

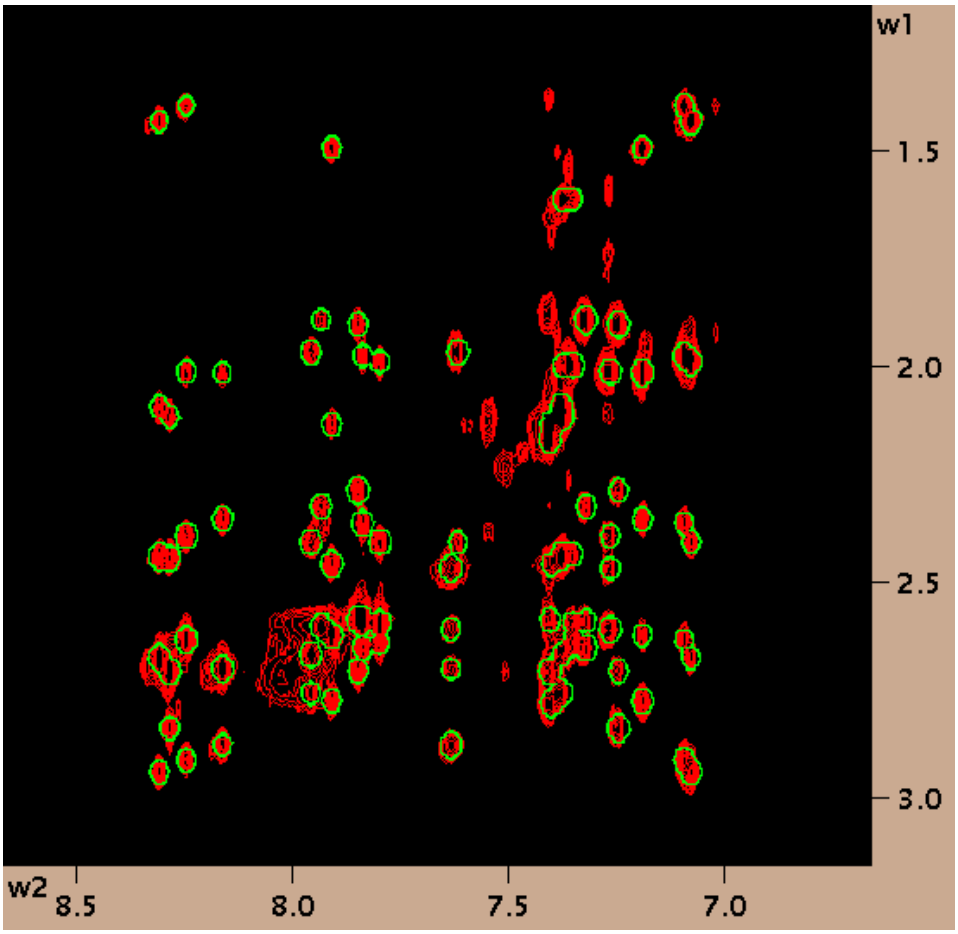

Figure S4. Portion of the back-calculated NOESY spectrum for the control DNA duplex. The observed spectrum is colored in red and the reconstructed one in green. Extra peaks in the observed spectrum correspond to unassigned resonances. The back-calculation was performed using a complete relaxation matrix analysis method (CORMA[2]) using a 80 ms mixing time and a theoretical isotropic correlation time of 2 ns. The low values of the sixth root squared R factor (0.128 ± 0.009 Å for the control DNA duplex, and 0.117 ± 0.007 Å for the CDV DNA duplex) reflects the high level of similarity between the experimental and back-calculated spectra.[3] The spectra overlay was generated using SPARKY (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

REFERENCES

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(3) Thomas, P. D.; Basus, V. J.; James, T. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1237-1241.