Supplementary Material (ESI) for Chemical Communications

Crystal Structure of Tricyclo-DNA: An Unusual Compensatory Change of Two Adjacent Backbone Torsion Angles

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Supporting Information

Space group	trigonal $P3_2$
Unit cell constants [Å]	a = 26.59, b = 26.59, c = 98.24
Resolution [Å]	1.75
No. of unique reflections	6,883
Completeness (1.79 – 1.75 Å) [%]	88.0 (60.2)
R-merge (1.79 – 1.75 Å) [%]	4.9 (15.7)
R-work (1.79 – 1.75 Å) [%]	17.6 (18.9)
R-free [%]	22.5
No. of DNA atoms	492
No. of waters	95
R.m.s. deviations bonds [Å]	0.007
R.m.s. deviations angles [°]	1.8
Average B-factor [Å^2]	23.5

Table S1. Selected crystal and refinement data.

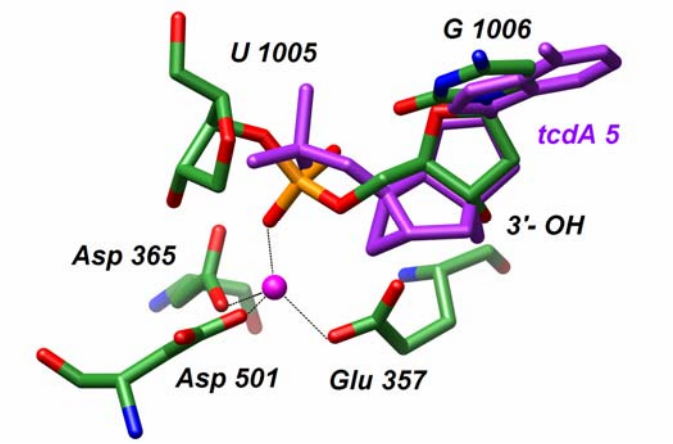

Figure S1. A potential steric origin of the nuclease resistance of tc-DNA. The tcdA nucleoside (magenta) superimposed on a 2′-*O*-(3-aminopropyl)-modified residue (2′-*O*-AP-G1006) in the crystal structure of the complex between a DNA fragment containing 3′-terminal 2′-*O*-AP-RNA residues and the *E. coli* DNA polymerase I Klenow fragment (PDB ID code 1d9d).[S1] The nucleobase of residue U1005 and the 2′-AP substituents have been deleted for clarity. Only one of the two metal ions (Zn^{2+}) at the active site of the Klenow fragment is depicted. The orientation of the tc-DNA residue shown here would result in a clash with the carboxylate of Glu357 and render impossible coordination of a water molecule that serves as the nucleophile in the phosphodiester hydrolysis reaction.

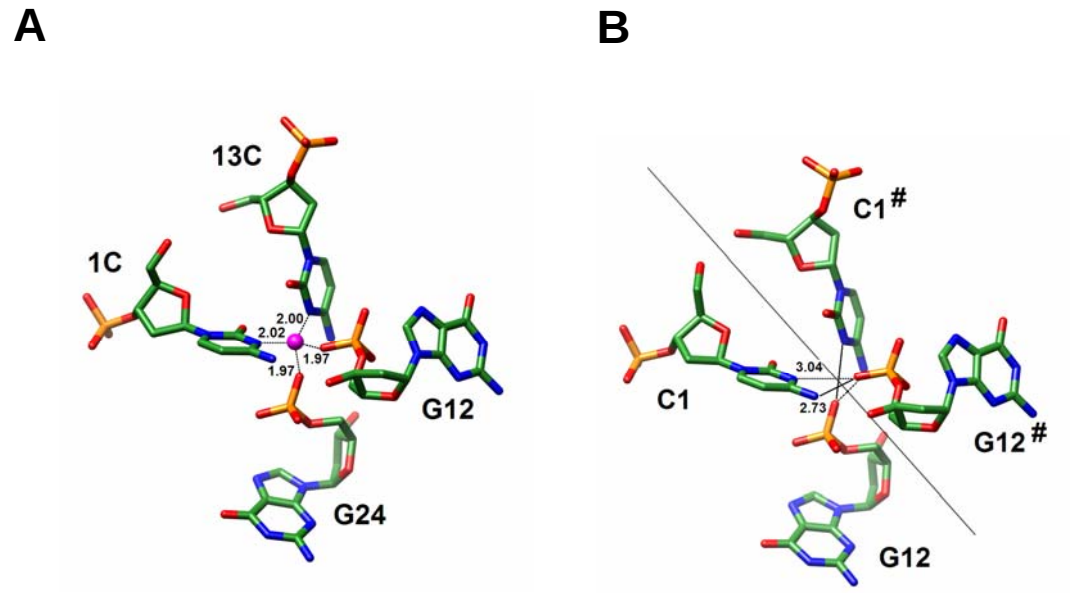

Figure S2. (A) Close-up view of the Zn^{2+} binding site in the crystal structure of the DDD with single tcdA modifications. (B) In the structure of the native DDD[15] the corresponding residues are engaged in direct interactions. The particular geometry indicates that cytidines are likely protonated at N3. Since the duplex in the native structure sits on a dyad (indicated by a thin line), the orientations of C1 and C13($C1^{\#}$) relative to the phosphates of G12 and G24($G12^{\#}$), respectively, are identical.

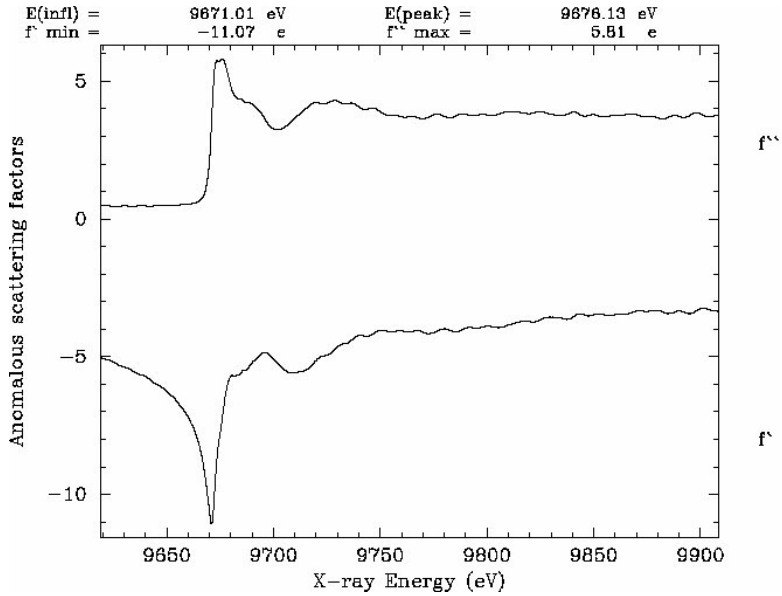

Figure S3. X-ray absorption spectrum of a crystal of [d(CGCGA)-tc(A)-d(TTCGCG)]$_2$.

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Experimental Section

Oligonucleotide Synthesis: The synthesis of the [(5′R,6′R)-2′-deoxy-3′,5′-ethano-5′,6′-methano-β-D-ribofuranosyl]thymine and –adenine nucleoside building blocks and the corresponding phosphoramidites was carried out according to published procedures.[S1] All oligonucleotides were synthesized as described previously[S2] on a 1.3 μM scale using a Pharmacia Gene Assembler Plus DNA synthesizer, and were purified by HPLC and analyzed by ESI+ mass spectrometry.

UV Melting Temperatures: We measured the stability of the native DDD and the tcdA-modified DDD under two conditions. At 1 M NaCl in 10 mM NaH_2PO_4, pH 7.0, and an 8 μM concentration of DNA (260 nm): tcdA-modified DDD Tm = 68.0°C and DDD = 67.0°C. At 150 mM NaCl in 10 mM NaH_2PO_4, pH 7.0, and a 4 μM concentration of DNA (260 nm): tcdA-modified DDD Tm = 53.0°C and DDD = 52.0°C.

Crystallization and Data Collection: Initial crystallization conditions of the tcdA-modified DDD were established using standard sparse matrix crystallization screens (Hampton Research, Aliso Viejo, CA). Crystals were grown at 18°C by the hanging drop vapor diffusion method. Droplets (2 μL) containing 0.75 mM oligonucleotide, 20 mM sodium cacodylate (pH 7.0), 6 mM spermine tetrahydrochloride, 6 mM NaCl, 40 mM KCl and 5% (v/v) 2-methyl-2,4-pentanediol (MPD) were equilibrated against a reservoir of 0.7 mL 35% MPD. Crystals of reasonable size appeared after ca. 1 week. For data collection, a single crystal was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120 K). X-ray diffraction data were collected on the X29 beamline (λ = 1.08 Å) of the National Synchrotron Light Source (NSLS, Brookhaven, NY), using a Quantum CCD detector. Data were integrated and merged in both Laue groups 3 and 3m using the program HKL2000.[S3] Selected crystal data and data quality and completeness are summarized in Table S1. X-ray absorption spectra were recorded on the DND-CAT 5-ID beamline at the Advanced Photon Source (APS, Argonne, IL).

Reference

S1 R. Steffens, C. J. Leumann, *Helv. Chim. Acta* 1997, **80**, 2426-2439.
S2 R. Steffens, C. J. Leumann, *J. Am. Chem. Soc.* 1997, **119,** 11548-11549.
S3 Z. Otwinowski, W. Minor, *Methods Enzymol*. 1997, **276**, 307-326.