Supplementary Material (ESI) for Chemical Communications
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Supporting Information

A Conformational Transition in the Structure of a 2′-Thiomethyl-Modified DNA Visualized at High Resolution

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Oligonucleotide synthesis: Both modified DNA oligonucleotides GCGTAU*ACGC and CGCGAAU*U*CGCG (U*=2′-*S*Me-rU) were synthesized via the solid-phase phosphoramidite approach and following published procedures.[S1] The 10mer and 12mer were purified by reverse phase HPLC and characterized by ES-MS and their purities were >95% as evaluated by capillary gel electrophoresis.

Crystallization and data collection: Crystals were grown by the hanging-drop vapor diffusion technique using the Nucleic Acid Miniscreen[S2] (Hampton Research, Aliso Viejo, CA). Crystals were mounted in nylon loops without further cryo-protection and frozen in liquid nitrogen. Diffraction data were collected on insertion beamlines at sector 21 of the Life Sciences Collaborative Access Team (LS-CAT) at the Advanced Photon Source (Agonne National Laboratory, Argonne, Illinois). Diffraction data were processed with the programs HKL2000[S3] (RNase-H complex; see below) or XDS[S4] (10 and 12-mer duplexes). Both the 10mer and 12mer crystals are of space group $P2_12_12_1$, whereby the asymmetric unit contained a single duplex and two independent duplexes, respectively.

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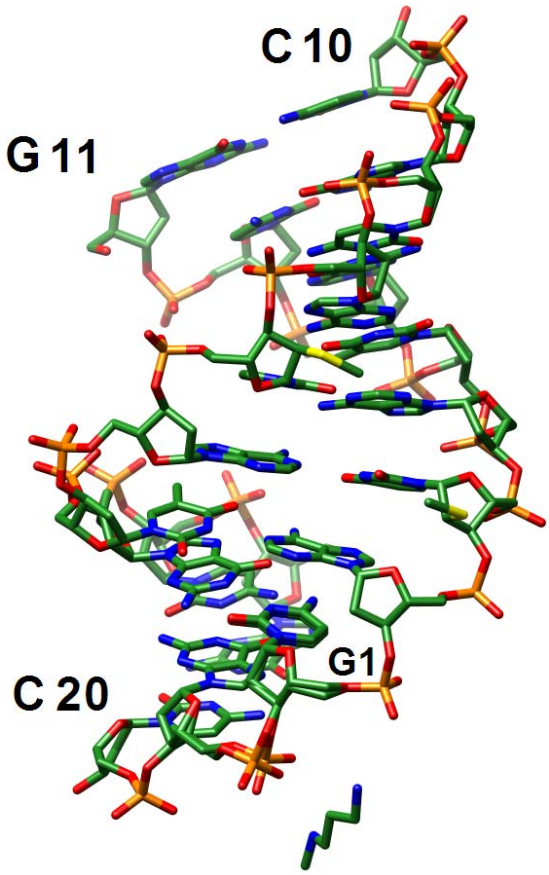

Fig. S1. Overall geometry of the A-form duplex formed by the decamer GCGTAU*ACGC. The view is into the central minor groove and a partial spermine molecule is visible near the G1:C20 terminus of the duplex. Local conformational flexibility in the backbones was resolved by applying dual occupancy for two phosphates in the crystallographic refinement.

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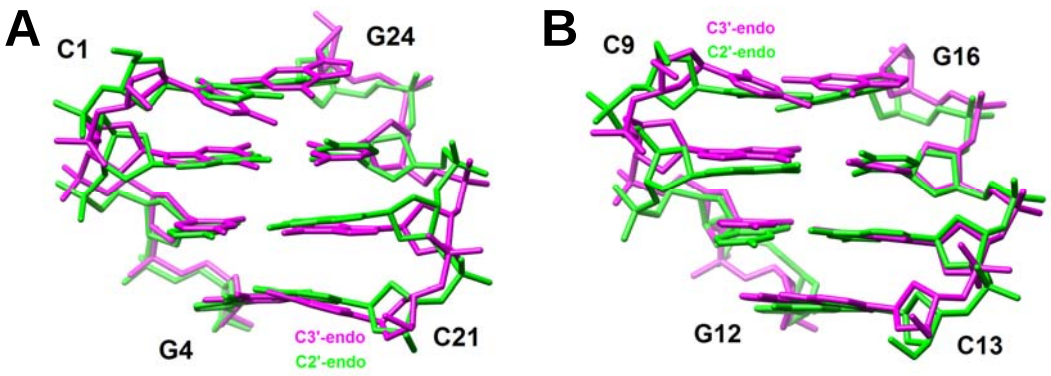

Fig. S2. Comparison between the geometries of G-tract regions in the native (green) and 2′-*S*Me-modified (purple) DDDs. Only the superimpositions of the (A) top and (B) bottom G-tracts for the first of the two modified DDD duplexes per asymmetric unit in the structure of $[CGCGAAU^*U^*CGCG]_2$ is shown. The deviations relative to the native DDD for the second duplex are very similar.

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Table S1. Selected crystal data and refinement parameters for the duplexes $[GCGTAU^*ACGC]_2$ and $[CGCGAAU^*U^*CGCG]_2$ and the complex between *B. halodurans* RNase H (*Bh*-RNase H) and $[CGCGAAU^*U^*CGCG]_2$ (U*=2′-*S*Me-U).

Parameter	**5′-CGCGAAU*U*CGCG-3′**	**5′-GCGTAU*ACGC-3′**	***Bh*-RNase H (D132N mutant)/ 5′-CGCGAAU*U*CGCG-3′**
Crystal data			
Space group	$P2_12_12_1$	$P2_12_12_1$	$C2$
No. of strands per asym. unit	4	2	1 RNase H and 1 single strand
Cell dimensions			
a, *b*, *c* (Å)	40.07 43.22 88.71	24.69 42.92 46.12	81.93 66.62 38.77
α, β, γ (°)	90.0 90.0 90.0	90.0 90.0 90.0	90.0 103.3 90.0
Data collection			
No. of reflections	43,276	23,179	26,499
Resolution (Å; last shell)	1.25 (1.33-1.25)	1.04 (1.09-1.04)	1.60 (1.66-1.60)
Completeness (%, last shell)	96.4 (92.2)	95.4 (88.6)	99.4 (96.9)
R-merge (%)	8.3	2.2	7.7
Refinement			
R-work / R-free	0.151/0.208	0.130/0.164	0.193/0.261
Number of atoms			
DNA / DNA and Protein	985	428	1,481
Water molecules	349	139	102
Ions/small molecules	7 Sr^{2+}, 2 MPD	1 partial spermine	glycerol
B-factors			
DNA / DNA and Protein ($Å^2$)	22.9	13.9	59.2
Water ($Å^2$)	39.2	14.6	45.2
R.m.s. deviations			
Bond lengths (Å)	0.013	0.016	0.009
Bond angles (Å; 1…3 dist.)	0.03	0.03	0.03

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