

## Supporting Information for:

### **Disagreement Between the Structure of the dTpT Thymine Pair Determined by NMR and Molecular Dynamics Simulations Using the Amber 14 Force Field**

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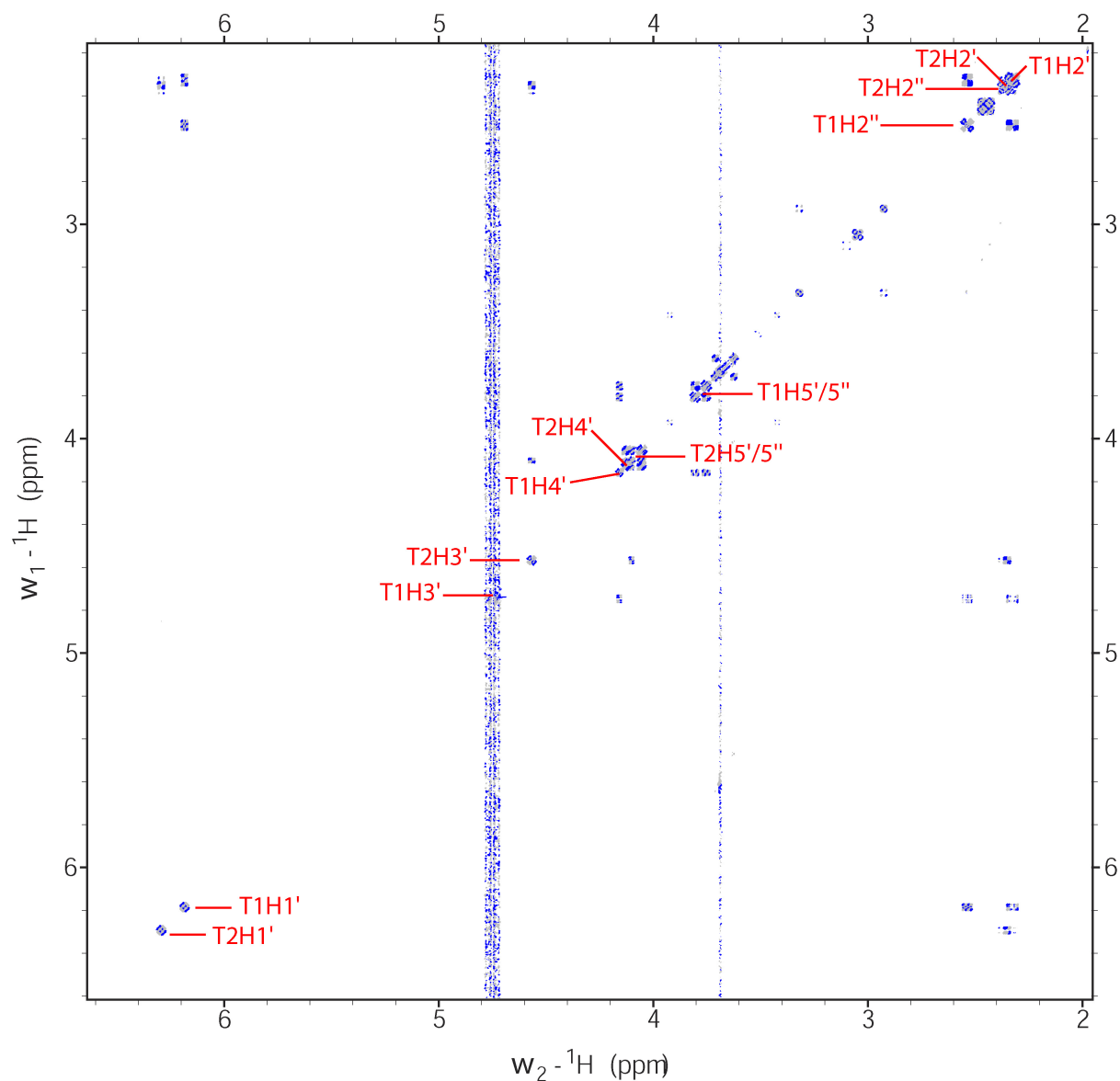
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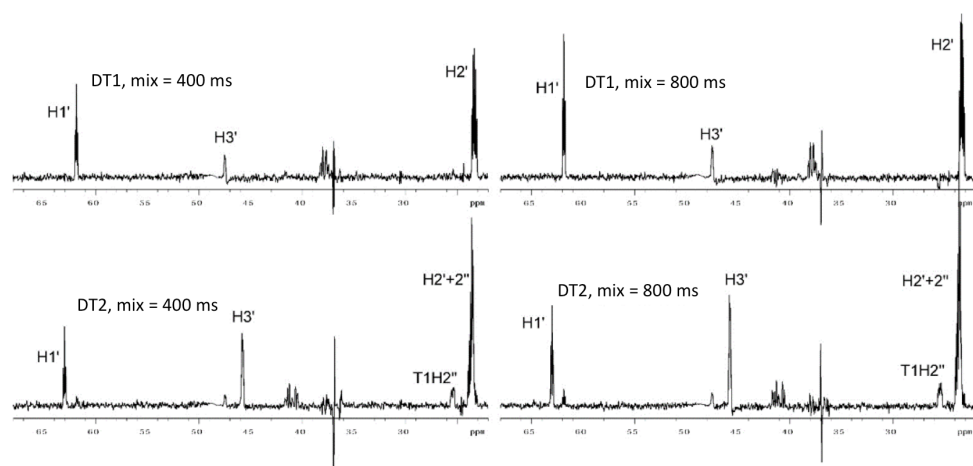
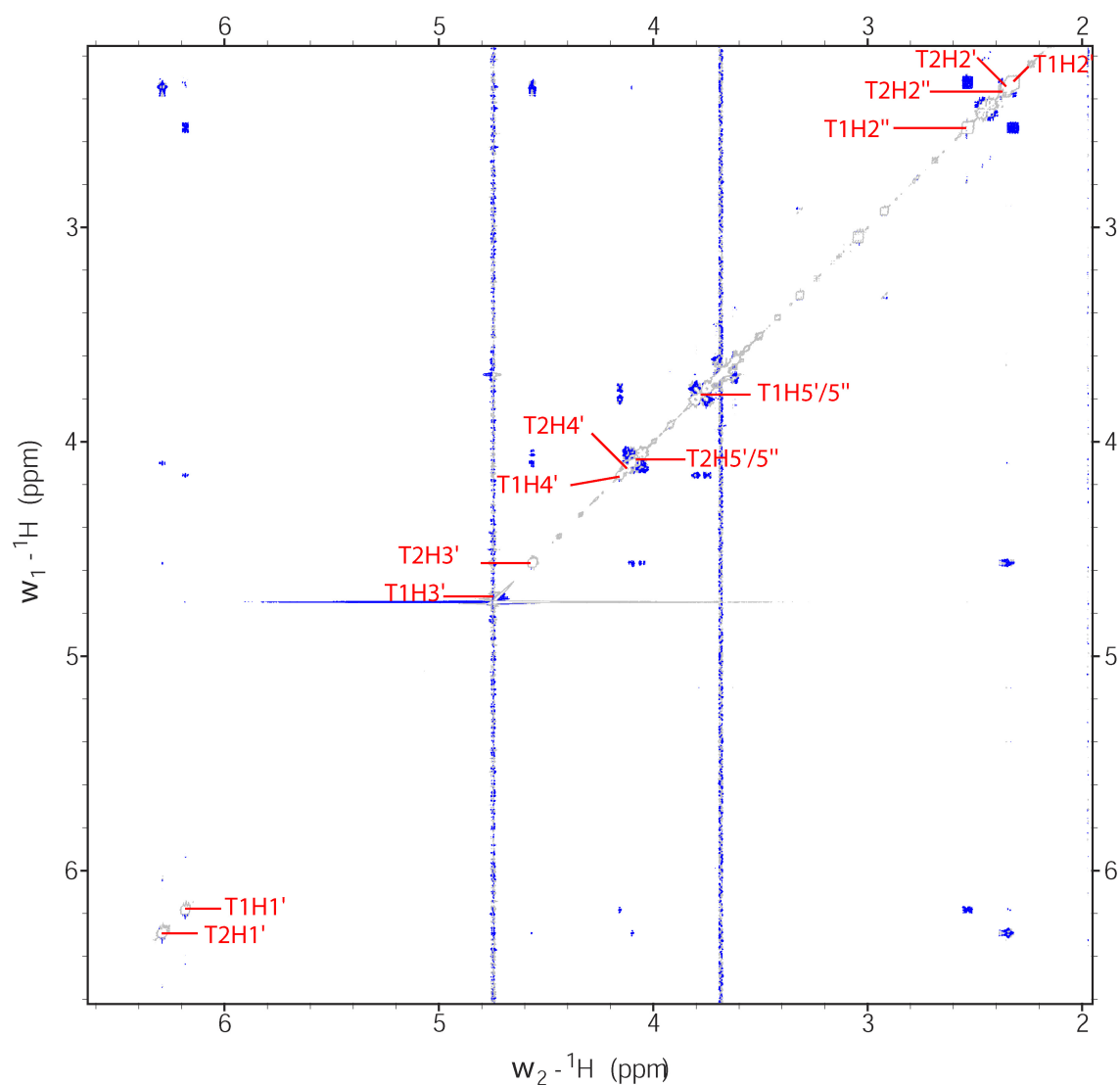
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**Table S1.** Sum of coupling constants measured from DQF-COSY and NOESY.  $\sum_{i'j'k''}$  is the sum of the spin coupling constant between  $\text{Hi}'-\text{Hj}'$  and  $\text{Hi}'-\text{Hk''}$ , where  $i'j'k''$  denotes the sugar proton  $\text{Hi}'$ ,  $\text{Hj}'$ ,  $\text{Hk''}$ , respectively. DT1 and DT2 denote the thymine 5' and 3' ends.

	$\sum_{1'2'2''}$	$\sum_{2'1'3'}$	$\sum_{3'2'2''4'}$	$\sum_{4'5'5''3'}$
	Hz			
DT1	13.53	14	13.4	11.2
DT2	13.6		14.8	9.4

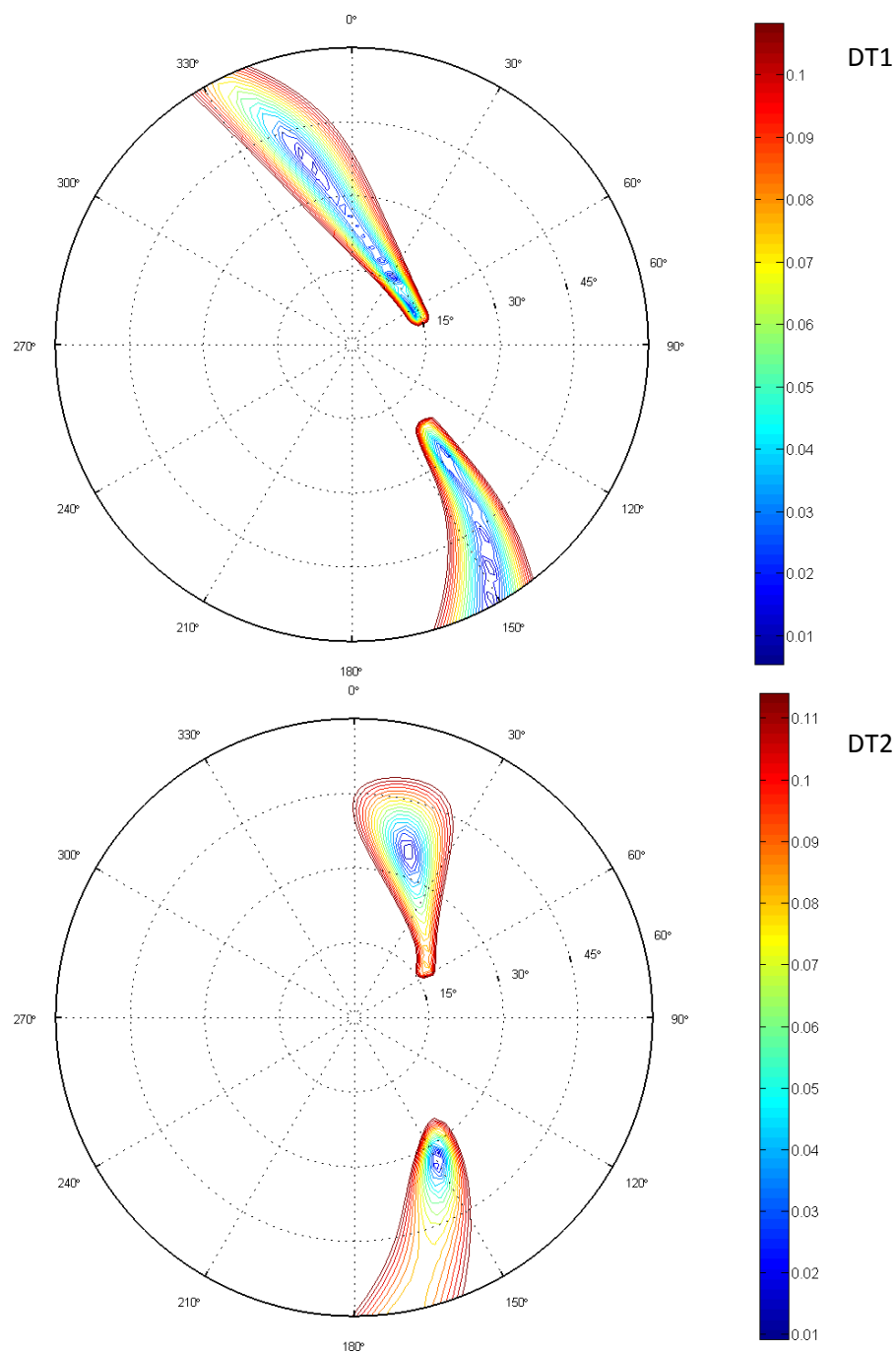


**Figure S1:** dTpT 2D DQF-COSY spectrum at 25° C. Used to assign sugar protons. Resonances of T1 are identified by the relatively upfield shift of  $\text{H5'}$  and  $\text{H5''}$ . Aromatic protons T1H6 and T2H6 are not included in this view. Note that here we label the 5' thymine protons with the “T1” prefix and the 3' thymine protons with “T2”.

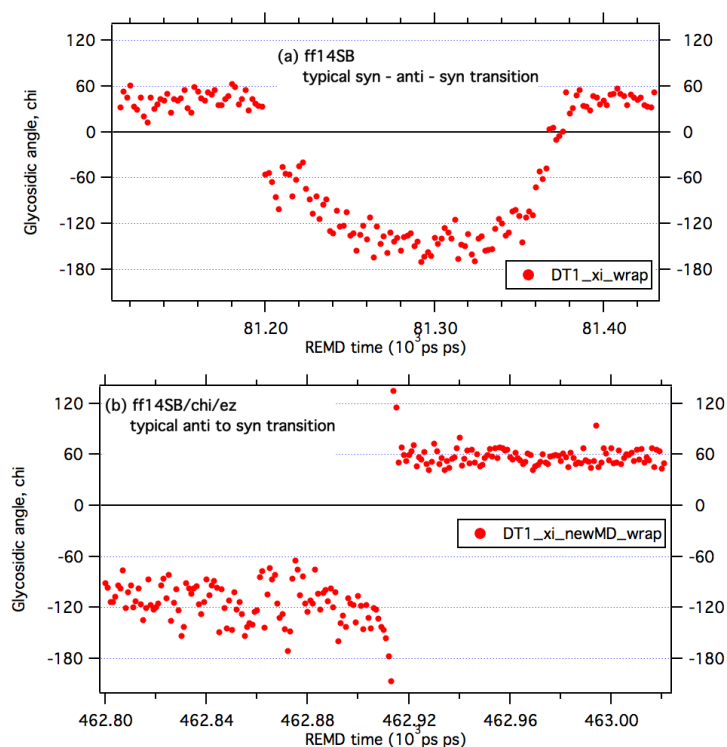


**Figure S2:** (top) Complete dTpT NOESY with mixing time of 800 ms at 25°C, (bottom) Slices from 1H-1H NOESY spectra of the thymine pair (dTpT), showing cross-peaks to the H6 protons. Each spectrum slice corresponds to H6 of either DT1, the 5' thymine, or DT2, the 3' thymine, with (left) 400 or (right) 800 ms mixing times. The peaks are labeled to indicate the atom producing the coupling to H6. The slices shown at the bottom correspond to horizontal slices through the two-

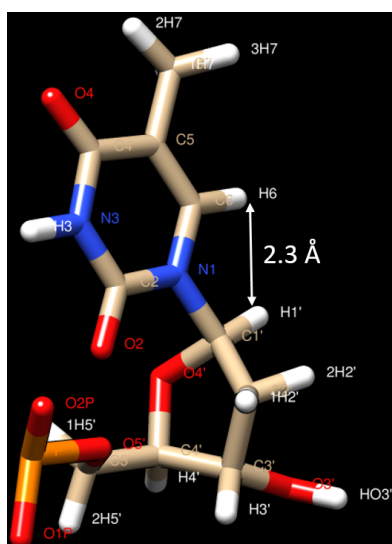
dimensional NOESY spectrum shown in the main text. Note that in the top panel, we label the 5' thymine protons with the “T1” prefix and the 3' thymine protons with “T2”.



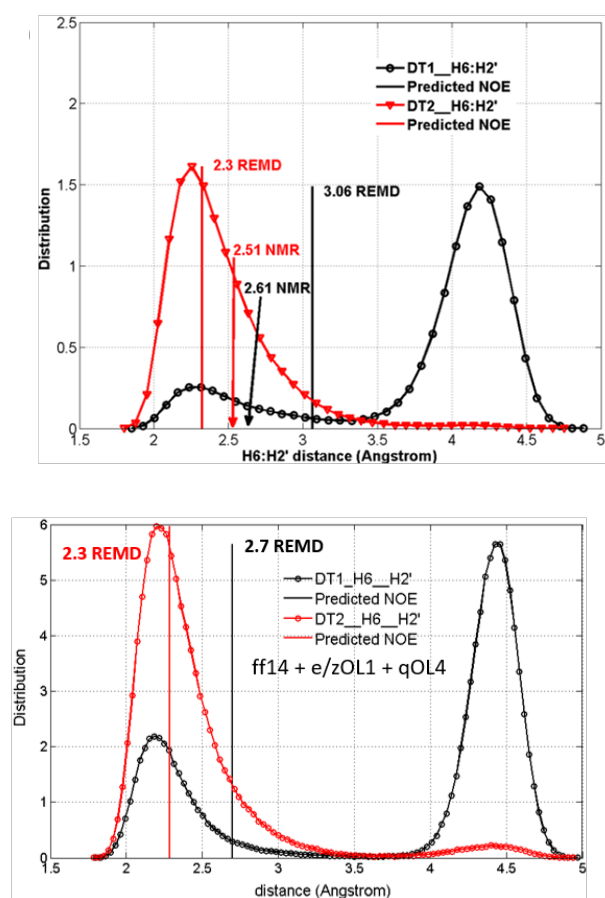
**Figure S3:** Pseudorotation wheel of the 5' (DT1) and 3' (DT2) ribose sugar in dTpT. Plots were obtained with the PSEUROT protocol implemented in the Matlab GUI of Hendrickx using the  $^3J_{HH}$  data from Table 1.<sup>1</sup> The contour line indicates the total RMSD of the scalar coupling of the sugar puckering in each nucleotide. The two sugars are predicted to be B-DNA form, with the smallest RMSDs occurring in the “south” at pseudorotations of 142° and 150°.



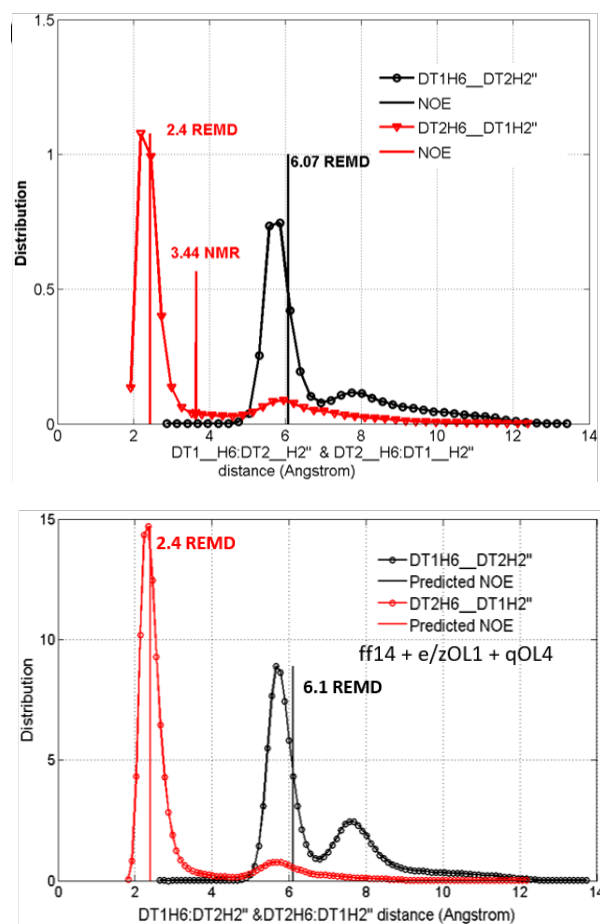
**Figure S4.** Typical examples of syn to anti flipping events occurring during the REMD simulations for (a) the ff14SB force field and for (b) the ff14SB/chi/ez force field. Note that in (a) the flipping occurs by crossing through  $\chi=0^\circ$ , due to the lower barrier at those angles in the old force field. The new force field, ff14SB/chi/ez, used in (b) imparts a large barrier at  $\chi=0^\circ$  and lowers the barrier around  $180^\circ$ , inducing flipping events that rapidly cross at  $\chi=0^\circ$ , i.e. where the thymine C2 carbonyl crosses over the ribose O4'.



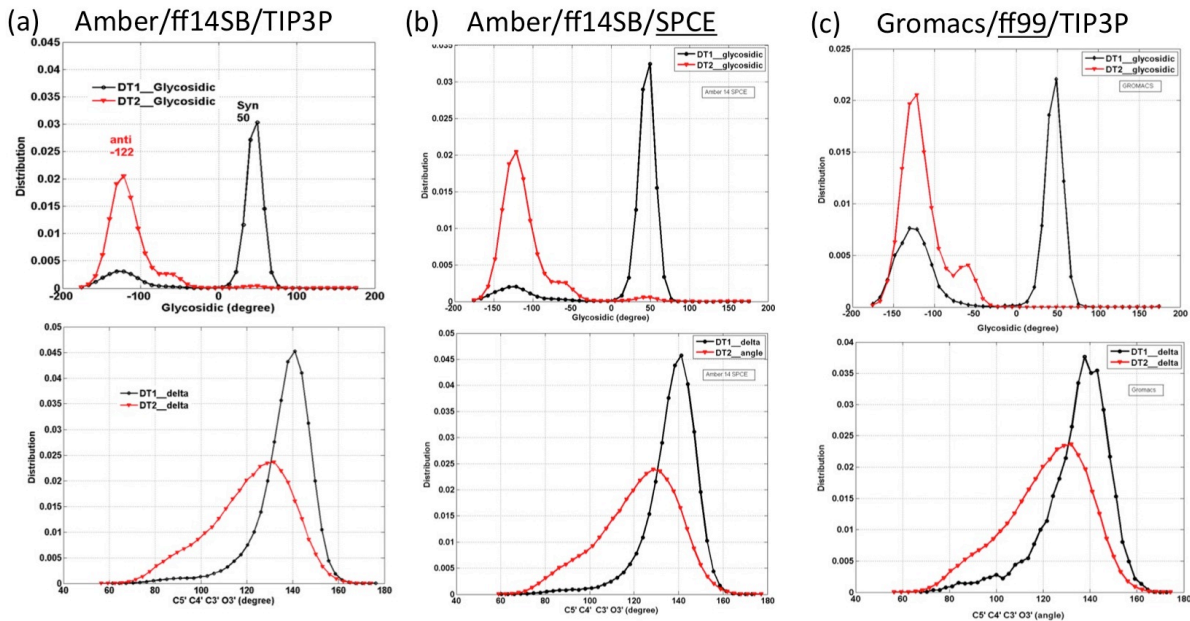
**Figure S5:** Schematic nucleotide showing the H6 and H1' protons in a *syn* conformation. The estimated proton proton distance is around 2.3 Å, which agrees with REMD folding structure Figure 6a. The structure was manually flipped from the native *anti* conformation in order to highlight the close proximity of H6 and H1'.



**Figure S6.** Population histograms of the H6:H2' distance from REMD simulations using the ff14SB (top) or ff14SB/chi/ez (bottom) force fields. Also indicated are distances derived from REMD (vertical bars) and from the NMR NOE couplings (arrows, top graph). Note that the DT1 REMD distances (3.06 and 2.7 Angstroms for ff14SB and ff14SB/chi/ez, respectively) are significantly larger than the distance from NMR (2.6 Angstroms). The REMD distance for DT2 agrees better with the NMR distance. DT1 and DT2 denote the thymine 5' and 3' ends, respectively. The vertical axis, "Distribution", is in units of probability per Angstrom.

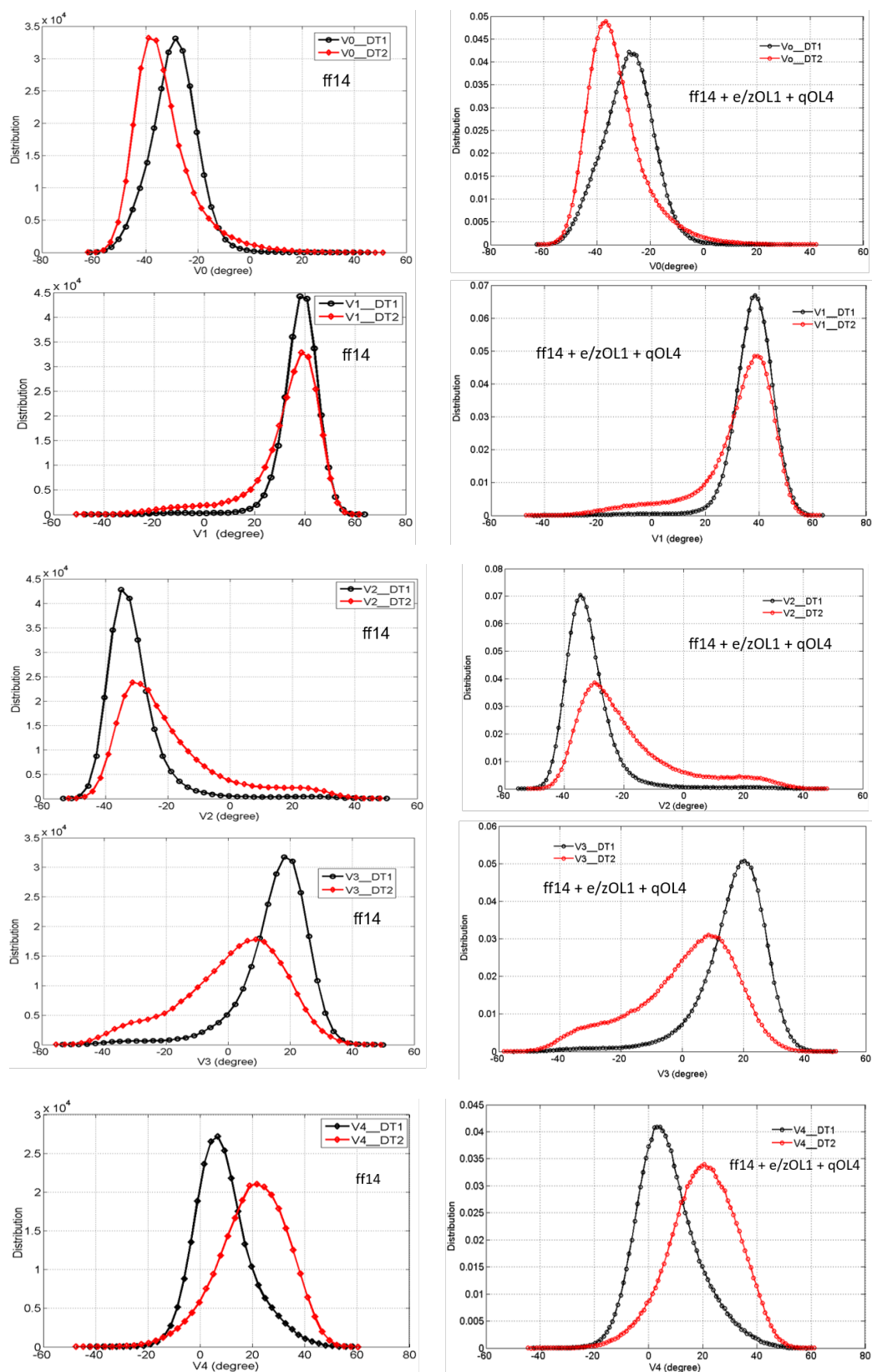


**Figure S7.** Population histograms of the H6(DTn):H2''(DTm) distance from REMD simulations using the ff14SB (top) or ff14SB/chi/ez (bottom) force fields. Also indicated are distances derived from REMD (vertical bars) and from the NMR NOE couplings (arrow at 3.44 Angstroms, top graph).

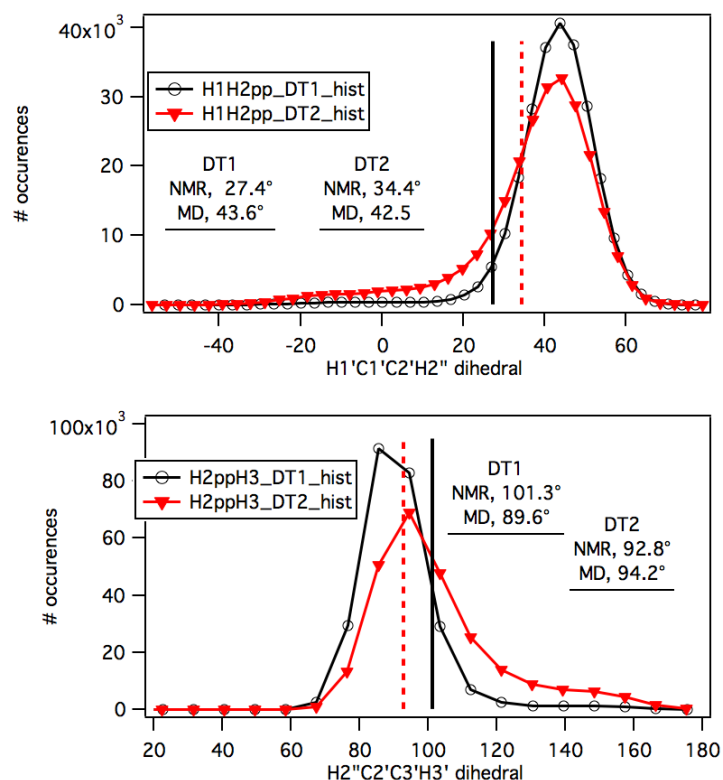


**Figure S8:** Statistics of the glycosidic and delta angle of the sugar pucker obtained from REMD using alternative water models and earlier force fields. The first column, (a), shows the results presented in the main text using Amber, the ff14SB force field and TIP3P water. The second column, (b), shows the results using a different water model, SPCE, but still running in Amber with ff14SB. In (c), we show results from using an older force field, ff99, but using the standard water, TIP3P. Note that (c) was performed in Gromacs by implementation of the “amber99sb\_ildn” parameters. Comparing (a) and (b) shows that the errors in the DT1 glycosidic angle distribution (i.e. a prediction that DT1 should be predominantly syn) are robust to changes in the water model. Although there is no change in the glycosidic dihedral potential between ff99 and ff14SB (only a change in the alpha and gamma potentials), there is still a slight change in the histogram for the DT1 glycosidic angle. The ff99 force field predicts a much larger probability of DT1 anti (~30%) than ff14SB (17%). Apparently, the bsc0 changes to the alpha and gamma potential have long distance implications on the glycosidic angle. The lower row of delta angle distributions shows that the sugar conformation is consistent despite changes in water model and force field.





**Figure S9.** Conformation of the five pseudorotation torsions  $v_0$ - $v_4$  of the sugar pucker in nucleotide DT1 and DT2. The torsional angles were obtained from REMD in TIP3P water with ff14SB (left) and ff14SB/chi/ez (right) force fields. These histograms highlight the different sugar conformations that MD predicts between the 5' (DT1) and 3' (DT2) ends of dTpT.



**Figure S10.** Dihedral angles to H2'' on the deoxyribose of DT1 (black) and DT2 (red). Top figure shows the dihedral angle between H1' and H2'', both of which point “down” on the side of the sugar opposite the thymine. The bottom figure shows the dihedral angle between H2'' and H3', which points “up” on the same side of the sugar as the nucleic acid. Given the stable tetrahedral geometry of the sugar carbons, these dihedral angles are redundant with those reported for H2' in the main text. Note that these histograms use a vertical axis of “number of occurrences” rather than the probability per degree reported in Figure 8.

#### References:

- (1) Hendrickx, P.; Martins, J. A User-Friendly Matlab Program and Gui for the Pseudorotation Analysis of Saturated Five-Membered Ring Systems Based on Scalar Coupling Constants. *Chemistry Central Journal* **2008**, 2, 20.