Supplemental Table S1. Parameters used for fitting R5a spectra measured at 25°C

| | $\bar{R}$ ($\times 10^7$ $s^{-1}$) | $R_{\|}/R_{\perp}$ [a] | $c_{20}$ | $S_{20}$ [b] | $\Delta^{(0)}$ (G) | *r.m.s.d* [c] |
|---|---|---|---|---|---|---|
| $dT_8$-$R_p$ | 8.47±0.27 | 0.2 | 1.49±0.02 | 0.331±0.005 | 0.53±0.18 | 0.017 |
| $dU_8$-$R_p$ | 9.29±0.15 | 0.2 | 1.34±0.01 | 0.297±0.002 | 0.34±0.08 | 0.026 |
| $dT_8$-$S_p$ | 8.19±0.09 | 0.2 | 1.22±0.01 | 0.271±0.002 | 0.26±0.11 | 0.020 |
| $dU_8$-$S_p$ | 8.33±0.13 | 0.2 | 1.23±0.01 | 0.274±0.003 | 0.35±0.10 | 0.017 |

[a] Simulations had little sensitivity to $R_{\|}/R_{\perp}$, which was fixed to 0.2
[b] $S_{20}$ was calculated using $c_{20}$ values as described in Methods.
[c] Root mean square deviation (*r.m.s.d*) between the measured spectrum and the corresponding simulated best-fit spectrum.

Supplemental Table S2. Label conformer distributions in simulations of d(CTACTG$_{pS}$C$_7$Y$_8$TTAG).d(CTAAAGCAGTAG)

($_{pS}$C$_7$ = phosphorothioate-dC; Y = T or U) labeled with R5a or R5 as an R$_p$ diastereoisomer at $_{pS}$dC$_7$.

| Simulation # | AMBER [a] | Geometry [b] | Spin Label | DNA dY$_8$ | t1 (%) [c] | | | t2 (%) [c] | | | t3 (%) [d] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | g+ | t | g- | g+ | t | g- | +100° | -100° |
| 1 | v. 8.0 | a | R5a | dT$_8$ | 50.2 | 9.1 | 40.7 | 0.0 | 100.0 | 0.0 | 99.2 | 0.8 |
| 2 | | b | | | 86.1 | 10.3 | 3.6 | 0.0 | 100.0 | 0.0 | 56.3 | 43.7 |
| 3 | | c | | | 54.5 | 21.8 | 23.7 | 1.7 | 98.3 | 0.0 | 40.4 | 59.6 |
| 37 | | d | | | 34.8 | 40.4 | 24.9 | 1.4 | 93.5 | 5.0 | 0.0 | 100.0 |
| 25 | v.10.0 | a | | | 22.0 | 0.0 | 77.8 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 26 | | b | | | 8.4 | 6.1 | 85.5 | 0.0 | 100.0 | 0.0 | 25.1 | 74.9 |
| 27 | | c | | | 54.7 | 0.5 | 44.8 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 38 | | d | | | 61.9 | 4.4 | 33.6 | 0.6 | 99.4 | 0.0 | 93.9 | 6.1 |
| 4 | v. 8.0 | a | R5a | dU$_8$ | 20.5 | 17.5 | 62.0 | 0.1 | 96.5 | 3.4 | 92.4 | 7.6 |
| 5 | | b | | | 26.3 | 41.7 | 32.1 | 0.1 | 99.4 | 0.5 | 15.4 | 84.6 |
| 6 | | c | | | 1.3 | 3.7 | 95.0 | 0.0 | 90.8 | 9.2 | 100.0 | 0.0 |
| 28 | v.10.0 | a | | | 15.1 | 24.2 | 60.7 | 9.1 | 90.8 | 0.1 | 48.4 | 51.6 |
| 29 | | b | | | 28.0 | 44.4 | 27.5 | 0.1 | 99.9 | 0.1 | 29.3 | 70.7 |
| 30 | | c | | | 15.1 | 11.8 | 73.1 | 7.9 | 84.4 | 7.7 | 59.7 | 40.3 |
| 13 | v. 8.0 | a | R5 | dT$_8$ | 20.5 | 34.4 | 45.0 | 14.9 | 67.7 | 17.4 | 61.2 | 38.8 |
| 14 | | b | | | 22.1 | 21.0 | 57.0 | 9.6 | 71.6 | 18.8 | 45.6 | 54.4 |
| 15 | | c | | | 13.0 | 16.1 | 70.9 | 1.9 | 78.9 | 19.3 | 51.5 | 48.5 |
| 16 | v. 8.0 | a | R5 | dU$_8$ | 7.9 | 27.2 | 64.9 | 21.2 | 74.7 | 4.1 | 62.8 | 37.2 |
| 17 | | b | | | 24.3 | 8.1 | 67.6 | 10.6 | 78.8 | 10.7 | 44.0 | 56.0 |
| 18 | | c | | | 10.5 | 15.2 | 74.2 | 8.2 | 47.4 | 44.4 | 47.2 | 52.8 |

[a] Molecular dynamics simulations were run on a Verari Systems computer with a Red Hat Linux 9.0 operating system using AMBER 8.0 or on a cluster with a Linux CentOS4 operating system using AMBER 10.0. All simulations used a parallelized version of AMBER running on 4 processors.

[b] Starting geometries were (a) t1=t2=t3=180°; (b) t1=60° (S$_p$) or -60° (R$_p$), t2=180°, t3=0°; (c) t1=60° (S$_p$) or -60° (R$_p$), t2=180°, t3=120° and (d) t1=180°, t2=120°, t3=180° (for definitions of t1, t2 and t3, see Figure 1A).

[c] g+: 0-120°; t: 120-240°; g-: 240-360°

[d] Torsion angle t3 adopts values centered on +100° and -100°. Each distribution was determined based on conformations with t3 > 0° and t3 < 0°, respectively, for a torsional angle range from +180° to -180°.

Supplemental Table S3. Label conformer distributions in simulations of d(CTACTG$_{pS}$C$_7$Y$_8$TTAG).d(CTAAAGCAGTAG)

($_{pS}$C$_7$ = phosphorothioate-dC; Y = T or U) labeled with R5a or R5 as an $S_p$ diastereoisomer at $_{pS}$dC$_7$.

| Simulation # | AMBER [a] | Geometry [b] | Spin Label | DNA dY$_8$ | t1 (%) [c] | | | t2 (%) [c] | | | t3 (%) [d] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | g+ | t | g- | g+ | t | g- | +100° | -100° |
| 7 | v. 8.0 | a | R5a | dT$_8$ | 63.0 | 35.2 | 1.8 | 0.1 | 91.2 | 8.7 | 58.3 | 41.7 |
| 8 | | b | | | 55.7 | 42.4 | 1.9 | 0.2 | 97.5 | 2.2 | 2.5 | 97.5 |
| 9 | | c | | | 57.0 | 41.2 | 1.8 | 7.2 | 84.8 | 8.0 | 51.0 | 49.0 |
| 39 | | d | | | 46.5 | 49.0 | 4.5 | 10.6 | 89.3 | 0.1 | 100.0 | 0.0 |
| 31 | v.10.0 | a | | | 35.8 | 63.2 | 1.0 | 12.9 | 86.6 | 0.5 | 100.0 | 0.0 |
| 32 | | b | | | 47.7 | 48.6 | 3.6 | 2.8 | 97.0 | 0.2 | 100.0 | 0.0 |
| 33 | | c | | | 47.4 | 50.9 | 1.7 | 5.0 | 91.5 | 3.6 | 95.2 | 4.8 |
| 40 | | d | | | 35.9 | 59.3 | 4.8 | 7.5 | 71.7 | 20.8 | 45.4 | 55.6 |
| 10 | v. 8.0 | a | R5a | dU$_8$ | 42.5 | 54.3 | 3.1 | 20.8 | 79.1 | 0.1 | 41.8 | 58.2 |
| 11 | | b | | | 46.5 | 49.9 | 3.6 | 2.0 | 90.4 | 7.5 | 8.4 | 91.6 |
| 12 | | c | | | 52.0 | 40.4 | 7.6 | 1.8 | 87.3 | 10.9 | 49.9 | 50.1 |
| 34 | v.10.0 | a | | | 34.0 | 62.4 | 3.6 | 0.0 | 99.7 | 0.2 | 100.0 | 0.0 |
| 35 | | b | | | 11.6 | 84.0 | 4.4 | 0.6 | 93.6 | 5.8 | 60.5 | 39.5 |
| 36 | | c | | | 35.5 | 63.2 | 1.2 | 3.9 | 95.7 | 0.4 | 100.0 | 0.0 |
| 19 | v. 8.0 | a | R5 | dT$_8$ | 39.9 | 56.2 | 3.8 | 21.1 | 61.8 | 17.2 | 48.7 | 51.3 |
| 20 | | b | | | 43.0 | 55.4 | 1.5 | 12.0 | 55.5 | 32.5 | 24.6 | 75.4 |
| 21 | | c | | | 75.1 | 24.3 | 0.6 | 30.5 | 61.9 | 7.6 | 65.5 | 34.5 |
| 22 | v. 8.0 | a | R5 | dU$_8$ | 53.1 | 46.0 | 0.9 | 9.5 | 63.9 | 26.6 | 48.7 | 51.3 |
| 23 | | b | | | 45.0 | 51.9 | 3.1 | 13.3 | 57.3 | 29.4 | 51.4 | 48.6 |
| 24 | | c | | | 18.3 | 78.5 | 3.2 | 4.0 | 52.8 | 43.2 | 61.8 | 38.2 |

[a] Molecular dynamics simulations were run on a Verari Systems computer with a Red Hat Linux 9.0 operating system using AMBER 8.0 or on a cluster with a Linux CentOS4 operating system using AMBER 10.0. All simulations used a parallelized version of AMBER running on 4 processors.

[b] Starting geometries were (a) t1=t2=t3=180°; (b) t1=60° ($S_p$) or -60° ($R_p$), t2=180°, t3=0°; (c) t1=60° ($S_p$) or -60° ($R_p$), t2=180°, t3=120° and (d) t1=180°, t2=120°, t3=180° (for definitions of t1, t2 and t3, see Figure 1A).

[c] g+: 0-120°; t: 120-240°; g-: 240-360°

[d] Torsion angle t3 adopts values centered on +100° and -100°. Each distribution was determined based on conformations with t3 > 0° and t3 < 0°, respectively, for a torsional angle range from +180° to -180°.

Supplemental Table S4. Distances from the $dT_8$ 5-methyl carbon atom to nitroxide label functional groups in $R_p$-R5a-$dT_8$ conformers.[a]

| Conformer (t1,t2,t3) | DNA[b] | R5a Count | R5a[c] Occupancy (%) | R5a[d] Pyrroline ring center (Å) | R5a[d] Closest ring methyl (Å) | R5a[d] Ring 4-Br (Å) | M[e] |
|---|---|---|---|---|---|---|---|
| g+,g+,-100 | $B_I$ | | | | | | |
| g+,g+,+100 | $B_I$ | | | | | | |
| g+,t,-100 | $B_I$ | 23 | 0.006 | 6.1 ± 0.8 | 4.8 ± 0.7 | 6.4 ± 1.2 | |
| g+,t,+100 | $B_I$ | 57 | 0.014 | 5.3 ± 0.7 | 4.2 ± 0.8 | 5.6 ± 0.9 | |
| g+,g-,-100 | $B_I$ | | | | | | |
| g+,g-,+100 | $B_I$ | | | | | | |
| t,g+,-100 | $B_I$ | 1004 | 0.251 | 10.8 ± 0.6 | 10.9 ± 0.7 | 8.8 ± 0.9 | |
| t,g+,+100 | $B_I$ | | | | | | |
| t,t,-100 | $B_I$ | 5349 | 1.337 | 10.5 ± 1.2 | 9.6 ± 1.4 | 10.0 ± 1.3 | |
| t,t,+100 | $B_I$ | 31 | 0.008 | 7.3 ± 1.7 | 5.9 ± 1.8 | 5.4 ± 1.7 | |
| t,g-,-100 | $B_I$ | | | | | | |
| t,g-,+100 | $B_I$ | | | | | | |
| g-,g+,-100 | $B_I$ | 4 | 0.001 | 7.0 ± 0.7 | 5.5 ± 0.9 | 9.1 ± 0.4 | |
| g-,g+,+100 | $B_I$ | | | | | | |
| g-,t,-100 | $B_I$ | 33031 | 8.258 | 6.3 ± 0.8 | 4.1 ± 0.8 | 8.9 ± 0.7 | |
| g-,t,+100 | $B_I$ | 111681 | 27.920 | 6.6 ± 0.8 | 5.8 ± 0.9 | 4.2 ± 0.5 | |
| g-,g-,-100 | $B_I$ | 889 | 0.222 | 9.3 ± 1.0 | 9.4 ± 1.2 | 7.8 ± 0.9 | |
| g-,g-,+100 | $B_I$ | 10 | 0.003 | 7.4 ± 1.2 | 7.6 ± 1.0 | 5.7 ± 1.5 | |
| g+,g+,-100 | $B_{II}$ | 2 | 0.001 | 5.4 ± 0.9 | 3.9 ± 0.5 | 6.1 ± 3.1 | |
| g+,g+,+100 | $B_{II}$ | 1 | 0.000 | 6.3 | 5.5 | 3.9 | |
| g+,t,-100 | $B_{II}$ | 54470 | 13.618 | 5.1 ± 0.7 | 4.3 ± 0.6 | 5.0 ± 1.2 | |
| g+,t,+100 | $B_{II}$ | 131753 | 32.938 | 4.9 ± 0.5 | 4.1 ± 0.5 | 4.9 ± 0.9 | |
| g+,g-,-100 | $B_{II}$ | | | | | | |
| g+,g-,+100 | $B_{II}$ | | | | | | |
| t,g+,-100 | $B_{II}$ | 843 | 0.211 | 10.4 ± 0.7 | 10.4 ± 0.7 | 9.2 ± 0.8 | |
| t,g+,+100 | $B_{II}$ | 1 | 0.000 | 8.9 | 7.0 | 9.6 | |
| t,t,-100 | $B_{II}$ | 32164 | 8.041 | 7.6 ± 1.1 | 6.9 ± 1.3 | 5.4 ± 1.3 | |
| t,t,+100 | $B_{II}$ | 6847 | 1.712 | 7.0 ± 1.1 | 5.0 ± 1.2 | 8.5 ± 0.9 | all |
| | | | 0.878 | 6.4 ± 0.9 | 4.1 ± 1.0 | 8.5 ± 1.5 | p1 |
| | | | 0.833 | 7.7 ± 2.3 | 5.8 ± 2.6 | 8.5 ± 1.5 | p2 |
| t,g-,-100 | $B_{II}$ | 1 | 0.000 | 6.6 | 4.7 | 8.1 | |
| t,g-,+100 | $B_{II}$ | 1 | 0.000 | 5.4 | 3.9 | 6.0 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| g-,g+,-100 | $B_{II}$ | 4 | 0.001 | 7.0 ± 1.5 | 5.5 ± 1.9 | 7.4 ± 1.6 | |
| g-,g+,+100 | $B_{II}$ | | | | | | |
| g-,t,-100 | $B_{II}$ | 12588 | 3.147 | 8.1 ± 1.4 | 6.0 ± 1.6 | 9.6 ± 1.1 | all |
| | | | 1.326 | 6.5 ± 1.2 | 4.2 ± 1.2 | 8.7 ± 1.2 | p1 |
| | | | 1.821 | 9.1 ± 1.3 | 7.1 ± 1.7 | 10.4 ± 1.2 | p2 |
| g-,t,+100 | $B_{II}$ | 6810 | 1.703 | 7.0 ± 0.8 | 6.4 ± 1.0 | 4.4 ± 0.8 | |
| g-,g-,-100 | $B_{II}$ | 1569 | 0.392 | 9.4 ± 0.8 | 9.0 ± 0.9 | 8.8 ± 0.8 | |
| g-,g-,+100 | $B_{II}$ | | | | | | |
| | | | | | | | |
| g+,t,-100 | g-,g+ | 1 | 0.000 | 4.5 | 4.3 | 4.1 | |
| | g-,g- | 5 | 0.001 | 5.8 ± 0.4 | 4.1 ± 0.3 | 6.9 ± 0.5 | |
| g+,t,+100 | t,t | 9 | 0.002 | 5.0 ± 0.3 | 4.1 ± 0.4 | 5.2 ± 0.7 | |
| | g-,g- | 11 | 0.003 | 5.1 ± 0.6 | 4.1 ± 0.6 | 5.0 ± 0.9 | |
| t,t,-100 | t,t | 40 | 0.010 | 7.9 ± 0.8 | 6.9 ± 0.9 | 7.1 ± 0.9 | |
| | g-,g- | 3 | 0.001 | 9.5 ± 0.7 | 8.4 ± 0.5 | 9.2 ± 0.9 | |
| t,t,+100 | t,t | 6 | 0.002 | 6.9 ± 0.3 | 5.8 ± 0.5 | 6.9 ± 1.0 | |
| g-,t,-100 | t,t | 383 | 0.096 | 6.3 ± 0.5 | 4.0 ± 0.6 | 8.6 ± 0.5 | |
| | g-,g- | 56 | 0.014 | 6.8 ± 1.2 | 4.6 ± 1.3 | 9.0 ± 0.9 | |
| g-,t,+100 | t,t | 228 | 0.057 | 6.5 ± 0.7 | 5.9 ± 1.0 | 3.9 ± 0.3 | |
| | g-,g- | 54 | 0.014 | 6.6 ± 0.8 | 6.0 ± 0.9 | 4.1 ± 0.5 | |
| g-,g-,-100 | t,t | 65 | 0.016 | 7.8 ± 0.6 | 7.6 ± 0.8 | 7.0 ± 0.6 | |
| | g-,g- | 6 | 0.002 | 8.8 ± 0.5 | 8.7 ± 0.7 | 7.6 ± 0.7 | |

[a] R5a adopted 37 conformers with at least one occurrence out of a possible total of 162 ($3^4$ [3 positions (g+, t, g-) for t1, t2, ε, ζ] × 2 [2 positions for t3 (-100, +100)].

[b] DNA backbone conformation at the $dG_{6\text{-}pS}dC_7$ dinucleotide. All possible entries with $B_I$ ((ε, ζ) = (t,g-)) and $B_{II}$ ((ε, ζ ) = (g-,t)) configurations are listed. Other entries are listed only if they have at least one occurrence, with the corresponding DNA configuration indicated by the (ε, ζ) pair.

[c] Percentage occupancy calculated from a total of 400,000 structures (eight 10 ns simulations).

[d] Average ± standard deviation of distance for each ensemble of conformers.

[e] M = multimodal. Some conformers gave an apparent multimodal distribution for the distances. The mean distance for each of these conformers is indicate by 'all' and the component means for subpopulations (based on Gaussian fits) are shown in smaller font and are indicated by p1, p2, etc.

Supplemental Table S5. Distances from the $dT_8$ 5-methyl carbon atom to nitroxide label functional groups in $R_p$-R5-$dT_8$ conformers. [a]

| Conformer (t1,t2,t3) | DNA [b] | R5 Count | R5 [c] Occupancy (%) | R5 [d] Pyrroline ring center (Å) | R5 [d] Closest ring methyl (Å) | M [e] |
|---|---|---|---|---|---|---|
| g+,g+,-100 | $B_I$ | | | | | |
| g+,g+,+100 | $B_I$ | | | | | |
| g+,t,-100 | $B_I$ | 51 | 0.034 | 5.9 ± 0.7 | 4.1 ± 0.5 | |
| g+,t,+100 | $B_I$ | 27 | 0.018 | 5.8 ± 0.6 | 4.0 ± 0.6 | |
| g+,g-,-100 | $B_I$ | 21 | 0.014 | 5.2 ± 0.3 | 3.9 ± 0.4 | |
| g+,g-,+100 | $B_I$ | | | | | |
| t,g+,-100 | $B_I$ | 957 | 0.638 | 10.4 ± 1.0 | 10.5 ± 1.1 | |
| t,g+,+100 | $B_I$ | 408 | 0.272 | 10.8 ± 0.8 | 9.5 ± 1.0 | |
| t,t,-100 | $B_I$ | 3926 | 2.617 | 9.6 ± 1.0 | 8.4 ± 1.2 | |
| t,t,+100 | $B_I$ | 8630 | 5.753 | 9.7 ± 1.0 | 8.7 ± 1.2 | |
| t,g-,-100 | $B_I$ | 9 | 0.006 | 11.2 ± 0.3 | 10.7 ± 0.4 | |
| t,g-,+100 | $B_I$ | 23 | 0.015 | 9.3 ± 0.9 | 9.7 ± 0.8 | |
| g-,g+,-100 | $B_I$ | 133 | 0.089 | 6.1 ± 0.5 | 4.1 ± 0.6 | |
| g-,g+,+100 | $B_I$ | 121 | 0.081 | 6.1 ± 0.3 | 3.9 ± 0.3 | |
| g-,t,-100 | $B_I$ | 24139 | 16.093 | 6.4 ± 0.9 | 4.2 ± 1.0 | |
| g-,t,+100 | $B_I$ | 14339 | 9.559 | 6.9 ± 1.6 | 6.1 ± 1.9 | all |
| | | | 4.219 | 5.3 ± 1.0 | 4.1 ± 0.9 | p1 |
| | | | 5.340 | 8.1 ± 1.6 | 7.5 ± 2.2 | p2 |
| g-,g-,-100 | $B_I$ | 4363 | 2.909 | 8.3 ± 1.3 | 8.1 ± 1.5 | all |
| | | | 0.172 | 5.7 ± 1.1 | 4.8 ± 1.5 | p1 |
| | | | 0.746 | 6.8 ± 1.3 | 6.9 ± 1.5 | p2 |
| | | | 1.990 | 8.9 ± 1.5 | 8.9 ± 1.7 | p3 |
| g-,g-,+100 | $B_I$ | 4171 | 2.781 | 8.9 ± 1.4 | 7.5 ± 1.6 | all |
| | | | 0.490 | 6.6 ± 1.2 | 4.9 ± 1.1 | p1 |
| | | | 2.290 | 9.4 ± 1.9 | 8.0 ± 2.6 | p2 |
| g+,g+,-100 | $B_{II}$ | 39 | 0.026 | 6.4 ± 0.5 | 4.4 ± 1.0 | |
| g+,g+,+100 | $B_{II}$ | 791 | 0.527 | 5.3 ± 0.8 | 4.2 ± 0.4 | |
| g+,t,-100 | $B_{II}$ | 13750 | 9.167 | 5.3 ± 0.7 | 4.2 ± 0.6 | |
| g+,t,+100 | $B_{II}$ | 13130 | 8.753 | 5.4 ± 0.7 | 4.0 ± 0.5 | |
| g+,g-,-100 | $B_{II}$ | | | | | |
| g+,g-,+100 | $B_{II}$ | | | | | |
| t,g+,-100 | $B_{II}$ | 3609 | 2.406 | 10.0 ± 0.7 | 10.2 ± 0.7 | |
| t,g+,+100 | $B_{II}$ | 5076 | 3.384 | 10.3 ± 0.7 | 9.6 ± 0.8 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| t,t,-100 | $B_{II}$ | 7326 | 4.884 | 8.8 ± 0.8 | 8.4 ± 1.1 | |
| t,t,+100 | $B_{II}$ | 5623 | 3.749 | 8.4 ± 1.2 | 6.7 ± 1.5 | all |
| | | | 0.863 | 6.9 ± 1.5 | 4.6 ± 1.4 | p1 |
| | | | 2.886 | 8.9 ± 1.6 | 7.1 ± 2.0 | p2 |
| t,g-,-100 | $B_{II}$ | 81 | 0.054 | 6.9 ± 0.6 | 4.8 ± 1.0 | |
| t,g-,+100 | $B_{II}$ | 12 | 0.008 | 8.8 ± 1.9 | 8.5 ± 2.9 | |
| g-,g+,-100 | $B_{II}$ | 713 | 0.475 | 7.4 ± 1.5 | 6.3 ± 1.8 | |
| g-,g+,+100 | $B_{II}$ | 1214 | 0.809 | 8.8 ± 0.7 | 6.9 ± 0.9 | |
| g-,t,-100 | $B_{II}$ | 7480 | 4.987 | 7.7 ± 1.5 | 5.7 ± 1.7 | all |
| | | | 2.448 | 6.3 ± 1.4 | 4.1 ± 1.2 | p1 |
| | | | 2.539 | 8.9 ± 1.6 | 7.1 ± 1.9 | p2 |
| g-,t,+100 | $B_{II}$ | 9951 | 6.634 | 8.4 ± 1.3 | 8.1 ± 1.5 | all |
| | | | 0.603 | 5.6 ± 1.4 | 4.5 ± 1.1 | p1 |
| | | | 6.031 | 8.7 ± 1.7 | 8.3 ± 2.3 | p2 |
| g-,g-,-100 | $B_{II}$ | 3695 | 2.463 | 9.4 ± 0.9 | 9.4 ± 1.0 | |
| g-,g-,+100 | $B_{II}$ | 15001 | 10.001 | 9.7 ± 0.7 | 9.1 ± 0.8 | |
| g+,t,-100 | t,t | 1 | 0.001 | 4.1 | 3.7 | |
| g+,t,+100 | t,t | 1 | 0.001 | 5.4 | 5.3 | |
| t,g+,+100 | t,t | 37 | 0.025 | 7.3 ± 0.5 | 5.5 ± 0.6 | |
| t,t,-100 | t,t | 10 | 0.007 | 7.4 ± 0.9 | 6.3 ± 1.4 | |
| | g-,g- | 1 | 0.001 | 9.7 | 9.0 | |
| t,t,+100 | t,t | 25 | 0.017 | 8.0 ± 0.8 | 6.8 ± 1.0 | |
| | g-,g- | 1 | 0.001 | 8.4 | 7.8 | |
| t,g-,+100 | t,t | 3 | 0.002 | 8.5 ± 0.5 | 8.8 ± 0.4 | |
| | g-,g- | 1 | 0.001 | 9.4 | 10.1 | |
| g-,g+,-100 | t,t | 85 | 0.057 | 5.7 ± 0.5 | 4.4 ± 0.4 | |
| | g-,g- | 1 | 0.001 | 6.0 | 3.8 | |
| g-,g+,+100 | g-,g- | 1 | 0.001 | 8.1 | 5.9 | |
| g-,t,-100 | t,t | 330 | 0.220 | 6.0 ± 0.5 | 3.9 ± 0.4 | |
| | g-,g- | 16 | 0.011 | 6.6 ± 1.2 | 4.5 ± 1.3 | |
| g-,t,+100 | t,t | 326 | 0.217 | 5.4 ± 0.8 | 4.6 ± 1.0 | |
| | g-,g- | 26 | 0.017 | 7.2 ± 1.9 | 6.6 ± 2.3 | |
| g-,g-,-100 | t,t | 137 | 0.091 | 6.9 ± 0.7 | 6.6 ± 0.9 | |

| | | | | | |
|---|---|---|---|---|---|
| | g-,g- | 4 | 0.003 | 8.2 ± 1.3 | 8.0 ± 1.3 |
| g-,g-,+100 | t,t | 176 | 0.117 | 8.4 ± 0.8 | 7.9 ± 1.1 |
| | g-,g- | 9 | 0.006 | 9.1 ± 0.8 | 8.2 ± 0.8 |

[a] R5 adopted 51 conformers with at least one occurrence out of a possible total of 162 ($3^4$ [3 positions (g+, t, g-) for t1, t2, ε, ζ] × 2 [2 positions for t3 (-100, +100)].

[b] DNA backbone conformation at the $dG_{6}\text{-}_{pS}dC_{7}$ dinucleotide. All possible entries with $B_I$ ((ε, ζ) = (t,g-)) and $B_{II}$ ((ε, ζ ) = (g-,t)) configurations are listed. Other entries are listed only if they have at least one occurrence, with the corresponding DNA configuration indicated by the (ε, ζ) pair.

[c] Percentage occupancy calculated from a total of 150,000 structures (three 10 ns simulations).

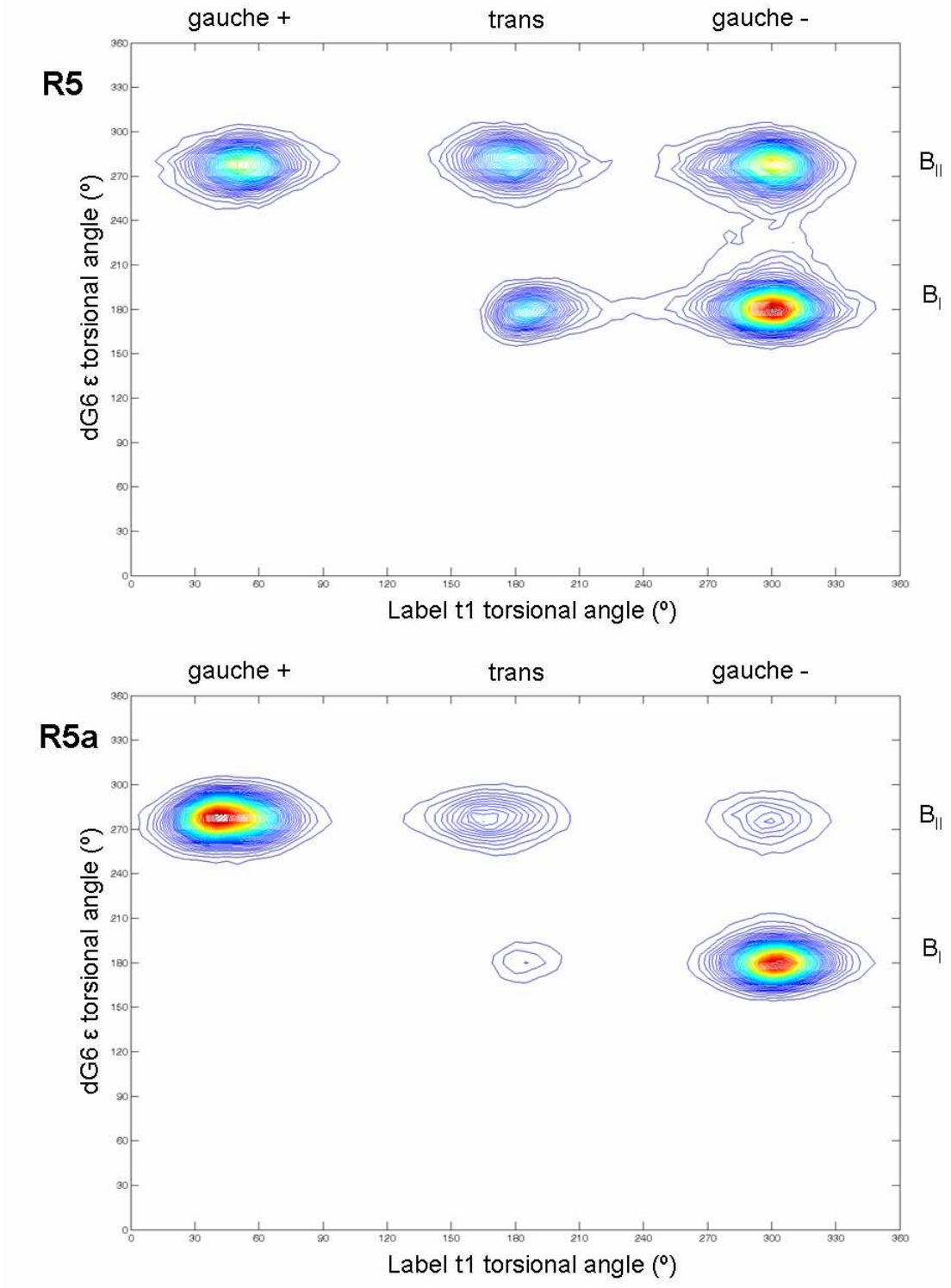
[d] Average ± standard deviation of distance for each ensemble of conformers.

[e] M = multimodal. Some conformers gave an apparent multimodal distribution for the distances. The mean distance for each of these conformers is indicate by 'all' and the component means for subpopulations (based on Gaussian fits) are shown in smaller font and are indicated by p1, p2, etc.

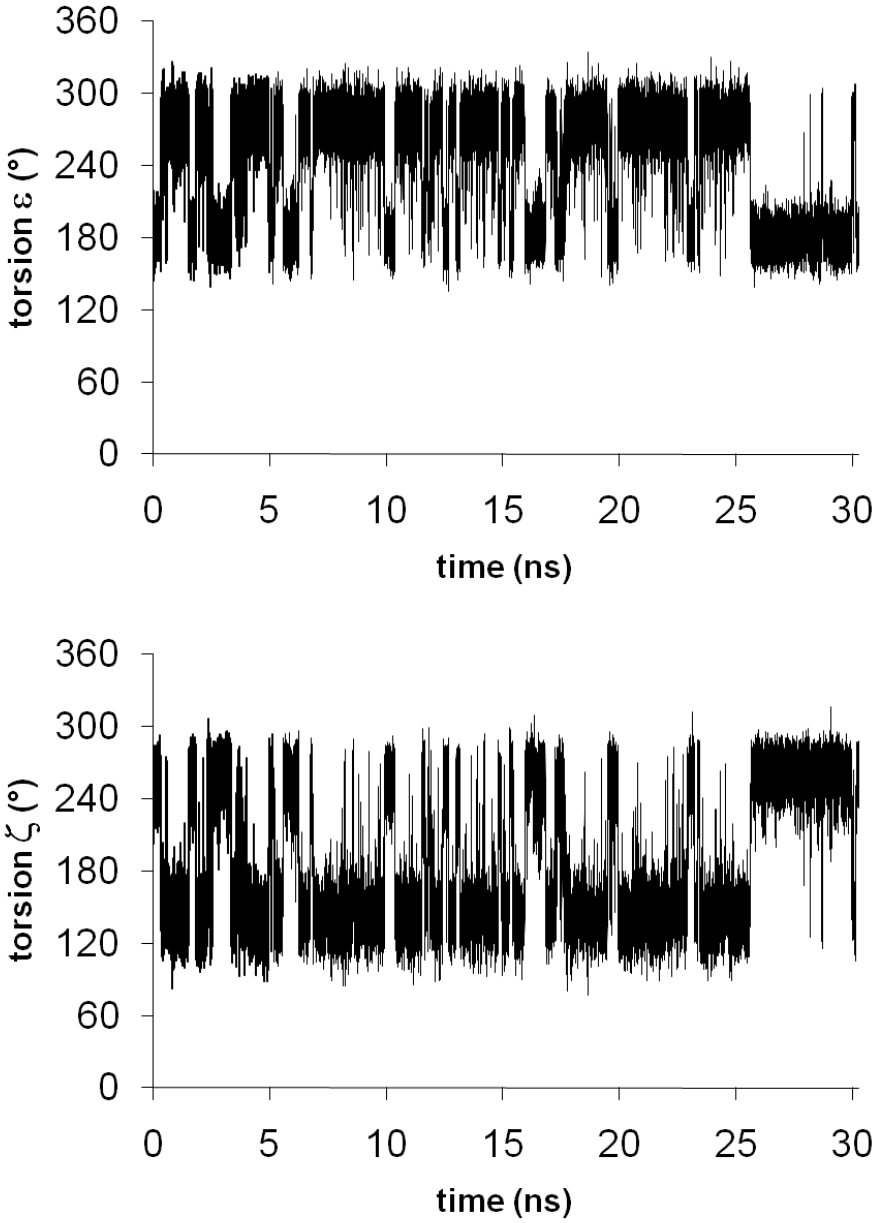
Supplemental Table S6. $B_I/B_{II}$ equilibria at the $dG_{6\text{-}pS}dC_7$ dinucleotide in simulations of a DNA duplex of sequence d(CTACT$G_{pS}C_7T_8$TTAG).d(CTAAAGCAGTAG) ($_{pS}C_7$ = phosphorothioate-dC) carrying a R5a or R5 label. Data are also shown for simulations of the unlabeled duplex d(CTACTG$C_7T_8$TTAG).d(CTAAAGCAGTAG).

| R5a Label | | | R5 Label | | | Unlabeled | | |
|---|---|---|---|---|---|---|---|---|
| Simulation # [a] | $B_I$ [b] | $B_{II}$ [c] | Simulation # [a] | $B_I$ [b] | $B_{II}$ [c] | Simulation # | $B_I$ [b] | $B_{II}$ [c] |
| 1 | 37.1% | 62.7% | 13 | 34.0% | 65.6% | 41 | 27.7% | 71.4% |
| 2 | 2.8% | 97.2% | 14 | 36.1% | 62.8% | 42 | 28.8% | 69.9% |
| 3 | 16.7% | 83.2% | 15 | 52.5% | 46.6% | 43 | 49.4% | 49.8% |
| 37 | 23.3% | 76.4% | | | | | | |
| 25 | 73.1% | 26.7% | | | | | | |
| 26 | 73.3% | 26.0% | | | | | | |
| 27 | 40.2% | 59.7% | | | | | | |
| 38 | 37.7% | 62.3% | | | | | | |
| Mean [d] | 38.0% | 61.8% | Mean [d] | 40.9% | 58.3% | Mean [d] | 35.3% | 63.7% |

[a] Details are given in Table S2
[b] $B_I$ at $dG_{6\text{-}pS}dC_7$ defined by ε >120° to ≤220° and ζ > 200° (see Figure S2 for the basis of these ranges)
[c] $B_{II}$ at $dG_{6\text{-}pS}dC_7$ defined by ε >220° and ζ >80° to ≤200° (see Figure S2 for the basis of these ranges)
[d] Average over all simulations. Note that the total for $B_I$ + $B_{II}$ is not 100% because of minor populations of other backbone conformers (ε, ζ = g-, g- or t, t; see Tables S4 (R5a) and S5 (R5))




Supplemental Figure S1. Correlation between t1 and $B_I/B_{II}$ at the $dG_{6}$-$_{pS}dC_{7}$ dinucleotide for simulations of $R_p$-R5-$dT_8$ and $R_p$-R5a-$dT_8$. The plots show the number of occurrences/total number of conformers for t1 and ε binned using intervals of 5°. Color code is from minimal (blue) to maximal (red) with 64 contour levels.




Supplemental Figure S2. Traces of the ε and ζ torsional angles for the $dG_6$-$dC_7$ dinucleotide in a 30-ns MD simulation of d(CTACTG$C_7$$T_8$TTAG).d(CTAAAGCAGTAG). Note that an initial 0.1-ns equilibration period is included in the plot to indicate that the duplex started (0 ns) in an initial $B_I$ conformation. The data in Table S6 are derived from the trace from 0.1 ns to 30.1 ns.