Binding of Topotecan to a Nicked DNA Oligomer in Solution

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Fig 1S. Sequence-specific chemical shift changes of nicked DNA on interaction with TPT.

Fig 2S. An example of a NOESY spectrum (pH 6, room temperature), with cross-peaks marked by arrows from Me-19 methyl group of TPT to nicked DNA (the 5' and 5'' cross-peaks were not assigned).
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<th></th>
<th>H1'</th>
<th>H2'</th>
<th>H2''</th>
<th>H3'</th>
<th>H4'</th>
<th>H6/8</th>
<th>H2/5/Me</th>
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a) TPT (lactone form) chemical shifts in the sample were found as follows (ppm from TSPA)

TPT-H7  - 8.424  TPT-H9  - 4.581
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<th>H2''</th>
<th>H3'</th>
<th>H4'</th>
<th>H6/8</th>
<th>H2/5/Me</th>
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<td>X-ray</td>
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<td>St1B</td>
<td>St2A</td>
<td>St2B</td>
<td>St3A</td>
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<td>St4A</td>
<td>St4B</td>
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<td>vw</td>
<td>vw</td>
<td>vw</td>
<td>vw</td>
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<td>m</td>
<td>st</td>
<td>st</td>
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<td>m</td>
<td>vw</td>
<td>vw</td>
<td>w</td>
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<td>vw</td>
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<td>st</td>
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<td>TPT-H17a / T5-Me</td>
<td>w</td>
<td>vw</td>
<td>vw</td>
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<td>m</td>
<td>st</td>
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<td>vv</td>
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<td>TPT-H17b / G6-H4'</td>
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<td>m</td>
<td>vw</td>
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<td>vv</td>
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<td>m</td>
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<td>TPT-H18a / G6-H1'</td>
<td>m</td>
<td>vv</td>
<td>vv</td>
<td>w</td>
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<td>vv</td>
<td>m</td>
<td>m</td>
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</table>

Table 3S NOE analysis.
The symbols represent % volume of the cross-peaks relative to the volume of the Cyt H5-H6 distance: St > 10%; 10% > m > 1%; 1% > w > 0.2%; 0.2% > vw. For computed structures the cross-peak volumes which conform with experimental cross-peak volumes are shown in bold.

**Table 4S. NOE analysis**

<table>
<thead>
<tr>
<th>Experimentally observed NOE effects</th>
<th>Exp. NOE</th>
<th>NOE effects for structures from molecular dynamics runs</th>
<th>X-ray</th>
</tr>
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<tbody>
<tr>
<td>TPT-H14 / A16-H2</td>
<td>7.2</td>
<td>St1A: 0.12 (0.41)  St1B: 0.15 (0.55)  St2A: 0.19 (0.06) St2B: 0.13 (0.05) St3A: 0.06 (0.03) St3B: 0.08 (0.02) St4A: 1.02 (1.50) St4B: 15.41 (17.97)</td>
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<td>TPT-H17a / T5-H2&quot;</td>
<td>8.3</td>
<td>St1A: 0.03 (0.04)  St1B: 0.06 (0.57)  St2A: 0.57 (0.84) St2B: 0.19 (0.24) St3A: 0.00 (0.00) St3B: 0.00 (0.00) St4A: 4.18 (7.45) St4B: 0.81 (1.50)</td>
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<td>St1A: 0.05 (0.09)  St1B: 0.04 (0.30)  St2A: 3.58 (14.04) St2B: 0.61 (1.39) St3A: 0.00 (0.00) St3B: 0.00 (0.00) St4A: 4.66 (8.88) St4B: 2.44 (3.71)</td>
<td>12.18</td>
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<td>0.01</td>
<td>0.26</td>
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<td>(0.09)</td>
<td>(0.35)</td>
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<tr>
<td>TPT-H17b / T5-H2’</td>
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<td>(0.08)</td>
<td>(2.50)</td>
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<td>(0.84)</td>
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<td>(0.04)</td>
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<td>(0.06)</td>
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<td>(0.00)</td>
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<td>Experimentally observed NOE effects</td>
<td>Exp. NOE</td>
<td>Back calculated NOE effects for structures from molecular dynamics runs</td>
<td>X-ray</td>
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<td>4.56 (12.19)</td>
<td>0.27 (0.94)</td>
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^a The values represent % volume of the crosspeaks relative to volume of the Cyt H5-H6 distance. The values in parentheses denote the standard deviation calculated for last 8 ns run (800 structures).

Table 5S. NOE analysis^a.
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<th>Volume (0.30)</th>
<th>Volume (0.61)</th>
<th>Volume (0.42)</th>
<th>Volume (0.61)</th>
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<td>TPT-H18a / A16-H2</td>
<td>&gt;1.0</td>
<td>0.37</td>
<td>0.73</td>
<td>0.82</td>
<td>0.70</td>
<td>0.62</td>
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<tr>
<td>TPT-H18b / A16-H2</td>
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<td>0.32 (0.38)</td>
<td>0.76 (0.28)</td>
<td>0.68 (0.34)</td>
<td>0.77 (0.34)</td>
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<td>0.43 (0.57)</td>
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<td>0.31 (1.30)</td>
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\(^a\)The values represent % volume of the crosspeaks relative to volume of the Cyt H5-H6 cross peak. The values in parentheses denote the standard deviation calculated for an entire trajectory (800 - 1000 structures). St4A-OH, St4A-NH and St4A-prot are 10 ns MD trajectories, where the starting point is the last geometry from St4A trajectory with modified TPT; St4A-NH and St4A-OH are the tautomers of the neutral molecule bearing proton on N and O atoms, respectively and St4A-prot is protonated TPT on nitrogen in the R\(^2\) = CH\(_2\)N(CH\(_3\))\(_2\) substituent.
Table 6S: Free energy analysis for DNA / TPT complexes.

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<td>15.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7S  Comparison of selected global and backbone parameters of the computed and X-ray\cite{6,8} complexes\textsuperscript{a}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rise (Å)\textsuperscript{b}</th>
<th>Rise (Å)</th>
<th>Twist (deg)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4/T5; T5/G6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5/G6; A16/C15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15/A14; A16/C15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Computed nicked decamer</strong>\textsuperscript{d}</td>
<td>3.49; 3.71</td>
<td>3.39; 3.63</td>
<td>-7.6; 2.3</td>
</tr>
<tr>
<td><strong>Nicked decamer, NMR</strong>\textsuperscript{e}</td>
<td>3.51; 2.77</td>
<td>2.44; 3.80</td>
<td>66.9; 60.4</td>
</tr>
<tr>
<td><strong>Nicked decamer, X-Ray</strong>\textsuperscript{f}</td>
<td>3.67; 3.17</td>
<td>2.49; 4.12</td>
<td>18.7; 33.8</td>
</tr>
<tr>
<td><strong>TPT complex, NMR</strong>\textsuperscript{g}</td>
<td>2.90; 8.67</td>
<td>3.12; 8.15</td>
<td>23.3; 26.4\textsuperscript{c}</td>
</tr>
<tr>
<td><strong>TPT complex, X-Ray</strong>\textsuperscript{h}</td>
<td>2.94; 7.31</td>
<td>2.60; 7.94</td>
<td>8.2; 12.7\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Parameters were calculated using the program CURVES\textsuperscript{[42]}

\textsuperscript{b}Global interbase parameter.

\textsuperscript{c}The average value for other pairs in a duplex is ca. 35° in both cases.

\textsuperscript{d}Computed structure of the nicked decamer, using native DNA geometry as a starting structure, as an average from last 8 ns MD run.

\textsuperscript{e}NMR structure of the nicked decamer, GCGTT↓GTCGC

\textsuperscript{f}X-Ray structure of a binary TopI-nicked DNA complex, GACTT↓TGAAA

\textsuperscript{g}This work, average from last 8 ns MD run

\textsuperscript{h}X-Ray – TPT structure, close environment of a nick in DNA, GACTT↓GGAAA
Scheme 1S  Starting structures for MD calculations
Fig. 3S Aromatic part expansion of a NOESY spectra of neat decamer (A, 500 MHz, mixing time 200 ms) and decamer titrated with TPT (B, 500 MHz, mixing time 300 ms). The longer mixing time was applied in a latter case to have better S/N on intermolecular NOE,s. Despite the change in mixing time both spectra have essentially the same chemical shift dispersion and crosspeaks.
Fig. 4S Minor groove views of the eight MD-derived structures of nicked decamer–TPT complexes. Only six base pair units flanking the nick are shown for clarity. Each structure represents the minimised average of structures from the last 1 ns out of a 10 ns MD run.