Supplementary Information of

DNA Binding of Tilorone; ^{1}H NMR and Calorimetric Studies of the Intercalation.

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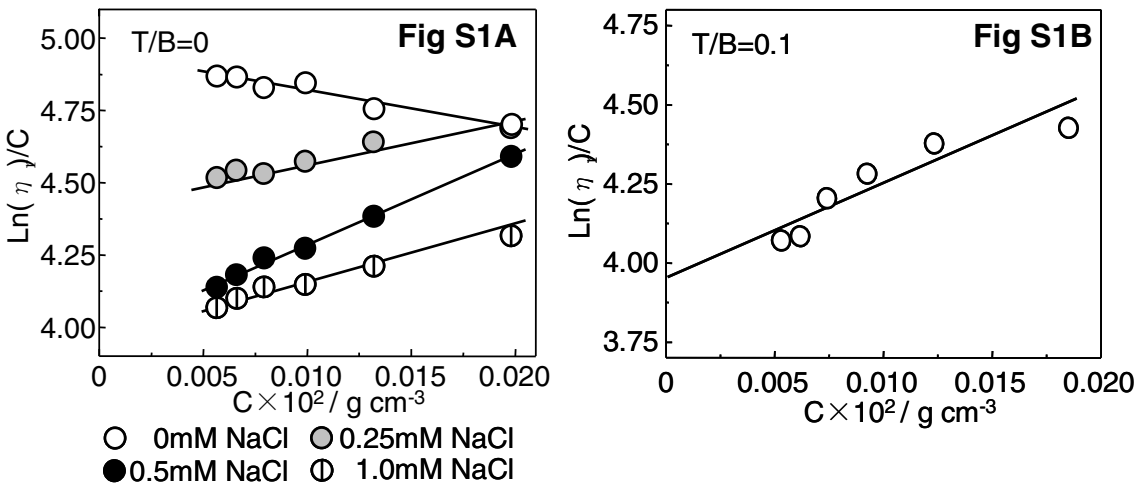
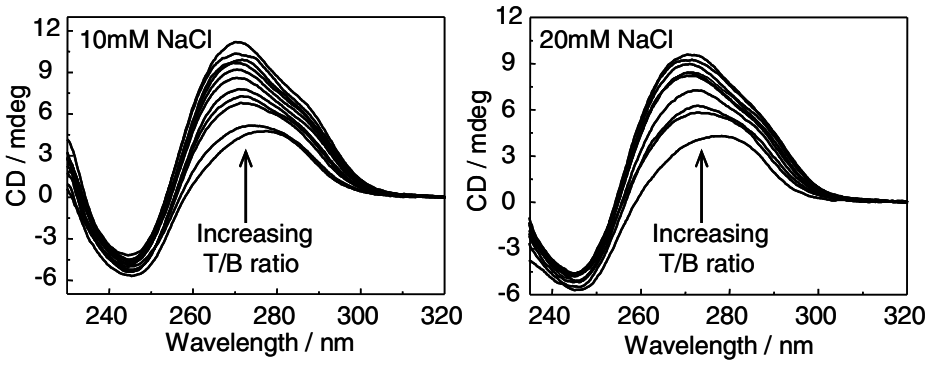
S1 Viscosity measurements

Polyelectrolyte solutions show abnormal up-turn of the relative viscosity at low polymer concentrations when the electrostatic shielding is not enough (i.e., low salt concentration). This feature makes impossible to extrapolate the relative viscosity to infinite dilution. Figure S1A shows the salt concentration dependence of $\ln \eta_r/c$ vs c plot. It can be seen that the extrapolation can be done when [NaCl] > 0.5 mM. Figure S1B shows an example of the determination of [η]. In this case, T/B =0.1.



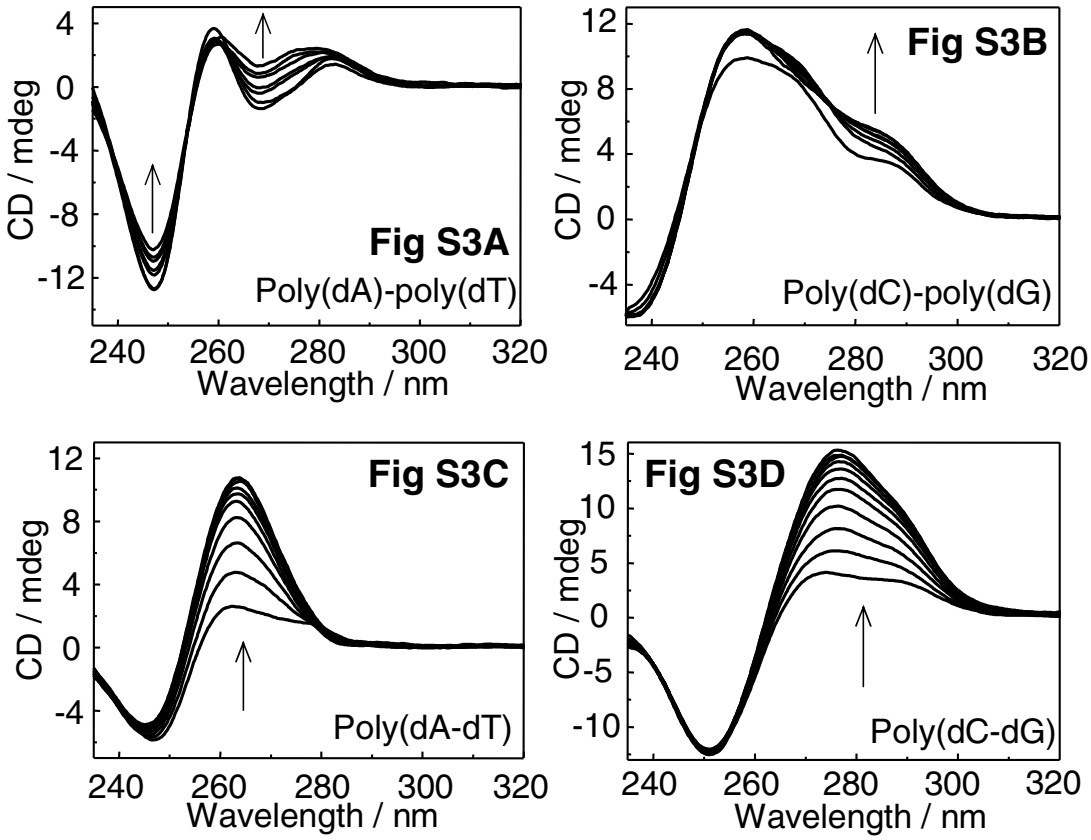
S2 CD spectral changes for [NaCl] = 10 mM and 20 mM

There was no upper limit in the CD spectral changes when [NaCl] = 0, as presented in Figure 5. On the other hand, there was upper limit observed when salts were present in the solution. Figure S2 show the CD changes when [NaCl] = 10 mM and 20 mM. The plots of Figure 6 were constructed from these spectra.



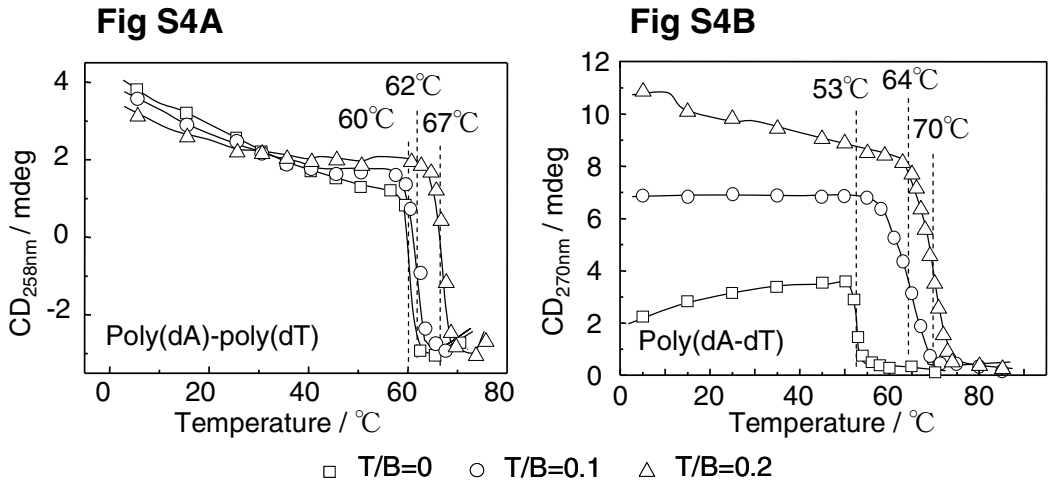
S3 CD spectral changes when tilorone was intercalated into AT or GC

In order to determine the melting temperature increment due to intercalation, we measured the CD changes for poly(dA)-poly(dT), poly(dC)-poly(dG), poly(dA-dT), and poly(dC-dG) upon the tilorone intercalation.



S4 Melting temperature increment due to intercalation

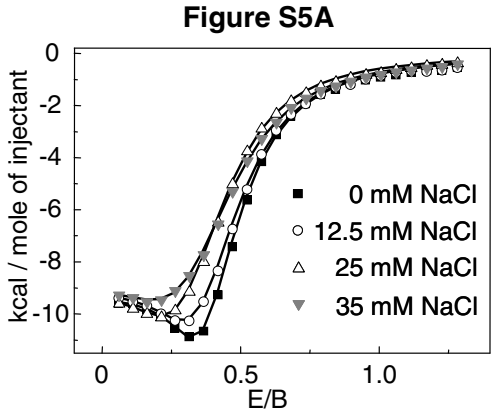
The melting temperature is increased with increasing T/B similarly to other intercalators(*1*).

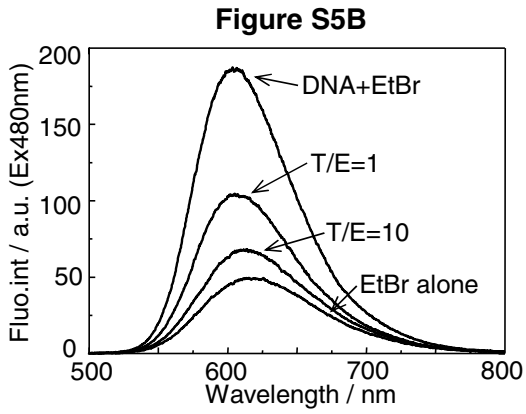


S5 Comparison with EtBr

Figure S5A presents the thermogram for different NaCl concentrations and the solid lines are the best fit in terms of the one site or two sites model (see the text). The resultant parameters are summarized in Table S2.

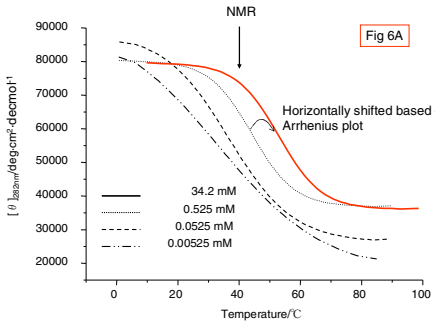
When compared the value of K_b between tilorone and EtBr, these are in the same magnitude. To confirm this fact, we added tilorone to the EtBr-intercalated DNA and measured the fluorescence intensity (Figure S5B). In this case, the fluorescence intensity is only caused by the intercalated EtBr. When tilorone was added at the equimol of EtBr (T/E = 1), the fluorescence intensity decreased by almost 50%. This is one of the strong evidence that tilorone has the same magnitude of K_b as EtBr.

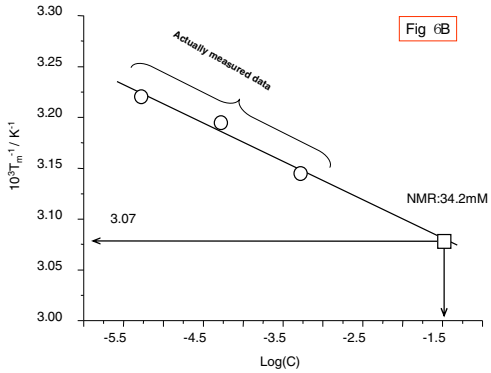




S6 NMR and Tm

For the sample used in NMR, direct measurement of Tm with CD was practically impossible owing to such a high concentration (34.2 mM). We measured CD melting curves at three concentrations (0.00525, 0.0525, and 0.525 mM) as presented in Fig 6A. From these data we evaluated Tm for each concentration and constructed the Arrhenius plot (Fig 6B). Three data points are fitted by a straight line and we estimated Tm at 34.2 mM (NMR concentration) to be 52 ^{o}C (325 K). This value is higher than the temperate at which NMR was recoded.

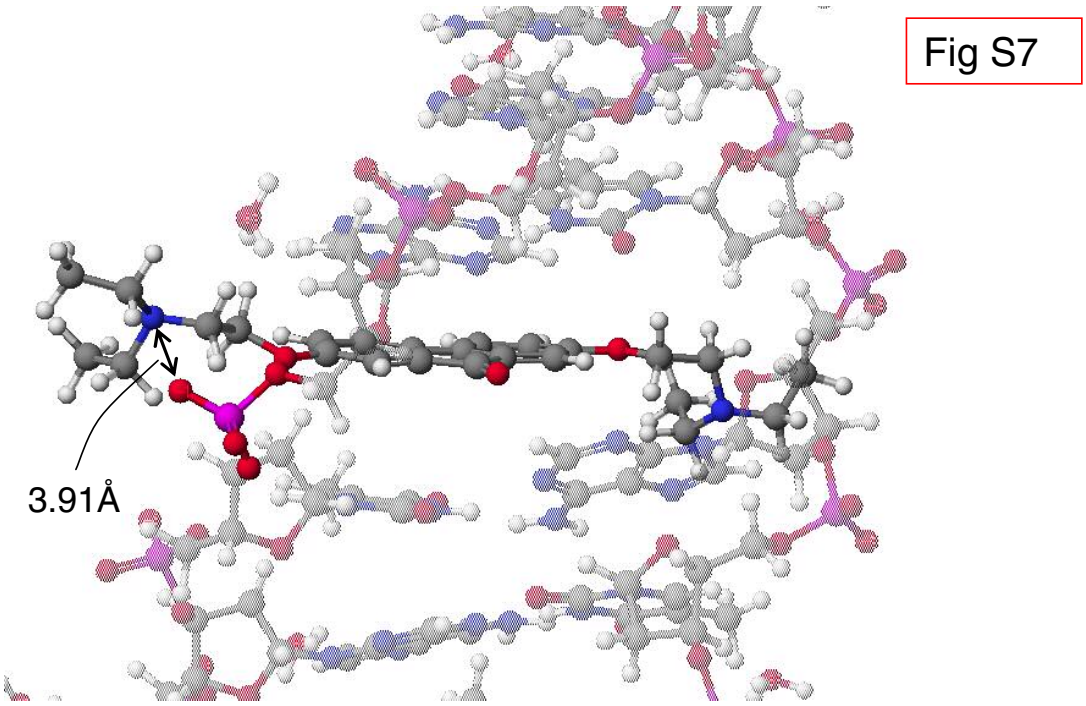




The red curve in Fig 6A presents the predicted melting curve at the concentration of 34.2 mM (NMR) and our NMR measurement was carried out 41 ^{o}C (indicated by the arrow). It can be seen that the most base pairs are hybridized at the concentration and temperature that we used for NMR.

S7 Molecular modeling

DNA conformation was constructed according to HyperChem (Ver 6.03) data base. Tilorone model was optimized with MOPAC AM1 and the resultant structure was inserted into AT/TA. After added 16 H_3O^+ ions as the counter of DNA phosphoric anions, MM3 (molecular mechanics) and then MOPAC AM1 were carried out to obtain the most stable structure (Fig 3).



From this model, the distance between O and N was evaluated to be 3.9 A. This distance is short enough to form an ionic interaction.

Supplementary Tables

Table S1. ^{1}H Chemical Shift assignments (ppm) for DNA

Nucleoside	H8/H6	H5/CH_3/H2	H1'	H2'	H2"	H3'	H4'	H5'/H5"
G1	7.984	--	5.874	2.243	2.683	4.876	4.263	3.769
C2	7.573	5.493	6.137	2.166	2.535	4.830	4.285	4.107
T3	7.439	1.754	6.037	2.683	2.777	4.876	4.208	4.083
A4	8.338	n.a.[a]	6.256	2.683	2.807	4.994	4.353	n.a.
T5	7.185	1.526	5.638	2.243	2.243	4.830	4.107	n.a.
A6	8.153	n.a.	6.024	2.683	2.807	4.994	4.402	4.172
G7	7.727	--	5.874	2.166	2.618	4.953	4.353	4.208
C8	7.439	5.493	6.202	2.535	2.535	4.497	4.083	n.a.

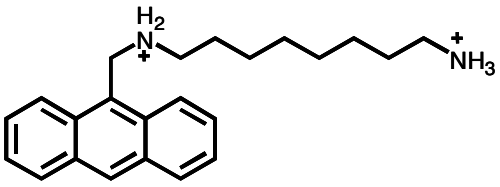
[a] Not assigned.

Table S2. Thermodynamic Parameters for the Association of EtBr with DNA at Different Salt Concentration

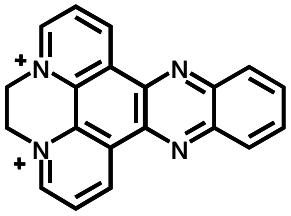
$[Na^+]$ / mM	ΔH_1 / kcalmol^{-1}	$T\Delta S_1$ / kcalmol^{-1}	ΔG_1 / kcalmol^{-1}	$K_{b1}\times 10^5$ / M^{-1}	n_1
0	-9.27	-0.66	-8.59	20.4	0.32
12.5	-9.04	-0.58	-8.45	16.0	0.29
25	-9.17	-0.69	-8.46	16.4	0.21
35	-8.84	-0.59	-8.23	11.1	0.21
$[Na^+]$ / mM	ΔH_2 / kcalmol^{-1}	$T\Delta S_2$ / kcalmol^{-1}	ΔG_2 / kcalmol^{-1}	$K_{b2}\times 10^5$ / M^{-1}	n_2
0	-32.54	-25.72	-6.80	0.99	0.11
12.5	-23.99	-17.14	-6.84	1.06	0.14
25	-17.38	-10.37	-6.97	1.32	0.20
35	-16.62	-9.69	-6.91	1.20	0.21

Table S3 Thermodynamic parameters for the first binding between Tilorone and sss-DNA.

$[Na^+]$ / mM	ΔH_1 / kcalmol^{-1}	$T\Delta S_1$ / kcalmol^{-1}	ΔG_1 / kcalmol^{-1}	$K_{b1} \times 10^6$ / M^{-1}	n_1
0	-4.14	5.30	-9.49	8.60	0.08
10	-4.46	4.83	-9.25	6.26	0.07
20	-4.50	7.12	-8.87	3.26	0.03



anthracene derivative



dppz derivative

Table S4. Thermodynamic Parameters for the Binding of anthracene derivative and dppz derivative

Ligand	ΔH / kcalmol^{-1}	$T\Delta S$ / kcalmol^{-1}	ΔG / kcalmol^{-1}	$K_b \times 10^4$ / M^{-1}	n
anthracene derivative	-4.1	3.6	-7.7	40.0	0.260
dppz derivative	-2.6	4.8	-7.4	5.4	0.283

References

(1) Ihtshamul, H., John, E. L., Babur, Z. C., and Terence, C. J. (1996) Molecular Anchoring of Duplex and Triplex DNA by Disubstituted Anthracene-9,10-diones: Calorimetric, UV Melting, and Competition Dialysis Studies. *J. Am. Chem. Soc. 118*, 10693-10701.