Supplementary Material (ESI) for Chemical Communications

Supporting Information

Evidence of Strong Hydrogen Bonding by 8-Amino-Guanine

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Hoogsteen Proton-Bound Homodimer of 8-aminoguanine

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Stoichiometry    C10H13N12O2(1+)
Framework group  C1[X(C10H13N12O2)]
Deg. of freedom   105
Full point group   C1      NOp   1
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Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Ångstroms) X	Y	Z
1	6	0	-5.194368	-0.219603	-0.101662
2	7	0	-4.326756	-1.285710	-0.104282
3	6	0	-2.916710	-1.205681	-0.034956
4	6	0	-2.478046	0.141658	0.027503
5	6	0	-3.443728	1.143922	0.019074
6	7	0	-4.782420	1.030940	-0.033376
7	8	0	-2.270777	-2.268357	-0.041514
8	7	0	-1.197771	0.695061	0.093198
9	7	0	-2.739164	2.324242	0.087080
10	6	0	-1.392666	2.005719	0.134204
11	1	0	-3.149691	3.244692	0.146319
12	1	0	-4.677322	-2.232007	-0.197881
13	7	0	-0.437955	2.954305	0.266257
14	1	0	-0.658696	3.897801	-0.012889
15	1	0	0.535024	2.670762	0.135534
16	6	0	2.499706	-0.103587	0.004215
17	6	0	3.415411	-1.138504	0.026919
18	7	0	2.672879	-2.308178	0.095743
19	6	0	1.337616	-1.990705	0.110269
20	7	0	1.213907	-0.655077	0.056025
21	1	0	3.063163	-3.239255	0.122642
22	6	0	2.963316	1.238419	-0.075715
23	8	0	2.338247	2.298134	-0.114486
24	7	0	4.383450	1.262037	-0.117386
25	1	0	4.768894	2.196129	-0.200741
26	7	0	0.343150	-2.865882	0.177481
27	1	0	-0.653217	-2.559885	0.107822
28	1	0	0.542868	-3.853498	0.177579
29	6	0	5.211491	0.166002	-0.085208
30	7	0	4.753135	-1.073623	-0.007788
31	1	0	0.284016	-0.124487	0.069040
32	7	0	6.544305	0.373788	-0.164564
33	1	0	7.138589	-0.434062	-0.057448
34	1	0	6.955312	1.284458	-0.038544
35	7	0	-6.523267	-0.486188	-0.221713
36	1	0	-7.133085	0.308049	-0.093472
37	1	0	-6.892198	-1.382755	0.055459

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Rotational constants (GHZ):        0.5638194    0.1152244    0.0958313
Standard basis: 6-31G(d,p) (6D, 7F)
There are   425 symmetry adapted basis functions of A   symmetry.

SCF Done:  E(RB+HF-LYP) = -1196.33343039         -V/T =  2.0092
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Proton shift TS in Hoogsteen Proton-Bound Homodimer of 8-aminoguanine

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Stoichiometry    C10H13N12O2(1+)
Framework group  C2[C2(H),X(C10H12N12O2)]
Deg. of freedom   53
Full point group  C2      NOp   2
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Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Ångstroms) X	Y	Z
1	6	0	-0.152426	5.146374	-0.172773
2	7	0	-1.241504	4.314492	-0.261215
3	6	0	-1.218490	2.900420	-0.173316
4	6	0	0.108142	2.423230	0.001839
5	6	0	1.135363	3.352851	0.079576
6	7	0	1.073999	4.691699	0.009664
7	8	0	-2.290137	2.287297	-0.257879
8	7	0	0.636963	1.129555	0.121132
9	7	0	2.290137	2.618098	0.252186
10	6	0	1.955763	1.282488	0.268782
11	1	0	3.216609	3.008319	0.347308
12	1	0	-2.167195	4.693400	-0.425970
13	7	0	2.855400	0.306478	0.451741
14	1	0	3.835044	0.536225	0.388679
15	1	0	2.595485	-0.664199	0.215887
16	6	0	-0.108142	-2.423230	0.001839
17	6	0	-1.135363	-3.352851	0.079576
18	7	0	-2.290137	-2.618098	0.252186
19	6	0	-1.955763	-1.282488	0.268782
20	7	0	-0.636963	-1.129555	0.121132
21	1	0	-3.216609	-3.008319	0.347308
22	6	0	1.218490	-2.900420	-0.173316
23	8	0	2.290137	-2.287297	-0.257879
24	7	0	1.241504	-4.314492	-0.261215
25	1	0	2.167195	-4.693400	-0.425970
26	7	0	-2.855400	-0.306478	0.451741
27	1	0	-2.595485	0.664199	0.215887
28	1	0	-3.835044	-0.536225	0.388679
29	6	0	0.152426	-5.146374	-0.172773
30	7	0	-1.073999	-4.691699	0.009664
31	1	0	0.000000	0.000000	0.112242
32	7	0	0.356795	-6.478780	-0.317385
33	1	0	-0.440657	-7.071328	-0.141219
34	1	0	1.270303	-6.886189	-0.195273
35	7	0	-0.356795	6.478780	-0.317385
36	1	0	0.440657	7.071328	-0.141219
37	1	0	-1.270303	6.886189	-0.195273

```
Rotational constants (GHZ):      0.5684964      0.1181191      0.0985323
Standard basis: 6-31G(d,p) (6D, 7F)
There are   213 symmetry adapted basis functions of A   symmetry.
There are   212 symmetry adapted basis functions of B   symmetry.
SCF Done:  E(RB+HF-LYP) =  -1196.32944490      -V/T =  2.0092
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Experimental Details

DNA Preparation

DNA was prepared on an Expedite Synthesizer using standard protocols. Deprotection was performed over 20 hr in concentrated aqueous ammonia with 2-mercaptoethanol as recommended by the manufacturer for the 8-amino-dG phosphoramidite (Glen Research).

Full length DNA was separated from truncation products by PAGE, excised from the gel and extracted by the crush-and-soak method. Desalting was effected by SPE. The identity of the oligonucleotide was confirmed by ESI-MS: found m/z=1922 (M-H), expected m/z=1922.

Solid phase extraction was performed on an ODS Sep-Pak Plus (Waters). The following solutions were pulled through the bed using a peristaltic pump at 1 ml/min. 10mL 100% MeCN; 10 ml 50% MeCN, 50% 100mM triethylammonium acetate, pH 7 (TEAA); 100% TEAA; and the salt-containing DNA in ca. 50mL TEAA. The bed was then washed twice with 5 ml portions of nanopure water. The DNA was then eluted with 5mL 40% aqueous acetonitrile, the volume was reduced by half on a vacuum centrifuge, and the remaining organic-depleted fraction was frozen and lyophilized.

The purified DNA was resuspended in nanopure and equilibrated over >100 equivalents of lithium sulfonate resin (Dowex 50X8). The resin was washed several times with nanopure water and the lithium-exchanged DNA was concentrated two-fold and then lyophilized.

Sample Preparation

The buffers used were 10 mM acetate or cacodylate with 100 mM metal chloride and were prepared at the desired pH at 5-10×. All samples were placed in boiling water for 5 min and annealed at 5× final concentration (50 μM total oligonucleotide, 50 mM buffer, 500 mM salt) at 4ºC overnight.

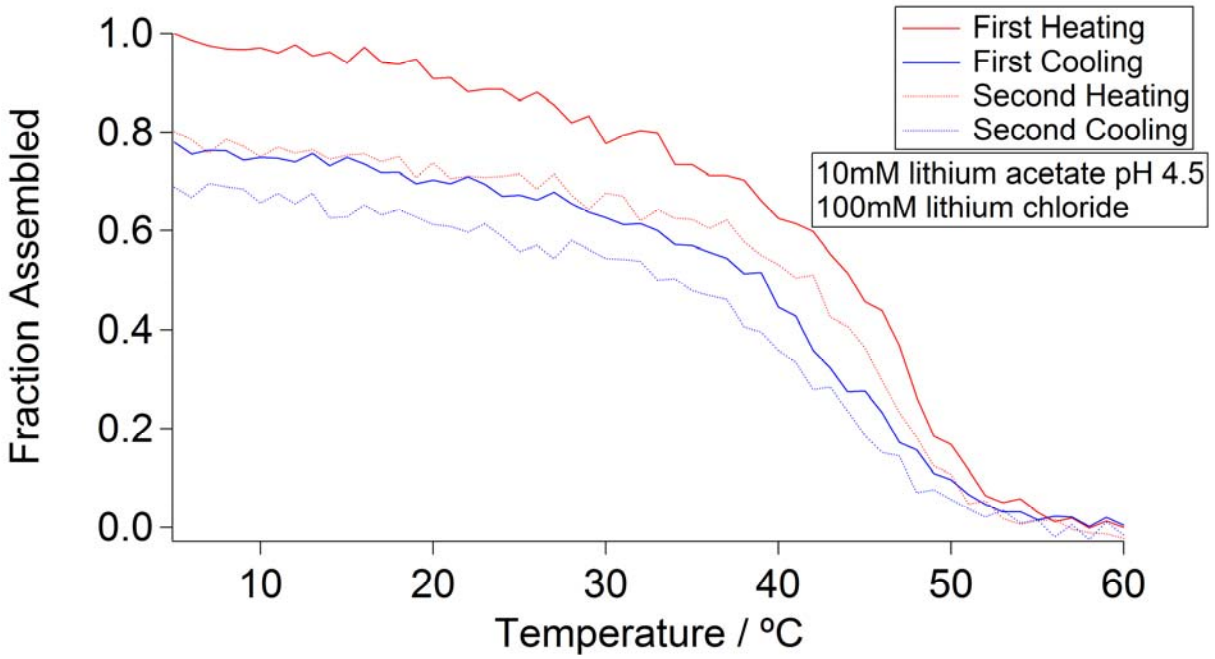
Circular Dichroism

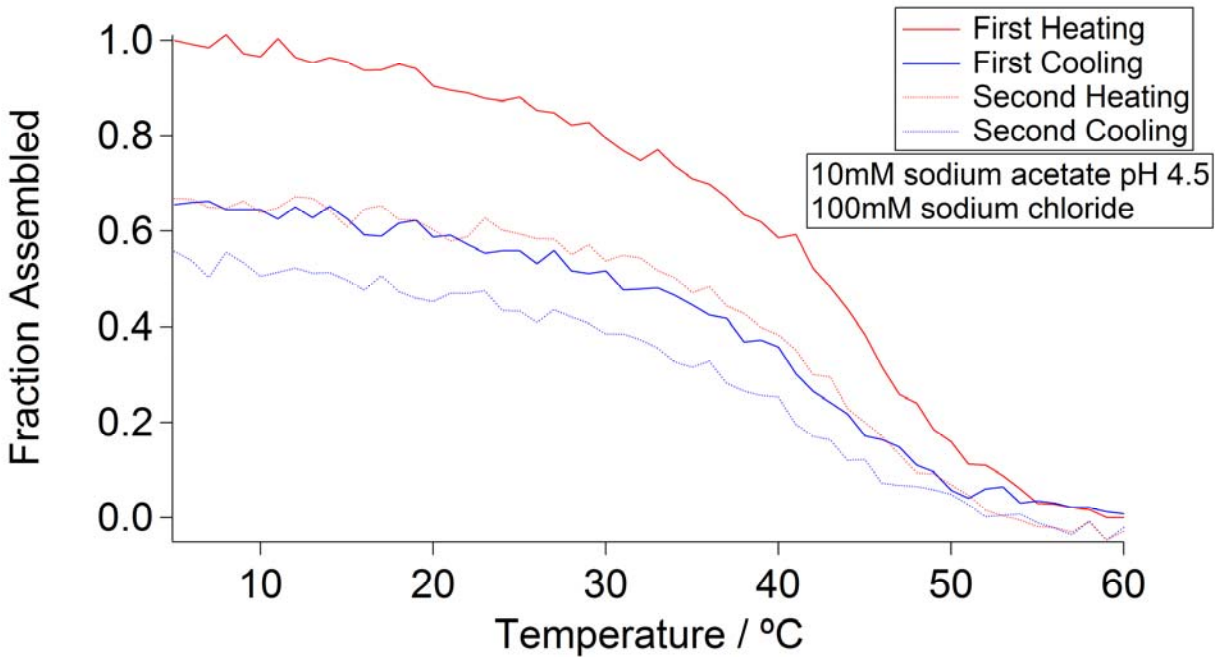
CD was performed on a JASCO J-810, at 10 μM total oligonucleotide, 10 mM buffer, 100 mM salt with 350-200 nm scans and 1ºC steps, with a temperature ramp rate of 0.16ºC - 1ºC/min. The full-wavelength spectrum at each temperature was fit to a two-state model, using the assumption that the 5ºC spectrum in the first heating corresponded to 100% duplex and the 60ºC spectrum corresponded to 0% duplex.

NMR

^{1}H-NMR spectra were collected on a Bruker DRX-500 at 275K (H_2O) or 280K (D_2O) and were the sum of 1024 transients. Spectra in 10% D_2O were observed using a 3-9-19 WATERGATE pulse sequence. Those in D_2O were 99.96% D and observed using a presaturation pulse.

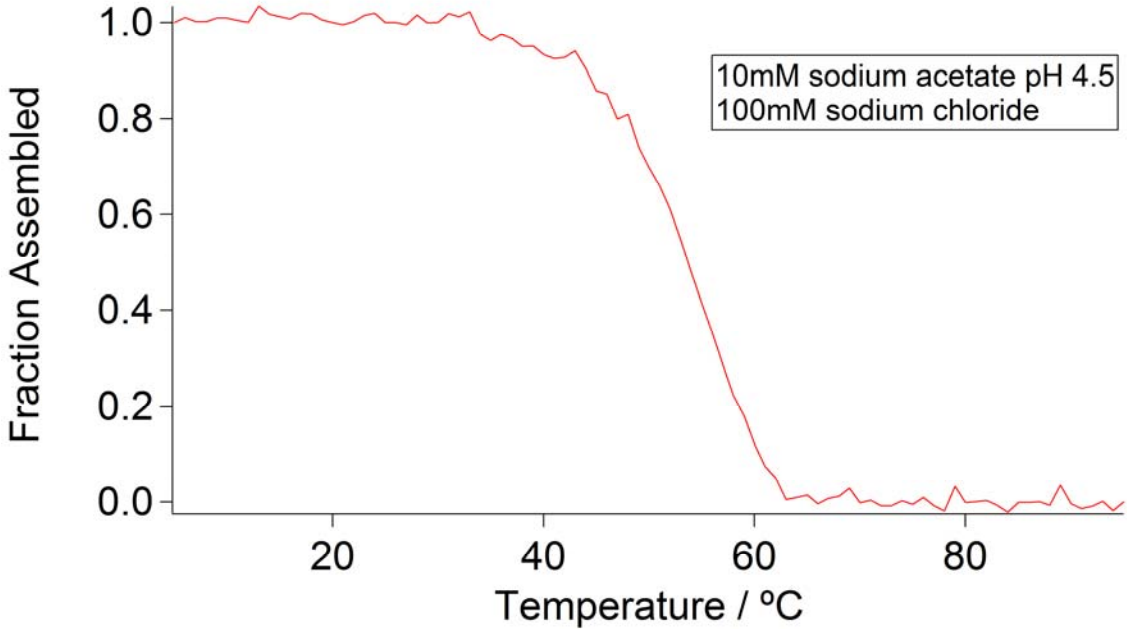
Circular Dichroism Studies of TX$_4$T Thermal Denaturation, Reversibility, and Ion Dependence





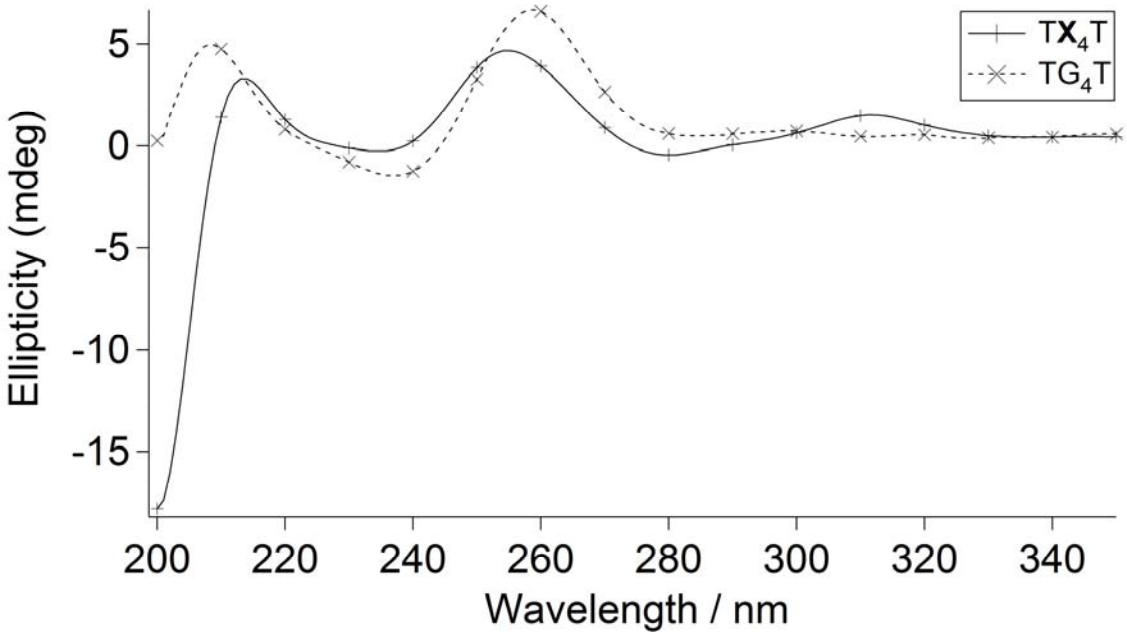
The partial loss of spectral intensity observed is not due to irreversible dissociation of a secondary structure, but, likely, partial depurination of d**X** residues. Extended heating resulted in further loss of ellipticity, supporting this hypothesis. The reaction appears to be salt-catalyzed, based on the cation dependence and lack of depurination in the salt-free NMR samples.

Circular Dichroism Studies of Thermal Denaturation of TG_4T



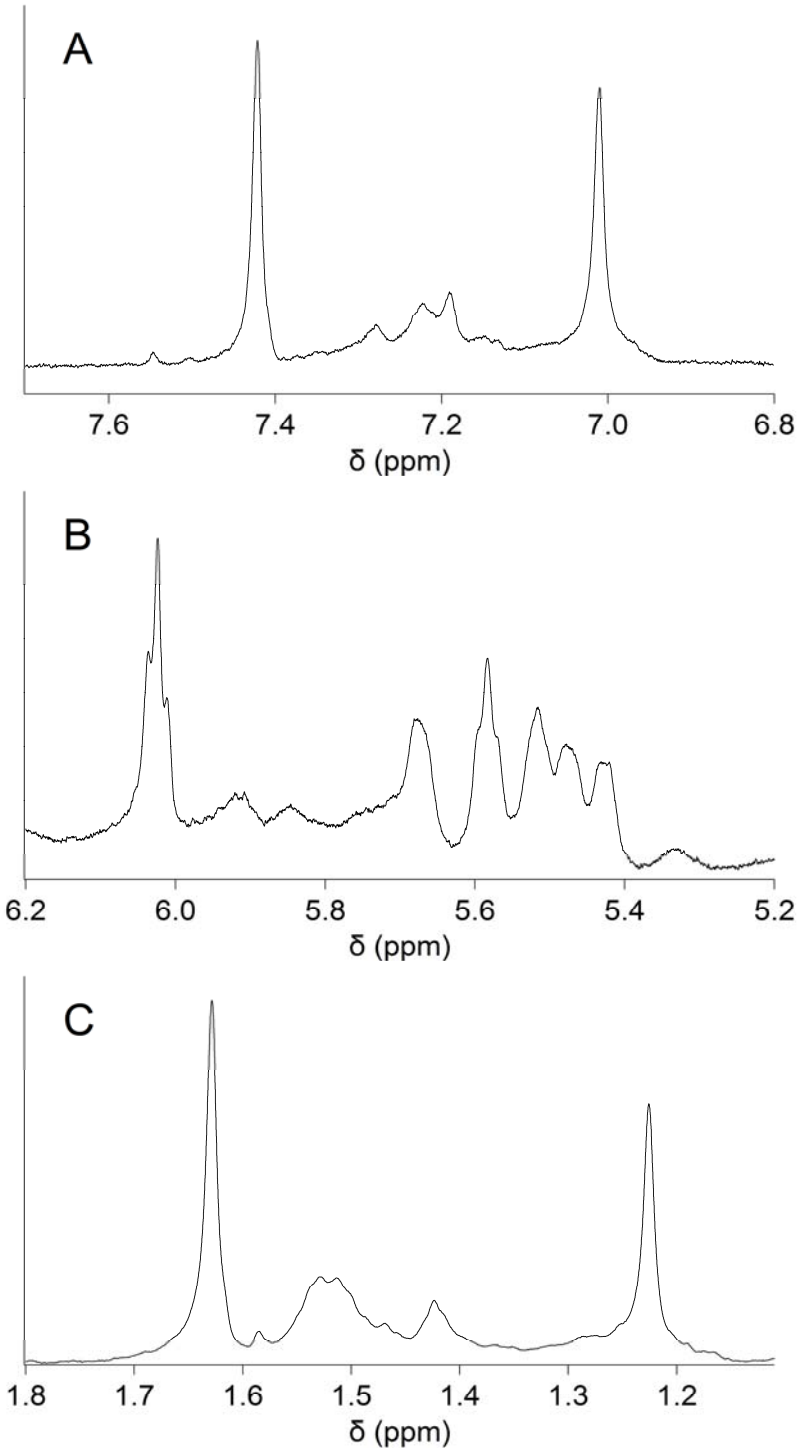
Irreversible changes in the CD spectrum persisted even after overnight incubation at 5ºC, due to the slow, tetramolecular association of the G-quadruplex.

CD spectra of TX_4T and TG_4T



Spectra were acquired at 278 K. Information on oligonucleotide and buffer concentrations is given above.

^{1}H NMR of TX_4T in D_2O



Consistent with a single predominant structure, two major aromatic (T1 and T6 H6) resonances (**A**), six H1′ resonances (**B**), and two methyl (T1 and T6 H5) resonances (**C**) are observed. 1mM lithium-exchanged T**X**$_4$T with no added salt in 99.96% D_2O, 280K, pD 5