Jakob T. Nielsen, Khalil Arar and Michael Petersen:

NMR solution structures of LNA (locked nucleic acid) modified quadruplexes

Supporting Information

Table S1: Chemical shift values of $d(TGLGLT)_4$ [a]

	H6/H8	CH_3	H1'	H2'	H2''	H3'	H4'	H5'	H5''	H6'	H6''	H1/H3	H21 [b]	H22 [b]	^{31}P
T1	7.309	1.372	5.843	1.974	2.271	4.644	4.024	3.652	3.593	-	-	10.810	-	-	−3.49
G2	8.089	-	6.211	2.915	2.816	5.057	4.408	4.098	4.000	-	-	11.416	9.63	6.32	−2.75
L3	7.444	-	5.832	5.059	-	4.669	-	4.77 [c]	4.529	4.374	4.441	11.146	9.31	6.23	−5.16
G4	7.861	-	6.192	2.791	2.791	5.092	4.419	4.504	4.257	-	-	11.177	9.31	6.23	−3.66
L5	7.499	-	6.155	4.77 [c]	-	4.751	-	4.382	4.273	4.103	4.155	11.437	9.45	6.76	−4.40
G6	7.532	1.491	6.078	2.057	2.275	4.427	4.065	4.160	4.053	-	-	10.570	-	-	-

[a] All values for the non-exchangeable protons were obtained from a 300 ms NOESY spectrum in D_2O, pH 6, 400 mM KCl and 3mM strand concentration at 25 °C. Values for the exchangeable protons were obtained from a 300 ms NOESY spectrum in 9:1 H_2O/D_2O, pH 6, 100 mM KCl and 0.8mM strand concentration at 5 °C. Proton chemical shifts in ppm are referenced relative to HDO. ^{31}P chemical shifts were obtained from a $^{1}H,^{31}P$ HSQC spectrum in D_2O, pH 6, 100 mM KCl and 0.8mM strand concentration at 25 °C. Values are referenced relative to inorganic phosphate.
[b] Approximate values due to very broad peaks.
[c] Assigned at 40 °C.

Table S2: Chemical shift values of $d(TL_4T)_4$ [a]

	H6/H8	CH_3	H1'	H2'	H2''	H3'	H4'	H5'	H5''	H6'	H6''	H1	H21	H22	^{31}P
T1	7.638	1.679	6.277	2.568	2.719	4.874	4.266	3.914	3.914	-	-	10.66 [b]	-	-	[c]
L2	7.785	-	5.623	5.407	-	5.026	-	4.406	4.367	4.080	4.233	10.770	[c]	6.058	−3.47
L3	7.387	-	6.031	5.410	-	4.891	-	4.410	4.328	4.157	4.268	10.825	9.343	7.105	−4.34
L4	7.580	-	6.242	5.248	-	5.050	-	4.262	4.324	4.147	4.147	11.155	9.516	6.308	−3.11
L5	7.462	-	6.149	4.730	-	4.683	-	4.250	4.327	4.180	4.143	11.482	9.384	7.477	−3.57
T6	7.548	1.526	6.116	1.990	2.313	4.475	4.104	4.057	4.104	-	-	11.08 [b]	-	-	-

[a] All values for the non-exchangeable protons were obtained from a 300 ms NOESY spectrum in D_2O, pH 6, 100 mM KCl and 1mM strand concentration at 25 °C. Values for the exchangeable protons were obtained from a 250 ms NOESY spectrum in 9:1 H_2O/D_2O, pH 6, 100 mM KCl and 0.25 mM strand concentration at 5 °C. Proton chemical shifts are referenced relative to HDO. ^{31}P chemical shifts were obtained from a $^{1}H,^{31}P$ HSQC spectrum in D_2O, pH 6, 100 mM KCl and 0.25 mM strand concentration at 25 °C. Values are referenced relative to inorganic phosphate.
[b] Not specific assignments.
[c] Not assigned.
[d] Assigned at 40 °C.

NMR experiments

The NMR experiments conducted are listed in Table S1. For experiments at 500 MHz (500 μL), the sample contents were 100 mM KCl, pH 6 and 0.25 mM strand concentration d(TL_4T) or 0.75 mM strand concentration d(TGLGLT). For experiments at 800 MHz (125 μL), the sample contents were 400 mM KCl, pH 6 and 1.0 mM strand concentration d(TL_4T) or 3.0 mM strand concentration d(TGLGLT).

Table S3. Experimental parameters for the 2D spectra applied in the assignment of resonances. [a]

Experiment	Field strength /MHz	T/°C	Mixing times /ms	Spectral width /ppm	Number of points (t_2)	Number of t_1 experiments	Number of transients	Delay /s
	d(TL_4T)							
NOESY	800	25	100,300	10.5	4096	322	80	2.244
WATERGATE NOESY [b]	500	5	250	15.0	4096	345	96	2.173
DQF-COSY	800	25	-	10.5	4096	330	64	1.744
^{1}H-^{31}P HSQC (decoupled)	500	25	-	10.0,2.5	2048	40	1408	1.705
	d(TGLGLT)							
NOESY	800	25	60,120,200,300	7.5	4096	465	32	3.141
Jump-return NOESY [c]	500	5	250	13.2	8192	512	48	3.141
WATERGATE NOESY [b]	500	5	250	13.2	4096	600	32	2.990
DQF-COSY	500	25	-	10.0	2048	430	48	2.025
TOCSY	500	25	90	10.0	2048	450	64	2.405
^{1}H-^{31}P HSQC (decoupled)	500	25	-	10.0,3.0	2048	67	1152	1.705
^{1}H-^{31}P HSQC (undecoupled)	500	50	-	10.0,3.0	2048	64	1888	1.000

[a] Experiments were performed on Varian Inova spectrometers operating at either 500 or 800 MHz. The 500 MHz spectrometer was equipped with a 5 mm triple resonance HCP probe and the 800 MHz spectrometer with a 3 mm triple resonance HCN probe.

[b] Includes a water flipback pulse to suppress water magnetization. A gradient is applied in t_1 to eliminate radiation damping effects.

[c] The jump-return delay was 100ms.

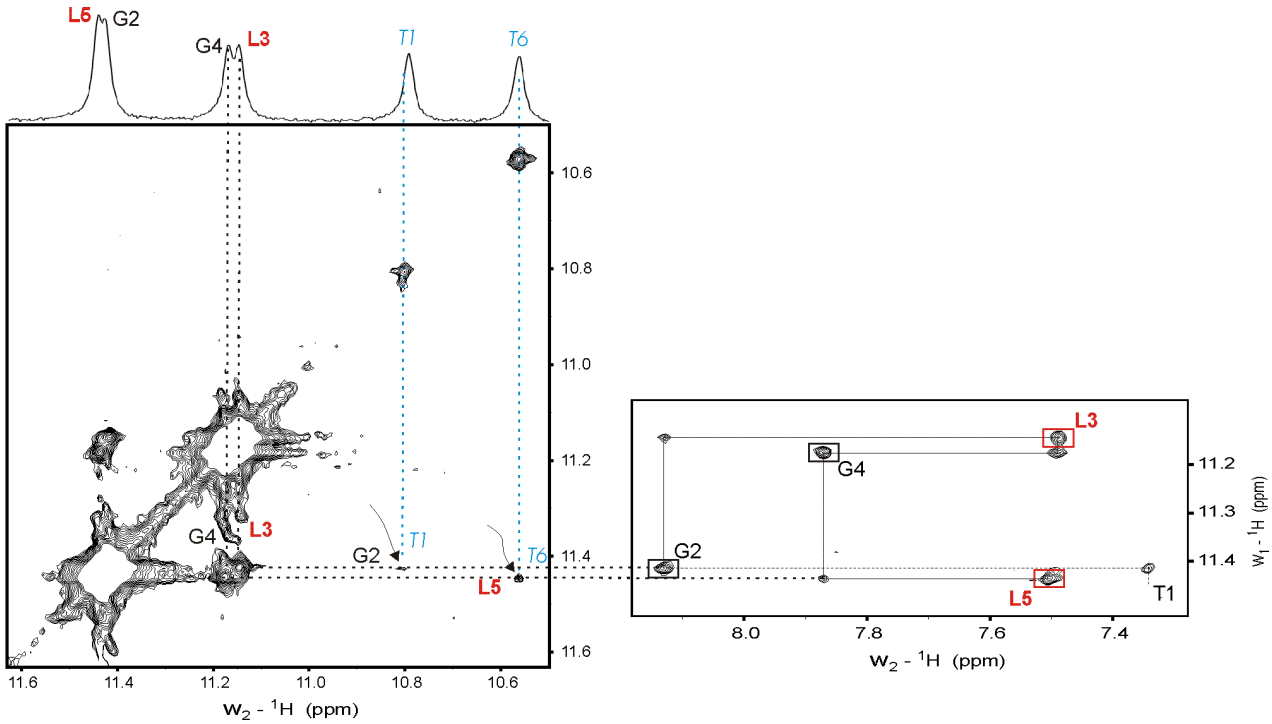

Figure S1: *Left* Imino to imino region of the 300 ms jump-return NOESY spectrum of d(TGLGLT)$_4$ at 5 °C in 9:1 H_2O/D_2O, pH 6, 100 mM KCl and 0.75mM strand concentration. The 1D spectrum is shown above the jump-return NOESY spectrum with assignments indicated. L denotes an LNA guanosine imino proton. T denotes a thymidine imino proton. Resonance positions are indicated by broken lines. The cross peaks G2H1-T1H3 and G5H1-T6H3 are marked with arrows. *Right* Imino to aromatic region of the 250 ms WATERGATE NOESY spectrum of d(TGLGLT)$_4$ at 5 °C in 9:1 H_2O/D_2O, pH 6, 100 mM KCl and 0.75mM strand concentration. Important intra-tetrad G(i)/L(i)H1-H8 cross peaks proving the parallel conformation are marked with boxes with the nucleotide specified next to the box. The unbroken lines connect the intra-tetrad cross peaks with the inter-strand cross peaks between the aromatic proton of a nucleotide and the imino proton of the 3'-flanking nucleotide of the neighbouring strand, *i.e.* G(i)/L(i)H8-G*(i+1)/L*(i+1)H1 cross peaks.

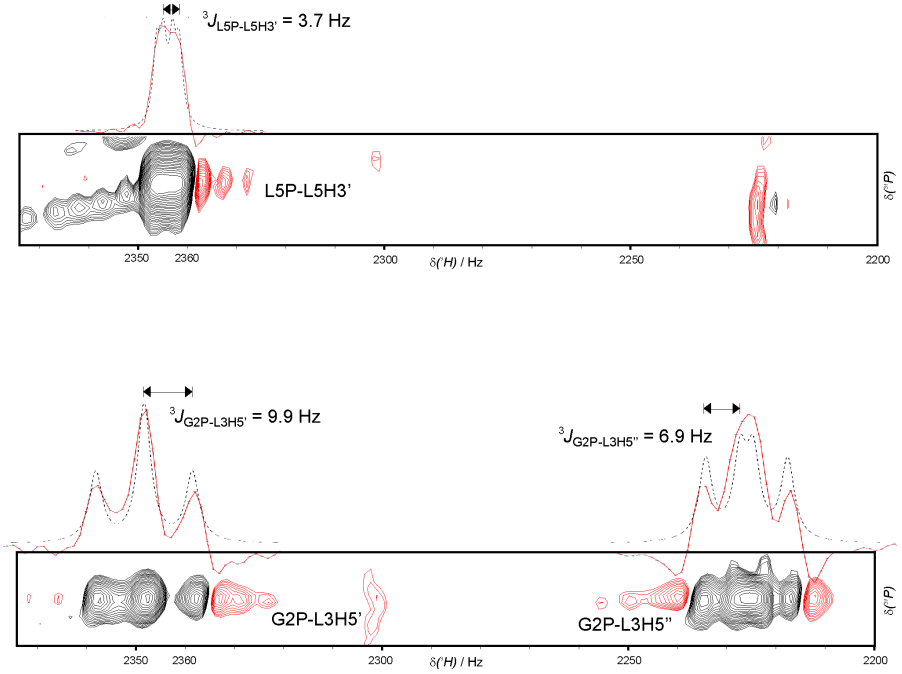

Figure S2. Two regions of the undecoupled $^{31}P,^{1}H$ HSQC of TGLGLT. 1D-traces are shown at the top of the 2D regions with red lines through the data points and fitted lineshapes included as broken curves. The fits are least squares fits to Lorentzian lineshapes. In the fitting procedure the coupling constants, line widths (all equal), chemical shifts, and peak intensities were varied. The following coupling constants were included in the fitting L5P-L5H3': $^3J_{L5P\text{-}L5H3'}$, $^3J_{L5H2'\text{-}L5H3'}$, G2P-L3H5': $^3J_{G2P\text{-}L3H5'}$, $^2J_{G2H5'\text{-}G2H5''}$, G2P-L3H5'': $^3J_{G2P\text{-}L3H5''}$, $^2J_{G2H5'\text{-}G2H5''}$.

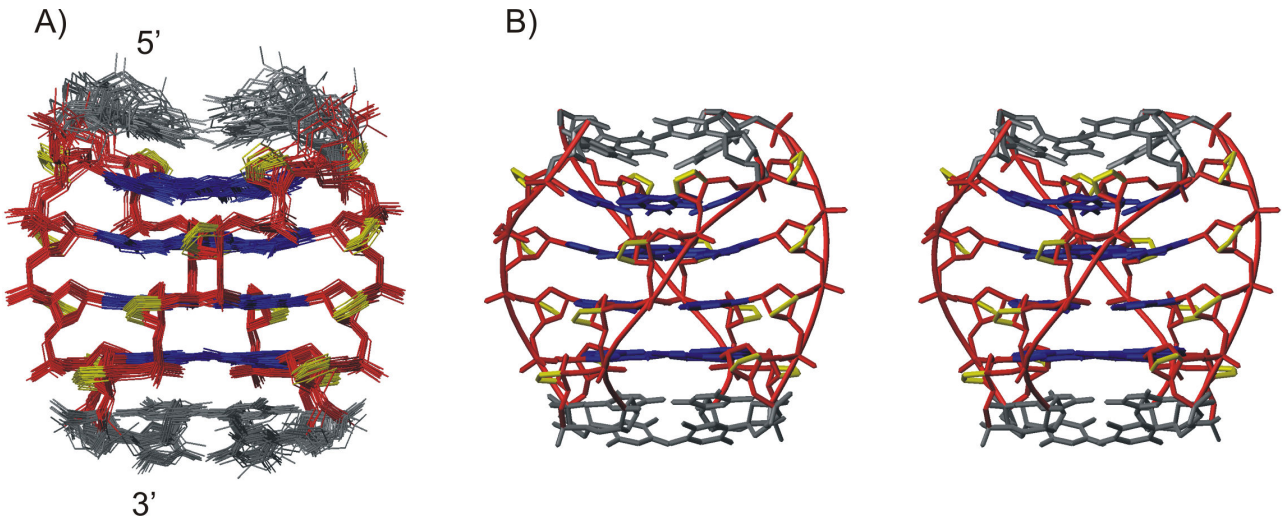

Figure S3. A) Overlay of the 20 structures calculated of the TL_4T quadruplex. B) Stereoview of one representative structure. The LNA oxymethylene bridges are shown in yellow. The sugar–phosphate backbone, the nucleobases and the thymines are shown in red, blue and grey, respectively. For clarity, hydrogen atoms are not shown. In figure B, a red ribbon is added to highlight the sugar–phosphate backbone.

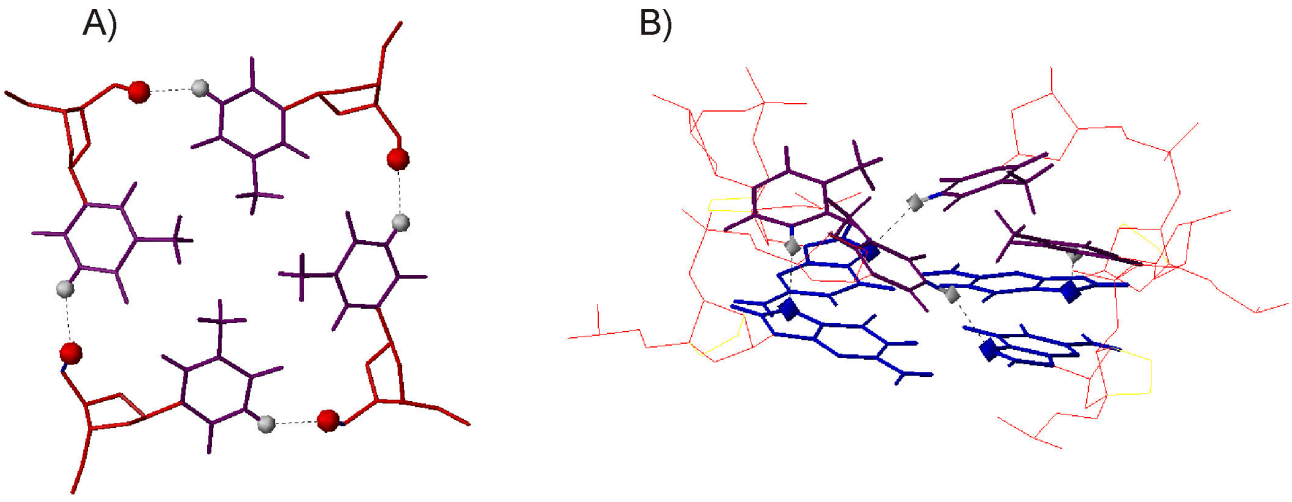

Figure S4. View of one of the structures of $d(TGLGLT)_4$ showing the hydrogen bonding of the thymine imino protons. Hydrogen bonds are shown with broken black lines. The colouring scheme is as in Figure S2 and thymine bases are shown in purple. A) View along the helix axis showing the hydrogen bonding pattern of the thymines at the 5’-end. T1 and symmetry related nucleotides are shown. A hydrogen bond is formed between T1H3 (H3 shown as a grey sphere) and T1O5’ of a neighbouring strand (O5’ shown as a red sphere). B) Hydrogen bonding pattern of the thymines at the 3’-end. The view is tilted slightly relative to being perpendicular to the helix axis. T6, G5 and symmetry related nucleotides are shown. A hydrogen bond is formed between T6H3 (H3 shown as a grey sphere) and G5N7 of a neighbouring strand (N7 shown as a blue sphere).

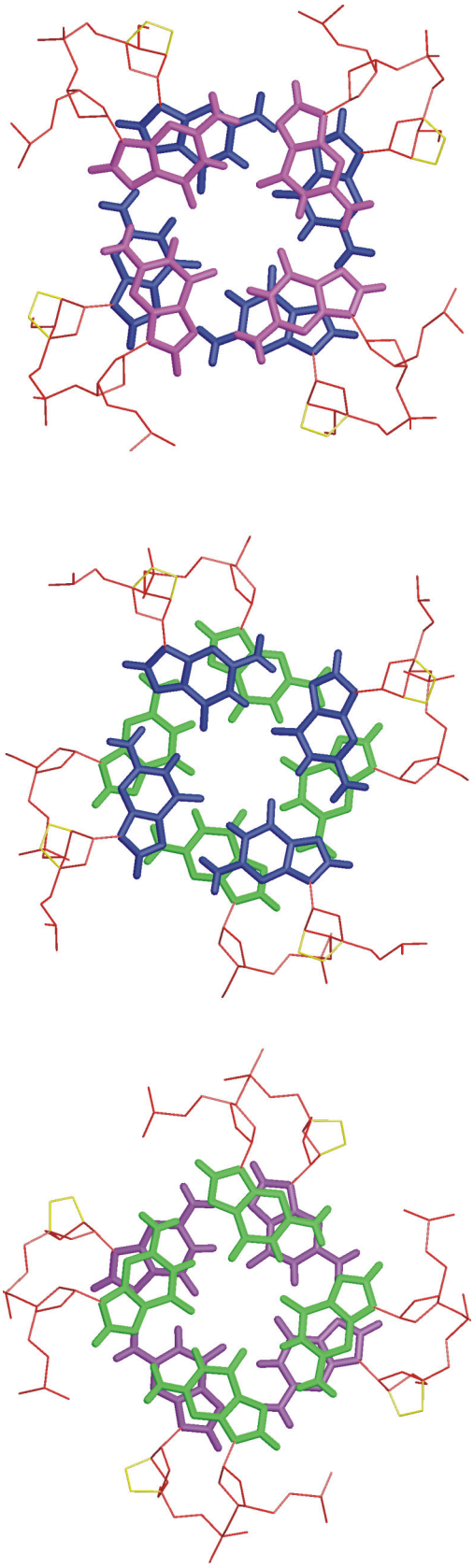

Figure S5. Consecutive stacking between pairs of tetrads. *Top* G2 and L3 tetrads, *middle* L3 and G4 tetrads and *bottom* G4 and L5 tetrads. The nucleobases are coloured according to the position in the sequence: G2: pink, L3: blue, G4: green, L5: violet.