Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

ELECTRONIC SUPPLEMENTARY INFORMATION

A novel equilibrium relating to the helix handedness in G-quadruplexes formed by heterochiral oligonucleotides with an inversion of polarity site

Antonella Virgilio,‡ Veronica Esposito,‡ Alfonso Mangoni, Luciano Mayol, and Aldo Galeone*

Department of Pharmacy, University of Naples "Federico II", Via D. Montesano, 49, 80131 Naples (Italy)

*Corresponding Author
galeone@unina.it

‡These authors contributed equally.

EXPERIMENTAL SECTION

Oligonucleotides synthesis and purification

The oligonucleotides **L33** and **L55** were synthesized on a Millipore Cyclone Plus DNA synthesizer using solid phase β-cyanoethyl phosphoramidite chemistry at 15 µmol scale. The synthesis of the 3'-5' tracts were performed by using 5'-dimethoxytrityl-β-L-deoxyguanosine (iBu)-3'-phosphoramidite and 5'-dimethoxytrityl-β-L-deoxythymidine-3'-phosphoramidite prepared according to Hurata *et al.* procedure (H. Urata, E. Ogura, K. Shinohara, Y. Ueda, M. Akagi, *Nucleic Acids Res.* **1992**, *20*, 3325-3332), whereas the 5'-3' tracts were synthesized by using 5'-phosphoramidites. For both ODNs, an universal support was also used.

The oligomers were detached from the support and deprotected by treatment with concentrated aqueous ammonia at 80°C overnight. The combined filtrates and washings were concentrated under reduced pressure, redissolved in H_2O, analyzed and purified by high-performance liquid chromatography (HPLC) on a Nucleogel SAX column (Macherey–Nagel, 1000-8/46); using buffer A: 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0), containing 20% (v/v) CH_3CN; buffer B: 1 M KCl, 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0), containing 20% (v/v) CH_3CN; a linear gradient from 0% to 100% B for 30 min and a flow rate of 1 mL/min were used. The fractions of the oligomers were collected and successively desalted by Sep-pak cartridges (C-18). The isolated oligomers proved to be >98% pure by NMR.

Circular dichroism

CD samples were prepared at a concentration of $1x10^{-4}$ M by using the buffer solution used for NMR experiments: KH_2PO_4/K_2HPO_4 (10 mM, pH 7.0), KCl (70 mM), and EDTA (0.2 mM). CD spectra of all quadruplexes were registered on a Jasco 715 CD spectrophotometer in a 0.1 cm pathlength cuvette; the wavelength was varied from 220 to 320 nm at 100 nm min^{-1} scan rate, and the spectra recorded with a response of 16 s, at 2.0 nm bandwidth and normalized by subtraction of the background scan (buffer alone). The temperature was kept constant at 10°C with a thermoelectrically-controlled cell holder (Jasco PTC-348).

UV thermal difference spectra (TDS)

UV samples of investigated oligonucleotides were prepared using a buffer solution: lithium cacodylate (10 mM, pH 7.2), KCl (70 mM). All experiments were performed on a Jasco V 530 UV/Vis spectrophotometer using quartz cuvettes with an optical path of 1 cm and at 40 µM strand concentration. Absorbance spectra were recorded in the 220-320 nm range, with a scan speed of 200 nm min^{-1} and with a data interval of 1 nm. The difference between the UV spectra at high (90°C) and low (10°C) temperatures was defined as the TDS; this represents the spectral difference between the unfolded and folded forms. The temperature (10 or 90°C) was kept constant with a thermoelectrically controlled cell holder (Jasco PTC-348). The thermal difference spectra were normalized (+1 for the highest positive peak).

Nuclear magnetic resonance experiments

NMR samples were prepared at a concentration of about 3 mM, in 0.6 mL (H_2O/D_2O 9:1 v/v), buffer solution having 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA (pH 7.0). All the samples were heated for 5-10 min at 80°C and slowly cooled (10-12 h) to room temperature. The solutions were equilibrated for several weeks at 4°C. The annealing process was assumed to be complete when ^1H NMR spectra were superimposeable on changing time. NMR spectra were recorded with Varian Unity INOVA 700 MHz and Varian Unity INOVA 500 MHz spectrometers. 1D proton spectra of the samples in H_2O were recorded using pulsed-field gradient DPFGSE for H_2O suppression (C. Dalvit, *J. Biomol. NMR* **1998**, *11*, 437-444). ^1H-chemical shifts were referenced relative to external sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Pulsed-field gradient DPFGSE sequences were used for NOESY (200 ms mixing time) and ROESY (200 ms mixing time) experiments in H_2O (T.L. Hwang, A.J. Shaka, *J. Magn. Reson.*, **1995**, *A 112*, 275-279; C. Dalvit, *J. Biomol. NMR* **1998**, *11*, 437-444). All experiments were recorded using STATES-TPPI procedure for quadrature detection (D. Marion, M. Ikura, R. Tschudin, A. Bax, *J. Magn. Reson.* **1989**, *85*, 393-399). In all 2D experiments, the time domain data consisted of 4096 complex points in t2 and 256-512 fids in t1 dimension. A relaxation delay of 1.5 s was used for all experiments.

Gel electrophoresis

Modified oligonucleotides were analyzed by non-denaturing PAGE. Samples in the NMR buffer (20 mM KH_2PO_4, 70 mM KCl and 0.2 mM EDTA, pH=7) were loaded on a 20% polyacrylamide gel containing Tris–Borate-EDTA (TBE) 2.5x and KCl 50 mM. The run buffer was TBE 1x containing 100 mM KCl. Single-strand samples were obtained by LiOH denaturation. For all samples, a solution of glycerol/TBE 1x-100mM KCl 2:1 was added just before loading. Electrophoresis was performed at 9.2 V/cm at a temperature close to 15°C. Bands were visualized by UV shadowing.

Thermal denaturation experiments

All experiments were performed in a 10 mM sodium cacodylate buffer at pH 7.2 containing 70 mM KCl. After denaturation at 95°C, the solutions were slowly cooled at room temperature and finally allowed to equilibrate at 4°C for 1 week at 4°C at 200–300 μM strand concentration. The tetramolecular complexes were then diluted to 40 μM strand concentration in potassium buffer. This long equilibration time at a relatively high strand concentration has been chosen because of the extreme kinetic inertia of tetramolecular complexes (Mergny, J.L., De Cian, A., Ghelab, A., Saccà,B. and Lacroix,L. Kinetics of tetramolecular quadruplexes. Nucleic Acids Res., 2005, 32, 81-94). Thermal denaturation curves were obtained with a Jasco V530 UV/Vis spectrophotometer using quartz optical cells of 1 cm pathlength the unfolding processes were recorded in the 20-80°C temperature range, using a scan rate of 0.5°C min^{-1} and following the variation of UV absorption at 295 nm.

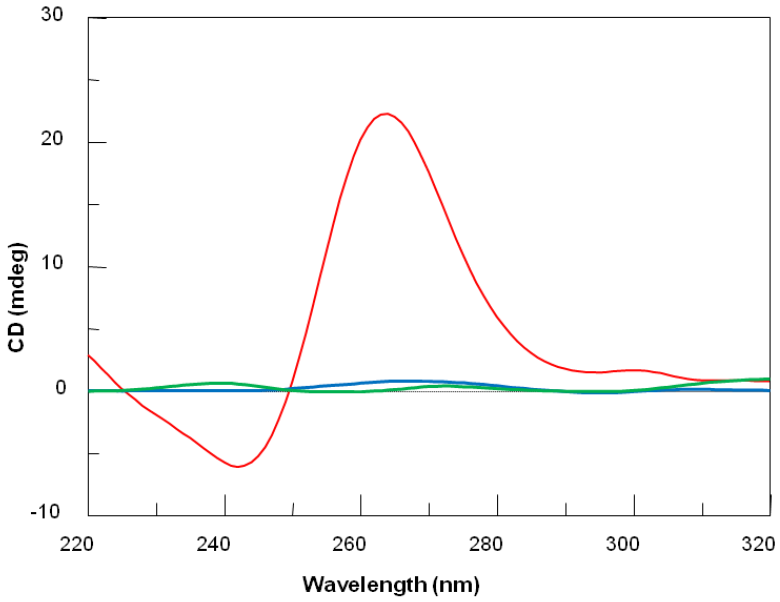

Figure S1: CD spectra of **L33** (blue), **L55** (green) and their natural counterpart $[d(TGGGGT)]_4$ (red) at 10°C (10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA, pH 7.0).

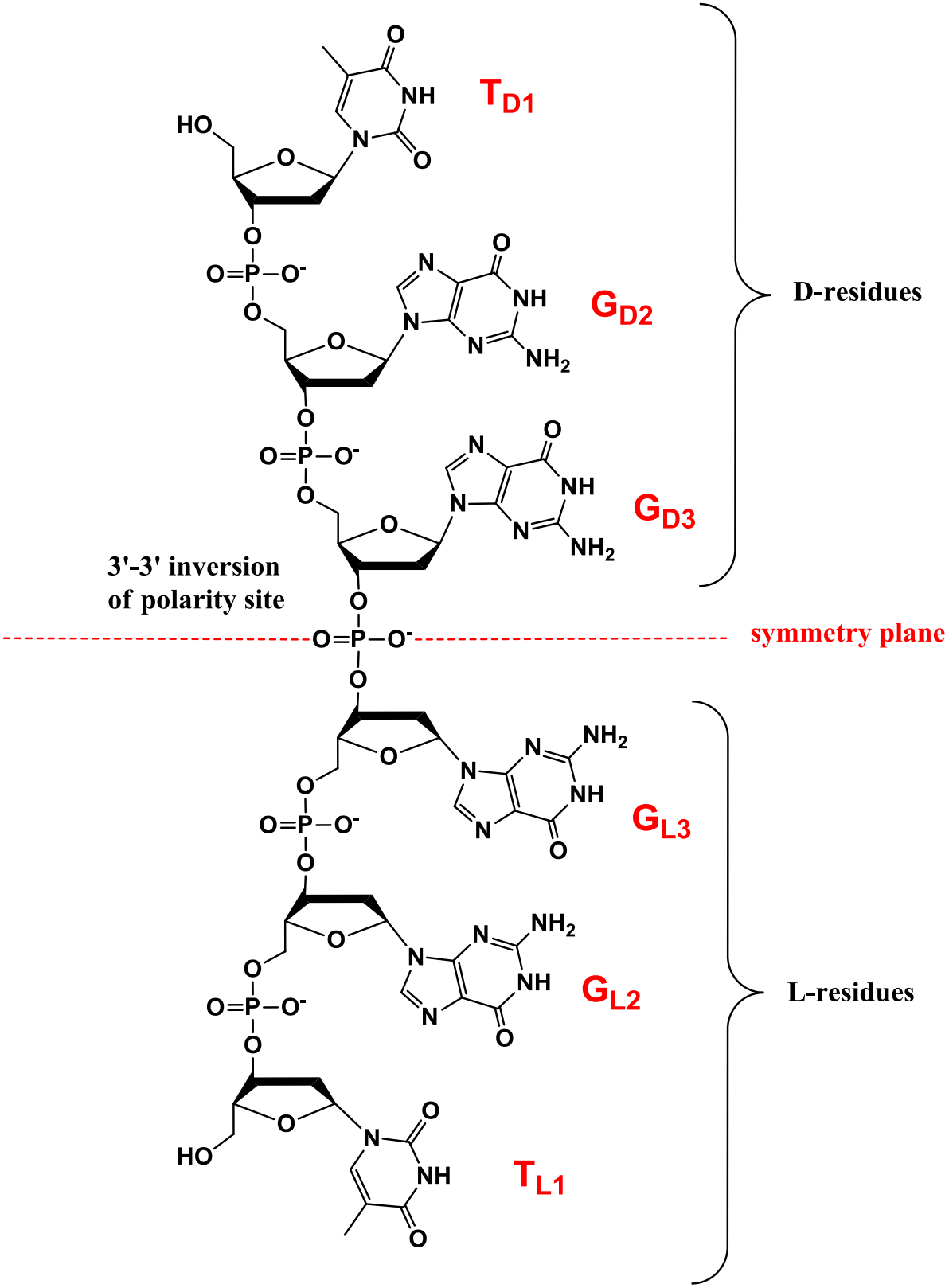

Figure S2: Chemical structure of D/L-ODN **L33**.

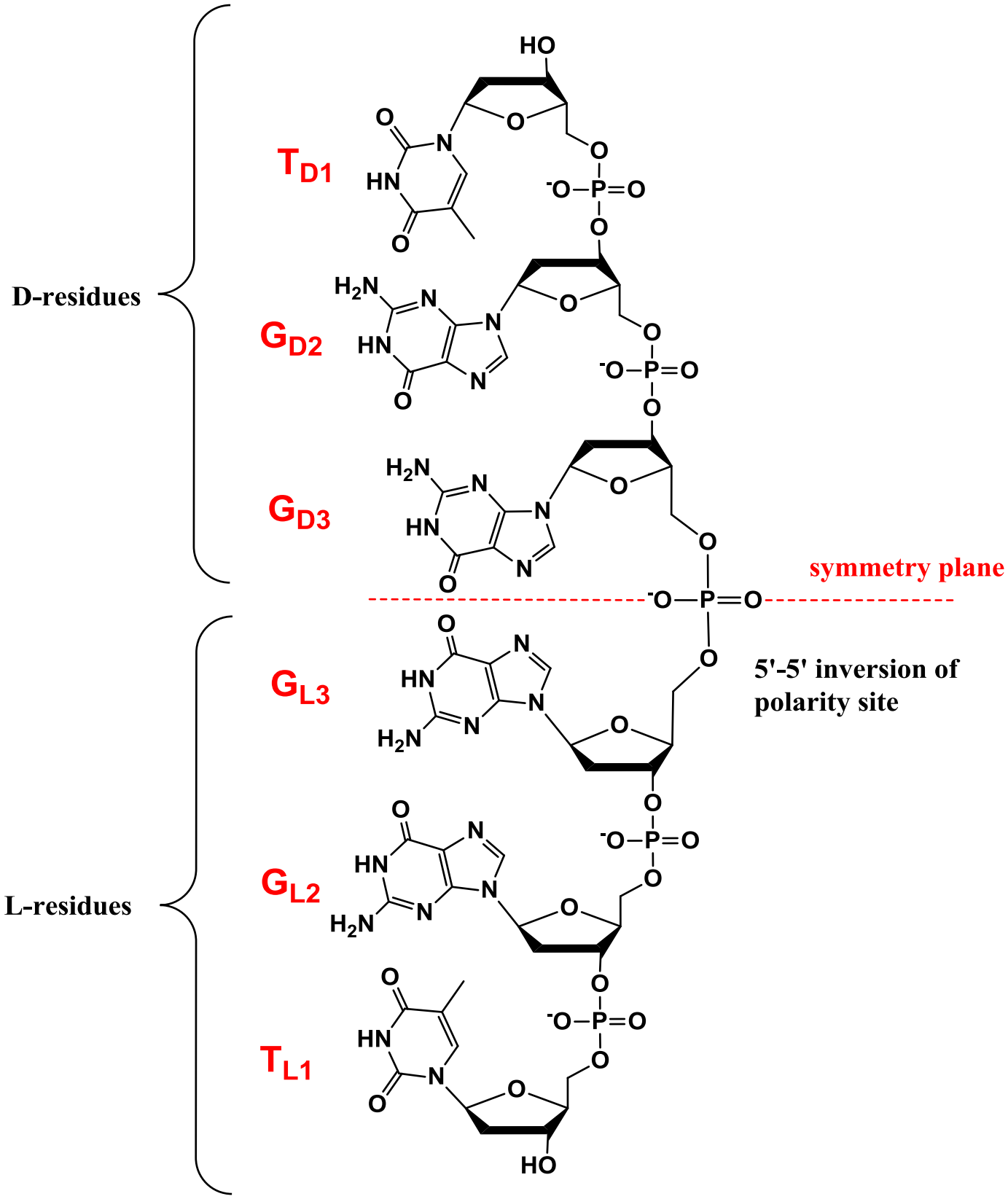

Figure S3: Chemical structure of D/L-ODN **L55**.

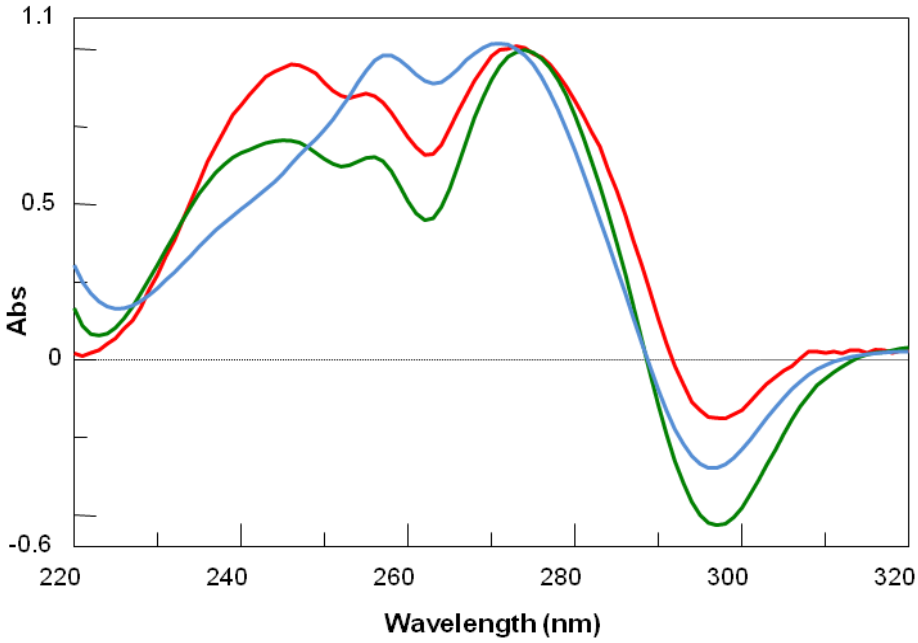

Figure S4: TDS profiles of **L33** (blue), **L55** (green) and $[d(TGGGGT)]_4$ (red). The TDS profile of **L55** is very similar to that of its natural counterpart $[d(TGGGGT)]_4$ and to those of other types of G-quadruplex structures. Particularly, positive peaks around 243 and 273 nm, peculiar of G-quadruplex structures, are evident. The TDS profile of **L33**, although preserves the positive peak around 273 nm, appears quite different from that of **L55** in the region 230-265 nm. These differences could be tentatively ascribed to the presence of a 3'-3' phosphodiester bond that, due to its more restricted flexibility compared to a canonical 3'-5' or a 5'-5' phosphodiester bond, could prevent the usual G-tetrad stacking for the guanosines adjacent to the 3'-3' inversion of polarity site.

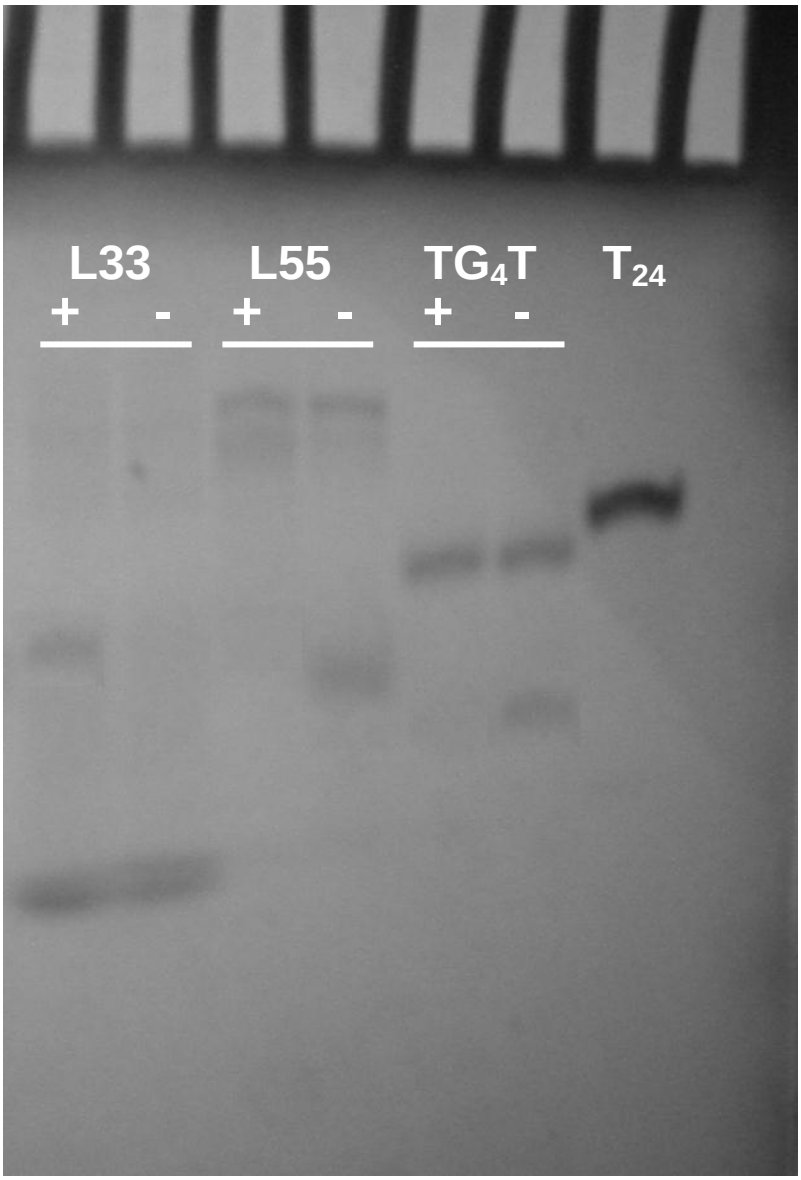

Figure S5: Non-denaturing PAGE. Samples were loaded on a non-denaturing gel, run at 15°C. Oligonucleotides were revealed by UV shadowing. Lanes marked by (-) correspond to the denatured sequences pre-treated with LiOH (0.1 M, 5' at 80°C) followed by neutralization by HCl 0.1 M and immediate loading on the gel. The native samples, indicated by (+), have been prepared in a buffer containing 10 mM KH_2PO_4, 70 mM KCl and 0.2 mM EDTA, pH=7. The behaviour of **L33** confirms its low thermal stability since the native sample (+) shows a less intense slower migrating band attributable to the quadruplex structure. On the other hand, the native sample (+) of **L55** doesn't show unstructured species, while the (-) lane displays a slower migrating band corresponding to the quadruplex structure re-forming during the gel run, probably due to its higher thermal stability compared to **L33**. ODN's TG_4T and T_{24} have been introduced as references.

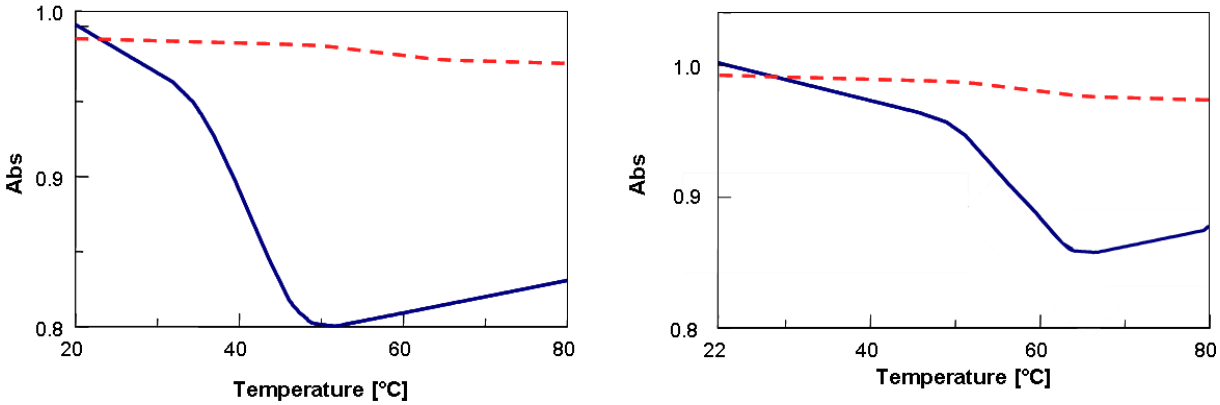

Figure S6: Normalized UV melting profiles of quadruplex structures formed by ODNs **L33** (left) and **L55** (right), compared with their natural counterpart $(TGGGGT)_4$ (red dashed line). In K^+ buffer $(TGGGGT)_4$ doesn't show an appreciable sigmoidal melting profile, thus indicating a $T_{1/2}$ higher than 80°C, in agreement with other authors (see J.-L. Mergny, A.-T. Phan, L. Lacroix, *Following G-quartet formation by UV-spectroscopy*, FEBS Letters 1998, 435, 74-78).

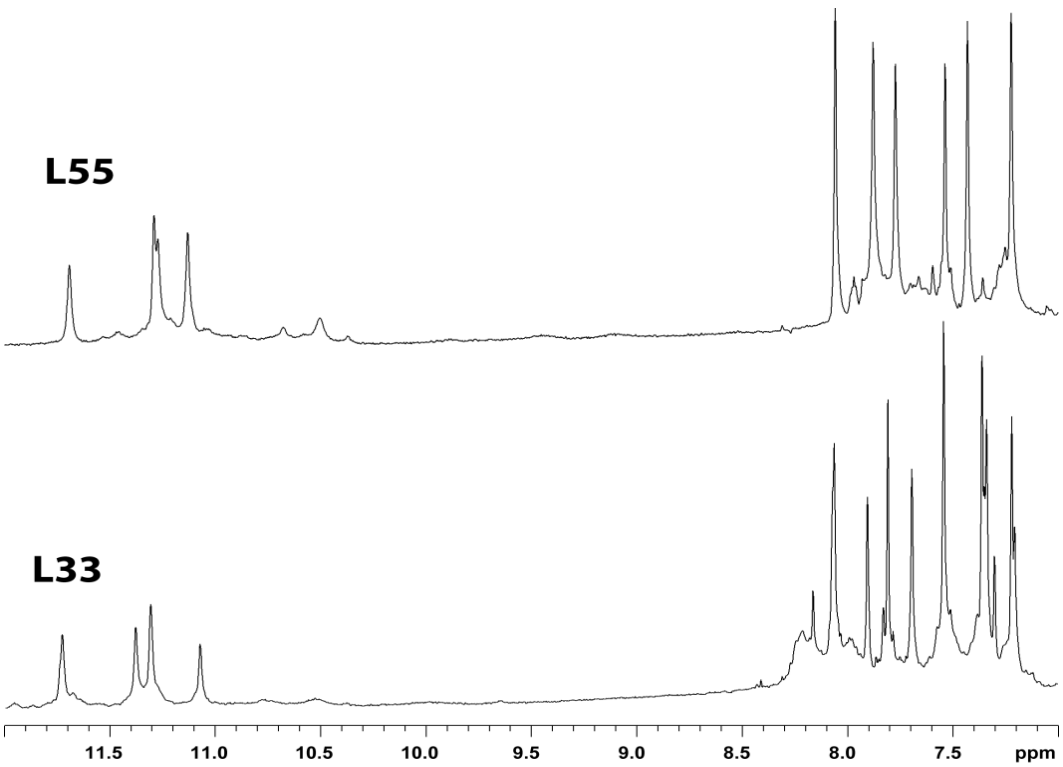

Figure S7. Aromatic and imino proton regions of the 1H-NMR spectra (700 MHz) of **L55** (10°C) and **L33** (7°C); 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA (pH 7.0) in H_2O/D_2O 9:1.

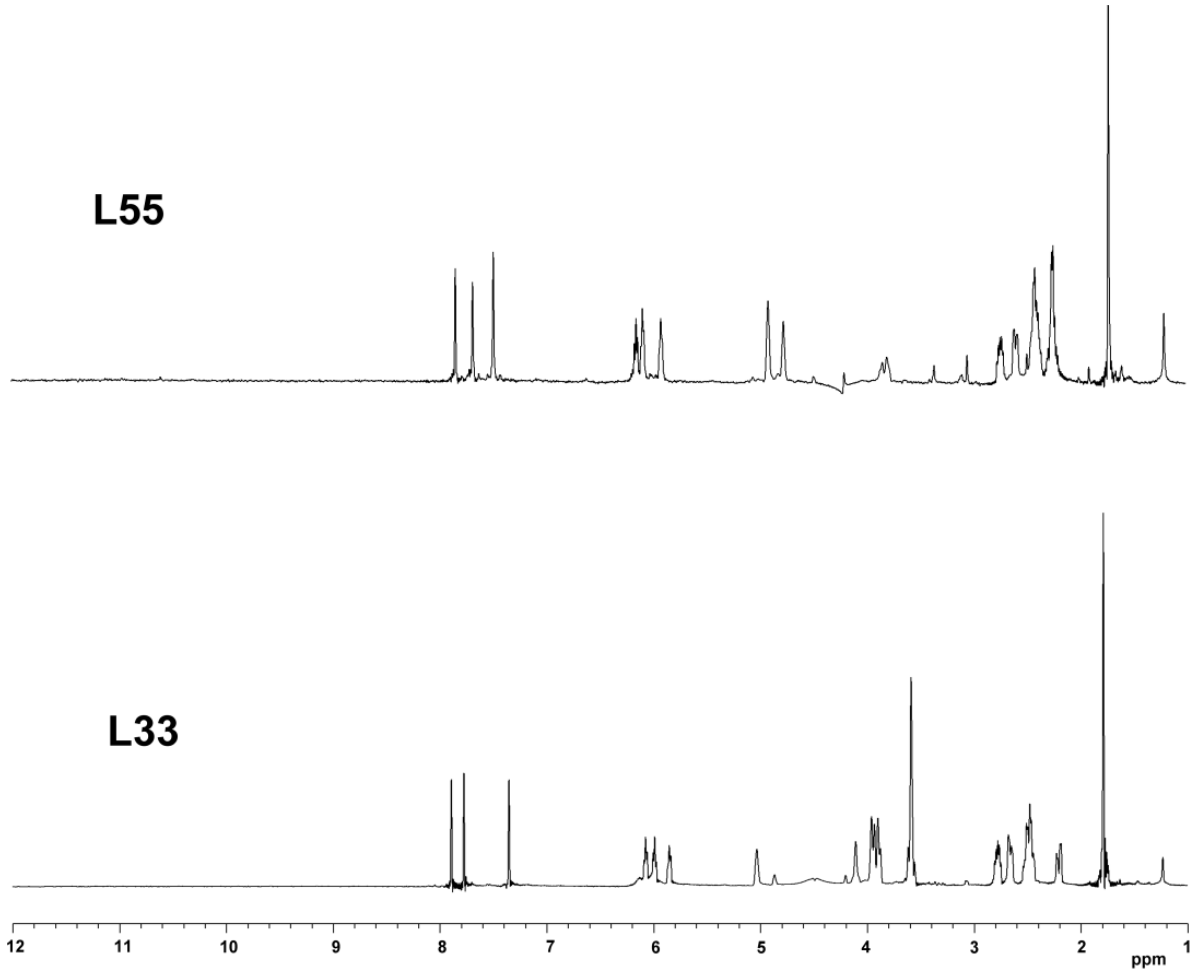

Figure S8: ^{1}H-NMR spectra (500 MHz) of **L55** (80°C) and **L33** (50°C); 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA (pH 7.0) in H_2O/D_2O 9:1.

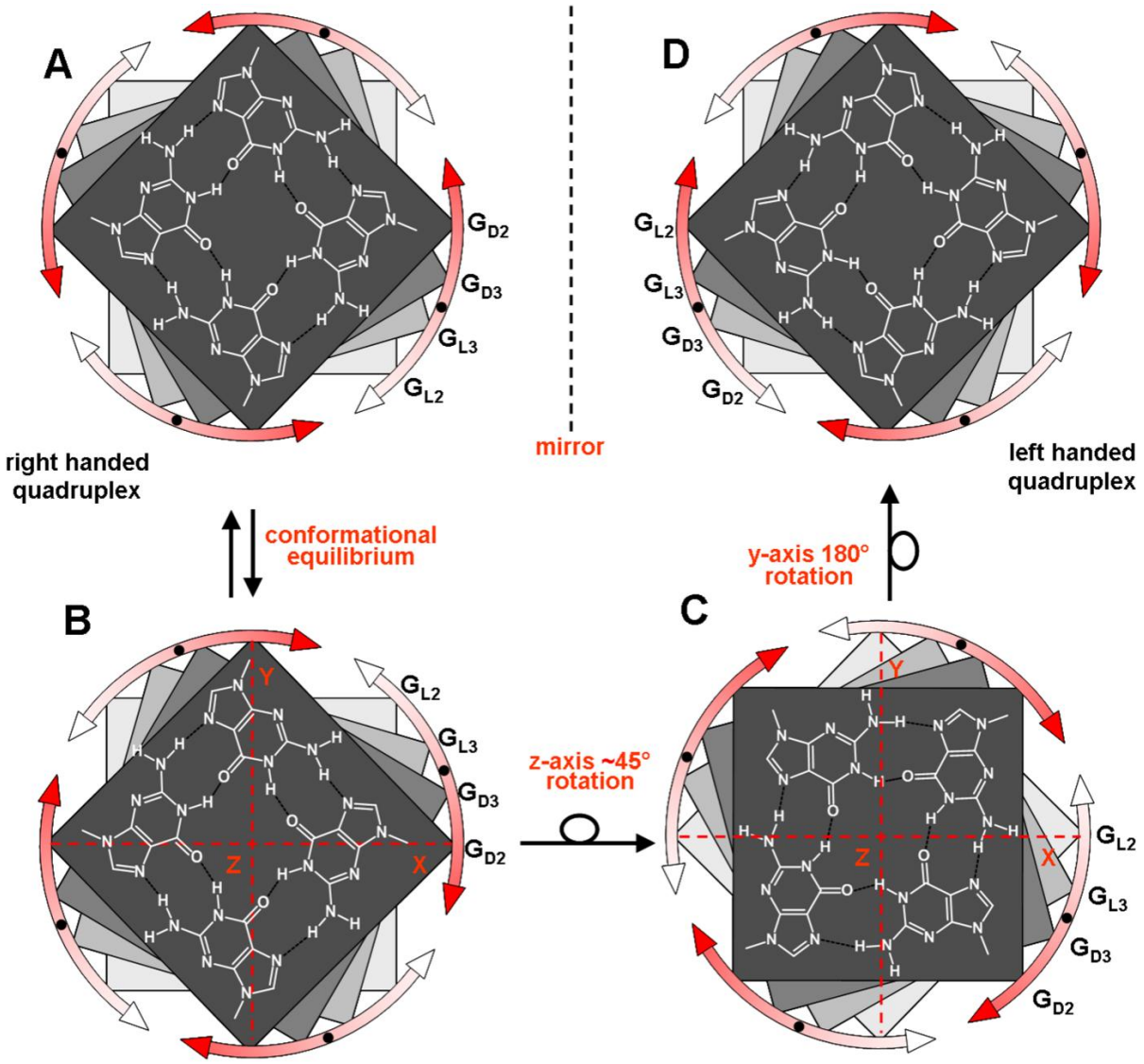

Figure S9: Schematic representation of the interconversion of the enantiomeric quadruplexes formed by **L55**. (**A**) Top view of the right-handed quadruplex. G-tetrads are represented by squares in shades of gray (the nearest in darker colour). Arrows indicate 3'-ends. Black-dots represent 5'-5' inversion of polarity sites. Strands are indicated by double-headed curved arrows with the nearest 3'-ends in red. (**B**) The quadruplex A after an anti-clockwise rotation of each tetrad respect to the adjacent ones, except the nearest one. (**C**) The same quadruplex after a Z-axis anti-clockwise rotation of about 45°. (**D**) The quadruplex after a Y-axis 180° rotation. Conformations **A** and **D** are enantiomeric. For clarity T-residues have been omitted and only G-residues for one strand have been labelled.

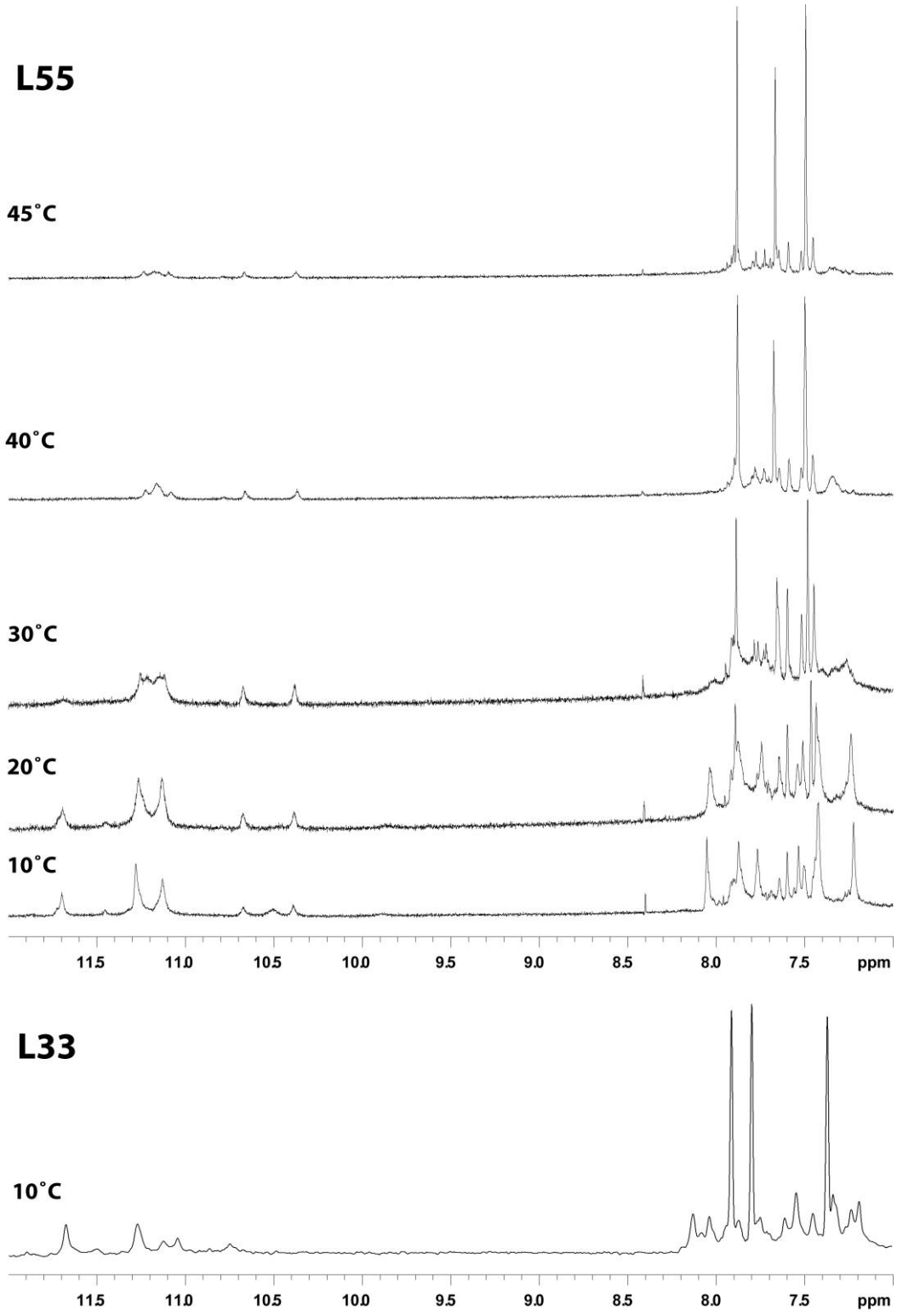

Figure S10: Aromatic and imino proton regions of the ^{1}H-NMR spectra (700 MHz) of **L55** at different temperatures and of **L33** at 10°C. Buffer solution 10 mM NaH_2PO_4/Na_2HPO_4, 70 mM NaCl and 0.2 mM EDTA (pH 7.0) in H_2O/D_2O 9:1. In the spectrum of **L33**, the aromatic region clearly shows the presence of three signals attributable to the unstructured species, thus pointing to a stability of the quadruplex structure in Na^+ buffer solution lower than in K^+ buffer solution.

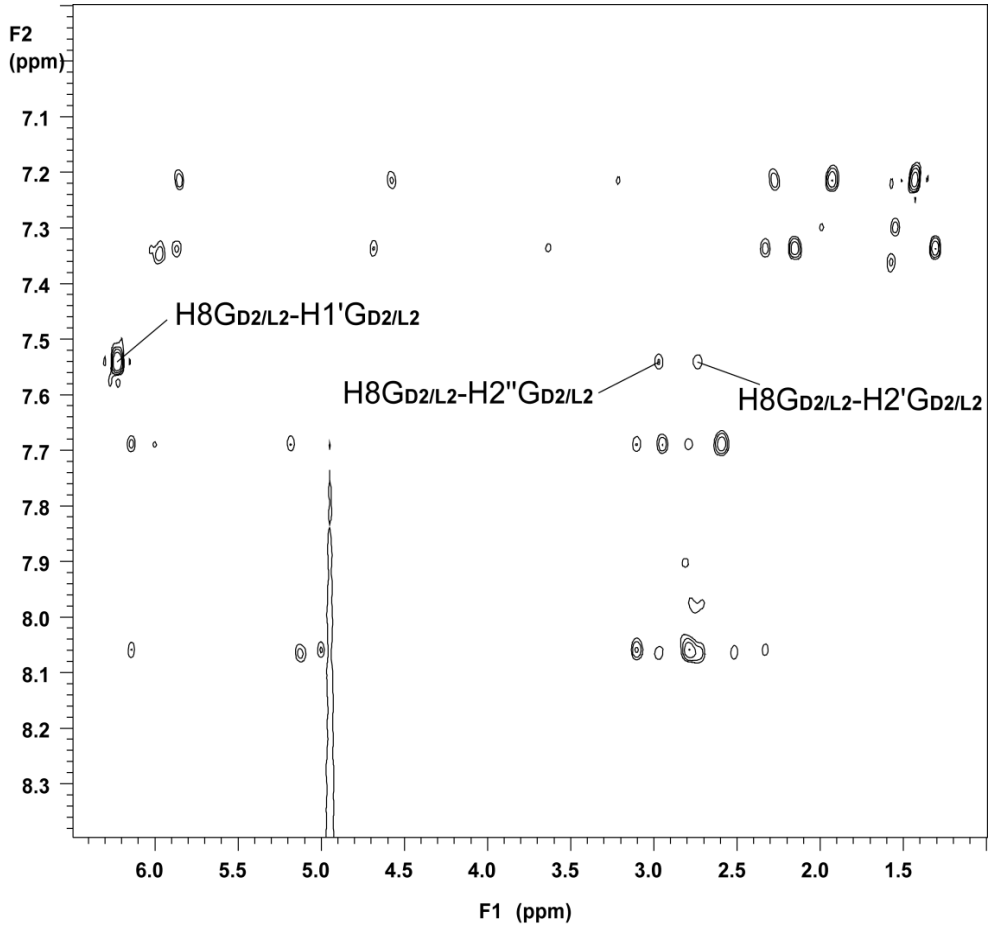

Figure S11: Expanded 2D NOESY spectrum of **L33** (700 MHz; 7°C; strand concentration 3 mM; 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA, pH 7.0 in H_2O/D_2O 9:1; total volume 0.6 ml; mixing time 180 ms) correlating aromatic base protons and sugar protons H1' and H2'/H2".

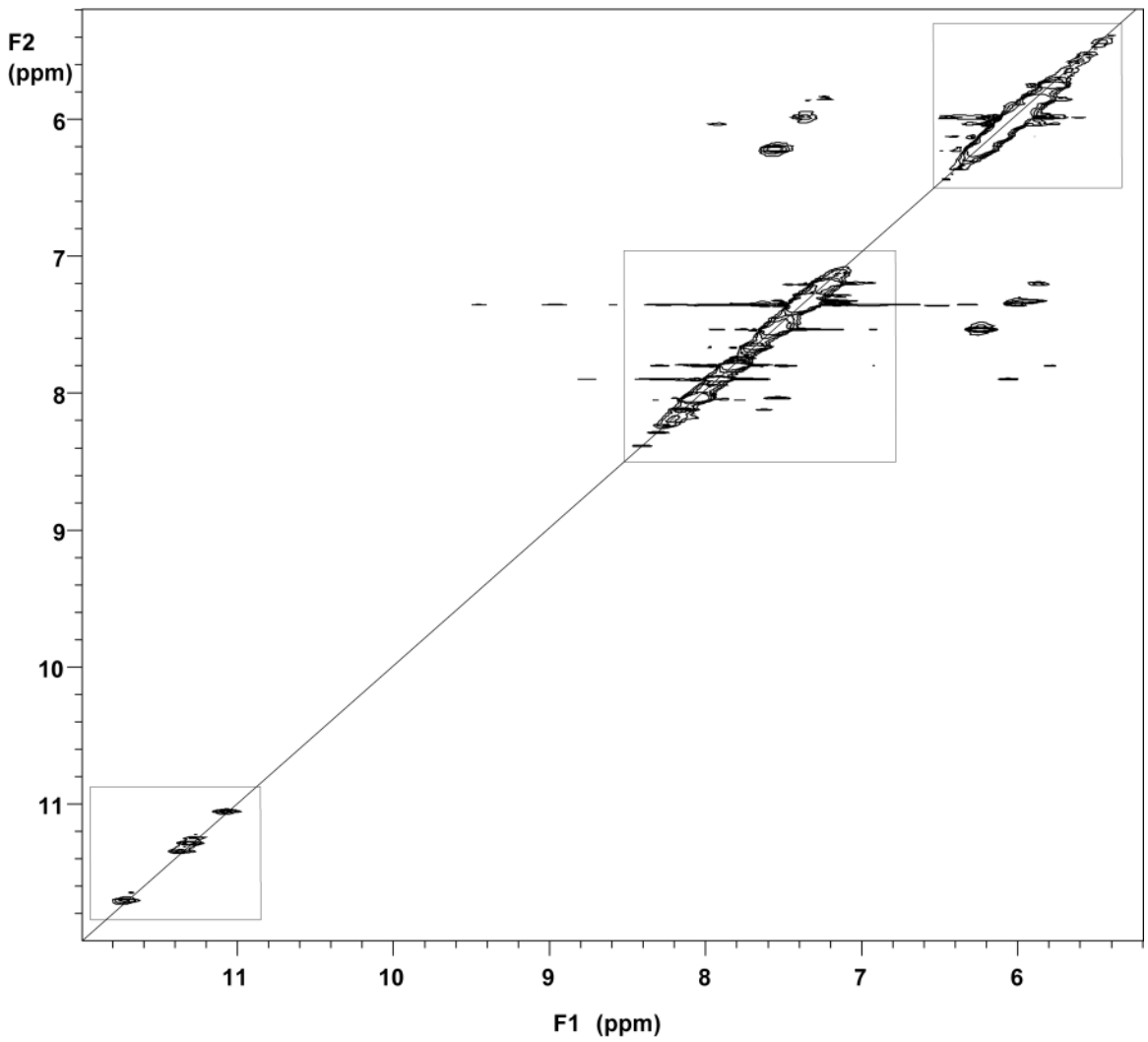

Figure S12: Expanded 2D ROESY spectrum of **L33** (700 MHz; 7°C; strand concentration 3 mM; 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA, pH 7.0 in H_2O/D_2O 9:1; total volume 0.6 ml; mixing time 180 ms). Boxes indicate the imino, aromatic and anomeric proton regions in which potential chemical exchange cross peaks should have been observed (compare with the ROESY spectrum of **L55** in Fig. 2).

L33 5'-$T_{D1}G_{D2}G_{D3}$-3'-3'-$G_{L3}G_{L2}T_{L1}$-5'								
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3	NH
$T_{D1/L1}$	7.34	5.87	2.15/2.33	4.69	4.03	3.63	1.31	-
$G_{D2/L2}$	8.06	6.15	2.79/3.10	5.00	4.26	4.18	-	11.72
$G_{D3/L3}$	7.69	6.00	2.59/2.95	5.18	N.D.	N.D.	-	11.07
$G_{L3/D3}$	8.07	6.00	2.51/2.77	5.12	N.D.	N.D.	-	11.37
$G_{L2/D2}$	7.54	6.23	2.74/2.97	5.13	N.D.	N.D.	-	11.30
$T_{L1/D1}$	7.22	5.86	1.92/2.28	4.57	3.83	3.31/3.21	1.43	-

L55 3'-$T_{D1}G_{D2}G_{D3}$-5'-5'-$G_{L3}G_{L2}T_{L1}$-3'								
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3	NH
$T_{D1/L1}$	7.44	6.11	2.08/2.20	N.D.	N.D.	N.D.	1.63	-
$G_{D2/L2}$	7.88	6.30	2.55/2.72	N.D.	N.D.	N.D.	-	11.13
$G_{D3/L3}$	7.55	6.08	2.75/2.84	N.D.	N.D.	N.D.	-	11.70
$G_{L3/D3}$	8.07	5.76	2.30/2.79	N.D.	N.D.	N.D.	-	11.28
$G_{L2/D2}$	7.78	6.09	2.45/2.60	N.D.	N.D.	N.D.	-	11.30
$T_{L1/D1}$	7.23	5.99	2.07/2.18	N.D.	N.D.	N.D.	1.63	-

Table S1. Proton chemical shifts assignment for quadruplexes formed by ODNs **L33** and **L55** (700 MHz) in 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl and 0.2 mM EDTA (pH 7.0, T = 7°C and 10°C for **L33** and **L55**, respectively). N.D. = not determined. Due to the symmetry of the sequences, the signals could not be assigned to a particular half (D- or L-tract) of the quadruplex.