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Supporting Information

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Ligand-Driven G-Quadruplex Conformational Switching by Using an Unusual Mode of Interaction

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Contents:

General Procedures:

Solvents were of HPLC or reagent grade quality and purchased commercially. Starting materials were purchased from Aldrich and used without further purification. Compounds were characterized using a Bruker DPX 400 MHz instrument. Spectra were recorded at 298 K and were referenced to solvent residual signals. ^{1}H NMR data are reported as follows: chemical shift in ppm on the δ scale, integration, multiplicity (s: singlet, d: doublet, t: triplet, m: multiplet, dd: doublet of doublets, bs: broad singlet, bd: broad doublet) and coupling constants (Hz). High-resolution (HR) electrospray ionization mass spectra were recorded on a Micromass Q-Tof (ESI) spectrometer. Products were purified by preparative HPLC using a *Varian Pro Star* equipped with a *Pursuit* C18 5μ column (250×212 mm) and TFA/MeCN/H_2O as mobile phase (0.1/20/80 until t = 5 min, then gradient 0.1/20/80 to 0.1/100/0 until t = 21 min) at a flow rate of 12 mL/min.

For CD experiments, DNA oligonucleotides were purchased from Sigma. CD spectra were recorded on an Applied Photophysics Chirascan circular dichroism spectropolarimeter using a 1-mm path length quartz cuvette. Scans were performed at 20°C over a wavelength range of 220-330 nm with response time of 0.5 s, 1 nm pitch and 1 nm bandwidth. Blank spectra of sample containing buffer and salts (where appropriate) were subtracted from collected data. The CD spectra represent an average of three scans, are zero-corrected at 330 nm and normalized (Molar ellipticity θ is quoted in 10^5 deg cm^2 dmol^{-1}). The human telomeric sequences telo24 d[TTAGGG]$_4$ was used at 10 μM in the presence or absence of buffer containing Tris-HCl (10 mM, pH 7.4) and 100 mM of KCl, NaCl or LiCl where appropriate. Samples were annealed where necessary by heating at 95 °C for 5 min and then cooled down to room temperature over 6-8 h.

For DNA-ligand interaction NMR studies, DNA oligonucleotides where purchased from Almac Sciences Ltd as HPLC purified and desalt samples. ^{1}H NMR spectra were recorded on a 500 MHz TCI-ATM Cryo instrument at 298 K. Water suppression was achieved using the Watergate W5 pulse sequence. Samples where prepared with a DNA concentration of 0.5 mM without buffer using a 1:9 mixture of D_2O / H_2O at pH = 6.5-7.0 (adjusted using < 1 mM aq. NaOH).

1. Synthesis of ligands 1 and 2:

N-(2-Aminoethyl)-N'-(9-anthracenylmethyl)-1,2-ethanediamine (1):
9-Anthraldehyde (1.00 g, 4.25 mmol) was refluxed in 100 ml of anhydrous chloroform for 2 h in the presence of diethylene triamine (2.62 ml, 24.24 mmol) and 25 g of dried 4 Å molecular sieves under argon. Molecular sieves were then removed by filtration and washed with 20 ml of anhydrous chloroform. The organic fractions were evaporated under reduced pressure. The crude residue was dissolved with 100 ml of anhydrous methanol and $NaBH_4$ (0.92 g, 24.24 mmol) was added in portions over 10 min under air atmosphere. The reaction mixture was stirred at rt for 2 h and then refluxed for 2 h under argon. The mixture was evaporated to dryness and the residue was then taken up with an appropriate mixture of $MeCN/H_2O$ containing 0.1% of TFA until the maximum of solid was dissolved. The insoluble solid fraction (presumably salts and dimer product) was removed using a Büchner funnel. The soluble fraction was purified by preparative HPLC (R_t = 16.25). The product obtained as a TFA salt was then crystallized from hot MeCN to yield the title product as a yellow powder (632 mg, 25%). ^{1}H NMR (400 MHz, D_2O) δ(ppm): 8.64 (1H, bs), 8.22 (2H, d, *J* = 8.0 Hz), 8.10 (2H, d, *J* = 8.0 Hz), 7.64 (2H, dd, *J* = 15.5, 8.0 Hz), 7.54 (2H, dd, *J* = 15.5, 8.0 Hz), 5.20 (2H, bs), 3.23 (2H, t, *J* = 6.0 Hz), 2.91 (2H, t, *J* = 6.0 Hz), 2.86 (2H, t, *J* = 6.0 Hz), 2.74 (2H, t, *J* = 6.0 Hz); ^{13}C NMR (100.6 MHz, CD_3CN) δ(ppm): 132.9, 132.8, 131.8, 130.8, 128.9, 127.1, 125.3, 123.8, 47.4, 46.9, 46.3, 44.8, 39.6; HRMS (ESI+) calculated for: $C_{19}H_{24}N_3$ $[M+H]^+$ (m/z): 294.1964, found: 294.1953.

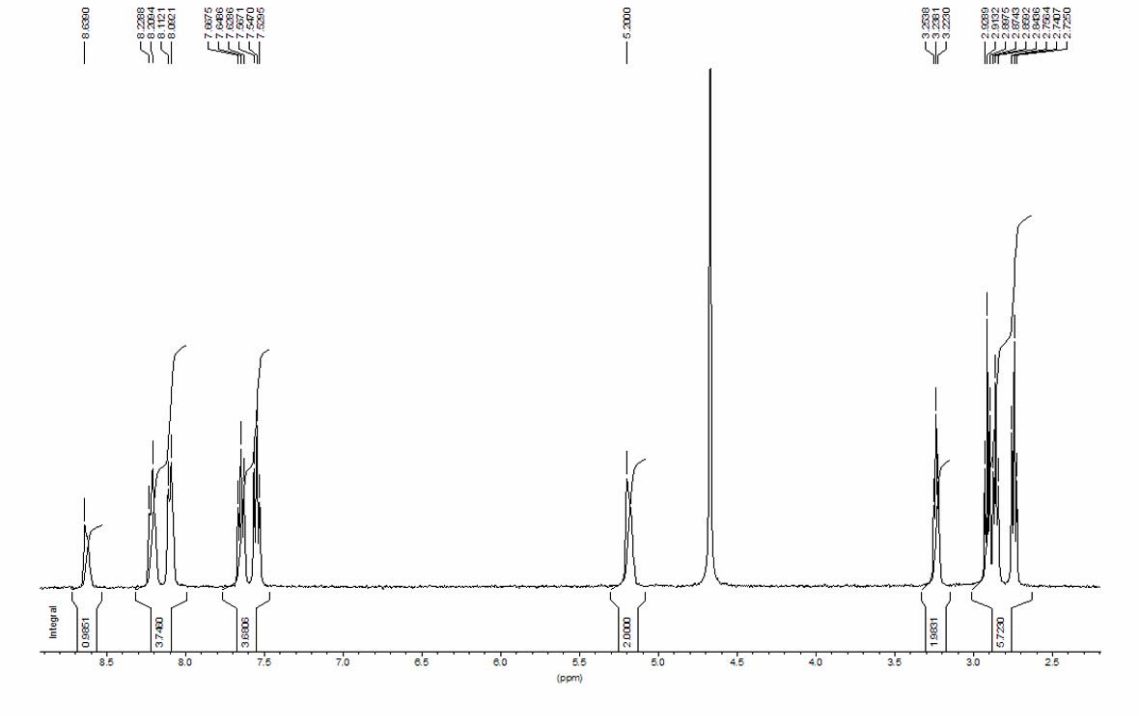

Figure S1. ^{1}H NMR spectra of **1** in D_2O recorded at 298 K.

N-[2-(2-Aminoethylamino)-ethyl]-anthracene-9-carboxamide (2):

Anthracene-9-carboxylic acid (500 mg, 2.24 mmol) was refluxed in 2.5 ml of neat $SOCl_2$ under argon for 3 h. The brown solution was then evaporated to dryness under reduced pressure and dissolved with 10 ml of freshly distilled DCM. This solution was added *via* cannula at 0 °C under argon to a 10 ml DCM solution of triethylamine (0.63 ml, 4.49 mmol) and diethylene triamine (0.49 ml, 4.49 mmol). The reaction mixture was slowly allowed to warm to room temperature and stirred for a further 16 h. After addition of 5 ml of triethyl amine, the mixture was stirred 15 min. The precipitate formed during the reaction was removed by filtration and the reaction mixture was evaporated under reduced pressure. The crude was then taken up with an appropriate mixture of MeCN/H_2O containing 0.1% of TFA until the solid was dissolved. After filtration to remove insoluble fraction, the soluble product was purified by preparative HPLC (R_t = 16.43) to yield the corresponding TFA salt as a brown solid (747 mg, 62%). ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.59 (1H, s), 7.50 (2H, d, *J* = 8.5 Hz), 7.30 (2H, d, *J* = 8.5 Hz), 7.22 (2H, dd, *J* = 15.0, 7.5 Hz), 7.06 (2H, dd, *J* = 15.0, 7.5 Hz), 3.66 (2H, t, *J* = 7.0 Hz), 3.32-3.36 (4H, m), 3.22 (2H, t, *J* = 6.5 Hz); ^{13}C NMR (100.6 MHz, D_2O) δ(ppm): 170.6, 128.7, 127.5, 127.2, 126.9, 125.7, 125.6, 124.0, 122.2, 45.2, 43.0, 34.7, 34.0; HRMS (ESI+) calculated for: $C_{19}H_{22}N_3O_1$ $[M+H]^+$ (m/z): 308.1757, found: 308.1748.

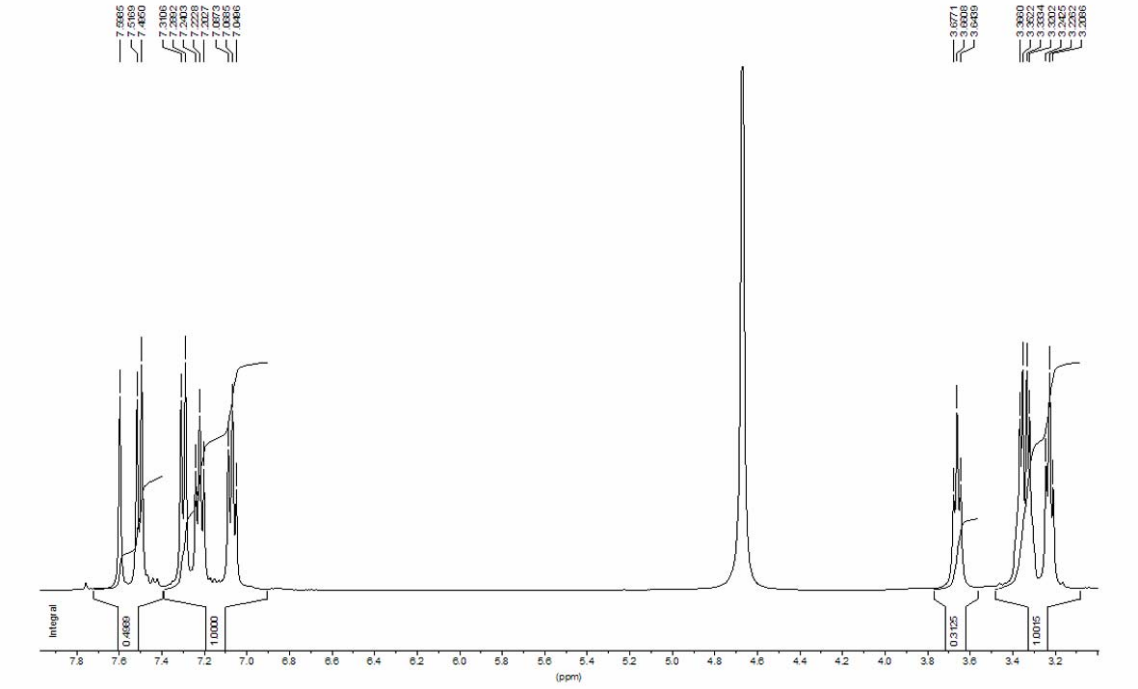

Figure S2. ^{1}H NMR spectra of **2** in D_2O recorded at 298 K.

2. CD experiments:

The *unstructured* DNA displays a residual positive CD signal at 257 nm (—). Upon addition of DETA (Δ), this signal disappeared consistent with the complete unfolding of the negatively charged DNA resulting from non specific electrostatic interactions with the positively charged ligand. The second experiment was carried out with up to 10 equivalents of **2** (+). At this concentration, the initial *unstructured* DNA CD signal remained present. No induction of a clear positive signal at 263 nm was observed. Therefore, **1** (o) and **2** *do not* bind to the DNA in the same manner.

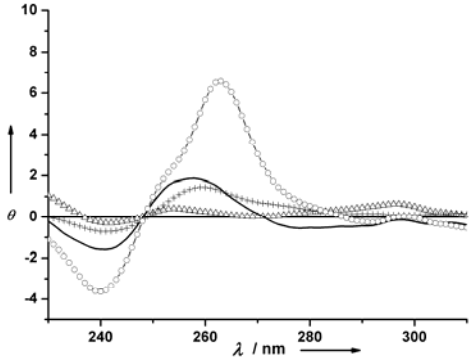

Figure S3. CD spectra of telo24 without ligand (—)**,** telo24 with 10 equivalents of **1** (o), telo24 with 20 equivalents of DETA (Δ) and telo24 with 10 equivalents of **2** (+). General conditions were telo24 (10 μM) in the absence of added cation at 20 °C in Tris-HCl (10 mM, pH 7.4).

3. ^{1}H-NMR experiments:

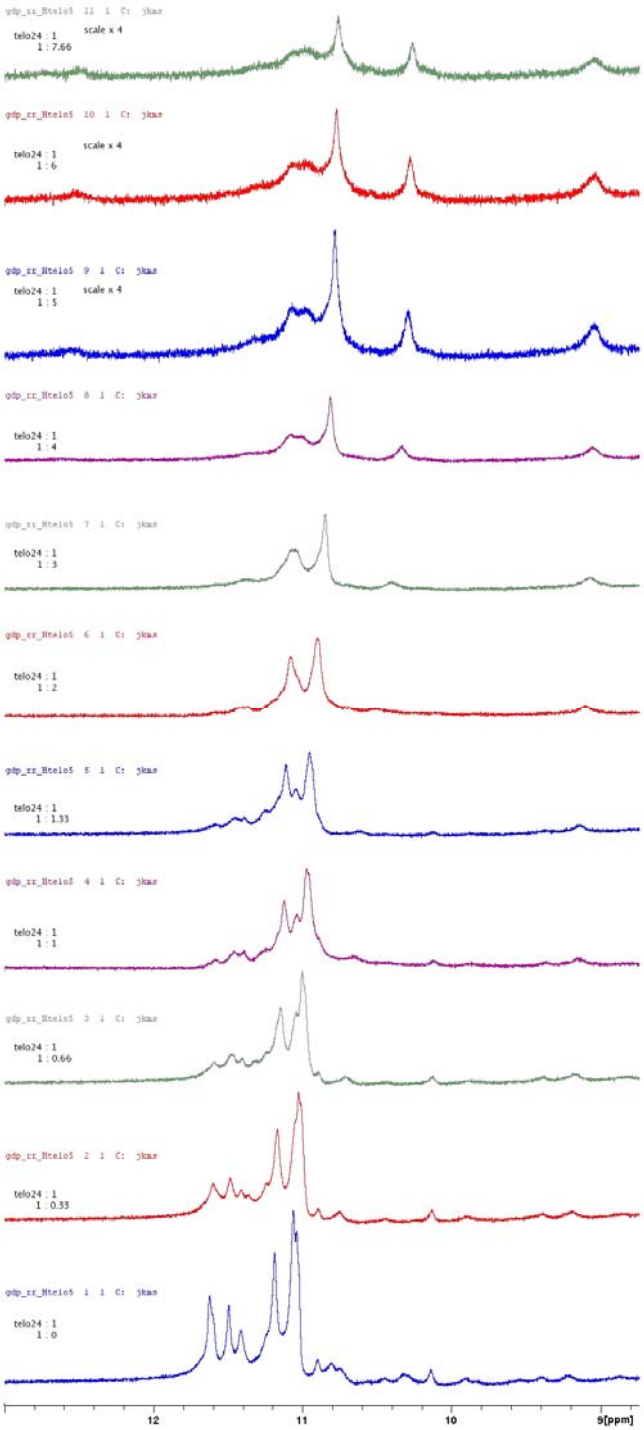

Figure S4. ^{1}H NMR stacked plot of the 9-13 ppm region of the telo24 spectrum (DNA annealed with 100 mM NaCl) in the presence of increasing amounts of **1**. Due to the highly dynamic character of the complex formed between **1** and the DNA, we did not observe NOESY correlations supportive of our model between exchangeable guanine imino protons and the CH_2 of **1**. This experiment is in agreement with G-quadruplex conformational switching from antiparallel to parallel conformation and removal of Na^+ from the central G-quadruplex cavity.