G-quadruplex Recognition by Bis-Indole Carboxamides

*Jyotirmayee Dash, Pravin S. Shirude and Shankar Balasubramanian**

The University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK, Fax: (+44) 1123-336-913; Tel: (+44) 1123-336347; E-mail: sb10031@cam.ac.uk

SUPPORTING INFORMATION for Chemical Communications

Contents

1.0 Bis-indole: G-quadruplex General conformations

The bis-indole core of **1** can adopt alternative conformations due to the geometrical freedom in compound **1** (Figure 1). The strong stabilization potential of ligand **1** might be attributed due to the comparable size of (Chem 3D) bis-indole core of **1** with the G-quartet (Figure 2). The end to end total length of the central core of **1** as calculated from energy minimized Chem 3D (**A**) is 13.5Å (**B**), which is equal to the distances of (13.6 Å) between the N_9 of the opposite guanine bases in the G-tetrad (**C**). Also the distances between other different carbons of the central core of **2** (9.3-12.8 Å) is similar to the distances (10.63-12.06 Å) between the two hydrogen atoms of the non-hydrogen-bonding G-NH_2 functional group of the opposite guanine bases in the G-tetrad.

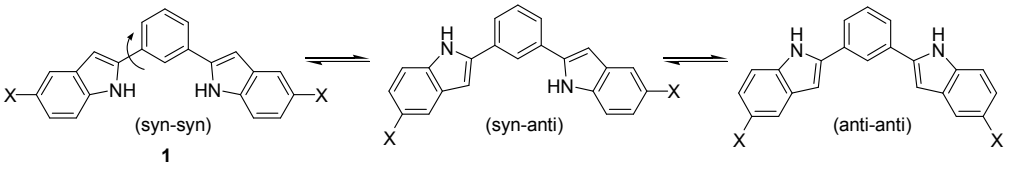


Figure 1: Different conformations of bis-indole core separated by a central benzene ring

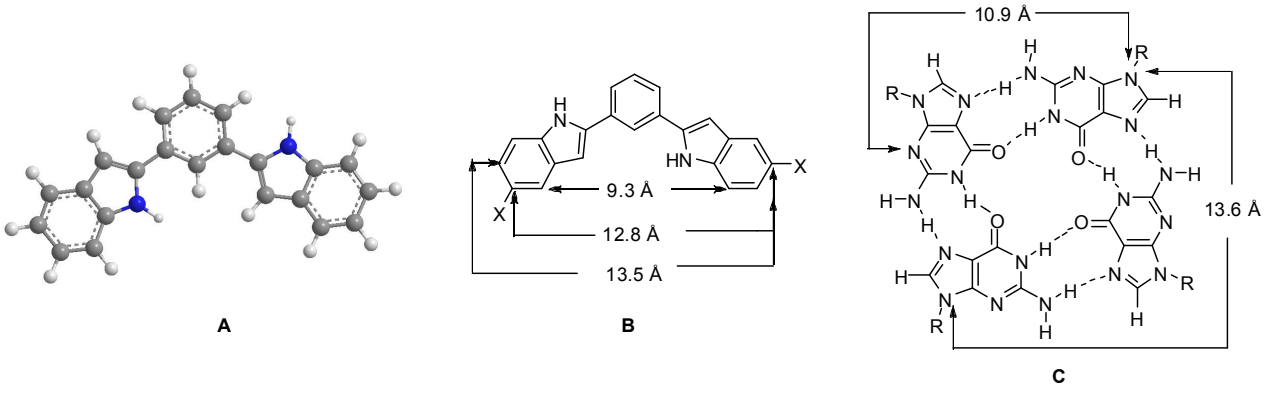


Figure 2. Energy minimized (Chem 3D Ultra 10.0, CambridgeSoft Corp., MA) of bis-indole core of **1** (A); Selected dimensions of **A** (B); dimensions of a G-quartet from X-ray structure.[1]

2.0 General Methods

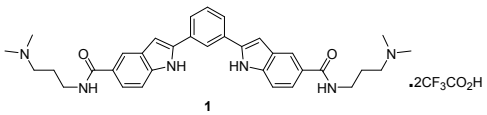
NMR spectra were acquired on Brucker DRX-500 instruments. Chemical shifts were relative to the deuterated solvent peak and are reported in parts per million (ppm). ^{1}H NMR spectra were recorded at 500 MHz in CD_3CN and data were reported as follows: chemical shift in parts per million from tetramethylsilane as an internal standard, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet or overlap of nonequivalent resonances), integration. ^{13}C NMR spectra were recorded at 125 MHz in CD_3CN and data were reported as follows: chemical shift in parts per million from tetramethylsilane with the solvent as an internal indicator ($CDCl_3$ δ 77.0 ppm), multiplicity with respect to proton (deduced from DEPT experiments, s=quaternary C, d=CH, t=CH_2, q=CH_3). The high resolution mass spectra were recorded on Micromass Q-Tof spectrometer using electrospray ionisation technique.

The compounds **1** and **2** were purified by HPLC using a Varian Pursuit C18, 5 μ column (250× 21.2 mm) and a gradient elution with water/acetonitrile 90:10 (0.1%TFA) to 50:50 in 40 min at a flow rate of 12.0 mL/min.

The ligands (**1** and **2)** solutions were prepared in MQ water, for biophysical experiments.

3.0 Characterization data of ligands 1 and 2:

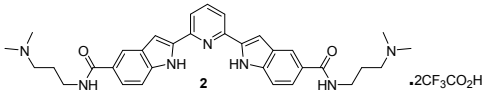
Bis-indole carboxamide 1:



1

^{1}H NMR (CD_3CN): δ = 10.91 (s, 2H, indole NH), 9.92 (s, 2H, amide NH), 8.42 (s, 1H), 8.18 (s, 2H), 7.81 (d, *J* = 7.7 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.70-7.68 (m, 2H, merged with aromatic proton, $CF_3CO_2\underline{H}$), 7.59 (d, *J* = 8.4 Hz, 1H), 7.56 (t, *J* = 7.7 Hz, 2H), 7.07 (s, 2H) 3.52 (q, *J* = 5.9 Hz, 4H), 3.11 (q, *J* = 5.9 Hz, 4H), 2.86 (s, 12 H), 2.04 (q, *J* = 5.9 Hz, 4H); ^{13}C NMR (CD_3CN): δ = 171.2 (s, 2C), 160.9 (q, $^2J_{C,F}$ = 35 Hz, $CF_3\underline{CO_2}H$), 140.3 (s, 4C), 133.5 (s, 2C), 130.6 (d), 129.4 (s, 2C), 126.1 (s, 2C), 125.7 (d, 2C), 122.8 (d), 122.2 (d, 2C), 121.3 (d, 2C), 117.3 (q, $^1J_{C,F}$ = 290 Hz, CF_3), 112.2 (d, 2C), 101.2 (d, 2C), 55.6 (t, 2C), 43.4 (q, 4C), 36.5 (t, 2C), 25.8 (t, 2C). HRMS (ESI): calcd for $C_{34}H_{40}N_6O_2$ $[M+H]^+$ 565.33061, found 565.3286.

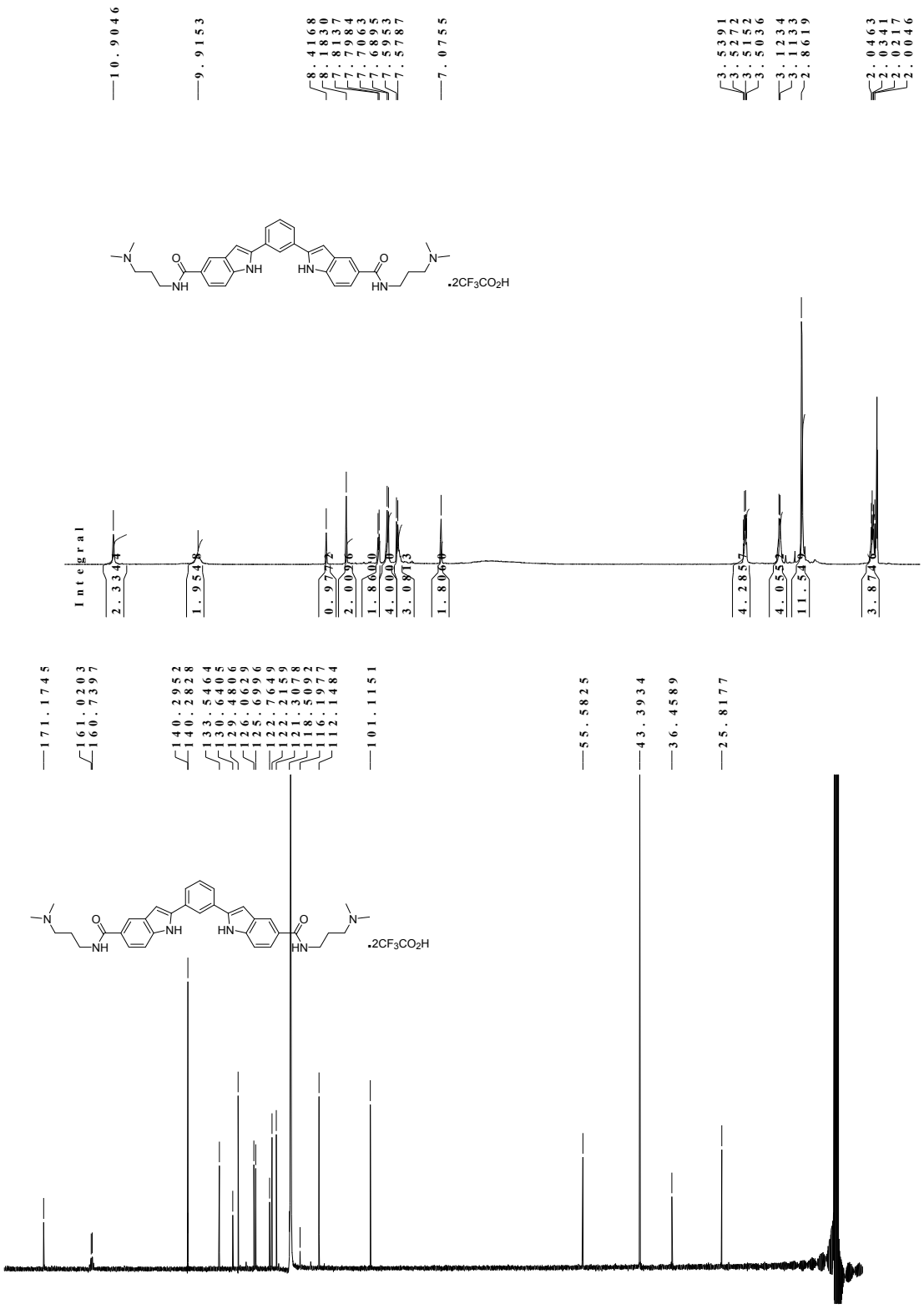
Bis-indole amide 2:



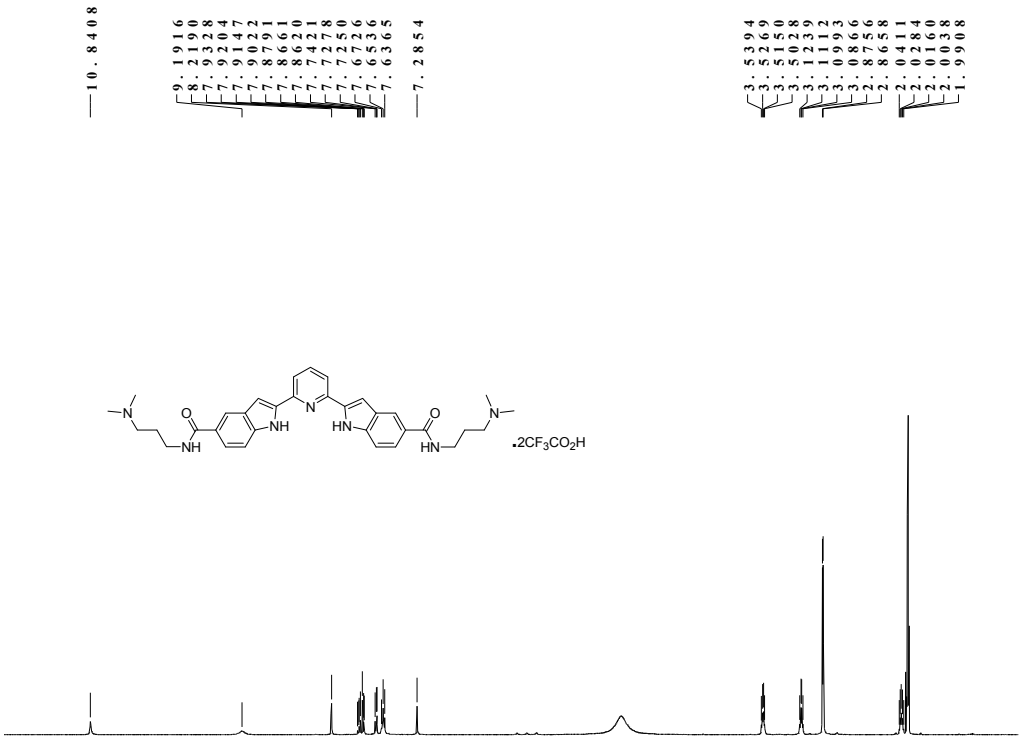
2

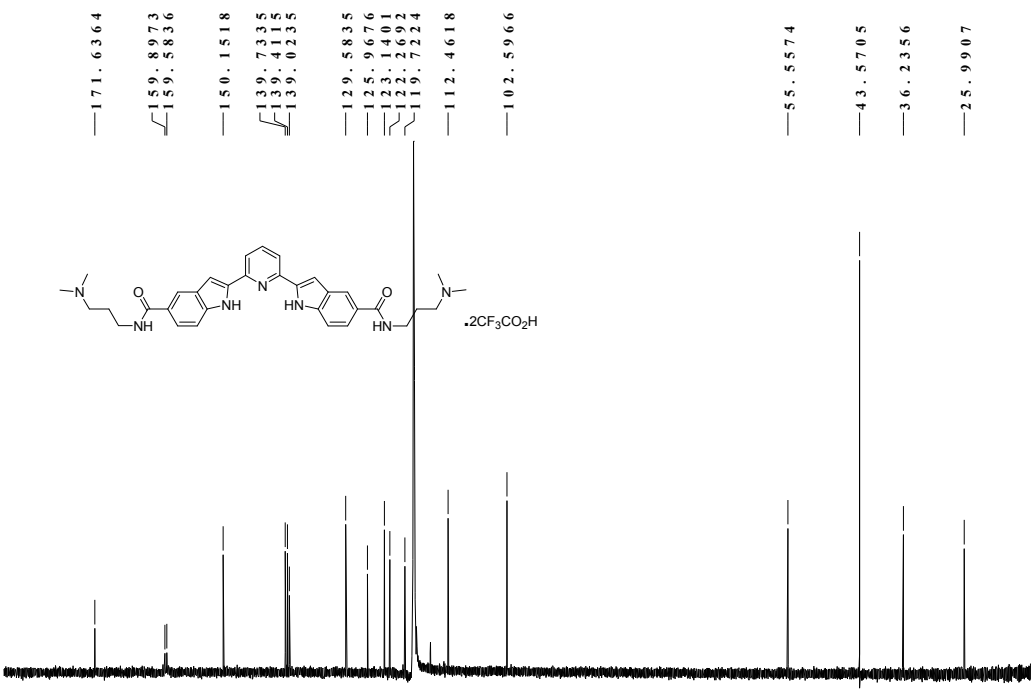
^{1}H NMR (CD_3CN): δ = 10.84 (s_{br}, 2H, indole NH), 9.19 (s_{br}, 2H, amide NH), 8.22 (s, 2H), 7.93-7.86 (m, 3H), 7.74-722 (dd, *J* = 8.6, 1.4 Hz, 2H), 7.67-7.63 (m, 2H), 7.65 (m, 2H, merged with aromatic proton, $CF_3CO_2\underline{H}$), 7.28 (d, *J* = 1.2 Hz, 2H), 3.52 (q, *J* = 6.1 Hz, 4H), 3.11 (q, *J* = 6.1 Hz, 4H), 2.88 (s, 6H), 2.87 (s, 6 H), 2.04 (quin, *J* = 6.2 Hz, 4H); ^{13}C NMR (CD_3CN): δ = 171.6 (s, 2C), 159.7 (q, $^2J_{C,F}$ = 39 Hz, $CF_3\underline{CO_2}H$), 150.2 (s, 2C), 139.7 (s, 2C), 139.4 (s, 2C), 139.0 (d), 129.6 (s, 2C), 126.0 (s, 2C), 123.1 (d, 2C), 122.3 (d, 2C), 119.7 (d, 2C), 116.6 (q, $^1J_{C,F}$ = 289 Hz, CF_3), 112.4 (d, 2C), 102.6 (d, 2C), 55.6 (t, 2C), 43.6 (q, 4C), 36.2 (t, 2C), 25.9 (t, 2C); HRMS (ESI): calcd for $C_{33}H_{39}N_7O_2$ $[M+H]^+$ 566.3238, found 566.3265.

3.1 ^{1}H and ^{13}C (CD_3CN) NMR for 1:



3.2 ^{1}H and ^{13}C (CD_3CN) NMR for 2:





4. Fluorescence Resonance Energy Transfer (FRET) Assay

All the oligonucleotides and their fluorescent conjugates (Eurogentec, Southampton, UK) were initially dissolved as a 100 μM stock solution in purified water; further dilutions were carried out in the relevant buffer. The ability of the compounds to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format. The labelled oligonucleotides h-telo: 5'-FAM-d(GGG[TTAGGG]3)-TAMRA-3', c-kit2: 5'-FAM-d(GGG CGG GCG CGA GGG AGG GG)-TAMRA-3', c-kit1: 5'-FAM-d(GGG AGG GCG CTG GGA GGA GGG)-TAMRA-3', c-myc: 5'-FAM-d($TGAG_3TG_3TAG_3TG_3TA_2$)-TAMRA-3', DNA duplex: 5'-FAM-d(TAT AGC TAT A-HEG-TAT AGC TAT A)-TAMRA-3'; donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine] were prepared as a 400 nM solution in a 60 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 90 °C for 2 min, followed by cooling to room temperature. Compounds were stored at -80 °C and dilutions were done with 60 mM potassium cacodylate buffer (pH 7.4) (1 mM stock solution of ligands **1** and **2** were made up in MQ water). The 96-well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 μl of the annealed DNA into each well, followed by 50 μl of the compound solutions using Beckman Coulter liquid-handling robot. For each compound, a minimum of 10 different concentrations were tested. Fluorescence melting curves were determined in a Roche light cycler 480, using a total reaction volume of 100 μl (ramp rate 4.4 °C/sec with 20 acquisitions per minute). Measurements were made in duplicate with excitation at 483 nm and detection at 533 nm. Final analysis of the data was carried out using Origin 7.5 (OriginLab Corp., Northampton, MA).

The FRET melting data of bis-indole amides **1** and **2** at 1 μM and 2 μM concentration for h-telo and c-kit2 quadruplex is summarized in Table 1. The ligands **1** and **2** show better selectivity for promoter quadruplexes over h-telo quadruplex DNA. In comparison to ligand **1**, the ligand **2** exhibits better discrimination between quadruplexes exhibiting higher stabilization for promtor quadruplexes (c-kit1, c-kit2 and c-myc) over

h-telo DNA. Also the stabilization potential for c-kit2 and c-myc by ligand **2** is the maximum at 2 μM concentration (saturates at 2 μM, Table 2).

The stabilization potential of the strongest quadruplex ligands BRACO-19, telomestatin and quinoline macrocycle are given for comparison (Table 1). The stabilization potential of ligand **1** is nearly equal to other potent ligands at 2 μM for h-telo quadruplex.

Table 1. Comparison of FRET data of ligands **1** and **2** with some of the reported ligands

Ligand	ΔT_m at 1 μM conc. (°C)			ΔT_m at 2 μM conc. (°C)	
	h-telo	c-kit2	dup	h-telo	c-kit2
1	21.5	21.7[b]	4.2	29.4	22.4
2	9.8	16.0	0.5	20.4	21.6
Telomestatin[2]	30.3	20.4	0	30.5	23.5
Quinoline macrocycle[2]	33.8	21.4	0	34.2	21.4

Table 2. DNA G-quadruplexes stabilization by **1** and **2** by FRET melting experiments

Ligand	ΔT_m at 2 μM conc (°C)			
	h-telo (59±1)[a]	c-kit1 (57±1)[a]	c-kit2 (71±1)[a]	c-myc (77±1)[a]
1	29.4	29.7	22.4	16.4
2	20.4	32.6	20.4	16.7
Conc. of ligand (μM) at ΔT_m = 15 °C				
1	0.58	0.27	0.52	0.70
2	1.41	0.63	0.92	1.34

[a] T_m in 60 mM potassium cacodylate buffer, pH 7.4 without ligand. [b] Maximum measurable ΔT_m that can be measured for ckit2 is ΔT_m 24 ºC (i.e. T_m = 95 ºC) and for c-myc is ΔT_m 18 ºC (i.e. *T*m = 95 ºC).

5. Circular Dichroism (CD)

CD spectra were recorded on a Applied Photophysics Chirascan Circular Dichroism Spectrophotometer (Applied Photophysics Ltd, UK) using a quartz cell of 1-mm optical path length and an instrument scanning speed of 100 nm/min with a response time of 2 s, and over a wavelength range of 200-330 nm and 200-450 nm. To provide further insight into the selective stabilization of G-quadruplex, we have used circular dichroism (CD) spectroscopy to elucidate ligand effects on c-kit1, c-kit2 and c-myc

promoter G-quadruplex structure in the absence of added salt. We used four different G-quadruplex forming oligonucleotides in the study, the human telomeric G-quadruplex DNA h-telo, d(AGGG[TTAGGG]$_3$TT, the two c-kit promoter G-quadruplex DNA c-kit1 d(GGG AGG GCG CTG GGA GGG AGG G) and c-kit2 d(GGG CGG GCG CGA GGG AGG GG) and c-myc promoter G-quadruplex DNA c-myc d(TGA GGG TGG GTA GGG TGG GTA A). All DNA samples were dissolved in Tris buffer (50 mM, pH 7.4). Where appropriate, the samples also contained 100 mM KCl. Ligands **1** and **2** (1 mM solution with MQ water) were titrated into the DNA samples at 0.5 mol equiv up to 7 mol equiv. The DNA strand concentrations used were 12.5 μM, and the CD data are a representation of three averaged scans taken at 23 °C. All CD spectra are zero corrected and baseline-corrected for signal contributions due to the buffer.

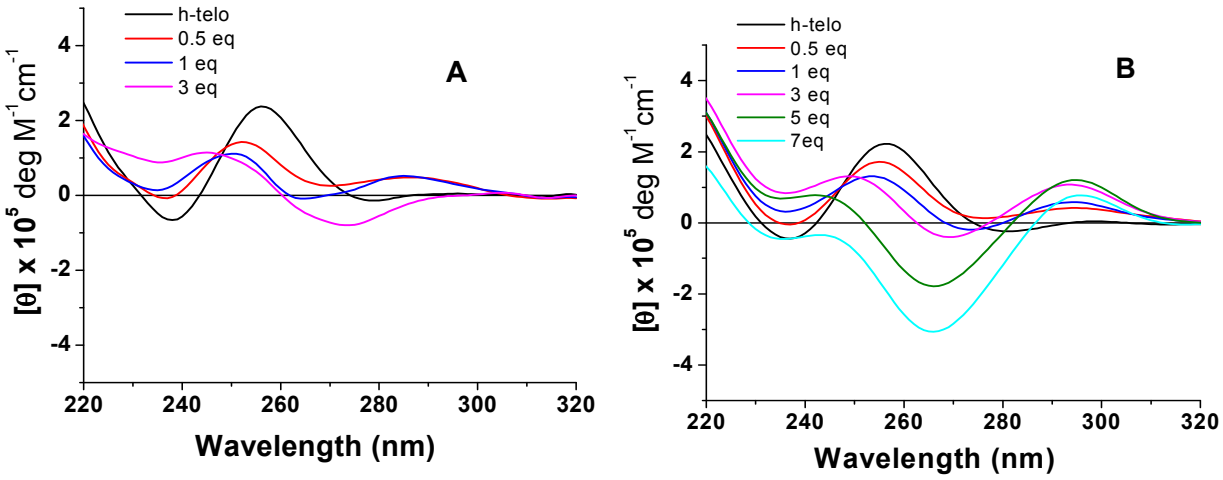


Figure 3. CD spectra of h-telo (12.5 μM) in Tris buffer (pH 7.4), 0-7 equivalent of ligands (**A**) **1** and (**B**) **2**.

CD titration with h-telo G-quadruplex DNA: The CD spectrum of the human telomeric d[A(G$_3$T$_2$A)$_3$G$_3$T] oligonucleotide exhibits a negative band centered at 235 nm and a major positive band at 256 nm (Figure 3). Upon addition of the ligands **1** (0.5-1 mol equiv) or **2** (0.5-3 mol equiv), the peak at 256 nm (parallel structure) decreased with the appearance of a weak peak at 293 nm (that of antiparallel structure).[3] No induction of G-quadruplex structure was observed at higher ligand concentration even after longer time (Figure 3). Overall there was no indication of ligand induced G-quadruplex folding.

CD titration with c-kit1 G-quadruplex DNA: In the absence of any ligand, the CD spectrum of the c-kit1 oligonucleotide was found to exhibit a negative band centered at 240 nm, and a major positive band at 260 nm, (Figure 4). Approximately 5 min after the addition of ligand **1** (1 mol equiv) and **2** (up to 3 mol equiv) to the c-kit1 quadruplex, a small increase in elliptic intensity at 260 nm was observed. At higher ligand concentration (2-3 eq in case of ligand **1** or 5-7 eq in case of ligand **2)**, the ellipticity at 260 nm was actually observed to decrease somewhat (Figure 4).

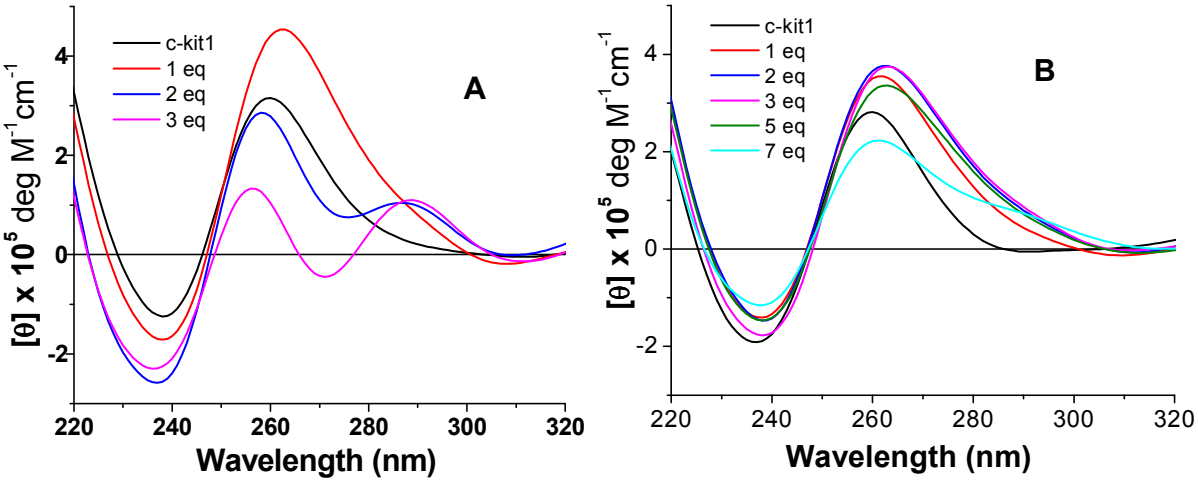


Figure 4. CD spectra of c-kit1 (12.5 μM) in Tris buffer (pH 7.4), 0-7 equivalent of ligands (**A**) **1** and (**B**) **2**.

CD titration with c-myc G-quadruplex DNA: In the absence of any ligand, c-myc G-quadruplex also shows a negative band centered at 240 nm, and a major positive maxima at 260 nm. Upon addition of ligands **1** (1 mol equiv) and **2** (up to 5 mol equiv) to this sequence, an increase in ellipticity at 260 nm was observed suggestive of a ligand induced folding to a G-quadruplex structure (Figure 5). However at higher ligand concentration, a decrease in elliptic intensity at 260 nm was observed.

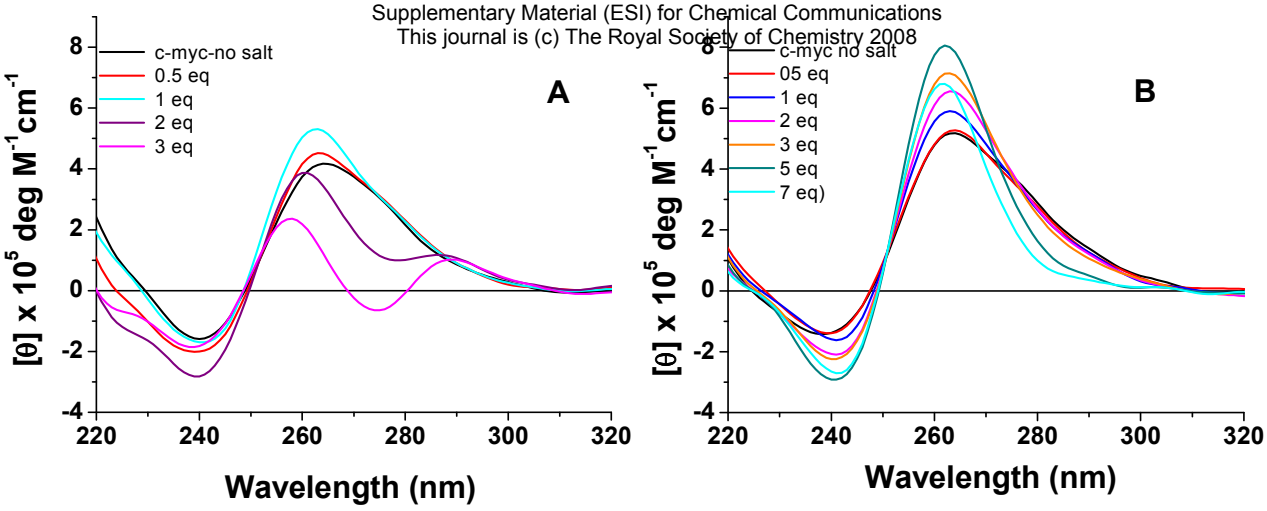


Figure 5. CD spectra of c-myc (12.5 μM) in Tris buffer (pH 7.4), 0-7 equivalent of ligands (**A**) **1** and (**B**) **2**.

CD experiments of c-kit2 G-quadruplex DNA annealed with ligand 2:

When ckit2 is annealed in the presence of a 1 equivalent of ligand **2**, a loss of the antiparallel signal (293 nm) was observed. With 5 fold excess of the ligand, a loss of the antiparallel signal (293 nm) was observed simultaneously with a significant increase of the band at 263 nm characteristic of a parallel conformation. Similar outcome was obtained as in the real time titration (Figure 3, manuscript). This indicates ligand **2** shows a thermodynamic as well as kinetic preference to induce a parallel G-quadruplex structure.

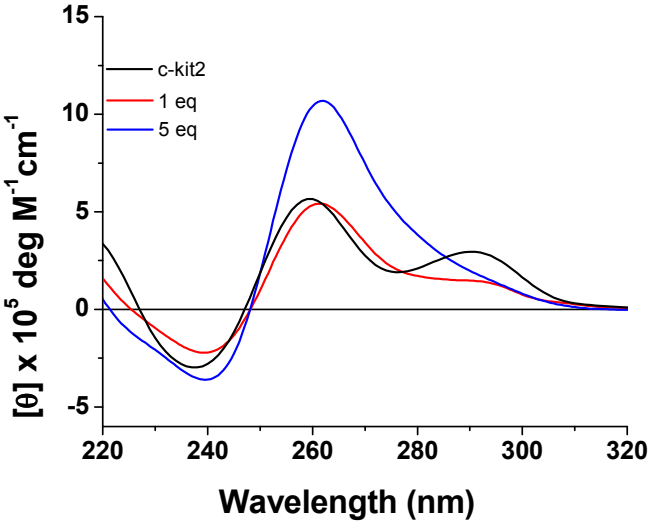


Figure 6: CD spectra of c-kit2 (12.5 μM) in Tris buffer (pH 7.4), annealed with 1 and 5 equivalent of ligand **2**.

CD titration with c-kit2 G-quadruplex DNA in the presence of salt:

The c-kit2 quadruplex exists as a predominantly parallel structure in K^+-containing buffer. Upon addition of excess ligand (up to 7 mol equiv) to ckit2 oligonucleotide preannealed with 100 mM K^+ could not further induce the ellipticity at 263 nm which was already stabilized by K^+ (Figure 7).

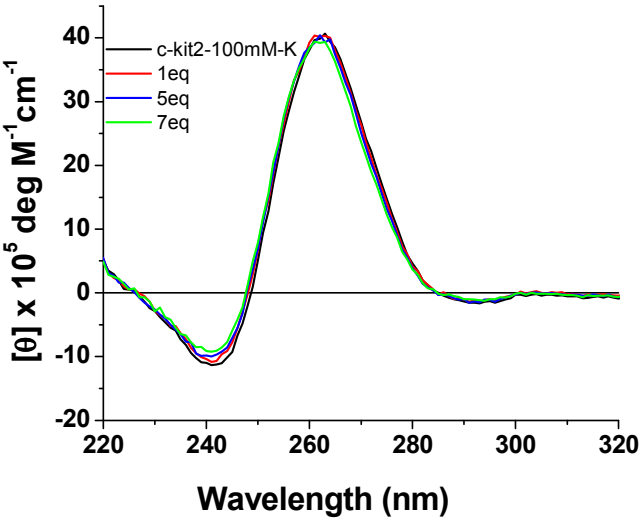


Figure 7. CD spectra of c-kit2 (12.5 μM) in K^+ containing buffer (50 mM Tris HCl pH 7.4, 100 mM KCl), 1-7 equivalent of ligand **2**.

Reference:

1 a) A. D. Cian, E. DeLemos, J.-L. Mergny, M.-P. Teulade-Fichou and D. Monchaud, *J. Am. Chem. Soc.*, 2007**, 129**, 1856. b) G. N. Parkinson, M. P. H. Lee and S. Neidle, *Nature*, 2002, **417**, 876.

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