Supplementary Material (ESI) for Chemical Communications

Diazonia- and Tetraazoniapolycyclic Cations as Motif for Quadruplex-DNA Ligands

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Electronic Supplementary Information (ESI)

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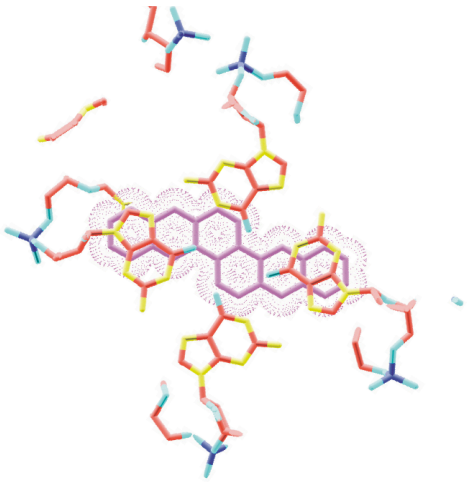
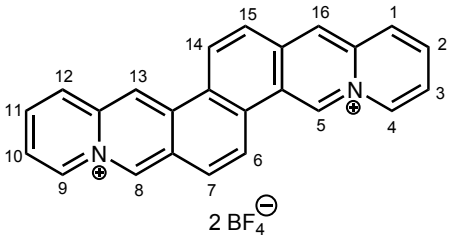
1. Calculated structures





1b

Figure S1. Superimposed structures of one G-quartet (yellow-red) of the human teleomere[1] and one molecule of **1a** (purple).

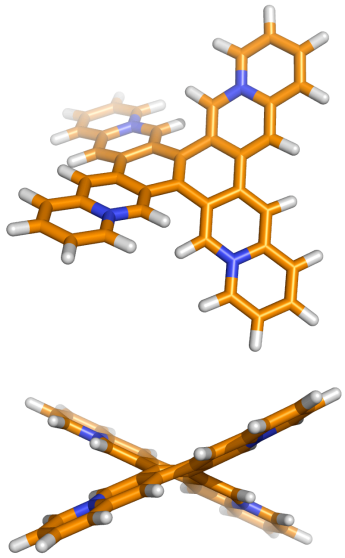


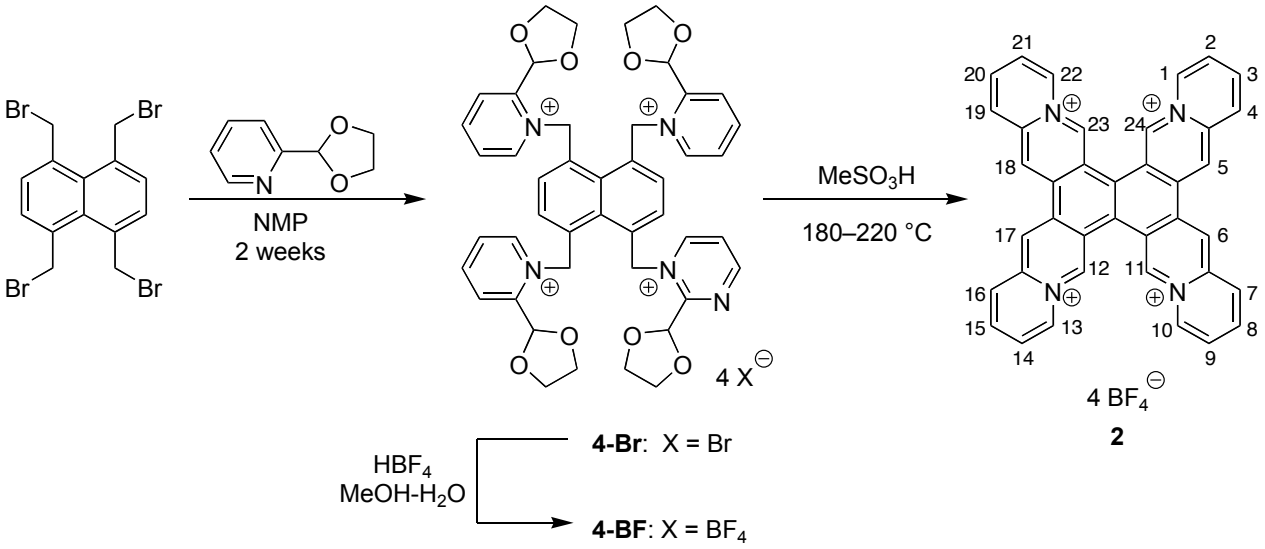
Figure S2. Energy-minimized structure of tetraazoniapolycycle **2**, as determined by energy-minimization calculation using a semi-empirical AM1 Hamiltionian, implemented in HyperChem 7.5 Software.

[1] K. N. Luu, A. T. Phan, V. Kuryavyi, L. Lacroix, and D. J. Patel, *J. Am. Chem. Soc.* 2006, **128**, 9963.

2. Synthesis of ligands 1a, 1b, and 2

The diazoniadibenzochrysene derivatives **1a** and **1b** (tetrafluoroborate salts) were synthesized according to published procedures.[2] *meso*-Tetra(*N*-methyl-4-pyridyl)porphyrin tetratosylate (**3**) was purchased from Fluka Chemie AG, Buchs, CH.

Synthesis of 10a,12a,22a,24a-tetraazoniapentapheno[6,7-*h*]pentaphene (2)



2

1,4,5,8-Tetrakis{[1-(1,3-dioxolan-2-yl)pyridinium]methyl}naphthalene tetrabromide (4-Br). A solution of 1,4,5,8-tetra(bromomethyl)naphthalene[3] (1.00 g, 2.00 mmol) and 2-(1,3-dioxolan-2-yl)pyridine[4] (1.81 g, 11.9 mmol) in *N*-methyl-2-pyrrolidinone (10 mL) was stirred under argon atmosphere at 60 °C for 3 days and subsequently at 45 °C for 10 days. The reaction mixture was poured in ethyl acetate (150 mL). The precipitate was separated and washed with ethyl acetate (20 ml) and Et_2O (3 × 10 mL). The crude product was dissolved in MeOH and precipitated with ethyl acetate to give **4-Br** as beige powder (1.95 g, 88%); m.p. 124–126 °C. ^{1}H-NMR (600 MHz, DMSO-d_6 / D_2O 30:1): δ = 4.02 (s, 16 H, CH(OC**H**$_2$)$_2$), 6.48 (s, 4 H, C**H**(OCH$_2$)$_2$), 6.72 (s, 4 H, Ar-H), 6.75 (s, 8 H, CH_2N^+), 8.30 (ddd, ^3J = 7.8 Hz, ^3J = 6.0 Hz, ^4J = 1.4 Hz, 4 H, Py-H), 8.41 (dd, ^3J = 8.0 Hz, ^4J = 1.4 Hz, 4 H, Py-H), 8.82 (ddd, ^3J = 8.0 Hz, ^3J = 7.8 Hz, ^4J = 0.9 Hz, 4 H,

[2] C. K. Bradsher and J. P. Sherer, *J. Heterocycl. Chem.* 1968, **5**, 253.
[3] T. Kamada and N. Wasada, *Synthesis* 1990, 967.
[4] C. K. Bradsher and J. C. Parham, *J. Org. Chem.* 1963, **28**, 83.

Py-H), 9.10 (dd, ^3J = 6.0 Hz, ^4J = 0.9 Hz, 4 H, Py-H); ^{13}C-NMR (100 MHz, DMSO-d_6 / D_2O 30:1): δ = 61.6 (CH_2N^+), 66.4 (CH(O**C**H_2)$_2$), 98.0 (**C**H(OCH_2)$_2$), 127.4 (CH), 128.3 (CH), 128.31 (CH), 130.1 (C_q), 132.6 (C_q), 148.5 (CH), 148.9 (CH), 153.4 (C_q).

1,4,5,8-Tetrakis{[1-(1,3-dioxolan-2-yl)pyridinium]methyl}naphthalene tetrakis(tetrafluoroborate) (4-BF). To a boiling solution of **4-Br** (239 mg, 216 mmol) in MeOH / H_2O (9 mL, 8:1) was added one drop of aq. HBF_4 (50%). The reaction mixture was cooled slowly to 4 °C and the resulting precipitate was separated. The crude product was heated in MeOH. The hot suspension was filtered, the solid was washed several times with MeOH and Et_2O and dried in vacuo, to give **4-BF** as a beige powder (139 mg, 57%); m.p. 160–163 °C. ^{1}H-NMR (600 MHz, DMSO-d_6 / D_2O 30:1): δ = 4.00 (s, 16 H, CH(OC**H**$_2$)$_2$), 6.39 (s, 4 H, C**H**(OCH_2)$_2$), 6.67 (s, 4 H, Ar-H), 6.75 (s, 8 H, CH_2N^+), 8.28 (dd, ^3J = 7.9 Hz, ^3J = 6.0 Hz, 4 H, Py-H), 8.40 (d, ^3J = 7.9 Hz, 4 H, Py-H), 8.81 (dd, ^3J = 7.9 Hz, ^3J = 7.9 Hz, 4 H, Py-H), 9.03 (d, ^3J = 6.0 Hz, 4 H, Py-H); ^{13}C-NMR (100 MHz, DMSO-d_6 / D_2O 30:1): δ = 61.0 (CH_2N^+), 65.9 (CH(O**C**H_2)$_2$), 97.4 (**C**H(OCH_2)$_2$), 127.0 (CH), 128.1 (CH), 129.4 (CH), 131.8 (C_q), 132.3 (C_q), 147.9 (CH), 148.4 (CH), 152.8 (C_q); anal. calcd. (%) for $C_{46}H_{48}N_4O_8B_4F_{16}$ × HBF_4 (1132.1): C 45.29, H 4.05, N 4.59; found: C 45.21, H 4.01, N 4.61.

10a,12a,22a,24a-Tetraazoniapentapheno[6,7-*h*]pentaphene tetrakis(tetrafluoroborate) (2). A mixture of the **4-BF** (197 mg, 0.17 mmol) and $MeSO_3H$ (2.5 mL) was heated in a teflon flask under argon-gas atmosphere. The solution was stirred at 180 °C (sand bath) for 3 days and at 220 °C for additional 3 days. After cooling the reaction mixture to room temperature, ice (8.00 g) was added carefully to the mixture followed by addition of a solution of $NaBF_4$ (750 mg, 6.82 mmol) in H_2O (5 ml). The resulting suspension was kept with an icebath at 0 °C for 1.5 h. The precipitated solid was separated, washed with cold water (2 x 10 mL) and recrystallized from water to yield compound **2** as brown crystalline solid (54.1 mg, 36%); m.p. > 270 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.51 (dd, ^3J = 7.0 Hz, ^3J = 7.7 Hz, 4 H, 3-H, 8-H, 15-H, 20-H), 8.74 (dd, ^3J = 7.7 Hz, ^3J = 8.7 Hz, 4 H, 2-H, 9-H, 14-H, 21-H), 8.94 (d, ^3J = 8.7 Hz, 4 H, 1-H, 10-H, 13-H, 22-H), 9.57 (d, ^3J = 7.0 Hz, 4 H, 4-H, 7-H, 16-H, 19-H), 10.35 (s, 4 H, 11-H, 12-H, 23-H, 24-H), 11.00 (s, 4 H, 5-H, 6-H, 17-H, 18-H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 122.9 (C_q), 124.3 (C-H, C-5, C-11, C-12, C-23), 125.7 (C-H, C-3, C-8, C-15, C-20), 127.9 (C-H, C-1, C-10, C-13, C-22), 128.0 (C_q), 133.0 (C_q), 137.7 (C-H, C-4, C-7, C-16, C-19), 138.5 (C-H, C-2, C-9, C-

14, C-21), 139.1 (C-H, C-5, C-6, C-17, C-18), 140.2 (C_q); anal. calcd. (%) for $C_{38}H_{24}N_4B_4F_{16} \times 2\ H_2O$ (919.88): C 49.62, H 3.07, N 6.09; found: C 49.20, H 3.07, N 5.99; UV/Vis (phosphate buffer): λ_{max} (log ε) = 233 nm (7.37), 264 nm (7.42), 365 nm (7.56), 415 nm (7.62); fluorescence emission (phosphate buffer, λ_{ex} = 415 nm): λ_{max} = 512 nm (ϕ_{fl} = 0.14).

3. DNA-binding studies

All buffer solutions were prepared from deionized water (Ω = 18 MW cm^{-1}) and biochemistry-grade chemicals. The buffer solutions were stored at 4 °C up to five days (cacodylate buffer) or two months (BPES buffer) and filtered through a PVDF membrane filter (pore size 0.45 μm) prior to use. The cacodylate buffer (10 mM sodium cacodylate, 100 mM LiCl or 90 mM LiCl and 10 mM KCl or 90 mM NaCl and 10mM LiCl, pH 7.2) was used for DNA melting experiments, CD-spectroscopic experiments, and intercalator displacement experiments (10 mM sodium cacodylate and 100 mM KCl). The BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0) was used for fluorimetric titrations.

Oligodeoxyribonucleotides (synthesis scale 200 nmol, purified by RP-HPLC) were purchased from Eurogentec S.A. (Seraing, Belgium); the quality was confirmed by mass spectrometric analysis data provided by the manufacturer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer; UV-visible spectra were recorded on a Varian Cary 100 double-beam spectrophotometer. For the CD-spectroscopic experiments a Chirascan CD spectrometer from Applied Photophysics was used. Concentrations of oligonucleotides are given in ODN units, i.e. mol per strand (determined by Eurogentec S.A.) and in base pairs for ds calf thymus DNA unless stated otherwise.

4. DNA melting experiments measured by FRET

Sample preparation:

The stock solutions of the oligonucleotide **F21T** (5'-fluorescein-GGGTTA-GGGTTAGGGTTAGGG-tetramethylrhodamine) (5 μM) and the ligands (50 μM) were prepared in a cacodylate buffer (10 mM sodium cacodylate, 100 mM LiCl, 90 mM LiCl and 10 mM KCl or 90 mM NaCl and 10mM LiCl, pH 7.2). The ligand solution was diluted to concentrations of 0.2, 0.5, and 1.0 μM, respectively. The samples were placed in an ultrasonic bath for 10 minutes. Subsequently, DNA (**F21T**, 5 μM) was added to obtain a DNA concentration of 0.2 μM. The samples were stirred and transferred to semi-micro quartz cells for fluorescence measurements.

Table 1. Sample Composition for FRET Measurements.

sample	*c*(DNA) / μM	*V*(DNA) / μL	*c*(ligand) / μM	*V*(ligand) / μL	*V*(buffer) / μL
1	0.2	40	0	0	960
2	0.2	40	0.25	5	955
3	0.2	40	0.5	10	950
4	0.2	40	1.0	20	940

Experimental settings:

To obtain the melting curves, the following parameters were used:

Excitation wavelength	470 nm
Emission wavelength	515 nm
Excitation slit	5 nm
Emission slit	5 nm

The following heating-cooling sequence was used:

1) heating from 20 °C to 90 °C at 2.5 °C min^{-1};

2) holding the max. temperature for 5 min;

3) cooling to 10 °C at 1.0 °C min^{-1};

4) heating from 10 °C to 99 °C at 0.2 °C min^{-1}; the fluorescence was detected during the latter ramp.

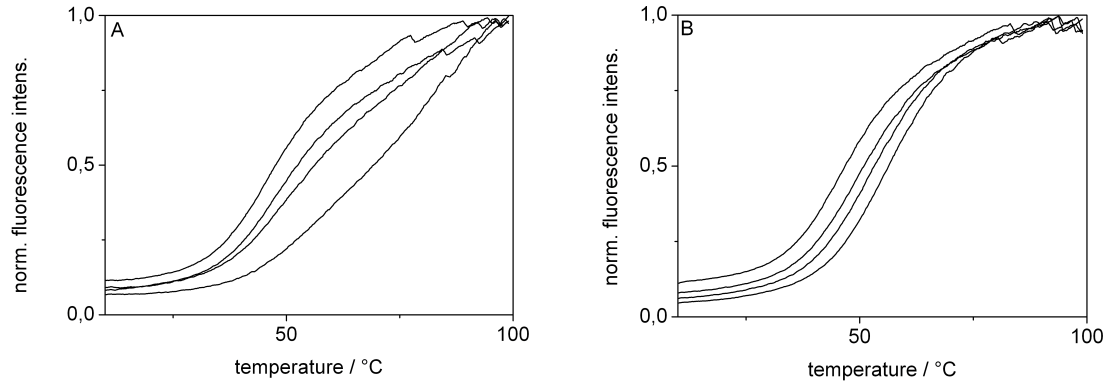


Figure S2. Melting profiles of **F21T** in the presence of **2** (A) and **1b** (B) at ligand-to-DNA ratios of 0, 1, 2.5 and 5 (detailed conditions are described above).

Determination of the melting temperature:

The normalized fluorescence intensities were plotted as a function of the temperature. The melting temperature was calculated by taking the maximum of the first derivative of the melting curve approximated by the Gaussian function. The shift of the melting temperature was calculated as $\Delta T_m = T_m$(DNA-Ligand) – T_m(DNA).

5. Fluorescent intercalator displacement titrations

Sample preparation

A solution of Thiazol Orange (**TO**, 10 µL, 50 µ M), a solution of DNA (**22AG**, *5′*-AGGGTTAGGGTTAGGGTTAGGG-*3′*, 12.5 µ L, 20 µ M), both in cacodylate buffer (100 mM KCl and 10 mM sodium cacodylate), and potassium cacodylate buffer (977.5 µ L) was equilibrated for 30 min at room temperature and transferred to semi-micro quartz cells for fluorescence measurements. Experiments were performed with double-stranded DNA (**ds17,** 5'-CCAGTTCGTAGTAACCC-3' : 3'-GGTCAAGCATCATTGGG-5') under identical conditions.

Experimental settings

To collect the data of the sample spectra following parameters were used:

Excitation wavelength	501 nm
Emission wavelength	510–750 nm
Excitation slit	5 nm
Emission slit	5 nm

After recording the fluorescence spectrum of the solution, the ligand was titrated into the solution (0.25 equivalents per titration step, after overall addition of 1.5 equivalents the amount of titrated ligand was increased). The equilibration time after each addition was 3 min.

Interpretation of the results

Every titration curve was integrated (area 510-750 nm), yielding values of the fluorescence area *FA*. These values were converted in percentage displacement *PD* (eq. 1).

$$PD = 100\text{-}[(FA / FA_0)\cdot 100] \qquad \text{(eq. 1)}$$

FA_0 is *FA* before addition of ligand. The values of PD were plotted as a function of the ligand concentration. The DC_{50} values were determined from intersect of the approximating curve with the line at PD = 50.

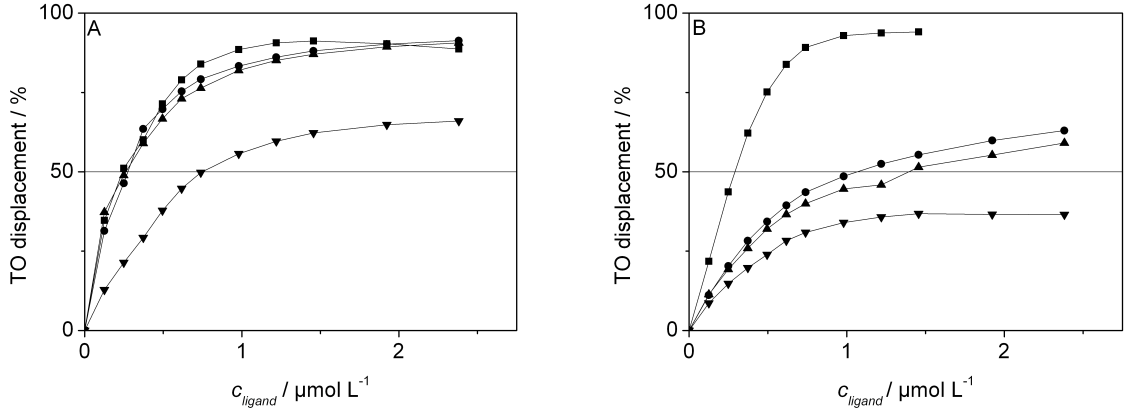


Figure S3. TO displacement from quadruplex DNA **22AG** (A) and duplex DNA **ds17** (B) for **1a** (●), **1b** (▲), **2** (▼) and **3** (■), (detailed conditions are described above).

6. Fluorimetric titrations

Sample preparation

The titrations were performed in BPES buffer at a ligand concentration of 5 µM. To avoid the dilution of the solution of the ligand during titration, the titrant solution contained the ligand at a concentration of 5 µM and DNA at a concentration of 500 µM. In fluorimetric titrations the excitation wavelength corresponded to an isosbestic point, which was determined by photometric titrations.

Experimental settings

Excitation wavelength	386 nm (**2**); 395 nm (**1b**)
Emission wavelength range	400-850 nm; 405-800 nm
Excitation slit	5 nm or 2.5 nm
Emission slit	5 nm

After recording the fluorescence spectrum of the solution of the ligand, the respective DNA or dG were added in 0.33 equivalents per step. The equilibration time after each addition was 3 min. After overall addition of 3 equivalents the volume of the aliquot was increased. When no changes in fluorescence intensity could be observed, the titration was finished.

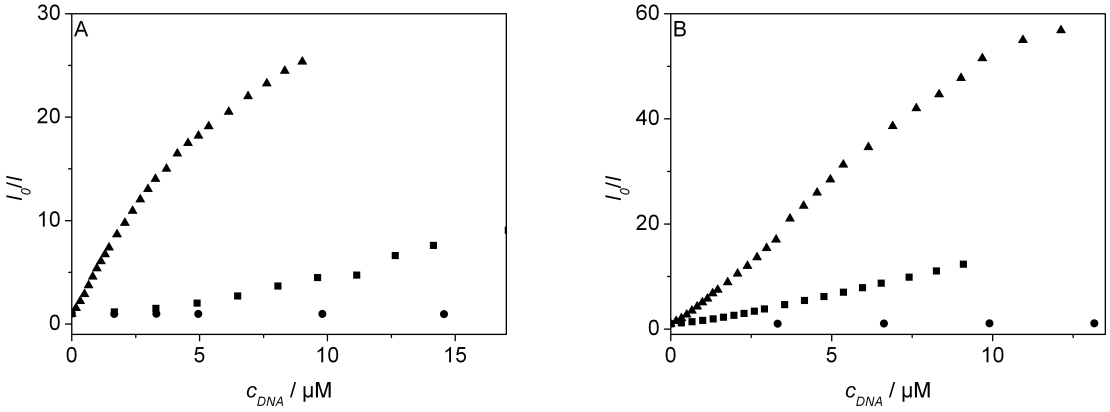


Figure S5. Stern-Volmer plot for the titrations of **1b** (A) und **2** (B) with quadruplex DNA **22AG** (▲), polydG (■) and dG(●); (detailed conditions are described above).

Determination of binding constants

The data were used to deduce the binding constants *K* of the ligands with the quadruplex DNA. For every step the fluorescence intensity *I* at a constant wavelength was determined. The values I/I_0 versus c_{DNA}/c_L were plotted and a numerical fitting to a 1:1 model (using equation 5-8) was performed to estimate the binding constant.

$$j = -(1-B)\cdot 0.5\cdot\left[A+1+x-\sqrt{(A+1+x)^2-4x}\right]+1 \qquad \text{(eq. 5)}$$

$$j = (I_0 - I)/(I_0 - I_{ende}) \qquad \text{(eq. 6)}$$

$$A = 1/K_a \cdot c_L \qquad \text{(eq. 7)}$$

$$B = I_{ende}/I_0 \qquad \text{(eq. 8)}$$

I_0 is the fluorescence intensity of the pure analyte solution, c_{DNA} ist the DNA concentration of every titration step, c_L ist the ligand concentration and I_{end} is the fluorescence intensity of the bound ligand.

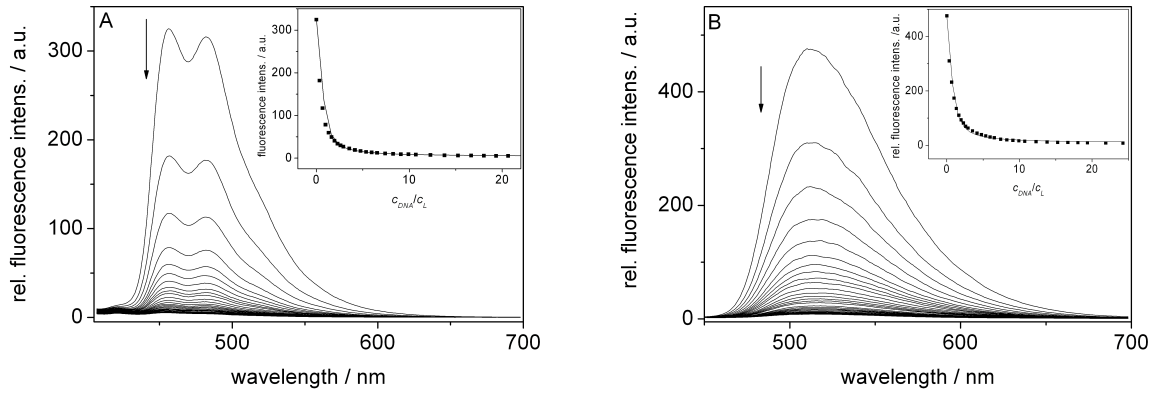


Figure S6. Spectra of the fluorimetric titrations of quadruplex DNA to **1b** (A) and **2** (B), (conditions are described above).

7. CD-spectroscopic experiments

Sample preparation

Solutions of DNA, the ligand and buffer (10 mM sodium cacodylate, 90 mM LiCl and 10 mM KCl) were measured in semi micro cells after an equilibration time of 30 minutes (Table 2).

Experimental settings

Firstly, a baseline of the buffer was collected. To collect the data of the sample spectra the following parameters were used:

Wavelength range	220–550 nm
Bandwidth	1.0 nm
Scan rate	1.0 nm/s
Time per point	0.5 s
Temperature	20 °C

Table 2. Sample Composition for CD-spectroscopic Experiments

sample	*c*(DNA) / μM	*V*(DNA) / μL	*c*(ligand) / μM	*V*(ligand) / µL	*V*(buffer) / µL
1A	20	70	0	0	280
2A	20	70	6	21	259
3A	20	70	10	35	245
4A	20	70	20	70	210
5A	20	70	40	140	140
1B	0	0	20	70	280
2B	6	21	20	70	259
3B	10	35	20	70	245
4B	20	70	20	70	210
5B	40	140	20	70	140

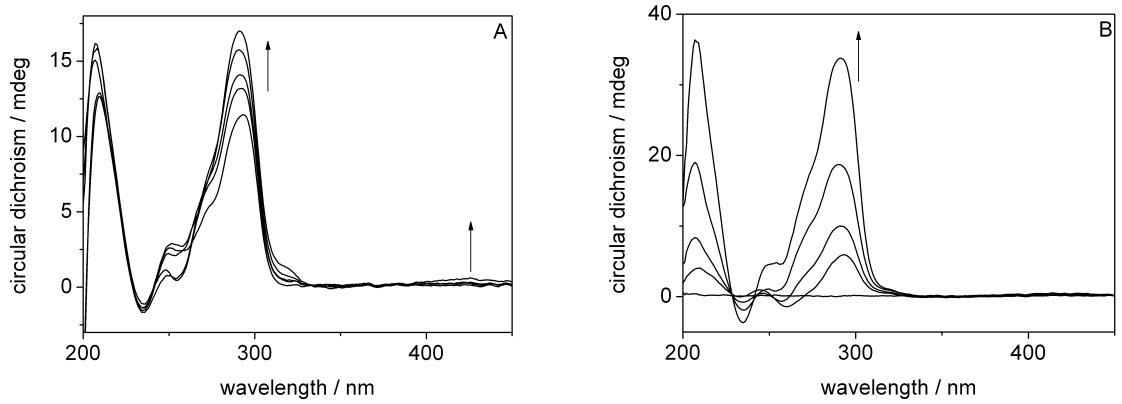


Figure S7. Spectra of the CD-spectroscopic experiments of quadruplex DNA to **1b** *r*(ligand/DNA) = 0, 0.3, 0.5, 1.0, 2.0 (A); *r*(DNA/ligand) = 0, 0.3, 0.5, 1.0, 2.0 (B), (conditions are described above).

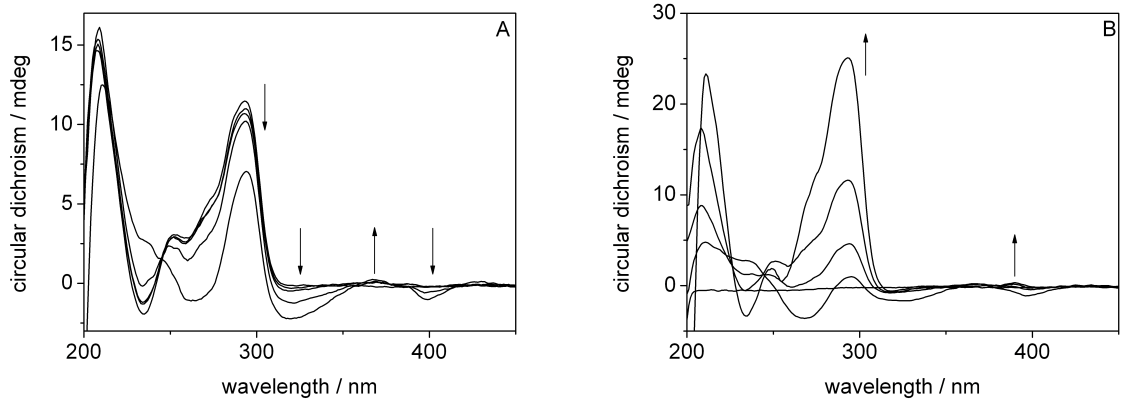


Figure S8. Spectra of the CD-spectroscopic experiments of quadruplex DNA to **2** *r*(ligand/DNA) = 0, 0.3, 0.5, 1.0, 2.0 (A); *r* (DNA/ligand) = 0, 0.3, 0.5, 1.0, 2.0 (B), (conditions are described above).

8. CD-spectroscopic displacement experiments

Sample preparation

A solution of thiazole orange (**TO**, 50 μ M), and DNA-solution (**22AG**, 20 μ M), both in cacodylate buffer (100 mM KCl and 10 mM sodium cacodylate), was equilibrated for 30 min at room temperature and transferred to semi micro cells.

Experimental settings

Firstly, a baseline of the buffer was collected. To collect the data of the sample spectra the following parameters were used:

Wavelength	220–550 nm
Bandwidth	1.0 nm
Scanrate	1.0 nm
Time per point	0.5 s
Temperature	20 °C

After recording the CD spectrum of the solution, a solution of **1b** was titrated into the analyte solution (1 or 2 equivalents per titration step). The equilibration time after each addition was 5 min.

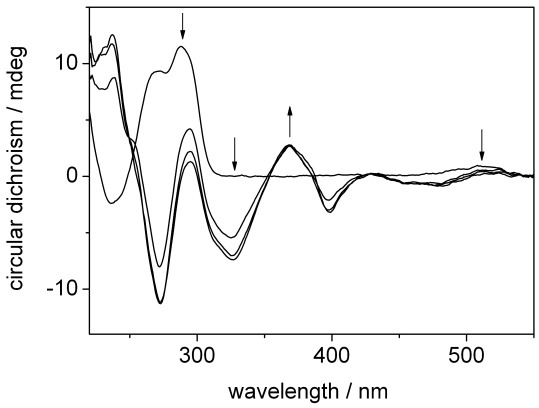


Figure S9. CD spectra of the intercalator displacement experiment with **2**; (detailed conditions are described above).